**The Effects of Picrotoxin, Atropine and Yohimbine
on Interictal Spikes and Postictal Spikes in the
SLOW and FAST Kindling Rat Strains**

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requirements for a Master of Science degree
in Psychology

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submitted by

Jenny A. Grabowski

in partial fulfillment of the requirements for

the degree of

Master of Science

Chairman, Department of Psychology

Thesis supervisor

Abstract

Although elusive, the interictal spike (IIS), a spontaneous neuronal burst seen on the electroencephalogram (EEG) between ictal episodes, has been an important tool for clinicians in their diagnosis of human epilepsy. The SLOW and FAST kindling rat strains, originally bred on the basis of their amygdala kindling rates, provide an excellent model for studying the IIS and postictal spike (PIS), as well as focal epileptogenicity. Although the IIS is a hallmark of epileptogenicity, surprisingly, the kindling resistant SLOW strain show significantly more IISs in all stages of kindling than the kindling prone FAST strain. The present study examined baseline differences in the development and progression of IISs and PISs in the two strains. In addition, the IIS, the convulsive seizure, and the PIS were investigated for their pharmacological sensitivity to the drugs picrotoxin, atropine and yohimbine in the SLOW and FAST strains.

Although each drug affected convulsive seizures in a different way, picrotoxin, atropine and yohimbine administration increased both IISs and PISs in both strains of rat examined. The fact that both IISs and PISs were affected in the same manner, indicates that they may be controlled by similar mechanisms.

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Introduction

1

Hughlings-Jackson first recognized partial epilepsy as a disorder localized to a discrete area of the cerebral cortex and consequently, designed a scientific approach for its study (Engel, 1995). It is largely as a result of his work that epilepsy became accepted as a disease of neurological, and not psychiatric origin. Today, epilepsy is defined as a chronic neurological disorder, characterized by recurrent seizures, due to excessive discharge of cerebral neurons (Gastaut, 1973). Approximately 3% of the population will have more than one seizure in a lifetime (Hauser & Kurland, 1975) although it appears that all individuals possess the potential to convulse (Hauser, 1982).

Seizures have been divided into two broad categories, namely, generalized and partial seizures (Baumann, 1982). Generalized seizures spread rapidly to involve both cerebral hemispheres simultaneously (Rodin, 1987), while partial seizures affect only a discrete region within the brain, referred to as the focus. Generalized seizures can be divided into grand-mal or tonic-clonic seizures, involving alternating spasm and relaxation of the muscles and petit-mal seizures, which consist of blinking or staring blankly. Partial seizures have also been subdivided into simple and complex partial seizures. Complex partial seizures are accompanied by a disruption of consciousness, while simple partial seizures are not. Seizures which begin in a focus and spread throughout the brain, to eventually produce generalized motor convulsions, are described as partial seizures with secondary generalization. Many complex partial seizures originate in the temporal lobe and, as a result, are referred to as temporal lobe seizures (Hauser & Kurland, 1975).

In the assessment and treatment of temporal lobe epilepsy, clinicians must first locate the epileptic focus which contains abnormally excitable neurons (Wyler, Ojemann & Ward, 1981). The interictal spike (IIS), a spontaneous burst, visible on the EEG between

ictal (clonic or rhythmic) episodes, is one of the hallmarks of the epileptic focus (Blume, Borghesi & Lemieux, 1993; Kanner, Morris, Luders, Dinner, Van Ness & Willie, 1993; Lieb, Woods, Siccardi, Crandell, Walter & Leake, 1978). Since the IIS is a defining feature of the abnormally excitable brain region, researchers believe that identifying the underlying mechanisms of the IIS, may lead to a deeper understanding of partial epilepsy (Fitz & McNamara, 1979).

In the absence of overt motor seizures in patients undergoing EEG recording, IISs are often instrumental in the diagnosis of epilepsy because, as mentioned, they are indicative of an abnormally excitable epileptic focus (Sundaram, Hogan, Hiscock & Pillay, 1990). In fact, some researchers have argued that IISs are the earliest manifestations of the epileptic process (Dichter & Ayala, 1987) and, as a consequence, could be used as markers of epileptogenicity in patients with a predisposition for epilepsy (genetic or post traumatic).

In the adult population, partial epilepsy is more common than the generalized form (Gastaut, 1975) and as many as half of the patients with partial seizures suffer from medically intractable epilepsy (Hauser & Kurland, 1975). Medically intractable epilepsy, which can not be adequately treated with the available anti-convulsant medication, often necessitates surgical intervention to decrease seizure frequency. In this regard, IISs have become invaluable for screening these individuals for surgery (Barry, Sussman, O' Connor & Harner, 1992; Barth, Sutherling, Engel & Beatty, 1984). Post surgical outcome, measured by seizure frequency in the year after surgery, appears to be correlated with how localized IISs are within the focus (Barry et al., 1992). Several regions within the focus may initiate IIS activity (Barth et al., 1984), but patients whose seizures are well controlled after surgical resection are invariably those whose IISs consistently arise from the same location within the epileptic region (Barry et al., 1992). Barry et al. reported that

patients who pre-surgically had well localized IISs, regardless of whether they were unilateral or bilateral, benefited most from surgery. Almost all patients with unilateral seizures were seizure free and 76% of patients with bilateral seizures were much improved. Conversely, only one third of patients whose IISs were not well localized benefited from surgery.

In addition to being functional in screening surgical candidates, IISs have also been used by clinicians to determine whether anti-epileptic medication should be discontinued (Gotman, 1991). A low rate or total lack of IISs suggests to the clinician that medication can safely be discontinued, since a belief prevails that IISs serve as a barometer of excitability within the epileptic brain. Although, clinicians assume that the IIS is representative of hyperexcitability, the validity of this hypothesis is not well documented in the literature.

It is uncertain whether clinicians should use IISs to guide their decisions about anti-convulsant medication, because anti-convulsant drugs may actually influence IIS rate in an opposite manner than originally assumed (Ludwig & Ajmone Marsan, 1975; Mulligan et al., 1983). For example, Ludwig et al. (1975) report that the withdrawal of anti-convulsant medication has, paradoxically, lead to an increase in IIS rates in some patients. However, it was reported by Mulligan et al. (1983) that acute injections of the anti-convulsant, phenytoin, decreased IIS rate in epileptic patients. Therefore, while evidence suggests that a relationship may exist between anti-convulsants and IISs, the nature of this relationship is somewhat ambiguous.

Finally, it has also been proposed that the pattern and frequency of IISs may be helpful in estimating seizure onset (Lange, Lieb, Engel & Crandell, 1983). Lange et al. (1983) report that 20 minutes preceding a seizure, IISs begin to occur in greater synchrony over larger areas of the brain. However, other researchers report that IIS rates

decrease in advance of seizures (Weiser, 1989; Lange et al., 1983), while still others have reported no change in IIS rate before seizure onset (Katz, Marks, McCarthy & Spencer, 1991; Gotman, 1991; Lange et al., 1983). These conflicting results have made conclusions regarding the relationship between IISs and seizure onset difficult to formulate. In contrast, the observations that IIS rate increases immediately following seizures (Gotman, 1991) and stays elevated for several days thereafter, have been more thoroughly documented (Gotman, 1985; Sundaram et al., 1990).

Although IISs are widely utilized by epileptologists for diagnostic purposes, as outlined above, few controlled human experiments have been undertaken to study these events. As a result, IISs are a topic of great controversy in the clinical literature. The available animal models of epilepsy, however, allow much more experimental control than is possible with clinical populations and consequently, may assist in providing answers to questions that have so far eluded clinicians.

Animal Models of Epilepsy

Numerous models have been employed to study IISs, but the three which will be discussed in this paper are the penicillin, the freeze lesion and finally, the kindling model. The penicillin model, an acute model of epilepsy, involves the direct application of penicillin onto the cerebral surface. Penicillin blocks post synaptic GABA receptors in the area of application (Dichter & Ayala, 1987) and, in so doing, interferes with inhibitory mechanisms (Anderson & Rutledge, 1979). Within minutes, the penicillin lesion produces paroxysmal activity, seen on the EEG as high voltage sharp negative waves or IISs in the centre of the lesion. The IIS activity lasts for several hours (Barth, 1989) and usually culminates in seizures (Dichter & Ayala, 1987). In this model, IIS activity is followed by GABA mediated refractory periods, which under normal circumstances, prevent further neuronal firing for up to 900 msec (Dorn & Witte, 1995). It has been suggested that a

weakening of these inhibitory processes may be responsible for the transition of IISs into ictal events (Dorn & Witte, 1995).

