

THE SELECTIVE BREEDING OF SEIZURE-PRONE VS. SEIZURE-RESISTANT RATS BASED  
ON AMYGDALOID KINDLING: BEHAVIORAL, ELECTROPHYSIOLOGICAL AND  
PHARMACOLOGICAL MEASURES

By

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KINDLING-PRONE vs KINDLING-RESISTANT RAT STRAINS

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### ABSTRACT

Repeated electrical stimulations of the brain via a chronically implanted electrode induce gradually stronger epileptiform responses until the animals show fully developed bilateral, clonic convulsions. This experimental epilepsy model is called kindling. Most of the experiments done on this phenomenon have compared kindled to non-kindled animals. Consequently, the changes observed are often secondary, rather than primary, to the developing epileptogenesis. /

As an alternative approach, two strains of rats were outbred from a common parent generation on the basis of amygdala kindling rates (the number of evoked epileptiform discharges (ADs) required to reach the fully developed seizure stage). After 5 generations, we succeeded in producing two groups of rats that demonstrated no overlap in amygdala kindling rates. Kindling-prone or FAST rats required 11.0 ADs, whereas kindling-resistant or SLOW rats required 42.1 ADs to develop fully generalized seizures.

We have used electrophysiological and pharmacological tests in an attempt to determine the mechanisms underlying the differences in epileptogenesis in these two strains.

The electrophysiological experiments were designed to test the hypothesis that FAST rats have either a greater plasticity in excitatory neural systems, or a faster rate of alteration in those systems. Both short-term and long-term potentiation effects were examined in 2 different forebrain pathways. The results did not support the

hypothesis. Rather, the results indicated that FAST rats may have lower levels of inhibition, as reflected in measures of paired-pulse depression.

Pharmacological experiments were performed in order to challenge specific inhibitory neurotransmitter systems. The results indicated that FAST rats had significantly lower thresholds for convulsants that are known to interfere with GABA-related inhibitory mechanisms. A preliminary study of GABA receptors, however, showed that [<sup>3</sup>H]-Muscimol binding did not differ between the strains.

It was argued that FAST rats may be deficient, relative to SLOW rats, in levels of inhibition mediated by other than GABA neurotransmitter systems, or that they may differ in membrane mechanisms that mediate late after hyperpolarizations.

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## CHAPTER I GENERAL INTRODUCTION

### A. HUMAN EPILEPSY

Epilepsy is among the most common of chronic disorders of the central nervous system (CNS), and it is estimated that at least 0.5-2% of the world's population suffers from epilepsy (Prince, 1978).

The extent and intensity of behavioral disturbance varies widely depending on the anatomical and physiological substrate of the seizure. There are two major groups of seizures: one is the 'primary generalized seizure' which involves the sudden onset of generalized attack with loss of consciousness, and the other is the 'partial attack', which may or may not evolve into generalized seizure (which would then be called 'secondary generalized seizure').

Grand mal is the most dramatic of the primary generalized seizure disorders. The sequence of motor events usually proceeds from a generalized muscular tonus, particularly in extensor systems, to clonic jerks, the entire process lasting about 2 minutes. Autonomic reactions include sweating, salivation, increased heart rate, respiratory difficulties, and emptying of the bladder.

The subject is likely to remain in a deep stupor for several minutes after the termination of a convulsion. Upon recovery from the stupor he may be confused and disoriented. Typically, the patient will sleep for several hours, then wake up with severe headache and aching muscles.

One of the mild forms of generalized seizure is called petit mal and is characterized by widespread, bilaterally synchronous three-per-

second spike and wave activity in the electroencephalogram (EEG). The attack often lasts no longer than 5 to 10 seconds, and the accompanying motor movements often involve only eyes and face. Other types of seizures, such as myoclonic seizures (arm jerks and trunk flexion) and atstatic seizures (loss of muscle tonus or postural control), may also occur with sudden onset and loss of consciousness, although these attacks are so brief that it is often impossible to determine the extent of any disturbance of consciousness. The sudden bilateral onset of primary generalized seizures suggests to some researchers that central brain regions are critically involved (Penfield and Jasper, 1954).

