**STATUS EPILEPTICUS INDUCED CHANGES
WITHIN THE LIMBIC SYSTEM**

by

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A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of
the requirements for the degree of

Master of Science

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ABSTRACT

In rare cases, especially in epileptic patients, seizure offset mechanisms fail and result in protracted seizures called 'status epilepticus' (SE). The causes of SE are unknown, but there is a theorized imbalance between the inhibitory and excitatory mechanisms. Thus, this experiment used an epileptic (i.e., kindled) brain to study SE-induced changes in extracellular concentrations of GABA and glutamate associated with SE induction and 24 hours thereafter. The first phase involved amygdala kindling with amygdala and hippocampal afterdischarge threshold (ADT) assessments pre- and post-amygdala kindling. The second phase, SE induction, involved continuous stimulation of the kindled amygdala for 60 min. During this procedure and for 6 hours thereafter, bilateral amygdala dialysates were collected using *in vivo* microdialysis, while behaviour and EEG were monitored for signs of SE. The next day, the amygdala and hippocampal ADTs were reassessed, while the behaviour, EEG and dialysis protocols continued.

It was observed that the more convulsive seizures expressed during the SE induction procedure, the greater the likelihood of developing SE. About 30% of the rats developed SE (SE-positive; SEP group), while the remaining did not (non-SE; NSE group). Although the NSE rats did not develop SE, their EEG suggested more serious seizures than were indicated by their behaviour. For example, many of the NSE subjects exhibited spontaneous convulsive seizures or 'EEG-only seizures' following the attempted SE induction phase.

During the SE induction phase, amygdala stimulation caused bilateral extracellular GABA and glutamate increases in the most severe SEP group. Shortly following the stimulation offset, however, the GABA and glutamate returned to baseline, independent of group membership or hemisphere (stimulated vs. non-stimulated). But, one hour later, GABA and glutamate levels again increased dramatically in the kindled amygdala in the more severe SEP subjects. The next day, the amygdala ADT test caused glutamate to increase in the non-kindled amygdala in the SEP group, even though no AD had occurred. By contrast, the dramatically increased hippocampal AD triggered a convulsive seizure in most NSE rats, which was associated with a bilateral decrease in GABA but bilateral increase in glutamate; at the same time, in the SEP group, there was an overall increase in both GABA and glutamate levels, especially in the kindled amygdala. This increased AD and/or convulsive seizure likely reflects increased excitability. Furthermore, this excitatory event can be correlated with the increased excitatory amino acid (i.e. glutamate) levels. Looking at the hemispheric and group differences in both GABA and glutamate, the differential changes possibly reflect less efficient release and re-uptake mechanisms which may, in whole or in part, be caused by the brain injury. Subsequently, the amino acid levels returned to baseline.

The brain injury evident at approximately 24 hours was positively correlated with SE severity in most structures. This was most notable in the kindled amygdala of the SEP subjects, where the stimulation, probe and cannula caused the majority of damage. In contrast, the injury in the cortex and hippocampus was uncorrelated and negatively correlated, respectively.

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TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF FIGURES.....	xi
LIST OF APPENDICES	xiv
INTRODUCTION	1
Epilepsy	1
Status Epilepticus	2
Kindling as model for temporal lobe epilepsy	5
Kindling-based SE model	5
Experimental SE types.....	9
SE damage	14
SE circuitry	17
Amino Acids	19

Inhibitory Amino Acid: GABA.....	19
Excitatory Amino Acids: Glutamate	20
Microdialysis.....	22
Capillary Electrophoresis	23
Purpose	24
MATERIALS AND METHODS	25
Subjects.....	25
Surgery.....	25
Kindling	26
Probes	26
SE Induction Procedure	29
Day Prior to SE induction	29
Day 1	29
Day 2.....	31
Day 1: Microdialysis.....	32
Day 2: Microdialysis.....	32
Data Analysis	37
Before SE	37

SE Induction Day.....	37
Day 2: 24 Hours After SE Induction	40
Data Analysis Exceptions	42
RESULTS	43
Data Presentation.....	43
Before SE.....	52
Pre-Kindling	52
Kindling	52
The Day before SE Induction.....	59
SE Induction Day (Day 1)	59
Baseline.....	59
SE Induction.....	62
Post-Induction Hour 1.....	70
Post-Induction Hours 2 to 6	82
Day 2: 24 hr After SE Induction	92
Baseline measurements before Amygdala ADT test on Day 2.....	92
Amygdala ADT and AD Duration.....	100

Baseline measurements before Hippocampal ADT test on Day 2	100
Ipsilateral Hippocampus ADT and AD Duration	103
Post-ADT	113
Day 2 Pathology	118
DISCUSSION	134
Before SE	134
SE Induction Day	135
Day 2	139
Amygdala ADT test	139
Ipsilateral Hippocampus ADT test	141
Post-ADT	144
Pathology	145
BIBLIOGRAPHY	159

LIST OF TABLES

TABLE	DESCRIPTION	PAGE
1	Racine's (1972) seizure classification scale.	7
2	Behaviour, EEG and neurological activity profiles for the four types of experimental SE.	11
3	Average ($\pm$ S.E.M.) initial amygdala ADT (mA) and AD duration for the NSE and SEP groups.	54
4	Average ($\pm$ S.E.M.) initial ipsilateral hippocampus ADT (mA) and AD duration (sec) for the NSE and SEP groups.	56
5	Average ($\pm$ S.E.M.) amygdala kindling rate for the NSE and SEP groups.	58
6	Average ($\pm$ S.E.M.) ipsilateral hippocampus ADT (mA) and AD duration (sec) for the NSE and SEP groups on the day before the SE induction procedure.	61
7	Average ($\pm$ S.E.M.) baseline GABA and glutamate concentrations (mM) from the kindled and non-kindled hemispheres in the NSE and SEP groups on Day 1.	64
8	Average ($\pm$ S.E.M.) number of convulsive seizures during SE induction for the NSE, ISE and ASE groups.	67
9	Average ($\pm$ S.E.M.) number of convulsive seizures for the NSE, ISE and ASE groups during the Post-Induction Hour 1.	75
10	Average ($\pm$ S.E.M.) number of EEG-only stage 5 seizures for the NSE, ISE and ASE groups during the Post-Induction Hour 1.	78
11	Average ($\pm$ S.E.M.) number of convulsive seizures for the NSE, ISE and ASE groups during the Post-Induction Hours 2 to 6.	87
12	Average ($\pm$ S.E.M.) number of EEG-only stage 5 seizures for the NSE and SEP groups during the Post-Induction Hours 2 to 6.	89

13	Average ($\pm$ S.E.M.) Pre-Amygdala ADT baseline GABA and glutamate concentrations (mM) from the kindled and non-kindled hemispheres in the NSE and SEP groups.	97
14	Average ($\pm$ S.E.M.) Pre-Hippocampus ADT baseline GABA and glutamate concentrations (mM) from the kindled and non-kindled hemispheres in the NSE and SEP groups.	105
15	Average ($\pm$ S.E.M.) ipsilateral hippocampus ADT (mA) for the NSE and SEP groups on Day 2.	108
16	Average ($\pm$ S.E.M.) ipsilateral hippocampus AD duration (sec) for the NSE and SEP groups on Day 2.	110
17	Average ($\pm$ S.E.M.) number of different stages of convulsive seizures at the ipsilateral hippocampus ADT for the NSE and SEP groups on Day 2.	112
18	Average ($\pm$ S.E.M.) Day 1 baseline GABA to Glutamate ratio (mM) from the kindled and non-kindled hemispheres in the NSE and SEP groups.	151
19	Average ($\pm$ S.E.M.) Pre-Amygdala ADT baseline GABA to Glutamate ratio (mM) from the kindled and non-kindled hemispheres in the NSE and SEP groups.	153
20	Average ($\pm$ S.E.M.) Initial, Pre-SE Induction and Day 2 contralateral hippocampus ADTs (mA) and AD duration (sec) for the NSE group (N = 3).	157

LIST OF FIGURES

Figure 1. Microdialysis probe with cannula.	28
Figure 2. Dialysate schedule for Day 1.	34
Figure 3. Dialysate schedule for Day 2.	36
Figure 4. Day 1 and Day 2 samples selected to be analyzed by capillary electrophoresis, labeled as they are referred to in the analyses.	39
Figure 5. Schematic diagram taken from Paxinos and Watson (1986) at bregma -3.30 mm showing the stimulating electrode tip locations in the amygdala, hippocampus, and thalamus.	45
Figure 6. Schematic diagram taken from Paxinos and Watson (1986) at bregma -5.60 mm showing the stimulating electrode tip location in the amygdala.	47
Figure 7. Schematic diagram taken from Paxinos and Watson (1986) at bregma -2.80 mm showing the microdialysis probe locations in both hemispheres.	49
Figure 8. Schematic diagram taken from Paxinos and Watson (1986) at bregma -3.80 mm showing the microdialysis probe locations in both hemispheres.	51
Figure 9. Average ($\pm$ S.E.M.) percent change from baseline GABA concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups during SE stimulation. (Note the different scales.).....	69
Figure 10. Average ($\pm$ S.E.M.) percent change from baseline glutamate concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups during SE stimulation. (Note the different scales.).....	72
Figure 11. Average ($\pm$ S.E.M.) percent change from baseline GABA concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour. (Note the different scales.).....	81
Figure 12. Average ($\pm$ S.E.M.) percent change from baseline glutamate concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour. (Note the different scales.).....	84

- Figure 13.** Average ($\pm$ S.E.M.) percent change from baseline GABA concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour, as well as for Hours 2 to 6. (Note the different scales.).....91
- Figure 14.** Average ($\pm$ S.E.M.) percent change from baseline glutamate concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour, as well as for Hours 2 to 6. (Note the different scales.).....94
- Figure 15.** Average ($\pm$ S.E.M.) percent change from Day 1 to Pre-Amygdala ADT baseline in GABA concentrations from the kindled and non-kindled hemispheres in the NSE group.99
- Figure 16.** Average ($\pm$ S.E.M.) percent change from Pre-Amygdala ADT baseline in glutamate concentrations from the kindled and non-kindled hemispheres in the NSE and SEP groups during the amygdala ADT procedure on Day 2. (Note the different scales.).....102
- Figure 17.** Average ($\pm$ S.E.M.) percent change from baseline in GABA concentrations from the kindled and non-kindled hemispheres in the NSE and SEP groups during the ipsilateral hippocampus ADT procedure on Day 2. (Note the different scales.)115
- Figure 18.** Average ($\pm$ S.E.M.) percent change from baseline in glutamate concentrations from the kindled and non-kindled hemispheres in the NSE and ASE groups during the ipsilateral hippocampus ADT procedure on Day 2. (Note the different scales.)117
- Figure 19.** Pathology revealed with fluoro-jade in and around the kindled and non-kindled amygdalae and piriform cortex for a NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).....120
- Figure 20.** Pathology revealed with cresyl violet in and around the kindled and non-kindled amygdalae and piriform cortex for an NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).....122
- Figure 21.** Pathology revealed with fluoro-jade in and around the ipsilateral and contralateral hippocampi for an NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).....125
- Figure 22.** Pathology revealed with cresyl violet in the ipsilateral and contralateral hippocampi for an NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).....127

- Figure 23.** Pathology revealed with fluoro-jade in the ipsilateral cortex for an NSE subject (A) and ipsilateral and contralateral cortex for a severe ASE subject (B and C). 129
- Figure 24.** Pathology revealed with cresyl violet in the ipsilateral (A) and contralateral (B) perirhinal fissure for a severe ASE subject. 131
- Figure 25.** Pathology revealed with fluoro-jade in the ipsilateral and contralateral thalamus for a severe ASE subject. 133

LIST OF APPENDICES

Appendix 1 – GABA TO GLUTAMATE RATIO	149
Appendix 2 – Contralateral Hippocampus.....	155
Appendix 3 – Ipsilateral Thalamus	158

INTRODUCTION

Epilepsy

Anyone can have a seizure, be it drug-, trauma- or electroshock-induced. When someone has *spontaneously recurring* seizures, as one in approximately 250 people do (Sato et al., 1990), these seizures are part of a common neurological syndrome called epilepsy. Some epilepsies have a known cause, such as trauma, stroke or tumor. Other epilepsies are idiopathic, or are without known causes. Despite the different causes, there are patterns that allow for the categorization of seizures based on their neurological and behavioural presentations.

The first distinction is neurological-based; a seizure episode that is isolated in one area of the brain is known as a partial seizure. On the other hand, a generalized seizure describes seizures that (a) involve the whole brain from the outset, or (b) evolve over time to engage the whole brain. The former is known as a primary generalized seizure, and the latter is a secondary generalized seizure. Whatever the neurological situation, consciousness is lost in the latter cases (Alloy et al., 1999). The behavioural manifestations of tonic-clonic or absence seizures are dramatically different. In tonic-clonic seizures, the person becomes rigid (tonic) and then rhythmic jerking (clonic) movement begins. During absence seizures, the person, usually a child, appears dazed

and “stares off into space.” It is the tonic-clonic seizures that most people associate with epilepsy, even though it is not the most common (Alloy et al., 1999).

Unlike generalized seizures, partial seizures do not involve the whole brain and consciousness is not always lost. If the person’s consciousness is not affected by the seizure, it is called a simple partial seizure; otherwise, it is complex (Alloy et al., 1999). Unless the epileptic activity reaches one of the brain’s output mechanisms, such as the motor cortex, the person may not even be aware that a simple partial seizure had occurred. When the limbic system is included within the seizure circuit, consciousness is invariably compromised. As the epileptic circuit incorporates other structures, increasingly complex behaviour results, and the distinction between partial and generalized becomes blurred. In many cases, a complex partial can develop into a secondary generalized seizure. Because the majority of complex partial seizures originate in the temporal lobe, this type of seizure is often called temporal lobe epilepsy (TLE) (Alloy et al., 1999). Complex partial seizures with secondary generalization are often studied using the kindling technique, which is described below (McIntyre & Kelly, 1998).

Status Epilepticus

Typically, a ‘normal’ seizure will self-terminate shortly after it begins due to the effect of inhibitory mechanisms. For approximately 50 people per 100,000 (Fountain, 2000), these offset mechanisms fail to stop the seizure. The protracted seizure that results is known as status epilepticus (SE) and is a medical emergency. Status epilepticus (SE)

is classically defined as a seizure or a series of seizures lasting for at least 30 minutes. More recently, a new five-minute definition is being considered because a seizure that lasts five minutes has a good chance of continuing past a half hour (Scott et al., 1998). As with discrete seizures, anyone can experience SE, but having epilepsy first is the greatest risk factor (Fountain, 2000).