Researchers using this model have generated data which suggests that the IIS is an excitatory event (Ayala et al, 1973; Dichter & Ayala, 1987), sometimes referred to as a "mini-seizure". Several lines of evidence support this hypothesis. First, the paroxysmal activity characteristic of the penicillin model, and a cellular counterpart of the IIS, is physiologically equivalent to an excitatory post synaptic potential (EPSP) (Ayala, Dichter, Matsumoto & Spencer, 1973). In addition, within minutes of the penicillin application, IISs lengthen, turn into so called "larval seizures" or afterdischarges (Ayala et al., 1973) and finally, progress into full blown seizures (Dichter & Ayala, 1987). Furthermore, the epileptic focus in rats with penicillin-induced epilepsy has been shown to be hypermetabolic, suggesting excitatory activity within the focus (Witte, Bruchl, Schlaug, Tuxhorn, Lahl, Villagran & Seitz, 1994). Many areas adjacent to the excitatory focus are inhibited or hypometabolic, producing an "inhibitory surround", similar to that found in PET studies of epileptic patients (Witte et al., 1994; Dichter & Ayala, 1987).

Despite the many conclusions drawn from research based on the penicillin model, the extent to which this model approximates the human condition is questionable. The tight relationship between IISs and paroxysmal activity in the penicillin model has not been documented between surface EEG recordings and intracellular bursts in clinical studies (Wyler et al., 1981). Furthermore, clinicians have seen no evidence of the IIS transition into rhythmic activity (seizures) indicated by the penicillin model. In fact, clinicians have determined that IIS rate most likely does not change prior to seizures (Gotman & Marciani, 1985; Gotman, 1991) and consequently, cannot corroborate the penicillin data. Finally, the penicillin model necessitates anaesthetizing the animal, in order to gather data. As a result, the loss of information from the environment may prevent normal

communication between various areas of the brain (Wyler et al., 1982). It has been postulated that this modification could lead to abnormal IIS synchronization and eventually to the seizures that characterize this acute model (Wyler et al., 1982). For these reasons, it can be argued that the penicillin model does not experimentally simulate the IISs observed in epileptic patients.

In direct contrast to the penicillin model, research into IIS mechanisms using the in vitro freeze lesion model, has suggested that the IIS represents an inhibitory event (Troyer et al., 1992). The freeze lesion model is an acute, post traumatic model of epilepsy, which attempts to simulate the dynamic changes that occur in individuals who experience head trauma and later come to develop partial epilepsy (Troyer, Blanton & Kriegstein, 1992). Lesions are produced by placing dry ice and ethanol on the cerebral surface. Using this model and patch clamp techniques, it has been determined that chloride dependent, GABA-mediated inhibitory outward currents recorded from pyramidal cells, occur simultaneously with IISs (Troyer et al., 1992). It was inferred that IISs are inhibitory in nature because they occurred in unison with the GABA mediated, inhibitory intracellular event.

To summarize, the two acute animal models discussed above illustrate that IISs are not simple phenomena, and may have different triggering mechanisms, depending on the agents used to induce them. However, neither model discussed is ideal for studying IISs, since both only allow for short term IIS monitoring, and thus are not practical for studying IIS development or progression. Finally, these two acute models produce IISs and epileptogenesis by inducing cell damage or frank lesions. Consequently, it may be difficult to generalize from such studies to the patient population where the idiopathy is often unknown (Hauser & Kurland, 1975).

IISs and the Kindling Model

The kindling model of temporal lobe epilepsy is characterized by the progressive development of electrographic afterdischarges, following repeated low intensity stimulation to various brain sites (Goddard, McIntyre & Leech, 1969). Brief stimulation initially produces short electrographic afterdischarges (ADs), which lengthen with daily stimulation and become associated with distinct behaviours. When electrographic activity is limited to the epileptic focus, eye blinking, mild chewing and salivating (stage 1) can be seen, these behaviours then develop into stronger chewing and head bobbing (stage 2). With continued daily stimulation convulsive motor seizures eventually appear and progress from unilateral forelimb clonus (stage 3) to bilateral forelimb clonus with rearing (stage 4) and, finally, to fully generalized stage 5 seizures which involve rearing and falling (Racine, 1972).

Many animal species have been kindled, including the rat (Goddard, McIntyre & Leech, 1969; Engel & Ackerman, 1980), cat (Gotman, 1984; Sobieszek, 1989), monkey (Delgado, 1971), mouse (Leech & McIntyre, 1976) and rabbit (Timofeeva & Peterson, in progress). While much of the brain, with the exception of the cerebellum, brain stem, and certain regions of the thalamus (Goddard et al., 1969), is responsive to kindling, the anatomical areas most thoroughly studied are the amygdala (Fitz & McNamara, 1979b; Engel & Ackerman, 1980; Gotman, 1984; Kairiss, Racine & Smith, 1984), hippocampus (Kairiss et al., 1984; Leung, 1988; Leung, 1990) and piriform cortex (Racine, Mosher & Kairiss, 1988).

Unlike the methods used to induce epileptogenesis in other models, the electrical stimulation employed in kindling does not produce evidence of gross damage to tissue or cells (Goddard & Douglas, 1975). In this way, kindling represents a reproducible model for chronic epileptogenesis and IISs. The model allows the researcher to study IIS genesis, progression and decline, in a freely moving animal, without the necessity of contamination

by drugs or toxins. Moreover, a broader knowledge of IISs may be acquired using this chronic model, since other features of the epileptogenic process, such as kindling rate (the number of stimulations required to elicit the first stage 5 seizure), which may be of relevance to an animal's propensity for IISs, can be studied simultaneously.

When IISs were first studied using the kindling paradigm, debate arose as to whether they were products of the kindling process or coincidental by-products, unrelated to the kindling phenomenon (Walters, 1970). The postulation that IISs were a motion artifact was ruled out, when it was shown that they could still be evoked in rats immobilized by curare (Walters, 1970). The hypothesis that IISs were due to metallic deposition from the electrode tip or from the implantation procedure was also abandoned because IISs were not seen in non-kindled rats with implanted electrodes. Moreover, IISs were not witnessed in rats stimulated using parameters that would not induce kindling (Walters, 1970). The fact that IISs were not evoked in stimulated, "non-kindled" animals was evidence that IISs were produced, not merely by stimulation per se, but were dependent upon the kindling process. In addition, researchers have not observed IISs in experimental animals prior to kindling or in unstimulated controls (Fitz & McNamara, 1979, Gotman, 1984; Leung, 1990), further suggesting that IISs are a direct consequence of the kindling process.

The point at which IISs are first observed during the kindling process varies and may depend on which structure is stimulated, the species or strain being studied and the experimental method employed. Four (Racine et al., 1988) and 6.6 stimulations (Fitz & McNamara, 1979) are required, on average, to elicit IISs from the amygdala in the rat, while 5 (Leung, 1990) to 7 (Wadman, Lopes da Silva & Leung, 1989) stimulations are required to produce IISs from the rat hippocampus.

Conflict exists in the literature regarding when IISs peak in relation to kindled seizures. Some researchers argue that IIS rate is highest minutes after seizure (Leung, 1990), while others observe a decrease in IIS rate immediately following seizures and an increase several hours later (Gotman, 1984). Gotman (1984) observed that, in kindled cats, IIS rate in the stimulated amygdala was reduced for 4-6 hours subsequent to the seizure and then peaked 6-8 hours later. Leung (1990) has reported that, in hippocampal kindled rats, IIS rate is high for 10-20 minutes post seizure, and declines thereafter, finally increasing to a stable level within 2-7 hrs. Most researchers argue that once IISs have been established their rate does not vary much throughout the kindling process (Gotman, 1984), however, some have reported that IIS rate is maximal during seizure development and declines as kindling progresses and seizures become generalized (Wada, Sato Corcoran, 1974; Lange, Tanaka & Naquet, 1977). It remains to be seen how important any of the above mentioned differences are to the overall understanding of IISs.

The permanence of the IIS has also been much debated. Kindling induced IISs appear to be long lasting (Gotman, 1984; Kairiss et al., 1984; Hiyoshi & Wada, 1990) and have been observed for up to nine months after cessation of kindling (Hiyoshi & Wada, 1990). However, some researchers report a sharp decline in IISs after 2 days without stimulation, with few observable IISs after 5 (Fitz & McNamara, 1979) to 10 (Walters, 1970) days. Once stimulation has been discontinued and IISs are no longer present in the EEG record, they can be retriggered, often with one kindling stimulation (Goddard et al., 1969). These observations suggest that IISs are permanent in much the same way that the kindling phenomenon is permanent.

In order to gain more insight into IIS mechanisms in the kindling model, much research has been focused on determining which areas of the brain are responsible for generating IISs. Several acute epilepsy models have demonstrated that the hippocampus is

a highly epileptogenic site and serves as a generator of IIS activity (Bragdon, Kojima & Wilson, 1992; Colom & Saggau, 1994). However, kindling data do not support this hypothesis. Instead, evidence indicates that the hippocampus is less reactive than other structures, by virtue of being less sensitive to the kindling process (McIntyre & Racine, 1986). The dorsal hippocampus kindles within 40 stimulations, which is much slower than the amygdala or piriform cortex, which kindle in 15 and 8 stimulations, respectively. The view that the hippocampus is the generator of IISs has also been challenged, since IISs evoked by hippocampal kindling rarely begin in this structure and are not as robust as those seen in other structures (Kairiss et al., 1984). Furthermore, research has revealed that IIS activity often begins initially in the piriform cortex (Racine et al., 1988), suggesting that the piriform cortex, and not the hippocampus, is the main IIS generator (Racine et al., 1988). In addition, after several stimulations, pre-kindling "transients" (events appearing as subtle changes in the EEG) develop into IISs in the piriform cortex (Racine et al., 1988), once again implicating this structure as the IIS initiator in the kindling model.

