A PHARMACOLOGICAL EXAMINATION OF $GABA_B$ RECEPTOR-MEDIATED INHIBITION IN THE AMYGDALA OF FAST AND SLOW KINDLING RAT STRAINS: *IN VIVO* AND *IN VITRO* STUDIES

by

RICK S. SHIN, M.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
(Neuroscience)

Department of Psychology
Carleton University
Ottawa, Ontario, Canada

May, 2005

ABSTRACT

In the central nervous system, γ-aminobutyric acid (GABA) has been the predominant neurotransmitter that mediates inhibitory synaptic transmission. It has long been suspected that a disruption of the GABAergic system is likely the cause of epilepsy. $GABA_B$ receptors have been implicated to play a role in this disorder, but to what extent are largely unknown. To this end, the goal of the present thesis is to investigate whether $GABA_B$ receptors contribute to seizure genesis and modulation in rat strains that are either seizure prone (Fast) or resistant (Slow). In experiment #1, chronic daily microinfusion of a potent $GABA_B$ antagonist, SCH 50911, into the amygdala along with other GABAergic pharmacological agents including baclofen were administered, through infusion or systemic injection, at various time periods during kindling. SCH 50911 and baclofen, administered via microinfusion, alone did not significantly alter afterdischarge thresholds and durations. However, a single systemic injection of baclofen completely abolished behavioural seizures in all Fast rats, and half of Slow rats. This same treatment in conjunction with infusion of SCH 50911 delivered the next day resulted in a substantial increase in the number of convulsive seizures, especially among Fast rats, compared to systemic baclofen administration alone. Distinct patterns of electroencephalographic recordings from administration of various GABAergic agents provide further evidence of seizure susceptibility and propagation. In experiment #2, the distribution of $GABA_B$ receptors, GAT1, and their co-localization at inhibitory terminals were explored using immunocytochemical techniques. No strain differences were evident. There was a slight tendency for increased GAT1 expression following kindling with no observable change in $GABA_B$ immunoreactivity. Assessment of *in vitro*

measurements from experiment #3 revealed that kindling of the amygdala resulted in 1) lower membrane resistance in Fast and Slow rats compared to sham controls; 2) greater and lower $GABA_B$ IPSP amplitudes in Fast and Slow rats, respectively; 3) increase and decrease of total charge transfer of the kindled (stimulated) amygdala and contralateral (non-stimulated) amygdala of Slow rats, respectively; 4) no changes in baclofen-induced slope conductance and reversal potential. The implications of these results suggest that $GABA_B$ receptor-mediated inhibition in the amygdala plays a modulatory role in kindling-induced epileptogenesis. An interplay between $GABA_A$ and $GABA_B$ receptors is critical for the regulation and dampening of neural network excitability, which differs between the Fast and Slow rats. Further elucidation of pre- and postsynaptic mechanisms that contribute to inhibition will ultimately be vital towards the development of more efficacious treatment protocols for epileptic patients.

To my lovely bride, Lisa; you are my inspiration, my hopes and dreams, my everything...

All this is for you.

Acknowledgements

I would like to offer my deepest appreciation to my advisor, Dr. Dan McIntyre, for his support and encouragement throughout the years. It has been an incredible journey and an absolute privilege to work alongside him. His dedication towards epilepsy is unparalleled, and the experience I have attained through his tutelage will remain with me always.

Thank you so much, Dr. Michael Poulter for welcoming me into your lab and answering my numerous questions on just about everything. I truly admire your passion for research. Thank you for the many lessons you have taught me and for your much appreciated assistance through this difficult hurdle.

To my beautiful wife, thank you for your endless support, for believing in me, and the many sacrifices you have made. I dedicate this thesis to you. My parents and brother, I can't thank you enough for your encouragement. I couldn't have done this without you. Last, but certainly not least, special thanks goes out to my in-laws for your prayers, support and love.

TABLE OF CONENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	Afterdischarge
ADT	Afterdischarge threshold
AP5	DL-2-Amino-5-phosphonopentanoic acid
Baclofen	4-Amino-3-(4-chlorophenyl) butanoic acid
BLA	Basolateral amygdala
CNS	Central nervous system
DNQX	6,7-Dinitroquinoxaline-2,3-dione
EEG	Electroencephalogram
GABA	Gamma-aminobutyric acid
G_{IRK}	G-protein coupled inward rectifying potassium
IIS	Interictal spikes
I.P.	Intraperitoneal
IPSC	Inhibitory postsynaptic current
IPSP	Inhibitory postsynaptic potential
LA	Lateral amgydala
mIPSC	Miniature inhibitory postsynaptic current
Muscimol	5-Aminomethyl-3-hydroxyisoxazole
NMDA	N-methyl-D-aspartate
Picrotoxin	1:1 mixture of picrotoxinin and picrotoxin
PC	Piriform cortex
PRh	Perirhinal cortex
PSF	Point spread function
R_i	Input resistance
SCH 50911	(2*S*)-(+)-5,5-Dimethyl-2-morpholineacetic acid
SR 95531 hydrobromide	6-Imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid hydrobromide
SEM	Standard error of the mean
TLE	Temporal lobe epilepsy
VCRT	Voltage/current ramp test

GENERAL INTRODUCTION

As we begin the twenty first century, the advancement in technological innovation in the field of medical sciences has launched a new era for studying many neurological diseases and disorders that continue to plague the human race. Despite greater understanding, improved management, and progressive developments in therapeutic approaches over the past several decades, the precise etiology of such diseases remain unknown. As scientists, one of our goals is to gain further insight and knowledge into the functional constituents of the normal brain. One simple question that may yield many complex answers is "How does the brain work"? Certainly the many discoveries will ultimately provide a framework to which specific behaviours are mediated by particular brain structures. Another goal is to understand the "abnormal brain" from a pathological perspective in hopes to eradicate diseases, or at least, develop effective treatment protocols to improve and prolong the quality of life. At what period do certain structures become pathological? What are the mechanisms responsible for the initiation, maintenance and termination of such disorders? It is without a doubt that these and other important questions must be addressed in order to elucidate the many mysteries of the brain.

Epilepsy

With a prevalence rate of approximately 1% of the population, human epilepsy is regarded as one of the most common neurological disorders known to man. It is a chronic and frequently debilitating medical condition produced by temporary changes in the electrical function of the brain, possibly culminating into convulsive seizures, where

awareness, movement, or sensations are often perturbed. At first glance, epilepsy is a condition that varies considerably in its etiology and pathophysiology of mechanisms involving spreading discharges, mode of onset, clinical manifestations, responsiveness to treatment, and there is no apparent justification for believing that a common, singular process underlies this brain dysfunction (Majkowski, 1999). Yet, despite obvious variability in the manifestation of epilepsy, there is considerable commonality in many facets of the varied symptomology that are shared among all species. With this in mind, investigations into the pathology of seizure genesis and maintenance will prove vital in our understanding of this ubiquitous disorder.

The classification of seizure types can be elaborate depending on its etiology, and manifestations. With approximately 40 different types of epilepsies and epileptic syndromes, the diversity of this disorder is far reaching. Nevertheless, a central feature of this classification system is the anatomical localization of seizure onset. In most cases, the origin of epileptic seizures is unknown, however, different types of epileptic seizures appear to arise from different underlying disturbances, whether they be environmentally or genetically influenced (Engel, 1990). Seizures are generally categorized into two broad categories: *idiopathic* and *symptomatic* epilepsies. Idiopathic disorders are of genetic origin and usually are not associated with other neurological pathologies, while symptomatic disorders may arise from a pathological substrate that may be genetic, such as tuberous sclerosis, or acquired, such as a traumatic scar (Engel, 1996). The most identifiable types of seizures are classified as *partial* versus *generalized* seizures. Partial seizures (simple or complex) occur when excessive electrical activity in the brain is restricted to a local area. Simple partial seizures encompass unusual sensations that often

translate into an aura, which can serve as a prelude to a more provocative seizure. Complex partial seizures, on the other hand, involve a loss of awareness accompanied by more robust behaviours. Generally, simple partial seizures do not involve an impairment of consciousness, but are often accompanied by staring and an arrest of motion, followed by altered responsiveness. The other main category involves generalized seizures (absence or tonic-clonic), which occur when the brain's highly synchronized electrical activity spread immediately to other areas, thus often affecting the entire body. Generalized absence seizures are most prevalent in childhood and tend to disappear toward adolescence. They are characterized by momentary lapse of consciousness often associated with staring into space. Generalized tonic-clonic seizures, as the name suggests, involve stiffening of the muscles (tonus) accompanied by jerking and twitching (clonus) of the body extremities.

One of the many challenges confronting epileptologists is to develop an animal model that mirrors many of the predisposing and precipitating causes of seizure activity seen in humans. With a clear understanding of epileptogenesis at its different stages: onset, persistence, termination, it is one step closer to answering the ultimate question, "why do seizures occur in some individuals and not in others"? To date, the best and most widely studied animal model of simple partial seizures and/or complex partial seizures that secondarily generalize is the kindling phenomenon. The most frequent form of this seizure type in humans originates in the temporal lobe, and is called temporal lobe epilepsy (TLE). TLE is also the most common form of human epilepsy and the most difficult to treat.

Kindling Mechanisms

Characteristics and Parameters

Kindling is an experimental model of epilepsy resulting from progressive activity-dependent changes in neuronal structure and function. Typically, kindling can be induced in one of two ways: chemically, in which repeated administration of subconvulsive doses of known convulsants can induce seizure genesis, or electrically, via deep electrodes into brain structures that are susceptible for triggering epileptic episodes. The former (chemical kindling) can be induced by a variety of drugs administered directly by local brain injection or systemically to mimic the kindling-like effects, while the latter (electrical kindling) is a phenomenon whereby repeated application of an electrical stimulus results in progressive development of electroencephalographic and behavioural seizures, culminating in a secondarily generalized convulsive seizure (McNamara, 1984). One essential requirement for the development of kindling is that the electrical stimulus must induce local afterdischarges (ADs), defined as rhythmic paroxysmal electrographic discharges that outlast the stimulus by two or more seconds (Racine, 1972a; McNamara, 1984). The kindling process consists of many fundamental features that involve several well-defined characteristics occurring over individual trials. The first of which is a reduction in the afterdischarge threshold (ADT) required to evoke the AD. Stimulation that is above or below the initial ADT will gradually lower this threshold on subsequent trials (Racine, 1972a; Handforth, 1984; McNamara, 1986; Post, 2002). Secondly, the progressive growth and propagation of AD to other anatomically related or distal structures are evidently shown in the form of increased AD frequency,

amplitude, complexity and duration. Further, successive administrations of this unvarying stimulus, which initially does not provoke a behavioural convulsion, will do so if it is repeated sufficiently (Goddard et al., 1969; Kulkarni and George, 1994). Once established, the stereotypic progression of seizure development (AD activity) will inevitably foster the propagation and generalization of subsequent seizures through the recruitment of a more spatially distributed neural network irrespective of the kindling site (Racine, 1972a; Ferland et al., 1998). When the necessary stimulus elicits a generalized full-blown convulsive seizure, the animal is then said to have reached the "kindled state". In the rat, the severity of convulsions typically develop through 5 stages (Racine, 1972a): (1) mouth and facial twitches, (2) clonic head movements, (3) mild forelimb clonus, (4) forelimb clonus with rearing, and (5) the latter with loss of postural control. All of these classes are associated with a reduced responsiveness to sensory stimuli, and the behaviour observed in classes 1 and 2 mimic similar behaviours found in human complex partial seizures; the behaviour in the latter three classes would be consistent with limbic seizures evolving to secondarily generalized convulsive seizures (McNamara, 1984).

The efficacy of kindling induction is dependent upon a variety of variables, such as, the strain of the animal, site of provocation, the number of stimulations, frequency, intensity and duration of the electrical stimulus. Typically, the standard paradigm used to elicit a convulsive seizure in the least amount of time (kindling rate) is to deliver a daily administration of a 2 s sine wave at a frequency of 60 Hz. Earlier studies have indicated that kindling can develop at comparable rates regardless of whether sine- or square waves are used, and regardless of whether train durations of 1 s or 60 s are used (Goddard et al., 1969). One of the more widely studied parameters of kindling induction involves varying

the interval between successive stimuli. Traditionally, kindling is best achieved using interstimulus intervals of 24 h or longer, whereas repeated electrical stimulation at intervals shorter than 30 min (rapid kindling) have been reported to suppress or retard kindling (Goddard et al., 1969; McIntyre et al., 1987, 1991b). Although seizures evoked using this rapid kindling protocol have been shown to result in a progressive, delayed development of the fully kindled state, similar morphological changes in response to stimulation via traditional kindling were reported, albeit arising from possible distinct underlying mechanisms (Penner et al., 2001). The manipulation of stimulation parameters under the rapid kindling paradigm allows for assessment of epileptogenesis during this procedure, and to permit its comparison to that of the standard kindling protocol. By allowing a 2-4 week recovery period following rapid kindling, it would be advantageous to test whether this procedure resulted in any increased susceptibility to the daily stimulation protocol. Such results may provide insights into the mechanisms of seizure genesis following a traumatic insult. To this end, the long lasting enhanced seizure sensitivity induced by the kindling process not only affects the behavioural and electrographic responses in the animal, but changes in neuronal stability that ranges from altered gene-expression in neurons to the loss of specific neuronal populations and the rearrangement of synaptic connections are often associated with this phenomenon (Kamphuis et al., 1990; Mody, 1999).

Advantages of the Kindling Model and its Clinical Relevance

Electrical kindling is currently the most commonly used experimental model of complex partial and TLE. Its versatility allows for studying epileptogenesis at various

defined points, especially during stages of development until the establishment of the epileptic state. This advantage is particularly relevant for drug testing since the severity of the seizures can be triggered at the investigator's convenience. For this reason, kindling is often regarded as the best-controlled model for chronic focal epilepsy. In addition, the ability to kindle in multiple sites permits an accurate assessment of connectivity and excitability in the contralateral homotopic region (mirror focus), or other sites in the ipsilateral hemisphere (McNamara, 1984; McIntyre and Poulter, 2001). Lastly, analyses of biochemical assays are made available without possible contamination from electrical kindling, unlike systemic administration of exogenous toxins, which will likely introduce a potential confound.

Wada (1978) introduced various criteria necessary to establish a valid animal model of human epilepsy: 1) experimental control over the anatomical areas and size of epileptogenic lesions; 2) create an epileptogenic site without identifiable destructive pathology; 3) precise control over the initiation and development of seizures; 4) ready induction of seizure by a discrete and identifiable epileptic event; 5) development of spontaneous recurrent seizures; 6) persistence of the epileptic state for many months. There are two lines of evidence to suggest a kindling-like mechanism in clinical epilepsy. First, the most effective animal models are those that imitate a slowly developing process of epileptogenesis. Thus, chronic models with long lasting focal neuronal discharges, as shown by encephalographic epileptic activity, may represent epilepsy in contrast to acute models, which represent seizures only (Majkowski, 1999). However, determining the extent to which clinical seizures are indeed progressive is no easy task. At the time of

evaluation and diagnosis, patients may have already experienced numerous seizures of which they may have no recollection.

Second, in humans, sudden head trauma or vascular lesions may damage brain tissue at a slow rate, depending on the severity of the injury. During the intervening period between the initial insult and the development of clinical seizures, scar tissue or bone fragments may still be present. As a consequence, focal hyperexcitability could induce epileptic discharges that may start the process of kindling. However, chronic recurrent seizures do not arise until a large area of the brain has been sufficiently perturbed or kindled (Engel and Rocha, 1992). Thus, kindling proves to be an invaluable model for studying epileptogenesis in humans by allowing detailed monitoring of the subject's symptoms and electrographical profile from the time of induction to its later stages.

Anatomical Substrates and Kindling Rates

The diversity of the kindling phenomenon is not restricted to any one species. In fact, mice, dogs, cats, and primates have also shown to be susceptible. This preferential sensitivity to kindling depends largely on the stimulated structure, stage of brain maturation, degree of brain dysfunction, and the species of animal studied (Majkowski, 1999). For example, kindling can be produced by electrical stimulation in a number of brain sites, but not all. Earlier studies have demonstrated that stimulation of the superior colliculus, reticular formation, or cerebellum, unlike other cortical and subcortical structures, does not result in kindling (Goddard et al., 1969; Racine, 1972b). It has been well established that seizure activity within the brain is not a random phenomenon, but

rather, the electrical discharge is generated and propagated by specific anatomical pathways that often can involve multiple interconnected brain structures (Sato et al., 1990a). Of these, the limbic system has been largely implicated in the initiation and maintenance of epileptic discharges. Thus, the induction of a local epileptic focus can be triggered, via kindling, 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 cortical and subcortical structures, such as the hippocampus, amygdala, piriform cortex (PC), perirhinal cortex (PRh), which are believed to be key structures in temporal lobe seizure genesis and propagation. However, the stereotypic progression in kindled seizure development occurs independently of the kindling site, suggesting that there is a common core of structures involved and/or recruited during kindling from all limbic system sites (Ferland et al., 1998).

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. This speaks directly to possible intimate connections between the kindled structure and efferent connections to drive brainstem and spinal cord motor reflexes. Within the limbic system, the amygdala has been known to kindle at a much faster rate than the hippocampus (Goddard et al., 1969; McIntyre et al., 1993). Such inherent differences in kindling development between these two anatomically distinct structures may be attributed 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., 1990a; Kulkarni and George, 1994). 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 centers (Kelly and McIntyre, 1996). As previously mentioned, one such structure may involve the PC. Located adjacent to the amygdala, the unique intrinsic associative fiber system of the PC and its various connections to and from other limbic nuclei allow for the facilitation of seizure generalization (Honack et al., 1991; McIntyre et al., 1993; McIntyre and Kelly, 2000). For example, the PC contains more susceptible neural circuits of all forebrain regions for electrical induction of limbic seizures, and that during stimulation of other limbic brain regions, broad and large ADs can be observed in the ipsilateral PC, indicating that the PC is activated early during the kindling process (Loscher and Ebert, 1996).

Recently, it has been suggested that the area immediately dorsal to the PC, the PRh, is a likely substrate for the expression of generalized clonic seizures in the late states of kindling development (Kelly and McIntyre, 1996; Ferland et al., 1998). In support of this hypothesis, the PRh has been shown to have the fastest kindling rates of any other site in the brain in that it only requires 3-4 kindling stimulations to produce bilateral forelimb clonus (McIntyre et al., 1993; Kelly and McIntyre, 1996). Furthermore, Kelly and McIntyre (1996) have demonstrated that the latency from the stimulus onset to the onset of a clonic convulsion was extremely brief. The significance of this finding indicates a

likely modification of local excitability and the pathways, which permit greater access for discharges to circuits controlling convulsive activity. Taken together, these data suggest that the progression of kindling to generalized convulsions requires the recruitment of the PRh cortex in combination with other structures, such as the PC or frontal cortices to activate motor systems in the spinal cord (McIntyre et al., 1999).

Kindling (Positive) Transfer

To understand the basic mechanisms underlying kindling, it is perhaps of initial interest to identify the spatial distribution of the altered network of neural circuits caused by this phenomenon. Since kindling has been implicated to involve modification of local networks surrounding the site of stimulation, it is likely that the progression of kindling will propagate to and reside in multiple structures within the brain. Consequently, these newly modified neural pathways and their efferent structures are said to have undergone some form of reorganization, and that kindling of one structure usually facilitate the subsequent kindling of another site, thus enabling recruitment of an entire neural network with great facility (Goddard et al., 1969; Racine, 1972b; McNamara, 1984; Geinisman et al., 1988; Sutula et al., 1988). Such facile access to the kindled network from a secondary site is termed 'positive transfer', and is measured in kindled animals as a positive savings in the number of ADs needed to evoke generalized convulsions in a secondary site (Racine, 1972b; Spiller and Racine, 1994). For instance, animals previously or primary site kindled from the PRh displayed significantly more advanced behavioural seizures during the early stages of secondary site amygdaloid kindling than either amygdala controls or those partially PRh kindled (Buchanan and Bilkey, 1997).

This finding suggests that primary PRh kindling may facilitate secondary amygdala access to systems responsible for the generation of motor seizures. It is likely that the enhanced responsiveness in the kindled network may represent a strengthening of excitatory connections within secondary sites that are monosynaptically or polysynaptically connected to the primary site (Racine, 1972b; McIntyre and Racine, 1986).

The precise mechanisms of transfer kindling are not fully understood, however, it has been suggested that the rapidity of seizure development in secondary sites reflect activation of circuits established by primary site kindling (Burnham, 1975). The efficacy of kindling transfer is largely dependent on the site of provocation. For example, kindling of structure 'A' followed by 'B' may not be equivalent to activation of 'B' followed by 'A'. This is to say that the efficacy of kindling is not always bi-directional, and that the propagation of ADs and transfer kindling effects reflect the extent to which seizures arising in two distinct regions share common underlying mechanisms (Buchanan and Bilkey, 1997; Michalakis et al., 1998). Because of the complex and highly modifiable integration of local and distal circuits together with specific networks associated with kindling, it may prove difficult to accurately assess the spatio-temporal patterns of discharge activation, which may differ with different kindling sites (Spiller and Racine, 1994).

Synaptic Plasticity

Interneuronal communication has been ubiquitously recognized as the sole basis for the acquisition, integration and transmission of information in the mammalian brain. A

proper balance between excitation and inhibition tightly regulates the maintenance of this communication in order to prevent circuits from either falling silent or generating epileptiform activity. The efficacy of this balance is largely dictated by the continual activity- and experience-dependent changes in neural circuitry, particularly the regulation of inhibition through changes in synapse numbers or synaptic strength (Kilman et al., 2002). The term synaptic plasticity refers to long lasting changes in neuronal activity encompassing synaptic growth, activation of silent synapses, and change in the effectiveness of existing synapses (Bliss and Lomo, 1973; Majkowski, 1999). This plasticity provides the nervous system with the possibility to store new information, but if not adequately controlled, it also can lead to changes in the dynamics of neuronal networks (Goddard et al., 1969; Racine, 1972b). Various neurological disorders, like epilepsy, can result from an overly increased excitatory drive that can induce pathologic discharges in many neurons at the network level (Mody, 1999). In recent years, research into the mechanisms of epileptogenesis has begun to unravel several cellular and molecular neuronal alterations common to human TLE and the many experimental models of epilepsy (Schwartzkroin, 1994). Insights into the long-term modification of cellular networks at the level of single neurons, excitatory and inhibitory amino acid receptors, and ligand-gated ion channels may elucidate factors responsible for the epileptogenesis of kindling.

GABAergic Neurotransmission and Epilepsy

In 1881, Sir William Gowers had postulated, "abnormal discharges are due to potentiation of excitatory mechanisms or to a failure of intrinsic cerebral inhibitory

systems". Today, the very same principle is the basic dogma of epileptogenesis. Accounting for 60-70% of all synapses, GABA, the key inhibitory neurotransmitter in the central nervous system (CNS), serves to maintain the inhibitory tone that counterbalances neuronal excitation (Treiman, 2001). When this balance is perturbed, seizures are likely to ensue. Localized primarily in short-axon interneurons that synapse on cell bodies and proximal axons, GABA formation by transamination of α-ketoglutarate to glutamic acid and subsequently to GABA is released into the synaptic cleft via two routes. One way is through vesicular release, which is calcium dependent, sensitive to tetanus toxin, and triggered by high potassium concentrations. The other method of GABA release is non-vesicular, whereby any efflux of GABA is calcium independent and occurs secondary to depolarization of the cell membrane and sodium influx (Treiman, 2001). Upon its release and following ligand gated binding to several types of GABA receptors (described below), GABA is rapidly removed by uptake into both glia and presynaptic nerve terminals, where it is further broken down and recycled. In the CNS, the efficacy of synaptic connections between neurons is modulated through a variety of mechanisms that affect the coupling between a presynaptic impulse and transmitter release at individual terminals (Behrends and Bruggencate, 1998). Recently, it has been shown that various neurotransmitters exert their synaptic actions through heterosynaptic interactions between different synaptic inputs converging on single target neurons (Yamada et al., 1999). Such interactions have been shown to involve the GABAergic system and that GABA can not only activate postsynaptic receptors, but also spill over synaptic gaps, thereby gaining access to non-synaptic targets (Yamada et al., 1999). This functional multiplicity further

illustrates the complexity and plasticity of the neurotransmitter systems regulating synaptic transmission.

It is well established that the use of excitatory amino acids, such as glutamate and aspartate, and the inhibitory amino acid GABA are widespread in the CNS. Since the 1960s, many convulsant drugs that were routinely used to generate experimental epilepsy were in fact antagonists of GABAergic synaptic transmission (Thompson, 1994). In view of these findings, the quest for numerous, more efficacious clinical antiepileptic drugs had begun. This brought forth an intensive investigation into the mechanisms of inhibitory transmission in the brain, and subsequently gave rise to the enduring hypothesis that a dysfunction of the GABA system is likely the fundamental cause of human epilepsy (Kapur et al., 1989; Olsen et al., 1999). Consequently, research into the role of GABAergic mediated inhibition in epilepsy has focused on five potential targets for therapeutics. They are: 1) GABA synthesis, 2) GABA release, 3) GABA transport and reuptake, 4) $GABA_A$ receptors, and 5) $GABA_B$ receptors (Olsen and Avoli, 1997). In this regard, our current understanding of GABA in epilepsy and epileptogenesis can be realized by its clinical and experimental findings: 1) genetic and acquired animal models of epilepsy display abnormalities of GABAergic function; 2) reductions in GABA-mediated inhibition and GABA levels as detected by microdialysis have been reported in studies of human epileptic brain tissue; 3) GABA agonists suppress seizures, while GABA antagonists facilitate seizures; 4) drugs that inhibit GABA synthesis induce seizures; 5) benzodiazepines and barbiturates enhance GABAergic-mediated inhibition; 6) drugs that increase synaptic GABA by inhibiting GABA catabolism, e.g. vigabatrin or tigabine are effective anticonvulsants (Treiman, 2001). A more thorough knowledge of

GABAergic processes will be required for understanding a wide variety of neuronal functions, including the pharmacology of GABA subunits, the integration of spatially and temporally patterned inputs, the coupling activity to learning-related changes in synaptic efficacy, and maintenance of the intricate balances between excitation and inhibition that are required for optimal function, while minimizing the risk of seizure activity (Kapur et al., 1997).

$GABA_A$ Receptor – Molecular Biology and Function

The ability of brain tissue to undergo specific structural or functional alterations is an essential property of the CNS (Malenka and Nicoll, 1999). Upon subsequent release into the synaptic cleft, GABA exerts its actions via $GABA_A$ and $GABA_B$ receptors. The $GABA_A$ receptors are ligand-gated chloride channels that mediate rapid inhibition, and possess modulatory binding sites for benzodiazepines, barbiturates, and anesthetics, all of which potentiate the response to GABA (MacDonald and Olsen, 1994; Johnston, 1996). These receptors are heteromeric and are assembled from a large family of 5 subunits: α, β, γ, δ, and ϵ. However, most $GABA_A$ receptors in the CNS are thought to contain both α and β subunits, with one or more of the γ, δ, and ϵ subunits. The many variations even within 1 subunit (e.g., α1, α2, α3, etc.) determine its pharmacological properties (Costa, 1998; Delaney and Sah, 1999). In general, the α subunit is critical for determining the sensitivity and pharmacological properties for benzodiapezines, while the β subunit appears to be important for determining the affinity of the receptor for GABA, the rate of desensitization, and the cooperativity of GABA binding (Sigel et al., 1990; Thompson, 1994).

The etiology of epileptic seizures is difficult to pinpoint, but often it is an acquired disorder, resulting from changes in the brain that are environmentally induced. $GABA_A$ receptor function can be persistently impaired in response to over-activity resulting from drugs, stress, trauma or kindling (Barbaccia et al., 1996). Because these particular receptors are often the molecular targets of convulsants and anticonvulsants, any impairment in $GABA_A$ receptor function has been hypothesized to play a role in seizure disorders. For example, an activity-dependent reduction in GABAergic inhibition has been observed in animals exposed to GABAergic drugs, such as benzodiazepines, barbiturates and alcohol (Hablitz et al., 1989; Kapur and Macdonald, 1997; Zeng and Tietz, 1999). Prolong tolerance to these drugs associated with abrupt cessation, facilitate seizures, similar to a kindling-like state. Thus, chronic administration of GABA enhancing drugs to animals produce hypofunctional $GABA_A$ receptors, and most likely lead to alterations in subunit mRNA expression (Tseng et al., 1994). Other mechanisms of epileptogenesis could involve a $GABA_A$ receptor "subunit switch" resulting in an over-expression of a particular type of receptor isoform (Banerjee et al., 1998). It has also been suggested that epilepsy may be caused by a mutation in a $GABA_A$ receptor gene, an unexpressed $GABA_A$ receptor, or a mutated gene for a product that regulates a $GABA_A$ receptor (Olsen et al., 1999). Recent reports have demonstrated unique subtype-specific expression of $GABA_A$ receptors with differential rearrangement of the $\alpha 1$, $\alpha 2$, $\alpha 3$ subunits in various cell populations of the human hippocampus (Loup et al., 2000). The significant reorganization of distinct receptor subtypes in surviving hippocampal neurons of TLE patients with hippocampal sclerosis underlines the potential for synaptic plasticity in the human GABA system.

Despite much evidence in support of $GABA_A$ receptor impairment in epileptogenesis, there exists some controversy over whether reductions in synaptic inhibition are a necessary component for the expression of hyper-excitability. Thus, the amplification of excitatory activity as a result of a reduction of inhibition in the epileptic foci, due to loss of GABAergic interneurons, or a reduction of GABA release or its effectiveness at its receptors, remains controversial (Bradford, 1995). Numerous obstacles may exist in an attempt to fully elucidate a deficit of GABAergic inhibition in epileptic tissue, including the multiplicity of GABAergic inhibitory pathways and the multiplicity of variables that characterize the potency of inhibition within each inhibitory pathway (Bernard et al., 2000). Nevertheless, further research into GABAergic-mediated inhibition in various regions of the brain coupled with molecular approaches to uncover subunit expression may provide important clues to understanding the mechanisms that initiate and regulate seizure genesis.

$GABA_A$ Receptor and Kindling

The generation of epileptic discharge, synchronized burst firing and interictal spikes (IIS), can be a consequence of alterations of $GABA_A$ receptor function. A result of GABAergic disinhibition is the formation of new polysynaptic pathways leading to networks of neurons that were previously not connected (Bureau et al., 1997). This type of plasticity within the CNS in association with cell loss is common in most patients with TLE. Epileptiform activity has been associated with lasting activity-dependent decreases in inhibition (Schwartzkroin, 1994). Yet, following chronic epilepsy induced by kindling, an *increase* occurs in paired pulse inhibition in the dentate gyrus that may last

for a few weeks (Tuff et al., 1983; Shin et al., 1985) with a concomitant increase in $GABA_A$ receptor binding and function (Otis et al., 1994). However, this increased inhibition ultimately decays with the absence of seizures. In contrast to results obtained from the dentate gyrus, $GABA_A$ receptor binding and function were reportedly *reduced* in the CA1 pyramidal cells (Kapur et al., 1989; Kamphuis et al., 1992; Lopes da Silva et al., 1995). Therefore, it is clear that a divergence of $GABA_A$ receptor-mediated inhibition exists even within one structure (CA1 vs. dentate gyrus of the hippocampus) after kindling.

The highly debated issue regarding the decrement of GABA release has been proposed as a basis for disinhibition in TLE, which is partially characterized by a loss of glutamate-stimulated GABA release that is secondary to a reduction in GABA transporters (During et al., 1995; Ueda and Willmore, 2000). This reduction of transporters in human TLE and in kindled animals could also be interpreted as part of a compensatory mechanism aimed at enhancing the efficacy of inhibition (Meldrum, 1995). These results support the more general concept of a loss of inhibition in chronic epilepsy models and probably in human epilepsies. To this end, synaptic $GABA_A$ receptors undergo significant structural reorganization that results in an increase in their numbers, and possibly their subunit composition. The altered properties of $GABA_A$ receptors as a result of kindling have been demonstrated to differ from those in control neurons. Such differences should contribute to understanding the processes whereby receptors or ion channels aid in the generation of epileptic discharges.

$GABA_B$ Receptor – Function

Located both presynaptically and postsynaptically, $GABA_B$ receptors (unlike $GABA_A$ receptors) are G-protein-linked receptors that mediate the late inhibitory postsynaptic potential, and thus hyperpolarize the neuron by increasing potassium conductance. In addition to activating potassium conductances, $GABA_B$ receptors have also been shown to inhibit calcium conductances in many types of neurons (Scholz and Miller, 1991; Swartz, 1993), and are further involved in presynaptic inhibition of neurotransmitter release (Doze et al., 1995; Olsen et al., 1999). The synaptic processes mediated by $GABA_B$ receptors have not been studied in great detail, however, it has been suggested that synaptically released GABA is believed to spill over and activate presynaptic $GABA_B$ receptors and transiently reduce the amount of GABA release during subsequent stimulation (Deisz and Prince, 1989; Davies et al., 1990; Jensen et al., 1999). The debate of whether $GABA_B$ receptor-mediated presynaptic inhibition is through modulation of potassium and/or calcium conductances remains controversial. Some reports have proposed that inhibition is mediated through potassium channels (Yoon and Rothman, 1991; Thompson and Gahwiler, 1992), while others proposed a mechanism involving direct modulation of calcium channels (Isaacson, 1998; Takahashi et al., 1998). However, other experiments indicate that $GABA_B$ receptors exert a novel action at excitatory synapses, independent from either potassium or calcium conductances, suggesting that the modulation of transmitter release does not necessarily require decreases of presynaptic calcium currents (Scanziani et al., 1992). Nevertheless, some, if not all, of the mechanisms of $GABA_B$ receptor-mediated presynaptic inhibition appears to be located at the terminal (Doze et al., 1995). The mediation of $GABA_B$ presynaptic

inhibition at the terminal follow three hypotheses: 1) $GABA_B$ receptor activation may directly modulate neurotransmitter release "downstream" from calcium channels (Scholz and Miller 1992; Silinsky and Solsona, 1992); 2) $GABA_B$ receptors may directly inhibit calcium currents necessary for action potential dependent release (Deisz and Lux, 1985; Scholz and Miller, 1991); 3) $GABA_B$ receptor activation of one of several potassium conductances may indirectly reduce calcium currents that are involved in transmitter release.

$GABA_B$ Receptor and Kindling

During GABAergic transmission, GABA can act presynaptically to regulate further release by binding and activating $GABA_B$ receptors (Diesz and Prince; 1989; Misgeld et al., 1995). It has been proposed that activation of postsynaptic $GABA_B$ receptors has an antiepileptic effect; by contrast, preventing GABA release via activation of presynaptic $GABA_B$ receptors can promote seizures (Pitler and Alger, 1994). Apparently, kindling-induced epileptogenesis reduces the sensitivity of presynaptic $GABA_B$ receptors, an effect that may contribute to the enhancement of excitatory transmission in kindled animals. Evidence in support of these findings came from various studies using the $GABA_B$ receptor agonist, baclofen as a means to elucidate its electrophysiological, behavioural and electroencephalographic characteristics. For example, baclofen has been shown to significantly increase the ADT in the amygdala, and consequently retard the rate of kindling and its behavioural symptoms (Wurpel, 1994) with a concomitant mild increase in AD duration (Sato et al., 1990b; Karlsson et al., 1992). Further, $GABA_B$ receptor activation was demonstrated to have potent inhibitory effects on both ictal and interictal-

like events as evidenced by the application of high frequency trains in the CA3 region of the hippocampus (Morrisett et al., 1993). These results suggest that baclofen may decrease the local excitability of the amygdala and retard the rate of seizure spread (or generalization) throughout the brain.

Electrophysiological data from *in vitro* studies from the hippocampus have produced similar results that further substantiate the functional role of $GABA_B$ receptors in kindling epileptogenesis. Days following kindling, paired pulse depression of inhibitory currents in the CA1 were shown to be significantly smaller in kindled rats compared to controls, which was likely attributed to reduced GABA autoinhibition after downregulation of presynaptic $GABA_B$ receptors (Wu and Leung, 1997). This downregulation after seizures may serve to prevent more recurrent seizures. There is also a possibility that chronic epilepsy is not the direct result of the loss of inhibitory drive, but rather due to plastic changes attributed to spontaneous 'kindling', possibly facilitated by loss of $GABA_B$-mediated inhibition (Wasterlain et al., 1996). In addition, different pharmacological properties of pre- and postsynaptic receptors, most noticeably in the amygdala and hippocampus, suggest two distinct populations of $GABA_B$ receptors whose long-lasting responses to kindling-induced seizures are different. To this end, in attempt to evaluate a therapeutic approach, it may be useful to involve antagonism of $GABA_B$ receptors on GABAergic nerve terminals combined with a postsynaptic agonist effect on cell bodies of $GABA_B$ receptors.

Kindling-Induced *In Vivo* Changes of GABA Levels

The relation between changes in extracellular amino acids, particularly GABA, and seizures has been tested in a number of experimental models of epilepsy. However, the lack of consistent data in support of an increase or decrease of GABA levels is both puzzling and frustrating. Such discrepancies among various findings can easily be attributed to methodology, area of sampling, the sensitivity of the equipment, or simply to the intricacies of the living brain. In an effort to study GABA levels as a consequence of kindling, several investigators have employed the microdialysis technique. 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, but when fully kindled, reached a seven-fold increase. This increase in GABA content in a kindled animal is thought to reflect its elevated metabolism during the induced hyperactive state (Kaura et al., 1995). The augmented inhibitory synaptic transmission may result from presynaptic factors such as increased transmitter release, or from the postsynaptic up-regulation of $GABA_A$ receptors (Kokaia et al., 1994; Otis et al., 1994). On the other hand, other investigators have indicated that the ratio of glutamate to GABA is significantly higher in fully kindled animals than partially kindled animals, thus supporting a kindling-induced deficit of GABA levels (Lothman et al., 1987; Buhl et al., 1996). In addition, Loscher and Schwark (1985, 1987) have reported decreases in GABA levels and disturbance of GABA metabolism that would be expected to be associated with a smaller GABA neurotransmitter pool in kindled brains. It is also possible that a reduction in GABAergic neurons or nerve terminals of a kindled rat could account for losses of GABA content (Callahan et al., 1991). The decay of GABAergic neurons was not limited to the

amygdala or hippocampus in response to amygdala kindling, but extended to include the PC, a structure already established to be highly susceptible to kindling and propagation of seizure activity (Lehmann et al., 1998). Thus, 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.

A Need for Genetic Models of Epilepsy

Unraveling the many mysteries of the brain has provided invaluable information into the physiological, anatomical and biochemical constituents that govern human behaviour. With the widespread availability of molecular and genetic techniques in recent decades, the possibility of developing new insight into the mechanisms responsible for epileptogenesis and various mental disorders was overwhelmingly optimistic. In an effort to better understand the genetic basis for many types of epilepsy, a number of animal models have been developed. Of these, two new strains of rats were developed through selective breeding of a Long Evans Hooded and Wistar rat cross for either fast or slow rates of amygdala kindling, culminating in a seizure-prone (Fast) and seizure-resistant (Slow) strain (Racine et al., 1999). The strains were designed as an animal model to test the hypothesis that there is a genetic predisposition for and against the development of complex partial seizures in humans (McIntyre et al., 2002a).

The apparent differences in the Fast and Slow strains are represented not only in their kindling rates (for which they were selected) but also in their local AD characteristics. This latter finding suggests that differences in innate seizure mechanisms

can operate at several levels, involving both local and distant networks (McIntyre et al., 1999). Further, it is interestingly that these two strains, aside from their kindling characteristics, show differential sensitivity to GABA modulators, such that the Fast rats are more sensitive than the Slow rats to negative modulators like bicuculline and picrotoxin, whereas the Slow rats are more sensitive to positive modulators such as pentobarbital and diazepam (McIntyre et al., 2002a).

