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**DIFFERENTIAL RELEASE OF AMINO ACIDS IN SEIZURE PRONE AND
RESISTANT RATS IN THE KINDLING MODEL OF EPILEPSY: A DUAL
PROBE MICRODIALYSIS STUDY**

by

RICK S. SHIN, B.Sc. Honours

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

Department of Psychology
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acceptance of the thesis.

**Differential Release of Amino Acids in Seizure Prone and Resistant Rats in the
Kindling Model of Epilepsy: A Dual Probe Microdialysis Study**

Submitted by

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in partial fulfillment of the requirements for the degree of
Master of Science

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ABSTRACT

A neurochemical basis for many of the epilepsies has long been suspected to result from an imbalance between excitatory and inhibitory neurotransmitter mechanisms. This imbalance likely also has a genetic predisposition. The initiation, maintenance and termination of seizure activity is largely modulated by the interaction of at least three amino acids, including GABA, glutamate, and aspartate, amongst other variables. Thus, it was important to ascertain the contribution of these three amino acids in seizure development using rats with a genetic predisposition for (Fast kindlers) or against (Slow kindlers) kindling epileptogenesis. First, using the kindling model of temporal lobe epilepsy, we replicated the electrophysiological and behavioural differences between these two rat strains. Second, using *in vivo* microdialysis, we assessed changes in amino acid levels from both amygdalae of the two strains before, during and after electrical stimulation of the amygdala (kindling). Our data revealed the expected strain differences in kindling rates, afterdischarge durations, and convulsion latency and durations. However, expected differences between the strains in GABA and glutamate levels were not seen, nor were there large increases in release associated with triggered seizures. Instead, amino acid levels tended to remain near baseline following focal seizure activity in the stimulated amygdala of the Slow rats and in both amygdalae of the Fast rats, while the contralateral amygdala showed a massive decrease in GABA in the Slow rats alone. By contrast, hippocampal stimulation caused a decrease in amino acid levels in both amygdalae of both hemispheres and in both strains. The implications of these results suggest that the physiological and biochemical mechanisms *for* and *against* epilepsy are

quite distinct. Understanding these mechanisms will ultimately be vital towards the treatment of epileptic patients.

**In loving memory of my grandmother
(1914-2000)**

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GENERAL INTRODUCTION

Epilepsy is a chronic brain disorder of various etiologies characterized by recurrent seizures as a result of excessive discharge of cerebral neurons (Sato et al., 1990). Since the prevalence rate of epileptics is one in one hundred of the population, it qualifies as the most common of the neurological diseases known to humans. As scientists, our obvious goal is not only to alleviate symptoms associated with the disease but to eradicate it completely. In order to achieve this feat, we must first understand what structures in the brain contribute to the initiation and propagation of seizure activity. The past half century has employed various physiological, pharmacological and in recent decades molecular methods in an attempt to unravel possible mechanisms that underlie epileptogenesis. Through the development of numerous experimental animal models as well as studies of patients undergoing surgical treatment, different types of epileptic seizures appear to arise from different underlying disturbances (Engel, 1990). Regardless of its origin, the consequence of recurrent seizures may result in massive convulsions accompanied by a loss of consciousness.

Although the types of epileptic seizures can be numerous, their classification is generally divided into two broad categories: idiopathic and symptomatic epilepsies. Idiopathic disorders are of genetic origin and usually are not associated with other neurological pathologies. Symptomatic disorders may arise from a pathological substrate that may be genetic, or acquired, such as traumatic scar (Engel, 1996). The two aforementioned types of epilepsies are further divided into partial and generalized seizures. Partial seizures originate in a restricted part of the cerebral cortex and therefore

are said to be localized. They usually do not involve an impairment of consciousness, but are often accompanied by staring and an arrest of motion, followed by altered responsiveness. When the electrical discharge produced by an epileptic event spreads to involve the limbic system, awareness and consciousness are often compromised. Over time the recruitment of other brain regions can evolve into secondarily generalized seizures. These generalized seizures produces increase muscle tonus followed by clonic motor manifestations, resulting in generalized tonic-clonic seizures. Such an event is referred to as a complex partial seizure.

Temporal Lobe Epilepsy

The various symptoms exhibited by patients correspond to the anatomical areas from which their seizures arise. Therefore, the initial onset of seizures can provide valuable information about the site of genesis. Induction of a local epileptic focus can be triggered in selective structures within the brain, and may preferentially propagate into the limbic system producing generalized convulsive seizures occasionally associated with widespread cellular damage. The severity of this pathology is dependent on the intimate connections between subcortical nuclei, such as the hippocampus and amygdala, which are believed to be key structures in temporal lobe seizure genesis and propagation. Indeed, seizure activity generated within the temporal lobe produces the most predominant and intractable type of epilepsy, temporal lobe epilepsy (TLE).

TLE is the most common of the epileptic syndromes and is characterized by complex partial seizures with ictal (rhythmic) and interictal discharges arising from limbic structures of the temporal lobe (Sutula, 1990). Our current understanding of the

mechanisms underlying epileptogenesis is largely based on information obtained from the hippocampus. Despite forty years of research, it still remains unclear as to which structures in the temporal lobe are responsible for the induction of the seizures and which are responsible for the arrest of seizures (Olivier, 1992). Earlier investigations have focused on the removal of mesial temporal lobe structures, including the hippocampus, as a means of eliminating recurrent seizures in human patients (Niemeyer, 1958). Since then, numerous surgical techniques have emerged to target other structures within the temporal lobe in an attempt to pin point a more precise location for resections. One of these techniques, called transventricular amygdalo-hippocampectomy, consisted of a selective removal of the hippocampus and the amygdala by a cortical incision through the second temporal gyrus (Olivier, 1991). Although this traditional technique appeared to serve as a temporary solution for alleviating symptoms, there are some associated limitations. Removal of the amygdala and/or hippocampus subsequently creates a widespread disconnectivity between the two structures possibly leading to cognitive, intellectual and some memory deficits (Olivier, 1983; Owen et al., 1996).

Recently, anterior temporal lobectomy has become an important option for treatment of TLE. This invasive method involves a more radical removal of the hippocampus and amygdala. Numerous studies have shown a high seizure free rate following this surgical protocol (Sperling et al., 1996; Eliashiv et al., 1997; Schuh et al., 1998). However, due to the extensive removal of subcortical tissue, other deficits associated with this procedure were apparent. For example, a decline in ability to encode new verbal information and other language based retrieval tasks have been reported (Helmstaedter and Elger, 1996; Bell and Davies, 1998). Although these surgical

techniques prove to be promising, the use of animal models and the need for refinement will continue to play dominant roles in our understanding of epileptogenesis.

Kindling and its Mechanisms

Kindling is a phenomenon whereby repeated administration of an initially subconvulsive electrical stimulation results in a progressive development of electrographic and convulsive motor seizures (Mohapel et al., 1996; Elmér et al., 1998). Since its discovery by Goddard et al. (1969), kindling has been studied extensively, and is now widely accepted as an experimental model for complex partial and TLE. Stimulation of limbic structures often elicits a focal electrographic discharge with no abnormal behavioural activity. Subsequent stimulations result in progressively increased seizure severity evolving through the following motor seizure classes as described by Racine (1972b); class (1) facial twitches, class (2) mastication with head nodding, class (3) forelimb clonus, class (4) rearing, class (5) rearing with loss of postural control. The number of stimulations required to kindle an animal depends greatly on the interval between successive stimuli. Kindling rates are optimized when a brief (1 or 2s) train of electrical pulses at 60Hz is applied once daily. The electrical stimulus must induce local afterdischarges (AD) in order for kindling to develop (Racine, 1972a; McNamara, 1984). An AD is a rhythmic paroximal electrographic discharge that outlast the stimulus by two or more seconds.

Two notable characteristics appear to develop as a result of kindling. The first is a reduction in the afterdischarge threshold (ADT) to elicit an AD. The ADT is defined as the minimum stimulus intensity to initiate an AD. Stimulation above or below the initial

ADT will gradually lower the ADT on subsequent sessions (Racine, 1972a). The second characteristic of kindling is the evolution and propagation of AD throughout the brain resulting in a progressive increase in amplitude and frequency of ADs (McIntyre and Racine, 1986). After an animal has been kindled, class 5 seizures can be consistently elicited even in the absence of a prolonged stimulation period (Lothman and Williamson, 1994). The induction of kindling by electrical stimulation may be accomplished in many structures in the brain and appears to be a permanent event. In an attempt to correlate the anatomical substrates to that of kindling, the rate at which a particular structure kindles or develops convulsive seizures can provide invaluable information to the development of generalized seizures. The amygdala is a well known fast kindling structure and requires relatively few stimulations to achieve the kindled state (McIntyre et al., 1993). This contrast with the hippocampus, which is considered to be a slow kindling structure. These anatomical differences between kindling rates may be due to 1) differential connections to the motor systems that drive the convulsive seizures, 2) differential connections to forebrain sites that may augment the seizure discharge or 3) differential reactivity of stimulation sites (Sato et al., 1990; Kulkarni and George, 1994).

The genesis of epileptic seizures is not an anatomically random event. Rather, the development and propagation of discharge activity follows selective routes that leads to generalized convulsions. In order to identify the pathways involved in this propagation, several anatomically linked structures must influence each other to facilitate this occurrence. The amygdala has been shown to be involved in a wide range of functions including emotion, memory and attention (Davis, 1992; Rogan and LeDoux, 1996). Damage to this structure appear to alter inhibitory circuitries that not only disrupt other

subdivisions within the amygdala, but more often corrupt the hippocampus and other brain structures (Swanson, 1995; Pitkanen et al., 1998). The inability of the amygdala and hippocampus to support clonic forelimb activity directly would suggest that seizures generated within these limbic structures must propagate, via intermediate structures, to sites that activate motor centres (Kelly and McIntyre, 1996). Following kindling, there is enhanced excitability of brain regions additional to the primary kindled focus.

Subsequent kindling of those secondary sites is greatly facilitated and is known as positive transfer (McIntyre and Goddard, 1973; Hiyoshi and Wada, 1992; Ferland and Applegate, 1999). It is a widespread phenomenon that occurs throughout the limbic system (Burnham, 1975). Recent evidence suggests that the efficacy of positive transfer greatly depends on the pathways and nuclei involved. Michael et al. (1998) had demonstrated amygdala kindling facilitated positive transfer to the perforant path, but no transfer was evident in the opposite direction, from perforant path to amygdala. This study suggest differences in primary and secondary site kindling are due to specific structures, and the extent of positive transfer must reflect differences in circuitry that trigger motor convulsions. Not only does the transfer phenomenon generally reduce the number of stimulations require to elicit an AD in the secondary site after kindling of the initial site, it appears to be long lasting in nature. Spiller and Racine (1994) reported a reduction in the kindling rate within the entorhinal cortex following a four week delay after primary kindling of the perforant path. These and other studies of positive transfer illustrate neural remodeling of secondary sites associated with primary site kindling. A relationship between chemical and electrical kindling has also been implicated to incorporate similar mechanisms to promote the positive transfer effect. Bidirectional

transhemispheric positive transfer has been demonstrated following administration of glutamate (Mori and Wada, 1989), picrotoxin (Cain, 1987), and NMDA (Croucher et al., 1995) in the amygdala. Clearly, the role of positive transfer suggests that seizures arising in two separate regions of the brain share common mechanisms involving transynaptic changes; these changes may underlie the kindling phenomenon.

Genetic Models of Epilepsy

A growing consensus over the past decade has implicated the importance of a genetic basis in several forms of epilepsy. The use of the kindling technique with various strains of experimental animals of diverse genetic background may provide insight into mechanistic differences in the development, propagation and maintenance of seizure activity. In order to study the pathological state of epilepsy, there is a need to develop an effective model to assess the genetic predisposition of epileptogenesis. In the mid-eighties, the development of the genetically epilepsy-prone rat uncovered new avenues of investigating inborn neurobiological abnormalities without the presence of a seizure provoking stimulus (Jobe and Dailey, 1991). Experiments using this model focused on acoustic stimulation in brainstem structures to elicit convulsions in an attempt to understand the neurochemical basis for brain stem seizure predisposition (Ribak et al., 1988; Mishra et al., 1989; Dailey et al., 1989). In recent years, a new approach to study complex partial epilepsy has been explored. Activation of forebrain seizures by daily kindling also influences brainstem circuitry and that genetic differences between seizure prone and seizure resistant strains may be important factors underlying human tonic-clonic seizures (Jobe et al., 1995; Coffey et al., 1996).