Other risk factors for SE include inadequate antiepileptic medication, young age - as 40% of new cases occur in children younger than 2 years old (Delgado-Escueta & Fong, 1997), genetic predisposition and head trauma (Fountain, 2000). The most affected age group is children under the age of 1 year, with febrile (fever-based) SE augmenting the incidence to 325 per 100,000. Adults over the age of 60 years form the next most affected group, with 100 per 100,000; stroke is the leading cause for this increase. Three to 15% of children and 15 to 22% of adults with SE die either from brain damage and/or systemic complications (Fountain, 2000).

SE produces a variety of acute systemic complications that can worsen brain damage and/or increase the probability of death, such as hyperthermia, pulmonary edema, irregular heartbeats, and cardiovascular collapse (Fountain, 2000). For those that survive, epilepsy, encephalopathy and neurological deficits are a few chronic conditions that can emerge from the brain damage, and approximately 13% re-experience SE (Fountain, 2000). These outcomes depend on the type and duration (Tanaka et al., 1996) of SE. As with epilepsy itself, there are many types of SE, and consciousness is not always affected in the milder forms.

The most common type, which includes 74% of SE cases (Fountain, 2000), is convulsive SE, where the unconscious patient is undergoing a continuous or series of convulsive seizure (Delgado-Escueta & Fong, 1997; Scott et al., 1998). Non-convulsive SE describes unconscious patients presenting with seizure activity on EEG and a continuous altered mental state - symptoms of a continuous absence or complex partial seizure (Delgado-Escueta & Fong, 1997; Kaplan, 1999). The latter is the most common form of non-convulsive SE (Delgado-Escueta & Fong, 1997) and comprises approximately 25% of SE cases (Fountain, 2000). In a rare form, *epilepsia partialis continua*, consciousness is not lost, while the patient is experiencing protracted partial seizures (Delgado-Escueta & Fong, 1997).

Paradoxically, epilepsy is both a common risk factor *and* an outcome of SE. With this in mind, one can use the kindling electrical stimulation model of TLE to produce an epileptic focus and increase the probability of developing SE, especially SE of the more severe forms (McIntyre et al., 1982; McIntyre et al., 1986). In a non-epileptic brain, as high as two-thirds of patients will develop epilepsy after a single episode of SE (Delgado-Escueta & Fong, 1997). This observation has been paralleled in chemical models of SE where agents, such as kainic acid, are injected systemically into a non-epileptic rat or mouse to produce SE. In most cases, after the SE ends, the rats become spontaneous epileptics several weeks or months later. Thus, SE can have a clear epileptogenic role.

Kindling as model for temporal lobe epilepsy

Kindling is an often used model of TLE with secondary generalization that does not involve chemical confounds. Further, the changes associated with kindling are permanent (Goddard et al., 1969; McIntyre et al., 1982). Through daily low intensity stimulation of a discrete forebrain site, progressively complex electrographic and behavioural seizures develop in response to each daily stimulation and eventually a generalized convulsion is triggered (Goddard et al., 1969). These generalized kindled convulsions are also known as stage 5 seizures, according to Racine's (1972) seizure classification scale (see Table 1).

Once the animal has experienced six stage-5 seizures following kindling stimulation, it is considered 'kindled' and has become permanently epileptic-prone. With continued stimulations, ultimately the animal will develop spontaneous seizures (McIntyre et al., 1991).

Kindling-based SE model

Electrical stimulation can be used to induce complex partial SE, although it is not the only means to induce this form of SE. Chemical models have been developed where agents are systemically injected into a non-epileptic rat or mouse. GABA antagonists, such as bicuculline and allylglycine, or glutamatergic drugs, such as kainic acid, or cholinergic drugs, like pilocarpine, all induce SE, and produce similar brain pathology

Table 1. Racine's (1972) seizure classification scale.

STAGE	BEHAVIOUR
1	Eye closure and chewing
2	Head bobbing
3	Forelimb clonus, without rearing
4	Forelimb clonus, with rearing
5	Generalized clonus while rearing and falling

involving the hippocampus, amygdala, thalamus, neocortex and cerebellum (Scott et al., 1998).

Like the chemical models, the electrical stimulation models of SE can be induced by various procedures. One such procedure involves 60 minutes of stimulation with one-minute interruptions every ten minutes to record EEG. Five minutes of stimulation followed by one minute on and one minute off for a total of 60 minutes is another design. The former paradigm was used with 60 Hz sine waves at low intensities, just slightly greater than the kindling stimulus (for example: McIntyre et al., 1982; McIntyre et al., 1986; McIntyre et al., 1991; Mohapel et al., 1996); the latter method, Handforth and Ackermann (1992) modified by using high intensity, 400 μ A, and 60 Hz bipolar square waves.

These designs have been used on both epileptic and non-epileptic brains. After exposure to 60 Hz sine waves at low intensities, none of the non-kindled and a half to two-thirds of the kindled animals developed SE (McIntyre et al., 1982; Mohapel et al., 1996). The low intensity stimulation *per se* produced no pathology. In contrast, the procedure of Handforth and Ackermann (1992) resulted in approximately half of the non-kindled and almost all kindled animals developing SE. Only rats that developed SE were used in their study, thus, no information about stimulus-related pathology was available.

Despite the different designs, there are strong intra-model similarities in the behaviours, anatomic activity and resulting brain pathologies, which most importantly are also similar to human SE (McIntyre et al., 1982; Handforth & Ackermann, 1992;

Pitkanen et al., 1998). The main problem with the chemical models is that the seizures are developed using neurotoxic substances. Thus, it may be difficult to distinguish SE-induced pathology from direct, exogenous, chemical-induced pathology. The electrically induced SE models avoid this chemical complication. With this in mind, the present experiment combines the electrical induction model with the kindling procedure to produce the 'desired' syndrome.

Experimental SE types

In the electrical induction models, animals develop one of four SE types: immobile, ambulatory, masticatory and generalized. Each form, discussed below, is not mutually exclusive but is associated with predominant behavioural, EEG and neurological activity profiles (McIntyre et al., 1991; McIntyre et al., in press). For example, an animal in masticatory SE can occasionally show ambulatory EEG and behaviour. These SE types are discussed in order of increasing SE severity (Handforth & Ackermann, 1992; Mohapel et al., 1996; McIntyre et al., in press) and are summarized in Table 2. Interestingly, the EEG activity is the best predictor of SE phenotype, while the anatomical induction site is independent of SE type (McIntyre et al., 1991; McIntyre et al., in press).

Immobile SE

Immobile SE is the mildest form of SE with the lowest EEG seizure frequency of less than 1 Hz, mildest behaviour and the least number of activated structures, as revealed

Table 2. Behaviour, EEG and neurological activity profiles for the four types of experimental SE.

SE TYPE	PREDOMINANT BEHAVIOUR	PREDOMINANT EEG	PRIMARILY ACTIVE STRUCTURES
Immobile	Awake and not moving	Less than 1 Hz, unilateral	<i>Ipsilateral nuclei:</i> AHA, and posterior medial and cortical amygdala
Ambulatory	Moving a lot; appears to be shuffling back and forth; turns primarily in one direction	1 to 3 Hz, bilateral	<i>Ipsilateral nuclei:</i> CE amygdala, endopiriform, posterior amygdalopiriform, and AHA; <i>Ipsilateral cortices:</i> deep insular, ventral entorhinal and perirhinal; <i>Bilateral nuclei:</i> BL amygdala and BNST
Masticatory	Continuous masticatory behaviour with drooling and head bobbing	4 to 5 Hz, bilateral	Same as ambulatory but bilateral; Hippocampus is now active
Generalized	Forelimb clonus; generalized convulsions, at times	5 to 10 Hz, bilateral	Same as masticatory; Plus, entire anterior neocortex, caudate putamen and ventral substantia nigra pars reticulata

through [^{14}C]2-deoxyglucose (2-DG). This non-convulsive status shows activity primarily in the ipsilateral amygdalo-hippocampal transition area (AHA), and posterior medial and cortical amygdala nuclei. Unilateral neurological activity is reflected in the ipsilateral EEG spiking activity (McIntyre et al., 1991; McIntyre et al., in press).

Ambulatory SE

A second type of SE is ambulatory, so named because the animal is continuously shuffling back and forth, while preferentially turning in one direction. Handforth and Ackermann (1992) have called this exploratory SE. Higher frequency epileptiform activity, more seizure-related behaviour and a greater neurological involvement make this form more severe than immobile SE. Normally, epileptiform spiking is a good predictor of metabolic activity, except during the initial ambulatory status profile. In the beginning, the EEG shows bilateral activity of 1 to 3 Hz, yet the 2-DG shows primarily unilateral activity. However, after five hours, 2-DG shows bilateral activity in the above structures, indicating a greater intensity of seizure activity has now included the contralateral side, while the ipsilateral activity decreases, likely reflecting brain damage. At ten hours, the only structures active are those seen in immobile SE (McIntyre et al., 1991; McIntyre et al., in press).

Masticatory SE

The next most severe form is the mild convulsive masticatory SE with a corresponding increase in EEG activity and greater neurological involvement. This form

of SE has also been called mild convulsive (Handforth & Ackermann, 1992).

Behaviourally, it is more complex with continuous stage 2 seizures, head bobbing and drooling. Both amygdalae show 4 to 5 Hz spiking. Bilateral activity is shown in the same structures in ambulatory SE, but this bilateral activity is evident at one hour and also includes the hippocampus. After five hours, all aspects of the masticatory profile are evident but have decreased, possibly reflecting brain damage (McIntyre et al., 1991; McIntyre et al., in press).

Generalized SE

The final, and most severe, type is generalized SE. It has also been named clonic convulsive SE (Handforth & Ackermann, 1992). Behaviour can range from generalized stage 3 to stage 5 seizures. Likewise, bilateral epileptiform spiking can range from 5 to 10 Hz. 2-DG reveals a large range of bilateral activity, spreading out from those involved in masticatory SE, including anteriorly to the frontal cortex, medially to the striatum and substantia nigra pars reticulata, and medially-dorsally to the thalamus. Interestingly, there are some areas that display decreased activity, including in the septum, diagonal band, globus pallidus and subthalamic nuclei. Generalized SE requires close supervision because the more severe the seizures, the greater chance of death. Thus, should an animal develop generalized SE, the seizure should be stopped within 3 hours using medication, such as Somnotol (McIntyre et al., 1991; McIntyre et al., in press).

SE damage

As with neurological activity, nuclear-specific pathology is also a good predictor of SE type (McIntyre et al., in press) and each type has its own predictable time-course of pathology. Necrotic and apoptotic mechanisms interact to produce the damage (Pitkanen et al., 1998; Scott et al., 1998; Fountain, 2000; Fujikawa et al., 2000).

Immobile SE

Very mild pathology is associated with this mildest SE. Damage, evident at 24 hours later, is isolated to a few structures in the ipsilateral amygdala, namely the lateral posterior cortical and posterior accessory basal amygdala nuclei (McIntyre et al., in press).

Ambulatory SE

Ambulatory SE, when stopped in 30 minutes with pentobarbital, induces only slight damage located around the stimulating electrode, similar to rats that did not develop SE (McIntyre et al., 1982). The majority of the damage associated with this form of SE appears within 3 to 4 hours in the ipsilateral temporal lobe (McIntyre et al., 1982; McIntyre et al., in press). Pathology was similar to that seen in immobile SE, plus additional damage extended to other amygdala nuclei, cortices and structures (McIntyre et al., in press).

The affected amygdala nuclei are the lateral, parvocellular division of the basal, and the accessory basal nuclei, while the medial, anterior cortical, central and intercalated nuclei are slightly damaged (Pitkanen et al., 1998; McIntyre et al., in press).

Interestingly, the magnocellular and intermediate divisions of the basal nucleus are more likely to survive SE (Pitkanen et al., 1998; McIntyre et al., in press). Pathology extends up to the orbital cortex through the perirhinal and agranular insular cortices. The most anterior damage, though, is evident in two layers of the prelimbic cortex, 6b and 5a, and continued to layer 3 of the taenia tecta through the infralimbic cortex. Certain layers of cortices are injured, namely layers 5/6 and entire deep layer 3 of the entorhinal, layers 3, 5A and 6B of the perirhinal, and entire deep layer 3 of the piriform. The other damaged nuclei include the ventral subiculum and endopiriform nuclei (McIntyre et al., in press).

Pathology remains relatively unchanged until 24 hours later. At this time, damage is also evident bilaterally in the CA1 and CA3 fields and slightly within the posterior dentate gyrus of the hippocampus; CA2 cells are relatively damage resistant (Pitkanen et al., 1998; Coulter, 1999). In the cortical layers, injury has spread to include layers 3 of the medial entorhinal and 2B of the piriform cortices. After 3 days, more extensive bilateral pathology is showing in the CA1 field of the hippocampus, and the deep layers of the posterior perirhinal and entorhinal cortices (McIntyre et al., in press). In two weeks, the net damage is present ipsilaterally (i) from the olfactory bulb to the ventral hippocampus throughout the amygdala-piriform area, (ii) medially to the thalamus and (iii) dorsally to the CA1 fields of the hippocampus. The latter can also show bilateral degeneration (McIntyre et al., 1982).

Another method to detect brain damage is to check for an elevation in the local afterdischarge threshold (ADT). In a study by McIntyre, Nathanson and Edson (1982), SE-induction was attempted in amygdala-kindled rats with 60 minutes of stimulation. Of those that developed ambulatory SE, some were injected with pentobarbital (Nembutal) at 30 minutes or 4 hours to stop the seizures while the remaining rats had no drug administered. All animals showed an elevation in amygdala ADT 24 hours post-stimulation. However, at this time, the rats that did not develop SE or had SE stopped at 30 minutes, triggered an ADT in the amygdala. Interestingly, only the former rats experienced a stage 5 at the ADT.

Masticatory SE

The mildly convulsive masticatory SE displayed a greater amount of damage than ambulatory SE. Where the latter has primarily unilateral damage, the former has bilateral damage. There is an exception, though, with the hippocampus. Masticatory SE, whether induced electrically in a kindled brain (McIntyre et al., in press) or chemically in a non-kindled brain (Kelly & McIntyre, 1994), actually produced less damage in the hippocampus than ambulatory or generalized SE. This difference hints at potentially SE type-specific neurological circuits and cascade of events that can trigger necrosis or apoptosis depending on the SE phenotype.

