In compliance with the
Canadian Privacy Legislation
some supporting forms
may have been removed from
this dissertation.

While these forms may be included
in the document page count,
their removal does not represent
any loss of content from the dissertation.

An Examination of Kainic Acid induced *Status Epilepticus* in seizure-prone (Fast-kindling) and seizure-resistant (Slow-kindling) rats.

By

Angelika Maria Goncalves DaSilva, B.Sc. Honours.

A thesis submitted to the Faculty of Graduate Studies and Research, and the Department of Psychology in partial fulfillment of the requirements for the degree of Masters of Science (Neuroscience)

Angelika Goncalves DaSilva © 2003

National Library
of Canada

Bibliothèque nationale
du Canada

Acquisitions and
Bibliographic Services

Acquisitions et
services bibliographiques

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 0-612-88769-3

Our file Notre référence

ISBN: 0-612-88769-3

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

Canada

The undersigned recommend to the
Faculty of Graduate Studies and Research
Acceptance of the thesis:

“An Examination of Kainic Acid induced *Status Epilepticus* in seizure-prone (Fast-kindling) and seizure-resistant (Slow-kindling) rats.”

Submitted by

Angelika Maria Goncalves DaSilva

In partial fulfillment of the requirements
for the degree of
Masters of Science (Neuroscience)

Chair

Thesis Supervisor, Carleton University

May, 2003

Abstract

Seizure susceptibility was examined in seizure-prone (Fast) and seizure-resistant (Slow) rat strains using the kainic acid (KA) model of temporal lobe epilepsy (TLE). Following a systemic injection of 20 mg/kg KA, behavioral outcomes, such as latency to and severity of status epilepticus (SE), were compared between the strains. Results demonstrated that Fast rats exhibited a shorter latency to SE, an increased number of convulsive episodes during SE, increased seizure severity and increased neuronal damage evident 4 weeks following the SE episode when compared to Slow rats. In a second experiment, analysis of EEG activity in four brain structures following KA injection demonstrated that although the two strains exhibited distinct behavioral profiles in response to KA, there was a certain level of homogeneity in the EEG pattern recorded. The results obtained from these studies further validate the use of these two strains to model the forces that promote versus oppose TLE, which will allow for better understanding of the mechanism(s) underlying this disease.

Acknowledgements

I am grateful to everyone who has helped me face and conquer the many challenges that have occurred during the two years that it has taken me to complete this thesis. I am indebted to both Dan McIntyre and Krista Gilby for their wonderful guidance and support during my graduate training at Carleton. I would especially like to thank Krista for giving me the opportunity of obtaining training at an international institution.

Over the past two years I have accomplished a lot and this is in part due to the great people who have helped me along the way. Thanks Tina, Pete and Theresa for all your help. You are amazing and I am grateful to have had the chance to work with you. I would also like to thank the girls, Andrea, Khara and Victoria, for all their help. Without you I would still be mounting sections. You'll all get your poems eventually!

I owe the most to my family because without their enduring support I would not have made it past my first day of school. Thanks Frankie for protecting me in the playground and making sure I am always safe. Eu tambien quero agradecer a minha mãe por toda a ajuda que ella me deu. A minha mãe estava sempre bem disposta para me ajudar en financial, emociunal e moral para eu ter curagen. A special thanks to R.A.B for always being available when I needed him, providing me with much-needed support and sugar loaded treats.

You need friends and family to survive and I am glad to have both. Thank you everyone!!!

Table of Contents

	Page
Acceptance Sheet	ii
Abstract	iii
Acknowledgements	iv
Table of Contents	v
List of Tables	vi
List of Figure	vii
List of Abbreviations	ix
Introduction	
Temporal Lobe Epilepsy	1
Experimental Models of Epilepsy	3
Fast and Slow Kindling Rats	7
Study's Objective	9
Experiment # 1	
Methods	10
Results	12
Experiment # 2	
Methods	35
Results	38
Discussion	60
References	70
Appendix 1	80

List of Tables

Table	Description	Page
1	The response profiles of naïve Slow and Fast rats after being systemically injected with 20mg/kg KA and the proportion of animals in each group.	16
2	The response profiles of Slow and Fast rats with implanted electrodes after being injected with 20 mg/kg KA and the proportion of animals in each group.	43
3	The distribution of c-Fos protein in Slow and Fast rats with implanted electrodes one hour after the termination of seizure activity.	58

List of Figures

Figure 1 – Average latency to SE ($\pm$ SEM, min) between Slow and Fast rats systemically injected with 20mg/kg Kainic acid.	 17
Figure 2 – Average number of wet dog shakes (WDS) in Slow and Fast rats before and after the onset of KA-induced SE.	 19
Figure 3 – Average number of entries into stage 3/4/5 behavioural seizures ($\pm$ SEM) seen in Slow and Fast rats during KA-induced SE.	 21
Figure 4 – Average percent duration of SE ($\pm$ SEM, %) in Slow and Fast rats.	 23
Figure 5 – Cresyl violet Photomicrograph of the dorsal hippocampus of Slow and Fast rats that were systemically injected with 20mg/kg KA but did not subsequently develop behavioural SE.	 25
Figure 6 – Cresyl violet photomicrograph of the hippocampus of Slow rats that were systemically injected with 20mg/kg KA and that subsequently developed SE.	 27
Figure 7 – Cresyl violet photomicrograph of the ventral hippocampus of Slow and Fast rats that were systemically injected with 20mg/kg KA and developed SE.	 29
Figure 8 - Cresyl violet photomicrograph of the hippocampus of Fast rats that were systemically injected with 20mg/kg KA and subsequently developed SE.	 31
Figure 9 – Fluoro-Jade photomicrograph of the piriform cortex of a LEH rat and a Slow rat that developed SE after being systemically injected with KA.	 33
Figure 10 – Schematic diagram taken from Paxinos and Watson (1986) showing the electrode tips of each rat in the first series of KA treated animals.	 44
Figure 11 – Schematic diagram taken from Paxinos and Watson (1986) showing the electrode tips of each rat in the second series of KA treated animals.	 46

Figure 12 - Average latency to SE ($\pm$ SEM, min) between Slow and Fast rats systemically injected with 20mg/kg Kainic acid with and without implanted electrodes.

..... 48

Figure 13 - Average number of entries into stage 3/4/5 behavioural seizures ($\pm$ SEM) seen in Slow and Fast rats with and without implanted electrodes during KA-induced SE.

..... 50

Figure 14 - Average percent duration of SE ($\pm$ SEM, %) in Slow and Fast rats with and without implanted electrodes during KA-induced SE.

..... 52

Figure 15 – The EEG patterns of Slow and Fast rats with implanted electrodes in the orbital/FMC, piriform cortex, amygdala and hippocampus.

..... 54

Figure 16 – A montage of region c-Fos protein expression from animals approximately one hour after SE termination.

..... 56

List of Abbreviations

AD	After discharge
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
BBB	Blood brain barrier
DG	Dentate gyrus
EEG	Electroencephalogram
GABA	γ -aminobutyric acid
GEPR	Genetically epileptic prone rat
i.p.	intraperitoneal
KA	Kainic acid
LEH	Long evans hooded
mRNA	messenger ribonucleic acid
NMDA	N-methyl-D-aspartate
PBS	Phosphate buffer saline
SE	Status Epilepticus
SEM	Standard error of the mean
TLE	Temporal Lobe Epilepsy
WDS	Wet dog shake
WF	Wistar Furth

Introduction

Epilepsy

Epilepsy is a neurological condition characterized by recurrent seizure activity within the brain, with an approximate prevalent rate of 1% of the general population (McNamara, 1994). Typically the acquisition of this disorder arises through heredity or as a consequence of prior head trauma, both of which are likely to contribute to the synchronous firing of neurons (Fisher, 1989). Regardless of its origin, seizures can be classified under two major categories: *partial* and *generalized* seizures (Gaustaut, 1973). Partial seizures initially involve only a single focus within a localized region of one hemisphere of the brain, and are demonstrated either by maintaining consciousness (simple partial seizures) or exhibiting a loss of consciousness (complex partial seizures) during the epileptic episode (Gaustaut, 1973). In contrast, generalized seizures recruit widespread involvement of both hemispheres from the onset of the epileptic episode, and often culminate in convulsive tonic-clonic seizures (repeated uncontrolled tonic and then rhythmic movements of the limbs).

A particularly unique and debilitating form of temporal lobe epilepsy (TLE), known as Status Epilepticus (SE), or simply *status*, can be elicited in a short amount of time, and is often characterized by prolonged and enduring periods of convulsive seizures, which in some cases has the ability to induce severe brain damage (Gaustaut, 1973). Generally, it is not uncommon for patients with SE to have seizures that last 30 minutes or more at various time intervals in their lives. In fact, depending on the

methodological approach used for data acquisition, approximately 1.3 - 16% of all epileptic cases are, unfortunately, diagnosed with SE (Hauser, 1983).

An epileptic patient can display and be diagnosed with either *convulsive* or *non-convulsive* status (Hauser, 1983). Convulsive status, as the name suggests, is manifested by behavioural seizures involving the peripheral motor system, whereby limbs make involuntary, uncontrolled movements over a prolonged period of time (over 30 minutes). Non-convulsive status can be subdivided into two categories: *absence* and *partial complex* status. Patients with absence status tend to exhibit longer moments of severe confusion, which can end abruptly with a brief generalized tonic-clonic seizure. Patients diagnosed with partial complex status show impaired consciousness with epileptiform activity that shifts from one hemisphere to the other. As previously mentioned, epilepsy is a condition that may take on many forms. However, an important point to address is precisely how these disorders evolve. A number of serious injuries, including major head trauma, stroke, infection, benign or malignant tumors can serve as potential triggers for epilepsy, particularly during the initial insult but also many months or years after, and have a tendency to compromise the integrity of limbic structures within the temporal lobes. Some of the symptoms often demonstrated by patients are complaints of visceral discomfort, flushing, tingling, cognitive-perceptual distortions such as *deja-vu* or vertigo, and in some cases, awareness and memory acquisition are perturbed (Gloor et al., 1982; King and Ajomne-Marsan, 1977). Clinical epilepsy research over the past century has greatly advanced our understanding of this disorder. Unfortunately, clinical research has always been hampered by ethical and experimental viability issues, which severely restrict answering basic fundamental questions about epilepsy. In this regard, the use of

animal models that mirror many features of human epilepsy has become a valuable tool for attempting to answer the ultimate question, “what causes epilepsy”? In an attempt to answer this question, recent development of animal models employing a variety of methods has greatly advanced our understanding of epilepsy.