The relationship between IISs and seizures has been an important and widely debated topic in the clinical and experimental literature. As mentioned earlier, Lange et al. (1983) have suggested that IISs may be helpful in estimating seizure onset in patients. Supporting this view, Pinel & Rovner (1978), using the kindling model, discovered that IIS rate increased in the amygdala contralateral to the stimulated amygdala in rats prior to spontaneous seizures. Gotman (1984), studying spontaneous seizures in cats, did not observe such a relationship, however, and noted that spontaneous seizures were as likely to occur when IIS rate was high as when it was low. He proposes that two separate mechanisms generate IIS and seizures, the seizure generating mechanism influencing the IIS generating mechanism, but not the reverse. Other kindling data also suggest that IISs

do not affect seizure onset, but instead that IISs are "time locked" with seizures in such a way that they occur at a high rate after the seizure (Leung, 1990; Gotman, 1984). Leung (1990) noted that the IIS rate declined when kindling was interrupted, and increased again when stimulations resumed. This data supports the hypothesis that IISs are directly regulated by and related to seizure occurrence (Gorman, 1984, 1991). However, the precise relationship between IISs and seizures remains to be determined.

Lastly, it is not obvious whether, in the kindling model, IISs represent excitatory or inhibitory events. Engel & Ackerman (1980) provide indirect evidence that IISs may represent inhibitory events since they observed that high rates of IISs were correlated with seizure suppression. More specifically, they noted that rats manifesting numerous IISs kindled more slowly than those with low IIS rates. Data from the McIntyre lab also demonstrates that IISs occur more frequently in a strain of rat that kindles slowly (SLOW) than in a strain that kindles quickly (FAST) (Dufresne, 1990). However, Pinel & Rovner (1978) found that there was no correlation between how soon animals manifested IISs and whether they subsequently experienced spontaneous seizures.

Genetic Model of Epilepsy Based on Kindling

A genetic model of epilepsy based on kindling, in which two strains of rat show differential excitability and predisposition for IISs, may directly address the nature of IISs and their relation to epileptogenesis. The SLOW and FAST rat strains were originally developed in the Racine lab at McMaster University (Steingart, 1983). It was possible to use individual differences in amygdala (basolateral nucleus) kindling rate of outbred rats to produce two genetically distinct rat strains, the kindling resistant (SLOW) strain and kindling prone (FAST) strain (Steingart, 1983). Rats that kindled slowly were mated, as were those who kindled quickly. This selection procedure was effected for several generations until no overlap between the kindling rates in the offspring of the "slow" and

"fast" kindling groups was apparent. After five generations the average kindling rate of the SLOW was 42 compared to 11 for the FAST strain (Steingart, 1983).

Differences between the two strains that may partially account for the differential amygdala excitability and IIS rates have been studied over the past several years. Originally, Steingart (1983) found that the SLOW strain had significantly elevated basal levels of norepinephrine compared to the FAST strain. Using more sophisticated techniques, Dufresne (1990) confirmed Steingart's findings that NE concentrations were higher in the brains of the SLOW strain. Dufresne further noted that, in the SLOW strain, NE levels were higher in the olfactory bulb than in the FAST strain and that concentrations of the NE metabolite, MHPG (3-methoxy, 4 hydroxy phenylethylenglycol), were also high in the basolateral amygdala of the SLOW strain. These data suggest that endogenous NE levels may modulate IISs and kindling susceptibility in the two strains.

In addition, other differences between the strains in neurotransmitter levels include higher levels of serotonin in the piriform cortex and medial amygdala in the SLOW strain, as well as higher levels of the dopamine metabolite, DOPAC (dihydroxyphenylacetic acid), in the CA1 region of the dorsal hippocampus in the SLOW strain as compared to the FAST (Dufresne, 1990). It has also been documented that rats of the SLOW strain are more successful in the T-maze (McLeod, 1995), radial arm maze and Morris water maze than FAST counterparts. These data suggest that acetylcholine (Ach), which has been shown to be important in learning and memory processes (Dunant, 1986; Hasslmo, 1995) may play a role in the strain differences. Finally, it is reasonable to postulate that GABA levels may also contribute to the epileptic predisposition of the strains, since rats of the SLOW strain are more sensitive to pentobarbital, a drug that involves the GABA channel, than the FAST strain (McIntyre, unpublished observations). In summary, there appear to

be several genetically determined differences in neurotransmitter levels between the strains, that could contribute to their epileptogenic and IIS variability.

Overwhelming evidence suggests that the neurotransmitters GABA, acetylcholine and noradrenaline affect kindling susceptibility. GABA most often has an inhibitory effect on kindled seizures (Kaimphuis & Lopes da Silva, 1990; Sato, Morimoto, Okamoto, Nakamura, Otsuki & Sato, 1990), as does noradrenaline, acting through its alpha-2 receptors (McIntyre, Rajala & Edson, 1987; Jimenez-Rivera, Voltura & Weiss, 1986). In contrast, acetylcholine seems to have a weak excitatory effect on kindling (Arnold, 1973). In addition, it has been documented, using selective cholinergic antagonists, that acetylcholine affects IIS rate in a kindled amygdala (Fitz & McNamara, 1979b) and the hippocampus (Leung, 1988). Fitz & McNamara (1979b) revealed that muscarinic cholinergic blockers, atropine and scopolamine increased rates of IISs in amygdala kindled rats. Leung (1988) also observed that scopolamine increased IIS rate in hippocampal kindled rats.

The purpose of the present research was to investigate the effects of the GABAergic, cholinergic and noradrenergic neurotransmitter systems on IISs, seizures and PISs in two rat strains with differing seizure susceptibility and propensity for IISs and PISs. Picrotoxin, a GABA_A antagonist, atropine, a cholinergic antagonist and yohimbine, an NE antagonist were administered to SLOW and FAST rats in an attempt to further elucidate the pathophysiological mechanisms associated with IISs and PISs, and their relationship to ictal seizures.

Methods

Subjects

A total of 44 male rats (19 SLOW, 25 FAST) weighing 250-350g at the time of surgery were used as subjects in this experiment. A subset of these 44 (n=18, 9 FAST and 9 SLOW) were used as subjects in the second part of this experiment involving drug manipulation. The animals were separated from the mother at 3 weeks of age and housed with other male siblings with food and water freely available. Animals were handled daily for one week prior to surgery.

Surgery

Under pentobarbital anesthesia (65 mg/kg for FAST, 55 mg/kg for SLOW) and sometimes with supplemental halothane animals were implanted with bilateral dorsal hippocampal and bilateral amygdala electrodes. The Krieg stereotaxic was used and the coordinates (Pellegrino, Pellegrino & Cushman, 1979) chosen were as follows: dorsal hippocampus: 2.0 mm posterior to bregma, 3.5 mm lateral to midline and 3.0 mm deep, and basolateral amygdala: 0.2 mm posterior to bregma, 4.5 mm lateral to midline and 8.2 to 8.5 mm deep.

Electrodes were constructed using 2 strands of twisted 0.127 mm diameter Diamel insulated, Nichrome wire which was attached to male amphenol pins. The electrodes were cut to the appropriate length, surgically implanted and the male pins were inserted into a plastic headplug. A reference electrode was secured to the skull at the frontal pole, attached to a jeweler's screw. The electrodes were then covered with dental acrylic following standard procedures in our laboratory (Molino & McIntyre, 1972).

AD Thresholds

One to two weeks after surgery, the afterdischarge (sustained rhythmic discharge) threshold at both amygdalae was ascertained in all rats. To determine the afterdischarge (AD) threshold (defined as the maximum stimulus intensity needed to provoke an AD that outlasts the stimulus by at least 2 seconds), a 2 second, 60 Hz sine wave frequency stimulus of progressively increasing intensity (15, 25, 35, 50, 75, 100, 150, 200 and 250 μ a peak to peak) was presented until an AD was triggered. The interval between stimulations was 1 minute and the interval between AD threshold determination for each amygdala was at least one hour. Kindling was initiated 24 hours after initial AD threshold assessment, and the amygdala to be stimulated was chosen on the basis of how representative the afterdischarge duration and threshold were for the amygdala, based on previous research in the McIntyre laboratory (Dufresne, 1990).

Kindling and Drugs

The initial kindling procedure involved stimulating the amygdala once each day at the AD threshold and observing IISs for 2 minutes before stimulating and PISs for 2 minutes following stimulation. In this way, interictal spike (IIS) and postictal spike (PIS) development and progression was observed. A sharp transient on the EEG was identified as an IIS if it was less than 100 msec in duration (and thus appeared as a single linear spike) and was approximately 1.5 times the amplitude of the background (after Fitz & McNamara, 1979b). Those spikes observed directly following an AD were defined as PISs.