Partial seizures affect motor and sensory functions localized to specific parts of the body. Such seizures may trigger uncontrollable motor movements in an orderly sequence (the Jacksonian march). Stereotyped deviations of posture, vocalization, or eye movements may also be triggered. The seizure may affect somatic sensory processing related to localized parts of the body, eliciting sensations of numbness, tingling, pins and needles, heat, water running over the skin, or a sense of movement. The seizures can also disturb visual, auditory and vestibular systems, and may elicit hallucinations. Other types of partial seizures are accompanied by olfactory hallucinations, gustatory sensations, visceral sensations or emotional symptoms such as happiness, fear, anxiety or rage reactions. These partial seizures may or may not develop into secondary generalized seizures.

Among seizures with a focal onset are those that originate in the temporal lobe. Temporal lobe epilepsy (TLE) is often characterized by the psychomotor attack, during which the patient appears to respond

in a limited fashion to his environment, although he later remembers nothing of what transpired during the attack. The patient behaves as if conscious but confused (automatism), and experiences a variety of emotions, illusions of increased reality, hallucinations, vivid-recall of the past and visceral sensations. At the onset of the attack, lip smacking, chewing, facial grimacing and swallowing movements are frequent. Such psychomotor automatisms rarely last more than 4 to 5 minutes, but the patient may experience prolonged periods of post-ictal confusion.

Even when controlled by anticonvulsant drugs, seizures are known to recur over many years. The mechanisms sustaining the properties of the neuronal substrate of chronic epilepsy may be quite different than those underlying acute seizures induced by fever or drug intoxication. Although the majority of seizures are spontaneous and recurrent in nature, some types of seizures can be evoked by specific stimuli. Such stimuli can be a flashing light (Jeavons & Harding, 1975), certain visual patterns (Wilkins, Darby & Binnie, 1979), auditory patterns (Servit, 1963), or even the emission of motor patterns such as speech (Lee, Sutherling, Persing & Butler, 1980) or eating (Robertson & Fariello, 1979).

Electrographic indices of seizure activity can be detected by EEG recordings from the skull surface, even when the clinical signs are ambiguous or undetectable. Primary generalized seizures are often characterized by an abrupt onset of spike-wave discharges, bilaterally synchronous from the onset, and recordable from all surface electrodes. The termination of such paroxysmal discharges is also abrupt. Partial

seizures, in contrast, have a relatively distinctive focus. The paroxysmal discharge appears to be generated within this focus and then to propagate to other regions. The quality of the seizure is determined by its specific anatomical substrate.

The temporal lobe, with its subcortical nuclei, appears to be a relatively common site for the development of epileptic foci. The paroxysmal discharges during temporal lobe epilepsy (TLE) are usually detected around the anterior temporal lobe by skull surface EEG recordings. The hippocampal formation and amygdala (Fig. 1) appear to be the two most critically and frequently involved structures in cases of TLE (Falconer, Serafetinides & Corsellis, 1964). Amygdaloid responses (evoked potentials, EPs) evoked by test-pulses applied to the ipsilateral hippocampal formation are well developed in patients with TLE but not in patients with an epileptic focus outside the temporal lobe (Buser, Bancaud & Talairach, 1973). This observation suggests that the excessive paroxysmal activity may result in a strengthening of or creation of functional connections within the limbic system.

Although causes for the formation of epileptic foci are often apparent, such as scar tissue formed by trauma or degenerative tissue produced by organic disease, many patients develop epileptic foci in the absence of detectable physical abnormalities (Ajmone-Marsan & Goldhammer, 1973). Some epileptic foci may contain neurons that are otherwise normal but excessively excitable due to functional abnormalities of the synapse or cell membrane.