The progressive development of focal and secondarily generalized seizures with repeated electrical stimulation of limbic structures or kindling can be induced in a variety of structures and within various species. In the rat, differences in kindling rates between and within strains have been reported in the basolateral amygdala (Loscher et al., 1998). The genetic basis for differences in kindling characteristics was shown recently by Racine et al. (1999), where they develop two new strains of rats through selective breeding that were seizure prone versus seizure resistant based on amygdala kindling rates. Not only did the kindling rates differ between the two strains, but their local ADT and its duration varied between structures, suggesting that the mechanisms involved in seizure genesis within these two models are associated with a genetic disposition for developing or resisting epilepsy at several levels, including both local networks and distant recruitment mechanisms (McIntyre et al., 1999). These studies clearly suggest a genetic basis may underlie the development of epilepsy originating in temporal lobe structures.

Glutamate and Aspartate

It has been hypothesized that seizure generation is a result of an imbalance between excitatory and inhibitory mechanisms leading to either an increased glutamatergic excitation or reduced GABAergic inhibition or both. Although epilepsy is reasonably well characterized, the varied mechanisms responsible for seizure development and propagation are still being discovered. In the past few decades, the role that putative amino acid neurotransmitters play in the development and maintenance of epilepsy have been explored. Two such amino acid candidates are glutamate, which is the

principal excitatory neurotransmitter in the brain, and to a lesser extent, aspartate. The possible involvement of glutamate and aspartate as triggers in seizure initiation, and the involvement of glutamatergic neurotransmission systems in the spread of epileptic seizures, has been suspected for quite sometime. In this vein, different mechanisms have been implicated for the increase in glutamatergic excitation during epileptogenesis. For example, excessive presynaptic release leading to high extracellular glutamate concentrations and/or impaired neuronal or glial uptake are two possibilities (Ueda and Tsuru, 1994; Obrenovitch et al., 1996).

The excitatory glutamatergic system may be implicated in epilepsy at three different levels: it may provide 1) low level excitatory discharges in the epileptic focus, 2) amplified excitatory activity that leads to recruitment of other excitatory neurons connected to the focus, and 3) facilitate spread of hyperactivity leading to motor convulsions (Bradford, 1995). Numerous studies have shown elevated glutamate and aspartate release during and shortly following seizures in humans (Ronne-Engström et al., 1992; Wilson et al., 1996), and in rat (Carlson et al., 1992) and cat models (Nakase et al., 1991). Ueda and Tsuru (1995) have reported increases in extracellular glutamate when dialysates were taken from both ventral hippocampi in response to amygdala kindling. Glutamate levels in partially kindled (stage 3) and fully kindled rats were three and five times higher than baseline, respectively. However, evidence demonstrating whether glutamate release was causally related to seizure initiation, rather than being a result of neuronal hyperactivity, remains controversial; although the current literature suggests the former as the more likely candidate (Kaura et al., 1995).

In recent years, the importance of glutamate transporters has been suggested as one of the contributing factors for the increase in glutamate seen prior to and during an epileptic event. Since seizure activity is known to be associated with excessive release of glutamate, alterations in glutamate receptors and/or transporters may favour epileptogenesis or at least to augment its pathological cascade leading to neurotoxicity (Chapman, 1998). Such alterations may include a supersensitivity developed in post-synaptic excitatory receptors at critical points in the circuitry, or an increase in their population numbers (Bradford, 1995). Thus, the local reorganization of neuronal circuitry as a result of kindling may lead to changes in neurotransmitter receptor populations. Simultaneous reductions in the effectiveness of inhibitory receptors would also provoke further excitation and the spread of epileptic seizures.

GABA (Gamma aminobutyric acid)

The amplification and synchronization of neuronal firing associated with an increase in severity of seizure episodes is also likely to involve a disruption of GABAergic inhibitory mechanisms. Although much research has focused on the failure of GABA-mediated inhibition as the principal catalyst in epileptogenesis (Tuff et al., 1983; Roberts, 1984; Meldrum, 1989), the evidence in support of this suggestion is not without controversy. In addition, the augmented activity of excitatory neurotransmitters within epileptic foci may play a role in the breakdown of inhibition by a reduction of GABA release, desensitization of its receptors or a loss of inhibitory interneurons. All these possibilities have yet to be clearly elucidated.

The central role played by the neurotransmitter GABA in suppressing seizure activity has been extensively documented using pharmacological, anatomical, electrophysiological and biochemical approaches over a number of years (Chapman, 1985). Kindling of the hippocampus has revealed a slight increase in GABA levels during an epileptic event, which accompanied an increase turnover ratio of glutamate to GABA (Lothman et al., 1987). Using microdialysis, Minamoto et al. (1992) have reported a three to four-fold increase in GABA levels in the hippocampus during early amygdala kindling in the rat, which, in fully kindled rats, reached a seven-fold increase. The increase in GABA content in a kindled animal likely reflects its elevated metabolism during the induced hyperactive state (Kaura et al., 1995). In contrast, other investigators have indicated that the ratio of glutamate to GABA is significantly higher in fully kindled animals than in early kindled animals (Lothman et al., 1987; Buhl et al., 1996). Discrepancies between these studies may arise from different kindling methods and variations in microdialysis parameters.

It has long been hypothesized that prolonged reductions in GABA mediated inhibition may account for the facilitatory effect of epileptogenesis seen in the kindled state. In support of this claim, a reduction in GABAergic neurons or nerve terminals of kindled rat amygdalae was reported together with an associated equivalent loss of GABA content (Callahan et al., 1991). The loss of GABAergic neurons was not limited to the amygdala or hippocampus in response to amygdala kindling, but extended to include the piriform cortex, a structure highly susceptible to kindling and propagation of seizure activity (Lehmann et al., 1998). Thus, further evaluation of genetic based variations in both excitatory amino acid and GABA release could provide important

information on neurotransmitter changes associated with a genetic predisposition for seizure genesis in TLE.

Microdialysis

The introduction of microdialysis in the early 1970's has launched a new era for monitoring the release of dynamic substances in the brain. Since then, there has been an increased interest in specific neurotransmitter release in attempt to discover their physiological, pharmacological, and pathological roles. The concept of microdialysis resides in the principle of diffusion of an artificial perfusion medium through a semipermeable membrane into localized brain tissue, and simultaneous extraction of molecules of restricted size from the associated extracellular space, directly into collection tubes for offline analysis of substance content (Connelly, 1999). The microdialysis probes are constructed to collect substances normally released from axons onto dendrites and cell bodies in the targeted area (Connelly, 1999). The probes behave similar to blood vessels in that there is no net loss of fluid from the sample tissue of interest (Lunte and Lunte, 1996). Microdialysis has been proven to be an effective technique for sampling fluids in the extracellular spaces of different tissues of freely moving animals (Tossman and Ungerstedt, 1986; Quan and Blatteis, 1989). It is particularly useful for studying changes in neurochemical events before, during and after electrical stimulation of specific brain areas. The technique can also be used to monitor the levels of drugs and endogenous compounds in systemic fluids and tissues (Scott et al., 1991).

Although microdialysis presents enormous advantages, it does offer some limitations when used in dialyzing specific brain areas. One concern is the recovery rate of the dialysate, which greatly depends on several factors, such as flow rate, length and diameter of membrane and the nature of the substance being collected. Low flow rates provide highly concentrated samples, but require a longer period of time to generate adequate sample volumes, which may cause complications in differentiating separate chemical events (Renno et al., 1998). On the other hand, high flow rates increase the rate of diffusion at the risk of diluting the dialysate, which makes analysis more difficult. Many types of flow rates can be employed, however, 1-2 $\mu\text{L}/\text{min}$ appear to be the conventional rate for sampling amino acids (Dawson, 1997). Improved sampling has been refined over the past decade due to the development of new materials for dialysis membranes and commercial availability of smaller, more consistently fabricated probes (Connelly, 1999).

Microdialysis sample analysis has conventionally favoured the use of high-performance liquid chromatography (HPLC) with electrochemical detection (Abercrombie and Finlay, 1991; Smolders et al., 1995). Despite its ability to determine various neurotransmitters, it does present certain disadvantages, namely long separation times and a requirement for relatively large injection volumes, resulting in poor temporal resolution of the dialysate (Dawson, 1997). The large volume required for HPLC does not appear to be universal enough to allow a sensitive and specific categorization of important compounds within a single collection analysis (Bert et al., 1996). Because of this drawback, the determination of various amino acids and catecholamines in a given sample, demand collection times of 20-30 min at flow rates greater than 2 $\mu\text{L}/\text{min}$.

In recent years, capillary electrophoresis with laser-induced fluorescence detection has been explored as an alternative to conventional analytical techniques for the determination of amino acids in brain microdialysis samples (Lada et al., 1997). This technique offers high sensitivity, high separation efficiency and rapid separations for a large number of compounds, simultaneously allowing several analyses on a single microdialysis sample (Robert et al., 1996). Capillary electrophoresis is well suited for the analysis of small molecules such as amino acids, particularly that of glutamate and aspartate (Hernandez et al., 1993; Lunte and O'Shea, 1994; Dawson et al., 1997). Under physiological conditions, the release of excitatory amino acids is tightly modulated, therefore, high temporal resolution sampling is necessary to assess the regulation of these neurotransmitters. As a result of numerous experiences, brain regions will inevitably express changes in the chemical balance between both excitatory and inhibitory amino acids. Therefore, it is important to obtain high recovery rate without compromising temporal resolution, but concomitantly reduce sample volume extraction. With capillary electrophoresis, it is possible to achieve these goals, and in recent years this technique has proven to be an invaluable tool for studying the temporal release of neurotransmitters and to help clarify their roles in epileptogenic mechanisms.

Objectives and Outline of Proposed Study

As presented earlier, the release of excitatory and inhibitory neurotransmitters has long been suspected to play an integral role in the initiation and termination of epileptic seizures. Using fast and slow kindling rat strains, the genetic based differences in neurochemical mechanisms affecting limbic system excitability and their modification

during epileptogenesis now can be studied. The purpose of this experiment is to examine strain differences in excitatory and inhibitory amino acid release in Fast and Slow rats before and after amygdala kindling. To accomplish this goal, dual probe microdialysis extraction of brain dialysates was employed from the two amygdalae in response to both amygdala and hippocampal stimulation. Dialysates were analyzed using capillary electrophoresis with laser-induced fluorescence detection to quantify the release in both pre- and post-kindled conditions. The release of amino acids in the ipsilateral and contralateral amygdalae before kindling will provide clues regarding the interaction and propagation patterns of ADs triggered either from the stimulated amygdala or hippocampus, and how this release is altered in the fully kindled state.

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 was approved by the Carleton University Animal Care Committee.

Animals

Ten genetically kindle-prone (Fast) and ten kindle-resistant (Slow) male rats were used in this study. The rats were developed through selective breeding based on the rate of amygdala kindling (Racine et al, 1999) and were housed at Carleton University, Ottawa, Ontario, Canada. The animals were maintained individually with food and

water available ad libitum in a constant temperature (21°C) and humidity (45-50%) controlled room with a 12h on 12h off light cycle.

Surgical Techniques

Rats weighing 250-500g at the time of surgery were anaesthetized with sodium pentobarbital (60mg/kg, i.p.) and implanted bilaterally with bipolar Nickel-Chromium stimulating/recording electrodes of 127 μ m in diameter into the two dorsal hippocampi and the amygdalae. Implantations were performed using a Krieg stereotaxic with coordinates determined from the rat brain atlas of Pellegrino et al. (1979). At the same time as electrode insertion, two microdialysis dummy cannulae probes (24 gauge) were inserted bilaterally into the 2 amygdalae. The following stereotaxic coordinates relative to bregma will be used: A -0.2; L $\pm$ 4.5; V 6.0 mm for the dummy probes; A -3.5; L $\pm$ 4.5; V 8.6 mm, angled caudo-rostrally at 20° for the amygdala electrodes; A -3.0; L $\pm$ 3.5; V 3.0 mm for the dorsal hippocampal electrodes. All measurements were made from the dural surface. All electrodes were connected to a 9 pin hole McIntyre head plug, and together with the dummy probes, were anchored to the skull by jeweler's screws and dental cement. The animals were allowed 7 days of postsurgical recovery before the start of experimentation.

