Epileptiform Synchronization of the Rat Insular and Perirhinal Cortices *in vitro*

Jessica Rosemary Sudbury

Department of Neurology and Neurosurgery
McGill University
Montreal, Quebec, Canada
February 2007

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science.

Your file Votre référence
ISBN: 978-0-494-32863-7
Our file Notre référence
ISBN: 978-0-494-32863-7

ACKNOWLEDGEMENTS

I would like to thank Dr. Massimo Avoli for giving me the opportunity to study under his supervision, and past and present members of his lab (especially Dr. Ruba Benini, Dr. Giulia Curia, Philip de Guzman and Thomas Papouin) for their support as colleagues and companions throughout my studies. I am indebted to Toula Papadopoulos, and the welcoming members of the MNI animal facilities, Neurophotography and Neuroelectronics teams for their help, and especially to the members of my advisory committee (Dr. Jean Gotman and Dr. David Ragsdale) and my mentor (Dr. Cecilia Flores) for their guidance throughout this experience. I would also like to thank Dr. Charles Bourque for his editorial assistance.

Above all, I wish to acknowledge my family and friends for their unconditional love and support, especially my parents (Harold and Betty Ann Sudbury) and my fiancé Patrick Allaire. Without them, I would not be pursuing my life's ambitions as I am today.

This thesis is dedicated to the curious and open-minded, who struggle for their art by reasons none other than to satisfy a thirst for knowledge.

TABLE OF CONTENTS

ABSTRACT

Temporal lobe epilepsy (TLE) is the most common form of adult epilepsy. Magnetic resonance imaging (MRI) and electroencephalography (EEG) studies have shown that the hippocampus plays a principle role in epileptogenesis. Recent clinical studies have additionally implicated extrahippocampal brain structures as important participants in TLE, including the perirhinal cortex (PC) and the insular cortex (IC). Modelling TLE with animals rendered epileptic (chronic models of TLE) have corroborated such clinical findings, and have enhanced our understanding of the pharmacology and network interactions that occur during sustained limbic seizures. Due to certain constraints associated with the chronic models, however, brain slices treated with pharmacological agents that induce synchronous neuronal network activity (acute models of TLE) have been popular systems with which to study epileptogenicity *in vitro*. These acute models have reproduced many findings from clinical and chronic animal studies, and have additionally revealed new and exciting concepts that have helped guide hypothesis-driven epilepsy research. The 4-aminopyridine (4AP) acute model of TLE has been especially useful in investigating GABAergic signaling during epileptiform network synchronization, and was therefore chosen to answer the questions asked

within this study. Using this model, I have provided the first evidence of epileptiform synchronicity among the PC and the IC *in vitro*, and have resolved some pharmacological and network mechanisms involved in sustaining such activity. Furthermore, I discovered that in the absence of glutamatergic signaling, GABAergic pathways can produce synchronous network activity in the PC and the IC that is modulated by the activation of μ-opioid receptors. The experiments presented here support clinical and chronic animal study findings concerning the involvement of the PC and the IC in TLE, and additionally provide interesting observations that may impact research that focuses on IC involvement in nociception.

ABRÉGÉ

L'épilepsie du lobe temporal (ELT) est le plus fréquent type d'épilepsie. Des études utilisant l'imagerie par résonance magnétique (IRM) et l'électroencéphalographie (EEG) ont démontré l'importance de l'hippocampe dans l'épileptogénèse. Récemment, diverses études cliniques ont démontré que des structures extra-hippocampales comme le cortex périrhinal (CP) et le cortex insulaire (CI) sont aussi des structures impliquées dans l'ELT. Ces études sont soutenues par des modèles épileptiques d'animaux et ont permises de mieux comprendre les interactions des différents réseaux ainsi que l'effet de divers agents pharmacologiques durant des crises limbiques soutenues. À cause des contraintes liées aux modèles chroniques, les modèles pharmacologiques où une drogue induit l'activité neuronale synchronisée des réseaux neuronaux se sont avérés plus précis et deviennent de plus en plus utilisés pour étudier l'épileptogénèse *in vitro.* Ces modèles précis ont, en effet, récapitulé les résultats obtenus cliniquement et dans les modèles chroniques, en plus de permettre de tester de nouvelles hypothèses. Entre autre, pour disséquer l'influence de la signalisation GABAergique durant les événements épileptiforme menant à une synchronisation des réseaux, le modèle ELT aigu 4-aminopyridine (4AP) c'est avéré

particulièrement utile. Pour cette raison j'ai utilisé ce modèle pour répondre aux questions émises dans mes études. Avec ce modèle, j'ai découvert qu'il y avait une synchronisation épileptiforme entre le PC et l'IC *in vitro*. De plus, j'ai trouvé que la signalisation GABAergique entre le PC et l'IC, en l'absence de signalisation glutamatergique, est régulée par l'activation du système μ-opioïde. En somme, mes études soutiennent les études cliniques et celles sur les modèles chroniques qui démontrent l'implication du PC et de l'IC dans l'ELT. De plus, elles suggèrent l'implication du système μ-opioïde dans l'ELT.

CONTRIBUTION OF AUTHORS

Research presented in section 2 of this thesis is original work that is in preparation to be submitted for publication. I performed the experiments and wrote the manuscript, while Dr. Avoli conceptualized the original hypotheses and general approaches used to guide this project.

LIST OF ABBREVIATIONS

4-aminopyridine (4AP)

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

Ammon's Horn (or Hippocampal) Sclerosis (AHS)

Anti-Epileptic Drugs (AEDs)

Basolateral/Lateral nuclei of the Amygdala (BLA)

Cornu Ammonis 1, 3 (CA1, CA3)

Electroencephalography (EEG)

Entorhinal Cortex (EC)

γ-aminobutyric Acid (GABA)

Insular Cortex (IC)

Kainic Acid (KA)

Magnetic Resonance Imaging (MRI)

Mesial Temporal Lobe Epilepsy (MTLE)

N-methyl-D-aspartate (NMDA)

Perirhinal Cortex (PC)

Rostral Agranular Insular Cortex (RAIC)

Status Epilepticus (SE)

Temporal Lobe Epilepsy (TLE)

Temporal Plus Epilepsy (TPE)

LIST OF FIGURES

1. INTRODUCTION

1.1 Introduction and Rationale

Epilepsy is a common neurological disorder with worldwide prevalence estimates ranging from 1-3% (Blumcke et al., 1999; McNamara, 1999; Shneker and Fountain, 2003). Temporal lobe epilepsy (TLE), the most common form of human epilepsy (Engel, 1996; Wiebe, 2000), refers to a subset of the 'symptomatic' epileptic syndromes as defined by the International League Against Epilepsy (ILAE, 1989) that result from an underlying, and often unknown, brain pathology. Patients with TLE frequently report histories of traumatic lesions, febrile seizures, family histories of seizures suggestive of a genetic component to TLE, or an unrelated genetic or acquired disease that secondarily results in TLE (ILAE, 1989; Engel, 1996). The common result of these conditions is recurrent partial seizures of focal origin within the temporal lobe, which can secondarily generalize to result in loss of consciousness and tonic-clonic convulsions. The TLE classification of the epileptic syndromes can be further subdivided into i) *mesial temporal lobe epilepsy* (MTLE), characterized by hippocampal sclerosis, ii) *lesional TLE*, which results from lesions in brain areas that project to temporal lobe structures, and iii) *cryptogenic TLE* which has no identifiable etiology (Engel, 1996). These three subtypes converge, however, since clinically the seizures they cause are often indistinguishable. For example,

auras consisting of epigastric rising sensations, intense emotional responses (e.g., fear), and olfactory and gustatory hallucinations that immediately precede seizure generalization are common across all three subtypes. MTLE is the most common form, estimated as 25-50% of all epilepsy patients, and has therefore been experimentally addressed extensively (Engel, 2001). Unfortunately, MTLE is also the most resistant to pharmacological interventions (Engel, 1996, 2001).

This project was conceptualized as part of an ongoing investigation into the involvement of brain structures outside of the hippocampus proper in TLE (or MTLE as the case may be) for reasons that will become clear throughout this introduction. I will first summarize the existing knowledge that has accumulated concerning TLE from clinical and experimental research, and then provide some background as regards the emerging importance of extrahippocampal structures in this syndrome (in particular, that of the perirhinal and insular cortices). Finally, I will finish with an introduction to the model that was chosen to address the present research questions, before conveying to you the results of my M.Sc. thesis work. Please note that here forth I will consider information pertaining to TLE in general, and MTLE specifically, as reflecting similar underlying principles, since distinctions among the two are often difficult to delineate. My reasons for this will become clear in section 1.2.4, since emerging evidence for

extrahippocampal involvement in TLE may be raising new questions concerning the accepted definition of TLE.

1.2 Temporal Lobe Epilepsy

1.2.1 TLE: Clinical Studies

> *"The clinical investigator must possess his mind with patience. He must be prepared to make records when he can, but his reward may well be great, for a man can give so much more valuable information than an experimental animal can. However, all too often when the golden opportunity presents itself, we lack the wit to ask the right question!"*
>
> *Penfield and Faulk, 1955, in: "The Insula: Further Observations on its Function"*

Determining the cause of TLE is particularly complicated, since the pathologies that can give rise to this affliction are as diverse as the people they affect. TLE is commonly associated with patient histories of febrile seizures, and less commonly with head and birth traumas, or childhood bacterial, viral or CNS infections (French et al., 1993; Blumcke, 2002). Interpretation of these results is difficult, however, since all of these causes whether known or unknown, converge to give a similar clinical picture. In general, it seems to be agreed upon that TLE is caused by trauma that leads to reactive changes in the brain and

hyperexcitability, which can lead to more extensive structural damage upon repetitive uncontrollable seizures.

In the vertebrate CNS, two neurotransmitters are largely responsible for creating the fine balance that exists between excitation and inhibition: *glutamate* and *γ-aminobutyric acid* (GABA), respectively. Glutamate mediates neuronal excitation mainly by interacting with three families of ligand-gated ionotropic receptors (N-methyl-D-aspartate, (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate), which open Na^+ and Ca^{2+} conductances to depolarize and hence excite postsynaptic membranes (Dingledine et al., 1999; Meldrum, 2000). GABA is the brain's principle inhibitory neurotransmitter (Krnjevic, 2004), and exerts its effects postsynaptically through ionotropic $GABA_A$ receptors that open Cl^-/HCO^{3-} conductances. This usually hyperpolarizes and thus reduces excitability in the postsynaptic membrane (Kaila, 1994; Macdonald and Olsen, 1994); however I will later discuss conditions under which $GABA_A$ receptor activation can result in postsynaptic membrane depolarization. GABA also induces inhibition by binding pre-, post- and peri-synaptic $GABA_B$ receptors, which either increase K^+ permeability or decrease Ca^{2+} permeability by activating GTP-binding proteins and setting into motion intracellular signaling cascades that regulate K^+ and Ca^{2+} channels (Scanziani, 2000; Bettler et al., 2004).

Glutamate-mediated excitation and GABA-mediated inhibition within the brain is highly regulated, and multiple compensatory systems are in place to maintain homeostatic balance among the two (Thompson et al., 1993; Liu, 2004). It is therefore not surprising that runaway hyperexcitability, as seen in epilepsy, has often been attributed to imbalances in this finely tuned system (McCormick and Contreras, 2001). Defects in glutamatergic neurotransmission have been detected in studies of epileptic patients and animals, contributed by changes in glutamate release, reuptake, and receptor expression levels or subunit composition (Dingledine et al., 1990; Avoli, 1991; Chapman, 2000). Prolonged glutamatergic signaling through NMDA receptors is also responsible for causing seizure-related neuronal death from excessive Ca^{2+} entry and subsequent excitotoxicity (Fujikawa, 2005). GABAergic signaling disturbances have also been extensively documented, including the loss of select GABAergic interneuron populations, alterations in GABA synthesis, release, and reuptake, and changes in GABA receptor subunit expression (Bernard et al., 2000; Treiman, 2001; Cohen et al., 2003; Sperk et al., 2004; Ben-Ari and Holmes, 2005; Cossart et al., 2005; Magloczky and Freund, 2005). Such arrays of disturbances that may occur create a complex clinical picture, no doubt contributed by individual differences in physiology, genetics, history and

environment. Patient diagnosis and treatment is therefore a highly individualized process that must take into account many variables to be successful.

Diagnosing TLE involves a multidimensional onslaught of tests, which nowadays routinely includes simultaneous video monitoring with electroencephalography (EEG) recordings, and magnetic resonance imaging (MRI). Pharmacoresistant neurosurgery candidates will also undergo depth and cortical surface electrode implantation in an attempt to locate the epileptogenic region. EEG diagnosis exploits the fact that neuronal networks tend to discharge hypersynchronously during and between seizures in epileptic patients. EEG recordings can detect abnormal "brain waves," which represent extreme changes in extracellular ionic gradients resulting from large neuronal network discharges. TLE results in two highly unusual electrographic events: i) *ictal discharges* and ii) *interictal spikes*. Both are usually lateralized to one mesial temporal lobe or the other, and occur focally; localization of these discharges aids in the detection of a patients' particular epileptogenic zone (Engel, 1996; Verma and Radtke, 2006). Interictal spikes, which have no obvious behavioural manifestation, are focal discharges lasting approximately 50-200 ms, and usually occur in clusters. On the intracellular level, they are large neuronal burst discharges characterized by rapid and successive action potentials that ride on a giant depolarizing envelope; this phenomenon is referred to as paroxysmal depolarizing shift (PDS; de Curtis

and Avanzini, 2001). Ictal discharges, on the other hand, are characterized by prolonged, rhythmic electrographic activity that initiates focally and rapidly spreads to nearby brain regions, and are associated with behavioural changes such as loss of consciousness and tonic-clonic convulsions (Engel, 1996; Verma and Radtke, 2006).

Consistent brain structural abnormalities in TLE have long been described in autopsy reports, however now clinicians can detect these irregularities in patients *in vivo* with the current widespread availability of MRI. A signature of TLE (65% of cases; Blumcke, 2002) is the presence of Ammon's Horn (or Hippocampal) Sclerosis (AHS). Post-surgical histopathological analysis of resected tissue has confirmed that AHS is associated with hippocampal neuronal loss, glial scarring, and axonal sprouting that leads to network reorganization (Fish and Spencer, 1995; Watson et al., 1997; Blumcke et al., 1999; Houser, 1999; Blumcke, 2002). Clinical studies have firmly established that such histopathological changes are associated with tissue volumetric reductions, detectable with MRI (Sutula et al., 2003). It remains a largely debated question, however, as to whether seizure activity causes brain damage, or whether brain damage precedes and thus causes seizures. Unfortunately, accumulating evidence supports the former hypothesis (Sutula et al., 2003). For example, it used to be thought that recurring febrile seizures were largely harmless, save for

episodes of exceedingly prolonged seizures (status epilepticus; SE). However, it has been shown that childhood onset TLE results in reduced brain volumes and cognitive deficits later in life (Hermann et al., 2002). Furthermore, animals subjected to single episodes or a short series of seizures experience cell loss and synaptic rearrangement (Bengzon et al., 1997; Holmes and Ben-Ari, 1998; Haas et al., 2001). Combined MRI and EEG studies of TLE patients show that sites of epileptiform discharge onset tend to localize to the same brain regions exhibiting volumetric reductions (Cendes et al., 1996; Watson et al., 1997; Cendes et al., 2000; Bernasconi et al., 2003; Vossler et al., 2004). Many have therefore revised old theories to suggest instead that an initial insult causing subtle reactive changes can lead to recurrent temporal lobe seizures, which subsequently cause the global structural changes commonly associated with TLE (Ben-Ari, 1985; Holmes and Ben-Ari, 1998). There is now a sense of increased urgency to pharmacologically treat recurring seizures early on, in the hopes of preventing progression of the disorder to untreatable stages (Sutula et al., 2003).