Extracellular recordings of single neurons in human epileptic cortical foci demonstrated high frequency burst responses (200-500



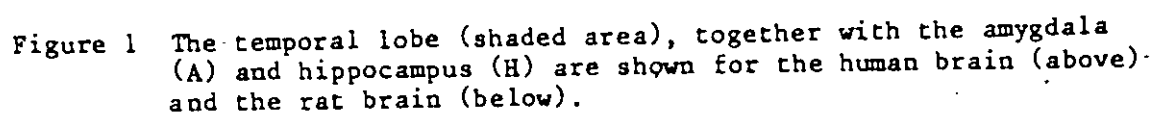
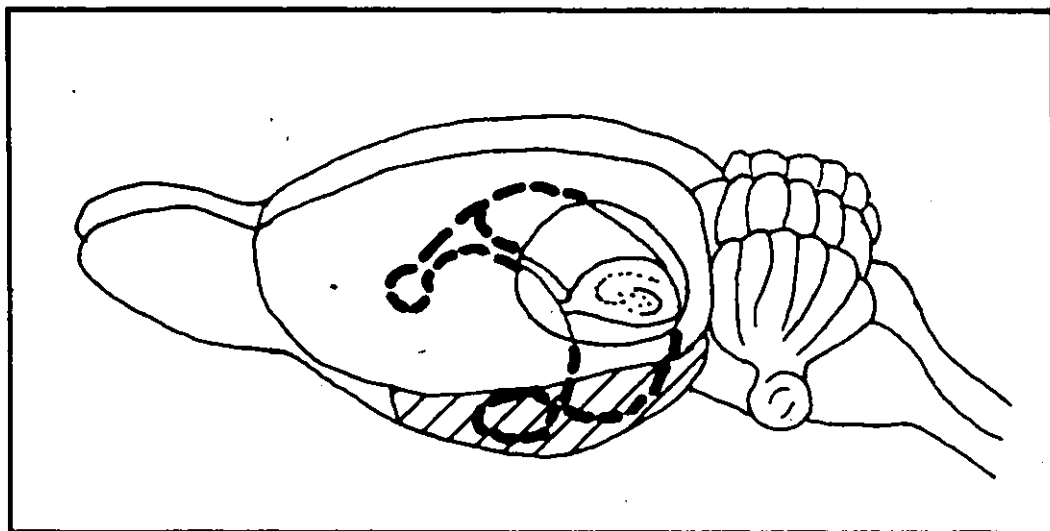
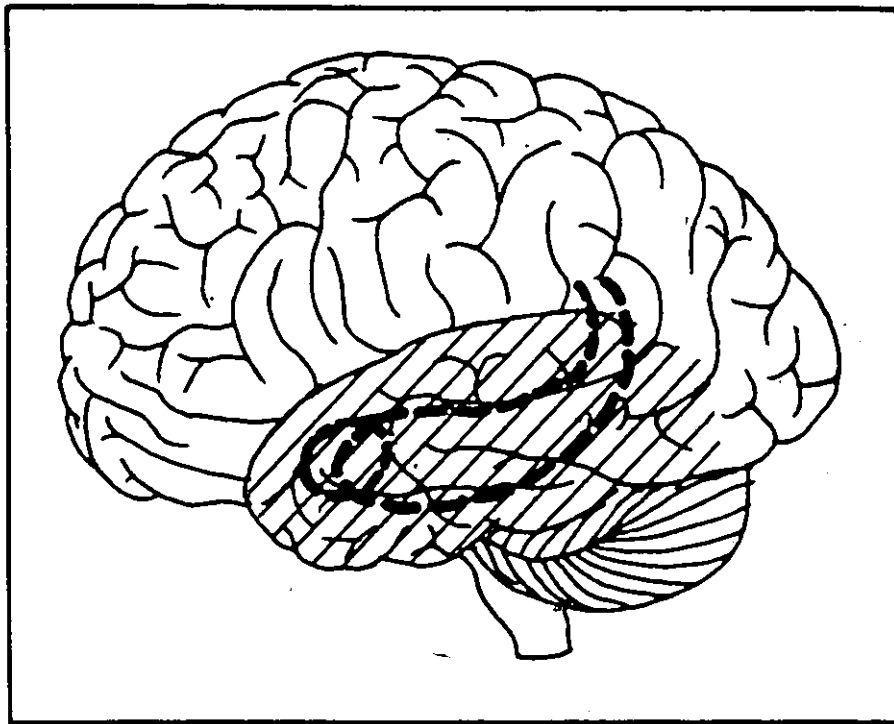


