January 2014

Differential effects of early-life seizures on neuronal inhibition in the hippocampus of seizure-prone and seizure-resistant rats

Amir Abbas Mohseni Zonoozi
The University of Western Ontario

Supervisor
Stan Leung
The University of Western Ontario

Graduate Program in Neuroscience

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

© Amir Abbas Mohseni Zonoozi 2014

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Other Neuroscience and Neurobiology Commons

Recommended Citation
https://ir.lib.uwo.ca/etd/1872

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact tadam@uwo.ca, wlswadmin@uwo.ca.
Differential effects of early-life seizures on neuronal inhibition in the hippocampus of seizure-prone and seizure-resistant rats

(Thesis Format: Monograph)

By

Amir A. Mohseni Zonoozi

Graduate Program in Neuroscience

A thesis is submitted in partial fulfilment Of the requirements for the degree of Master of Science

School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

© Amir A. Mohseni Zonoozi 2013
ABSTRACT

The aim of this study is to investigate the long-term physiological consequences of early-life seizures in rats of different genetic backgrounds. Rats bred to be prone (FAST) or resistant (SLOW) to amygdala kindling were induced with status epilepticus (SE) on postnatal day (PND) 10 by injecting 3 mg/kg i.p. kainic acid; SE consisted of seizures for 2 h, including stage 5 seizures (lying down with four limb tonic-clonic convulsions). Littermates injected with the same volume of saline i.p. served as controls. On PND 40-55, population spikes (PSs) were recorded at the CA1 pyramidal cell layer in hippocampal slices in vitro following paired-pulse stimulation of the stratum radiatum. As compared to the saline-injected control FAST rats, seizure FAST rats showed a larger paired-pulse index (PPI = ratio of second PS to first PS) at 4 x threshold stimulus intensity, and 20-100 ms interpulse interval (IPI) (repeated measures ANOVA, P<0.05). By contrast, seizure SLOW rats as compared to control SLOW rats showed a smaller PPI at 20-100 ms IPI (P<0.05). For control rats, PPI at 20-100 ms was lower in FAST than SLOW rats (P<0.05); for seizure rats, PPI was higher in FAST than SLOW rats (P<0.05). We conclude that FAST and SLOW rats have different PPI in CA1 that responded differently to early-life seizures. A small PPI (< 1) at 20-100 ms is likely mediated by GABA-A receptor-mediated inhibition. In a separate experiment, field excitatory postsynaptic potentials (pEPSPs) were recorded in stratum radiatum in CA1 following stimulation of the same layer; pEPSPs were recorded in control and seizure rats, before and after perfusion of GABA-B receptor agonist baclofen (20 µM) in the bath. In either FAST or SLOW rats, baclofen suppressed the pEPSPs less in seizure than their respective control rats (P<0.05). FAST and SLOW rats did not differ in their pEPSP response (at 2 x threshold stimulus intensity) to baclofen in the control groups or in the seizure groups. We conclude that the efficacy of presynaptic GABA-B receptors on glutamatergic terminals of CA1 pyramidal cells was reduced for at least 30 days after early-life seizures, without significant difference between FAST and SLOW rats. Thus, it appears that GABA-A postsynaptic inhibition, but not GABA-B presynaptic inhibition, in hippocampal CA1 area is differentially affected by early-life seizures in FAST and SLOW rats.
ACKNOWLEDGEMENT

I am deeply grateful to my supervisor, Dr. Stan Leung, for his excellent supervision, and his patient and constant help during the writing this manuscript. I am also very grateful to my lab members, Min-Ching Kuo, Justin Arcaro, and Ravnoor Singh for their various means of support ranging from assistance with techniques to encouragement and motivation. I would especially like to thank Dr. Jingyi Ma for her essential help during my project and always being available to answer questions or discuss concepts.

I would like to express my advisory committee, Dr. Michael Poulter, and Dr. Seyed Mirsattari, for their valuable comments and helpful advice. A special thanks to Ms. Carol Anderson for her personal and administrative support.

Finally, I would like to thank you my family, especially my parents, for their continuing support and thoughtfulness throughout the period of my study.
TABLE OF CONTENTS

Differential effects of early-life seizures on neuronal inhibition in the hippocampus of seizure-prone and seizure-resistant rats

ABSTRACT

ACKNOWLEDGEMENT

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

Chapter 1. Introduction

1.1 Introduction

1.2 The Hippocampus

1.2.1 Anatomy

1.2.2 Basic hippocampal circuitry

1.2.3 Hippocampal CA region

1.2.4 Interneurons

1.3 GABA receptors function and synaptic transmission

1.3.1 GABA-A receptors and function

1.3.2 GABA-B receptors and function

1.4 GABA-A and GABA-B receptors function in immature brain

1.4.1 GABA-A receptors

1.4.2 GABA-B receptors

1.5 GABA-A and GABA-B receptor function before and after seizure

1.5.1 GABA-A receptors

1.5.2 GABA-B receptors

1.5 Extracellular potential
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6 Seizure prone (FAST) and Seizure resistant (SLOW) rat</td>
<td>18</td>
</tr>
<tr>
<td>1.7 Kainic Acid</td>
<td>19</td>
</tr>
<tr>
<td>1.8 Rationale and aims of the study</td>
<td>20</td>
</tr>
<tr>
<td>Chapter 2. Materials and Methods</td>
<td>22</td>
</tr>
<tr>
<td>2.1 Animals</td>
<td>22</td>
</tr>
<tr>
<td>2.2 Kainic Acid induced seizure</td>
<td>22</td>
</tr>
<tr>
<td>2.3 Brain slices preparation</td>
<td>23</td>
</tr>
<tr>
<td>2.4 Electrophysiology recording</td>
<td>23</td>
</tr>
<tr>
<td>2.5 Temperature measurement</td>
<td>26</td>
</tr>
<tr>
<td>2.6 Data Analysis</td>
<td>26</td>
</tr>
<tr>
<td>2.6.1 Electrophysiological analysis</td>
<td>26</td>
</tr>
<tr>
<td>2.6.2 Statistical analysis</td>
<td>27</td>
</tr>
<tr>
<td>Chapter 3. Results</td>
<td>28</td>
</tr>
<tr>
<td>3.1 Kainic acid-induced seizure</td>
<td>28</td>
</tr>
<tr>
<td>3.2 Paired-pulse paradigm in the CA1 hippocampus</td>
<td>28</td>
</tr>
<tr>
<td>3.2.1 Changes in excitatory postsynaptic potential slope and population spike evoked by single pulse</td>
<td>29</td>
</tr>
<tr>
<td>3.2.2 Changes in paired pulse population spike response at the pyramidal cell layer</td>
<td>31</td>
</tr>
<tr>
<td>3.2.3 Changes in the EPSP paired-pulse index in pyramidal cells</td>
<td>35</td>
</tr>
<tr>
<td>3.2.4 Comparing FAST rats versus SLOW rats</td>
<td>37</td>
</tr>
<tr>
<td>3.3 Hippocampal perfusion of baclofen</td>
<td>38</td>
</tr>
<tr>
<td>3.3.1 Field EPSP in CA1 stratum radiatum of seizure-treated and control FAST and SLOW rats</td>
<td>39</td>
</tr>
<tr>
<td>3.3.2 Field EPSP in the CA1 region of seizure-treated and control FAST and SLOW rats after baclofen perfusion</td>
<td>40</td>
</tr>
<tr>
<td>Chapter 4: Discussion</td>
<td>43</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

FIGURE 1. SCHEMATIC DIAGRAM OF TRANSVERS SECTION THROUGH THE HIPPOCAMPAL FORMATION DEPICTING MAJOR CIRCUITRY AND LAYERS.................................................................1

FIGURE 2. SCHEMATIC ILLUSTRATION OF GABAERGIC INHIBITORY SYNAPSE WITH PRE- AND POST-SYNAPTIC PROCESS INVOLVED IN GABAERGIC TRANSMISSION. ..............................11

FIGURE 3. SCHEMATIC DIAGRAM TO ILLUSTRATE THE FIELD POTENTIAL RECORDING. ..............................................1


FIGURE 5. INPUT/OUTPUT CURVE OF MEAN POPULATION EPSP (E1) RESPONSE (+/- S.E.M), RECORDED AT THE CA1 CELL LAYER, EVOKED BY THE FIRST PULSE FROM SEIZURE-TREATED (RED LINE) AND CONTROL (BLUE LINE) FAST AND SLOW RATS. STIMULATION INTENSITIES WERE AT 1, 1.5, 2, 3 AND 4 TIMES RESPONSE THRESHOLD. .................................................................1

FIGURE 6. INPUT/OUTPUT CURVES OF POPULATION SPIKE (P1) RESPONSE (MEAN +/- S.E.M), RECORDED AT THE CA1 CELL LAYER, EVOKED BY THE FIRST PULSE IN SLICES FROM SEIZURE-TREATED (RED LINE) AND CONTROL (BLUE LINE) FAST AND SLOW RATS. STIMULATION INTENSITIES WERE AT 1, 1.5, 2, 3 AND 4 TIMES RESPONSE THRESHOLD. .............1

FIGURE 7. INPUT/OUTPUT CURVE OF MEAN POPULATION EPSP (E1) RESPONSE (+/- S.E.M) RECORDED AT STRATUM RADIATUM EVOKED BY THE FIRST PULSE FROM SEIZURE-TREATED (RED LINE) AND CONTROL (BLUE LINE) FAST AND SLOW RATS. .................................................................1

FIGURE 8. OVERLAID TRACES OF PAIRED-PULSE POPULATION SPIKES RECORDED FROM CA1 REGION OF REPRESENTATIVE FAST RATS, AT 20 TO 200 MS IPI AND 4 TIMES STIMULUS THRESHOLD INTENSITY. .................................................................1

FIGURE 9. FAST RATS PAIRED-PULSE POPULATION SPIKE RATIO (PS2/PS1) IN CA1 (MEAN +/- S.E.M) AS A FUNCTION OF INTER-PULSE INTERVAL (IPi) IN KAINIC ACID -INDUCED SEIZURE AND SALINE-INJECTED CONTROLS RECORDED 30-45 DAYS AFTER EARLY-LIFE TREATMENT; STIMULUS INTENSITY WAS 4 TIMES STIMULUS THRESHOLD.........................................................1

FIGURE 10. FAST RATS INPUT/OUTPUT CURVES OF THE PAIRED-PULSE INDEX (PS2/PS1 RATIO) OF THE POPULATION SPIKE (MEAN +/- S.E.M) EVOKED BY PAIRED PULSES AT 20 MS IPI FROM
SEIZURE-TREATED (RED LINE) AND CONTROL (BLUE LINE) RATS. STIMULATION INTENSITIES WERE AT 1, 1.5, 2, 3 AND 4 TIMES RESPONSE THRESHOLD. .................................................................1

FIGURE 11. OVERLAID TRACES OF REPRESENTATIVE IN VITRO HIPPOCAMPAL SLICES OF SLOW RATS SHOWING PAIRED-PULSE POPULATION SPIKES RECORDED FROM CA1 REGION AT 20 TO 200 MS IPI AND 4 TIMES STIMULUS THRESHOLD INTENSITY. ..............................................................................1

FIGURE 12. SLOW RATS PAIRED-PULSE POPULATION SPIKE RATIO (PS2/PS1) IN CA1 (MEAN +/- S.E.M) AS A FUNCTION OF INTER-PULSE INTERVAL (IPI) IN KAINIC ACID –INDUCED SEIZURE AND SALINE-INJECTED CONTROLS RECORDED 30-45 DAYS AFTER EARLY-LIFE TREATMENT; STIMULUS INTENSITY WAS 4 TIMES STIMULUS THRESHOLD. ..............................................................................................1

FIGURE 13. SLOW RATS INPUT/OUTPUT CURVES OF PAIRED-PULSE INDEX (PS2/PS1 RATIO) OF POPULATION SPIKE (MEAN +/- S.E.M) EVOKED BY PAIRED PULSES AT 20 MS IPI FROM SEIZURE-TREATED (RED LINE) AND CONTROL (BLUE LINE) RATS. STIMULATION INTENSITIES WERE AT 1, 1.5, 2, 3 AND 4 TIMES RESPONSE THRESHOLD. ..............................................................................................1

FIGURE 14. FAST AND SLOW RATS PAIRED-PULSE POPULATION EPSP RATIO (E2/E1) RECORDED AT CA1 PYRAMIDAL CELL LAYER AS A FUNCTION OF INTER-PULSE INTERVAL (IPI), 30-45 DAYS AFTER EARLY-LIFE TREATMENT.........................................................................................1

FIGURE 15. FAST AND SLOW RATS INPUT/OUTPUT CURVES OF PAIRED-PULSE INDEX (E2/E1 RATIO) OF POPULATION EPSP (MEAN +/- S.E.M) EVOKED BY DIFFERENT STIMULUS INTENSITY, 30-45 DAYS AFTER EARLY-LIFE TREATMENT.........................................................................................1

FIGURE 16. PAIRED-PULSE INDEX OF POPULATION SPIKE (PS2/PS1 RATIO) AS A FUNCTION OF INTER-PULSE INTERVAL (IPI) WAS DIFFERENT BETWEEN SLICES DERIVED FROM SLOW (RED LINE) AND FAST CONTROL (SALINE-TREATED) RATS (BLUE LINE).........................................................................................1

FIGURE 17. INPUT/OUTPUT CURVE OF THE POPULATION EPSP (E1) AND POPULATION SPIKE (P1) RESPONSE (MEAN +/- S.E.M) EVOKED BY THE FIRST PULSE WAS NOT DIFFERENT BETWEEN SLOW CONTROL RATS (RED LINE) AND FAST CONTROL RATS (BLUE LINE). .........................................................................................1