There exist attractive treatment options for epilepsy patients in anti-epileptic drugs (AEDs), which are available in seemingly endless varieties. Though the mechanism(s) of action are not well understood for some, AEDs have single or multiple effects which may act to i) block voltage-gated sodium channels in a use-dependent manner (e.g., phenytoin, carbamazepine,

oxcarbazepine, lamotrigine, topiramate, zonisamide, felbamate), ii) potentiate GABAergic inhibition (e.g., vigabatrin, tiagabine, benzodiazepines, valproate), iii) block Ca^{2+} channels (e.g., ethosuximide, gabapentin, pregabalin, valproate), and iv) alter glutamatergic neurotransmission (e.g., topiramate, felbamate; Perucca, 2005). Choices in medication can be tailored to each individual's history and clinical features. Regrettably, from 20 to 40% of patients with epilepsy are "medically intractable," meaning unresponsive to pharmacological treatment, and most of these are cases of TLE (Engel, 2001; Engel et al., 2003). Surgical intervention is becoming the best alternative for these patients, since up to 80% of TLE patients become seizure-free with resection of temporal structures (Engel, 2001; Wiebe et al., 2001; Engel et al., 2003; Cohen-Gadol et al., 2006). While unilateral removal of hippocampal and amygdalar structures is often sufficient and successful, there is evidence that seizures originating within extrahippocampal structures may account for the lack of success in the remaining 20% that do not respond to surgery (Ryvlin and Kahane, 2005). In support of this, general mesial temporal atrophy and sclerosis that extends beyond the hippocampus is found in a subset of cases (Lee et al., 1998; Moran et al., 2001). This may include atrophy in the amygdala, entorhinal cortex (EC), the perirhinal cortex (PC), and in more rare cases, the insular cortex (IC) (Cendes et al., 1993; Bernasconi et al., 1999; Bernasconi et al., 2000;

Salmenpera et al., 2000; Jutila et al., 2001; Salmenpera et al., 2001; Bernasconi et al., 2003; Bauer et al., 2006). Since clinical studies have only just begun to address this issue, investigators have turned to animal models of TLE to further investigate these observations.

1.2.2 TLE: Chronic Models

Due to obvious ethical constraints against experimental studies in epileptic patients, researchers have had to circumvent this by modelling epilepsy in animals. Reproducing TLE in animals has been appreciably difficult due to the range of etiological factors that lead to the common set of symptoms making up this clinical syndrome. After years of experimentation, two general approaches to this problem have emerged: i) producing epileptiform network activity in normal brain tissue (acute models), and ii) replicating the behavioural and etiological aspects of TLE, namely an initial brain insult followed by the behavioural pattern of recurrent seizures (chronic models).

Chronic models, such as the kindling and post-SE (or SE) models in particular, have proven very informative. The kindling model of TLE has long been a first choice by investigators due to its ability to re-create an epileptogenic focus that can eventually generate spontaneous generalized seizures. Here, a stimulating electrode is chronically implanted in a chosen brain region, and low

voltage current is delivered repetitively over several trials during EEG monitoring. Initially, the stimulus does not elicit epileptiform discharges; however, focal discharges develop that increase in amplitude and duration over repeated trials. These discharges eventually "kindle" surrounding brain structures to become epileptogenic, and spontaneous generalized seizures eventually occur in the absence of any stimulation (Racine, 1972b, a). Surprisingly, however, no detectable morphological changes have been reported as a result of kindling, (Morimoto et al., 2004). Despite this, the kindling model has been extremely useful in the study of network interactions and pharmacological mechanisms that give rise to seizure activity *in vivo*.

SE models, on the other hand, cause a traumatic insult to the brain resulting in an immediate state of prolonged seizure activity (an episode of SE). By an incompletely understood mechanism, this renders neuronal networks epileptogenic and produces remarkably similar patterns of brain damage as seen in human TLE (Ben-Ari, 1985). This model also reproduces the temporal characteristics of TLE, whereby the initial SE episode is followed by a seizure free period lasting several weeks; eventually spontaneous persistent seizures begin (Racine, 1972b, a; Ben-Ari, 1985). The initial insult can be induced in a variety of ways, however. Systemic injections of convulsants such as the glutamatergic receptor agonist kainic acid (KA), and the cholinergic receptor

agonist, pilocarpine, have proven to be very effective in producing chronically epileptic animals. Remarkably, both kindling and SE models produce pharmacologically similar seizures, and have been extensively used to develop and test AEDs in a controlled setting (Loscher, 2002; Morimoto et al., 2004). Not surprisingly, however, these models are imperfect. Not only are they labour intensive and thus expensive, but the initial SE is difficult to control and can result in death of the animal. Furthermore, the induced neural damage is often more severe than that seen in humans, and individual reactions to the protocol among animals makes drawing generalized conclusions problematic (Morimoto et al., 2004). Of interest here, nonetheless, these models have corroborated clinical findings for extrahippocampal involvement in TLE including that pertaining to the PC and the IC.

1.2.3 Extrahippocampal Involvement in TLE: The Perirhinal Cortex

The PC forms a part of the parahippocampal region which is thought to act as a gateway that selectively filters inputs from the neocortex to the hippocampus (de Curtis and Pare, 2004). The PC also functions as an object memory and recognition structure (Buckley, 2005; Murray et al., 2005). Importantly, the PC is connected with other structures involved in TLE, namely the EC (the major input and output structure for the hippocampus), the hippocampus (CA1 and

subiculum), the amygdala, and the IC (Burwell et al., 1995; Burwell and Witter, 2002).

As alluded to earlier, several clinical studies have implicated the PC in TLE due to observations of atrophy in this region in a subset of TLE patients (Bernasconi et al., 2000; Salmenpera et al., 2000; Jutila et al., 2001; Bernasconi et al., 2003). Furthermore, concurrent volumetric reductions and neuronal loss were recently reported in the PC of pilocarpine-treated rats (Nairismagi et al., 2006). However, most evidence for the involvement of the PC in TLE has come from kindling studies. Kindling rate, the number of stimulation trials required to produce full motor seizures, has often been used as an assessment of the importance of a structure in seizure-generating circuits (Kelly and McIntyre, 1996; Morimoto et al., 2004). The PC has been shown in numerous cases to be the fastest to kindle among all structures tested, and with extremely short latencies between stimulus delivery and motor convulsion (McIntyre et al., 1993; Mohapel et al., 1996; Sato et al., 1998; Mohapel et al., 2001; Barnes et al., 2005; for recent reviews see Kelly and McIntyre, 1996; McIntyre and Kelly, 2000). Further, lesions in the PC prevent the development of kindled seizures, lending more support for its importance in epileptogenesis (Kelly and McIntyre, 1996; McIntyre and Kelly, 2000). Given these trends, the PC has been proposed to be part of a pathway that amplifies and rapidly propagates seizure activity to motor and

cortical areas, giving rise to secondary generalized convulsive seizures (Kelly and McIntyre, 1996; McIntyre and Kelly, 2000). Interestingly, studies that compared kindling rates among the PC and the IC demonstrated similar kindling profiles (Kodama et al., 2001; Mohapel et al., 2001) implying that the IC may also be hyperexcitable and important in producing kindled seizures.

1.2.4 Extrahippocampal Involvement in TLE: The Insular Cortex

The IC has long been suspected as an important participant in pain processing; however only recently it has been proven as a key player. Specifically, the rostral agranular region of the IC (the RAIC) is part of a pain processing pathway that alters nociception in a top-down manner. Though the exact mechanism is unknown, IC outputs chronically regulate detection thresholds for painful stimuli in the periphery via GABAergic signals (Jasmin et al., 2003; Jasmin et al., 2004). In addition, an unusually dense staining region for μ-opioid receptors (primary receptor targets for analgesia-producing opiates such as morphine) is present in the rostral agranular region (Delfs et al., 1994; Burkey et al., 1996). Of interest here, the IC is connected with other structures involved in TLE, such as the anterior hippocampus, the PC and the amygdala. Importantly, the RAIC is a principal output target for fibres originating within the PC (Flynn et al., 1995; Augustine, 1996; Delatour and Witter, 2002).

The role of the IC in TLE remains largely unaddressed; however, a few recent studies have indicated it may be deserving of more attention. While it appears to have similar kindling characteristics as the PC (Kodama et al., 2001; Mohapel et al., 2001), a few studies have shown significantly increased *c-fos* mRNA expression and Fos protein levels in the IC of PC-kindled rats, which are indicators of neuronal excitation (Ferland et al., 1998; Sato et al., 1998). Further, infusion of NMDA receptor antagonists into the IC of rats *in vivo* can protect against the development of kindled seizures (Holmes et al., 1992; Kodama et al., 2001). Finally, a recent study reported substantial neuronal loss in the IC in pilocarpine-treated rats (Chen et al., 2006), while a clinical case study reported volumetric reduction in the IC (Bauer et al., 2006).

The most compelling evidence for IC involvement in TLE comes from a neurosurgical team that systematically implanted depth electrodes in the IC of intractable TLE patients undergoing pre-surgical evaluation. The IC had been suspected for some time to contribute in part to non-responsiveness in some patients to temporal lobectomy, but it hadn't yet been directly shown to do so. Remarkably, these investigators found that seizure activity invaded the IC in every recorded temporal lobe seizure, most often from the hippocampus. Further, they found that the IC exhibited interictal spikes that were sometimes synchronized with other temporal structures. Importantly, in two cases the IC

initiated ictal activity which then spread to other temporal lobe structures to mimic a typical temporal lobe seizure. Perhaps not surprisingly, upon temporal lobe resection (which leaves the IC intact), the two patients with IC ictal foci were not remedied of their seizures (Isnard et al., 2000), while nearly all other patients were. Further investigation by this group revealed that some of the somatosensory and visceral sensations that typically occur during typical TLE seizures can be attributed to IC involvement in epileptiform discharges, and that a tailored resection that includes a portion of the IC was more effective in controlling seizures with focal IC onset (Isnard et al., 2004). Other studies have corroborated these results. One group reported involvement of the IC in 60% of TLE seizures using positron emission tomography (PET; Bouilleret et al., 2002), while assorted case studies of patients with insular lobe seizures report similar visceral and somatosensory sensations during ictal discharges (Rossetti et al., 2005; Ryvlin, 2006). Such findings have prompted the proposal for an alternative epileptic syndrome designation, "Temporal Plus Epilepsy" (TPE). TPE is meant to describe the subset of patients that have prominent temporal lobe ictal involvement and other clinical features suggestive of TLE, but whose seizures actually initiate outside temporal structures (Ryvlin and Kahane, 2005). The IC may contribute toward many cases of TPE, since sensations experienced during insular lobe seizures closely mirror those reported by TLE patients (Ryvlin,

2006). This designation may help identify patients who are likely to benefit from individualized surgical procedures that resect more extrahippocampal tissue.

Given the mounting clinical evidence for extrahippocampal involvement in TLE for both the PC and the IC, it seemed appropriate to define their involvement experimentally. The present research questions were addressed with an acute model for TLE, however, due to aforementioned restrictions associated with the TLE chronic models, and for reasons that will become clear in the following sections.

1.3 Acute Models: Capturing Network Synchronization

1.3.1 General Findings: *In Vitro* Epileptiform Activity

Another solution for modelling TLE is to induce extreme synchronicity in otherwise normal neuronal networks to recreate the situation seen during epileptic seizures. As such, this kind of neuronal behaviour can then be experimentally manipulated. While these models do not recreate epilepsy *per se*, they have been useful in delineating how networks become recruited in synchronized discharges, helping to form hypotheses that can later be tested in epileptic animals. To induce network synchrony, brain slices are generally prepared acutely from animals, and then bathed in a medium that shifts

excitation and inhibition balances to result in *in vitro* epileptiform activity. Commonly used convulsants include i) high extracellular K^+, which enhances neuronal excitability by shifting the resting membrane potential closer to firing threshold while relieving NMDA receptor channels of the Mg^{2+} block that is present at normal resting membrane potentials (Rutecki et al., 1985), ii) increasing NMDA receptor conductance by removing extracellular Mg^{2+} (Anderson et al., 1986) or bath-applying the glutamatergic receptor agonist KA (Fisher and Alger, 1984), iii) reducing GABA-mediated inhibition by antagonizing $GABA_A$ receptors with picrotoxin or bicuculline (Hablitz, 1984; Ives and Jefferys, 1990), iv) augmenting cholinergic neurotransmission by bath application of pilocarpine, a cholinergic agonist (Nagao et al., 1996), and v) bath-applying the drug 4-aminopyridine (4AP), which enhances Ca^{2+} conductances (Thesleff, 1980; Segal and Barker, 1986) and blocks A- and D-type K^+ channels to interfere with neuronal repolarization (Rudy, 1988; Hille, 1992) and result in an increase of neurotransmitter release at both excitatory and inhibitory synaptic terminals (Thesleff, 1980; Buckle and Haas, 1982; Rutecki et al., 1987; Perreault and Avoli, 1989). Traditionally, such convulsants were applied to hippocampal slices, which conveniently allowed for the study of network interactions during epileptiform discharges at the system or single cell level due to the laminar network organization. However, as clinical evidence mounted to implicate more

widespread network involvement in TLE, slices were made to preserve connections among the hippocampus and other surrounding structures such as the EC and amygdala, and more recently, the PC. Several years of study later, a few general trends have emerged.

While epileptiform interictal-like (hereafter termed *interictal*) discharges could be induced with convulsants in hippocampus, it was rare that ictal-like (hereafter termed *ictal*) discharges were observed (Anderson et al., 1986; Rafiq et al., 1993; Avoli et al., 2002). Area CA3 of the hippocampus is unique, in that its pyramidal cells are endowed with intrinsic burst-generating properties, can generate rapid successions of dendritic Ca^{2+} spikes, and have extensive recurrent connections, all resulting in a neuronal network with very low endogenous synchronization thresholds (Miles and Wong, 1986, 1987; Traub and Jefferys, 1994; McCormick and Contreras, 2001). Upon application of even low concentrations of convulsant agents on hippocampal slices, interictal discharges are generated primarily in CA3 region which subsequently spread to other hippocampal subdivisions (Fisher and Alger, 1984; Nagao et al., 1996; Barbarosie et al., 2000; Avoli et al., 2002). AMPA-mediated neurotransmission is required for generating interictal discharges, while NMDA receptor activity, associative intercellular connections and non-synaptic interactions generate afterdischarges following an initial burst of activity during ictal discharges (Lee

and Hablitz, 1989; Jones and Lambert, 1990; Traub and Jefferys, 1994; de Curtis et al., 1999; de Curtis and Avanzini, 2001). Interestingly, surrounding limbic structures such as the EC and basolateral/lateral nuclei of the amygdala (BLA) generate prolonged ictal discharges when included in the slice preparation, which can subsequently propagate into the hippocampus through the perforant and temporoammonic pathways (Barbarosie and Avoli, 1997; Barbarosie et al., 2000; Avoli et al., 2002; Benini et al., 2003). *In vitro* ictal discharges initiating in the EC, the PC or BLA require NMDA receptors (Walther et al., 1986; Swartzwelder et al., 1987; Jones and Lambert, 1990; Nagao et al., 1996; Lopantsev and Avoli, 1998; Stoop and Pralong, 2000; Benini et al., 2003; de Guzman et al., 2004). Since convulsant-induced epileptiform discharges appear to be pharmacologically similar to those that occur in chronic animal models of TLE, human epileptic tissue *in vitro* and perhaps even epileptic discharges *in vivo* (Dingledine et al., 1990; Avoli, 1991; Chapman, 2000; Cohen et al., 2002), these models have proven successful at capturing features of TLE-like epileptiform synchronicity for experimental study.

1.3.2 Acute Models and TLE

Currently, debates are ongoing as to how close these models recreate actual epileptic synchronicity, since some contradictory trends have emerged *in*

vitro that may or may not reflect the situation in TLE. For example, a big unanswered question in epilepsy research is: *why do interictal discharges occur in epilepsy, and what are their effects?* A common assumption among clinicians is that interictal discharges in TLE are pro-convulsive, since they often occur within the same epileptogenic zones that initiate ictal discharges; interictal discharges are therefore useful for locating the epileptogenic zone by EEG (Bautista et al., 1999; Avoli et al., 2006). In addition, cases of TLE with frequent interictal spikes tend to be more severe than cases with infrequent interictal spikes (Rosati et al., 2003). In support of this, interictal discharge frequency tends to positively correlate to the number of kindling trials administered in rats (Pinel and Rovner, 1978). However, others have observed that, in general, interictal spike frequency does not change immediately prior to seizure onset in patients according to some (Gotman, 1991; Katz et al., 1991), or decreases preceding seizures according to others (Lange et al., 1983; de Curtis and Avanzini, 2001), and increases immediately following ictal discharges (Gotman and Marciani, 1985; Gotman, 1991). Furthermore, post-interictal spike depression associated with reductions in high frequency oscillations and increased discharge thresholds have been reported in epileptic patients (de Curtis et al., 2005; Urrestarazu et al., 2006); this implies not only that interictal

spikes reduce excitability, but that they may even be neuroprotective (de Curtis and Avanzini, 2001).