All baseline IIS and PIS data for both strains was gathered throughout the experiment to assess differences between the strains in terms of the development and progression of IISs and PISs. Once IISs were reliably established in the stimulated amygdala (usually by 17 stimulations) saline/vehicle and drug injections were initiated and

IIS and PIS rates on experimental vs. control days could be compared. Three drugs, in doses which have been previously shown to affect both seizure susceptibility and IISs were used in this study and injected i.p. The GABA_A antagonist, picrotoxin, was administered at a dose (0.5 mg/kg), which has been previously determined as optimal for influencing IIS rate, without provoking seizures (Grabowski, pilot data). The muscarinic cholinergic antagonist, atropine was administered at a dose (25 mg/kg) also previously shown to influence IIS rate in rats (Fitz & McNamara, 1979b). Finally, the noradrenergic antagonist (specific to the α -2 receptor), yohimbine was administered at a dose (10 mg/kg) previously shown to be effective for influencing seizure susceptibility (McIntyre, Kelly & Dufresne, 1991). Separate vehicle injections (saline for picrotoxin and atropine, dimethyl sulphoxide (DMSO) for yohimbine) were administered approximately 6 days prior to the drug injection and used as the control condition. Rats were randomly assigned to one of three groups, and every group consisted of 3 rats from each strain. Animals received all three drugs (and vehicle injections) in an order that was determined for each group, using counterbalancing techniques.

On experimental and control days, baseline IIS rates were recorded for 2 minutes prior to experimental manipulations. Subsequently, either drug or vehicle was administered and IISs were recorded for 2 minutes intervals during 4 time periods following the injection. The 4 time periods were as follows: directly post injection, and at 5 min., 10 min., and 20 min. following the injection. During these intervals the animals' behaviour (such as immobility, walking and grooming) was also observed and recorded. After the final 2 minute recording session (20 min. post injection) animals were stimulated at the AD threshold and the seizure profiles, including latency to clonus, duration of clonus, total duration of AD and behavioural stage of the seizure, were recorded.

Subsequent to the seizure IISs (referred to as PISs) were recorded for a duration of 5 minutes.

Histology

At the end of the experiment, under deep anesthesia, all rats were perfused intracardially with saline followed by 10% formalin. One day post perfusion, electrodes were removed and brains immersed into 10% formalin solution for at least one week before sectioning. Frozen sections, 40 μ m in thickness, were made through the brain at the depth of the electrode tract, placed onto gelled slides and stained with cresyl violet to identify electrode placements.

Data analysis

Baseline differences: Kindling rates for SLOW vs. FAST rats was analyzed using a one way ANOVA. Comparisons of IIS and PIS development and progression (such as differences in initial IIS and PIS rates) in the strains were also analyzed using a one way ANOVA.

Drug manipulation: Overall effects of the drug vs. control conditions on IIS and PIS rates were analyzed using repeated measures MANOVA. Drug by time interactions were also analyzed using repeated measures MANOVA. Multiple comparisons were analyzed using a paired t- test and α levels were adjusted depending on the number of comparisons involved in the comparison.

Seizure characteristics such as, latency to clonus, duration of clonus, and duration of AD for control vs. drug conditions were all analyzed with repeated measures analysis of variance, with exception of the atropine condition in the SLOW strain. The data for this condition was ordinal (seizure stage) and consequently, the non-parametric Wilcoxon signed-rank test was performed.

SLOW Strain

Histology:

All electrode placements for the SLOW strain (N=19) were verified as being within the amygdala-piriform region (refer to Figure 1) and hippocampus.

Kindling:

The mean kindling rate, the number of stimulations necessary in order to elicit a fully generalized seizure, for the SLOW strain was 32.0 ± 1.2 stimulations for all 19 subjects and 30.1 ± 3.2 for the subset of 9 rats used in the drug experiments. The present kindling rates are very similar to those reported by Dufresne (1990) who reported a kindling rate of 31.2 ± 4.9 for the SLOW strain.

Baseline IIS and PIS Characteristics:

Interictal spikes (IISs) appeared in this strain after a mean of 5.4 ± 0.7 stimulations. IIS rates were initially low (less than 1/min in the kindled site) during the first 10 stimulations but increased to a steady rate within 15-20 stimulations and remained steady until the end of the experiment (Figure 2). Once IISs were established the mean IIS rate per minute (counting only the 1st minute of EEG recording) for the SLOW strain was 9.5 ± 1.0 (for trials 15-50). As kindling progressed IISs increased in amplitude (data not shown) and became associated with a short cessation of activity and staring. While most animals manifested maximal IIS rates in the kindled structure, two rats, subjects SJ-14 and SJ-1, were unusual in that more IISs were observed in the contralateral amygdala.

As in the case of IISs, the PIS rates were low (less than 1 / min) during the first 10-15 stimulations but stabilized thereafter. PISs appeared more quickly than IIS, after a mean of 2.8 ± 0.3 stimulations. PIS rates were low in the first minute following

stimulation but increased gradually, peaking at 3 min post stimulation and decreasing thereafter (Figure 3). PIS rates were always highest in the kindled amygdala, regardless of which structure had the highest IIS rate.

Picrotoxin:

Behaviour: Within 5-10 minutes of the drug injection, animals became immobile, with eyes partially closed. Often by 20 min following the injection SLOW rats began to show rhythmic nose twitching, which was sometimes followed by more vigorous twitching of the face, and several rats experienced mild myoclonic jerks.

IIS: IISs were similar in form to those observed prior to drug administration, although in most rats the amplitude of the IISs observed during the picrotoxin trial was higher, suggesting the involvement of more neurons (Figure 4). After the administration of picrotoxin IISs were not limited to the stimulated structure but occurred in all recorded structures. In addition, picrotoxin was often associated with enhanced spindles in the EEG (especially in the stimulated channel).

In the SLOW strain, picrotoxin administration was not associated with an overall change in IIS rate (main effect). However, a significant drug by time interaction [$F(4,24) = 3.2$, $p < .05$, Figure 5] indicated a significant difference in IIS rates between picrotoxin and saline trials. Multiple comparisons revealed that picrotoxin was associated with markedly elevated IIS rates at 20 min. post injection compared to the saline condition, with IIS rates of 15.6 ± 2.6 for the picrotoxin trial compared to 9.4 ± 2.2 for saline [$t(2, 8) = 4.9$, $p < .01$].

Ictal event: Although latency to clonus was reduced when animals were administered picrotoxin $9.3 \pm 1.7s$ compared to $14.0 \pm 2.1s$ this difference was not statistically significant [$F(1,6) = 1.1$, $p=0.1$, Figure 6]. In addition, picrotoxin had no effect on clonus duration or after discharge (AD) duration.

PIS: Picrotoxin did not significantly alter PIS rates, as there was no significant overall drug vs. vehicle effect [$F(1,6) = 1.9, p > .05$, Figure 7].

In summary, picrotoxin was associated with a significant increase in IIS activity by 20 minutes post injection, but had little or no effect on any characteristics of the kindled AD or the rate of PISs.

Atropine:

Behaviour: Within 5-10 minutes of drug injection animals began to circle the kindling chamber in a stereotyped manner, sometimes freezing for several seconds at one corner.

IIS: Atropine administration was associated with an overall increase in the amplitude of the EEG (Figure 8). The IIS appearance was variable between subjects and, in many rats, atropine was associated with IISs that were higher in amplitude and/or broader than those seen following saline (Figure 8). As expected, atropine was associated with a significant increase in IIS rate (Figure 9). There was a significant overall difference between atropine and saline treatment with a mean IIS rate of 11.8 ± 2.1 for atropine vs. 7.9 ± 1.5 for saline [$F(1,6) = 7.3, p < .05$]. There was also a significant drug by time interaction [$F(4,24) = 4.4, p < .05$]. Multiple comparisons revealed that IIS rates of atropine treated rats were elevated at 10 and 20 min. post injection compared to saline (refer to Table 1).

Ictal event: Since too few SLOW rats experienced reliable stage 5 seizures following atropine administration, it was impossible to gather an adequate sample size to analyze differences in latency to clonus, clonus duration or AD duration. However, an analysis of seizure stage (Racine, 1972) revealed that atropine tended to decrease the severity of the seizure when compared to the control condition, although this effect did not reach statistical significance in the SLOW strain [$z(1, 13) = -1.6, p = 0.1$]. This

inhibition of motor response was observed in over half of the SLOW subjects. In the remainder of the rats atropine had no effect on seizure severity.

PIS: In addition, as with IISs, atropine significantly increased PIS rate compared to the saline condition (Figure 10). There was a significant overall effect of atropine vs. saline with a mean PIS rate for saline of 19.9 ± 2.6 and 30.1 ± 3.5 for atropine, [$F(1,6) = 15.4, p < .01$].

Yohimbine:

Behaviour: Within 5-10 minutes of injection, animals would lie flat on their stomach and, from time to time, would crawl around the kindling chamber salivating, with mouths open.