As previously mentioned, epilepsy may arise from an alteration in the normal functioning of the GABA system, particularly involving $GABA_A$ receptor function, and specific $GABA_A$ subunit differences that may contribute to functional changes. In this regard, differences in $GABA_A$-mediated inhibitory neurotransmission in various genetic models may elucidate predispositional factors in the development of TLE. To further explore this possibility, Poulter et al. (1999) have shown that the genetically seizure prone Fast rats highly expressed the $\alpha 2$, $\alpha 3$, and $\alpha 5$ $GABA_A$ subunits compared to controls, while the $\alpha 1$ subunit was significantly under-expressed. Conversely, the seizure resistant Slow rats showed a significant under-expression of the $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits, but an over-expression of the $\alpha 1$ compared with controls and Fast rats. The composition of the $GABA_A$ α subunit receptors is differentially expressed throughout development. For example, the $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits (over-expressed in Fast rats) are highly expressed early in development, but diminish substantially in adulthood and are replaced by the $\alpha 1$ subunit (over-expressed in Slow rats) (Poulter et al., 1992; McIntyre et al., 2002b). Consistent with this theme, the pattern of subunit expression in the seizure prone Fast rats is one of immaturity (juvenile), whereas the seizure resistant Slow rats exhibit a mature (adult) subunit expression. The significance of these findings speaks strongly to a

stunted development of the $GABA_A$ receptor system in the Fast rats and this may be a critical underlying molecular correlate to the Fast kindling phenotype (Poulter et al., 1999). Consistent with this hypothesis, it has been suggested that brain trauma/injury causes a re-expression of embryonic GABAergic subunits, leading to increased seizure susceptibility (Brooks-Kayal et al., 1998). Such explorations into the differences between the two kindling rat strains hopefully will provide insight into possible mechanisms underlying epileptogenesis.

Physiology of GABAergic Neurons

Our knowledge of intrinsic membrane properties of single cells is vast. However, the mechanisms by which neuronal networks operate in synchrony are less clear. Nevertheless, electrophysiological research in recent decades has generated a plethora of studies in attempt to provide a greater understanding of synaptic inhibition. This in turn will undoubtedly lead to more promising developments of better, more selective drug treatments for epilepsy and other disorders.

The physiological response patterns of neurons typically arise from two broad categories of cell types: principal cells and interneurons. Given the importance of GABAergic inhibition in normal and pathological conditions, it seems crucial to better understand the properties of individual synapses made by various types of GABAergic interneurons and principal cells. The ability of these cells to communicate with each other to form a well-adapted pattern of activity is often identified in the recruitment of neuronal networks in epileptic tissue. Differences between principal cells and interneurons may be distinguished not only on the basis of their morphology, but also on

the basis of their obvious physiological properties. Interneurons have the ability to maintain a high rate of action potential discharge during sustained depolarizing current injection, and have only brief after-hyperpolarizations following trains of action potentials (Thompson, 1994). Pyramidal cells (a type of principal cells), in contrast, display adaptation in their firing rate during similar depolarization, and have a small after-hyperpolarization following single action potentials, but a pronounced after-hyperpolarization following trains of action potentials (Thompson, 1994). Synaptically, GABAergic interneurons receive both excitatory glutamatergic and inhibitory GABAergic synaptic inputs. Direct excitatory inputs arise from both afferent fibers, mediating 'feedforward' inhibition, and from recurrent axon collaterals of principal cells, mediating 'feedback' inhibition (Ribak, 1992; Bradford, 1995; Douglas et al., 1995). This modulation of convergent inputs allows for the integration of information with different processing capabilities. The extensive connectivity among interneurons through $GABA_A$ synapses of various properties may underlie complex network oscillations at different frequencies.

It has been suggested that interneurons are thought to provide the necessary timing mechanism for both low and high frequency firing of principal cells (Bragin et al., 1995; Cobb et al., 1995; Jefferys et al., 1996). This is achieved by forming a divergent synaptic arrangement on principal cells that permits the synchronization of a large number of these neurons to initiate neuronal network oscillations that appear to be essential for memory processing and seizure genesis (Lupica et al., 2001). Such interneuronal networks have the ability to sustain a range of oscillation frequencies without any requirement for fast excitatory synaptic drive, and by sustaining such oscillations, may participate in higher

cognitive functions (Hajos and Mody, 1997). It is likely that these oscillations are dependent on the kinetic properties of $GABA_A$ receptor-mediated events in interneurons and on the nature of the connectivity among them.

Inhibitory Postsynaptic Currents and Potentials

Voltage-dependent channels are ultimately responsible for regulating the amount or pattern of neurotransmitter released from neurons. However, the contributions that these ion channels make to the process of neurotransmitter release in most neuronal networks are not completely understood. Synaptic communication between neighboring and distant cells is dependent on the efficacy and strength of their connections. In this regard, the strength of synaptic inhibition at GABAergic terminals is determined by a number of factors. First, the receptors opposite each bouton are thought to be saturated by the release of a single vesicle of transmitter, so that the number of available receptors for GABA binding rather than the amount of transmitter released determines the quantal amplitude of the response (Jonas et al., 1993; Nusser et al., 1997; Ling and Benardo, 1998). Second, the number of active zones per synapse and the time course of the inhibitory postsynaptic current (IPSC) are largely determined by the receptor kinetics and the release process (Glavinovic and Rabie, 1998; Wierenga and Wadman, 1999).

GABA-mediated IPSCs and inhibitory postsynaptic potentials (IPSPs) have been widely investigated in mammalian systems using the slice preparation from the hippocampus (Barker and Harrison, 1988; Kapur et al., 1997; Isokawa, 1998), amygdala (Smith and Dudek, 1996; Shoji et al., 1998; Delaney and Sah, 1999; Yamada et al., 1999), PRh (Ziakopoulos et al., 2000; D'Antuono et al., 2001) and PC (Libri et al., 1998;

Patil and Hasselmo, 1999). The heterogeneity of GABAergic responses is both intricate and elaborate. Several inhibitory circuits may exist in which different interneuron populations communicate with principal cells via biochemically/pharmacologically distinct receptors. Thus, spontaneous currents and evoked potentials generated at synapses on or near the soma of pyramidal and interneurons provide an excellent opportunity to employ whole cell patch clamping techniques to determine the time course and amplitude of the underlying inhibitory currents that govern receptor channel opening. There is evidence to suggest that $GABA_A$ mediated IPSCs are generated in apical dendrites as well as cell bodies, and that $GABA_A$ inhibition consists of fast and slow components with a greater proportion of the slow component residing in distal dendrites than cell bodies (Kanter et al., 1996). For example, within the pyramidal layer of the hippocampus, IPSCs evoked by minimal stimulation of principal neurons ($GABA_{A,fast}$) have been shown to maintain rapid rising and decay kinetics, whereas GABA currents evoked in more distal dendritic layers ($GABA_{A,slow}$) are slow to rise and decay (Banks et al., 1998). The kinetics of these evoked currents is differentially expressed because they are produced by receptors with different kinetic and pharmacological properties. The time course of IPSCs is partly determined by the concentration of neurotransmitter acting on postsynaptic receptors and by the kinetics of $GABA_A$ receptors. Thus, slower time course of $GABA_{A,slow}$ suggests an extended presence of neurotransmitter or slow diffusion of transmitter to extrasynaptic receptors (Lambert et al., 1996). These findings are in agreement with other studies suggesting that inhibitory dendritic conductances are subjected to greater filtering than in or near the soma (Hausser, 2001). This in turn will lead to a prolongation of IPSC rise and decay times than their proximal counterparts

(Ling and Benardo, 1998). To this end, inhibition mediated by the slow $GABA_A$ conductance component appears to be more effective than the fast $GABA_A$ component for regulating excitatory responses, especially of the N-methyl-D-aspartate (NMDA) type (Kapur et al., 1997).

One of the more studied tools for investigating synaptic inhibition has been the technique of pharmacologically isolated monosynaptic IPSPs. These IPSPs are obtained by blocking all excitatory amino acid receptors with high concentrations of NMDA and non-NMDA receptor antagonists, such as AP5 and CNQX/DNQX, sufficient to eliminate feedforward and feedback excitation of interneurons (Thompson, 1994). These manipulations allow for direct activation of cell bodies and/or axons, which can subsequently trigger GABA release without altering the excitation of the interneuron.

Since normal functioning of the CNS is dependent on relative strengths of excitatory and inhibitory events, any limitations on the magnitude of fast and slow inhibition could have significant consequences that lead to excitable states such as epilepsy. One issue that remains to be elucidated is whether fast and slow inhibition is mediated presynaptically, due to a limit on GABA release from inhibitory interneurons, or postsynaptically, consequent to saturation of $GABA_A$ and $GABA_B$ receptors on principal cells (Ling and Benardo, 1998).

Objectives and Outline of Proposed Study

Kindling-induced epileptogenesis can involve a number of mechanisms interacting in synchrony to permit the brain to manifest a seizure, which is the hallmark of epilepsy. In this regard, differential expression and subunit arrangement of the GABA receptor

complex have been hypothesized to underlie various profiles of GABAergic inhibition, including synchronization and oscillation patterns of neural networks. In keeping with this presumption, any disruption or failure of GABA-mediated inhibition may result in the abnormal expression of certain GABA receptor subunits leading to various neurological disorders such as epilepsy. This phenomenon has been demonstrated in a number of epileptic models whereby the efficacy of inhibitory synaptic transmission is often compromised leading to a heightened excitation and/or a hypo-functional inhibition. The purpose of this thesis is to better understand GABAergic-mediated inhibitory processes, particularly through $GABA_B$ receptors, in two rat strains that have a predisposition either for or against epilepsy. These strains were created for their rate of amygdala kindling development, and so properly named Fast (seizure prone) and Slow (seizure resistant) kindling rats.

The thesis is divided into three separate experiments designed to delineate and further understand $GABA_B$ receptor function in naïve, as well as, kindled Fast and Slow rat strains. In vivo assessments of $GABA_B$ receptor involvement during kindling development, and in the kindled state, are measured by changes in afterdischarge threshold, its duration, and kindling rates. Localization of these receptors within the amygdala complex will provide important information regarding the abundance and distribution of local circuits and inhibitory networks. From this, we can further study the physiological properties of individual cells, as they provide clues regarding ion channel conductivity, input resistance, and firing patterns. All recordings were taken from the basolateral amygdala (BLA), which is known to be an important structure involved in the initiation and propagation of seizure activity. The experiments provide novel insight into

the factors responsible for seizure initiation, maintenance and termination in seizure prone versus seizure resistant phenotypes.

The first of these experiments examined the chronic effects of a $GABA_B$ antagonist, SCH 50911, on kindling epileptogenesis of the Fast and Slow rats. There is ample evidence to suggest that $GABA_B$ receptors may play a modulatory role in the maintenance of seizure activity. We wished to assess how $GABA_B$ pharmacology has an impact on the development of such seizures. Furthermore, in the kindled rat, a comparison between systemic administration and local infusion of GABAergic agents was made to assess the influence of one system (focal vs. distal networks) over the other. Additional $GABA_A$ and $GABA_B$ agonists and antagonists administered to kindled Fast and Slow rats alone or in combination were used to compare the impact of these pharmacological agents on amygdala ADT and duration.

The second experiment focused on the identification and localization of $GABA_B$ receptors in the amygdala using immunocytochemical techniques. It is important to characterize the distribution of these receptors, as their location within the synaptic terminal or on dendritic spines (extrasynaptically) determine their functionality, including their binding affinities and subunit composition. This technique was applied to identify $GABA_B$ receptors from both naïve and kindled brains of the Fast and Slow rats.

Finally, the last experiments examined the kinetics and other electrophysiological properties of principal cells within the BLA from an *in vitro* preparation. This is the first attempt to examine the intrinsic properties, synaptic currents and potentials, and conductance underlying $GABA_B$ receptors of the Fast and Slow strains. With the use of various $GABA_B$ receptor agonists and antagonists, we sought to gain new insights into

the mechanisms that govern GABA release, and the efficacy, as well as the magnitude of $GABA_B$-mediated synaptic transmission.

GENERAL METHODS AND MATERIALS

The treatment of animals was conducted in accordance with the guidelines of the Canadian Council on Animal Care and all protocols were approved by the Carleton University Animal Care Committee.

Animals

Originally developed from an outbred parent population of Wistar and Long-Evans hooded cross, the seizure prone (Fast) and seizure resistant (Slow) rat strains were selectively bred for their differential rates of amygdala kindling. Housed in the Life Sciences Research Building of Carleton University, 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.

Experiment #1 – Kindling with Microinfusion and Systemic Administration of GABAergic Agents

Synopsis

In recent years, an evaluation of a possible role of $GABA_B$ receptor involvement in kindling epileptogenesis has ignited an exciting area of pharmacological and electrophysiological research. In this regard, the exploration of a number of GABAergic

agents has provided new insights into possible mechanisms that mediate local and global recruitment of epileptogenic circuits, which if sufficiently activated can easily trigger seizure onset. In the BLA, administration of the $GABA_B$ agonist, baclofen, has been shown to significantly increase ADTs, shorten AD duration, and retard the rate of kindling in rats, suggesting that postsynaptic activation of these receptors has an anticonvulsant/antiepileptic effect (Sato et al., 1990b; Wurpel, 1994). However, only a handful of investigators have examined the chronic effects of $GABA_B$ antagonists on ADT, duration and kindling rates. To this end, the purpose of the current experiment was to determine the long-term behavioural and electrographical effects of daily administration of the $GABA_B$ antagonist, SCH 50911, on amygdala seizure threshold, duration, and kindling rates.

The important points addressed in this experiment are simply to assess whether $GABA_B$ receptors are involved in seizure susceptibility, and determine if the antagonist would accelerate kindling by lowering its threshold for seizure generation. If so, then activation of $GABA_B$ receptors would likely cause a disturbance or alteration in the seizure network. We would predict that our Fast and Slow kindling rat strains may show increased seizure susceptibility by exhibiting a lowered ADTs, increased AD durations, and decreased kindling rates, perhaps more pronounced in the Fast rats. Further, differential expression of these characteristics between the strains may suggest that $GABA_B$ receptor activation may be inherently different. To further assess potential differences that may exist between the Fast and Slow rats, it was necessary to examine their electrophysiological properties using an *in vitro* preparation. Experiment #3 will address this issue. The answers brought forth from Experiment #1 will undoubtedly

provide valuable clues regarding $GABA_B$ receptor contribution and sensitivity to epileptogenesis.

METHODS AND MATERIALS

Animals

Originally, 15 Fast and 15 Slow male rats underwent surgery for electrode and cannula implantation. Due to unforeseen circumstances during the experiment such as lost of head cap and plugged cannula, 4 Fast and 4 Slow rats were removed from the experiment.

Drugs

(2*S*)-(+)-5,5-Dimethyl-2-morpholineacetic acid, *SCH 50911*; (*R*)-4-Amino-3-(4-chlorophenyl) butanoic acid, *(R)-Baclofen*; 5-Aminomethyl-3-hydroxyisoxazole, *muscimol*; 1:1 mixture of picrotoxinin and picrotoxin, *picrotoxin*; 5-Aminomethyl-3-hydroxyisoxazole, *muscimol* were purchased from Tocris, MO. (*RS*)-4-Amino-3-(4-chlorophenyl) butanoic acid, *(RS)-Baclofen* was purchased from Sigma (St. Louis, MO.).

Surgery

Under sodium pentobarbital anesthesia (65mg/kg, i.p.) a single Nickel-Chromium stimulating/recording bipolar electrode of 127 μm diameter wire was implanted into the left BLA of adult male Fast and Slow rats (250-350g). A second electrode was glued

alongside a 26G steel cannula with its tip extending 0.5 mm more ventral than the tip of the cannula. Together, the cannula/electrode guide was implanted into the right BLA. An obturator capped the cannula until the first day of pharmacological infusions. Implantations were performed using a Krieg stereotaxic with coordinates determined from the rat brain atlas of Pellegrino et al. (1979). The following stereotaxic coordinates relative to bregma were used for both the single electrode and electrode/cannula: A -0.0; L +4.5; V -8.5 mm. Depth measurements were made from the dural surface. The cannula and electrodes were anchored to the skull by 7 jeweler's screws and a covering of dental cement. The Amphenol pins from the electrodes were inserted into a 9 pin 'McIntyre head plug'. All subjects were allowed 5-7 days of postsurgical recovery before the beginning of the experiment.

Kindling and Drug Administration

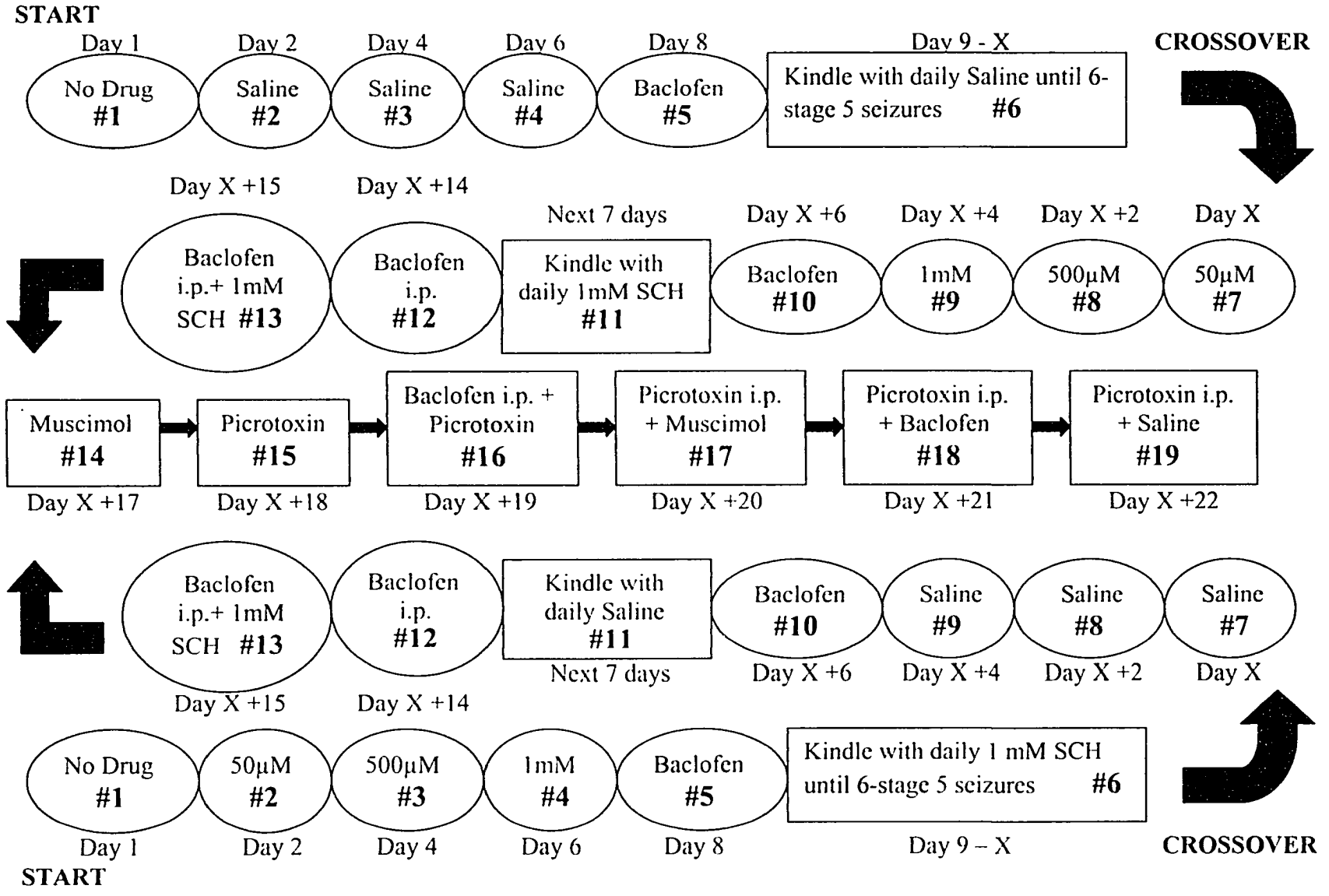
On day 1 of the experiment, a subthreshold electrical stimulation (60 Hz sine wave, 2s duration) was presented to the right amygdala (associated with the cannula) every two minutes with increasing intensity until an AD was elicited. Stimulation intensities were delivered in the ascending order of 15, 25, 35, 50, 75, 100, 150, 200, 300, and in 100 μA increments thereafter. An AD was defined as a traditional spike-and-wave discharge that outlasts the stimulus by 2 or more seconds. Upon triggering an AD, its duration was recorded and the behavioural activity characterized by Racine's amygdala kindling 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. This initial

baseline AD assessment on Day 1 was in the absence of SCH 50911 or saline infusions via the cannula.

The design of the study then separated the rats into 2 basic groups for the rest of the experiment: control (saline) and experimental (drug, SCH 50911) groups (and of course Fast and Slow strains). Please refer to Figure 1 for the complete timeline of drug administrations and kindling schedules. A bolded number for each manipulation and the corresponding day are labeled for easy reference throughout the results section. Starting on day 2, and on every other day for the next 2 testing trials, rats in the experimental group experienced microinfusion into their right amygdala (one dose increment for each testing day) of SCH 50911 (50 μM, 500 μM or 1 mM), while the control group received only the vehicle, saline -- delivered via a 10 μL Hamilton syringe. ADTs were re-determined on those 3 testing days 10 min after the end of infusion. The infusion needle was left attached to the cannula apparatus for 5 additional minutes following the cessation of pharmacological administration. All drugs were dissolved in sterile 0.9% saline and delivered at a flow rate of 0.2 μL/min for a total of 7.5 min, amounting to a total volume of 1.5 μL. Following this assessment, a single dose of 50 μM of (R)-baclofen was infused into the amygdala and the ADT re-assessed. Daily kindling was begun the next day using the 1 mM dose SCH 50911 (experimental group) or saline (control group). In order to easily follow changes in ADTs over time, each rat was stimulated on each daily trial using an intensity one increment lower than the one that was required to elicit the AD on the previous day. If an AD was triggered, the stimulus intensity and duration of the AD were recorded. If no AD was elicited, the intensity

Figure. 1 – Complete timeline of kindling and drug administration schedules for Fast and Slow rats. At the top and bottom left corner labeled "start", two individual groups received either saline or SCH 50911 treatment over the course of kindling. The numbers in bold represented individual treatments for easy reference and identification (also indicated in the results section). Treatments **#2, #3, #4** (before crossover), and **#7, #8, and #9** (after crossover) represented increasing dosages of SCH 50911. All drug administration without "i.p." as a suffix was delivered via microinfusion into the amygdala. For treatment **#6**, the "X" in Day 9 –X represented the number of days for the rat to acquire 6 stage-5 seizures (varies from rat to rat). Day X (treatment **#7**) was the first day after the 6^{th} stage-5 seizure (crossover). Notice all rats were pooled into 1 treatment group at the start of $GABA_A$ manipulations (treatment **#14**).

Note: SCH = SCH 50911



was increased one increment until an AD was produced. Thus changes in ADTs during the kindling process were detected using the lowest stimulus intensity possible without unnecessary stimulations. This procedure continued until 6 stage-5 seizures were accumulated. One day after the last stage-5 seizure, there was a crossover of the drug (SCH 50911) and saline conditions. That is, the animals that previously received SCH 50911 during kindling now were infused with only saline (vehicle). Similarly, the animals that previously received saline infusions were switched over to 3 SCH 50911 treatments (3 doses as in the original assessment for the drug group). On this day, and on every other day for the next 2 testing days, the ADT was *re-assessed* from the lowest intensity until an AD was elicited. This was followed by a single (R)-baclofen infusion as before kindling. Then for the next 7 days, the identical procedure in ADT assessment as described during kindling was followed (described above, including 1 mM SCH 50911 or saline as used during initial kindling). Thus, the crossover treatment applied to fully kindled rats (both groups) lasted a total of 10 days. After this time, the treatments applied to the two original groups (experimental and control) were *identical* (see Figure 1) and continued with single treatments of *systemic* or i.p. (RS)-baclofen (5 mg/kg) administered on one day, and *systemic* (RS)-baclofen coupled with 1 mM SCH 50911 cannula infusion on the following day to all rats. These two treatments assessed the influence of $GABA_B$ mechanisms on the broad kindled network and its interface with the kindled focus, respectively, in both strains. In accordance with the literature, a baclofen dosage of 10 mg/kg was initially used in this experiment. However, a few animals became extremely lethargic and eventually died. Therefore, the empirical dosage for systemic baclofen was decided at 5 mg/kg.

To assess several other important questions, all kindled rats were subjected to various $GABA_A$ agonists and antagonists delivered via microinfusion or systemic injection during the next 5 days. The control and experimental group were pooled for these manipulations. The compounds were given in the following sequence on separate days: 1) $GABA_A$ agonist, muscimol (10 mM) by focal infusion; 2) $GABA_A$ antagonist, picrotoxin (500 μM) by focal infusion; 3) (RS)-baclofen systemically with focal picrotoxin infusion; 4) picrotoxin (0.5 mg/kg) systemically with focal muscimol infusion; 5) picrotoxin systemically with focal (R)-baclofen infusion. The final trial consisted of systemic picrotoxin with a focal saline infusion. As described earlier, ADT re-assessment was performed 10 min following focal muscimol, picrotoxin, and baclofen infusions. However, following systemic baclofen and picrotoxin administration, ADTs were re-determined 60 and 20 min, respectively. At the completion of the experiment, the animals were subsequently perfused intracardially with 0.9% saline, followed by 4% paraformaldehyde. Brains were later exposed to 30% sucrose solution for 5 days before sectioning on a freezing microtome in 40 μm sections. This was followed by Cresyl violet staining to confirm electrode and cannula placements.

RESULTS

$GABA_B$ Manipulations - Effects of SCH 50911 on Afterdischarge Thresholds and Afterdischarge Durations Early in Kindling, i.e., *Partially Kindled* Rats

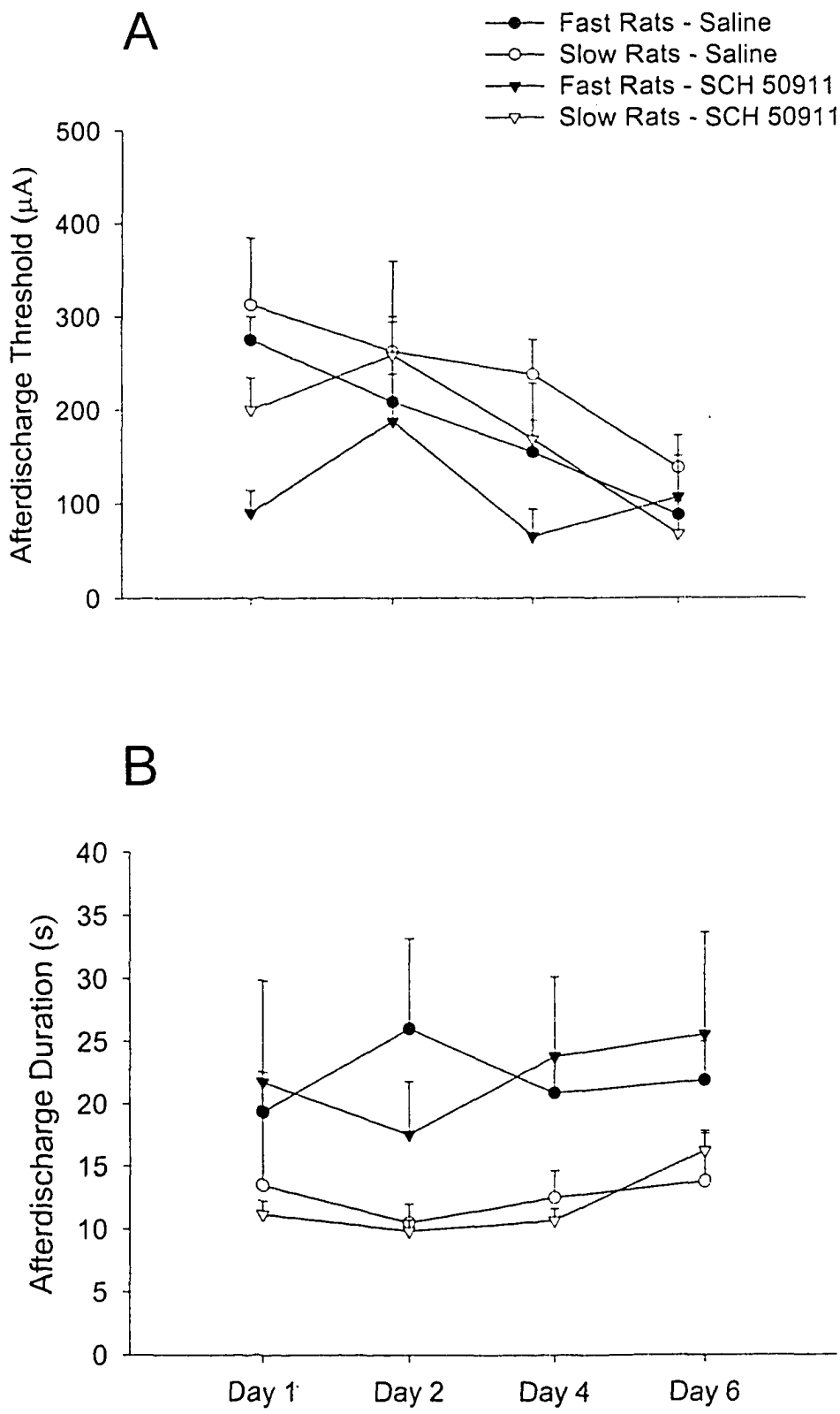
Afterdischarge Thresholds (ADTs)

Three increasing dosages (50 μM, 500 μM, 1 mM) of the $GABA_B$ receptor antagonist, SCH 50911, or saline were administered through an infusion cannula on every other day following the initial ADT assessment (see Figure 1 – timeline for treatment numbers). The examination of the initial ADT trial (Day 1, treatment **#1**) in the absence of infusion compared to the ADT on the first SCH 50911 dose (50 μM, treatment **#2**) or saline administration (Day 2, treatment **#2**) revealed no significant Strain (Fast vs. Slow), Condition (Drug vs. No Drug), or Trial (Days) differences. Thus the two strains had similar amygdala ADTs, and the lowest dose of SCH 50911 or saline did not change that threshold. The second analysis, assessing ADTs over the 3 increasing SCH 50911 dosages or saline (Days 2, 4, 6; treatments **#2**, **#3**, and **#4**), showed a gradual decrease in ADTs yielding a main effect of Trial ($F_{2,30} = 7.84$, $p<.01$) (Figure. 2, A). Follow-up comparisons confirmed that the decrease was observed with both strains and drug treatments. The importance of this assessment was to determine whether SCH 50911 would significantly alter seizure threshold to favour facilitation of a longer AD, which it did not.

Afterdischarge Durations

The AD durations associated with the triggered thresholds were analyzed in an identical manner. In the first analysis (Day 1 vs. Day 2, treatment **#1** vs. **#2**), longer AD durations were evident in the Fast rats, regardless of whether SCH 50911 (50 μM) or saline were administered, compared to the Slow rats ($F_{1,16} = 5.66$, $p<.05$). The second

Figure. 2 – Seizure profiles of the basolateral amygdala of Fast and Slow rats following saline or $GABA_B$ antagonist infusion. The average (± SEM) afterdischarge thresholds (A) and durations (B) of partially kindled Fast and Slow rats following amygdala infusion of saline or 3 ascending concentrations of SCH 50911 (50 μM, 500 μM, and 1 mM) on Days 2, 4, 6; (treatments **#2, #3, and #4**), respectively were assessed. On Day 1, the ADT and duration were recorded in the absence of drug/saline infusion.

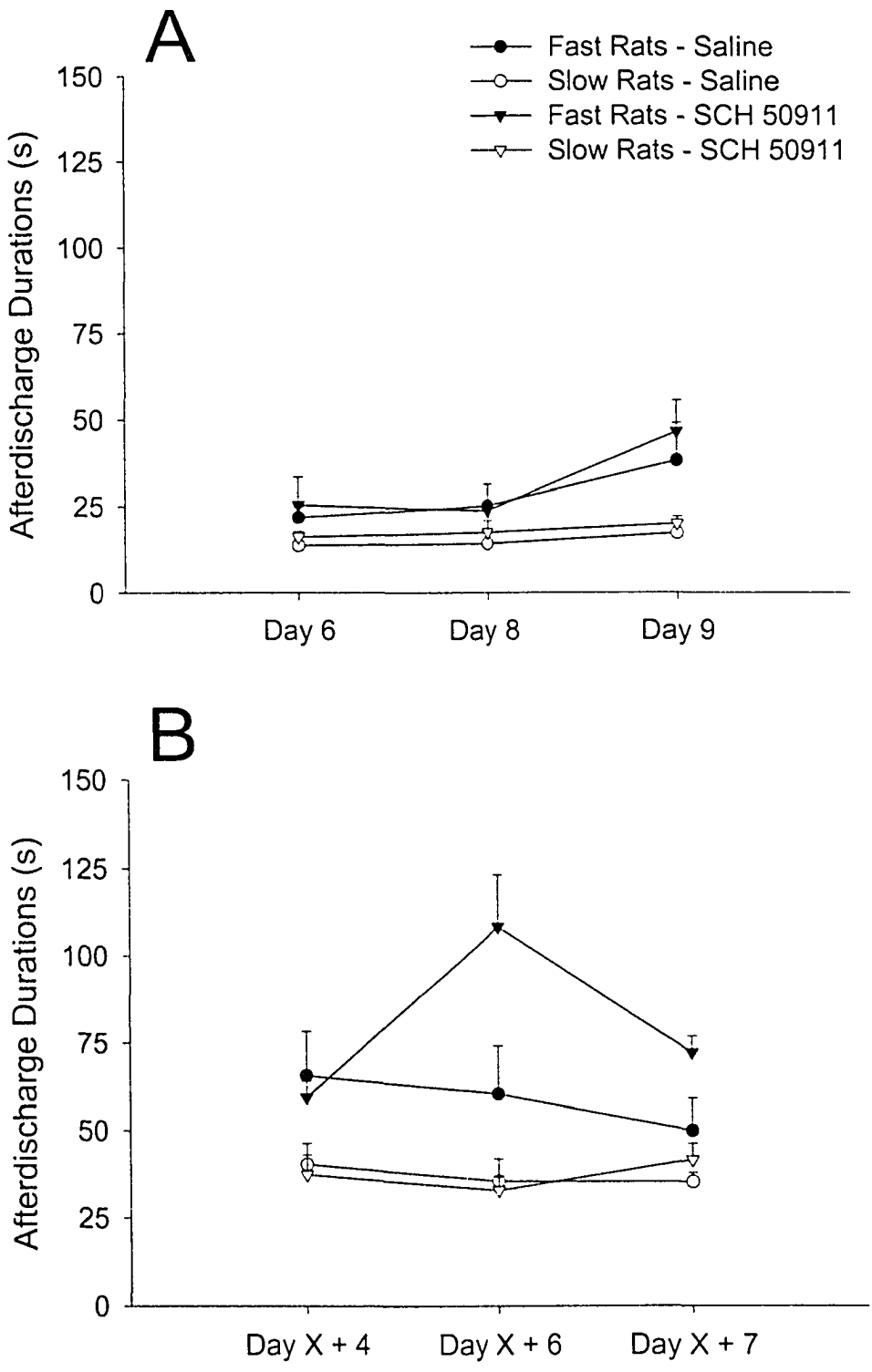


analysis with the 3 increasing dosages of SCH 50911 or saline infusion (Days 2, 4, 6; treatments **#2**, **#3**, and **#4**) produced similar results ($F_{1,16} = 8.63$, $p<.01$) (Figure 2, B). Taken together, while Fast rats exhibited longer AD durations over Slow rats, SCH 50911 focally applied via infusion into the amygdala failed to alter AD durations compared to saline infused rats.

Baclofen Infusion into Partially Kindled and Fully Kindled Rats

The effects of the well-known $GABA_B$ agonist, baclofen, on ADTs and durations were assessed in both partially kindled and fully kindled Fast and Slow rats. In reference to Figure 1 (timeline), focal infusion of baclofen occurred on Day 8 (partially kindled rats, treatment **#5**) and again on Day X + 6 (when the same rats were fully kindled, treatment **#10**). In partially kindled rats, the baclofen infusion occurred 2 days following the last of the 3 increasing dosages of SCH 50911 (1 mM) or saline (Day 6, treatment **#4**). Saline or SCH 50911 treatment resumed the day after baclofen administration (Day 9, treatment **#6**) and throughout kindling (represented by "X", which is idiosyncratic for each rat). In this regard, we compared the ADT and its duration in the presence of baclofen with the trial prior to and following the infusion (Day 6, 8, 9; treatments **#4**, **#5**, **#6**). The analysis revealed an overall significant decline in ADTs over the 3 trials (4 day period) ($F_{2,30} = 12.26$, $p<.001$). However, focal baclofen administration had no significant influence on ADTs in either strain. Examination of the AD durations again revealed longer durations in Fast than Slow rats ($F_{1,15} = 11.86$, $p<.01$), but no significant changes were evident as a result of baclofen administration.

Figure. 3 – A comparison of the effects of baclofen infusion into the basolateral amygdala on afterdischarge durations in partially vs. fully kindled Fast and Slow rats. In partially kindled Fast and Slow rats (A), the average (± SEM) afterdischarge durations following infusion of 50 μM baclofen (Day 8, treatment **#5**), with saline or 1 mM SCH 50911 administered 2 days prior (Day 6, treatment **#4**) and again 1 day following baclofen infusion (Day 9, treatment **#6**) were assessed. Similarly, average (± SEM) afterdischarge durations of fully kindled (B) Fast and Slow rats following infusion of 50 μM baclofen (Day X + 6, treatment **#10**), with saline or 1 mM SCH 50911 administered 2 days prior (Day X + 4, treatment **#9**) and again 1 day following baclofen infusion were determined (Day X + 7, treatment **#11**).



Surprisingly, however, following baclofen infusion (Day 9, treatment **#6**), the Fast rats of saline and SCH 50911 groups exhibited incrementally longer AD durations than the Slow rats, yielding a main effect of Trial ($F_{2,30} = 7.49$, $p<.01$), and a Strain x Trial interaction ($F_{2,30} = 3.68$, $p<.05$) (Figure 3, A).

In fully kindled rats, shortly after the 'crossover' from saline to SCH 50911 and vice versa, focal infusion of baclofen (Day X + 6, treatment **#10**) directly into the amygdala again resulted in no significant ADTs differences in both strains. As expected, the associated AD durations were significantly longer in the Fast rats than the Slow rats ($F_{1,16} = 19.12$, $p<.001$). Interestingly, the Fast rats that previously received SCH 50911 (1 mM) 2 days before baclofen infusion (Day X + 4, treatment **#9**) exhibited longer AD durations in the presence of baclofen (Figure 3, B). Consequently, AD durations assessed the following day (Day X + 7, treatment **#11**) were once again lowered to pre-baclofen values resulting in a Strain x Trial interaction ($F_{2,32} = 5.44$, $p<.01$). Subsequent analyses further revealed a significant Condition x Trial interaction ($F_{2,32} = 4.32$, $p<.05$). The purpose of the above assessments was to examine whether direct focal infusion of baclofen into the amygdala would significantly reduce seizure threshold and duration in both partially kindled and fully kindled Fast and Slow rats. Further, it was also of great interest to observe the effects of a focally applied $GABA_B$ agonist into the amygdala that had been previously treated with a $GABA_B$ antagonist, compared with saline, on kindling development.

