GABA receptor signaling and epileptiform synchronization during optogenetic activation of parvalbumin-positive interneurons in the mouse limbic system

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Abstract

Mesial temporal lobe epilepsy (MTLE) is the most common form of refractory focal epilepsy, and is characterized by recurring seizures. Theta oscillations (4-10 Hz) are associated with physiological functions such as memory formation, exploratory and visual navigation as well as with the generation of seizures (i.e. ictogenesis). Parvalbumin (PV)-positive interneurons, which release the inhibitory neurotransmitter GABA, play an important but controversial role in ictogenesis and the exact contribution of $GABA_A$ signaling to the generation of theta rhythms and epileptiform synchronization remains elusive. In my experiments, I used the 4-aminopyridine (4AP) *in vitro* model to study the effects of optogenetic stimulation of PV-positive interneurons at theta frequency on seizure generation in temporal lobe structures such as the hippocampus and entorhinal cortex.

Trains of optogenetic stimuli were delivered at 8 Hz for 5 s with 30 s intervals in brain slices obtained from adult PV-ChR2 mice, containing channelrhodopsin-2 on the PV-positive interneurons. This experimental protocol triggered theta oscillations and interictal-like spikes in the CA3 and dentate area of the hippocampus as well as in the entorhinal cortex. Moreover, optogenetic stimulation also induced ictal-like events with electrographic features similar to the low-voltage fast onset ictal-like events that occurred spontaneously. My results further support the notion that interneuron activity, and more specifically PV-positive interneurons as well as the subsequent activation of $GABA_A$ signaling, facilitates the generation of epileptiform interictal spikes and ictal discharges in the 4AP *in vitro* model.

Résumé

L'épilepsie temporale mésiale est la forme la plus courante et la plus réfractaire d'épilepsie focale, et se caractérise par des crises récurrentes. Les oscillations thêta (4-10 Hz) sont liées à des fonctions physiologiques telles que la mémoire et la navigation exploratoire, mais contribuent aussi à la production de crises épileptiques (c'est-à-dire l'ictogénèse). Les interneurones positifs à la Parvalbumine (PV), qui libèrent le neurotransmetteur inhibiteur GABA, jouent un rôle important mais complexe dans l'ictogénèse. En effet, la contribution exacte du $GABA_A$ à la production des rythmes thêta et à la synchronisation épileptiforme reste incertaine. Au cours de mes expériences, j'ai utilisé le modèle in vitro à la 4-aminopyridine (4AP) afin d'étudier les conséquences de la stimulation optogénétique des interneurones PV-positifs sur les oscillations thêta et la production de crises épileptiques dans les structures du lobe temporal telles que l'hippocampe et le cortex entorhinal.

Des trains de stimulations optogénétiques ont été délivrés à une fréquence de 8 Hz pendant 5 s avec des intervalles de 30 s sur des tranches de cerveau obtenues à partir de souris PV-ChR2 adultes, qui possèdent la channelrhodopsine 2 sur les interneurones PV-positifs. Ce protocole expérimental a déclenché des oscillations thêta et des pointes intericales dans la région CA3 de l'hippocampe, le gyrus dentelé ainsi que dans le cortex entorhinal. De plus, la stimulation optogénétique a déclenché des événements ayant des caractéristiques électrographiques similaires aux événements ictaux à hautes fréquences et basse amplitude se produisant spontanément. Mes résultats appuient l'hypothèse selon laquelle l'activité des interneurones, et plus particulièrement celle des interneurones PV-positifs, ainsi que la transmission $GABA_A$, facilitent la production de pointes intericales et des décharges ictales dans le modèle in vitro à la 4AP.

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I've always wanted to write a book. Every time I finish one, I always find myself reading the acknowledgments at the end, even though they have nothing to do with the contents of the book itself. It gives me insight on who the writer is as a person and in their life, rather than just as a voice in my head, reading words. Even though this is not a book, I am happy to be acknowledging every person who stood by me during these past two years as I completed this project and produced this body of work.

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Contribution of Authors

All parts of this manuscript were written by Cristen Kfoury. Revisions and corrections were provided by supervisor Dr. Massimo Avoli.

Experiments were performed by both Cristen Kfoury and Siyan Wang. Dr. Avoli originated the idea for this project and designed the protocol. Siyan Wang wrote the MATLAB code for analysis and designed the figures. Dr. Maxime Lévesque and Siyan Wang provided lab training. All other work shown in this thesis was performed by Cristen Kfoury.

Chapter 1: Introduction

In this chapter, a brief introduction of mesial temporal lobe epilepsy (MTLE) and its various experimental models is made. This is followed by an overview of the effect of pathological neural network oscillations and a summary of the mechanisms of theta oscillations. Finally, we look into the role of PV-positive interneurons and the neurotransmitter γ-aminobutyric acid (GABA) in epileptogenesis.

1.1. Mesial temporal lobe epilepsy

Epilepsy is a neurological condition defined by recurring seizures that are caused by excessive neuronal synchronization. Seizures can be focal or generalized (Engel, 2005). Focal seizures occurring in humans are resistant to pharmacological treatment in about one third of cases; hence, when all available pharmacological strategies fail to halt the development of seizures (i.e ictogenesis), surgical resection of the epileptic focus remains the only alternative (Blume and Parrent, 2006; Wiebe, 2004).

Mesial temporal lobe epilepsy is the most common and refractory form of focal epilepsy, and it is characterized by recurring seizures that initiate from temporal structures such as the amygdala, the hippocampus and the entorhinal cortex (EC). It is usually associated with hippocampal sclerosis that is characterized by neuronal loss in the CA1 and CA3 subfields of the hippocampus, the dentate hilus, layer III of the entorhinal cortex and the amygdala (Gloor, 1990). MTLE patients also present with cognitive deficits which include impairments in memory, executive functioning and language (Helmstaedter et al., 2003; Bell et al., 2011).

1.2. *In vivo* animal models of MTLE

Over the years, researchers have used several animal models to study ictogenesis and epileptogenesis. Clinical studies are also conducted with the use of depth electrodes for EEG recordings in epileptic patients (Spencer, 1994). It is very difficult, however, to fully understand the complex underlying mechanisms in epilepsy solely through clinical trials. Although no experimental model encompasses all the symptoms of MTLE, various animal models have been developed to mimic the changes seen in the epileptic brain (Kandratavicius, 2014). Seizures have both electrographic and behavioral characteristics and thus, animal models aim to mimic the neuropathological, behavioral and neurophysiological alterations that are seen in patients with epilepsy (Kandratavicius, 2014).

In vivo animal models can be categorized as those induced by electrical stimulation (such as kindling) (Goddard et al., 1969) as well as *status epilepticus* (SE)-induced epileptic models; these include pilocarpine and kainic acid (KA)-induced SE animal models. The KA and pilocarpine animal models are similar; they are characterized by an initial SE that is first followed by a latent period marked by lack of seizures and reorganization of the neuronal network excitability (i.e. epileptogenesis) (Pitkänen and Sutula, 2002) and then by spontaneous seizure recurrence. These models can and have been used in a variety of species through systemic, intrahippocampal or intra-amygdaloid administration (Ben-Ari et al., 1979a; Turski et al., 1989; Curia et al., 2008; Lévesque and Avoli, 2013; Lévesque et al., 2019). As for the kindling model, this involves continuous electrical stimulation of a specific brain area, leading to a chronic permanent hyperexcitable state (McNamara, 1984; see for review Morimoto et al., 2004). These models allow researchers to study ictogenesis, the mechanism that leads to the occurrence of spontaneous seizures (ictal discharges) and epileptogenesis.

1.3. *In vitro* animal models are essential for understanding the underlying mechanisms of ictogenesis

Ictogenesis can also be studied *in vitro* using brain slices containing the hippocampus and entorhinal cortex. Perfusion with artificial cerebrospinal fluid (ACSF) and the use of convulsants or a zero Mg^{2+} environment is necessary to induce epileptiform synchronization (see for review Avoli et al., 2002). The potassium channel blocker 4-aminopyridine (4AP) enhances neurotransmitter release from both glutamatergic and GABAergic neurons (Buckel and Haas, 1982; Perreault and Avoli, 1991; see for review Avoli et al., 2002, Avoli and de Curtis, 2011). The application of 4AP induces ictal discharges that mainly initiate from the entorhinal and perirhinal cortices and interictal spikes that occur in any temporal lobe structure.

Two types of interictal spikes are observed in this model: the first is initiated in the CA3 area, occurs at a rate of 0.3-1.3 sec^{-1}, with each discharge lasting 0.02-0.07 s and has been therefore termed “fast”. The second type is seen in all limbic areas, occurs at a frequency of 0.036 sec^{-1}, lasts longer (0.4-1 s) and has been termed “slow”. (Perreault and Avoli, 1992; Voskyul and Albus, 1985; Avoli et al., 2002; Avoli and de Curtis, 2011). Slow interictal discharges have been shown to be sensitive to $GABA_A$ receptor antagonists and thus, these spikes presumably mirror the responses of principal, glutamatergic neurons’ to GABA release by interneuron firing (Avoli et al., 2013). Moreover, NMDA and non-NMDA glutamatergic and $GABA_A$ receptors are responsible for the emergence of ictal discharges (Avoli et al., 2013). In several areas of the limbic brain in slice preparations, a slow interictal spike is seen shortly before the onset of the ictal discharge, suggesting that it could initiate the ictal discharge (Avoli et al., 1996; Sudbury and Avoli, 2007; Panuccio et al., 2009; Avoli et al., 2011). Transient increases in extracellular potassium ($[K^+]_o$) occur during interictal spiking and even larger increases during ictogenesis.