Experimental Models of Epilepsy

Since epilepsy comes in many forms, there are numerous models that reflect it. For example, models of simple partial seizures use topical convulsants such as penicillin, acute electrical stimulation, or cortically implanted metals such as alumina hydroxide, while models of complex partial seizures can involve systemic injections of kainic acid or central application electrical kindling (Fisher, 1989, McNamara, 1994, Racine et al., 1999). With the recent advancements in cellular and molecular biology, there is increasing evidence to suggest that several forms of epilepsy may have a genetic origin, and various models have been developed to look at the genetic components (Kanekos et al., 2002; Willmore et al., 2002).

Genetic models of Epilepsy

Various murine models of inheritable epilepsy have been developed using well-established breeding techniques (Noebels, 1986; Seyfried et al., 1986, Seyfried and Glaser, 1985). The DBA/2 strain has audiogenic seizures produced by loud, high-frequency sound, while the EL strain has generalized seizures that occur after gentle handling, tossing or even spontaneously. Curiously, the EL strain was developed in 1954 from ddY mice that did not seize spontaneously or from handling (Imaizumi and Nakamoto, 1964). An advantage to these mice models is the fact that they already show a

predisposition to seizure, allowing for investigation of seizure genesis using additional manipulations such as kindling and systemic kainic acid. When DBA/2 mice are kindled, they show an accelerated kindling rate as compared to control animals suggesting a relation between seizure susceptibility and accelerated kindling rate (Leech and McIntyre, 1976; Green et al., 1991). Other murine models of generalized epilepsy are ‘stargazer’ (Noebels et al., 1990), ‘tottering’ (Noebels and Sidman, 1979), ‘lethargic’ (Hosford et al., 1992), and BXD (Collins, 1972).

Similarly to the murine models, various rat models of inheritable epilepsy have been developed. For example, the genetically epilepsy prone rats (GEPR) were derived from Sprague Dawley rats at the University of Arizona. There are two distinct GEPR lines; selection for the GEPR-9 line started in 1957, while selection for the GEPR-3 did not start until 1971. Seizure induction in the GEPR commonly occurs through either an auditory stimuli or the gas flurothyl. The delineation between GEPR-9 and GEPR-3 is in reference to Jobe’s severity scale (1981), whereby GEPR-9 tend to have severe generalized convulsion to a high intensity acoustic input, i.e., attaining a score of 9, when in the GEPR-3 the same stimuli causes submaximal seizures, i.e., score of 3 (Jobe, 1981; Faingold, 1988).

Kindling Model

Kindling is a phenomenon whereby repeated administration of an initially subconvulsive electrical stimulus to various brain structures results in a progressive development of electrographic and convulsive motor seizures (Goddard et al., 1969). For the past four decades, we have witnessed a plethora of studies attempting to explore the many mechanisms underlying kindling in a variety of species, including rats, frogs,

primates, and even humans (Velasco et al., 1987; Wada et al., 1978; Morrell et al., 1976). Traditionally, kindling can be induced with a 2s 60Hz sine wave stimulus delivered once daily into limbic sites, such as the amygdala, hippocampus or perirhinal cortex (Goddard et al., 1969; McIntyre et al., 1993). However, not all structures are equally susceptible to kindling. For example, the amygdala has been shown to kindle with greater facility than the hippocampus, while the perirhinal cortex is considered one of the fastest kindling structures within the forebrain. Differential kindling rates (the number of stimulations required to elicit a convulsive seizure) even within one structure, or between structures, may speak directly to the efficacy of recruiting local and distal circuits that serve to drive a motor convulsion. Over time, the progressive behavioural manifestations of kindling typically follow these 6 developmental stages (Racine, 1972): stage 0, immobility; stage 1, freezing with staring and mastication; stage 2, severe jaw mastication and drooling; stage 3, mild to severe bilateral fore and hind limb clonus without rearing; stage 4, rearing with back extended in addition to severe forelimb clonus; stage 5, rearing and falling with the addition of severe forelimb clonus. Once the animal has experienced a convulsive (stage-5) seizure, it is said to have reached the “kindled” state, and the ensuing changes in brain function and excitability are relatively permanent.

To develop an effective animal model of epilepsy, it must mirror some or all of the characteristics seen in man. In this regard, the evolution of kindling used in experimental research and human clinical trials share striking similarities that include: (1) behavioural and EEG patterns, (2) sensitivity to anticonvulsants, (3) spontaneous seizures after kindling and (4) a permanent change in brain function. To this end, the kindling model offers numerous advantages to studying many of the epilepsies. Of these, the three

major benefits of the kindling model are: 1) the permanence of convulsions, 2) no ensuing tissue damage or degeneration, and 3) prolonged kindling may result in spontaneous recurrent seizures (Goddard et al., 1969; McIntyre, et al., 1993).

Kainic Acid Model

Kindling, as described above, is an example of an ‘electrical’ stimulus driven model of epilepsy. Although it has been well documented that seizure induction using this approach is highly effective, kindling-like effects can also be generated by pharmacological interventions. One popular ‘chemical’ model is to employ an excitotoxin known as Kainic Acid (KA). KA, a cyclic analogue of the major excitatory neurotransmitter glutamate, binds to a distinct class of excitatory amino acid receptors in the CNS (Olney et al., 1974; Golden et al., 1995). It is a well-known excitotoxin capable of inducing seizures in animals following systemic injection or intracerebral infusions (Olney et al., 1974; Fisher, 1989; Revuelta et al., 2001). Regardless of its route of administration, the effects of KA in animal studies reproduce many behavioural and histopathological features of human temporal epilepsy with high accuracy (Ben-Ari et al., 1981; Ben-Ari., 1985; Lothman and Colin, 1981). In fact, there has been convincing evidence demonstrating a very characteristic pattern of KA-induced cellular damage in the hippocampus, parietal cortex, perirhinal cortex, piriform cortex, olfactory cortex and amygdala, followed by a decrease in seizure threshold with spontaneous seizures (Collins et al., 1983; Kelly and McIntyre, 1994; Fisher, 1989; Golden et al., 1995).

Although both the kindling and KA models of epilepsy are widely used by epileptologists, there is considerable debate as to which model is more appropriate to address fundamental questions regarding the etiology and maintenance of this broad

disorder. In fact, those in support of an electrical stimulation model have argued that the use of chemoconvulsants inadequately controls epileptogenesis, unlike electrical kindling, which can be studied at various defined points in its evolution. Further, it is difficult to predict the type of seizure induced by chemoconvulsants, not to mention the gliosis and pathology in selected cell populations that are associated with injection or infusion. In such pharmacological studies, it is imperative to delineate between the results due solely to the effects of the drug *per se* or effects that are a consequence of the ensuing seizure activity. Thus, chemical and electrical kindling have both pros and cons, but taken together provide great versatility for elucidating possible mechanisms underlying seizure genesis.

Fast and Slow kindling rats

Undoubtedly the development of genetic models of epilepsy can provide novel insights into the mechanisms that govern the initiation and maintenance of seizure genesis. With this mind, the quest for an effective genetic animal model with an inherent predisposition for and against epilepsy was begun. To address this need, animals can be bred to be either seizure prone or seizure resistant in some manner because of some underlying genetic mechanism.

Development of Fast (seizure-prone) and Slow (seizure-resistant) kindling rats was originally started by Dr. Racine at McMaster University. The Fast and Slow rats were selectively bred based on differing amygdala excitability and kindling rates. Animals with either fast or slow kindling rates (i.e., the total number of stimulations to the first generalized stage-5 motor convulsion) in each generation were used as breeders

to develop the two strains (Racine et al., 1999). The kindling-based selection ended at the 11th generation. Now, in their 50th generation, the Fast and Slow kindling rats have remained two distinct, stable populations.

Presently, Slow rats take an average of 45 daily amygdala stimulations to reach stage-5 generalized seizures, whereas Fast rats only take an average of 10 daily stimulations to produce the same stage-5 generalized seizure (McIntyre et al., 1999a). Clearly, the Fast and Slow rat strains developed via the kindling model demonstrate that seizure genesis in the temporal lobe is under some level of genetic control.

The Fast and Slow rats differ in various respects, and the extent of this variation is currently under investigation. So far it has been shown that the two strains vary in their response to GABAergic modulators; Slow rats respond to much lower doses of positive modulators, such as sodium pentobarbital or diazepam, than do Fast or Long Evans Hooded (McIntyre et al., 1999a). All of the mechanisms underlying these physiological differences between the Fast and Slow strains are still to be fully determined. Thus far, it has been found that the strains differ considerably in their GABA_A subunit expression in several of the temporal lobe structures, specifically the amygdala, piriform and perirhinal cortex. Examination of the GABA_A subunits using *in situ* hybridization shows that the Fast rats over express the embryonic α_2 , α_3 , and α_5 GABA_A subunits compared to controls and Slow rats, whereas the Slow rats over express the adult α_1 GABA_A subunit (Poulter et al., 1999). Differential display and microarray analysis of the Fast and Slow rats also shows various differentially expressed genes, such as the AMPA receptor GluR4 subunit, Plasminogen activator inhibitor and cytochrome C oxidase (Gilby, 2001 personal communication).

The genetic differences in the two strains are being discovered, but there are also clear behavioural differences as well, and the links between the genetics and the behaviour are currently being investigated. Indeed, Fast and Slow rats show marked differences in fear/anxiety, learning, habituation, open-field activity and impulsivity (McIntyre and Anisman, 2001; Mohapel and McIntyre, 1998; McIntyre et al., 1999a, McIntyre et al., 1999c).

Study's objective

The Fast and Slow rats offer a wonderful model for the exploration of the underlying mechanisms in KA-induced status epilepticus (SE). It is known that the Fast and Slow rats differ remarkably in the kindling model of epilepsy by which they were selected, but it has yet to be determined if the Fast and Slow strains would show the parallel differential seizure profiles when challenged with chemoconvulsants. Since the Fast and Slow strains have never been examined in an excitatory acid chemoconvulsant paradigm, their seizure profiles in two experiments were determined after systemic injection of KA. The information obtained from these experiments will aid in understanding epilepsy, and further research could shed light on the mechanisms involved in seizure genesis.

EXPERIMENT # 1

Materials and Methods

Animal Treatment

Subjects: Male Fast and Slow rats weighing between 250-400 grams, on the day of KA injection, were removed from their home cages and singly housed.

Seizure induction: On the day of the experiment the animals received a 12, 15 or 20mg/kg (i.p.) injection of kainic acid (Sigma & Ocean Pacific). Animals were observed during the entire experiment and had two hours of steady status epilepticus (SE), which was scored using Racine's behavioural seizure scale (Appendix 1). At the end of two hours of status or if there was a risk of death, the seizure was shut down with an injection of a sedative dose of sodium pentobarbital (0.3-0.4cc). After the status epilepticus, the animals were returned to their home cages and given fresh food and water.

KA recovery: After their status episodes, all animals were monitored for behaviour once daily for another four weeks, before being sacrificed for histological examination. Any animals that showed difficulty recovering from the status episode were hand fed mash and water, or else sacrificed if it looked like they would not survive.