Amygdala Kindling Rates

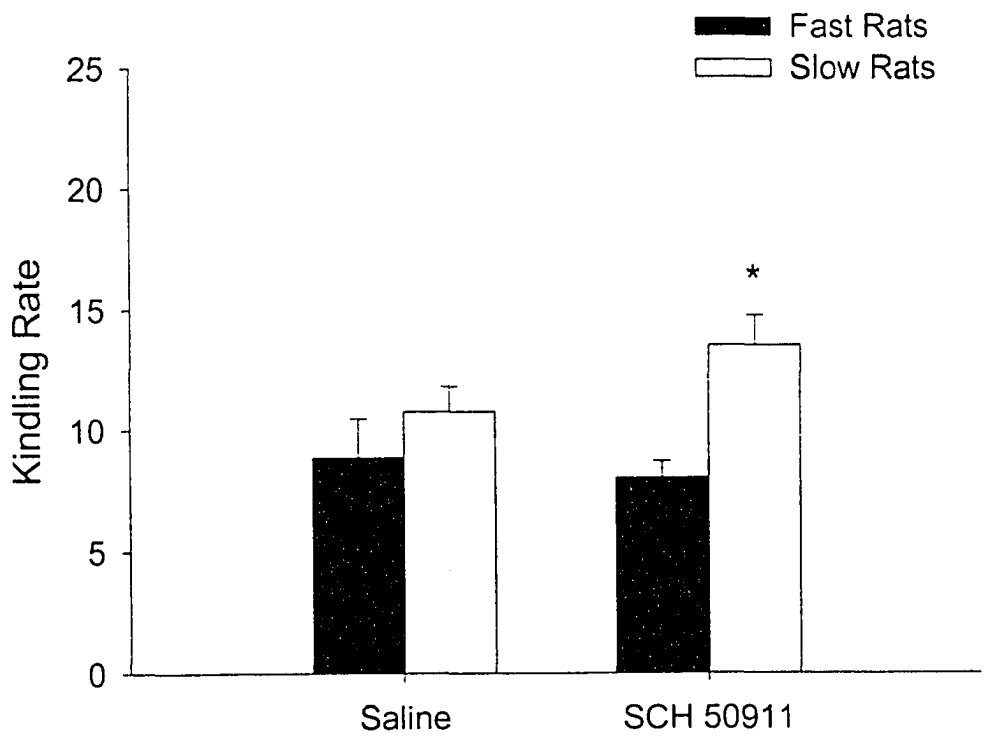
Analysis of the kindling rate in the Fast and Slow rats with saline vs. 1 mM SCH 50911 treatment showed a significant main effect of Strain ($F_{1,16} = 7.38$, $p<.05$). The follow-up comparisons, very unexpectedly, confirmed no strain differences in the kindling rate under saline infusion, but SCH 50911 treatment progressively lengthened the kindling rate of the Slow rats, while the Fast rats remained indifferent (Figure 4). This demonstrates remarkably that the cannula/electrode apparatus and/or chronic infusion of a liquid directly into the amygdala preferentially accelerate the kindling process in only the Slow rats. The significance of this finding will be addressed in the discussion section.

Kindled Convulsive Seizure Profiles

A series of 6 fully kindled convulsive seizures (before the crossover treatment) comprising of the following measurements: 1) ADT, 2) latency to forelimb clonus, 3) duration of clonus, and 4) total afterdischarge durations were recorded to assess the impact of kindling and drug interactions on these parameters. In the presence of 1 mM SCH 50911, ADTs across 6 convulsive kindling trials were not statistically significantly different between the Fast and Slow rats compared to saline groups. However, ADTs in the Slow rats were elevated following the first convulsive seizure, while the Fast rats maintained lower threshold values (Figure 5, A).

The duration of time from the onset of stimulation to forelimb clonus is represented as the convulsion's *latency*. As seen in Figure 5 (B), the Fast rats under both saline and 1 mM SCH 50911 have longer latencies than the Slow rats during the first 2

Figure. 4 – Rate of amygdala kindling of the Fast and Slow rats. This figure demonstrated the average (± SEM) number of stimulations required to elicit the first stage-5 seizure (kindling rate) of the Fast and Slow rats under daily saline or 1 mM SCH 50911 infusion into the basolateral amygdala. '*' = significantly different than SCH 50911 treated Fast rats, $p < .05$.



stimulation trials ($F_{5,80} = 16.70$, $p<.001$), but they quickly declined thereafter. This differential response of the Fast and Slow rats over 6 convulsive trials was reflected in a significant Strain x Trial interaction ($F_{5,80} = 4.97$, $p<.001$) (Figure 5, B).

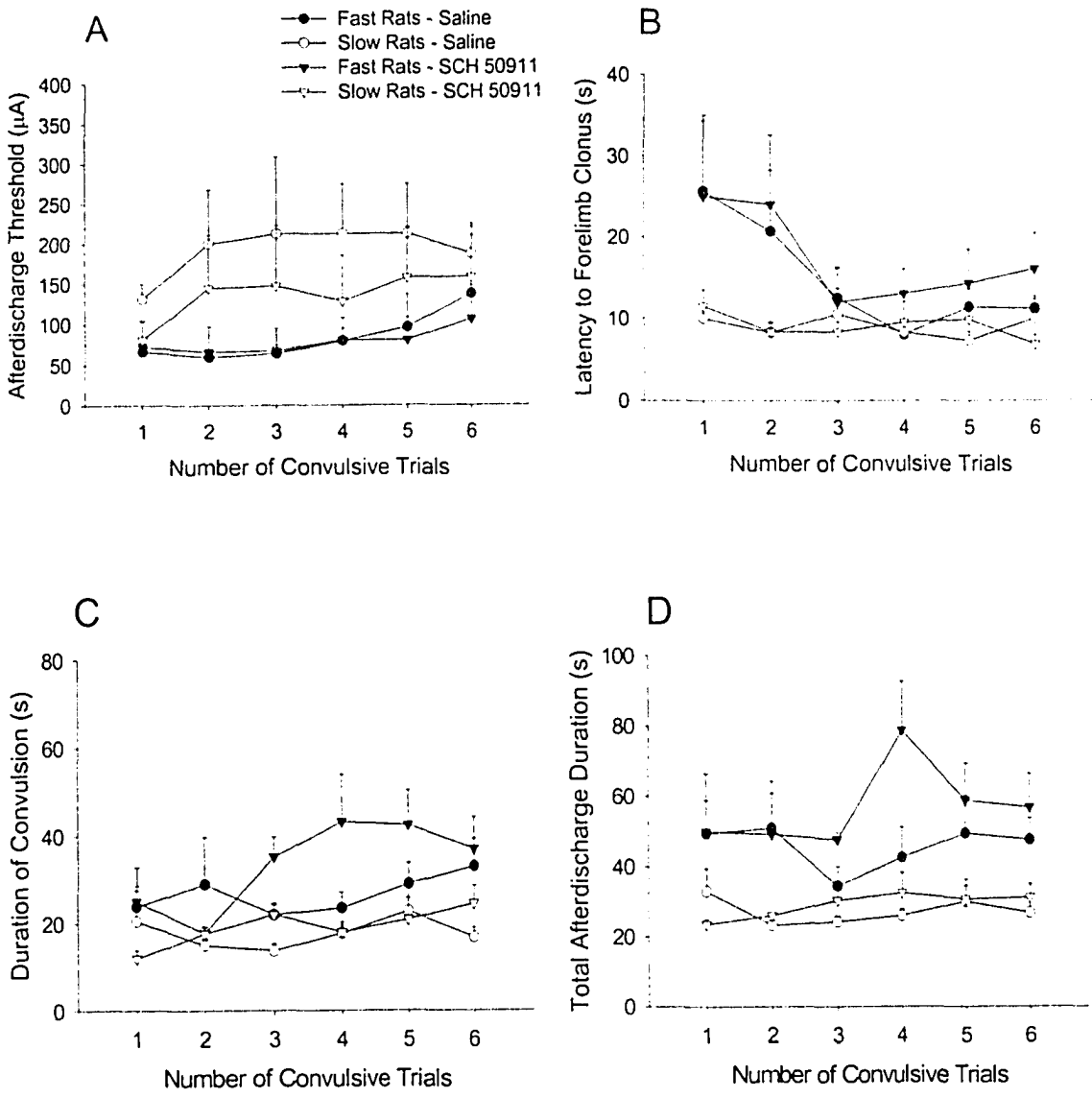
The duration of clonic motor convulsion was significantly longer in the Fast rats compared to the Slow rats ($F_{1,16} = 13.78$, $p<.01$). Over the course of 6 convulsive trials, there was a gradual lengthening of motor seizure in the SCH 50911 treated Fast rats compared to both saline controls and Slow rats, ultimately resulting in a main effect of Trial ($F_{5,80} = 2.45$, $p<.05$) (Figure 5, C).

The total AD duration on those convulsive trials is never shorter than the motor seizure and is often considerably longer. Similar to motor convulsion assessment, the total AD duration was significantly longer in the Fast rats than Slow rats ($F_{1,16} = 20.14$, $p<.001$). In addition, the Fast rats that were treated with SCH 50911 sustained longer durations than the saline group (Figure 5, D).

$GABA_B$ Manipulations - Effects of SCH 50911 on Afterdischarge Thresholds and AD Durations in *Kindled* Rats

One day following the last stage-5 seizure, both saline and drug groups underwent a 'crossover' in treatment administration. Fast and Slow rats that were previously treated with saline infusion were given 3 increasing dosages of SCH 50911 (50 μM, 500 μM, 1 mM) on every other day (Day X, Day X + 2, Day X + 4; treatments **#7**, **#8**, **#9**), identical to the procedure described for the partially kindled condition as shown in the Figure 1 timeline. Similarly, rats that were initially on SCH 50911 now received focal infusions

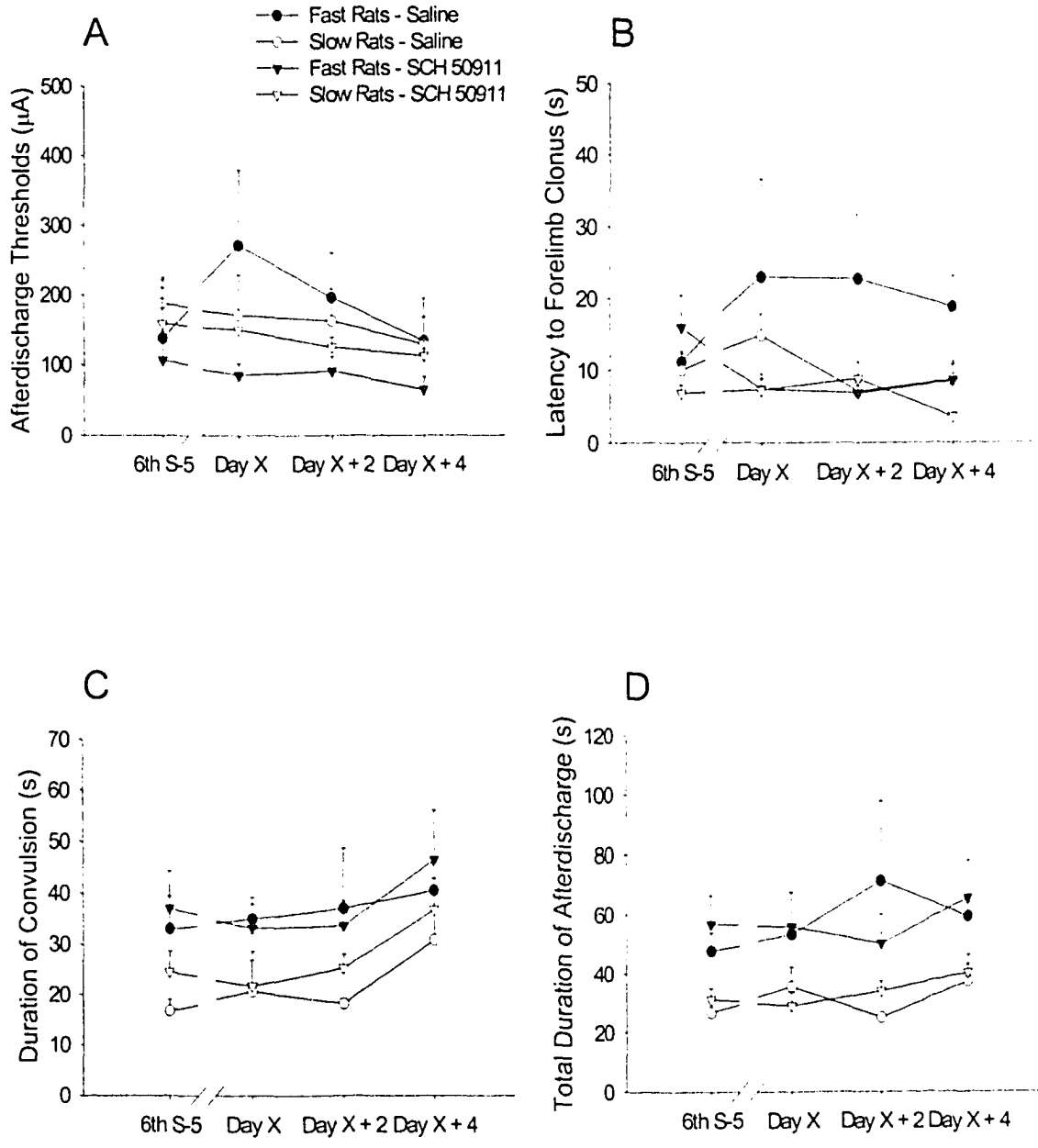
Figure. 5 – Seizure convulsion trials of the Fast and Slow rats. These figures showed a profile of 6 convulsive seizure trials before the crossover (Day 9 – X, treatment **#6**) measuring average (± SEM) afterdischarge threshold (A), latency to forelimb clonus (B), duration of convulsion (C), and total AD duration (D) of Fast and Slow kindled rats following daily infusion of saline or 1 mM SCH 50911.



of saline. The purpose of this assessment was to test the focal impact of the $GABA_B$ antagonist on kindled rats that were previously subjected daily to focal saline infusion and vice versa to test whether the treatment would significantly alter the previously established seizure expression. We examined possible changes in ADTs and durations immediately after the 'crossover'. Thus, the repeated measures analyses focused on 2 comparisons. The first examined a comparison of the trial (last stage-5 kindled seizure) before, and the trial after the crossover (50 μM SCH 50911 or saline; Day X (treatment #7)). The second investigated 3 consecutive trials of increasing dosages of SCH 50911 (described above) or saline. In Figure 6 (A), the Fast rats that were previously under daily SCH 50911 infusion exhibited higher ADTs the following day when given local infusions of saline. However, the same strain that had been on saline treatment showed a slight decrease in ADT in the presence of the drug (various dosages). This analysis yields a significant Condition x Trial interaction ($F_{1,16} = 5.90$, $p<.05$). The application of the 3 SCH 50911 dosages or saline infusion resulted in a significant overall decrease in ADT over 3 consecutive trials ($F_{2,32} = 5.70$, $p<.01$). No differences were reported in the Slow rats from both saline and SCH 50911 treatments.

In the Fast rats, administration of 50 μM SCH 50911 resulted in a dramatic decrease in latency to convulsion from the previous saline trial (Figure 6, B). On the other hand, Fast rats that were previously on SCH 50911 showed increased latency in the presence of saline. Taken together, the analysis revealed a significant Condition x Trial interaction ($F_{1,14} = 7.20$, $p<.05$). Examination of the 3 consecutive dosages of SCH 50911 or saline resulted in longer latencies for the Fast rats compared to Slow rats

Figure. 6 – Impact of the saline/drug crossover on afterdischarge threshold and durations. These figures represented seizure profiles of 3 saline or 3 ascending dosages of SCH 50911 (50 μM, 500 μM, and 1 mM, treatments **#7, #8, #9**) following the crossover compared with a single convulsive seizure trial assessed 1 day before the crossover (separated by the break). The seizure profile measurements were: average (± SEM) afterdischarge threshold, (A), latency to forelimb clonus (B), duration of convulsion (C), and total AD duration (D) of Fast and Slow kindled rats. Please note that the kindling trial before the crossover was represented in opposition of the illustrated symbol legend. For example, if the symbol indicates Fast - saline group, the actual representation was Fast - SCH 50911 treated group.



($F_{1,13} = 6.63$, $p<.05$).

Not surprisingly, the Fast rats once again exhibited significantly longer convulsions ($F_{1,14} = 6.31$, $p<.05$) (Figure 6, C) and total AD durations ($F_{1,16} = 9.02$, $p<.01$) (Figure 6, D) than the Slow rats when assessed before compared to after the crossover. No other differences were observed throughout the assessment.

Systemic Baclofen Administration

In the following analyses, we tested the effects of 1) baclofen administered systemically and; 2) the combination of systemic baclofen and focal infusion of 1 mM SCH 50911 on ADTs and durations in *kindled* Fast and Slow rats. One day after local infusion of either saline or 1 mM SCH 50911 (Day X + 13, treatment **#11**), *all* rats were given a single i.p. injection of (RS)-baclofen (Day X + 14, treatment **#12**). The next day (Day X + 15, treatment **#13**), the effects of systemic (RS)-baclofen in conjunction with focal infusion of 1 mM SCH 50911 were assessed in *all* groups. Thus, the data were analyzed as repeated measures for these 3 consecutive days (saline/SCH 50911, systemic baclofen, systemic baclofen with focal SCH 50911 infusion). As seen in Figure 7 (A), there was a drastic reduction in ADTs of saline treated rats with systemic baclofen injection, which continued the next day even in combination with SCH 50911 ($F_{2,20} = 8.69$, $p<.01$). On the other hand, in the crossover SCH 50911 treated animals, Slow rats demonstrated a modest increase followed by a sharp decline in ADT under systemic baclofen, and systemic baclofen with focal infusion of 1 mM SCH 50911, respectively. Interestingly, the Fast rats exhibited no changes in ADT across the conditions. Taken together, these data resulted in a Condition x Trial interaction ($F_{2,20} = 7.44$, $p<.01$).

To further explore the systemic effects of baclofen and better understand the immediate relationship with seizure activity, it was important to determine the number of rats that exhibited generalized seizures in the presence of this potent $GABA_B$ agonist administered alone through systemic injection or in combination with the $GABA_B$ antagonist via infusion into the amygdala. Following the crossover, nearly all Fast and Slow rats that received only daily amygdala infusion of 1 mM SCH 50911 and saline demonstrated generalized convulsions upon triggering an AD. However, in the presence of systemic baclofen, all of the SCH 50911 treated Fast rats also failed to generalize, while such seizures in half of the Slow rats were also suppressed. Similarly, all the crossover saline infused Fast rats failed to elicit convulsive seizures in the presence of systemic baclofen. In addition, all the saline treated Slow rats also failed to show convulsive seizures, unlike those treated previously with 1 mM SCH 50911 (described above). Interestingly, most of the SCH 50911 treated Fast rats given systemic baclofen in conjunction with focal SCH 50911 infusion resumed behavioural convulsions tested 1 day later (Day X + 15, treatment **#13**). On the other hand, the majority of the SCH 50911 treated Slow rats did not produce a generalized convulsion at their ADT. In saline treated animals, both the Fast and Slow rats showed similar occurrence of convulsions (50%) in the presence of focally infused SCH 50911 and systemic baclofen. These results, summarized in Table 1, demonstrate that the impact of systemic $GABA_B$ manipulation on kindled seizure activity in the Fast and Slow strain is quite distinct.

Figure. 7 – Systemic baclofen administration in the Fast and Slow rats. A comparison of the average (± SEM) afterdischarge thresholds (A) and durations (B) was made between kindled Fast and Slow rats following administration of saline or SCH 50911 infusion (Day X + 13, treatment **#11**), systemic baclofen (Day X + 14, treatment **#12**), and systemic baclofen with 1 mM SCH 50911 infusion (Day X + 15, treatment **#13**) delivered over 3 consecutive days.

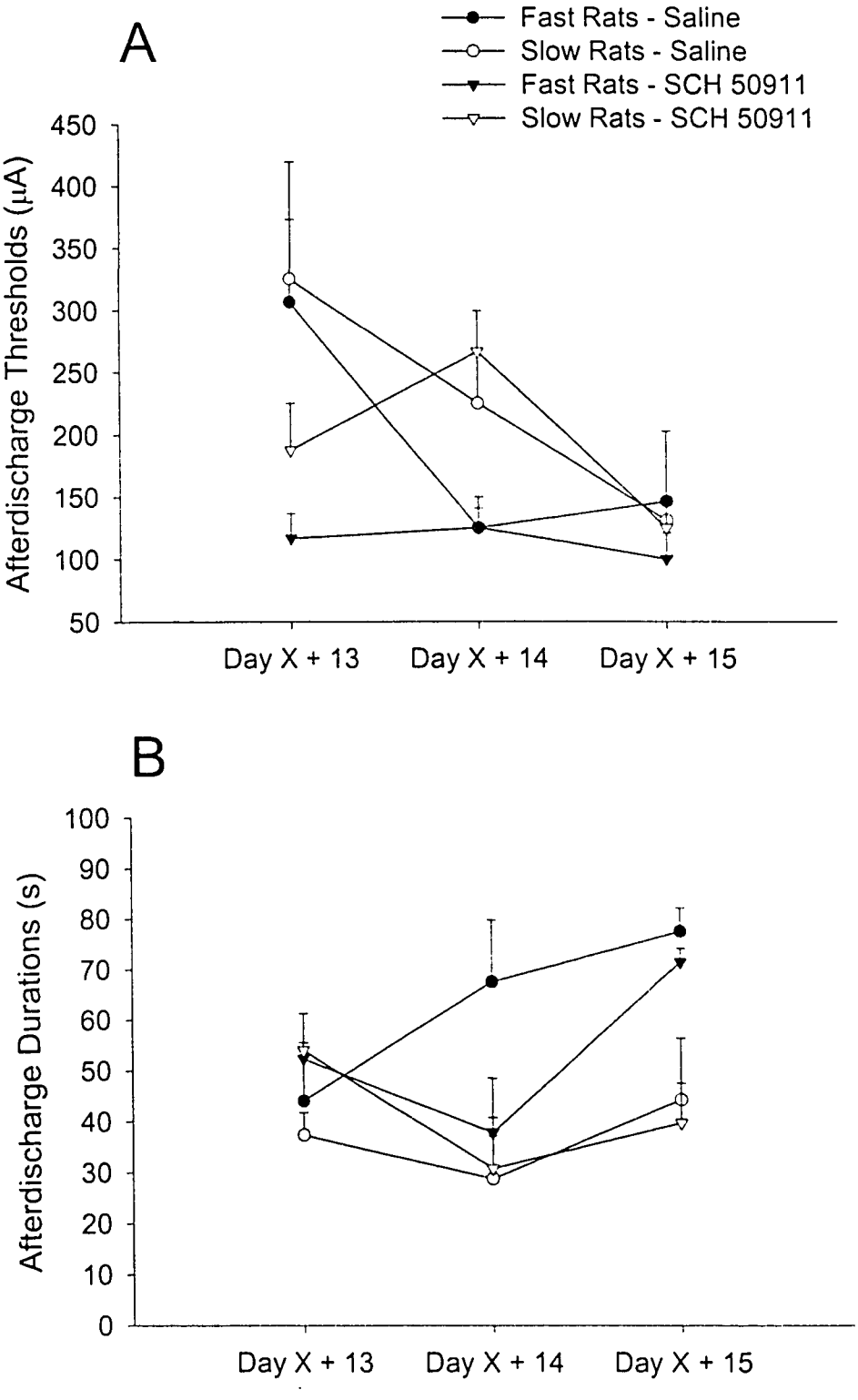


Table. 1 – This table showed the average (± SEM) percentage of Fast and Slow rats that experienced a stage-5 seizure upon triggering an AD at threshold in the presence of saline or 1 mM SCH 50911 infusion, systemic baclofen, and systemic baclofen with 1 mM SCH 50911 infusion over 3 consecutive days. Systemic baclofen, and 1 day later another systemic baclofen injection with SCH 50911 infusion were administered to all rats regardless of treatment groups. Fast and Slow rats on the trial prior to systemic baclofen administration were either given saline or SCH 50911 amygdala infusion.

	SCH 50911	BACLOFEN I.P.	BAC I.P.+ SCH50911	SALINE	BACLOFEN I.P.	BAC I.P.+ SCH50911
Fast Rats	83% (N =5/6)	0% (N =0/5)	80% (N = 4/5)	75% (N =3/4)	0% (N =0/4)	50% (N=2/4)
Slow Rats	100% (N =4/4)	50% (N =2/4)	33% (N =1/3)	75% (N=3/4)	0% (N =0/5)	50% (N=2/4)

The administration of systemic baclofen alone lengthened the AD durations in the Fast rats previously treated with saline compared to the prior trial (day before). However, the SCH 50911 treated Fast and Slow rats exhibited a slight truncation of the AD duration. The following day, in the presence of systemic baclofen injection with focal SCH 50911 infusion, the Fast rats of both saline and SCH 50911 treated group showed even longer durations than the previous trials ($F_{2,24} = 4.04$, $p<.05$) (Figure 7, B). However the same treatment did not alter AD durations in the Slow rats. This not only resulted in a significant Strain effect ($F_{1,12} = 8.39$, $p<.05$), but also a Strain x Trial interaction ($F_{2,24} = 3.45$, $p<.05$).

$GABA_A$ Manipulations

After 1 day of rest following systemic baclofen administration with simultaneous focal SCH 50911 infusions, all kindled animals regardless of initial drug/saline treatment were pooled according to their strain. Over a period of 6 consecutive days, $GABA_A$ agonists and antagonists were administered either through focal infusion and/or via systemic injection. The purpose of these manipulations was to examine the interaction between focal amygdala GABA mechanisms (infusion) and network GABA mechanisms (systemic) involving $GABA_A$ and $GABA_B$ treatments on amygdala kindled convulsions.

The first analysis over three days focused on the focal effects of muscimol, a $GABA_A$ agonist, (Day X + 17, treatment **#14**), followed the next day by focal picrotoxin, a $GABA_A$ antagonist (Day X + 18, treatment **#15**), and then systemic baclofen coupled with focal picrotoxin infusion (Day X + 19, treatment **#16**) on AD thresholds and durations. No significant ADT differences were evident in both strains across all 3 drug

trials. With focal muscimol infusion, 56% (5/9) of Fast rats experienced convulsive seizures, while 33% (3/9) did not. In contrast, an equal number (43% (3/7)) of Slow rats produced and also failed to produce a convulsive seizure with muscimol infusion. In addition, the ADT in 1 Fast and 1 Slow rats could not be determined. Although not statistically significant, local infusion of picrotoxin suggested a shorter latency to convulsion onset compared to administration of muscimol the previous day. Upon examination of convulsive profiles, the number of Fast and Slow rats that experienced a generalized convulsion vs. no convulsion was nearly identical to the findings under previous focal muscimol infusion. However, 1 Slow and 2 Fast rats failed to elicit an AD even at the highest stimulation intensity (1250 μA). Total duration of the electrographic seizure duration between focal muscimol and picrotoxin infusions were not significant. Interestingly, one day following picrotoxin infusion, the administration of systemic baclofen and 50 min later with focal picrotoxin infusion blocked the initiation of convulsive seizures in only 29% of Slow rats, a finding quite different with previous SCH 50911 infusion with systemic baclofen, where approximately 50-67% failed to elicit a convulsion. However, the same treatment blocked convulsive seizures in 71% of Fast rats, an opposite finding compared with previous SCH 50911 infusions with systemic baclofen. These results were obviously quite different from earlier administration of systemic baclofen alone in the absence of picrotoxin, whereby, 50% of Slow rats, and none of the Fast rats exhibited a convulsive seizure at a stimulus intensity sufficient to trigger an AD. No significant strain differences were evident in the assessment of convulsion duration and total AD duration. The significance of this finding speaks

directly to the efficacy of a focally applied $GABA_A$ vs. $GABA_B$ antagonist on seizure expression even in the face of a potent centrally acting $GABA_B$ agonist.

The second analysis examined the effects of systemic picrotoxin coupled with focal infusion of muscimol (Day X + 20, treatment **#17**), baclofen (Day X + 21, treatment **#18**), and finally saline (Day X + 22, treatment **#19**) administered over 3 consecutive days. These manipulations assessed whether $GABA_A$ or $GABA_B$ agonists focally applied to the amygdala in the presence of systemic picrotoxin will alter seizure ADT and seizure severity in Fast and Slow kindled rats. Focal application of muscimol, baclofen or saline delivered via cannula infusion in conjunction with systemic picrotoxin did not affect ADT on these 3 trials. Further, no significant differences were reported in latency to forelimb clonus in all 3 trials, despite longer latencies in the Fast compared to Slow rats in the presence of muscimol, but not baclofen or saline. Assessment of motor seizure durations demonstrated that Fast rats exhibit longer convulsions than Slow rats in the presence of systemic picrotoxin and baclofen infusion ($F_{1,4} = 9.33$, $p<.05$).

The convulsive profiles in the presence of systemic picrotoxin with focal infusion of muscimol or baclofen revealed that the majority of Fast (57%, (4/7)) and Slow (71%, (5/7)) exhibited generalized convulsions upon stimulation of the amygdala at threshold. Meanwhile, only 14% (1/7) of both Fast and Slow rats failed to initiate convulsions at threshold. Finally, systemic picrotoxin with focal infusion of saline produced similar results.

EEG Measurements

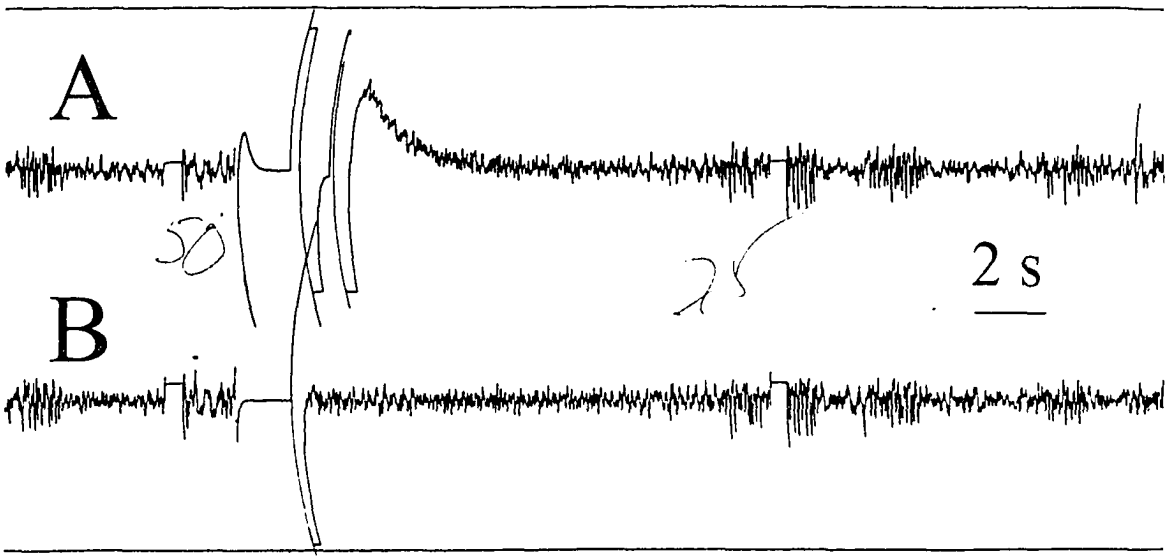
Electrographic events were recorded from both amygdalae to document seizure progression and severity as a consequence of systemic drug injections/focal infusions and subsequent electrical stimulation. The administration of $GABA_A$ and $GABA_B$ agents resulted in unique electrographic seizure patterns before stimulation and in between multiple ADT stimulations of Fast and Slow kindled rats. Each EEG pattern was described, labeled and categorized according to the type of drug administered. IIS is an important characteristic of seizure development, propagation, and recruitment, and has been considered by many as the 'hallmark' of epilepsy. In this regard, the frequency of IIS was measured and recorded before stimulation of the ipsilateral amygdala, between sub-threshold stimulations during ADT testing, and if applicable, the stimulation trial immediately prior to the elicitation of an AD.

Systemic Baclofen Administration

Sixty minutes following systemic injection of baclofen (Day X + 14, #12), IIS and specific discharge patterns were assessed. There was little evidence of abnormal discharge activity prior to and between stimulations of Slow rats leading up to the ADT determination, except for 2 rats that elicited average IIS discharge frequencies of 1.0 ± .08 Hz. The Fast rats, on the other hand, did not show any IIS activity either measured before daily stimulations or between stimulations during ADT testing.

In a perhaps related vein, 'spindles' were seen in many of the EEG records. Interestingly, 56% (5/9) of Fast rats, and only 38% (3/8) of Slow rats exhibited multiple

Figure. 8 – EEG recordings from the basolateral amygdala of Fast (A,B) and Slow (C,D) kindled rats were taken 60 min following systemic baclofen (5 mg/kg) injection. Notice the bilateral appearance of spindles between sub-threshold stimulations in both the right (stimulated) (trace A and C) and left (non-stimulated) (trace B and D) amygdala. The written numbers represent stimulation intensities in μA. Calibration = 2 seconds.



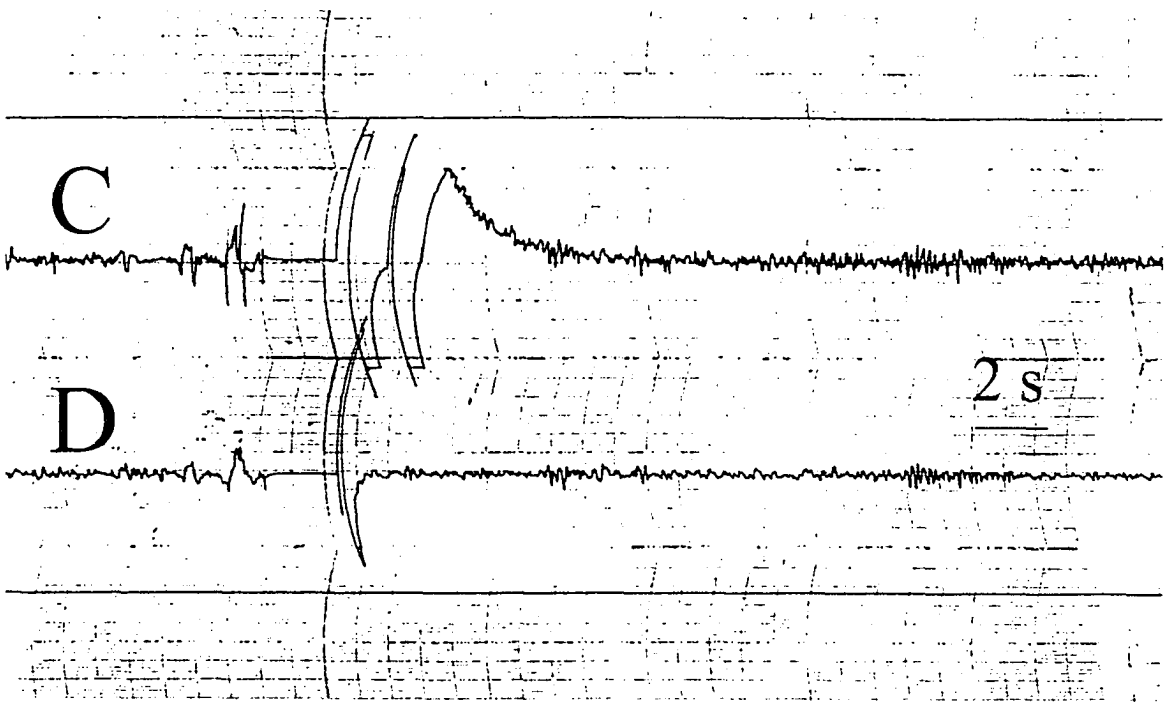
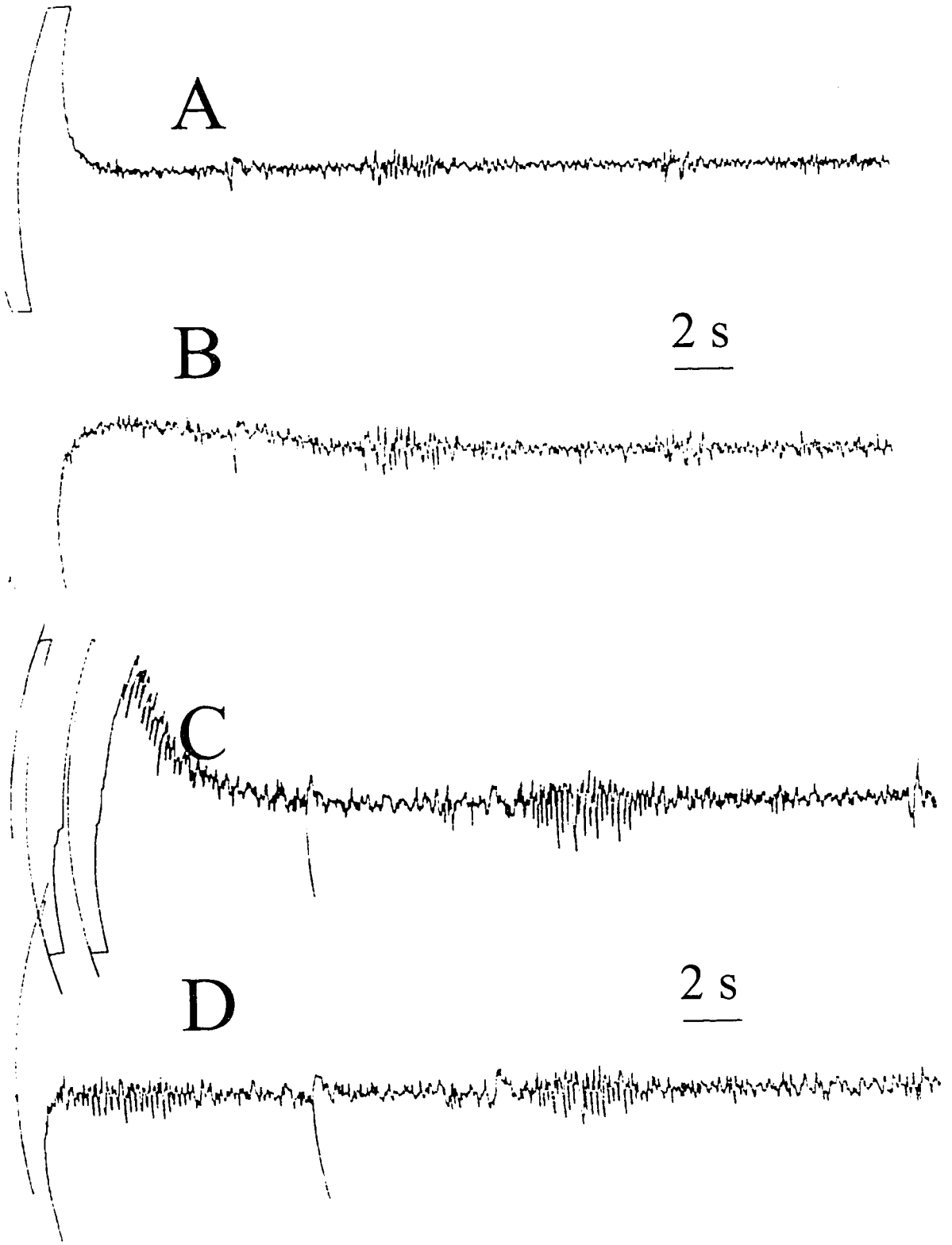


Figure. 9 - EEG recordings from the basolateral amygdala of Fast (A,B) and Slow (C,D) kindled rats were taken 60 min and 10 min following systemic baclofen (5 mg/kg) injection and 1 mM SCH 50911 infusion, respectively. SCH 50911 was infused 50 min following systemic injection of baclofen. Despite different times of drug administration, EEG recordings were taken simultaneously. Again, the appearance of spindles was evident from both amygdalae (stimulated amygdala, A and C; contralateral (non-stimulated amygdala, B and D)). Calibration = 2 seconds.