Generalized SE

The greatest pathology arises from the most severe form, namely generalized SE. At 3 hours, in addition to the damage seen in masticatory SE, mild bilateral degeneration is evident in the CA1 field and posterior dorsal dentate gyrus granule cell field of the hippocampus, and layer 5 of the medial frontal cortex. Three cortices, the posterior parietal, temporal and perirhinal, all show serious damage to layer 3. Posterior and medial to the amygdala, the substantia nigra reticulata no longer exists (McIntyre et al., in press).

After 24 hours, more pathology is evident within the hippocampal CA1 field, and cortical damage extends from the parietal to the visual cortex through layer 4 and into layer 5A of the retrosplenial cingulate cortex. Within 3 days, degeneration includes the globus pallidus (McIntyre et al., in press).

Since SE activity and pathology spreads through the brain via various connections and epilepsy is a risk factor, it would be reasonable to assume that SE uses the same circuitry as the epileptic seizures. Interestingly, this may not be the case.

SE circuitry

Handforth and Ackermann (1992) argue that the same behaviours are occurring during SE and kindling, thus, the seizures are accessing the same circuitry. The main difference is SE seizures result from failed seizure-terminated mechanisms within the circuits. On the other hand, certain structures that are important for kindling are not

important for SE, and vice versa (Mohapel et al., 1996). During kindling, the kindling rate can be used as an indicator of anatomical access to the motor cortex and/or anatomical access to other sites that promote seizure activity and/or enhanced excitability (Sato et al., 1990; Mohapel et al., 1996). For example, the piriform and perirhinal cortices have the lowest kindling rate (Sato et al., 1990; Mohapel et al., 1996). However, there is a difference found between these two structures in the latency to forelimb clonus. As with kindling rate, clonus latency can be used as a measure of anatomical connection to the motor cortex. The perirhinal cortex has the shortest latency of (1.8 ± 0.4) sec compared to (22.0 ± 4.2) sec for the piriform cortex (Mohapel et al., 1996).

If speed of kindling is related to SE induction and connectivity to motor cortex, the piriform and perirhinal cortices should develop SE faster than other kindled sites. However, this is not the case. Continuous stimulation produced SE in only one quarter of those stimulated from the perirhinal cortex and none from the piriform cortex. In contrast, the basolateral amygdala was one of the slower kindling limbic structures, but continuous stimulation of it produced SE in all rats. This has led researchers to conclude that although the perirhinal cortex may be vital for the development of kindled seizures, the basolateral amygdala is required for the initiation and maintenance of SE (Mohapel et al., 1996). Thus, kindled seizure circuitry may differ from SE circuitry.

During SE, neurological activity spreads as SE severity increases. The basolateral amygdala, bed nucleus of the stria terminalis, and piriform and perirhinal cortices are active even during the mild forms of SE. As SE progresses to the more severe forms, the

activity spreads through the ipsilateral limbic system to the hippocampus and surrounding cortex and can become bilateral (McIntyre et al., 1991). This is also seen in humans (Pitkanen et al., 1998). The contralateral hemisphere can be accessed by the hippocampal commissure and/or through the ipsilateral medial prefrontal cortex's projections to the contralateral basolateral nucleus (McIntyre et al., 1991) and/or the amygdala commissure (Pitkanen et al., 1998). After time, seizure activity in the contralateral limbic structures becomes independent (McIntyre et al., 1991).

Amino Acids

Seizure afterdischarge (AD) produces many chemical changes, despite its short duration. Thus, a protracted seizure discharge will have a vast amount of chemical changes, changes that will depend on many factors such as duration, energy supply, transport mechanisms and brain damage. Previous studies have investigated these changes in various structures using different models. The more commonly studied neurotransmitters are the inhibitory amino acid, GABA, and excitatory amino acid, glutamate. These amino acids are targeted because (1) there is a theorized interaction between the excitatory and inhibitory amino acids that is vital to the development and maintenance of SE, and (2) they are abundant in the brain.

Inhibitory Amino Acid: GABA

Kindling causes some permanent changes to the GABAergic systems (Sato et al., 1990; Mohapel et al., 1996); other transient changes are responses to the stimulation,

thus, may not be part of the kindling process (Sato et al., 1990). Certain findings indicate the importance of GABA in SE. First, the loss of GABAergic inhibition in the hippocampus is correlated with the initiation of SE (Coulter & DeLorenzo, 1999; Macdonald & Kapur, 1999). The degeneration of the highly vulnerable GABAergic, somatostatin positive neurons in the hilar area of the dentate gyrus contributes to this decrease in inhibition (Sherwin, 1999; Pitkanen & Jolkkonen, 1999). Second, SE can be induced with GABA antagonists, such as bicuculline and allylglycine, and the resulting pathology is very similar to that seen after human SE (Scott et al., 1998). Third, baseline GABA levels are lower at the epileptic focus (Sherwin, 1999). And, fourth, there is emerging evidence that GABA has neurotoxic effects (Xu et al., 2000). Similar to glutamate, GABA does increase during a seizure, albeit more slowly (Scott et al., 1998; Sherwin, 1999). Unlike the excitatory amino acids, though, the non-epileptic side showed higher levels of GABA (Sherwin, 1999). However, this contralateral effect was not found in a seizure-resistant rat strain. In fact, in these rats, GABA levels dropped in the non-kindled side during discrete focal seizures (Shin, 2000). Thus, genetic predisposition for or against epilepsy may affect GABA release or its extracellular concentration during a seizure.

Excitatory Amino Acids: Glutamate

Excitatory amino acids appear to play an important role in the maintenance and damage resulting from SE (Macdonald & Kapur, 1999). Certain findings point to their importance: (1) prolonged exposure to the excitatory amino acids has neurotoxic effects

(McIntyre et al., 1986; Fountain, 2000), (2) when glutamate is administered to *in vitro* models of SE, the resulting damage to hippocampal slices is very similar to *in vivo* models (Scott et al., 1998), (3) glutamatergic drugs, such as kainic acid, also produce very similar results (Scott et al., 1998; Sloviter, 1999), (4) glutamate has been found to be involved in the maintenance of SE (Macdonald & Kapur, 1999), and (5) the focus has a higher resting extracellular concentration of glutamate compared to the contralateral non-epileptic side (Scott et al., 1998; Sherwin, 1999), although this point has not been replicated in all models. For example, kindling, which produces an epileptic focus, does not appear to change glutamate release, reuptake or receptors (Sato et al., 1990; Shin, 2000).

Another controversy involves the seizure-related release of amino acids. Several studies have found that prior to a seizure, glutamate concentrations increase to potentially neurotoxic levels (Sechi et al., 1997; Scott et al., 1998; Sherwin, 1999) and remain elevated during the seizure (Scott et al., 1998; Sherwin, 1999). This is especially true of the focus because it releases more glutamate and takes longer to return to baseline levels than the non-epileptic side (Scott et al., 1998; Sherwin, 1999). These results, though, were not shown when a discrete seizure was triggered in a kindled focus (Shin, 2000), or when SE was induced in non-epileptic brains (Tanaka et al., 1996). Clearly, more research is needed to reach a deeper understanding of the amino acid release during discrete seizures and SE. One commonly used technique to study transmitter release is microdialysis.

Microdialysis

Microdialysis can be used to measure the *in vivo* changes of neurotransmitters released over time in a structure like the amygdalae before, during and after SE. This technique relies on diffusion of the transmitter into the extracellular fluid where it can be collected; however, it cannot be used to estimate neurotransmitter concentrations within the synaptic cleft or in vesicular storage (Sankar et al., 1997). To effect microdialysis, an artificial perfusion medium, namely, artificial cerebral spinal fluid (ACSF), is pumped through an implanted, intracerebral probe into and through a semi-permeable membrane. As the ACSF flows through the membrane into brain tissue, extracellular molecules of a restricted size flow from the brain tissue into the probe (Connelly, 1999). Fortunately, the net flow of fluid is zero, so intracranial pressure does not increase, nor does the localized tissue loose fluid (Lunte & Lunte, 1996).

Within the probe itself, the physics of the microdialysis probe and pump require close monitoring so the dialysate contains the right concentration of the desired substance, facilitating interpretation (Dawson, 1997). The types of substance collected, and the volume and collection area of the membrane, dictate an ideal flow rate (Renno et al., 1998); for amino acids, it's 1 to 2 $\mu\text{L}/\text{min}$ (Dawson, 1997). Should the flow rate be higher than the ideal, the dialysate will be diluted (Dawson, 1997), or if lower than ideal, concentrated (Renno et al., 1998). Once the dialysate is collected, it is frozen and is analyzed at a later date.

One concern about inserting a probe into live brain tissue is the potential brain damage and its alteration of kindling properties. Fortunately, in a previous study, probe insertion into the stimulating site did not appear to affect the local amygdala's ADT or its AD duration (Shin, 2000). Thus, the local cellular and circuit properties, such as local and circuit excitability, remain substantially intact.

Capillary Electrophoresis

Capillary electrophoresis with laser-induced fluorescence is a highly sensitive technique that rapidly and simultaneously separates many compounds within a dialysate sample (Robert et al., 1996). Each compound's concentration relative to the artificial perfusion medium is then determined. Thus, a single dialysate collection can yield a lot of information.

Another technique, high-performance liquid chromatography, requires a large volume, a long collection time and a flow rate of more than 2 $\mu\text{L}/\text{min}$, which gives poor temporal resolution and is not suitable for amino acids (Dawson, 1997). On the other hand, capillary electrophoresis requires only a small volume of dialysate that can be collected over a short time. Also, flow rate is more flexible and it can be adjusted for amino acids. As a result, a highly accurate and detailed account of an amino acid's concentration and temporal fluctuation is possible.

Purpose

Our knowledge about the relationship between amino acid release and the induction of SE and the development of brain damage is quite vague at best. In spite of this fact, much speculation has been presented over the years that suggests a strong relationship between increased glutamate release, seizure induction and brain damage (e.g., Tapia et al., 1999). More recently, even the inhibitory transmitter GABA has emerged as another neurotransmitter that potentially has neurotoxic properties (Xu et al., 2000).

The purpose of the present thesis, therefore, was to determine the relationship between the induction of SE, by electrical activation of the kindled amygdala, and the focal release of glutamate and GABA in the kindled amygdala as compared to the non-kindled amygdala, over time. As changes in amino acid release could be associated with either induction of SE, or various conditions following induction, including development of brain damage, dialysates were collected continuously from both amygdalae before and during SE induction, as well as the first hour thereafter, and once every hour for the remainder of the 6 hours post-induction period, including several epochs the next day.

MATERIALS AND METHODS

Treatment of subjects was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care, and has been approved by the Carleton University Animal Care Committee.

Subjects

Twenty male LEH rats (Canadian Breeding Farms), weighing 250 to 300g at the time of surgery, were housed singly with free access to food and water in a constant temperature (21°C) and humidity (45 – 50%) controlled room with a 12 hour on/12 hour off light cycle.

Surgery

After 1 week of daily handling, all animals were anesthetized with sodium pentobarbital (Somnotol; 60 mg/kg, i.p.) and implanted with 4 bipolar Nickel/Chromium stimulating/recording electrodes of 127 μ m in diameter into both hippocampi and amygdalae using a Krieg stereotaxic, and 2 cannulae for dialysis probes in both amygdalae. The following coordinates (Pellegrino et al., 1979) relative to bregma were used: A 0.0 mm, L $\pm$ 4.5 mm, V 9.0 mm for the cannulae; A -3.5 mm, L $\pm$ 4.5 mm, V 8.5 mm angled caudo-rostrally at 24° for the amygdala electrodes; A -3.0 mm, L $\pm$ 3.5 mm, V

3.0 mm for the hippocampal electrodes. The cannulae were sealed when not in use.

After two weeks of recuperation, each animal was kindled as described below.

Kindling

The afterdischarge threshold (ADT) for either the left or the right amygdala was found by stimulating the amygdala for 2 seconds with a 60 Hz sine wave at 15 μ A, then 25, 35, 50, 75, 100, 150, 200, 300 μ A, until an afterdischarge (AD) occurred. Each stimulation was separated by one minute. Five minutes after finding the amygdala ADT, the ipsilateral hippocampus ADT was found using the same procedure. Twenty-four hours later, kindling of the amygdala began. To do so, each animal was stimulated once daily at the ADT until six stage-5 seizures were accumulated (see Table 1). Each animal then rested for at least 20 days, and then prepared for dialysis and the SE experiment. To receive the dialysis probes (see below), the rats were re-anesthetized with Halothane and the bilateral probes inserted.

Probes

A probe was made of 24 GSS, polyethylene tubing, glass silica tubing and a semi-permeable membrane, sealed at the tip with glue. Ideally, only the tip with the semi-permeable membrane (6000 molecular mass cutoff) extended 1.5mm past the cannula, into the amygdala (see Figure 1). Using a Harvard infusion pump, artificial CSF (Ringer solution: 120 mM NaCl, 3 mM KCl, 1.2 mM CaCl_2 , 1.5 mM MgCl_2 ; pH 7.2) was pumped at a rate of 1 μ L/min into each probe. The ACSF takes the following path:

Figure 1. Microdialysis probe with cannula.

pump, polyethylene tubing, dual channel swivel (Instech Laboratories, Pennsylvania), 24 GSS, and then the semi-permeable membrane. Through the semi-permeable membrane, extracellular fluid diffuses into the ACSF. The dialysate then flows into the fused-silica tubing (75 μm I.D. $\times$ 150 μm O.D., Polymicro Technologies, Arizona) and into a collection tube. The collection tube with dialysate was frozen over dry ice, stored at -80°C and then analyzed at a later date using capillary electrophoresis.

After one to two hours of recuperation, the SE induction procedure began with a fully conscious, freely moving animal (see below).

SE Induction Procedure

Day Prior to SE induction

On the day before SE induction, *only* the ADT for the ipsilateral hippocampus was re-assessed. This procedure was used to avoid provoking amygdala seizure aftereffects that might compromise the induction of SE from the amygdala the next day. The focal hippocampal seizure does not create such a disruptive milieu.

Day 1

Although the SE induction procedure occurred on Day 1, it was preceded by insertion of the dialysis probes. To do so, the rat was anesthetized with Halothane and the bilateral microdialysis probes were inserted. After one to two hours of recuperation, 10-

minute of baseline dialysate samples were taken, and then the SE induction procedure began.