FIGURE 18. PAIRED-PULSE INDEX OF THE PEPSP SLOPE (E2/E1 RATIO) (MEAN +/- STANDARD ERROR OF THE MEAN) WITH NORMAL ACSF PERFUSION, 30 TO 45 DAYS AFTER EARLY-LIFE TREATMENT IN SEIZURE-TREATED (RED LINE) AND CONTROL SLICES (BLUE LINE) OF FAST AND SLOW RATS. .........................................................................................1
FIGURE 19. EARLY-LIFE KAINIC ACID SEIZURE INDUCTION REDUCED THE SUPPRESSIVE EFFECT OF BACLOFEN ON THE FIELD POULATION EXCITATORY POSTSYNAPTIC SPIKES (PEPS) IN CA1 OF FAST RATS.
LIST OF ABBREVIATIONS

aCSF: Artificial cerebrospinal fluid
ANOVA: Analysis of Variance
CA1: Cornu ammonis, region 1
E1/E2: Population EPSP following 1st and 2nd pulses, respectively
EPI: Excitatory postsynaptic inhibition
EPSP: Excitatory postsynaptic potential
GABA: Gamma-aminobutyric acid
GAD: Glutamic acid decarboxylase
i.c.v.: Intracerebroventricular
i.p.: Intraperitoneal
IPI: Interpulse interval
IPSP: Inhibitory postsynaptic potential
KCC: Potassium chloride cotransporter
NKCC: Sodium chloride cotransporter
pEPSP: Population excitatory postsynaptic potential
PND: Post natal day
PPF: Paired-pulse facilitation
PPI: paired-pulse inhibition indicated by PS2/PS1 ratio, see below
PS: population spike
PS1: population PS following 1st pulse
PS2: population PS following 2nd pulse
SEM: Standard error of the mean
TLE: Temporal lobe epilepsy
Chapter 1. Introduction

1.1 Introduction

For many years, convulsive disorders have been one of the most common neurological disorders in human beings. Just in the United States, over 3,000,000 people suffer from seizures and epilepsy every year. About 150,000 adults and children are admitted to medical facilities due to various seizure disorders (Hauser et al., 1995) at an estimated direct and indirect cost of $17.6 billion per year.

In the normal human brain, the neurons connect to each other and other organs, such as muscles and glands by generating electrical impulses. However, if any abnormality arises in the brain this may result in disrupted neuronal activity. Such disruptions can have an impact on our thoughts, feelings and actions. One of these problems that can arise is seizures in which neurons and/or nerve cells generate abnormal signals. However, we should consider that this interaction is a dynamic system and instability (in this case seizure) is caused by transient changes of either excitation or inhibition (Leung et al., 2000).

Research has shown that seizures occurs more in the immature rather than adult brain, due to the presence of more susceptible immature neurons in brain (Hauser, 1995; Holmes and Ben-Ari, 2001). In addition, immature seizures compared to adult seizures can cause different kinds of brain damage (Sarkisian, 1997; Holmes and Ben-Ari, 2001).

Despite being one of the most common childhood problems, the long-term consequences of seizures is still a controversial issue. Interestingly, more than 50% of epilepsies start in infancy or early childhood (Hauser, 1995). Epilepsy in early-life is also known as a cause of various postictal changes of different time courses (Todd’s phenomena). In fact, some retrospective studies showed an association between long-term problems and childhood seizure (Maher and McLachlan, 1995), however prospective studies did not support them (Shinnar and Glauser, 2002).
Retrospective studies have shown hippocampal sclerosis after early childhood convulsion (Cendes et al., 1993), and MRI studies demonstrated hippocampal injury after long lasting febrile seizures in some children (Scott et al., 2003). It has been reported that 40% of adult patients with hippocampal sclerosis-associated temporal lobe epilepsy (TLE) have a past history of febrile seizure (Abou-Khalil et al., 1993); however, the epileptogenesis effect of early-life seizure is still controversial (Berg and Shinnar, 1997). Research has shown that the immature brain is much more resistant to seizure consequences, as compared with the adult brain (Maytel et al., 1989). The literature reveals that both short- and long-term reported consequences of early-life seizure varies among different models, suggesting that each model can have specific effects on the brain (Cilio et al., 2003). One well established animal model for inducing seizures is the Kainic Acid model. However, despite years of study using this model, the long lasting effect of early-life seizure induced by kainic acid still remains unknown. Thus, more research is necessary to completely understand this seizure model.

1.2 The Hippocampus

The hippocampus is one of a group of structures forming the limbic system and is a part of the hippocampal formation, which also includes the dentate gyrus, subiculum, and entorhinal cortex. Extensive evidence implicates the hippocampus and related structures in the formation of episodic memories in humans (Aggleton and Brown 1999) and in consolidating information into long-term declarative memory (Mumby et al., 2001). The initial insights on the role of the hippocampus came from studies of human amnesia following removal of the hippocampus plus neighboring medial temporal structures (Scoville and Milner 1956).

As research has shown, seizure can cause different kinds of changes in many areas of the brain. The hippocampus is one of the most vulnerable parts of the brain to seizure-induced injury such as cell loss and synaptic reorganisation (Holmes and Ben-Ari, 2001; Tsai and Leung, 2006).
1.2.1 Anatomy

The hippocampus is one the most studied areas in mammalian brain. It is one of the largest parts of limbic system. The limbic lobe, which is a ring of gray matter on the medial aspect of each hemisphere, was described in 1878 by Broca. The limbic lobe consists of three large components: the hippocampus, parahippocampal gyrus and cingulate gyrus (Kiernan, 2013). The limbic system was defined later by J. W. Papez in 1938 and includes hippocampal formation, cingulate cortex, hypothalamus, nucleus accumbens and amygdala (Issacson, 1980; Morgane et al., 2005). From an evolutionary point of view, the hippocampal formation is a part of archicortex and is simpler than the neocortical structures. The hippocampus appears as a C-shaped structure with its axis extending from the rostromedial septal nuclei to the caudoventral medial aspect of temporal lobe (Amaral and Witter, 1989) and its cell bodies and their connections are beautifully arranged in orderly layers. Based on the cytoarchitectonic determination, hippocampus in humans includes six distinct regions: the dentate gyrus (DG), cornu ammonis (CA1, CA2, CA3, CA4) and the subiculum (Lorente de No, 1933; Amaral and Witter, 1989; Lorente de No, 1933).

In the rodent brain, the CA region is divided to 3 separate regions (CA1, CA2, CA3) based on different anatomical and connectional characteristics. CA1, an area containing densely packed triangular shaped pyramidal cells, is located at the most dorsal aspect. CA2 is a smaller area than CA1. Finally, CA3 is a larger area with less densely packed cells located on the curve of the hippocampus (Knowles, 1992).

In humans, a CA4 area usually described as a region within the DG, but in rodents it is referred to as the dentate hilus (Lopes da Silva et al., 1990; Knowles, 1992). Despite both structural and cellular differences between human and rodents’ hippocampus, the circuitry and function are the same.

Due to its characteristic cell organisation and interconnections, the hippocampus has been described as a lamellar structure. It has 4 layers, which start from a superficial layer called stratum oriens, followed by stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare respectively (Fig. 1B). The stratum pyramidale contains a dense layer of neural cell
bodies of pyramidal cells. The dendrites of pyramidal cells project to both directions, with the larger dendritic arbor, the apical dendrites, projecting towards the stratum radiatum and the smaller one, basal dendrites, projecting towards stratum oriens. The stratum lacunosum-moleculare receives fibers from the perforant pathway which form synapses with apical dendrites of pyramidal cells (Amaral and Witter, 1989; Amaral and Lavenex, 2007). Within the thinnest layer, the stratum lucidum defined for CA3 only, mossy fibers from the granule cells of dentate gyrus form synapses on the proximal apical dendrites of CA3 pyramidal cells.

**Figure 1.** Schematic diagram of transverse section through the hippocampal formation depicting major circuitry and layers. **A:** The hippocampal formation. Information enters via the perforant path from the entorhinal cortex and exits via the axons of the CA1 pyramidal cell neurons. **B:** Organization of the layers within the CA1 region of the hippocampus (Originally illustrated by Megias et al., 2001)

Within the hippocampus there are two main cell types, pyramidal cells and granule cells. Pyramidal cells are large triangular neurons with cell bodies in stratum pyramidale (Knowles, 1992; Turner et al., 1998). Granule cells are the principal cells of DG with cell bodies within the stratum granulosum layer and possess a single dendritic arbour found in the molecular layer (Knowles, 1992).

In addition to the principal cells, there are different kinds of interneurons classified based on their shape, location and neurophysiology. These include basket cells, oriens/alveus interneurons (Freund and Buzsaki, 1996; Knowles, 1992). These interneurons have a critical role
in modulating cellular activities by their inhibitory effect. They inhibit cells in both somatic and dendritic regions by presynaptic and postsynaptic inhibition (Megias et al., 2001).

1.2.2 Basic hippocampal circuitry

A trisynaptic circuit, a unidirectional progression of excitatory pathways to link different regions of hippocampus in a lamella, was described by Per Andersen in 1971 (Andersen et al., 1971). Later on, other researchers suggested that the hippocampal formation is more complex and multi-directional circuit, with inter-region feedback within the hippocampal formation. However, most of the in vitro studies on the hippocampus were done based on the lamellar hypothesis (Amaral and Witter, 1989).

The trisynaptic circuit of the hippocampus is described as mainly excitatory (Andersen et al., 1971). Entorhinal cortex (EC) projects their axons via the perforant path that synapses with cells in the dentate gyrus, CA3 and CA1. The granule cells of the DG give rise to the mossy fibers which terminate on the proximal apical dendrites of the CA3 pyramidal cells in stratum lucidum. Axons of CA3 cells, named Schaffer collaterals, project to other layers of CA3 and the CA1 region. The CA3 axons have two different paths, the associational path terminates on the ipsilateral side, and the commissural path terminates on the contralateral side, in stratum oriens and stratum radiatum of CA1 (Buzsaki and Eidelberg, 1992; Brivanlou et al., 2004). Finally, the majority of CA1 pyramidal cell axons connect with subiculum and the minority of those axons project directly to the EC. Hippocampal projection to EC first goes to the deep EC layer and then project to the surface EC layer. The EC also projects to some of neocortical regions which also project back to EC (Amaral and Witter, 1989) (Fig. 1A).

In addition to aforementioned connections, the pyramidal cells of CA1 receive information from the amygdala and medial septal nucleus. The ventral part of CA1 receives a direct input from the amygdala, and medial septal nucleus projects to the hippocampus through fimbria-fornix pathway (Hershman, Freedman and Bickford, 1995; Miller and Freedman, 1995).
1.2.3 Hippocampal CA region

The hippocampus Cornu Ammonis (CA) based on the different cytoarchitecture of its cells is divided into 3 sub regions in the rodents: CA1, CA2 and CA3 (Lorente de No, 1934). The laminar organization is almost similar for all CA regions. Pyramidal cells which are characterised by a compact grey triangular cell bodies form the principal cell layer in the hippocampus, also known as the pyramidal cell layer. Below this layer there is a relatively cell free zone called the stratum oreins which contains the basal dendrites of pyramidal cells. Above the pyramidal cell layer is stratum radiatum which also contains the apical dendrites of pyramidal cells. Schaffer collaterals, as the axon collaterals of the CA3 pyramidal cells go through both stratum radiatum and stratum oriens and bring input from CA3 to CA1. The most superficial layer is stratum lacunosum-molecularare.

In addition to the principal cells, there are also various types of interneurons in CA1 region which mostly use $\gamma$-aminobutyric acid (GABA) as neurotransmitter (Freund and Buzsaki, 1996). Despite the fact that CA1 pyramidal cells do receive inhibitory input from interneurons, such as basket cells, the inhibition in CA1 region compare with DG or CA3 region is not enough strong (Schwartzkroin and McIntyre, 1997). As a result, the CA1 region of hippocampus is likely more prone to epileptiform activity and related damages.

1.2.4 Interneurons

Interneurons make up the main inhibitory circuit in the brain and play a critical role in modulating cellular activity in different regions. Interneurons in the brain are classified into different groups based on shape, location and neurophysiology (Freund and Buzsaki, 1996). Despite the small number of the interneurons compared to principal cells in the hippocampus (5-10% of total cells), there are a vast number of synaptic connections between interneurons and principal cells; 8-40 interneurons make contact with each pyramidal cell and they can provide powerful inhibition (Miller and Freedman, 1995; Nurse and Lacaille, 1997).
In the CA1 region, every part of the pyramidal cells, including the soma, dendrite branches and the axon hillock, receives inhibitory synapses from interneurons (Benes and Berretta, 2001). Interneurons are classified into three groups according to their synaptic targets: basket cells, axo-axonic (chandelier) cells and bistratified cells. Basket cells are located near the pyramidal cells soma and form synapses with the somata of pyramidal cells (Benes and Berretta, 2001). Chandelier cells make axo-axonic synapses onto the initial segment of the axon and have a strong control on action potential initiation (Benes and Berretta, 2001). Bistratified cells make synaptic contact with the apical and basal dendritic branches of pyramidal neurons. The dendrites of all aforementioned cell types project into both stratum radiatum and stratum oriens and may receive input from Schaffer collateralss, commissural-associated fibers, and feedback from pyramidal neurons in the local region of the interneurons (Buhl et al., 1996; Halasay et al., 1996).