Figure 1 The temporal lobe (shaded area), together with the amygdala (A) and hippocampus (H) are shown for the human brain (above) and the rat brain (below).



action potentials per sec) during the inter-ictal period. These bursts often showed characteristic stereotyped intraburst patterns (Calvin, Ojemann & Ward, 1973) with variable interburst frequencies. Such observations led Prince (1976) to speculate that the burst responses of neurons in epileptic foci are the reflection of functionally abnormal neurons synchronously bursting in stereotypical fashion, rather than normal neurons being driven to burst by an abnormally synchronous synaptic input. Another characteristic of the epileptic focus is the progressive increase in strength of the pathological response over time. This increase could reflect a type of plasticity, or simply a progressive increase in degeneration - as in Parkinson's disease. A clinical observation (Perrin & Hoffman, 1979) indicates that the symptoms are more likely to persist, after surgical excision of the epileptic focus, in patients with a longer history of paroxysmal discharge. The continuous excessive bombardment by discharges from the epileptic focus may induce functional alterations in adjacent normal neurons which then become epileptic.

The standard treatment for epilepsy is the use of anticonvulsant drugs. Most of these are found to enhance the activity of putative inhibitory neurotransmitter systems. Intravenous or oral administration of such drugs appears to suppress the paroxysmal activity within the focus and/or limit the propagation of discharges. This has suggested to some that the underlying mechanisms of epilepsy may involve a deficit in inhibitory neurotransmitter systems (e.g. Meldrum, 1975).

As a summary, human epilepsy with focal onset presents several important features to be investigated experimentally. First, the

bursting neurons within the epileptic focus may be abnormal in their response properties, or they may receive abnormally synchronous synaptic input, resulting in stereotypical synchronous bursts. Second, the background inhibition within the focus or along the propagation route may be depressed and so fail to suppress the paroxysmal discharges. Third, the repeated activation of propagation pathways appears to potentiate the synaptic transmission in target sites. And finally, there may be a central brain region which, when activated, is responsible for synchronizing the discharges bilaterally.

#### B. EXPERIMENTAL MODELS OF EPILEPSY

In order to facilitate the investigation of mechanisms of human epilepsy, a number of experimental animal models of epilepsy have been developed. Seizures or seizure sensitivity are manipulated in these models by thermal or electrical stimulation, chemical stimulation or genetically.

Physical stimulation such as local freezing of the brain surface is known to produce regular low-frequency discharges within a few hours of application (Goldensohn & Purpura, 1963). The intracellular recordings from such epileptic foci revealed that EEG spikes and sharp waves are temporally related to prolonged membrane depolarization, accompanied by high frequency cell firing, and subsequent after-hyperpolarization.

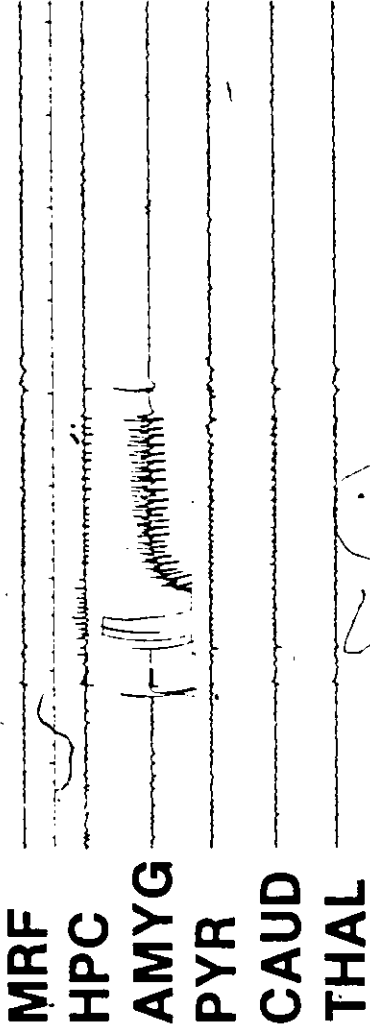
One can also induce seizures by applying an electroconvulsive shock (ECS) through earclip electrodes (Nutt, 1981). ECS seizures vary in severity from those "characterized by facial clonus and rhythmic