Electrophysiological Measurement and Brain Microdialysis

Pre-Kindling

Eight days after surgery, each rat was allowed to habituate in a test chamber (40x40x29cm) over a 15 minute interval for 4 consecutive days. No electrical stimulation was delivered during this experience. The day after the habituation experience, the animal was briefly anaesthetized with halothane and the dummy probes removed. In their place, microdialysis probes were positioned, which extended ventrally to 1.5 mm below the cannulae. The dialysis probes were connected by polyethylene tubing (PE20) to a dual channel swivel (Instech Laboratories, Pennsylvania) and continuously perfused with Ringer solution (120 mM NaCl, 3 mM KCl, 1.2 mM CaCl₂, 150mM MgCl₂; pH 7.2) using a Harvard infusion pump. Collection of samples was made possible with an active membrane length of 1.5mm with a molecular mass cutoff 6000. Dialysates were extracted into collection vials through fused-silica tubing (75 μ m I.D. $\times$ 150 μ m O.D., Polymicro Technologies, Arizona). The animals were conscious and freely moving for both dialysis and EEG measurements. All dialysate collection was done at a rate of 1 μ L/min. All dialysates were frozen immediately in dry ice, and kept at -80°C until capillary electrophoresis analysis.

The time-line of various measurements was presented in Figure 1, and described with their rationale below. Upon insertion of the microdialysis probes, 6x10 min baseline dialysates were first taken during a 60 min stabilization period. A 2 min sample from the last 10 min of the 60 min stabilization period was used as a baseline.

Following this period, a subthreshold stimulation for AD was presented to the right amygdala. The electrical stimulations (60 Hz sine wave, 2s duration) were

Figure 1. Microdialysates in both amygdalae are collected for various time (minutes) intervals. Two minute dialysate time windows chosen for analysis in response to amygdala (A1-A5) and hippocampal stimulation (H1-H5) are shown (circled). Same dialysates were taken after kindling.

Microdialysis Time Line

delivered every two minutes to the right amygdala until an AD was elicited. The ADT was recorded. Stimulation intensities were delivered in the ascending order of 15, 25, 35, 50, 75, 100, 150, 200 μ A. Two subthreshold, one AD, and one post 10 min AD samples were taken (once again in 2 minute bins). When the AD was triggered, the duration of electrographic discharge was measured and the behavioural activity characterized by Racine's scale (Racine, 1972b): stage 0, immobility; stage 1, mouth and facial twitches; stage 2, mastication and head nodding; stage 3, forelimb clonus; stage 4, rearing with forelimb clonus; stage 5, rearing and falling on side or back with forelimb clonus. One hour following the initial amygdala ADT assessment, the right dorsal hippocampus was subjected to the same ADT assessment procedure already described for the amygdala. Dialysates from the amygdala were recorded as above.

The important points addressed by the amygdala and hippocampal ADT procedures are related to the consequences each manipulation has for the network. The direct provocation of the amygdala by the ADT stimulus usually results in a local AD that does not propagate from the stimulated amygdala (and associated dialysis probe) either to the contralateral amygdala (where the second probe is collecting dialysates) or the hippocampi. By contrast, the AD triggered from the hippocampus will propagate to both amygdalae (and probes) with equal facility. Thus, we can assess the release of amino acids that are associated with direct local AD or transynaptic propagated AD by these 2 manipulations.

Kindling and Post-Kindling

Following the initial AD tests of the amygdala and hippocampus, the animals were kindled once daily in the right amygdala using the ADT intensity determined the previous day. This procedure continued daily until a stage-5 seizure was produced. Then, after 5 stage-5 seizures, the ADTs in both structures were redetermined. The next day, the microdialysis probes were reimplanted and samples collected using exactly the same procedure as during pre-kindling measurements, including ADT measurements of the amygdala and hippocampus. The probes were then removed. Finally, the ADTs were redetermined once again one day after probe removal. These manipulations were necessary to assess whether reinsertion or removal of the probes change the neural network activity, and thus, potentially alter the stimulation threshold required to elicit an AD.

Histology

After removal of the dialysis probes and determination of final thresholds, the animals were sacrificed and perfused intracardially with saline followed by 4% paraformaldehyde. The brains were removed at least 6 hours after perfusion and placed in post-fixative for a minimum of 3 days followed by a 30% sucrose solution for another 3 days prior to sectioning. Frozen coronal sections were taken at 40 μm and stained with Cresyl violet to verify probe and electrode placements.

Amino Acid Analysis

Detection and quantitation of amino acids were carried out by capillary

electrophoresis with laser-induced fluorescence detection (Beckman Instruments, P/ACE system 5010, Mississauga, Ontario). Excitation was performed by a Argon Ion laser at a wavelength of 488nm. Separations were carried out with fused-silica capillary (Polymicro Technology, Phoenix, Arizona) of 50 μ m inner diameter and 375 μ m outer diameter having a total length of 48.5cm. The capillary was sequentially flushed with 0.1M sodium hydroxide prior to use. Hydrodynamic injections were made by nitrogen at 80psi for 3 seconds.

Capillary electrophoresis was carried out with 25mM sodium carbonate buffer (pH 9.4) and a 25kV voltage was applied with a 25-30mA current.

Data Analysis

The data were analyzed parametrically using Analysis of Variance (ANOVA) with the STATISTICA program, and when appropriate, post-hoc group comparisons were made with Scheffe's test. The dependent measures associated with seizures are: 1) the initial AD threshold and duration for both the amygdalae and hippocampi; 2) the kindling rate, defined as the number of electrical stimulations required to elicit the first stage 5 convulsion; 3) the profile of the six stage 5 convulsions, including the convulsion latencies (amount of time from stimulus onset to forelimb clonus) and duration of convulsions; 4) the total electrographic AD duration; 5) AD threshold and duration for both amygdalae and hippocampi before and during the second dialysis sampling following kindling; 6) AD threshold and duration for both amygdalae and hippocampi one day following second dialysis sampling.

Data analyses for the amino acids are described in the results section.

RESULTS

Histological evaluation confirmed stimulating/recording electrode placements in the stimulated amygdala and hippocampus (Figures 2 and 3). The contralateral electrodes are not shown.

Kindling Analysis

A 2x2 repeated measures design was used to assess group differences (Fast vs. Slow strains is the between factor) in AD thresholds and their associated electrographic durations acquired from the amygdala and hippocampus (within factor), tested at 2 time intervals: before kindling (T1), and on the 6th stage-5 seizure (after kindling) (T2). Similarly, a 2x3 repeated measures design was used to analyze group (strain) differences at 3 time intervals following kindling: T2; on the day of reimplantation of microdialysis probes (T3), and 1 day following reimplantation (T4).

Amygdala AD Thresholds and Durations

Analysis of amygdala AD thresholds before and after kindling revealed no significant group differences or interactions between Fast and Slow strains (Table 1) either before or after kindling. Thus, reimplantation of the probes after kindling did not affect the AD thresholds or thresholds assessed the following day. (Table 1).

Although differences in amygdala AD thresholds between the Fast and Slow strains were not significant, large strain differences in AD duration were observed

Figure 2. Schematic diagram taken from Paxinos and Watson (1986) showing the stimulating electrode tip locations in the amygdala and hippocampus in Fast (filled symbols) and Slow (unfilled symbols) rats at bregma -3.6mm . Non stimulating electrodes are not shown.

Figure 3. Schematic diagram taken from Paxinos and Watson (1986) showing the stimulating electrode tip locations in the amygdala and hippocampus in Fast (filled symbols) and Slow (unfilled symbols) rats at bregma -4.3mm . Non stimulating electrodes are not shown.

Bregma -4.30 mm

Table 1. Mean ($\pm$ S.E.M.) amygdala AD thresholds (μ A) in Fast and Slow rats across 4 separate time intervals.

GROUP	T1	T2	T3	T4
Fast	36.8 ± 5.0	34.5 ± 4.7	38.6 ± 4.5	44.5 ± 6.3
Slow	40.0 ± 4.4	58.8 ± 14.1	65.0 ± 13.1	59.6 ± 13.1

Figure 4. Mean ($\pm$ S.E.M.) amygdala AD durations between Fast and Slow rats across 4 separate time trials. Each time point is different at the $p<0.05$ level.

before and after kindling ($F_{1,21} = 33.41$, $p < 0.00001$) (Figure 4). Not only were there differences among the 2 strains, a significant main effect of time trial ($F_{1,21} = 110.04$, $p < 0.000001$), and group x time trial interaction were also found ($F_{1,21} = 11.05$, $p < 0.004$). Analysis of the 3 time trials after kindling revealed a significant main effect of group ($F_{1,21} = 36.76$, $p < 0.000005$), where the Fast strain displayed longer AD durations over the course of kindling. There was no main effect of time, nor was there a significant group x time interaction. Furthermore, as shown in Figure 4, the AD duration after kindling was not significantly affected by reimplantation of microdialysis probes in either strain.

Kindling Rate and Convulsion Measures

All animals were kindled by a daily 2 second, 60Hz sine wave, until 6 stage-5 seizures had been elicited. If an AD was not evoked by a particular stimulus, the intensity was increased by one increment one minute later until an AD was triggered. The kindling rate was the number of stimulations required to elicit the first stage-5 seizure. As expected, the Fast rats exhibited fewer stimulations to achieve a generalized convulsion than the Slow rats ($F_{1,21} = 31.93$, $p < 0.00002$) (Figure 5). Clearly, differences in kindling rates among the 2 strains represent distinct profiles of recruitment and propagation patterns of seizure activity.

Throughout the kindling process, 3 measures of convulsion performance were recorded: latency, the interval of time from stimulation stimulus onset to forelimb clonus; duration of convulsion, i.e., the length of clonic activity; and, electrographic AD duration (from stimulus onset to the end of electrographic discharge). A 2x6

Figure 5. Mean ($\pm$ S.E.M.) amygdala kindling rate between Fast and Slow rats.
* Significantly different from the Fast rats, $p < 0.05$.

repeated measures design was used to analyze these 3 sets of data. The 2 strains were tested on 6 kindling trials, representing successive stage-5 seizures over a period of 6 days.

First, analysis of latency revealed no significant differences among the Fast and Slow strains, but a main effect of time was evident ($F_{5,105} = 6.94$, $p < 0.00002$). In both strains, significantly longer latencies were apparent in the first and second stage-5 seizures, after which there was a gradual shortening of latency from stimulus onset to a generalized convulsion (Figure 6). There was no significant group by time interaction.

Second, assessment of convulsion duration revealed a significant difference between the strains ($F_{1,21} = 27.08$, $p < 0.00004$) (Figure 7). Multiple comparisons showed that the Fast rats displayed significantly longer convulsions at the second, third, and fifth stage-5 seizure compared to the Slow strain. There was no main effect of time and no significant group x time interaction.

Third, analysis of the electrographic AD duration during the 6 stage-5 seizures showed a significant main effect of group ($F_{1,21} = 19.23$, $p < 0.0003$) (Figure 8). Further assessment indicated there were differences between Fast and Slow strains on all trials except on the first stage-5 seizure. No significant main effect of trials or groups x trials interaction were found.

Hippocampal AD Thresholds and Durations

One hour following the initial determination of each amygdala AD threshold, assessment of the hippocampal AD threshold occurred, and followed the same

Figure 6. Mean ($\pm$ S.E.M.) latency across 6 stage-5 seizures during amygdala kindling in Fast and Slow rats.

Figure 7. Mean ($\pm$ S.E.M.) AD convulsion duration across 6 stage-5 seizures in Fast and Slow rats.

Figure 8. Mean ($\pm$ S.E.M.) electrographic AD convulsion duration across 6 stage-5 seizures in Fast and Slow rats.

protocol. Analysis of the hippocampal AD thresholds before and after kindling revealed no significant group differences among the Fast and Slow strains. However, a main effect of time was evident ($F_{1,21} = 8.64$, $p < 0.008$). As seen in Figure 9, only the Fast strain exhibited a significantly higher AD threshold after kindling compared to before kindling ($F_{1,10} = 17.8$, $p < 0.002$). In addition, there was a significant group x time trial interaction ($F_{1,21} = 6.83$, $p < 0.02$). Analysis of the 3 time intervals after kindling showed no significant group differences between the 2 strains. There was, however, a main effect of time ($F_{2,42} = 5.64$, $p < 0.007$), but no significant group x time interaction was seen. These results imply that kindling of the amygdala substantially increases the AD threshold in the hippocampus, but only in the Fast rats. However, reimplantation of the probes resulted in lower AD thresholds in the Fast strain, while a slight increase was seen in the Slow strain. Furthermore, one day following reimplantation, hippocampal AD thresholds rose in both strains. The results suggests that after amygdala kindling reinserting the probes increases excitability in the hippocampus of the Fast rats, but this effect is time labile.