Behavioural Scoring

Behavioural seizure profiles, including latency to SE onset, number and duration of stage 3/4/5 generalized seizures and the number of wet dog shakes pre- and post-SE, for each animal were scored from tapes by three observers. The latency to seizure onset was determined to be the time from KA injection to the 1st stage 5 generalized seizure. KA induced behavioural difference were analyzed by performing a two-tailed

independent samples t-test and paired samples t-test, where significance was attained at $p < 0.05$.

Histology

Four weeks after SE, under deep anesthesia with sodium pentobarbital, the rats were perfused intracardially with 120 mL of 0.9% saline and 120 mL of Lana's fixative (200ml of 20% Paraformaldehyde, 320ml of 0.1M Phosphate Buffer and 140ml Saturated Picric acid). Brains were removed 24 hours after perfusion and placed in Lana's fixative for at least one week, 2-4 days in 10% sucrose in PB and 2-4 days in 30% sucrose in PB in order to cryoprotect the brain. Fixed whole brains were fast frozen using compressed CO₂, and sectioned at 26µm using a microtome (American Optical Company). A proportion of the animals were sacrificed 24 hours after SE and that tissue was used in another study.

Cresyl Violet

Some sections were stained with Cresyl violet to ascertain the amount of damage 4 weeks after SE. Every 12th section was mounted on a gelatin-coated slide. Sections were dehydrated in a series of increasing ethanol steps (70, 75, 95, 100%), defatted with Clearene, rehydrated in decreasing ethanol steps and stained with 0.1% Cresyl violet acetate (Sigma). The sections were rinsed in distilled water, followed by acetic acid and dehydrated in an increasing series of ethanols and washed again with Xylene. The sections were cover slipped using permount and cellular damage was visualized at 4.5x and 10x magnification using a Leica light microscope.

Results

Behaviour

Once injected with KA, rats that were behaviourally responsive to it showed a consistent slow progression in their behaviour from their initial drug response (a pronation response) to fore- and hindlimb clonic-tonic convulsions (generalized stage-5 response), which ultimately led to *status epilepticus* (SE); the latter was characterized by many repetitions of the generalized event. In this experiment a total of 60 animals was injected with 20mg/kg KA. Of these 60 animals, 27 were Slow rats and 33 were Fast rats.

At a KA dose of 20mg/kg, Fast animals were more prone to violent seizures that ultimately resulted 24% of the Fast animals dying in the first 24 hours compared to only 3% in the Slow rats; this difference, however, was not significant ($\chi^2 = 3.0$, $df = 1$, $p > 0.01$). Shortly after injection, each strain equally displayed the KA pronation response (Table 1). Interestingly, however, having a pronation response did not perfectly predict the animal successfully entering into SE. Of the Slow and Fast rats that were injected with KA, only 7.4% and 12.1%, respectively, showed a pronation response and then did not go on to develop SE. By contrast, only 14.8% of the Slow and 9.1% of the Fast rats had *no* pronation response, but subsequently went on to develop SE

Animals were also monitored for their latency to develop SE from the time of injection. Here there was a significant difference between the strains in the length of time to develop SE ($t_{(2\text{-tailed})} = 9.48$; $df = 34$; $p < 0.01$). On average, the Fast rats took 44.7 ± 4.2 minutes to enter into SE, whereas the Slow rats took ~ 3 times longer with a latency of 140.4 ± 8.3 minutes (Figure 1). Interestingly, during this latency period, the Slow rats displayed a significantly higher number of wet dog shakes (WDS) as compared to the

Fast rats ($t_{(2\text{-tailed})} = 3.95$; $df = 34$; $p < 0.01$). Slow rats had an average number of 93.1 ± 20.1 WDS before entering into SE, which was 24.5 times more than the average number of 3.8 ± 1.9 WDS in the Fast rats (Figure 2). When the difference in latency time between the strains is accounted for by turning the WDS scores into a proportion of the time elapsed, there is still a difference between the strains ($\chi^2 = 21.6$, $df = 1$, $p < 0.01$), where the Slow rats had significantly more WDS than the Fast rats. After entering SE, there was also a significant decrease in the number of WDS in the Slow rats from their pre-SE production ($p < 0.01$), but not so in the Fast rats where there was no change in the number of WDS observed before and during SE. Once the animals had entered status, however, there remained a significant difference between the strains in the number of WDS, similar to that seen before entering SE ($t_{(2\text{-tailed})} = 2.4$; $df = 34$; $p < 0.05$) (Figure 2).

During SE, animals also were monitored for the number and percent duration of generalized stage 3/4/5 seizures. Throughout SE, repeated entries into stage 3/4/5 seizure indicated a significant increase in frequency in the Fast rats as compared to Slow rats ($t_{(2\text{-tailed})} = -7.86$; $df = 34$; $p < 0.01$) (Figure 3). On average, in the 2 hour window allotted for SE, before its termination with pentobarbital, Fast rats had 22.7 ± 2.3 entries into stage 3/4/5 seizures, which is approximately four times the number of entries seen in the Slow rats (5.6 ± 0.6). Not surprising, therefore, it was also found that Fast rats spent significantly more time in stage 3/4/5 seizures as compared to Slow rats ($t_{(2\text{-tailed})} = -8.45$; $df = 34$; $p < 0.01$) (Figure 4). However, the amount of time spent in stage 3/4/5 seizures was calculated as a percentage of the overall time spent in SE, since the duration of SE varied between animals. Fast rats spent on average 26.3 ± 2.8 % of their total SE duration in generalized seizures, compared to only 4.4 ± 0.7 % shown by the Slow rats. In

summary, it may be said that Fast rats had a relatively quick onset to SE, which was filled with many episodes of severe generalized seizures. In contrast, the Slow rats show a delayed progression to SE, but once in SE, they too had equally strong generalized seizures, albeit fewer in number. Thus, these two strains showed clear and significant behavioural seizure differences in response to a 20mg/kg dose of KA.

Histology

Slow and Fast rats sacrificed 4 weeks after SE had varying amounts of neuronal damage as seen in their brain tissue stained with cresyl violet. Animals that were injected with KA and that did not subsequently develop SE did not show any cellular damage in any brain regions examined. Figure 5 is a photomicrograph of the right hippocampus of a typical Slow (A, B) and a Fast (C, D) rat that received a 20mg/kg KA injection, but did not show any obvious signs of behavioural seizures. There was no damage in any of the hippocampal regions (CA1, CA2, CA3 or DG), as seen by broad, darkly stained and normal appearing cellular layers. Examination of tissue from Slow and Fast rats that had undergone KA-induced behavioural seizures, however, showed cellular damage that was largely isolated to the hippocampus in both strains. There was a distinct difference in the amount of hippocampal cellular damage evident between Slow (N = 13) and Fast (N = 3) rats that was related to the severity of their SE. The dorsal hippocampus of four Slow rats that had behavioural seizures showed an overall faint staining with discrete regions of cells that were pyknotic and darkly stained (Figure 6, A, B, E & F). Examination of more caudal hippocampal sections, additionally containing the ventral hippocampus, showed bilateral cellular damage in the CA3 regions. Patches of cells in the CA3 region of the

ventral hippocampus in 10 of 13 Slow rats were absent, and glia had infiltrated the region (Figure 7 A & B). In 2 of the Slow rats, their seizures arrested spontaneously (without pharmacological intervention) and subsequent histological examination showed an absence of damage in all limbic structures. The cellular damage seen in the 3 Fast rats that survived the 4 week recovery period was also bilateral, but was more pronounced in the CA3 region of the dorsal hippocampus, with the CA3b region being devoid of neurons and substantially infiltrated with glia (Figure 8 A, D, E & F). Therefore it is not surprising that animals with more severe SE, as assessed by their SE duration scores, showed increased cellular damage as compared to less severe SE cases (Figure 8 C versus E) independent of strain. Thus Slow and Fast animals that showed similar SE severity/duration also showed similar cellular damage (Figure 7 A, B versus C,D). The dorsal hippocampus of both the Slow and Fast rats with similar SE severity scores had similar pyknotic cells. By the same token, caudal sections containing the ventral hippocampus CA3 region, contained comparable amounts of bilateral cellular damage and microglial infiltration in both the Slow and Fast rats (Figure 7 A, B versus C, D). However, because of the large strain difference in the duration of generalized SE behavior, the amount of cellular damage that occurred in the Slow and Fast rats was positively related to the differences in SE severity.

The neurons that remained in the hippocampus were not actively dying since there was an absence of Fluoro-Jade positive cells in any of the regions examined. Figure 9 shows a fluoro-jade stained piriform cortex of a Slow animals having had generalized SE compared to a naïve LEH which was sacrificed during the throes of electrically induced SE.

Table 1

The response profiles of naïve Fast and Slow rats after being systemically injected with 20mg/kg KA and the proportion of animals that make up each of the experimental groups.

Animal N = 60	Developed SE and lived	Developed SE and died	Did not develop SE
Slow N = 27	20/27 (74.1%)	1/27(3%)	6/27 (22.2%)
Fast N = 33	18/33 (54.5%)	8/33 (24%)	7/33 (21.2%)

Figure 1 – Average latency to SE ($\pm$ SEM, min) between Slow and Fast rats systemically injected with 20mg/kg Kainic acid. It takes significantly longer for the Slow animals to enter SE than the Fast animals ($t_{(2\text{-tailed})} = 9.48$; $df=34$; $p < 0.01$).

Figure 2 – Average number of wet dog shakes (WDS) in Slow and Fast rats before and after the onset of KA-induced SE. There is a significantly larger number of WDS seen in the Slow rats before and after the onset of SE as compared to the Fast rats ('a' $\sim t_{(2\text{-tailed})} = 3.95$; $df=34$; $p < 0.01$ and 'b' $\sim t_{(2\text{-tailed})} = 2.4$; $df=34$; $p < 0.05$). There was also a significant drop in WDS in the Slow rats after the commencement of SE ('c' $\sim t_{(\text{paired}; 2\text{-tailed})} = 2.81$; $df=15$; $p < 0.01$).

Figure 3 – Average number of entries into stage 3/4/5 behavioural seizures ($\pm$ SEM) seen in Slow and Fast rats during KA-induced SE. During SE Slow rats enter 3/4/5 seizures to a significantly lesser degree than Fast rats ($t_{(2\text{-tailed})} = -7.86$; $df=34$; $p < 0.01$).

Figure 4 – Average percent duration of SE ($\pm$ SEM, %) spent in stages 3/4/5 for Slow and Fast rats. The percent duration of SE for Slow rats was significantly less than that of Fast rats ($t_{(2\text{-tailed})} = -8.45$; $df=34$; $p < 0.01$).