1.3 GABA receptors function and synaptic transmission

In the hippocampus, similar to other parts of the brain, inhibition is accomplished by the release of GABA (Bettler et al., 2004). GABA is a principal inhibitory neurotransmitter in the brain, and is synthesized within GABAergic axon terminals. GABA is formed by transamination of α-ketoglutarate to glutamic acid, which is then decarboxylated to GABA by glutamic acid decarboxylase (GAD) (Fig. 2). In 90% of the hippocampal GABAergic cells, GAD is localized with GABA (Benes and Barretta, 2001). GABA is stored in small vesicles which fuse with the cellular membrane of the axon terminals to release GABA into the synaptic cleft. Then the released GABA binds to GABA receptors and acts on them. Finally, it is taken up by GABA transporters, or degraded by enzymes (Isaacson et al., 1993). There are two main types of GABA receptors, GABA-A and GABA-B, in the hippocampus; the third type, of GABA-C receptor, is not abundant in the hippocampus.

GABA-A and GABA-B receptors mediate inhibitory postsynaptic potentials (IPSPs). GABA-A receptors open Cl- conductance and GABA-B receptors open a K+ conductance
(Newberry and Nicoll, 1985). In addition, GABA-B receptors affect the presynaptic terminals by decreasing the Ca++ influx and neurotransmitter release.

1.3.1 GABA-A receptors and function

GABA-A receptors belong to a large family group of ligand-gated ion channels that are heteromeric pentamers (Benarroch, 2007). GABA-A receptors are formed by different subunits: α1-6, β1-4, γ1-3, ε, δ, θ, Λ, and ρ1-3. Each of these subunits has a large extracellular amino-terminal, four transmembrane domains (TM1-4) and an intracellular domain (Unwin, 1993; Moss and Smart, 2001). In the hippocampus, most of the GABA-A receptors contain two α subunits and two β subunits, together with either γ or δ subunit (Barnard et al., 1998).

Different composition of these subunits determines the electrophysiological and pharmacological characteristics of GABA-A receptors. The α subunit specifies the affinity and the sensitivity of receptor to GABA and/or other modulators, such as steroids, ethanol and pharmacological agents like benzodiazepines, barbiturates and general anesthetics (Barnard et al., 1998). The γ subunits, in addition to the similar effects of α type, regulate the trafficking and clustering of GABA-A receptors at postsynaptic sites (Essrich et al., 1998; Henschel et al., 2008). The δ subunits can cause GABA-A receptors to have a high affinity for GABA, but insensitive to benzodiazepines. It has been shown that δ-contained GABA-A receptors play an important role in GABA-A receptor-dependent tonic inhibition in dentate gyrus cells of the hippocampus (Nusser and Mody, 2002).

The GABA-A receptors are inotropic channels which mediate two types of inhibition in the nervous system by directly gating Cl⁻ phasic and tonic inhibition. The phasic inhibition is produced by postsynaptic GABA-A receptors which have low affinity to GABA. These postsynaptic receptors are activated by rapid and transient releasing of GABA from presynaptic interneurons and produce fast inhibitory postsynaptic potential (IPSP) that acts for 150 ms or less in the brain (Alger, 1991; Olsen and Avoli, 1997) by increasing Cl⁻ conductance (Fig. 2) (Newbury and Nicoll, 1985). Tonic inhibition, a persistent form of inhibition, is regulated by
extrasynaptic GABA-A receptors which have high affinity for GABA. This inhibition is produced in the presence of tens of nanomolar to few micromolar of GABA which is enough to cause continued activation of GABA-A receptors (Lerma et al., 1986; Kennedy et al., 2002; Wu et al., 2003). Tonic inhibition has been found in several parts of the brain, such as the cerebellum (Wall and Usoicz, 1997), thalamus (Porcello et al., 2003), cerebellar cortex (Yamada et al., 2007) and hippocampus (Bai et al., 2001). In general, GABA-A receptor activation reduces the cell excitability by inducing membrane hyperpolarization due to increasing cell membrane conductance.

In addition to the aforementioned physiological modulations, GABA-A receptor-mediated postsynaptic inhibition can be blocked by exogenous GABA-A antagonists such as bicuculline methiodide and picrotoxin. These receptors are also modulated by barbiturate, benzodiazepine (e.g., diazepam) through positive allosteric modulation of receptor subunits. In addition, neurosteroids have both agonist and antagonist effects. Muscimol is a GABA-A agonist which binds to the same site as the GABA neurotransmitter.

1.3.2 GABA-B receptors and function

GABA-B receptors are G-coupled metabotropic receptors and consist of heterodimers R1 and R2 subunits, each with splice variants. GABA-B receptors modulate indirect inhibition through the inhibition of adenylyl activity in brain tissue (Hill, 1985; Bowery, 1993). These receptors are found in different brain regions such as thalamic nuclei, molecular layer of cerebellum, cerebral cortex, interpeduncular nucleus and laminae II and III of spinal cord (Bowery et al., 1987).

GABA-B receptors are widely located on both the presynaptic axonal terminal and postsynaptic membrane (Fig. 2). They are also found in extra synaptic membranes (Fischer et al., 1999). In the hippocampus, GABA-B receptors are located mainly on the dendrites of the hippocampal neurons and interneurons (Bowery and Pratt, 1992; Bowery, 1993). In the postsynaptic cells, GABA-B receptors, compared to the GABA-A receptors, elicit slower and
longer IPSPs (150-500+ ms) (Bowery, 1993; Nurse and Lacaille, 1999) due to multi step process set off G-protein coupled receptors that open G-protein gated inward–rectifying K (GIRK) channels (Andrade et al., 1986; Misgeld et al., 1995). Presynaptic GABA-B receptors are located on axon terminals (Pittaluga et al., 1987; Morrisett et al., 1991; Bowery, 1993). These GABA-B receptors suppress N- and P/Q type Ca\(^{++}\) channels via their G-protein cascade and cause presynaptic inhibition on both glutamatergic and GABAergic terminals (Davies et al., 1991; Lambert and Wilson, 1994).

The time course of presynaptic GABA-B receptors mediated inhibition is different from the postsynaptic inhibition and peaks later and lasts longer (Issacsan, Solis and Nicoll, 1993). Presynaptic GABA-B receptors have inhibitory effect on both glutamatergic and GABAergic terminals by decreasing the Ca\(^{++}\) influx (Bowery, 1993) and neurotransmitter release. These receptors which are located at glutamatergic terminals are called GABA-B heteroreceptors and their activation causes inhibition of glutamate release and results in decreased excitation (Scanziani et al., 1992; Parker et al., 2004). The other group of presynaptic GABA-B receptors at GABAergic (inhibitory) terminals are named GABA-B autoreceptors and their action decreases the release of GABA (Mody et al., 1994).

The GABA released into the synaptic cleft can have a feedback effect on GABA-B receptors located on both the activated and neighbouring terminals. Under this effect, the presynaptic GABA-B receptors can suppress subsequent release of GABA, leading to smaller GABA-A and GABA-B receptor-mediated IPSP. For instance, it has been shown that the reduction of the second IPSP during paired pulse stimulation can be prevented by a GABA-B antagonist (Davies at al., 1991). This provides an important mechanism to control and regulate both GABA-A and GABA-B receptor-mediated inhibition.

In the hippocampus, GABA-B receptors are localised both in the CA region and the DG based on radio-labeled baclofen as a GABA-B receptor agonist (Bowery, 1985). In the CA1 region, GABA-B receptors are found at the dendrites (Bowery et al., 1987). GABA-B receptors mediated IPSPs in both pyramidal cells (Alger and Nicoll, 1982) and interneurons (Misgeld et al., 1989; Lacaille, 1991) in the CA1.
**Figure 2.** Schematic illustration of GABAergic inhibitory synapse with pre- and postsynaptic process involved in GABAergic transmission. 1: GABA is synthesized within GABAergic terminals by the transmination of Glutamate (GluA), which is then decarboxylated by glutamic acid decarboxylase (GAD) to make GABA. 2: GABA is restored into vesicles, that travel to and fuse with the cell membrane to release GABA into the synaptic cleft. 3: GABA acts on postsynaptic GABA-A receptors, where a fast IPSP is generated mainly from inward chloride ion conductance. 4: GABA also acts on postsynaptic GABA-B receptors, which generate SLOW outward potassium ion conductance. 5: GABA diffuse back to activate presynaptic GABA-B receptors, which initiate SLOW inward calcium ion conductance. 6: GABA is removed from the synaptic cleft by GABA transporters on the neurons and glial cells. In the presynaptic terminals, the GABA is recycled and transferred to the vesicle packing process. The glial cells break down GABA until it returns to glutamate, which can be reused by the neurons. 7: Extra GABA is broken down into succinic semialdehyde, succinic acid and glutamate.
1.4 GABA-A and GABA-B receptors function in immature brain

For many years it has been well-known that the function of GABAergic inhibitory system is markedly different between mature and immature rats’ brain. This is because of several factors including different ion concentration and different times of some inhibitory system maturation. For instance, maturation of the postsynaptic GABA-A receptors and presynaptic GABA-B receptors happens earlier than postsynaptic GABA-B receptors in hippocampal CA3 and CA1 (Nurse and Lacaille, 1997).

1.4.1 GABA-A receptors

Normally, in the mature brain the activation of GABA-A receptors results in suppression of cell firing by neuronal hyperpolarization and shunting. However, GABA-A receptors activation during the first postnatal week of rats induces membrane depolarisation and cell firing (Muller et al., 1984, Zhang et al., 1991 Ben-Ari et al., 1998). This depolarization happens as a result of high intracellular concentration of Cl⁻ due to different K-Cl co-transporter system in immature brain (Zhang et al., 1991, Rivera et al., 1999). GABA-A receptor-mediated depolarisation disappears after the first postnatal week in rats, and the GABA-A receptor-mediated IPSP becomes hyperpolarizing (Zhang et al., 1991). GABA-A receptor-mediated depolarization can contribute to the greater excitability of the immature brain and thus lead to an enhanced chance of seizure susceptibility or early-life seizure.

In addition to the GABA-A receptor activity, it has been shown that the molecular structure of GABA-A receptors changes during the brain development (Paysan et al., 1998; Swanwick et al., 2006). In rodents at the birth time, the expression of GABA-A receptor α1 subunit is very low but the amount gradually increases with age (Laurie et al., 1992; Brooks-Kayal et al., 1998).
1.4.2 GABA-B receptors

It has been shown that the hippocampus CA3 region matures earlier when compared to the CA1 region (Nurse and Lacaille, 1997). In this regard, the postsynaptic GABA-B receptors in the CA3 are mature and their postsynaptic inhibition can be observed in pyramidal cells around PND 6 (Swann et al., 1989; Ben-Ari et al., 1994), but in the CA1 region GABA-B receptor paired-pulse depression was not observed during the first postnatal week (Caillard, et al., 1998). Also, the postsynaptic GABA-B currents in the CA3 region reach adult magnitude at PND 7 (Ben-Ari et al., 1994; Gaiarsa et al., 1995a). In CA1 region, GABA-B currents reach approximately 10% of adult magnitude by PND 12-14 (Nurse and Lacaille, 1997). Other studies have shown that the number of GABA-B receptors reaches its peak at PND 3 in CA3, PND 7 in CA1 and DG, PND 14 in neocortex and thalamus, and PND 21 in the medial geniculate in rodents. However, the number of GABA-B receptors decreases to adult level by PND 28 following those aforementioned regionally specific peaks (Turgeon and Albin, 1994).

Like hippocampus, the time course of GABA-B maturation varies in different brain regions. In the neocortex, postsynaptic GABA-B receptor-mediated inhibition becomes mature after the 3rd postnatal week (Fukuda et al., 1993). But, presynaptic GABA-B receptor maturation occurs earlier as compared to the postsynaptic ones (Gaiarsa et al., 1995b). Also, in rodents’ substantia nigra, the density of GABA-B receptors is almost three-fold higher at PND 15 as compared to adults (Garant et al., 1992).

Using different agonist and/or antagonist have revealed more about GABA-B receptors. Age-dependent difference in in vivo response to GABA-B antagonist CGP35348 has been reported (Veliskova et al., 1996). Intraperitoneal (i.p.) administration of CGP35348 (600 mg/kg) has a proconvulsant effect on pentylentetrazol-induced seizure in PND 15 rat pups, but not in the adult rats (Veliskova et al., 1996). Another study has been shown that baclofen, a GABA-B receptor agonist, can inhibit GABA release in CA3 region by activation of GABA-B receptors (Caillard et al., 1998).

During development, GABA-B receptors are also regulated at the molecular level. An increase in the amount of both GABA-B receptor subunits, GABA-B1 and GABA-B2, was
observed during rat pup development (Correa et al., 2004). Also, it has been shown that the two known GABA-B receptor splice variants, GABA-B1a and GABA-B1b, have different expression profiles. The GABA-B1a express predominantly at birth, whereas GABA-B1b is predominant in adult brain (Fritschy et al., 1999).