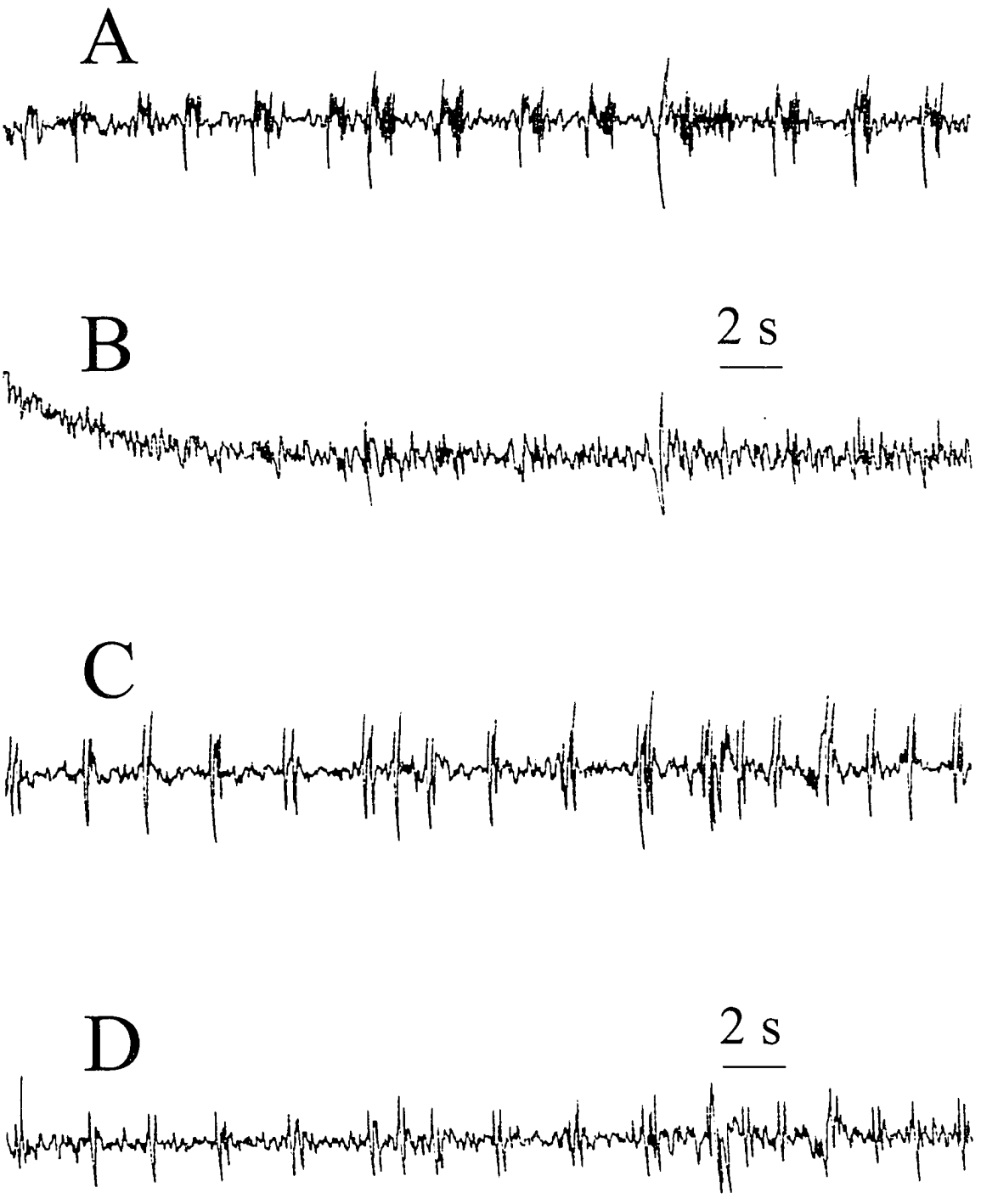


2 s spindles between successive stimulations (Figure 8). The amplitude of these spindles, evident in both hemispheres and occurred with equal propensity, was highly correlated with increasing stimulation intensity. The meaning of the spindles is not clear, but it represents highly synchronize activity.

Systemic Baclofen Administration with 1 mM *SCH 50911* Focal Infusion

One day following systemic baclofen injection, all rats were given the same treatment in combination with 1 mM infusion of SCH 50911 into the right amygdala (Day X + 15, treatment **#13**). Surprisingly, unlike the previous treatment with only systemic baclofen administration, which failed to elicit any IIS in the Fast rats, 44% (4/9) of these same rats exhibited spiking of 1.5 ± .27 Hz *prior* to stimulation. In fact, with subsequent sub-threshold stimulations, the Fast and Slow rats displayed an average of 1.3 ± .78 and .99 ± .22 Hz, respectively (Figure 9). The presence of bilateral spindles was evident again in 56% (5/9) of the Fast rats, but did not necessarily occur in the same 5 rats. In other words, prior treatment with systemic baclofen did not predict identical spindle activity when treated with systemic baclofen and SCH 50911 the following day. Further, in 2 Fast rats, we observed a truncated AD (~ 7 s) on the trial before the induction of a generalized seizure. On the other hand, the appearance of bilateral spindles was more robust and numerous in 71% (5/7) of Slow rats with longer (~3-4 s) bouts and greater frequency when coupled with higher stimulation intensities.

Figure. 10 – EEG recordings from the basolateral amygdala of Fast (A) and Slow (B) kindled rats taken 10 min following picrotoxin (500 μM) infusion into the amygdala. A very consistent and frequent spiking pattern was evident from both strains and amygdalae (stimulated amygdala, A and C; contralateral (non-stimulated amygdala, B and D)). Calibration = 2 seconds.



Muscimol Infusion

The administration of muscimol (Day X + 17, treatment **#14**) resulted in quiescent EEG activity. There was some evidence of bilateral IIS between stimulations with an average of 0.71 ± 0.13 Hz in 50% (3/6) of the Slow rats, while the Fast rats showed no apparent IIS patterns before stimulation trials and sporadic spiking between subthreshold stimulations.

Picrotoxin Infusion

Infusion of the $GABA_A$ antagonist, picrotoxin (Day X + 18, treatment **#15**), resulted in significant changes in the EEG activity in both amygdalae. A distinctive and consistent "double spiking" pattern, indicative of a mild form of spike and wave discharge, was evident shortly following focal infusion into the kindled amygdala (Figure 10). Interestingly, 57% (4/7) of Fast rats demonstrated this spiking pattern bilaterally, while only 43% (3/7) of Slow rats showed similar bilateral patterns. Upon closer qualitative examination, EEG from the contralateral (non-stimulated) side of Fast rats expressing the spiking pattern showed it was significantly attenuated and somewhat fewer in number of spikes compared to the ipsilateral (stimulated) side. Furthermore, the EEG of rats that initially showed ipsilateral spiking became more bilateral towards threshold for eliciting convulsive seizures during the ADT test. The IIS frequency measured between sub-threshold stimulations was 1.6 ± 0.13 Hz, 1.7 ± 0.19 Hz, for the Fast and Slow rats, respectively. Prior to stimulation, the frequency of IIS were 1.2 ± .14

in Fast rats, and 1.7 ± .01 Hz for the Slow rats, but only 2 Slow rats demonstrated pre-stimulation activity.

Systemic Baclofen with Focal Picrotoxin Infusion

The distinctive EEG pattern exhibited by the Fast and Slow rats as a consequence of picrotoxin infusion demonstrated that local manipulation of the amygdaloid complex with this $GABA_A$ antagonist could induce unique spiking patterns evident from both amygdalae. The introduction of systemic baclofen in conjunction with local picrotoxin infusion allowed for the examination of whether focal manipulation of local circuits by a potent $GABA_A$ antagonist could alter the behaviour and firing patterns in the presence of a strong, centrally administered $GABA_B$ agonist. The most apparent pattern from this assessment consisted of a single spike immediately followed by high frequency bursting (Figure 11). Interlaced between these IIS was the appearance of spindles in both amygdalae, characteristic of earlier administration of systemic baclofen alone described previously. The frequency of IIS measured before stimulation and between sub-threshold stimulations in the Fast rats was .82 ± .12 and .70 ± .12 Hz, respectively. In the Slow rats, the same IIS measurements pre-stimulation and between sub-threshold stimulations were .86 ± .22 and .75 ± .27 Hz, respectively. Further, in contrast to picrotoxin infusion alone described above, the present treatment showed that the occurrence of IIS was bilateral in 71% (5/7) of Fast rats, while 67% (4/6) of Slow rats adopted a unilateral pattern. These differences may be attributed to the interaction of picrotoxin with a systemically administered GABAergic agonist, and to strain specificity.

Figure. 11 – EEG recordings from the basolateral amygdala of Fast (A,B) and Slow (C,D) kindled rats taken 60 min and 10 min following systemic baclofen (5 mg/kg) and picrotoxin (500 μM) infusion, respectively. Picrotoxin was infused 50 min after systemic baclofen injection. Despite different times of drug administration, EEG recordings were taken simultaneously. A unique spiking pattern was apparent in the Fast rats of the stimulated amygdala (A), but more sporadic/random in the Slow rats (C). Notice EEG spikes from the contralateral (non-stimulated) side of each amygdala (B and D) were more muted than the ipsilateral (stimulated) side (A and C). Calibration = 2 seconds.

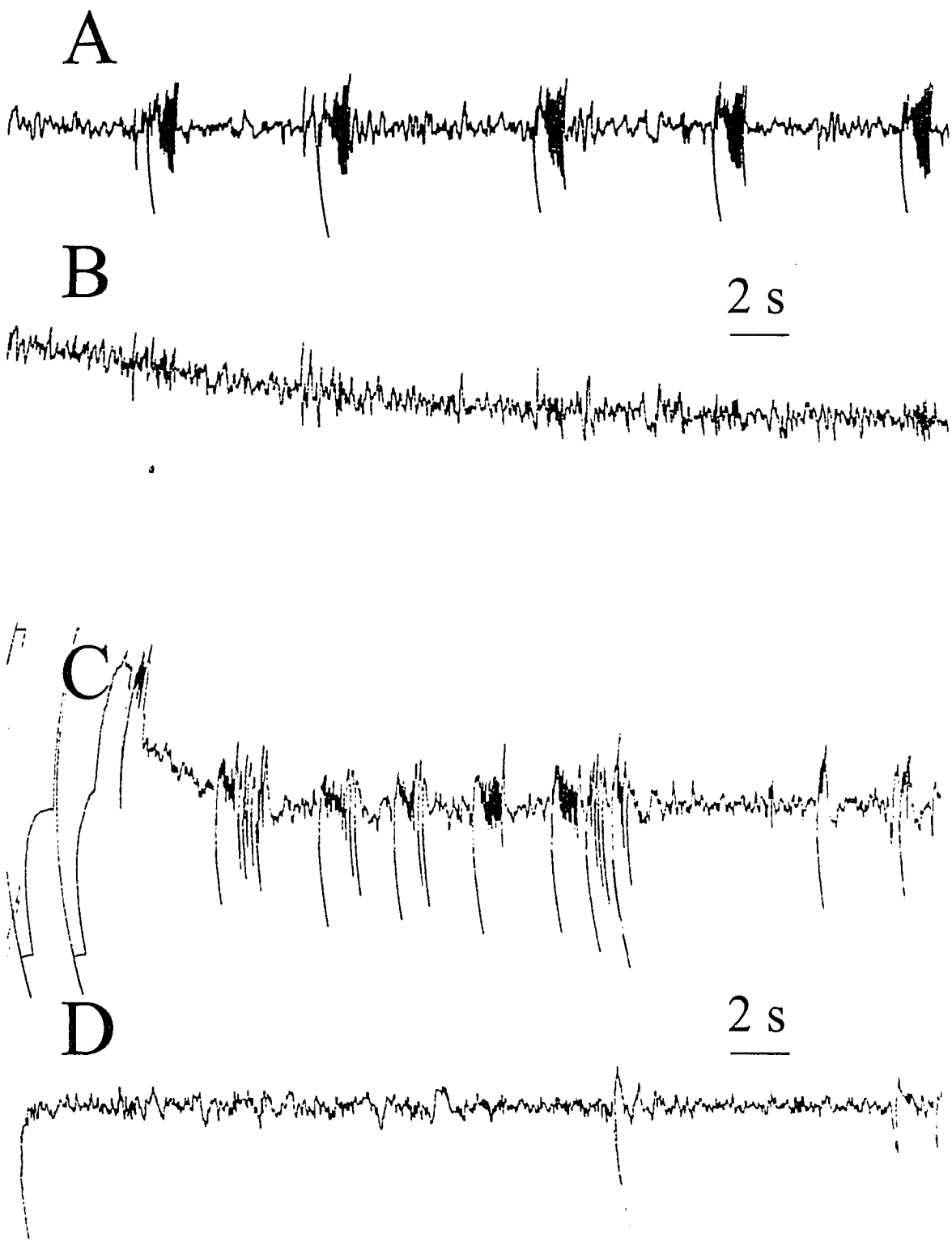
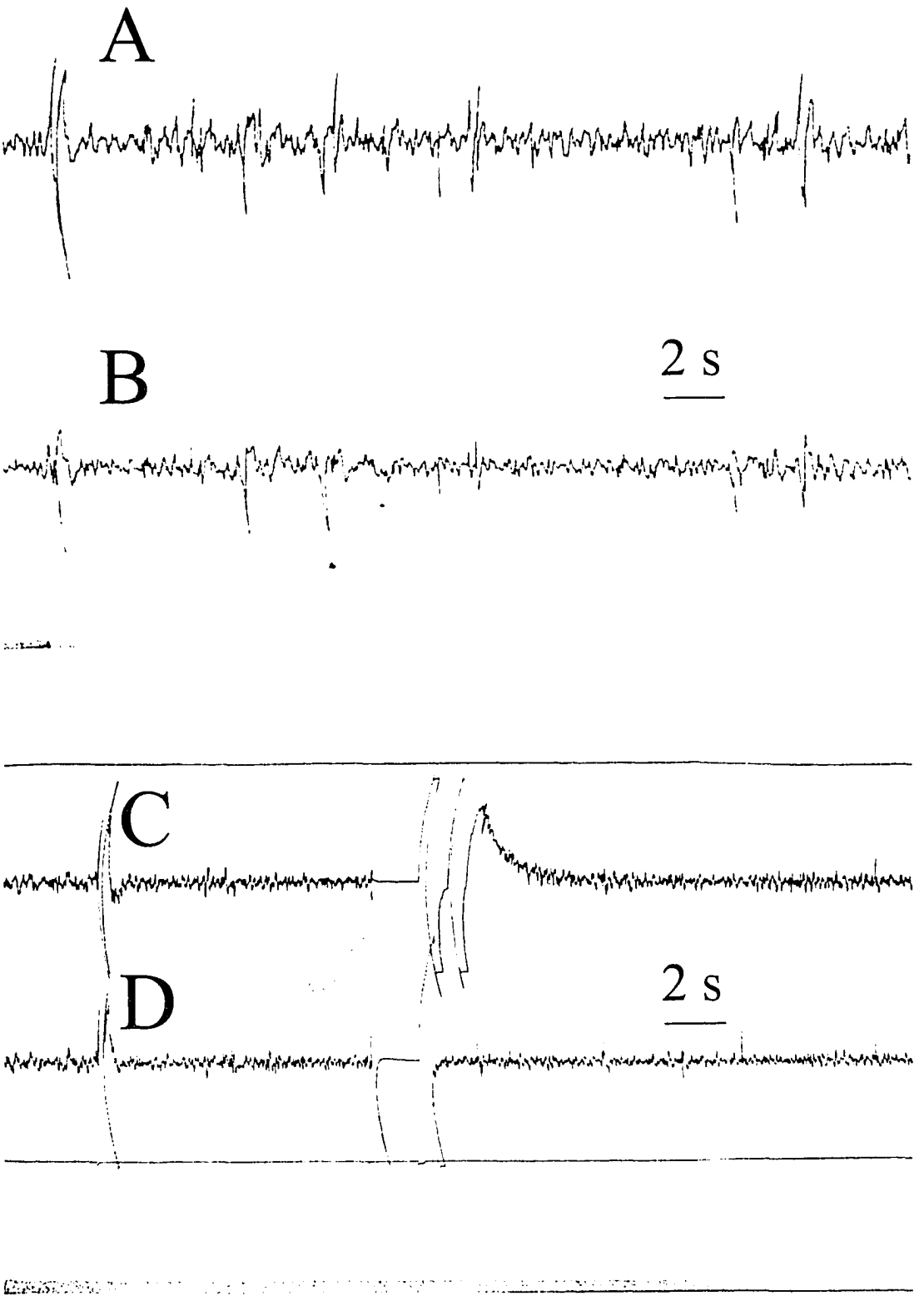


Figure. 12 – EEG recordings from the basolateral amygdala of Fast (A,B) and Slow (C,D) kindled rats taken 20 min and 10 min following systemic picrotoxin (.5 mg/kg) and muscimol (10 mM) infusion, respectively. Muscimol was infused 10 min after systemic picrotoxin injection. Despite different times of drug administration, EEG recordings were taken simultaneously. Interictal spikes were fewer in number from both hemispheres (stimulated amygdala, A and C; contralateral (non-stimulated amygdala, B and D)) and in both strains. The written number represented stimulation intensity in μA. Calibration = 2 seconds.



Systemic Picrotoxin with Muscimol Infusion

A combination of systemic picrotoxin and local infusion of muscimol (Day X + 20, treatment #17) into the amygdala resulted in sporadic and bilaterally occurring IIS with an average frequency of .95 ± .13 and 1.0 ± .08 Hz in the Fast and Slow rats, respectively (Figure 12). The rate of IIS between successive stimulations in both strains was directly proportional to the intensity delivered. There was some evidence of IIS in both strains measured before stimulation, but were very infrequent.

Systemic Picrotoxin with Baclofen or Saline Infusion

Administration of baclofen directly infused into the amygdala in the presence of systemic picrotoxin (Day X + 21, treatment #18) resulted in EEG patterns similar to the above treatment with muscimol. Focal infusion of saline into the amygdala in combination of systemic picrotoxin (Day X + 22, treatment #19) did not show any apparent abnormalities in EEG profiles, but did display some periods of IIS in the Fast and Slow rats before stimulation.

DISCUSSION

This main focus of experiment #1 was to assess the chronic effects of a daily-administered $GABA_B$ antagonist, SCH 50911, on seizure genesis and development in Fast and Slow kindling rats. In addition, EEG and behavioural profiles were examined in response to the administration of a $GABA_B$ agonist, baclofen, as well as a $GABA_A$ agonist, muscimol, and antagonist, picrotoxin. The efficacy of these compounds in altering seizure profiles was strongly dependent on the route of administration: 1)

systemically (i.p.) or 2) microinfusion through a cannula directly into the amygdala. We explored the pharmacological effects of both treatments on amygdala-kindled seizures. The experiment provides answers to some challenging questions directed toward the importance and involvement of both $GABA_A$ and $GABA_B$ receptors in temporal lobe epilepsy.

Due to the obvious complexity of this experiment, the treatment comparisons and their corresponding treatment numbers from Figure. 1 (timeline) will be indicated in the text. Each section of the discussion will follow the sequential order in which the results were presented. However, only the results that we deemed the most significant will be presented and discussed.

In regards to the time course of the present study, we acknowledge that the series of daily drug manipulations may provide a basis for confounds due to possible carryover effects on various dependent measures, such as ADT, durations, and severity of the seizures. Thus, concerns may be raised to challenge the validity of these manipulations and to the extent they reflect the human condition. Be it known that the treatment of clinical epilepsy requires administration of repeated pharmacological agents to patients that likely have a history of epileptic episodes. It is often the case that many types of antiepileptic medications be administered before settling on the most effective. In this regard, numerous prior drug treatments to an epileptic patient mirror the present experiment with repeated drug applications into an epileptic (kindled) rat. Therefore, the validity and justification of current procedures are certainly warranted.

Microinfusion of SCH 50911 and Baclofen into Partially Kindled Rats

The current discussion is in reference to the administration of SCH 50911 (timeline: treatment #2 onwards until the crossover) and a single administration of baclofen through microinfusion (treatment #5). The main findings of these two $GABA_B$ manipulations failed to reveal any significant or obvious $GABA_B$ effects (involving the agonist, baclofen or antagonist, SCH 50911) on ADTs and AD durations when infused directly into the amygdala of Fast and Slow rats either before or during the early stages of amygdala kindling. To our knowledge, this was the first investigation that employed microinfusion of SCH 50911 in any known seizure models, and even studies that have examined seizure properties of baclofen via microinfusion were few in number. Most, if not all, of the experiments in the literature employed systemic injections of SCH 50911 or baclofen at doses that range from 100 – 300 mg/kg and 2 – 30 mg/kg, respectively. The results obtained from the current study suggest that repeated, local infusion of a $GABA_B$ antagonist did not obviously change the inhibitory tone within the amygdala to significantly alter AD propagation leading progressively during kindling to generalized seizures. Similarly, microinfusion of baclofen failed to significantly alter kindling parameters during partial kindling, where it was hypothesized it might increase ADTs and/or shorten AD durations. Despite our narrow focus on the physiological measurements of ADT and durations, which did not change, possible focal changes in receptor density, kinetics, and expression, as a consequence of daily SCH 50911 infusion, cannot be ruled out. However, if such changes did occur from the repeated administration of SCH 50911, they did not have an impact on the physiological measurements. In addition, it may be possible that the dosage used in this experiment

was not sufficient to offset the ADT for triggering seizures at the local level. Other studies have similarly shown an absence of behavioural or EEG abnormalities with intracortical microinfusion of another $GABA_B$ antagonist, phaclofen, into the somatocortex of rats during GABA withdrawal syndrome (Brailowsky et al., 1995). Further, the $GABA_B$ receptor antagonist CGP 35348 (1 mM), administered for 20 min via microdialysis into the cortex, was without effect on spontaneous GABA-mediated acetylcholine release (Giorgetti et al., 2000). In this regard, lower to moderate dosages through microinfusion appear ineffective to induce global changes in seizure expression. On the other hand, exposure of a larger area of the CNS to CGP 35348 through intracerebroventricular injection has been shown to increase hippocampal triggered AD durations, and seizure severity in a dose dependent manner (Leung et al., 2005). It was further suggested that $GABA_B$ receptor blockade increases seizure susceptibility by reducing AD threshold and increasing the spread of hippocampal AD (Leung et al., 2005). Such discrepancies between studies could be attributed to the method of drug delivery and distribution, time of AD assessment, and also the brain structure from which the seizures were triggered. Certainly the hippocampus is one of the most vulnerable areas of the brain to seizure induction, witnessed by its ADT, compared to various neocortices (McIntyre et al., 1999).

$GABA_B$ receptor antagonists delivered *systemically* have demonstrated pro-convulsive effects, but only at extremely high dosages (Vergnes et al., 1997). In fact, dosages as high as 300 mg/kg of SCH 50911 were considered non-convulsive (Carai et al., 2002). Despite a very low inhibitory concentration value (IC_{50}) of 1.1 μM, SCH 50911, which is approximately 60 times more potent than the more common $GABA_B$

antagonist CGP 35348, needs to be administered with high potency to exert its considerable convulsive effects (Badran et al., 1997). The reason for this has not been fully elucidated, however, it may be due to poor penetration through the blood-brain barrier when injected systemically, and thus the effective amount of binding at the receptors would be considerably reduced and delayed over time (Olpe et al., 1993). Clearly, any discrepancies that arise between focal and systemic application of compounds could be due to differences in potency, distribution, binding affinity and receptor availability and occupancy.

To this end, our data indicated that the highest dosage of SCH 50911 (1 mM), administered daily through microinfusion, was not sufficient to significantly perturbate the local amygdala networks in either strain and impact the ADT, or affect recruitment from that focus and alter the kindling process. The precise mechanisms by which SCH 50911 exerts its effects have not been fully elucidated. However, we suspect that the drug may favour postsynaptic $GABA_B$ receptors, which when occupied, would suppress late IPSPs. This putative action should ultimately provide increased sensitivity in the local networks and translate into slight increases in AD durations in some of the SCH 50911 treated trials, which we observed in many of the Fast rats. If this proposal was the case, daily microinfusion of SCH 50911 into the amygdala might subtly and locally up-regulated $GABA_B$ receptors both synaptically and extrasynaptically. However, it is difficult to determine whether changes in GABA release, binding affinity or uptake mechanisms are greatly affected by this chronic manipulation. In fact, it has been shown from radioligand assays that $GABA_B$ antagonists display flat inhibition curves, while $GABA_B$ agonists show normal inhibition curves with higher binding affinity (Bittiger et

al., 1993). These data may partially explain the relative invariant nature of the seizure network in response to SCH 50911 in the Fast and Slow strains.

Kindling Rates

One of the more puzzling findings in this experiment was the kindling rate of our rat strains. Traditionally, the number of stimulations required for BLA kindling in the Fast and Slow rats are typically 8-12, and 40-50, respectively (McIntyre et al., 1999). On the other hand, our data show that with saline infusion directly into the amygdala, the kindling rate of the Slow rats was dramatically reduced to rates comparable to the Fast rats! This was a stunning and unexpected observation, which tells us that there is something unique about the Slow rats with respect to this manipulation. SCH 50911 infusion (dissolved with saline) produced a similar, but less robust pattern in the Slow rats. The reasons for this are unclear. The presence of electrodes in the amygdala over time exert a pro-kindling effect by lowering the ADT, which should provide greater recruitment and faster access to motor network to trigger stage-5 kindled convulsions (Loscher et al., 1995, 1999). How daily infusion of isotonic saline, albeit a small volume, directly into brain tissue accelerated that process is speculative but may have increased interstitial pressure, which could lead to an acceleration of the kindling process, perhaps by increasing gap junction communication or ephaptic transmission, but preferentially in the Slow rats. This suggest, however, that adequate perturbation of local networks within the amygdala of a seizure resistant rat (Slow rat in this case) may render the local area more vulnerable to subsequent stimulations, and provide a local kindling environment similar in important ways to the seizure prone Fast rat. Thus, these observations provide

further evidence that aggravation of brain tissue, via mechanical invasion (insertion of cannula) or fluid injection, in a kindling susceptible structure may push the local network closer to the threshold for recruiting and triggering generalized seizures. Traumatic brain injured patients associated with brain lesions or a scar tissue may express a similar fate. The addition of SCH 50911 to the infusion did little to alter the kindling progression, however, there was a slight suggestion of retardation (compared to saline alone) in the Slow rats.

Baclofen Microinfusion in Kindled Rats

Although the $GABA_B$ antagonist SCH 50911 had little effect on the progression of kindling or partial seizure development, our data point to a sensitivity to infusion of the $GABA_B$ agonist, baclofen in *kindled* Fast rats (treatment #10). Earlier results indicated no significant strain differences in ADTs and durations as a consequence of baclofen infusion into the amygdala of *partially kindled* rats (treatment #5) compared to previous saline/SCH 50911 trials (treatment #4). However, repeated daily treatment with SCH 50911 before the crossover phase (rats that received saline after the crossover) elicited a dramatic increase in AD duration following baclofen infusion in kindled Fast rats shortly following the crossover. This result suggests that the Fast rats were perhaps uniquely sensitized by the antagonist (becoming manifest after its withdrawal), which was indicated by an exuberant response to the agonist. This finding again indicates the importance of the focus in driving the recruiting response, witnessed by a much-lengthened AD duration. Others have reported similar pro-convulsive findings of baclofen using systemic or focal administration. Karlsson et al. (1992) indicated an

increase in AD durations after *systemic* administration. Similar epileptogenic effects have also been seen after intracortical microinjections of baclofen *in vivo* (van Rijn et al., 1987). Further Brailowsky et al. (1995) have shown that baclofen infused directly into the somatomotor cortices induced 2 types of paroxysmal discharges: 1> consisting of a fast rising monophasic spike followed by a biphasic slow wave: 2> consisting of a high frequency burst of high voltage, fast monophasic spikes, where both patterns peaked ~70 min after the injection. In both cases, no behavioural correlates were associated with the abnormal EEG patterns. Compared to the present experiment, microinfusion of baclofen into the amygdala did not trigger a similar fast spiking EEG profile, but did corroborate an absence of behavioural seizures with a concomitant increase in AD duration. Discrepancies between our results and the above study, of course, can be attributed to the site of drug administration (amygdala vs. somatomotor cortex), concentration (50 μM, amygdala; 10 mM, somatocortex) and other variables.

One of the more important questions that need to be addressed is whether SCH 50911 and baclofen bind to pre- and/or postsynaptic receptors to create their effects. In the previous discussion (above), we had alluded to the fact that chronic, daily microinfusion of SCH 50911 may cause change in regulation of $GABA_B$ receptors, resulting in increased seizure responsiveness of local networks. It has been suggested that the autoreceptor-mediated release of GABA is increased by interactions of $GABA_B$ receptor antagonists at presynaptic receptors (Waldmeier et al., 1992; Sohal and Hasselmo, 1998). However, at postsynaptic sites, these antagonists suppress the late IPSPs during both *in vitro* and *in vivo* studies (Bittiger et al., 1993). Presumably, $GABA_B$ receptor agonists operate in an opposite fashion, where activation of the

presynaptic responses would decrease GABA release and increase seizure behaviour, while a postsynaptic agonist would increase late IPSPs and decrease seizure suppression. Because baclofen has multiple post-synaptic sites of action on both inhibitory and excitatory neurons, and affects both synaptic and extrasynaptic $GABA_B$ receptors, it is difficult to interpret the precise mechanisms of drug action with great accuracy (Karlsson et al., 1992), making its assessment largely empirical (rather than speculative). Nevertheless, speculatively, if baclofen has a preferential affinity to presynaptic $GABA_B$ receptor binding, then it is conceivable that previous repeated infusions of SCH 50911 into a "kindled" network may have "primed" or increased the sensitivity of resident presynaptic $GABA_B$ receptors to baclofen. This in turn might have increased activity in $GABA_B$ autoreceptors, resulting subsequently in reduced GABA availability in and around the synapse. How this would affect the efficacy of local GABA transporters will be addressed in experiment #2.

In the hippocampus, baclofen has been shown to promote inhibition of both calcium channels and quantal release of GABA presynaptically (Jarolimek and Misgeld, 1992, 1997). With respect to our data, the lack of increase in AD duration of previously treated saline Fast rats in response to focal microinfusion of baclofen support our initial hypothesis that SCH 50911 may have sensitized postsynaptic $GABA_B$ receptors, a finding that was absent with saline manipulation (Figure 1, treatment #10 compared to treatment #5). The invariant nature of the kindled Slow rats in response to focally infused $GABA_B$ agonists and antagonists demonstrate that the $GABA_B$ receptor system within the amygdala of this strain is less responsive to $GABA_B$ manipulations and likely requires higher doses to induce significant changes at the cellular and physiological

levels. Taken together, these findings indicate a small overall effect of $GABA_B$ treatments delivered directly into the kindled amygdala of the Fast rats compared to virtually no effect in Slow rats. These data might suggest that the $GABA_B$ receptor system in humans who are *susceptible* to epilepsy could play a more significant role in seizure suppression and maintenance than was previously thought.

Systemic Baclofen Administration

The administration of *systemic* baclofen (treatment #12) in Fast and Slow *kindled* rats produced profound effects on ADT and duration. Fast rats previously treated with focal SCH 50911 (after crossover) failed to exhibit significant changes in ADT as a consequence of both systemic baclofen and the combination of systemic baclofen with focal SCH 50911 administered the next day (Figure 1, treatments #12 and #13). On the other hand, the SCH 50911 treated Slow rats showed minimal increase in ADT as a result of systemic baclofen injection (treatment #12), but thresholds dropped dramatically the following day in combination focal SCH 50911 (treatment #13). Saline infused Fast and Slow rats (after crossover) exhibited a different ADT profile. Systemic baclofen dramatically reduced the ADTs of Fast and Slow rats, with the Slow rats showing yet a further reduction following the combination of baclofen with SCH 50911. The reasons for a preferential decrement in ADT of previously treated saline rats compared to SCH 50911 assessed immediately following systemic injection of baclofen are not clear. We hypothesized earlier that the increase in AD duration during focal baclofen microinfusion into the amygdala might be attributed to a change in sensitization resulting from earlier SCH 50911 treatments. The possible up-regulation of postsynaptic $GABA_B$ receptors

resulting from a possible receptor blockade by SCH 50911 might enhance local neuronal excitability by effecting a lowered ADTs, especially among the seizure prone Fast rats. Furthermore, an enhanced presynaptic effect of baclofen is likely to reduce GABA release, which should increment excitability. As discussed previously, this hypothetical increase in excitability was nevertheless insufficient to drive the network toward seizure expression, both behaviourally and electrographically. In this vein, one of the more important observations in this study was realized after the crossover, in that saline treated Fast and Slow rats (previously SCH 50911 treated prior to crossover) exhibited a gradual, but significant increase in ADTs over approximately 10 kindling trials compared to those treated with SCH 50911 (previously saline treated prior to crossover). These findings strongly suggest that $GABA_B$ antagonists, like SCH 50911, maintains a disinhibitory tone of the neural network upon daily infusion into the kindled amygdala.

It is known that the $GABA_B$ agonist, baclofen, is a muscle relaxant acting at the spinal level, but many of its physiological/pharmacological effects are still somewhat unclear. Studies have shown that baclofen (5 mg/kg) produced severe sedation, vomiting and ataxia along with diffuse paroxysmal EEG discharges (Morimoto et al., 1993). Interestingly, the effects of baclofen on seizure profiles are vastly different *during* kindling development compared to the fully kindled state. Karlsson et al. (1992) have shown that during kindling baclofen prolongs AD duration while retarding the development of behavioural symptoms. Once the fully kindled state has been achieved, differences in AD durations and severity ceased to exist with baclofen administration (Sato et al., 1990b; Wurpel et al., 1990). In the present study, a reduction of ADT along with a concomitant increase in AD duration in the *saline* treated Fast rats may seem

paradoxical since baclofen has been regarded as a agent that enhances GABAergic activity in the CNS. Apparently, this result was completely opposite to the findings from focal microinfusion of baclofen into the kindled amygdala, where large increase in AD duration was reported in *SCH 50911* treated Fast rats. Since systemic baclofen binds pre- and postsynaptic sites and both synaptic and extrasynaptic $GABA_B$ receptors (Karlsson et al., 1992), the proposed up-regulation of $GABA_B$ receptors by chronic infusion of SCH 50911 may result in less disinhibition. In other words, presynaptic activation of $GABA_B$ receptors by baclofen will likely limit GABA release into the synapse. At the same time, more postsynaptic $GABA_B$ receptors may be available for baclofen (as a consequence of SCH 50911) favouring a reduction in seizure expression. Conversely, the large increase in AD duration in saline treated Fast rats as a result of systemic baclofen is likely attributed to the same mechanism except that the number of postsynaptic $GABA_B$ receptors is not as abundant because of the absence of SCH 50911. These differential effects of focal vs. systemic administration of baclofen, in saline and SCH 50911 treated Fast rats, speak strongly to the modulatory influence of the $GABA_B$ receptor system in seizure expression and maintenance.

Despite the increased focal AD activity seen in saline treated Fast rats in response to systemic baclofen, the motor manifestations of generalized kindled seizures of both strains were absent, suggesting a clearly retardation in the *effective* spread of seizure beyond the primary focus. How is this possible when the focal AD duration was not truncated? The presence of AD, however, does not indicate the magnitude of the network that is involved in that local seizure response. As McIntyre et al. (1991a) previously showed, the utilization of glucose of a unilaterally amygdala kindled rats was entirely

different in the two amygdala during electrically induced status epilepticus, yet, remarkably, the AD in the two amygdalae during the status event was identical. Thus the EEG response appeared to be far more sensitive to the presence of focal seizure activity, but did not discriminate its 'magnitude' or intensity like the glucose measurements. In the present context, the long AD durations triggered in the kindled amygdala associated with systemic baclofen do not necessarily mean that the amygdala output was intense but only that it was present. It was certainly sufficiently weak to fully recruit the network and trigger a generalized stage-5 convulsive seizure, as other provocations did. These results are in agreement with other reports that have supported an antiepileptic role of baclofen as evidenced by a lengthening of kindling rates and reduction of seizure severity following its administration (Wurpel et al., 1990; Wurpel, 1994). However, using outbred rats, other studies have also shown that moderate doses of baclofen have no anticonvulsant action on any parameters of kindling, including AD duration and severity of seizures in the kindled amygdala (Morimoto et al., 1993). Yet, baclofen has been demonstrated to increase electrographic epileptiform activity in strains of rats with spontaneous, generalized spike-wave discharges of thalamocortical origins (Vergnes et al., 1984) *in vivo* and *in vitro* (Mott et al., 1989; Burgard and Sarvey, 1991). It is clear from our data that systemically administered baclofen not only exhibited anticonvulsant properties, but also facilitated electrographic activity in our genetically seizure prone Fast rat, and that this phenomenon appear to be strongly dependent on previous pharmacological manipulations (drug vs. saline). This variant nature of seizure sensitivity to baclofen may be attributable to inherent strain differences within the

GABAergic system, including receptor efficacy, density, and subunit composition (Poulter et al., 1999; Racine et al., 2003).

In conclusion, baclofen delivered via cannula vs. i.p. injection yields significantly different effects on amygdala kindled ADT and durations. Moreover, previous daily infusion into the amygdala of SCH 50911 interacts with local networks and subsequently induces changes in excitability expressed at the focus, which subsequently affects the kindled network. It is also conceivable that the different pharmacological properties of pre- and postsynaptic receptors in the amygdala may give rise to two distinct populations of $GABA_B$ receptors whose responses to kindling-induced seizures are dissimilar, and that, differential coupling of presynaptic and postsynaptic $GABA_B$ receptors to effector mechanisms may also exist (Asprodini et al., 1992; Pitler and Alger, 1994).

Systemic Baclofen with Focal Microinfusion of SCH 50911

Lastly, injection of baclofen combined with SCH 50911 infusion (treatment #13) resulted in a substantial increase in the number of convulsive seizures in previously treated saline and SCH 50911 Fast rats, and previously saline treated Slow rats compared to baclofen administration alone the previous day. The only exception was the SCH 50911 treated Slow rats, who exhibited a slight reduction in seizure frequency (see Table 1). These results clearly demonstrate that focally applied SCH 50911 directly into the amygdala was sufficient to overcome the enhanced inhibitory tone exerted by the powerful anticonvulsant properties of systemic baclofen. The ability of the kindled focus to recruit the kindled motor networks in the presence of focal SCH 50911 (during systemic baclofen) demonstrates a new efficacy of the antagonist, which was absent

when administered alone prior to kindling. As previously suggested, it is possible that daily SCH 50911 might exert a disinhibitory tone on the seizure network by lowered ADTs compared to saline controls. The most obvious example in support of this hypothesis was immediately following systemic baclofen with SCH 50911, in which previously treated SCH 50911 Fast rats (treatment #13) (80%) exhibited significantly longer and more frequent generalized convulsions than the Slow rats (33%) or saline group (50%). These results not only suggest that $GABA_B$ receptor antagonism of kindled brains has epileptogenic effects, but may also affect the strains differently. It is also important to note that a decrement in ADTs does not always equal a concomitant increase in seizure durations. These different measures may well be reflecting very different mechanisms. In this vein, our data, despite demonstrating a large decrease in ADTs of saline treated Fast and Slow rats following baclofen and baclofen with SCH 50911, do not show substantial increases in seizure expression. Our findings were in contrast to those found in other studies (Karlsson et al., 1992) that showed rats given both systemic injections of baclofen and a $GABA_B$ antagonist, CGP 35348, for 12 consecutive days demonstrated large increases in AD durations at seizure stage 3 and 4, with none of the rats achieving stage 5 seizures. This result provides evidence that baclofen can *increase*, rather than suppress AD durations during kindling development (Karlsson et al., 1992). It appears that the effects of the two drugs (baclofen vs. CGP 35348) in that study were mutually antagonistic, especially during stages 1-2, but at later stages of kindling CGP 35348 failed to overcome the threshold for the initiation of generalized convulsions. Clearly, the differences in the two studies were attributed to the kindled state vs. partially kindled, strain of the animals, and route of administration of the $GABA_B$ antagonist.

As mentioned above, we also found that Slow rats, previously treated with SCH 50911 after the crossover, in response to systemic baclofen with SCH 50911 infusions demonstrated fewer convulsive seizures compared to only systemic baclofen administration the day before. This finding was in complete opposite to the Fast rats. It is likely that SCH 50911 infused into the amygdala of Slow rats did not adequately recruit motor networks to drive generalized convulsions in the presence baclofen. Another possibility may arise from a greater binding affinity for systemic baclofen to $GABA_B$ receptors than focal SCH 50911 in the Slow rats. In either case, the above manipulation demonstrated the importance of the amygdala focus in determining the initiation of seizure activity even in the face of a potent $GABA_B$ agonist. Furthermore, the increase in seizure expression and severity largely evident only in the Fast rats, suggest that the heightened responsiveness to $GABA_B$ antagonism is strain specific.

$GABA_A$ Manipulations

Muscimol Microinfusion in Amygdala Kindled Fast and Slow Rats

Microinfusion of the $GABA_A$ agonist, muscimol, into the kindled amgydala (treatment #14) failed to significantly alter any measurable parameters of kindling in the Fast and Slow rats, including ADTs, AD durations and convulsion profiles. Approximately half of the Slow rats, and the majority of Fast rats experienced generalized convulsions when triggered with a kindling stimulus at their ADT. In addition, EEG recordings showed little evidence of IIS measured before and during subthreshold stimulations. The large majority of Fast rats did not exhibit IISs prior to

stimulation, while most Slow rats demonstrated some IISs, with low frequency. Subsequently, between subthreshold stimulations, IISs remained sporadic but now appeared in both strains, while the frequency was increased slightly with higher stimulation intensities and appeared in both hemispheres. Taken together, these data suggest that the agonist of $GABA_A$ type receptor, muscimol, focally administered into the amygdala did not alter the expression of either IISs or the mechanisms recruiting and manifesting the motor seizures emanating from the kindled amygdala.

