Impaired tuning of afferent excitatory synapses onto hippocampal fast-spiking interneurons by acute early life seizures

by

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Abstract

During the first year of life, newborn infants' brains have a developmentally elevated ratio of excitation to inhibition and this critical period have the highest incidence rates for ELS. Patients with early-life seizures (ELS) are often refractory to anti-epileptic drugs (AED). The current knowledge gap on the effects of seizure on the immature brain has motivated us to find novel cellular targets of ELS in the immature brain. Using a combination of electrophysiology, voltage sensitive dye imaging and immunohistochemistry in an ELS mouse model, I completed a comprehensive investigation on the effects of ELS on hippocampal interneurons in the developing brain. I demonstrated for the first time that ELS impaired the excitatory synaptic inputs into a specific subtype of interneurons, the FS interneurons. FS interneurons play an important role in mediating the excitation/inhibition balance in the neural network by providing strong and precise perisomatic inhibition to various brain regions. ELS significantly altered the probability of release and the readily releasable pools at the presynaptic membrane of the excitatory inputs into FS interneurons. This changed the short-term plasticity at these excitatory inputs and FS interneurons during repetitive excitatory activity. Additionally, ELS significantly depressed asynchronous neurotransmitter release at the excitatory inputs, resulting in impaired postsynaptic spike timing and temporal fidelity of the FS interneurons. These findings highlight ELS-induced target specific modulations in FS interneurons and provided a novel cellular mechanism by which ELS alter the immature brain and disrupt the excitation/inhibition balance.

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Abbreviations

Pentylenetetrazol	PTZ
Parvalbumin	PV
Antiepileptic drug	AED
Gamma-aminobutyric acid	GABA
N-methyl-D-aspartate receptor	NDMAR
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor	AMPAR
N-methyl-D-aspartate receptor 1	NMDAR1
N-methyl-D-aspartate receptor 2	NMDAR2
N-methyl-D-aspartate receptor 3	NMDAR3
Glutamate Receptor 2	GluR2
Post-natal day #	P # (ex. P10)
Hypoxic-ischemic encephalopathy	HIE
Hypoxic ischemic	HI
Electroencephalogram	EEG
Central nervous system	CNS
Feedforward	FF
Feedback	FB
Glutamic acid decarboxylase	GAC
Cholecystokinin	CCK
Embryonic stage #	E# (ex. E12)
Excitatory Post Synaptic Current	EPSC

Sharp Wave Ripples	SWR
Generalized epilepsy with febrile seizures plus	GEFS+
Early Life Seizures	ELS
Dravet Syndrome	DS
West Syndrome	WS
Action potential	AP
Paired pulse ratio	PP
Excitatory Postsynaptic Current	EPSC
Inhibitory Postsynaptic Current	IPSC
Excitatory Postsynaptic Potential	EPSP
Inhibitory Postsynaptic Potential	IPSP

1. Introduction

1.1 Epilepsy.

The neurological disease epilepsy was first documented by Dr. Berger in the 1920s, and has remained highly prevalent and problematic since, affecting populations of all ages. The definition of epilepsy and epileptic treatments have undergone many revisions over the years; however, due to the complexity and heterogeneity of the disease, many of the underlying mechanisms of the disease are still unknown. In 2005, the International League Against Epilepsy (ILAE) defined epileptic seizures as "a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity of the brain" (Falco-Walter et al., 2018). Electrical signals of the brain during a seizure episode have a clear beginning and end that can be detected with electroencephalogram (EEG) monitoring under a clinical setting (Falco-Walter et al., 2018). The basic definition of an epileptic patient is a person who has had two or more unprovoked seizures more than 24 hours apart; and it has been found that a single seizure will raise the likelihood of having another by more than 60% (Fisher et al., 2018). The disease is considered resolved in a patient if they are seizure-free for 10 years (Fisher et al., 2018).

The underlying etiology of epilepsy is highly diverse, and understanding these variations is crucial for effective epilepsy management and prognostic counseling. Currently there are six recognized diagnostic categories: 1) Structural, 2) Genetic, 3) Infectious, 4) Metabolic, 5) Immune and 6) Unknown (Fisher et al., 2018). Epilepsy is also classified into different types, as follows: 1) Focal 2) Generalized 3) Combined generalized and focal 4) Unknown (Fisher et al., 2018). This classification system applies to seizures in adults and children but excludes infantile seizures in which a separate classification is used (Falco-Walter et al., 2018). Currently our scientific

understanding of the etiologies and types of seizures is insufficient for a classification based on underlying neurological manifestations of seizures. The current classification system is mainly based on physical seizure signs and symptoms (Fisher et al., 2018).

As the medical and research fields gain more knowledge on the disease, epilepsy is now commonly identified as a disease beyond seizures due to the numerous associated comorbidities and cognitive deficits (Salpekar and Mula, 2018). Recently, various population and animal studies have begun to identify the negative adverse effects of a single or recurrent seizure on the neuronal network (Katsarou et al., 2017; Lee et al., 2000; Minjarez et al., 2017; Oh et al., 2017). Common comorbidities with epilepsy, including mood disorders (e.g. depression) and cognitive deficits (e.g. learning and memory deficits), are often reported to be more detrimental to the quality of life than recurrent seizures themselves (Salpekar and Mula, 2018). Recent clinical findings noted that around 60% of adult patients can be effectively treated with a single antiepileptic drug (AED) if they are accurately diagnosed in a timely manner, and the other 40% who do not respond to initial treatment, may require polytherapy (Ben-Menachem et al., 2010). The success rate of epilepsy management overall is on the incline, due to better diagnostic tools/techniques. However, further research into the mechanisms and pathophysiology of the disease is required, in order to develop more effective AEDs.

1.1.1 The Immature Brain & ELS.

The early life period is characterized as a period of maximal synaptogenesis and plasticity, which is in part modulated by a developmentally heightened ratio of excitation to inhibition in the immature brain (Sanchez and Jensen, 2001). The glutamate receptors, N-methyl-D-aspartate receptor (NDMAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) are transiently overexpressed in the immature brain, and are thought to contribute to

the high level of synaptic plasticity in the developing neuronal circuits (Silverstein and Jensen, 2007). In the remainder of this introduction, the brain regions of focus will be in the neocortex and the hippocampus, unless stated otherwise.

The subunit composition of these receptors is also different in the mature brain versus the immature brain. The NMDAR is a tetrameric complex that consists of many different possible subunit compositions. The subunits of NMDARs are: NR1, NR2 (A-D), and NR3 (A-B) (Pachernegg et al., 2012; Sanderson et al., 2016). The expression of NMDAR peaks in the first postnatal week in the neocortex and the hippocampus (Pachernegg et al., 2012). In the immature brain, NMDAR with NR2B subunit is preferentially expressed over the NR2A found in adult brains (Silverstein and Jensen, 2007). NMDAR with NR2B subunit is found to facilitate longer current decay time, which leads to a higher potential for depolarization and excitability (Sanderson et al., 2016). The NR2C, NR2D and NR3A subunits expression is also increased in the first two weeks of life in the immature brain, which results in a reduced sensitivity to magnesium blockade (Silverstein and Jensen, 2007).

The AMPAR is also a tetrameric complex, composed of a combination of 4 subunits, glutamate receptor 1-4 (GluR1, GluR2, GluR3, or GluR4), and is often associated with fast-acting excitatory synaptic transmission. AMPAR expression peaks in the second postnatal week (Hanley et al., 2018; Wang et al., 2010). In adults, most AMPAR contain the GluR2 subunit and are calcium impermeable. However, Ca^{2+} permeable GluR2-lacking AMPARs have larger single channel conductance that mediates larger postsynaptic potentials than their GluR2 containing counterparts. GluR2-lacking AMPARs are preferentially expressed in the immature brain (Sanchez and Jensen, 2001). Therefore, the two developmentally regulated excitatory ionotropic receptors, unique to the immature brain, together mediate enhanced excitability in the neuronal network.

Consequently, various studies have also reported differences in metabotropic glutamate receptors, gamma-aminobutyric acid (GABA) receptors and voltage-gated ion channels in the immature brain (Sanchez and Jensen, 200; Silverstein and Jensen, 2007; Zilberter, 2016). All these factors result in an increased ratio of excitation to inhibition, which also increases the immature brain's susceptibility to seizures.

Additionally, other early life risks can cause the onset of seizures; a common one being hypoxic-ischemic encephalopathy (HIE), which is likely to occur during complications at birth (Kasahara et al., 2018; Ronen et al., 1999). In a population study conducted by Ronen et al., in 1999, they found that around 42% of infants had seizures following an HIE event. Other common causes of ELS include infections of the central nervous system, maternal drug abuse and rare epilepsy syndromes (Volpe, 2001). Also, the prevalence rate of seizures in preterm infants is much higher than full-term infants and these seizures tend to be less responsive to conventional AEDs (Spagnoli et al., 2018).

Currently, AED treatments that are relatively effective for adults have much lower success rates in treating ELS (Silverstein and Jensen, 2007; Van Rooji et al., 2013). This is likely due to the physiological and functional differences between the immature brain and the mature brain. Novel AED therapeutics that take into account the unique physiology of the immature brain are much needed in order to increase the efficacy rate of treatments and decrease the side effects.

1.1.2 Diagnosis.

Recent studies have shown that the duration and the number of seizures is highly correlated with adverse prognosis, suggesting that fast and accurate diagnosis is crucial for effective ELS treatments (Kasahara et al., 2018). The most common clinical diagnostic tool for ELS is the use of non-invasive electroencephalogram (EEG) technology to detect distinct electrographic features of

ELS (Spagnoli et al., 2018; Fisher et al., 2018). EEG has been used for decades as a tool to detect and diagnose seizures and still remains as one of our best tools for detecting abnormal neuronal activity and predicting outcomes (Rowe 1985; Spagnoli et al., 2018). Even after the diagnosis and treatment of seizures, it is often recommended to continue EEG monitoring in order to quantify "seizure burden" and gain better prognosis information in infants (Insel et al., 1990; Lawrence and Inder, 2010; Spagnoli et al., 2018). The use of EEG is relatively safe; however, the cost of EEG equipment and maintenance, as well as the variability in EEG result interpretation by the clinical staff, present some limitations (Lawrence and Inder, 2010). Other forms of neuroimaging approaches used to diagnose and monitor seizures in infants include cranial ultrasound scans and MRI scans (Spagnoli et al., 2018).

1.1.3 Treatments.

Treatment of ELS is a highly debated topic, especially since the early life period is critical for synaptogenesis and neuronal development; therefore, is extremely sensitive to perturbations. A major concern is whether the use of AEDs, especially prolonged AED treatment, can impair normal activity-dependent development of the immature brain and result in greater adverse burdens than the seizures themselves (Kaushal et al., 2016; Sanchez and Jensen, 2001). This is especially controversial since the success rate of AED is relatively low in infants (Sanchez and Jensen, 2001).

Currently, AEDs used to treat ELS are largely the same as those used to treat adults. These drugs have three main mechanisms of action: (i) inhibit repetitive neuronal firing by blocking voltage-dependent sodium channels (e.g. Phenytoin) (ii) enhance GABA inhibition through various mechanisms (eg. Benzodiazepines and phenobarbital) and (iii) blockade of glutamatergic excitatory transmission (e.g. Valproate) (Bezaire et al., 2013; Bittigau et al., 2002; Forcelli et al.,

2011). According to the World Health Organization guidelines, phenobarbital is the first-line AED treatment (Bezaire et al., 2013; Bittigau et al., 2002; Forcelli et al., 2011). Benzodiazaepine, phenytonin or lidocaine will be used as the second-line agent in the scenario that a maximum dose of phenobarbital is ineffective at stopping the seizures (Bezaire et al., 2013; Bittigau et al., 2002; Forcelli et al., 2011). Studies have shown that less than 50% of ELS were suppressed by phenobarbital and phenytoin (Silverstein and Jensen, 2007; Kasahara et al., 2018). If an infant does not display seizures for more than 72 hours, indicated by EEG monitoring, then AED treatment can be stopped.

Various animal studies have found that these AEDs may cause apoptotic neurodegeneration in various regions of the developing brains at plasma concentrations equivalent to the dose used for seizure control in humans. (Bittigau et al., 2002; Forcelli et al., 2010; Forcelli et al., 2011). Lidocaine has been reported as an effective treatment for refractory ELS, but there are concerns with cardiac toxicity at the loading dose (Silverstein et al., 2007). Recently newer antiepileptic medications, i.e. levetiracetam, have been tested clinically; however, their safety and pharmokinetic data are lacking (Komour et al., 2014; Lawrence and Inder, 2010). There has been some progress on understanding the developmental mechanisms underlying seizure responsiveness to AEDs. However, the treatment of ELS has changed significantly over the last few decades, and currently there is no definitive consensus for the best choice in regard to AED treatments (Silverstein and Jensen, 2007; Van Rooji et al., 2013).

1.1.4 Prognosis and Comorbidities.

Population studies have shown that seizure-induced infant mortalities have decreased; however, the comorbidities of epilepsy have increased. (Forcelli et al., 2011; Salpekar and Mula, 2018; Sanchez and Jensen, 2001) It is estimated that around 3 in 1000 infants are affected by ELS

and 16% of those infants will experience later life epilepsy and/or various other comorbidities that are in part mediated by the seizures (Sogawa et al., 2001; Oh et al., 2017). Preterm infants, especially those with low birth weight, have higher risks of poorer prognoses (Van Rooji et al., 2013; Silverstein and Jensen, 2007; Spagnoli et al., 2018). Additionally, patients that experience ELS are found to be at a much higher risk for developing epilepsy, intellectual disabilities, delayed milestones, and mental retardation in comparison to those with later onset seizures (Oh et al., 2017). Children and adolescents with later onset seizures are more at risk for developing psychiatric and behavioral disorders, mood disorders, migraines and headaches (Takano et al., 2014). The prognoses of ELS and their associated comorbidies are difficult to predict but have been shown to be highly dependent on the underlying etiology of the seizures; time of diagnosis, response to treatment, severity of seizures, proper EEG monitoring and the rate of recurrence of seizures (Rowe, 1985). From population studies, it has also been found that hypoglycemic seizures occurring in the first week of life, or simple febrile seizures in childhood, have a benign effect on later-life cognitive functions (Sanchez and Jensen, 2001). However, seizures caused by cerebral dysgenesis and global hypoxia-ischemia are associated with poorer developmental outcomes (Silverstein and Jensen, 2007). Finally, the number of seizure episodes could possibly determine the prognosis of the patient: for instance, prolonged febrile convulsions in a small fraction of infants have been found to develop acute hippocampal edema and delayed hippocampal atrophy (Chang et al., 2008).

Certain underlying etiologies of seizure could induce additional neuronal injury, those being: 1) the increase in brain temperature and metabolic demands, 2) the generation of mediators (e.g. Reactive Oxygen Species) and 3) the disruption of endogenous proteins and repair

mechanisms (Silverstein and Jensen, 2007). Protective measures against these factors could have potential beneficial effects on the prognosis of the patient.

1.1.5 Animal models.

Due to the many confounds and limitations of clinical/population studies, animal models provide a great platform to investigate the underlying molecular and cellular mechanisms of epilepsy. In addition, the latent period before the occurrence of a seizure, and to explore novel targets for potential treatments (Kasahara et al., 2018). A key limitation faced by virtually all models is the precise correlation of developmental stages between immature rodents and humans, as well as the translational accuracy from the animal model to the disease phenotypes in humans (Silverstein and Jensen, 2007).

Seizure Induction Methods. The accuracy and usefulness of the animal model employed to study ELS is highly dependent on the developmental stage at which it occurs. In an across-species comparison, age P8-10 mice can be compared to full term human newborn and are often used to study ELS (Forcelli, et al., 2010). For the purpose of studying the mechanisms and pathophysiologies of seizures alone, seizures are often induced by excitotoxins (e.g. Pentylenetetrazol) or by kindling (Fricker and Miles, 2000). Chemoconvulsants often act on the excitatory or inhibitory pathway as an AMPAR agonist or GABA(A)R antagonist (Adesnik and Nicoll, 2007; Khoshkhoo, et al., 2016; Kleen, et al., 2012). These agents have been proven to reproduce the EEG signatures of a seizure, state of chronic neuronal hyperexcitability, and various epilepsy-associated cognitive and behavioral defects (Khoshkhoo, et al., 2016). The cognitive impacts of both prolonged and acute seizures do not appear to be model specific, indicating that the seizure induce pathologies may be a universally attribute to early onset seizures (Fricker and Miles 2000).

Kindling is another commonly utilized method to induce seizures by repeated electrical stimulation or through repeated chemical kindling at subconvulsive doses (Adesnik and Nicoll, 2007). The kindling method induces sub-seizure levels of hyperactivity in the brain over a period of time, until the maximal seizure threshold is reached (Adesnik and Nicoll, 2007). It has been noted that seizure thresholds to chemoconvulsants and kindling are much lower in the neonatal animals (Cossart, et al., 2001).

Key Findings from Animal models. Through these animal models, researchers have gained key insights into ELS. Although the immature brain has a greater propensity for seizures, in many animal models, seizure induced cell death was found to be non-existent or present to a much less extent compared to the adult rodent brain (Kasahara et al., 2018; Sanchez and Jensen, 2001). Severe seizures often induce extensive cell death in the mature hippocampus, amygdala and entorhinal cortex (Sanchez and Jensen, 2001; Silverstein and Jensen, 2007). This observation initially led to the conclusion that the immature brain was inherently protected against seizure induced neuronal injury and cell death (Sanchez and Jensen, 2001; Silverstein and Jensen, 2007; Lawrence and Inder, 2010; Minjarez et al., 2017.) However, as we continually gain more knowledge of the disease, this theory seems highly implausible.

Although the immature brain seems to be less vulnerable to seizure-induced neuronal death, with some exceptions, recurrent and even acute seizures can permanently alter the neuronal network in several ways. These changes can be broadly characterized into three categories: 1) physical dendritic and neuronal alterations 2) more subtle molecular and receptor reorganizations and 3) network connectivity and plasticity changes (Botterill et al., 2017; Lawrence and Inder, 2010; Minjarez et al., 2017; Swann, 2002).

A recent study has found that seizures altered the subunit compositions of the ionotropic glutamate receptors, NMDAR and AMPAR (Sogawa et al., 2001). In the CA3 region of the hippocampus, the number of GluR1 subunit of the AMPARs in P20 rats and NR1 subunit of NMDARs in P35 rats were increased after recurrent seizures (Sogawa et al., 2001). Another study has found similar changes at the glutamatergic synapses after a single acute seizure (Cornejo et al., 2007). Seizure induced changes in the synaptic potency, mediated by AMPAR of pyramidal cells in the CA1 region of the hippocampus, are reportedly increased in the immature brain (Rakhade et al., 2008). This was associated with the rapid increase in phosphorylation at GluR2 S845/S831 and GluR2 S880 sites due to the increased activity of various protein kinases that mediate the phosphorylation of these AMPAR subunits (Rakhade et al., 2008). In addition, other studies have also investigated the effects of ELS on the inhibitory network and interneuron population (Andre et al., 2001); neuronal connectivity (Silverstein and Jensen, 2007); pathological alterations in oscillatory patterns (Cornejo et al., 2007); and changes in long-term potentiation (LTP) and long-term depression (LTD) (Cornejo et al., 2007). In a study conducted by Jensen et al., in 1998, ELS was found to induce long-lasting changes in the inhibitory synaptic transmission in rat hippocampal slices, resulting in long-lasting hyperexcitability in pyramidal cells. This chronic increase in excitability could substantially lower the seizure threshold and increase the likelihood of epileptogenesis (Jensen et al., 1998). However, the research in the underlying mechanisms of ELS is far from complete and extensive future research is required to clarify the overall effects in an immature brain.

1.2 Inhibitory Interneurons

The GABAergic inhibitory interneuron population is a highly diverse and dynamic system that provides the majority of the inhibition in numerous brain regions. A hallmark of interneurons is their functional and morphological diversity (Roux and Buzsaki, 2015). The numbers may vary depending on brain region, but overall interneurons constitute 10-20% of the total number of cortical neurons (Magueresse and Monyer, 2013). Different subcategories of interneurons target different regions of excitatory pyramidal cells and other interneurons, and these subgroups have highly diverse division of labour, functioning to control and orchestrate the gain, timing, tuning, and bursting properties of their postsynaptic cell targets (Butler and Paulsen, 2015; Cardin et al., 2009; Chiovini et al., 2014; Roux and Buzsaki, 2015). In the hippocampus alone, at least twenty different subtypes of interneurons have been identified; however, categorization and discovery of new interneuron subtypes is still an active area of research (Roux and Buzsaki, 2015). The entire surface of the pyramidal cell membrane, from the tips of the dendrites to the axon hillock, is covered by innervations from different subgroups of interneurons (Magueresse and Monyer, 2013). At the network level, via their temporally and spatially precise dynamic interactions, interneurons form a comprehensive neuronal assembly for efficient transmission of information and routing of excitatory and inhibitory signals, usually in the form of brain oscillations (Roux and Buzsaki, 2015).

The neurotransmitter GABA is the primary inhibitory neurotransmitter synthesized by nearly all cortical interneurons. GABA evokes fast inhibition via ionotropic GABA(A)R conductance of the anion chloride. Glutamic acid decarboxylase (GAD) is a major enzyme involved in the synthesis of GABA by converting glutamate into GABA (Brooks-Kayal et al., 2001). GAD is expressed in two isoforms with different functions, GAD65 and GAD67 (Andre et al., 2001). GAD67 is inherently active, producing around 90% of all CNS neurotransmitter GABA,

and is expressed in the cell bodies of inhibitory interneurons (Brooks-Kayal et al., 2001). GAD65 is only transiently active in the axon terminals during periods of intense network activity and facilitates rapid conversions of glutamate to GABA (Brooks-Kayal et al., 2001). Therefore, GAD65 antibodies are a good indicator for transient fluctuations of GABA levels and GAD67 antibodies are more commonly used for interneuron quantification and indication of interneuron survival or loss (Brooks-Kayal et al., 2001).

The interneuron populations provide inhibition to the pyramidal cells in various different ways that can be categorized into feedforward (FF) and feedback (FB) inhibition. FF inhibition is a form of disynaptic inhibition, where the interneuron receives afferent excitatory inputs and exerts inhibitory outputs onto pyramidal cells downstream (Buzsaki, 1984; Isaacson and Scanziani, 2011). FF inhibition influences the precision of spike timing in pyramidal cells (Roux and Buzsaki, 2015). There are several other mechanisms in place that shorten the time in which a pyramidal cell could respond in order to achieve high temporal precision of evoked spikes. Two key mechanisms include a lowered firing threshold in subpopulations of interneurons and larger excitatory synapses on interneurons (Buzsaki, 1984). Long-term changes in FF afferent paths have been found to cause deficits in temporal associative memory (Buzsaki, 1984).

FB inhibition (or recurrent inhibition) is initiated by the firing of pyramidal neurons, which would recruit the downstream postsynaptic inhibitory interneuron (Isaacson and Scanziani, 2011; Roux et al., 2013). In turn, interneurons send inhibitory outputs back to the pyramidal cell that the interneuron had initially received excitatory inputs from. FB inhibition allows the interneuron to modulate excitation and prevent excessive discharge from pyramidal cells in their neural network. This form of inhibition is prevalent in groups of interneurons associated with pacemaker activities (Brooks-Kayal et al., 2001). Another related form of inhibition is known as lateral inhibition where

an interneuron, activated by an excitatory pyramidal cell, inhibits the activity of a similar type of pyramidal cell (Isaacson and Scanziani, 2011). This form of inhibition plays a critical role in neuronal group selection and assembly competition (Isaacson and Scanziani, 2011).