Figure 5 – Cresyl violet Photomicrograph of the dorsal hippocampus of Slow and Fast rats that were systemically injected with 20mg/kg KA but did not subsequently develop behavioural SE. A) An overall view of the dorsal hippocampus from a Slow rat. B) A magnified view of (A), the arrows point to the CA2/3 regions. C) An overall view of the dorsal hippocampus from a Fast rat. D) A magnified view of (B), the arrows point to the CA2/3 hippocampal region. (Small scale bar = 400 μ m, Large scale bar = 200 μ m).

Figure 6 – Cresyl violet photomicrograph of the hippocampus of Slow rats that were systemically injected with 20mg/kg KA and that subsequently developed SE. A) A view of the hippocampus from the Slow rat that developed the most severe SE. B) A magnified of (A), the arrows point to darkly stained pyknotic cells. C) An overall view of the dorsal hippocampus from a Slow animal that had mild SE. D) A magnified view of (C), in clockwise order, the first arrow points to darkly stained pyknotic neurons in the CA2 region, while next arrow points to healthier neurons in the CA3 regions. E) An overall view of the dorsal hippocampus from a Slow animal that had mildest form of SE. F) A magnified view of (E), the arrow points to darkly stained pyknotic cells. (Small scale bar = 400 μ m, Large scale bar = 200 μ m).

Figure 7 – Cresyl violet photomicrograph of the ventral hippocampus of Slow and Fast rats that were systemically injected with 20mg/kg KA and developed SE. A) A view of the ventral hippocampus from a Slow rat that had the most severe SE. B) A magnified view of (A), the arrows point to the glial cells that have infiltrated the region. C) A view of the ventral hippocampus from a Fast rat that had the least severe SE. E) A magnified view of (C), the arrows point to the glial cells that have infiltrated the region. (Small scale bar = 400 μ m, Large scale bar = 200 μ m).

Figure 8 - Cresyl violet photomicrograph of the hippocampus of Fast rats that were systemically injected with 20mg/kg KA and subsequently developed SE. A) The dorsal hippocampus of the Fast rat that developed SE that was similar in severity to that of the Slow rat in figure 6A. B) A magnified view of (A), in clockwise order, the first arrow points to darkly stained pyknotic cells in the CA2 regions while the second arrow points to a region of cell loss in the CA3a region. C) An overall view of the dorsal hippocampus from a Fast rat that had the most severe SE. D) A magnified view of (C), there is a total loss of CA3b neurons plus the infiltration of glial cells. E) An overall view of the dorsal hippocampus from a Fast rat that had a less severe SE than seen in (C). D) A magnified view of (E), there is also a loss of CA3b neurons plus the infiltration of glial cells. (Small scale bar = 400 μ m, Large scale bar = 200 μ m).

Figure 9 – Fluoro-Jade photomicrograph of the piriform cortex of a LEH rat and a Slow rat that developed SE after being systemically injected with KA. A) An overall view of the piriform cortex of a LEH rat sacrificed in the throes of electrically induced SE. B) A magnified view of (A), the arrows point to the fluoro-jade expressing neurons. C) An overall view of the piriform cortex of a Slow rat that was sacrificed 4 weeks after having behavioural SE induced by an injection 20mg/kg KA. D) A magnified view of (C), as evident from the picture there are no fluoro-jade positive cells in this animal.

EXPERIMENT # 2

Materials and Methods

Animal Treatment

Subjects: Fast and Slow rats weighing between 250-300 grams at the time of surgery were used in this experiment. All animals were singly housed with food and water liberally available.

Surgery: Under pentobarbital anesthesia (60mg/kg, i.p.), the rats were implanted with stimulating/recording electrodes into the following brain structures: left amygdala, left hippocampus, right frontal motor cortex and right piriform cortex. The stereotaxic coordinates (Paxinos and Watson, 1986) chosen for the four structures were 4.8 mm anterior to bregma, 2.5 mm lateral to midline and 2.5 mm deep for Frontal motor cortex; 4.5 mm lateral to bregma and 8.3 mm deep for amygdala; 3.0 mm posterior to bregma, 3.5-3.7 mm lateral to midline and 3.0 mm deep for dorsal hippocampus; and 4.0 mm lateral to midline angled at 10 degrees and 8-9 mm deep for piriform cortex.

The recording electrodes were constructed of two twisted strands of 0.127 mm diameter Nichrome wire, Diamel insulated, and attached to male Amphenol pins. These electrodes were cut to length, surgically implanted and secured to the rat's skull with 5 to 7 jeweller's screws. A covering of dental acrylic was applied to the head according to standard protocols in our laboratory and animals had a 1 to 2 week recovery period (Molino and McIntyre, 1972).

Seizure induction: On the day of the experiment animals were attached to an EEG recording apparatus and a two-minute baseline was recorded. The animals were then injected with 20mg/kg (i.p.) of kainic acid (Sigma & Ocean Pacific) and monitored

continually from the point of injection. Animals had 90 minutes of steady status epilepticus (SE), followed by an injection of a sedative dose of sodium pentobarbital (0.3-0.4cc) to shut down the seizure. The EEG patterns following the sodium pentobarbital injection were recorded for about 30 minutes to 1 hour.

Behavioural Scoring

Behavioural seizure profiles for each animal were scored from video monitoring. As in experiment #1, the same seizure behaviours were scored. In this experiment the electrographic seizures were also examined and compared to the behavioural seizure.

Histology

After the 30 minutes to 1 hour following the sodium pentobarbital injection, animals were perfused intracardially with 120 mL of 0.9% saline and 120 mL of Lana's fixative (200ml of 20% Paraformaldehyde, 320ml of 0.1M Phosphate Buffer and 140ml of Saturated Picric acid). Brains were removed 24 hours after perfusion and placed in Lana's fixative for at least one week, 2-4 days in 10% sucrose in PB and 2-4 days in 30% sucrose in PB. Fixed whole brains were fast frozen using compressed CO₂, and sectioned at 26µm using a microtome (American Optical Company).

C-Fos staining

A rostral-caudal series of sections was examined for the level of c-fos protein expression one hour after SE. Sections were removed from cryoprotectant and placed in a 0.1M PBS- 0.2% Triton-X 100 wash over night at 4 degrees with constant shaking. The next day the sections were transferred into a new 0.1M PBS – 0.2% Triton-X 100 and washed three times at room temperature for 10 minutes with constant shaking. This was followed by 1% H₂O₂ in 0.1M PBS-0.2% Triton-X 100 wash for 20 minutes. The

sections were put in fresh 0.1M PBS- 0.2% Triton-X 100 for three ten minute washes. The sections were then blocked with 3% goat serum (Vector Laboratories) for 30 minutes with constant shaking. This was followed by an overnight incubation in the primary antibody, C-Fos rabbit polyclonal (1/10000 dilution, Santa Cruz biotechnology inc.) with shaking at 4 degrees.

The next day the sections were placed in fresh 0.1M PBS- 0.2% Triton-X 100 for three ten-minute washes, followed by 1 hour incubation at room temperature in the secondary antibody, biotinylated goat anti-rabbit (1/500 dilution, Vector Laboratories). The sections were placed in fresh 0.1M PBS- 0.2% Triton-X 100 for three ten-minute washes and this was followed by 1 hour incubation in avidin-biotin-horseradish peroxidase complex (ABC) (Vector Laboratories). After this, incubation sections were washed in fresh 0.1M PBS- 0.2% Triton-X 100 for three ten-minute intervals. In order to visualize the c-fos, sections were incubated in diaminobensidine-tetrachloride (DAB) for approximately 5 minutes and washed again in 0.1M PBS- 0.2% Triton-X 100 for three ten-minute intervals. The sections were finally mounted on gelatin-coated slides and allowed to dry overnight before being dehydrated through a series of ethanols (70%-100%) and cleared. The sections were cover slipped using permount and c-fos staining was visualized at 4.5x and 10x magnification using a Leica light microscope.

Data Analysis of c-fos staining

The distribution of c-fos positive neurons in the rat brains after KA seizure was determined. The distribution of Fos-stained cells were rated by direct visual examination, with a '+' representing positive staining in a Fast rat while a '*' was used for Slow rats. The semiquantitative rating of c-fos labeling, based on intensity of immunostaining in the

limbic structures, provides information about the origin and/or circuitry of the seizure, but statistical analysis of these results would be inappropriate.

Results

Behaviour

In this experiment, we examined the electrographic activity of four limbic structures (the orbital/frontal cortex [FMC], the piriform cortex [PMC], the hippocampus and the amygdala) and the associated seizure behavior of Fast and Slow rats following a systemic injection of 20 mg/kg of KA. Figures 10 and 11 show the electrode placements for each of the structures analyzed. Similar to the first experiment, the presence of a good KA pronation response following the injection had no ability to predict the development of generalized SE. The overall injection success for this response was similar between the Slow and Fast rats, since there were equal numbers of injected rats in both groups that showed the pronation response ($\chi^2 = 0.55$, $df = 1$, $p > 1$)(Table 2). Subsequently, with the development of SE, there was a 16.0% mortality rate in the Slow rats following the KA injection, which was not significantly higher than the 5.6% mortality rate seen in the Fast rats ($\chi^2 = 2.09$, $df = 1$, $p < 0.20$)(Table 2). In addition, there was also a significant difference between the strains in terms of the development of generalized convulsions, which were significantly harder to induce in the Slow rats as compared to the Fast rats ($\chi^2 = 9.26$, $df = 1$, $p < 0.01$). Only 16.7% of the Slow rats systemically injected with 20mg/kg KA entered generalized SE, as defined in experiment # 1; on the other hand, and interestingly, there were more Slow rats than Fast rats that developed masticatory and ambulatory SE. Of the Slow animals injected with KA, 27.8% entered

masticatory/ambulatory SE, while none of the Fast rats exhibited this type of status ($\chi^2=5.81$, $df=1$, $p<0.025$).

As in experiment #1, we assessed the behavioural response to systemic KA injection in the Slow and Fast rats, but with the addition of EEG monitoring. As mentioned above, few Slow rats entered generalized convulsions ($N=3$) when compared to Fast rats ($N=12$); with such a small sample size in the Slow population, it is hard to make statistical generalizations based on the results obtained for this group. In spite of this short-coming, differences in seizure susceptibility between the Slow and Fast rats compared to the first experiment were observed. The latency to SE for the Slow rats in this experiment was significantly shorter than that of experiment #1 ($t_{(2\text{-tailed})}=4.85$; $df=21$; $p<0.01$) (Figure 12). Slow rats in the first experiment had a latency to SE of 140.4 ± 8.3 minutes, which was ~ 5 times greater than the latency for the Slow rats in experiment #2 that had undergone surgical electrode implantation (28.7 ± 20.7 minutes). Although a significant difference was observed in latency to onset of SE, it should be noted that the sample sizes being compared are very different, $N=20$ for the first experiment and $N=3$ for the second experiment. Contrary to the Slow rats, the Fast rats showed no significant difference in latency to SE between the first experiment and the second experiment, with latencies of 44.7 ± 4.2 and 34.2 ± 7.5 minutes, respectively ($t_{(2\text{-tailed})}=1.29$; $df=26$; $p>0.05$) (Figure 12).