SE Induction

Each animal was hooked up to the stimulation/recording leads and exposed to 9 minutes of stimulation of the kindled amygdala followed by one minute of no stimulation (to allow for EEG recording) for a period of one hour. The stimulus for SE induction was set at 50 μ A above the original amygdala ADT. The occurrence and duration of the ensuing seizures were recorded. During the one minute of no stimulation, the EEG was recorded to look for electrographic signs of SE, i.e. spiking (see below). If regular spiking at 1 Hz or above was observed, stimulation stopped and SE induction was assumed; otherwise the stimulation continued for the full one hour.

Post-SE Induction

Following the 60 min of stimulation to induce SE, EEG and behaviour (see below) were monitored continuously for one additional hour. Subsequently, the EEG was monitored every half-hour, while behaviour was monitored continuously for the next 5 hours.

Overnight

The probes were left in the rat overnight, while being continuously pumped with ACSF. The EEG wires during this time, however, were detached. The cage was cleaned, and food and water was left with the animal in the stimulation cage over night.

Day 2

After reattaching the stimulation/EEG leads, the ADT was assessed for the previously stimulated amygdala and then, one hour later, the ipsilateral hippocampus. Based on previous experiments, it was anticipated that the stimulated amygdala would be unresponsive, while the hippocampus would be hyper-responsive.

Amygdala ADT

The starting stimulation intensity for the amygdala ADT test was just below the original ADT. With no AD response, the ADT test intensities went to 300 μ A, 400, 600 and then 1000 μ A. This ended the amygdala testing series.

Hippocampal ADT

One hour after the amygdala ADT test, the ipsilateral hippocampal ADT was assessed using the same method to find the original hippocampal ADT.

Post-Hippocampal ADT

All rats were carefully monitored for convulsive activity for an additional 30 minutes following the hippocampal AD provocation. Most of the rats were then perfused.

Day 1: Microdialysis

A maximum of three 10-minute baseline microdialysis samples were obtained prior to brain stimulation. Subsequently, during stimulation, 6 x 9-minute samples were collected corresponding to the 9 minutes of stimulation, punctuated by one minute periods with no stimulation. No samples were taken during the one-minute intervals when brain stimulation was stopped. During the first one hour post-stimulation period, 6 x 9-minute samples again were collected, interspersed by one minute periods of no collection. Then for the next 5 hours, a single 9-minute sample was obtained at the beginning of each hour. See Figure 2.

Day 2: Microdialysis

A maximum of two 10-minute baseline samples were obtained prior to amygdala ADT stimulation. A sample was also taken corresponding to the period of time required to test the amygdala for its possible ADT. Fifty minutes after the amygdala ADT test was completed, another 10-minute baseline sample was obtained prior to hippocampal ADT stimulation. Afterwards, a sample, associated with the period of time required to obtain the hippocampal ADT, was gathered. After finding the hippocampal ADT, a one-minute interval of no sample collection occurred followed by a second 9-minute sample collection. Thirty minutes after finding the hippocampal ADT, a final 9-minute sample was obtained. See Figure 3.

Figure 2. Dialysate schedule for Day 1.

Figure 3. Dialysate schedule for Day 2.

1		(Minutes)	9	1						9	1					9
Baseline 1				Baseline 2							Amygdala ADT*					

50 minutes later...

												30						9
Baseline 3				Hippo ADT*				Post ADT #1				Post ADT #2						

* - this time will vary

Data Analysis

Before SE

Although each subject's group membership of course was not known before SE induction, the rats and their data were grouped before SE induction according to the type of SE performance that was to develop during the SE induction procedure. These groups included those that did not develop SE (NSE group), and those that did (SEP group; SE positive); this latter group consists of rats showing either the Immobile (ISE) or the Ambulatory (ASE) type of SE as their predominate form. However, for the principle reason of a small sample size, we combined these two types to form a single SEP group. Thus, the initial between groups factor was NSE vs. SEP. If there was a significant difference between these two main groups, the SEP group was further divided into ISE and ASE rats. Following this, the statistical tests were redone to determine the differences in SE type (NSE, ISE and ASE). This comparison will be further outlined where appropriate.

SE Induction Day

Due to equipment constraints, capillary electrophoresis cannot analyze all dialysate samples, thus, specific samples had to be selected. These selected samples are indicated in the appropriate section and are illustrated in Figure 4. From these dialysates, GABA and glutamate concentration scores were determined using capillary electrophoresis. Analysis of the baseline levels used the raw scores. Conversely,

Figure 4. Day 1 and Day 2 samples selected to be analyzed by capillary electrophoresis, labeled as they are referred to in the analyses.

DAY 1																			
1	(Minutes)	0	1																
										Day 1 Baseline									
Stimulation																			
0 to 9 min						20 to 29 min								50 to 59 min					
Post-Stimulation																			
Hour #1																			
0 to 9 min						20 to 29 min								40 to 49 min					
Hour #2				Hour #3				Hour #4				Hour #5				Hour #6			
Hour 2								Hour 4								Hour 6			

DAY 2																		
1	(Minutes)	0	1															
		Pre-Amygdala ADT Baseline			Amygdala ADT*			50 minutes later...										
Pre-Hippocampus ADT Baseline		Hippocampus ADT*		Post ADT #1		<table border="1"> <tr> <td>30</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>0</td> </tr> </table>				30								0
30								0										
Post ADT #2																		
* - this time will vary																		

analysis of the changes in amino acid levels over time involved converting (1) each baseline score to a percent of the baseline's average and (2) the amino acids raw scores to 'percent of baseline' values. These conversions were accomplished by using the

following equations: $100 * \frac{raw\ score_i}{mean_{baseline_i}}$ and $100 + 100 * \left(\frac{sample - baseline}{baseline} \right)$, respectively.

The GABA and glutamate scores and percent change values were then analyzed using repeated measure designs. This was done to assess group differences between the stimulated and non-stimulated hemispheres and, where appropriate, over time.

Day 2: 24 Hours After SE Induction

Baseline Measurements Before Day 2 Amygdala ADT Test

The baseline (Pre-Amygdala ADT Baseline) measurements on Day 2 were analyzed using the same ANOVA as Day 1 Baseline. An additional analysis was done to compare this baseline to Day 1 Baseline by calculating the percent change from Day 1 to Pre-Amygdala ADT Baseline, for both kindled and non-kindled amygdalae.

Amygdala ADT and AD Duration

One minute after collecting the Pre-Amygdala ADT Baseline, the amygdala ADT test began and dialysates were collected throughout this test.

Baseline Measurements Before Day 2 Hippocampal ADT Test

This baseline (Pre-Hippocampus ADT Baseline) was analyzed using the same ANOVA as Day 1 Baseline. An additional analysis was done to compare the two baselines on Day 2 by calculating the percent change from Pre-Amygdala to Pre-Hippocampus ADT Baseline, for both kindled and non-kindled amygdalae.

Hippocampal ADT and AD Duration

One minute after collecting the Pre-Hippocampus ADT Baseline, the ipsilateral hippocampus (or contralateral hippocampus or ipsilateral thalamus in some rats – see Appendices 2 and 3, respectively) ADT test began and dialysates were collected throughout the test.

Post-ADT

One minute after collecting the dialysates for the ipsilateral hippocampal ADT procedure (or contralateral hippocampus or ipsilateral thalamus in some rats), the first 9-minute Post-ADT dialysate (Post-ADT Sample 1) was collected from the kindled and non-kindled hemispheres. Then, 30 minutes after the last stimulation of the hippocampal ADT procedure, the second 9-minute Post-ADT dialysate (Post-ADT Sample 2) was collected.

Data Analysis Exceptions

ADT and AD Durations

The ADT and AD duration analyses were done for the amygdala, ipsilateral hippocampus, and contralateral hippocampus. The hippocampal electrode in 5 rats was actually in the ipsilateral thalamus (N = 4) and ventricle (N = 1). The ADTs and AD duration values for these 5 rats were excluded from the 'hippocampal' analysis.

Amino Acids

Some amino acid concentrations were undetectable by the capillary electrophoresis machine. Rather than entering a concentration of zero, the minimum concentration each test could detect was used. At times, the baseline was undetectable, thus, percent change from baseline could be very large (for example, above 10,000%). The reverse was also true in that the baseline was detectable but the following sample was not, thus, the percent change could be very small (for example, below 0.0001%). These large and small values were excluded from analysis.

Other values were lost due to error during the experiment or due to problems with the capillary electrophoresis machine. The missing sample's concentration was calculated to be the average of the samples immediately before and immediately following, under the assumption that amino acid release does not fluctuate widely from sample to sample during a specific, *unchanging* procedure. This assumption does not

apply when the missing sample occurred during a procedure different from the samples immediately before or after.

RESULTS

Histological results. Histological evaluation confirmed stimulating/recording electrode placements in the kindled amygdala, ipsilateral hippocampus, and in some rats the thalamus, ventricle and contralateral hippocampus (Figures 5 and 6). Also confirmed were bilateral microdialysis probe placements in the amygdala (Figures 7 and 8). The placements of the electrodes that were not used to stimulate the brain (e.g., contralateral amygdala) are not shown.

Data Presentation

The results are presented in the temporal order in which they were collected. Further they are organized in sections: Before SE, SE Induction Day, and post SE. The first section, Before SE, includes the Pre-Kindling and Kindling data, namely ADT, AD durations and kindling rates. Then, SE Induction Day (or Day 1) includes the rats' behaviour, EEG and amino acid levels obtained during the 60 min SE induction procedure and for 6 hours thereafter. The next day, post SE (or Day 2), includes the rats' behaviour, EEG and amino acids associated with the amygdala and hippocampal ADT

Figure 5. Schematic diagram taken from Paxinos and Watson (1986) at bregma -3.30 mm showing the stimulating electrode tip locations in the amygdala, hippocampus, and thalamus.

Bregma -3.30 mm

Interaural 5.70 mm

Figure 6. Schematic diagram taken from Paxinos and Watson (1986) at bregma -5.60 mm showing the stimulating electrode tip location in the amygdala.

Figure 7. Schematic diagram taken from Paxinos and Watson (1986) at bregma -2.80 mm showing the microdialysis probe locations in both hemispheres.

Figure 8. Schematic diagram taken from Paxinos and Watson (1986) at bregma -3.80 mm showing the microdialysis probe locations in both hemispheres.

procedures followed by the histological evidence of pathology at that time (24 hours after the SE induction procedure).

Before SE

Pre-Kindling

Before beginning kindling, the amygdala ADTs were determined and their AD durations were recorded (Table 3). Five minutes later, the same procedure was repeated for the ipsilateral hippocampus (Table 4) [or the contralateral hippocampus in some rats (see Appendix 2)].

A one-way ANOVA revealed no differences between NSE and SEP groups in the initial amygdala and ipsilateral hippocampus ADTs and AD durations.

Kindling

The day after the amygdala ADTs were determined, amygdala kindling began and continued until six stage 5 seizures had been recorded. The kindling rate, that is the number of stimulations before the first stage 5 seizure, was recorded (Table 5). A one-way ANOVA revealed no group differences (NSE vs. SEP) in the kindling rate.

Table 3. Average ($\pm$ S.E.M.) initial amygdala ADT (μ A) and AD duration (sec) for the NSE and SEP groups.

GROUP	ADT	AD DUR	N
NSE	142.50±25.68	12.57±1.50	14
SEP	108.33±13.94	9.50±0.40	6

Table 4. Average ($\pm$ S.E.M.) initial ipsilateral hippocampus ADT (μ A) and AD duration (sec) for the NSE and SEP groups.

GROUP	ADT	AD DUR	N
NSE	95.00±31.65	43.38±9.21	8
SEP	90.00±24.07	26.25±2.56	4

Table 5. Average ($\pm$ S.E.M.) amygdala kindling rate for the NSE and SEP groups.

GROUP	KINDLING RATE	N
NSE	8.00±1.39	14
SEP	8.17±1.82	6

The Day before SE Induction

Three weeks after amygdala kindling, the day preceding the SE induction procedure, the ADT and AD duration were reassessed for the ipsilateral hippocampus (Table 6), but not the amygdala.

Tests showed that the ADT and AD durations did not differ between SE groups or from the values obtained prior to kindling.

SE Induction Day (Day 1)

The day of SE induction first involved the implantation of dialysis probes followed by baseline dialysis accumulations (see methods). This was followed by one hour of amygdala stimulation to the kindled site (Induction period), followed by measurements for 6 hours thereafter: continuously for one hour (Post-Induction Hour 1) and then intermittently from 2 to 6 hours of post SE recordings (Post-Induction Hours 2 to 6). Throughout these 4 measurement periods, the subject's behaviour and EEG were monitored for seizure-related activity, while bilateral dialysates were collected.

Baseline

Behaviour and EEG

Prior to starting the SE induction stimulation, the rats showed 'normal' EEG activity, although some rats had occasional single high voltage spikes and others had occasional bursts of 'spindles', which are often seen in non-kindled rats. The behaviour

Table 6. Average ($\pm$ S.E.M.) ipsilateral hippocampus ADT (μ A) and AD duration (sec) for the NSE and SEP groups on the day before the SE induction procedure.

GROUP	ADT	AD DUR	N
NSE	103.13±19.15	33.50±4.12	8
SEP	90.00±24.07	31.00±5.05	4

associated with this EEG was quite normal; indeed, most of the rats had fallen asleep by the time of the SE induction procedure.

Amino Acids

GABA and Glutamate

Analysis of the baseline amino acids before SE induction revealed no significant differences between Side (hemisphere) or between SE group for GABA or glutamate (Table 7).

SE Induction

Behaviour and EEG

During the SE induction phase, each subject's kindled amygdala was stimulated for one hour at 50 μ A above their personal ADT. One-way ANOVA revealed no group differences between NSE and SEP in their kindled amygdala ADTs at the onset of the induction period (data not shown).

Of course, after the SE induction procedure, the two main NSE and SEP groups were formed. During the induction procedure, the *behaviour* of the rats that would form the NSE group was highly variable in that it ranged from non-convulsive to convulsive; by definition, however, the convulsive activity did not persist in these NSE rats. The EEG, rather than behaviour, appeared to more accurately predict the ultimate grouping because, in the case of the NSE rats, consistent, enduring EEG did not occur. On the

Table 7. Average ($\pm$ S.E.M.) baseline GABA and glutamate concentrations (μ M) from the kindled and non-kindled hemispheres in the NSE and SEP groups on Day 1.

SIDE	NSE	SEP
GABA		
	N = 11	N = 3
Kindled Side	15.8±4.4	21.4±8.5
Non-Kindled Side	15.1±4.0	25.7±7.7
Glutamate		
	N = 13	N = 6
Kindled Side	9.2±2.5	7.1±3.7
Non-Kindled Side	9.4±4.1	5.5±6.0

other hand, consistent spiking at ~1 Hz *during SE induction* was seen in 5 of the 6 subjects that ultimately did develop SE. In these cases, both the behaviour and EEG predicted the SE type.