In summary, the different forms of inhibition modulate the activity of the pyramidal cells through different distinct mechanisms of actions. FF inhibition provides a precise temporal integration of excitatory inputs by limiting the postsynaptic pyramidal cells' excitatory postsynaptic currents (EPSC) summation, while FB inhibition modulates local excitatory output and can result in the generation of neuronal oscillation.

Currently, the exact inhibitory contacts and circuits across different subgroups of interneurons are not fully elucidated due to their extensive and diverse nature. Future computational modeling and imaging are needed to disentangle the complexity of these circuits.

1.2.1 Types of interneurons.

The classification of the highly functional and physiologically diverse GABAergic interneuronal populations is a key challenge in contemporary neuroscience. To date, no widely accepted taxonomy exists (Roux and Buzaki, 2015). The current classification schemes are typically based on genetic, morphological, functional or developmental characteristics (Roux and Buzaki, 2015). Different classifications can be considered based on the nature of the research or on the level of understanding as well as on the region of focus, since interneuron populations vary greatly between different brain regions. Since the main function of interneurons is to modulate excitatory pyramidal neuron activity, a classification scheme based on their axonal targets will be elucidated. Unlike pyramidal neurons that commonly form only a single synaptic contact with their postsynaptic targets, interneurons tend to form connections with multiple downstream targets, making them even more difficult to categorize (Bezaire and Soltessz, 2013; Cossart et al., 2001).

A common categorization scheme focuses on the postsynaptic neuron region that interneurons preferentially target. This consists of four main divisions, 1) the perisomatic group, 2) the dendrite-targeting group, 3) the interneuron specific group and 4) the long-range group (Botterill et al., 2017).

The perisomatic group is the largest family of interneurons comprised of basket cells (Fast-spiking (PV+) and CCK cells) and axo-axonic (chandelier) cells. This family of interneurons controls the spiking output of pyramidal cells and is critical for the precise timing and synchrony of pyramidal cells' outputs by implementing strong inhibition onto the axon hillock of their pyramidal cell targets. This in turn generates gamma and other oscillations throughout the neuronal network (Freund and Katona, 2007).

The second group of interneurons are the dendritic targeting interneurons, which are divided into subgroups that innervate the same dendritic domains (Cornejo et al., 2007). This makes this group highly variable and the contributions of these interneurons are not well understood. Overall, these interneurons control the strength and plasticity of dendritic glutamatergic inputs to various domains (Freund and Katona, 2007). An example of dendritic-targeting group is the somatostatin interneurons (Cossart et al., 2001).

The third group are the interneuron-specific interneurons. These cells synapse onto other interneurons but avoid pyramidal cells, thereby exerting indirect effects on pyramidal cells by modulating their level of inhibition (Cossart et al., 2001).

The final group of interneurons is the long-range group (Botterill et al., 2017). They are morphologically diverse and can have axon trees spanning across two or more anatomical brain regions. Due to their extensive axonal reach, they provide quick communication between different cortical regions of the brain (Botterill et al., 2017).

Understanding the great diversity of these interneurons and their functions is a challenge that has baffled researchers for decades. Although we are far from a complete picture of this convoluted network, recent studies have shown that innervations are far from random, but rather pertain to highly specific sub-circuits.

1.2.2 Fast-spiking basket cells.

FS (i.e. PV+) interneurons are often identified by their unique action potential patterns, and by the expression of the Ca2+- binding protein parvalbumin (PV) (Hu et al., 2010). They are known for their unique ability to convert excitatory input into strong and precise inhibitory output signal within milliseconds due to their fast-spiking action potentials (Hu et al., 2010). Recent research has found that these interneurons play a much more complicated role than simple inhibition, including critical regulation of neural circuit dynamics, oscillations and maintenance of the excitatory-inhibition balance throughout the brain (Botterill et al., 2017; Chevaleyre and Piskorowski, 2014). Improper function of these interneurons is associated with a variety of diseases, including epilepsy (Andre et al., 2001; Del Pino et al., 2013; Shiri et al., 2014). To better understand the properties of this subgroup of interneurons, we will review their morphology, development, synaptic properties, and their functional roles in the hippocampal circuit.

Morphology of PV Interneurons. The morphology, dendritic input, and axonal output of FS interneurons are quite notable. In 1999, Gulayas and others used light microscopy to map out the complete dendritic trees of three functionally distinct interneuron groups, one of them being FS interneurons. The geometry, total length and laminar distribution of these interneurons were identified. It was found that FS interneurons possessed the most extensive dendritic tree (4,300 μm) with the thickest dendrites, and also had the largest soma diameter, in comparison to the other two groups of interneurons studied. More notably, the density of afferent excitatory and inhibitory

inputs was several times higher on FS interneurons than the two other interneuron groups. The majority of afferents were excitatory inputs (93.6%) (Cossart et al., 2001). The large laminar distribution of the FS interneuron confers them the ability to receive inputs from a large population of pyramidal cells. The axon of the FS interneurons typically shows extensive arborization up to 46mm in the hippocampal CA1 region, allowing the FS interneurons to generate a wide and divergent inhibitory output (Chiovini et al., 2014; Cornejo et al., 2007). The morphology of FS interneurons alone suggests that this group of interneurons can integrate a large number of excitatory inputs and generate powerful and widespread inhibitory outputs.

Parvalbumin Protein in Development. In animal rodent models, the protein PV is highly specific to FS interneurons and is an effective marker of this unique population of interneurons. However, this protein is not universally expressed during the pre- and post-natal periods, and its expression follows a developmental trajectory. The protein PV can first be detected during embryonic day 13 (E13) in the rat brain in highly localized areas and will be sequentially expressed rapidly during the following days (Solbach and Celio, 1991). In the hippocampus, the PV protein is first detected in postnatal animals at P7-10 (Roux et al., 2013) and lags behind the development of GABA immunoreactivity by around one to two weeks (Solbach and Celio, 1991). The onset of PV expression is correlated with the maturation of the cell's physiological activity and in the cerebellum, it coincides with the arrival of excitatory synaptic input and onset of spontaneous activity (Solbach and Celio, 1991).

Perisomatic Inhibition. Perisomatic inhibition is defined as inhibitory output that targets the region or nearby regions of the axon hillock of the downstream neuron. This form of inhibition is provided mainly by FS interneurons and Cholecystokinin (CCK) interneurons in numerous brain regions, such as the hippocampus and the cortex (Freund and Katona, 2007; Takacs et al., 2015).

The two populations of interneurons, also commonly coined as “basket cells” have unique roles in the network oscillations associated with proper cognitive functions: FS interneurons are generally thought to control rhythm through high-frequency presynaptic inhibition; and CCK interneurons play more of a fine-tuning role through asynchronous presynaptic action potentials (AP) (Evstratova et al., 2014; Freund and Katona, 2007). Common pathologies associated with disruption in these subgroups of interneurons include, but are not limited to, epilepsy (FS interneurons) and anxiety (CCK+ interneurons) (Freund and Katona, 2007). In the neocortex, around 25-30% of inhibitory boutons are provided by the basket cells, whereas in certain regions of the hippocampus, the percentage can be as high as 80% (Freund and Katona, 2007). FS interneurons are found to receive as much as three times more local glutamatergic inputs and 7.5 times larger EPSC peak amplitude than their perisomatic peer, the CCK interneurons (Freund and Katona, 2007). The large synaptic events of the FS interneurons are generated through cell-type specific unique postsynaptic glutamate receptor compositions (Fuchs et al., 2007). Also, FS interneurons’ short membrane time constants, and the unique dendritic potassium channels, allows these cells to fire fast AP and faithfully follow high frequency repetitive stimulations (Freund and Katona, 2007).

1.2.3 FS Interneuron: in vivo and in vitro experiments.

One of the key roles of the hippocampus is to facilitate the cognitive processes related to short-term and long-term memory consolidation in order to encode higher order representations of the external world (Roux and Buzsaki, 2005). The hippocampal network activity and function is generated by oscillations through a complex interplay between excitation and inhibition. Within the hippocampus and neocortex, there are three well-characterized types of oscillations based on their frequency phenotypes (Butler and Paulsen, 2015). The first type is the rhythmic activity

generated in the theta frequency range (4-12 Hz) (Stark et al., 2013). This oscillation often occurs during movement and rapid eye movement during sleep in mammals (Butler and Paulsen, 2015; Stark et al., 2013). Faster frequencies ranging between 30-100Hz are characterized as gamma oscillations, which can be further divided into slow gamma (40Hz), medium gamma (80Hz) and fast gamma (120Hz) (Butler and Paulsen, 2015). The behaviour patterns associated with gamma waves overlap with those associated with theta waves. This also include navigation sensory associations, short-term memory maintenance, and certain functions implicated in some models of working memory (Butler and Paulsen, 2015; Fuchs et al., 2007). These two oscillations occur during a high cholinergic tone and are thought to prime the hippocampus for an 'online processing' mode (Stark et al., 2013). During low levels of acetylcholine levels, present in rest- and slow-wave sleep, irregular bursts of high frequency activities known as sharp wave ripples (SWRs) are observed. SWRs are associated with reactivation of neuronal ensembles and are thought to be critical in information processing and consolidation in the hippocampus, which is then propagated to the neocortex for long-term memory formation (Butler and Paulsen, 2015; Chiovini et al., 2014). SWRs' activation originates from the hippocampal CA3 region and it has been observed that in vitro acute hippocampal slices can generate spontaneous SWRs (Butler and Paulsen, 2015). SWRs are generated by transforming input activity of CA3 neurons downstream to neuronal outputs clustered into small dendritic subunits called the "hot spots", which help form synchronized cell assembly patterns (Chiovini et al., 2014). It is highly likely that perisomatic interneurons can initiate these oscillations, suggested by the recent finding that perisomatic interneuron firing precedes the sequential activation of excitatory cells (Chiovini et al., 2014). These network oscillations could be detected using various neural imaging tools such as EEG, and can also be recorded through electrophysiology as local field potentials that reflects ion level changes within

the extracellular regions of the brain (Butler and Paulsen, 2015). Each region of the hippocampus contains its own oscillation generator and the information is then transferred to downstream regions (Butler and Paulsen, 2015). In human EEG recording studies of the hippocampus, significant reduction in different oscillation power is associated with specific hippocampal memory and cognitive deficits (Fuchs et al., 2007).

Hippocampal FS interneurons are also found in the dentate gyrus, stratum oriens and pyramidal layers of the CA3-CA1 subfields of the hippocampus. Input synapses from all layers of the hippocampus are received by these interneurons (Botterill et al., 2017). In order to study the role of these interneurons in shaping higher hippocampal functions, numerous *in vivo* and *in vitro* experiments have been conducted. In *in vivo* study of FS interneurons, found that the activity of FS interneurons in the hippocampus changes in accordance with change during the theta, gamma and SWRs network oscillations (Lapray et al., 2012). This study observed that during theta oscillations, action potential frequency was markedly increased; and that during SWRs, the firing frequency was increased by more than one order of magnitude (Lapray et al., 2012). *In vivo* manipulations and interference of FS interneurons is also highly effective at correlating the roles of these interneurons to different neuronal oscillations. Utilizing contemporary experimental techniques/tools, such as optogenetics, researchers are able to transiently enhance or inhibit the function of specific cell groups with high temporal and spatial precision (Magnus et al., 2011). Stimulation of only FS interneurons at different frequencies was able to induce theta spikes in the CA1 pyramidal cells and gamma spikes in the somatosensory cortex (Cardin et al., 2009; Stark et al., 2013). Furthermore, Fuchs et al., in 2007 found that reduced excitatory drive into FS interneuron excitatory drive resulted in persistent reduction of gamma oscillation power in CA3 and abnormalities in spatial working memory and novel object exploration in mice. FS

interneurons were also found to be a key player in generating and regulating SWRs (Chiovini et al., 2014). Through 3D-photon imaging and caged glutamate experimental techniques, FS interneuron dendrites were found to be critical in generating calcium spikes originating from the previously mentioned dendritic "hot spots" (Chiovini et al., 2014). These findings highlight only some of the critical roles that FS interneurons play in neuronal network oscillations (Fuchs et al., 2007; Chiovini et al., 2014; Freund and Katona, 2007; Stark et al., 2013; Cardin et al., 2009). Permanent knockdown of FS interneuron function through genetic ablation of AMPAR or NMDAR is also highly effective at studying disease models associated with the dysfunction of one subgroup of interneuron (Roux and Buzsaki, 2005). Disease models associated with FS interneuron dysfunction will be highlighted in the following section.

1.2.4 Interneuronopathies.

Interneuronopathies, first proposed by Kato and Dobyns in 2005, as a term to describe disorders that result from improper development, migration or function of interneurons (Kleen et al., 2012). Currently, interneuronopathies, such as the deregulation of specific interneuron regulatory genes, have been found to be linked to several early-life disorders such as epilepsy and several other neurodevelopmental disorders (Katsarou et al., 2017; Marin, 2012). Examples of interneuronopathy-induced epileptic syndromes include West syndrome (WS), DS, generalized epilepsy with febrile seizures plus (GEFS+) and lissencephaly (Katsarou et al. 2017). Beyond epilepsy, diseases caused by interneuron dysfunction include autism spectrum disorders (ASD) and schizophrenia (Katsarou et al., 2017; Marin, 2012). Much insight on the functions of interneurons can be gained from studying interneuronopathies in both human and animal models.

A severe form of drug refractory epilepsy is present in 70-80% of DS patients (Katsarou et al. 2017). DS results from the missense mutation of SCN1A gene (Katsarou et al. 2017). The SCN1A

gene encodes for the α-subunit of the neuronal voltage-gated sodium ion channel, type1 (NaV 1.1), and loss of function of this channel has been found to severely and selectively impair the sodium currents in hippocampal interneurons and prevent them from firing AP (Katsarou et al. 2017). No changes are found in excitatory pyramidal cells' activity (Catterall et al., 2010). The missense mutation in NavV 1.1 is also associated with the development of GEFS+ (Katsarou et al. 2017). A mouse model studied by Dutton et al. (2012) showed that the selective deletion of SCN1A gene in only FS interneurons replicated the DS disease phenotype, suggesting a critical role of this group of interneurons in this disorder. WS is another infantile epileptic disease characterized by severe seizures and poor neurodevelopment and epilepsy prognosis (Kato, 2006). The genetic basis for this disease is heterogeneous and associated with numerous different gene mutations. Such genes include ARX and LIS1, which both are associated with the development of GABAergic interneurons (Kato, 2006).

Numerous recent studies have found that FS interneuron deregulation, as well as overall interneuron population loss, leads to various forms of epilepsy. Schwaller et al., in 2004 found that the chemoconvulsant PTZ was able to induce seizures in both PV-/- and PV+/+ mouse models, but the severity of the seizures was much greater in PV-/- mice. The reduction in quantity of hippocampal FS (PV+) interneurons cells was associated with a significant reduction in pyramidal cell inhibition, resulting in an increase in seizure susceptibility and epileptogenesis. Another study quantified the number of FS interneurons through immunoreactivity and found a significant decrease in hippocampal samples from human epileptic patients with and without sclerosis (Cossart et al., 2001). Other non-epileptic cognitive disorders associated with interneuronopathies include ASD and schizophrenia. The etiologies of both are considered very heterogeneous, with

various genetic and environmental factors involved. Animal models are highly useful in elucidating the role of interneurons in these disorders (Katsarou et al., 2017).

1.2.5 FS Interneurons synaptic transmission properties.

As previously highlighted, FS interneurons provide quick and reliable perisomatic GABAergic inhibition to a wide range of pyramidal cells and are required for the generation of synchronized oscillations in the brain (Povysheva et al., 2006). This reliable form of inhibition is highly dependent on the potent and temporally precise excitatory afferent inputs onto these interneurons (Povysheva et al., 2006). Deregulation at these excitatory synapses through presynaptic and/or postsynaptic mechanisms could disrupt proper FS interneurons' function and could lead to serious impairments in the E/I balance in the neuronal circuit. The excitatory postsynaptic potentials in these FS interneurons, in general, have larger amplitudes and a faster time course, which lower the threshold and shorten the latency for excitatory postsynaptic potential (EPSP) (Cecilia et al., 1999; Povysheva et al., 2006, Fricker and Miles, 2000; Gieger et al., 1997). In this regard, they are often coined as coincidence detectors versus temporal integrators (Cecilia et al., 1999; Konig et al., 1996). Most neurons can operate in one of two distinct ways, i.e. as temporal integrators or coincidence detectors, and based on these classifications they are primed to perform different computational tasks (Gieger et al., 1997). This categorization is based on the duration of the interval over which they summate the incoming synaptic signals (Konig et al., 1996). Temporal integrators summate over a period of time with low reliability, whereas coincidence integrators relay synchronized input into outputs that precisely correlate with the input patterns, over a short integration interval (Galarreta and Hestrin, 2001; Konig et al., 1996). Coincidence detectors also have a very low signal-to-noise ratio and are able to discard unrelated stimuli (Galarreta and Hestrin, 2001). As coincidence detectors, FS interneurons are able to

generate oscillations from their selective integration of excitatory inputs that are converted to precise and strong inhibitory outputs onto a large population of pyramidal cells (Gieger et al., 1997).

Excitatory afferent input on FS interneurons: Postsynaptic membrane. In order to better understand the excitatory signal transmissions received by the FS interneurons, we must investigate the presynaptic and postsynaptic membrane properties of the excitatory synapses on FS interneurons. Generally, NMDARs and AMPARs are the two main ionotropic receptors that differentially mediate postsynaptic excitation and long-term potentiation/depression. At the postsynaptic membrane of FS interneuron, the majority of AMPARs are GluR2-lacking AMPARs, and low levels of NMDARs are present (Cecilia et al., 1999). NMDAR-EPSCs in comparison to AMPAR-EPSCs are much longer lasting, therefore, the high AMPAR to NMDAR ratio in FS interneurons allow these neurons to display fast synaptic activation that precisely follows presynaptic cell activity pattern (Galarreta and Hestrin, 2001; Rotaru et al., 2013). Additionally, calcium permeable, GluR2-lacking AMPARs have faster AMPAR-EPSCs and can induce deactivation up to two times faster and desensitization up to three times faster than GluR2-containing AMPARs (Gieger et al., 1997; Rotaru et al., 2013).

GluR2-lacking AMPARs are also calcium permeable, which enables temporally accurate local calcium influx through these AMPARs into the postsynaptic dendrites, which could trigger postsynaptic calcium-dependent plasticity cascades at sub-threshold membrane potentials (Topolnik et al., 2005). This unique form of LTP helps FS interneurons function as stable timing devices at specific oscillatory frequencies (Camire et al., 2018). Influx of calcium could also result in LTD induction through the Ca^{2+} -induced Ca^{2+} release via ryanodine receptors in response to certain excitatory input frequencies (Camire et al., 2018). These postsynaptic properties endow the

FS interneurons with a remarkably fast synaptic activation, and enhanced sensitivity to synchronized inputs (Sanderson et al. 2016).

Excitatory afferent input on FS interneurons: Presynaptic membrane. There are various presynaptic mechanisms to take into consideration in order to better assess the efficacy and strength of the synaptic communication. Three main mechanisms of neurotransmitter release have been commonly established; synchronous, asynchronous and spontaneous neurotransmitter release (Kaeser and Regehr, 2015). Most neuronal communication relies on action potential induced synchronous neurotransmitter release, which is dependent on the fast and brief opening of the voltage-gated calcium channels. This form of release is temporally precise and most of the vesicles, consisting of the readily releasable pools, fuse to the presynaptic membrane within hundreds of microseconds of the arrival of the AP (Kaeser and Regehr, 2015). The key differentiating factor between synapses that endow unique properties to each synapse, is the number of readily releasable pools of vesicles available (Kaeser and Regehr, 2015). At most synapses, synchronous release accounts for almost all (>90%) NT release at low-frequency stimulations (Kaeser and Regehr, 2015).

Another form of neurotransmitter release is asynchronous release, wherein neurotransmitters are released with a longer, highly variable delay period after an action potential. This form of release is highly variable depending on the particular synapse and has been found to be prominent at specialized synapses. Usually asynchronous release occurs at high stimulation intensities, depending on the synapses (Kaeser and Regehr, 2015). A recent study found that asynchronous release usually dominates during repetitive trains at higher frequencies of >20Hz (Kaeser and Regehr, 2015). Numerous roles have been proposed for asynchronous release, such as reducing the relative refractory period which can play a role in controlling post-spike

excitability, as well as contributing to the precision of action potential firing and hence rendering the synaptic communication more reliable (Iremonger and Bains, 2016; Evstratova et al., 2014). Other studies have also found that changes in asynchronous release are associated with pathological conditions, such as epileptogenesis (Kaeser and Regehr, 2015). Many characteristics and underlying mechanisms of asynchronous release remain unclear; however, it has been established that this release is also dependent on calcium, but with another type of Ca2+ sensor (Kaeser and Regehr, 2015).

The third type of neurotransmitter release is spontaneous release. Their synaptic currents are often referred to as miniature excitatory postsynaptic currents (mEPSC). A number of functional roles have been hypothesized; however, their exact roles and function remains highly debated (Kaeser and Regehr, 2015). Some of the leading hypotheses include regulating the excitability of neurons, preventing loss of dendritic spines by activating ionotropic receptors and synaptic stabilization (Kaeser and Regehr, 2015). Together, these three forms of neurotransmitter release modulate the synaptic strength and are critical in proper synaptic function.

At the excitatory synapses, short-term synaptic changes that result from recent activity are facilitation, augmentation, and depression. These are short-term changes that last for various time intervals, and often determine the information processing and response, molding the efficacy of neural circuits (Zucker, 1989). The presynaptic strength is often assessed by two key parameters: the size of the readily releasable pool (RRP); and the probability that the vesicle in the pool will undergo exocytosis upon APs at the synaptic terminal. These variables control the synaptic plasticity at individual neuron terminals and are crucial to understanding the strength of the neural circuit (Ariel et al., 2013). Short-term plasticity of these synapses is often dependent on presynaptic mechanisms: residual elevation of presynaptic calcium can result in facilitation; while depression

is most commonly attributed to depletion of the pool of readily releasable vesicles (Zucker and Regehr, 2002; Dittman et al., 2000). Synapses that show a high level of facilitation, defined as successive spikes at high frequency evoked postsynaptic potential of increasing amplitude, are often called low probability synapses (Dobrunz and Stevens, 1997). High probability release synapses often show depression or significantly less of a difference in amplitude between the first spike and successive spikes at high frequencies (Dobrunz and Stevens, 1997). Decrease in strength can also arise from various other pathways such as from the release of modulatory substances from activated presynaptic terminals, or desensitization of ligand-gated receptors making the postsynaptic neuron less sensitive to neurotransmitters (Zucker and Regehr, 2002). Statistical analysis showed that depression reveals a reduction in either the probability of release of releasable quanta, or number of releasable quanta, or both (Zucker and Regehr, 2002). The interplay between facilitation and depression dictates synaptic strength and variability during repetitive stimulation. Various studies have designed numerous mathematical models to study the probability of release, number of physical release sites, size of the readily releasable pool and the role of residual calcium at the presynaptic membrane (e.g. Dobrunz and Stevens, 1997; Dittman et al., 2000; Ariel et al., 2013).