Interestingly, examination of the number of entries with 3/4/5 seizures and percent duration of SE did not show any significant differences between the first and second experiments for the Slow rats (Figures 13 and 14). In the second experiment, Slow rats enter 3/4/5 seizure 11.0 ± 3.5 times, which is about twice as many as was seen

in the first experiment (5.6 ± 0.6 entries) ($t_{(2\text{-tailed})} = 1.50$; $df = 21$; $p > 0.05$). Similarly, the percent duration of SE was somewhat longer for Slow rats in the second experiment ($9.1 \pm 3.6\%$ versus $4.4 \pm 0.7\%$) ($t_{(2\text{-tailed})} = -1.29$; $df = 21$; $p > 0.05$). Unlike the Slow rats, there was a slight decrease in the number of entries into 3/4/5 seizure and the percent duration of SE for Fast rats in the second experiment compared to Fast rats in the first experiment. Fast rats in the second experiment had 19 ± 2.2 entries into 3/4/5 seizures, which was not significantly less than the 22.7 ± 2.3 entries seen in the first experiment ($t_{(2\text{-tailed})} = 1.12$; $df = 26$; $p > 0.05$). Accordingly, there was a significant decrease in the percent duration of SE, whereby Fast rats from the first experiment had a larger percent SE duration, $26.3 \pm 2.8\%$, versus that seen in the second experiment, $17.7 \pm 2.7\%$ ($t_{(2\text{-tailed})} = 2.16$; $df = 26$; $p < 0.05$). Based on the difference observed between the first and second experiment, it seems that each experiment must be examined in isolation, since the addition of electrodes fundamentally altered the seizure behaviours observed in the two strains. This is further substantiated by the odd change in WDS behaviour in this experiment. In the second experiment, there was no significant difference between the number of WDS Fast and Slow rats perform in the pre and post SE conditions ($t_{(2\text{-tailed})} = 0.26$; $df = 13$; $p > 0.05$ and $t_{(2\text{-tailed})} = 0.22$; $df = 13$; $p > 0.05$, respectively). In fact, in both conditions, the Slow rats performed fewer WDS than the Fast rats (7.67 ± 7.67 versus 15.17 ± 13.813 , and 58.67 ± 35.62 versus 76.58 ± 38.25), and there was a great number of WDS performed after SE onset in the Fast rats, which is opposite to what occurred in the first experiment.

In this second experiment, there were no significant differences between the Fast and Slow rats in any of the behaviours observed. Interestingly, the latency to SE for the Slow rats was slightly shorter than that of the Fast rats (28.7 ± 20.7 versus 34.2 ± 7.5

minutes ($t_{(2\text{-tailed})} = -0.31$; $df = 13$; $p > 0.05$). Examination of the other behaviours showed that, similar to the first experiment, the Slow rats had fewer entries into stage 3/4/5 seizures and a lower percent duration of SE. Slow rats entered into 3/4/5 seizure 11.0 ± 3.5 times compared to the 19.0 ± 2.2 entries seen in the Fast rats ($t_{(2\text{-tailed})} = 1.66$; $df = 13$; $p > 0.05$) and spent 9.1 ± 3.6 % of their time in SE versus the 17.7 ± 2.7 % that Fast rats spent ($t_{(2\text{-tailed})} = 1.46$; $df = 13$; $p > 0.05$).

EEG Behaviour

The latency to the first electrographic AD in the Slow rats was 395.3 ± 94.7 seconds, which was not significantly shorter than the 692.4 ± 185.1 seconds latency seen in the Fast rats ($t_{(2\text{-tailed})} = 0.81$; $df = 21$; $p > 0.05$). In Figure 15, the EEG patterns in the hippocampus, FMC, amygdala and PC of an injected rat that did not develop SE, a severe generalized SE rat and an ambulatory/masticatory SE rat are presented. Although the behavioural manifestations of seizure are different between the strains, the EEG patterns observed in rats of both strains one hour after the KA injection showed a continuous pattern of firing in all brain regions regardless of strain.

There was no significant difference between the Fast and Slow rats for first structure showing seizure activation. The first structure activated was the FMC, which occurred equally in both the Fast and Slow rats ($\chi^2 = 0.525$, $df = 1$, $p > 1$). Examination of the latency to activation of the other three structures also did not show any significant difference between the Fast and Slow rats (Hippocampus, $\chi^2 = 0.875$, $df = 1$, $p > 1$; Amygdala, $\chi^2 = 1.05$, $df = 1$, $p > 0.20$; PC, $\chi^2 = 0.39$, $df = 1$, $p > 1$).

Histology

Examination of neuronal activation using c-fos immunohistochemistry showed that two hours after SE, either generalized or ambulatory/masticatory, most limbic regions for both Fast and Slow rats were activated (Table 3, Figure 16). In addition, paradoxically, there seemed to be an increase in c-Fos protein in the amygdala region (as indicated by arrowheads) of ambulatory/masticatory Slow rats, when compared to Fast and Slow rats that exhibited the behaviourally and electrographically more severe generalized SE, but this apparent difference was not assessed quantitatively.

Table 2

The response profiles of Fast and Slow rats with implanted electrodes after being systemically injected with 20mg/kg KA and the proportion of those animals that make up each of the experimental groups.

Animal N = 36	Developed SE and lived	Developed SE and died	Did not develop SE
Slow N = 18	Generalized 3/18 (16.7%) Amb./Mast. 5/18 (27.8%)	4/18 (16.0%)	6/18 (33.3%)
Fast N = 18	12/18 (66.7%)	1/18 (5.6%)	5/18 (27.8%)

Figure 10 – Schematic diagram taken from Paxinos and Watson (1986) showing the electrode tips of each rat in the first series of KA treated animals. Electrode tips are located in the orbital/FMC cortex, piriform cortex, amygdala and dorsal hippocampus with a few exceptions. Filled circles represent the electrode tips of Slow rats while filled squares represent the electrode tips of Fast rats. (FMC = black; Piriform cortex = red; Amygdala = blue; Hippocampus = green).

Figure 11 – Schematic diagram taken from Paxinos and Watson (1986) showing the electrode tips of each rat in the second series of KA treated animals. Electrode tips are located in the orbital/FMC cortex, piriform cortex, amygdala and dorsal hippocampus with a few exceptions. Filled circles represent the electrode tips of Slow rats while filled squares represent the electrode tips of Fast rats. (FMC = black; Piriform cortex = red; Amygdala = blue; Hippocampus = green).

Placement Legend

- Frontal motor cortex
 - Slow
 - Fast
- Piriform cortex
 - Slow
 - Fast
- Amygdala
 - Slow
 - Fast
- Hippocampus
 - Slow
 - Fast

Figure 12 - Average latency to SE ($\pm$ SEM, min) between Slow and Fast rats systemically injected with 20mg/kg Kainic acid with and without implanted electrodes. It takes significantly longer for the Slow animals to enter SE than the Fast animals in the first experiment. There is a significant difference between the Slow rats without implanted electrodes and those with implanted electrodes (* $\sim t_{(2\text{-tailed})} = 4.85$; $df = 21$; $p < 0.01$). There is no significant difference between the Fast rats with and without electrode implantation ($t_{(2\text{-tailed})} = 1.29$; $df = 26$; $p > 0.05$). There was no significant difference between the electrode implanted Slow and Fast rats ($t_{(2\text{-tailed})} = -0.31$; $df = 13$; $p > 0.05$).

Figure 13 - Average number of entries into stage 3/4/5 behavioural seizures ($\pm$ SEM) seen in Slow and Fast rats with and without implanted electrodes during KA-induced SE. During SE Slow rats without implanted electrodes enter 3/4/5 seizures to a significantly lesser degree than Fast rats (* $\sim t_{(2\text{-tailed})} = -7.86$; $df=34$; $p < 0.01$). There was no significant difference in the number of entries into stage 3/4/5 behavioural seizures between either the Slow or Fast with or without electrode implantation ($t_{(2\text{-tailed})} = 1.50$; $df = 21$; $p > 0.05$ and $t_{(2\text{-tailed})} = 1.12$; $df = 26$; $p > 0.05$ respectively). There was no significant difference between the Slow and Fast rats with implanted electrodes ($t_{(2\text{-tailed})} = 1.66$; $df = 13$; $p > 0.05$).

Figure 14 - Average percent duration of SE ($\pm$ SEM, %) in Slow and Fast rats with and without implanted electrodes during KA-induced SE. The percent duration of SE for Slow rats without implanted electrodes was significantly less than that of Fast rats (*' $\sim t_{(2\text{-tailed})} = -8.45$; $df = 34$; $p < 0.01$). There was no significant difference between Slow rats with and without implanted electrodes ($t_{(2\text{-tailed})} = 1.50$; $df = 21$; $p > 0.05$). There was a significant difference in the Fast rats with and without implanted electrodes ('a' $\sim t_{(2\text{-tailed})} = 2.16$; $df = 26$; $p < 0.05$). There was no significant difference between the Slow and Fast rats with implanted electrodes ($t_{(2\text{-tailed})} = 1.46$; $df = 13$; $p > 0.05$).

Figure 15 – The EEG patterns of Slow and Fast rats with implanted electrodes in the orbital/FMC, piriform cortex, amygdala and hippocampus. (A) An EEG taken from a KA injected rat that did not show any behavioural or electrographic seizures. (B) An EEG taken from a KA injected Slow rat that developed masticatory/ambulatory SE. (C) An EEG taken from a KA injected Fast rat that developed generalized SE. (1= hippocampus; 2 = FMC; 3 = amygdala; 4 = piriform cortex).

A **B**

5 seconds

C

5 seconds

Figure 16 – A montage of region c-Fos protein expression from animals approximately one hour after SE termination. (A) The left side shows the FMC of a Slow rat while the right side shows that of a Fast rats. (B) The left side shows the hippocampus, amygdala and piriform cortex of a Slow rat while the right shows the same regions from a Fast rats. The arrowhead point towards the increase c-Fos expression observed in the masitactory/ambulatory SE Slow rats. (Scale bar = 500 μ m)

Table 3

The distribution of c-fos positive neurons in rat brains after KA induced seizure

Region	c-fos positive neurons for		
	<i>Fast generalized SE</i>	<i>Slow generalized SE</i>	<i>Slow Masti/Amb SE</i>
<i>Cortex</i>			
Frontal	+++	***	***
Orbital	+++	*_*	*_*
Cingulate	+++	***	***
Temporal	+++	*_*	***
Insular	+++	*_*	***
Piriform	+++	***	***
Entorhinal	+++	***	***
Occipital	+++	*_*	***
Parietal	+++ ---	*_*	***
<i>Hippocampus</i>			
Subiculum	--+	*_*	***
CA1 sector d/v	+/-+/-+/-+	*/_-/*/*	*/_-/*/*
CA2 sector d/v	+/-+/-+/-	*/_-/*_-	*/_-/*_-
CA3 sector d/v	+/-+/-+/-+	*/_-/*/*	*/_-/*/*
<i>Amygdala</i>			
Lateral nuc.	+++	***	***
Basolateral nuc.	+++	***	***
Basomedial nuc.	+++	***	***
Central nuc.	+++	***	***
Medial nuc.	+++	***	***
Cortical nuc.	+++	***	***
<i>Thalamus</i>			
Dorsomedial nuc.	---	---	---
Midline nuclei	---	*_*	*_*
Ventral nuclei	+--	---	---
Lateral nuclei	+--	*_*	*_*

Each '+' will represent one fast animal showing c-fos positive neurons.
 Each '*' will represent one Slow animal showing c-fos positive neurons.