In addition to the EEG and behaviour, the average number of convulsive seizures experienced by each rat during SE induction also predicted the ultimate group assignments (Table 8). A one-way ANOVA showed that the SEP group during the induction period had a significantly greater number of convulsive seizures than the NSE group, $F(1,18) = 19.5$, $p < 0.00034$. Consequently, the SEP group was further divided into ISE and ASE, and another one-way ANOVA was performed with NSE, ISE and ASE as the between-group factors. Again a significant effect of group was found, $F(2,17) = 13.5$, $p < 0.00031$, and subsequent multiple comparisons indicated that only the ASE group had a significantly greater number of seizures than the NSE group.

Amino Acids

GABA

The SE induction procedure resulted in a significant 3-way interaction between group, side and time, $F(1.9, 16.7) = 6.2$, $p < 0.012$. Furthermore, as there was a significant effect of group, $F(1,9) = 11.9$, $p < .008$, the next step was to check for differences between the NSE, ISE and ASE groups (Figure 9). Unfortunately, the ISE group had only 1 subject so it had to be excluded. This new analysis between the NSE and ASE groups showed a significant three-way interaction, $F(2,16) = 3.8$, $p < 0.044$, between side, time and group, and a significant effect of group, $F(1,8) = 11.2$, $p < 0.011$.

Table 8. Average ($\pm$ S.E.M.) number of convulsive seizures during SE induction for the NSE, ISE and ASE groups.

* – significantly greater number of seizures than the NSE group, $p < 0.00034$.

α – significantly greater number of seizures than the NSE group, $p < 0.05$.

GROUP	SEP TYPE	NUMBER OF CONVULSIVE SEIZURES	N
NSE		2.9 ± 0.3	14
SEP		$6.2 \pm 0.9^*$	6
	ISE	4.5 ± 0.5	2
	ASE	$7.0 \pm 1.1^\alpha$	4

Figure 9. Average ($\pm$ S.E.M.) percent change from baseline GABA concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups during SE stimulation. (Note the different scales.)

* - significantly greater percent change from Baseline to Third Stimulation on the kindled side in the ASE group than the NSE group, $p < 0.05$.

α - significantly greater percent change from Baseline to Third Stimulation on the non-kindled side in the ASE group than the NSE group, $p < 0.05$.

GABA - During Stimulation

69

The latter result was due to the ASE group having a higher percent change from baseline than the NSE group independent of time and side. Further testing showed that the NSE group had no significant changes either over time or between hemispheres. But, the kindled side in the ASE group was significantly greater than the NSE group after 20 minutes of stimulation. After 50 minutes, the kindled side was almost significantly greater than the NSE group ($p < 0.090$). In contrast, the ASE group's non-kindled side was significantly greater than the NSE group at this time.

Glutamate

The effect of SE stimulation on glutamate levels created a significant effect of group, $F(1,13) = 5.3$, $p < 0.038$. As a result, analysis focused on differences between the NSE, ISE and ASE groups (Figure 10). This new analysis also found a significant effect of group, $F(2,14) = 7.0$, $p < 0.009$. The post-hoc tests showed that the ASE group had a significantly greater percent change from baseline than the NSE group in *both* the kindled and non-kindled amygdalae during the last 9 minutes. The other two groups, NSE and ISE, showed no differences over time, between sides or between groups.

Post-Induction Hour 1

Behaviour and EEG

Once the stimulation stopped, each subject's EEG and behaviour were closely monitored for the first hour. During this interval, the subject's SE type was based on the dominant EEG and behavioural profile. The subjects with mostly normal behaviour and

Figure 10. Average ($\pm$ S.E.M.) percent change from baseline glutamate concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups during SE stimulation. (Note the different scales.)

* - significantly greater percent change from Baseline to the Last Stimulation in the ASE group than the NSE group, $p < 0.05$.

Glutamate - During Stimulation

EEG, of course, made up the NSE group. In contrast, if regular and consistent EEG spiking was seen in at least the kindled amygdala, these subjects were assigned to the SEP group. However, not all SEP subjects were the same. For instance, spiking frequency in two SEP subjects was around 1 Hz and they expressed immobile SE (ISE). Interestingly, spiking was seen in only the kindled amygdala of one ISE subject while spiking was in all recording structures of the other ISE subject. Thus, there was EEG variability within the ISE group ($n=2$). The second SE group had 1 to 2 Hz spiking in all recording structures and constant ambulation involving repetitive patterns, especially circling. These rats, of course, were labeled as ambulatory SE rats (ASE; $n = 4$).

Just as in the induction stimulation phase, EEG and behavioural seizures continued to occur in many rats, but the difference in this phase was that not all subjects had convulsive seizures. The average number of convulsive seizures in each group for this first hour is shown in Table 9. A one-way ANOVA indicated that the SEP group had a significantly greater number of seizures than the NSE group, $F(1,18) = 6.8$, $p < 0.019$. Consequently, another one-way ANOVA was done with NSE, ISE and ASE as the between-groups factor. Again, a significant effect of Group was found, $F(2,17) = 4.7$, $p < 0.024$, and subsequent multiple comparisons indicated that the most severe SEP group, ASE rats, had a significantly greater number of seizures than the NSE group. Interestingly, the NSE group periodically expressed some intermittent seizure activity.

The average number of seizures seen during the Post-Induction Hour 1 may actually be higher than the values provided in Table 9. Typically, the first signs of

Table 9. Average ($\pm$ S.E.M.) number of convulsive seizures for the NSE, ISE and ASE groups during the Post-Induction Hour 1.

* – significantly greater number of seizures than the NSE group during the Post-Induction Hour 1, $p < 0.019$.

α – significantly greater number of seizures than the NSE group during the Post-Induction Hour 1, $p < 0.05$.

GROUP	SEP TYPE	NUMBER OF CONVULSIVE SEIZURES	N
NSE		1.0 ± 0.4	14
SEP		$3.8 \pm 1.5^*$	6
	ISE	2.0 ± 0.0	2
	ASE	$4.8 \pm 2.1^{\alpha}$	4

seizure activity occur in the EEG, not in the rat's behaviour. It is only when the EEG seizure propagates to motor areas that there is any behavioural evidence of seizure activity (e.g., a stage 2-5 convulsion). In this particular period of time in this experiment (Hour 1), there were times when the EEG activity looked identical to that occurring during a stage 5 convulsion, but without behavioural correlates. These EEG seizures I have labeled *EEG-only seizures*. If those EEG-only seizures were compared to those associated with a stage 5, there would be no obvious difference. These EEG-only seizures, however, were not included in the number of stage 5 seizures shown above.

To study this new phenomenon, the average number of EEG-only seizures was examined (Table 10). A one-way ANOVA showed that there was a significant difference between the NSE and SEP groups, $F(1,19) = 17.5, p < .002$. As a result, the NSE, ISE and ASE groups were compared. This test also showed a significant effect of group, $F(2,19) = 9.3, p < 0.003$. Multiple comparisons indicated that the ASE group had a significantly greater number of EEG-only seizures than the NSE group. Interestingly, this parallels the result from the convulsive seizures.

Closer examination of the EEG records did show a difference between the *convulsive* stage 5 event and the *EEG-only* seizures. In all groups, the electrographic seizures began in the kindled amygdala during the majority of both the convulsive and EEG-only seizures. Once the spontaneous EEG seizure began in the kindled amygdala, a second, third and fourth structure was recruited into the seizure, but the order varied. Apart from the kindled amygdala, the only other structure frequently associated with a

Table 10. Average ($\pm$ S.E.M.) number of EEG-only stage 5 seizures for the NSE, ISE and ASE groups during the Post-Induction Hour 1.

* – significantly greater number of EEG-only seizures than the NSE group, $p < 0.002$.

α – significantly greater number of EEG-only seizures than the NSE group, $p < 0.05$.

GROUP	SEP TYPE	NUMBER OF EEG-ONLY STAGE 5 SEIZURES	N
NSE		0.50 ± 0.20	14
SEP		$4.67 \pm 1.50^*$	6
	ISE	3.50 ± 0.50	2
	ASE	$5.25 \pm 2.29^{\alpha}$	4

spontaneously *convulsive seizure* was the ipsilateral hippocampus. In contrast, the *EEG-only seizures* often started in the non-kindled amygdala, or the ipsilateral or contralateral hippocampus in both NSE and SEP groups.

Remarkably, these EEG-only seizures have never been reported before, thus, it is not clear if they are a product of SE induction *per se* and/or if the physical insult to the brain (i.e., cannulae and/or probes) created the phenomenon by preventing the full propagation pattern of the local amygdala seizure, as it blocked the normal recruitment of other limbic structures, preventing the discharge from reaching convulsive motor centers.

Amino Acids

GABA

For the first hour after the stimulation offset, there was a significant effect of group, $F(1,10) = 13.3$, $p < 0.006$, so the next step was to compare the NSE, ISE and ASE groups (Figure 11). Unfortunately, the ISE group had only 1 subject so it had to be excluded. This new analysis between the NSE and ASE groups showed a significant two-way interaction between side and group, $F(1,9) = 8.6$, $p < 0.018$, and a significant effect of group, $F(1,9) = 6.2$, $p < 0.035$. Further testing of the interaction showed that the NSE group had no changes from baseline after the stimulation stopped, but the *non-kindled* side in the most severe status subjects (i.e. ASE) was significantly greater than the NSE group throughout this first post-stimulation hour.

Figure 11. Average ($\pm$ S.E.M.) percent change from baseline GABA concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour. (Note the different scales.)

* - significantly greater percent change from Baseline on the non-kindled side in the ASE group than the NSE group, $p < 0.05$.

GABA - Post-Stimulation

81

Glutamate

The initial analysis to compare the NSE and SEP groups showed no significant differences between groups and hemispheres or over time in glutamate levels. To see if this finding is due to the ISE group adding too much variability, the analysis was re-done with only the NSE and ASE groups (Figure 12). The Time*Side interaction was approaching significance, $F(1.3,14.3) = 3.6$, $p < 0.071$; the group effect was not significant. Further testing showed that only the kindled side in the ASE group was significantly greater than baseline for the first 9 minutes after the stimulation stopped.

Post-Induction Hours 2 to 6

Behaviour and EEG

For the remainder of SE induction day, Post-Induction Hours 2 to 6, each subject's behaviour was monitored continuously, while the EEG was monitored during dialysate collection and intermittently between these collection times. The EEG and behaviour of the NSE groups was roughly unchanged from the one hour assessment, remaining predominantly normal. Regular and consistent spiking of 1 Hz was now seen in both ISE rats in all structures. This spiking continued in all structures for the rest of the day, although the amplitude gradually decreased towards the end of the day. For the ASE group, the amplitude and frequency of the spiking gradually decreased, which was associated with a decrease in movement. In 3 of the 4 ASE subjects, the spiking decrease to the point that SE stopped, and normal behaviour emerged. In the 4th ASE subject, the frequency of spiking remained around 1 Hz, and ambulatory behaviour continued.

Figure 12. Average ($\pm$ S.E.M.) percent change from baseline glutamate concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour. (Note the different scales.)

* - significantly greater than Baseline, $p < 0.05$.

Glutamate - Post-Stimulation

84

Similar to the one hour post-induction period, some subjects continued to have convulsive seizures (Table 11). However, there were no longer differences between groups even though this interval had a similar number of convulsive seizures as the Post-Induction Hour 1.

Just like the Post-Induction Hour 1, EEG-only seizures occurred, thus, the actual number of seizures may be more numerous than indicated in Table 11. Unfortunately, the EEG was not recorded continuously during this interval, thus, the real number of EEG-only seizures was unavailable; however, there is a minimum number available for each group based on the sampling procedure that was effected (Table 12).

Amino Acids

GABA

For GABA (see Figure 13), only the NSE and ASE groups were analyzed because the ISE group had only 1 subject. Using these two groups, the side by group interaction was significant, $F(1,8) = 10.6$, $p < 0.013$, and the group effect was significant, $F(1,8) = 7.2$, $p < 0.029$. Further testing showed that the former result was due to the kindled side in the ASE group having a significantly greater percent change from baseline than the NSE group.

Glutamate

The analysis of glutamate levels revealed a significant 3-way interaction between time, side and group for Hours 2 to 6, $F(1.2,15.7) = 4.4$, $p < 0.048$. Additional analysis of

Table 11. Average ($\pm$ S.E.M.) number of convulsive seizures for the NSE, ISE and ASE groups during the Post-Induction Hours 2 to 6.

GROUP	NUMBER OF CONVULSIVE SEIZURES	N
NSE	1.8 ± 0.5	14
SEP	3.5 ± 1.6	6

Table 12. Average ($\pm$ S.E.M.) number of EEG-only stage 5 seizures for the NSE and SEP groups during the Post-Induction Hours 2 to 6.

GROUP	NUMBER OF EEG-ONLY STAGE 5 SEIZURES	N
NSE	1.00±0.65	14
SEP	4.83±2.37	6

Figure 13. Average ($\pm$ S.E.M.) percent change from baseline GABA concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour, as well as for Hours 2 to 6. (Note the different scales.)

* - significantly greater percent change from Baseline on the non-kindled side in the ASE group than the NSE group, $p < 0.05$.

α - kindled side significantly greater than the NSE group, $p < 0.005$.

GABA - Post-Stimulation

91

glutamate then focused on the differences between NSE and ASE; ISE was excluded because there was only one subject (Figure 14). This new analysis also showed a significant three-way interaction between side, time and group, $F(1.2,14.8) = 6.4$, $p < 0.019$, along with a significant main effect of group, $F(1,12) = 19.1$, $p < 0.002$. Further testing showed that the NSE group had no significant differences either between hemisphere or over time. The ASE group, however, showed a significant effect of Side, $F(1,3) = 51.816$, $p < 0.007$, because the percent change from baseline in the kindled side was higher than the non-kindled side. Furthermore, the ASE group's kindled side had a significantly greater percent change from baseline than the NSE group's kindled side for Hours 2, 4 and 6.

Day 2: 24 hr After SE Induction

Day 2 started approximately 24 hours after the Induction phase and consisted of baseline measurements followed by finding ADTs, first in the kindled amygdala and, 60 minutes later, the ipsilateral hippocampal (or contralateral hippocampal/ipsilateral thalamus in some rats).