1.5 GABA-A and GABA-B receptor function before and after seizure

1.5.1 GABA-A receptors

Studies using chemo-convulsant models have shown that altered hippocampal expression of GABA-A receptors has an important role in seizure induction (Raol et al., 2006). Other studies shown that GABA-A receptor antagonist can induce seizure, and GABA-A receptor agonists act as anticonvulsant due to their effect on enhancing GABA-A receptor-mediated synaptic inhibition (Gale, 1992; Macdonald and Olsen, 1994). In addition, during the past decades, plenty of GABA-A receptor mutations associated with different type of seizures have been identified. A mutation in $\alpha_1$ (A322D) subunit by causing a reduction in surface expression and an increase in deactivation of GABA-A receptors is linked to juvenile myoclonic epilepsy and childhood absence epilepsy not associated with febrile seizure (Macdonald et al., 2004; Krampfl et al., 2005; Maljevic et al., 2006). Different mutations of GABA-A receptor gene which code the $\gamma_2$ subunit such as R43Q, K289M and Q351X, have been linked with childhood absence epilepsy and generalized epilepsy with febrile seizure plus (GEFS+) (Mizielinska et al., 2006; Mohler, 2006; Benarroch, 2007). These mutation decrease GABA-mediated inhibition by affecting different aspects of GABAergic signaling, such as impairing receptor trafficking, decreasing receptor expression and accelerating receptor deactivation (Kang et al., 2006; Eugene et al., 2007). Moreover, an increase in GABA-A- receptor-mediated inhibition after a prolonged hyperthermia seizure in immature rats has been observed (Chen et al., 1999). A recent study has shown that febrile seizure has a persistent effect on the composition of granule cell layer in the dentate gyrus (DG) in which these cells display a long-lasting increase in GABA-A receptor
expression; however, the functional effect of this increase is not clear (Swijsen et al., 2012). In addition, seizure or epilepsy may affect GABA-A receptor expression, function and modulation in both patients and animals (Loup et al., 2000). For instance, seizure can cause a reduction in $\alpha_1$ subunit expression in the CA1 region and a reduction in $\beta$ and $\gamma_2$ subunits colocalization in DG (Loup et al., 2000). Another study, by combining patch-clamp recording and single-cell mRNA amplification (aRNA) techniques in single dentate granule cells, demonstrated that expression of GABA-A receptor subunit mRNAs is substantially altered in neurons from epileptic rats (Brooks-Kayal et al., 1998). One study suggests that increasing GABA-A receptor activity during different postnatal stages in rats can have variable results. In the first postnatal week this increase can enhance the interneuronal size, however in the second week reduce the cell size and number of primary dendrites (Marty et al., 1996).

1.5.2 GABA-B receptors

In rats, malfunction of both pre- and postsynaptic GABA-B receptor-mediated inhibition after spontaneous seizure generation in the CA1 area of hippocampus has been described by Mangan and Lothman (1996). It has been reported that GABA-B receptor antagonists can provoke partial and generalized seizure (Badran et al., 1997) and drugs which increase GABA induced inhibition have been used as effective anticonvulsants (Macdonald and McLean, 1986). In the adult rats, intracerebroventricular (i.c.v) injection of GABA-B antagonist induces seizure in the hippocampus and neocortex, which is characterized by jumping, running, forelimb clonus and wet-dog shake (Leung, Canning and Shen, 2005). On the other hand, therapeutic dose of baclofen, as a GABA-B receptor agonist, in a patient free of epilepsy can provoke seizure (Rush and Gibberd, 1990). Also, toxic dose of baclofen can induce seizure in nonepileptic patients (Lipscomb and Meredith, 1980). In rat hippocampal slices, baclofen perfusion elicits rhythmic sharp waves in all regions (Lewis et al., 1989). Baclofen also can facilitate epileptiform activity in the DG (Burgard and Sarvey, 1991). The GABA-B receptors have a dual effect in seizure where they have an important role in controlling partial and tonic-clonic seizures; on the other hand, they can enhance absence seizure. In absence seizure, blocking GABA-B receptors in the
Thalamus inhibits the spike waves while GABA-B receptor agonists provoke absence seizure (Vergnes et al., 1997). Knocking out the GABA-B receptor gene in mice resulted in spontaneous complex partial seizure after 12 days of age, and then developed into generalized tonic-clonic seizure that resulted in early death (Prosser et al., 2001; Schular et al., 2001). In humans, GABA-B1 receptor polymorphism is associated with temporal lobe epilepsy (Gambardella et al., 2003).

It has been reported that GABA-B receptor function can be changed by various types of seizures. Recurrent seizures in immature brain provoke long-term excitability, maybe due to making changes during the growth and development of GABA-B receptor-mediated inhibitory mechanisms (Tsai and Leung, 2006; Tsai et al, 2008). In adult rats, synaptic plasticity and spatial performance were decreased up to four weeks after partial kindling, accompanied by down regulation of GABA-B autoreceptors and heteroreceptors (Wu and Leung, 1997; Poon et al., 2006), despite an initial increase in GABA-B expression following a seizure (Leung et al., 2005, Sperk et al., 1994). In addition, an increase in postsynaptic GABA-B receptor currents in CA1 pyramidal cells was reported after partial hippocampal kindling (Liu and Leung, 2003). In vitro studies have shown a decrease in function of presynaptic and interneuronal GABA-B receptors in the DG after kainic acid seizure induction (Haas et al., 1996; Chandler et al., 2003).

Related to early-life seizure, one study has been shown that inducing early-life seizures by blocking GABA-B receptors causes long term GABA-B receptor hypofunction (Tsai et al., 2008). In addition, early-life induced seizures facilitated hippocampal kindling and this can be an evidence of decreasing the GABA-B receptor-mediated inhibition in the adult hippocampus (Tsai et al., 2008; Qu et al., 2010). In humans, recurrent seizures in TLE cause a reduction in GABA-B receptor binding and mRNA levels in the DG and pyramidal cell layers (Billinton et al., 2001).

### 1.5 Extracellular potential

The extracellular space can be considered as a volume conductor because ions can flow throughout the volume. In some areas of the brain, neuronal activity can create extracellular field
potential with spatial gradients that result in measurable current flows. Extracellular field potential can be produced by a single or a group of neurons (Leung et al., 2010).

The hippocampus, due to its highly laminar organization of the cell bodies and dendrites of pyramidal cells and their afferents and efferents, is an ideal structure for neuroanatomical and electrophysiological studies. An evoked field potential can be recorded in the dendrites of hippocampal pyramidal cells by a single pulse stimulation via an electronic stimulator. This stimulation gives rise to a population excitatory postsynaptic potential (pEPSP) in pyramidal cells. The pEPSP is characterised as a dipole field, with extracellular currents flowing from a distant location (source) to the dendrite as the point of activation (sink). If the EPSPs are strong enough, nearly synchronous action potentials may be generated at the somata of pyramidal cells, result in a population spike (PS).

Paired-pulse stimulation is a method to study facilitation and inhibition of EPSPs and PSs. Two stimulus pulses with different interpulse interval (IPI) are used to evoke pEPSP and PS, the first pulse is a conditioning pulse and the second one is test pulse. The first PS (P1) can provide a measure of the tonic excitability of the neuronal population, whereas the second PS (P2) is a measure of the excitability during the period of inhibition caused by the first pulse. P2/P1 < 1 indicates paired-pulse inhibition (PPI), while P2/P1 > 1 indicates paired pulse facilitation (PPF). Paired-pulse response of PS in the hippocampus is an indicator of GABA mediated inhibition (Steffenson and Henriksen, 1991; Leung et al., 2008) and can be observed in the DG and CA1. The largest population spike was recorded by electrodes placed at the CA pyramidal cell layer. It has been shown that GABA-A receptor antagonist can block early paired pulse inhibition (< 100 ms) of the PSs (Tuff et al., 1983; Steffenson and Henriksen, 1991), while GABA-B receptor antagonist can block late paired pulse inhibition (> 100 ms) of PSs (Olpe et al., 1993; Wasterlain et al., 1996; Canning and Leung, 2000; Leung et al., 2008), suggesting that early PPI is mediated by GABA-A receptors which is consistent with the fast ionotropic nature of the receptors, and GABA-B slow metabotropic receptors are responsible for the late PPI.

PPF of the EPSP (E2/E1 > 1) is also observed in the hippocampus (Leung and Fu, 1994; Zhao and Leung, 1991). Also, PPI of the pEPSPs were reported following strong afferent stimulation due to GABA-A and GABA-B receptor-mediated inhibition (Leung et al., 2008). One in vitro study on the CA1 hippocampal slices was shown that a test EPSP 200 ms following
a conditioning tetanus is smaller as compared with a test EPSP alone (Issacson et al., 1993). The advantage of studying EPSP which are generated by dendrites is that the pEPSP field is extensive and can be easily recorded with electrodes.

1.6 Seizure prone (FAST) and Seizure resistant (SLOW) rat

The growing need for a complex partial seizure animal model based on the different genetic predisposition led Dr. Racine and his colleagues to develop two new strains of rats based on their rates of amygdala kindling (Racine et al., 1999). The two new strains of rats were derived from parents belonging to two strains - Long Evans Hooded and Wistar rats. Rats were selected for the fastest or the slowest rate to achieve amygdala kindling with stage-5 convulsive seizure, and then bred within the FAST and the SLOW groups. The new rat strains were examined both for local and propagating seizure profiles from F6 to F10 to assure that their kindling characteristics are consistent during several breeding. Finally, after 11 generations, the strains were introduced as kindling-prone (FAST) and kindling-resistant (SLOW) (Racine et al., 1999).

The FAST rats are approximately 40% faster, whereas SLOW rats 200-400% slower, to kindle to fully generalized seizure from the basolateral amygdala, compared to their original parent population (Dufresne et al., 1989). Consistent with the fact that noradrenaline has an anticonvulsant effect in amygdala kindling and other epilepsy models (Mason and Corcoran, 1979; McIntyre, 1980), the basal level of noradrenaline in the amygdala of SLOW rats is significantly higher than FAST rats (Dufresne, 1990).

These FAST and SLOW lines, selected for their different susceptibility to amygdala kindling, also differed in their kindling rats in other limbic structures such as the hippocampus and piriform/perirhinal cortex (McIntyre, Kelly and Dufresne, 1999). It has been shown that the expression of GABA-A subunits differs in most parts of the limbic system except the hippocampus (Poulter et al., 1999). They showed that in FAST rats compared to control and SLOW rats, the α2, 3 and 5 subunits are highly up-regulated, however the α1 subunit is
significantly down regulated. Since previous studies showed that the α2, 3 and 5 subunits are highly expressed early in development and replaced by the α1 subunit in adulthood (Poulter et al., 1999), the juvenile expression of GABA-A subunits in the FAST rats are presumed to be associated with retained juvenile-like brain qualities and a behavioral profile that includes hyperactivity and impulsivity in adulthood (McIntyre et al., 2002).

FAST and SLOW rats also differed in their behavioral responses. Different behavioral tasks revealed that the FAST rats have poor habituation to the environment. In the learning task such as the Morris water maze, the FAST rats can be easily more distracted by irrelevant cues than SLOW rats (Anisman and McIntyre, 2002). Despite the fact that mating behavior is normally highly dependent upon the male’s response to various sensory cues shown by female (Beach, 1976), the FAST males immediately approach to the females without having those appropriate cues (McIntyre et al., 2002).

Conversely, almost at all ages, SLOW rats show a more adult-typical behavioral phenotype. It has been shown that the amygdala in the SLOW rats releases more noradrenaline in in vivo stress paradigms compare to the FAST rats (Mohapel and McIntyre, 1998). Other studies suggested that the SLOW rats are more expressively fearful than FAST rats. For instance, in familiar and novel environments, in reaction to foot-shock and in fear-based learning tasks, the SLOW rats show greater fear- and anxiety-related response, such as: inhibitory and active avoidance, than FAST rats (Mohapel and McIntyre, 1998).

1.7 Kainic Acid

Kainic acid (KA) was first isolated from Japanese seaweed as an ascaricide. KA has been used extensively in the study of neurobiological diseases for many years. KA is a cyclic analogue of glutamate which is one of the major neurotransmitters in nervous system. Administration of KA to experimental animals, regardless of its route of administration, produces neurotoxic effects by binding to glutamate kainate receptors in the brain, thus increasing neuronal excitation. This neuronal over-excitation induces acute status epilepticus of limbic system in the
animal models (Holmes et al., 1988), which reproduces the behavioral and histological feature of human temporal lobe epilepsy (TLE). However, different responses were reported after systemic administration of KA within a single rat strain (Sperk, 1994). It was also shown that the response to intracranial KA injection varies in different strain of rats (McGeer et al., 1978). Thus variability in genetic factors in the rats influences the degree of sensitivity or resistance to KA-induced seizure. Other studies have shown that intracranial injection of KA in rats can destroy specific neurons with kainate receptors while sparing axons and synaptic terminals (McGeer et al., 1978; Schwarcz and Coyle, 1977).

1.8 Rationale and aims of the study

Seizures are one of the most common neurological disorders that still remain unknown in many aspects. While seizure can occur at any age in at least 2% of the population, they are more common in childhood (~5%) than adulthood. Different kind of seizures has been linked to structural and functional changes in different region of the brain. However, the long term consequence of early-life seizure is not understood completely yet. Understanding these consequences may result in the development of possible interventions and/or pharmacological prevention/treatment.

A decrease in GABA-A and GABA-B receptor-mediated inhibition is a common cause of seizure, however the long term effect of early-life seizure on different GABA-A and GABA-B receptor-mediated inhibition is not completely clear yet. In addition, the effect of different genetic background on different consequences has remained unknown. The purpose of this thesis is to investigate the long-term physiological consequences of early-life seizure in the rat plus the differences among these consequences based on genetic background. For these reasons, we used two strains of Long-Evans rats bred respectively for FAST and SLOW kindling of the amygdala.

It was hypothesized that early-life seizure can induce different long lasting changes in the hippocampal CA1 region in seizure-treated rat pups as compared to controls and the changes may be different for FAST than SLOW kindling strain of rats. It was further hypothesized that
the efficacy of presynaptic GABA-B receptors on glutamatergic excitation on the Schaffer collaterals synapsing with the apical dendrites of CA1 pyramidal cells decrease after early-life seizure.

Synaptic transmission through the major afferents to the CA1 region of the hippocampus was studied by means of paired pulse extracellular recording in vitro. Two set of experiments were designed to test the effect of early-life kainic acid induced seizure in two different genetically background of immature rats.

The first experiment is to study the GABAergic receptor mediated inhibition in the CA1 region of the hippocampal slices at 20 to 45 days after kainic acid induced status epilepticus (SE) in PND 10 rats. It is hypothesized that both GABA-A and GABA-B neuronal inhibition in the hippocampus are altered after the SE in immature rats.