These increases are observed with the application of 4AP as well as of ionotropic glutamatergic receptor blockers, showing that they may be due to $GABA_A$ release from interneurons (Avoli et al., 2013; Barolet and Morris, 1991). According to this hypothesis, blocking $GABA_A$ receptors abolishes these ionotropic glutamatergic-independent slow interictal events (Avoli et al, 1996). Thus, this $GABA_A$-mediated increase in $[K^+]_o$ during interictal spikes may facilitate synchronization of the network, leading to ictal discharges (Avoli et al., 1996, 2013, 2016).

1.4. Optogenetics as a novel tool for manipulating specific neuronal subtypes

Recently, the development of optogenetics, a novel approach in the field of neuroscience, has greatly improved our ability to study the specific roles of neurons. Indeed, network optogenetics has allowed us to investigate and control various neuronal populations in a quick and precise manner (Deisseroth et al., 2006; Deisseroth, 2011 Chen et al., 2018). This technique involves the introduction of a light-sensitive opsin that can be used to either depolarize or hyperpolarize the target cells. To this end, two types of opsins are used: (1) channelrhodopsin-2 (ChR2) and its variants, which are non-selective cation transporters, allow the depolarization of the cells, causing action potential generation, (2) and halorhodopsin-2, a chloride pump, and its variants hyperpolarize the cells (Oesterhelt and Stoeckenius, 1971; Matsuno-Yagi and Mukohata, 1977; Nagel et al., 2002; Boyden et al., 2005; Deisseroth, 2015).

The opsins are introduced into the rodents and into specific cell types through either a viral delivery, where Cre is expressed in a target cell type and allows for the opsin activation in transgenic mice, or through transgenic technology where the opsin is expressed in a target cell type and is maintained throughout future generations. The opsin is then activated when illuminated with a specific wavelength, (around 470 nm for ChR2) (Zhang et al., 2006), causing the opening of ion

channels, thus leading to either a depolarization or hyperpolarization of the neurons. Optogenetic manipulation has, over the years, proven to be extremely effective as it is more precise than electrodes and faster acting than drugs (Deisseroth, 2011). Although it is a relatively new technique, optogenetic manipulation has immensely contributed to the field of neuroscience.

1.5. Pathological neural oscillations are the key for understanding the pathologies underlying ictogenesis

Although extensive research has been done on both animal and human subjects, the mechanisms underlying ictogenesis and the specific roles of the neurons and network remain, to some extent, elusive. Thus, further research is vital to understand the roles of the neuronal populations involved in ictogenesis.

The extent of the role of neural oscillations is also an important topic to consider. Indeed, MTLE is characterized by pathological oscillations which are involved in ictogenesis and epileptogenesis. For instance, high frequency oscillations (HFOs) are categorized into ripple (80-200 Hz) and fast ripples bands (250 Hz-500 Hz) in temporal lobe epilepsy (Bragin et al., 1999; Jacobs et al., 2012; Lévesque et al., 2017). These pathological HFOs have been shown to be associated with epileptogenic areas and seizures onset zones (Jacobs et al., 2008; Jiruska et al., 2010; Jefferys et al., 2012; Lévesque et al., 2012), and are linked with MTLE's pathological substrate, hippocampal sclerosis. HFOs are also associated with different types of seizure onsets. Low-voltage fast (LVF) and Hypersynchronous (HYP) represent the two types of seizures onsets usually recorded from MTLE patients (Spencer et al., 1992; Lee et al., 2000; Velasco et al., 2000; Ogren et al., 2009) and animal models (Bragin et al., 2005b; Lévesque et al., 2012). LVF seizures are characterized by a single positive or negative spike followed by low-amplitude, high-frequency

activity whereas HYP seizures are characterized by immediate high-frequency activity (Velasco et al., 2000; Ogren et al., 2009; Perucca et al., 2014). Indeed, ripples predominate LVF seizures (Lévesque et al., 2012, Panuccio et al., 2012) whereas fast ripples predominate HYP seizures (Bragin et al., 2005b; Salami et al., 2015). Pathological oscillations such as HFOs are thought to mirror pathophysiological changes occurring in the epileptic brain and thus, they are important markers of underlying mechanisms in ictogenesis (Salami et al., 2014; Cotic et al., 2015; Lévesque et al., 2012). Moreover, HFOs reflect the dysfunctional neural network that underlies epileptogenesis (Bragin et al., 2004; Bragin et al., 2005a; Jacobs et al., 2009). Indeed, the presence of HFOs during the latent period is a strong indication that seizures will occur (Bragin et al., 2004) and is representative of the neural modifications that occur in the transition from the latent to chronic period. More specifically, the presence of fast ripples may indicate an increase in excitatory activity which may lead to seizures (Lévesque et al., 2018).

Other oscillatory bands, such as theta oscillations and gamma oscillations, are also impaired in the epileptic hippocampus. Understanding how these oscillations differ between their physiological and pathological characteristics, and how they impact seizure generation, is vital to further our current understanding of this neurological condition.

1.6. Mechanisms of physiological theta oscillations

Theta, 4-10 Hz, oscillations are observed in the EEG of mammals and have been associated with many physiological functions, including exploratory and visual navigation, as well as memory formation (see for review Buzsáki, 2002). The mechanisms underlying the generation of theta oscillations are complex as many subcortical nuclei may be involved in the rhythmic generation of theta oscillations through either a permissive (where neurons release

neurotransmitters allowing the emergence of network oscillations in the hippocampus) or a pacemaker function (where neurons provide a coherent theta output) (Vanderwolf, 1969; Buzsáki et al., 1986; Kocsis et al., 1999; Buzsáki, 2002). The minimum requirement, however, for the emergence of theta oscillations is the proper connection between the medial diagonal band of Broca (MS-DBB) and the hippocampus (Petsche et al., 1962).

The MS-DBB is a highly interconnected brain region that receives input from the hippocampus, the amygdala, the thalamus, the supra-mammillary nuclei and the ventral tegmental area, among others (Borhegyi and Freund, 1998; Fuhrmann et al., 2015). In return, it projects back to the entire hippocampus, the amygdala, the ventral tegmental area and the hypothalamus (Fuhrmann et al., 2015; Swanson and Cowan, 1979). Inputs from the MS-DBB include cholinergic, glutamatergic and GABAergic projections. The sum of the activity of these neurons results in hippocampal theta oscillations (Tóth et al., 1997; Buzsáki, 2002; Hajszan et al., 2004). More specifically, cholinergic neurons innervate glutamatergic pyramidal neurons whilst glutamatergic neurons cause a strong excitatory drive on both cholinergic and GABAergic neurons (Manseau et al., 2005). Importantly, GABAergic neurons synchronize the network in the medial septum and pace the cells at theta frequency (Fuhrmann et al., 2015). The GABAergic and glutamatergic neurons in the medial septum project mainly to other GABAergic interneurons in the hippocampus (Freund and Antal, 1988).

The MS-DBB is the ultimate rhythm generator or “pacemaker” of theta oscillations. This means that the MS-DBB is mainly responsible for the emergence of the oscillatory pattern and frequency of theta oscillations and that lesion of this structure in the medial septum completely halts hippocampal theta oscillations (Chrobak et al., 1989; Givens and Olton, 1990; Buzsáki, 2002). In the hippocampus, a dipole is created between the distal dendrites, which receive

excitatory input from cholinergic cells in the entorhinal cortex and CA3 subfield of the hippocampus (Schaffer collaterals), and the soma, where inhibitory inputs are received from GABAergic basket and chandelier cells in the MS-DBB (Konopacki et al., 1987; Bland, 1986; Buzsáki, 2002; Vertes et al., 2004). A study by Goutagny et al. (2009), however, has shown that the CA1 subfield of the hippocampus is able to generate theta oscillations intrinsically, without the need of an extrinsic rhythm generator such as the MS-DBB. Indeed, the intrinsic properties of pyramidal cells and interneurons also play an important role in the generation of theta oscillations through voltage-gate and ionic currents such as the cyclic nucleotide gated hyperpolarization activated ion channels (HCN) (Leung and Yim, 1991; Buzsáki, 2002).

1.7. Pathological theta oscillations affect seizures and cognitive dysfunction in MTLE

Recently, theta oscillations have been shown to be involved in seizure generation. A study by Sedigh-Sarvestani et al. (2014) shows that theta oscillations had actually preceded 81% of spontaneous seizures in a rat model of tetanus toxin-induced temporal lobe epilepsy. Indeed, studies have shown that theta oscillations are abundant during the transition to seizures (Broggini et al., 2016; Grasse et al., 2013). Interestingly, these pathological theta oscillations that occur in the 100 s preceding seizures (i.e. pre-ictally) differ from those occurring in interictal periods as there is a narrowing of the frequency band from 4-10 Hz to about 5-8 Hz (Grasse et al., 2013). Moreover, the tempo of these oscillations changes from a slow tempo during the interictal phase to a faster, more rhythmic tempo pre-ictally (see for review Moxon et al., 2019). It has also been shown that there is a significant reduction of theta power following both acutely induced SE and chronic spontaneous and recurrent seizures (Karunakaran et al., 2016; Moxon et al., 2019). These

data support the view that theta oscillations may play a role in epileptiform network synchronization.