Baseline measurements before Amygdala ADT test on Day 2

Behaviour and EEG

Prior to performing the amygdala ADT test, baseline EEG and behaviour were again recorded. At the end of 6 hours on Day 1, one ASE and two ISE subjects continued

Figure 14. Average ($\pm$ S.E.M.) percent change from baseline glutamate concentrations from the kindled and non-kindled hemispheres in the NSE, ISE and ASE groups for the first post-stimulation hour, as well as for Hours 2 to 6. (Note the different scales.)

* - significantly greater than Baseline, $p < 0.05$.

α - significantly greater percent change from Baseline on the Kindled Side than the Non-Kindled Side in the ASE group, $p < 0.007$.

β - significantly greater percent change from Baseline in the ASE group than the NSE group on the Kindled Side, $p < 0.008$.

Glutamate - Post-Stimulation

94

to display SE. However, by the time Day 2 began, these 3 subjects were no longer in SE. As a result, the EEG and behaviour appeared to be void of any signs of SE in all subjects.

Amino Acids

GABA

For the Pre-Amygdala ADT Baseline scores, *only 1* SEP subject had detectable GABA levels, while the remaining SEP rats had levels of GABA that were too low to be considered reliable at baseline. This was not true of the NSE rats. Analysis of the Pre-Amygdala ADT Baseline scores for the NSE subjects revealed that the kindled side had more GABA than the non-kindled side, $F(1,9) = 5.4$, $p < 0.047$ (Table 13).

Analysis of the GABA percent change from Day 1 Baseline in the NSE group showed a significant effect of Side, $F(1,9) = 5.7$, $p < 0.042$ due to a significantly greater percent change on the kindled side compared to the non-kindled side (Figure 15). In addition, a t-test showed that the kindled side had significant increase from the Day 1 Baseline, $p < 0.05$.

Glutamate

Analysis of the Pre-Amygdala ADT Baseline on Day 2 revealed no significant differences between Side or between SE group for glutamate (Table 13). Although, this baseline had an effect of Side that was approaching significance, $F(1,14) = 3.954$, $p < 0.080$. Glutamate also didn't show any significant differences between hemisphere or group in percent change from the Day 1 Baseline (data not shown).

Table 13. Average ($\pm$ S.E.M.) Pre-Amygdala ADT baseline GABA and glutamate concentrations (μ M) from the kindled and non-kindled hemispheres in the NSE and SEP groups.

* - kindled side significantly greater than the non-kindled side in the NSE group, $p < 0.047$.

β - only 1 SEP subject had detectable GABA levels.

SIDE	NSE	SEP
GABA		
	N = 10	N = 1 ^b
Kindled Side	31.3±8.9*	93.8
Non-Kindled Side	19.3±5.2	30.8
Glutamate		
	N = 11	N = 4
Kindled Side	9.2±3.0	11.6±4.9
Non-Kindled Side	6.1±1.2	4.2±2.0

Figure 15. Average ($\pm$ S.E.M.) percent change from Day 1 to Pre-Amygdala ADT baseline in GABA concentrations from the kindled and non-kindled hemispheres in the NSE group.

* - significant increase from Day 1 Baseline on the kindled side, $p < 0.05$.

α - kindled side significantly greater than the non-kindled side, $p < 0.05$.

Amygdala ADT and AD Duration

Behaviour and EEG

No subjects had an amygdala ADT below the 400 μ A cutoff (data not shown).

Amino Acids

GABA

Statistical tests revealed no significant change in GABA levels during the amygdala ADT procedure (data not shown).

Glutamate

Glutamate levels showed a significant 2-way interaction between Side and Group, $F(1,10) = 9.1$, $p < 0.014$ (Figure 16). This interaction was due to the non-kindled side having a significantly greater percent change from baseline in the SEP group than the NSE group.

Baseline measurements before Hippocampal ADT test on Day 2

Behaviour and EEG

Approximately 50 minutes after the last amygdala stimulation, the EEG and behaviour were again monitored. These appeared to be similar to the Pre-Amygdala Baseline with no evidence of seizure spiking.

Figure 16. Average ($\pm$ S.E.M.) percent change from Pre-Amygdala ADT baseline in glutamate concentrations from the kindled and non-kindled hemispheres in the NSE and SEP groups during the amygdala ADT procedure on Day 2. (Note the different scales.)
* - non-kindled side's increase significantly greater in the SEP group than the NSE group, $p < 0.05$.

Amino Acids

GABA

Analysis of the Pre-Hippocampus ADT Baseline revealed no significant differences between Side or between Group for GABA (Table 14). Nor were there any significant percent changes between Side or Group from Pre-Amygdala ADT Baseline (data not shown).

Glutamate

Analysis of the Pre-Hippocampus ADT Baseline on Day 2 revealed no significant differences between Side or between Group for glutamate (Table 14). Although, this baseline had an effect of Side that was approaching significance, $F(1,14) = 5.522$, $p < 0.067$.

Analysis of the glutamate percent change from Pre-Amygdala to Pre-Hippocampus ADT Baseline showed no significant differences between Group or Side (data not shown). Furthermore, power does not appear to be a concern.

Ipsilateral Hippocampus ADT and AD Duration

After observing the EEG and behaviour, ADT and AD durations were assessed in the ipsilateral hippocampus (or contralateral hippocampus or ipsilateral thalamus in some rats - see Appendices 2 and 3, respectively).

Table 14. Average ($\pm$ S.E.M.) Pre-Hippocampus ADT baseline GABA and glutamate concentrations (μ M) from the kindled and non-kindled hemispheres in the NSE and SEP groups.

SIDE	NSE	SEP
GABA		
	N = 9	N = 2
Kindled Side	21.7±6.0	43.0±12.7
Non-Kindled Side	35.7±19.0	29.6±40.3
Glutamate		
	N = 11	N = 4
Kindled Side	9.3 ± 2.7	10.8 ± 4.4
Non-Kindled Side	5.6 ± 1.0	3.8 ± 1.6

Behaviour and EEG

The tests showed no effect of Group or Time for ipsilateral hippocampal ADTs (Table 15). On the other hand, the duration of AD that was triggered at the threshold did show a significant effect of Time, $F(2,20) = 7.3$, $p < 0.005$ (Table 16). Multiple comparisons revealed that the Day 2 AD duration was significantly longer than both Initial ADT duration and the duration on the Day Before Induction in both the NSE and SEP groups.

The longer AD duration on Day 2 reflects a little known phenomenon that we have called the 'Hippocampal Release Phenomenon'. Despite both main groups showing this phenomenon, the response was more provocative in the NSE group than the SEP group. In this experiment, 7 of the 8 NSE subjects experienced a full stage-5 convulsive seizure at their hippocampal AD threshold and the eighth subject had a stage 2 seizure, whereas none of the SEP subjects had a stage 5 seizure and only 1 ISE subject had a stage 2 seizure at their hippocampal AD threshold (Table 17). Using Logistic Regression, the NSE group had a greater probability of experiencing seizure activity than the SEP group ($\chi^2 = 9.0$, $p < .0028$).

Amino Acids

GABA

During the ipsilateral hippocampus ADT procedure, which triggered an AD in all rats, a significant effect of Group was observed, $F(1,4) = 15.5$, $p < 0.018$, and the Side x

Table 15. Average ($\pm$ S.E.M.) ipsilateral hippocampus ADT (μ A) for the NSE and SEP groups on Day 2.

GROUP	PRE-KINDLING	DAY BEFORE SE	DAY 2	N
NSE	95.00±31.65	103.13±19.15	93.75±15.49	8
SEP	90.00±24.07	90.00±24.07	100.00±20.41	4

Table 16. Average ($\pm$ S.E.M.) ipsilateral hippocampus AD duration (sec) for the NSE and SEP groups on Day 2.

* - the Day 2 AD duration was significantly longer than the Pre-Kindling AD duration, $p < 0.05$

α - the Day 2 AD duration was significantly longer than the Day Before SE AD duration, $p < 0.05$

GROUP	PRE-KINDLING	DAY BEFORE SE	DAY 2 ^{*,α}	N
NSE	43.38 $\pm$ 9.21	33.50 $\pm$ 4.12	89.75 $\pm$ 8.72	8
SEP	26.25 $\pm$ 2.56	31.00 $\pm$ 5.05	53.75 $\pm$ 31.30	4

Table 17. Average ($\pm$ S.E.M.) number of different stages of convulsive seizures at the ipsilateral hippocampus ADT for the NSE and SEP groups on Day 2.

* - significantly different from the NSE group, $p < .0028$.

GROUP	NUMBER OF SEIZURES AT ADT			N
	TOTAL	Stage 2	Stage 5	
NSE	8	1	7	8
SEP	1 *	1	0	4

Group interaction was approaching significance, $F(1,4) = 6.1$, $p < 0.071$ (Figure 17).

Further tests showed that the SEP group had a greater percent change from baseline than the NSE group on the kindled side. This SEP group's kindled side also had an increase from baseline that was approaching significance, $p < 0.06$.

Glutamate

Glutamate showed a non-significant bilateral increase in both groups during this sample (Figure 18). Although this increase was not significant, the figure was included to make comparisons with another experiment (i.e. Shin, 2000) easier (see Discussion).

Post-ADT

Behaviour and EEG

For 10 minutes and 30 to 40 minutes following the last stimulation, the behaviour and EEG were monitored. Behaviour was mostly normal; some had fallen asleep. EEG activity, though, appeared to be slightly lower than baseline because most subjects had only occasional spikes in all structures.

Amino Acids

Once the ADT procedures were finished, GABA and glutamate showed no differences between Group or Side, or over Time (data not shown).

Figure 17. Average ($\pm$ S.E.M.) percent change from baseline in GABA concentrations from the kindled and non-kindled hemispheres in the NSE and SEP groups during the ipsilateral hippocampus ADT procedure on Day 2. (Note the different scales.)
* - significantly greater percent change from baseline on the kindled side in the SEP group than the NSE group, $p < 0.05$.

Figure 18. Average ($\pm$ S.E.M.) percent change from baseline in glutamate concentrations from the kindled and non-kindled hemispheres in the NSE and ASE groups during the ipsilateral hippocampus ADT procedure on Day 2. (Note the different scales.)

Day 2 Pathology

Within an hour of the last stimulation, 9 NSE, 1 ISE and 3 ASE subjects were perfused for histological assessment of brain damage. Gliosis and injured cells at this time could be identified with cresyl violet and fluoro-jade (F-J) stains, respectively. Indeed, variable gliosis and injury were observed in the amygdala, piriform cortex, dorsal endopiriform nucleus, perirhinal and entorhinal cortices, hippocampus, parietal cortex, thalamus and neocortex of rats in different groups.

Amygdala

In the amygdala, most of the damage was the result of the cannulae and probes plus the SE induction stimulation, particularly in the SEP group. On the non-stimulated side, however, there was very little probe-related damage in any group (see Figures 19 and 20).

For all subjects, dead and injured cells were found in the ipsilateral basal, lateral, cortical, amygdala-hippocampal transition and amygdala-piriform transition nuclei. The medial nucleus, on the other hand, had dead and injured cells only in the SEP group and only injured cells in the NSE group. One subject that had the most serious form of SEP (ASE) showed *bilateral* dead and injured cells in several of these nuclei.

Hippocampus

For the most part, the NSE group had no signs of damage in the hippocampus, represented as gliosis or fluoro-jade. The *most severe* SEP subjects, however, showed

Figure 19. Pathology revealed with fluoro-jade in and around the kindled and non-kindled amygdalae and piriform cortex for a NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).

Various arrows point out fluoro-jade positive areas for each subject:

Arrow 1: As a typical 'stimulation control', the NSE subject showed that the combination of a probe, 60 min of stimulation and number of seizures but not SE can cause injury to the kindled amygdala (A). In contrast, the non-stimulated side had only very mild probe-related damage (B). Inducing status resulted in additional damage to the kindled amygdala but the affected nuclei are hard to identify because most of the damaged area was likely very fragile and was removed with the probe (C and E). This is most dramatic with the hole in the kindled amygdala of the ASE subject (E). On the other hand, the contralateral sides (D and F) show mostly probe-related damage, much like the NSE subjects.

Arrow 2: Some of the fluoro-jade positive amygdala nuclei were not removed with the probe and remained in the brain, namely the lateral nuclei. Both SEP subjects had ipsilateral lateral amygdala nucleus pathology (C and E), but only the ASE subject had contralateral damage (F).

Arrow 3: Both SEP subjects had ipsilateral piriform cortex damage, although the ISE had very few damaged cells (C), while the ASE subject had much more extensive pathology (E).

Arrow 4: Only the ASE subject had injured cells in the ipsilateral dorsal endopiriform nucleus (E).

Arrow 5: In these particular pictures, only the ASE subject showed bilateral pathology in the perirhinal cortex layers (E & F). In reality, however, more cortical damage had occurred in this rat [Figures 21(B) and (C)].

Small arrow: Gliosis around the electrode in the non-kindled amygdala (F).

Ipsilateral

Contralateral

120

NSE

ISE

ASE

Figure 20. Pathology revealed with cresyl violet in and around the kindled and non-kindled amygdalae and piriform cortex for an NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).

Various arrows are used to point out areas for each subject:

Arrow 1: As the stimulation control, the NSE subject showed that the probe, SE stimulation and seizures can cause injury to the kindled amygdala (A); note the pale color compared to the contralateral side (B). In contrast, the non-stimulated side (B) has only very mild probe-related damage. Inducing status caused additional damage to the kindled amygdala area (C and E). This induction damage is most dramatic with the hole in the kindled amygdala of the ASE subject (E), while the milder ISE subject's kindled amygdala showed milder damage (C). The contralateral sides (D and F), however, show only probe-related damage, like the NSE subjects.

Arrow 2: Only the SEP subjects had ipsilateral piriform cortex damage, but in differing amounts. The ISE subject had pathology isolated to a small area identifiable by a break in the continuous line (C), while the ASE subject's remaining piriform cortex appears blurred (E). In contrast, the contralateral piriform cortex in both subjects showed a continuous, dense two layer cell field (D and F).

Arrow 3: The ASE subject had no ipsilateral dorsal endopiriform nucleus (E), while the contralateral side's nucleus appears unaffected (F).

Small arrow: Gliosis around the electrode in the non-kindled amygdala (F).

Ipsilateral

Contralateral

122

NSE

ISE

ASE

some hippocampal pathology because gliosis was isolated primarily within the ipsilateral CA3 field at 24 hours, but there were no F-J positive cells. In puzzling contrast, the *mild* SEP subject (i.e. the only ISE subject perfused at Day 2) showed the greatest amount of gliosis in the ipsilateral CA2 and CA3 fields (see Figures 21 and 22), and damage bilaterally in the CA1 fields of the ventral hippocampus (not shown).