IIS have been regarded as the hallmark of epilepsy, and are indicative of an abnormally excitable epileptic focus (Sundaram et al., 1990). Therefore, it is of no surprise that the occurrence of IIS is a direct consequence of the kindling process since they are not observed in experimental animals prior to kindling (Gotman, 1984, Leung, 1990). However, the mechanism and meaning of IIS are highly controversial. IIS are characterized as synchronized burst of action potentials generated by recurrent excitation, followed by a period of hyperpolarization (McCormick and Contreras, 2001). Thus, the nature of the IIS may represent periods of faulty inhibition of the network that would account for the appearance of discrete excitatory spikes. It has been shown that IISs occur more frequently in Slow rats compared to Fast rats during normal development of amygdala kindling (Dufresne, 1990). To explain this apparently paradoxical result of IISs in the seizure resistant Slow rats, we suggest that they might normally manifest spontaneous or triggered shunting of excitation from a focus during and after kindling, resulting in enhanced inhibition of ictal seizure generating mechanisms (slower kindling) but higher IIS rates, while Fast rats, with fewer and less sustained IISs, maintain a more facilitated seizure network (faster kindling) (Grabowski, 1996).

The lack of significant effects of muscimol against kindled and other seizures has been supported by other studies. For example, muscimol perfusion via microdialysis into the hippocampus failed to significantly inhibit glutamate increase induced by various depolarizing agents (Tanaka et al., 2003). Infusion studies reported that muscimol aimed at the anterior substantia nigra of amygdala-kindled rats did not elicit an increase in seizure thresholds or reduce seizure severity and duration (Gernert and Loscher, 2001). Additionally, centrally administered muscimol failed to attenuate kindled seizures even at doses that produced pronounced side effects (Loscher and Schwark, 1985).

Taken together, despite the relatively quiescent baseline IIS activity from both amygdalae, the results from this and other experiments demonstrate that microinfusion of muscimol directly into various seizure susceptible structures has little consequence on the blockage of kindled seizures. The mechanisms underlying this phenomenon could originate from several possibilities. First, microinfusion of muscimol directly into the amygdala may cause an initial desensitization of postsynaptic $GABA_A$ receptors, which would lead to their partial inactivity (Anwyl and Narahashi, 1980). This in turn would prevent endogenous GABA from binding to postsynaptic sites, and a subsequent reduction of inhibition will likely ensue. Therefore, upon triggering an AD at threshold, generalized seizures may occur. In the present study, this desensitization hypothesis may be very likely because our total duration of microinfusion of muscimol lasted 7.5 min. However, the rate of desensitization may depend on many factors especially the type of receptor. For the GABA receptor, concentration of the drug, number of receptors, binding affinity and areas of the brain all play a role. Second, a more plausible explanation for the inadequate blockade of generalized seizures from muscimol

microinfusion is the possibility that muscimol directly acts on inhibitory interneurons or on the GABAergic nerve terminal (Chesnut and Swann, 1989). Such a mechanism may promote or at least maintain disinhibition of the amygdala that allows for unimpeded generalized kindled seizures, a condition that appears more favourable among the seizure prone Fast rats since the majority were able to elicit convulsions when triggered with an adequate stimulus at threshold.

Picrotoxin Microinfusion in Amygdala Kindled Fast and Slow Rats

Microinfusion of picrotoxin into the kindled amygdala (treatment #15) also did not elicit any clear behavioural abnormalities. However, the most important finding from this manipulation was the appearance of very distinct and nearly rhythmic pattern of rapid double spikes. This type of IIS was clearly of higher frequency than those elicited from muscimol or baclofen administration. EEG recordings showed evidence of IIS measured before stimulations, but were dominated by the high frequency spikes between subthreshold stimulations. In other studies, picrotoxin spikes are often associated with myoclonic jerks indicative of convulsive seizures (Medvedev et al., 1996). However, the spikes reported in other studies have used a picrotoxin dosage 3 times the dose of our experiment (Medvedev et al., 1996; MacKenzie et al., 2004).

Typically, the spikes are in the range of 5-7 Hz and often accompanied by bilateral, synchronous high amplitude bursts (van Luijtelaar and Coenen, 1986). The proposed mechanism by which picrotoxin exerts its synchronizing effects is via the disinhibition of GABAergic interneurons, which under normal circumstances serve to modulate and inhibit principal cells (Thompson, 1994). The lack of myoclonic jerks associated with

the focal spikes in our Fast and Slow rats was not surprising considering the focal method by which the drug was administered compared to the more centrally activating systemic routes used in other studies. Interestingly, however, the dosage used in our experiment was enough to induce measurable EEG abnormalities in the amygdala, but did not trigger the severe behavioural seizures characterized by systemic application. However, in the present study, the unilateral infusion was sufficient to induce bilateral spiking, which occurred with equal propensity in Fast and Slow rats, an effect that became more apparent with increased stimulation intensities during the subsequent ADT test. The *bilateral* appearance of the spikes was likely attributed to a powerful drive radiating from the infused focus to other structures, including the propagation of impulses through callosal pathways to the contralateral amygdala (Medvedev et al., 1996).

Although it has been suggested that picrotoxin causes a decrease in recurrent inhibition, both in the induction of IIS and in ictal seizure facilitation (Dichter and Ayala, 1987), to our surprise, a near equal number of Fast and Slow rats (approximately 50%) failed to produce a generalized kindled convulsive seizure upon triggering an AD in the kindled focus in the presence of focal picrotoxin. This finding suggests that picrotoxin, delivered directly into the amygdala through microinfusion, *failed* to create a ubiquitous hyperexcitable environment that unabashedly allowed a focally triggered AD from readily generalizing into the kindled network to drive a kindled motor seizure. Why this should happen is not clear. It may be possible that the dosage used in this experiment was simply too low to allow for that outcome or to induce behavioural changes and/or a change in the kindled ADTs and their respective durations. Another possibility is that the 'pattern' of triggered activity is critically important in allowing for generalization to

occur, and picrotoxin perturbated the network and upset the balance between excitation and inhibition, but also created a milieu such that the 'necessary pattern' for generalization was not easily induced by the triggered AD.

In the present experiment, focal microinfusion of picrotoxin did not show any preferential effects for one strain over the other, unlike previous reports, using *systemic administrations,* which demonstrated that Fast rats were far more sensitive to GABAergic antagonists like picrotoxin than Slow rats (Racine et al., 2003). Taken together, the results from microinfusion of picrotoxin directly into the amygdala showed that it produced very strong, IISs that synchronized both amygdalae of Fast and Slow rats bilaterally, and that the occurrence of these spikes did not clearly predict the spontaneous spread of excitation beyond the focus to other structures, or the secondary generalization of focally triggered ADs that typically occurs during the threshold triggering of kindled convulsions.

Systemic Baclofen with Focal Picrotoxin Infusion

The primary purpose of the systemic baclofen and picrotoxin microinfusion drug combination was to assess whether focal application of a $GABA_A$ antagonist would offset the inhibitory effects of a centrally administered $GABA_B$ agonist. Further, this focal $GABA_A$ antagonist, picrotoxin, was specifically selected to compare with the previously used focal $GABA_B$ antagonist, SCH 50911 (treatment #13), on seizure thresholds, durations and severity. The most significant finding from this manipulation was that microinfusion of picrotoxin (treatment #16) produced opposite convulsive profiles compared with microinfusion of SCH 50911 (treatment #13) in the presence of systemic

baclofen among Fast and Slow rats. More specifically, focal administration of picrotoxin with systemic baclofen did not unmask the ability of systemic baclofen to block the expression of convulsive seizures in Fast rats. Conversely, the same combination treatment in Slow rats resulted in the convulsive generalization in many cases. This result has two important messages. The first speaks to the difference between the two strains with respect to focal $GABA_A$ mechanisms and their interaction with the network when exposed to a $GABA_B$ agonist. The second speaks to the importance of local networks and their control of seizure initiation mechanisms in one strain compared to the other. The ability of systemic baclofen to significantly block convulsive seizures in the Fast rats, even in the face of a potent focal $GABA_A$ antagonist, demonstrates not only a greater sensitivity of the $GABA_B$ system in the network of this strain (perhaps owing to a greater number of $GABA_B$ receptors) but also the dominance of the global network excitability over the focal seizure discharge as a synchronizer of the network. This finding was consistent with our earlier systemic administration of baclofen (treatment #12), which blocked the initiation of convulsive seizures in all Fast rats and all Slow rats, depending upon the previous day's treatment (treatment #11, saline versus SCH 50911). In the majority of Slow rats, the ability of the focal AD to generate convulsions clearly suggests that manipulation of local network with a potent $GABA_A$ antagonist in that strain can overcome the powerful inhibitory effects in the global network under the influence of systemic baclofen. How might this be achieved? It may be possible that the focal amygdala of Slow rats is more under the influence of $GABA_A$ mediated inhibition than the Fast rats, while the global network is more under the influence of $GABA_B$ mediated mechanisms in the Fast compared to Slow rats. Certainly, the Slow rats seem

far less sensitive to $GABA_B$ manipulations, either administered systemically or directly into the focus than Fast rats. Despite the natural differential seizure expression between the Fast and Slow rats (longer convulsion latencies and durations and longer AD durations in Fast rats), the combination of baclofen and picrotoxin failed to induce significant changes in the ADTs, or the profile of the ensuing convulsive seizure, when it occurred.

Systemic baclofen with focal application of picrotoxin changed EEG attributes but failed to uncover *strain differences*. Although the electrographic profiles differed from the previous infusion of picrotoxin, spike frequency measured before stimulation and during IIS was substantially reduced and more disorganized when baclofen was onboard. Clearly systemic baclofen had a profound effect on the timing and frequency of picrotoxin-induced spiking in the amygdala. The similarities in EEG characteristics between the strains during these manipulations might suggest that $GABA_A$ mechanisms relevant to IIS are similar between the two strains but the recruitment of the network from a focal AD is not.

In the last 2 manipulations (treatments #17-19), in parallel to the immediately previous manipulations, we wished to further explore whether infusion into the amygdala of $GABA_A$ or $GABA_B$ agonists would alter the spread of seizures from the focus in the presence of a disinhibited network under the influence of systemic picrotoxin.

Systemic Picrotoxin with Focal Infusion of Muscimol or Baclofen

In the last 2 manipulations, picrotoxin was systemically administered in conjunction with either muscimol or baclofen infusion into the kindled amygdala. There were no

obvious abnormalities in behaviour or significant electrographic patterns during these treatments (treatments #17 and #18). As expected, the presence of IIS was infrequent before and during subthreshold stimulations. Administered separately, baclofen and muscimol through infusion, and picrotoxin via systemic injection elicited generalized convulsive seizures in nearly all of the Fast and Slow rats. Although baclofen exerts its effects on different receptors than muscimol, the ability of either agonist to suppress the recruitment of seizure spread from the focus in the presence of systemic picrotoxin was lacking. This was of no surprise, as these two agonists did not change the seizure profiles of the rats when they were infused singly at an earlier time (treatments #10 and #14). These manipulations were more to complete the parallelism of procedures, and to look for larger changes in convulsive profiles (perhaps shorter latencies and longer seizures) when the network was disinhibited with picrotoxin.

In summary, the data addressed the overall contribution and efficacy of $GABA_A$ versus $GABA_B$ pharmacology on seizure development when administered centrally via systemic injection compared to focal application directly into the amygdala. The activation and/or blockage of one receptor type over another suggest the relative importance of each GABA receptor system in kindling epileptogenesis and the subsequent modulation of inhibition within the amygdala during the kindled state. Given that the amygdala is a common origin of seizure activity in humans, elucidating the many possible cellular mechanisms in rats that possess a genetic background for or against epilepsy should ultimately provide novel insights into the development of more efficacious treatments against temporal lobe epilepsy.

Experiment #2 – Immunocytochemical Identification and Localization of $GABA_B$ Receptors and GAT1 Transporters

Synopsis

The predominant neuronal GABA transporter GAT1 is localized in GABAergic axons and nerve terminals, where it is thought to influence GABAergic synaptic transmission by modulating extracellular GABA concentration (Bernstein and Quick, 1999; Jensen et al., 2003). GABA levels, in turn, modulate chronic changes in GABA transport in a time and dose dependent manner. This powerful feedback mechanism governs and maintains the brain's inhibitory tone. In epilepsy, it has been postulated that an up-regulation of GAT expression may result in lowered GABAergic transmission and therefore contribute to the generation of seizures (Gadea and Lopez-Colome, 2001). To this end, one part of the present experiment was conducted to quantify and assess GAT1 distribution in the BLA of Fast and Slow rats. The second part of the study was to identify $GABA_B$ receptors in the BLA, and also assess whether these receptors co-localized with GAT1 within inhibitory terminals. Previous studies have shown that $GABA_B$ receptors are located on pre- and postsynaptic sites, primarily on the extrasynaptic membrane of spines and dendritic shafts of principal cells (Kulik et al., 2003). As a result, their activation is primarily dependent on spillover of GABA, and not directly associated with GABAergic synapses per se. Nevertheless, $GABA_B$ receptors are important in determining enhanced and/or simultaneous activity of GABAergic interneurons, particularly during oscillations of neuronal populations in the epileptic brain.

The present study attempted to examine whether the seizure prone Fast rats may show a preferential increase in co-localization of $GABA_B$ and GAT1 (indicative of loss in inhibition) within the inhibitory terminal of GABAergic neurons compared to Slow rats. Furthermore, in the kindled amygdala, we hypothesized that GAT1 immunoreactivity of Fast rats would escalate and postsynaptic $GABA_B$ receptors to down-regulate, thereby, favouring a decrease in GABAergic transmission across the synapse.

METHODS AND MATERIALS

Ten non-kindled and 10 kindled Fast and Slow male rats were used in this experiment. Kindled animals were sacrificed at least 1 week following the last convulsive seizure. The present study sought to: 1) examine the independent distribution of $GABA_B$ (R1) receptor and GAT1 protein within the basolateral amygdaloid complex and; 2) examine the co-localization of these proteins at specific inhibitory nerve terminals.

Intracardial perfusion of both non-kindled and kindled brains was made possible with 70/30% acetone/methanol solution for increased resolution during immunocytochemical fixations. The brains were placed in acetone/methanol for a minimum of 3 days at 4°C before subjected to cryoprotection in 30% sucrose in PB for an additional 5 days. Whole brain coronal sections of 10 μm were made possible using a cryostat maintained at -30°C. The tissue was kept in a -20°C freezer until processing for immunocytochemistry. The mounted sections were permeabilized with PBS for a total of 3 runs at 5 min each. PBS with 0.2% Triton X-100 was then applied for an additional 30 min. A solution containing guinea pig polyclonal primary antibody (1:5000, Chemicon)

raised against synthetic peptides of the rat sequences of $GABA_B$ receptors, and rabbit anti-GABA transporter-1 (GAT-1) polyclonal antibody (1:50, Chemicon) was applied to the tissue and allowed to incubate overnight at 4°C. The next day, after rinsing in 0.2% Triton X-100 in PBS for 3 runs (10 min each), the sections were incubated in Cy3-conjugated donkey anti-guinea pig (1:1000, Jackson Immunoresearch Labs) together with Alexa Fluor 488 goat anti-rabbit (1:1000, Molecular Probes) secondary antibodies for 2 hr at room temperature in minimal light. The slices were then rinsed with Triton X-100 in PB for 3 runs (10 min each) followed by a 3 min incubation period using antifade with DAPI equilibration buffer (Molecular Probes). After rinsing off the excess antifade buffer, the sections were subjected to 1% Sudan Black. Slices were then perfused 3 times with 70% alcohol then mounted using PBS with glycerol mixture and placed in -20°C until examination under a fluorescent microscope. Negative controls consisted of sections processed as described above but in the absence of primary antibody.

Image Acquisition and Analysis

A series of stacked photos were taken from a Leica DMXRA wide-field fluorescence microscope at 100x magnification to visualize immunoreactivity. A Photometrics *CoolSnap fx* CCD camera (1300 x 1030 pixels) in conjunction with the "Openlab" software (Improvision) was used to capture these images. The optical resolution (OR) of an image was calculated based on the wavelength of each fluorescent channel and the numerical aperture (NA) of the objective using the formula, $OR = \lambda/(2 \times NA)$. The NA for the 100x objective was 1.3. The following colour channels were used in this experiment: DAPI (stain DNA, λ=440 nm, OR=169 nm), Rhodamine (identify CY3

fluorescence, $GABA_B$, λ=590 nm, OR=227 nm), FITC (identify CY2 fluorescence, GAT1, λ=540 nm, OR=208 nm). Thirty photos in total (10 for each fluorescent channel) with a z spacing of 0.2 μm covering a total depth of 2 μm were obtained. The individual TIFF files were converted to raw files using the IPLAB software (Scanalytics). The files were then de-convolved using a series of mathematical algorithms, while incorporating multiple "point spread functions" (PSFs) to resolve out of focus photos into fine detailed images. Fluorescently labeled Styrofoam beads (Molecular Probes) with a radius of 0.175 μm were used for the PSFs. Following de-convolution, the images were assessed for co-localization using IPLAB whereby images from each colour channel were merged and selected for co-localization based on a signal ratio of 1.5x background. When appropriate, an area was selected for a given region of interest, and the amount of immunoreactivity was analyzed accordingly. The presence of co-localization was explored in 3 separate groups of rat within the basolateral complex; naïve (non-kindled) and kindled ((stimulated amygdala), and contralateral (non-stimulated amygdala)). All co-localization was expressed as a percent of the availability of $GABA_B$ and GAT1 in 1) the whole photo (53.5 x 71.5 μm area), 2) the soma (approximately 13.2 x 15.4 μm area), and 3) the neuropil (approximately 23.3 x 26 μm area). In addition, the total area of immunoreactivity, as represented by the number of pixels (764 x 1020), of $GABA_B$ and GAT1 was also examined.

Analysis

As mentioned above, the expression of co-localization was presented as a percent based on a ratio of co-localization to the density of $GABA_B$ and GAT1. Using ANOVA,

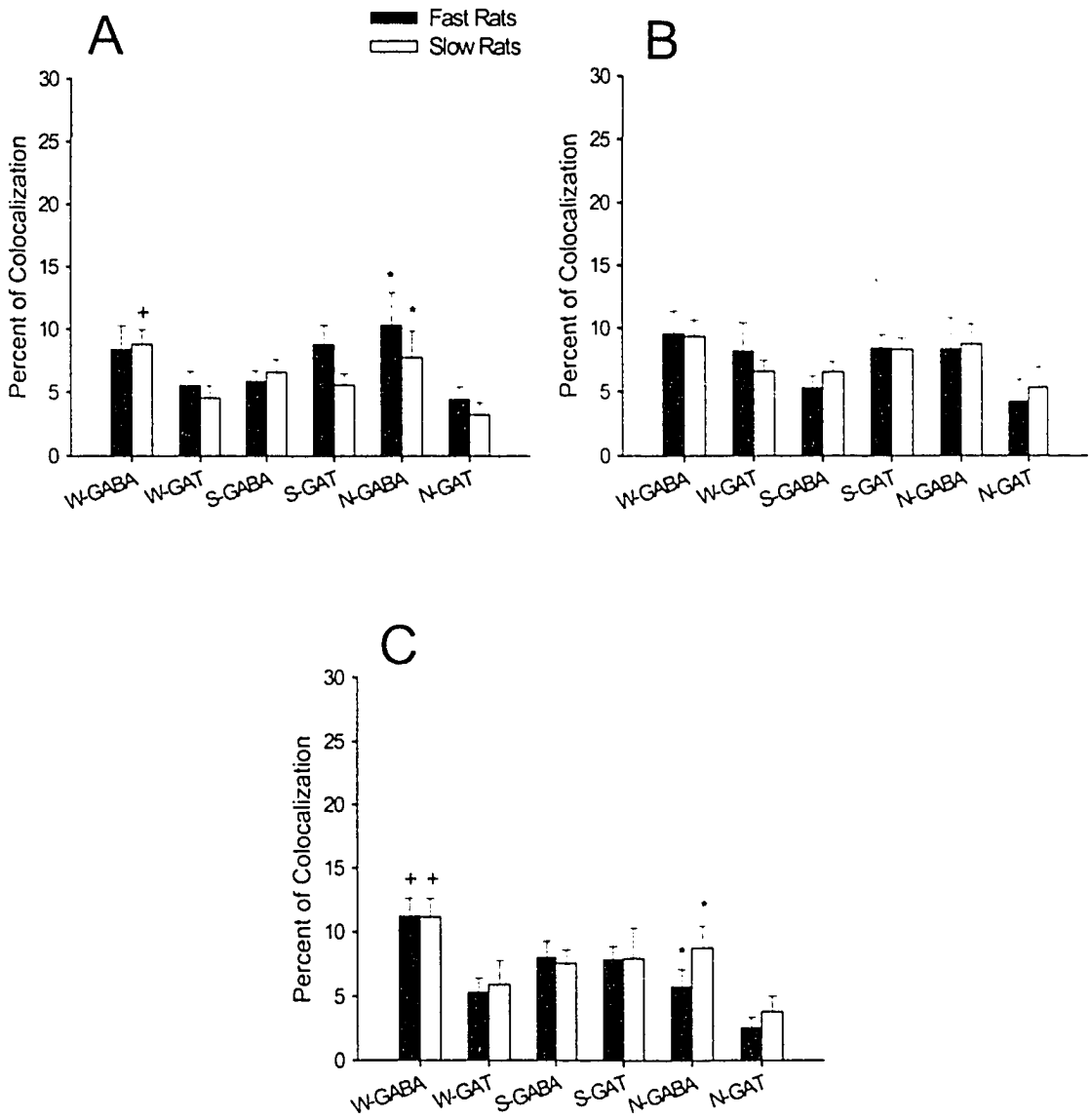
the Fast and Slow rats represented between groups factors, as did kindling manipulations (naive vs. kindled animals, (Group)). The within group factors were analyzed according to region of interest (whole photo, soma, neuropil).

RESULTS

As seen in Figure 13, co-localization of $GABA_B$ receptor and GAT1 immunoreactivity within the basolateral complex was minimal (~5-10%). The overall analysis found no strain differences between naive and kindled rats. As expected, there was a greater amount of GAT1 immunoreactivity compared to $GABA_B$ in both the entire photo ($F_{1,52} = 27.05$, $p<.001$), and within the neuropil ($F_{1,53} = 39.20$, $p<.001$). In the soma, there was no significant difference in the intensity of co-localization between $GABA_B$ and GAT1.

The total pixelated area of antibody immunoreactivity was examined to measure the absolute amount of $GABA_B$ and GAT1 present irrespective of co-localization. This analysis was necessary to explore possible changes in synaptic efficacy or receptor density as a consequence of kindling. The analysis showed a significant increase in whole photo GAT1 compared to $GABA_B$ ($F_{1,53} = 32.22$, $p<.001$). However, there were no strain or kindling effects. Although statistical insignificant, there was a slight tendency for a greater increase in GAT1 of both strains in the kindled (stimulated) amygdala compared to the contralateral (non-stimulated) amygdala and naive rats (Figure 14). Assessment of the somatic distribution of total $GABA_B$ and GAT1 immunoreactivity revealed a significant main effect of Group ($F_{2,150} = 5.48$, $p<.01$) and Group x Antibody interaction ($F_{1,150} = 4.14$, $p<.05$). The follow-up comparisons

Figure. 13 – Co-localization of $GABA_B$ and GAT1 immunoreactivity in the basolateral amygdala of the Fast and Slow rats. These series of figures represented an average (± SEM) percentage of co-localization of $GABA_B$ and GAT1 immunoreactivity in the Fast and Slow strains. Each percentage was represented as a ratio of co-localization to the amount of $GABA_B$ or GAT1 assessed in whole photo (W), soma (S), and neuropil (N). Very similar profiles existed between naive (non-kindled) (A), contralateral (non-stimulated) amygdala (B), and kindled (stimulated) amygdala rats (C).
'*' = significantly different than N-GAT of the same strain, $p<.05$.
'+' = significantly different than W-GAT of the same strain, $p<.05$.



confirmed that the immunoreactivity of somatic $GABA_B$ in the contralateral (non-stimulated) amygdala was significantly higher than naïve rats. Further, the kindled (stimulated) and contralateral (non-stimulated) amygdala maintained a higher density of total GAT1 in the somatic region compared to naive rats. Again, total GAT1 expression in the neuropil was significantly higher than $GABA_B$ ($F_{1,53} = 48.89$, $p<.01$). A photomicrograph shown in Figure 15 displays typical $GABA_B$ and GAT1 immunoreactivity. The majority of $GABA_B$ staining was located in cell bodies with GAT1 in the immediate vicinity and also scattered throughout the neuropil.

DISCUSSION

The results of the present experiment did not confirm the original hypothesis, which suggested that the seizure prone Fast rats might express higher levels of co-localization of $GABA_B$ and GAT1. In fact, although the percentage of co-localization was remarkably low, it was consistent across all 3 groups (naïve, stimulated, non-stimulated amygdala). This indicates that $GABA_B$ receptors may not play a pivotal role in regulating GABA release at inhibitory terminals of GABAergic neurons, and that kindling does not significantly alter $GABA_B$ expression at both pre- and postsynaptic sites within the BLA. Although statistically insignificant the kindled (stimulated) amygdala in the whole photo showed a slightly larger percentage of co-localization (expressed as a ratio to overall $GABA_B$) compared to non-kindled rats, suggesting a minor up-regulation of $GABA_B$ receptors on inhibitory terminals as a result of kindling. The data was further examined for total $GABA_B$ and GAT immunoreactivity in the entire photo irrespective of co-

Figure. 14 – A measure of total $GABA_B$ and GAT1 immunoreactivity in the basolateral amygdala of the Fast and Slow rats. The average (± SEM) total amount of $GABA_B$ and GAT1 immunoreactivity in the Fast and Slow strains were assessed in naive rats (non-kindled) (A), contralateral (non-stimulated) amygdala (B), and kindled (stimulated) amygdala (C) irrespective of co-localization. Each total was examined in whole photo (W), soma (S), and neuropil (N). Notice the slight increase of GAT1 immunoreactivity taken from the whole photo of Fast and Slow kindled rats (C).

'*' = significantly different than N-GABA of the same strain, $p<.05$.

'+' = significantly different than W-GABA of the same strain, $p<.05$.

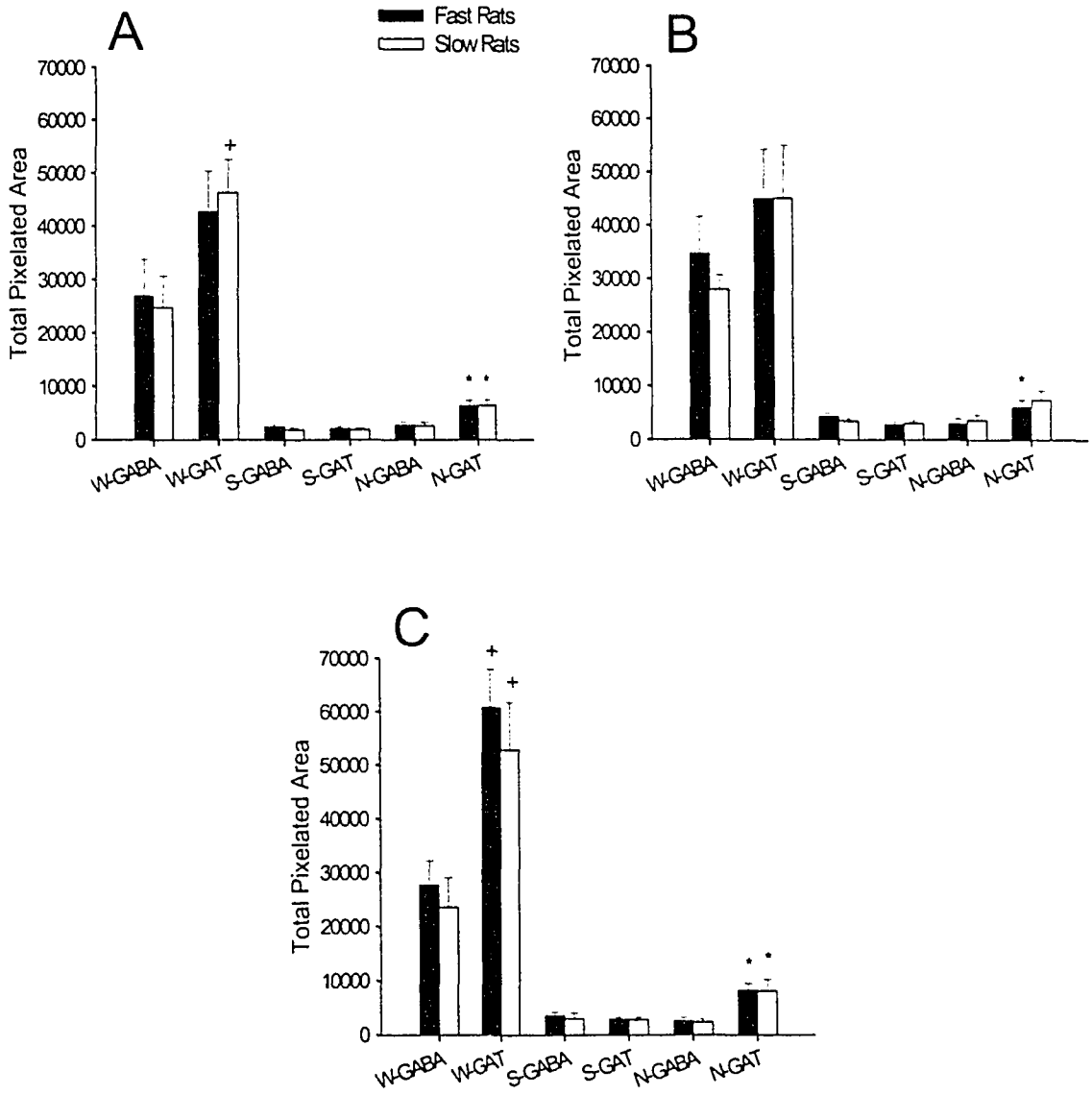
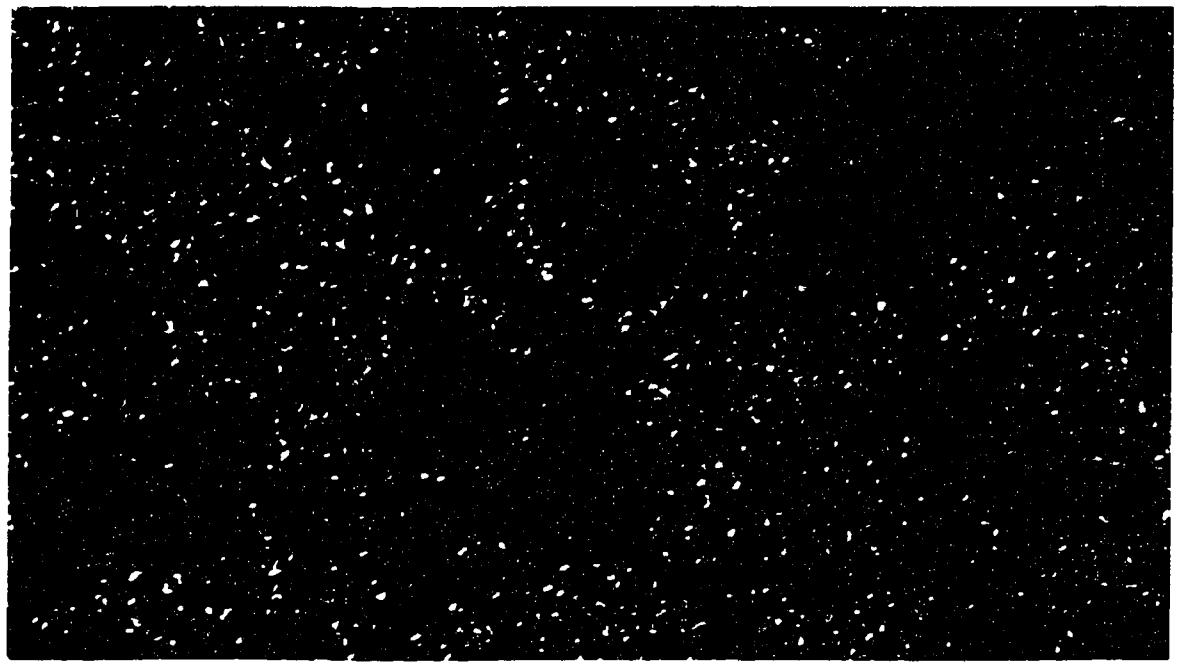
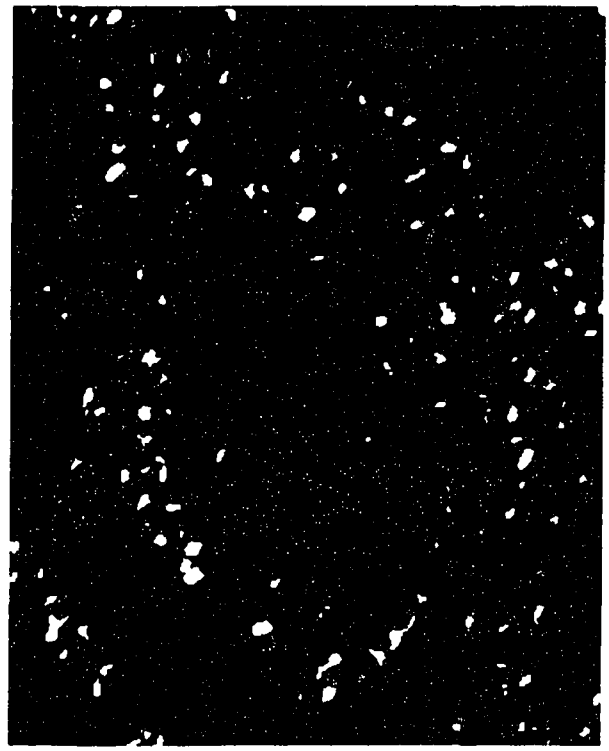


Figure. 15 – Co-localization of $GABA_B$ and GAT1 immunoreactivity from pyramidal cells of the basolateral amygdala under 100x magnification. This was a representative photomicrograph taken from the contralateral (non-stimulated) amygdala of a kindled Fast rat that showed modest co-localization of $GABA_B$ and GAT1 immunoreactivity (yellow). Largest concentration of GAT1 (green) was scattered in the neuropil (N), along dendrites of pyramidal cells, and the region surrounding the outer membrane of the soma (S) that housed the majority of $GABA_B$ receptors (red). There was also the appearance of $GABA_B$ staining present on dendritic branches. The presence of the nucleus was confirmed with a DAPI stain (blue). The bottom photomicrograph was a magnified image from an ideal cell (blue boxed area), which showed that the presence of co-localization usually resided in between $GABA_B$ and GAT1 immunoreactivity (blue arrows).





localization. The kindled (stimulated) amygdala of Fast and Slow rats demonstrated greater expression of GAT1 compared to the contralateral (non-stimulated) amygdala and naïve rats, but again not statistically significant. Similarly, somatic GAT1 was significantly higher in the kindled rats compared to naïve controls. There was, however, no significant change in overall $GABA_B$ immunoreactivity.

One of the more important points that must be addressed in this discussion is the modulation of GAT1 transporter function by a balanced feedback mechanism for controlling GABA levels at the synapse. The amount of transmitter at synaptic and extrasynaptic sites ultimately determines the number of functional receptors. For example, changes in extracellular GABA concentrations induce changes in GAT1 activity in a dose and time dependent manner (Bernstein and Quick, 1999). In fact, this modulation occurs through the action of GABA on the transporter directly (not mediated by GABA receptors), and that the regulation of transporter levels are proportional to the rate of transporter internalization (Bernstein and Quick, 1999). In the context of epilepsy, studies have shown that antiepileptic drugs have been used to down-regulate neuronal and glial GAT1 protein expression, thereby increasing the extracellular GABA concentration (Ueda and Willmore, 2000). Our results are in agreement with the hypothesis that an up-regulation of GAT expression in epilepsy may result in lowered GABAergic transmission and therefore contribute to the generation of seizures (Gadea and Lopez-Colome, 2001). Since activation of both presynaptic and postsynaptic $GABA_B$ receptors is dependent on spillover of GABA from inhibitory synapses, the increased efficacy and number of GAT1 transporters, along with a slight rise in $GABA_B$ presynaptic receptors, may be sufficient to dampen the inhibitory tone. Although the

present findings suggest an elevation of inhibitory terminals as a consequence kindling, the amount of postsynaptic $GABA_B$ immunoreactivity remain relatively unchanged. One explanation may be that the presence and efficacy of GAT1 transporters after kindling at synaptic and extrasynaptic sites are sufficiently available to lower GABA concentration to levels that activate minimal number of $GABA_A$, $GABA_B$ autoreceptors and heteroreceptors (Mitchell and Silver, 2000). This in turn will have little impact on the number and density of pre- and postsynaptic $GABA_B$ elements, and may even promote down-regulation of postsynaptic sites over a prolonged period. As a result, more GABA may be released into the synapse via other mechanisms due to the brain's attempt to maintain an inhibitory balance. One such mechanism may involve a calcium independent, non-vesicular efflux of GABA via GAT1 transporters that may help to compensate for the GABA deficit. It has been proposed that this mechanism is related to pathological conditions like epilepsy (Meldrum, 1995). During a severe seizure, neuronal depolarization reaches threshold with great facility and is sufficient to reverse GABA transport (Ma et al., 2001). It is possible that the over-expression of GAT1 may be compensatory and serves to facilitate the calcium independent GABA release, thus suppressing further spread of seizure activity and maintaining a balance between excitatory and inhibitory processes. However, this particular mechanism of GABA release may not apply to the present results because immunoreactivity in the kindled rats was not assessed until at least a week following the last convulsive seizure. The present data suggest that kindling does not induce a permanent, robust change in $GABA_B$ receptor distribution and expression. We propose that the availability of GABA in the synaptic cleft of kindled rats, regardless of strain, was not sufficient to induce a long

lasting change in the number of postsynaptic $GABA_B$ receptors. It is unclear in this study whether the minor increase of GAT1 expression in the Fast and Slow rats after kindling was due to the above mechanism and/or other external factors such as presynaptic $GABA_B$ modulation of glutamatergic transmission.

Are $GABA_B$ Receptors Important in Epilepsy?

Presynaptically, $GABA_B$ receptors reside in the extrasynaptic membrane and presynaptic membrane specialization of glutamatergic, and to a lesser extent, GABAergic axon terminals (Kulik et al., 2003). These receptors are known to inhibit neurotransmitter release by depressing calcium influx via voltage activated calcium channels (Wu and Saggau, 1995; Olsen et al., 1999). Postsynaptically, $GABA_B$ receptors are localized to the extrasynaptic plasma membrane of spines and dendritic shafts of principal cells and shafts of interneuron dendrites (Kulik et al., 2003). Their function is to generate and maintain delayed, long lasting inhibitory postsynaptic potentials. There are many reports suggesting that $GABA_B$ plays a minor role in neuronal signaling. For example, ambient GABA levels in brain slices at moderate concentrations have been shown to activate postsynaptic $GABA_A$ receptors and presynaptic $GABA_B$ receptors to depress GABA release, while failing to activate postsynaptic $GABA_B$ receptors (Yoon and Rothman, 1991). On the other hand, Jensen et al. (2003) demonstrated from immunocytochemical studies that GAT1 knockout mice exhibited no changes in the numbers of $GABA_A$ and $GABA_B$ receptors at inhibitory terminals compared to wild type controls. In fact, despite several fold higher external GABA concentrations in GAT1 knockout slices compared to wild type, no presynaptic $GABA_B$ tone was found in these

animals (Jensen et al., 2003). These experiments demonstrate that $GABA_B$ receptors quietly modulates neurotransmission via inhibitory synapses, and perhaps may not become fully functional until the balance between excitation and inhibition is offset by seizure activity. If this was true, the severity of the seizure (partially kindled, fully kindled, recurrent seizures or status epilepticus) may then account for the degree at which the GABAergic system is able to compensate for this pathological state.