IIS: The appearance of IISs following yohimbine administration was similar in form to that following vehicle administration, but often the amplitude of the IISs was higher in the yohimbine condition (Figure 11). Although there was no overall effect of yohimbine on IIS rate, a significant difference in IIS rate between yohimbine and vehicle, at different time intervals post injection, was revealed (i.e. significant interaction) [$F(4,24) = 6.7, p < .05$, Figure 12]. Multiple comparisons revealed that, at 20 min. post injection, the rate of IIS during the yohimbine condition was significantly elevated compared to the vehicle condition. IIS rate for the yohimbine was 19.3 ± 3.7 compared to 7.9 ± 1.7 for the vehicle [$t(2,8) = 3.78, p < .01$].

Ictal event: Although the difference was not statistically significant, yohimbine tended to decrease latency to clonus (Figure 13). The mean latency to clonus following yohimbine administration was 7.7 ± 1.6 seconds compared to 13.7 ± 2.8 s following administration of vehicle [$F(1,6) = 1.5, p = 0.1$]. There were also no differences detected in duration of clonus or AD duration.

PIS: Finally, yohimbine did not increase the overall PIS rate significantly, but a significant drug by time interaction was detected [$F(4,24) = 4.8$, $p < .05$, Figure 14). However, multiple comparisons revealed no significant differences at any of the time periods sampled.

FAST Strain

Histology:

The electrode placements for all the FAST rats ($N=25$) were verified as being within the amygdala piriform region (Figure 15) and hippocampus.

Kindling:

The mean kindling rate for the FAST strain was 8.6 ± 0.9 ($N=25$), while the kindling rate for the subset of 9 animals used in the drug experiment was 9.11 ± 1.16 . These kindling rates are similar to those observed by Dufresne (1990) who reported a kindling rate of 8.8 ± 1.5 in the FAST strain.

Baseline IIS and PIS Characteristics:

Few IISs appeared during the first 10 stimulations. However, between the 10th and 15th stimulation, IIS rates increased slightly, and then remained fairly stable throughout the experiment (Figure 2). IIS appeared after a mean of 5.4 ± 0.4 stimulations. Mean IIS rate (counting only the 1st minute of EEG recording once IISs had stabilized) for the FAST strain was 1.7 ± 0.2 per minute. IISs usually began in the stimulated amygdala, but unexpectedly, within approximately 10 stimulations, higher rates were observed in the two hippocampi than in the stimulated amygdala and remained so for the rest of the experiment. There was very little change in amplitude of IISs or IIS rate as kindling progressed, however, when kindling was interrupted IISs decayed rapidly in the stimulated structure, with few IISs present after 3-4 days.

After a mean of 2.8 ± 0.4 stimulations PISs began to appear and their rate became stable within 5-10 stimulations. PIS rate was low in the first minute post AD but peaked during the second minute and decreased rapidly thereafter (Figure 16).

Picrotoxin:

Behaviour: Within 5 minutes of the picrotoxin injection, rats became immobile and began to experience rhythmic nose and facial movements. This behaviour was often followed by myoclonic jerks (usually at 10 min. post injection), which occurred in unison with the IISs.

IIS: The IISs observed following picrotoxin administration were similar in form but sometimes higher in amplitude than those following saline administration. (Figure 17). Picrotoxin significantly increased IIS rate in the FAST strain (Figure 18), since there was a significant overall main effect of picrotoxin vs. saline [$F(1,6) = 75.8, p < .0001$]. Moreover, there was also a significant drug by time interaction [$F(4,24) = 9.1, p < .05$]. Multiple comparisons revealed that, at 10 and 20 min. post injection following picrotoxin administration, IIS rate were notably increased compared to that following the administration of saline (refer to Table 2).

Ictal event: Picrotoxin facilitated kindling by significantly decreasing the latency to clonus. (Figure 19). The mean latency to clonus following picrotoxin administration was 6.7 ± 1.2 compared to a latency of 15.1 ± 3.9 following saline administration. This difference was statistically significant [$F(1,6) = 8.7, p < .05$]. Other features of the kindling profile, such as duration of clonus and AD duration, were not significantly affected by picrotoxin.

PIS: No significant differences in PIS rate were detected between picrotoxin and saline trials, although PIS rate was slightly higher at 2 and 3 minutes post AD (Figure 20).

Atropine:

Behaviour: Within 5-10 minutes of the atropine injection animals began to circle the kindling chamber, moving from corner to corner, in a stereotyped manner. In some FAST rats hoarse breathing was witnessed following atropine administration.

IIS: Atropine administration was associated with an overall increase in the amplitude of the EEG as well as an increase in amplitude and breadth of IISs (Figure 21). As expected, atropine significantly increased IIS rate (Figure 22). There was both a significant main effect of drug and a significant drug by time interaction. The mean overall rate of IIS for the saline condition was $3.5 \pm .9$ compared to 9.4 ± 1.8 for the atropine condition [$F(1,6) = 49.2, p < .0001$]. The interaction between drug and time was also highly significant [$F(4,24) = 23.7, p < .0001$]. Multiple comparisons revealed that at 5, 10 and 20 min. after the atropine injection, IIS rates were significantly elevated when compared to control values (refer to Table 3).

Ictal event: Atropine significantly increased latency to clonus (Figure 23), thus acting in a suppressive manner on the seizure by delaying recruitment of the AD into its convulsive form. The mean latency to clonus for the atropine condition was 24.9 ± 4.9 s compared to 12.6 ± 3.4 s for the saline trial [$F(1,6) = 20.6, p < .01$]. No significant differences with respect to clonus or AD duration were apparent.

PIS: In addition, atropine significantly increased PIS rate in the FAST strain (Figure 24). A significant overall effect of drug was detected [$F(1, 6) = 25.2, p < .01$] with the mean PIS rate for the atropine condition of 29.6 ± 2.9 compared to 18.6 ± 2.3 for the saline condition. A significant drug by time interaction was also detected [$F(4, 24) = 17.5, p < .001$] and multiple comparisons revealed that PIS rates for the atropine treatment were

significantly greater than PIS rates associated with the saline treatment, at 3, 4 and 5 min. post AD (refer to Table 4).

Yohimbine:

Behaviour: Within 5-10 minutes of injection animals were often seen lying on their stomachs, salivating with mouths open. In addition, they would sometimes crawl around the kindling chamber on their bellies.

IIS: IISs following yohimbine administration were of the same form, but usually higher in amplitude than those associated with the vehicle condition (Figure 25). Yohimbine significantly increased IIS rate in the FAST strain (Figure 26). The overall effect (main effect) of yohimbine vs. vehicle was significant [$F(1,6) = 28.6, p < .01$] with mean IIS rates of 5.3 ± 0.8 and 10.0 ± 1.4 , for the vehicle and yohimbine trials, respectively. A significant drug (yohimbine vs. vehicle) by time (1, 5, 10, 20 min) was also detected [$F(4,24) = 4.8, p < .05$]. Multiple comparisons revealed that 20 min. post injection, IIS rate was increased after the yohimbine, but not the vehicle injection [$t(2, 8) = 5.9, p < .001$]. The mean IIS rate was 10.0 ± 1.6 and 5.3 ± 1.4 for the yohimbine and vehicle, respectively.

Ictal event: Yohimbine significantly decreased the latency to clonus [$F(1,6) = 5.9, p < .05$, Figure 27). The mean latency to clonus for yohimbine was 7.8 ± 1.5 seconds as compared to 15.2 ± 3.7 seconds for the vehicle condition. No other significant differences in the seizure profile were apparent.

PIS: Yohimbine was not associated with an overall increase in PIS rate, but a significant drug by time interaction was revealed [$F(4,24) = 8.9, p < .01$, Figure 28). Mean PIS rates tended to be decreased at 1 and 2 min. post AD and increased at 3, 4 and 5 min. post AD. Multiple comparisons revealed that at 4 and 5 min. post AD the PIS rate for the

yohimbine condition was significantly increased compared to the vehicle condition (refer to Table 5).

Strain Comparison

Kindling:

The kindling rate was 32 ± 1.2 and 8.6 ± 0.1 , for the SLOW and FAST rat strain, respectively. Thus, as expected, the kindling rate for the SLOW strain was significantly retarded compared to the FAST strain [$F(1,43) = 77.2, p < .0001$].

Baseline IIS and PIS Frequencies:

Once IISs had been reliably established the mean IIS rate in the SLOW strain was 9.5 ± 1.0 compared to the mean for the FAST strain which was 1.7 ± 0.2 . This difference in IIS rate was highly significant [$F(1, 25) = 40.8, p < .0001$]. In the SLOW strain, the initial IIS rate was similar to that of the FAST strain. However, within 10-15 stimulations IIS rates in the SLOW strain increased dramatically and remained high for the rest of the experiment (at least 60 stimulations). In the FAST strain, the IIS rate was very low initially and within 10 stimulations increased slightly, remaining at this low rate for the rest of the experiment. As kindling progressed, the amplitude of the IISs from the stimulated structure often increased in the SLOW, but seldom in the FAST strain. In contrast to the SLOW strain, which manifested the highest rate of IISs in the stimulated structure, the FAST strain experienced more IISs in both hippocampi than in the two amygdalae. IISs were rarely observed in the hippocampi of the SLOW strain.