Perirhinal/Parietal/Entorhinal cortex

The perirhinal, parietal and entorhinal cortices had various amounts of F-J positive cells in all groups (Figure 23). The NSE group had none to a small amount of positive cells in one bilateral layer of parietal cortex (A). In contrast, the SEP subjects' damage included several layers of different structures, either ipsilateral or bilateral (see Figure 23). Gliosis, on the other hand, was found ipsilaterally only in the most severe SEP subjects (see Figure 24).

Thalamus

The thalamus had no obvious signs of gliosis. However, many fluoro-jade positive cells were evident. There were no positive cells in the NSE group. In contrast, the SEP subjects showed a wide range of positive cells, which were associated with the severity of the SE; some of the damage was ipsilateral and some bilateral (see Figure 25).

Figure 21. Pathology revealed with fluoro-jade in and around the ipsilateral and contralateral hippocampi for an NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).

The NSE subject had no damage on either side in the hippocampus (A and B). Various arrows are used to point out fluoro-jade results for the two SEP subjects (C and E):

Arrow 1: The subject with the mildest form of SE, ISE, has the most severe pathology in this field. These sick cells are clearly indicated with fluoro-jade on the ipsilateral side (C). In contrast, the most severe SE, the ASE subject, also showed no F-J pathology on either side (E and F). Although, the ipsilateral medial CA1 field showed mild positivity (E). However, as F-J positive cells tend to glow brilliantly like in the ISE subject (C), the mild positivity in (E) was viewed as a staining artifact.

Arrow 2: The ISE subject continued to have the most severe hippocampal pathology because it was the only subject to show positive cells in the ipsilateral CA2 field damage-resistant area (C) (Pitkanen et al., 1998; Coulter, 1999).

Arrow 3: The ISE subject was the only subject to show positive cells in the ipsilateral CA3 field (C).

Arrow 4: Bilateral positive cells were found only in the ISE subject in the dendrites below the CA1 field (C and D).

Arrow 5: Only the ISE subject had a few positive cells in the ipsilateral dentate gyrus (C).

Arrow 6: In (E), part of the thalamic damage unique to the ASE subject is evident; the specific nucleus shown here is the mediodorsal lateral posterior. See Figure 25 for more thalamic pathology.

Long thin arrow: Hippocampus electrode tract (all figures).

Short thin arrow: Amygdala electrode tract (B and F).

Ipsilateral

Contralateral

125

NSE

ISE

ASE

Figure 22. Pathology revealed with cresyl violet in the ipsilateral and contralateral hippocampi for an NSE subject (A and B), an ISE subject (C and D), and a severe ASE subject (E and F).

The NSE subject had no damage on either side in the hippocampus (A and B). The most severe SE, seen in the ASE subject, also showed no pathology on either side (E and F). In contrast, only the ipsilateral CA2 (Arrow 1 in C) and CA3 (Arrow 2 in C) fields in the ISE subject had signs of gliosis because these fields were less dense than their contralateral counterparts. These finding may normally be suspect but they are supported by the fact that these same fields have fluoro-jade positive cells [Figure 19(C)].

Long thin arrow: Hippocampus electrode tract (all figures).

Short thin arrow: Amygdala electrode tract (B and F).

Ipsilateral

Contralateral

127

NSE

ISE

ASE

Figure 23. Pathology revealed with fluoro-jade in the ipsilateral cortex for an NSE subject (A) and ipsilateral and contralateral cortex for a severe ASE subject (B and C).

Arrow 1: The NSE subject showed very mild parietal cortical damage in layer 4. This pathology was bilateral but only the ipsilateral side is shown (A). The ASE subject had additional damage, which included different cortical areas, thus, both sides are shown. The ipsilateral perirhinal cortex had a larger amount of pathology, extending from the top of the figure to the bottom of the rhinal fissure (B), while the contralateral side had lesser damage, involving fewer layers (C).

Arrow 2: Only the ASE subject had fluoro-jade positive cells adjacent to the external capsule in layer 6B. Just like Arrow 1, the ipsilateral cortex showed a larger amount of pathology, starting much higher (B) than the other side (C). Unlike Arrow 1, both sides had injured cells extending through to bottom of the rhinal cortex.

Arrow 3: Layer 3 of the perirhinal cortex was damaged only on the ipsilateral side in the ASE subject (B).

Arrow 4: The ASE subject showed bilateral amygdala pathology but just the top of the lateral nuclei are shown here (B and C). For the rest of the amygdala see Figures 17(E) and (F).

Ipsilateral

129

Ipsilateral

Contralateral

Figure 24. Pathology revealed with cresyl violet in the ipsilateral (A) and contralateral (B) perirhinal fissure for a severe ASE subject. Arrow 1 shows that layers 3B and 5A of the ipsilateral perirhinal cortex are damaged, while the contralateral layers are unaffected. There is also no cellular sign of the ipsilateral dorsal endopiriform nucleus, but the top of the contralateral nucleus is visible

Ipsilateral

Contralateral

ASE

Figure 25. Pathology revealed with fluoro-jade in the ipsilateral and contralateral thalamus for a severe ASE subject.

This subject had ipsilateral pathology in the lateral posterior thalamic nucleus (Arrow 1), but bilateral damage in the mediodorsal thalamic nucleus (Arrow 2 and 4) and zona incerta (Arrow 3 and 5); the contralateral injury was always less extensive than the ipsilateral injury.

Discussion

In this study, we examined the electrographic and convulsive behaviour associated with the induction of SE produced by protracted electrical stimulation of the amygdala in amygdala-kindled rats. Of critical interest were the changes in the levels of extracellular amino acids, measured by microdialysis samples from both amygdalae, that were associated with the SE induction phase, and up to 24 hours thereafter. The important question was whether increased amygdala amino acids were associated with the appearance of SE and its ensuing pathology.

Before SE

The ADTs and associated AD durations for both the amygdala and ipsilateral hippocampus did not differ between two SE groups (NSE and SEP) either before kindling or after kindling. Similarly, there were no differences between the two groups in their kindling rates. Thus, the behavior of the rat during SE induction was independent of its earlier amygdala ADT or kindling profile prior to the SE induction procedure. In other words, the excitability of the amygdala and/or its ability to access the convulsive seizure circuitry were independent of SE development, as has been reported previously (Sato et al., 1990; Mohapel et al., 1996). These findings agree with an earlier experiment

(McIntyre et al., 1982) and support the hypothesis that kindling and SE may not be accessing the same circuitry for their expression (Mohapel et al., 1996).

SE Induction Day

Behaviour and EEG

During the induction of SE, variations in the intensity of current (50 μ A above the ADT) necessary to drive the periodic convulsive seizures did not determine group membership. However, group membership was related to the convulsive performance of the rat during the stimulation period. As was seen in other experiments (McIntyre et al., 1982; Mohapel et al., 1996), the number of convulsive seizures expressed during the stimulation period was significantly greater in the rats that developed SE, and predicted the ultimate SEP group membership. This higher rate of convulsive seizure expression during stimulation in the SEP group continued during the first post-stimulation hour, but then abated thereafter. Unexpectedly, however, after the SE induction period, spontaneous seizures intermittently appeared in 11 of the 14 *NSE* rats! This finding in *NSE* has not been observed previously. As a result, that outcome suggests that even the rats that we have defined as *NSE* subjects experienced a mild form of SE. It is possible that this heightened sensitivity in the *NSE* rats immediately following SE induction efforts results from a sort of 'irritation' to the kindled amygdala tissue created by the adjacent dialysis probe and its fluid extraction procedure. If this outcome had been anticipated, kindled control rats without dialysis probes and such extraction procedures would have clarified this possibility.

Further to this point, we raise the specter of the previously unseen finding of *EEG-only* seizures in both NSE and SEP rats, which were similar in form to those associated with stage-5 convulsive responses. It is not clear whether the EEG-only seizures were a product of the SE procedure and/or microdialysis probe activity, either of which might have prevented (or augmented) the normal recruitment of the focal seizures; clearly, however, those EEG seizures did not propagate into motor output structures that drive the convulsive response.

In addition to the SE predictive value of the number of convulsive seizures during induction, the frequency of the EEG spiking during that period was predictive of the SE group membership. If a rat developed consistent spiking behaviour early during stimulation, it also developed SE. By contrast, during the induction period, the *general* behaviour did not predict SE group, as it ranged broadly from non-convulsive to convulsive in all subjects.

Once the SE induction stimulation stopped, the NSE subjects exhibited normal behaviour and EEG (except for the bursts of EEG-only seizures), and remained so for the rest of the day. In contrast, the behaviour of the SEP group and their EEG changed over the day, changes that depended on the SE type and, possibly, the development of neuropathology. For instance, the ISE subjects had an ongoing seizure spiking frequency of approximately 1 Hz. Their behavioural immobility and EEG spiking continued for the rest of the day, although the EEG and spiking amplitude gradually decreased towards the end of the day. On the other hand, the EEG of the ASE rats was characterized by 1 to 2

Hz spiking, while the behaviour was constant movement. However, as the day progressed, the amplitude and frequency of their spiking gradually decreased, along with an associated decrease in movement. In all but one of the ASE subjects, the spiking decreased to the point that seizure spiking stopped altogether, and normal behaviour emerged. In the remaining ASE subject, the frequency of spiking continued at ~ 1 Hz and the ambulatory behaviour remained ongoing.

The EEG spiking frequencies seen in our SEP groups appeared higher than other studies from our laboratory (for example, McIntyre et al., 1991; McIntyre et al., in press). According to these and other studies, our higher spike frequencies should have been associated with more severe SE phenotypes (like masticatory and generalize SE). As indicated earlier, the behavioural expression of the SE may have been truncated because of the location of the dialysis probes and the ongoing dialysis perfusion of the area, which may have affected the local recruitment and propagation of the ongoing seizures, resulting reduced in behavioural seizure expression.

Amino Acids

Prior to starting the SE induction, both amygdalae had similar extracellular concentrations of GABA and glutamate in both the NSE and SEP groups. Thus, the development of SE was independent of the initial amygdala amino acid concentrations.

During the SE induction procedure, however, GABA (Figure 9) and glutamate (Figure 10) showed changes that depended on the eventual group membership. In the NSE group, neither amino acid showed any significant changes over time or between

hemispheres from baseline. As for those that developed the most severe status (i.e. ASE), GABA and glutamate had different changes. For GABA, the changes were hemisphere-dependent. Namely, the kindled side's extracellular GABA increased faster than the non-kindled side. On the kindled side, the ASE group was greater than the NSE group within 20 minutes of stimulation and essentially remained so for the rest of the stimulation. The non-kindled side had a much slower increase in the ASE group as it took approximately 50 minutes to become greater than the NSE group. At this window of time, glutamate showed its first difference in the ASE group, where it was greater than the NSE group in both amygdalae. The overall picture appears to be that the induction procedure and the resulting seizure activity was associated with a gradual and continuous bilateral increase in extracellular glutamate, while GABA shows a dramatic increase in the kindled amygdala while the non-kindled amygdala slowly increasing its extrasynaptic levels. Whether these changes cause status or are the result of a brain that is inherently susceptible to status is unclear. Although, a possible conclusion is that the amino acid release and/or reuptake is different in those that develop status versus those that do not.

Once the induction stimulation was terminated, glutamate (Figure 12) was briefly above baseline in the kindled amygdala in the ASE group; within 20 minutes, it had declined back to baseline. In contrast, GABA (Figure 11) only showed a significant difference in the non-kindled side of the ASE group: for the whole first hour after the stimulation, the non-kindled side had more extrasynaptic GABA in the ASE group than the NSE group.

Then, inexplicably, within 50 to 60 minutes after the stimulation, there was a dramatic change in the amino acid levels, where the kindled side showed another increase in both GABA (Figure 13) and glutamate (Figure 14) only in the most severe SE subjects, ASE. Furthermore, these levels remained high for the four hours thereafter. In contrast, both sides in the NSE group and the non-kindled side in the ASE group did not show that protracted elevation. Because elevated levels of glutamate (for example, McIntyre et al., 1986; Scott et al., 1998; Sloviter, 1999; Fountain, 2000) and GABA (Xu et al., 2000) have been reported to produce neurotoxicity, these results might well have significance for the neuropathology in the ASE group that was to follow by the next day.

Day 2

Amygdala ADT test

Behaviour and EEG

Approximately 24 hours after the SE induction phase, the EEG and behaviour appeared to be similar to pre-induction levels. The increased amygdala ADT, though, can be used as an indication that the brain was not back to normal. The amygdala ADT increase may be due to local disruption or damage within this nucleus, the cause of which could be stimulation-related and/or based on some reversible or irreversible changes to the network making it 'refractory' for recruiting an AD at that time. The elevation in amygdala ADTs seen in the SEP group, however, are permanent, while the elevations in the NSE group return to normal within about 7 days (Sato et al., 1990).

Amino Acids

The changes in amino acid levels from the baseline values on Day 1 to the baseline values on Day 2 were small and significant for GABA but not glutamate. More specifically, GABA levels showed a significant increase from Day 1 baseline on the kindled/stimulated side of the NSE group, while the changes were seen on the non-kindled/non-stimulated side of the SEP group (data not shown). These results might suggest that the elevated ADTs described above for the NSE group at this time were perhaps based on a higher than normal GABA concentration in the kindled amygdala. By contrast, the elevated ADTs of the kindled amygdala in the SEP group were more likely related to brain damage effects and not to extracellular GABA concentrations.

Interestingly, however, stimulation of the amygdala during the ADT testing was not without effect on amino acid levels. One minute after collecting the baseline samples, described earlier, the amygdala ADT test occurred. Even though no AD was triggered during these ADT tests, there was an obvious change in the levels of glutamate triggered in the contralateral amygdala, which involved a significantly greater increase from baseline in the SEP group compared to the NSE group. This result was unexpected. Presumably, the discrete stimulations in the kindled amygdala sufficiently activated the non-kindled amygdala via inter-amygdala connections, e.g., the amygdala commissure (Pitkanen et al., 1998). The kindled amygdala, on the other hand, was perhaps too injured itself to release glutamate with direct stimulation. In contrast, stimulation remote from the amygdala by way of the hippocampus increased the extrasynaptic glutamate concentration in both amygdalae, perhaps because of strong synaptic input to the two

amygdalae from the hippocampus, which were activated by the triggered hippocampal AD (see below). The fact that direct activation of the damaged amygdala did not trigger an AD suggests that the stimulation most probably caused a release of amygdala glutamate from glial origins. By contrast, there was no effect of direct amygdala stimulation on the extrasynaptic levels of GABA.