movements of vibrissae, jaws and ears", to the maximal seizure pattern consisting of tonic extension (Vernadakis & Woodbury, 1969).

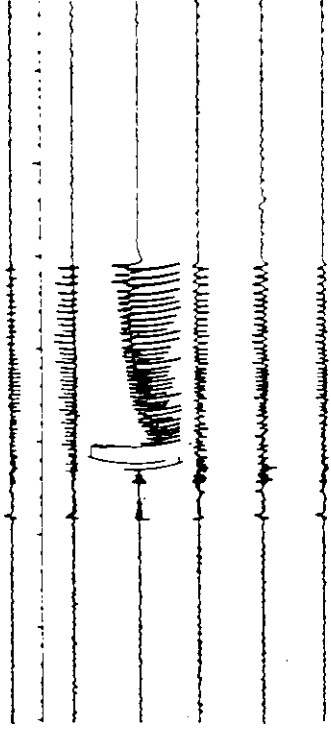
Kindling is another model which employs electrical stimulations. Unlike ECS, the kindling stimulation is applied to certain brain structures via chronically implanted electrodes. Initially it does not induce convulsions (except from the motor cortex) although it may trigger brief focal epileptiform afterdischarges (ADs - Figure 2) which outlast the offset of the kindling stimulation. With the spaced repetition of stimulation, however, the ADs become prolonged and complex. Behavioral seizures begin to occur during ADs and become increasingly strong in response to the same kindling stimulation. Amygdaloid kindling in the rat, for example, develops through the following five stages (Racine, 1972): 1. facial clonus, 2. head nodding, 3. forelimb clonus, 4. rearing, and 5. loss of postural control. Once the animals reach stage 5, the absence of stimulation for as long as 3 months will not reverse the seizure susceptibility to the kindling stimulation (Racine, 1969). The kindling effect was reported to last for at least 12 months in cats (Wada, Sato & Corcoran, 1974). If kindling stimulations are continued beyond stage 5, the animals eventually develop spontaneous motor seizures (although several hundred stimulations are required). These spontaneous motor seizures may then recur for as long as 7 months after the discontinuation of kindling stimulation (Pinel & Rovner, 1978). Therefore, kindling has elements of both chronic and acute epilepsy. Kindling as an epilepsy model will be discussed more thoroughly in a subsequent section.

Figure 2 An example of epileptiform afterdischarges (ADs) evoked by amygdala (AMYG) stimulation. The 1st, 3rd and 8th ADs are shown. Other sites are pyriform cortex (PYR), hippocampus (HPC), caudate nucleus (CAUD), thalamus (THAL) and mesencephalic reticular formation (MRF).