Discussion

Systemic administration or intracerebral infusion of kainic acid (KA) is known to cause *status epilepticus* (SE) in most animals. Subsequently, many of the animals that experience SE develop spontaneous seizures days or weeks later. As a result, KA is commonly often used to model temporal lobe epilepsy (TLE) (Olney et al., 1974; Ben-Ari et al., 1985). In the present experiment, the objective was to determine the seizure susceptibility, measured by the induction of SE, of the Slow and Fast rats to systemically injected KA.

Historically, in comparison to Slow rats, Fast rats have lower kindling rates, shorter latencies to forelimb clonus, longer convulsion durations and longer after-discharge durations (Racine et al., 1999). In the present experiment, Fast and Slow rats were systemically injected with KA and monitored for the subsequent development of generalized seizures. Of the 60 animals injected with 20mg/kg KA in the first experiment, 77.8% of the Slow rats and 78.8% of the Fast rats developed *generalized* SE as described by Kelly and McIntyre (1994). In the second experiment, 16.7% of the Slow and 66.7% of the Fast rats injected with 20mg/kg KA developed similar generalized seizures. Contrary to the first experiment, a high number of Slow rats (27.7%) injected with 20mg/kg developed *ambulatory/masticatory* SE, as defined by Hanforth and Ackermann (1988). Yet, following the development of SE, the Fast rats had a higher overall mortality rate than the Slow rats, observed as 20% and 11.1%, respectively when both experiments are taken into consideration. As such, the Fast rats were much more sensitive to the convulsive effect of KA, which was further substantiated directly by the seizure behaviour observed.

In order to interpret the results obtained in this experiment, it is important to ascertain what level of KA-induced seizure susceptibility the original two parental strains exhibited, since the Fast and Slow rats were derived from a hybridization of two different parent populations. The two parental strains, Wistar-Furht (WF) and Long Evans Hooded (LEH) rats, reportedly have latencies to SE of 91 min and 132 min, respectively, when injected systemically with 12mg/kg of KA (Golden et al., 1995). The latencies to SE described by Golden et al. (1995) are not directly comparable to the latencies to SE described in the present study due to methodological differences. The important point of their finding, however, is the fact that the parental strains show inherent differences to KA-induced SE, which existed prior to them being involved in our selection protocol to develop the Fast and Slow strains. In an even earlier study by Golden et al. (1991), showed that not only did WF have shorter latencies to develop SE, but WF also spent more time in seizure activity than did the LEH rats, which were clearly much less vulnerable to KA. Subsequently, full KA dose response curves again showed that WF were more susceptible than LEH to the SE inducing effects of KA at all doses, where the maximal dose examined was 14mg/kg.

In our pilot study, it became apparent that, similar to the parent population, there was a dichotomy in the KA dose required to induce SE. At lower KA doses (12 or 15mg/kg), Fast rats would enter into status without difficulty, but at similar doses, generalized seizures or SE were seldom observed in the Slow rats. Indeed, to develop SE in most rats, this required that we increase the dose of KA to 20mg/kg in all animals. At 20mg/kg, 74.1% of the Slow rats and 54.5% of the Fast rats developed generalized SE

and lived in the first experiment. But as was mentioned previously, there was a much higher mortality rate in the Fast rats, and those percentages do not include the rats that died.

Fast and Slow rats also showed significant differences with respect to their overall SE severity. Not only did the Fast rats enter SE more quickly, but they also had much more severe SE as assessed by the percent duration of stage 3/4/5 generalized seizures. Fast rats spent 26.3 ± 2.8 % of their total SE time having severe generalized seizures, while Slow rats only spent 4.4 ± 0.7 % of their total time engaged in similar seizures. Intermittently between severe seizures, the Slow rats performed more masticatory behaviour, punctuated with freezing and circling, usually associated with masticatory and ambulatory SE (Hanforth and Ackermann, 1988; McIntyre et al., 1991). By comparison, Fast rats alternated between generalized stage 3/4/5 seizures more often than Slow rats, and were rarely observed exhibiting masticatory or ambulatory SE. Based on our observations, we suggest that the process of selective breeding of the Fast and Slow strains based on amygdala excitability accentuated historical differences in KA sensitivity between the original parental strains. .

One of the first major behavioural differences observed between the Fast and Slow rats was the latency to develop SE, which for this experiment was defined as the period of time, in minutes, from the i.p. injection of KA to the first behavioural stage 5 seizure. The Fast rats entered SE at a significantly faster rate than the Slow rats. On average, it took about 3 times longer for a Slow rat to achieve generalized convulsions. This difference in latency time to SE in the Fast and Slow rats is analogous to their differing kindling rates (Racine et al., 1999). On average it normally takes ~45 electrical

stimulations to reach the first stage 5 in Slow rats, while it only takes ~12 electrical stimulations to achieve a similar seizure in Fast rats. However, in the KA paradigm, latency to SE could be a product of the diffusion rate of KA across the blood-brain barrier (BBB) (Willoughby et al., 1997; Berger et al., 1986). Scherer-Singler and McGeer (1979) found that ~ 0.07 and 0.25% of radioactive KA administered systemically was present in the brain 30 and 60 minutes later, thus concluding that neither KA nor its metabolite are easily incorporated into the brain. It is possible that the difference in SE latency observed between the Fast and Slow rats is related to physiological differences in BBB dynamics. In fact the significant drop in latency to SE seen in the Slow animals during the second experiment could be a consequence of BBB damage induced by the electrode surgery. However, arguing against this is the fact that the latency to SE did not significantly change in the Fast rats between the two studies, and there is no *a priori* reason to suspect a significant, surgically-based BBB compromise in one strain but not the other. On the other hand, based on the behavioural results, it seems that the presence of electrodes in the brain significantly altered several aspects of the animals' response to KA, which were clearly different from the action of KA in a naïve nervous system. Indeed, Loscher et al. (1993) reported in kindled and implanted control rats, similar alterations in seizure thresholds and excitatory amino acid concentrations in brain regions at the electrode tip after prolonged electrode implantation. Clearly implanted electrodes *per se* increase brain excitability.

Since BBB dynamics were not assessed in this study, further experiments examining the transport rates of KA across the BBB in the Fast and Slow rats could further our understanding of the mechanisms underlying SE latency. One should also take

into consideration the fact that administration of KA has been shown to cause breaks in the BBB (Berger et al., 1986; Saija et al., 1992; Pont et al., 1995). Thus, in a naïve system such as that seen in the first experiment, it is possible that lower concentrations of KA could cause breaks in the BBB of Fast rats, ultimately leading to a faster rate of diffusion for KA across the BBB and the quicker manifestation of generalized seizures.

In concordance with this idea of a heightened action of KA in the Fast rats is the observation that Fast rats showed significantly fewer WDS before entering SE in the first experiment. WDS are characterized by paroxysmal shaking of the head, neck and trunk (Frush and McNamara, 1986). Either electrical or chemical stimulation of the limbic structures can lead to the production of WDS, the induction of which is often associated with a delayed progression to seizure (Engel et al., 1980; Dagci et al., 2002). Using the KA paradigm, Dagci et al. (2002) showed that the disappearance of WDS was associated with the commencement of seizure activity. In the present experiment, Fast rats went directly into SE, having only a few WDS, whereas the Slow rats spent prolonged periods of time having WDS without the manifestation of any other seizure behaviour. Grimes et al. (1988) reported that the dentate gyrus (DG) is responsible for the WDS following KA treatment and disinhibition of the GABAergic input from the DG to the hippocampal CA regions has been proposed in the initiation of epileptogenic activity in various animals (Frerking et al., 1999; Min et al., 1999). Based on our behavioural results, the increased number of WDS seen in the Slow rats seems to suggest an increased ability to suppress seizure activity, possibly mediated through the DG. When WDS behaviour is no longer present, seizures appear. In this context, the Fast rats have a significantly lower DG kindling rate and longer DG after-discharge durations (ADD), suggesting inherently

reduced inhibition compared to Slow rats (McIntyre et al., 1999). Also, recent unpublished morphological data show an increased volume of DG in Slow rats as compared to Fast rats. Clearly a reduction in the size of the DG in Fast rats may indicate that the anatomy of that structure is different in them compared to Slow rats, and may even imply that the structure is compromised in some unknown way.

Although, the mechanisms underlying the differences in seizure susceptibilities in the Fast and Slow rats remain largely unknown, it is evident that the Fast and Slow rats show different hippocampal damage profiles after a 20mg/kg KA i.p. injection, and that such damage is known to be associated with epileptogenesis. For example, it is well known that KA is a potent excitatory analogue of glutamic acid (Glu) and has a high affinity for the AMPA and KA receptor subset of excitatory receptors in the CNS (Johnston et al., 1974; Nadler et al., 1978; Boulter et al., 1990; Sato et al., 1993; Lason et al., 1997). Brain regions with high levels of AMPA and KA receptors tend to succumb to the effects of KA more readily than other structures (Balchen et al., 1993; Yang et al., 1998). As such, there is a very specific hierarchy to damage in the CNS, with the hippocampal areas being the most vulnerable followed by other limbic structures (Nadler et al., 1978). In the present study, analysis of hippocampal damage in Fast and Slow rats four weeks following SE showed a distinct pattern of damage. Damage in both strains was isolated to the CA3 region of the hippocampus (which has the highest concentration of KA receptors). However, damage was much more prominent in the Fast rats that had undergone severe SE as compared to the Slow rats. The CA3b region of the Fast rats that had severe SE was devoid of neurons, which were replaced by glial cells, whereas Slow rats had less CA3 damage that involved a loss of neurons. Instead, the cell damage to the

dorsal hippocampus of Slow rats was indicated as darkly stained cells that were pyknotic. It was only in the ventral regions of the hippocampus that the Fast and Slow rats showed similar damage profiles, and only in animals that had similar forms of SE with equal severity. Also the hippocampal and other brain damage that was evident in the two strains was much less than that which is normally present four weeks following severe generalized SE (Kelly and McIntyre, 1994). It is possible that the limited amount of damage observed in this study resulted from the seizures being terminated two hours after SE onset, a procedure that was effected if seizure-induced death was imminent. Still it must be remembered that there remained a difference between the strains in their KA-induced damage profiles.