Ipsilateral Hippocampus ADT test

Behaviour and EEG

One hour later, when stimulating the ipsilateral hippocampus during its ADT test, a dramatic increase in AD duration was seen, which resulted in very increased seizure behaviour, including frank *convulsions*. This phenomenal result clearly showed that the excitability of the hippocampal and/or parahippocampal network was altered at this time. The defining feature of this unusual phenomenon is the high probability of triggering a *convulsive* seizure during hippocampal ADT test, a response that would never otherwise occur from this structure unless it had been directly kindled previously. Even the contralateral hippocampus showed increased electrographic seizure durations (but no convulsions; not shown). The main difference between the two sides of the hippocampus appears to be that the ipsilateral side more readily accesses the kindled circuitry, established by ipsilateral amygdala kindling, to trigger a convulsion. In contrast, the contralateral hippocampus is forced to use its ipsilateral connections to a non-kindled hemisphere, which resulted in increased local seizure activity but no convulsion (Kelly and McIntyre, 1996). Furthermore, this phenomenon may be isolated to the hippocampus

and its associated networks because ipsilateral thalamic stimulation (in misplaced hippocampal electrodes; not shown) did not trigger AD or convulsive seizures on the Day 2 ADT test.

This dramatic effect of hippocampal stimulation on Day 2 was most evident in the NSE rats. Little change in behavior was evident on the hippocampal ADT test from the SEP rats. These differences between the NSE and SEP groups are likely due to SE-related injury in the SEP group (Kelly and McIntyre, 1996) combined with altered amino acid release and reuptake mechanisms. For example, the neurocircuitry involved in spreading the seizure from the hippocampus to the motor system may be damaged in the SEP group, but not the NSE group. As a result, the full motor component of a strong seizure (i.e., a stage 5) would not emerge in the SEP rats. In addition, the increased neurological excitability can be correlated with an increase in the excitatory amino acid, glutamate (discussed below). Consequently, the differential neuropathology in the two groups may have altered the seizure circuitry and the amino acid release and reuptake mechanisms, which could interact to produce this phenomenon.

Amino Acids

Both GABA and glutamate levels were unchanged from the earlier amygdala ADT baseline. Consequently, these amino acid results prior to the hippocampal ADT test could not predict the subsequent occurrence of the hippocampal stage-5 convulsion in the NSE group.

In the collection period associated with that dramatic seizure response in the NSE group, a significant increase in extrasynaptic GABA and glutamate occurred primarily in the kindled amygdala, but not in the NSE group, only in the SEP group. Again, provoked activity in the amygdala, in this case triggered via the ipsilateral hippocampus, effected an amino acid release in the SEP rats that was not seen in the NSE group. The only other experiment of this kind was reported by Shin (2000), where the *identical* ipsilateral hippocampus ADT procedure was used in amygdala kindled rats of the Fast and Slow kindling strains. In both of those cases, large bilateral *decreases* in both glutamate and GABA were observed. Interestingly, those rats had not undergone the SE induction procedure, and can be considered to be amygdala kindled controls. Of course, Shin's rats did not show the hippocampal convulsive response that we described above. It appears from these two studies that normal activation of the amygdala from the nearby hippocampus results in a clearance of amino acids from the extracellular space (seen as decreased levels). However, if the tissue has been challenged previously by the SE induction procedure, the amino acid response will be either a slight increase or decrease in the NSE rats compared to a large increase in the damaged tissue of the SEP group.

The differences between the SE groups and the asymmetry between the two amygdalae might reflect altered GABA and glutamate release and reuptake efficiency, possibly due to pathology. In other words, the areas that had little SE-related pathology (i.e. both amygdalae in the NSE group) had more efficient amino release and reuptake mechanisms, while the areas that had SE-related pathology (i.e. both amygdalae in the SEP group, especially the kindled amygdala) had mechanisms that were seriously

affected. Thus, GABA levels decreased bilaterally in the NSE group, but increased in the SEP group, while glutamate levels increased more in the SEP group than the NSE group. Moreover, these differences were most notable in the SEP rats on the kindled side.

In contrast to the ipsilateral (and contralateral) hippocampus but similar to the amygdala, no AD occurred from stimulation to the ipsilateral thalamus (see Appendix 3). Even though this structure was stimulated at increasing intensities, there were no changes in extracellular GABA or glutamate provoked within either amygdala. Thus, discrete brain stimulations unrelated to the amygdala were not enough to induce amino acid release changes in either amygdalae. Only direct amygdala stimulation or propagating AD may be required.

Post-ADT

Behaviour, EEG and Amino Acids

Following the hippocampal ADT procedure, the behaviour of all rats was mostly normal. The EEG activity of the rats, though, appeared to be slightly lower in frequency than baseline, possibly because most were asleep.

For the first 10 minutes after the ADT procedures on Day 2, the extrasynaptic concentrations of GABA and glutamate returned to basal levels, where they remained 30 minutes later. Thus, the earlier convulsive seizure and/or AD caused only a transient, reversible change in extrasynaptic GABA and glutamate concentrations.

Pathology

The majority of the SE-related pathology revealed with cresyl violet and fluoro-jade was positively correlated with SE severity. This relationship was found in the amygdala, dorsal endopiriform nucleus, thalamus and piriform, perirhinal and entorhinal cortices. The same cannot be said, however, for the hippocampus and neocortex.

The pathology seen in the amygdala appeared to be correlated with SE severity at 24 hours after the SE induction stimulation, although most of the damage was the result of the cannulae, probes and the induction stimulus itself. As a result, the damage was most notable on the ipsilateral side, especially in the SEP group. Presumably, the physical integrity of the neural network in the kindled amygdala was so poor that when the probe was removed, many of the adjacent cells were extracted along with the probe. Apart from the probe-related damage, there was some amygdala pathology that was SE-related. For example, only the SEP group had gliosis in the medial nucleus. Then, as SE severity increased, gliosis became bilateral in the medial, lateral and BSTIA nuclei, and injured cells were seen bilaterally in the lateral, basal and amygdala-piriform transition nuclei (see Figures 19 and 20 for examples of fluoro-jade and cresyl violet, respectively).

As mentioned, other limbic system structures had pathology that was positively correlated with SE severity. The milder SEP subjects showed gliosis (see Figure 20) and F-J positive cells (see Figure 19) in the ipsilateral dorsal endopiriform nucleus. As severity increased, more of this nucleus showed gliosis and injured cells. Then, in the most severe SEP subjects, injured cells (but not gliosis) could be found bilaterally. The

perirhinal and entorhinal cortices showed the same pathological trend *only* with F-J positive cells (see Figure 23). In contrast, gliosis was only seen ipsilaterally within this cortical area in the most severe cases (see Figure 24). Another limbic cortical area, the piriform cortex, showed ipsilateral gliosis (see Figure 20) and injured cells (see Figure 20) ranging from moderate to extensive. Even parts of the limbic thalamus (dorsal medial nucleus, reuniens nucleus) had injured cells in the ipsilateral nuclei for the mild SEP subjects and bilaterally in the more severe SEP cases (see Figure 25).

Unlike the other limbic structures, the pathology in the hippocampus at the 24 hour window appeared to be negatively correlated with the SE severity. This was demonstrated in the findings that the most severe SEP subjects (ASE) had relatively mild ipsilateral damage, while more extensive ipsilateral and some bilateral pathology was found in the subject with milder SE (ISE) (see Figures 21 and 22 for fluoro-jade and cresyl violet examples, respectively). Important to note, the statement of 'negatively correlated' applies only to the results in this experiment. Other research indicates the SE types that are more severe than ASE, masticatory (MSE) and generalized (GSE), will show greater hippocampal damage than the mild SE form, ISE. MSE and GSE did not occur in this experiment. It was proposed by McIntyre et al. (in press) that hippocampal damage may be related to the intensity and length of time that the synaptic drive emanates from the temporal cortex. If the drive is sustained, then the hippocampus is damaged. If the drive is disrupted (e.g., by damage in the temporal cortex), the hippocampal damage is less. In the present cases, the damage to the temporal cortex (piriform area) was less in the ISE rat, who registered more cell injury in the

hippocampus than the ASE rats. By contrast, the latter rats showed much more piriform damage than the ISE rat, and much less hippocampal damage.

The final structure examined was the neocortex. In contrast to all the other structures showing damage, the cortical injury appeared to be independent of SE type.

Despite inserting a probe into each amygdala, the SE-related pathology seen in the amygdala, thalamus, endopiriform nucleus and cortices associated with different forms of SE was consistent with previous experiments (McIntyre et al., 1982; McIntyre et al., in press). The hippocampal pathology, however, was not. Using a silver staining method for identifying pathology, previous experiments showed that ISE produced no hippocampal damage (McIntyre et al., in press). In this experiment, hippocampal damage was seen in the ISE subject, and indicated a pattern that was more typical of the ASE rat. Although the ASE group in the present experiment had less hippocampal pathology than the ISE subject, its temporal lobe damage was more extensive and very typical of that condition. The reason for the difference in hippocampal damage is unknown, but the most likely explanation is related to the staining technique. Previous identification of damaged cells was made using the silver impregnation or argyrophilic technique of Nadler and Evenson (1983). We suspect that fluoro-jade may be a more sensitive technique for identifying injured cells than silver impregnation, and that the ISE injury can be reversible in the hippocampus. This latter statement is supported by the finding that an ISE subject perfused 8 days after the Induction had no F-J positive cells in these areas. Such injury also occurs in the hippocampus in the ASE rats, but it represents a

delayed cell death via apoptosis, which begins ~3 days after the SE experience (McIntyre et al., 1982). Our rats, of course, were killed for histology after only one day. All other aspects of the neuropathology in the ISE and ASE phenotypes, however, were identical to those previously reported (McIntyre et al., in press).

Appendix 1 – GABA TO GLUTAMATE RATIO

Apart from primary changes in inhibitory and excitatory amino acids, their relative concentrations appear to be different. In fact, a transient imbalance may be a major contributor to the spread of seizure activity seen in amygdala-kindled rats (Ueda & Tsuru, 1995). After time, though, the focus may develop a permanent imbalance with a greater amount of excitatory than inhibitory amino acids compared to a non-epileptic area (Labiner et al., 1999). As a result, the ratio of GABA to glutamate provides an indication as to the balance of these amino acids in a structure.

This ratio was determined by dividing the GABA concentration score by the glutamate score, ($\frac{GABA}{Glutamate}$), for all samples. Analysis of the GABA/glutamate ratio paralleled those done for the amino acids.

Analysis of the Day 1 Baseline ratio before SE induction revealed no significant differences between Side or between SE group in the ratio of GABA to glutamate (Table 18). Furthermore, the ratio showed no significant changes from baseline during the SE induction procedure nor after stimulus offset (data not shown).

Only 1 SEP subject had detectable GABA levels for the Pre-Amygdala ADT Baseline, thus, analysis focused on the NSE group. No differences were found between Side for the NSE group (Table 19). Clearly, these values do not differ from the Day 1

Table 18. Average ($\pm$ S.E.M.) Day 1 baseline GABA to Glutamate ratio (μ M) from the kindled and non-kindled hemispheres in the NSE and SEP groups.

SIDE	NSE; N = 11	SEP; N = 3
Kindled Side	3.6±1.0	2.6±2.0
Non-Kindled Side	3.6±0.9	3.7±1.7

Table 19. Average ($\pm$ S.E.M.) Pre-Amygdala ADT baseline GABA to Glutamate ratio (μ M) from the kindled and non-kindled hemispheres in the NSE and SEP groups.
* - only 1 SEP subject had detectable GABA levels

SIDE	NSE; N = 10	SEP; N = 1*
Kindled Side	3.8 ± 0.8	2.8
Non-Kindled Side	3.2 ± 0.6	4.4

Baseline ratios (Table 18). The subsequent tests also had no significant differences between group, time or side, where appropriate (i.e. Amygdala ADT, Pre-Amygdala to Pre-Hippocampus ADT Baseline, Pre-Hippocampus ADT Baseline, Hippocampus ADT, and post ADT samples) (data not shown).

Thus, the relative concentrations of GABA and glutamate, as revealed by their ratio, did not predict SE development. Furthermore, the presence or lack of SE didn't affect their relativity. Since there were significant changes in GABA and glutamate, the ratio results imply that these amino acids changed at the same rate.

Appendix 2 – Contralateral Hippocampus

Due to a misplaced ipsilateral hippocampus electrode, data was collected from the contralateral hippocampus for three NSE rats; the same analysis methods were used to find differences between hemispheres and, where appropriate, over time. The Pre-Kindling, Pre-SE Induction, and Day 2 ADTs and AD durations are provided in Table 20. Throughout this experiment, the ADT showed no changes just like the ipsilateral hippocampus. Further paralleling the ipsilateral hippocampus, the AD duration had a significant main effect of Time, $F(2,4) = 84.5$, $p < 0.002$. The effect was due to a significant increase in duration on Day 2 from pre-Kindling. In addition, 1 subject even had a stage 2 seizure. Consequently, even the contralateral hippocampus and/or parahippocampal circuitry can show increased excitability, but not to the same extent as the ipsilateral circuitry.

Tests revealed that an AD from the contralateral hippocampus resulted in no significant change in GABA release, glutamate release or in the ratio of GABA to glutamate within either amygdalae (data not shown).

Table 20. Average ($\pm$ S.E.M.) Initial, Pre-SE Induction and Day 2 contralateral hippocampus ADTs (μ A) and AD duration (sec) for the NSE group (N = 3).
* - the Day 2 AD duration was significantly longer than the Initial duration

TIME	ADT	AD DUR
Pre-Kindling	66.67±8.33	29.33±1.2
Pre-SE Induction	66.67±8.33	26.33±2.03
Day 2	75.00±14.43	46.67±1.76*

Appendix 3 – Ipsilateral Thalamus

Due to a misplaced ipsilateral hippocampus electrode, data was also collected from the ipsilateral thalamus. On Day 2, no subjects (NSE = 4; SEP = 1) had an ADT below the 400 μ A cutoff. Further testing showed that this day's ADT did not differ from Pre-Kindling and Day Before Induction (data not shown).

Only 2 NSE subjects had valid amino acid data, thus, no between groups analysis could be performed and the subsequent repeated measures tests were the same as the contralateral hippocampus. These tests showed that attempting to find the ipsilateral thalamic ADT resulted in no significant changes in GABA release, glutamate release or GABA to glutamate ratio within either amygdala (data not shown).

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