Postictal spike (PIS) rate was higher in the FAST strain during the first 10-15 stimulations. However, after the initial stimulations, PIS rates became similar in the two strains during the first minute post stimulation. The FAST strain experienced a peak in PIS rates in the second minute post stimulation and PIS rates subsequently declined more rapidly than in the SLOW strain. In the SLOW strain PIS rates peaked at 3 minutes post

stimulation and declined gradually, stabilizing within minutes to a rate similar to that seen 24 hrs. later (baseline IIS rate).

Figure 1. A schematic representation of electrode placements in the SLOW strain.
The shaded areas represent the regions in which electrode placements were located.

Figure 2. Baseline EEG recordings from the SLOW (a) and FAST (b) strains showing IISs, indicated by the small triangles above the trace. The asterix denotes the stimulated channel (LH = left hippocampus, RA = right amygdala, LA = left amygdala, RH = right hippocampus).

SLOW

FAST

Figure 3. Mean ($\pm$ S.E.M.) PIS rate (for trial 15- 50) in the SLOW strain at 1 - 5 min. post AD.

Figure 4. EEG recording from SLOW subject SJ-14, showing IISs and spindles at 20 min. post injection of saline (left trace) and picrotoxin (right trace). The asterix denotes the stimulated channel.

SLOW

SALINI:

PICROTOXIN

Figure 5. Mean ($\pm$ S.E.M) IIS rates per minute for 5 time intervals (-1 = baseline rate, 1 = directly post injection, 5 = 5 min. post injection, 10 = 10 min. post injection, 20 = 20 min. post injection) in the SLOW strain. Saline and picrotoxin trials are represented by open and closed circles, respectively. The double asterices denote a highly significant effect, $p < 0.01$.

Figure 6. Mean ($\pm$ S.E.M) latency to forelimb clonus (in seconds) in the SLOW strain. Vehicle and picrotoxin trials are represented by the solid and shaded regions, respectively.

Figure 7. Mean ($\pm$ S.E.M.) PIS rate (per minute) in the SLOW strain for saline and picrotoxin trials, at 1 - 5 minutes post AD. Saline and picrotoxin trials are represented by the open and closed circles, respectively.

Figure 8. EEG recording of SLOW subject SJ-6 showing IISs at 20 min. post injection of saline (left trace) and atropine (right trace). The asterix denotes the stimulated channel (LH = left hippocampus, RA = right amygdala, LA = left amygdala, RH = right hippocampus).

SLOW

SALINE

ATROPINE

Figure 9. Mean ($\pm$ S.E.M.) IIS rate (per minute) at 5 time intervals (-1 = baseline rate, 1 = directly post injection, 5 = 5 min. post injection, 10 = 10 min. post injection, 20 = 20 min. post injection). Saline and atropine trials are represented by the open and closed circles, respectively. Asterix denotes a significant effect, $p < 0.05$. The double asterices denote highly significant effect, $p < 0.01$.

Figure 10. Mean ($\pm$ S.E.M) PIS rate (per minute) in the SLOW strain for saline and atropine trials, at 1-5 minutes post AD. Saline and atropine trials are represented by the open and closed circles, respectively.

Figure 11. EEG recording for SLOW subject SJ- 21, showing IISs at 20 min. post injection of vehicle (left trace) and yohimbine (right trace). The asterix denotes the stimulated channel (LH = left hippocampus, RA = right amygdala, LA = left amygdala, RH = right hippocampus).

SLOW

VEHICLE

YOHIMBINE

LH

RA*

LA

RH

Figure 12. Mean ($\pm$ S.E.M.) IIS rate (per minute) for the SLOW strain during vehicle and yohimbine trials, at 5 time intervals (-1 = baseline rate, 1 = directly post injection, 5 = 5 min. post injection, 10 = 10 min. post injection, 20 = 20 min. post injection). Vehicle and yohimbine trials are represented by the open and closed circles, respectively. The double asterices denote highly significant effects, $p < 0.01$.

Figure 13. Mean ($\pm$ S.E.M) latency to forelimb clonus (in seconds) in the SLOW strain, during the vehicle and yohimbine trials. Vehicle and yohimbine trials are represented by the solid and shaded regions, respectively.

Figure 14. Mean ($\pm$ S.E.M) PIS rate (per minute) in the SLOW strain for vehicle and yohimbine trials, at 1-5 minutes post AD. Vehicle and yohimbine trials are represented by the open and closed circles, respectively.

Figure 15. A schematic representation of electrode placements in FAST strain.

The shaded region represents the border of electrode placements.

Figure 16. Mean ($\pm$ S.E.M.) PIS rate (for trial 15-50) in the FAST strain at 1 - 5 min. post AD.

Figure 17. EEG recording for FAST subject FJ-24 , showing IISs at 20 min. post injection of saline (left trace) and picrotoxin (right trace). The asterix denotes the stimulated channel (LH = left hippocampus, RA = right amygdala, LA = left amygdala, RH = right hippocampus).

FAST

PICROTOXIN

	SALINE	
LII		
RA		
* LA		
RII		

Figure 18. Mean ($\pm$ S.E.M.) IIS rate (per minute) in the FAST strain at 5 time intervals (-1 = baseline rate, 1 = directly post injection, 5 = 5 min. post injection, 10 = 10 min. post injection, 20 = 20 min. post injection). Saline and picrotoxin trials are represented by the open and closed circles, respectively. The double asterices denote a highly significant effect, $p < 0.01$.

Figure 19. Mean ($\pm$ S.E.M) latency to forelimb clonus (in seconds) in the FAST strain. Saline and picrotoxin trials are represented by the solid and shaded regions, respectively. The asterix denotes a significant effect, $p < 0.05$.

Figure 20. Mean ($\pm$ S.E.M) PIS rate (per minute) in the FAST strain for saline and picrotoxin trials, at 1-5 minutes post AD. Saline and picrotoxin trials are represented by the open and closed circles, respectively.

Figure 21. EEG recording for FAST subject FJ-17 , showing IISs at 20 min. post injection of saline (left trace) and atropine (right trace). The asterix denotes the stimulated channel (LH = left hippocampus, RA = right amygdala, LA = left amygdala, RH = right hippocampus).

FAST

	SALINE	ATROPINE
LII		
RA		
* LA		
RII		

Figure 22. Mean ($\pm$ S.E.M.) IIS rate (per minute) in the FAST strain at 5 time intervals (-1 = baseline rate, 1 = directly post injection, 5 = 5 min. post injection, 10 = 10 min. post injection, 20 = 20 min. post injection). Saline and atropine trials are represented by the open and closed circles, respectively. The double asterices denote a highly significant effect, $p < 0.01$.

Figure 23. Mean ($\pm$ S.E.M) latency to forelimb clonus (in seconds) in the FAST strain. Saline and atropine trials are represented by the solid and shaded regions, respectively. The double asterisks denote a highly significant effect, $p < 0.01$.

Figure 24. Mean ($\pm$ S.E.M) PIS rate (per minute) in the FAST strain for saline and atropine trials, at 1-5 minutes post AD. Saline and atropine trials are represented by the open and closed circles, respectively. The double asterices indicate a highly significant effect, $p < 0.01$.

Figure 25. EEG recording for FAST subject FJ-23 , showing IISs at 20 min. post injection of vehicle (left trace) and yohimbine (right trace). The asterix denotes the stimulated channel (LH = left hippocampus, RA = right amygdala, LA = left amygdala, RH = right hippocampus).

FAST

	VEHICLE	YOHIMBINE
LH		
RA [*]		
LA		
RH		

Figure 26. Mean ($\pm$ S.E.M.) IIS rate (per minute) in the FAST strain at 5 time intervals (-1 = baseline rate, 1 = directly post injection, 5 = 5 min. post injection, 10 = 10 min. post injection, 20 = 20 min. post injection). Vehicle and yohimbine trials are represented by the open and closed circles, respectively. The double asterices denote a highly significant effect, $p < 0.01$.

Figure 27. Mean ($\pm$ S.E.M) latency to forelimb clonus (in seconds) in the FAST strain. Vehicle and yohimbine trials are represented by the solid and shaded regions, respectively. The asterix denotes a significant effect, $p < 0.05$.

Figure 28. Mean ($\pm$ S.E.M) PIS rate (per minute) in the FAST strain for vehicle and yohimbine trials, at 1-5 minutes post AD. Vehicle and yohimbine trials are represented by the open and closed circles, respectively. The asterix denotes significant effect, $p < 0.05$.

Double asterices indicate a highly significant effect, $p < 0.01$.

Table 1. Results of multiple comparisons of IIS rate between saline and atropine conditions in the SLOW strain. The table shows the significant time periods following drug administration, the associated mean ($\pm$ S.E.M.) IIS rates for the two conditions, as well as the probability and t- statistic for the effects.

TABLE 1

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Time period post injection	Mean IIS rate $\pm$ SE		Probability	t statistic
	saline	atropine		
10	7.4 $\pm$ 1.1	14.3 $\pm$ 2.2	< 0.01	t(2,8) = 3.7
20	9.5 $\pm$ 1.8	18.3 $\pm$ 3.0	< 0.05	t(2,8) = 2.8

Table 2. Results of multiple comparisons of IIS rate between saline and picrotoxin conditions in the FAST strain. The table shows the significant time periods following drug administration, the associated mean ($\pm$ S.E.M.) IIS rates for the two conditions, as well as the probability and t- statistic for the effects.