The second experiment was to study the effect of early-life seizure on GABA-B receptor-mediated inhibition in the CA1 region specifically by using baclofen as a GABA-B receptor agonist. It was hypothesized that the presynaptic GABA-B receptor-mediated inhibition is decreased after kainic acid induced seizure at PND 10.
Chapter 2. Materials and Methods

2.1 Animals

All experiments conducted were approved by the Animal Use Committee at Western University (London, Ontario, Canada) based on the guidelines set by the Canadian Council for Animal Care. In this set of experiments, litters of seizure prone (FAST) and seizure resistant (SLOW) rats were bred and raised at Western University. These two new strains of rats were derived from parents belonging to two strains - Long Evans Hooded and Wistar rats. Rats were selected for the fastest or the slowest rats to achieve amygdala kindling with stage-5 convulsive seizure, and then bred within the FAST and the SLOW groups. After 11 generations, the strains were introduced as kindling-prone (FAST) and kindling-resistant (SLOW) (Racine et al., 1999). The colonies of FAST and SLOW rats were transferred to Dr. Leung’s lab by Dr. McIntyre. Pups were housed with their mother until weaning at PND 21. Animals had ad libitum access to water and standard rodent chow and maintained on a 12hr:12hr light:dark cycle commencing at 7:00am. Both male and female pups were used.

2.2 Kainic Acid induced seizure

Seizure was induced in immature rats at PND 10 by injection of kainic acid intraperitoneally (i.p). The rats’ brain at this age can be considered an equivalent to the human brain at age 1-years (Dobbing and Sands, 1973). Both male and female pups with similar weight from the same litter were chosen and assigned as either Control or Seizure group. The rats in the seizure group were injected with 3 mg/kg i.p. dose of kainic acid diluted in saline, and then placed back with their mother. Control rats were injected with the same amount of saline as the Seizure group rats and were also placed back with their mother.
Behaviors were observed in the rat pups from 20 minutes after kainic acid injection (earliest time of seizure onset) to 2 hours after showing a first behavioral seizure. Observed seizures were classified in one of six stages based on their severity (modified from Racine, 1972 and Haas et al., 1990): (I) mouth clonus or chewing, (II) head bobbing, (III) unilateral, alternative or bilateral forelimb clonic jerks, (IV) bilateral forearm clonus, (V) lying down with supine position and 4 limb tonic clonic convulsion, (VI) squeaking and jumping. After 2 hours, the seizure was stopped by injecting diazepam (5 mg/kg i.p.) Seizure-treated rats were marked with a small cut in their left ear, and Control rats were marked with a small cut in their right ear, to make identification easier in adulthood.

2.3 Brain slices preparation

Brain slices were prepared from both male and female seizure-prone and seizure resistant rats at postnatal 30-55 days old, 20-45 days after treatment with kainic acid or saline. Briefly, following deep isoflurane anesthesia, rats were decapitated, the brain was retrieved and placed in cold, oxygenated artificial cerebrospinal fluid (aCSF) which consisted of (in mM): NaCl 124, NaHCO₃ 26, D-glucose 10, KCl 2.4, MgSO₄·7H₂O 2, NaH₂PO₄·H₂O 1.25, and CaCl₂·6H₂O for 1 minute. Then the hippocampus was rapidly dissected (<3 min) and sliced with a tissue chopper. Slices of 450 µm thick were incubated at room temperature (~ 25°C) in oxygenated aCSF. In the next step, the slices were placed in a pre-chamber containing aCSF. Slices used for recording were later transferred onto a nylon mesh in a liquid/gas interface chamber where their undersurfaces were perfused with aCSF (warmed to 32 °C) at a rate of 1-2 ml/min while their upper surfaces were exposed to a humidified 95% O₂ /5% CO₂ gas mixture.

2.4 Electrophysiology recording

Two to four slices were transferred to the recording chamber. Extracellular recordings were taken from CA. Extracellular recording was started 1 hour after the pre-incubation period.
Glass micropipettes (impedance 5-20 Mohm) filled with aCSF were used for recording at the CA1. For CA1 cell body layer and apical dendrite layer recording, the stimulating electrode, a concentric stainless steel bipolar electrode (MCE-100, Rhode Medical), was placed in the stratum radiatum of CA1, near CA3 (Fig. 3A).

**Figure 3.** Schematic diagram to illustrate the field potential recording. A: Stimulating electrode was in the stratum radiatum of CA1 and recording electrode was in the CA1 pyramidal cell layer. B: Stimulating electrode was in the stratum radiatum of CA1 and recording electrode was in the apical dendrite of pyramidal cells (Originally illustrated by Poon et al., 2006).

The stimulus threshold was defined as the lowest stimulus intensity producing a consistently identifiable response. The population spikes (PSs) were recorded followed by activation of commissural Schaffer collaterals from CA1 pyramidal cell layer. PS amplitude was measured as the vertical line dropped from a tangent line connecting the two positive peaks surrounding the negative spike transient (Fig. 4A). Extracellular population excitatory postsynaptic potentials (pEPSPs) were recorded. Following this the activation of Schaffer collaterals synapsing on either CA1 pyramidal cell layer or their basal-dendrites were recorded. The pEPSPs at the cell layer were measured as a maximal slope over 1 ms from its onset to the positive peak (Fig. 4A); at the dendritic layer, the pEPSPs were measured as a maximal negative
slope (1 ms duration) from onset to the maximal negative peak (Fig. 4B). Paired-pulse stimulations were given at interpulse intervals (IPIs) of 20, 30, 50, 100, 150, 200 ms. The signals were amplified and filtered by AXON-1A amplifier, then digitalized and stored in a computer for further analysis offline by a custom program: Integrated Program for Electrophysiological Experiments (IPEE) written by C.Y Yim.

![Figure 4](image)

**Figure 4.** A typical evoked response illustrating the measurements of the slope of the population EPSP and the population spike

In the next set of experiments, extracellular recordings were also made from the apical dendrites of CA1 neurons. Like the previous study, recording started one hour after preincubation period. Glass micropipettes (impedance 5-20 Mohm) filled with aCSF were used for recording population excitatory postsynaptic potentials (pEPSPs) at the stratum radiatum (apical dendritic layer) of hippocampal CA1 neurons. A concentric stainless steel bipolar stimulating electrode (MCE-100, Rhode Medical) was placed in the stratum radiatum, about 250 µm from the recording micropipette (Fig. 1B). Typically, stratum radiatum was stimulated by a pair of pulses to assess paired pulse facilitation of response. The field excitatory postsynaptic potentials (pEPSPs) had to be stable for >15 min in normal aCSF before aCSF containing baclofen (20 µM) was perfused. The pEPSP threshold (minimal stimulus intensity to visually detect an evoked pEPSP) was adjusted to be 30 µA for all slices, by changing the stimulus pulse.
duration. Evoked responses (average of 4 sweeps of pEPSP) were digitalized and stored at 10 KHz by using a 12-bit analog to digital converter, using custom software.

Input–output curves of the extracellular pEPSPs in response to various stimulus intensities (1, 1.5, 2, and 4 times threshold intensity) were obtained during baseline (in normal artificial cerebrospinal fluid (aCSF)) and at 5, 10, 15 and 20 min after the perfusion of the aCSF contained baclofen. After 20 minutes of baclofen perfusion, the drug was washed out with normal aCSF perfusion for 30 minutes. Paired-pulse facilitation (PPF) of the pEPSP was defined and measured by the ratio of the second EPSP slope (E2) to the first one (E1).

2.5 Temperature measurement

The temperature was recorded by a temperature controlling device which was directly connected to the recording chamber. The temperature was maintained between 30-34°C during the whole recording sessions for the all slices.

2.6 Data Analysis

2.6.1 Electrophysiological analysis

The extracellular field potential consists of population of excitatory postsynaptic potential (pEPSP) and population spike (PS) (Wu and Leung, 2001). The population spike amplitude was measured as the vertical line dropped from a tangent line connecting the two positive peaks surrounding the negative spike transient. The population EPSP at the cell layer was measured as a maximal slope over 1 ms from its onset to the positive peak before the onset of PS at the dendritic layer. The first population spike (P1) and the second population spike (P2) were
respectively measured as the PS amplitudes following the 1\textsuperscript{st} and the 2\textsuperscript{nd} pulse, whereas E1 and E2 were respectively the slopes of EPSPs following the 1\textsuperscript{st} and the 2\textsuperscript{nd} pulse.

The ratio of P2 to the P1 was calculated as an estimate of paired pulse inhibition or paired pulse facilitation. When P2 is larger than P1, P2/P1 will be larger than 1 and indicate paired-pulse facilitation (PPF), in contrast when P2 is smaller than P1, the ratio of P2 to P1 will be less than 1 and will be indicative of paired-pulse inhibition (PPI). P1 and P2 amplitudes (in mV) were analyzed from typically average of four sweeps of evoked potential.

2.6.2 Statistical analysis

Data were analysed from 1 to 3 slices for each individual rat, and data were averaged for each rat. All the data is presented as mean ± standard error of the mean (SEM). A Two-Factor Repeated Measures ANOVA was performed (IPI or stimulus intensity as one factor, and rat group as another factor). When necessary, a significant ANOVA was followed by post-hoc Newman-Keuls multiple comparison test. Statistical significance was set at P < 0.05. Comparisons were made between seizure treated (test group) and saline treated (control group).
Chapter 3. Results

3.1 Kainic acid-induced seizure

Kainic acid (3 mg/kg i.p.) was administered to 23 immature rats of PND 10, consisting of 11 FAST rats and 12 SLOW rats, to induce seizure. 23 immature control rats were given equal volume injection of saline, consisting of 11 FAST rats and 12 SLOW rats, to induce seizure. Observation usually began at about 15-20 minutes after injection. In the KA injected rat pups, seizures started with wet dog shakes, forelimb and head clonus at 39.7 ± 1.2 min (n=10) and 23.5 ± 0.6 min (n=10) in SLOW and FAST rats, respectively. The seizure onset times were significantly different between FAST and SLOW rats (t=12.3, n= 14.76; p<0.0001). The seizure immediately progressed to stage IV and V, which were characterized by bilateral forelimb clonus while rearing and lying down in supine position with four-limb tonic-clonic convulsions, respectively.

3.2 Paired-pulse paradigm in the CA1 hippocampus

Recordings were made from in vitro hippocampal brain slices derived from rats 20 to 35 days after kainic acid and saline injection. A total of 46 slices from 26 rats were used for paired-pulse studies, among which 23 slices were derived from FAST rats (10 slices from 6 seizure-treated rats and 13 slices from 7 control rats) and 23 slices were derived from SLOW rats (12 slices from 7 seizure-treated rats and 11 slices from 6 control rats).

Following the delivery of a single stimulus pulse to the Schaffer collaterals, an evoked field response at the hippocampal pyramidal cell layer (stratum pyramidale) was observed, consisting of a single population spike superimposed on a positive wave named as the population
excitatory postsynaptic potential (pEPSP). In some slices the pEPSP was small and at noise level (<0.1 mV/ms), therefore the pEPSP was not measured from all the slices. The stimulus threshold intensity to evoke a visually detectable response for all the slices was adjusted to 30 µA intensity by changing the stimulus pulse duration (range 0.01 to 0.1 ms).

### 3.2.1 Changes in excitatory postsynaptic potential slope and population spike evoked by single pulse

Input-output curves at a fixed inter-pulse interval (IPI) indicated that increasing the stimulus intensity significantly enhanced both the EPSP slope (E1) and the population spike (P1) evoked by a single pulse, in either seizure-treated or control slices of FAST and SLOW rats.

For both the FAST and the SLOW rats group, E1 as a function of stimulus intensity did not show any significant difference between seizure-treated and control slices in both the FAST rats (two factor ANOVA group effect; F(1,11)=1.13, p=0.31; Fig. 5A) and the SLOW rats (two factor ANOVA group effect; F(1,11)=0.31, p=0.59; Fig. 5 B) groups.

---

**Figure 5.** Input/output curve of mean population EPSP (E1) response (+/− S.E.M), recorded at the CA1 cell layer, evoked by the first pulse from seizure-treated (red line) and control (blue line) FAST and SLOW rats. Stimulation intensities were at 1, 1.5, 2, 3 and 4 times response threshold. **A:** population EPSP for FAST rats (7 seizure-treated and 6 control rats; two factor ANOVA group effect; F(1,11) = 1.13, p = 0.31); **B:** population EPSP for SLOW rats (6 seizure-treated and 7 control rats; two factor ANOVA group effect; F(1,11) = 0.12, p = 0.59).
The population spike (P1) versus stimulus intensity plots shows a sigmoid shape curve. The P1 did not show any significant difference between seizure-treated and control slices in the FAST (two factor ANOVA group effect $F(1,11) = 0.86, p = 0.77$; Fig. 6A) and the SLOW (two factor ANOVA group effect $F(1,11) = 0.62, p = 0.45$; Fig. 6B) rats.

**Figure 6.** Input/output curves of population spike (P1) response (mean +/- S.E.M), recorded at the CA1 cell layer, evoked by the first pulse in slices from seizure-treated (red line) and control (blue line) FAST and SLOW rats. Stimulation intensities were at 1, 1.5, 2, 3 and 4 times response threshold. **A:** population spike for FAST rats (7 seizure-treated and 6 control rats; two factor ANOVA group effect; $F(1,11) = 0.86, p = 0.77$); **B:** population spike for SLOW rats (6 seizure-treated and 7 control rats; two factor ANOVA group effect; $F(1,11) = 0.62, p = 0.45$).

At the apical dendrites, stimulation of stratum radiatum evoked a negative field response in the same layer. Similar to the pEPSP slope (E1) at pyramidal cell layer, extracellular recording from apical dendrites showed that the E1 slope evoked by a single pulse increased with stimulus intensity in either the seizure-treated or the control slices, in both the FAST and the SLOW rats. The E1 slope did not show any significant difference between the seizure-treated slices and the
control slices in either the FAST rats (two factor ANOVA group effect F(1,11) = 0.54, p = 0.47; Fig. 7A) or the SLOW ones (two factor ANOVA group effect F(1,9) = 0.12, p = 0.74; Fig. 7B).