Assessment of hippocampal AD durations before and after kindling demonstrated no significant group differences, or no group x time interaction. Further, analysis of the three time trials after kindling revealed no significant main effect of group, even though upon reimplantation of the probes the Fast strain showed longer AD durations than the Slow strain ($F_{1,21} = 5.76$, $p < 0.03$) (Figure 10). In addition, a significant main effect of time was evident ($F_{2,42} = 5.52$, $p < 0.008$). In the Fast strain, post hoc analysis revealed a significant increase in AD duration when the probes were reimplanted compared to the day before ($p < 0.05$), and the day after ($p < 0.03$). Further, a significant group x time

Figure 9. Mean ($\pm$ S.E.M.) hippocampal AD threshold (μ A) in Fast and Slow rats across 4 time intervals.

Figure 10. Mean ($\pm$ S.E.M.) hippocampal AD duration in Fast and Slow rats across 4 separate time intervals.

interaction was assessed ($F_{2,42} = 3.92$, $p < 0.03$). These results again suggest that probe re-entry into both amygdalae promotes excitability in the hippocampal network of Fast rats, an effect that is reversible over days.

AMINO ACIDS

Histological evaluation of microdialysis probes confirmed bilateral placements in the central and basolateral nuclei of the amygdalae (Figures 11, 12). Rats with probes completely outside of the amygdala area, e.g. ventral striatum, were eliminated from the amino acid analyses ($n = 2$ rats).

Analysis of Amino Acids

The analyses involving one inhibitory amino acid (GABA), and two excitatory amino acids (glutamate, aspartate) were divided into 5 sections. The first section presents average 'raw' values of the amino acids in μM concentrations. These values form the baseline scores that are used in the subsequent sections to derive '*percent of baseline*' values, which constitute all data sets for subsequent analyses. This basic procedure is consistent with other studies employing microdialysis, and its need is derived from the fact that there is very high variability between rats within a group in the concentrations of amino acids collected from their individual, unique probes and unique CNS placements; the percent of baseline values normalize the data.

In section 2, 4 dialysate samples, collected during and following amygdala stimulation, were compared to the baseline sample taken prior to stimulation. Similarly, one hour following amygdala stimulation, amygdala dialysate samples were again

Figure 11. Schematic diagram taken from Paxinos and Watson (1986) showing microdialysis probe locations in both hemispheres of the amygdala of the Slow rats at bregma -2.8mm . Non-stimulating electrodes are not shown.

Figure 12. Schematic diagram taken from Paxinos and Watson (1986) showing microdialysis probe locations in both hemispheres of the amygdala of the Fast rats at bregma -2.8mm . Non-stimulating electrodes are not shown.

collected, but during hippocampal stimulation; the 4 samples collected at this time were compared to the baseline sample taken immediately prior to hippocampal stimulation. In section 3, the same analyses as above are presented, but immediately *after* amygdala kindling has been completed. In section 4, the impact of kindling *per se* on amino levels in each strain is assessed. Finally, in section 5, the rats were segregated into those that did versus did not experience a generalized convulsion during the final assessment after kindling to determine the impact of (or predisposition to) a convulsive seizure on amino acid levels. In each section, amino acid results are presented in the same order, i.e., GABA, glutamate and aspartate, respectively.

Section 1: Baseline Amino Acid Values

A 3-way ANOVA was used to assess the baseline values of GABA, glutamate, and aspartate in both the stimulated and non-stimulated hemispheres of Fast and Slow rats. The baseline dialysates were collected from both the ipsilateral and contralateral amygdalae prior to amygdala stimulation and then prior to hippocampal stimulation. These measurements were taken both before and after amygdala kindling.

Baseline GABA Levels

Although there were no significant differences in baseline extrasynaptic GABA levels between the two strains or the two hemispheres either before or after amygdala kindling, there was a tendency for the Fast rats to show reduced GABA levels one hour

Figure 13. Average ($\pm$ S.E.M.) baseline GABA concentrations from both the stimulated and non-stimulated hemisphere in the Slow and Fast rats before stimulation of the amygdala and hippocampus.

Figure 14. Average ($\pm$ S.E.M.) baseline glutamate concentrations from both the stimulated and non-stimulated hemisphere in the Slow and Fast rats before stimulation of the amygdala and hippocampus.

Figure 15. Average ($\pm$ S.E.M.) baseline aspartate concentrations from both the stimulated and non-stimulated hemisphere in the Slow and Fast rats before stimulation of the amygdala and hippocampus.

after amygdala stimulation, at the onset of the hippocampal stimulation trials. This tendency was more apparent after rather than before amygdala kindling (see Figure 13). This result suggests that in the Fast rats GABA levels after amygdala stimulation do not readily recover to their original baseline concentrations.

Baseline Glutamate and Aspartate Levels

By contrast, baseline levels of glutamate showed no differences associated with strain, hemisphere or state of kindling (Figure 14). Levels of aspartate, however, presented a slightly different profile. Like GABA levels in Fast rats, levels of aspartate showed a small, non-significant reduction one hour after amygdala stimulation in both strains, both before as well as after amygdala kindling (Figure 15). Thus, although glutamate levels recover to baseline values after amygdala stimulation in both strains, aspartate levels remained slightly depressed.

Section 2: Changes in Amino Acid Concentrations During Electrical Stimulation

Before Amygdala Kindling

To assess changes in amino acid levels during stimulation delivery to the amygdala (and then, 60 min later, in the ipsilateral hippocampus), a 3-way ANOVA was used to assess strain, hemisphere and stimulation trials. Four dialysate collection samples (2 subthreshold, one AD, one post AD) and a baseline sample were included in most analyses. The results were divided into 2 separate comparisons. The first comparison was between the 2 subthreshold samples and the AD sample (thus 3 stimulation trials).

The second comparison was between baseline sample and the post AD sample (10 min post stimulation trial). These comparisons were incorporated into a 2-way ANOVA to further assess differences *within* each strain.

GABA: Amygdala Stimulation – Before Kindling

First we questioned the percent change in GABA levels from baseline in the 2 amygdalae resulting from the 3 brain stimulations to one amygdala, the last of which ultimately produced an AD, and then 10 min later (post-AD sample). This question and procedure was repeated one hour later, stimulating the ipsilateral hippocampus. The results are presented in Figure 16. First, the 2 sub-AD threshold and one supra-AD threshold trials were analyzed to examine changes in amino acid levels immediately prior to and during the elicitation of an AD. The analysis revealed a significant main effect of hemisphere ($F_{1,19} = 5.62$, $p < 0.03$) and a significant strain x hemisphere interaction ($F_{1,19} = 8.34$, $p < 0.01$). In further analyses, a 2-way ANOVA of hemisphere by trials was used to examine the profile of amino acid release within each strain. It was clear that amygdala stimulation in the Slow rats was associated with a dramatic decrease in GABA levels from baseline in the non-stimulated hemisphere, but little or no change in the stimulated hemisphere (Figure 16, top left). After inspection of the figure, it was not surprising that the analysis showed a highly significant main effect of hemisphere ($F_{1,10} = 77.2$, $p < 0.00001$). By contrast, in the Fast rats, there were no significant changes from baseline in GABA levels resulting from amygdala AD threshold determinations (Figure 16, bottom left).

Figure 16. Changes in GABA levels of the Slow (top) and Fast (bottom) rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in both hemispheres before kindling.

GABA – Before Kindling

Slow Rats

Amygdala

Hippocampus

Fast Rats

Amygdala

Hippocampus

One hour rest

GABA: Amygdala Post Stimulation – Before Kindling

Analysis of the sample taken 10 min after the amygdala AD (post AD trial) compared to the baseline sample revealed a significant main effect of strain ($F_{1,20} = 4.73$, $p < 0.05$) and trial ($F_{1,20} = 4.91$, $p < 0.04$). In addition, significant 2-way interactions between strain and hemisphere ($F_{1,20} = 6.58$, $p < 0.02$) and strain and trial ($F_{1,20} = 4.73$, $p < 0.05$) were found. The 3-way interaction was also significant ($F_{1,20} = 6.58$, $p < 0.02$). All of these effects were based on the Slow rats' behavior, as the 2-way ANOVA in Slow rats showed highly significant main effects of hemisphere ($F_{1,10} = 46.0$, $p < 0.00005$), trial ($F_{1,10} = 21.9$, $p < 0.0009$) and hemisphere x trial interaction ($F_{1,10} = 46.0$, $p < 0.00005$), but no effects in the Fast rats; the post AD trial of the Fast rats also showed GABA levels that were similar to their baseline.

GABA: Hippocampal Stimulation and Post AD – Before Kindling

Sixty minutes after amygdala stimulation, determination of new amino acid release baseline levels in each amygdala was made. This was followed immediately by hippocampal AD threshold stimulation, exactly as was done previously using the ipsilateral amygdala.

Again, as brain stimulation was applied, a dramatic decrease from baseline was apparent in GABA levels in both strains (see Figure 16, top and bottom right). Interestingly, the decrease was greater in the Slow rats than in the Fast rats, but there was no difference between the hemispheres in either strain. This outcome is quite unlike the results from these same Slow rats during amygdala stimulation. The GABA release

during the 3 stimulation trials and the post-AD trial were statistically similar in both strains.

Collectively these results suggest that direct electrical stimulation of the amygdala near the dialysis probe results in release of extrasynaptic GABA that is largely unchanged from baseline in both Fast and Slow rats. In the Fast rats, the same response is also seen in the non-stimulated amygdala, while a dramatic decline in GABA occurs in the Slow rats. A similar decline was observed in both amygdalae in both strains following hippocampal stimulation. The results suggest that the 'greater' or more direct the activation of the amygdala by the electrical stimulus, the greater the extrasynaptic GABA release.

Glutamate: Amygdala Stimulation and Post AD – Before Kindling

Applying these exact comparisons to the samples for glutamate levels revealed similar but smaller effects to those reported above for GABA. As can be seen in Figure 17 (top and bottom left), there was a significant main effect of trials ($F_{2,38} = 5.96$, $p < 0.006$), where the levels of glutamate decreased from baseline following amygdala stimulation. However, there was no observed interaction of trials with strain or hemisphere. Further, the post AD glutamate level remained unchanged from the previous AD trial - a result similar to that seen with GABA.

Glutamate: Hippocampal Stimulation and Post AD – Before Kindling

As can be seen in Figure 17 (top and bottom right), a slight gradual decline of glutamate levels from the amygdalae of both strains was apparent with repeated

Figure 17. Changes in glutamate levels of the Slow (top) and Fast (bottom) rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in both hemispheres before kindling.

Glutamate – Before Kindling

Slow Rats

Amygdala

Hippocampus

Fast Rats

Amygdala

Hippocampus

One hour rest

stimulations to the hippocampus, resulting in a significant main effect of trials ($F_{2,22} = 4.22$, $p < 0.03$). No significant trials with strain or hemisphere interactions were evident. The pattern of glutamate release in the stimulation trials was similar to that of GABA. As in the amygdala stimulation trials, the post AD glutamate levels were identical to the previous AD trial.

Aspartate: Amygdala Stimulation and Post AD – Before Kindling

Upon stimulation of the amygdala, aspartate levels were observed to follow the same decline in both hemispheres as with the previous 2 amino acids, although the loss was much more subtle. As can be seen in Figure 18 (top and bottom left), the analysis revealed no significant interactions of strains with hemispheres or trials.

Analysis of the post AD trial did not significantly differ from the previous AD trial suggesting that aspartate levels after stimulation did not fully recovered to baseline.

Aspartate: Hippocampal Stimulation and Post AD – Before Kindling

As indicated in Figure 18 (top and bottom right), after the large drop in aspartate levels associated with the first AD, there were clearly no further effects among the stimulation trials. Again, similar to GABA and glutamate, comparable reductions of aspartate were observed in both strains.

Lastly, the aspartate levels during the post AD trial did not significantly differ from either the previous AD trial or the other subthreshold AD trials.

Figure 18. Changes in aspartate levels of the Slow (top) and Fast (bottom) rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in both hemispheres before kindling.

Aspartate – Before Kindling

Slow Rats

Amygdala

Hippocampus

Fast Rats

Amygdala

Hippocampus

One hour rest

Section 3: Changes in Amino Acid Concentrations During Electrical Stimulation

After Amygdala Kindling

After the animals were fully kindled, microdialysis probes were reimplanted into the two amygdalae. Dialysis samples were taken in the same manner as before kindling, and the procedure and data analyses performed to assess between group and within group differences were identical.