An interesting framework proposed by Moxon et al. (2019) suggests that instead of epilepsy mirroring solely a hyperexcitable state, it actually alternates between a hyper- and hypo-synchronous state. This depends on the varying tempos of neural oscillations involved in this condition, including theta oscillations. It proposes that cognitive dysfunction, a symptom of MTLE, arises due to periods of hypo-synchrony in the network. In this period, the neural network is desynchronized and thus cannot properly perform cognitive functions. Conversely, in periods of hypersynchrony, seizures are likely to occur as the network synchronizes enough to cause hyperexcitability (Moxon et al., 2019). Indeed, this framework encompasses the pathologies of epilepsy since it takes into consideration not only ictogenesis but also the generation of cognitive deficits. As explained earlier, theta oscillations are vital for the generation of certain physiological functions and thus, it could be plausible that the generation of both seizures and cognitive deficits may have a similar underlying pathological source in theta oscillations and their influence on the network. Perhaps increasing our knowledge about the role of neural oscillations, and specifically theta oscillations, could help us develop treatment strategies that would treat all epileptic symptoms - both electrographic and behavioral.

1.8. GABAergic interneurons and their various subtypes

Another unsettled topic when it comes to seizure initiation is the interplay between interneurons and principal cells and thus, the interplay between inhibition and excitation. For a long time, it was believed that ictogenesis is caused by excess excitation and concomitant decrease in inhibition, with studies proving decreases in interneuron activity in some experimental models

of epilepsy (Ribak and Reiffenstein, 1982). Recently, however, inhibitory interneurons have been shown to play a role in seizure initiation. (Davenport et al., 1990; Avoli et al, 1993; Esclapez et al, 1997; Prince and Jacobs, 1998; Lévesque et al., 2019; Chen et al., 2018; Avoli and de Curtis, 2011; Nahar et al., 2021). These experimental data have been recently confirmed by single-unit recordings in human epileptic patients (Elahian et al., 2018).

GABAergic interneurons are a diverse group of cells which are involved in many aspects of cortical function (Pelkey et al., 2017). Indeed, there are more than 20 different kinds of inhibitory GABAergic interneurons, varying in their morphology, intracellular molecular markers and in specific synaptic outputs. Based on their specific molecular markers, interneurons can be divided as parvalbumin-, somatostatin-, neuropeptide Y-, cholecystokinin- and vasoactive intestinal peptide-positive interneurons. In particular, PV-positive interneurons are basket and chandelier cells. Further, these interneurons can be classified based on their physiological properties as fast-spiking interneurons, non-fast spiking interneurons, adapting interneurons, irregular spiking interneurons, intrinsic bursting interneurons, and accelerating interneurons (Ascoli et al., 2008; DeFelipe et al., 2013; Nahar et al., 2021)

PV-positive interneurons make up around 50% of the cortical interneurons, which provide inhibitory input to pyramidal cells in the cortex (Nahar et al., 2021). They also seem to inhibit other interneurons upon which they strongly synapse, thus disinhibiting the network. In the cortex, PV-positive basket cells synapse onto the soma and proximal dendrites whereas PV-positive chandelier cells synapse onto axons of postsynaptic pyramidal neurons (Ferguson and Gao, 2018). In the hippocampus, PV-positive interneurons are essential in memory consolidation (Nahar et al., 2021), however, they make up only about 10-15% of the total neuronal population (Pelkey et al., 2017). In the CA1 subfield of the hippocampus, 24% percent of the GABAergic neurons are PV

fast spiking interneurons (Deng et al., 2019) and are important in the generation of gamma oscillations (Cardin et al., 2009). As the excitatory-inhibitory balance is altered in the hippocampus in MTLE, it is important to understand whether PV-positive interneurons play an important role in this brain disease (Nahar et al., 2021).

1.9. γ-aminobutyric acid and GABA receptors

Most of the interneurons in the forebrain release the inhibitory neurotransmitter γ-aminobutyric acid or GABA which activates pre- and postsynaptic receptors as well as extrasynaptic receptors (Farrant and Kaila, 2007). There are two main types of GABA receptors: A and B (Avoli and de Curtis, 2011). The $GABA_A$ receptor is ionotropic whilst the $GABA_B$ receptor is metabotropic. Presynaptic $GABA_B$ receptors control neurotransmitter release in both excitatory and inhibitory neurons, a role not yet established for $GABA_A$ receptors (Draguhn et al., 2008).

$GABA_A$ receptor activation opens channels that are permeable to Cl^- and, to a lesser extent, to HCO_3^- (Kaila and Voipio, 1987; Kaila et al, 1989; Hamidi and Avoli, 2015). Activation of the $GABA_A$ receptor induces Cl^- influx whilst $GABA_B$ receptor activation causes K^+ efflux. Both of these events hyperpolarize the postsynaptic cell and cause a shunting effect, effectively shunting the cells away from their action potential threshold (Ben-Ari et al., 2007; Pelkey et al., 2017). $GABA_A$ receptor-mediated current is hyperpolarizing due to low levels of intracellular Cl^- ($[Cl^-]_i$) maintained by cation chloride cotransporters, secondarily active transporters that transport chloride through cation gradients (Rivera et al., 1999; Hamidi and Avoli, 2015). Of these, the KCC2 cotransporter extrudes Cl^- along with K^+ and NKCC1 controls Cl^- uptake (Rivera et al., 1999; Rivera et al., 2005; Viitanen et al., 2010; Hamidi and Avoli, 2015). Changes in these mechanisms

in diseases like MTLE alter the role of $GABA_A$ receptors (Huberfield et al., 2007; Miles et al. 2012). It is therefore essential to understand what role GABA plays in epileptogenesis and how it can be modulated.

1.10. PV-positive interneurons and their role in the generation of interictal spikes

Importantly, GABAergic interneurons are involved in the generation of interictal spikes (Zhang et al., 2012; see for review Avoli and de Curtis, 2011). Interictal spikes are brief hypersynchronous events occurring in the periods between seizures and observed in patients and animal models of MTLE (de Curtis and Avanzzini, 2001). Interictal spikes are considered important biomarkers of epilepsy. As explained in section 1.3 above, it has been shown that slow interictal spikes are associated with hyperpolarizing-depolarizing intracellular potentials and transient increases in $[K^+]_o$ which are $GABA_A$ receptor-dependent (Avoli, 1996). Excess activation of $GABA_A$ receptors is believed to lead to accumulation of $[Cl^-]_i$ and consequent activation of the KCC2 cotransporter, thus leading to increase in $[K^+]_o$ (Viitanen et al., 2010).

During the spike that precedes the onset of the ictal discharge, a larger increase in $[K^+]_o$ is induced through the increased activity of interneurons, and consequently a decrease in the activity of principal cells, leading to the initiation of ictal discharges. Again, KCC2 is the culprit as the hypersynchronization of interneurons induces an excess in $[Cl^-]_i$ causing KCC2 to extrude both Cl^- and K^+, leading to the hypersynchronization of neighboring cells and thus, seizures (Avoli and de Curtis, 2011). *In vitro* studies on mouse brain slices have shown that down-regulating KCC2 activity through the channel blockers VU0240551 or VU046327 abolished the ictal discharges induced by the application of 4AP and simultaneously increased interictal discharges (Hamidi and Avoli, 2015; Chen et al., 2019). Indeed, the role of KCC2 in the generation of epileptiform activity

helps us further understand the effect of GABAergic transmission. The role of GABA in the initiation of epileptiform discharges, including interictal spikes, has, in fact, changed over the years and throughout the literature.

1.11. The role of PV-positive interneurons in ictogenesis

As mentioned in section 1.10 above, ictal discharges are characterized by an initial long-lasting depolarization similar to a slow interictal spike but with a higher increase in $[K^+]_o$ (Uva et al., 2009; Carriero et al., 2010; see for review Avoli and de Curtis, 2011). Indeed, some data show that ictal discharges seem to occur due to $GABA_A$-mediated synchronization (Köhling et al., 2000; Gnatkovski et al., 2008). Other studies, however, have challenged this idea and instead have shown that ictal discharges may occur due to exhaustion of presynaptic release of $GABA_A$ during interictal periods (Zhang et al., 2012). Indeed, interneurons have been shown to undergo a depolarization block during the ictal discharge whilst principal cells maintain action potential discharges (Ziburkus et al., 2006).

Moreover, studies using optogenetic techniques have also uncovered a complex role for PV-positive interneurons and $GABA_A$ in ictogenesis. Studies from our laboratory have shown that a brief optogenetic activation of PV-positive interneurons at 0.2 Hz *in vitro* does induce ictal discharges (Shiri et al., 2015), while optogenetic activation of PV-positive interneurons at 1 Hz decreases the rate of ictal discharges (Shiri et al. 2016). Other studies have also revealed a more complex role for PV-positive interneurons in ictogenesis *in vivo*. For instance, activation of PV-positive interneurons at seizure onset decreases the duration of ictal discharges (Krook-Magnuson et al., 2013). Activation of PV-positive interneurons during pilocarpine induced SE also decreases seizure duration (Magloire et al., 2019). Lévesque et al. (2019), however, have shown that in the

pilocarpine model, optogenetic activation of PV-positive interneurons in the CA3 area of the hippocampus at a frequency of 8 Hz triggers the occurrence of ictal discharges during the stimulation (Lévesque et al., 2019). This controversial data point at a complex picture for the role of PV-positive interneurons, GABA, as well as theta oscillations in ictogenesis.

1.12. Research rationale and brief summary of results

In order to understand the role of PV-positive interneurons and thus the role of GABA in the generation of theta oscillations as well as in ictogenesis, we used the 4-aminopyridine (4AP) *in vitro* model and optogenetic tools. Brain slices from adult PV-ChR2 mice, which contain channelrhodopsin-2 on the PV-positive interneurons, were bathed in 4AP and were then subjected to an optogenetic stimulation protocol targeting the CA3 and dentate gyrus (DG) subfields of the hippocampus and the entorhinal cortex. This optogenetic protocol consisted of 15 bouts of an 8 Hz stimulation for 5 s with a 30 s interval in between, before and after the application of 4AP. The 8 Hz stimulation was chosen because, first, it is within the theta frequency range (4-10 Hz) and second, because it has been shown to be the most effective frequency to induce theta oscillations in complete hippocampus preparations (Amilhon et al., 2015).