In convulsant-treated slices, the picture is also mixed. Support has been gathered in favour of a pro-convulsant role for interictal discharges, since a phenomenon labelled "interictal-to-ictal transition" has been described. Here, interictal afterdischarges become increasingly longer, eventually transitioning into full ictal discharges (Dzhala and Staley, 2003). By contrast, it has been shown *in vitro* that the presence of interictal activity within a neuronal network, or interictal activity propagating into an adjacent network, reduces excitability in the affected region to prevent the development of ictal discharges. This has been demonstrated within the hippocampus (Swartzwelder et al., 1987), and when interictal activity propagates from the hippocampus to the EC, BLA and the PC (Barbarosie and Avoli, 1997; D'Antuono et al., 2002; Benini et al., 2003; Librizzi and de Curtis, 2003; de Guzman et al., 2004; D'Arcangelo et al., 2005). Direct application of low amplitude current in hippocampal slices has been shown to suppress convulsant-induced ictal discharges without enhancing excitability (Warren and Durand, 1998), and "substituting" interictal discharge inputs with low frequency stimulation has the same effect (Barbarosie and Avoli, 1997; Benini et al., 2003; D'Arcangelo et al., 2005). Finally, it has been shown that discharge thresholds are increased *in vitro* immediately following interictal discharges (de

Curtis et al., 2001). Clearly, the interictal-to-ictal question demands further investigation with chronic models of TLE to be resolved.

An important revelation made with the help of convulsant-treated slices, is that GABA can act as a depolarizing neurotransmitter under certain conditions (Michelson and Wong, 1991, 1994). It appears that GABA is not simply an "inhibitory" neurotransmitter (Bernard et al., 2000; Ben-Ari and Holmes, 2005). Seizures are unlikely due to a simple loss of GABAergic inhibition, since pathologically depolarizing GABA may also contribute to the generation of epileptic seizures *in vivo* (Cohen et al., 2003; Ben-Ari and Holmes, 2005; Cossart et al., 2005). Most acute models of TLE induce epileptiform activity by interfering with glutamatergic and especially GABAergic neurotransmission; however the 4AP model has proven to be very useful in circumventing this problem. Leaving the principle excitatory and inhibitory systems intact, it lends well to the study of pharmacological mechanisms in network synchronization (Perreault and Avoli, 1989), including those mediated by GABA.

1.3.3 GABA-Mediated Synchronicity and 4-Aminopyridine

GABAergic interneurons are not simply passive inhibitory bystanders to their pyramidal cell counterparts in the brain as previously believed. Rather, they are active and central participants in coordinating synchronous activity among

large neuronal networks (Buzsaki et al., 1992; Cobb et al., 1995; Freund and Buzsaki, 1996; Csicsvari et al., 1999; Whittington and Traub, 2003; Somogyi and Klausberger, 2005). Currently, there is strong evidence favouring GABA, not glutamate, as the important excitatory neurotransmitter coordinating neuronal network maturation during development by inducing giant depolarizing potentials (GDPs; Ben-Ari et al., 1989; Kasyanov et al., 2004; Krnjevic, 2004; Ben-Ari and Holmes, 2005). GABA has also been observed to exert excitatory postsynaptic effects in adult hippocampal slices in response to high frequency stimulation or intense dendritic activation (Staley et al., 1995; Lamsa and Taira, 2003; Krnjevic, 2004), and in the presence of 4AP (Perreault and Avoli, 1989; Aram et al., 1991; Michelson and Wong, 1991; Lamsa and Kaila, 1997).

Interestingly, similar mechanisms give rise to depolarizing actions of GABA in the juvenile brain and with 4AP. In young animals, intracellular Cl^- concentrations are high, causing depolarizing $GABA_A$ receptor responses; upon *de novo* expression of the Cl^--extruding KCC2 co-transporter later in development, intracellular Cl^- concentrations decrease to adult levels, and GABA exerts its "typical" hyperpolarizing postsynaptic effects (Rivera et al., 1999). It was recently shown that a similar process may contribute toward the ability of 4AP to induce depolarizing GABA responses. The KCC2 co-transporter is downregulated following periods of prolonged network activity such as during

convulsant-induced epileptiform discharging (Rivera et al., 2004), and in response to kindling *in vivo* (Rivera et al., 2002). Examinations of resected human epileptic tissue have also revealed an excitatory GABAergic component to spontaneous epileptiform discharges in subicular and neocortical tissue, suggesting similar defects in Cl^- regulation (Kohling et al., 1998; Cohen et al., 2002; Cohen et al., 2003; Avoli et al., 2005). 4AP-induced synchronized interneuronal discharges, recorded as spontaneous GABA-mediated potentials, have been observed in rat hippocampus and neocortex *in vitro* (Perreault and Avoli, 1989, 1992; Avoli et al., 1994; Michelson and Wong, 1994; Avoli, 1996; Avoli et al., 1996; Lamsa and Kaila, 1997), providing a convenient model for the study of GABA-driven interneuronal network synchronization. These potentials are $GABA_A$ receptor-dependent, implying that depolarizing GABA contributes to these large-scale network responses. Furthermore, GABA-mediated potentials have been shown to play a role in initiating 4AP-induced ictal discharges in combined rat hippocampus-EC slices (Barbarosie et al., 2002). Pathological GABAergic mechanisms clearly contribute toward epileptiform synchronicity *in vitro*; exactly how remains to be determined.

In summary, the aim of my M.Sc. studies was to investigate the network interactions of the hippocampus, the PC and the IC during epileptiform synchronous activity. The 4AP acute model of TLE was chosen since it allows

for thorough pharmacological manipulation *in vitro* without direct interference with GABAergic and glutamatergic neurotransmission, the two major neurotransmitter systems that contribute to epileptic seizures *in vivo*. Results from this study, in combination with studies employing other available models of TLE, will no doubt help guide future studies in clarifying the respective roles of extrahippocampal structures and GABAergic signaling in producing epileptic seizures.

2. EPILEPTIFORM SYNCHRONIZATION IN THE RAT INSULAR AND PERIRHINAL CORTICES IN VITRO

2.1 Abstract

The hippocampus plays a primary role in temporal lobe epilepsy, a common form of partial epilepsy in adults. Recent studies, however, indicate that extrahippocampal areas such as perirhinal cortex and insular cortex may represent important participants in this epileptic disorder. By employing field potential recordings in the *in vitro* 4-aminopyridine model of temporal lobe epilepsy we have investigated here the contribution of glutamatergic and GABAergic signaling to epileptiform activity in these structures. First, we provide evidence of epileptiform synchronicity between the perirhinal cortex and insular cortex, and resolve some pharmacological and network mechanisms involved in sustaining the interictal- and ictal-like discharges recorded in these areas. Second, we report that in the absence of ionotropic glutamatergic transmission, GABAergic networks produce synchronous potentials that spread between the perirhinal and insular cortices. Finally, we have established that such activity is modulated by activating μ-opioid receptors.

In conclusion, our findings support clinical and experimental evidence concerning the involvement of perirhinal cortex and insular cortex networks in

temporal lobe epilepsy, and additionally provide observations that may impact research focusing on the role of the insular cortex in nociception.

2.2 Introduction

The insular cortex (IC) is a 6-layered, higher-order, neocortical processing area implicated in many functions (Flynn et al., 1995; Augustine, 1996; Aleksandrov and Fedorova, 2003). The rostral agranular IC, in particular, is important in regulating peripheral nociceptive thresholds via opioidergic, dopaminergic and GABAergic signaling (Burkey et al., 1996; Burkey et al., 1999; Jasmin et al., 2003; Jasmin et al., 2004). In addition, the rostral agranular IC is reciprocally connected with the perirhinal cortex (PC) (Delatour and Witter, 2002), a structure central in object memory and recognition (Buckley, 2005; Murray et al., 2005). Both the IC and PC receive and project dense connections from and to several brain regions implicated in temporal lobe epilepsy (TLE), such as the entorhinal cortex, the amygdala and the hippocampus (Burwell et al., 1995; Flynn et al., 1995). TLE - an epileptic syndrome characterized by spontaneous recurring seizures originating within the temporal lobe - is often associated with mesial temporal sclerosis that extends beyond the hippocampus (Lee et al., 1998; Moran et al., 2001). Accordingly, histological and volumetric changes in PC and IC have been reported from studies in TLE patients and in

animal models of this epileptic disorder (Bernasconi et al., 1999; Bernasconi et al., 2000; Salmenpera et al., 2000; Jutila et al., 2001; Bernasconi et al., 2003; Bauer et al., 2006; Chen et al., 2006; Nairismagi et al., 2006). While the PC is well established as important in the generalization of limbic kindled seizures (McIntyre and Kelly, 2000; Morimoto et al., 2004), IC involvement in epilepsy was only recently identified in a group of TLE patients implanted with IC depth electrodes during pre-surgical evaluations (Isnard et al., 2000; Isnard et al., 2004). These studies have shown that electrical stimulation within the IC and insular focal ictal discharges produce similar visceral and somatosensory sensations to those reported during TLE seizures. Thus, insular seizures may be misdiagnosed as true TLE leading to surgical treatment failures since temporal lobectomy does not typically include the IC (Isnard et al., 2000; Isnard et al., 2004; Ryvlin and Kahane, 2005; Ryvlin, 2006).

Electrographic epileptiform events similar to the interictal and ictal discharges seen in TLE patients can be induced in brain slices maintained *in vitro* by bath application of convulsants such as 4-aminopyridine (4AP). These studies have revealed that interictal-like (hereafter termed *interictal*) discharges originate within the hippocampal CA3 area (Fisher and Alger, 1984; Anderson et al., 1986; Nagao et al., 1996; Barbarosie et al., 2000; Stoop and Pralong, 2000) while ictal-like (hereafter termed *ictal*) events resembling electrographic limbic

seizures, initiate within the entorhinal cortex, the PC or the amygdala (Walther et al., 1986; Avoli, 1996; Barbarosie and Avoli, 1997; Benini et al., 2003; de Guzman et al., 2004; Uva et al., 2005). Among available drugs that induce *in vitro* epileptiform activity, 4AP lends particularly well to study the contribution of GABAergic mechanisms to network synchronization since it enhances both glutamatergic and GABAergic transmission (Perreault and Avoli, 1989). Indeed, spontaneous $GABA_A$ receptor-mediated interictal events have been reported in human epileptogenic brain tissue removed during surgery (Kohling et al., 1998; Cohen et al., 2002). GABA release is in part regulated by the action of metabotropic μ-opioid receptors localized to GABAergic interneurons in hippocampus (Cohen et al., 1992; Capogna et al., 1993; McQuiston and Saggau, 2003). In addition, disruptions of the hippocampal opioidergic system have been described in TLE animal models (Rocha et al., 1994; Rocha and Maidment, 2003; Skyers et al., 2003; Sanabria et al., 2006). *In vitro*, μ-opioid receptors have further been established as important regulators of network synchronicity since a specific μ-opioid agonist abolishes 4AP-induced spontaneous GABA-mediated network events in CA3 and entorhinal cortex (Barbarosie et al., 1994; Avoli et al., 1996; Barbarosie et al., 2002). Given that μ-opioid receptors similarly localize to interneurons in cortex (Taki et al., 2000; Ferezou et al., 2006), we hypothesized that a similar mechanism might regulate network synchronicity in

the PC and the IC as well. We therefore sought to characterize network interactions among these structures and the hippocampus in response to 4AP using a novel slice preparation (Inaba et al., 2006), and investigated the involvement of μ-opioid receptors in regulating GABA-mediated synchronization within the PC and IC.

2.3 Methods

Adult male Sprague-Dawley rats (250-300 g) were decapitated under halothane anaesthesia according to Canadian Council of Animal Care guidelines. The brains were quickly removed and placed in cold (1-3 °C), oxygenated artificial cerebrospinal fluid (ACSF). Horizontal slices (450 μm thick) were cut ventrally with a vibratome along a postero-superior/antero-inferior plane tilted by a 10 ° angle and were transferred to a tissue chamber where they lay in an ACSF-humidified gas (95% O_2/5% CO_2) interface at 34-35 °C. As shown in Fig. 1A, slices at approx. -7 mm from the bregma (Paxinos and Watson, 2005) included hippocampus, the PC and the IC. ACSF composition (pH 7.4) was (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10. 4AP (50 μM), 3,3-(2-carboxypiperazine-4-yl)propyl-1-phosphonate (CPP, 10 μM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), picrotoxin (PTX, 50 μM), [D-Ala^2,N-Me-Phe^4-,Gly-ol^5]enkephalin (DAGO, 10 μM) and

naloxone (20 μM) were bath perfused. Chemicals were acquired from Sigma-Aldrich Canada (Oakville, Ontario, Canada) and Tocris Cookson (Ellisville, MO, USA). PC-IC separation cuts (see Fig. 2) were performed in some experiments using a microknife while slices were in the tissue chamber. Once cut, tissue was gently separated by a minimum distance of 100 μm.

Field potential recordings were made with ACSF-filled glass microelectrodes (resistance 2–10 MΩ) connected to a Cyberamp 380 amplifier (Molecular Devices, Sunnyvale, CA, USA). Signals were fed to a computer interface (Digidata 1322A, Molecular Devices) and digitized using the pCLAMP 8.0 software (Molecular Devices). Microelectrodes were fixed in place throughout each experiment within CA3, PC and IC at the sites indicated in Fig. 1A. The posterior and the anterior rhinal fissures were used as guides for identifying the PC and the IC, respectively (see Fig. 1A). Slice stimulation was performed with a concentric bipolar electrode (FHC, Bowdoinham, ME, USA); stimulus intensity was 100 μA and pulse width 50 μs.

Data analysis was conducted using the Clampfit 9.2 software (Molecular Devices). Traces were processed offline with an electrical filter with a 60 Hz reference frequency, followed by lowpass filtering set at a 100 Hz cutoff frequency. Interictal events were arbitrarily defined as discharges shorter than 3 s in duration, while ictal events were defined as discharges exceeding 3 s in

duration. The site of initiation for 4AP-induced synchronous activity was determined by measuring latency times starting from the first baseline deflection between traces recorded from each brain region. Measurements throughout this study are represented as mean±SEM, where the mean represents an averaged value of all means from all slices; the *n* value represents the number of slices analyzed and the number of means included in the measurement. Data sets for pharmacological manipulations were compared with the Student's *t* test; means were considered significantly different if $p < 0.05$.

2.4 Results

2.4.1 4AP-Induced Epileptiform Activity in Connected Hippocampus-PC-IC Slices

Bath application of 4AP (50 μM) induced within 45-60 min, two distinct patterns of epileptiform activity. In slices illustrating the first case (n= 15), fast interictal discharges (duration= 126.7±10.1 ms, interval of occurrence= 928.9 ± 39.2 ms; n=15 slices) (arrows in Fig. 1B) interjected with less frequent, longer-duration field potentials (asterisk in Fig. 1B) occurred in CA3. In these experiments we also recorded interictal discharges (duration= 1.0±0.1 s, interval of occurrence= 14.3±2.7 s, n=15 slices) along with prolonged, synchronous ictal

events (duration= 38.8±7.8 s, interval of occurrence= 263.5±45.7 s, n=15 slices) that propagated among the PC and IC (Fig. 1B). In slices characteristic of the second pattern of 4AP-induced epileptiform activity (n= 9), CA3 remained as described above, exhibiting fast interictal discharges (duration= 186.8±18.7, interval of occurrence= 787.0±63.0; n=9 slices) intermixed with slow field potentials. However, the PC and the IC responded to 4AP application with interictal discharges only (duration= 971.6±283.4 ms, interval of occurrence= 4.7±0.9 s, n=9 slices), i.e., no ictal discharges occurred (Fig. 2A-C, left panels).

Latency times of propagation for synchronous interictal discharges were measured in slices exhibiting such activity, and it was revealed that the majority of these initiated in PC. The time histogram in Fig. 1C, which shows the distribution of the latency of 406 synchronous interictal events from 24 slices, indicates that 69.7% of them initiated in PC. The mean latency time of spread of interictal discharges from PC to IC and from IC to PC were 34.9±1.1 ms (283 events, n= 18 slices) and 33.3±1.6 ms (123 events, n= 10 slices), respectively. Ictal discharges also initiated more frequently in the PC and propagated to the IC with a mean latency time of 31.1±1.6 ms (49 events, n= 13 slices) while the opposite was true in 6 experiments with a mean latency time of spread from IC to the PC of 35.6±4.2 ms (29 events, n= 6 slices). The time histogram in Fig. 1D illustrates the distribution of the latency obtained from 78 events and indicates

that 62.8% of these ictal discharges initiated in PC. In some experiments, ictal and interictal discharges entered the hippocampus from the PC (PC to CA3 latency time= 97.8±6.6 ms; 90 events, n= 5 slices), which caused a readily visible and transient decrease in the interval between interictal events in CA3 (Fig. 2B, asterisks).