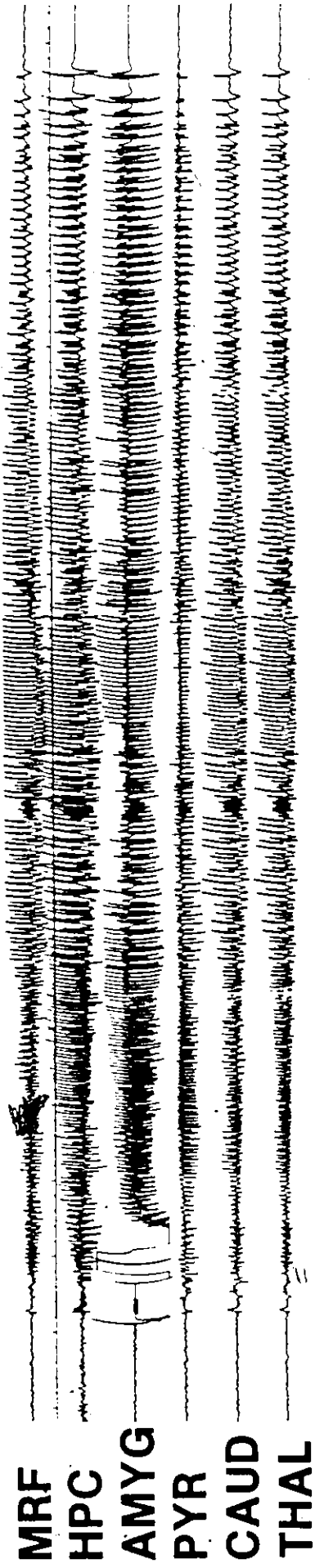
**AD No. 1**



**AD No. 3**



**AD No. 8**



Certain chemical agents are epileptogenic when applied systemically (e.g. pentylenetetrazol, picrotoxin, etc.) or topically (e.g. penicillin, alumina). Other chemicals that produce acute epileptic foci when applied topically are strychnine (Chang & Kaada, 1950; Cobb, Cowan, Powell & Wright, 1955), picrotoxin (Usunoff, Atsev & Tchavdarov, 1969), acetylcholine (Ferguson & Jasper, 1971), tungstic acid (Meshner, Wyler & Neafsey, 1978), ouabaine (Bignami & Palladini, 1966; Lewin, 1971), isoniazid (Sakai, Yoshikawa, Kinoshita, Oshika & Nakai, 1964), thiosemicarbazide (Baker & Wratkey, 1967) and conjugated estrogens (Marcus, Watson & Goldman, 1966). Penicillin is known to induce synchronous epileptiform discharges from neurons underlying the region of topical application, typically the cortical surface (Prince, 1978). The penicillin focus remains epileptic for 2 to 6 hours after one application (Schwartzkroin & Wyler, 1980). Swelling of dendrites and pyknosis of neurons were found in the cortical penicillin focus (Okada, Ayala & Sung, 1971), while no significant morphological changes have yet been found in hippocampal foci (Schwartz, Broggi & Pappas, 1970). Intracellular recordings from neurons in chemically induced foci showed high amplitude prolonged membrane depolarizations, commonly referred to as depolarization shifts (DSs), which gave rise to bursts of action potentials (Matsumoto, Ayala & Gumnit, 1969; Wong & Prince, 1979). In one study, over 90% of the sampled neurons exhibited alterations in membrane potentials and spike generation during surface epileptiform events (Matsumoto, & Ajmone-Marsan, 1964).

Intracortical injection of aluminum hydroxide (alumina) to the sensorimotor cortex in monkeys produces slow waves and spikes by 3-5



weeks. Subarachnoid injection triggers similar activity but requires 2 months (Harris, 1975). Partial motor seizures in the upper extremities begin in 6-8 weeks (intracortical injection) or 4-6 months (subarachnoid injection) and persist thereafter. The morphological abnormalities of neurons at the intracortical injection site parallel those found in some human epileptic foci. These abnormalities include granuloma, a mild to moderate depopulation of neurons, and deformations in dendritic structure in and around the epileptic foci. The amplitudes of surface spikes as well as cellular  $\text{pSs}$  are considerably smaller in chronic alumina foci compared to acute penicillin foci (Prince & Futamachi, 1970). Only about half of the sampled neurons in penicillin foci exhibit alterations in firing pattern during surface epileptiform waves. Pyramidal tract neurons in alumina foci show structured bursts with a stereotypical long-first-interval intraburst pattern (Calvin, Sybert & Ward, 1968). This burst structure has not been found in penicillin foci (Mesher & Wyler, 1976), but has been reported from human epileptic cortex (Calvin, Ojemann & Ward, 1973).