We found that, in all three regions recorded, optogenetic stimulation of PV-positive interneurons at 8 Hz triggered theta field oscillations under both control (i.e., normal artificial cerebrospinal fluid, ACSF) and 4AP conditions. Moreover, optogenetically-induced theta oscillations also triggered ictal discharges with similar electrographic features as those occurring spontaneously. Interictal spikes were also induced by the optogenetic stimulation and contained an 8 Hz field oscillation after the initial spike component, a feature not seen in spontaneous

interictal discharges. Thus, our study highlights the important role of PV-positive interneurons and theta oscillations in seizure generation.

Chapter 2: Materials and Methods

Animals - All procedures were approved by the McGill University Animal Care Committee and performed according to the protocols and guidelines of the Canadian Council on Animal Care. We used 31 slices from 20 PV-ChR2 mice. These mice contained the channelrhodopsin-2 on PV-positive interneurons. PV-ChR2 pups were generated from cross-breeding PV-Cre [B6;129P2-*pvalb*$^{tm1(cre)Arbr}$/J, The Jackson Laboratory; RRID: IMSR_JAX:008069] with Ai32 mice [R26-lox-stop-lox-ChR2(H134R)-EYFP, The Jackson Laboratory; RRID IMSR_JAX:012569]. Both lines were maintained in-house. Histology and staining for channelrhodopsin-2 expression in PV-positive interneurons were not repeated here as we used the same strains of mice previously used in work from our laboratory (Lévesque et al., 2019; Shiri et al., 2017).

Slice preparation and maintenance- Mice were deeply anesthetized with inhaled isoflurane and then decapitated at P30-P140. Brains were then quickly removed and transferred into ice-cold ACSF containing 124 mM of NaCl, 2 mM of KCl, 10 mM of D-glucose, 26 mM of $NaHCO_3$, 2 mM of $CaCl_2$, 2 mM$MgSO_4$and 1.25 mM of KH_2PO_4. ACSF was oxygenated with 95% O_2 and 5% CO_2. Horizontal slices of the brain were obtained with a vibratome (VT1000S; Leica, Wetzlar, Germany) slicing at a 10 degree angle. 450 μm thick slices, containing the hippocampus and entorhinal cortex, were transferred to an interface chamber where they were maintained in warm (32 ± 1 °C) ACSF (pH 7.4, 305 mOSM/kg) and humidified gas (O_2/CO_2, 95%/5%). Epileptiform activity was then induced, following a recovery period of approximately 1 h, by continuous bath application of 4AP (50-100 μM; Sigma-Aldrich, Oakville, ON, Canada) at a flow rate of 1-2

ml/min. Recordings from CA3, EC and DG were obtained during 4AP application for periods of up to 150 min.

Electrophysiological recording– Recordings were made with ACSF-filled glass pipettes (1B150F-4; World Precision Instruments, Sarasota, Florida, USA; tip diameter <10 μM, resistance 5-10 MΩ) (Figure 1A). Signals were sampled at 5000 Hz, amplified with a high impedance amplifier and digitized (Digidata 1322A, Molecular Devices, Palo Alto, CA, USA). PCLAMP software (Molecular Device) was used to visualize the signals on a computer.

Optogenetic stimulation - Blue light pulses (450 nm, 25 mW, 20 ms pulse duration) from a laser diode fiber light source (Doric lenses, Quebec, Canada) were delivered onto the mouse brain slices. The optogenetic stimulation protocol used in this study was as follows: 15 rounds of light pulses (20 ms) at 8 Hz for 5 s ON followed by a 30 s rest (OFF) was administered once before the application of 4AP and once again 30 min to 1 hour after (Figure 1B). For this protocol, an 8 Hz frequency stimulation was chosen because 8 Hz stimulation of PV-positive interneurons is the optimal frequency to drive theta field oscillations (Amilhon et al., 2015).

Chapter 3: Results

In this chapter, the results of the experiments conducted for this project are presented.

3.1. Effect of optogenetic stimulation of PV-positive interneurons at 8 Hz

In order to understand the effect of theta oscillations on ictogenesis, we optogenetically stimulated PV-positive interneurons at 8 Hz. We shone the optogenetic light on the entire combined slice (hippocampus and entorhinal cortex) to include the three regions of interest: the CA3 and DG subfields of the hippocampus and the EC. We also inserted glass pipettes in these regions for recording (Figure 1A). Two rounds of optogenetic stimulation were applied; one before the application of 4AP and one after. Each round of stimulation consisted of 15 bouts of 5 s of stimulation (ON), with a 30 s interval in between each bout (OFF) (Figure 1B). As expected, this stimulation protocol resulted in an 8 Hz field oscillation in all three brain regions under control conditions (Figure 1 C,D) as well as under 4AP. Thus, by optogenetically stimulating PV-positive interneurons at 8 Hz, we were able to induce theta oscillations in the brain slices.

3.2. 8 Hz optogenetic stimulation caused epileptiform discharges under 4AP

First, we wanted to establish whether theta oscillations contributed to epileptiform discharges. Knowing that optogenetically stimulating PV-positive interneurons at 8 Hz mimicked theta oscillations in brain slices *in vitro* (Figure 1), we used this stimulating protocol to assess the induced epileptiform activity. Optogenetic stimulation of PV-positive interneurons induced ictal and interictal discharges in all three limbic regions studied (Figure 2A). Both spontaneous (Figure 2a,b) and induced (Figure 2c,d) epileptiform discharges were observed. Both spontaneous and induced ictal discharges presented with a low-voltage fast onset (Figure 2Ba,c).

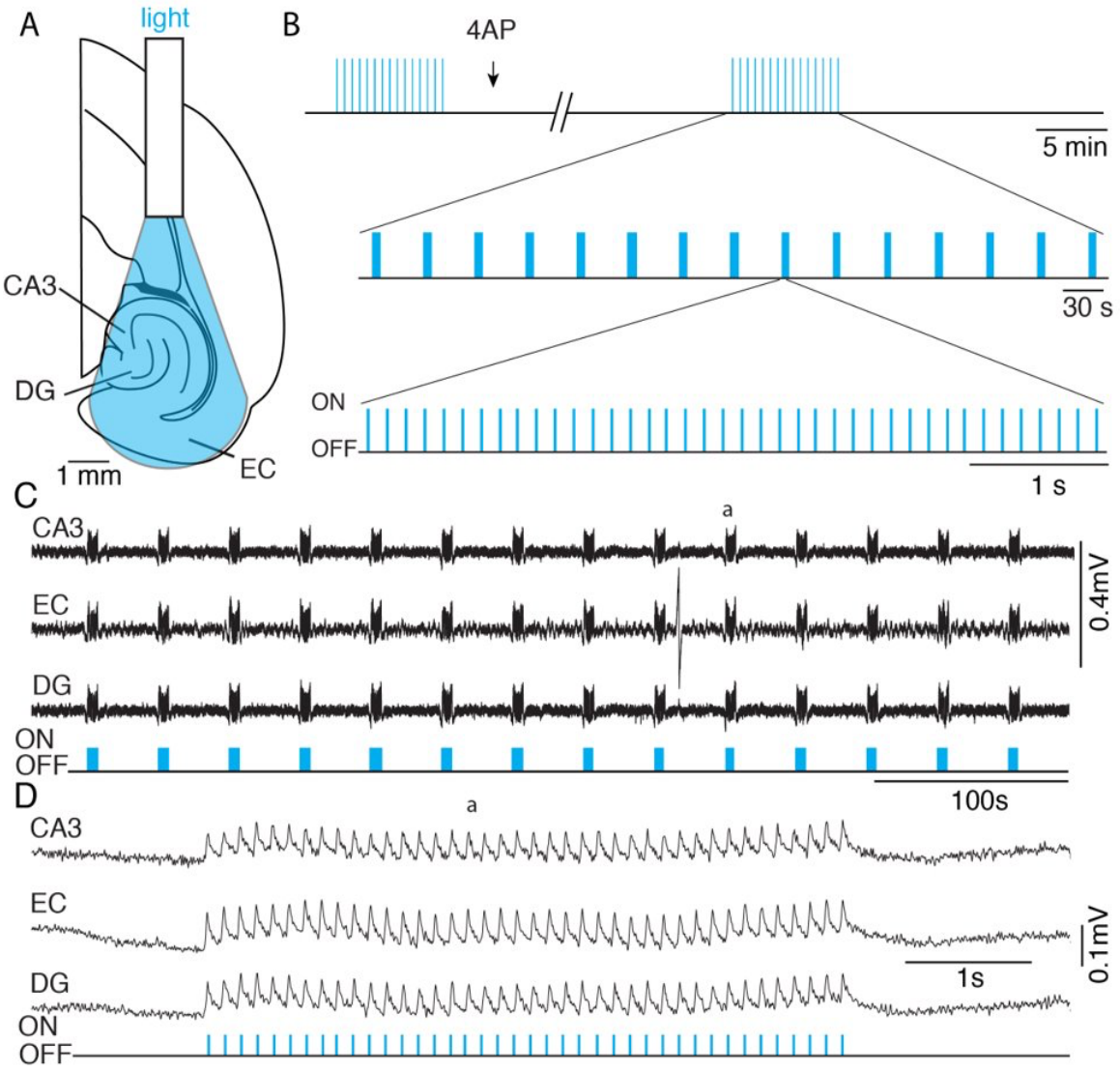

Figure 1 - Optogenetic protocol and effect on target brain regions. A: Image showing half a brain slice exposed to the blue optogenetic light (450 nm, 25 mW, 20 ms pulse duration). The light was shone on the entire combined slice and on all three brain regions where electrodes were inserted for recording (CA3, DG and EC). **B:** Optogenetic stimulation protocol. Each round consisted of 15 bouts of 5 s ON and 30 s OFF. **C:** Representative traces showing effect of 8 Hz optogenetic stimulation on CA3, EC and DG. **D:** Enlargement of a in C showing detailed 8 Hz field oscillation in CA3, EC and DG.