**Figure 7.** Input/output curve of mean population EPSP (E1) response (+/- S.E.M) recorded at stratum radiatum evoked by the first pulse from seizure-treated (red line) and control (blue line) FAST and SLOW rats. Stimulation intensities were at 1, 1.2 and 1.5 times response threshold. **A:** population EPSP for FAST rats (7 seizure-treated and 6 control rats; two factor ANOVA group effect; F(1,11) = 0.54, p = 0.47); **B:** population EPSP for SLOW rats (6 seizure-treated and 5 control rats; two factor ANOVA group effect; F(1,9) = 0.12, p = 0.74).

### 3.2.2 Changes in paired pulse population spike response at the pyramidal cell layer

For FAST rats, control slices showed paired-pulse depression of the population spike at all IPIs with paired pulse stimulation, as shown by a representative slice (Fig. 8I). A representative seizure-treated slice from the FAST rat group shows a slight paired-pulse depression at 20 ms IPI, but paired-pulse facilitation at 30 to 200 ms IPI (Fig. 8II). In all the seizure-treated and the control groups of slices, at 4 times threshold stimulus intensity, PPI (= P2/P1 ratio) generally increased from 10 to 100 ms IPI and then decreased slightly from 100 to 200 ms IPI. However, the PPI was significantly larger in seizure-treated slices compared to
control ones (two factor ANOVA group effect; F(1,11)=5.20, p<0.05; Fig. 9). Post hoc Newman-Keuls tests revealed that the PPI of seizure-treated and control rats differed significantly from each other at 20 and 30 ms IPIs. In addition, input-output curve at a fixed IPI of 20 ms showed that the PPI decreased with increasing the stimulus intensity (Fig. 10). The decline of PPI was not significantly different between seizure-treated and control groups, (IPI=20 ms; two factor (group x stimulus intensity) ANOVA group effect; F(1,11) = 0.08, p=0.77) but there was a significant decrease of PPI with stimulus intensity (stimulus intensity effect (F(4,44) =14.43, P<0.0001; Fig. 10).

**Figure 8.** Overlaid traces of paired-pulse population spikes recorded from CA1 region of representative FAST rats, at 20 to 200 ms IPI and 4 times stimulus threshold intensity from A: Control slice, B: Seizure-treated slice. Horizontal line indicates the amplitude of the first population spike (PS1). Dots indicate stimulus artifacts.

**Figure 9.** FAST rats paired-pulse population spike ratio (PS2/PS1) in CA1 (mean +/- S.E.M) as a function of inter-pulse interval (IPI) in kainic acid –induced seizure and saline-injected controls recorded 30-45 days after early-life treatment; stimulus intensity was 4 times stimulus threshold. Seizure and control groups were significantly different from each other (two factor ANOVA group effect; F(1,11) = 5.2, P < 0.05; 7 seizure-treated and 6 control rats; * indicates P<0.05 following Newman-Keuls posthoc test; Inset: Trace of paired-pulse population spikes (PS1, and PS2) was shown at 20ms IPI.
For the SLOW rats at a fixed stimulus intensity of 4x threshold, control slices showed a small paired-pulse depression of population spike at 20 and 30 ms IPI and paired-pulse facilitation at 50 to 200 ms IPI (Fig. 11I). In seizure-treated group, a representative slice shows depression at all IPIs compared with control slices (Fig. 11II). The PPI in seizure-treated group slices was significantly smaller than control group slices (two factor ANOVA group effect; F(1,10) = 6.16, p < 0.05; Fig. 12). Post hoc tests revealed that the PPI of seizure-treated and control rats differed significantly from each other at 20 and 30 ms IPIs. Similar to the FAST rats group at a fixed IPI, PPI declined with the stimulus intensity and the decrease of PPI in the seizure-treated rats was not significantly different from control group (IPI=20 ms; two factor ANOVA group effect; F(1,11) = 0.89, p = 0.36; but PPI was significantly different between IPIs (F(5,55) =13.40, P <0.0001; Fig. 13).

Figure 10. FAST rats input/output curves of the paired-pulse index (PS2/PS1 ratio) of the population spike (mean +/- S.E.M) evoked by paired pulses at 20 ms IPI from seizure-treated (red line) and control (blue line) rats. Stimulation intensities were at 1, 1.5, 2, 3 and 4 times response threshold (7 seizure-treated and 6 control rats; two factor ANOVA group effect; F(1,11) = 0.08, p = 0.77).
Figure 11. Overlaid traces of representative in vitro hippocampal slices of SLOW rats showing paired-pulse population spikes recorded from CA1 region at 20 to 200 ms IPI and 4 times stimulus threshold intensity. A: Control slice, B: Seizure-treated slice. Horizontal line indicates the amplitude of the first population spike (PS1). Dots indicate stimulus artifacts.

Figure 12. SLOW rats paired-pulse population spike ratio (PS2/PS1) in CA1 (mean +/- S.E.M) as a function of inter-pulse interval (IPI) in kainic acid –induced seizure and saline-injected controls recorded 30-45 days after early-life treatment; stimulus intensity was 4 times stimulus threshold. Seizure and control groups were significantly different from each other (two factor ANOVA group effect F(1,11) = 6.16, p < 0.05; 5 seizure-treated and 7 control rats* indicates P<0.05 following Newman-Keuls posthoc test).
3.2.3 Changes in the EPSP paired-pulse index in pyramidal cells

In both FAST and SLOW rats, at fixed stimulus intensity, the EPI (= E2/E1 ratio) did not change with increasing IPI. In the FAST rats, data showed no difference in EPI between the seizure-treated and the control groups (two factor ANOVA group effect; F(1,11) = 2.1, p = 0.17), but EPI was significant different between IPIs (F(5,55) = 2.56, P<0.05; Fig. 14A). In the SLOW rats, collected data did not show any significant difference between seizure-treated slices and the control ones (two factor ANOVA group effect; F(1,11) = 1.81, p = 0.2; Fig. 14B).

At a fixed IPI, the EPI was almost constant across stimulus intensity in all FAST and SLOW rats slices and data did not show any significant difference between the seizure-treated slices and the control slices (Fig. 15A, 15 B).

Figure 13. SLOW rats input/output curves of paired-pulse index (PS2/PS1 ratio) of population spike (mean +/- S.E.M) at 20 ms IPI did not show a significant difference between seizure-treated (red line) and control (blue line) rats. Stimulation intensities were at 1, 1.5, 2, 3 and 4 times response threshold (5-seizure-treated and 7 control rats; two factor ANOVA group effect; F(1,11) = 0.89, p = 0.36).
Figure 14. FAST and SLOW rats paired-pulse population EPSP ratio (E2/E1) recorded at CA1 pyramidal cell layer as a function of inter-pulse interval (IPI) was not significantly different between kainic acid-induced seizure and saline injected controls. Population EPSPs recorded at 4 times stimulus threshold, 30-45 days after early-life treatment. A: E2/E1 ratio for FAST rats (7 seizure-treated and 6 control rats; two factor ANOVA group effect, F(1,11)=2.1, p=0.17); Inset: Trace of paired-pulse population EPSP (E1, and E2) was shown at 20ms IPI. B: E2/E1 ratio for SLOW rats (5 seizure-treated and 7 control rats; two factor ANOVA group

Figure 15. FAST and SLOW rats input/output curves of paired-pulse index (E2/E1 ratio) of population EPSP (mean +/- S.E.M) evoked by different stimulus intensity did not show significant difference between seizure-treated (red line) and control (blue line) FAST and SLOW rats. Recordings made at a fixed paired pulse IPI of 20 ms using stimulation intensities at 1, 1.5, 2, 3 and 4 times respond threshold, on 30-45 days after early-life treatment. A: E2/E1for FAST rats (7 seizure-treated and 6 control rats; two factor ANOVA; F= (1,11), P=0.49). B: E2/E1 for SLOW rats (5 seizure-treated and 7 control rats; two factor ANOVA; F(1,10)=0.31, P=0.59).
3.2.4 Comparing FAST rats versus SLOW rats

Data were collected from total of 13 saline injected FAST and SLOW rats (6 SLOW rats and 7 FAST rats). At fixed stimulus intensity of 4 x threshold response, in the FAST rats the PPI increased with IPI from 20 to 150 ms and then it showed a slight decrease from 150 to 200 ms. In the SLOW rats, the PPI increased with IPI up to 100 ms and then declined at 100 to 200 ms. Data indicated a significant difference between the control FAST rats slices compared to the control SLOW rats slices (IPI=20 ms; two factor ANOVA group effect; F(1.11) = 4.98, p < 0.05; Fig. 16).

As mentioned previously, input-output curves at a fixed IPI showed that increasing the stimulus intensity enhanced both E1 and the P1 evoked by a single pulse in either the FAST rats or the SLOW rats slices. Neither E1 as a function of stimulus intensity (two factor ANOVA group effect F(1,11) = 0.03, p = 0.85; Fig. 17A) nor P1 as a function of stimulus intensity (F(1.11) = 0.03, p = 0.85; Fig. 17B) was different between control FAST and SLOW groups.

Figure 16. Paired-pulse index of population spike (PS2/PS1 ratio) as a function of inter-pulse interval (IPI) was different between slices derived from SLOW (red line) and FAST control (saline-treated) rats (blue line). Stimulus pulse was at 4 times response threshold, recorded 30-45 days after early-life treatment. (7 SLOW rats and 6 FAST rats; two factor ANOVA group effect F(1,11) = 4.98, p <0.05).
3.3 Hippocampal perfusion of baclofen

Data were recorded 20 to 35 days after kainic acid and saline injection from a total of 38 slices from 20 rats, of which 18 slices were from FAST rats (10 slices from 5 seizure-treated rats and 10 slices from 5 control rats) and 20 slices were from SLOW rats (10 slices from 5 seizure-treated rats and 8 slices from 5 control rats). Data were analysed from 1 to 2 slices for each individual rat.

In the CA1 region of the hippocampus, a low-intensity single stimulus at stratum radiatum evoked a negative wave at the same layer which corresponds to the pEPSP (Leung and Au, 1994). The stimulus threshold intensity to evoke a detectable response for all the slices was set to 30 µA intensity by adjusting the stimulus duration (effectively ~0.1 ms duration) during normal aCSF perfusion and then kept the same during baclofen perfusion.
3.3.1 Field EPSP in CA1 stratum radiatum of seizure-treated and control FAST and SLOW rats

Hippocampal slices were recorded, while perfused with normal aCSF and the pEPSP slope (E1) was measured at 2 and 4 times response threshold intensity (60 and 120 µA) for both the FAST and SLOW rats. In the slices from FAST rats, the average pEPSP slope (E1) at 2 times threshold stimulus intensity was 4.37 mV/ms for seizure-treated slices and 3.47 mV/ms for control group slices. At 4 times threshold stimulus intensity, the average E1 was 4.88 mV/ms and 3.74 mV/ms for seizure-treated and control slices, respectively. However, there was not any significant difference between control and seizure-treated groups.

In the SLOW rats, the average of pEPSP slope (E1) evoked by 2 times threshold stimulus intensity was 3.43 for seizure-treated and 4.36 mV/ms for control group slices. At 4 times thresholds stimulus intensity, the average E1 was 2.81 mV/ms and 3.19 mV/ms for seizure-treated and control group slices, respectively.

At a fixed 50 ms IPI, the E2/E1 ratio at different stimulus intensities (30, 45, 60, 90 and 120 µA) was measured for both the seizure-treated and the control slices in either the FAST or the SLOW rats. E2/E1 was not significantly different between seizure and control groups for the FAST rats (two factor (group x IPI) ANOVA group effect; F(1,8) = 0.11, p = 0.74), but EPI was significant different among the IPIs (IPI effect F(4,40) =5.49, P<0.05; Fig. 18A). Similarly, E2/E1 was not significantly different between seizure and control groups for the SLOW rats (two factor ANOVA group effect F(1,8) = 1.69, p = 0.24) but EPI was significant different between IPIs (IPI effect F(4,40) =8.23, P<0.05; Fig. 18B).
3.3.2 Field EPSP in the CA1 region of seizure-treated and control FAST and SLOW rats after baclofen perfusion

In the CA1 neurons of the hippocampus, baclofen (20 µM) perfusion decreased the pEPSP evoked at 2 and 4 times threshold stimulus intensity in either the seizure-treated or the control slices of FAST and SLOW rats. In the FAST rats, by 5 minutes after perfusion, the slope of EPSP decreased until it reached to a minimum level at 10 to 25 minutes in either the seizure-treated or the control group slices. In the control group slices, the E1 value recorded 5 to 25 minutes after the baclofen perfusion was significantly smaller than the baseline in seizure-treated slices (P<0.05, 2 way ANOVA test). 15 minutes after baclofen perfusion the E1 value of control group slices reached to a level of 31.31% of the baseline E1 value but in seizure-treated group the value reached to 61.47% of the baseline. In addition, group data showed that the time course of the change in pEPSP slope after baclofen perfusion (pEPSP slope measured at 5, 10, 15, 20,
25 min after 20 µM baclofen and 30 min after washing out) was significantly different between the seizure-treated and the control group of slices at 2 times threshold stimulus intensity (two factor (group x time) repeated measures ANOVA; group x time interaction F(4,40) = 7.46, p=0.0002; time effect F(4,40) = 2.68, p < 0.001; Fig. 19A). Newman-Keuls post hoc tests revealed that the field EPSP slope of seizure-treated and control rats differed significantly from each other at 5 and 20 minutes after baclofen (P<0.05). The results was significantly different at 4 times threshold intensity (two factor repeated measures ANOVA; time effect F(5,45) = 2.53, p < 0.05; Fig. 19B)