GABA: Amygdala Stimulation and Post AD – After Kindling

Analysis of the 3 amygdala stimulation trials revealed a significant strain x hemisphere interaction ($F_{1,19} = 4.65$, $p < 0.05$). As can be seen in Figure 19 (top and bottom left), stimulation of the amygdala in the Slow rats was associated with a higher level of GABA release from the stimulated amygdala than the contralateral amygdala, while the opposite effect occurred in the Fast rats.

Assessment GABA levels during the post AD trial compared to the baseline levels showed a significant main effect of trial ($F_{1,20} = 10.9$, $p < 0.004$), where the average levels of GABA decreased over time. However, as seen in Figure 19 (top and bottom left), after the elicitation of an AD, GABA levels continued to rise slightly in the stimulated hemisphere of the Slow rats, while the much lower levels of GABA in the Fast rats remained unchanged. By contrast, both strains showed a decline in GABA levels in the non-stimulated hemisphere following an AD in the amygdala, which obviously contributed to the overall significant main effect of trials described above. Multiple

Figure 19. Changes in GABA levels of the Slow (top) and Fast (bottom) rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in both hemispheres after kindling.

GABA – After Kindling

Slow Rats

Amygdala

Hippocampus

Fast Rats

Amygdala

Hippocampus

One hour rest

comparisons of the post AD trial between hemispheres revealed a significant hemisphere $\times$ trial interaction ($F_{1,10} = 5.80$, $p < 0.04$) only in the Slow rats.

GABA: Hippocampal Stimulation and Post AD – After Kindling

As a result of stimulation of the hippocampus after amygdala kindling, a dramatic decline in GABA levels was observed in the two strains in both hemispheres (Figure 19, top and bottom right); there were no significant differences between strains or hemispheres. This large decrease in GABA during stimulation trials was virtually identical to that which was seen before kindling. Similarly, GABA levels during the post AD trial remained low and unchanged from the previous stimulation trials.

Glutamate: Amygdala Stimulation and Post AD – After Kindling

Analysis of glutamate levels in response to amygdala stimulation after amygdala kindling revealed no significant main effects or interactions. As can be seen in Figure 20 (top and bottom left), both strains showed a small decrease in glutamate levels at the onset of stimulation of the amygdala, after which the levels remained largely unchanged.

In comparison to baseline levels, the post AD trial showed a significant main effect of trial ($F_{1,21} = 30.0$, $p < 0.00002$). Interestingly, the profile of the post AD trial in the two strains was almost identical to that of GABA. The glutamate levels in the two hemispheres of the Slow rats diverge from each other in that the stimulated side and recovered towards baseline while the non-stimulated side dropped (Figure 20, top left). However, the post AD trial did not differ significantly from the previous AD trial in either strain.

Figure 20. Changes in glutamate levels of the Slow (top) and Fast (bottom) rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in both hemispheres after kindling.

Glutamate – After Kindling

Slow Rats

Amygdala

Hippocampus

Fast Rats

Amygdala

Hippocampus

One hour rest

Glutamate: Hippocampal Stimulation and Post AD – After Kindling

Once again, a large decline of glutamate levels was observed in response to hippocampal stimulation, similar to that seen with GABA. No main effects or interactions were found, although the loss of glutamate was somewhat greater in the Fast strain (Figure 20, bottom right). Interestingly, it should be remembered that this loss in the Fast rats was against a baseline (at the beginning of the hippocampal stimulation trials) that was already depressed from the original baseline recorded before the amygdala stimulation trials.

Glutamate levels in the post AD trial did not change from the stimulation trials.

Aspartate: Amygdala Stimulation and Post AD – After Kindling

No significant main effects or interactions were present across the amygdala stimulation trials. An apparent general decline in aspartate levels in both hemispheres of the Fast and Slow rats was noted (Figure 21, top and bottom left). However, due to the large variability associated with these data, any consistent pattern in aspartate release during amygdala stimulation was not observed.

A significant decline from baseline was seen in the post AD trial ($F_{1,9} = 12.8$, $p < 0.006$), but there was no interaction with strain or hemisphere. Aspartate levels in the post AD trial did not differ significantly from those of the previous stimulation trials.

Aspartate: Hippocampal Stimulation and Post AD – After Kindling

During hippocampal stimulation trials, levels of aspartate followed the same decreasing profile as glutamate.

Figure 21. Changes in aspartate levels of the Slow (top) and Fast (bottom) rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in both hemispheres after kindling.

Aspartate – After Kindling

Slow Rats

Amygdala

Hippocampus

Fast Rats

Amygdala

Hippocampus

One hour rest

As expected, a large trial effect was observed in both strains during the post AD trial compared to baseline ($F_{1,16} = 140.2$, $p < 0.0000001$). As seen in Figure 21 (top and bottom right), after triggering an AD, there was no recovery towards baseline levels in either strain.

Section 4: Changes in Amino Acid Concentrations Within Each Strain as a Function of Amygdala Kindling

GABA: Slow Rats – Amygdala Stimulation

In the Slow rats, amygdala kindling dramatically altered the profile of GABA release between the 2 hemispheres during amygdala threshold stimulation. While GABA levels remained close to baseline in the stimulated hemisphere both before and after kindling, GABA levels in the non-stimulated hemisphere showed a large decline in at the onset of stimulation before kindling but only small decline after kindling. The analysis to directly compare the effects of kindling on GABA release in the two hemispheres during the 3 stimulations trials in Slow rats showed not only a significant main effect of hemisphere ($F_{1,9} = 54.7$, $p < 0.00005$), but a significant kindling x hemisphere interaction ($F_{1,9} = 22.6$, $p < 0.001$). This outcome can be seen when comparing Figures 16 and 19.

Before kindling (Figure 16, top left), levels of GABA in the stimulated hemisphere began to decrease after the second sub-threshold trial, and continued to decline in the post AD trial. By contrast, after kindling (Figure 19, top left), GABA levels rose after the second sub-threshold trial and remained elevated above baseline. These observations were only seen in the stimulated hemisphere. In the non-stimulated

hemisphere. steady and very low GABA levels were seen throughout stimulation before kindling; on the other hand, after kindling, the GABA levels, now near baseline, declined only slowly during stimulation trials. After the AD was triggered, however, a much larger decline in GABA levels was observed in the non-stimulated hemisphere, which resulted in a significant interaction between hemisphere and trial ($F_{1,10} = 24.6$, $p < 0.0006$).

GABA: Slow Rats – Hippocampal Stimulation

Analysis of the changes in amygdala GABA levels following the hippocampal stimulation trials did not show any significant differences associated with kindling. GABA levels remained low and unchanged in both hemispheres from the onset of hippocampal stimulation to the post AD trial. Of course, there was a large significant main effect of trials comparing baseline and the post AD trial ($F_{1,9} = 106.8$, $p < 0.000003$), but the GABA decline was similar between the pre-kindled and post-kindled assessments.

GABA: Fast Rats – Amygdala and Hippocampal Stimulation

Similar comparisons before or after kindling in the Fast rats revealed no significant differences among the 3 stimulation trials or between baseline and the post AD trial associated with kindling.

Kindling also did not affect GABA levels in the amygdala during hippocampal stimulations or during the post AD trial.

Glutamate: Slow Rats – Amygdala and Hippocampal Stimulation

Although there was a tendency for kindling to differential change the release pattern in the two hemispheres of the Slow rats during the stimulation trials, the kindling x hemisphere interaction was not significant ($p < 0.08$). On the other hand, the release pattern during the post AD trial revealed a small, significant kindling x trial interaction ($F_{1,11} = 4.99$, $p < 0.05$), where the decrease in release after kindling was slightly greater than before kindling.

Hippocampal stimulation provoked nearly identical release patterns of GABA before and after kindling. After the onset of stimulation, glutamate levels declined immediately, and remained unchanged throughout the 3 stimulation trials and one post AD trial.

Glutamate: Fast Rats – Amygdala and Hippocampal Stimulation

The profile of glutamate release in Fast rats was unaffected by amygdala kindling. This apparent indifference to kindling was evidenced during the amygdala stimulation trials and the post AD assessment (see Figures 17, 20, bottom left). On the other hand, during hippocampal stimulation, a significant kindling x trial interaction ($F_{2,6} = 5.18$, $p < 0.05$) was observed, where kindling was associated with a larger decline of glutamate levels after than before kindling. The pattern of decline was the same in both hemispheres (see Figures 17, 20, bottom right).

Further, comparing the baseline and the post AD trial, there also was a kindling x trial interaction ($F_{1,7} = 25.7$, $p < 0.002$), which was mostly due to a greater decline in release after kindling.

Aspartate: Slow Rats – Amygdala and Hippocampal Stimulation

Analysis of the amygdala and hippocampal stimulation trials, as well as the post AD trial in Slow rats, revealed no significance difference in aspartate levels due to kindling.

Aspartate: Fast Rats – Amygdala and Hippocampal Stimulation

As in the Slow rats, analysis of the amygdala and hippocampal stimulation trials, as well as the post AD trial in the Fast rats, revealed no significance difference in aspartate levels due to kindling.

Section 5: Do Generalized Convulsions *per se* Affect Amino Acid Levels?

The previous section focused on changes in amino acids in response to both amygdala and hippocampal stimulation in both Fast and Slow rats, and as a function of kindling. However, not all animals after kindling experienced a stage-5 seizure on the day when the probes were reimplanted; thus, the amino acid concentrations on that day could vary depending upon the seizure severity (generalized versus focal). In this section, we examined the levels of the 3 amino acids during the *amygdala* AD threshold stimulation procedure, depending upon the presence or absence of a generalized convulsive seizure. Of course, rats during *hippocampal* stimulation procedures, only focal seizures are triggered in amygdala kindled rats (similar to that observed before amygdala kindling).

A 4-way ANOVA was used, with strain and the presence or absence of a generalized convulsion as between factors, and hemisphere and trials as within factors, to assess percent change in amino acid levels from baseline. A 3-way ANOVA was subsequently employed to assess differences within each strain. As in section 2, the 2 sub-threshold trials and the AD trial constituted one analysis, while the baseline and the post AD trial made up the second analysis.

GABA - Amygdala Stimulation

Analysis of GABA levels after kindling on the stimulation trials revealed a significant strain x convulsion interaction ($F_{1,16} = 8.29$, $p < 0.02$), a strain x hemisphere interaction ($F_{1,16} = 6.74$, $p < 0.02$) and a convulsion x hemisphere interaction ($F_{1,16} = 6.35$, $p < 0.03$). The strain x hemisphere difference is clearly seen when comparing Figures 22 and 23 (top versus bottom), where the Slow rats with generalized seizures showed higher GABA levels in the kindled hemisphere, but lower GABA levels in the non-stimulated hemisphere than the Fast rats. Further, in single strain assessment, GABA levels were significantly higher in the stimulated hemisphere in Slow rats that had a generalized seizure than those without a convulsion. Such a difference, however, was not evident in the contralateral, non-stimulated hemisphere.

The analysis of the post AD trial compared to baseline in the Slow rats revealed a significant convulsion x hemisphere interaction ($F_{1,9} = 11.6$, $p < 0.008$), a hemisphere x trial interaction ($F_{1,9} = 9.82$, $p < 0.02$), and a convulsion x hemisphere x trial interaction ($F_{1,9} = 11.6$, $p < 0.008$). These data clearly suggests that in the Slow rats, which

Figure 22. Changes in GABA levels of the Slow rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in the stimulated hemisphere (top), and non-stimulated hemisphere (bottom).

GABA – Slow Rats

Stimulated Hemisphere

Amygdala

Hippocampus

Non-stimulated Hemisphere

Amygdala

Hippocampus

One hour rest

Figure 23. Changes in GABA levels of the Fast rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in the stimulated hemisphere (top), and non-stimulated hemisphere (bottom).

GABA – Fast Rats

Stimulated Hemisphere

Amygdala

Hippocampus

Non-stimulated Hemisphere

Amygdala

Hippocampus

One hour rest

experienced a generalized convulsion, GABA levels deviate less from baseline than rats without a convulsion, in the stimulated hemisphere only.

In the Fast rats, an opposite phenomenon was observed. Administration of a suprathreshold AD stimulus to the amygdala of the Fast rats after kindling resulted in a slight increase in GABA levels in the stimulated hemisphere (Figure 23, top left). In fact, both hemispheres of the Fast rats showed little reliable change in GABA levels depending upon the presence or absence of a generalized convulsion. Analysis of the 3 stimulation trials of Fast rats showed a main effect of hemisphere ($F_{1,8} = 5.89$, $p < 0.05$), where the GABA levels were slightly lower in the stimulated versus non-stimulated hemisphere. No interactions were significant. The post AD trial compared to baseline did not show any significant deviation from baseline levels.