Moreover, the induced interictal spikes contained an 8 Hz field oscillation that followed the wave component (Figure 2Bd), a characteristic not seen in the spontaneous interictal discharges (Figure 2Bb) and which further proved the activation of PV-positive interneurons by the stimulation protocol. We can assume that the activated PV-positive interneurons caused network synchronization leading to the generation of interictal spikes that were associated with field oscillations. Overall, these results prove that optogenetic activation of PV-positive interneurons at theta frequency was sufficient to induce ictal and interictal discharges in mouse brain slices and that the ictal discharges produced had the same LVF onset as spontaneous seizures.

3.3. Induced ictal discharges had similar characteristics to spontaneous ictal discharges

Ictal discharges were more likely to occur when the optogenetic stimulation was ON compared to when it was not (Figure 3A). We calculated the ictal rate (events/hour) during and after the optogenetic stimulation and found that the rate of ictal discharges was significantly higher during the optogenetic stimulation. More specifically, looking at the 35 s timespan where the optogenetic stimulation was ON for the first 5 s and OFF for the remaining 30 s, the induced ictal discharges were more probable to occur during the 5 s of optogenetic stimulation than during the 30 s intervals in between (Figure 3B). These results show that ictal discharges were presumably induced by the optogenetic stimulation. As mentioned above, the induced and spontaneous ictal discharges were similar in their onset features (Figure 2B). Additionally, we calculated the duration of both induced and spontaneous ictal discharges (Figure 3C) and found that the induced ictal discharges, which occurred during the optogenetic stimulation, and the spontaneous ictal discharges, which occurred before the optogenetic stimulation, were similar in duration (Figure 3C).

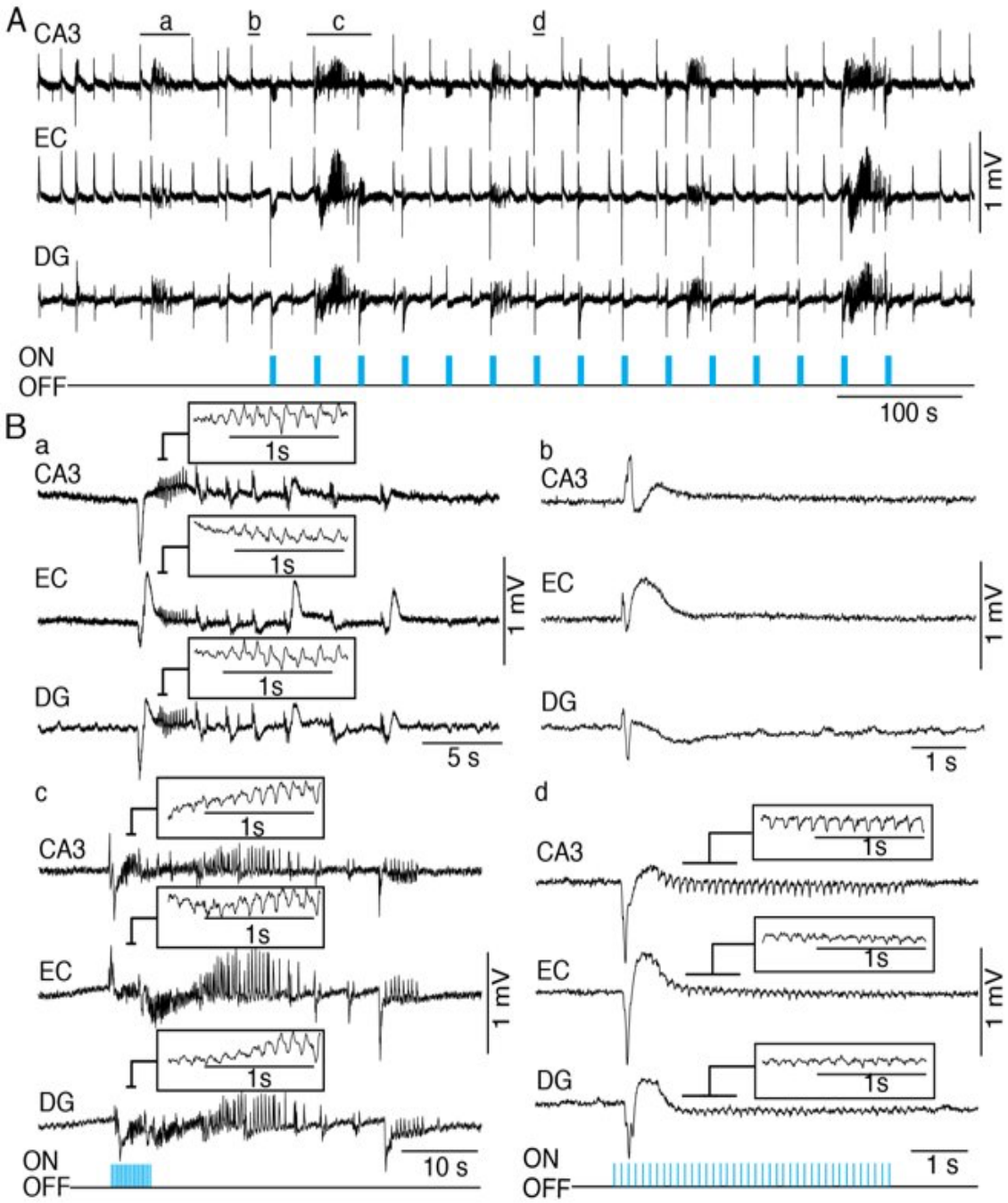

Figure 2 - Effect of optogenetic stimulation on PvChR2 mouse brain slices. A: Representative traces showing spontaneous ictal (a) and interictal (b) discharges and optogenetically induced ictal (c) and interictal (d) discharges. **B:** Enlargements of epileptiform discharges from A. Note that both spontaneous and induced ictal discharges showed an LVF onset (a,c) as well as that induced interictal spike contained an 8 Hz field oscillation during the falling phase (d).

These results show that optogenetically activating PV-positive interneurons at theta frequency did induce ictal discharges similar to those occurring spontaneously in the hippocampus and EC.

3.4. 8 Hz optogenetic stimulation of PV-positive interneurons induced interictal spikes

Interictal discharges as well were more likely to occur when the optogenetic stimulation was ON (Figure 4A). We calculated the probability of an interictal spike occurring when the optogenetic stimulation was ON or OFF in all three target brain regions and found that the probability was significantly higher when the optogenetic stimulation is ON. Moreover, looking at the 5 s timespan when the optogenetic stimulation was ON, the distribution of the spike count is significantly skewed towards the onset, showing that most interictal spikes occurred right at the onset of the optogenetic stimulation (Figure 4B). Conversely, when looking at a random 5 s timespan when the optogenetic stimulation was OFF, the distribution is even (Figure 4B), which shows that interictal spikes occurred evenly during the 5 s. This finding suggests that the induced interictal spikes were provoked by the optogenetic stimulation of PV-positive cells and occurred right at the onset in all three limbic regions studied. Furthermore, we calculated the 8 Hz power when an interictal spike was present and when it was not. We found that the 8 Hz power was significantly higher when interictal spikes occurred compared to when they did not (Figure 4C). These results demonstrate that optogenetic stimulation of PV-positive interneurons at theta frequency induced interictal spikes in the CA3, DG and EC and that they occurred right at the onset of the optogenetic stimulation.