TABLE 2

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Time period post injection	Mean IIS rate $\pm$ SE		Probability	t statistic
	saline	picROTOXin		
10	4.0 $\pm$ 0.9	6.8 $\pm$ 1.4	< 0.01	t(2,8) = 4.0
20	3.7 $\pm$ 1.0	9.8 $\pm$ 3.0	< 0.01	t(2,8) = 3.8

Table 3. Results of multiple comparisons of IIS rate between saline and atropine conditions in the FAST strain. The table shows the significant time period following drug administration, the associated mean ($\pm$ S.E.M.) IIS rates for the two conditions, as well as the probability and t- statistic for the effects.

TABLE 3

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Time period post injection	Mean IIS rate $\pm$ SE		Probability	t statistic
	saline	atropine		
5	4.0 $\pm$ 1.1	12.0 $\pm$ 2.5	< 0.01	t(2.8) = 4.0
10	4.4 $\pm$ 1.0	12.2 $\pm$ 2.1	< 0.001	t(2.8) = 5.0
20	5.1 $\pm$ 0.8	13.8 $\pm$ 2.4	< 0.001	t(2.8) = 5.8

Table 4. Results of multiple comparisons of PIS rate between saline and atropine conditions in the FAST strain. The table shows the significant time periods following the AD, the associated mean ($\pm$ S.E.M.) PIS rates for the two conditions, as well as the probability and t- statistic for the effects.

TABLE 4

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Time period post AD	Mean PIS rate $\pm$ SE		Probability	t statistic
	saline	atropine		
3	22.2 $\pm$ 2.5	35.2 $\pm$ 2.7	< 0.001	t(2,8) = 4.8
4	17.9 $\pm$ 2.2	37.0 $\pm$ 2.0	< 0.001	t(2,8) = 7.0
5	15.5 $\pm$ 2.4	36.0 $\pm$ 2.5	< 0.001	t(2,8) = 7.2

Table 5. Results of multiple comparisons of PIS rate between vehicle and yohimbine conditions in the FAST strain. The table shows the significant time periods following the AD, the associated mean ($\pm$ S.E.M.) PIS rates for the two conditions, as well as the probability and t- statistic for the effects.

TABLE 5

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Time period post AD	Mean PIS rate $\pm$ SE		Probability	t statistic
	vehicle	yohimbine		
4	12.4 $\pm$ 1.5	21.1 $\pm$ 2.3	< 0.01	t(2.8) = 3.4
5	10.8 $\pm$ 1.0	17.7 $\pm$ 2.3	< 0.05	t(2.8) = 3.0

The present study confirmed that the SLOW and FAST kindling rat strains differ remarkably in terms of their seizure generating capabilities. The seizure resistant SLOW strain required 4 times more stimulation than the FAST strain to develop fully generalized seizures. In addition, during the course of kindling, SLOW rats experienced many more partial seizures before becoming generalized than the FAST rats, whose seizures progressed quickly into a generalized form with few observable partial seizures. These observations are in agreement with Dufresne (1990) and suggest that the SLOW strain have suppressed local seizure recruitment and/or are less capable of recruiting motor systems for triggering convulsive behaviour (Dufresne, 1990).

Moreover, it was documented that, in agreement with Dufresne (1990), the kindling resistant SLOW strain maintains a much higher interictal spike (IIS) rate than the kindling prone FAST strain. Although Dufresne observed that the FAST strain could not sustain IISs, the present long term IIS monitoring revealed that after numerous stimulations, the FAST strain can maintain low, yet stable IIS rates. Additionally, new information was gathered regarding the development and patterns of progression of IISs and postictal spikes (PISs) in both strains.

In both strains IISs developed after a similar number of stimulations; however, once they were established, IIS progression differed between the SLOW and FAST rats. Initial IIS rates were low in both strains, but after approximately 10-15 stimulations IIS rates increased dramatically to 10/min in the SLOW strain, while they remained at 2/min in the FAST strain. After 10-15 stimulations, the FAST strain had already experienced fully generalized seizures, while the SLOW strain had not. This fact may help to explain why IIS rates remain low in the FAST strain, since it is possible that the establishment of a

broader seizure network might conceivably recruit forms of inhibition that interfere with the ability of this strain to generate IISs.

The pattern of IIS progression was also different in the two strains examined. The SLOW strain demonstrated the more traditional pattern of IIS progression reported in outbred rats (Fitz & McNamara, 1979a; Gotman, 1984). IISs in the SLOW strain invariably began (and soon appeared at a maximal frequency) in the stimulated amygdala, subsequently spread to the contralateral amygdala, then to the ipsilateral hippocampus, and finally to the contralateral hippocampus. Contrary to this traditional (SLOW) pattern, in FAST rats, IISs often appeared initially in the hippocampus ipsilateral to the stimulated amygdala and, only after further stimulation, became apparent in the kindled amygdala. Additionally, as kindling progressed in the FAST strain, IIS rates remained much higher in both hippocampi than in the stimulated amygdala. Thus, in the FAST strain it appears that direct stimulation of the amygdala does not produce high IIS rates in this structure. Instead, in FAST rats, high IIS rates are achieved in the hippocampus through indirect activation via amygdala propagated seizures. Racine et al. (1984) have observed that direct stimulation of the hippocampus does not generate reliable IISs in outbred rats. However, it is possible that in the FAST strain, amygdala activation was able to access networks of local circuits in the hippocampus that might otherwise not be activated through direct hippocampal stimulation. These observations suggest a considerable difference between the two strains in both the anatomy and physiology of IISs and their network.

Although certain aspects of the IIS network may be different within the strains, basic network similarities have also been demonstrated, since in both strains IISs follow the neuronal activation produced by the kindling procedure (Walters, 1970; Fitz & McNamara, 1979). In the SLOW strain, IISs appear to be a product of local neuronal

activation around the electrode tip, since they are first seen in the stimulated channel, decay (to a low rate) in the absence of stimulation and are easily reestablished following only one kindling stimulation. Similarly, in the FAST strain, IISs decay in the absence of the kindling stimulation and can be retriggered within one stimulation, suggesting that they are likewise influenced by the kindling stimulation.

In addition to differences and similarities involving IISs, it became apparent that the strains also displayed different patterns of development and progression of PISs. Perhaps as a result of their suppressed seizure network, SLOW rats displayed fewer PISs than FAST rats during the first few stimulations. However, as kindling progressed, the PISs became more robust and longer lasting in SLOW rats relative to their FAST counterparts. In the SLOW strain, PIS rate peaked at 3 minutes post stimulation, decreased very gradually, remaining high at one hour post stimulation. In the FAST strain, PIS rate was maximal at 2 minutes post stimulation but, in contrast to the SLOW, declined steeply thereafter, often decreasing to the low rate observed 24 hours after stimulation (IIS rate) within the hour. It has been hypothesized, therefore, that IISs are weaker versions of PISs and that over a course of minutes or hours, PISs transform into IISs. Thus, with the present results in mind one might propose that the FAST strain has reduced IIS rates (measured 24 hrs post stimulation) compared to the SLOW, simply because the PISs decay is accelerated in the FAST strain. In other words, the data suggest that the FAST strain lacks the capability for long term PIS maintenance of the SLOW strain, and this may lead to decreased IIS rates in the FAST rats.

Following the assessment of IIS and PIS characteristics in the two strains, the effects of three pharmacological manipulations on IISs, convulsive seizure and PISs in SLOW and FAST rats were investigated. A discussion of the effects of picrotoxin, atropine and yohimbine will follow.

Picrotoxin

The GABA_A antagonist, picrotoxin, significantly increased IIS rate in both strains. The IIS rate was significantly increased at 10 and 20 min post injection in the FAST strain and at 20 min post injection in the SLOW strain. Furthermore, consistent with reports of its convulsive properties (Kalichman & Burnham, 1980), picrotoxin also facilitated convulsive seizures. More specifically, picrotoxin aided in the recruitment of the AD to produce motor convulsions by decreasing latency to clonus, thus acting in a proconvulsant manner. The observations that picrotoxin increased IIS rate and facilitated seizures are in agreement with research employing penicillin. Penicillin, like picrotoxin, interferes with the fast acting chloride-mediated GABA_A channel (Dichter, 1987), and this hinders recurrent inhibition (Anderson, 1979). It has been suggested that this decrease in recurrent inhibition is involved, both in the induction of IISs and in seizure facilitation (Dichter, 1987). Additionally, topical application of penicillin seems to further synchronize IISs by increasing recurrent excitatory connections, which in turn recruit surrounding neurons (Dichter, 1987). Therefore, it seems likely that the behavioural effects demonstrated following picrotoxin administration may have involved both decreased inhibition and facilitated excitation.