1.2.6 Plasticity of FS interneurons in the hippocampal circuits.

In the hippocampus, there are two main forms of afferent inputs to the fast spiking interneurons, feedforward (FF) inputs and feedback (FB) inputs, both of which play an important role and have unique 'learning' or LTP rules due to the distinct postsynaptic glutamate receptors found at these synapses (Roux et al., 2013). For a long time, the excitatory synapses of the interneurons were thought to be static and non-plastic, however, recent studies have disputed this

claim (Murase, 2014; Gulya et al., 1999). In these FF and FB synapses, unique calcium permeable AMPARs are found on the postsynaptic FS interneurons that confer a unique form of anti-Hebbian LTP. Anti-Hebbian LTP relies on coincident presynaptic membrane activation and postsynaptic hyperpolarization and synaptic activation, unlike the typical Hebbian, NMDAR- dependent LTP (Camire et al., 2018; Cecilia et al., 1999; Roux et al., 2013). The FF and FB unique phenotypes allow them to be differentially tuned upon high frequency afferent excitatory input, such that a high frequency stimulation of >20Hz selectively potentiates FB afferents, but not FF afferents (Fricker and Miles, 2000; Galarreta and Hestrin, 2001; Roux et al., 2013). The stratum (st.) radiatum interneurons mainly recruit FF Schaffer collateral inputs, whereas the st. oriens region mainly recruits FB inputs (Roux et al., 2013). It was also found that calcium-permeable, GluR2-lacking AMPARs facilitated LTP is found in both FF and FB synapses, but it is more abundant in the FF synapses (Roux et al., 2013).

In the CA1 region, FF inputs include Schaffer collaterals from CA3 pyramidal cells and entorhinal inputs from afferent path and inputs from the limbic brain region; whereas FB inputs mainly originate from neighbouring pyramidal cells (Fricker and Miles, 2000). The type of excitatory inputs was found to segregate according to hippocampal layers: in the st. radiatum layer, most inputs originate from Schaffer collaterals; whereas in the st. oriens layer, a more heterogeneous mixture of inputs are found, consisting of ~50% inputs from recurrent CA1 pyramidal cell layer and less than 20% from direct entorhinal inputs (Fricker and Miles, 2000).

2. Objectives

Epilepsy continues to be one of the most prevalent and challenging neurological diseases to treat in infants, and ELS are often refractory even to the best state-of-the-art medical treatments.

Therefore, there is an urgent need to identify novel targets and develop more effective treatment strategies to combat ELS. The onset of seizure activity is thought to occur when the delicate balance of excitation and inhibition in the brain is dysregulated. Currently, the main focuses in the epilepsy field are on the dysfunction of different ion channels, increased activity and excitatory output from pyramidal neurons, deregulation of inhibitory outputs from GABAergic interneurons and neuronal cell death (Blair et al., 2004; Nusser et al., 1998; Obenaus et al., 1993; Murase, 2013; Loo et al., 2019; Jensen et al., 1998; Hansen et al., 2014). Also, the majority of research on epilepsy and seizures have been done in adult animal models. Little to no research has investigated the effects of ELS, and its potential effects on the excitatory inputs onto different populations of interneurons in the immature and developing brain. In my study, I will be focusing on the function of a specialized group of interneurons—the FS interneurons—due to the important perisomatic inhibition they provide and their role in maintaining the excitation/inhibition balance. I hypothesize that acute seizures could alter the excitatory synaptic inputs onto the FS interneurons, while having minimal effects on the excitatory inputs on other interneuron subtypes. The following aims and experiments are designed to test this hypothesis.

Aim 1: To characterize the properties of FS and non-FS interneurons in the immature CA1 region of the hippocampus.

Many studies have characterized key functional and phenotypic traits of mature FS interneurons in the hippocampus that distinguish them from other interneuron subtypes (Kaiser et al., 2016; Tsukamoto, et al., 2004; Hua et al., 2014). However, these characteristics may not be displayed by immature hippocampal FS interneurons. Prior to studying how ELS can affect the hippocampal interneuron subgroups, I want to first identify and characterize the properties of FS

and non-FS interneurons from the hippocampal CA1, stratum oriens region, in control P10-12 mice pups.

Experiment 1: Identify whether FS interneurons express parvalbumin protein in the immature hippocampus. One key difference between the mature and immature FS interneurons is the expression of parvalbumin protein, which almost all FS interneurons in the mature hippocampus express. First, I will utilize immunohistochemistry to determine whether FS interneurons express PV in my brain region of interest, at the earliest age point of our study-P10, in order to determine whether we can utilize the available transgenic animals.

Experiment 2: Identify the intrinsic membrane properties of FS and non-FS interneurons. Next, I want to determine the intrinsic membrane properties of the immature FS and non-FS interneurons and determine whether they are distinguishable from each other. I will examine resting membrane potential; input resistance; the action potential (AP) amplitude, threshold and duration; and input-output function using whole cell current clamp recordings in control slices.

Experiment 3: Characterize and compare the AMPAR-mediated excitatory inputs into FS and non-FS interneurons. Finally, I will characterize the function of the excitatory synapses onto FS and non-FS interneurons. I will examine the spontaneous AMPAR-mediated currents, determine the presynaptic probability of release with pulse ratios (PPR) tests at different interstimulus intervals (ISI) of 20, 30, 50, 100,200, 400 and 500ms and determine short-term plasticity of these synapses with repetitive stimulation trains at different frequencies: 1, 5, 10, 20 and 50 Hz. Recordings will be done under whole-cell voltage clamp.

Aim 2: To investigate whether acute ELS affect the non-FS interneurons.

I hypothesize that different interneuron populations receive characteristically different excitatory inputs and these inputs can be affected differently by ELS. I will first evaluate whether ELS modulates non-FS interneurons and the excitatory inputs onto these cells.

Experiment 1: Can ELS affect the intrinsic membrane properties of non-FS interneurons? I will examine intrinsic membrane properties, with the measurements mentioned previously in Aim 1: Experiment 2, of non-FS interneurons in the stratum oriens region of the CA1 hippocampus from ex-vivo slices from 1h-post seizure mouse pups and littermate controls. Recordings will be done under whole-cell current clamp.

Experiment 2: Can ELS modulate the excitatory afferent inputs onto non-FS interneurons? Next, I will assess the spontaneous AMPAR mediated excitatory inputs in non-FS interneurons in slices from controls and ELS animals. Then the excitatory synaptic function of non-FS interneurons will be assessed through PPR tests. Recordings will be under whole-cell voltage clamp.

Aim 3: To investigate whether ELS affect excitatory afferent synapses of the FS interneurons.

I hypothesize that ELS can deregulate the excitatory inputs onto FS interneurons in a cell-type-specific manner. Furthermore, the function of FS interneurons may be compromised.

Experiment 1: Can ELS affect the intrinsic membrane properties of FS interneurons? I will examine intrinsic membrane properties, using the measurements previously mentioned from Aim 2: Experiment 1, of FS interneurons in the stratum oriens region of the CA1 hippocampus from ex-vivo slices from 1h-post seizure mouse pups and littermate controls. Recordings will be done under whole-cell current clamp mode.

Experiment 2 Can ELS modulate the excitatory afferents inputs into FS interneurons? First, I will assess the excitatory inputs into FS interneurons first by analyzing the spontaneous AMPAR-mediated sEPSC. Next, the presynaptic probability of release (PPR test) and short-term plasticity (repetitive stimulation test) at the excitatory synapses onto FS interneurons will be measured to tests for possible ELS induced changes. Recordings will be done under whole-cell voltage clamp. (Both PPR and repetitive train tests were highlighted with more detail in Aim 1: Experiment 3).

If ELS induced changes at the excitatory afferent inputs are found, I will further investigate the underlying mechanisms, with mathematical modeling to estimate the number of readily releasable vesicles and the probability of release at the excitatory synapses onto FS interneurons.

Experiment 3: Can ELS affect asynchronous neurotransmitter release, and subsequently modulate the spike timing and fidelity in hippocampal FS interneurons? Asynchronous neurotransmitter release has been found to be important in modulating postsynaptic cells' excitability and enhancing the cell's AP precision and reliability (Iremonger and Bains, 2016). Since FS interneurons are critical in providing highly reliable and temporally precise perisomatic inhibition in the brain, ELS could potentially disrupt their function by deregulating the presynaptic asynchronous release. I will first determine whether the frequency of asynchronous EPSCs (aEPSCs) is disrupted by ELS. Then I will assess for possible ELS induced changes in FS interneurons action potential response, to repetitive stimulations at different frequencies (1Hz, 5Hz, 10Hz, 20Hz & 50Hz).

3. Methods

3.1. Experimental Animals

Male and female C57BL/6J mouse pups from age postnatal(P) 10-12 were used for all experiments. During this age window, the neonatal mouse brain undergoes a critical period of developmental plasticity and a developmentally regulated imbalance between excitation and inhibition, which makes the immature brain susceptible to early life seizures (ELS) (Silverstein and Jensen, 2007). All mice were maintained in a 12 h light/dark cycle schedule with a room temperature of 22°C. All mice had free access to food and water and nestlets were provided. All experimental procedures were approved by and in accordance with the guidelines of the Animal Care and Use Committee at Carleton University (Protocol #106036). Efforts were made to minimize animal suffering and the number of animals used.

3.2. Early life Seizure (ELS) Induction

ELS were induced in P10-12 mouse pups through i.p. injection of chemoconvulsant, pentylenetetrazol (PTZ, 60 mg/kg). The severity of the seizures was monitored by the experimenter according to a revised Racine scale (Table 1, Lüttjohann et al. 2009). Animals were sacrificed at 1h post ELS. Littermate controls were injected with saline and sacrificed at the same time as the ELS mouse pups.

Table 1. Criteria to assess the severity of acute ELS in P10-12 mouse pups

Seizure Stages	Physical Symptoms
0	No change in behaviour
1	Sudden movement, arrest, freezing
2	Head nodding
3	Forelimb clonus, erect tail
4	Forelimb clonus, rearing and falling movements
5	Tonic-clonic activity with posture tone loss, wild jumping and jerking, often results in death

3.3 Hippocampal slice Preparation

Hippocampal slices were prepared from P10-12 mice pups as described previously (Sun et al., 2018). Mouse pups were rapidly decapitated with procedures in accordance with guidelines set by institutional animal care and use committee. Animal brains were rapidly dissected from the skull and placed in ice-chilled oxygenated cutting solution containing (mM): 119 choline chloride, 5 KCl, 4 $MgSO_4$, 1.25 NaH_2PO_4, 0.8 $CaCl_2$, 26 $NaHCO_3$, 18 glucose, and 5 sucrose. The brain sections were obtained using a Leica VT1000S vibratome (Leica Microsystems VT1000S). Coronal hippocamp slices (300μm thickness) of the middle third of the hippocampus were selected and used for recording. The selected slices were then transferred from the vibrotome to slice holding chamber and incubated in oxygenated artificial cerebral spinal fluid (ACSF) containing (mM): 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 1.2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 glucose, kept at 35°C for 30 minutes and room temperature for at least 1h before any electrophysiological recordings. For recording, the slices were transferred to a 2.5mL recording chamber placed in an upright Zeiss Axio Scope A1 microscope or a NIKON FN1 upright microscope equipped with infrared and differential interference contrast imaging devices and were perfused with oxygenated ACSF, while kept at room temperature (22-24°C).

3.4. Whole-cell patch clamp recording

Whole-cell patch clamp recordings were made from interneurons of the CA1 stratum oriens region in the hippocampal brain slices using an Axopatch 700B amplifier or a Axopatch 200B amplifier (Axon Instruments, Molecular Devices), as previously described (Sun et al., 2013; Sun et al., 2018; Zhou et al., 2011). The patch-pipette intracellular solution contains (mM): 130 K-Gluconate, 2 MgCl2, 0.6 EGTA, 10 HEPES, 5 KCl, 2 ATP-Mg(Na2), pH 7.3. Filled electrodes

with a resistance of 6-11MΩ were prepared from borosilicate glass capillaries with a Narishige micropipette puller (Model PC-100, Tokyo, Japan). Spontaneous EPSCs (sEPSCs) and evoked EPSCs (eEPSCs) were recorded in neurons held at a -60mV holding potential to isolate AMPAR mediated currents from NMDAR mediated currents. Additionally, picrotoxin (PTX) (100 μM), a GABAA receptor blocker, was administered to block all GABAA receptor mediated inhibitory postsynaptic currents. Access resistance was monitored throughout the experiments and cells were abandoned if changes of access resistance were >25%. Signals were filtered at 2kHZ, digitized at 20kHz by a Digidata 1320A or Digidata 1550B interface, acquired by the pClamp 9.2 or pClamp 10.7 software, and analysed with the Clampfit 10.7 program (Molecular Devices). Digitized data were saved on disk and imported for analysis and graphing into commercial software (Graphpad, Prism v.8.).

3.5. Intrinsic Membrane Properties

To examine the intrinsic membrane properties of CA1 stratum oriens neurons, a sequence of rectangular hyperpolarizing and depolarizing pulses (500ms duration, from -200 to 200pA, 20pA/step) was used to evoke membrane voltage responses under the current clamp mode and bridge balance was applied. All evaluations of intrinsic membrane properties were made from stable recordings at least 5 mins after the whole-cell configuration was achieved. Neurons with resting membrane potentials higher than -55mV were discarded from analysis. The AP properties including AP amplitude, half-width, and threshold were determined from current clamp recordings that only one or two APs were evoked by 500ms depolarizing current injections. AP amplitude was measured by the difference between the peak voltage of the AP and initial membrane potential at which the membrane potential started to rise rapidly (inflection point). The AP threshold was

measured by the membrane potential at which only one or two spikes are generated. The AP half-width was determined at 1/2 amplitude of the AP. To determine the input resistance, membrane potential changes evoked by 500ms hyperpolarizing current injections (0 to -80pA, 20pA/step) were measured between the baseline membrane potential and the peak hyperpolarization to generate current–voltage (I–V) curves. The slope of the I–V curve was essentially linear and calculated to determine the input resistance of the cell membrane.

3.6. AMPAR mediated spontaneous excitatory postsynaptic responses (sEPSCs)

AMPAR-mediated sEPSCs were recorded over a period of at least 10 minutes at a holding potential of -60mV. sEPSC amplitude and frequency were analysed further using Clampfit 10.7. sEPSC events were detected automatically using Clampfit 10.7. The threshold for detection of sEPSC events was set at 6-10pA depending on the noise level. This threshold remained constant throughout analysis for all recordings in the CA1 stratum oriens region from both control and ELS mice. All detected sEPSCs were visually confirmed for a clear monotonic rising phase and an exponential decay time course. sEPSC frequency and amplitude histograms were constructed using Graphpad program.

3.7. AMPAR mediated evoked excitatory postsynaptic responses (eEPSCs)

eEPSCs were evoked with 0.1ms square pulse extracellular stimulation in the stratum oriens, CA1 pyramidal neuron layer, or Schaffer collateral pathway with the use of a bipolar concentric tungsten electrode through a WPI A365 stimulator. The distance between the stimulation electrode and the recording electrode was around 80-100 μm. The stimulation intensity was adjusted for each cell to elicit ~50% of the maximum amplitude of evoked EPSCs. The cell membrane potential was clamped at -60mV throughout the recording in the voltage clamp mode. eEPSCs amplitudes were measured from basal line to the peak of each EPSC event.

3.8. Evoked excitatory postsynaptic potentials (eEPSPs)

eEPSPs were evoked with 0.1ms square pulse extracellular stimulation by a stimulation electrode placed around the same region as mentioned in the above section *2.9.7.* The stimulation intensity was carefully adjusted to a minimal intensity level necessary to evoke one action potential on top of the EPSP. The 1st spike latency (FSL) was measured from the stimulation artifact to the peak of the 1st spike.

3.9. Immunohistochemistry

Standard protocols were used for immunohistochemistry (IHC) as described previously (Sun et al., 2013). The procedure is briefly described below.

***Perfusion*.** Mice were anesthetized with sodium pentobarbital (200mg/Kg, Euthasol®, Virbac USA) and perfused with 1ml of 0.9% saline, followed by 50 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). Whole brains were extracted from the perfused animals and kept in 4% PFA overnight at 4°C. The following day the tissue was washed two times with 10% sucrose in PB solution for the duration of 4 hours each time. The brain is then transferred into 30% sucrose in PB + azide solution for a least 2 days and kept at 4°C until tissue sectioning.

***Tissue Sectioning*.** The tissue was first flash frozen with Fisherbrand Super Friendly Freeze-it (Fisher cat # 23022524) prior to cutting. The brain was then mounted and sectioned into 40µm thick slices using Thermo scientific cryostat (Fisher) and kept in 0.1 M PB + azide and stored at 4°C until staining.

Immunohistochemistry. Free-floating sections from mouse hippocampus were first rinsed 2 x 5 mins in 10mM Phosphate buffered saline (PBS). The slices were then blocked for 1 hour in 5% Normal Goat Serum (NGS, Sigma cat# G9023) and 0.3% Triton-X in PBS. The blocker was removed, and the slices incubated in PV primary antibody (mouse IgY; 1:5000; Aves Labs Inc.) overnight at room temperature in a primary dilution solution (2% BSA in PBS, and blocker). The following day the slices were rinsed 3 x 5 minutes in PBS. Next, the slices were incubated in anti-PV fluorescent secondary Alexa Fluor 488 (anti-mouse IgG; 1:1000; Invitrogen Alexa Fluor series) dissolved in the secondary dilution solution (NGS, 10% Triton-x, BSA, and PBS) for 2-3 hours. Once again, the slices were rinsed 3 x 5 minutes in PBS followed by mounting onto positively charged slides (Thermo Fisher cat# 12-550-15) and coverslip (Thermo Fisher cat# 12-545M) with Flouromount containing DAPI (4',6-diamidino-2-phenylindolel; Thermo Fisher # 00-4959-52). Slices were left to air dry overnight and sealed with nail polish the next day. They were finally stored at -20°C until imaging. False-positive staining was excluded by incubating control sections without primary antibodies, adding only the secondary antibodies.

Imaging. All fluorescent images were captured using the ZEISS Axioimager2 microscope with the digital camera HAMAMATSU C10600 attached and XXX software (Zeiss Canada, ON).

3.10. Voltage sensitive dye imaging

Hippocampal slices were prepared as described above in section *2.3.* Hippocampal slices were stained with 0.2 mM the fast voltage-sensitive dye Di-4-ANEPPS (Di-4-ANEPPS, Biotium, CA) for 30 min in the incubation chamber and then transferred into the recording chamber. Imaging was performed at room temperature (22-24°C). Excitation light emitted by a shuttered green LED (LEX2, Brainvision, Tokyo, Japan) was reflected toward the cells through an excitation

filter (531 nm wavelength). Emitted fluorescence signals that passed through an absorption filter (580 nm wavelength) was imaged (0.5ms frame rate; 6-8mins time-lapse period) by a MiCam05 CMOS-based camera (SciMedia) with a Leica Plan APO 5x objective (NA: 0.5, Leica Microsystems, Wetzlar, Germany). The imaging data were acquired and analyzed using Brainvision Analysis Software (Brainvision). Fluorescence intensity changes (ΔF/F) were normalized to baseline fluorescence recorded during the initial 10ms of each recording and represented by pseudo-colors. Red color indicated a membrane depolarization, while blue color indicated a membrane hyperpolarization.

3.11. Statistical analysis

All numerical averages were presented as means ± SEMs. First, my data was tested for normality using the Shapiro-Wilk normality test. Then, statistical significance was assessed using a Student's t test, a Kolmogorov-Smirnov test or a one-way or two way ANOVA test when the comparison of data had normal distributions. Post hoc Bonferroni test, and a Mann-Whitney or Wilcoxon matched-pairs signed rank test for comparisons of data that were not normally distributed. The minimum criterion for statistical significance was set at $P<0.05$.

4. Results

In the present study, I used a combination of electrophysiology, voltage-sensitive dye imaging, and immunochemistry approaches to systematically examine whether ELS affects different populations of interneurons in the developing hippocampal CA1 stratum oriens region. Overall, whole-cell current and voltage clamp recordings were obtained from 261 hippocampal

CA1 stratum oriens interneurons: 139 interneurons from control animals (102 non-FS interneurons and 37 FS interneurons) and 112 interneurons from ELS animals (77 non-FS interneurons and 35 FS interneurons, Figure 1D). All neurons included in this thesis had a stable resting membrane potential more negative than -55 mV (mean: -60.810±7.176 mV) throughout the recordings.

4.1. Characterization of FS and non-FS interneurons in the stratum oriens of the CA1 in the developing hippocampus.

My first set of experiments were designed to characterize the physiological properties of FS and non-FS interneurons in stratum oriens region of the hippocampal CA1 region in the immature brain. I measured and compared the intrinsic membrane properties and characteristics of the excitatory afferent inputs onto the two different groups of interneurons.

4.1.1. Identification of FS and non-FS interneurons in CA1 stratum oriens.

FS interneurons in adult brain are often characterized and identified by the expression of the calcium binding protein, parvalbumin (PV). Therefore, available transgenic reporter mouse lines expressing tdTomato (Kaiser et al., 2016), EYFP (Pvalb-ChR2-EYFP) (Zhao et al., 2011), and Pvalb-Cre mice (Wang et al., 2018) in PV positive FS interneurons have provided very useful tools to study physiological function of this crucial group of interneurons in the adult brain. However, it is important to note that PV expression is developmentally regulated and is not fully expressed in FS interneurons until P16-21 in the mouse hippocampus (De Lecea et al., 1995). Therefore, I first examined whether PV is expressed in FS interneurons in the P10-12 mouse hippocampus using immunohistochemistry staining. As shown in Figure 1A, no detectable PV staining was found in the CA1 region of the hippocampus in P10 mice (n=3 mice). However, in the CA3 region of the same section, PV staining was detected in a few neurons (Figure 1B).

Furthermore, our PV antibody performance and target specificity were verified using mature hippocampal sections from P60 mice (Figure 1C, n=2). These data strongly support the delay of PV expression in CA1 interneurons in the developing hippocampus.

As an alternative approaching rather than using PV transgenic reporter lines, I was able to distinguish FS interneurons from non-FS interneurons in CA1 stratum oriens from P10-12 mice according to their distinct physiological properties. Consistent with previous studies in adult mice (Casale, et al., 2015; Tsukamoto, et al., 2004), compared to non-FS interneurons, FS interneurons exhibited high frequency spike trains (39.909 ± 4.037Hz in response to 200pA current injection, n=15) with little adaptation in response to depolarizing current steps, in contrast to non-FS interneurons that showed lower spike frequency and large spike adaptation (18.833 ± 3.280Hz in response to 200pA current injection, n=13, $P<0.001$, Figure 1N). In addition, the duration of action potentials at ½ AP amplitude of FS interneurons was significantly shorter (1.460 ± 0.074ms, n=15) than those of non-FS interneurons (1.862 ± 0.112ms, n=13, $P=0.002$, Figure 1J). However, compared to non-FS interneurons, FS interneurons did not show significant differences in the resting membrane potential (FS interneurons 62.390 ± 2.310mV, n=17 vs non FS interneurons 60.920 ± 1.652mV, n=14, $P=0.945$, Figure 1H), the input resistance (FS interneurons 371.600 ± 54.230MΩ, n=10 vs non-FS interneurons 379.800 ± 31.460MΩ, n=14, $P=0.841$, Figure 1I), AP amplitude (FS interneurons 80.070 ± 2.386mV, n=16 vs non-FS interneurons 86.710 ± 3.954mV, n=14, $P=0.062$, Figure 1G), AP threshold (FS interneuron -41.390 ± 1.017mV, n=14 vs non-FS interneurons -45.590 ± 1.759mV, n=14, $P=0.140$, Figure 1K). These results support distinct physiological properties in FS interneurons compared to non-FS interneurons in CA1 stratum oriens during the critical period of development.