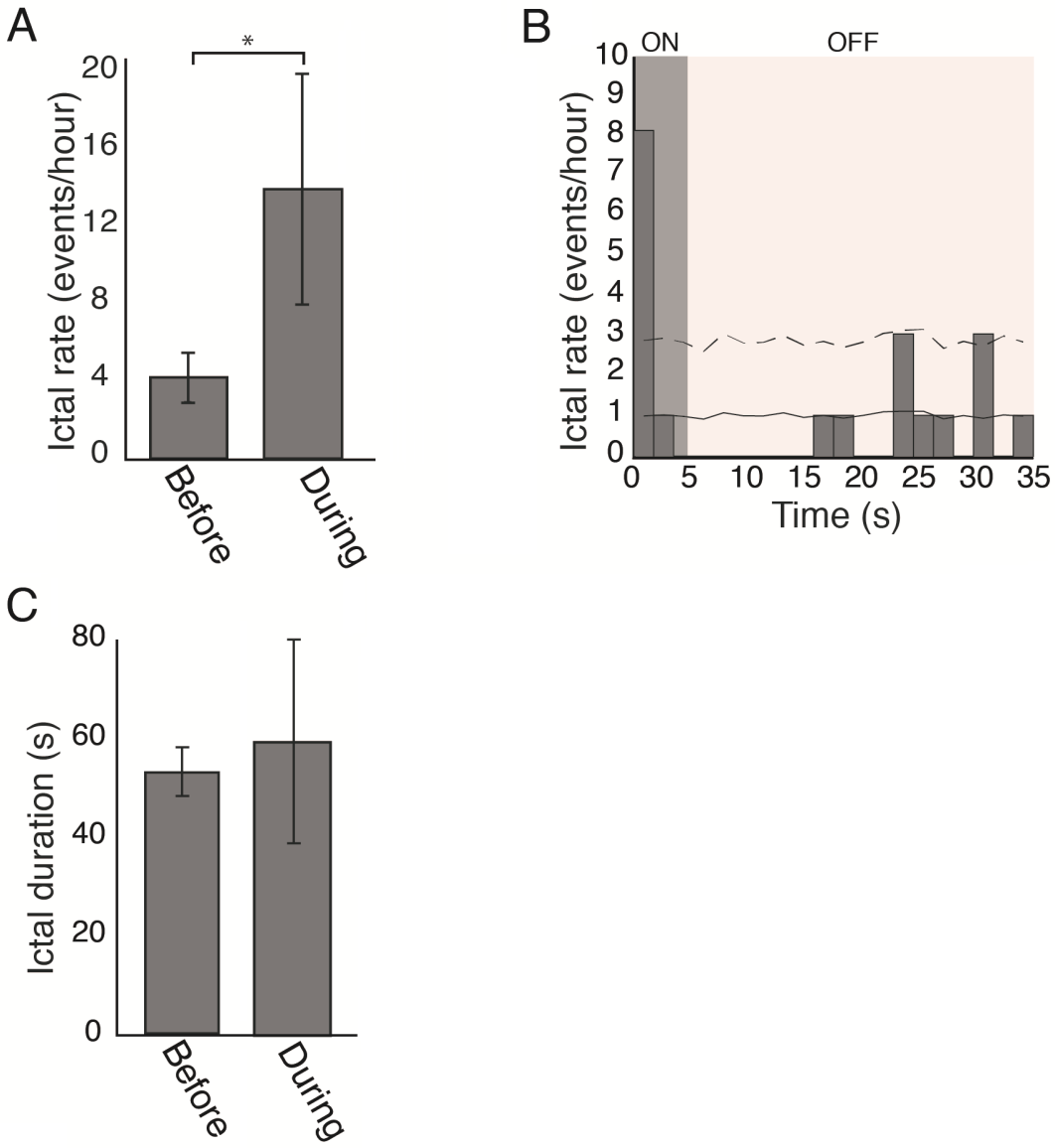

Figure 3 - Ictal discharges are more likely to occur during the optogenetic stimulation. A: Ictal rate (events/hour) before and during optogenetic stimulation. Ictal rate is significantly higher during the stimulation compared to before. **B:** Ictal rate during a time span of 35 s with the optogenetic stimulation ON for the first 5 s and OFF for the next 30 s. Ictal events are more likely to occur during the first 5 s when the optogenetic stimulation is ON. **C:** Ictal duration (s) for ictal discharges occurring before and during the stimulation. No significant differences were found.

We then looked at the difference in amplitude of the induced (ON) and spontaneous (OFF) interictal discharges. We found that the amplitude of interictal spikes was significantly higher in the CA3 and DG compared to EC only when the optogenetic stimulation was ON (Figure 5A). No such differences were seen when the optogenetic stimulation was OFF. We also looked at the percentage onset (%) for each of the three target regions and found that whether the optogenetic stimulation was ON or OFF, induced and spontaneous interictal spikes seemed to initiate equally from all three regions since there was no outstanding onset zone (Figure 5B).

3.5. Optogenetic stimulation had no effect on PV-Cre mouse brain slices

Finally, to prove that these results were in fact due to activation of PV-positive interneurons through the optogenetic effect on PvChR2 mice, we used PV-Cre mice as controls. These mice did not contain the channelrhodopsin-2 on the PV-positive interneurons. As shown in Figure 6, brain slices from these mice did not respond to the optogenetic stimulation. Epileptiform discharges induced by 4AP occurred spontaneously and were not affected by the optogenetic stimulation. The interictal spikes that occurred during the optogenetic stimulation (Figure 6Ac) did not contain the 8 Hz field oscillation seen in in PvChR2 mice and thus, we can assume that the PV-positive interneurons were not activated in these mice. Further, we looked at the probability of an interictal spike occurring when the optogenetic light is ON or OFF in all three brain regions (Figure 6B) and found that there was no significant difference. Finally, the spike count distributions over the 5 s timespan when the optogenetic light was ON were even, showing that the stimulation was not able to trigger spikes (Figure 6C). Therefore, these results prove that the results found in the PvChR2 mouse brain slices were accurate and that stimulation of PV-positive interneurons induced epileptiform discharges in those slices.

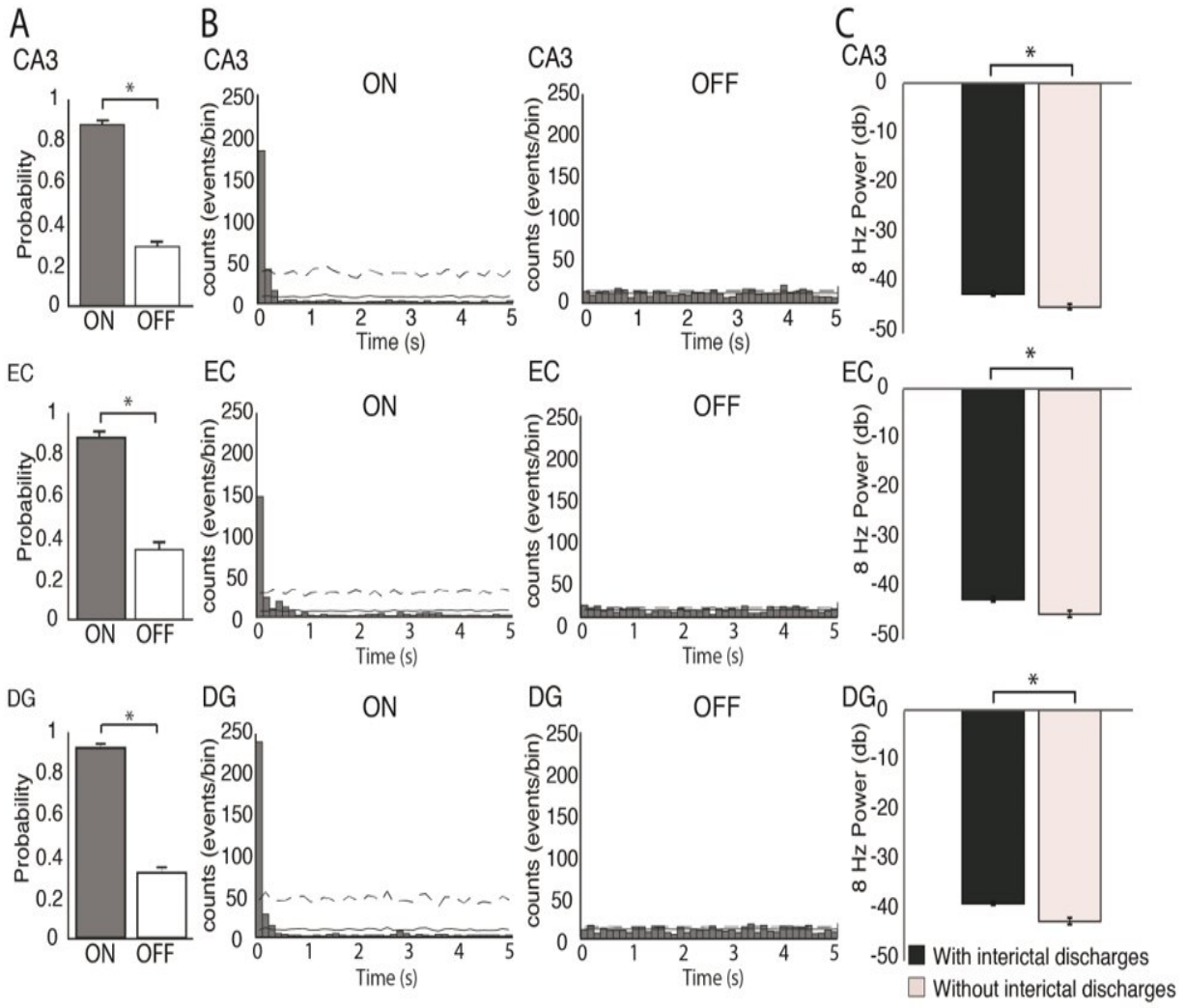

Figure 4 - Interictal discharges are more likely to occur during the optogenetic stimulation. **A**: Probability of an interictal discharge occurring when the optogenetic stimulation is ON or OFF in the three target regions. Interictal spikes are significantly more likely to occur when the optogenetic stimulation is ON. **B**: Distribution of interictal counts (events/bin) during 5 s when the optogenetic stimulation is ON and a random 5 s when it is OFF. When the stimulation is ON, interictal spikes occur at the onset of the stimulation. **C**: 8 Hz Power (db) comparison with interictal discharges and without in all three target regions. Note that the 8 Hz power is stronger when interictal discharges are induced.