A potential explanation for the observed differences in CA3 damage between the strains could be due to different receptor composition in this region. Kainic acid acts on the excitatory receptors, namely NMDA, AMPA and kainate (Choi, 1988; Hicks and Conti, 1996). KA has the highest affinity for kainate receptors, which are formed from five subunits; GluR5, GluR6, GluR7, KA1 and KA2 (Lerma, 1999). The kinetics and functionality of a channel ultimately lies in its subunit composition, since homomeric and heteromeric channels can be formed. GluR6, like GluR5 and 7 can form homomeric and heteromeric functional channels, while KA1 and KA2 can only form heteromeric channels when combined with one of the aforementioned subunits (Liliu et al., 2002; Joels et al., 1996; Paternain et al., 2000). The diversity of KA receptors is further enhanced by subunit variants due to mRNA alternative splicing and mRNA editing processes that regulate KA receptor channels conductance and calcium permeability (Liliu et al., 2002). The primary subunits expressed in the CA3 pyramidal cells are

GluR6, KA1 and KA2 (Paternain et al., 2000; Malva et al., 1998). It is possible that further examination of the CA3 region in Fast and Slow rats would show differences in KA subunit expression. If this were the case, it might explain the difference in damage observed four weeks after KA induced seizure, since excitotoxic stimulation of these receptors is known to increase calcium permeability, which results in the activation of apoptotic and necrotic pathways.

Activation of KA receptors can also have a downstream effect on GABA induced inhibition since the GluR5 and 6 subunits coexist in unique populations of hippocampal interneurons (Paternain et al., 2000). Approximately 24-27% of GABAergic interneurons of the pyramidal cellular layer for CA1, CA3 and the stratum oriens of the CA3 express the above-mentioned subunits. It is possible that the differences observed in latency to SE in the Fast and Slow rats could be related to a difference in the release of inhibition in combination with differing susceptibilities to excitation.

As mentioned above, the DG plays a role in inhibition and is thought to act as a filter, slowing down the spread of seizure activity (Alger and Teyler, 1976; McNaughton et al., 1981). The DG expresses GluR 6, GluR7, KA1 and KA2 mRNA, but unlike the CA3 regions, the DG has no KA mediated synaptic responses in either control or kindled animals (Wisden and Seeburg, 1993; Lerma et al., 1997; Behr et al., 1998). As such, it is thought that modulation of GABAergic inhibition is brought about via the activation of presynaptic KA receptors on the terminals of inhibitory interneurons. Kindling can further enhance KA receptor mediated depression of GABAergic inhibition. Since the Fast and Slow rats were selected from a parent population of rats that already expressed mild differences in seizure susceptibility, one can speculate that kindling induced

enhancement of KA mediated depression of GABAergic inhibition occurred with each successive breeding selection. Evidence shows that the Fast and Slow rats express different GABAergic subunits constitutively, whereby Fast have lower levels of α_1 subunit and higher levels of α_2 , α_3 and α_5 subunit than Slow rats in the amygdala, piriform, endopiriform and perirhinal cortices. Hippocampal examination of both GABAergic and KA subunit expression would be the next step in understanding the behavioural differences observed in this experiment. Once the question of subunit expression is answered, there still remains the conundrum of the dose-induced effect KA has on GABAergic inhibition. Since a 5 μ M concentration of KA depresses GABAergic transmission, whereas a submicromolar concentration (0.3 μ M) of KA enhances GABAergic transmission (Kullmann, 2001). Perhaps the difference in behaviours and cellular damage observed between the Fast and Slow rats is related ultimately to whether the dose given was high enough in both brains to act in a similar manner. Or, perhaps regardless of dose, the difference between the Fast and Slow rats is their ability to behaviourally manifest convulsions. The EEG of both Fast and Slow rats that have obtained either ambulatory/masticatory and/or generalized SE shows similar levels of activation, which is further substantiated by the similarity in c-Fos protein expression. However, detailed analysis of the EEG patterns between the Fast and Slow rats is required to ascertain what, if any, functional neurocircuitry differences might exist between them.

The results obtained in this experiment clearly show KA-induced behavioural differences between the Fast and Slow rats. The mechanism(s) for the behavioural differences remains unknown. However, differences in seizure susceptibility that were

apparent in the kindling paradigm were corroborated in the KA paradigm, and add further validity to the use of these two strains as a good models for susceptibility and resistance to TLE. It is possible that the difference in KA-induced seizure behaviour and the subsequent damage in the strains are related to blood-brain- barrier (BBB) permeability, regional receptor differences and/or inhibitory and excitatory network differences. Understanding how these mechanisms differ between the strains will further epileptic research, allowing for better diagnosis and treatment of the disease.

References

- Alger, B. E. and Teyler, T. J.** (1976). Long-term and short-term plasticity in CA1, CA3 and dentate region of the rat hippocampal slice. *Brain Research* **110**, 463-480.
- Balchen, T., Berg, M. and Diemer, N. H.** (1993). A paradox after systemic kainate injection in rats: lesser damage of hippocampal CA1 neurons after higher doses. *Neuroscience Letters* **163**, 151-154.
- Behr, J., Lyson, K. J. and Mody, I.** (1998). Enhanced propagation of epileptiform activity through the kindled dentate gyrus. *Journal of Neurophysiology* **79**, 1726-1732.
- Ben-Ari, Y.** (1985). Limbic seizures and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14**, 375-403.
- Ben-Ari, Y., Tremblay, E., Riche, D., Ghilini, G. and Naquet, R.** (1981). Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline, or pentetrazole; metabolic mapping using deoxyglucose method with special reference to the pathology of epilepsy. *Neuroscience* **6**, 1361-1391.
- Berger, M. L., Lefauconnier, J. M., Tremblay, E. and Ben-Ari, Y.** (1986). Limbic seizures induced by systemically applied kainic acid: How much kainic acid reaches the brain? *Advances in Experimental medicine and biology* **203**, 199-209.
- Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. and Heinemann, S.** (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. *Science* **249**, 1033-7.

- Choi, D. W.** (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1, 623-634.
- Collins, R., Lothman, E. and Olney, J.** (1983). Status epilepticus in the limbic system: biochemical and pathological changes. *Advances in Neurology* 34, 277-288.
- Collins, R. L.** (1972). Audiogenic seizures. In *Experimental models of epilepsy: A manual for the laboratory worker*, eds D. P. Purpura D. P. Penry D. Tower D. M. Woodbury and R. Walter), pp. 346-372. New York: Raven Press.
- Dagci, T., Tan, M., Koylu, E. O., Tan, U. and Pogun, S.** (2002). The effect of octreotide on kainate-induced wet dog shakes and seizure activity in male and female rats. *international Journal of Neuroscience* 112, 829-839.
- Engel, J., Ackermann, R. F. J., Caldecott-Hazard, S. and Kuhl, D. E.** (1980). Epileptic activation of antagonistic systems may explain paradoxical features of experimental and human epilepsy: a review and hypothesis. In *Kindling 2*, (ed. J. A. Wada), pp. 193-218. New York: Raven press.
- Faingold, C.** (1988). The genetically epilepsy-prone rat. *General Pharmacology* 19, 331-338.
- Fisher, R. S.** (1989). Animal models of the epilepsies. *Brain Research reviews* 14, 245-278.
- Frerking, M., Petersen, C. C. and Nicoll, R. A.** (1999). Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* 96, 12917-12922.

- Frush, D. P. and McNamara, J. O.** (1986). Evidence implicating dentate granule cells in wet dog shakes produced by kindling stimulations of entorhinal cortex. *Experimental Neurology* **92**, 102-113.
- Gastaut, H.** (1973). Dictionary of Epilepsy. Geneva: World Health Organization.
- Gloor, P., Olivier, A., Quesney, L., Andermann, F. and Horowitz, S.** (1982). The role of the limbic system in experimental phenomena of temporal lobe epilepsy. *Annals in Neurology* **12**, 129-144.
- Goddard, G. V., McIntyre, D. C. and Leech, C. K.** (1969). A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology* **25(3)**, 295-330.
- Golden, G., Smith, G., Ferraro, T. and Reyes, P.** (1995). Rat strain and age differences in kainic acid induced seizures. *Epilepsy Research* **20**, 151-159.
- Golden, G. T., Smith, G. G., Ferraro, T. N., Reyes, P. F., Kulp, J. K. and Fariello, R. G.** (1991). Strain differences in convulsive response to the excitotoxin kainic acid. *NeuroReport* **2**, 141-144.
- Green, R. C. and Seyfried, T. N.** (1991). Kindling susceptibility and genetic seizure predisposition in inbred mice. *Epilepsia* **32**, 22-26.
- Grimes, L., McGinty, J., McLain, P., Mitchell, C., Tilson, H. and Hong, J.** (1988). Dentate granule cells are essential for kainic acid-induced wet dog shakes but not for seizure. *Journal of Neuroscience* **8**, 256-264.
- Handforth, A. and Ackermann, R. F.** (1988). Functional [¹⁴C]2-deoxyglucose mapping of progressive states of status epilepticus induced by amygdala stimulation in the rat. *Brain Research* **460**, 94-102.

- Hauser, W. A.** (1983). Status epilepticus: frequency, etiology, and neurological sequelae. In *Advances in Neurology: Status epilepticus*, vol. 34 eds A. V. Delgado-Escueta D. M. Treiman and R. J. Porter), pp. 3-14. New York: Raven.
- Hicks, T. P. and Conti, F.** (1996). Amino acids as the source of considerable excitation in cerebral cortex. *Canadian journal of physiological pharmacology* **74**, 341-61.
- Hosford, D. A., Clark, S., Cao, Z., Wilson, W. A. J., Lin, F. H., Morrisett, R. A. and Huin, A.** (1992). The role of GABA-B receptor activation in absence seizures of lethargic(Ih/Ih) mice. *Science*, 398-401.
- Imaizumi, K. and Nakano, T.** (1964). Mutant stocks. Strains: *El. Mouse Newsletter* **31**, 57.
- Jobe, P. C., Brown, R. D. and Dailey, J. W.** (1981). Effect of Ro 4-1284 on audiogenic seizure susceptibility and intensity in epilepsy-prone rats. *Life Science* **28**, 2031-2038.
- Joels, M., Bosman, A., Hendriksen, H., Diegenbach, P. and Kamphuis, W.** (1996). Corticosteroid actions on the expression of kainate receptor subunit mRNAs in rat hippocampus. *Molecular Brain Research* **37**, 15-20.
- Johnston, G. A., Curtis, D. R., Davies, J. and McCulloch, R. M.** (1974). Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. *Nature* **248**, 804-5.
- Kaneko, S., Okada, M., Iwasa, H., Yamakawa, K. and Hirose, S.** (2002). Genetics of epilepsy: current status and perspectives. *Neuroscience Research* **44**, 11-30.