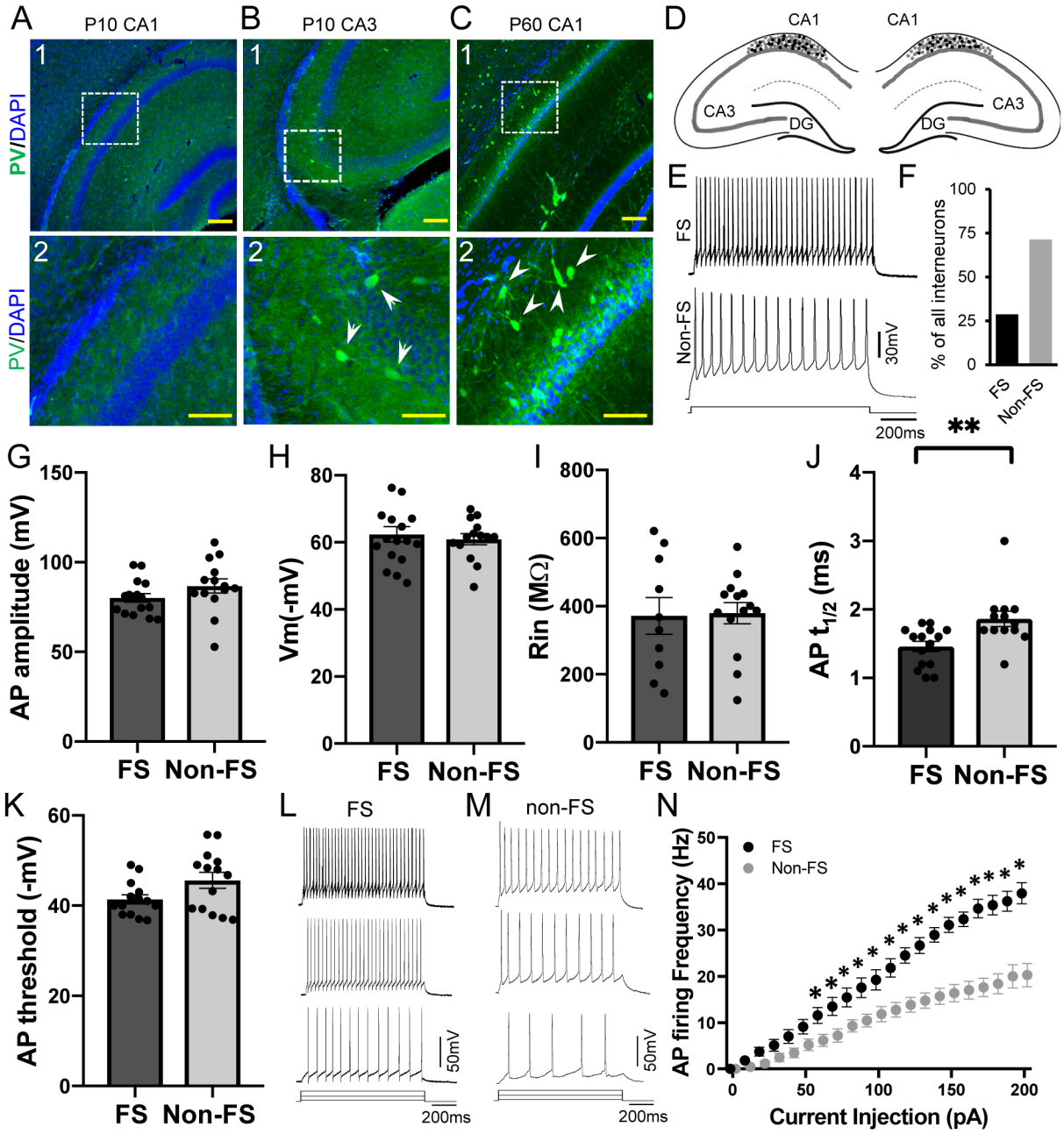

Figure 1. Characterization of FS and non-FS interneurons in CA1 stratum oriens of the developing hippocampus. **(A-C)** Delayed PV expression in CA1 FS interneurons in the developing hippocampus at P10. Coronal hippocampal sections labelled with PV (green) and DAPI (blue) showed a lack of PV staining in CA1 (**A1 and 2**), but a few cells with PV staining in CA3 from the same section as shown **A** (**B1** and **2**). Mature hippocampus section from a P10 mouse demonstrated strong PV expression in CA1 (**C1** and **2**). **D.** Schematic diagram of the hippocampal CA1 stratum oriens region highlighting the total number of FS interneuron and non-FS interneuron recorded in this study. **E.** Representative whole-cell current clamp recording traces of FS (top) and non-FS interneuron (bottom) in response to twice-threshold current injection. **F.** Percentage of FS and non-FS interneurons in all interneurons recorded from the stratum oriens in the developing hippocampus. **(G-K)** Comparisons of the intrinsic membrane properties of FS and non-FS interneurons including the AP amplitude (**G**), resting membrane potential (**H**), input resistance (**I**), AP $t_{1/2}$ (**J**), and AP threshold (**K**), n=15, 13. **P<0.0001. **(L-N)** (left) Representative traces of FS (**L**) and non-FS interneuron (**M**) in response to depolarizing current injections. Input-output function of FS and non-FS interneurons showed significant higher firing frequency in FS interneurons (**N**). n=15, 13 from 10 and 6 mice. **P <0.01. Data are expressed as mean ± SEM.

4.1.2. Determination of the optimal stimulation site to study the excitatory afferent inputs into FS and non-FS interneurons in CA1 stratum oriens

Next, the excitatory afferents onto FS and non-FS interneurons in CA1 stratum oriens were examined. It is well known that hippocampal CA1 interneurons in the stratum oriens receive a mixture of afferent excitatory inputs from 1) Schaffer collaterals from CA3 pyramidal neurons, 2) CA1 pyramidal neurons, and 3) entorhinal inputs (Fricker and Miles, 2000). To determine the the optimal stimulation site in our hippocampal slice preparation to study the excitatory afferent inputs into FS and non-FS interneurons in the stratum oriens, I used the powerful voltage sensitive dye imaging to monitor the spatiotemporal properties of the activity of the populations of neurons in the striatum oriens in *ex vivo* hippocampal slices by stimulating different sites. As shown in Figure 2 (6 slices from 3 mice), a bipolar stimulating electrode was positioned at stratum oriens (Figure 2A and B), stratum pyramidal neuron layer (Figure 2C and D), or stratum radiatum (Figure 2E and F) to activate the excitatory synapses onto FS and non-FS interneurons in CA1 stratum oriens while blocking synaptic inhibition with a $GABA_A$ receptor blocker picrotoxin. As shown in the pseudocolor map in Figure 2B, stimulating the stratum oriens initiated response within 1-2ms. While the activity spread across the slice to cover the regions of stratum pyramidale and stratum radiatum, the activity peaked within 5-6ms in the stratum oriens presumably representing synaptic excitation and generation of a population of spikes in CA1 interneurons. The activity in the stratum oriens can also be evoked by stimulating stratum pyramidal neuron layer (Figure 2D), or stratum radiatum (Figure 2F). Compared to the activity spreading with stimulating stratum pyramidal neuron layer (Figure 2D), or stratum radiatum (Figure 2F), the activity associated with the stimulation site in the stratum oriens (Figure 2B) has very short time course and strong activity in the stratum oriens. Therefore, in all subsequent experiments, excitatory afferent inputs to CA1 FS

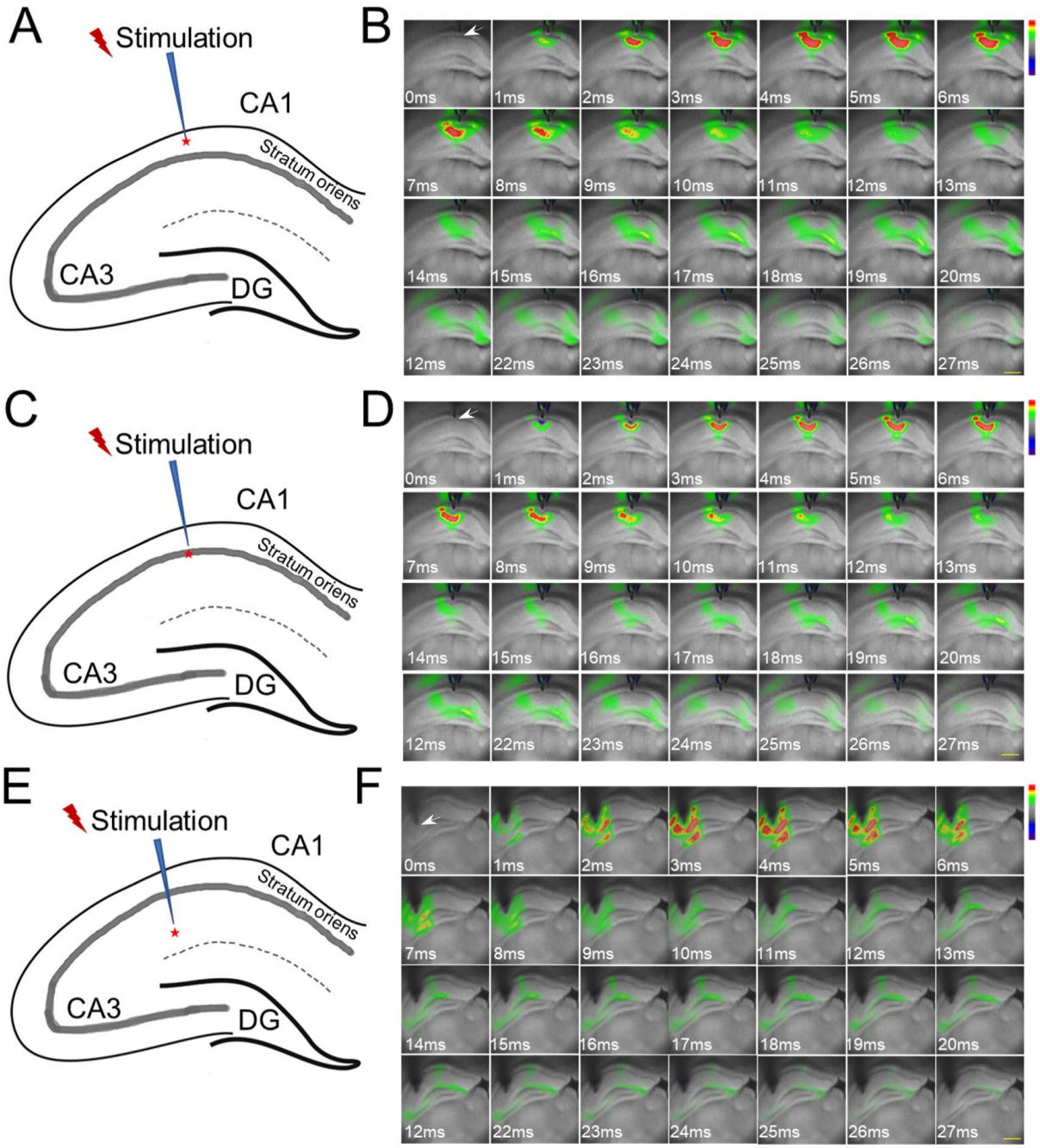

Figure 2. Determination of the optimal stimulation site to examine the excitatory afferent inputs of interneurons in the CA1 striatum oriens of the developing hippocampus. (A-B) Schematic diagram shows the stimulation site (red star) in the hippocampal CA1 stratum oriens (**A**). Representative optical responses recorded from a hippocampal slice stained with Di-4-ANNEPS for 30mins are presented here as pseudo-color images. The stimulation site is indicated by white arrowhead in the baseline image (0ms). Red color illustrates membrane depolarization. Time course of changes and spreading in fluorescence in response to a single electrical stimulation is captured in ACSF (**B**). (**C-D)** Schematic diagram shows the stimulation site (red star) in the hippocampal CA1 striatum pyramidale (**C**). Time course of optical responses in response to a single electrical stimulation (white arrowhead at 0ms) is captured in ACSF (**D**). (**E-F)** Schematic diagram shows the stimulation site (red star) in the Schaffer collateral pathway (**E**). Time course of optical responses in response to a single electrical stimulation (white arrowhead at 0ms) is captured in ACSF (**F**).

and non-FS interneurons were evoked by stimulation adjacent sites in the stratum oriens.

4.1.3 Excitatory afferent inputs of FS and non-FS interneurons in CA1 stratum oriens.

The biophysical properties of glutamatergic excitatory synapses have been known to be postsynaptic target specific *(*Bao et al., 2010; Gozlan et al., 2003; Maccaferri et al., 1998; Scanziani et al., 1998*).* In the following experiments, I determined whether there are any differences between the excitatory synaptic connections onto FS interneurons and non-FS interneurons in the immature CA1 stratum oriens. To achieve this purpose, my first set of experiments were designed to assess the AMPAR-mediated spontaneous postsynaptic currents (sEPSCs) using whole-cell voltage clamp recordings in FS and non-FS interneurons in CA1 stratum oriens. AMPAR-mediated sEPSCs were pharmacologically isolated by blocking inhibitory $GABA_A$ receptors using 100μM picrotoxin and at holding potential of -60mV, at which magnesium blocks the NMDARs. I found no significant difference between AMPAR sEPSC amplitude for FS interneurons 28.550 ± 2.793pA, n=10, vs non-FS interneurons 23.620 ± 1.106pA, n=29, *P*=0.122 (Figure 3A, B, and C). In addition, there was no significant difference in AMPAR sEPSC frequency between FS interneurons 0.915 ± 0.280Hz, n=10, vs non-FS interneurons 0.618 ± 0.142Hz, n=27, *P*=0.506 (Figure 3A, B and D). It is known that AMPAR-mediated sEPSCs in FS interneurons in mature brain show faster decay kinetics in comparison to non-FS interneurons, in part due to the prevalent postsynaptic GluR2-lacking AMPARs (Akgul and McBain, 2016; Isaac, 2007). Therefore, I measured the kinetics of AMPAR sEPSCs in both FS and non-FS interneurons and found that there was no significant difference in the rise time for AMPAR sEPSCs in FS interneurons 1.177 ± 0.193ms, n=9, compared with non-FS interneurons 1.305 ± 0.168ms, n=29, *P*=0.953 (Figure 3E). More interestingly, the AMPAR sEPSC decay time in FS interneurons 4.214 ± 0.684ms, n=9, was not significantly different from those in non-FS interneurons 4.328 ± 0.412ms, n=29, *P*=0.968

(Figure 3F). Collectively, these data do not identify any statistically significant differences in AMPAR sEPSC amplitude, frequency, rise time and decay time between FS and non-FS interneurons in CA1 stratum oriens at this age.

To further evaluate the properties of excitatory synapses in FS and non-FS interneurons, I tested the presynaptic properties of these synapses by examining the pair-pulse ratios (PPRs: a ratio of the 2nd eEPSC amplitude over the 1st eEPSC amplitude) at different interstimulus intervals (ISI) of 20, 30, 50, 100, 200, 400 and 500ms. The stimulation intensity was adjusted to evoke ~50% of the maximum EPSCs of the recorded cells. I found no significant difference in PPRs between FS interneurons and non-FS interneurons at any of the ISIs (The PPR at ISI of 20ms for FS interneurons 2.638 ± 0.245, n=9 vs non-FS interneurons 2.868 ± 0.345, n=18, *P*=0.856; The ISI of 30ms for FS interneurons 2.250 ± 0.268, n=9 vs non-FS interneurons 2.692 ± 0.336, n=20, *P*=0.417; The ISI of 50ms for FS interneurons 2.331± 0.334, n=9 vs non-FS interneurons 2.426 ± 0.326, n=20, *P*=0.982; ISI of 100ms for FS interneurons 1.880 ± 0.256, n=9 vs non-FS interneurons 1.787 ± 0.161, n=20, *P*=0.711; ISI of 200ms for FS interneurons 1.352 ± 0.079, n=9 vs non-FS interneurons 1.537 ± 0.110, n=21, *P*=0.449; ISI of 400ms for FS interneurons 1.178 ± 0.040, n=9 vs non-FS interneurons 1.204 ± 0.046, n=20, *P*=0.982; ISI of 500ms for FS interneurons 1.181 ± 0.053, n=8 vs non-FS interneurons 1.073 ± 0.031, n=18, *P*=0.417, Figure 3G and H). Therefore, no detectable difference between the probability of release at the excitatory synapses onto both FS and non-FS interneurons were found.

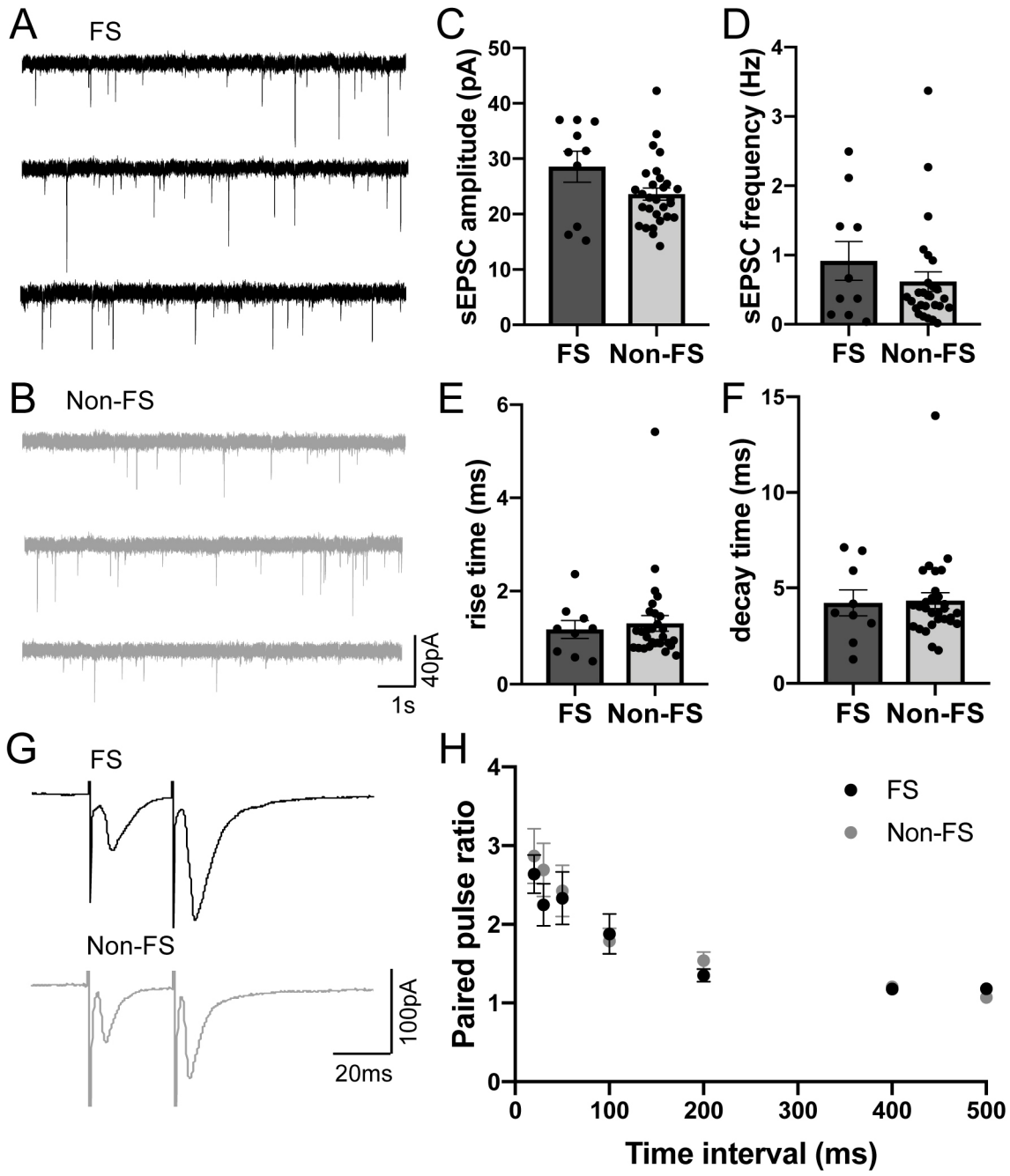

Figure 3. Comparison of the AMPAR function between FS and non-FS interneurons in striatum oriens in the immature hippocampus. (**A-B**) Representative traces of AMPAR-mediated sEPSCs, pharmacologically isolated by blocking NMDA and $GABA_A$ receptors, in FS (**A**) and non-FS (**B**) interneurons in hippocampal ex vivo slices from P10-12 mice. (**C-D**) AMPAR sEPSC amplitude (**C**) and frequency (**D**) showed no significant difference between FS and non-FS interneurons, n=10, 29 from 8 and 20 mice $P > 0.05$. (**E-F**) AMPAR sEPSC rise time (**E**) and decay time (**F**) did not show significant difference between FS and non-FS interneuron, n=9, 29, from 8, 20 mice, $P > 0.05$. (**G**) Representative traces of paired pulse at ISI of 30ms EPSC recordings in FS (top trace) and non-FS interneurons (bottom trace) in CA1 striatum oriens. **(H)** Group data showed that no significant differences in paired-pulse ratio during different stimulation intervals between FS and non-FS interneurons in CA1 striatum oriens, n=10, 18 from 7 and 14 mice, $P > 0.05$**.** Data are expressed as mean ± SEM.

4.1.4 Excitatory synapses onto FS and non-FS interneurons in CA1 stratum oriens display different short-term plasticity during high frequency repetitive stimulation.