There have been a number of *in vitro* studies that have reported a decrease in presynaptic $GABA_B$ receptor efficacy after kindling (Asprodini et al., 1992; Wu and Leung, 1997). On the other hand, an increase in presynaptic inhibition has also been documented (Gloveli et al., 2003). However, it is not known whether kindling can induce changes in $GABA_B$ receptors at the cellular level in vivo. Numerous experiments have confirmed a decrease in GABA-immunoreactivity in kindled animals (Kamphuis et al., 1986; Lehmann et al., 1998; Callahan et al., 1991), which may be directly attributed to a down-regulation of GABA receptors that would account for the loss of GABA-mediated inhibitory postsynaptic potentials in seizure prone areas like the amygdala (Gean et al., 1989). In contrast, under a mass kindling stimulation protocol of 40 trials delivered to the ventral hippocampus every 5 minutes, Kokaia and Kokaia (2001) found no changes in GABA immunoreactivity at 6, 12 hours, and 4 weeks in the amygdala. Interestingly, in all other areas examined (entorhinal cortex, perirhinal), except the piriform cortex, changes in $GABA_B$ receptor immunoreactivity were transient suggesting that $GABA_B$ receptors do not contribute to the long-term maintenance of the kindled state (Kokaia and Kokaia, 2001). Tuunanen et al. (1997) confirmed that the density of GABA

immunoreactive neurons in the stimulated amygdala did not differ from the contralateral (non-stimulated) amygdala 6 months after the last evoked seizure.

Taken together, the results of this experiment revealed no overall significant differences in $GABA_B$ expression of the Fast and Slow rats from the BLA. Future studies should be directed to identify the molecular subunits and distribution of $GABA_B$ in these strains at different stages of kindling. $GABA_A$ alpha subunits from the Fast and Slow rats have already been identified and exhibit differential expression of juvenile and adult forms, suggesting that abnormal expression of these receptors may underlie epileptogenesis (Poulter et al., 1999; McIntyre et al., 2002). The effect of kindling, from the present data, does not support a robust change in GAT1 or $GABA_B$ immunoreactivity in the amygdala. There was a slight tendency toward increased GAT1 and $GABA_B$ expression in kindled rats, suggesting enhanced presynaptic uptake of GABA from synaptic and extrasynaptic sites resulting in decreased inhibitory neurotransmission indicative of seizure activity. However, this effect was subtle at best.

Experiment #3 – Electrophysiological Correlates of $GABA_B$ Receptor-Mediated Inhibition of Fast and Slow Rats

Synopsis

Synaptic transmission, particularly through inhibitory networks can be studied in a variety of ways. One approach is to elicit polysynaptic IPSCs and IPSPs from principal cells via stimulation of an afferent pathway or a group of nuclei innervating these cells. Depending on the placement of the stimulating electrode, viable recordings can be

obtained from direct activation of cell bodies and/or axons of the inhibitory neurons through feedforward or feedback inhibition (Thompson, 1994). One of the more important parameters worth investigating is the postsynaptic conductance change at GABAergic synapses as a result of kindling or pharmacology. Differences in conductance values may stem from variations in the strength of inhibitory inputs. For example, an ion channel with a small conductance would suggest relatively few to moderate number of channels that would have been available to open at the peak of the IPSC (Collingridge et al., 1984). Further, maximal IPSC conductance attained from stimulation may stem from one of two sources: 1) a limit on GABA release from inhibitory interneurons; or 2) saturation of $GABA_A$ receptors on principal cells (Ling and Benardo, 1998). Therefore, understanding IPSC and IPSP kinetics in the Fast and Slow rats as a consequence of evoked stimulation should provide valuable information regarding GABA release and the inherent differences that favours or resists epilepsy in these two strains.

The modulation and inhibition of GABA release from synaptic terminals of interneurons is, to some extent, presynaptically controlled by $GABA_B$ receptors, albeit the mechanisms are not fully understood. Postsynaptically, it has been demonstrated that activation of $GABA_B$ receptors may impose an antiepileptic effect by increasing the ADT and rate of kindling (Bowery, 1989; Wurpel, 1994). Further, activation of $GABA_B$ receptors retards the influx of calcium ions into the terminals, thereby reducing the evoked release of excitatory amino acids and possibly other transmitters (Davidoff, 1985). In contrast, by preventing GABA release, activation of presynaptic $GABA_B$ receptors can promote seizures (Pitler and Alger, 1994). To further understand the

electrophysiological properties and responses of these receptors and its role in epileptogenesis, several investigators have used baclofen as one approach for studying frequency-dependent depression of GABAergic IPSCs (Mott et al., 1993; Doze et al., 1995; Jensen et al., 1999). Others have employed the paired pulse depression paradigm with the hypothesis that short interval periods between successive stimulations is largely due to the $GABA_B$-dependent reduction in GABA release (Pitler and Alger, 1994; Wu and Leung, 1997). It has been found that baclofen significantly attenuates IPSCs in a concentration-dependent manner with no effect on the frequency of miniature IPSCs (mIPSCs) (inhibitory currents in the absence of stimulation) recorded from CA1 pyramidal neurons (Doze et al., 1995). Conversely, baclofen attenuated the frequency, but not the mean amplitude of mIPSCs recorded from the BLA (Doze et al., 1995). These results suggest that activation of $GABA_B$ receptors by baclofen may be modulated by different presynaptic mechanisms that underlie the physiological properties of neurons in a particular region of the brain. Such regional differences may speak directly to the importance and involvement of $GABA_B$ receptor-mediated inhibition in the modulation and maintenance of seizures within the limbic system. Irrespective of the methodology used to activate and study $GABA_B$ receptors, the information gathered from electrophysiological and pharmacological studies will likely provide important clues regarding its role in both pre- and postsynaptic inhibition.

The purpose of this experiment is to determine the time course and amplitude of the underlying inhibitory currents and potentials in the Fast and Slow strain in response to afferent stimulation. We wish to explore the kinetics of $GABA_B$ receptor-mediated responses in the amygdala of principal cells. The relationship between kinetic properties

of the IPSC, IPSP and conductance is a major determinant of inhibitory strength and efficacy of neurotransmission. If $GABA_B$ receptors played a role in seizure genesis and expression, we would expect large differences in our Fast and Slow strains, suggesting that these receptors acting alone or in combination with $GABA_A$ receptors mediate seizure susceptibility and maintenance.

METHODS AND MATERIALS

Slice Preparation

Patch-clamp recordings were performed on brain slices isolated from adult Fast and Slow male and female rats (85-250 d of age). The number of rats used in the experiment was as follows: kindled group (stimulated amygdala, 20 Fast, 18 Slow), (non-stimulated amygdala, 20 Fast, 20 Slow); sham group (5 Fast, 14 Slow). The number of cells attempted, and of that group, the actual number of cells used for analysis (shown in brackets) were as follows: kindled group (stimulated amygdala – Fast 66(13), Slow 55(7)), (non-stimulated amygdala – Fast 62(18), Slow 46(8)); sham group – Fast 25(10), Slow 91(22). Taken together, this amounts to a 24% average success rate for eliciting $GABA_B$ responses from the BLA. Before decapitation, the rat was heavily anesthetized (sodium pentobarbital, 65mg/kg, i.p.) and perfused intracardially with an ice-cold Ringer's solution (composition in mM: 110 $C_5H_{14}NOCl$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 KCl, 2.4 $C_3H_3NaO_3$, 1.3 L-ascorbate, 20 glucose, 7 $MgCl_2$, 0.5 $CaCl_2$; pH 7.2) in which choline replaced sodium for the purpose of reducing sodium influx coincident with the tendency for neurons to depolarize during initial anoxia that follows decapitation,

dissection, and slicing. Immediately following perfusion, the brain was rapidly removed and placed in ice-cold choline aCSF for approximately 2 min before a sagittal dissection along the midline. One hemisphere was glued at a time, on its posterior surface, to a vibratome tray. Subsequently, coronal slices (300 μm) were obtained and immediately incubated in choline aCSF maintained at 34°C for 30 min. Finally, the slices were transferred to a room temperature choline bath until needed for recording. Choline solution was constantly bubbled with 95% O_2 - 5% CO_2 mixture of humidified gas. During electrical recordings, regular aCSF solution was similar to choline aCSF, but with pyruvate, ascorbate and $MgCl_2$ removed, and choline was replaced with 120 mM NaCl, with the addition of $CaCl_2$ and $MgSO_4$ in 2 mM concentrations. All recordings were made in a submersion chamber with each slice supported by a nylon net anchor. The appropriate drugs, described below, were delivered via a gravity perfusate system at a rate of approximately 3 mL/min.

Drugs

(2*S*)-(+)-5,5-Dimethyl-2-morpholineacetic acid, *SCH 50911*; (*R*)-4-Amino-3-(4-chlorophenyl)butanoic acid, *(R)-Baclofen*; 6-Imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid hydrobromide, *SR 95531* hydrobromide (Gabazine); 6,7-Dinitroquinoxaline-2,3-dione, *DNQX*; DL-2-Amino-5-phosphonopentanoic acid, *DL-AP5* were purchased from Tocris, MO.

Whole-Cell Recordings

In this experiment, only pyramidal (principal) cells from the basolateral amygdala were recorded for their $GABA_B$ profiles. Recordings from interneurons were not possible because of the inability to isolate pure, clean $GABA_B$ responses in the presence of appropriate glutamatergic and $GABA_A$ blockers. Recordings were made possible with an upright microscope (Zeiss Axioskop, Germany) and a 40X water immersion objective attached to a CCD camera to project cells of interest onto a Hitachi television monitor. Initially, baseline whole cell recordings were made at a resting membrane potential (V_m) of −58 mV. Series resistance and whole cell capacitance were monitored and adjusted during the experiments using the "PULSE" software program in conjunction with an EPC 9 Amplifier (Heka Instruments). The recordings were filtered at 2 kHz and sampled at 5 kHz. The absolute threshold for accepting a viable recording was at least −55 mV, series resistance (R_s) of ≤20 MΩ, membrane input resistance (R_{in}) of ≥100 MΩ, and series compensation at approximately 60%. Events with poor baselines and unstable V_m were excluded from the analyses. All recordings were made at 32°C. The junction potential was calculated to be -2 mV, and all subsequent measurements were adjusted accordingly.

Potassium gluconate patch pipettes of 3-8 MΩ were filled with an internal electrode solution containing (in mM): 145 gluconate, 3 KCl, 0.5 $CaCl_2$, 1 EGTA, 2 MgATP, 0.3 GTP, 10 glucose, and 10 HEPES (~300 mOsm; pH adjusted to 7.2), with 0.3% Lucifer Yellow for pyramidal classification of cells based on their somatic, dendritic, and axonal morphology.

Both voltage-clamp and current-clamp recordings were used to resolve evoked IPSCs and IPSPs. Stimulation was made possible by a bipolar electrode placed in the

lateral amygdaloid nucleus (LA). Isolated $GABA_B$ IPSCs and IPSPs were recorded in the presence of the glutamatergic antagonists 10 μM DNQX, 50 μM AP5, the $GABA_A$ antagonist, SR 95531 (20 μM) in the bathing solution. Baclofen (10, 20 μM) was applied to the bathing solution to assess changes in current in the absence of stimulation. The $GABA_B$ receptor antagonist, SCH 50911 (20 μM), was added to the perfusion medium for a selected number of cells to confirm the abolishment of $GABA_B$ responses.

IPSP and IPSC Measurements and Analysis

The rats used in this experiment were divided into 3 groups (Treatment): 1) sham (amygdala electrodes with no stimulation); 2) stimulated kindled amygdala; 3) non-stimulated (contralateral) amygdala. The initial voltage clamp assessment was designed to ensure the cell had sufficiently strong inward Na^+ current (> 1 nA) indicative of a healthy, tightly sealed cell. The next recording employed the current clamp technique to examine dynamic and steady state changes in voltage as measured by the peak amplitudes of both traces. Further, the spiking characteristics of each cell were recorded and documented. Following these 2 initial recordings, evoked $GABA_B$ responses were isolated in the presence of DNQX, AP5, and SR95331. Starting with a membrane potential held at –50 mV, $GABA_B$ IPSPs and IPSCs (at –58 mV) were evoked using a stimulation intensity of 20 V via a pencil point profile stimulation electrode (FHC, Maine) connected to a PSIU6 photoelectric isolation unit with pulses controlled by a Grass Instrument S88 stimulator. All stimulation pulses were 2 ms duration and delivered at 5 s intervals. Subsequent changes in amplitude were reflected at current intensities held at the corresponding membrane potential of –58, -68, -78, -88, -98, -108

mV. $GABA_B$ responses during IPSP recordings were examined for time to peak, amplitude (assessed at peak), and the duration of response. Conversely, responses during IPSC recordings with V_m held at –58 mV examined the total charge transfer, as measured by the area under the curve from baseline, determined by the experimenter and analyzed with a default macros function in the IGOR Pro software program. The evoked IPSCs and IPSPs for each membrane potential were averaged over 5 individual traces. Immediately following the series of stimulated-evoked responses, a Voltage/Current Ramp Test (VCRT) was conducted, which consisted of a linear drop in V_m from –55 mV to –100 mV over a period of 2 s to measure changes in voltage, and subsequently conductance.

Baclofen Administration

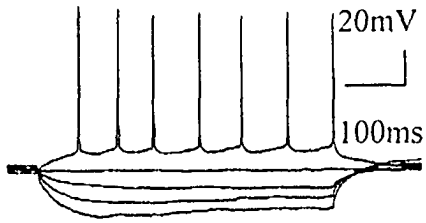
Following 1 min of baseline whole cell recordings (after the VCRT) held at –58 mV, 2 different doses (10, 20 μM) of the $GABA_B$ agonist were introduced into the aCSF perfusate in the absence of stimulation to assess changes in current flow for duration of 3 min. At the end of this assessment, a second VCRT was conducted, followed by another 2 min of baclofen with 9 min of washout. In this manipulation, the time to baclofen onset, peak of response, time of washout, and reversal potential were recorded. The conductance for each experimental group was calculated from the slope of the responses during the second VCRT (peak of baclofen) and subsequently subtracting the baseline responses during the initial VCRT.

RESULTS

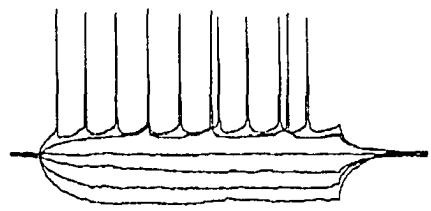
Input Resistance of Fast and Slow Rats

Basic spiking properties of the Fast and Slow rats in response to hyperpolarizing currents are characterized in Figure 16. Regardless of strain and treatment group, all pyramidal cells exhibited regular spiking pattern with an average threshold for triggering action potentials of approximately -50 mV. The average overshoot was approximately 30 mV. Under current clamp, standard I-V curves were generated to assess current-voltage relationship at both peak amplitude and steady state of sham, and kindled (stimulated and non-stimulated amygdala) Fast and Slow rats. A total of 4 currents (3 hyperpolarized (-150, -100, -50 pA) and 0 pA) were used to examine intrinsic voltage responses in the absence of external stimulation. As seen from Figure 17, sham rats of both strains showed slightly larger inward rectifying currents compared to kindled rats (stimulated and non-stimulated amygdala). The corresponding averaged voltage amplitudes in response to the 3 hyperpolarizing pulses are shown in Figure 18. At the most hyperpolarizing pulse (-150 pA), significant differences were reported between the Treatment groups (sham vs. kindled) ($F_{2,71} = 6.65$, $p<.01$). The follow-up comparisons confirmed that the Slow rats from the sham group exhibited significantly higher amplitudes at both peak and steady state compared to the Slow kindled rats. However, no main effect of Strain or Trial (steady state vs. peak) was reported. At the second hyperpolarizing pulse (-100 pA), significant differences were again evident between the Treatment groups ($F_{2,71} = 6.80$, $p<.01$). This dissimilarity was again attributed to a greater amplitude response among sham controls. Lastly, the weakest hyperpolarizing current of –50 pA yielded the lowest amplitude. A main effect of Treatment group was consistent with the responses from previous pulses ($F_{2,71} = 7.98$, $p<.001$).

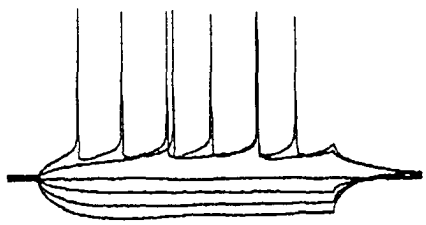
Figure. 16 – Spiking characteristics of basolateral pyramidal cells of the Fast and Slow rats. Typical examples of current-voltage relationships in sham and kindled (stimulated and non-stimulated amygdala) Fast and Slow rats showing prominent spiking properties of pyramidal cells of the basolateral amygdala assessed under current clamp in response to hyperpolarizing (-150, -100, -50, 0 pA) and depolarizing (50, 100 pA) steps of current.



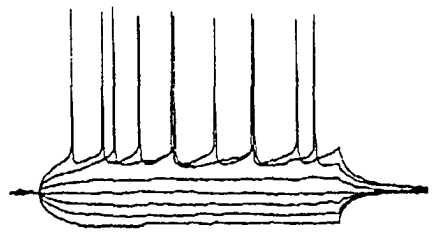
Fast Rats -Sham



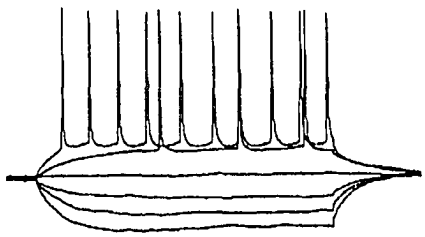
Slow Rats - Sham



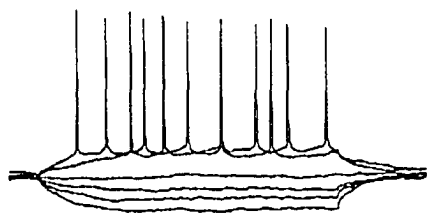
Fast Rats - Contralateral



Slow Rats - Contralateral

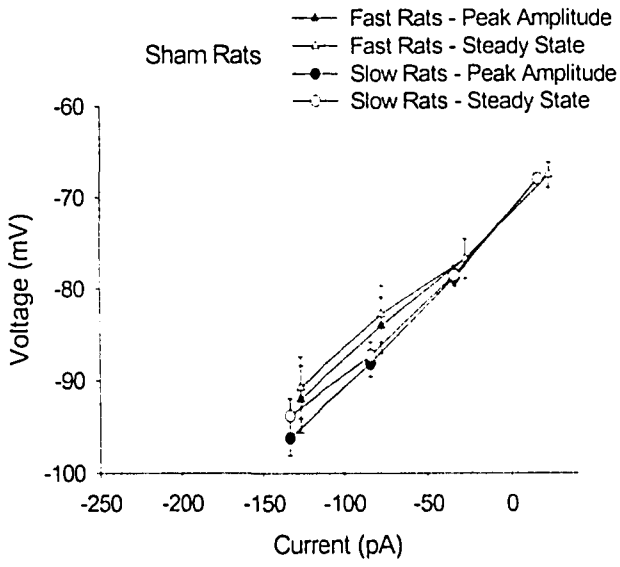


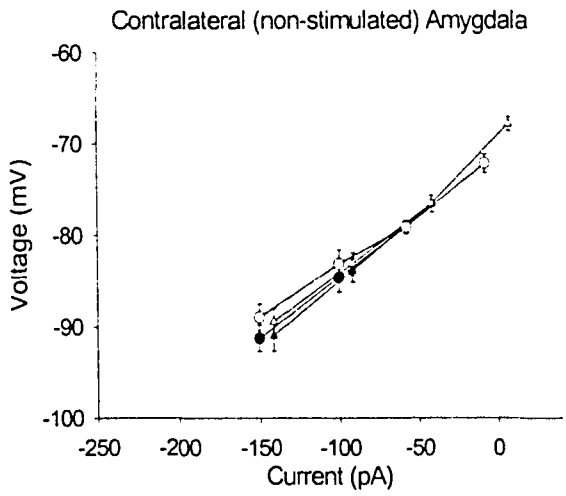
Fast Rats - Kindled



Slow Rats - Kindled

Figure. 17 – Typical plots of current-voltage relationships, obtained from Figure 16 (above), of sham and kindled (stimulated and non-stimulated amygdala) Fast and Slow rats. The slope of each experimental condition was used to calculate input resistance assessed at peak and steady state.





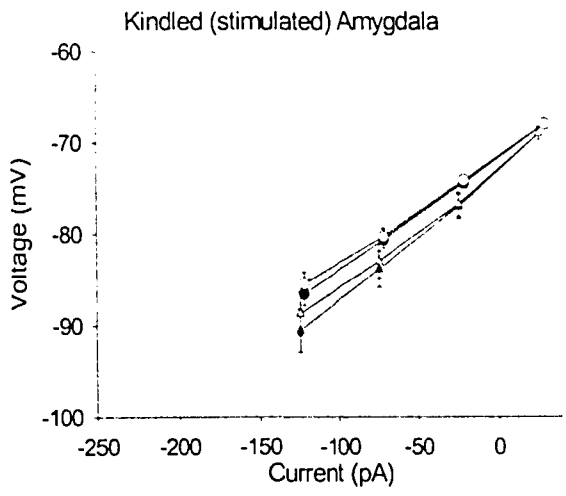
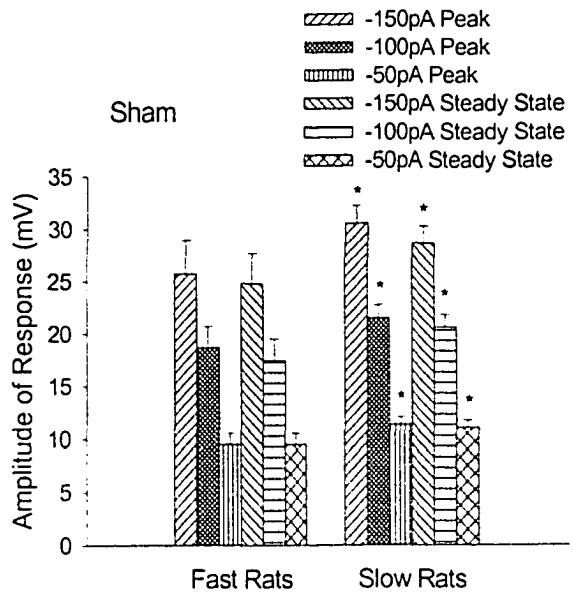
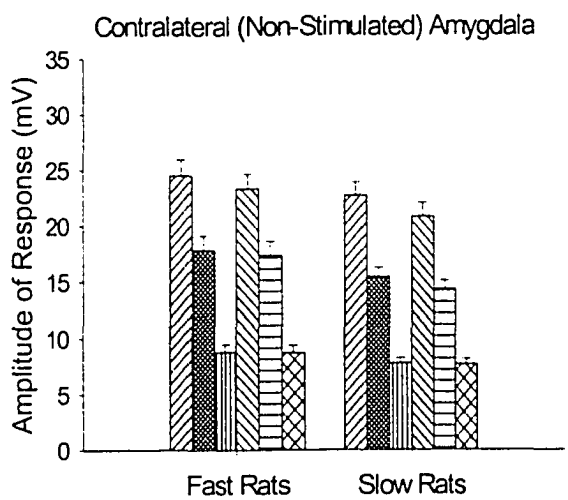


Figure. 18 – Average (± SEM) voltage amplitude recordings taken at peak and steady state in response to hyperpolarizing steps of current (-150, -100, -50 pA) in sham (top) rats, contralateral (non-stimulated) amygdala (bottom left), and kindled (stimulated) amygdala (bottom right) of the Fast and Slow rats. '*' = significantly different than the Slow kindled (stimulated and non-stimulated) amygdala of the same hyperpolarized current, $p<.05$.





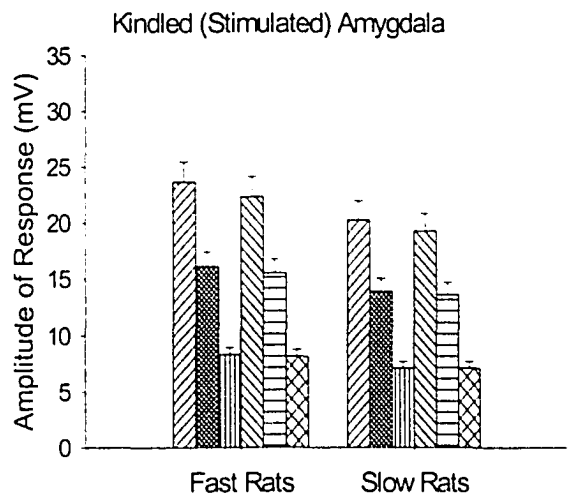


Table. 2 – This table represented average (± SEM) input resistance in MΩ, as measured by the slope of the current-voltage plot, in sham, contralateral (non-stimulated) amygdala, and kindled (stimulated) amygdala groups of the Fast and Slow rats assessed at peak and steady state.

'*' = significantly different than Sham group of the same strain and treatment, $p<.05$.

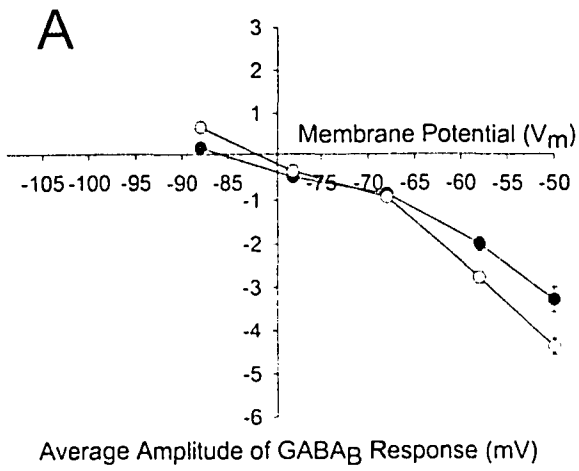
	Fast Rats Peak	Slow Rats Peak	Fast Rats Steady State	Slow Rats Steady State
Sham	161.0± 18.2 MΩ (N = 10)	187.9± 11.8 MΩ (N = 22)	151.0± 17.3 MΩ (N = 10)	172.2± 11.4 MΩ (N = 22)
Contralateral	152.8± 9.6 MΩ (N = 18)	135.3± 7.1* MΩ (N = 8)	141.4± 8.4 MΩ (N = 18)	117.5± 8.3* MΩ (N = 8)
Kindled	139.4± 13.6 MΩ (N = 13)	122.3± 9.0* MΩ (N = 7)	129.2± 13.9 MΩ (N = 13)	115.6± 8.4* MΩ (N = 7)

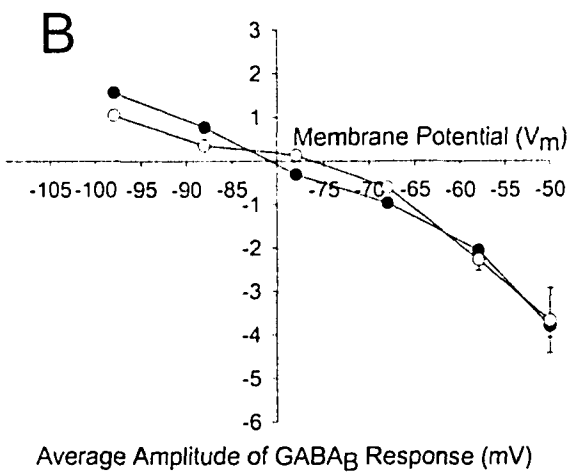
The input resistance of peak and steady state in the Fast and Slow rats are indicated in Table 2. The overall ANOVA showed no strain differences, but yielded a significant main effect of Treatment group ($F_{2,69} = 5.27$, $p<.01$). Follow-up comparisons confirmed that cells from the kindled (stimulated) and contralateral (non-stimulated) amygdala of Slow rats exhibited lower input resistance compared to sham controls. Differences in input resistance between treatment groups (kindled vs. sham) did not reach statistical significance in the Fast rats. Input resistance between peak and steady state were again robust resulting in a significant main effect of Trial ($F_{1,92} = 76.6$, $p<.001$). The average resting membrane potential of all groups among the Fast and Slow rats was very consistent at $-69.9 \pm .57$ mV.

Evoked $GABA_B$ Inhibitory Postsynaptic Potentials - Amplitude

Evoked IPSP amplitudes were elicited at various membrane potentials described in the methods section. The time to peak and duration of the entire evoked $GABA_B$ response were also recorded (described in the next section). Under current clamp, at the most depolarized potential of –50 mV, stimulation-induced $GABA_B$ responses revealed a Strain x Treatment group interaction ($F_{2,287} = 12.13$, $p<.001$). Post-hoc analyses showed that cells recorded from the kindled (stimulated) amygdala of Fast rats demonstrated larger $GABA_B$ amplitudes compared to Slow rats, and sham controls. Conversely, recordings of $GABA_B$ amplitudes taken from the kindled amygdala of Slow rats were smaller than sham controls (Figure 19). As the membrane potential was shifted to –58 mV, Slow sham rats maintained greater $GABA_B$ amplitudes over Slow kindled rats. Meanwhile, the Fast kindled (stimulated) amygdala group maintained greater amplitudes

Figure. 19 – Current clamp assessment of $GABA_B$ IPSP amplitudes of the Fast and Slow rats. Stimulus-evoked average (± SEM) $GABA_B$ IPSP amplitudes from the basolateral amygdala of the Fast and Slow rats assessed at membrane potentials of –50, -58, -68, -78, -88, -98, -108 mV were represented in sham (A), contralateral (non-stimulated) (B), and kindled (stimulated) amygdala (C) groups.





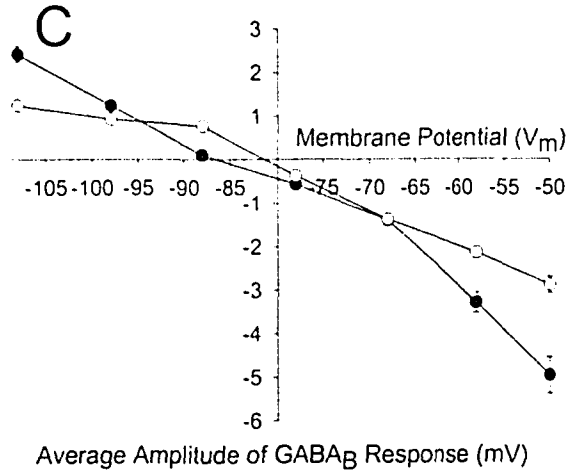
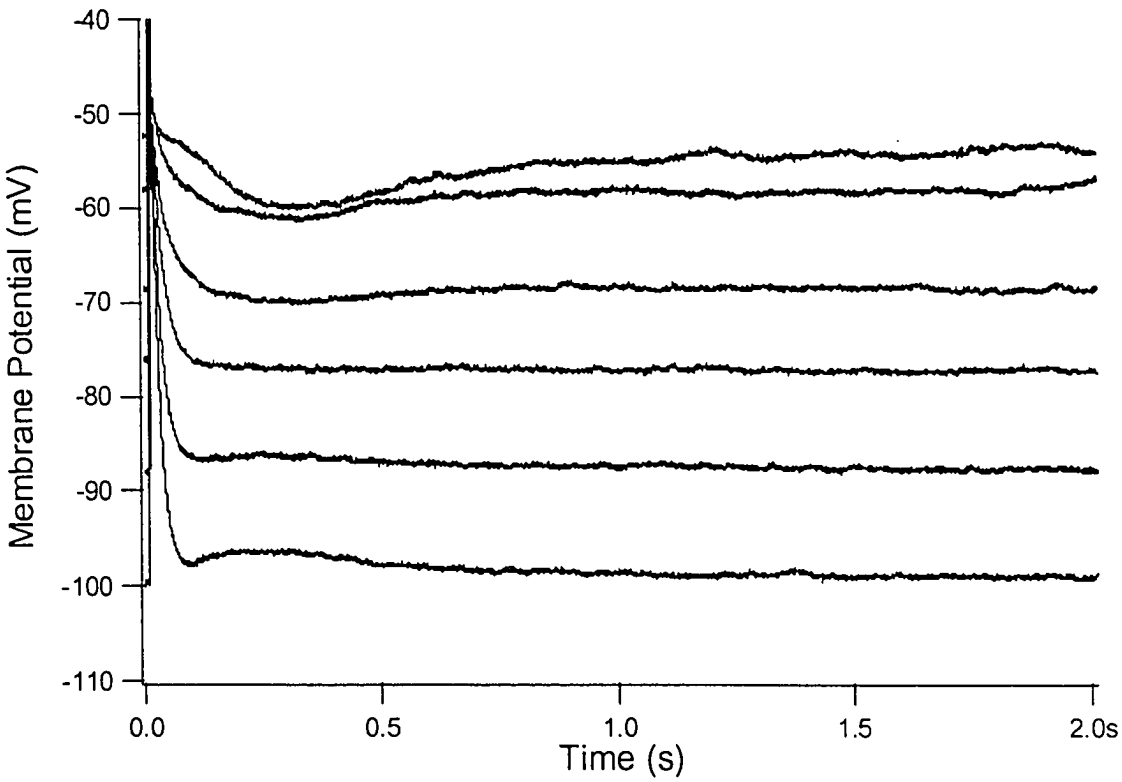


Figure. 20 – Representative stimulus-evoked $GABA_B$ IPSPs recorded from the basolateral amygdala at various membrane potentials assessed under current clamp. The reversal potential for this particular cell was approximately –80 mV.

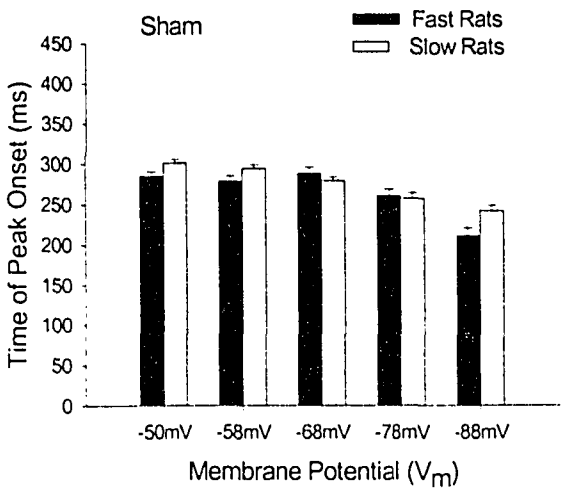


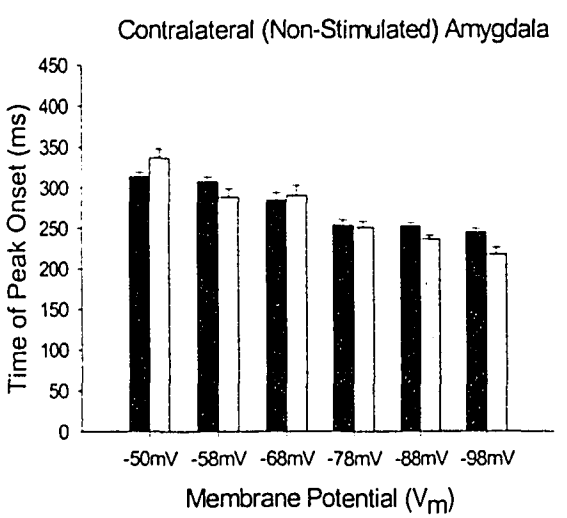
over sham and contralateral (non-stimulated) amygdala groups. Taken together, these results revealed a main effect of Treatment group ($F_{2,323} = 3.62$, $p<.05$). On the other hand, the different $GABA_B$ IPSP amplitude profiles in the kindled amygdala vs. sham rats resulted in a significant Strain x Treatment group interaction ($F_{2,323} = 11.61$, $p<.001$). The assessment of $GABA_B$ amplitudes measured at –68 mV indicated a proportional decrease in both strains across the different treatment groups ($F_{2,325} = 7.44$ $p<.001$), with cells from the kindled amygdala maintaining higher amplitudes compared to the contralateral amygdala and sham controls. At –78 mV, the membrane potential was slowly approaching the reversal potential of $GABA_B$ neurons. The Slow rats showed smaller amplitude compared to Fast rats across all conditions ($F_{1,290} = 5.25$, $p<.05$). Interestingly, our results indicated that the contralateral (non-stimulated) amygdala maintained the smallest $GABA_B$ amplitudes compared to the kindled amygdala and sham controls ($F_{2,290} = 5.59$, $p<.01$). As the membrane potential became more hyperpolarized (-88, -98, -108 mV), the Fast rats maintained significantly larger $GABA_B$ responses over the Slow rats. Typical $GABA_B$ responses under current clamp measured at different membrane potentials are displayed in Figure 20. The reversal potential for all cells was approximately –85.8 mV.

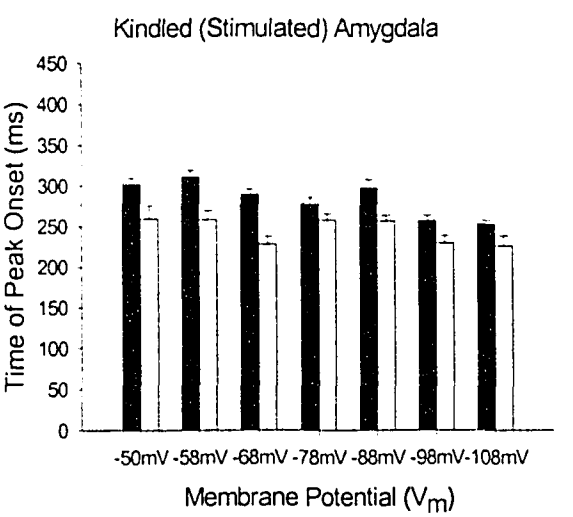
Time to Peak Onset of $GABA_B$ Responses

The time to peak onset of evoked $GABA_B$ responses represents the time from stimulation to maximum amplitude assessed under current clamp. At –50 mV membrane potential, the analyses revealed a significant main effect of Treatment group ($F_{2,287} =$

Figure. 21 – Average (± SEM) time to achieve peak amplitude of the stimulus-evoked $GABA_B$ IPSPs measured at various membrane potentials in sham (top), contralateral (non-stimulated) amygdala (bottom left), kindled (stimulated) amygdala (bottom right) groups of the Fast and Slow rats.





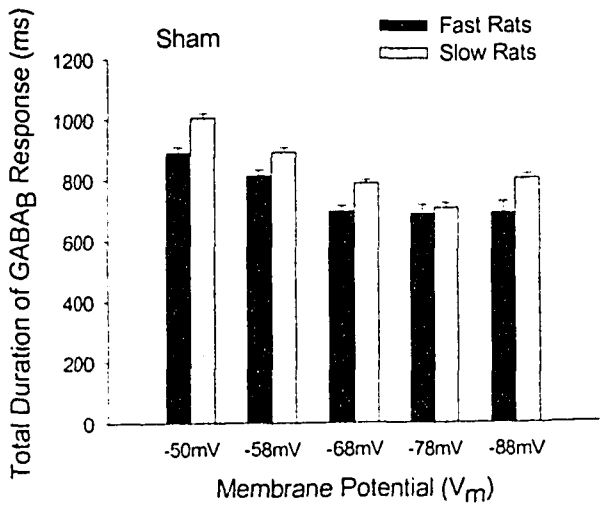


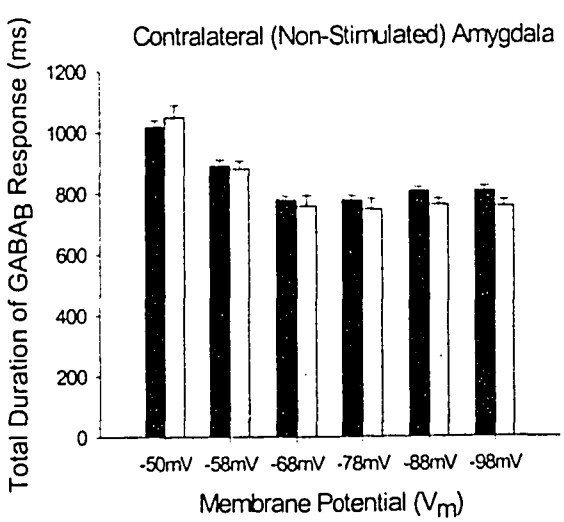
18.35, p<.001) and a Strain x Treatment group interaction ($F_{2,287}$ = 9.84, p<.001). As a result of kindling, the time of onset assessed from the stimulated amygdala of Slow rats was markedly reduced compared to Fast rats, a finding opposite to the contralateral amygdala (Figure 21). At –58 mV, the Fast rats overall exhibited longer onset times compared to Slow rats ($F_{1,287}$ = 7.09, p<.01), with the kindled (stimulated) amygdala showing the largest difference. The analysis also showed a significant main effect of Treatment group ($F_{2,287}$ = 5.59, p<.01), and a Strain x Treatment group interaction ($F_{2,287}$ = 4.86, p<.01) (Figure 21). At more hyperpolarized membrane potentials, the time of onset steadily diminished in all treatment groups and in both Fast and Slow rats. However, recordings from the kindled (stimulated) amygdala of the Fast rats maintained longer onset times compared to Slow rats, coincident with greater $GABA_B$ amplitudes described previously.