We also found that ictal discharges could be induced in slices exhibiting interictal activity only, by performing a complete transection separating the PC from IC (see top insets in Fig. 2 for schematic). Following such a cut, ictal discharges occurred (i) asynchronously in both structures (Fig. 2A right panel; n=4 slices), (ii) rarely in the PC only (Fig. 2B right panel; n=2 slices), (iii) and most often in the IC only (in 2 cases with an absence of interictal discharges, Fig. 2C right panel; n=7 slices). Closer examination of the epileptiform activity recorded from intact slices revealed that the site of ictal discharge occurrence following slice transection was related to the site of initiation of interictal discharges prior to the PC/IC cut. In slices that later developed ictal discharges in both PC and IC, there was no preferential site of interictal initiation under control conditions since 57.5% of interictal discharges propagated from the PC to the IC (out of 20 samples taken from each of 4 slices; Fig. 2D). In slices that later developed ictal discharges in the PC only following transection, only 7.5% of interictal discharges propagated from the PC to IC in control (out of 20 samples

taken from each of 2 slices; Fig. 2D). Finally, in slices that disclosed ictal discharges in the IC only following PC/IC cuts, 90.0% of interictal discharges in controls propagated in the PC to IC direction (out of 20 samples taken from each of 7 slices; Fig. 2D), demonstrating a predominant site of initiation for interictal events in the PC. Upon stimulation of isolated IC slices, we discovered that ictal discharges were abolished at stimulation frequencies of 0.5 Hz and 1 Hz, but not at 0.2 Hz (n=3 slices; Fig. 2E). These results thus imply that interictal input activities from the PC to the IC at such similar frequencies prevented the development of ictal discharges prior to slice transection.

2.4.2 Participation of NMDA and $GABA_A$ Receptors in Network Synchronization

Ictal discharges were abolished upon application of the NMDA receptor antagonist CPP (10 μM, n= 5; Fig. 3, +CPP). While ictal discharges ceased, synchronized interictal activity continued to propagate among the PC and the IC at a slightly increased but non-statistically significant interval of occurrence; discharges in the hippocampus remained unchanged. Further application of the $GABA_A$ receptor antagonist PTX (50 μM) synchronized all three structures (Fig. 3, +PTX); this recurrent interictal activity was driven by CA3 and propagated through the PC to the IC (latency time from CA3 to the PC= 167.8 ± 7.8 ms, n=5 slices).

2.4.3 Synchronous GABAergic Events are Modulated by μ-Opioid Receptors

Ictal and interictal activity ceased upon simultaneous addition of NMDA- and non-NMDA glutamatergic receptor antagonists (CPP and CNQX, both 10 μM) to ACSF containing 4AP. Instead relatively infrequent field potentials occurred in CA3 (duration= 1.4±0.1 s; interval of occurrence= 24.3±2.8 s; n=10 slices) independently from similar field events that appeared often synchronously in PC (duration= 680.3±94.7 ms, interval of occurrence= 42.4±8.0 s, n=10 slices) and IC (duration= 1.0±0.1 s; interval of occurrence= 40.1±7.7 s; n=10 slices) (Fig. 4A, +CPP+CNQX). There was no preferential site of initiation among the PC and IC for these glutamatergic-independent field events as revealed by measurements of propagation latency times for slices disclosing synchronous potentials (236.1 ± 13.5 ms from the PC, n=8 slices; and 192.8 ± 16.3 ms from the IC, n=8 slices) (Fig. 4A, histogram and Fig. 4B insets). Similar discharges (duration= 1.3±0.4 s, interval of occurrence= 48.4±15.3 s; n=4 slices) also occurred in isolated IC slices upon application of 4AP, CPP and CNQX (data not shown).

These potentials were confirmed to be mainly GABA receptor-mediated in the PC and the IC since they were fully abolished upon further application of the $GABA_A$ receptor antagonist PTX (50 μM, n=3 slices) (Fig. 4B). Moreover, addition of the μ-opioid agonist DAGO (10 μM, n= 8 slices) to ACSF containing 4AP, CPP

and CNQX, significantly reduced the occurrence of these presumptive GABA receptor-mediated events in all three structures (Fig. 5A). This effect was reversed by further application of the μ-opioid receptor antagonist naloxone (20 μM; Fig. 5A). The normalized changes in the interval of occurrence of these events analyzed in CA3, PC and IC during DAGO and after naloxone application are summarized in Fig. 5B. A statistically significant increase in field potential amplitude during DAGO also occurred in the IC (from 0.75±0.15 mV to 1.2±0.2 mV, $p<0.01$), but not in the PC (from 0.74±0.15 mV to 0.58±0.13 mV, $p>0.1$) or CA3 (from 1.0±0.13 mV to 1.1±0.17 mV, $p>0.5$; all n=8 slices).

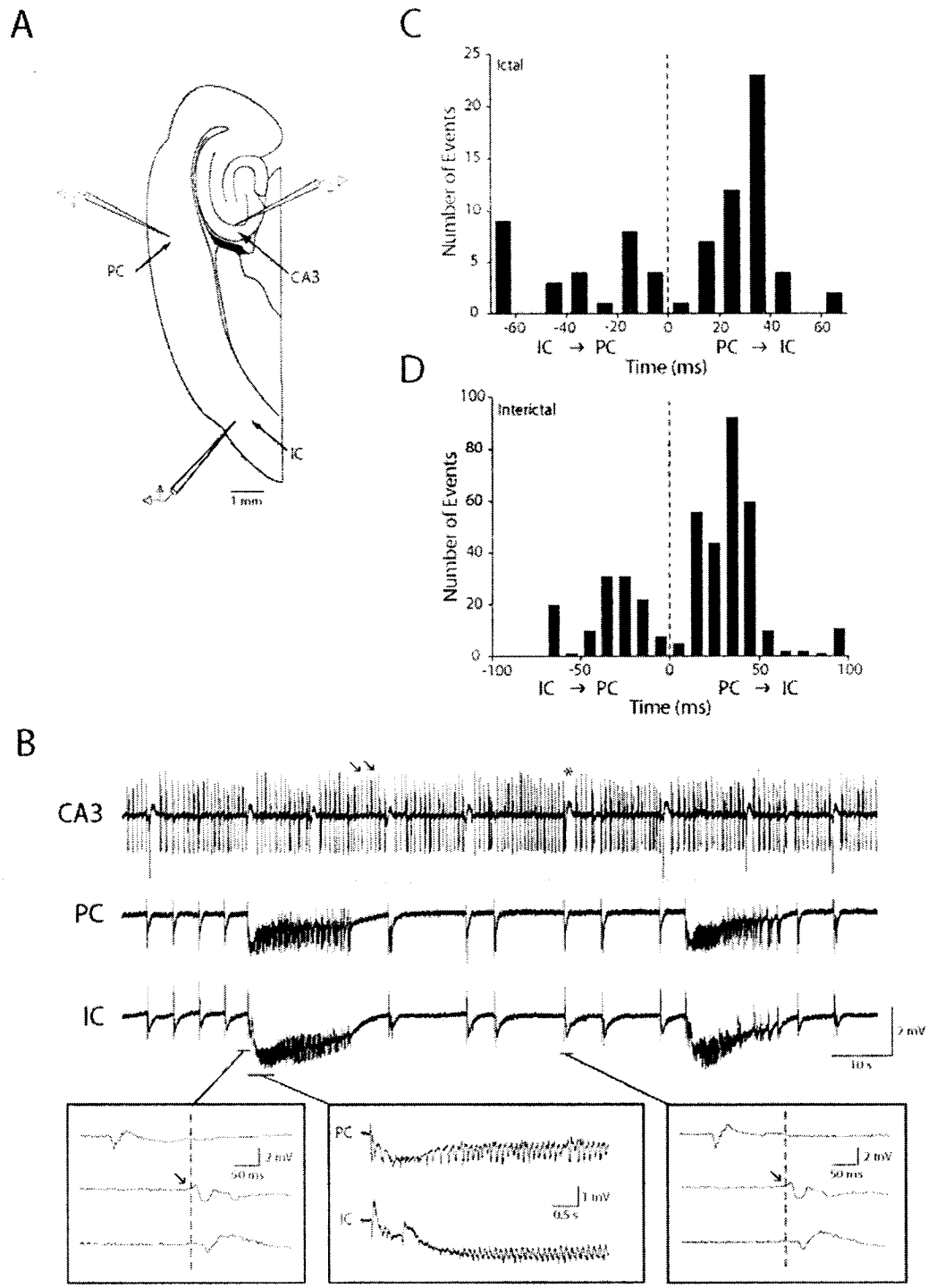
A
PC
CA3
IC
1 mm
C
Ictal
Number of Events
25
20
15
10
5
0
-60
-40
-20
0
20
40
60
IC → PC
PC → IC
Time (ms)
D
Interictal
Number of Events
100
80
60
40
20
0
-100
-50
0
50
100
IC → PC
PC → IC
Time (ms)
B
CA3
PC
IC
2 mV
10 s
2 mV
50 ms
PC
1 mV
0.5 s
IC
2 mV
50 ms

Figure 1 - (A) Schematic of a hippocampus-PC-IC combined slice and positioning of field potential recording electrodes. (B) 4AP application discloses fast (arrows) and slow (asterisk) interictal discharges in CA3, along with synchronous ictal and interictal activity in PC and IC. Insets show expansions of representative interictal and ictal discharges; note that both ictal and interictal events initiate in the PC. In this and following figures the initiation site of a discharge is highlighted by dashed lines and arrows. (C) Distribution histogram of latency times for interictal discharges that initiated in IC and propagated to PC (IC→PC), or that initiated in PC and propagated to IC (PC→IC); data were obtained from 24 slices. (D) Distribution histogram of latency times for ictal discharges in 19 slices that initiated in IC and propagated to PC (IC→PC) or initiated in PC and propagated to IC (PC→IC). Note that the majority of interictal and ictal events initiate in PC.

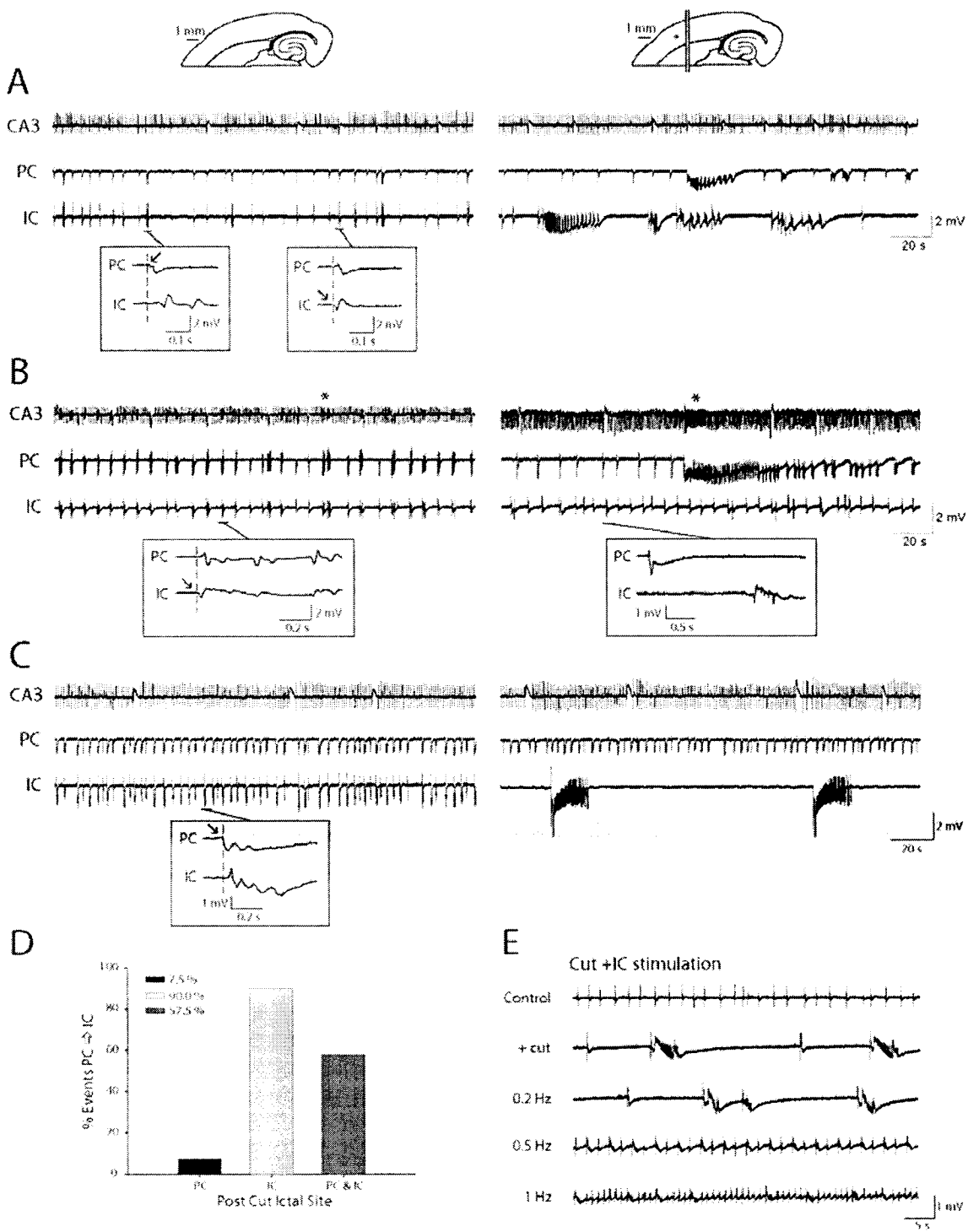
A
1 mm
1 mm
CA3
PC
IC
2 mV
20 s
PC
IC
2 mV
0.1 s
B
CA3
PC
IC
2 mV
20 s
1 mV
0.5 s
0.2 s
C
CA3
PC
IC
2 mV
20 s
1 mV
0.2 s
D
% Events PC → IC
Post Cut Ictal Site
PC
IC
PC & IC
E
Cut +IC stimulation
Control
+ cut
0.2 Hz
0.5 Hz
1 Hz
1 mV
5 s

Figure 2 - (A-C) Ictal discharges can be induced *de novo* in 4AP-treated slices by cutting the connections between PC and IC. Schematics of brain slices before (left) and after (right) transection and separation at the location indicated by the lines, are shown on top. Insets represent expansions of synchronous interictal discharges recorded from PC and IC before or after transection. (A) 31% of slices exhibited interictal discharges initiating in either PC or IC; upon transection asynchronous ictal discharges were disclosed in both structures (n=4). (B) 15% of slices exhibited synchronous interictals that initiated most often in IC; upon transection ictal discharges were disclosed in the PC only (n=2); note also that in this experiment PC interictal and ictal discharges influence the CA3 activity (asterisks). (C) 54% of slices exhibited interictal events initiating more frequently in PC; upon transection ictal discharges were disclosed in the IC only (n=7). (D) Proportion of interictal events (20 samples counted per slice) that propagated from the PC to the IC under control conditions in slices that developed ictal discharges in the PC (n=2) or the IC only (n=7), or in both areas (n=4) upon transection. (E) Stimulation with a bipolar electrode at the site indicated by the asterisk in the slice schematic on the right, abolished ictal discharges in isolated IC slices at stimulation frequencies of 0.5 and 1 Hz.