Short-term plasticity, regulated in a brain region and synapse dependent manner during development, have critical roles in activity-dependent wiring of different neuronal circuits in the immature brain. Distinctive synaptic plasticity endows different cell types with unique capabilities to filter and respond to excitatory or/and inhibitory afferent inputs (Zucker and Regehr, 2002). In order to assess the short-term plasticity of the excitatory afferents into FS and non-FS interneurons in immature CA1 stratum oriens, I evaluated the responses of FS and non-FS interneurons to trains of repetitive stimuli (20 pulses) at different frequencies (1Hz, 5Hz, 10Hz, 20Hz and 50Hz) using whole-cell voltage clamp recordings at a holding potential of -60mV. The stimulation electrode was placed at stratum oriens ~80-100μm away from the recorded cells. I found that repetitive stimulation at 1 and 5 Hz did not evoke significant facilitation or depression in both FS and non-FS interneurons (Figure 4B and C). Repetitive stimulation at 10Hz induced similar mild facilitation in FS and non-FS interneurons at the 20th pulse for FS interneuron 184.137 ± 33.234%, n=16, vs non-FS interneuron 113.958 ± 22.795%, n=15, *P*=0.792 (Figure 4D). Stimulation at 20 and 50Hz produced significant differences, where FS interneurons showed facilitation compared to non-FS interneurons that showed depression (Figure 4E and F). During stimulation trains of 20Hz the normalized EPSC amplitude of the 20th stimuli was significantly different between FS interneuron 296.428 ± 65.389%, n=13, vs. non-FS interneuron 109.451 ± 12.892 %, n=26, *P*=0.005; last 5 normalized EPSC values for FS interneuron were 305.400 ± 2.436%, n=13, and for non-FS interneuron were 119.300 ± 2.657%, n=26, *P*=0.008 (Figure 4A and E). During stimulation trains of 50Hz, the normalized EPSC amplitude for the 20th stimuli for FS interneuron was 356.545± 57.215%, n=9, and for non-FS interneuron was 97.559±23.284%, n=14, *P*=0.0036; last 5

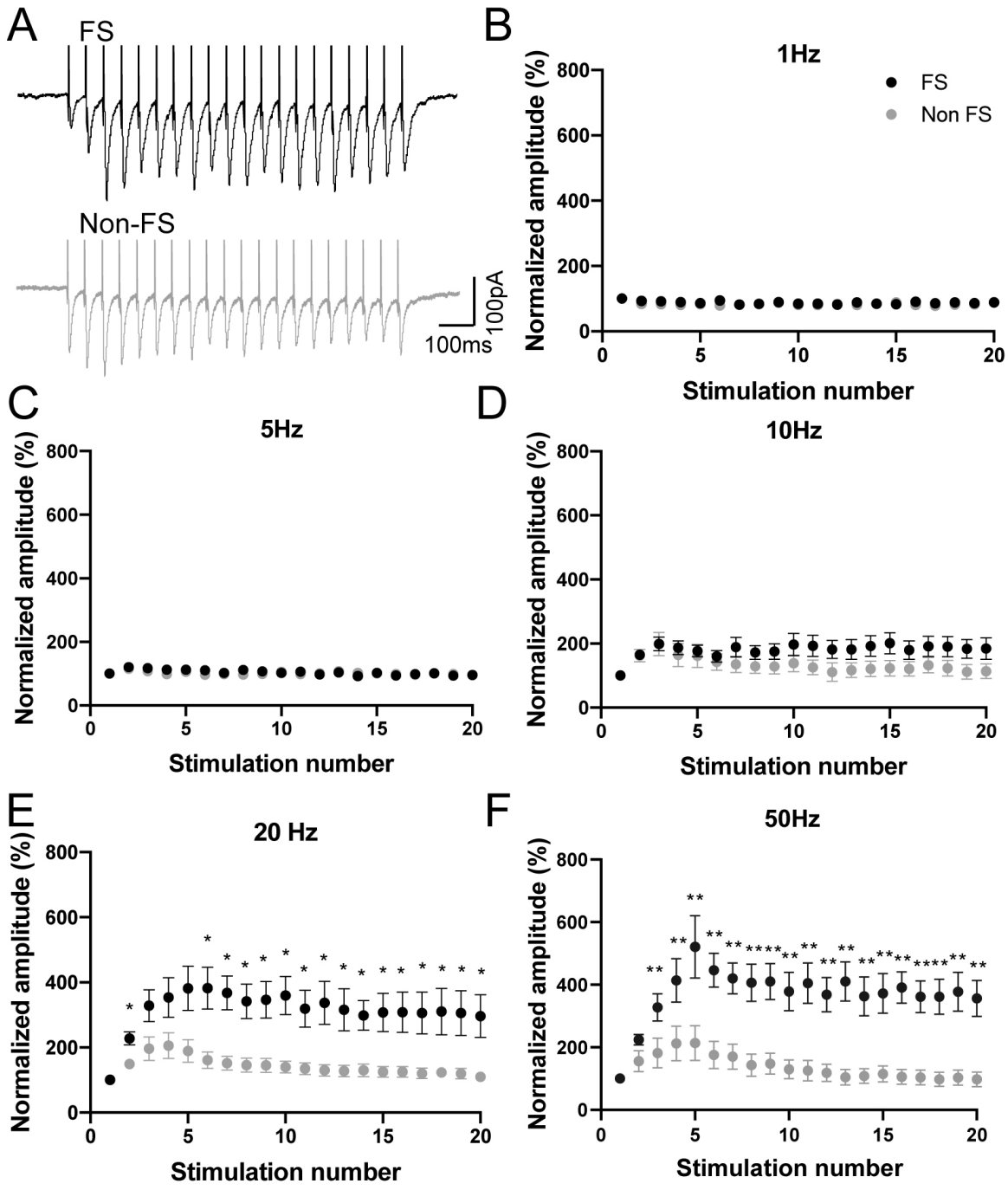

Figure 4. FS and non-FS interneurons display different forms of short-term synaptic plasticity at high frequency stimulations. **(A)** Representative traces of averaged trains of eEPSC elicited by 20 Hz repetitive stimulation train in FS (top) and non-FS (bottom) interneurons from hippocampal ex vivo slices. **(B-F)** Group data of EPSCs normalized to the first EPSC amplitude of FS interneuron and non-FS interneurons during 1, 5, 10, 20 & 50 Hz trains. At stimulation of 1, 5, and 10 Hz, there was no significant difference between short-term plasticity at excitatory afferent inputs onto FS and non-FS interneuron, n=13, 26 from 9 and 17 mice, $P > 0.05$ **(B-D)**. At stimulation of 20 and 50 Hz, there were significant differences in short-term facilitation between excitatory inputs onto FS and non-FS interneurons, n=7, 16 from 7 and 10 mice, $*P<0.05$, $**P<0.001$ **(E-F)**. Data are expressed as mean ± SEM.

normalized EPSC values for FS interneuron were 369.8 ± 6.399%, n=9, and for non-FS interneuron were 101.5 ± 1.697%, n=14, *P*=0.008 (Figure 4F). Thus, these data reveal a diversity of short-term plasticity at excitatory synapses onto FS vs. non-FS interneurons in CA1 stratum oriens, suggesting distinct frequency-dependent modulation of these synapses.

4.2. Acute effects of ELS on non-FS interneurons in CA1 stratum oriens.

Considering the distinct biophysiological properties of FS and non-FS interneurons in the immature CA1 stratum oriens, I next examined how ELS during the critical period of development affect these two group of interneurons. First, I determined whether ELS acutely affect the function of non-FS interneurons in CA1 stratum oriens.

4.2. 1 ELS did not affect the intrinsic membrane properties of non-FS interneurons in CA1 stratum oriens.

To investigate the acute effects of ELS on the intrinsic membrane properties of non-FS interneurons in CA1 stratum oriens, I performed whole-cell current clamp recordings in CA1 non-FS interneurons in *ex vivo* hippocampal slices removed from P10-12 1h post-ELS mice and littermate control mice. I found that there were no significant changes in intrinsic membrane properties in non-FS interneurons from control mice (the resting membrane potential: -62.350 ± 1.103mV, n=14; the input resistance: 379.800 ± 31.460ΩM, n=14; the AP amplitude: 86.710 ± 3.954 mV, n=14; the AP threshold: -45.590 ± 1.759mV, n=14; the AP1/2time: 1.862 ± 0.112ms, n=14) compared with recordings from mice with ELS (the resting membrane potential: -60.670 ± 1.632mV, n=11, *P*=0.380; the input resistance: 434.900 ± 39.190ΩM, n=11, *P*=0.572; the AP amplitude: 81.550 ± 4.517mV, n=11, *P*=0.527; the AP threshold: -44.350 ± 1.360 mV, n=11,

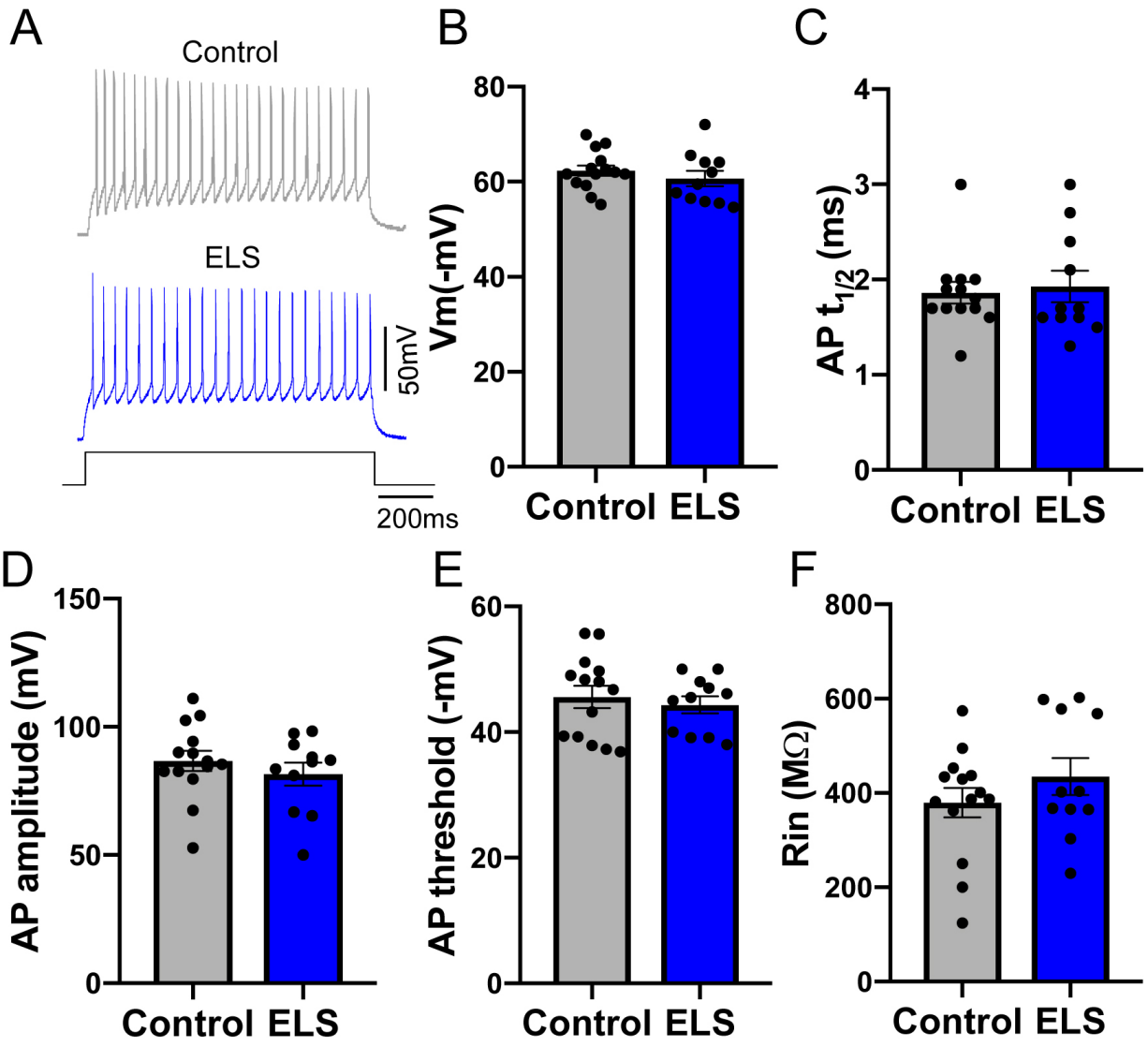

Figure 5. ELS did not significantly modulate intrinsic membrane properties in non-FS interneurons. **(A)** Representative current clamp recordings in response to hyperpolarizing and depolarizing current steps in non-FS interneurons from control (top) and ELS (bottom) conditions**. (B-F)** No significant difference between the intrinsic properties between non-FS interneurons in control and ELS conditions, resting membrane potential **(B),** AP $t_{1/2}$ **(C**), AP amplitude **(D)**, AP threshold **(E)**, and input resistance **(F),** n=13, 11 from 6 and 4 mice, P >0.05. Data are expressed as mean ± SEM.

P=0.657; the AP1/2time: 1.927± 0.165ms, n=11, *P*=0.635, Figure 5A-F). My results indicated that ELS did not significantly alter the intrinsic membrane properties of non-FS interneurons in CA1 stratum oriens in the developing hippocampus.

4.2.2 ELS did not significantly alter the excitatory afferent inputs into non-FS interneurons in CA1 stratum oriens.

To determine whether ELS affected the excitatory afferent inputs into non-FS interneurons in CA1 stratum oriens, I first assessed the AMPAR mediated sEPSCs in non-FS interneurons using whole-cell voltage-clamp recordings at a holding potential of -60mV in *ex vivo* hippocampal slices removed from P10-12 1h post-ELS mice and littermate control mice. I found that there was no significant difference in AMPAR-mediated sEPSC amplitude in non-FS interneurons from the control mice 23.620 ± 1.106pA, n=29, compared with recordings from the 1h post ELS mice 23.200 ± 1.090pA, n=21, *P*=0.965, (Figure 6A-C). There was no significant difference in AMPAR-mediated sEPSC frequency between the control non-FS interneurons 0.651 ± 0.191Hz, n=28, and post-ELS non-FS interneurons 0.483 ± 0.159Hz, n=21, *P*=0.338 (Figure 6A, B &D). Moreover, I did not observe significant changes in the kinetics of AMPAR-mediated sEPSCs in non-FS interneurons from control mice (rise time: 1.305 ± 0.168ms, n=29; decay time: 4.328 ± 0.419ms, n=29) compared with recordings from post-ELS mice (rise time: 1.587 ± 0.352ms, n=20, *P*=0.204, Figure 6E; decay time: 4.842 ± 0.435ms, n=20, *P*=0.101, Figure 6F). In addition, no significant changes in PPRs was observed between non-FS interneurons in hippocampal slices from post-ELS mice and littermate control mice at this age, indicating that the presynaptic probability of release was not altered at these excitatory inputs (ISI of 20ms: control 2.868 ± 0.345, n=18 vs ELS 2.706 ± 0.294, n=14, *P*=0.640; ISI of 30ms: control 2.692 ± 0.336, n=20 vs ELS 2.477 ± 0.254, n=20, *P*=0.995; ISI of 50ms: control 2.426 ± 0.326, n=20 vs ELS 2.226 ± 0.225,

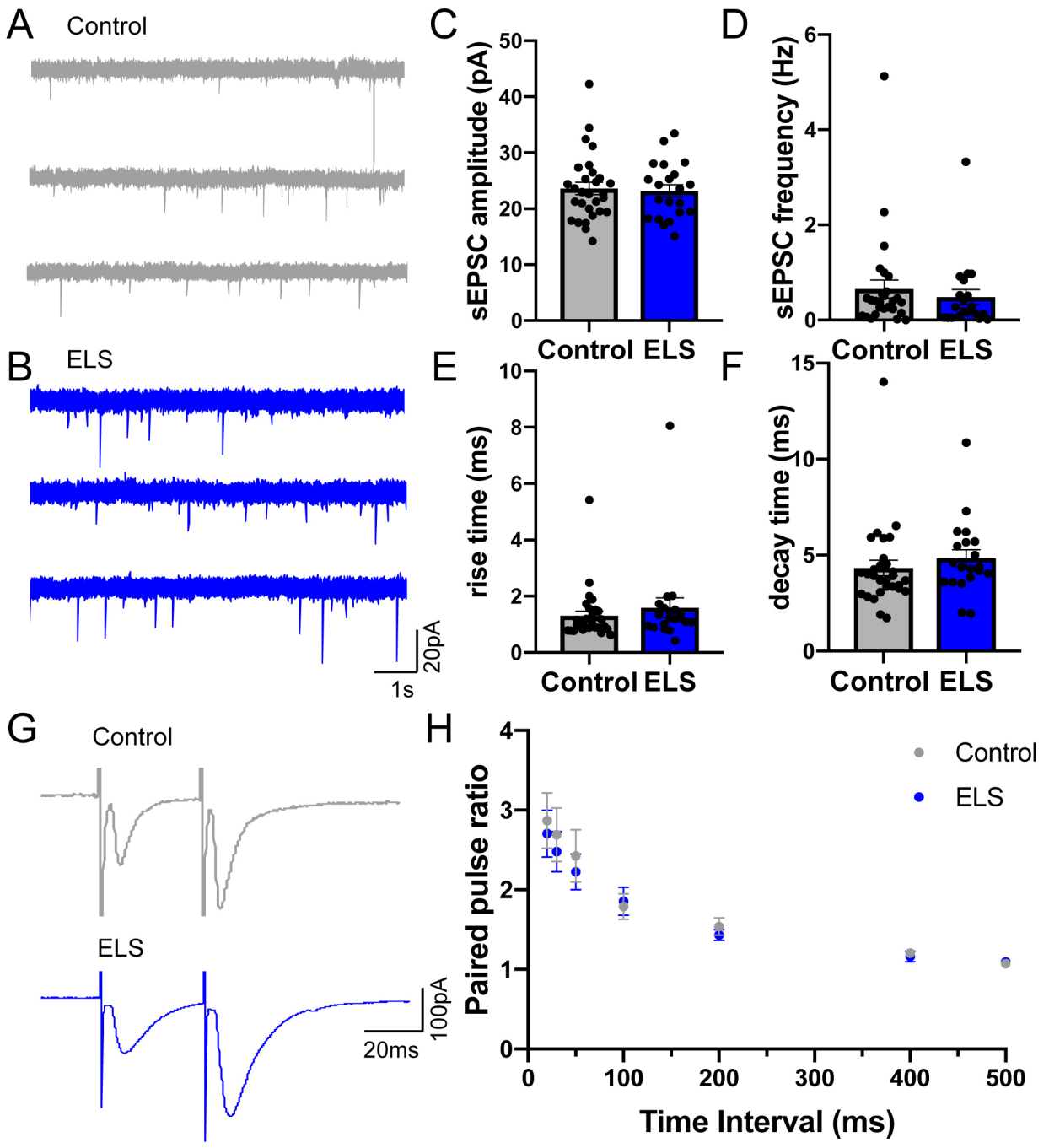

Figure 6. ELS did not significantly alter AMPAR function in non-FS interneurons. (**A-B**) Representative traces of AMPAR-mediated sEPSCs in non-FS interneurons, pharmacologically isolated by blocking NMDA and $GABA_A$ receptors, in control (**A**) and ELS (**B**) conditions, in hippocampal ex vivo slices from P10-12 mice pups. **(C-F)** AMPAR sEPSC amplitude (**C**), frequency (**D**), rise time (**E**) and decay time (**F**) did not show significant difference between non-FS interneurons from control and ELS conditions, n=29, 20 from 20 and 12 mice, $P>0.05$. (**G**) Representative traces of paired pulse EPSC recordings in non-FS interneurons from control (top) and ELS (bottom) conditions at ISI of 30ms. Cells were voltage clamped at -60mV and stimulation electrode placed ~100um away, both at CA1 stratum oriens. **(H)** Group data showed that no significant differences in paired-pulse ratio at any stimulation ISIs between non-FS interneurons from control and ELS conditions, n=18, 29 from 14 and 20 mice, $P>0.05$. Data are expressed as mean ± SEM.

n=17, *P*=0.892; ISI of 100ms: control 1.787 ± 0.161, n=17 vs ELS 1.855 ± 0.177, n=18, *P*=0.782; ISI of 200ms: control 1.537 ± 0.110, n=21 vs ELS 1.431 ± 0.670, n=18, *P*=0.989; ISI of 400ms: control 1.204 ± 0.046, n=20 vs ELS 1.160 ± 0.066, n=17, *P*=0.460; ISI of 500ms: control 1.073 ± 0.031, n=18 vs ELS 1.094 ± 0.050, n=14, *P*=0.587; Figure 6G and H). These results suggest that ELS did not significantly modulate the excitatory afferent inputs into non-FS interneurons in CA1 stratum oriens in the developing hippocampus.

4.3. Acute effects of ELS on FS interneurons in CA1 stratum oriens.

Given that ELS did not affect the excitatory afferent inputs in the non-FS interneurons, I next evaluated whether ELS modulates the excitatory afferent inputs into FS interneurons in CA1 stratum oriens in a target specific manner.

4.3.1 Acute effects of ELS on intrinsic membrane properties of FS interneurons in CA1 stratum oriens.

Before investigating the effects of ELS on excitatory inputs onto FS interneurons in CA1 stratum oriens, I determined if the intrinsic membrane properties of FS interneurons were acutely altered by ELS, using whole-cell current clamp recordings in CA1 FS interneurons in *ex vivo* hippocampal slices removed from P10-12 1h post-ELS mice and littermate control mice. I found that there were no significant changes in intrinsic membrane properties in FS interneurons from control mice (the resting membrane potential: -62.390 ± 1.881mV, n=17; the input resistance: 371.600 ± 54.230ΩM, n=10; the AP amplitude: 75.680 ± 1.788mV, n=17; the AP threshold: -41.390 ± 1.017mV, n=14; the AP1/2time: 1.462 ± 0.069ms, n=13) compared with recordings from mice with ELS (the resting membrane potential: -59.310 ± 1.527mV, n=18, *P*=0.291; the input resistance: 340.300 ± 46.700ΩM, n=15, *P*=0.683; the AP amplitude: 77.180 ± 3.025mV, n=15,

P=0.574; the AP threshold: -41.770 ± 0.890mV, n=13, *P*=0.572; the AP1/2time: 1.617 ± 0.077ms, n=18, *P*=0.215, Figure 7A-F). My results indicated that ELS did not significantly alter the intrinsic membrane properties of FS interneurons in CA1 stratum oriens in the developing hippocampus. In addition, I further examined the input-output relationship of AP firing frequency evoked by depolarizing current steps in FS interneurons from the control and ELS groups. I found that the firing frequency of FS interneurons in response to lower depolarizing current injections did not show significant differences between from the control and ELS groups. However, 200pA current injections evoked significantly lower firing frequency in FS interneurons from post-ELS mice (28.833 ± 2.150Hz, n=18) compared with FS interneurons from the control mice (37.940 ± 2.282Hz, n=18; *P*=0.028, Figure 7G-I). These results suggest that ELS might be impairing the ability of FS interneurons to response to strong excitatory inputs.

4.3.2 Effects of ELS on the excitatory afferent inputs into FS interneurons in CA1 stratum oriens.

Next, I assessed the effects of ELS on the excitatory afferent inputs into FS interneurons in CA1 stratum oriens by first measuring the AMPAR-mediated sEPSCs using whole-cell voltage-clamp recordings at a holding potential of -60mV, in *ex vivo* hippocampal slices removed from 1h post-ELS mice and littermate control, P10-12 mice. There was no significant difference in AMPAR sEPSC amplitude in FS interneurons between the control group 28.550 ± 2.793pA, n=10 and the ELS group 19.870 ± 1.208pA, n=10, *P*=0.051 (Figure 8A-C). However, FS interneurons in slices from mice with ELS showed significantly smaller AMPAR sEPSCs frequency 0.091 ± 0.026Hz, n=10, compared with FS interneurons from littermate controls 0.915 ± 0.280Hz, n=10, *P*=0.0016 (Figure 8A, B and D). There was no significant difference in AMPAR sEPSC rise time

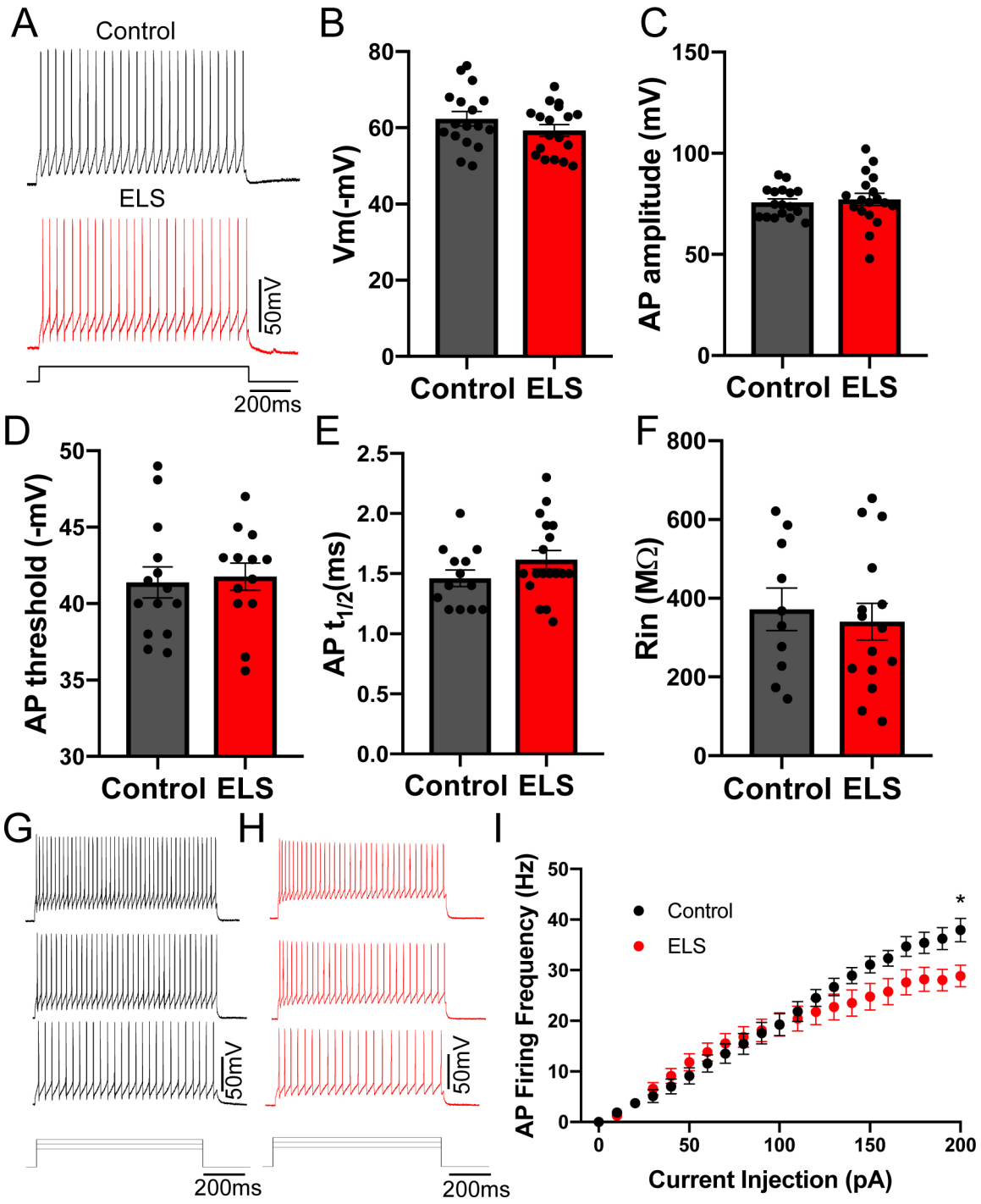

Figure 7. Effects of ELS on intrinsic membrane properties of FS interneurons. **(A)** Representative action potential recordings during depolarizing current injections in FS interneurons from control (top) and ELS (bottom) groups. **(B-F)** No significant difference between the intrinsic properties between FS interneurons from control and ELS conditions, resting membrane potential (**B**), AP amplitude **(C)**, AP threshold **(D)**, AP $t_{1/2}$ (**E**) and input resistance (**F**), n=15, 18 from 10 and 15 mice, P>0.05. **(G-I)** Representative traces of FS interneurons from control (**G**) and ELS (**H**) conditions, in response to depolarizing current injection steps. **(I)** Input-output function of FS interneurons in control and ELS conditions show significant difference in firing frequencies during 200pA current injection step. n=15, 15 from 10 and 12 mice. *P <0.05. Data are expressed as mean ± SEM.

and decay time between FS interneurons from the control group (rise time 1.177 ± 0.193ms, n=9; decay time 4.214 ± 0.684ms, n=9) and FS interneurons from the ELS group (rise time 1.743 ± 0.200ms, n=9, *P*=0.051; decay time 6.187 ± 0.788ms, n=10, *P*=0.079, Figure 8E and F).