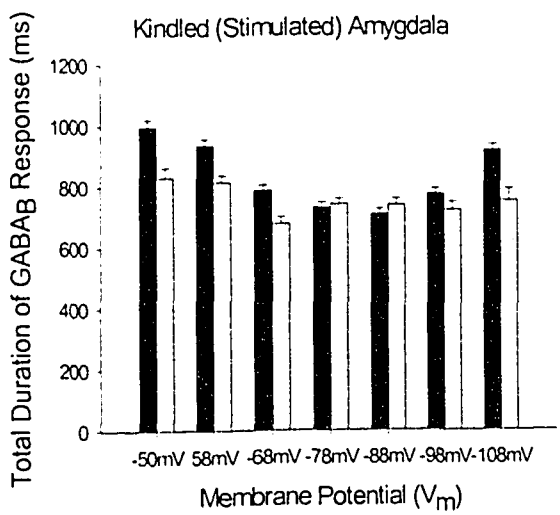
Total Duration of $GABA_B$ Responses

A final measurement from evoked $GABA_B$ recordings under current clamp assessed the total duration of these responses. At the most depolarized membrane potential of –50 mV, the analysis showed a significant main effect of Treatment group ($F_{2,287}$ = 12.26, p<.001). In the kindled (stimulated) amygdala group, Fast rats showed significantly longer total durations than Slow rats, while the opposite effect was evident in the sham control group ($F_{2,287}$ = 15.80, p<.001) (Figure 22). This finding supports the argument of kindling-induced augmentation of evoked $GABA_B$ responses in the Fast rats, but not in Slow rats. At a membrane potential of –58 mV the results were almost identical to the

Figure. 22 – Average (± SEM) total duration of stimulus-evoked $GABA_B$ IPSPs measured at various membrane potentials in sham (top), contralateral (non-stimulated) amygdala (bottom left), kindled (stimulated) amygdala (bottom right) groups of the Fast and Slow rats.







prior assessment except that the total duration of $GABA_B$ responses in both strains from the contralateral (non-stimulated) amygdala were substantially decreased compared to responses at –50 mV resulting in a significant Strain x Treatment group interaction ($F_{2,334}$ = 7.40, p<.001). At –68 mV and subsequent hyperpolarized membrane potentials, total $GABA_B$ durations were relatively stable across all treatment groups. The only exception was at –108 mV where the kindled (stimulated) amygdala of the Fast rats elicited significantly longer durations than the ipsilateral Slow kindled rats ($F_{1,13}$ = 19.17, p<.001) (Figure 22).

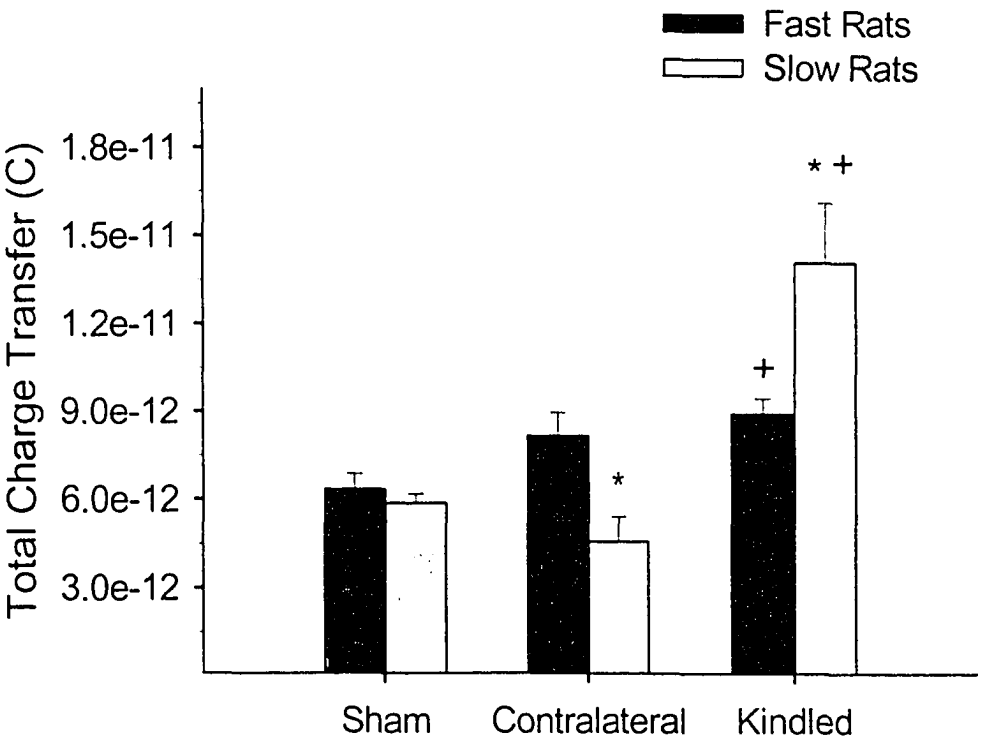
Total Charge Transfer

The amount of evoked current over a period of approximately 1 s was measured in the Fast and Slow rats with the membrane potential clamped at –58 mV. The overall ANOVA revealed significant greater charge transfer in the kindled (stimulated) amygdala compared to sham and contralateral (non-stimulated) amygdala ($F_{2,84}$ = 24.47, p<.001). Further, a Strain x Treatment group interaction was also reported ($F_{2,84}$ = 12.72, p<.001). The follow-up comparisons showed that the Slow rats exhibited greater charge transfer in the kindled (stimulated) amygdala compared to Fast rats, and sham controls. The Fast kindled amygdala group also demonstrated significantly greater charge transfer compared to shams. In contrast, the Slow rats showed less charge transfer compared to Fast rats from the contralateral (non-stimulated) amygdala (Figure 23).

Figure. 23 – Average (± SEM) total charge transfer, measured as the area under the stimulus-evoked IPSC curve, assessed during voltage clamp at membrane potential of –58 mV in sham, contralateral (non-stimulated) amygdala, and kindled (stimulated) amygdala groups of the Fast and Slow rats.

'*' = significantly different than Fast rats, $p<.05$.

'+' = significantly different than sham controls, $p<.05$.



Baclofen Administration

Following the assessment of $GABA_B$ recordings at various membrane potentials, baclofen was administered to the perfusate to assess changes in current, conductance and reversal potential induced by the $GABA_B$ agonist. Cells that have had no previous evoked $GABA_B$ response and no baclofen response were excluded from the analysis.

Baclofen Onset, Peak Current, and Washout

The speed of which baclofen took effect to induce changes in measured current was examined in the Fast and Slow rats, and among treatment groups. Significant differences were reported between Treatment groups ($F_{3,37} = 7.05$, $p<.001$), with follow-up comparisons confirming that the sham rats have a faster time of onset compared to the kindled group (Figure 24, A). At the height of the baclofen response, the peak current was measured in all groups. No significant differences were reported between Treatment groups and Strain. However, sham controls showed slightly higher peak current than kindled rats (Figure 24, B). The time required for baclofen to washout was slightly longer in the kindled (stimulated) amygdala compared to the contralateral amygdala yielding a significant main effect of Treatment group ($F_{3,36} = 3.99$, $p<.05$) (Figure 24, C).

Peak Conductance During Baclofen Administration

At the peak of baclofen recording, a measure of $GABA_B$ membrane conductance was taken to assess whether kindling impacted on the conductivity of ion channels in the Fast and Slow rats. Surprisingly, there were no correlation between the concentration of baclofen (10 vs. 20 μM) and conductance. Also, no significant differences were reported

Figure. 24 – Baclofen administration induces outward current. Average (± SEM) time for baclofen to take effect following its application into the perfusate (A), average (± SEM) increase in current induced by 5 min of baclofen (B), and average (± SEM) time required for baclofen washout (C) in sham, contralateral (non-stimulated) amygdala, and kindled (stimulated) amygdala groups of the Fast and Slow rats.

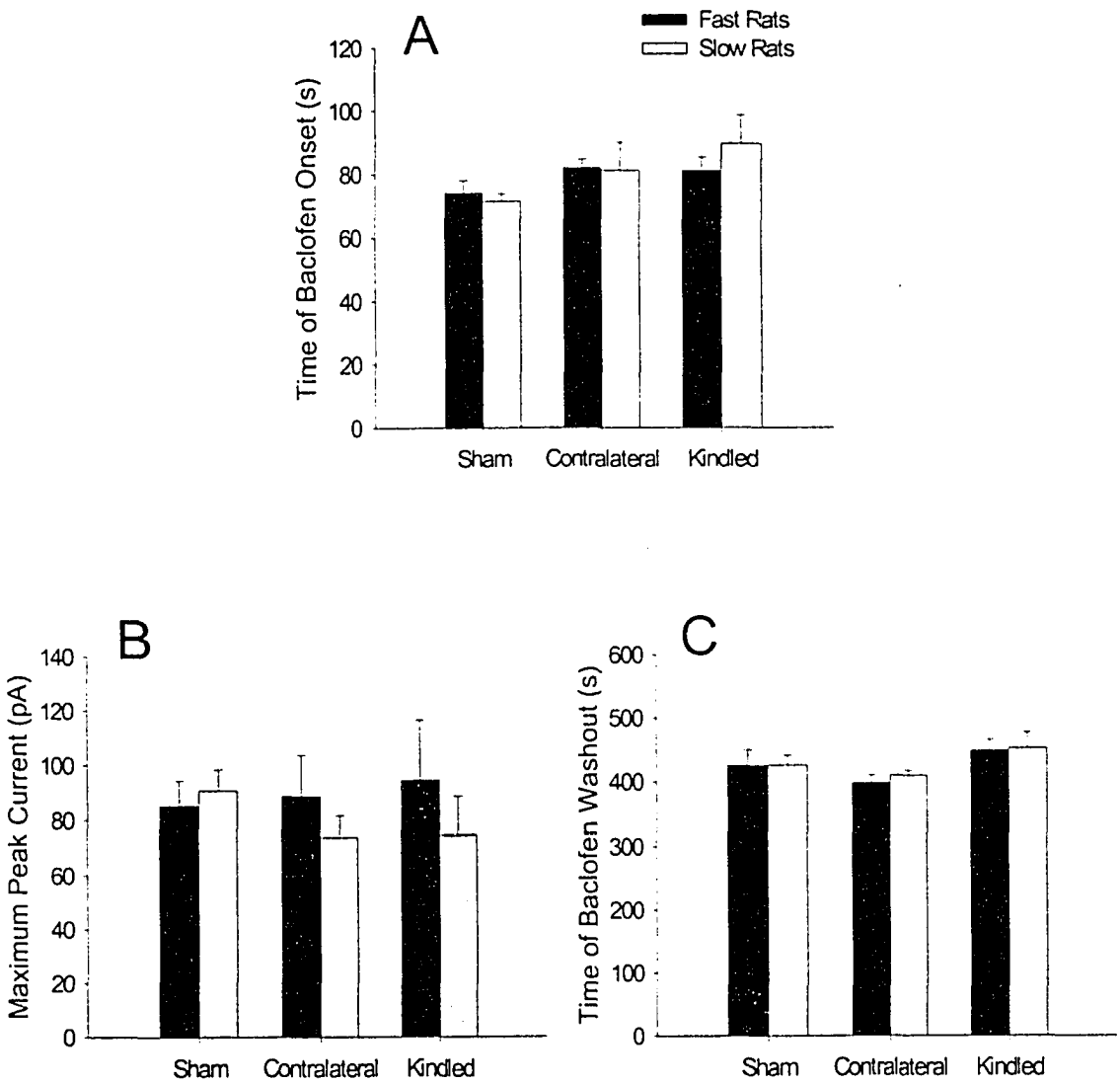


Figure. 25 – An average (± SEM) measurement of $GABA_B$ membrane conductance assessed at the peak of baclofen administration. Conductance was calculated by subtracting the slope of the voltage-current ramp test (VCRT) during baseline recordings from the slope of the VCRT during peak baclofen response. These were recordings from basolateral amygdala pyramidal cells of Fast and Slow rats that previously had synaptically driven $GABA_B$ responses compared to those that did not.
'*' = significantly different than Fast rats with no previous $GABA_B$ response, $p<.05$.

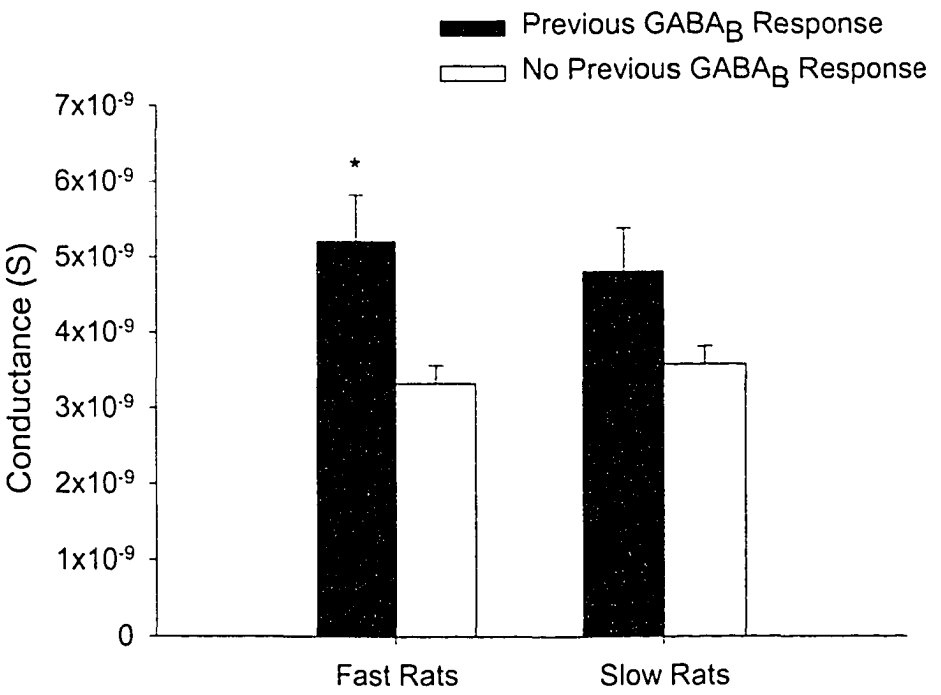


Figure. 26 – Typical baclofen-induced current response curve over a total period of 12.5 min (750 s) measured at a membrane potential clamped at –58 mV. Baseline recordings were taken for 1 min then baclofen (20 μM) was administered into the perfusate for 5 min, after which was the washout.

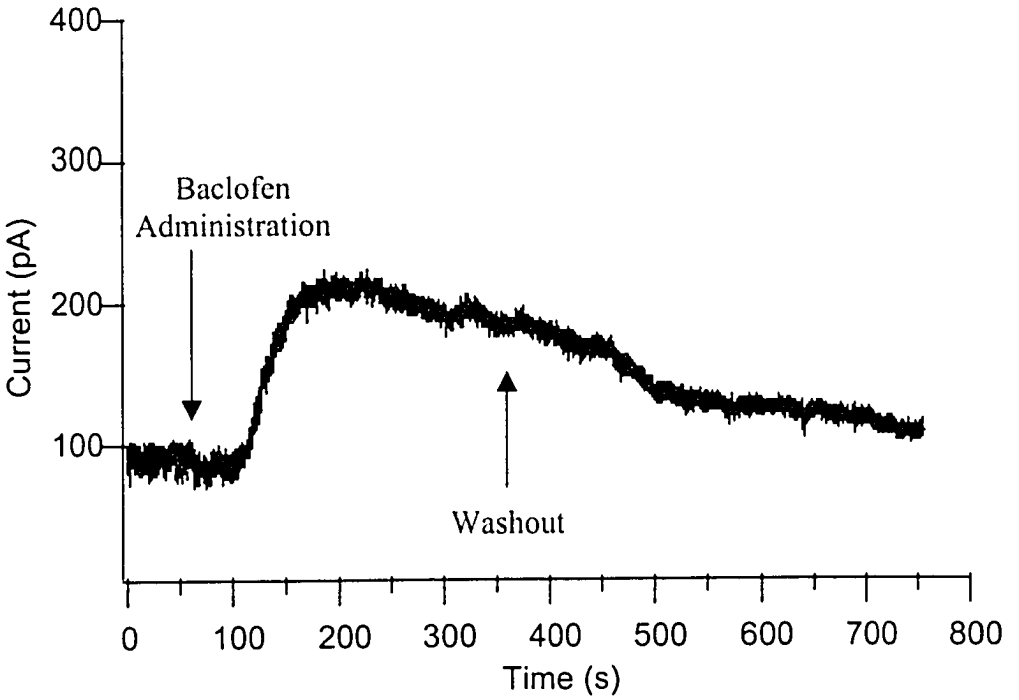
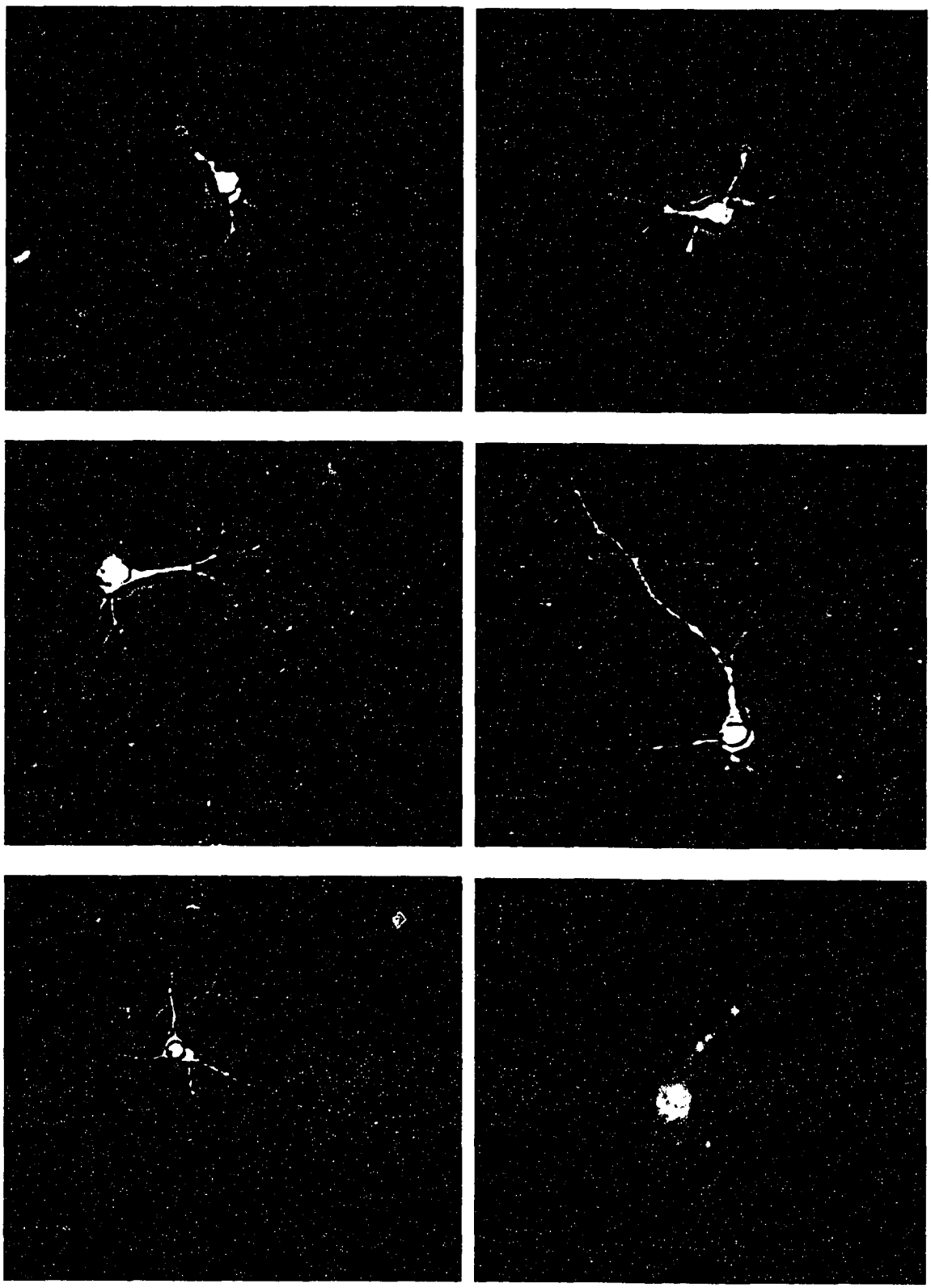


Figure. 27 – Deconvolved photomicrographs of basolateral amygdala pyramidal cells stained with 0.3% Lucifer Yellow. Fast sham (A), Slow sham (B), Fast contralateral (non-stimulated) amygdala (C), Slow contralateral (non stimulated) amygdala (D), Fast kindled (stimulated) amygdala (E), Slow kindled (stimulated) amygdala (F) groups are shown. Notice the presence of basal and apical dendrites radiating away from the soma. The size of the soma was calculated to be approximately 5 μm in diameter.



between the Fast and Slow rats, and across the treatment groups. However, the strength of $GABA_B$ conductance was strongly dependent upon prior evoked $GABA_B$ response recorded at various membrane potentials under current clamp. Therefore, the data was pooled into 2 groups for analysis: 1) prior evoked $GABA_B$ response, 2) no evoked $GABA_B$ response, and analyzed according to strain. The analysis showed that cells with prior $GABA_B$ response maintained greater conductance over cells without any detectable $GABA_B$ response regardless of the strain ($F_{1,93} = 8.61$, $p<.01$) (Figure 25).

The reversal potential recorded during peak baclofen administration was the final measurement in this experiment. No significant differences across treatment groups, and among the Fast and Slow rats were evident. The overall average reversal potential was -84.9 ± 1.27 mV.

Morphology of Basolateral Neurons

Lucifer Yellow impregnated BLA neurons were identified based on their morphology (soma size, appearance of dendrites). All of our representative photomicrographs showed pyramidal characteristics with soma size of approximately 20 μm in diameter. The presence of basal and apical dendrites that branched away from the soma to distances of several hundred micrometers was evident in most cells (Figures 27).

DISCUSSION

The present study has provided evidence that kindling of the basolateral amygdala 1) reduced the apparent membrane input resistance (R_i) of both Fast and Slow rats compared to sham controls, this reduction was greater in the Slow than in the Fast strain;

2) resulted in greater $GABA_B$ amplitude in Fast rats compared to Slow rats, and also sham controls; 3) caused a reduction of $GABA_B$ amplitudes measured in current clamp in the Slow rats compared to sham controls; despite 4) an increase in total charge transfer of Slow from the kindled (stimulated) amygdala, and a decline in charge transfer from the contralateral (non-stimulated) amygdala of Slow rats measured using voltage clamp; 5) did not elicit any change in the estimated slope conductance measured at the peak of baclofen administration.

Input Resistance of Fast and Slow Rats

We found that the apparent input resistance (R_i) of neurons in both Slow and Fast rats was significantly decreased after kindling. This was estimated from the current-voltage relationship obtained from hyperpolarizing pulses delivered in current clamp mode. Both R_i estimated at the peak and steady state voltage deflections were altered. This result may be due to a number of possible mechanisms including alteration in leak conductances, inward rectification and background synaptic noise (D'Aguanno et al., 1989; O'Brien et al., 2002). Input resistance can have a profound affect on cell excitability as the synaptic input sums in parallel with this resistance. In this regard, the apparent input resistance measured here is known to be attributable to neuronal size, degree of innervation onto the soma, and the number of ion channels, (Liu et al., 1996; Magee, 2000). As this parameter can have an effect on the efficacy of synaptic responses (Magee, 2000), our results demonstrating that kindling significantly lowers this resistance in both Fast and Slow rats compared to sham controls suggests that cell excitability may be altered. This further implies that kindled neurons of the BLA would require a stronger

and/or higher frequency excitatory input to drive the membrane toward threshold for generating an action potential. Alternatively kindled cells may be less efficiently inhibited by local interneurons as the increased conductance may shunt the membrane. It is not clear from these studies what accounts for the change in R_i. Possible explanations include increase in soma surface area and/or a change in the expression of channels responsible for this resistance.

A second possible explanation may arise from inward rectification properties. Our data indicated a divergence from linearity of the I-V curve at approximately –77 mV regardless of treatment groups (kindled vs. non kindled), and strain. Negative current injections produced hyperpolarized responses with an initial peak voltage trace that was followed by a depolarizing "sag". In agreement with other studies (Rainnie et al., 1991a), the present data showed at membrane potentials greater than –75 mV, a time-independent rectification (measured at peak) was evident in BLA neurons. Further, a time-dependent rectification (measured at steady state) at membrane potentials more negative than –80 mV was also reported. Taken together, both contributing factors played a role in the observed decreased input resistance of kindled Fast and Slow BLA neurons, which functionally would act to strengthen inhibition in the hyperpolarizing direction (Rainnie et al., 1991a). This is characteristic of a hyperpolarizing current, which serves to modulate membrane properties that influence excitability and inhibitory processes.

Unlike the present study, other reports have demonstrated that kindling of the BLA failed to induce changes in the firing frequency, input resistance, and resting membrane potential of pyramidal neurons (Asprodini et al., 1992; Rainnie et al., 1992; Shoji et al., 1998). The reason for this is likely attributed to the recording technique. For example,

intracellular recordings, adopted by the above studies, typically produce much lower input resistance than whole cell recordings. As a result, modest changes in membrane excitability or ion channel expression during intracellular recordings may not be easily detectable compared to whole cell recordings.

Evoked $GABA_B$ IPSPs - Amplitude

In the BLA there is a high concentration of $GABA_B$ receptors along the dendrites of pyramidal neurons and particular subpopulations of interneurons (Washburn and Moises, 1992). Activation of these receptors produces a slow, prolonged hyperpolarization via the activation of inwardly rectifying potassium channels (Thompson and Gahwiler, 1992). Local interneurons are the most abundant source of GABA activating these receptors through feedforward inhibition, but a direct inhibition from GABAergic LA interneurons has also been shown to contribute as well (Rainnie et al., 1991b; Rainnie, 1999, Katona et al., 2001). $GABA_B$ receptors on pyramidal cells and interneurons are mainly activated when many GABAergic neurons fire simultaneously, in association with rhythmic oscillations (Traub et al., 1996). The subsequent release of GABA into the extracellular space, if sufficient, may activate extrasynaptic receptors and exert an inhibitory postsynaptic effect in the adjacent postsynaptic neuron.

During current clamp assessment of evoked $GABA_B$ IPSPs, the measured amplitude elicited from Fast rats of the kindled (stimulated) amygdala at membrane potentials of –50 and –58 mV was significantly greater than the amplitude of the contralateral (non-stimulated) amygdala, Slow rats, and sham controls. Although the results of the present study showed that kindling significantly increases $GABA_B$ amplitude of the Fast rats,

other studies have not found similar findings in outbred rats. For example, in the presence of DNQX and APV, no differences were reported in $GABA_A$ or $GABA_B$ IPSPs elicited from control and kindled neurons of the BLA in response to LA stimulation (Shoji et al., 1998). On the other hand, kindling of the amygdala has been shown to reduce GABA receptor-mediated inhibitory transmission (Gean et al., 1989; Asprodini et al., 1992). More specifically, amygdala kindling has been suggested to induce a pathway specific loss of LA evoked feedforward GABAergic synaptic transmission (Rainnie et al., 1992). Our data from these different strains of rat show a different outcome. The increase in $GABA_B$ IPSP amplitude in Fast rats following kindling of the amygdala might be due to a strengthening of inhibitory input onto pyramidal cells necessary to activate the receptors. As our data show from experiment #2 that most $GABA_B$ immunoreactivity is not associated with synaptic markers (GAT1), this implies that extrasynaptic $GABA_B$ receptors are activated by a larger extracellular accumulation of GABA after kindling (Mody et al., 1994; Thomson and Destexhe, 1999). The outcome of this may be to act as a compensatory mechanism for further limiting the spread of seizures in the Fast rats. Functionally, the potentiation of inhibition could serve to modulate the balance between excitation and inhibition, favouring a shift towards the latter. In any case, an augmentation of $GABA_B$ inhibition may be due to several possibilities that include an increase in receptor density and/or innervation resulting in more GABA release. Also increase efficacy of GABA binding to $GABA_B$ receptors or increase expression of G protein-coupled inward rectifying potassium (G_{IRK}) channels may all contribute to enhanced $GABA_B$ receptor-mediated inhibition. The significance of reduced $GABA_B$ IPSP responses in the Slow rats will be discussed below.

Evoked $GABA_B$ IPSPs – Time of Onset and Total Duration

In the kindled (stimulated) amygdala, it was observed in seizure resistant Slow rats that the time to peak onset and duration, along with amplitude, of the evoked $GABA_B$ IPSPs were considerably reduced especially at more depolarized membrane potentials compared to the contralateral (non-stimulated) amygdala, Fast rats, and sham controls. These results contrasted to those found in Fast rats, which demonstrated longer onset time, broader and longer lasting IPSP response. In contrast the total charge transfer due to $GABA_B$, assessed under voltage clamp, increased after kindling in the Slow rats, and to a lesser extent in the Fast rats, measured at a membrane potential of –58 mV. These apparently conflicting results may be accounted for by the fact that R_i decreased. In current clamp the change in R_i would decrease the charging time constant and shorten the potential of the $GABA_B$ response, but in voltage clamp this effect would not have been seen because only the total charge would be manifested irrespective of any changes in R_i. Overall these data suggest that $GABA_B$ function is enhanced in the Slow strain due to kindling but that the decrease in R_i prevents this outcome from manifesting itself as an increased IPSP. Although it has not been ruled out that decreased $GABA_B$ mediated IPSPs may arise from a reduction in GABA synthesis, or neuronal depletion of GABA (Rainnie et al., 1992).

Effects of Baclofen

Application of baclofen into the perfusate resulted in roughly equivalent increase of outward currents recorded from the basolateral amygdala regardless of strain or treatment groups. The $GABA_B$ conductance measured at the peak of baclofen response did not

differ between the Fast and Slow rats, or between kindled and sham controls. Cells that had a synaptic response maintained a larger baclofen response than those that did not have a synaptic driven GABA response. These findings imply that a direct connection from the lateral amygdala to BLA neurons is necessary for $GABA_B$ mediated transmission. Baclofen exerts its effects on both synaptic and extrasynaptic $GABA_B$ receptors and so it is not clear if the smaller response reflects a subset of cells that have relatively little $GABA_B$ receptors or we were simply unable to stimulate the direct inhibitory path to these cells. In those that had both a synaptic response and baclofen response we estimated that charge transfer from the synaptic receptors was only about 5% of the baclofen response. This result clearly indicates a minor involvement of $GABA_B$ receptors at the synapse, and that baclofen-induced increase in K^+ conductance largely activates extrasynaptic $GABA_B$ receptors of the Fast and Slow rats.

For the past many years, there has been considerable debate as to whether $GABA_B$ agonists, such as baclofen, have a preferential binding for pre- or postsynaptic sites. It has been suggested that the activation of pre- and postsynaptic $GABA_B$ receptors is dependent on the amount of GABA released into the synaptic cleft (Yamada et al., 1999). In this regard, activation of presynaptic $GABA_B$ receptors with a low baclofen concentration (<10 μM) has been shown to attenuate excitatory and/or inhibitory synaptic transmission (Wu and Leung, 1997; Garden et al., 2002; Hefft et al., 2002) by inhibition of axonal terminal Ca^{2+} current (Dunlap and Fischbach, 1981; Thompson and Gahwiler, 1992). On the other hand, $GABA_B$ receptor activation of postsynaptic receptors with higher baclofen concentrations (>10 μM) produced outward currents accompanied by increased membrane conductance (Lambert and Wilson, 1993). These findings likely

suggest that pre- and postsynaptic $GABA_B$ mechanisms are mediated by G proteins that couple to different effector substrates (Pitler and Alger, 1994). The aim of the present study was bounded by its limitations to specifically assess the mechanisms involved in $GABA_B$ binding, or the pre- or postsynaptic effects of baclofen. However, in our slice preparation, we believe that baclofen exerted a postsynaptic effect as evidenced by increased K^+ conductance following its application. In addition, the increase in outward current with baclofen administration from the present study support the findings of other experiments, which demonstrated no differences of the K^+ current between kindled and naïve controls (Gloveli et al., 2003; Liu and Leung, 2003).

In summary, this experiment demonstrated some important characteristics of the Fast and Slow rats as a result of kindling. First, lower input resistance in the Fast rats and more so in the Slow rats after kindling, compared to sham controls, may account for changes in cell excitability or $GABA_B$ channel expression. Second, the evoked $GABA_B$ IPSPs in kindled Fast rats were significantly greater than Slow rats and sham controls. This could be attributed to increased inhibitory innervation and/or greater number of $GABA_B$ extrasynaptic receptors that may serve as a compensatory mechanism for generating further seizures. The Slow rats, on the other hand, exhibited smaller IPSP amplitudes compared to Fast rats and sham controls. This apparent reduction of $GABA_B$ response coupled with an increase in charge transfer from the kindled amygdala suggests that the low input resistance of Slow rats may mask the enhanced inhibition. Lastly, the administration of baclofen showed that an increase in K^+ conductance is generated by the activation of extrasynaptic, rather than synaptic $GABA_B$ receptors. Taken together, these results suggest that $GABA_B$ receptor mediated inhibition of the Fast and Slow rats within

the basolateral amygdala is altered after kindling, but favours an inhibitory tone. From this and other studies, seizure genesis, propagation and termination are likely to involve $GABA_B$ receptors, but to what extent are presently unknown.

GENERAL DISCUSSION AND CONCLUSIONS

The current thesis provided a comprehensive examination into the effects of kindling on $GABA_B$ receptor-mediated inhibition in the amygdala of seizure prone (Fast) and seizure resistant (Slow) rats. Three separate experiments were performed to answer one ultimate question: What role does the $GABA_B$ system plays in amygdala kindling-induced epileptogenesis, and does that system differentially affect the Fast and Slow rats in the spread of such seizures? With the administration of various GABAergic agents delivered via systemic injection and/or direct focal infusion into the amygdala, we investigated the extent to which these compounds, separately, or in combination affected the threshold, duration and severity of stimulation-induced seizures. In addition, the administration of various $GABA_A$ and $GABA_B$ agents elicited distinct EEG patterns, including interictal spikes and in some cases spindles from both amygdalae. The *in vivo* assessment of these GABAergic manipulations provided important answers regarding drug efficacy, seizure genesis, interaction of focal and global networks, and the synchronization of inhibition within the amygdala. Given the utmost importance of receptor binding and its subsequent effects on GABA transmission, it was of great interest to the current thesis to assess the localization and distribution of $GABA_B$ receptors and the GABA transporter (GAT1) in both the non-kindled and kindled amygdala. The information gathered from these results speaks directly to receptor

availability and to the amount of transporters present within inhibitory terminals of interneurons that impinge on pyramidal cells. The possible up- or down-regulation of $GABA_B$ receptors and GAT1 transporters regulate the availability of GABA in the synaptic cleft. Consequently, using immunocytochemistry, our data provided an evaluation to the extent of GABAergic inhibitory transmission in the amygdala. Lastly, the biophysical properties of $GABA_B$ receptor-mediated inhibition were studied using *in vitro* preparations from sham, and kindled Fast and Slow rats. Inherent membrane characteristics, basic spiking properties, evoked $GABA_B$ potentials and currents, and the effects of baclofen on membrane conductance were all examined from pyramidal cells of the basolateral amygdala. The importance of these recordings was three-fold. First, the interpretation of the results, including possible cellular mechanisms of $GABA_B$ mediated inhibition could be compared directly to the findings in the whole animal preparation (experiment #1). Second, the efficacy and magnitude of cell-to-cell synaptic transmission were easily examined. Third, assessment of ion channel behaviour and conductivity of K^+ ions provided important information regarding the extent to which $GABA_B$ receptors regulate epileptic seizures.

Activation of $GABA_A$ and $GABA_B$ receptors allow for the direct modulation and control of seizure genesis, maintenance, and termination in many brain structures that are susceptible to epilepsy, including the amygdala and other limbic structures. With this in mind, our Fast and Slow rat strains provide a genetic backdrop to these GABA manipulations that may uncover similar or dissimilar mechanisms by which local circuits synchronize and recruit more global networks in generating convulsive seizures.

Our data not only provided further evidence that both $GABA_A$ and $GABA_B$ receptors are involved in epilepsy, but $GABA_B$ receptor activation appears to affect the seizure prone (Fast) rats with greater propensity compared to seizure resistant (Slow) rats. Both our *in vivo* and *in vitro* studies support this notion. This might suggest that the number of $GABA_B$ receptors may be more abundant in the Fast rats or perhaps $GABA_B$ receptors receive more inhibitory inputs from interneurons as a compensatory mechanism to further limit seizure development. It is difficult to conclude from the present study with absolute certainty the precise concentration and location (synaptic vs. extrasynaptic) of $GABA_B$ receptors that rest at pre- and postsynaptic sites, but none the less, clear differences between the strains exhibit in the behaviours of those systems recorded in this thesis. Regardless, from our *in vitro* experiments and immunocytochemistry data, it appears that a large majority of $GABA_B$ receptor activation takes place extrasynaptically, suggesting that a significant amount of GABA must be released from presynaptic receptors to exert its inhibitory action on postsynaptic sites. Our data from electrophysiological findings, in part, showed that kindling induced an increase in $GABA_B$ receptor-mediated inhibition, which appeared to be more prevalent in the Fast rats. Ultimately, the expression of epileptic seizures will greatly depend on the binding affinities for these receptors and subsequent activation of G-coupled proteins that are likely to induce a cascade of events that will govern inhibition or disinhibition of the amygdala, and the global network.

One of the more important disclosures from the current study was the intimate association between local and global seizure networks. Our initial measurement of daily focal infusion of a $GABA_B$ antagonist, SCH 50911, into the amygdala early in kindling of both Fast and Slow rats did not affect ADTs and AD durations. Similarly, in the same

rats during subsequent kindling trials, continuous daily treatment of this drug failed to further elicit robust changes in seizure expression. In fact, it was only after a systemic injection of baclofen, a powerful $GABA_B$ agonist, that we observed an inability of Fast and Slow rats to elicit generalized convulsions when triggered with a stimulus at the seizure threshold. This finding provided clear evidence of the anticonvulsive properties of baclofen, which were only manifested through systemic administration, and not through focal infusion. The very next day, the ability of the kindled amygdala to generate convulsive seizures in the presence of the focally applied SCH 50911 with systemic injection of baclofen was tested. The majority of Fast rats, when given adequate stimulation intensity, elicited generalized convulsions, while behavioural seizures in a large number of Slow rats were suppressed. Taken together, these results demonstrate a greater $GABA_B$ sensitivity of the Fast rats, compared to Slow rats, in response to focal manipulation of local amygdala networks. If this was the case, it would mean that amygdala $GABA_B$ receptors as a whole are important players in regulating inhibition, perhaps more so in the Fast than Slow rats, and adequate blockage of these receptors (postsynaptically) may constitute the predominant factor for triggering and recruiting epileptic seizures beyond the focus.

Another important revelation uncovered in this thesis worth discussing is the differential expression of seizure genesis in response to focal applications of $GABA_A$ and $GABA_B$ antagonists. Previously, it was shown that focal application of a $GABA_B$ antagonist, SCH 50911, into the amygdala of Fast rats overcame the strong anticonvulsive effects of systemic baclofen leading to generalized convulsions. Several days following this assessment, we tested the focal effects of a potent $GABA_A$ antagonist,

picrotoxin, with systemic administration of baclofen. This allows for a direct comparison between $GABA_A$ and $GABA_B$ efficacy on seizure genesis. Our results indicated that the majority of Fast rats failed to elicit convulsive seizures, while most Slow rats experienced generalized convulsions. This finding was in stark contrast to previous $GABA_B$ antagonist infusion in the presence of systemic baclofen. The implication of these results suggest 1) that the amygdala of Slow rats have more receptive $GABA_A$ receptors and/or bind GABA with greater efficacy than Fast rats, and/or 2) that the focal amygdala of Slow rats is more controlled by $GABA_A$ receptor-mediated inhibition than Fast rats, whereas $GABA_B$ receptor-mediated inhibition is more globally organized. By this same measure, this hypothesis also suggests that there may be more *functional* $GABA_B$ receptors in the amygdala of Fast rats than Slow rats.