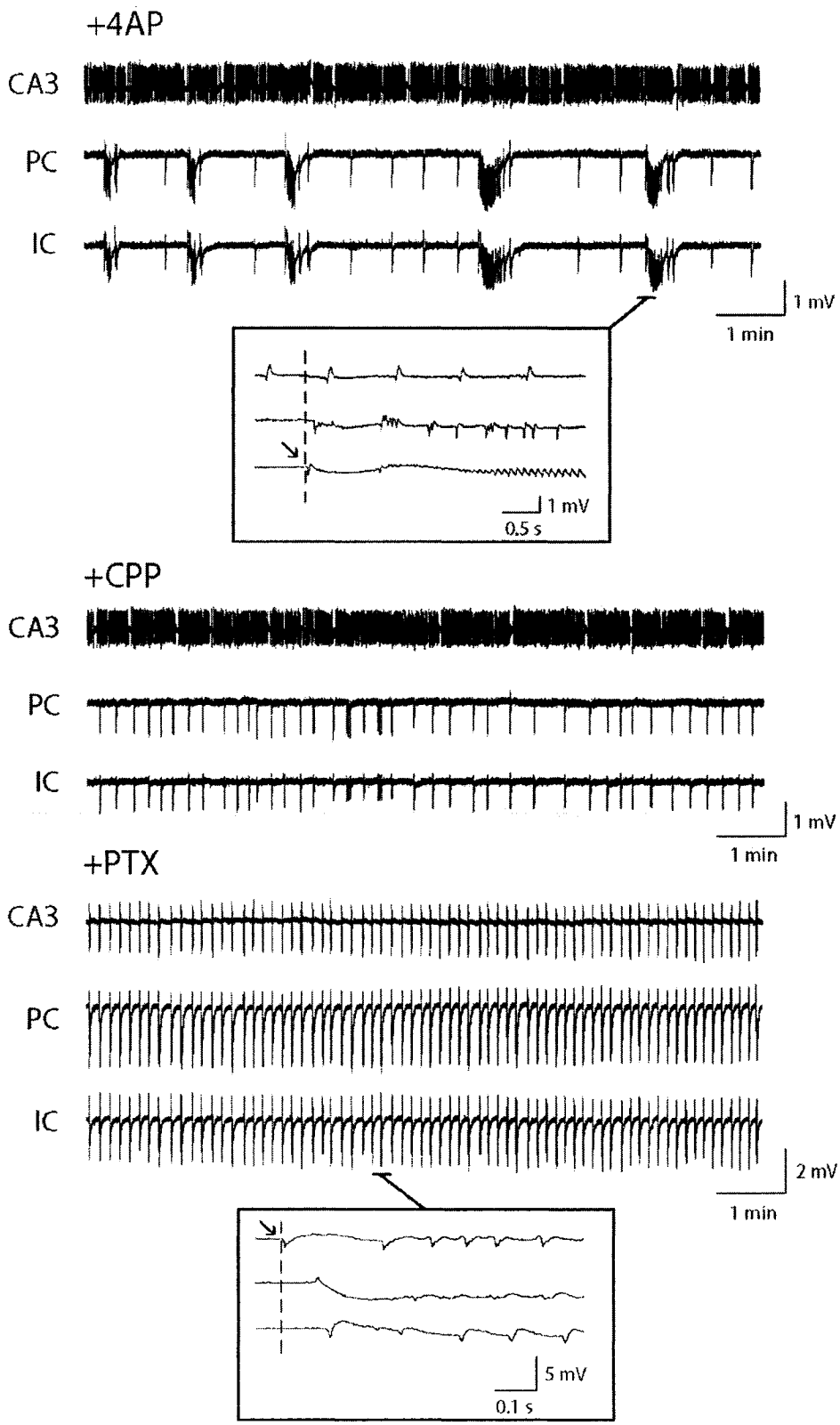
+4AP
CA3
PC
IC
1 mV
1 min
1 mV
0.5 s
+CPP
CA3
PC
IC
1 mV
1 min
+PTX
CA3
PC
IC
2 mV
1 min
5 mV
0.1 s

Figure 3 - 4AP-induced ictal discharges are abolished upon application of the NMDA receptor antagonist CPP while further addition of the $GABA_A$ receptor antagonist PTX synchronizes the interictal activity generated within CA3, PC and IC. Insets represent expansions of an ictal discharge that initiated under control conditions in IC without effect on hippocampal interictal activity, and of an interictal discharge recorded during PTX application showing initiation in CA3.

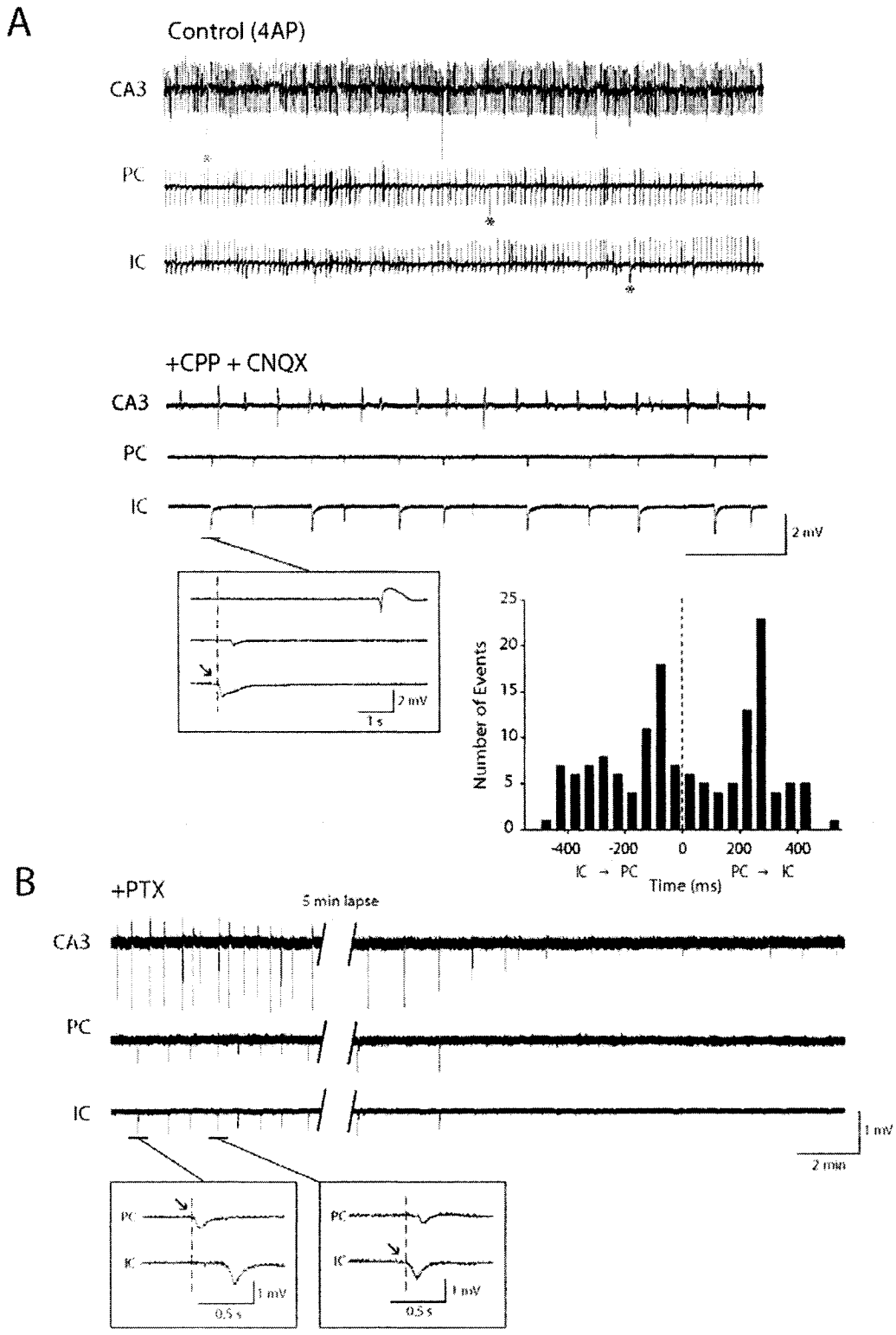
A
Control (4AP)
CA3
PC
IC
+CPP + CNQX
CA3
PC
IC
2 mV
2 mV
1 s
Number of Events
25
20
15
10
5
0
-400
-200
0
200
400
IC → PC
PC → IC
Time (ms)
B
+PTX
5 min lapse
CA3
PC
IC
1 mV
2 min
PC
IC
1 mV
0.5 s
PC
IC
1 mV
0.5 s

Figure 4 – GABA receptor-mediated potentials are disclosed upon application of NMDA and non-NMDA glutamatergic receptor antagonists in 4AP-treated slices. (A) CPP and CNQX application discloses slow potentials that occur independently in CA3 from those seen synchronously in PC and IC; inset shows an expanded example of such potential originating in IC. Graph represents latency times of potentials recorded in 10 slices that initiated in IC and propagated to PC (IC→PC) or that initiated in PC and propagated to IC (PC→IC). (B) PTX addition to ACSF containing 4AP, CPP and CNQX abolishes GABA receptor-mediated potentials in all areas. PTX application began at the onset of the trace. Trace expansions in the insets show that initiation occurred in either PC or IC.

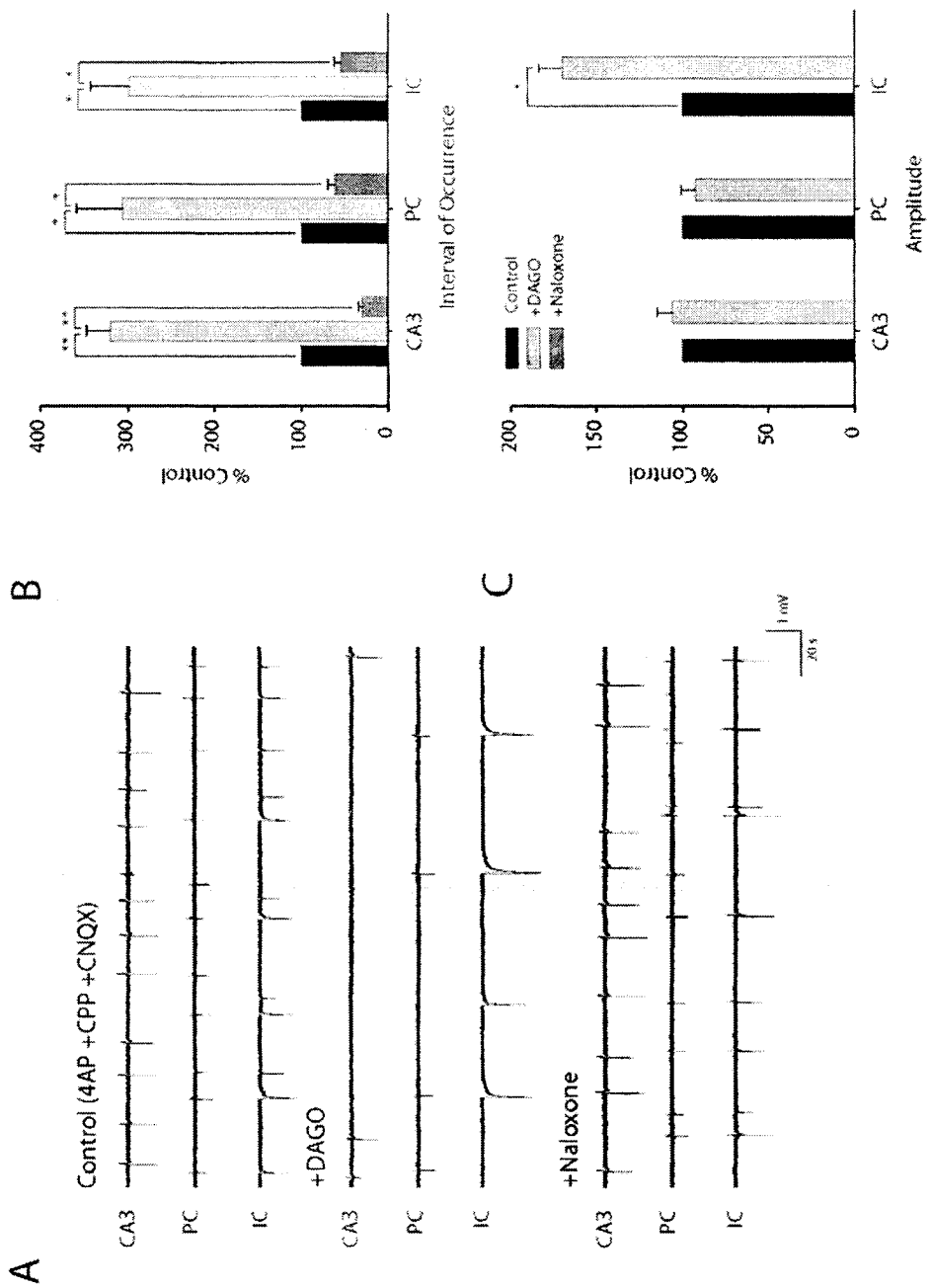
A
Control (4AP +CPP +CNQX)
CA3
PC
IC
+DAGO
CA3
PC
IC
+Naloxone
CA3
PC
IC
1 mV
50 s
B
% Control
400
300
200
100
0
CA3
PC
IC
Interval of Occurrence
C
% Control
200
150
100
50
0
Control
+DAGO
+Naloxone
CA3
PC
IC
Amplitude

Figure 5 - (A) Application of the μ-opioid receptor agonist DAGO reduces the occurrence of the GABA receptor-mediated potentials in CA3, the PC and the IC; such effect is reversed by naloxone. (B) Normalized changes in the interval of occurrence of the GABA receptor-mediated potentials upon serial application of DAGO and naloxone. (C) Normalized changes in the amplitude of the GABA receptor-mediated potentials upon serial application of DAGO and naloxone. * = $p < 0.01$, ** = $p < 0.001$.

2.5 Discussion

2.5.1 Glutamatergic Receptor-Dependent Cortical Discharges Occur Independently of Hippocampal Network Activity in Hippocampus-PC-IC Slices in Response to 4AP

We have shown that epileptiform discharges in 4AP-treated hippocampus-PC-IC slices are largely mediated by non-NMDA glutamatergic receptors; however, ictal discharges in the PC and the IC were selectively NMDA receptor-dependent. Excessive NMDA receptor activation is associated with seizure activity and neuronal death in TLE (for reviews, see Chapman, 2000; Fujikawa, 2005). Previous studies in TLE animal models have shown that injection of NMDA antagonists into the PC or the IC of rats is able to protect against the development of kindled seizures (Holmes et al., 1992; Kodama et al., 2001; Raisinghani and Faingold, 2005), and that NMDA receptors are important in mediating network hyperexcitability within the non-epileptic IC (Inaba et al., 2006). AMPA receptors also appear to be important in the propagation of kindled and convulsant-induced seizure activity in these structures in rats *in vivo* (Tortorella et al., 1997; Kodama et al., 2001). Interictal discharges require fast, AMPA-mediated neurotransmission in human epileptic tissue (Cohen et al., 2002) and when induced pharmacologically *in vitro* (de Curtis and Avanzini,

2001). In addition, previous studies with convulsant-treated slices have demonstrated similar ictal discharge selectivity for NMDA receptors in the PC and the entorhinal cortex (Dreier and Heinemann, 1991; Lopantsev and Avoli, 1998; de Guzman et al., 2004). These studies combined with our results suggest that epileptiform network activity occurs via distinct mechanisms within hippocampal and extrahippocampal cortical structures *in vitro*.

We have further demonstrated that synchronized 4AP-induced epileptiform discharges within the PC and the IC occurred independently of activity within the hippocampus. *In vitro*, the PC and the entorhinal cortex primarily initiate ictal discharges, which can subsequently invade the hippocampus through the temporoammonic or perforant pathways (Barbarosie and Avoli, 1997; Barbarosie et al., 2000; de Guzman et al., 2004). Similarly, we found here that the PC primarily, and the IC secondarily, initiated ictal discharges that could propagate to CA3 probably depending on the number of preserved afferents in the slice. This supports previous findings in acute models of TLE that the PC may be particularly excitable, since ictal discharges preferentially initiate in the PC compared to other interconnected limbic structures (McIntyre and Plant, 1993; de Guzman et al., 2004). In addition, PC kindling produces generalized motor seizures after relatively few stimulation trials, implying that it acts as a primary route for seizure propagation from limbic to neocortical

structures (McIntyre et al., 1993; Kelly and McIntyre, 1996; Sato et al., 1998; Barnes et al., 2005). Interestingly, the IC exhibits comparable kindling rates to the PC, and presents similar EEG profiles during kindled seizures (Kodama et al., 2001; Mohapel et al., 2001). Furthermore, the RAIC is a principle target for PC efferents (Delatour and Witter, 2002). Since we report epileptiform discharge synchronicity among these structures *in vitro*, the IC may form an important part of the PC seizure generalization pathway proposed based on kindling studies (McIntyre and Kelly, 2000).

2.5.2 $GABA_A$ Receptors Gate Hippocampal Outputs and Increase Local Network Synchrony in Hippocampus-PC-IC Slices

Here, 4AP-induced CA3 interictal discharges remained contained within the hippocampus proper. We have previously reported that hippocampal outputs are capable of entraining surrounding EC, amygdalar and PC networks with 4AP in slice preparations that did not include the IC (D'Antuono et al., 2002; Benini et al., 2003; de Guzman et al., 2004). Due to the inclusion of the IC, slices here were prepared at slightly more dorsal depths, which may have differently preserved connections among parahippocampal and hippocampal networks. Further, previous studies were performed with slices from mice, which may have resulted in subtle differences in network arrangements. Our results highlight the

importance of maximizing connectivity in slice preparations when addressing network phenomena, however, since important interactions may emerge upon preservation of larger networks.