Significant decrease in AMPAR-mediated sEPSC frequency suggest a potential presynaptic mechanism by which ELS modulated the excitatory afferent synapses of FS interneurons. In order to confirm the ELS-induced presynaptic modulation of the excitatory afferent inputs of FS interneurons, I assessed the PPRs at different ISIs of 20, 30, 50, 100, 200, 400 and 500ms in FS interneurons, in *ex vivo* hippocampal slices removed from 1h post-ELS mice and littermate control, P10-12 mice. Indeed, my PPR results confirm significant changes in presynaptic neurotransmitter release probability as evidenced by significant decreases in PPRs in FS interneurons from the ELS group compared to the control group (PPR at ISI of 20ms: control 2.638 ± 0.245, n=9 vs ELS 1.503 ± 0.087, n=9, *P*=0.003 PPR at ISI of 30ms: control 2.250± 0.268, n=9 vs ELS 1.272 ± 0.065, n= 9, *P*=0.016; PPR at ISI of 50ms: control 1.786 ± 0.164, n=9 vs ELS 1.333 ± 0.081, n=9, *P*=0.118; PPR at ISI of 100ms: control 1.713 ± 0.251, n=9 vs ELS 1.232 ± 0.097, n= 9, *P*=0.177; PPR at ISI of 200ms: control 1.352 ± 0.079, n=9 vs ELS 1.074 ± 0.100, n=7, *P*=0.165; PPR at ISI of 400ms: control 1.178± 0.040, n=9 vs ELS 1.058 ± 0.044, n=7, *P*=0.175; PPR at ISI of 500ms: control 1.161 ± 0.051, n=9 vs ELS 1.114 ± 0.162, n=6, *P*=0.749; Figure 8G and H).

Collectively, these data suggest that ELS acutely modulates the excitatory afferent inputs into FS interneurons in CA1 stratum oriens by decreasing AMPAR sEPSC frequency through modulating the presynaptic probability of neurotransmitter release.

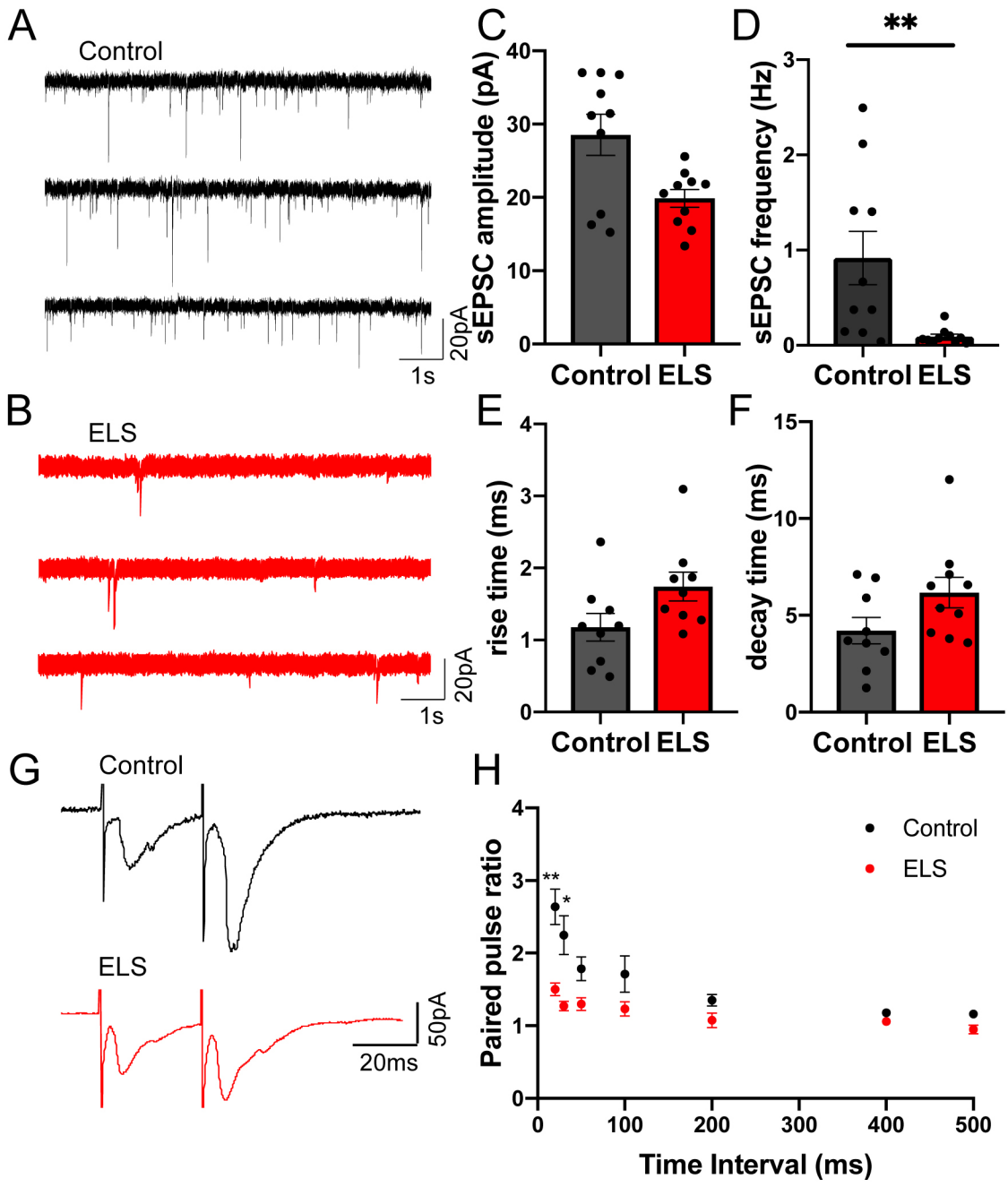

Figure 8. ELS significantly modulated AMPAR function in FS interneurons. (**A-B**) Representative traces of AMPAR-mediated sEPSCs in FS interneurons, pharmacologically isolated by blocking NMDA and $GABA_A$ receptors, under control (**A**) and ELS (**B**) conditions, in hippocampal ex vivo slices from P10-12 mice pups. **(C-F)** AMPAR sEPSC amplitude (**C**) had no significant change, n=10, 10, from 8 and 7 mice, $P > 0.05$. AMPAR frequency (**D**) was significantly decreased by ELS in FS interneurons, n=10, 10, from 8 and 7 mice, $^{**}P < 0.01$. (**E-F**) AMPAR sEPSC rise time (**E**), and decay time (**F**) showed no significant difference between FS interneurons from control and ELS conditions, n=9, 10, from 8 and 7 mice $P > 0.05$. (**G**) Representative traces of paired-pulse EPSC recordings in FS interneurons from control (top) and ELS (bottom) conditions at ISI of 30ms. Cells were voltage clamped at -60mV and stimulation electrode placed ~100um away, both in stratum oriens hippocampal CA1 region. (**H**) Group data showed that ELS significantly decreased the paired-pulse ratio at ISI of 20ms and 30ms in FS interneurons in CA1 striatum oriens, n=9, 9 from 7 and 8 mice. Data are expressed as mean ± SEM. $^{**}P < 0.01$.

4.3.3 ELS impairs short-term plasticity of excitatory afferent synapses onto FS interneurons in CA1 stratum oriens.

Next, I investigated whether ELS impairs short-term plasticity at the excitatory afferent inputs into FS interneurons evoked by trains of repetitive stimulations (20 pulses), in *ex vivo* hippocampal slices removed from 1h post-ELS mice and littermate control P10-12 mice. Repetitive stimulation at 1 and 5 Hz did not evoke significant facilitation or depression in FS interneurons from both control and post-ELS groups (Figure 9B & C). Stimulation at 10 and 20Hz evoked similar short-term facilitation in FS interneurons in the control group (the normalized EPSC amplitude of the 20th stimuli for FS interneuron at 10Hz is 184.137 ± 33.234%, n=16; 20Hz is 296.428 ± 65.289%, n=13) compared with the post-ELS group, (the normalized EPSC amplitude of the 20th stimuli for FS interneuron at 10Hz is 178.594 ± 38.550%, n=11 P=0.914, (Figure 9D); 20Hz is 216.537 ± 34.129%, n=7, P=0.889 (Figure 9D & E)). However, during the high frequency repetitive stimulation at 50 Hz, the level of the short-term facilitation at the excitatory synapse onto FS interneurons was significantly decreased in the ELS group compared to the control group (Figure 9A & F). During 50 Hz stimulation, FS interneurons from the control mice showed 378.768 ± 61.718% (n=9) for the normalized EPSC amplitude at the 20th stimuli and 376.200 ± 4.792% for the last 5 AMPAR eEPSCs (n=9). In contrast, FS interneurons from the post-ELS mice exhibited 132.628±16.894% at the 20th stimuli (n=9, P=0.002) and 149.2 ± 4.665% for the last 5 AMPAR eEPSCs (n=9, P=0.008) (Figure 9A & F). These data further support ELS impairs short-term plasticity and alters the frequency-dependent information processing in FS interneurons. The mechanisms underlying short-term plasticity can be dependent on presynaptic alterations of neurotransmitter release or/and postsynaptic modifications of receptor numbers (Zucker, 1989). My previous findings (Figure 8) showed that ELS decreased AMPAR-mediated sEPSC

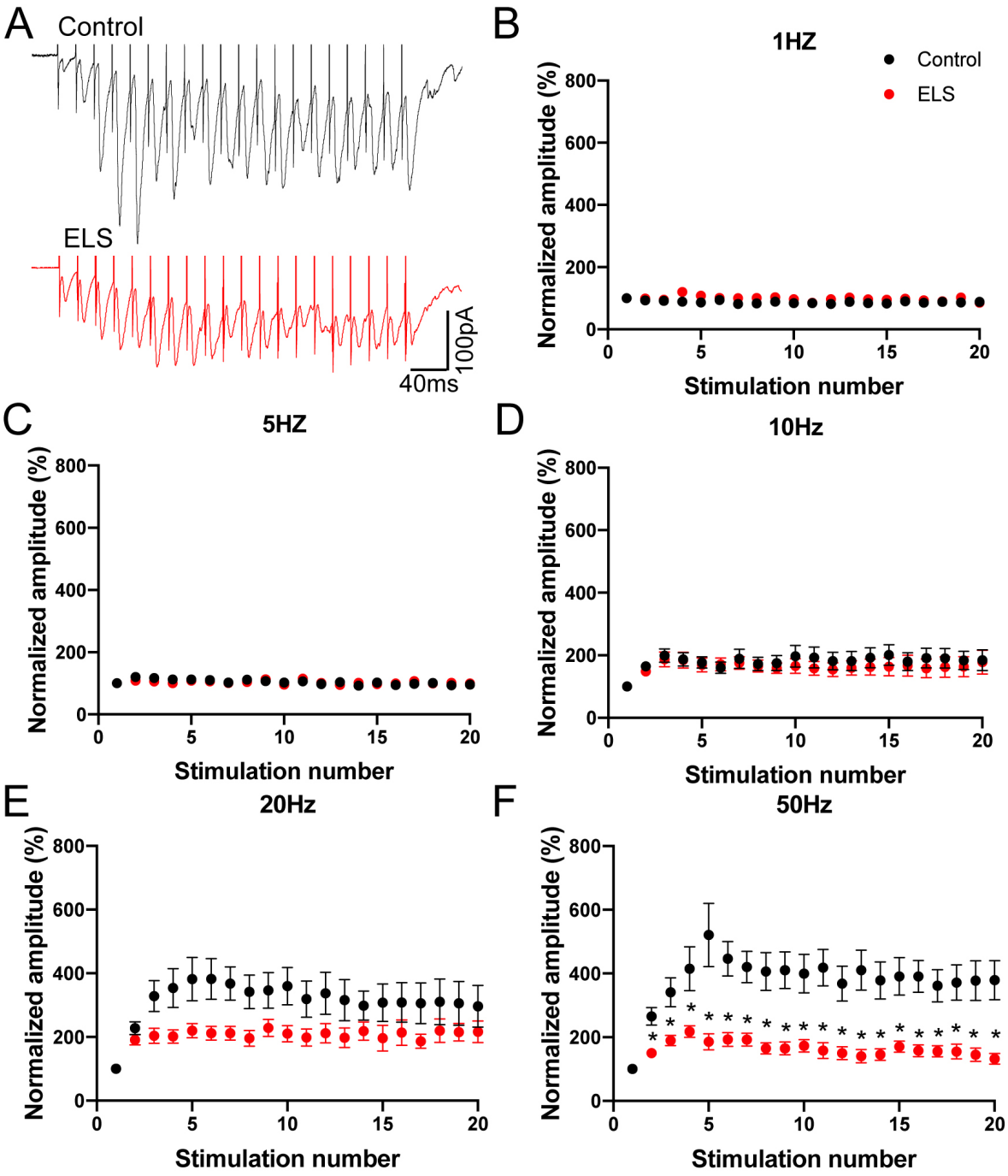

Figure 9. ELS significantly modulated short-term plasticity in FS interneurons in striatum oriens from the developing hippocampus. (**A**) Representative traces of averaged trains of eEPSC elicited by 50 Hz repetitive stimulation train, FS interneurons in control (top) and ELS (bottom) conditions from hippocampal *ex vivo* slices. **(B-F)** Group data of EPSCs normalized to the first EPSC amplitude of FS interneuron from control and ELS conditions during 1, 5, 10, 20 & 50 Hz trains. At stimulation frequencies of 1, 5, 10 & 20Hz ELS did not significantly alter short-term plasticity at excitatory afferent inputs onto FS interneurons n=17, 12, from 12 and 8 mice, $P>0.05$ **(B-E)**. At stimulation frequency of 50Hz, ELS significant depressed short-term facilitation at excitatory afferent inputs onto FS interneurons, n=9, 8 from 8 and 8 mice **(F)**. *$P<0.05$. Data expressed as mean ± SEM.

frequency without affecting AMPAR-mediated sEPSC amplitude and PPR change, suggesting that ELS-induced impairment in the short-term plasticity is mediated through a presynaptic mechanism at excitatory afferent synapses onto FS interneurons in CA1 stratum oriens. Therefore, I further investigated how ELS affect presynaptic function of these excitatory afferents.

4.3.4 ELS altered the excitatory afferent inputs into FS interneurons in CA1 stratum oriens by decreasing the presynaptic readily releasable pool (RRPs).

Short-term plasticity can be modulated at the presynaptic membrane through two key factors, the probability of neurotransmitter release and the availability of readily releasable pool, which can be depleted during high frequency stimulation (Zucker and Regehr, 2002). Availability of presynaptic vesicles pools are critical in initiating the flow of information in neural networks and play crucial roles in determining the synaptic strength (Zucker and Regehr 2002; Regehr, 2002; Fioravante and Regehr, 2011). To assess whether ELS alters the availability of the presynaptic vesicle pool and initial release probability in FS interneurons, I adapted the synaptic plasticity modelling according to previous work (Dittman et al., 2000; Yang and Xu, 2008) to calculate the number of RRPs at the presynaptic membrane when transmission strength reaches a steady level during a train of repetitive stimulations.

In my mathematical model, the amplitude of any excitatory postsynaptic current can be calculated through this equation: $EPSC = q * N_{RRP} * P_{r.}$

1) $\boldsymbol{q}$ represents the quantal size. In this study, I used the mean amplitude of AMAPR sEPSCs to estimate $\boldsymbol{q}$ as no spontaneous action potentials were detected during prolonged current recordings in FS interneurons in CA1 stratum oriens at this age and action potentials cannot be blocked to identify the FS interneurons.
2) $\boldsymbol{N_{RRP}}$ represents the number of the ready releasable vesicles or the size of the RRPs.

3) $\boldsymbol{P_r}$ represents the release probability of presynaptic ready releasable vesicles.

Considering the short-term facilitation behaviour of excitatory synapses onto FS interneurons (see Figure 9) during a train of repetitive stimulations, I calculated the number of RRPs (N_{RRP}) at the presynaptic membrane when EPSC responses reach to a steady state during a train of simulations. As shown in Figure 10, an example of the approach is illustrated to estimate the N_{RRP} and the release probability P_r. As shown in Figure 10A, during a train of repetitive stimulation, the EPSC amplitude initially fluctuates reflecting the short-term plasticity and the changes between RRP, the rate of replenishment and probability of release. However, the steady state of EPSC responses was achieved later in the train when these presynaptic factors stabilize and remains constant. Therefore, the cumulative release plot (Figure 10C) is generated by plotting the accumulated peak EPSC amplitudes against the stimulation numbers based on the recordings shown in Figure 10A. The back-extrapolating line from the steady responses of the cumulative EPSC graph was used to calculate the I_{RRP} which is the N_{RRP} multiplied by the quantal size q and the initial probability of release Pr. My results showed that the number of the ready releasable vesicles, $N_{RRP,}$ at excitatory afferent synapses of FS interneurons was significantly smaller in slices from post-ELS mice 18.680 ± 5.373, n=6, compared with the slices from littermate control mice 43.710 ± 8.165, n=9, P=0.036 (Figure 10D). In addition, the initial probability of release at excitatory afferent synapses of FS interneurons of FS interneurons was significantly larger in slices from post-ELS mice 0.353 ± 0.133, n=6, compared with the slices from littermate control mice 0.0520 ± 0.009, n=9, P<0.001 (Figure 10E). These data strongly support that ELS modified the excitatory afferent synapses of FS interneurons by decreasing the size of effective readily releasable pool and increasing the initial probability of neurotransmitter release in the presynaptic terminal.

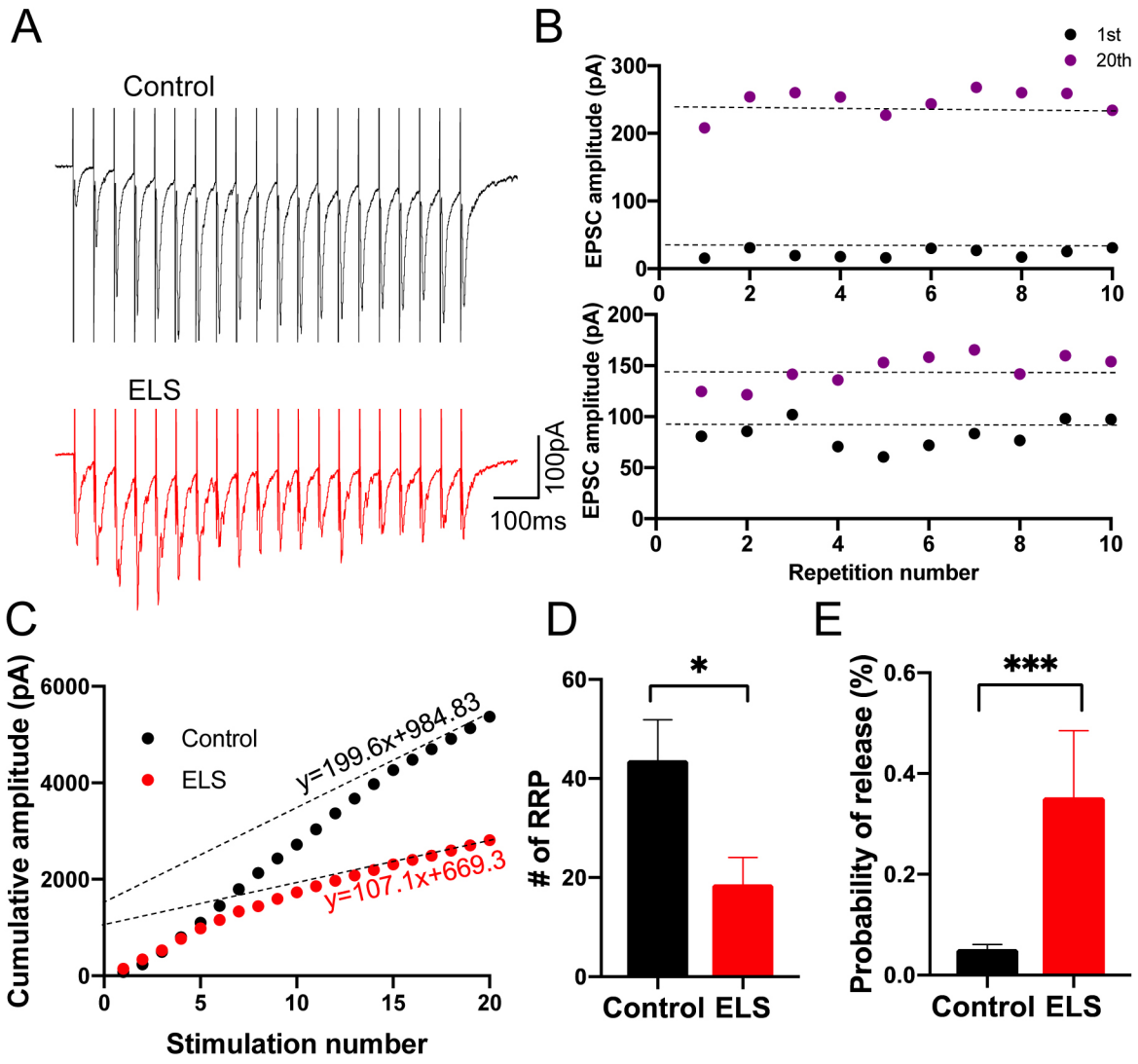

Figure 10. ELS induced changes at the excitatory afferents of FS interneurons by modulating the presynaptic readily releasable pools size. (**A**) Representative traces of averaged trains of eEPSC elicited by 20 Hz repetitive stimulation train in FS interneurons from control (top) and ELS (bottom) groups, in hippocampal ex vivo slices from P10-12 mice. (**B**) Top (control) and bottom (ELS) plots represents EPSC amplitude from the **first** stimulation (black) and plot of the EPSC amplitude from the **last** stimulation (purple) for 10 repeated sweeps. **(C)** Example of a cumulative plot of the EPSC amplitudes from a train of 20 repetitive pulses, with the line of best fit for the last 3-5 EPSC amplitudes for FS interneurons in control and ELS conditions. **(D-E)** Bar graph showing that the average number of presynaptic RRPs (**D**) and probability of release for the first stimuli **(E)** for FS interneurons was significantly between control and ELS groups n=9, 6 from 8 and 6 mice. *P <0.05, ***P <0.001. Data expressed as mean ± SEM.