Lastly, our electrophysiological data points to the conclusion that kindling does alter cell excitability. Evidence in support of this notion came from changes in input resistance of kindled Fast and Slow rats compared to sham controls. Furthermore, the increase in evoked $GABA_B$ IPSP amplitudes of the Fast rats following kindling provides further evidence that this strain may be more sensitive to $GABA_B$ manipulations than the Slow rats. However, the lack of increase in evoked $GABA_B$ IPSP amplitudes of Slow rats is likely due to lowered input resistance, which likely attenuated $GABA_B$ responses that may otherwise be more pronounced. The large increase in total charge transfer in the kindled amygdala of Slow rats, compared to Fast rats, supports this possibility.

In conclusion, the findings of the current thesis provided some invaluable answers to the contributions of $GABA_B$ receptor-mediated inhibition of the Fast and Slow rats within the basolateral amygdala. The primary role of $GABA_B$ receptors appears to be

one of modulation. In order to fully appreciate and elucidate the precise mechanisms of action of the $GABA_B$ receptor and its role in epilepsy, further questions must be addressed in future experiments regarding $GABA_B$ receptor subunit distribution, competitiveness and specificity of $GABA_B$ agonists and antagonists, binding affinities, all of which may affect GABA release and the overall inhibitory tone of the amygdala. Although it is still too early to know when blockade or stimulation of $GABA_B$ receptors might be of clinical use, certainly $GABA_B$ agonists and antagonists hold promise as therapeutic agents for the treatment of epilepsy in the many years to come.

REFERENCES

Anwyl, R., Narahashi, T. (1980) Desensitization of the acetylcholine receptor of denervated rat soleus muscle and the effect of calcium. *Br J Pharmacol.*, 69(1):91-98.

Asprodini, EK., Rainnie, DG., Shinnick-Gallagher, P. (1992) Epileptogenesis reduces the sensitivity of presynaptic gamma-aminobutyric acidB receptors on glutamatergic afferents in the amygdala. *J Pharmacol Exp Ther.*, 262(3):1011-1021.

Badran, S., Schmutz, M., Olpe, HR. (1997) Comparative in vivo and in vitro studies with the potent GABAB receptor antagonist, CGP 56999A. *Eur J Pharmacol.*, 333(2-3):135-142.

Banerjee, PK., Tillakaratne, NJ., Brailowsky, S., Olsen, RW., Tobin, AJ., Snead, OC 3rd. (1998) Alterations in GABAA receptor alpha 1 and alpha 4 subunit mRNA levels in thalamic relay nuclei following absence-like seizures in rats. *Exp Neurol.*, 154(1):213-223.

Banks, MI., Li, TB., Pearce, RA. (1998) The synaptic basis of GABAA,slow. *J Neurosci.*, 18(4):1305-1317.

Barbaccia, ML., Roscetti, G., Trabucchi, M., Mostallino, MC., Concas, A., Purdy, RH., Biggio, G. (1996) Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. *Neuroendocrinol.*, 63(2):166-172.

Barker, JL., Harrison, NL. (1988) Outward rectification of inhibitory postsynaptic currents in cultured rat hippocampal neurones. *J Physiol.*, 403:41-55.

Behrends, JC., ten Bruggencate, G. (1998) Changes in quantal size distributions upon experimental variations in the probability of release at striatal inhibitory synapses. *J Neurophysiol.*, 79(6):2999-3011.

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

Bernstein, EM., Quick, MW. (1999) Regulation of gamma-aminobutyric acid (GABA) transporters by extracellular GABA. *J Biol Chem.* 274(2):889-895.

Bittiger, H., Froestl, W., Mickel, S., Olpe, HR. (1993) GABAB receptor antagonists: from synthesis to therapeutic applications. *Trends Pharmacol Sci.*, 14(11):391-394.

Bliss, TV., Lomo, T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol.*, 232(2):331-356.

Bowery, N. (1989) GABAB receptors and their significance in mammalian pharmacology. *Trends Pharmacol Sci.*, 10(10):401-407.

Bradford, HF. (1995) Glutamate, GABA and epilepsy. *Prog Neurobiol.*, 47(6):477-511.

Bragin, A., Jando, G., Nadasdy, Z., Hetke, J., Wise, K., Buzsaki, G. (1995) Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci.*, 15:47-60.

Brailowsky, S., Montiel, T., Meneses, S., Di Scala, G. (1995) Effects of GABAB receptor antagonists on two models of focal epileptogenesis. *Brain Res.*, 702(1-2):126-132.

Buchanan, JA., Bilkey, DK. (1997) Transfer of epileptogenesis between perirhinal cortex and amygdala induced by electrical kindling. *Brain Res.*, 771(1):71-79.

Buhl, EH., Otis, TS., Mody, I. (1996) Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science.*, 271(5247):369-373.

Bureau, M., Laschet, J., Minier, F., Chauvel, P. (1997) Intervention of GABAergic neurotransmission in partial epilepsies. *Rev Neurol (Paris).*, 153 Suppl 1:S46-54.

Burgard, EC., Sarvey, JM. (1991) Long-lasting potentiation and epileptiform activity produced by GABAB receptor activation in the dentate gyrus of rat hippocampal slice. *J Neurosci.*, 11(5):1198-1209.

Burnham, WM. (1975) Primary and "transfer" seizure development in the kindled rat. *Can J Neurol Sci.*, 2(4):417-428.

Callahan, PM., Paris, JM., Cunningham, KA., Shinnick-Gallagher, P. (1991) Decrease of GABA-immunoreactive neurons in the amygdala after electrical kindling in the rat. *Brain Research*, 555:335-339.

Carai, MA., Brunetti, G., Lobina, C., Serra, S., Vacca, G., Minardi, G., Colombo, G., Gessa, GL. (2002) Proconvulsive effect of the GABA(B) receptor antagonist, SCH 50911, in rats undergoing ethanol withdrawal syndrome. *Eur J Pharmacol.*, 445(3):195-199.

Chesnut, TJ., Swann, JW. (1989) Disinhibitory actions of the GABAA agonist muscimol in immature hippocampus. *Brain Res.*, 502(2):365-374.

Cobb, SR., Buhl, EH., Halasy, K., Paulsen, O., Somogyi, P. (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature.*, 378(6552):75-78.

Collingridge, GL., Gage, PW., Robertson, B. (1984) Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. *J Physiol.*, 356:551-564.

Costa, E. (1998) From GABAA receptor diversity emerges a unified vision of GABAergic inhibition. *Annu Rev Pharmacol Toxicol.*, 38:321-350.

D'Aguanno, A., Bardakjian, BL., Carlen, PL. (1989) A system model for investigating passive electrical properties of neurons. *Biophys J.*, 55(6):1169-1182.

D'Antuono, M., Biagini, G., Tancredi, V., Avoli, M. (2001) Electrophysiology of regular firing cells in the rat perirhinal cortex. *Hippocampus.*, 11(6):662-672.

Davidoff, RA. (1985) Antispasticity drugs: mechanisms of action. *Ann Neurol.*, 17(2):107-116.

Davies, CH., Davies, SN., Collingridge, GL. (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J Physiol.*, 424:513-531.

Deisz, RA., Lux, HD. (1985) gamma-Aminobutyric acid-induced depression of calcium currents of chick sensory neurons. *Neurosci Lett.*, 14;56(2):205-210.

Deisz, RA., Prince, DA. (1989) Frequency-dependent depression of inhibition in guinea-pig neocortex in vitro by GABAB receptor feed-back on GABA release. *J Physiol.*, 412:513-541.

Delaney, AJ., Sah, P. (1999) GABA receptors inhibited by benzodiazepines mediate fast inhibitory transmission in the central amygdala. *J Neurosci.*, 19(22):9698-9704.

Dichter, MA., Ayala, GF. (1987) Cellular mechanisms of epilepsy: a status report. *Science.*, 237(4811):157-164.

Douglas, RJ., Koch, C., Mahowald, M., Martin, KA., Suarez, HH. (1995) Recurrent excitation in neocortical circuits. *Science.*, 269(5226):981-985.

Doze, VA., Cohen, GA., Madison, DV. (1995) Calcium channel involvement in GABAB receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *J Neurophysiol.*, 74(1):43-53.

Dufresne, C. (1990) Seizure development in kindling fast and kindling slow rat strains. Master's thesis, Carleton University, Ottawa.

Dunlap, K., Fischbach, GD. (1981) Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J Physiol.*, 317:519-535.

During, MJ., Ryder, KM., Spencer, DD. (1995) Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature.*, 376(6536):174-177.

Engel, J. Jr. (1990) Functional explorations of the human epileptic brain and their therapeutic implications. *Electroencephalography and Clinical Neurophysiol.*, 76:296-316.

Engel, J Jr., Rocha, LL. (1992) Interictal behavioral disturbances: a search for molecular substrates. *Epilepsy Res Suppl.*, 9:341-349.

Engel, J. Jr. (1996) Excitation and inhibition in epilepsy. *Can J of Neurol Sci*, 23:167-174.

Ferland, RJ., Nierenberg, J., Applegate, CD. (1998) A role for the bilateral involvement of perirhinal cortex in generalized kindled seizure expression. *Exp Neurol.*, 151(1):124-137.

Gadea, A., Lopez-Colome, AM. (2001) Glial transporters for glutamate, glycine, and GABA: II. GABA transporters. *J Neurosci Res.*, 63(6):461-468.

Garden, DL., Kemp, N., Bashir, ZI. (2002) Differences in GABAergic transmission between two inputs into the perirhinal cortex. *Eur J Neurosci.*, 16(3):437-444.

Gean, PW., Shinnick-Gallagher, P., Anderson, AC. (1989) Spontaneous epileptiform activity and alteration of GABA- and of NMDA-mediated neurotransmission in amygdala neurons kindled in vivo. *Brain Res.*, 494(1):177-181.

Geinisman, Y., Morrell, F., deToledo-Morrell, L. (1988) Remodeling of synaptic architecture during hippocampal "kindling". *Proc Natl Acad Sci.*, 85(9):3260-3264.

Gernert, M., Loscher, W. (2001) Lack of robust anticonvulsant effects of muscimol microinfusions in the anterior substantia nigra of kindled rats. *Eur J Pharmacol.*, 432(1):35-41.

Giorgetti, M., Bacciottini, L., Giovannini, MG., Colivicchi, MA., Goldfarb, J., Blandina, P. (2000) Local GABAergic modulation of acetylcholine release from the cortex of freely moving rats. *Eur J Neurosci.*, 12(6):1941-1948.

Glavinovic, MI., Rabie, HR. (1998) Monte Carlo simulation of spontaneous miniature excitatory postsynaptic currents in rat hippocampal synapse in the presence and absence of desensitization. *Pflugers Arch.*, 435(2):193-202.

Gloveli, T., Behr, J., Dugladze, T., Kokaia, Z., Kokaia, M., Heinemann, U. (2003) Kindling alters entorhinal cortex-hippocampal interaction by increased efficacy of presynaptic GABA(B) autoreceptors in layer III of the entorhinal cortex. *Neurobiol Dis.*, 13(3):203-212.

Goddard, G., McIntyre, DC., Leech, C. (1969) A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurol.*, 25:295-330.

Gotman, J. (1984) Relationships between triggered seizures, spontaneous seizures, and interictal spiking in the kindling model of epilepsy. *Experimental Neurol.*, 84:259-273.

Grabowski, JA. (1996) The effects of picrotoxin, atropine and yohimbine on interictal spikes and postictal spikes in the slow and fast kindling rat strains. *Master's Thesis, Carleton University.*

Hablitz, JJ., Tehrani, MH., Barnes, EM Jr. (1989) Chronic exposure of developing cortical neurons to GABA down-regulates GABA/benzodiazepine receptors and GABA-gated chloride currents. *Brain Res.*, 501(2):332-338.

Handforth, A. (1984) Implications of stimulus factors governing kindled seizure threshold. *Exp Neurol.*, 86(1):33-39.

Hajos, N., Mody, I. (1997) Synaptic communication among hippocampal interneurons: properties of spontaneous IPSCs in morphologically identified cells. *J Neurosci.*, 17(21):8427-8442.

Hausser, M. (2001) Synaptic function: dendritic democracy. *Curr Biol.*, 11(1):R10-12.

Hefft, S., Kraushaar, U., Geiger, JR., Jonas, P. (2002) Presynaptic short-term depression is maintained during regulation of transmitter release at a GABAergic synapse in rat hippocampus. *J. Physiol.*, 539(Pt 1):201-208.

Honack, D., Wahnschaffe, U., Loscher, W. (1991) Kindling from stimulation of a highly sensitive locus in the posterior part of the piriform cortex. Comparison with amygdala kindling and effects of antiepileptic drugs. *Brain Res.*, 538(2):196-202.

Isaacson, JS. (1998) GABAB receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *J Neurophysiol.*, 80(3):1571-6.

Isokawa, M. (1998) Modulation of GABAA receptor-mediated inhibition by postsynaptic calcium in epileptic hippocampal neurons. *Brain Res.*, 810(1-2):241-250.

Jarolimek, W., Misgeld, U. (1992) On the inhibitory actions of baclofen and gamma-aminobutyric acid in rat ventral midbrain culture. *J Physiol.*, 451:419-443.

Jefferys, JG., Traub, RD., Whittington, MA. (1996) Neuronal networks for induced '40 Hz' rhythms. *Trends Neurosci.*, 19(5):202-208.

Jensen, K., Lambert, JD., Jensen, MS. (1999) Activity-dependent depression of GABAergic IPSCs in cultured hippocampal neurons. *J Neurophysiol.*, 82(1):42-9.

Jensen, K., Chiu, CS., Sokolova, I., Lester, HA., Mody, I. (2003) GABA transporter-1 (GAT1)-deficient mice: differential tonic activation of GABAA versus GABAB receptors in the hippocampus. *J Neurophysiol.* 90(4):2690-2701.

Johnston, GA. (1996) GABAA receptor pharmacology. *Pharmacol Ther.*, 69(3):173-98.

Jonas, P., Major, G., Sakmann, B. (1993) Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. *J Physiol.*, 472:615-663.

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

Kamphuis, W., Wadman, WJ., Buijs, RM., Lopes da Silva, FH. (1986) Decrease in number of hippocampal gamma-aminobutyric acid (GABA) immunoreactive cells in the rat kindling model of epilepsy. *Exp Brain Res.*, 64(3):491-495.

Kamphuis, W., Huisman, E., Dreijer, AM., Ghijsen, WE., Verhage, M., Lopes da Silva, FH. (1990) Kindling increases the K(+)-evoked Ca2(+)-dependent release of endogenous GABA in area CA1 of rat hippocampus. *Brain Res.*, 511(1):63-70.

Kamphuis, W., Gorter, JA., Wadman, WJ., Lopes da Silva, FH. (1992) Hippocampal kindling leads to different changes in paired-pulse depression of local evoked field potentials in CA1 area and in fascia dentata. *Neurosci Lett.*, 141(1):101-105.

Kanter, ED., Kapur, A., Haberly, LB. (1996) A dendritic GABAA-mediated IPSP regulates facilitation of NMDA-mediated responses to burst stimulation of afferent fibers in piriform cortex. *J Neurosci.*, 16(1):307-312.

Kapur, J., Bennett, JP Jr., Wooten, GF., Lothman, EW. (1989) Evidence for a chronic loss of inhibition in the hippocampus after kindling: biochemical studies. *Epilepsy Res.*, 4(2):100-108.

Kapur, J., Macdonald, RL. (1997) Rapid seizure-induced reduction of benzodiazepine and Zn2+ sensitivity of hippocampal dentate granule cell GABAA receptors. *J Neurosci.*, 17(19):7532-7540.

Kapur, A., Pearce, RA., Lytton, WW., Haberly, LB. (1997) GABAA-mediated IPSCs in piriform cortex have fast and slow components with different properties and locations on pyramidal cells. *J Neurophysiol.*, 78(5):2531-2545.

Karlsson, G., Klebs, K., Hafner, T., Schmutz, M., Olpe, HR. (1992) Blockade of GABAB receptors accelerates amygdala kindling development. *Experientia.*, 48(8):748-751.

Katona, I., Rancz, EA., Acsady, L., Ledent, C., Mackie, K., Hajos, N., Freund, TF. (2001) Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci.*, 21(23):9506-9518.

Kaura, S., Bradford, HF., Young, AM., Croucher, MJ., Hughes, PD. (1995) Effect of amygdaloid kindling on the content and release of amino acids from the amygdaloid complex: in vivo and in vitro studies. *J Neurochem.*, 65(3):1240-1249.

Kilman, V., van Rossum, MC., Turrigiano, GG. (2002) Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. *J Neurosci.*, 22(4):1328-1337.

Kokaia, M., Pratt, GD., Elmer, E., Bengzon, J., Fritschy, JM., Kokaia, Z., Lindvall, O., Mohler, H. (1994) Biphasic differential changes of GABAA receptor subunit mRNA levels in dentate gyrus granule cells following recurrent kindling-induced seizures. *Brain Res Mol Brain Res.*, 23(4):323-332.

Kokaia, Z., Kokaia, M. (2001) Changes in GABA(B) receptor immunoreactivity after recurrent seizures in rats. *Neurosci Lett.*, 315(1-2):85-88.

Kulik, A., Vida, I., Lujan, R., Haas, CA, Lopez-Bendito, G., Shigemoto, R., Frotscher, M. (2003) Subcellular localization of metabotropic $GABA_B$ receptor subunits $GABA_{B1a/b}$ and $GABA_{B2}$ in the rat hippocampus. *J. Neurosci.*, 23(35):11026-11035.

Kulkarni, SK, George, B. (1994) Kindling model of epilepsy. *Methods Find Exp Clin Pharmacol.*, 16(10):735-745.

Lambert, JD., Fueta, Y., Roepstorff, A., Andreasen, M. (1996) Analysis of the kinetics of synaptic inhibition points to a reduction in GABA release in area CA1 of the genetically epileptic mouse, El. *Epilepsy Res.*, 26(1):15-23.

Lambert, NA., Wilson, WA. (1993) Discrimination of post- and presynaptic GABAB receptor-mediated responses by tetrahydroaminoacridine in area CA3 of the rat hippocampus. *J Neurophysiol.*, 69(2):630-635.

Lehmann, H., Ebert, U., Loscher, W. (1998) Amygdala-kindling induces a lasting reduction of GABA-immunoreactive neurons in a discrete area of the ipsilateral piriform cortex. *Synapse.*, 29(4):299-309.

Leung, LS. (1990) Spontaneous hippocampal interictal spikes following local kindling: time-course of change and relation to behavioural seizures. *Brain Res.*, 513:308-314.

Leung, LS., Canning, KJ., Shen, B. (2005) Hippocampal afterdischarges after GABA(B)-receptor blockade in the freely moving rat. *Epilepsia.*, 46(2):203-216.

Libri, V., Constanti, A., Postlethwaite, M., Bowery, NG. (1998) Blockade of GABA(B) receptors facilitates muscarinic agonist-induced epileptiform activity in immature rat piriform cortex in vitro. *Naunyn Schmiedebergs Arch Pharmacol.*, 358(2):168-174.

Ling, DS., Benardo, LS. (1998) Synchronous firing of inhibitory interneurons results in saturation of fast GABA(A) IPSC magnitude but not saturation of fast inhibitory efficacy in rat neocortical pyramidal cells. *Synapse.*, 28(1):91-102.

Liu, RH., Yamuy, J., Engelhardt, JK., Xi, MC., Morales, FR., Chase, MH. (1996) Cell size and geometry of spinal cord motoneurons in the adult cat following intramuscular injection of adriamycin: comparison with data from aged cats. *Brain Res.*, 738(1):121-130.

Liu, X., Leung, LS. (2003) Partial hippocampal kindling increases GABAB receptor-mediated postsynaptic currents in CA1 pyramidal cells. *Epilepsy Res.*, 57(1):33-47.

Lopes da Silva, FH., Kamphuis, W., Titulaer, M., Vreugdenhil, M., Wadman, WJ. (1995) An experimental model of progressive epilepsy: the development of kindling of the hippocampus of the rat. *Ital J Neurol Sci.*, 16(1-2):45-57.

Loscher, W., Schwark, WS. (1985) Evaluation of different GABA receptor agonists in the kindled amygdala seizure model in rats. *Exp Neurol.*, 89(2):454-460.

Loscher, W., Schwark, WS. (1987) Further evidence for abnormal GABAergic circuits in amygdala-kindled rats. *Brain Res.*, 420(2):385-390.

Loscher, W., Wahnschaffe, U., Honack, D., Rundfeldt, C. (1995) Does prolonged implantation of depth electrodes predispose the brain to kindling? *Brain Res.*, 697(1-2):197-204.

Loscher, W., Ebert, U. (1996) The role of the piriform cortex in kindling. *Prog Neurobiol.*, (5-6):427-481.

Loscher, W., Honack, D., Gramer, M. (1999) Effect of depth electrode implantation with or without subsequent kindling on GABA turnover in various rat brain regions. *Epilepsy Res.*, 37(2):95-108.

Lothman, EW., Bennett, JP., Perlin, JB. (1987) Alterations in neurotransmitter amino acids in hippocampal kindled seizures. *Epilepsy Res.*, 1(6):313-320.

Loup, F., Wieser, HG., Yonekawa, Y., Aguzzi, A., Fritschy, JM. (2000) Selective alterations in GABAA receptor subtypes in human temporal lobe epilepsy. *J Neurosci.*, 20(14):5401-5419.

Lupica, CR., Bell, JA., Hoffman, AF., Watson, PL. (2001) Contribution of the hyperpolarization-activated current (I(h)) to membrane potential and GABA release in hippocampal interneurons. *J Neurophysiol.*, 86(1):261-268.

Ma, Y., Hu, JH., Zhao, WJ., Fei, J., Yu, Y., Zhou, XG., Mei, ZT., Guo, LH. (2001) Overexpression of gamma-aminobutyric acid transporter subtype I leads to susceptibility to kainic acid-induced seizure in transgenic mice. *Cell Res.*, 11(1):61-67.

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

Mackenzie, L., Pope, KJ., Willoughby, JO. (2004) Physiological and pathological spindling phenomena have similar regional EEG power distributions. *Brain Res.*, 1008(1):92-106.

Magee, JC. (2000) Dendritic integration of excitatory synaptic input. *Nat Rev Neurosci.*, 1(3):181-190.

Majkowski, J. (1999) Kindling: clinical relevance for epileptogenicity in humans. *Adv Neurol.*, 81:105-113.

Malenka, RC., Nicoll, RA. (1999) Long-term potentiation--a decade of progress? *Science.*, 285(5435):1870-1874.

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

McIntyre, DC., Racine, RJ. (1986) Kindling mechanisms: current progress on an experimental epilepsy model. *Prog Neurobiol.*, (1):1-12.

McIntyre, DC., Rajala, J., Edson, N. (1987) Suppression of amygdala kindling with short interstimulus intervals: effect of norepinephrine depletion. *Exp Neurol.*, 95(2):391-402.

McIntyre, DC., Don, JC., Edson, N. (1991a) Distribution of [14C]2-deoxyglucose after various forms and durations of status epilepticus induced by stimulation of a kindled amygdala focus in rats. *Epilepsy Res.*, 10(2-3):119-133.

McIntyre, DC., Kelly, ME., Dufresne, C. (1991b) Suppression of amygdala kindling with massed stimulation: effect of noradrenaline antagonists. *Brain Res.*, 561(2):279-284.

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

McIntyre, DC., Kelly, ME., Dufresne, C. (1999) FAST and SLOW amygdala kindling rat strains: comparison of amygdala, hippocampal, piriform and perirhinal cortex kindling. *Epilepsy Res.*, 35(3):197-209.

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

McIntyre, DC., Poulter, MO. (2001) Kindling and the mirror focus. *Int Rev Neurobiol.*, 45:387-407.

McIntyre, DC., Poulter, MO., Gilby, K. (2002a) Kindling: some old and some new. *Epilepsy Res.*, 50(1-2):79-92.

McIntyre, DC., Hutcheon, B., Schwabe, K., Poulter, MO. (2002b) Divergent GABA(A) receptor-mediated synaptic transmission in genetically seizure-prone and seizure-resistant rats. *J Neurosci.*, 22(22):9922-9931.

McNamara, JO. (1984) Kindling: an animal model of complex partial epilepsy. *Ann Neurol.*, 16 Suppl:S72-76.

McNamara, JO. (1986) Kindling model of epilepsy. *Adv Neurol.*, 44:303-318.

Medvedev, A., Mackenzie, L., Hiscock, JJ, Willoughby, JO. (1996) Frontal cortex leads other brain structures in generalized spike-and-wave spindles and seizure spikes induced by picrotoxin. *Electroencephalography and Clin Neurophysiol.*, 98:157-166.

Meldrum, B. (1995) Epilepsy. Taking up GABA again. *Nature.*, 376(6536):122-123.

Michalakis, M., Holsinger, D., Ikeda-Douglas, C., Cammisuli, S., Ferbinteanu, J., DeSouza, C., DeSouza, S., Fecteau, J., Racine, RJ., Milgram, NW. (1998) Development of spontaneous seizures over extended electrical kindling. I. Electrographic, behavioral, and transfer kindling correlates. *Brain Res.*, 793(1-2):197-211.

Minamoto Y, Itano T, Tokuda M, Matsui H, Janjua NA, Hosokawa K, Okada Y, Murakami TH, Negi T, Hatase O. (1992) In vivo microdialysis of amino acid neurotransmitters in the hippocampus in amygdaloid kindled rat. *Brain Res.*, 573(2):345-348.

Misgeld, U., Bijak, M., Jarolimek, W. (1995) A physiological role for GABAB receptors and the effects of baclofen in the mammalian central nervous system. *Prog Neurobiol.*, 46(4):423-462.

Mitchell, SJ., Silver, RA. (2000) GABA spillover from single inhibitory axons suppresses low-frequency excitatory transmission at the cerebellar glomerulus. *J Neurosci.*, 20(23):8651-8658.

Mody, I., De Koninck, Y., Otis, TS., Soltesz, I. (1994) Bridging the cleft at GABA synapses in the brain. *Trends Neurosci.*, 17(12):517-525.

Mody, I. (1999) Synaptic plasticity in kindling. *Adv Neurol.*, 79:631-643.

Morimoto, K., Sanei, T., Sato, K. (1993) Comparative study of the anticonvulsant effect of gamma-aminobutyric acid agonists in the feline kindling model of epilepsy. *Epilepsia.*, 34(6):1123-1129.

Morrisett, RA., Lewis, DV., Swartzwelder, HS., Wilson, WA. (1993) Antiepileptic effects of GABAb receptor activation in area CA3 of rat hippocampus. *Brain Res.*, 600(2):235-242.

Mott, DD., Bragdon, AC., Lewis, DV., Wilson, WA. (1989) Baclofen has a proepileptic effect in the rat dentate gyrus. *J Pharmacol Exp Ther.*, 249(3):721-715.

Mott, DD., Xie, CW., Wilson, WA., Swartzwelder, HS., Lewis, DV. (1993) GABAB autoreceptors mediate activity-dependent disinhibition and enhance signal transmission in the dentate gyrus. *J Neurophysiol.*, 69(3):674-691.

Nusser, Z., Cull-Candy, S., Farrant, M. (1997) Differences in synaptic GABA(A) receptor number underlie variation in GABA mini amplitude. *Neuron.*, 19(3):697-709.

O'Brien, BJ., Isayama, T., Richardson, R., Berson, DM. (2002) Intrinsic physiological properties of cat retinal ganglion cells. *J Physiol.*, 538(Pt 3):787-802.

Olpe, HR., Steinmann, MW., Ferrat, T., Pozza, MF., Greiner, K., Brugger, F., Froestl, W., Mickel, SJ., Bittiger, H. (1993) The actions of orally active GABAB receptor antagonists on GABAergic transmission in vivo and in vitro. *Eur J Pharmacol.*, 233(2-3):179-186.

Olsen, RW., Avoli, M. (1997) GABA and epileptogenesis. *Epilepsia.*, 38(4):399-407.

Olsen, RW., DeLorey, TM., Gordey, M., Kang, MH. (1999) GABA receptor function and epilepsy. *Adv Neurol.*, 79:499-510.

Otis, TS., De Koninck, Y., Mody, I. (1994) Lasting potentiation of inhibition is associated with an increased number of gamma-aminobutyric acid type A receptors activated during miniature inhibitory postsynaptic currents. *Proc Natl Acad Sci.*, 91(16):7698-7702.

Patil, MM., Hasselmo, ME. (1999) Modulation of inhibitory synaptic potentials in the piriform cortex. *J Neurophysiol.*, 81(5):2103-2118.

Pelligrino, LJ., Pellegrino, AS., Cushman, AJ. (1979) A stereotaxic atlas of the rat brain. Plenum Press, New York.

Penner, MR., Pinaud, R., Robertson, HA. (2001) Rapid kindling of the hippocampus protects against neural damage resulting from status epilepticus. *Neuroreport.*, 12(3):453-457.

Pitkanen, A., Tuunanen, J., Kalviainen, R., Partanen, K., Salmenpera, T. (1998) Amygdala damage in experimental and human temporal lobe epilepsy. *Epilepsy Res.*, 32(1-2):233-253.

Pitler, TA., Alger, BE. (1994) Differences between presynaptic and postsynaptic GABAB mechanisms in rat hippocampal pyramidal cells. *J Neurophysiol.*, 72(5):2317-2327.

Post, RM. (2002) Do the epilepsies, pain syndromes, and affective disorders share common kindling-like mechanisms? *Epilepsy Res.*, 50(1-2):203-219.

Poulter, MO., Barker, JL., O'Carroll, AM., Lolait, SJ., Mahan, LC. (1992) Differential and transient expression of GABAA receptor alpha-subunit mRNAs in the developing rat CNS. *J Neurosci.*, 12(8):2888-2900.

Poulter, MO., Brown, LA., Tynan, S., Willick, G., William, R., McIntyre, DC. (1999) Differential expression of alpha1, alpha2, alpha3, and alpha5 GABAA receptor subunits in seizure-prone and seizure-resistant rat models of temporal lobe epilepsy. *J Neurosci.*, 19(11):4654-4661.

Racine, RJ. (1972a) Modification of seizure activity by electrical stimulation: I. After-discharge threshold. *Electroencephalography and Clinical Neurophysiol.*, 32:269 279.

Racine, RJ. (1972b) Modification of seizure activity by electrical stimulation: II. Motor

seizure. *Electroencephalography and Clinical Neurophysiol.*, 32:281-294.

Racine, RJ., Steingart, M., McIntyre, DC. (1999) Development of kindling-prone and kindling-resistant rats: selective breeding and electrophysiological studies. *Epilepsy Res.*, 35(3):183-195.

Racine, RJ., Steingart, M., Bureau, Y., McIntyre, DC. (2003) Differential sensitivity of genetically Fast vs. Slow kindling rats strains to GABAergic convulsive agents. *Neuropharmacology.*, 45(7):918-924.

Rainnie, DG, Asprodini, EK, Shinnick-Gallagher, P. (1991a) Excitatory transmission in the basolateral amygdala. *J Neurophysiol.*, 66(3):986-998.

Rainnie, DG, Asprodini, EK, Shinnick-Gallagher, P. (1991b) Inhibitory transmission in the basolateral amygdala. *J Neurophysiol.*, 66(3):999-1009.

Rainnie, DG, Asprodini, EK, Shinnick-Gallagher, P. (1992) Kindling-induced long-lasting changes in synaptic transmission in the basolateral amygdala. *J Neurophysiol.*, 67(2):443-454.

Rainnie, DG. (1999) Serotonergic modulation of neurotransmission in the rat basolateral amygdala. *J Neurophysiol.*, 82(1);69-85.

Ribak, CE. (1992) Local circuitry of GABAergic basket cells in the dentate gyrus. Epilepsy Res Suppl., 7:29-47.

Sato, K., Morimoto, K., Okamoto, M., Nakamura, Y., Otsuki, S., Sato, M. (1990b) An analysis of anticonvulsant actions of GABA agonists (progabide and baclofen) in the kindling model of epilepsy. *Epilepsy Res.*, 5(2):117-124.

Sato, M., Racine, RJ., McIntyre, DC. (1990a) Kindling: basic mechanisms and clinical validity, *Electroencephalography and Clinical Neurophysiol.*, 76:459-472.

Scanziani, M., Capogna, M., Gahwiler, BH., Thompson, SM. (1992) Presynaptic inhibition of miniature excitatory synaptic currents by baclofen and adenosine in the hippocampus. *Neuron.*, 9(5):919-927.

Scholz, KP., Miller, RJ. (1991) GABAB receptor-mediated inhibition of Ca2+ currents and synaptic transmission in cultured rat hippocampal neurones. *J Physiol.*, 444:669-686.

Scholz, KP., Miller, RJ. (1992) Inhibition of quantal transmitter release in the absence of calcium influx by a G protein-linked adenosine receptor at hippocampal synapses. *Neuron.*, 8(6):1139-1150.

Schwartzkroin, PA. (1994) Role of the hippocampus in epilepsy. *Hippocampus.*, 4(3):239-242.

Shin, C., Pedersen, HB., McNamara, JO. (1985) gamma-Aminobutyric acid and benzodiazepine receptors in the kindling model of epilepsy: a quantitative radiohistochemical study. *J Neurosci.*, (10):2696-2701.

Shoji, Y., Tanaka, E., Yamamoto, S., Maeda, H., Higashi, H. (1998) Mechanisms underlying the enhancement of excitatory synaptic transmission in basolateral amygdala neurons of the kindling rat. *J Neurophysiol.*, 80(2):638-646.

Sigel, E., Baur, R., Trube, G., Mohler, H., Malherbe, P. (1990) The effect of subunit composition of rat brain GABAA receptors on channel function. *Neuron.*, 5(5):703-711.

Silinsky, EM., Solsona, CS. (1992) Calcium currents at motor nerve endings: absence of effects of adenosine receptor agonists in the frog. *J Physiol.*, 457:315-28.

Smith, BN., Dudek, FE. (1996) Amino acid-mediated regulation of spontaneous synaptic activity patterns in the rat basolateral amygdala. *J Neurophysiol.*, 76(3):1958-1967.

Sohal, VS., Hasselmo, ME. (1998) GABA(B) modulation improves sequence disambiguation in computational models of hippocampal region CA3. *Hippocampus.*, 8(2):171-193.

Spiller, AE., Racine, RJ. (1994) Transfer kindling between sites in the entorhinal cortex-perforant path-dentate gyrus system. *Brain Res.*, 635(1-2):130-138.

Sundaram, M., Hogan, T., Hiscock, M., Pillay, N. (1990) Factors affecting interictal spike discharges in adults with epilepsy. *Electroencephalogr Clin Neurophysiol.*, 75(4):358-360.

Sutula, T., He, XX., Cavazos, J., Scott, G. (1988) Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science.*, 239(4844):1147-1150.

Swanson, TH. (1995) The pathophysiology of human mesial temporal lobe epilepsy. *J Clin Neurophysiol.*, 12(1):2-22.

Swartz, KJ. (1993) Modulation of Ca2+ channels by protein kinase C in rat central and peripheral neurons: disruption of G protein-mediated inhibition. *Neuron.*, 11(2):305-320.

Takahashi, T., Kajikawa, Y., Tsujimoto, T. (1998) G-Protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *J Neurosci.*, 18(9):3138-3146.

Tanaka, S., Tsuchida, A., Kiuchi, Y., Oguchi, K., Numazawa, S., Yoshida, T. (2003) GABAergic modulation of hippocampal glutamatergic neurons: an in vivo microdialysis study. *Eur J Pharmacol.*, 465(1-2):61-67.

Thomson, AM., Destexhe, A. (1999) Dual intracellular recordings and computational models of slow inhibitory postsynaptic potentials in rat neocortical and hippocampal slices. *Neuroscience.*, 92(4):1193-1215.

Thompson, SM., Gahwiler, BH. (1992) Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. *J Physiol.*, 451:329-345.

Thompson, SM. (1994) Modulation of inhibitory synaptic transmission in the hippocampus. *Prog Neurobiol.*, 42(5):575-609.

Traub, RD., Whittington, MA., Colling, SB., Buzsaki, G., Jefferys, JG. (1996) Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J Physiol.*, 493(Pt 2):471-484.

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

Tseng, YT., Wellman, SE., Ho, IK. (1994) In situ hybridization evidence of differential modulation by pentobarbital of GABAA receptor alpha 1- and beta 3-subunit mRNAs. *J Neurochem.*, 63(1):301-309.

Tuff, LP., Racine, RJ., Mishra, RK. (1983) The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. II. Receptor binding. *Brain Res.*, 277(1):91-98.

Tuunanen, J., Halonen, T., Pitkanen, A. (1997) Decrease in somatostatin-immunoreactive neurons in the rat amygdaloid complex in a kindling model of temporal lobe epilepsy. *Epilepsy Res.*, 26(2):315-327.

Ueda, Y., Willmore, LJ. (2000) Hippocampal gamma-aminobutyric acid transporter alterations following focal epileptogenesis induced in rat amygdala. *Brain Res Bull.*, 52(5):357-361.

van Luijtelaar, EL., Coenen, AM. (1986) Two types of electrocortical paroxysms in an inbred strain of rats. *Neurosci Lett.*, 70(3):393-397.

van Rijn, CM., Van Berlo, MJ., Feenstra, MG., Schoofs, ML., Hommes, OR. (1987) R(-)-baclofen: focal epilepsy after intracortical administration in the rat. *Epilepsy Res.*, 1(6):321-327.

Vergnes, M., Marescaux, C., Micheletti, G., Depaulis, A., Rumbach, L., Warter, JM. (1984) Enhancement of spike and wave discharges by GABAmimetic drugs in rats with spontaneous petit-mal-like epilepsy. *Neurosci Lett.*, 44(1):91-94.

Vergnes, M., Boehrer, A., Simler, S., Bernasconi, R., Marescaux, C. (1997) Opposite effects of GABAB receptor antagonists on absences and convulsive seizures. *Eur J Pharmacol.*, 332(3):245-255.

Wada, JA. (1978) Kindling as a model of epilepsy. *Electroencephalogr Clin Neurophysiol Suppl.*, (34):309-316.

Waldmeier, PC., Stocklin, K., Feldtrauer, JJ. (1992) Systemic administration of baclofen and the GABAB antagonist, CGP 35348, does not affect GABA, glutamate or aspartate in microdialysates of the striatum of conscious rats. *Naunyn Schmiedebergs Arch Pharmacol.*, 345(5):548-552.

Washburn, MS., Moises, HC. (1992) Inhibitory responses of rat basolateral amygdaloid neurons recorded in vitro. *Neuroscience.*, 50(4):811-830.

Wasterlain, CG., Shirasaka, Y., Mazarati, AM., Spigelman, I. (1996) Chronic epilepsy with damage restricted to the hippocampus: possible mechanisms. *Epilepsy Res.*, 26(1):255-265.

Wierenga, CJ., Wadman, WJ. (1999) Miniature inhibitory postsynaptic currents in CA1 pyramidal neurons after kindling epileptogenesis. *J Neurophysiol.*, 82(3):1352-1362.

Wu, C., Leung, LS. (1997) Partial hippocampal kindling decreases efficacy of presynaptic GABAB autoreceptors in CA1. *J Neurosci.*, 17(23):9261-9269.

Wu, LG., Saggau, P. (1995) GABAB receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by reduction of presynaptic Ca2+ influx. *J Physiol.*, 485 (Pt 3):649-657.

Wurpel, JN., Sperber, EF., Moshe, SL. (1990) Baclofen inhibits amygdala kindling in immature rats. *Epilepsy Res.*, 5(1):1-7.

Wurpel, JN. (1994) Baclofen prevents rapid amygdala kindling in adult rats. *Experientia.*, 50(5):475-478.

Yamada, J., Saitow, F., Satake, S., Kiyohara, T., Konishi, S. (1999) GABA(B) receptor-mediated presynaptic inhibition of glutamatergic and GABAergic transmission in the basolateral amygdala. *Neuropharmacol.*, 38(11):1743-1753.

Yoon, KW., Rothman, SM. (1991) The modulation of rat hippocampal synaptic conductances by baclofen and gamma-aminobutyric acid. *J Physiol.*, 442:377-390.

Zeng, XJ., Tietz, EI. (1999) Benzodiazepine tolerance at GABAergic synapses on hippocampal CA1 pyramidal cells. *Synapse.*, 31(4):263-277.

Ziakopoulos, Z., Brown, MW., Bashir, ZI. (2000) GABAB receptors mediate frequency-dependent depression of excitatory potentials in rat perirhinal cortex in vitro. *Eur J Neurosci.*, 12(3):803-809.