A gating mechanism in the hippocampus was the most likely contributor to this network discharge independence, however. Recently, it was demonstrated that a $GABA_A$ receptor-mediated mechanism localized to the subiculum can prevent the spread of hippocampal discharges toward the EC in 4AP-treated slices (Benini and Avoli, 2005). In support of this, we found that $GABA_A$ receptor blockade during 4AP application caused hippocampal entrainment of perirhinal and insular cortical networks in synchronous discharges. Furthermore, ictal activity was not observed in the PC and the IC with $GABA_A$ receptor blockade, supporting previous proposals that epileptiform afterdischarges are dependent on GABAergic signaling (Traub and Jefferys, 1994; de Curtis and Avanzini, 2001; Avoli et al., 2002).

2.5.3 Reciprocal Cortical Inputs Control the Development of Ictal Discharges

We have shown that ablating connections between the PC and the IC in 4AP-treated slices induces ictal discharges within the neighbouring structure. While the precise role of interictal spikes in TLE remains unknown, some have suggested that they are capable of reducing excitability and increasing discharge

thresholds within the affected region (de Curtis and Avanzini, 2001; de Curtis et al., 2005; Urrestarazu et al., 2006) and thus may exert neuroprotective effects in the brain against excessive network excitation (de Curtis and Avanzini, 2001). Previous studies with connected hippocampal and limbic networks demonstrated that propagated interictal discharges can inhibit the development of ictal discharges (D'Antuono et al., 2002; Benini et al., 2003; de Guzman et al., 2004). Furthermore, direct application of current at low amplitudes and frequencies can similarly reduce excitability and suppress convulsant-induced ictal discharges in slices (Warren and Durand, 1998; D'Arcangelo et al., 2005). We have provided further evidence for this phenomenon here since i) separation of the PC and the IC induced ictal discharges, and ii) stimulation of isolated IC slices at low frequencies abolished ictal discharges. In general, the PC initiated most propagated interictal discharges in these slices, which likely accounts for the tendency of PC/IC cuts to result in ictal discharges in the IC alone. Bidirectionally propagated interictal discharges thus may also account for the complete absence of ictal discharges seen in several slices. Prominent connectivity is seen among these structures in tract tracing studies (Delatour and Witter, 2002; for reviews, see Burwell et al., 1995; Flynn et al., 1995), which we have confirmed here experimentally.

2.5.4 μ-Opioid Receptor Modulation of Synchronous Glutamate-Independent Network Activity in the PC and the IC

Here, slow $GABA_A$ receptor-dependent potentials were induced with 4AP in the PC and the IC upon blockade of NMDA and non-NMDA glutamatergic receptors. In the hippocampus and neocortex, these potentials are the result of synchronized discharges of interneuronal networks (Avoli et al., 1994; Michelson and Wong, 1994; Avoli et al., 1996; Lamsa and Kaila, 1997), and appear to play a role in the initiation of *in vitro* ictal discharges in the EC (Barbarosie et al., 2002). We found that these potentials occur within the PC and the IC synchronously without a preferential site of initiation. Similar results have been described within and among hippocampal and EC networks (Perreault and Avoli, 1992; Avoli et al., 1996); however, this is the first such demonstration of GABA-mediated synchronous events among adjacent cortical structures. The extent of this synchronicity among the PC and the IC may reflect the widespread interconnectivity and distribution of interneurons throughout and across cortical columns in bordering cortical regions (Markram et al., 2004). Overall, these results implicate a contribution of GABAergic signaling in producing network synchronicity among the IC and the PC in the absence of glutamatergic neurotransmission in this model of TLE. Normally, GABAergic interneuronal networks serve to coordinate oscillatory network activity (for reviews, see Freund

and Buzsaki, 1996; Whittington and Traub, 2003); however under pathophysiological conditions, GABAergic networks may aberrantly contribute to network hypersynchronicity during epileptic seizures (Ben-Ari and Holmes, 2005; Magloczky and Freund, 2005).

Opioids are important regulators of GABAergic neurotransmission and network excitability in the hippocampus, whereby they interact primarily through somatic and dendritic μ-opioid receptors on inhibitory interneurons (Cohen et al., 1992). Opioid binding thus effectively hyperpolarizes and silences interneurons, preventing GABA release onto pyramidal cell $GABA_A$ receptors and allowing widespread disinhibition (Drake and Milner, 2002; McQuiston and Saggau, 2003). Not surprisingly, there are several lines of evidence that implicate defects in hippocampal opioidergic signaling in various models of TLE (Rocha et al., 1994; Rocha and Maidment, 2003; Skyers et al., 2003; Sanabria et al., 2006). We have previously shown *in vitro* that μ-opioid receptor agonism abolishes isolated 4AP-induced GABA-mediated field potentials in CA3 of the hippocampus (Avoli et al., 1996; Barbarosie et al., 2002). No studies until now had investigated whether similar such mechanisms regulate network excitability in neocortex, however. Similarly to hippocampus, neocortical μ-opioid receptors localize to GABAergic interneurons (Taki et al., 2000; Ferezou et al., 2006). We found that μ-opioid receptor activation decreased the occurrence of GABA-

mediated potentials in the PC and the IC, indicating similar mechanisms of opioidergic regulation over GABAergic neurotransmission among these structures and the hippocampus.

GABA-mediated potentials were not completely abolished in the PC and the IC, however. In hippocampus, μ-opioid receptor agonists cause simultaneous somatic hyperpolarization and synaptic disinhibition of interneurons; the inhibitory effect on their output predominates in response to weak network activation, however (Madison and Nicoll, 1988). Thus, the hyperpolarizing effects of μ-opioid receptor activity in interneurons can be overridden if there is sufficiently strong synaptic excitation to result in large synchronized postsynaptic responses in the hundreds of pyramidal cells each interneuron can contact (Somogyi et al., 1983). 4AP application results in downregulation of the KCC2 co-transporter in hippocampal slices, resulting in intracellular Cl^- accumulation and hence depolarizing postsynaptic responses to GABA (Rivera et al., 2004). It is thus possible that these potentials were not completely abolished despite the presence of DAGO due to strong somatic excitation mediated by interneuron recurrent collateral release of depolarizing GABA, to lead to large synchronized interneuronal network discharges (Michelson and Wong, 1994). Increased field potential amplitudes within the IC that occurred during μ-opioid receptor activation may have reflected a greater

extent of synchronization of these interneuronal networks. While the PC has some involvement in opiate-mediated antinociception (d'Amore et al., 1991), pain studies have reported a particular abundance of μ-opioid receptors in the RAIC that control nociception thresholds *in vivo* (Delfs et al., 1994; Burkey et al., 1996; Jasmin et al., 2003). The increased abundance of μ-opioid receptors within the IC therefore may account for differences in response to DAGO between the PC and the IC, however more experimentation would be necessary to determine precisely how these phenomena relate. In general, we have implicated the existence of similar opioidergic mechanisms regulating GABA-mediated synchronicity among the PC, the IC and the hippocampus, while these pathways may be particularly influential over network synchronicity within the IC.

2.5.5 Conclusions

This study represents the first characterization of *in vitro* epileptiform network interactions among the hippocampus, the PC and the IC. We have confirmed that the PC is a particularly hyperexcitable structure within this model and slice preparation, and we have provided further evidence that interictal discharges or low frequency stimulation can decrease network excitability *in vitro*. Recent studies have shown that brain stimulators placed within an epileptogenic zone in an attempt to control temporal focal seizures can reduce the occurrence

of epileptiform discharges (Vonck et al., 2002; Yamamoto et al., 2002). However, more investigation is needed to determine where chronically implanted stimulators should be placed to obtain maximal seizure relief in patients. Our results combined with previous studies suggest that implantation in an input structure adjacent to the epileptogenic zone may provide effective control over the generation of ictal discharges; however, testing in chronically epileptic animals will no doubt be needed to fully resolve this issue. Interestingly, our results also suggest the presence of similar opioidergic mechanisms of control over network excitability among hippocampal and cortical networks. Specific localization of μ-opioid receptors combined with a direct assessment of effects on GABAergic neurotransmission at the synaptic level within the PC and the IC will more specifically resolve the mode of opioid receptor action within these structures, however. Interestingly, direct application of opioids or locally increasing GABA concentrations within the RAIC both result in long-lasting analgesia in rats (Burkey et al., 1996; Jasmin et al., 2003); however the manner by which these signaling pathways interact remains unknown. We have provided preliminary evidence that a similar mechanism to that in hippocampus occurs within the RAIC, although precisely how μ-opioid receptors modulate GABAergic neurotransmission within the RAIC *in vivo* will require a more multidisciplinary approach. Opioidergic mechanisms regulating network excitability may thus be

particularly influential over excitability within the IC given the potent opioid receptor responses that occur normally. Obtaining answers to such questions will surely have implications not only for TLE, but also for understanding mechanisms of pain regulation.

3. GENERAL CONCLUSIONS

As a result of my M.Sc. studies, I can draw the following conclusions:

1) 4AP induces highly synchronous epileptiform activity within the PC and the IC that occurs largely independently from hippocampal activity

2) PC inputs preferentially propagate toward and thereby reduce excitability within IC networks

3) Hippocampal GABAergic mechanisms gate outputs that can entrain PC and IC networks

4) μ-Opioid receptors regulate GABA-mediated network synchrony within the PC and the IC

My results provide support for studies that employed multiple model systems to show that the PC is a hyperexcitable structure in TLE. Given the similarity in responses among the PC and the IC from kindled animals, and here with the 4AP model, it will be interesting to see whether these structures are also as closely associated in chronically epileptic animals. In addition, GABAergic

mechanisms in the hippocampus are perhaps in place to prevent the spread of epileptiform activity into surrounding structures such as the PC and the IC; this issue also deserves further investigation with chronic models of TLE. Concerning the function of interictal discharges in TLE, results obtained here favour the side of the debate defending a neuroprotective role by way of ictal discharge suppression. In general, there is no consensus among both clinical and experimental studies as to why interictal discharges occur, and whether they represent a pathogenic or homeostatic reactive process. Resolving this debate may prove to be useful, since the development of brain stimulators to control network excitability would provide an exciting alternative for intractable TLE patients that otherwise must undergo invasive and risk-laden neurosurgery to reach seizure freedom. Finally, I have provided preliminary evidence that opioid receptors are important regulators of GABAergic synchronicity within the PC and the IC. These results may have interesting ramifications, since this study bridges concepts developed from research in epilepsy, and from research on pain mechanisms.

Clearly, the relevance of results obtained with any model of TLE in isolation will only become apparent when confirmed in multiple systems, since none captures every facet of the human affliction in one single animal or preparation. Existing models draw on particular strengths and have their own

individual weaknesses. Practical constraints inherent within each model unfortunately leave true multifactorial study as the rare but ideal approach. More importantly, combining existing models in unique and innovative ways may prove to be the best use of what we currently know, while we seek to more accurately model this infinitely complex syndrome. My hope is that I will have provided one more perspective on the mechanisms underlying epileptiform synchronization to aid in addressing the problem of TLE as a whole.

REFERENCES

Aleksandrov VG, Fedorova KP (2003) Structure of the insular region of the rat neocortex. Neurosci Behav Physiol 33:199-202.

Anderson WW, Lewis DV, Swartzwelder HS, Wilson WA (1986) Magnesium-free medium activates seizure-like events in the rat hippocampal slice. Brain Res 398:215-219.

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

Augustine JR (1996) Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Brain Res Rev 22:229-244.

Avoli M (1991) Excitatory amino acid receptors in the human epileptogenic neocortex. Epilepsy Res 10:33-40.

Avoli M (1996) GABA-mediated synchronous potentials and seizure generation. Epilepsia 37:1035-1042.

Avoli M, Biagini G, de Curtis M (2006) Do interictal spikes sustain seizures and epileptogenesis? Epilepsy Curr 6:203-207.

Avoli M, Louvel J, Pumain R, Kohling R (2005) Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol 77:166-200.

Avoli M, Mattia D, Siniscalchi A, Perreault P, Tomaiuolo F (1994) Pharmacology and electrophysiology of a synchronous GABA-mediated potential in the human neocortex. Neuroscience 62:655-666.

Avoli M, Barbarosie M, Lucke A, Nagao T, Lopantsev V, Kohling R (1996) Synchronous GABA-mediated potentials and epileptiform discharges in the rat limbic system in vitro. J Neurosci 16:3912-3924.

Avoli M, D'Antuono M, Louvel J, Kohling R, Biagini G, Pumain R, D'Arcangelo G, Tancredi V (2002) Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. Prog Neurobiol 68:167-207.

Barbarosie M, Avoli M (1997) CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. J Neurosci 17:9308-9314.

Barbarosie M, Louvel J, Kurcewicz I, Avoli M (2000) CA3-Released entorhinal seizures disclose dentate gyrus epileptogenicity and unmask a temporoammonic pathway. J Neurophysiol 83:1115-1124.

Barbarosie M, Louvel J, D'Antuono M, Kurcewicz I, Avoli M (2002) Masking synchronous GABA-mediated potentials controls limbic seizures. Epilepsia 43:1469-1479.

Barbarosie MT, Nagao T, Avoli M (1994) Control of 4-aminopyridine-induced synchronous activity by adenosine A1 and mu-opioid receptor agonists in adult rat hippocampus. Neurosci Lett 182:208-212.

Barnes SJ, Hua JM, Pinel JP, Takahashi A, Wig GS (2005) Conditioned effects of kindling three different sites in the hippocampal complex of the rat. Behav Neurosci 119:1572-1579.

Bauer G, Gotwald T, Dobesberger J, Embacher N, Felber S, Bauer R, Seppi K, Walser G, Trinka E, Poewe W (2006) Transient and permanent magnetic resonance imaging abnormalities after complex partial status epilepticus. Epilepsy Behav 8:666-671.

Bautista RE, Cobbs MA, Spencer DD, Spencer SS (1999) Prediction of surgical outcome by interictal epileptiform abnormalities during intracranial EEG monitoring in patients with extrahippocampal seizures. Epilepsia 40:880-890.

Ben-Ari Y (1985) Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 14:375-403.

Ben-Ari Y, Holmes GL (2005) The multiple facets of gamma-aminobutyric acid dysfunction in epilepsy. Curr Opin Neurol 18:141-145.

Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. J Physiol 416:303-325.

Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O (1997) Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc Natl Acad Sci U S A 94:10432-10437.

Benini R, Avoli M (2005) Rat subicular networks gate hippocampal output activity in an in vitro model of limbic seizures. J Physiol 566:885-900.

Benini R, D'Antuono M, Pralong E, Avoli M (2003) Involvement of amygdala networks in epileptiform synchronization in vitro. Neuroscience 120:75-84.

Bernard C, Cossart R, Hirsch JC, Esclapez M, Ben-Ari Y (2000) What is GABAergic inhibition? How is it modified in epilepsy? Epilepsia 41 Suppl 6:S90-95.

Bernasconi N, Bernasconi A, Andermann F, Dubeau F, Feindel W, Reutens DC (1999) Entorhinal cortex in temporal lobe epilepsy: a quantitative MRI study. Neurology 52:1870-1876.

Bernasconi N, Bernasconi A, Caramanos Z, Andermann F, Dubeau F, Arnold DL (2000) Morphometric MRI analysis of the parahippocampal region in temporal lobe epilepsy. Ann N Y Acad Sci 911:495-500.

Bernasconi N, Bernasconi A, Caramanos Z, Antel SB, Andermann F, Arnold DL (2003) Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. Brain 126:462-469.

Bettler B, Kaupmann K, Mosbacher J, Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev 84:835-867.

Blumcke I (2002) Ammon's horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. Brain Pathol 12:199-211.

Blumcke I, Beck H, Lie AA, Wiestler OD (1999) Molecular neuropathology of human mesial temporal lobe epilepsy. Epilepsy Res 36:205-223.

Bouilleret V, Dupont S, Spelle L, Baulac M, Samson Y, Semah F (2002) Insular cortex involvement in mesiotemporal lobe epilepsy: a positron emission tomography study. Ann Neurol 51:202-208.

Buckle PJ, Haas HL (1982) Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. J Physiol 326:109-122.

Buckley MJ (2005) The role of the perirhinal cortex and hippocampus in learning, memory, and perception. Q J Exp Psychol B 58:246-268.

Burkey AR, Carstens E, Jasmin L (1999) Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception. J Neurosci 19:4169-4179.

Burkey AR, Carstens E, Wenniger JJ, Tang J, Jasmin L (1996) An opioidergic cortical antinociception triggering site in the agranular insular cortex of the rat that contributes to morphine antinociception. J Neurosci 16:6612-6623.

Burwell RD, Witter MP (2002) Perirhinal structure and connectivity. In: The Parahippocampal Region (Witter MP, Wouterlood F, eds): Oxford Press.

Burwell RD, Witter MP, Amaral DG (1995) Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. Hippocampus 5:390-408.

Buzsaki G, Horvath Z, Urioste R, Hetke J, Wise K (1992) High-frequency network oscillation in the hippocampus. Science 256:1025-1027.