4.3.5 ELS suppressed asynchronous neurotransmitter release at excitatory afferents in CA1 FS interneurons.

There are two forms of action potential evoked neurotransmitter release: fast synchronous release and delayed asynchronous release. Fast synchronous release is triggered in phase with presynaptic action potentials and provide rapid communication between neurons (Hua et al., 2013; Evstratova et al., 2014). Slow asynchronous release occurs after the termination of presynaptic action potentials and provides a prolonged modulation of the excitability of postsynaptic neurons (Evstratova et al., 2014; Rozov et al., 2019). Next, I aimed to determine whether ELS could affect slow asynchronous release at excitatory afferents in CA1 FS interneurons. To achieve this purpose, I stimulated the excitatory afferents to CA1 FS interneurons using a 20 Hz repetitive train with 20 pulses and 1s duration (repeated 10 times with 30s interval). Clearly, many EPSC events were prominent during the decay phase of the last EPSC and could be observed up to 1s after the last stimulation (Figure 11A), confirming the asynchronous release at excitatory afferents in CA1 immature FS interneurons for the first time in literature. To quantify the asynchronous release, the number of these EPSC events were counted 200 ms after the last stimulation pulse within a 1s period. The sEPSC events before the first stimulation within a 1s period was counted to be used as baseline to accurately calculate asynchronous EPSCs (aEPSCs) using the equation: aEPSCs=(2 aEPSCs+sEPSCs)−(1 sEPSCs). As shown in Figure 11, FS interneurons in slices from age-matched P10-12 control mice exhibited a sEPSC frequency of 1.120 ± 0.471Hz, n=10 before the first stimulation, and a sEPSC+aEPSC (EPSCs) frequency of 4.740 ± 0.723Hz, n=10, 200 ms after the last stimulation pulse, yielding an aEPSC frequency of 3.620 ± 0.7126Hz, n=10 (Figure 11A and C). In contrast, FS interneurons in slices removed from p10-12 mice 1h after ELS

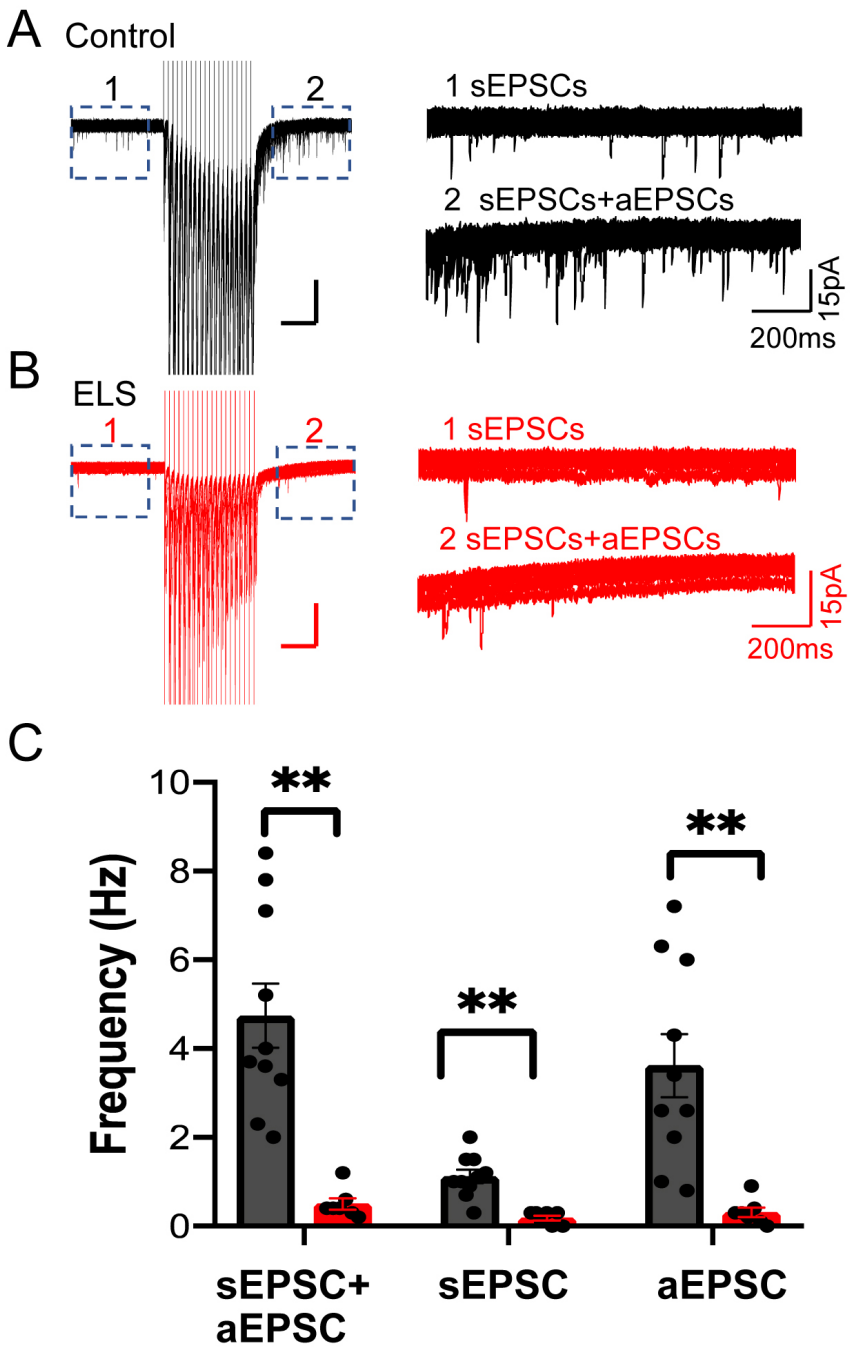

Figure 11. ELS significantly decreased asynchronous neurotransmitter release at excitatory afferent inputs of FS interneurons. (A-B) Representative traces of sEPSCs before (**1**) and aEPSCs+aEPSCs after (2) the 20 stimuli delivered at 20Hz in FS interneurons from control **(A)** ELS **(B)** groups. Inserts show expanded one second regions (**1**) and (**2**). **(C)** Bar graph showing that the average frequency of sEPSC+aEPSC, sEPSC, and aEPSC in FS interneurons was significantly different between control and ELS groups. n=10, 7 from 8 and 6 mice. Data expressed as mean ± SEM. **P <0.001.

showed a significantly lower sEPSC frequency of 0.186 ± 0.050Hz, n=7, *P*=0.0004, before the first stimulation, and a smaller sEPSC+aEPSC (EPSC) frequency of 0.500 ± 0.125Hz, n=7, *P*=0.0001, 200 ms after the last stimulation pulse, yielding an aEPSC frequency of 0.314 ± 0.110Hz, n=7, *P*=0.0002 (Figure 11B and C). These data strongly support that ELS significantly suppressed asynchronous neurotransmitter release at excitatory afferents in CA1 FS interneurons.

4.3.6 ELS acutely impaired action potential temporal precision and fidelity in CA1 FS interneurons.

Asynchronous neurotransmitter release has been shown to critically determine action potential timing and control postsynaptic signal integration (Scheuss et al., 2007; Evstratova et al., 2014; Rozov et al., 2019). Since I showed that asynchronous neurotransmitter release at excitatory afferents onto CA1 FS interneurons was suppressed by ELS, I further examined whether action potential temporal precision and fidelity are impaired by ELS.

To investigate the effects of ELS on action potential precision, I did a series of experiments in which I used 10 trials of repetitive stimulation (20 pulses) at a frequency of 1, 5, 10, and 20Hz (Figure 12). The action potential precision was quantified using the spike jitter, which is defined as the standard deviation of individual spike latencies in response to 10 trials of 20 pulse repetitive stimulus. As shown in Figure 12, I first quantified the action potential jitter averaged over spikes in response to **each pulse** over 10 stimulation trains and found that CA1 FS interneurons in slices removed from p10-12 mice 1h after ELS showed significant decreases in action potential precision in response to 1, 5, 10, and 20Hz repetitive stimulation compared to age-matched P10-12 control mice (1Hz, from 1st pulse to 20th pulse: control action potential jitter range from 0.406 to 1.016ms, n=6 vs ELS from 2.201 to 5.291ms, n=6, $p<0.05$ for 1st, 5th, 7th, 16th, and 19th pulses, Figure 12A, B and C; 5Hz: from1st pulse to 20th pulse: control action potential jitter range from 0.200 to

0.479ms, n=6 vs ELS from 1.384 to 3.979ms, n=6, $P<0.05$ for 1st and 14th pulses, Figure 12 A, B and D; 10Hz: from1st pulse to 20th pulse: control action potential jitter range from 0.679 to 2.129ms, n=5 vs ELS from 2.800 to 5.066ms, n=6, $P<0.05$ for 1st, 6th, 7th, 11th, 12th, and 14th pulses, Figure 12 A, B and E; 20Hz: from1st pulse to 20th pulse: control action potential jitter range from 0.394 to 1.831ms, n=4 vs ELS from 3.212 to 9.165ms, n=4, $P<0.05$ for 2nd, 3rd, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th, 15th, 16th, 17th, 18th, 19th, and 20th pulses, Figure 12 A, B and F). Furthermore, the overall action potential jitter averaged over **all 20 pulses** during each challenge period in CA1 FS interneurons in slices removed from p10-12 mice 1h after ELS showed significantly increases in response to repetitive stimulus at frequencies of 1, 5, 10, and 20Hz compared to age-matched P10-12 control mice (1Hz: control 0.609 ± 0.289ms, n=6 vs ELS 3.66 ± 1.46ms, n=6, $P=0.026$; 5Hz: control 0.322 ± 0.213ms, n=6 vs ELS 2.327 ± 0.899ms, n=6, $P=0.009$; 10Hz: control 1.180 ± 0.511ms, n=5 vs ELS 3.603 ± 0.713ms, n=6, $P=0.017$; 20Hz: control 0.861 ± 0.389ms, n=4 vs ELS 5.241 ± 0.930ms, n=4, $P=0.029$, Figure 12G). These results demonstrated that ELS acutely impaired action potential temporal precision in CA1 FS interneurons in the developing hippocampus.

To investigate the effects of ELS on action potential fidelity, I quantified the fidelity rate, i.e., when an action potential was successfully evoked by a stimulation pulse. I did a series of experiments in which I also used 10 trials of repetitive stimulation (20 pulses) at a frequency of 1, 5, 10, 20, and 50Hz. As shown in Figure 13, I first quantified the fidelity rate over spikes in response to **each pulse** over 10 stimulation trains and found that excitatory afferents to CA1 FS interneurons in slices removed from both control and ELS P10-12 mice were able to reliably

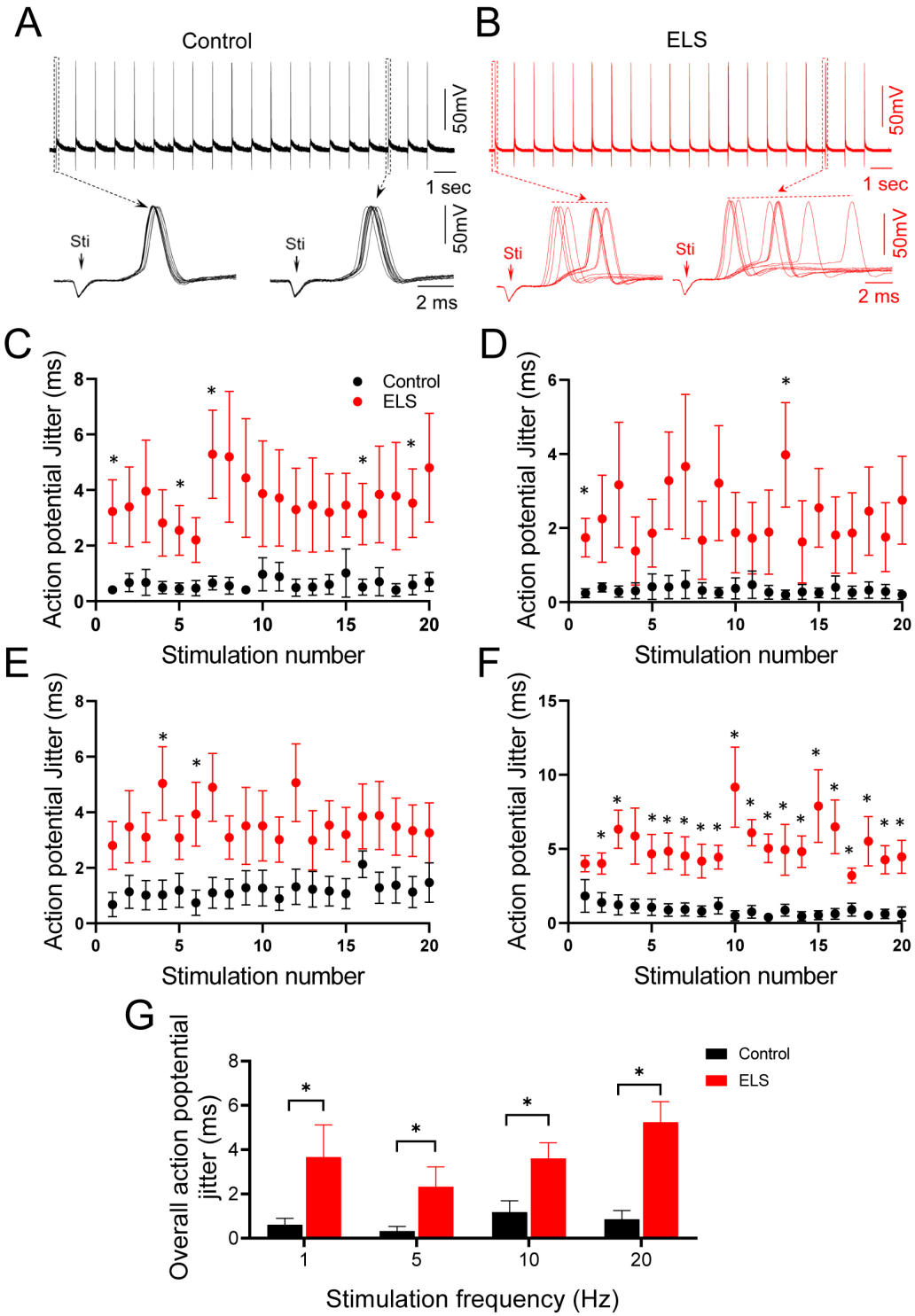

Figure 12. ELS acutely impairs action potential precision in FS interneurons in CA1 striatum oriens from the developing hippocampus. (**A-B**) Representative postsynaptic action potentials evoked by 1Hz repetitive stimulation in CA1 FS interneurons in hippocampal *ex vivo* slices from a P10 control mouse (**A**) and a P10 post-ELS mouse (**B**). (**C-F**) Action potential jitter as a function of the stimulation number during sustain repetitive stimulation at 1Hz (**C**), 5Hz (**D**), 10Hz (**E**), and 20Hz (**F**). Note that significant decreases in action potential jitter at 1, 5, 10 and 20Hz repetitive stimulation following ELS. n=5-6. *p<0.05. (**G**) Comparisons of the overall action potential jitter at 1, 5, 10, and 20Hz stimulation frequency in FS and non-FS interneurons. n=5-6. *p<0.05. Error bars indicate S.E.M.

evoke action potential with a high fidelity at 1 and 5 Hz stimulation (1Hz, from1st pulse to 20th pulse: control fidelity rate range from 88.33% to 98.33%, n=6 vs ELS fidelity rate from 73.331% to 93.332%, n=6, P>0.05 for all pulses, Figure 13A,B and C; 5Hz: from1st pulse to 20th pulse: control fidelity rate range from 88.333% to 100%, n=6 vs ELS fidelity rate from 93.333% to 100%, n=6, P>0.05 for all pulses, Figure 13 A, B and D). At 10, 20 and 50 Hz, excitatory afferents to CA1 FS interneurons in slices removed from P10-12 ELS mice showed significantly lower fidelity rate compared to age-matched P10-12 control mice (10Hz: from1st pulse to 20th pulse: control fidelity rate range from 75% to 100%, n=6 vs ELS fidelity rate range from 66.67% to 80.00%, n=6, P<0.05 for 11th, 12th, 13th, and 20th pulses, Figure 13A, B and E; 20Hz: from1st pulse to 20th pulse: control fidelity rate range from 70.00% to 100%, n=5 vs ELS fidelity rate range from 57.50% to 70%, n=5, P<0.05 for 16th, 17th, 18th, and 20th pulses, Figure 13 A, B and F; 50Hz: from 1st pulse to 20th pulse: control fidelity rate range from 47.50% to 72.50%, n=5 vs ELS fidelity rate range from 10.00% to 40.00%, n=3, P<0.05 for 3rd, 5th, 9th, 16th, and 20th pulses, Figure 13 A, B and G). Furthermore, I quantified the overall action potential fidelity rate over **all 20 pulses** during each challenge period. The CA1 FS interneurons in slices removed from p10-12 mice 1h after ELS showed significantly decreases in overall spike fidelity rate at frequencies of 1, 10, 20, and 50Hz compared to age-matched P10-12 control mice (1Hz: control 92.170 ± 1.770%, n=6 vs ELS 82.250 ± 3.070%, n=6, P=0.006; 5Hz: control 95.250 ± 1.080%, n=6 vs ELS 96.000 ± 1.430%, n=6, P=0.675; 10Hz: control 86.400 ± 2.880%, n=6 vs ELS 73.250 ± 4.390%, n=6, P=0.021; 20Hz: control 84.380 ± 2.360%, n=5 vs ELS 67.750 ± 5.040%, n=5, P=0.017; 50Hz: control 56.250 ± 3.790%, n=5 vs ELS 27.250 ± 3.580%, n=3, P=0.0005; Figure 13H). These results confirm that ELS acutely impaired action potential fidelity during sustained high frequency stimulation in CA1 FS interneurons in the developing hippocampus.

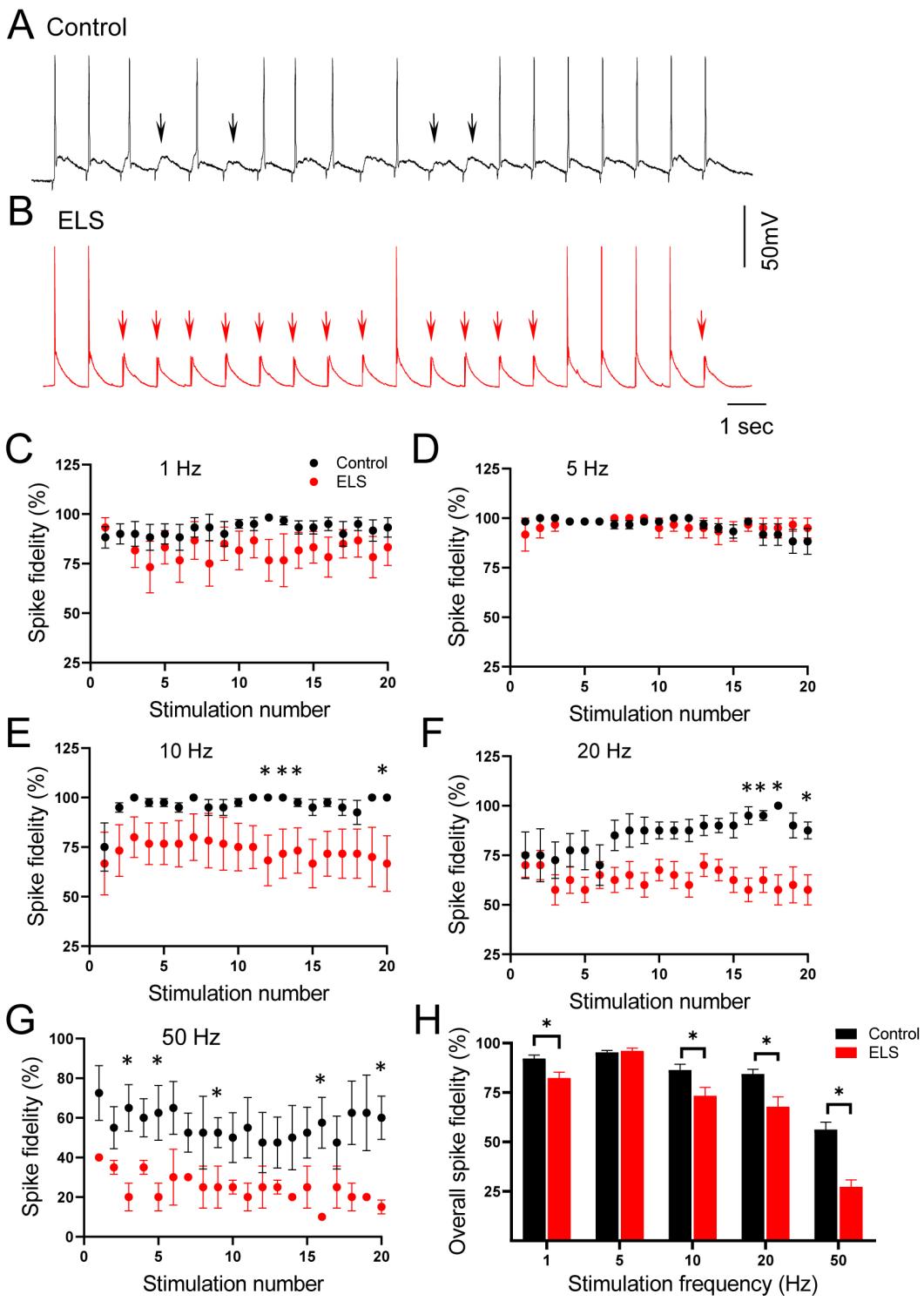

Figure 13. ELS acutely impairs action potential fidelity in FS interneurons in CA1 striatum oriens from the developing hippocampus. (A-B) Representative postsynaptic action potentials evoked by 1Hz repetitive stimulation in CA1 FS interneurons in hippocampal *ex vivo* slices from a P10 control mouse (A) and a P10 post-ELS mouse (B). (C-G) Spike fidelity as a function of the stimulation number during sustain repetitive stimulation at 1Hz (C), 5Hz (D), 10Hz (E), 20Hz (F), and 50Hz (G). Note that significant reduction of spike fidelity at 10, 20 and 50 Hz repetitive stimulation following ELS. n=6, 6. *p<0.05. (H) Comparisons of the overall spike fidelity at 1, 5, 10, 20 and 50Hz stimulation frequency in FS and non-FS interneurons. n=3-6. *p<0.05. Error bars indicate S.E.M.