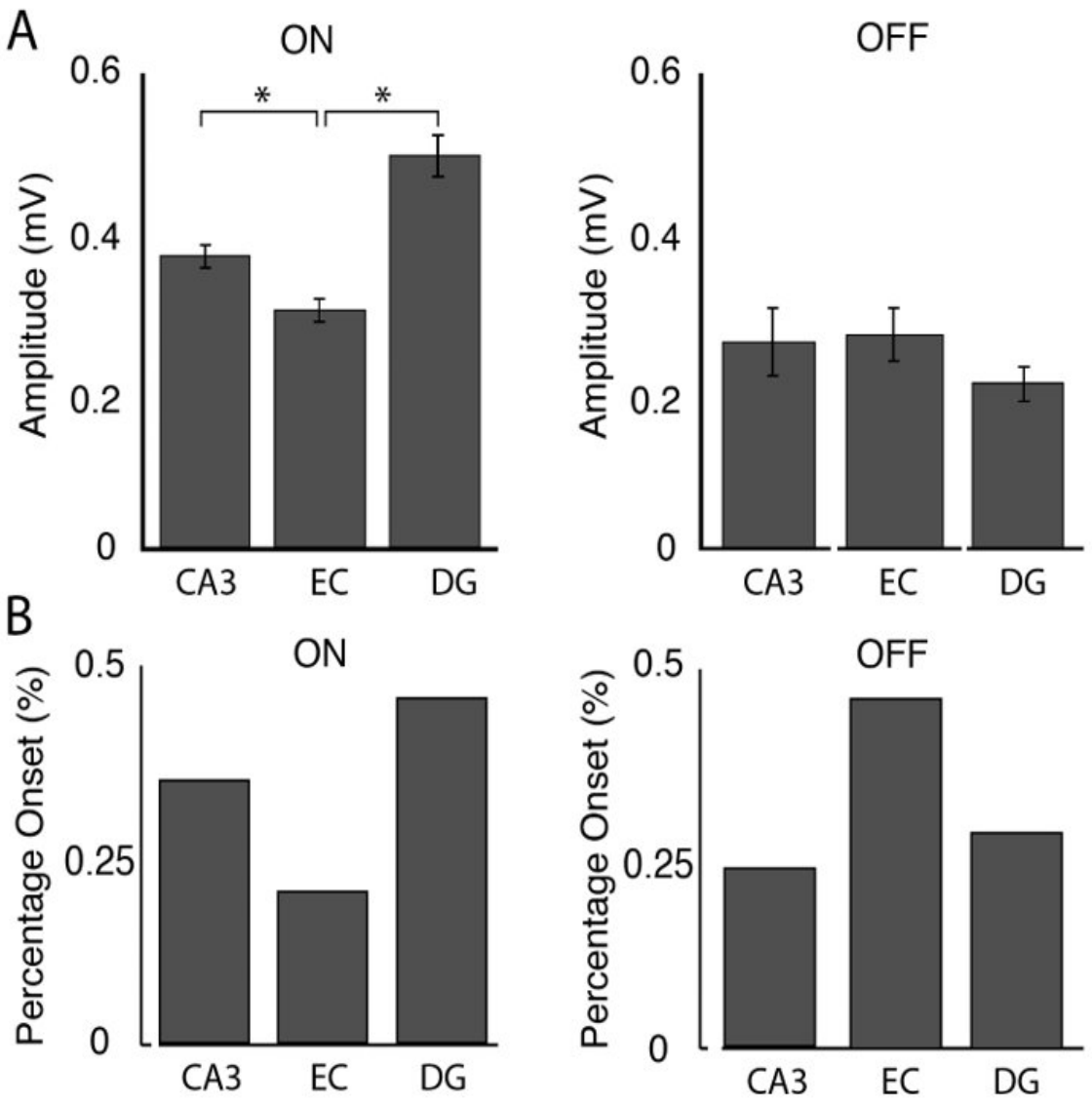

Figure 5 - Induced and spontaneous interictal spikes characteristics. A: Amplitude (mV) of interictal spikes in the three target regions when the optogenetic stimulation is ON and OFF. Note that only when the optogenetic stimulation is ON, the amplitude of interictal spikes is significantly higher in the CA3 and DG. **B:** Percentage Onset (%) when the optogenetic stimulation is ON or OFF in the three target regions. No significant differences were found.

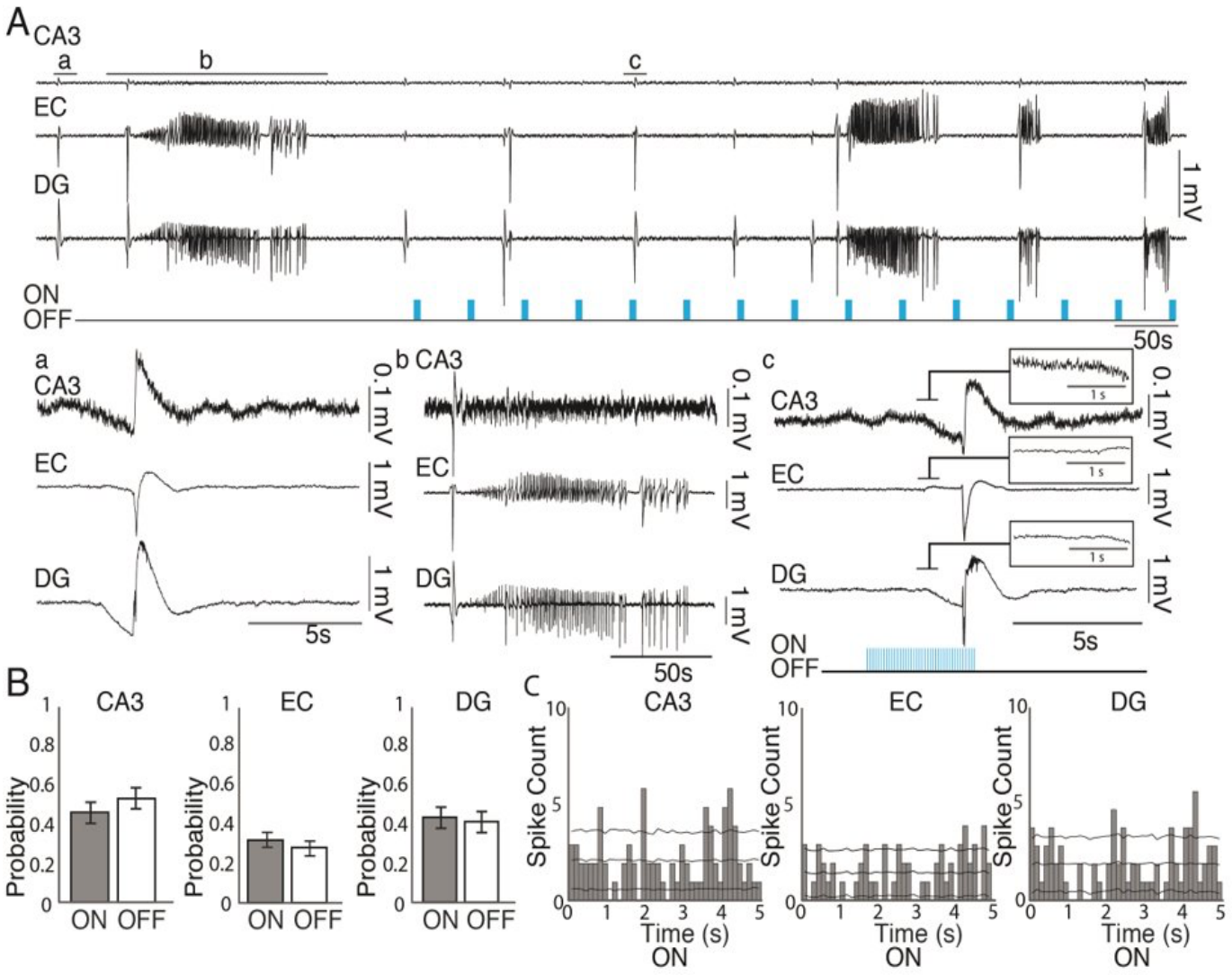

Figure 6 - Optogenetic stimulation has no effect on control PvCre mice. A: Representative traces for CA3, EC and DG with interictal (a) and ictal discharges (b) before the optogenetic stimulation and an interictal discharge (c) during. a, b, c: enlargements of the events. Note that in c, the interictal discharge occurring during the stimulation does not have the 8 Hz field oscillation seen in PvChR2 mice. **B:** Probability of an interictal spike occurring when the optogenetic stimulation is ON or OFF in the three target regions. No significant differences seen. **C:** Spike count distribution for a 5 s timespan when the stimulation is ON for the three target regions. Note that distribution is even over the 5 s timespan.

Chapter 4: Discussion

The main findings of our study can be summarized as follows: (1) optogenetic stimulation of PvChR2 brain slices at 8 Hz induced oscillations at similar frequency under both control (i.e., normal ACSF) and 4AP application; (2) both ictal and interictal discharges were induced by this pattern of optogenetic stimulation; (3) the ictal discharges induced by optogenetic stimulation shared similar electrographic features with spontaneously occurring LVF seizures caused by 4AP, (4) optogenetically-induced interictal discharges contained an 8 Hz field oscillation during their late phase.

4.1. 8 Hz optogenetic stimulation modulates PV-positive interneuron firing

PV-positive interneurons are believed to play an important role in the generation of theta oscillations in the hippocampus as they fire in phase with hippocampal theta oscillations (Varga et al., 2012). In this study, by stimulating PV-positive interneurons at 8 Hz, we were able to produce an 8 Hz oscillation in the hippocampus and EC under both ACSF (Figure 1D) and 4AP. This confirmed the finding from Amilhon et al. (2015) that an 8 Hz stimulation is optimal for producing theta oscillations in intact hippocampal brain slice preparation. In their study, hippocampal slices were stimulated at various frequencies ranging from 2 to 20 Hz, with an 8 Hz stimulation causing the peak power (Amilhon et al., 2015). Our study proves that by stimulating PV-positive interneurons, we were able to mimic theta oscillations in brain slices *in vitro* and that 4AP did not affect this effect.