GABA – Hippocampus

One hour following stimulation of the amygdala, the hippocampus ADT was determined in all rats. Although the analysis of the 3 stimulation trials revealed no significant main effects of strain or interactions, in general, a sharp decrease in GABA levels was present in both hemispheres of the Fast rats. The loss was more pronounced in the non-stimulated hemisphere after triggering a generalized convulsion (Fig 23, bottom right versus top right). Several minutes following an AD in the hippocampus, the post AD trial was compared to baseline to assess recovery levels. The analysis showed a main effect of trial ($F_{1,13} = 10.8$, $p < 0.006$). In general, the post AD trial showed a decline of GABA levels from baseline in both hemispheres and also in both the presence or absence of a generalized convulsion.

In the Slow rats, a convulsion x hemisphere x trial interaction was evident ($F_{2,16} = 4.27$, $p < 0.04$) in the assessment of the 3 amygdala stimulation trials. In response to stimulation of the hippocampus, a slightly greater loss of GABA was reported in both hemispheres with rats that experienced a convulsion one hour earlier during the amygdala trials (Fig 22, top and bottom right). As expected, differences between baseline and the post AD trial was evident. The analysis showed a significant main effect of trial ($F_{1,8} = 63.3$, $p < 0.00005$). The hippocampal results suggest that even an hour following an AD in the amygdala, stimulation of the hippocampus produced a large decline in GABA levels of both strains and in both hemispheres. Furthermore, following provocation of an AD in the hippocampus, GABA levels taken from both amygdalae do not appear to deviate significantly from each other in rats that had either a generalized convulsion or no convulsion.

Glutamate and Aspartate – Amygdala and Hippocampal Stimulation

Changes in glutamate and aspartate levels, related to stimulation of the amygdala in Fast or Slow rats with or without a generalized convulsion, were not significant (Appendix 1-4). The amino acid levels on the post AD trial also did not significantly differ between groups from the AD trial or vary with the presence or absence of a convulsion, but remained suppressed in comparison with baseline.

A similar scenario was registered during the hippocampal stimulation trials. Again the presence or absence of a convulsion did not differentiate changes in levels of glutamate and aspartate in either the Fast or Slow rats.

The above results suggest that there are differences in amino acids between amygdala kindled rats that have experienced a generalized convulsion in response to provocation of an amygdala AD and those that did not, but that the differences were strain related and were most apparent in the inhibitory transmitter, GABA. The data also suggest that the two hemispheres differ in this regard. In the Slow strain, GABA levels in the *stimulated* hemisphere were progressively elevated during stimulation trials, while the opposite occurred in the Fast strain. In the non-stimulated hemisphere, both strains showed decreased GABA levels, with a greater decrease in the Slow rats.

By contrast, GABA release during hippocampal stimulation in amygdala kindled rats was similar in the two strains and two hemispheres.

DISCUSSION

There are many ways that seizure activity can be developed in the central nervous system. One of the more obvious ways is by pharmacological manipulations of the brain's chemistry. Particularly important in that chemistry are the brain levels and activity of the amino acids GABA, glutamate and aspartate. In general, GABA has been implicated as a critical inhibitory transmitter, dampening excitability, while glutamate and aspartate are important excitatory transmitters. Because knowledge of these transmitters is crucial to our understanding of seizure genesis, in this project we have studied their extrasynaptic release during epileptogenesis, engendered by the kindling procedure. Further, as epilepsy is believed to have strong genetic underpinnings, we also

have used our epileptic prone (Fast kindling) and resistant (Slow kindling) rats strains to assess the relationship of the amino acids to kindling development. The levels of the amino acids were assessed by microdialysis in the two amygdalae before and after development of an amygdala kindled site.

Because these special rats strains were selected for their amygdala excitability (Racine et al., 1999), we anticipated differences between them in their amygdala amino acid response during amygdala kindling. In addition, because their kindling profiles from the dorsal hippocampus did not reflect the same excitability difference between them as amygdala kindling (McIntyre et al., 1999), we gathered information from the same rats about their amygdala amino acid levels during hippocampal stimulation before and after amygdala kindling as a control or comparison procedure. We felt that the amino acid release from the amygdalae before, during, and after electrical stimulation, and consequently, kindling, will provide important clues about how that structure modulates its chemical release at and around the synaptic cleft during epileptogenesis.

Historically, the microdialysis procedure has required perfusate through the extracellular space collected over many minutes (typically 20 min). However, the neurochemical basis for initiation and maintenance of an epileptic seizure more likely resides in only small windows of time (1-3 min), and would be altered considerably by long collection periods. Fortunately, a procedure for chemical analysis is now available (and was employed in this experiment) where amino acid levels using short sample times can be determined. Thus, because of our short collection windows, we anticipated that our results might differ importantly from other experiments using the kindling procedure, discussed below.

Prior to a discussion of the amino acid findings in the two strains, however, we will first discuss their seizure data, including amygdala and hippocampal AD thresholds and durations, as well as several measures of convulsion performance during amygdala kindling. This will be followed by a discussion of the associated amino acid levels before, during and after amygdala and hippocampal brain stimulation in the two strains.

Amygdala/Hippocampal AD Thresholds and Durations

Due to their high excitability and strong connectivity to other regions of the brain necessary for recruitment and initiation of an epileptic event, the amygdala and hippocampus are two structures that are most widely studied in temporal lobe epilepsy. Since the generation of seizures is highly dependent on the excitability of these structures in response to electrical stimulation, and consequently kindling, determination of AD thresholds and their associated durations, in the Fast and Slow rats, provides valuable information regarding the genetic contribution to those structures and to seizure propagation patterns.

Our results indicate upon initial stimulation of the amygdala, little difference in pre-kindling AD thresholds in both strains, even though there was tendency for the Slow rats to show higher AD thresholds both before and after kindling. These observations are consistent with Racine et al. (1999), who demonstrated AD thresholds between strains that were similar even after the 10th breeding generation. By the 40th generation, however, McIntyre et al. (1999) reported slightly higher AD thresholds in the Slow rats but only within the medial nucleus of the amygdala and not the basolateral complex, and only after kindling. By contrast, McIntyre et al. (1999) reported significantly higher AD

thresholds in the dorsal hippocampus of Slow compared to Fast rats. Such differences were not evident in the present study. A clear difference between these two studies is related to the fact that McIntyre et al. (1999) tested rats which only had hippocampal electrodes, and never previously received amygdala stimulation. In our study, of course, the rats first had amygdala dialysis probes inserted, then had AD thresholds of the amygdala determined, which was followed one hour later by the hippocampal AD threshold assessment. Clearly, these earlier manipulations to the amygdala could have altered the 'real' or naive threshold responses of the hippocampus in the two strains, as were reported by McIntyre et al. (1999). Significant interactions between the amygdala and hippocampus are likely, and are described later.

McIntyre et al. (1999) reported that the AD durations associated with the amygdala threshold discharges were dramatically different between the Fast and Slow strains. We observed that outcome as well. Not only did the Fast rats exhibit much longer AD durations before kindling than the Slow rats, but the magnitude of this increase during kindling was approximately 1.5-2 fold. Thus, the excitability of the neuronal network in the amygdala of the Fast rats appears greater than in the Slow rats. Whether this excitability difference is correlated to different levels of GABA and/or glutamate remains to be determined and is addressed in the amino acid section.

One important theoretical/methodological point raised by these AD threshold experiments concerns the impact of the dialysis probe on the neural network. Since the original insertion and reimplantation of the probes were in close proximity to the amygdala kindling electrode, one might suspect that the excitability at that electrode would be altered. When examining the rats after kindling, the effect of reimplantation of

the probe on local excitability was clear. In this examination, first the rats' AD thresholds and durations were determined on one day (T2 in Table 1 and Figure 4), and then again the next day, one hour after reimplantation of the probe (T3 in Table 1 and Figure 4). Interestingly, although reimplantation and removal of the probes may add insult to the brain, and thus, might alter the network attributes that generate and maintain both focal and propagating seizure, the amygdala AD thresholds and durations remarkably were not affected by the reimplantation. By contrast, however, the hippocampal AD thresholds were affected. In this case, reimplantation of the amygdala probes, *only* the seizure prone Fast rats showed a lowered AD threshold in the hippocampus and transiently increased its focal seizure duration. This finding is remarkably similar to a recent observation by Farrell and McIntyre (in progress), where disruption of the amygdala by protracted (60 min) electrical stimulation in amygdala kindled Long-Evans hooded rats (rather than probe insertion) temporarily and reversibly increased hippocampal excitability. It is likely, therefore, that the hippocampus and amygdala have reciprocal relationships, mostly through intermediary structures like the perirhinal and entorhinal cortices, where serious provocation of one structure can significantly alter the behaviour of the other. This point will be revisited when discussing the amino acid results below.

Kindling Rate and Convulsion Measures

As a result of kindling, the rate necessary to achieve the first stage-5 seizure, and several other measures of convulsion performance were examined. These measures include kindling rate, latency to clonus, duration of clonus and electrographic AD duration.

Kindling Rate

Electrical stimulation of the amygdala results in a localized or focal AD. Repetitive stimulation to the same structure consequently triggers seizure discharges that increase progressively and propagate through the recruited neuronal networks to ultimately generate tonic and clonic generalized convulsions. The difference in amygdala kindling rates between the two strains was clear (see Figure 5). Adopting the once daily stimulation protocol to the amygdala, kindling rates were achieved on average in 13 stimulations for the Fast rats and about 33 for the Slow rats. Similar differences in kindling rates between the two strains were also reported previously for other limbic structures, including the CA1 hippocampus and dentate gyrus, the piriform cortex and the perirhinal cortex (McIntyre et al., 1999). However, in those studies the differences between the Fast and Slow rats were most pronounced in the amygdala. This result suggests that synaptic connections and/or chemistry involving the amygdala and other forebrain and motor structures are considerably different between the strains.

Latency

Once convulsions have been kindled, the latency to forelimb clonus is a valuable measure to indicate the speed of recruitment of the motor system by the focal discharge. Previously, McIntyre et al. (1993) and Mohapel et al. (1996) have shown that the amygdala kindles more slowly, and its latency to clonus are longer than adjacent cortical structures, such as the piriform and perirhinal cortices. It is possible that the amygdala provides less innervation to the required motor structures, and/or is more anatomically remote than those particular cortical structures, and thus, requires more time to recruit

and synchronize the neuronal structures responsible for the initiation of forelimb clonus (Kelly and McIntyre, 1996).

Generally, over the course of 6 stage-5 seizures, there were no differences between the two strains in latency. However, there was a slight tendency for the Fast strain to exhibit longer latencies than the Slow strain. This pattern was most apparent during the first two stage-5 seizures, after which, the longer latencies progressively shortened, and remained unchanged between the strains. The gradual shortening of the convulsion latencies is indicative of a modification of local excitability, which facilitates the speed at which the motor system is accessed to generate a convulsion. These results are very similar to those of McIntyre et al. (1999), who also found no significant difference between the strains in latency from the basolateral amygdala. However, in that study, the Fast rats had much longer latencies than Slow rats from the medial amygdala. Most of the kindling electrodes in the present study were located in the basolateral amygdala.

Convulsive and AD Duration

The convulsions of the Fast rats were longer than the Slow rats and, of course, so were the associated ADs. This result is the same as McIntyre et al. (1999) and suggests that the Fast rats have a greater disposition for maintaining both focal and propagating seizure activity in the limbic system than the Slow rats.

Thus the Fast and Slow kindling rats used in the present experiment provided seizure characteristics that were nearly identical to previous kindling reports (Racine et al., 1999; McIntyre et al., 1999) on these two strains. The reliability of the kindling

development and profiles in the strains gives one confidence in the reliability of the seizure backdrop against which the amino acid responses from the two amygdalae are assessed.

Amino Acids

Over the past several decades, the biochemical regulation of neuronal excitability in pathological mechanisms of seizure induction, propagation and termination has focused on the involvement of excitatory and inhibitory amino acids (Meldrum, 1994; Sierra-Paredes et al., 1998). The present study sought to examine the profile of the amino acids prior to, during and following stimulation of the amygdala and hippocampus in the Fast and Slow strains both before and after amygdala kindling.