- Kelly, M. E. and McIntyre, D. C.** (1994). Hippocampal kindling protects several structures from the neuronal damage resulting from kainic acid-induced status epilepticus. *Brain Research* **634**, 245-256.
- King, D. and Ajmone-Marsan, C.** (1977). Clinical features and ictal patterns in epileptic patients with EEG temporal lobe foci. *Annals in Neurology* **2**, 138-147.
- Kullman, D. M.** (2001). Presynaptic kainate receptors in the hippocampus: slowly emerging from obscurity. *Neuron* **32**, 561-564.
- Lason, W., Turchan, J., Przewlocka, B., Labuz, D., Mika, J. and Przewlocki, R.** (1997). Seizure-related changes in glutamate R2 and R5 receptor genes expression in the rat hippocampal formation. *Journal of Neural Transmission* **104**, 125-133.
- Leech, C. K. and McIntyre, D. C.** (1976). Kindling rates in inbred mice: an analog to learning? *Behavioural Biology* **16**, 439-452.
- Lerma, J.** (1999). Kainate receptors. In *Handbook of experimental pharmacology: ionotropic glutamate receptors in the CNS*, vol. 141 eds P. Jonas and H. Monyer), pp. 275-307. Berlin: Springer.
- Lerma, J., Morales, M., Vicente, M. A. and Herreras, O.** (1997). Glutamate receptors of the kainate type and synaptic transmission. *Trends in Neuroscience* **20**, 9-12.
- Lilliu, V., Perrone-Capano, C., Pernas-Alonso, R., Trelles, R. D., d'Amato, G. L. C., Zuddas, A. and Porzio, U. d.** (2002). Ontogeny of kainate receptor gene expression in the developing rat midbrain and striatum. *Molecular Brain Research* **104**, 1-10.
- Losher, W., Horstmann, D., Honack, D., Rundfeldt, C. and Wahnschaffe, U.** (1993). Transmitter amino acid levels in rat brain regions after amygdala-kindling

or chronic electrode implantation without kindling: evidence for a pro-kindling effect of prolonged electrode implantation. *Neurochemistry Research* **18**, 775-81.

Lothman, E., Collins, R. and Ferrendelli, J. (1981). Kainic acid-induced limbic seizures: electrophysiologic studies. *Neurology* **31**, 806-812.

Lothman, E. W. and Collins, R. C. (1981). Kainic acid induced limbic seizures: metabolic, behavioural, electroencephalographic and neuropathological correlates. *Brain Research* **3**, 299-318.

Malva, J. O., Carvalho, A. P. and Carvalho, C. M. (1998). Kainate receptors in hippocampal CA3 subregion: evidence for a role in regulating neurotransmitter release. *Neurochemistry International* **32**, 1-6.

McIntyre, D., Kelly, M. E. and Dufresne, C. (1999a). Fast and Slow amygdala kindling rat strains: comparison of amygdala, hippocampal, piriform and perirhinal cortex kindling. *Epilepsy Research* **35**, 197-209.

McIntyre, D. C., Kelly, M.E., Armstrong, J.N., (1993). Kindling in the perirhinal cortex. *Brain Research* **615**, 1-6.

McIntyre, D. C. and Anisman, H. (2000). Anxiety and impulse control in rats selectively bred for seizure susceptibility. In *Neurobiological foundations of aberrant behaviours contemporary issues in modeling psychopathology*, (ed. M. S. M. a. I. Weiner), pp. 29-43. Boston: Kluwer academic publishers.

McIntyre, D. C., Don, J. C. and Edson, N. (1991). Distribution of [¹⁴C]2-deoxyglucose after various forms and durations of status epilepticus induced by stimulation of a kindled amygdala focus in rats. *Epilepsy Research* **10**, 119-33.

- McIntyre, D. C., Kent, P., Hayley, S., Merali, Z. and Anisman, H.** (1999c). Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. *Brain Research* **840**, 65-74.
- McIntyre, D. C. and Plant, J. R.** (1993). Long-lasting changes in the origin of spontaneous discharges from amygdala-kindled rats: piriform versus perirhinal cortex in vitro. *Brain Research* **624**, 268-276.
- McNamara, J. O.** (1994). Cellular and molecular basis of epilepsy. *Journal of Neuroscience* **14**, 3413- 3425.
- McNaughton, B. L., Barnes, C. A. and Andersen, P.** (1981). Synaptic efficacy and EPSP summation in granule cells of rat fascia dentata studied in vitro. *Journal of Neurophysiology* **46**, 952-66.
- Min, M. Y., Melyan, Z. and Kullman, D. M.** (1999). Synaptically released glutamate reduces gamma-aminobutyric acid (GABA)ergic influences on periventricular somatostatin neurons. *Proceedings of the National Academy of Science of the United States of America* **96**, 9932-9937.
- Mohapel, P. and McIntyre, D. C.** (1998). Amygdala kindling-resistant (Slow) or prone (Fast) rat strains show differential fear responses. *Behaviour Neuroscience* **112**, 1402-1413.
- Molino, A. and McIntyre, D. C.** (1972). Another inexpensive headplug for the electrical recording and or stimulation of rats. *Physiol. Behav.* **9**, 273-5.
- Morrell, F., Tsuru, N., Hoepfner, T.J., Morgan, D., Harrison, W.H.,** (1976). Secondary epileptogenesis in frog forebrain: Effect of inhibition of protein synthesis. In *Kindling*, (ed. J. A. Wada), pp. 41-60. New York: Raven Press.

- Nadler, J., Perry, B. and Cotman, C.** (1978). Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. *Nature* **271**, 676-677.
- Noebels, J. L.** (1986). Mutational analysis of inherited epilepsies. In *Basic mechanism of epilepsy: Molecular and cellular approaches*, eds A. V. Delgado-Escueta A. A. Ward D. M. Woodbury and R. J. Porter), pp. 97-113. New York: Raven press.
- Noebels, J. L., Qiao, X., Bronson, R. T., Spencer, C. and Davisson, M. T.** (1990). Stargazer: a new neurological mutant on chromosome 15 in the mouse with prolonged cortical seizures. *Epilepsy Research* **7**, 129-135.
- Noebels, J. L. and Sidman, R. L.** (1979). Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. *Science* **204**, 1334-1336.
- Olney, J., Rhee, V. and Ho, O.** (1974). Kainic acid: a powerful neurotoxic analogue of glutamate. *Brain Research* **77**, 501-512.
- Paternain, A. V., Herrera, M. T., Nieto, M. A. and Lerma, J.** (2000). GluR5 and GluR6 kainate receptor subunits coexist in hippocampal neurons and coassemble to form functional receptors. *The Journal of Neuroscience* **20**, 196-205.
- Paxinos, G. and Watson, C.** (1986). The rat brain in stereotaxic coordinates. NY: New York Academic press.
- Pont, F., Collet, A. and Lallement, G.** (1995). Early and transient increase of rat hippocampal blood-brain barrier permeability to amino acids during kainic acid-induced seizures. *Neuroscience Letters* **184**, 52-54.
- Poulter, M. O., Brown, L. A., Tynan, S., Willick, G., William, R. and McIntyre, D. C.** (1999). Differential expression of alpha 1, 2, 3 and 5 GABA_A receptor subunits in

- seizure-prone and seizure resistant rat models of temporal lobe epilepsy. *The Journal of Neuroscience* **19**, 4654-4661.
- Racine, R.** (1972). Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogram Clinical Neurophysiology* **32**, 281-294.
- Racine, R., Steingart, M. and McIntyre, D. C.** (1999). Development of kindling-prone and kindling-resistant rats: selective breeding and electrophysiological studies. *Epilepsy Research* **35**, 183-195.
- Revuelta, M., Castano, A., Venero, L., Machado, A. and Cano, J.** (2001). Long-lasting induction of brain-derived neurotrophic factors in restricted to resistant cell populations in an animal model of status epilepticus. *Neuroscience* **103**, 955-969.
- Saija, A., Princi, P., Pisani, S. A., Santoro, S. G., Pasquale, R. D., Massi, M. and Costa, G.** (1992). Blood-brain barrier dysfunctions following systemic injection of kainic acid in the rat. *Life Science* **51**, 467-477.
- Sato, K., Kiyama, H. and Tohyama, H.** (1993). The differential expression patterns of messenger RNAs encoding non-N-methyl-aspartate glutamate receptor subunits (GluR1-4) in the rat brain. *Neuroscience* **52**, 515-539.
- Scherer-Singler, U. and McGeer, E. G.** (1979). Distribution and persistence of kainic acid in brain. *Life Science* **26**, 1015-22.
- Seyfried, T. N. and Glaser, G. H.** (1985). A review of mouse mutants as genetic models of epilepsy. *Epilepsia* **26**, 143-50.
- Seyfried, T. N., Glaser, G. H., Yu, R. K. and Palayoor, S. T.** (1986). Inherited convulsive disorders in mice. In *Basic mechanisms of the epilepsies: molecular*

and cellular approaches, eds A. V. Delgado-Escueta A. A. J. Ward D. M.

Woodbury and R. J. Porter), pp. 115-33. New York: Raven Press.

Velasco, F., Velasco, M., Ogarrio, C. and Fanghanel, G. (1987). Electrical stimulation of the centromedian thalamic nucleus in the treatment of convulsive seizures: a preliminary report. *Epilepsia* **28**, 421-30.

Wada, J., Mizoguchi, T. and Osawa, T. (1978). Secondary generalized convulsive seizures induced by daily amygdaloid stimulation in rhesus monkeys. *Neurology* **28**, 1026-1036.

Willmore, L. J. and Ueda, Y. (2002). Molecular biology and genetics of epilepsy. *Acta Med Okayama* **56**, 57-68.

Willoughby, J. O., Mackenzie, L., Medvedev, A. and Hiscock, J. J. (1997). Fos induction following systemic kainic acid: early expression in hippocampus and later widespread expression correlated with seizure. *Neuroscience* **77**, 379-92.

Wisden, W. and Seeburg, P. H. (1993). A complex mosaic of high-affinity kainate receptors in rat brain. *Journal of Neuroscience* **13**, 3582-3598.

Yang, Y., Tandon, P., Liu, Z., Sarkisian, M., Stafstrom, C. E. and Holmes, G. L. (1998). Synaptic reorganization following kainic acid-induced seizures during development. *Developmental brain research* **107**, 169-177.

Appendix 1

Behavioural seizure scale (Racine et. al, 1972)

Stage	Behaviour
1	Freezing with staring and mastication
2	Severe jaw mastication and drooling
3	Mild to severe bilateral fore and hind limb clonus without rearing
4	Rearing with back extended in addition to severe forelimb clonus
5	Rearing and falling with the addition of severe forelimb clonus