Capogna M, Gahwiler BH, Thompson SM (1993) Mechanism of mu-opioid receptor-mediated presynaptic inhibition in the rat hippocampus in vitro. J Physiol 470:539-558.

Cendes F, Li LM, Watson C, Andermann F, Dubeau F, Arnold DL (2000) Is ictal recording mandatory in temporal lobe epilepsy? Not when the interictal electroencephalogram and hippocampal atrophy coincide. Arch Neurol 57:497-500.

Cendes F, Dubeau F, Andermann F, Quesney LF, Gambardella A, Jones-Gotman M, Bizzi J, Olivier A, Gotman J, Arnold DL (1996) Significance of mesial temporal atrophy in relation to intracranial ictal and interictal stereo EEG abnormalities. Brain 119 (Pt 4):1317-1326.

Cendes F, Andermann F, Gloor P, Evans A, Jones-Gotman M, Watson C, Melanson D, Olivier A, Peters T, Lopes-Cendes I, et al. (1993) MRI

volumetric measurement of amygdala and hippocampus in temporal lobe epilepsy. Neurology 43:719-725.

Chapman AG (2000) Glutamate and epilepsy. J Nutr 130:1043S-1045S.

Chen S, Kobayashi M, Honda Y, Kakuta S, Sato F, Kishi K (2006) Preferential neuron loss in the rat piriform cortex following pilocarpine-induced status epilepticus. Epilepsy Res.

Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. Nature 378:75-78.

Cohen-Gadol AA, Wilhelmi BG, Collignon F, White JB, Britton JW, Cambier DM, Christianson TJ, Marsh WR, Meyer FB, Cascino GD (2006) Long-term outcome of epilepsy surgery among 399 patients with nonlesional seizure foci including mesial temporal lobe sclerosis. J Neurosurg 104:513-524.

Cohen GA, Doze VA, Madison DV (1992) Opioid inhibition of GABA release from presynaptic terminals of rat hippocampal interneurons. Neuron 9:325-335.

Cohen I, Navarro V, Le Duigou C, Miles R (2003) Mesial temporal lobe epilepsy: a pathological replay of developmental mechanisms? Biol Cell 95:329-333.

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R (2002) On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 298:1418-1421.

Cossart R, Bernard C, Ben-Ari Y (2005) Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. Trends Neurosci 28:108-115.

Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G (1999) Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. J Neurosci 19:274-287.

d'Amore A, Lorenzini P, Massotti M (1991) Antinociceptive action of opiates and opioid peptides after unilateral microinjection into area tempestas in rats. J Pharmacol Exp Ther 259:1308-1315.

D'Antuono M, Benini R, Biagini G, D'Arcangelo G, Barbarosie M, Tancredi V, Avoli M (2002) Limbic network interactions leading to hyperexcitability in a model of temporal lobe epilepsy. J Neurophysiol 87:634-639.

D'Arcangelo G, Panuccio G, Tancredi V, Avoli M (2005) Repetitive low-frequency stimulation reduces epileptiform synchronization in limbic neuronal networks. Neurobiol Dis 19:119-128.

de Curtis M, Avanzini G (2001) Interictal spikes in focal epileptogenesis. Prog Neurobiol 63:541-567.

de Curtis M, Pare D (2004) The rhinal cortices: a wall of inhibition between the neocortex and the hippocampus. Prog Neurobiol 74:101-110.

de Curtis M, Radici C, Forti M (1999) Cellular mechanisms underlying spontaneous interictal spikes in an acute model of focal cortical epileptogenesis. Neuroscience 88:107-117.

de Curtis M, Librizzi L, Biella G (2001) Discharge threshold is enhanced for several seconds after a single interictal spike in a model of focal epileptogenesis. Eur J Neurosci 14:174-178.

de Curtis M, Tassi L, Lo Russo G, Mai R, Cossu M, Francione S (2005) Increased discharge threshold after an interictal spike in human focal epilepsy. Eur J Neurosci 22:2971-2976.

de Guzman P, D'Antuono M, Avoli M (2004) Initiation of electrographic seizures by neuronal networks in entorhinal and perirhinal cortices in vitro. Neuroscience 123:875-886.

Delatour B, Witter MP (2002) Projections from the parahippocampal region to the prefrontal cortex in the rat: evidence of multiple pathways. Eur J Neurosci 15:1400-1407.

Delfs JM, Kong H, Mestek A, Chen Y, Yu L, Reisine T, Chesselet MF (1994) Expression of mu opioid receptor mRNA in rat brain: an in situ hybridization study at the single cell level. J Comp Neurol 345:46-68.

Dingledine R, McBain CJ, McNamara JO (1990) Excitatory amino acid receptors in epilepsy. Trends Pharmacol Sci 11:334-338.

Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7-61.

Drake CT, Milner TA (2002) Mu opioid receptors are in discrete hippocampal interneuron subpopulations. Hippocampus 12:119-136.

Dreier JP, Heinemann U (1991) Regional and time dependent variations of low Mg2+ induced epileptiform activity in rat temporal cortex slices. Exp Brain Res 87:581-596.

Dzhala VI, Staley KJ (2003) Transition from interictal to ictal activity in limbic networks in vitro. J Neurosci 23:7873-7880.

Engel J, Jr. (1996) Introduction to temporal lobe epilepsy. Epilepsy Res 26:141-150.

Engel J, Jr. (2001) Intractable epilepsy: definition and neurobiology. Epilepsia 42 Suppl 6:3.

Engel J, Jr., Wiebe S, French J, Sperling M, Williamson P, Spencer D, Gumnit R, Zahn C, Westbrook E, Enos B (2003) Practice parameter: temporal lobe and localized neocortical resections for epilepsy. Epilepsia 44:741-751.

Ferezou I, Hill EL, Cauli B, Gibelin N, Kaneko T, Rossier J, Lambolez B (2006) Extensive Overlap of {micro}-Opioid and Nicotinic Sensitivity in Cortical Interneurons. Cereb Cortex.

Ferland RJ, Nierenberg J, Applegate CD (1998) A role for the bilateral involvement of perirhinal cortex in generalized kindled seizure expression. Exp Neurol 151:124-137.

Fish DR, Spencer SS (1995) Clinical correlations: MRI and EEG. Mag Res Im 13:1113-1117.

Fisher RS, Alger BE (1984) Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. J Neurosci 4:1312-1323.

Flynn DD, Ferrari-DiLeo G, Levey AI, Mash DC (1995) Differential alterations in muscarinic receptor subtypes in Alzheimer's disease: implications for cholinergic-based therapies. Life Sci 56:869-876.

French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD (1993) Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol 34:774-780.

Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6:347-470.

Fujikawa DG (2005) Prolonged seizures and cellular injury: understanding the connection. Epilepsy Behav 7 Suppl 3:S3-11.

Gotman J (1991) Relationships between interictal spiking and seizures: human and experimental evidence. Can J Neurol Sci 18:573-576.

Gotman J, Marciani MG (1985) Electroencephalographic spiking activity, drug levels, and seizure occurrence in epileptic patients. Ann Neurol 17:597-603.

Haas KZ, Sperber EF, Opanashuk LA, Stanton PK, Moshe SL (2001) Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. Hippocampus 11:615-625.

Hablitz JJ (1984) Picrotoxin-induced epileptiform activity in hippocampus: role of endogenous versus synaptic factors. J Neurophysiol 51:1011-1027.

Hermann B, Seidenberg M, Bell B, Rutecki P, Sheth R, Ruggles K, Wendt G, O'Leary D, Magnotta V (2002) The neurodevelopmental impact of childhood-onset temporal lobe epilepsy on brain structure and function. Epilepsia 43:1062-1071.

Hille B (1992) Ionic channels of excitable membranes, 2nd Edition. Sunderland, Mass.: Sinauer Associates.

Holmes GL, Ben-Ari Y (1998) Seizures in the developing brain: perhaps not so benign after all. Neuron 21:1231-1234.

Holmes KH, Bilkey DK, Laverty R (1992) The infusion of an NMDA antagonist into perirhinal cortex suppresses amygdala-kindled seizures. Brain Res 587:285-290.

Houser CR (1999) Neuronal loss and synaptic reorganization in temporal lobe epilepsy. In: Jasper's Basic Mechanisms of the Epilepsies, Third Edition:

Advances in Neurology (Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ, eds). Philadelphia: Lippincott Williams & Wilkins.

ILAE (1989) Proposal for Revised Classification of Epilepsies and Epileptic Syndromes. Epilepsia 30:389-399.

Inaba Y, de Guzman P, Avoli M (2006) NMDA receptor-mediated transmission contributes to network 'hyperexcitability' in the rat insular cortex. Eur J Neurosci 23:1071-1076.

Isnard J, Guenot M, Sindou M, Mauguiere F (2004) Clinical manifestations of insular lobe seizures: a stereo-electroencephalographic study. Epilepsia 45:1079-1090.

Isnard J, Guenot M, Ostrowsky K, Sindou M, Mauguiere F (2000) The role of the insular cortex in temporal lobe epilepsy. Ann Neurol 48:614-623.

Ives AE, Jefferys JG (1990) Synchronization of epileptiform bursts induced by 4-aminopyridine in the in vitro hippocampal slice preparation. Neurosci Lett 112:239-245.

Jasmin L, Burkey AR, Granato A, Ohara PT (2004) Rostral agranular insular cortex and pain areas of the central nervous system: a tract-tracing study in the rat. J Comp Neurol 468:425-440.

Jasmin L, Rabkin SD, Granato A, Boudah A, Ohara PT (2003) Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. Nature 424:316-320.

Jones RS, Lambert JD (1990) Synchronous discharges in the rat entorhinal cortex in vitro: site of initiation and the role of excitatory amino acid receptors. Neuroscience 34:657-670.

Jutila L, Ylinen A, Partanen K, Alafuzoff I, Mervaala E, Partanen J, Vapalahti M, Vainio P, Pitkanen A (2001) MR volumetry of the entorhinal, perirhinal, and temporopolar cortices in drug-refractory temporal lobe epilepsy. AJNR Am J Neuroradiol 22:1490-1501.

Kaila K (1994) Ionic basis of GABAA receptor channel function in the nervous system. Prog Neurobiol 42:489-537.

Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. Proc Natl Acad Sci U S A 101:3967-3972.

Katz A, Marks DA, McCarthy G, Spencer SS (1991) Does interictal spiking change prior to seizures? Electroencephalogr Clin Neurophysiol 79:153-156.

Kelly ME, McIntyre DC (1996) Perirhinal cortex involvement in limbic kindled seizures. Epilepsy Res 26:233-243.

Kodama M, Yamada N, Sato K, Sato T, Morimoto K, Kuroda S (2001) The insular but not the perirhinal cortex is involved in the expression of fully-kindled amygdaloid seizures in rats. Epilepsy Res 46:169-178.

Kohling R, Lucke A, Straub H, Speckmann EJ, Tuxhorn I, Wolf P, Pannek H, Oppel F (1998) Spontaneous sharp waves in human neocortical slices excised from epileptic patients. Brain 121 (Pt 6):1073-1087.

Krnjevic K (2004) How does a little acronym become a big transmitter? Biochem Pharmacol 68:1549-1555.

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

Lamsa K, Taira T (2003) Use-dependent shift from inhibitory to excitatory GABAA receptor action in SP-O interneurons in the rat hippocampal CA3 area. J Neurophysiol 90:1983-1995.

Lange HH, Lieb JP, Engel J, Jr., Crandall PH (1983) Temporo-spatial patterns of pre-ictal spike activity in human temporal lobe epilepsy. Electroencephalogr Clin Neurophysiol 56:543-555.

Lee JW, Andermann F, Dubeau F, Bernasconi A, MacDonald D, Evans A, Reutens DC (1998) Morphometric analysis of the temporal lobe in temporal lobe epilepsy. Epilepsia 39:727-736.

Lee WL, Hablitz JJ (1989) Involvement of non-NMDA receptors in picrotoxin-induced epileptiform activity in the hippocampus. Neurosci Lett 107:129-134.

Librizzi L, de Curtis M (2003) Epileptiform ictal discharges are prevented by periodic interictal spiking in the olfactory cortex. Ann Neurol 53:382-389.

Liu G (2004) Local structural balance and functional interaction of excitatory and inhibitory synapses in hippocampal dendrites. Nat Neurosci 7:373-379.

Lopantsev V, Avoli M (1998) Participation of GABAA-mediated inhibition in ictallike discharges in the rat entorhinal cortex. J Neurophysiol 79:352-360.

Loscher W (2002) Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res 50:105-123.

Macdonald RL, Olsen RW (1994) GABAA receptor channels. Annu Rev Neurosci 17:569-602.

Madison DV, Nicoll RA (1988) Enkephalin hyperpolarizes interneurones in the rat hippocampus. J Physiol 398:123-130.

Magloczky Z, Freund TF (2005) Impaired and repaired inhibitory circuits in the epileptic human hippocampus. Trends Neurosci 28:334-340.

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

McCormick DA, Contreras D (2001) On the cellular and network bases of epileptic seizures. Annu Rev Physiol 63:815-846.

McIntyre DC, Plant JR (1993) Long-lasting changes in the origin of spontaneous discharges from amygdala-kindled rats: piriform vs. perirhinal cortex in vitro. Brain Res 624:268-276.

McIntyre DC, Kelly ME (2000) The parahippocampal cortices and kindling. Ann N Y Acad Sci 911:343-354.

McIntyre DC, Kelly ME, Armstrong JN (1993) Kindling in the perirhinal cortex. Brain Res 615:1-6.

McNamara JO (1999) Emerging insights into the genesis of epilepsy. Nature 399:A15-22.

McQuiston AR, Saggau P (2003) Mu-opioid receptors facilitate the propagation of excitatory activity in rat hippocampal area CA1 by disinhibition of all anatomical layers. J Neurophysiol 90:1936-1948.

Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130:1007S-1015S.

Michelson HB, Wong RK (1991) Excitatory synaptic responses mediated by GABAA receptors in the hippocampus. Science 253:1420-1423.

Michelson HB, Wong RK (1994) Synchronization of inhibitory neurones in the guinea-pig hippocampus in vitro. J Physiol 477 (Pt 1):35-45.

Miles R, Wong RK (1986) Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. J Physiol 373:397-418.

Miles R, Wong RK (1987) Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. J Physiol 388:611-629.

Mohapel P, Dufresne C, Kelly ME, McIntyre DC (1996) Differential sensitivity of various temporal lobe structures in the rat to kindling and status epilepticus induction. Epilepsy Res 23:179-187.

Mohapel P, Zhang X, Gillespie GW, Chlan-Fourney J, Hannesson DK, Corley SM, Li XM, Corcoran ME (2001) Kindling of claustrum and insular cortex: comparison to perirhinal cortex in the rat. Eur J Neurosci 13:1501-1519.

Moran NF, Lemieux L, Kitchen ND, Fish DR, Shorvon SD (2001) Extrahippocampal temporal lobe atrophy in temporal lobe epilepsy and mesial temporal sclerosis. Brain 124:167-175.

Morimoto K, Fahnestock M, Racine RJ (2004) Kindling and status epilepticus models of epilepsy: rewiring the brain. Prog Neurobiol 73:1-60.

Murray EA, Graham KS, Gaffan D (2005) Perirhinal cortex and its neighbours in the medial temporal lobe: contributions to memory and perception. Q J Exp Psychol B 58:378-396.

Nagao T, Alonso A, Avoli M (1996) Epileptiform activity induced by pilocarpine in the rat hippocampal-entorhinal slice preparation. Neuroscience 72:399-408.

Nairismagi J, Pitkanen A, Kettunen MI, Kauppinen RA, Kubova H (2006) Status epilepticus in 12-day-old rats leads to temporal lobe neurodegeneration and volume reduction: a histologic and MRI study. Epilepsia 47:479-488.

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates, 5th Edition. Amsterdam ; Boston: Elsevier Academic Press.

Penfield W, Faulk ME, Jr. (1955) The insula; further observations on its function. Brain 78:445-470.

Perreault P, Avoli M (1989) Effects of low concentrations of 4-aminopyridine on CA1 pyramidal cells of the hippocampus. J Neurophysiol 61:953-970.

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

Perucca E (2005) An introduction to antiepileptic drugs. Epilepsia 46 Suppl 4:31-37.

Pinel JP, Rovner LI (1978) Experimental epileptogenesis: kindling-induced epilepsy in rats. Exp Neurol 58:190-202.

Racine RJ (1972a) Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 32:281-294.

Racine RJ (1972b) Modification of seizure activity by electrical stimulation. I. After-discharge threshold. Electroencephalogr Clin Neurophysiol 32:269-279.

Rafiq A, DeLorenzo RJ, Coulter DA (1993) Generation and propagation of epileptiform discharges in a combined entorhinal cortex/hippocampal slice. J Neurophysiol 70:1962-1974.