4.4 Comparison of the effects of ELS on FS interneurons non-FS interneurons in CA1 stratum oriens.

Central to understanding the heterogeneity of synaptic systems is the elucidation of physical, chemical and biological factors that determine synapse performance. FS interneurons differ from the non-FS interneurons in several aspects: resistance to synaptic fatigue, low failure rate and exquisite temporal precision. These properties provide extremely rapid and temporally precise signally required for FS interneurons precise and strong inhibition. Here, I compared the differences of responses to ELS between FS and non-FS interneurons in CA1 stratum oriens (Figure 14). As shown in Figure 14, FS and non-FS interneurons showed clear distinctions in response to ELS at P10-12. There was significant differences in ELS-induced alterations in AMPAR sEPSC amplitude and frequency in FS interneurons (changes in amplitude -30.420 ± 4.232%, n=10; changes in frequency -90.06 ± 2.881%, n=10) compared with non-FS interneurons (changes in amplitude -1.747 ± 4.614%, n=21, *P*=0.0003, Figure 14A; changes in frequency -25.850 ± 24.460, n=21, *P*=0.0036, Figure 14B). There were no significant difference in ELS-induced changes in AMPAR sEPSC rise time between FS interneuron 48.070 ± 17.000%, n=9 vs. non-FS interneurons 21.600 ± 26.940%, n=20, *P*=0.083, or ELS-induced changes in AMPAR sEPSC decay time in FS interneurons 46.800 ± 18.690%, n=10, compared with non-FS interneurons 11.870 ± 10.050%, n=20, *P*=0.082, (Figure 14C & D). In addition, ELS-induced changes in PPRs is significantly between FS interneurons and non-FS interneurons at ISI of 20, 30 &100ms. PPR at ISI of 20ms: -43.05 ± 3.311%, n=9; ISI of 30ms: -43.47 ± 2.873%, n=9; ISI of 50ms: -27.240 ± 4.905%, n=9; ISI of 100ms: -28.08 ± 5.653%, n=9; ISI of 200ms: -20.55 ± 7.367%, n=7; ISI of 400ms: -10.17 ± 3.705%, n=7; ISI of 500ms: -9.640 ± 8.712%, n=6) compared with non-FS interneurons changes in PPR at ISI of 20ms: -

5.657±10.24%, n=18, *P*=0.0009; ISI of 30ms: -7.989 ± 9.431%, n=18, *P*=0.0029; ISI of 50ms: -8.234±9.268%, n=18, *P*=0.1332; ISI of 100ms: 3.809 ± 9.906%, n=18, *P*=0.0268; ISI of 200ms: -4.737 ± 4.179%, n=16, *P*=0.06; ISI of 400ms: -15.300 ± 8.083%, n=17, *P*=0.664; ISI of 500ms: 1.971 ± 4.643%, n=14, *P*=0.2391) shown in Figure 14E. Furthermore, ELS-induced changes in responses to repetitive stimulations at different frequencies is significantly different in FS interneurons (overall changes in responses at 1Hz: 0.829 ± 0.694, n=19; 5Hz: -0.713 ± 1.634, n=19; 10Hz: -9.592 ± 1.536, n=19; 20Hz: -35.586 ± 1.692, n=19; 50Hz: -35.014 ± 1.746, n=19) compared with non-FS interneurons (changes in responses at 1Hz: 5.843 ± 0.536, n=19, *P*<0.00001; 5Hz: 6.473 ± 1.804, n=19, *P*=0.0075; 10Hz: 17.770 ± 3.942, n=19, *P*<0.000001; 20Hz: 48.457 ± 3.89, n=19, *P*<0.000001; 50Hz: 19.751 ± 3.458, n=19, *P*<0.000001, Figure 14K). These results demonstrate ELS induced target specific changes in the excitatory afferent inputs to FS interneurons.

5. Discussion

Using a combination of electrophysiology, voltage sensitive dye imaging and immunohistochemistry, I systematically studied the effects of ELS on different groups of interneurons in the hippocampal CA1 region during the critical period of development. To the best of my knowledge, this is the first comprehensive investigation examining the effects of ELS on the excitatory afferents on CA1 interneurons in the developing brain. In this study, I show for the first time that ELS can selectively affect the CA1 FS interneurons and impair the tuning of afferent excitatory synapses onto hippocampal CA1 FS interneurons through modulating the size of the presynaptic ready release vesicle pool. Furthermore, I found that asynchronous release was

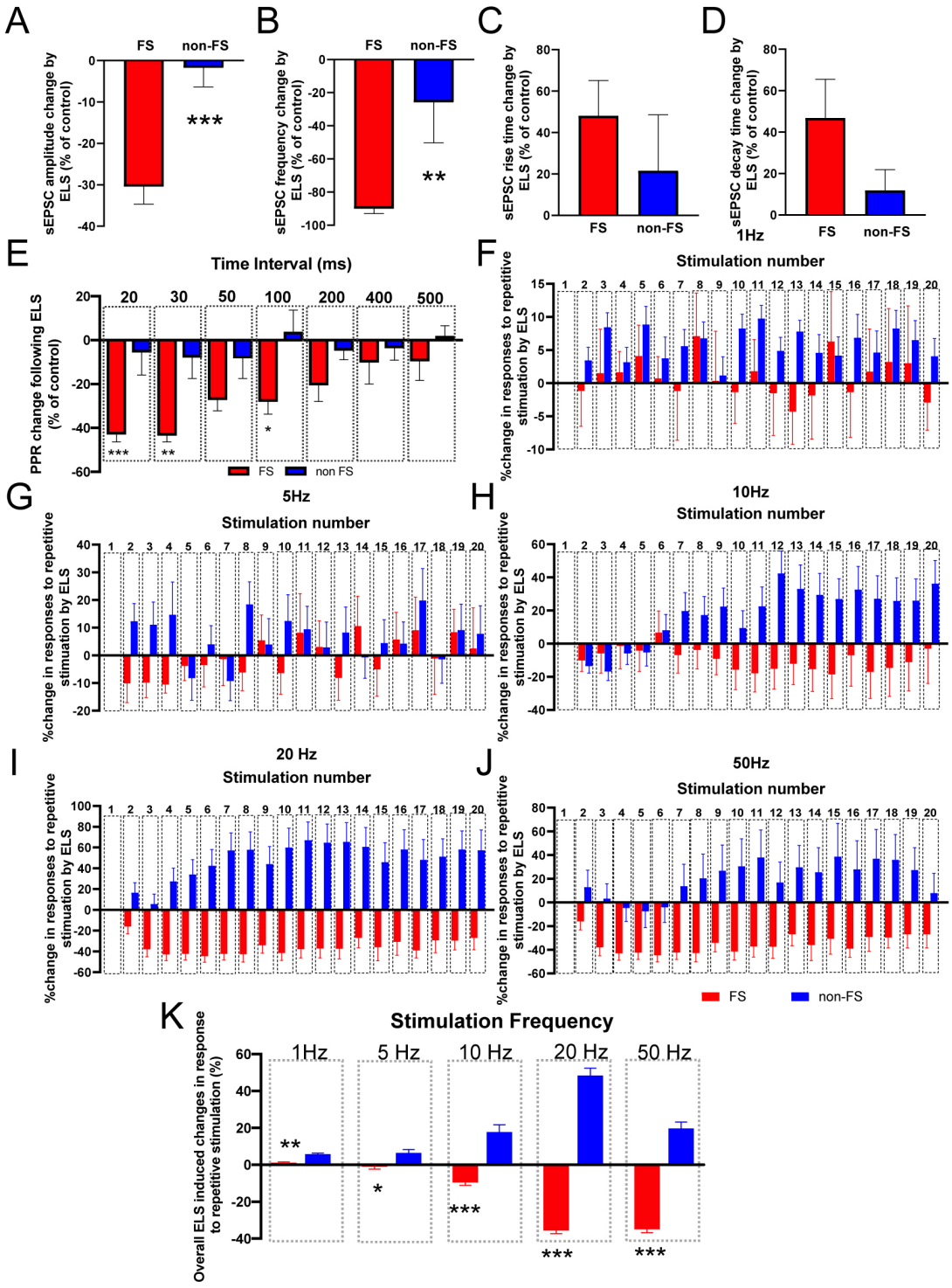

Figure 14. Comparison of the effects of ELS between FS and non-FS interneurons in striatum oriens from the developing hippocampus. A-D: Comparison of ELS-induced changes in AMPAR-mediated sEPSC amplitude (**A**), frequency (**B**), rise time (**C**), and decay time (**D**) between CA1 FS and non-FS interneurons. n=10, 21, **P<0.01, ****P*<0.001. **E:** Comparison of ELS-induced changes in PPR at different interstimulus interval between CA1 FS and non-FS interneurons. n=8, 16, *p<0.05, **P<0.01, ****P*<0.001. **F-J:** Comparison of ELS-induced changes in responses to repetitive stimulation at 1Hz (**F**), 5Hz (**G**), 10Hz (**H**), 20 Hz (**I**), and 50Hz (**J**) between CA1 FS and non-FS interneurons. n=7-11, 11-20, *P<0.05, **P<0.01. (**K**) Comparisons of the overall ELS induced changes in response to repetitive stimulation at 1, 5, 10, 20 and 50Hz in FS and non-FS interneurons. *P<0.05, *P<0.001, ***P<0.0001. Error bars indicate S.E.M.

also significantly modulated at excitatory afferents in CA1 FS interneurons, which surprisingly impairs action potential temporal precision and fidelity. To date, effects of acute seizures on the excitatory synapses on different subtypes of interneurons in the immature brain are completely unknown. My study highlights novel targets by which acute seizures can selectively modulate the excitatory inputs into immature FS interneuron population, as well as deregulation of the FS interneurons function. Our study provides a better understanding of the precise physiological alterations by which ELS can disrupt the excitation/inhibition balance in the developing hippocampal circuits and reveal potential therapeutic targets to treat ELS.

Characteristics of immature vs. mature hippocampal FS and non-FS interneurons

Prior to studying the effects of ELS on FS and non-FS interneurons, I characterized and compared FS to non-FS interneurons in the immature brain under control conditions. The results from my experiments indicated that PV protein was not expressed in FS interneurons in the stratum oriens region of the CA1 hippocampal region but was faintly expressed in the hippocampal stratum oriens CA3 region, in P10 mice pups. Other than the implications this had on how I identified immature FS interneurons in my experiments, I also considered how the function of FS interneurons, in particular their ability to receive excitatory inputs, can be affected by the developmentally delayed expression of PV protein. PV protein is a slow calcium buffer that can mediate the amplitude and time course of intracellular calcium transients at FS interneurons' terminals and dendrites. This protein has been found to be highly critical in regulating the short-term plasticity at inhibitory synapses and neurotransmitter release (Caillard, at al., 2000). At the proximal apical dendrite level, FS interneurons that express PV protein shapes the local CA2+ signals near transiently activated Ca2+ permeable, GluA2 lacking AMPARs by reducing the

amplitude and prolonging the decay phases of the calcium transient (Aponte et al. 2008). PV proteins' modulation of calcium signals in FS interneurons may be have implications for the excitatory input signals at the glutamatergic synapses, especially in long-term potentiation at these synapses (Aponte et al. 2008). With these implications in mind, the effects of ELS on the excitatory afferents on immature FS interneurons can be unique and highly dependent on the developmental period.

While I could not identify the immature FS interneurons from my study through PV expression, I distinguished FS interneurons using electrophysiology as these immature FS interneurons displayed hallmark traits of FS interneurons such as high frequency AP with little adaptation, short and AP duration (Casale, et al., 2015; Hu et al., 2014 ; Okaty, et al., 2009; Tsukamoto, et al., 2004). However, the intrinsic membrane properties of the immature FS interneurons and non-FS interneurons from my study, did not directly correspond to electrophysiological properties of mature hippocampal interneurons, characterized from previous studies. The resting membrane resistance of immature FS and non-FS interneurons were significantly higher (~3X higher) than the input resistance of mature FS and non-FS interneurons *(*Tsukamoto, et al., 200*4).* The AP duration at ½ amplitude was ~ 2X longer in immature FS and non-FS interneurons than mature counterparts (Tsukamoto, et al., 2004; Kawaguchi, et al., 1987). However, the AP duration at ½ amplitude of the immature FS interneurons was still significantly shorter than that of immature non-FS interneurons. I also found that immature FS and non-FS interneurons fire at a slower frequency than their mature counterparts (Tsukamoto, et al., 2004; Kawaguchi, et al., 1987). However, the immature FS interneuron was still firing at a significantly higher frequency (~2X) than immature non-FS interneurons. These differences found between mature and immature FS and non-FS interneurons are not surprising, since many properties of the

developing interneurons, such as the ion channels are not yet fully formed.

Ion channels, in particular the voltage-gated K+ channels, endow FS interneurons with unique electrophysiological phenotypes, such short AP duration and high AP firing properties, that distinguish FS interneurons from pyramidal cells and other interneurons types (Tansey, et al., 2002; Rudy, et al., 2001; Martina, et al., 2000). However, there are significant differences between the expression of these ion channels in immature FS interneurons from mature FS interneurons. For example, potassium channels, Kv3.2 and Kv3.1b at P7-8 are only weakly expressed, their expression does not stabilize until much later, P21 and P40 respectively (Tansey, et al., 2002; Du, et al., 1996). Other functional and physiological differences between immature and mature FS interneurons can also be present, since thousands of genes of FS interneurons, belonging to different functional classes, undergo profound regulations during the first 4 postnatal weeks (Okaty, et al., 2009).

Most interestingly, I found significant differences between the short-term plasticity at the excitatory synapses onto immature FS vs. non-FS during repetitive stimulations frequencies of 20Hz and 50Hz. Short-term plasticity at different synapses are often tuned in a target specific manner (Bao et al., 2010; Maccaferri et al., 1998; Scanziani et al., 1998), which endows different functional properties and regulates the information input into different cell types. These phenomena are generally associated with complex presynaptic mechanisms and synaptic proteins (Jackman et al., 2017; Muller et al., 2018; Skyler and Regehr, 2017). In my study, immature FS interneuron displayed synaptic facilitation, a form of short-term plasticity that enhances synaptic transmission during 20Hz and 50Hz frequency stimulation, while immature non-FS interneurons displayed synaptic depression, where synaptic transmission weakens during repeated activation. These results suggest that there already might be significant differences between information

transfer between pyramidal cells and FS interneurons versus non-FS interneurons during this critical developmental period. These differences may due a number of different factors, like synaptic proteins and presynaptic and postsynaptic receptors (Jackman et al., 2017; Xu et al., 2012; Scott et al., 2012).

ELS selectively modulated FS interneuron excitatory afferent

In the immature hippocampus, interneurons are critical in the regulation of neuronal circuits and are implicated in the promotion of early network oscillations (Dugladze et al., 2012; Wu et al., 1996). Interneurons display AMPAR-mediated synaptic currents much earlier than pyramidal cells, since pyramidal cells form synaptic targets onto interneurons prior to connections with other pyramidal cells (Durand et al., 1996; Tyzio et al., 1999; Gozlan et al., 2003; Hennou et al., 2002). In the mature hippocampus, FS interneurons are one of the most densely innervated interneurons, with the majority of these innervations being excitatory inputs from pyramidal cells (Gulya et al. 1999; Geiger et al. 1997). The early excitatory inputs onto FS interneurons are highly critical in these cells ability to pace and control the activity of large neural ensembles and regulate the excitatory/inhibitory balance in the immature brain.

In my current study, I found that ELS selectively modulated the excitatory afferent inputs onto FS interneurons, while no significant ELS induced changes of the excitatory afferents on non-FS interneurons were found. My results indicated that ELS significantly reduced the frequency of AMPAR-mediated excitatory currents into FS interneurons through a presynaptic mechanism and significantly depressed the short-term plasticity at these synapses, during high frequency repetitive stimulation of 50Hz. Short-term depression has been commonly associated with presynaptic vesicle depletion from previous studies (Zucker and Regehr 2002; Regehr, 2002; Fioravante and

Regehr, 2011). Other factors can also contribute to synaptic depression, such as a decrease in presynaptic calcium currents (Xu and Wu, 2005) and residual presynaptic calcium (Dittman, et al. 2000); however, these factors are unique to different synapse types, while vesicle depletion has been found to be a universal contributor to synaptic depression (Zucker and Regehr 2002). FS interneurons responded to the initial stimuli robustly, and the probability of release underwent various plasticity induced changes. Progressively, the synaptic transmission stabilizes, and the transmission strength and probability of release eventually reach a steady state, in which release is perfectly balanced by refilling. At this steady state of release, I estimated the number of RRPs and found that ELS induced depression at the excitatory synapses onto FS interneurons by decreasing the available presynaptic RRP during repetitive high frequency challenge periods. The readily releasable vesicle pools are associated with presynaptic physiology in other ways as well, particularly in regard to synaptic initial release probability (Murthy, et al., 1997). The initial probability of release has been found to be able to modulate short-term plasticity (Jackman and Regehr, 2017). Upon further investigation, I found that ELS modulated the initial presynaptic release and significantly increased the initial probability of release in response to the first stimuli.

Previous studies have found that synapses that exhibit facilitation often also exhibit low initial probability of release and synapses with high initial probability of release usually exhibit use-dependent depression (Jackman and Regehr, 2017). My data shows that ELS modulated the low probability release excitatory synapses of FS interneurons to high initial probability release, with use-dependent depression synapses. I hypothesize ELS changes the initial probability of release in FS interneurons, results in a large fraction of vesicles to fuse during the initial action potentials and thus depleting the RRP. Later synaptic responses are depressed until the RRPs are able to be replenished again.

The disruption of excitatory afferent inputs onto FS interneurons by ELS can result in the improper information transfer from pyramidal neurons into FS interneurons. The short-term plasticity characteristics of the excitatory inputs into FS interneurons have important roles in determining how synapses transmit information and regulate the strength of inputs as a function of past activity (Fuhrmann et al., 2002). Facilitation has also been found to be crucial for information transfer during high-frequency burst firing, as it has been shown that synapses that facilitate during these bursts maintains intact synaptic transmission (Xu et al., 2012). Therefore, ELS induced change in synaptic facilitation to depression at the excitatory inputs can strongly impair the FS interneurons ability to 'sense' the ongoing activity of neuronal ensembles and their ability to properly modulate excitation with strong and precise perisomatic inhibition.

Disruption of excitatory inputs onto FS interneurons through selective knockout/knockdown of glutamatergic receptors can induce a persistent reduction of gamma oscillation power in the hippocampus as well as abnormalities in spatial working memory and novel object exploration in adult mice (Fuchs et al., 2007). The implications my findings on a behavioural level can be associated with the critical roles hippocampal FS interneurons have in facilitating cognitive processes associated with memory formation and information processing (Roux and Buzsaki, 2005). Important network oscillations, generated through an interplay between excitation and inhibition, are highly dependent on proper functioning of FS interneurons (Chiovini et al., 2014; Fuchs et al., 2007; Lapray et al., 2012). Therefore, the disruption in the excitatory inputs onto hippocampal FS interneurons not only can disrupt the E/I balance, which can further predispose the immature brain to seizures, but can also inhibit the crucial neuronal oscillations in the immature brain, resulting in other cognitive and memory deficits, often associated with ELS (Oh et al., 2017).

ELS alters the FS interneurons high temporal precision and spike fidelity response to excitatory inputs

In addition to AP-triggered synchronous neurotransmitters, some excitatory and inhibitory synapses also display another form of delayed neurotransmitter release, known as the asynchronous neurotransmitter release (Evstratova, et al., 2014). While synchronous neurotransmitter release provides rapid communication between presynaptic cells to their postsynaptic targets, asynchronous release during high frequency activity modulates postsynaptic excitability, by overcoming post-spike afterhyperpolarization and shortening the refractory period. This permits the postsynaptic cell to generate highly reliable and temporally precise action potentials and ensure reliable information transfer over an extended period of time (Iremonger and Bains, 2016; Evstratova, et al., 2014). Although my previous experiments have found that presynaptic synchronous neurotransmitters release have been altered by ELS, a different set of molecular machinery regulates asynchronous neurotransmitter release (Goda and Stevens, 1994; David & Barrett, 2003; Otsu et al. 2004; Yang & Xu-Friedman, 2010). Therefore, I wanted to determine whether ELS also modulated this form of neurotransmitter release. A recent study found that asynchronous release usually dominates during repetitive trains at higher frequencies of >20Hz (Kaeser and Regehr, 2015), therefore, I measured the frequency of asynchronous events after a 20Hz repetitive stimulation train using a method elucidated by Evstratova et al., 2014. My results indicated that ELS compromised and significantly reduced asynchronous neurotransmitter release at excitatory synapses onto FS interneurons. This can have significant consequences on postsynaptic FS interneurons ability to fire reliable and temporally precise APs (Iremonger and Bains, 2016; Evstratova et al., 2014).

Action potential probability and timing are two key parameters of synaptic information coding, and there has been compelling evidence that the reliability in spike timing is highly dependent on the structure of the excitatory stimuli (Fricker and Miles, 2000). FS interneurons in different animal models have been found to respond to afferent excitatory input with reliable and temporally precise APs in comparison to pyramidal cells (Simons, 1978). FS interneurons are often coined as coincidence detectors vs. temporal integrators. Coincidence detectors have a very low signal-to-noise ratio and relay synchronized inputs into output that precisely correlate with input patterns over a short integration period (Galarreta and Hestrin, 2001; Konig et al., 1996).Under control conditions, I found that the immature FS interneurons were also able to respond to repetitive excitatory inputs of varying frequencies with highly precise and reliable APs. However, ELS significantly decreased in the probability of postsynaptic spike generation (i.e. spike fidelity) during high frequency stimuli (10Hz-50Hz), as well as the precision of spike timing (i.e. AP jitter), measured by the variance of the temporal precision of AP in response to stimulus, at all frequencies.

The precise and reliable APs in FS interneurons are critical factors that enable FS interneurons to produce strong and highly precise inhibitory output (Simons, 1978; Simons and Carvell, 1989; Swadlow, 1989; Galarreta and Hestrin, 2001; Maccaferri and Dingledine, 2002). ELS induced deregulation of AP precision could decrease the reliability of information transfer from FS interneurons and the decrease in temporal precision could reduce the inhibitory output from FS interneurons. The abnormalities described here, along with the changes at the excitatory input into FS interneurons, can contribute to the disruption of the E/I balance in the immature brain and the cognitive symptoms associated with ELS (Cornejo et al., 2007; Cossart et al., 2001; Katsarou et al., 2017; Schwaller et al., 2004; Volpe, 2001).

In summary, our results discovered novel cell-type-specific targets by which a single acute seizure can significantly modulate neuronal network excitability in the immature brain. Although we do not know how long these changes at the excitatory synapses onto FS interneurons will last, our results indicated that these changes also deregulated the function of FS interneurons themselves and have the potential to at least temporarily modulate the E/I balance in the immature brain. Further *in vivo* recordings from freely behaving mice to detect local field potentials is required to detect whether acute seizures impair FS interneurons to generate oscillations in the hippocampus, such as SWRs which is critical in hippocampal dependent learning and memory (Butler and Paulsen, 2015; Chiovini et al., 2014; Korotkova, et al., 2010). Further behavioral experiments will allow us to assess whether acute ELS can induce acute and prolonged cognitive and memory deficits. Additionally, I cannot assume that the excitatory afferent inputs into the FS interneurons are uniformly affected by ELS. During the developmental period, different FS interneurons mature at different rates, as seen from the variability in PV expression in FS interneurons from CA3 vs CA1 region from our staining results. Also, the heterogeneity of excitatory inputs from heterogeneous pyramidal neurons to FS interneuron subtypes can result in differential changes following ELS. Therefore, further in-depth investigations on the specific pyramidal-interneuron connections that are affected by ELS will give us further understanding of the pathophysiology of early life epilepsy.

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