Facilitated excitation is further suggested by the fact that picrotoxin administration was associated with IISs that were higher in amplitude than those seen during saline trials. This increase in IIS amplitude suggests that, in the presence of picrotoxin, more neurons around the electrode tip were synchronously activated. In turn, very high amplitude IISs were associated with contractions of the entire body, which often induced a jump or "myoclonic jerk", especially in the FAST strain. Those researchers studying the role of GABA within local circuits propose that picrotoxin works by disinhibiting GABAergic interneurons which, under normal circumstances, inhibit principal cells (Thomson, 1994).

If this is the case, then it is possible that in the FAST strain this decrease of local inhibition may have allowed the principal cells to synchronize periodically, and drive projection neurons to synchronize the motor system. The resulting activation of cells in the motor cortex, may have produced the myoclonic jerks previously mentioned.

Atropine

It was previously documented with amygdala and hippocampal kindled outbred rats (Fitz & McNamara, 1979; Leung, 1989) that IIS rates are increased following atropine administration. It came as no surprise, therefore, that atropine was also associated with an increase in IIS rate in the present experiment. Within 5 min of the atropine injection, FAST rats demonstrated significantly higher rates of IISs than during their saline trial, while such a difference was observed after 10 min in SLOW rats. Furthermore, atropine was also associated with IISs that were higher in amplitude and broader in form than those seen during the saline trial. This increase in amplitude suggests that atropine, like picrotoxin, activates a greater number of neurons in the vicinity of the electrode tip. On the other hand, the mechanism responsible for the broadening of the IIS is presently unknown.

In addition to increasing IIS rate, atropine appeared to act as an anticonvulsant. However, the anticonvulsant effect of atropine was manifested in a qualitatively different manner in each strain. In the FAST strain, atropine delayed recruitment of the AD into its convulsive form, as evidenced by a longer latency to the clonic or behavioural seizure, while in the SLOW strain, atropine reduced the severity of the seizure, with no clear effect on seizure recruitment. These anticonvulsant effects of atropine are consistent with the evidence that acetylcholine (ACh) and its agonists facilitate kindling (Pelletier & Corcoran, 1993), probably via a mechanism that suppresses GABA (Hasselmo, 1995).

However, the function of Ach is by no means straight-forward, since Ach appears to have very diverse and sometimes opposing influences within the brain. It has been documented that Ach agonists increase the activity of GABA interneurons, while at the same time decreasing the synaptic transmission from these neurons (Hasselmo, 1995). Researchers have demonstrated in whole cell clamp recordings that carbachol, an Ach agonist, simultaneously suppresses spontaneous GABA_A inhibitory synaptic potentials and increases the number of miniature synaptic potentials from the same inhibitory neurons (Hasselmo, 1995). The net result is facilitated excitation. Thus, if an Ach agonist has these effects on inhibitory neurons, then perhaps an Ach antagonist such as atropine might have a net inhibitory effect, producing an increase in synaptic transmission from the GABA interneurons. This observation may help explain why atropine was associated with inhibitory effects, such as decreased seizure severity and increased latency, in the present study.

Finally, with this in mind, our data indicate the presence of a negative relationship between Ach and PIS maintenance. This is presumed since atropine, in addition to dramatically increasing PIS rates in the FAST strain, was also associated with a drastic change in the typical pattern of PIS decay during the 5 minute post seizure interval, since it prevented the usual PIS decay. Although the length of the post seizure test period was too short to make a complete assessment, it appeared that the effect of atropine on PIS rate was a relatively long-lasting phenomenon. This long term increase in PIS rates in the FAST rats might imply that, by antagonizing Ach, it was possible to prevent PIS decay in this strain and, consequently, influence long term maintenance of PISs. Perhaps this long term maintenance of PISs may also help to sustain higher IIS rates in this strain, particularly if the mechanisms of PISs and IISs are similar.

Yohimbine

The final pharmacological manipulation, involving the noradrenergic antagonist (specific to the α -2 receptor) yohimbine, also led to an increase in IIS rate. In both strains, IIS rates increased within 20 minutes of the yohimbine injection. In addition, yohimbine also increased the amplitude of IISs in many rats, once again suggesting that a larger number of neurons around the electrode tip were being synchronously activated.

Although a statistical evaluation was not performed, yohimbine did not appear to produce any differential effects on IIS rates in the two strains. This observation is interesting since Dufresne (1990) reported that basal norepinephrine (NE) and NE metabolite concentrations differed between the SLOW and FAST strains. As a result of these biochemical differences, it was hypothesized that NE might be responsible for mediating certain differences between the two strains, including those involving IIS rate (Dufresne, 1990). However, as mentioned above, the NE antagonist yohimbine, had no differential effects on IIS rate in the present investigation.

On the other hand, yohimbine had a less surprising outcome with regard to convulsive seizure. Many previous studies have indicated that NE, utilizing the post synaptic, α -2 noradrenergic receptors, has a suppressive effect on amygdala kindling (McIntyre et al., 1991; Pelletier & Corcoran, 1993), probably through the inhibition of excitatory transmission (Hasselmo, 1995). Consistent with this research, yohimbine had a proconvulsant effect on the triggered seizures. This proconvulsant effect was evidenced by shortened latencies to forelimb clonus, suggesting a facilitated recruitment of the AD into its convulsive form.

Finally, the effect of yohimbine on PIS rate was biphasic in nature. In the presence of yohimbine, the PIS rate was decreased for the first 2 min after the seizure, but increased for the remaining 3 min of testing. This biphasic effect was not observed with either

picrotoxin or atropine and, consequently, may be due to the large amounts of NE released by the convulsive seizure (Kokaia et al., 1989). This large phasic release of NE might be sufficient to override the antagonizing effects of yohimbine for a short period of time, as witnessed for the initial 2 min post seizure, but as this effect subsides, the behavioural effects of yohimbine are reestablished.

General Discussion

The present study did not directly address the mechanisms involved in the generation and maintenance of IISs and PISs, nonetheless the results may shed some light on the controversy between those who believe that IISs lead to enhanced neuronal excitation and others who propose that they enhance inhibition. The notion that IISs are indicative of hyperexcitability has been the traditional approach, since IISs have always been seen as the "hallmark" of epilepsy. Several lines of evidence involving clinical research, indicating increased glucose utilization (Handforth et al., 1994) and increased seizure susceptibility associated with high IIS rates (Lange et al., 1983; Blume, 1993), have led to the hypothesis that IISs reflect augmented excitation. However, this view seems at odds with the present data which demonstrates that the kindling resistant SLOW strain manifests much higher IIS rates than the kindling prone FAST strain.

Several lines of evidence, consistent with the kindling data for the SLOW strain, suggest that IISs are not representative of excitatory events (Engel, 1995b). The observation that humans rarely experience seizures during periods of intense IISs (Engel, 1995 a,b; Gotman, 1991) is consistent with an inhibition hypothesis for the IISs. Furthermore, other clinical data and animal evidence reveals that IISs (Gotman & Marciani, 1985) and absence seizures, which consist of spike-wave transients similar in

form to IISs, can be provoked by GABA agonists and anti-epileptic drugs (Engel, 1995a). The increase in IIS rate in the presence of drugs that decrease excitability suggests that, unlike the ictal seizures which these drugs are meant to subdue, IISs are controlled by counter mechanisms. This evidence has led to the hypothesis (Engel, 1995b) that IISs are reflective of a hyperexcitability or hypersynchronization of inhibitory neurons.

However, the fact that, in both strains, IISs were provoked by the GABA antagonist, picrotoxin and that these IISs were associated with one decidedly excitatory event, namely the myoclonic jerk, is inconsistent with the view that IISs represent purely inhibitory events. Rather, this evidence suggests that IISs in fact represent excitatory events, which in the case of the SLOW strain, may shunt excitation, in normal circumstances, resulting in enhanced inhibition of the ictal seizure generating mechanisms. On the other hand, the FAST strain have fewer IISs and have a facilitated seizure network. Therefore, it is feasible that at least one factor that results in the inhibited seizure genesis of SLOW animals is the constant shunting of excitation from the epileptic focus or kindled site. When pharmacologically challenged by a convulsive agent such as picrotoxin, this shunting mechanism may intensify, evidenced by the increased IIS frequency. However, eventually the shunting mechanism becomes insufficient to subdue the convulsant effects of the drug and prevent seizure expression.

Finally, it is also possible that the controversy in the literature regarding the underlying mechanisms of IISs is a result of the fact that more than one type of IIS exists. Consistent with this view, Wadman et al. (1983) report that progressive hippocampal kindling is associated with a transition from one type of IIS, most likely associated with hyperpolarization, to another associated with depolarization. The evidence that the IISs associated with atropine, which inhibited ictal seizures, were different in appearance from those produced by the drugs that facilitated seizures may indicate that the two

morphologically different IISs were regulated by two separate, perhaps opposing mechanisms.

In conclusion, it appears that the physiological mechanisms influencing IISs and PISs are more complex than originally believed. The SLOW and FAST strain represent one of the few models in which animals with genetically determined IIS propensities can be compared. Thus, we propose that these two strains provide a model of IISs and PISs that may be useful in elucidating the complex underlying mechanisms controlling these two events.

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