Basal Levels of Amino Acids

GABA

The concept of microdialysis is dependent on direct sampling of brain extracellular fluid. Briefly following stimulation of a neuron, the neurotransmitters released are subjected to active clearing processes such as efflux, uptake, and enzymatic degradation, which are responsible for efficient removal of the neuroactive compounds from the synaptic cleft. This implies that dialysis probes only collect minor amounts of neurotransmitter that has been “spilled over” from reuptake and degradation mechanisms. Thus, the neurotransmitter output or release is a reflection on the efficacy of these

clearing mechanisms. Under experimental manipulations, it is logical to assume fluctuations in amino acid levels are due to a temporary impairment in sequestering these transmitters back into the presynaptic terminal. However, changes in amino acid levels are dependent on further processes such as, synthesis, metabolism, degradation, and site of microdialysis collection. We will discuss these issues in the sections below.

In our results, we have reported slightly but non-significantly higher basal GABA levels collected from the amygdala in the Fast rats before kindling, but reduced levels one hour following stimulation of the amygdala. The opposite pattern was true of the Slow rats. Not only were these patterns apparent before kindling, almost identical patterns were evident after kindling (see Figure 13).

Many studies have examined the profile of amino acid release associated with kindling in structures, such as the hippocampus (Zhang et al., 1991; Ueda and Tsuru, 1993), neocortex (Fabisiak and Schwark, 1982; Leach et al., 1985), and raphe nucleus (During et al., 1992), but very few investigators have studied this phenomenon in the amygdala. One study by Kaura et al. (1995) employed *in vivo* microdialysis within the basolateral amygdala to analyze changes in amino acid levels before, during and after kindling. Using outbred rats, they found that by administering a single supratherapeutic stimulus to elicit an AD in the amygdala, no significant change in GABA concentration was evident in both the ipsilateral and contralateral hemisphere compared to basal levels. These results demonstrated that a locally applied stimulus to the amygdala failed to disrupt the GABAergic system. Our results differ slightly in that one hour following stimulation, the GABA levels rose and fell somewhat from baseline in the Slow and Fast rats, respectively. This potentially implies a greater breakdown of GABA mediated

inhibition after a stimulation in the Fast rats than the Slow rats. Alternatively, the sustained reduction in GABA levels seen only in the Fast strain after stimulation could be due to reduced GABA synthesis. Conversely, the relative increase in GABA levels in the Slow rats could be due to increased synthesis and release of the neurotransmitter coupled with decreased utilization. All of these possibilities need to be assessed in future experiments.

Glutamate

While GABA levels fluctuated in the amygdala and hippocampus between the two strains, basal glutamate levels remain unchanged in both hemispheres prior to and following kindling. Kaura et al. (1995) reported similar findings in basal levels and also after delivery of a single suprathreshold stimulation. However, following kindling their glutamate results were remarkably different from ours. They found large increases in both basal glutamate levels and after elicitation of a stage-5 seizure. Discrepancies between the two studies may reside in the temporal order in which the samples were collected. In their study, following stimulation of a non-kindled and kindled brain, dialysates were examined ten minutes post AD. In our assessment of basal levels, we analyzed dialysates sixty minutes post AD. The quantity of glutamate concentrations detected in dialysates is a result of quantity of release and reuptake by high-affinity transport. It may be plausible that in our data unchanged levels of glutamate before and after kindling may signify a more effective reuptake mechanism in our strains, or that glutamate may only be a minor player in the initiation and maintenance of seizure activity compared to GABA in the Fast and Slow strain.

Aspartate

Aspartate, like glutamate, is an excitatory amino acid. Although much of the literature has focused on the involvement of glutamate as the principal excitatory neurotransmitter in various animal models and in man, aspartate has been shown to play a considerable role towards our understanding of the neurochemical basis for epilepsy. Previous studies have alluded to the fact that microinjections of aspartate alone, or in conjunction with glutamate, serves as a chemical model of kindling; it may cause disrupted neuronal activity leading to a breakdown of GABAergic inhibition, and subsequently excitotoxic cell death (Croucher and Bradford, 1989; Mori et al., 1990; Mori, 1993).

In our study, we reported a slight decrease in basal aspartate levels 60 min following the elicitation of an AD in the amygdala. No differences were shown between the two hemispheres, and these values after kindling were almost identical to the pre-kindling condition in both the Fast and Slow rats. Interestingly, similar to the pattern found in basal GABA levels, after a local AD and also following a seizure, we observed a persistent drop in aspartate levels. Again, in comparison to the study by Kaura et al. (1995), they found a minor decrease in basal aspartate levels or following stimulation of the amygdala. Taken together, these results suggest that aspartate likely plays a minimal role in the induction of seizure activity, prior to and following kindling. Furthermore, a lack of a significant reduction in aspartate levels suggests rapid inactivation by reuptake mechanisms.

Changes in Amino Acid Levels in Response to Brain Stimulation

Following assessment of basal amino acid levels, the Fast and Slow rats were subjected to subthreshold stimulations until an AD had been provoked. Dialysates were collected in two minute samples prior to AD, during AD and ten minutes following an AD. Similarly, one hour after amygdala stimulation, the ipsilateral hippocampus was subjected to the same stimulation and collection protocol as the amygdala. Following kindling, this procedure was repeated to assess changes in amino acid concentrations as a function of kindling.

GABA

It is well established that GABA is the predominant inhibitory neurotransmitter throughout the brain. The hypothesis that a dysfunction in the mechanisms of GABAergic inhibition contributes to the initiation and maintenance of seizures has been well documented in animal and human models of epilepsy. In order to further investigate the integrative role that GABA and other amino acids play in seizure generation, it is necessary to monitor their temporal release patterns in limbic structures that participate in seizure induction.

Upon amygdala stimulation of the naive rats, we observed a dramatic 60% decrease in GABA levels from baseline in the non-stimulated hemisphere of the Slow rats. However, dialysates collected ipsilateral to the stimulating electrode remained at or slightly above baseline levels for both Fast and Slow rats. No hemispheric differences were observed in the Fast rats. One hour later, stimulation of the ipsilateral hippocampus

resulted in a large decline in GABA levels in both strains. These data imply that in the Slow rats, electrical stimulation delivered to the amygdala does not result in a complementary release/utilization of GABA in both hemispheres. Moreover, these unique ipsilateral and contralateral levels remain unchanged even after an AD and ten minutes post AD. Weeks later, after kindling and reimplantation of the dialysis probes, hemispheric differences in the Slow rats were less apparent. Again GABA levels collected ipsilateral to the stimulating electrode did not change from baseline during stimulation, whereas the contralateral hemisphere displayed a gradual decrease over the course of stimulation. In the Fast rats, kindling did not have a significant effect on the GABA levels in either hemispheres. However, again stimulation of the hippocampus resulted in a decrease of GABA levels in both amygdalae.

In other experiments, kindling and different seizure induction procedures induced changes in GABA levels within the same structure and between structures, producing inconsistent results. Rowley et al. (1995) used maximum electroshock to induce a single generalized seizure, and found an increased in GABA levels in the ventral hippocampus during the first five minutes, after which, the levels fell below basal values. Slightly higher GABA levels were reported following repeated seizures. Similar results obtained from the ventral hippocampus were reported by Ueda and Tsuru (1995). Upon initial stimulation of the amygdala, no change in GABA levels up to ninety minutes were reported in both hemispheres. Following a partial seizure, and a stage-5 seizure, however, they observed a 100% increase in GABA concentrations from baseline for both hippocampi. In contrast, in another study, dialysis collected from the kindled amygdala yielded a reduction of 67% and 56% in the ipsilateral and contralateral side, respectively,

compared to controls (Kaura et al., 1995). Taken together, these results and our data suggest that GABA release is not only dependent on the structures from which the dialysates are collected, but also the method used to trigger the seizure.

Unfortunately, microdialysis does not allow conclusions to be drawn about the influence of seizures on the separate metabolic processes of synthesis, storage, release or catabolism. However, there has been some evidence to suggest that elevation or depression of GABA levels are attributed to alterations in transporter mechanisms. Hippocampal studies examining GABA transporter immunoreactivity in humans using microdialysis indicated that during periods of interictal neurotransmission (between ictal or rhythmic seizure bouts), a decrease in GABA transporters may increase the transmitter availability in the extracellular space, enhancing GABAergic function (Mathern et al., 1999). Similarly, the same authors proposed that during seizure activity, the reduction in reverse transporter-mediated GABA release might reduce GABAergic inhibition, especially in neighbouring glutamatergic synapses, and promote seizure spread. Since glutamate is closely related to the GABA system, any increase in extracellular GABA may signify a compensatory response to suppress the firing of glutamatergic neurons after stimulation (Ueda and Tsuru, 1995). Following kindling, this GABAergic compensatory function may decline, leading to a collapse in GABA receptor mediated inhibition.

As mentioned previously, microdialysis only detects chemicals extrasynaptically. Any one of the following mechanisms could have contributed to this excess transmitter availability: increase in GABA synthesis and/or exocytosis, a decrease of GABA utilization or reuptake in neurons and/or glial cells and/or a reduced rate of breakdown of

GABA. Thus it is possible that stimulation of the amygdala in the Slow rats, producing a gradual elevation of GABA concentrations, may represent increased release from the presynaptic terminal. Upon provocation of an AD, these levels decreased and continued to decline well after our next sampling trial of 10 minutes post AD. However, one hour following stimulation of the amygdala, our data showed elevated GABA levels, above baseline levels prior to hippocampal stimulation. This outcome might imply that a loss of GABAergic inhibition was not long lasting in Slow rats. Nevertheless, following hippocampal stimulation, the GABA levels again declined *bilaterally*, and remained depressed. These results speak to relatively unilateral nature or hemispheric asymmetry associated with amygdala kindling versus the bilateral nature or symmetrical hemispheric changes associated with hippocampal stimulation (McIntyre and Kelly, 1993).

After kindling was completed, subthreshold amygdala stimulations produced much more similar GABA release in the two amygdalae of the Slow rats. However, after provocation of an AD, GABA levels began to rise slightly in the stimulated hemisphere, and declined in the non-stimulated hemisphere. In general, however, kindling in Slow rats resulted in GABA release from both hemispheres that were quite similar to baseline levels. It is not uncommon that kindling produced an enhancement of GABA release that could lead to a desensitization and/or down regulation of the GABA receptor, and thus result in an increase of seizure activity (Minamoto et al., 1992; Ueda and Tsuru., 1995).

The Fast rats did not show such large hemispheric differences. Not only was GABA almost identical between hemispheres before kindling, but it also remained similar to baseline levels. This result suggests that a single localized seizure in the amygdala of Fast rats did not immediately affect the release/degradation of GABA, and

change the rate of recovery by microdialysis. This finding is perplexing since we might expect that the Fast (seizure prone) rats should endure some rapid loss of GABA as a result of triggering an AD. However, one hour after the amygdala AD threshold tests, the baseline level of GABA in the Fast rats was quite reduced compared to Slow rats. This might represent a long term change in local excitability.

In another related difference between the two strains, Poulter et al (1999) have suggested that seizure proneness in the Fast rats may arise from arrested development of the GABA_A receptor system. Using immunocytochemistry and *in situ* hybridization techniques, they have reported an under expression of the α_1 (adult) subunit mRNA, and concomitantly an over expression of the α_2 , α_3 , α_5 (embryonic) subunits in the Fast rats. The converse was true for the Slow rats. This would suggest that a failure to complete a normal switch from the GABA_A subunits expressed during development to those expressed in adulthood may be a predispositional factor for temporal lobe epilepsy (Poulter et al., 1999). If this was the case, then GABA_A-mediated inhibitory neurotransmission may be very different between the Fast and Slow strains, independent of transmitter levels. Indeed, a small loss of GABA levels before or after kindling in the Fast rats may have a large effect on excitability, resulting from possibly a decreased density of GABA_A receptors in the synapses of Fast rats or if their subunits are inefficiently assembled.

Glutamate and Aspartate

We have reported no hemispheric changes in glutamate or aspartate levels prior to and following kindling. More importantly, the decline from baseline of both strains was

subtle. The role of the principal excitatory neurotransmitter, glutamate, in the development of epileptic seizures had been under intensive investigation for many decades. However, its involvement as a trigger for seizure activity remains controversial. Although it has been proposed that high levels of extracellular glutamate, as a result of excessive presynaptic release and/or altered glutamate uptake, is epileptogenic, the evidence to support such a claim is somewhat trivial. Obrenovitch et al. (1996) used several methods, including picrotoxin-induced seizures, and a glutamate uptake inhibitor to detect changes in glutamate levels associated with an epileptic event in the hippocampus. They reported no changes in glutamate levels after picrotoxin exposure, but a twenty fold increase in extracellular glutamate, but was not indicative of excessive excitation. They hypothesized that seizure activity is not necessarily accompanied by an increase in extracellular concentration of glutamate. Furthermore, even in the presence of prolonged status epilepticus, adequate to induce both neuronal injury and cell death, no elevations of glutamate measured by microdialysis were detected (Tanaka et al., 1996).