In the SLOW rats, by 5 minutes after baclofen perfusion, the slope of pEPSP in control group of slices declined until it reached a steady level at 10 to 25 minutes. But, in the seizure-treated slices, after a decrease in the pEPSP slope in the first 10 min, the pEPSP slope increased from 10 to 20 minutes after baclofen until it reached a steady level at 25 minutes. The E1 value recorded from control slices reached a level of 27.6% of the baseline E1 value compared to 73.03% of the baseline E1 value seizure-treated slices. Also, the time course of pEPSP change after baclofen perfusion (pEPSP measured at 5, 10, 15, 20, 25 min after 20 µM baclofen) was significantly different between control and seizure-treated slices at 2 times threshold stimulus intensity (two factor (group x time) repeated measures ANOVA group x time interaction, F(4,40)=10.98, p<0.0001; time effect F(4,40) = 26.52, p < 0.001; Fig. 19C). Newman-Keuls post hoc tests revealed that the pEPSP slope of seizure-treated and control rats differed significantly from each other at 5 and 25 minutes (P<0.05). The results was also different at 4 times threshold intensity (two factor, repeated measures ANOVA; group x time interaction F(4,40) = 2.97, p < 0.05; time effect F(4,40)=5. 2, p<0.05; Fig. 19D).
Figure 19. Early-life kainic acid seizure induction reduced the suppressive effect of baclofen on the field population EPSPs in CA1 of FAST rats. Group data in both FAST and SLOW rats showed that E1 was suppressed by baclofen perfusion more in control slices (blue line) than seizure-treated slices (red line) at 2 times threshold stimulus intensity, recording was done at 30-45 days after kainic acid or saline injection at PND 10. Slope of pEPSP was normalized to 100% for each slice before baclofen perfusion (time 0). A: Normalized pEPSP for FAST rats at 2x threshold (5 seizure-treated and 5 control rats; two factor, repeated measures ANOVA; group x time interaction, F(4,40)=7.46, p=0.0002; Time effect, F(4,40) = 2.68, p < 0.0001). Inset, representative slices from kainic acid seizure-treated slice and control group slice of FAST rats. B: Normalized pEPSP for FAST rats at 4x threshold (5 seizure-treated and 4 control rats; two factor, repeated measures ANOVA; Time effect, F(5,45) = 2.53, p < 05). C: Normalized pEPSP at 2x threshold for SLOW rats (5 seizure-treated and 5 control rats; two factor, repeated measures ANOVA; group x time interaction F(4,40) = 10.98, p < 0.0001; time effect F(4,40)=26.52, p<0001). D: Normalized pEPSP at 4x threshold for SLOW rats (5 seizure-treated and 5 control rats; two factor, repeated measures ANOVA; group x time interaction F(4,40) = 2.97, p < 0.05; time effect F(4,40)=5. 2, p<05).
Chapter 4: Discussion

The objective of this thesis was to investigate the long-term changes of early-life seizure on the evoked extracellular paired-pulse responses in the hippocampus of the two genetically different strains of rats, seizure resistant (SLOW) and seizure prone (FAST), after kainic acid induced seizure in immature rats, 10 days after birth, as a model of seizure in children. It was hypothesised that there would be a breakdown in paired-pulse inhibition in the hippocampus due to effects of early-life seizure. It was further hypothesised that consequences could vary based on the different genetic backgrounds.

Results from this study suggest that early-life seizure can affect the functioning of neurons in the CA1 region of the hippocampus. The present study demonstrated that early-life kainic acid-induced seizures had long lasting effect on hippocampal electrophysiology in both FAST and SLOW rats of ~PND 40-55 and this effect was different based on the different genetic background. An examination of paired-pulse evoked response at CA1 region showed that PPI was affected by kainic acid-induced seizure differently in the hippocampal CA1 of the FAST rats compared to the SLOW rats. Early-life seizure did not significantly change the EPI in either the FAST or the SLOW rats.

4.1 Seizure induced by kainic acid in immature rats

The present results confirm that kainic acid-induced prolonged behavioral seizures in immature rats. The majority of induced seizures were quite severe, rated as stage four (bilateral forelimb clonus while rearing) and stage five (four limb tonic-clonic convulsion while supine) in both FAST and SLOW rats. However, by injecting the same amount of kainic acid the mortality rate in FAST rats was higher than SLOW rats. The seizure onset latency was significantly different between immature FAST rats compared to SLOW rats, which is consistent with the result of adult FAST and SLOW rats (Gilby et al., 2005). Previous studies indicated that early postnatal seizure have effects on the development circuitry and can modify development of
synaptic connections in hippocampal circuits (Durand et al., 1996). It was shown that early postnatal (P1-P15) kainic acid-induced seizure disrupted the normal neuronal activity in dentate gyrus by inducing a long-term increase in paired-pulse inhibition (Lynch et al., 2000). In adult rats, two weeks after kainic acid injection, both early and late inhibition was enhanced in the dentate gyrus, but kainic acid injection in rats at PND 16 induced no subsequent change in inhibition (Haas et al., 2004). In contrast to a spatial maze performance deficit induced by kainic acid injection in immature rats (Lynch et al., 2000), another study reported no evidence of cognitive and morphological consequences of kainic acid-induced seizure even by multiple doses of kainic acid in rats younger than day 26 (Sarkisian et al., 1997). Maytal and colleagues (1989) reported that children who experienced status epilepticus have better survival rate as compared to adults who experienced status epilepticus. Even children with repeated seizures have a low mortality rate of about 3-4% in childhood and 6-7% twenty years after diagnosis (Camfield and Camfield, 2002).

It is also important to consider some variability between kainic acid injected rats in their development of seizure stages. Some rats achieved stage four and five seizures quickly, while others, even from the same litter, took a long time to reach these stages. Compared to kindling, which induced seizure through electrical stimulation of the brain, our model does not require surgery to implant stimulating electrodes. Thus, inducing seizures with kainic acid is an expedient and effective method of inducing seizure.

It is well understood that the immature brain is not simply a small version of the adult brain. Although the immature brain is more prone to acute seizure compared to the adult brain, it is less susceptible to pathological damage of seizure in both animal models and human being. Pronounced cell loss in the hippocampus, amygdala, piriform and entorhinal cortex was reported after status epilepticus induced by kainic acid or pilocapine in adults but not in immature rats before the second postnatal week (Nitecka et al., 1984; Sperber et al., 1991). The present animal model used PND 10 immature rats, which corresponded with age 1 to 3 years in children (Avishai-Eliner et al., 2002). Thus, kainic acid induced seizures in PND 10 rats may be an appropriate model for early-life seizures in children.
4.2 Changes in population EPSPs

In both the CA1 cell layer and apical dendrites, the rate of rise of the pEPSP evoked by the first pulse (E1) did not show any significant difference between seizure-treated and control groups, in either FAST or SLOW rats. As expected, E1 increased significantly with increasing the stimulus intensity in both seizure-treated and control slices of both FAST and SLOW rats. An increase in the E1 in CA1 following increasing single pulse stimulation has been previously reported (Leung and Fu, 1994).

Similarly, at fixed IPI, the paired-pulse EPSP ratio (E2/E1) in relation to stimulus intensity did not show any significant difference between kainic acid-induced seizure and control rats, for either FAST or SLOW rats. In addition, no significant difference was found in the E2/E1 ratio recorded at the CA1 pyramidal cell layer, for IPIs ranging between 20-200 ms. Presynaptic facilitation of EPSPs in vitro was typically reported at all IPIs <200 ms (Muller and Lynch, 1989; Zhao and Leung, 1991; Leung and Fu, 1994). The unaltered E2/E1 ratio suggests a lack of change in presynaptic facilitation of EPSPs between seizure-treated and control rats in the present study. The relative lack of changes of paired-pulse EPSP after our seizure model contrasts with general increase in paired-pulse pEPSP at the basal and apical dendrites of CA1 after kainic acid seizures in normal Long-Evans adult rats recorded under urethane anesthesia in vivo (Wu and Leung, 2003).

4.3 Changes in population spike responses

The amplitudes of P1 at a fixed stimulus intensity with respect to response threshold was not significantly changed in seizure-treated rats compared to control groups, in either FAST or SLOW rats. While preparation of in vitro slices may alter the threshold and response of individual neurons that participated in E1 and P1, the results on P1 and E1 discussed above suggest that orthodromic excitation and the spike threshold of CA1 neurons were not greatly altered by early-life seizures.
The ratio of P2 to P1 was used to examine paired-pulse inhibition and paired-pulse facilitation (Fu and Leung, 1994). At 30-45 days after treatment, paired-pulse responses in CA1 were different between rats that had kainic acid-induced early-life seizure as compared to control rats, in either FAST or SLOW rats. In the FAST rats, a significant decrease in early PPI (20-30 ms IPIs) in CA1 was found after early-life seizure. In the hippocampus, GABA-A receptors are responsible for FAST (<100 ms) postsynaptic inhibition, and increase in P2/P1 at <100 ms IPI was found after blockade of GABA-A receptors (Fu and Leung, 1994; Steffensen and Henriksen 1991; Leung et al., 2008). Thus, we interpret that the higher P2/P1 ratio in the seizure-treated FAST rats compared to the controls was caused by a decrease in postsynaptic GABA-A receptor inhibition. In the SLOW rats, an opposite result was found for PPI of <50 ms, in that seizure-treated rats showed a lower P2/P1 ratio as compared to control rats. This is interpreted as an increase in the postsynaptic GABA-A receptor inhibition after early-life seizures in SLOW rats.

At fixed 20 ms IPI, the input-output curve of the P2/P1 ratio did not show any significant difference between seizure-treated and control slices in either FAST or SLOW rats. The lack of difference between seizure and control slices indicates that the change of PPI with stimulus intensity was not different between seizure and control rats, and this effect may obscure the change in PPI following high-intensity stimulation at 20 ms IPI. Only high-intensity stimulation was able to induce paired-pulse inhibition that was different between seizure and control groups. At low intensity, paired-pulse facilitation of the pEPSP was an important factor of increasing P2/P1 ratio, and recruitment of synchronously firing neuronal units likely increased P2 more than P1, causing even larger PPI than EPI (Leung and Au 1994, Leung and Fu 1994). At high intensity stimulation, all the units were fired during P1, however P2 can still be larger due to increase in synchrony among unit firing during P2 (Leung and Au, 1994; Leung and Fu, 1994).

The Cl- mediated GABA IPSP reached its peak action at 10-50 ms and lasted about 100 ms duration (Avoli and Curtis, 2011). A change in GABA-A receptor-mediated responses can be compensatory, i.e., it may repair or restore normal function or it may lead to postictal dysfunction such as cognitive dysfunction or epileptogenesis. Loss of GABAergic interneurons is a hallmark of pathology in some types of focal seizure (Briggs and Galanopoulou, 2011). Many studies have shown that prolonged seizure can lead to interneuronal loss; however such effects are age dependent, i.e., even three episodes of seizures during first week of rats’ life do
not injure GABAergic neurons (Galanopoulou, 2008), but cell death becomes a progressive phenomenon feature as the age increase (Leite et al., 1996; Haas et al., 2001; Nitecka et al., 1984). Even without loss of GABAergic interneurons, early-life seizure can disrupt the physiological function of GABA-A receptor system.

Unlike the adult, in which the physiology of GABA-A receptor-mediated signaling has been well studied, developmental research in the brain maturation period is more complicated because of evolving changes which normally occur during that period (Laurie et al., 1992). Different types of seizures selectively affect the expression of GABA-A receptors subunits. It was reported that kainic acid-induced seizure at PND9 rats causes the preservation of immature pattern of GABA-A receptors complex (less α1, more α2 and α3 subunits) on the third postictal week, and the immature inhibition was characterized by slower IPSC and less sensitivity to benzodiazepines (Leuren et al., 2005). Similarly, a decreased α1 subunit expression and decreased amplitude of GABA-A receptor-mediated IPSCs were reported after recurrent flurothyl-induced seizures at PND1-10 (Isaeva et al., 2006; Ni et al., 2005). The age at onset of seizure may have a key role in defining the final composition of GABA-A receptors. Lithium-pilocarpine status epilepticus at PND 10 enhanced α1 subunit expression in the dentate granule cells in adulthood; but status epilepticus at PND 20 decreases α1 subunit expression in adulthood (Zhang et al., 2004; Raol et al., 2006). Alpha subunits affect the affinity of GABA-A receptors for benzodiazepines ligands, such that receptors which contain α1,2,3, and 5 show a high affinity for a number of benzodiazepines, such as flunitrazepam, diazepam and clonazepam (Pritchett et al., 1989; Von Blankenfeld et al., 1990), while α4 containing receptors have no affinity for benzodiazepines binding site agonists (Wisden et al., 1991). In addition, it was reported that pilocarpine-induced status epilepticus increased α1 and decreased α4 GABA-A receptor subunits (Brooks-Kayal et al., 1998). Hippocampal GABA-A receptor gene expression alteration was reported in kainic acid-induced seizure in adult rats (Friedman et al., 1994). Hippocampal kindling in adult rats was inferred to induce GABA-A receptors loss of function in CA1 without affecting late GABA-B receptor-mediated inhibition (Tsai et al., 2008).

Previous studies on differential expression of GABA-A receptor subunits revealed that the expression profile of α subunits in the FAST rats is similar to the late embryonic or early postnatal period of normal rats, which show high expression α2, α3 and α5 subunits and low
expression of \( \alpha_1 \) subunits (Laurie et al., 1992; Poulter et al., 1992). In contrast, in SLOW rats, there was an under-expression of \( \alpha_2, \alpha_3, \) and \( \alpha_5 \) subunits and an over-expression of the \( \alpha_1 \) subunit (Poulter et al., 1999). However, further studies revealed that there are no significant differences in \( \alpha \) subunits expression of GABA-A receptors in the hippocampus of adult FAST and SLOW rats (Poulter et al., 1999).