Raisinghani M, Faingold CL (2005) Evidence for the perirhinal cortex as a requisite component in the seizure network following seizure repetition in an inherited form of generalized clonic seizures. Brain Res 1048:193-201.

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

Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila S, Payne JA, Minichiello L, Saarma M, Kaila K (2004) Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. J Neurosci 24:4683-4691.

Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M (2002) BDNF-induced TrkB activation down-regulates the K+-Cl- cotransporter KCC2 and impairs neuronal Cl- extrusion. J Cell Biol 159:747-752.

Rocha L, Maidment NT (2003) Opioid peptide release in the rat hippocampus after kainic acid-induced status epilepticus. Hippocampus 13:472-480.

Rocha L, Maidment NT, Evans CJ, Ackermann RF, Engel J, Jr. (1994) Microdialysis reveals changes in extracellular opioid peptide levels in the amygdala induced by amygdaloid kindling stimulation. Exp Neurol 126:277-283.

Rosati A, Aghakhani Y, Bernasconi A, Olivier A, Andermann F, Gotman J, Dubeau F (2003) Intractable temporal lobe epilepsy with rare spikes is less severe than with frequent spikes. Neurology 60:1290-1295.

Rossetti AO, Mortati KA, Black PM, Bromfield EB (2005) Simple partial seizures with hemisensory phenomena and dysgeusia: an insular pattern. Epilepsia 46:590-591.

Rudy B (1988) Diversity and ubiquity of K channels. Neuroscience 25:729-749.

Rutecki PA, Lebeda FJ, Johnston D (1985) Epileptiform activity induced by changes in extracellular potassium in hippocampus. J Neurophysiol 54:1363-1374.

Rutecki PA, Lebeda FJ, Johnston D (1987) 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. J Neurophysiol 57:1911-1924.

Ryvlin P (2006) Avoid falling into the depths of the insular trap. Epileptic Disord 8 Suppl 2:37-56.

Ryvlin P, Kahane P (2005) The hidden causes of surgery-resistant temporal lobe epilepsy: extratemporal or temporal plus? Curr Opin Neurol 18:125-127.

Salmenpera T, Kalviainen R, Partanen K, Pitkanen A (2001) Hippocampal and amygdaloid damage in partial epilepsy: a cross-sectional MRI study of 241 patients. Epilepsy Res 46:69-82.

Salmenpera T, Kalviainen R, Partanen K, Mervaala E, Pitkanen A (2000) MRI volumetry of the hippocampus, amygdala, entorhinal cortex, and perirhinal cortex after status epilepticus. Epilepsy Res 40:155-170.

Sanabria ER, D'Andrea Vieira I, da Silveira Pereira MF, Faria LC, da Silva AC, Cavalheiro EA, da Silva Fernandes MJ (2006) Pro-epileptic effect of alfentanil in rats subjected to pilocarpine-induced chronic epilepsy. Brain Res Bull 69:535-545.

Sato T, Yamada N, Morimoto K, Uemura S, Kuroda S (1998) A behavioral and immunohistochemical study on the development of perirhinal cortical kindling: a comparison with other types of limbic kindling. Brain Res 811:122-132.

Scanziani M (2000) GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. Neuron 25:673-681.

Segal M, Barker JL (1986) Rat hippocampal neurons in culture: Ca2+ and Ca2+-dependent K+ conductances. J Neurophysiol 55:751-766.

Shneker BF, Fountain NB (2003) Epilepsy. Dis Mon 49:426-478.

Skyers PS, Einheber S, Pierce JP, Milner TA (2003) Increased mu-opioid receptor labeling is found on inner molecular layer terminals of the dentate gyrus following seizures. Exp Neurol 179:200-209.

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

Somogyi P, Smith AD, Nunzi MG, Gorio A, Takagi H, Wu JY (1983) Glutamate decarboxylase immunoreactivity in the hippocampus of the cat: distribution of immunoreactive synaptic terminals with special reference to the axon initial segment of pyramidal neurons. J Neurosci 3:1450-1468.

Sperk G, Furtinger S, Schwarzer C, Pirker S (2004) GABA and its receptors in epilepsy. Adv Exp Med Biol 548:92-103.

Staley KJ, Soldo BL, Proctor WR (1995) Ionic mechanisms of neuronal excitation by inhibitory GABAA receptors. Science 269:977-981.

Stoop R, Pralong E (2000) Functional connections and epileptic spread between hippocampus, entorhinal cortex and amygdala in a modified horizontal slice preparation of the rat brain. Eur J Neurosci 12:3651-3663.

Sutula TP, Hagen J, Pitkanen A (2003) Do epileptic seizures damage the brain? Curr Opin Neurol 16:189-195.

Swartzwelder HS, Lewis DV, Anderson WW, Wilson WA (1987) Seizure-like events in brain slices: suppression by interictal activity. Brain Res 410:362-366.

Taki K, Kaneko T, Mizuno N (2000) A group of cortical interneurons expressing mu-opioid receptor-like immunoreactivity: a double immunofluorescence study in the rat cerebral cortex. Neuroscience 98:221-231.

Thesleff S (1980) Aminopyridines and synaptic transmission. Neuroscience 5:1413-1419.

Thompson SM, Capogna M, Scanziani M (1993) Presynaptic inhibition in the hippocampus. Trends Neurosci 16:222-227.

Tortorella A, Halonen T, Sahibzada N, Gale K (1997) A crucial role of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid subtype of glutamate receptors in piriform and perirhinal cortex for the initiation and propagation of limbic motor seizures. J Pharmacol Exp Ther 280:1401-1405.

Traub RD, Jefferys JG (1994) Simulations of epileptiform activity in the hippocampal CA3 region in vitro. Hippocampus 4:281-285.

Treiman DM (2001) GABAergic mechanisms in epilepsy. Epilepsia 42 Suppl 3:8-12.

Urrestarazu E, Jirsch JD, LeVan P, Hall J, Avoli M, Dubeau F, Gotman J (2006) High-frequency intracerebral EEG activity (100-500 Hz) following interictal spikes. Epilepsia 47:1465-1476.

Uva L, Librizzi L, Wendling F, de Curtis M (2005) Propagation dynamics of epileptiform activity acutely induced by bicuculline in the hippocampal-parahippocampal region of the isolated Guinea pig brain. Epilepsia 46:1914-1925.

Verma A, Radtke R (2006) EEG of partial seizures. J Clin Neurophysiol 23:333-339.

Vonck K, Boon P, Achten E, De Reuck J, Caemaert J (2002) Long-term amygdalohippocampal stimulation for refractory temporal lobe epilepsy. Ann Neurol 52:556-565.

Vossler DG, Kraemer DL, Haltiner AM, Rostad SW, Kjos BO, Davis BJ, Morgan JD, Caylor LM (2004) Intracranial EEG in temporal lobe epilepsy: location of seizure onset relates to degree of hippocampal pathology. Epilepsia 45:497-503.

Walther H, Lambert JD, Jones RS, Heinemann U, Hamon B (1986) Epileptiform activity in combined slices of the hippocampus, subiculum and entorhinal cortex during perfusion with low magnesium medium. Neurosci Lett 69:156-161.

Warren RJ, Durand DM (1998) Effects of applied currents on spontaneous epileptiform activity induced by low calcium in the rat hippocampus. Brain Res 806:186-195.

Watson C, Jack CR, Cendes F (1997) Volumetric magnetic resonance imaging: clinical applications and contributions to the understanding of temporal lobe epilepsy. Arch Neurol 54:1521-1531.

Whittington MA, Traub RD (2003) Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. Trends Neurosci 26:676-682.

Wiebe S (2000) Epidemiology of temporal lobe epilepsy. Can J Neurol Sci 27 Suppl 1:S6-10; discussion S20-11.

Wiebe S, Blume WT, Girvin JP, Eliasziw M (2001) A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 345:311-318.

Yamamoto J, Ikeda A, Satow T, Takeshita K, Takayama M, Matsuhashi M, Matsumoto R, Ohara S, Mikuni N, Takahashi J, Miyamoto S, Taki W, Hashimoto N, Rothwell JC, Shibasaki H (2002) Low-frequency electric cortical stimulation has an inhibitory effect on epileptic focus in mesial temporal lobe epilepsy. Epilepsia 43:491-495.

APPENDIX A: Animal Subject Use Certificate

Same as previous year's application:

Project is still ongoing and research is going well. We have published over 20 papers, during the last five years, based on this work

In particular we will continue to analyze the cellular, pharmacological and molecular mechanisms underlying:
- limbic seizures that are encountered in patients presenting with TLE. These studies are performed on brain slices obtained by control and pilocarpine-treated animals. The latter represents a well established model of TLE.
- epileptic seizures that occur in Fragile X patients. These studies are performed on brain slices obtained by wild type and fmr1 knockout animals.
- epileptic seizures in acute model of epilepsy.

5 d) List the section / subsection numbers where significant changes have been made

5 e) KEYWORDS: Using keywords only, list the procedures used on animals (e.g. anaesthesia, breeding colony, injection IP, gavage, drug administration, major survival surgery, euthanasia by exsanguination, behavioural studies). For a more complete list of suggested keywords refer to Appendix 1 of the Guidelines (www.mcgill.ca/research/compliance/animal/forms).

Anesthesia (isoflurane gas inhalation, ketamine-xylazine i.p. injection)
Intracardiac perfusion
Decapitation
Brain tissue collection
Intraperitoneal injection (saline, scopolamine, pilocarpine, diazepam)
Breeding colony
Drug administration

6. Animals Use data for CCAC

6 a) Purpose of Animal Use (Check most appropriate one):

1. ☒ **Studies of a fundamental nature/basic research**
2. ☐ **Studies for medical purposes relating to human/animal diseases/disorders**
3. ☐ **Regulatory testing**
4. ☐ **Development of products/appliances for human/veterinary medicine**
5. **If for Teaching, use the Animal Use Protocol form for Teaching (www.mcgill.ca/research/compliance/animal/forms)**

6 b) Will field studies be conducted? NO ☒ YES ☐ If yes, complete "Field Study Form"
Will the project involve the genetically altering animals? NO ☒ YES ☐ If yes, complete SOP #5 or #6
Will the project involve breeding animals? NO ☐ YES ☒ If breeding transgenics or knockouts, complete SOP#4

7. Animal Data

7 a) Please justify the need for live animals versus alternate methods (e.g. tissue culture, computer simulation)
The study of the cellular mechanisms of epilepsy (including live seizures) necessitates the methods outlined in our proposal

7 b) Describe the characteristics of the animal species selected that justifies its use in the proposed study (consider characteristics such as body size, species, strain, data from previous studies or unique anatomic/physiological features)
Our previous studies as well as those reported in the literature pertaining with our work have dealt with rats and mice

7 c) Description of animals

Quality Control Assurance: To prevent introduction of infectious diseases into animal facilities, a health status report or veterinary inspection certificate may be required prior to receiving animals from all non-commercial sources or from commercial sources whose animal health status is unknown or questionable. Quarantine and further testing may be required for these animals.

If more than 6 columns are needed, please attach another page

MAR 2006

	Sp/strain 1	Sp/strain 2	Sp/strain 3	Sp/strain 4	Sp/strain 5	Sp/strain 6
Species	Rat	Rat	Mice	Mice		
Supplier/Source	Charles River	Charles River	Charles River	in-house		
Strain	Sprague-Dawley	Sprague-Dawley	C57BL/6J	C57BL/6J fmrp-1 knock out		
Sex	Male	Male	Male	Male-Female		
Age/Wt	7 to 20 days old	adult/200-300 g	adult/20-30 g	adult/20-30 g		
# To be purchased	30	30	30			
# Produced by in-house breeding				30		
# Other (e.g.field studies)						
#needed at one time	1	1	1	1		
# per cage	2	2	2	2		
TOTAL# /YEAR	30	30	30	30		

7 d) **Justification of Animal Usage: BASED ON THE EXPERIMENTAL OBJECTIVES OF THE PROJECT, describe the number of animals required for one year. Include information on experimental and control groups, # per group, and failure rates. For breeding, specify how many adults are used, number of offspring produced, and how many offspring are used in experimental procedures. Use the table below when applicable. The arithmetic explaining how the total of animals for each column in the table above is calculated should be made clear.** *(Space will expand as needed)*

PILOCARPINE PROJECT: (Sp/Strain 2)
A control group of 8 animals will be injected with saline solution (2 ml/Kg animal i.p.). The experimental group consists of 12 animals as well. They will be injected with Scopolamine (1 mg/Kg i.p.) and Pilocarpine (410 mg/Kg i.p.). It can happen that some animals do not survive during a very severe breif-long convulsive seizure (usually up to 20%). The animals that do not experience at least 30 minutes of status epilepticus (usually up to 30%) will be sacrified by decapitation after anaesthesia because they can't be considered as control or model of cronic epilepsy. The last 50% will be used for in vitro experiments.
8 control animals + 12 pilocarpine animals = 20 animals.

FRAGILE X PROJECT: (Sp/Strain 3-4)
Wild type animals obtained from Charles River Canada will be used like control group. Knockout animals from in-house breeding are our experimental model. Because with this particular knockout colony, the fertility rate is not very high, we usually keep 6-8 adult rats for breeding. We can obtain about 0-6 puppies each time and we will use for our in vitro experiments. Animals older than 6 months (usually 10%) will be sacrified by decapitation after anaesthesia because out of the range of age (compatible with our type of study and technique) and useless for breeding.
30 control animals + 30 knockout animals = 60 animals (30 Strain 3 + 30 Strain 4)

BASIC MECHANISMS OF EPILEPSY: (Sp/Strain 1-2)
We will anaesthetize the animals by isolflurane gas inhalation and we will decapitate them. After that we will remove the brain and obtain slices. We will performe in vitro experiments in control condition and in acute model of epilepsy. At least five different experimental protocols will be carry out during in vitro recordings. Six animals are required in each strain, to obtain statistically significant results.
8 animals x 5 experimental protocol = 40 animals (30 Strain 1 + 10 Strain 2)

For all the projects this number of animals is required in each strain, to obtain statistically significant results. Therefore, a total of 120 rats are needed.

7d table) The following table may help you explain the animal numbers listed in the 7c table:

(Table will expand as needed)	Sp/strain 1	Sp/strain 2	Sp/strain 3	Sp/strain 4	Sp/strain 5	Sp/strain 6
Test agents or procedures						

MCGILL UNIVERSITY
UNIVERSITY ANIMAL CARE COMMITTEE

Standard Operating Procedure #UACC-2 November 2003 version

GENERAL ANAESTHESIA IN ADULT EXPERIMENTAL ANIMALS

1. INTRODUCTION

Standard Operating Procedures (SOPs) provide a detailed description of commonly used procedures. SOPs offer investigators an alternative to writing detailed procedures on their protocol forms. Any deviation from the approved procedures must be clearly described and justified in the Animal Use Protocol form (AUP). Approval of the protocol indicates approval of the deviation from the SOP for that project only. A signed SOP cover page must be attached to the Animal Use Protocol form. The relevant SOP number must be referred to in the Procedures section.

2. USE THIS SOP IF NOT USING *'RODENT SURGERY SOP#10'* OR *'STEREOTAXIC SURVIVAL SURGERY SOP#13'*

3. INFORMATION REQUIRED

3.1 Species/strain(s): ***(must refer to the Sp/strain column # of the table in "Description of animals" section in main protocol)***
Sp/Strain 1: Rat Sprague Dawley, male, newborn to 20 days old, from Charles River Canada
Sp/Strain 2: Rat Sprague Dawley, male, adult, 200-300 g, from Charles River Canada
Sp/Strain 3: Mouse C57BL/6J, male, adult, 20-30 g, from Charles River Canada
Sp/Strain 4: Mouse C57BL6/J fmrp-1 knock out, male, adult, 20-30 g, from in-house breeding

3.2 Anaesthesia chosen:

Agent:
Isoflurane gas (rats Sp/Strain 1-2) before decapitation
Ketamine-Xylazine solution (mice Sp/Strain 3-4)

Dose (mg/kg):
2-3% (Isoflurane gas)
150 mg-10 mg/Kg (Ketamine-Xylazine solution)

Route:
Inhalation (Isoflurane gas)
i.p. injection (Ketamine-Xylazine solution)

Duration of anaesthesia:
2 minutes (Isoflurane gas)
5 minutes (Ketamine-Xylazine)

Frequency of administration:
once (Isoflurane gas)
once (Ketamine-Xylazine solution)

3.3 There are changes to this SOP indicated in the AUP form: ☐ **Yes** ☒ **No**

3.4 If species other than rabbit and rodent, supply references used (such as CCAC *Guide to the Care and Use of Experimental Animals*)