A number of studies have attempted to examine the changes in glutamate concentrations in the hippocampus during and after an amygdala kindled seizure. Ueda and Tsuru (1993) reported a rapid release of glutamate in both hemispheres, in a partial and generalized seizure, but only lasting four minutes before returning to baseline levels. If the release and sequestering mechanisms were also that rapid in the amygdala, then our results would not have shown an increase ten minutes post AD. It is noteworthy that any change in glutamate levels from many other studies were minute, and the dialysates were often represented in nM amounts. The amino acid results in our study were all represented in μ M amounts. Moreover, it is important to keep in mind that although

synaptic release and uptake mechanisms provide logical vehicles to explain seizure activity, other contributing factors, such as density of glutamate receptors and enhanced synaptic efficacy (spreading of additional excitatory synapses increasing the network connectivity) are critically important (Obrenovitch et al., 1996).

The glutamate and aspartate levels in the Slow rats prior to kindling was similar but smaller in magnitude than the GABA levels. Dialysis collected in the stimulated amygdala resulted in glutamate levels that were unchanged from baseline, while the non-stimulated amygdala showed a decline of glutamate from baseline. As a result of kindling, several minutes following an AD, there was a recovery to baseline levels only in the stimulated hemisphere of the Slow rats, while further decline was evident in the non-stimulated hemisphere. The opposite hemispheric pattern was seen in the Fast rats. The non-stimulated hemisphere of the Fast rats before kindling showed an increase in glutamate back to baseline levels ten minutes following an AD. No such attempt was made in the stimulated hemisphere. As a result of kindling, levels of glutamate in the Fast rats during the AD did not differ from levels assessed several minutes later.

Following hippocampal stimulation, glutamate levels in both amygdalae declined in both strains, before and after kindling. This was very much in concert with the GABA profile. As was mentioned earlier, it was of no surprise that the amino acid response in both hemispheres were virtually identical during hippocampal stimulation.

Although, aspartate may be important in the induction of seizure activity, its efficacy, however, is small in comparison to glutamate. It has also been shown that high doses of aspartate alone into the amygdala causes rapid kindling of full limbic seizures

(Croucher and Bradford, 1989). This suggest that an extracellular threshold must be reached before chemical kindling is initiated by aspartate.

The profile of aspartate in our data did not vary significantly from glutamate. Both strains exhibited approximately 20% loss in both hemispheres, before and after kindling. This suggests that stimulation of the amygdala, and provocation of an AD did not produce a significant change in aspartate levels from baseline before, and after kindling. The mechanisms are unclear, however, it may involve rapid inactivation of aspartate by high affinity reuptake systems.

Again, we reported a decline in aspartate levels in the amygdala following hippocampal stimulation. Similar mechanisms that act on glutamate may be present here.

Changes in Amino Acid Levels During a Generalized Convulsion

GABA

To further address the question of whether amino acid levels in the Fast and Slow rats were affected by kindling, we examined their release patterns on the day of reimplantation of the probes by separating them on the basis of whether triggering an AD in the amygdala resulted in a presence or absence of a generalized (stage-5) convulsion. We observed in the stimulated hemisphere of Slow rats that had experienced a convulsion, GABA levels rose above baseline and continued to slightly increase ten minutes following the AD. Conversely, GABA levels diminished at the onset of amygdala stimulation in the Slow rats that had no convulsion. In the Fast rats, GABA levels did not predict a convulsion. Interestingly, a drop in GABA levels was apparent at

the onset of stimulation, but gradually rose back to baseline ten minutes following a convulsion. In the absence of a convulsion, the opposite pattern was observed.

A very different profile was seen in the non-stimulated hemisphere. In the Slow rats, the onset of amygdala stimulation resulted in a substantial decline in GABA levels for both groups, and remained unchanged even at ten minutes post AD. However, the Fast rats that had a convulsion were only subjected to a 20% loss of GABA throughout amygdala stimulation.

Our results provide evidence to support the notion that the mechanisms responsible for GABA release and removal at and around the synaptic cleft are remarkably different between the Slow and Fast strains. From our data, it was apparent that the loss of GABA between hemispheres of both strains did not parallel each other. Activation of the ipsilateral amygdala by electrical stimulation promoted higher than normal GABA levels during a generalized convulsion, while no convulsion resulted in a loss of GABA in the Slow rats. During a convulsive seizure, the GABA system may be challenged to upregulate the production and release of GABA into the synapse. Since the Slow kindling rats have a higher synaptic density of GABA receptors, they may be better able to prevent a collapse of GABA-mediated inhibition (Poulter et al., 1999). In contrast, our data also showed a loss of GABA in the Slow rats with no convulsion. This would suggest that in the absence of a generalized convulsion, there may be a reduction in the release of GABA from the presynaptic terminals, resulting in less GABA pickup by the dialysis probes. In the non-stimulated hemisphere of the Slow rats, a reduction in GABA levels in the presence or absence of a convulsion could again be attributed to a reduction of presynaptic release. This data suggest that the Slow rats show a greater

hemispheric *asymmetry* than the Fast rats, owing to a remarkably different profile of GABA release. Thus, their GABA release in both hemispheres provide further evidence that the Fast rats are better able to recruit other structures and promote faster hemispheric transfer of seizure activity than the Slow rats during epileptogenesis.

Because the Fast rats were associated with longer AD duration and convulsion duration, it was reasonable to assume a breakdown of inhibition would contribute to a reduction in GABA release. However, it has been shown that the elevation of GABA in kindled amygdala is likely to reflect an elevated metabolism owing to a hyperactive state (Kaura et al., 1995). This raised tissue content of GABA in kindled amygdala may be linked to lower extracellular GABA levels detected by microdialysis. If this was true, then the provocation of a convulsive seizure could lead to an excessive efflux of GABA into the synaptic cleft and more readily picked up by the probes. This is precisely what our data showed in the Fast rats.

One hour following the amygdala AD, stimulations to the ipsilateral hippocampus resulted in a general loss of GABA levels in both strains compared to baseline. The presence or absence of a convulsion did not predict any significant change of GABA concentration levels. However, it is worth mentioning that at the onset of an AD from the hippocampus, there was a reduction in GABA levels only in the non-stimulated hemisphere of both strains even after ten minutes post AD. On the other hand, the stimulated hemisphere did not show such a change. This suggests that in a kindled brain, triggering an AD from the hippocampus propagates to both amygdalae with equal efficacy in both strains.

The profile of glutamate and aspartate did not yield any conclusive pattern of their release in the presence of absence of a generalized convulsion. Their possible roles and mechanisms of action before, during and after an AD were discussed in the previous section.

In summary, the present experiment suggests that differences in amino acid release, particularly GABA, exist between the Fast and Slow rats during localized stimulation and kindling of the amygdala. As shown previously in other studies, these strains not only differed in their rate of seizure genesis, local excitability, propagation patterns and recruitment, but now this thesis has shown they also differ in their amino acid release. The mechanisms responsible for the amino acid release are still under investigation. However, future considerations to precisely measure neurotransmitter levels in the synaptic cleft during a seizure, instead of collecting the overflow of synaptic neurotransmission and/or glial metabolism, would provide a better understanding into the neurochemical basis for and against limbic seizures.

Appendix 1. Changes in glutamate levels of the Slow rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in the stimulated and non-stimulated hemisphere.

* denotes a generalized convulsion. S = stimulated hemisphere, NS = non-stimulated hemisphere.

APPENDIX 1

Hemisphere	Structure	Baseline	Sub-Threshold 1	Sub-Threshold 2	AD	Post AD
S	Amy *	100±0	76±10	73±12	71±10	81±10
	Amy	100±0	81±5	88±11	83±6	94±10
	Hippo *	100±0	62±17	61±11	56±14	72±19
	Hippo	100±0	78±11	73±10	89±13	79±10
	Amy *	100±0	95±6	94±13	70±15	52±11
	Amy	100±0	81±11	84±14	86±11	75±12
	Hippo *	100±0	73±25	60±9	65±14	58±14
	Hippo	100±0	67±9	68±8	56±10	56±12
NS						

Appendix 2. Changes in glutamate levels of the Fast rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in the stimulated and non-stimulated hemisphere.

* denotes a generalized convulsion. S = stimulated hemisphere, NS = non-stimulated hemisphere.

APPENDIX 2

Hemisphere	Structure	Baseline	Sub-Threshold 1	Sub-Threshold 2	AD	Post AD
S	Amy *	100±0	73±13	58±4	73±9	74±8
		100±0	77±9	76±18	82±6	81±10
	Hippo *	100±0	34±5	34±4	32±5	29±6
		100±0	46±.5	42±6	39±.5	54±3.5
	Amy *	100±0	78±10	80±16	62±8	64±15
		100±0	84±19	83±15	83±17	84±20
	Hippo *	100±0	67±20	60±18	74±30	48±13
		100±0	42±6	43±5	36±0	44±4
NS	Amy *	100±0	73±13	58±4	73±9	74±8
		100±0	77±9	76±18	82±6	81±10
	Hippo *	100±0	34±5	34±4	32±5	29±6
		100±0	46±.5	42±6	39±.5	54±3.5
	Amy *	100±0	78±10	80±16	62±8	64±15
		100±0	84±19	83±15	83±17	84±20
	Hippo *	100±0	67±20	60±18	74±30	48±13
		100±0	42±6	43±5	36±0	44±4

Appendix 3. Changes in aspartate levels of the Slow rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in the stimulated and non-stimulated hemisphere.

* denotes a generalized convulsion. S = stimulated hemisphere, NS = non-stimulated hemisphere.

APPENDIX 3

Hemisphere	Structure	Baseline	Sub-Threshold 1	Sub-Threshold 2	AD	Post AD
S	Amy *	100±0	67±8	68±12	75±13	77±13
	Amy	100±0	84±13	93±20	85±16	98±19
	Hippo *	100±0	43±8	41±11	40±9	45±10
	Hippo	100±0	57±19	58±21	70±14	61±20
	Amy *	100±0	91±14	76±13	79±13	61±7
	Amy	100±0	73±24	67±21	61±23	60±23
	Hippo *	100±0	39±6	51±9	49±11	40±7
	Hippo	100±0	55±11	60±10	49±15	50±15
NS	Amy *	100±0	67±8	68±12	75±13	77±13
	Amy	100±0	84±13	93±20	85±16	98±19
	Hippo *	100±0	43±8	41±11	40±9	45±10
	Hippo	100±0	57±19	58±21	70±14	61±20
	Amy *	100±0	91±14	76±13	79±13	61±7
	Amy	100±0	73±24	67±21	61±23	60±23
	Hippo *	100±0	39±6	51±9	49±11	40±7
	Hippo	100±0	55±11	60±10	49±15	50±15

Appendix 4. Changes in aspartate levels of the Fast rats during amygdala stimulation, and one hour later, hippocampal stimulation. Dialysates collected at 2 sub-threshold stimulations, AD, and post AD stimulation were represented as a percent change from baseline in the stimulated and non-stimulated hemisphere.

* denotes a generalized convulsion. S = stimulated hemisphere, NS = non-stimulated hemisphere.

APPENDIX 4

Hemisphere	Structure	Baseline	Sub-Threshold 1	Sub-Threshold 2	AD	Post AD
S	Amy *	100±0	89±28	54±13	98±21	100±22
		100±0	75±24	75±36	81±30	79±30
	Hippo *	100±0	34±13	34±11	33±9	30±11
		100±0	36±1.5	32±6	28±0	33±2
	Amy *	100±0	77±10	86±25	55±15	68±16
		100±0	116±32	114±40	94±26	102±33
	Hippo *	100±0	54±17	55±16	57±25	38±14
		100±0	30±1	32±1.5	25±3	34±3.5
NS	Amy *	100±0	89±28	54±13	98±21	100±22
		100±0	75±24	75±36	81±30	79±30
	Hippo *	100±0	34±13	34±11	33±9	30±11
		100±0	36±1.5	32±6	28±0	33±2
	Amy *	100±0	77±10	86±25	55±15	68±16
		100±0	116±32	114±40	94±26	102±33
	Hippo *	100±0	54±17	55±16	57±25	38±14
		100±0	30±1	32±1.5	25±3	34±3.5

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