In another study, lithium-pilocarpine induced \textit{status epilepticus} in adult animals was shown to depolarize E-GABA by enhancing the ratio of NKCC1 over KCC2 activity and altering it to immature pattern of KCC balance as a long-term effect (Rivera et al., 2004; Li et al., 2008). In the neonatal rat hippocampus, one hour after kainic acid injection, KCC2 is reshuffled toward the plasma membrane and increase its capacity to export Cl- (Khirug et al., 2010) that results more negative E-GABA, which can contribute to the ability of neurons to stop seizures. Early-life seizure can change the direction of GABA-receptor maturation. That may result in a long-term change of the strength of GABA-A receptor-mediated inhibition (Briggs and Galanopoulou, 2011).

Changed PPI in different regions of hippocampus was also reported in other seizure models of both adult and immature rats. Adult kindling increased the PPI in dentate gyrus (Jonge and Racine, 1983; Tuff et al., 1983), but decreased it in either CA1 or CA3 regions of hippocampus (Zhao and Leung, 1991, 1992; Kamphuis et al., 1988; Tsai et al., 2008). In neonatal rats, an enhanced PPI in the dentate gyrus was reported after early-life kainic acid induced-seizure (Lynch et al., 2000). It was suggested that increase in dentate gyrus inhibition can be a compensatory response to seizures (Lothman et al., 1992; Wu and Leung, 2001).

The present study found that early paired-pulse inhibition in CA1 was low in control SLOW rats and high in control FAST rats. Conversely, paired-pulse inhibition in CA1 was high in seizure SLOW rats and low in seizure FAST rats. The early paired-pulse inhibition was blocked by GABA-A receptor antagonist. In addition, further studies are needed to identify the underlying mechanism of seizure-induced changes in paired-pulse inhibition in both FAST and SLOW rats. Direct recordings of GABA-A receptor-mediated IPSP/IPSC may provide confirmatory evidence of opposite changes in GABA-A inhibition in FAST and SLOW rats.
4.4 Alteration of GABA-B receptor function after early-life seizure

We showed that the effect of 20 µM baclofen on suppressing pEPSP in CA1 hippocampal neurons was reduced in kainic acid-induced seizure model as compared to control rats in either FAST or SLOW rats. We found that this reduction at 60 and 120 µA stimulus intensities at 2 and 4 times threshold stimulus intensity, more than 45 days after seizure induction. These results are consistent with a previous study in our lab, in which 10 µM baclofen induced a decrease in efficacy of presynaptic GABA-B receptors on 1 or 21 days after partial hippocampal kindling (Poon et al., 2006). Baclofen affects both postsynaptic and presynaptic GABA-B receptors of the CA1 pyramidal cells (Poon et al., 2006; Olpe et al., 1982). Baclofen hyperpolarized the postsynaptic membrane by opening the postsynaptic K+ channels (Poon et al., 2006). The latter effect on CA1 pyramidal cell GABA-B receptor-mediated conductance was likely small in comparison to the presynaptic inhibition of EPSPs (Poon et al., 2006). In addition, various studies have concluded that baclofen’s main effect in suppressing the pEPSP was presynaptic (Ault and Nadler, 1982; Olpe et al., 1982; Poon et al., 2006). A decrease in presynaptic GABA-B receptor-mediated efficiency in CA1 was reported after other models of status epilepticus, i.e., a decrease in presynaptic but not postsynaptic GABA-B receptor function at glutameric terminals was reported after partial hippocampal kindling (Buhl et al., 1996; Wu and Leung, 1997; Poon et al., 2006) and twenty four hours after pilocarpine-induced status epilepticus (Chandler et al., 2003). Similarly, the same decrease of efficacy was shown in the basolateral amygdala after amygdala full kindling (Asprindini et al., 1992). Disruption in both presynaptic and postsynaptic GABA-B receptor-mediated inhibition was shown in CA1 in vitro studies after a status epilepticus model which was induced by continuous hippocampal kindling (Mangan and Lothman, 1996). Partial hippocampal kindling can induce a decrease in presynaptic GABA-B autoreceptor efficacy (Wu and Leung, 1997) and an increase in electrically evoked postsynaptic GABA-B receptor current in CA1 (Liu and Leung, 2003). In dentate gyrus, status epilepticus induced by kainic acid in adult rats reduced GABA-B function in inhibitory neurons and enhanced paired-pulse inhibition (Haas et al., 1996). In one study, data did not show any change in dentate inhibition after kainic acid-induced seizure in immature rats PND16 (Haas et al., 2001), but in another one an enhancement of GABAergic dentate inhibition was reported.
after kainic acid-induced seizure in immature rats on PND1, 7, and 14 (Lynch et al., 2000). Increase in GABA-B receptor-mediated IPSCs was reported after CGP55845A-induced seizures in immature rats (Qu et al., 2010). It has been reported that there is indeed a relationship between losses of GABA-B receptor-mediated inhibition and chronic epilepsy (Wasterlain et al., 1996).

At a molecular level, GABA-B receptor expression was found to be decreased in several seizure models. Using in situ hybridization and immunocytochemical staining in the kainic acid-induced seizure model, was shown that mRNA levels of GABA-B receptor subunits in the hippocampus were attenuated at 6-12 hours after seizure, and gradually returned to control level after 24 hours to 30 days (Furtinger et al., 2003a). In addition to alteration of expression of GABA-B receptors in experimental seizure models, GABA-B receptor binding and mRNA levels were decreased in CA subregions and dentate gyrus, while increased in subiculum in human TLE patients (Furtinger et al., 2003b; Billinton et al., 2001a; Billinton et al., 2001b). Kainic acid seizure induction in adult mice produced rapid (1 day) and long-lasting (3 months after seizure) decrease in GABA-B receptor subunits expression in the CA1 and CA3 areas of the hippocampus (Straessle et al., 2003). Hyperthermia in immature rats caused a decrease of expression GABA-BR2 receptors which was accompanied with decreased binding of GABA-BR1a and –R2 subunits that persisted for 30 days (Han et al., 2006).

Conversely, other experimental seizure models have suggested an increase of expression of GABA-B receptors. Electroshock induced seizure in rats upregulated GABA-B 1b mRNA level (Billinton et al., 2000), and hippocampal kindling in rats caused a transient increase in GABA-B receptor-1 and 2 mRNA in the hippocampus which returned to control level 4 weeks after the seizure (Kokaia et al., 2001).

As mentioned above, disruption in both presynaptic and postsynaptic GABA-B receptor-mediated inhibition was reported in CA1 following different seizure models and it has been postulated that there is indeed a causal relationship between loss of GABA-B receptor-mediated inhibition and seizure. However, there are several important factors that should be considered to interpreting the results together. Firstly, it should be noted that a decrease in GABA-receptor function may not correspond to a loss in receptor expression, since the function and expression have not been shown to relate directly to each other (Francis et al., 1999). Secondly, the signalling pathway of GABA-B receptor in different seizure models has not been systemically
studied (Couve et al., 2000). Finally, previous studies of GABA-B receptor expression did not distinguish between presynaptic and postsynaptic receptors or whether receptors are expressed on principal cells or interneurons. The results of this study established a loss of efficacy of presynaptic GABA-B receptors, however detailed mechanism of the kainic acid-induced decrease in efficacy of the presynaptic GABA-B receptor is still not well known and further studies are required.

4.5 Long-lasting changes of inhibition by early-life seizures

This study showed a long lasting decrease (up to 45 days after early-life seizure) in presynaptic GABA-B receptor-mediated inhibition in the hippocampal CA1, based on the electrophysiology studies in vitro after early-life seizures induced by kainic acid.

Although there is still some debate regarding a long lasting decline in GABA-mediated activities in epilepsy (Wu and Leung, 1997), there is some evidence which suggest recurring seizures in both adult and immature brain can change inhibition in the hippocampus by affecting GABA functioning for a long time. In adult animals, repeated hippocampal afterdischarges applied in a partial kindling model caused a decrease in efficacy of GABA-B autoreceptors in the CA1 region (Wu and Leung, 1997). Recurrent seizures produced by kindling were also shown to cause a long-lasting reduction in CA1 GABAergic inhibition (Kapur et al., 1989). In immature rats, the evidence for long-lasting alteration in GABAergic inhibition is less common. However, an enhanced excitability in the hippocampus has been reported following seizure, which is assumed to be as a result of decreased inhibition (Wasterlain and Shirasaka, 1994). In addition, it has been shown that seizures in the immature brain increase seizure susceptibility and epileptogenesis (Cilio et al., 2003; Tsai et al., 2008). Increasing evidence from various types of animal studies suggests that adverse effects of seizures in early age are primarily due to their interference with brain development rather than cell loss, as developing networks are quite resistant to brain damage have long lasting changes (Ben-Ari and Holmes, 2006). Many cognitive and/or behavioral changes, like long-term changes in learning and memory, due to changing in brain coactivity, dendritic morphology, and receptor and ion channels differences are
not paralleled by cell loss (Holmes, 2005). Some studies suggested mossy fiber sprouting and other alteration in neuroreactive connections caused by recurrent immature seizure are not accompanied by cell loss (Ben-Ari and Holmes, 2006). This could indicate that perhaps construction of network pattern or migration of receptors is affected by seizure rather than structural changes (Ben-Ari and Holmes, 2006).

In human, it was suggested that decreased postsynaptic GABA-B receptor function in the brain increases epileptogenesis (Leung et al., 2005a; Tsai et al., 2008). There are increasing evidence from studies on immature animals suggesting that repeated seizures may induce long-lasting changes (Ben-Ari and Holmes, 2006). These long-lasting changes may include dysfunction in GABA-B receptor-mediated inhibition.

4.6 Conclusion

In conclusion, this study has found significant long-term changes occur in the hippocampal CA1 following early-life kainic acid-induced seizure, and these changes seem to involve both GABA-A and presynaptic GABA-B receptor-mediated inhibition. In addition, our study demonstrated that the long-lasting effect of seizure on GABA-A receptor-mediated inhibition could be different based on different genetic background of the SLOW and the FAST rats.

Long-term extracellular field potential recording at the CA1 region of hippocampus indicated that while GABA-A receptor-mediated inhibition was decreased in the seizure-treated FAST rats; it was increased in the SLOW rats. However, the effect of 20 µM baclofen perfusion on both FAST and SLOW rats was the same, and baclofen activated stronger presynaptic GABA-B receptor-mediated inhibition in control groups as compared with seizure-treated groups, in either FAST or SLOW rats.

This study demonstrated alteration in GABAergic mediated-inhibition following early-life seizure. This result suggests a cause-effect relationship between seizure and long-lasting physiological changes in GABAergic function in the hippocampus.
4.7 Future studies

While this thesis has advanced the understanding of the long-lasting consequences of early-life seizures on GABA-A receptors, the mechanisms whereby kainic acid–induced seizures may change GABA-Aergic synaptic transmission in immature brain are unclear. In addition, it will be interesting to study the changes in GABA-A subunits differences between seizure-treated and control rats in future. Furthermore, behavioral studies can be helpful to make better understanding of long-lasting effect of early-life seizures on memory and learning.
References


Mason, S. T., Corcoran, M. E. (1979). Seizure susceptibility after depletion of spinal or cerebellar noradrenaline with 6-OHDA. Brain Research, 166(2), 418-421.


Muller, D., Lynch, G. (1989). Evidence that changes in presynaptic calcium currents are not responsible for long-term potentiation in hippocampus. Brain Research, 479(2), 290-299.


Curriculum Vitae

Amir A. Mohseni Zonoozi

Research Interests:
Seizure, Neuroelectrophisiology, Regenerative medicine, Spinal cord injury, Movement disorders

Educations:

I. University of Western Ontario, London, Ontario, Canada  
   M.A.Sc., Neuroscience (Jan, 2012- Jan, 2014)  
   GPA: 85/100 (Until now)

II. University of Uppsala, Uppsala, Sweden  
    M.A.Sc., Medical Science (2010-2012) (Thesis was defended at 2013)  
    GPA: NA

III. Medical school of Azad University, Tehran, Iran  
     M.D., Doctorate of Medicine (2001-2008)  
     Internship GPA: 17.32 / 20  
     Total GPA: 15.98 / 20  

Honors & Awards & Grants:

- Western Graduate Research Scholarship (WGRS, $10,800/y), 2011- Present
- Western Stipend Scholarship ($8,500/y), 2011-Present
- UGSBR (Uppsala Graduate School of Biomedical Research) (75,000 SEK/y), 2011
- Tehran Azad Medical University entrance exam for M.D., ranked 121 among nearly 6000 participants (2001)
- National University entrance exam for M.D in Veterinary, total ranked 910 among nearly 300,000 participants (2001)

Research Experiences:

1. I worked on exploring long term consequences of early life status epilepticus in both seizure-prone and seizure-resistant pup rats by electrophysiology recording of hippocampus slices.
2. I worked as a project student at cancer and vascular biology department of Rudbeck institute, Uppsala University (June, 2011- Jan, 2012). I was interested to investigate the role of microglia and inflammatory monocytes in glioma tumor progression. Moreover, I investigated the involvement of semaphorins (axon guidance molecules) in glioma tumor progression.

3. I worked as a project student at Regenerative Neurobiology group in department of neuroscience, Uppsala University since February until June 2011. The overall aim of our group researches is to promote functional recovery following nervous system injuries by using stem cells.

4. My M.D thesis is: “The prevalence of Neuropathy in Patients with Chronic Renal Failure”.

Referees:

- Dr. Stan Leung . Professor, University of western Ontario sleung@uwo.ca
- Dr. Paul Gribble. Professor, University of western Ontario paul@gribblelab.org