Moreover, as explained previously, the emergence of theta oscillations depends on the proper connection between the MS-DBB and the hippocampus. This connection is the source of physiological theta oscillations, which are necessary for cognitive function and movement, as well

as of pathological theta oscillations, which are seen during the transition to seizures (Moxon et al., 2019). In our study, this septohippocampal connection was severed in the brain slices used. Thus, spontaneous theta oscillations could not be produced. By optogenetically stimulating PV-positive interneurons at theta frequency, however, we were able to mimic pathological theta oscillations which also led to the emergence of seizures (Figure 2Bc; Figure 3). This shows that, in brain slices where the septohippocampal connection is lost, optogenetically stimulating PV-positive interneurons at 8 Hz was sufficient to induce theta oscillations.

4.2. GABA and GABAergic signaling contribute to the generation of seizures

Studies investigating the cause of seizures have, over the years, produced controversial data. It used to be common belief 40 years ago that seizures were caused by decreased inhibition. Early studies led to the hypothesis that uncontrolled excess excitation due to a significant decrease in inhibition led to seizures (Ben-Ari et al., 1979b; Ayala et al., 1970). Later studies, however, have uncovered an important role for GABA-releasing interneurons in seizure initiation (Perez-Velasquez and Carlen, 1999; Zhang et al., 2011). Now, we know that interneurons can not only control inhibition in brain networks (Freund and Buzsáki, 1996) but also paradoxically initiate seizures (Avoli, 1996; Gnatkovsky et al., 2008; Grasse et al., 2013; Schevon et al., 2012; Truccolo et al., 2011; Chang et al., 2018; Elahian et al., 2018). In this study, by showing that both ictal and interictal discharges could be induced by solely activating PV-positive interneurons, we show that GABA and GABAergic interneurons likely modulate ictogenesis.

As for the intracellular mechanisms underlying the generation of ictal discharges, it has been shown that sustained membrane depolarizations contribute by inducing first, action potential firing characterized as “tonic”, followed by a “clonic” phase with action potential burst discharges

(Lopantsev and Avoli, 1998). $GABA_A$ conductance seems to be responsible for the emergence of these ictal discharges in the EC as the reversal potential is more negative than what would be expected from an excitatory postsynaptic potential (EPSP) (Johnston and Brown, 1981; Gutnick et al., 1982; Avoli et al., 2002). In fact, $GABA_A$ receptor-mediated inhibition seems to contribute to both the initiation and termination of ictal discharges as enhanced Cl^--dependent inhibition follows the progression of ictal discharges (Lopantsev and Avoli, 1998). Indeed, our study highlights the important role of GABA in epileptiform synchronization as the activation of PV-positive interneurons leads to GABA release. We have shown that modulating this activity at theta frequency led to a significant increase in the number of LVF seizures induced (Figure 3A) as well as to a significant increase in interictal discharges (Figure 4A).

4.3. Optogenetically-induced seizures have similar properties as spontaneous seizures

Our optogenetic stimulation protocol (8 Hz stimulation, 5 s ON and 30 s OFF) induced ictal discharges similar to those occurring spontaneously (Figure 2Bc; Figure 3). The generation of these discharges was likely due to activation of PV-positive interneurons at theta frequency, which seemed to synchronize the surrounding neurons in the network. Moreover, the induced ictal discharges had similar electrographic properties as those occurring spontaneously.

The two types of seizure onsets, LVF and HYP, differ by distinct patterns of HFOs whereby ripples dominate LVF seizures and fast ripples are more present in HYP seizures (Lévesque et al., 2012). Ripples emerge due to the sum of inhibitory postsynaptic potentials of principal cells from inhibitory interneurons and thus, they are modulated by GABAergic activity. LVF seizures are mainly caused by GABAergic-mediated synchronization (Salami et al., 2015). As stated above, 4AP enhances GABAergic activity and thus, it is likely to induce spontaneous LVF seizures

(Figure 2Ba). Similarly, optogenetic stimulation activating PV-positive interneurons at theta frequency also induced seizures with LVF characteristics (Figure 2Bc). Moreover, both spontaneous and induced ictal discharges had similar durations (Figure 3C). In fact, significantly more seizures were caused by the photostimulation of PV-positive interneurons than by 4AP. Indeed, when looking at a 35 s timespan, with the first 5 s being when the optogenetic light was ON and the next 30 s being the interval between bouts, significantly more seizures occurred during the first 5 s (Figure 3B). We can therefore conclude that the optogenetic stimulation of PV-positive interneurons at 8 Hz induced ictal discharges similar to those occurring spontaneously and that these induced seizures were more likely to occur.

4.4. Optogenetic stimulation of PV-positive interneurons induces interictal discharges

The 4AP *in vitro* model also induces interictal discharges, both fast and slow. Slow interictal spikes occur in all limbic structures and at lower rates than those occurring in the CA3 subfield of the hippocampus (see for review Avoli et al., 2002, Avoli and de Curtis, 2011). These interictal spikes do not seem to have a fixed site of origin, as we've also proven in this study (Figure 5B) and, as mentioned in the Introduction, they can also be referred to as "$GABA_A$ receptor-mediated, slow interictal potentials" as they occur due to the response of principal cells to GABA release from interneurons (Avoli et al., 2002; Avoli and de Curtis, 2011). They are characterized by long-lasting depolarization but minimal action potential firing (Avoli et al., 2002). As already mentioned, interictal discharges are also associated with transient increases in $[K^+]_o$ due to the depolarizing effect of GABA release from interneurons, an effect caused by excess $[Cl^-]_i$ and activity of KCC2 cotransporter. Thus, here we highlight the important role of the neurotransmitter GABA in the generation of both types of epileptiform discharges (i.e ictal and

interictal discharges). Indeed, GABA-mediated synchronization leads to neuronal excitability due to increases in $[K^+]_o$, a positive shift in the GABA receptor-mediated postsynaptic inhibition which depolarizes the neighboring neurons and disinhibits excitatory postsynaptic activity (Avoli et al., 2002; Avoli and de Curtis, 2011).

Interestingly, the optogenetically-induced interictal discharges differed from the spontaneous spikes in that they contained an 8 Hz oscillation during the falling phase (Figure 2Bd). This finding proves that the optogenetic stimulation did activate the PV-positive interneurons and that, after they synchronized to form the interictal spike, they continued firing at an 8 Hz frequency. Thus, theta oscillations contributed to both ictal and interictal discharges by synchronizing the neuronal network.

Chapter 5: Conclusions

As previously discussed, theta oscillations were shown to precede both chemically induced acute seizures *in vivo* (Butuzova and Kitchigina, 2008) and spontaneous seizures in status epilepticus models in rodents *in vivo* (Broggini et al., 2016; Moxon et al., 2019). In this study, we showed that by stimulating PV-positive interneurons at theta frequency, we were able to induce both ictal and interictal discharges in mouse brain slices *in vitro* during application of 4AP.

Furthermore, the role of PV-positive interneurons in the generation of ictal discharges is complex. *In vitro* (Shiri et al., 2015; 2016) and *in vivo* (Krook-Magnuson et al., 2013; Magloire et al., 2019; Lévesque et al., 2019) studies have shown a controversial role for PV-positive interneurons in seizure initiation which was either pro-epileptogenic or anti-epileptogenic. In my experiments, I have confirmed that PV-positive interneurons have a pro-ictogeneic role when stimulated at theta frequency. The mechanisms underlying this controversial role are elusive, however we could suppose that the changes in the intensity and the timing of the stimulation of these interneurons could be key factors. We know that the activation and synchronization of PV-positive interneurons leads to an increase in $[K^+]_o$, which causes the synchronization of surrounding principal cells, resulting in seizures (Avoli and de Curtis, 2011). We could thus assume that different intensities of photostimulation of PV-positive interneurons could lead to changes in the extent of the increase in $[K^+]_o$ and therefore different stimulations could either promote or impede the generation of ictal discharges. Moreover, the timing of the bouts of stimulation is important as activating the interneurons too often could overwhelm the network and lead to fatigue; on the contrary applying a stimulation protocol with a wider interval between bouts could favor network synchronization. This study also highlighted the important role of GABA and

GABAergic signaling in ictogenesis. Indeed, the activation of these interneurons led to the release of GABA and to the emergence of epileptiform discharges.

Theta oscillations have been shown to be important in the preictal phase of seizure generation (Karunakaran et al., 2016; Moxon et al., 2019). These pathological theta oscillations differ from the physiological oscillations in various ways described above. Interestingly, during the oscillation, interneurons involved in its generation differ in their activity. Indeed, some interneurons are more active during theta oscillations (theta-on) whilst some decrease their activity (theta-off) (Bland et al., 1999; Grasse et al., 2013; Toyoda et al., 2015; Karunakaran et al., 2016). Since theta oscillations seem to be important not only in the generation of seizures but also in that of cognitive deficits in MTLE, it would be essential to further understand the role of the specific neurons involved and how they are modulated. Thus, further studies should concentrate on the roles of theta-on and theta-off interneurons, but also on the differences between PV-positive interneurons, SOM-positive interneurons, basket and chandelier cells as well as the involvement of various receptors. This could be done through pharmacological manipulation with the application of various agonists and antagonists and could help us further understand the specifics occurring in the neural network during the transition to seizures. Moreover, similar experiments could be performed by recording intracellularly interneurons; this approach would aid in further understanding the role of specific neurons. To understand specifically the role of each of the three areas studied, the CA3, the DG and the EC, experiments could also be performed after shielding all areas other than that of interest. These further studies could expand and elaborate on the results found in this thesis.

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