Other agents, such as cobalt (Payan, 1967; Dow, McQueen & Townsend, 1972) and methionine sulfoximide (Hrebicek & Kolousek, 1968), are known to produce chronic epileptic foci when applied topically. Pure metals, antimony and nickel, produced severe necrotizing lesions following intracortical implantation, and occasional spontaneous clinical seizures were seen for 1 to 6 months (Chusid & Kopeloff, 1962). Other metals such as bismuth, cadmium, iron, mercury, molybdenum, tantalum, titanium tin, tungsten, vanadium and zirconium induced

subclinical (not behavioral) EEG epileptic activities (Chusid & Kopeloff, 1962).

Another group of experimental models of epilepsy employs systemic application of convulsant chemical agents, and generally provide acute rather than chronic preparations. Pentylenetetrazol (PTZ, or Metrazol) is the most frequently used convulsant drug, inducing seizures which consist of the following three phases: myoclonus manifested as a whole-body twitch, clonic spasms involving forelimbs and jaw, and clonic-tonic hindlimb extension which is usually lethal (Orloff, Williams & Pfeiffer, 1949). PTZ is often used to assess the strength of anticonvulsants (Desmedt, Niemegeers, Lewi & Janssen, 1976), but the exact mechanisms underlying PTZ seizures are yet to be determined.

Most of the remaining convulsants manifest their epileptogenic effects by suppressing specific inhibitory neurotransmitter systems, or interfering with the transport of cations across neuronal membranes. For example, strychnine suppresses hyperpolarization and depression of spinal neurons induced by glycine (a putative inhibitory neurotransmitter in spinal cord), without affecting that induced by another inhibitory neurotransmitter,  $\gamma$ -amino butyric acid (GABA) (Curtis, Duggan & Johnston, 1971). The convulsions induced by systemic administration of strychnine start immediately and consist of tonic extension and extensor jerks that can be triggered by any sensory stimuli (Esplin & Zablocka-Esplin, 1969). All voluntary muscles engage in full contraction, and respiration ceases leading to frequent death by brain hypoxia (Gilman, Goodman & Gilman, 1980).

The convulsants which interfere with the GABA system are also well studied, and the mechanisms underlying their convulsant effects are to some extent documented. Bicuculline, for example, has been shown to block the postsynaptic inhibitory action of GABA (Simmonds, 1980), as well as GABA transport (uptake) systems (DeFeudis, Maitre, Ossola, Elkouby & Mandel, 1979). The systemic administration of bicuculline induces body jerks within two minutes, and at high doses the jerks evolve directly into a full tonic seizure followed by a clonic phase (personal observation). Picrotoxin, another drug that affects GABA systems, interferes with GABA-induced inhibition presynaptically (Eccles, Schmidt & Willis, 1963) and postsynaptically (Bruggencate & Engberg, 1971). At convulsant dose levels, body jerks appear within 10 minutes after systemic administration. The jerks gradually intensify over the next few minutes, and the generalized seizure suddenly occurs with tonic extension of the limbs followed by forelimb clonus and head nodding (personal observation). Finally, isonicotinic acid hydrazide (INH or isoniazid) is also a GABA inhibitor. It interferes with the activity of glutamic acid decarboxylase (GAD), the rate-limiting enzyme for GABA synthesis (Horton, Chapman & Meldrum, 1979). The systemic administration of INH induces seizures within an hour. These consist of rearing and forelimb clonus, followed by loss of postural control with continuing limb clonus (personal observation).

Ouabain is one of a group of convulsant drugs which interferes with cation transport. At high concentrations, ouabain is capable of total inhibition of the active transport of sodium and potassium (Donaldson, St. Pierre, Minnich & Barbeau, 1971). The intraventricular

injection of ouabain in rats induces seizures that consist of running fits and leaping or jumping (Izumi, Donaldson, Minnich & Barbeau, 1973).

Rather than inducing acute or chronic epileptic animals by exogenous agents, the genetic models of epilepsy provide inbred strains that are genetically susceptible to epileptogenic stimuli as well as stimuli that would not normally be epileptogenic, such as loud noise, flashing light and postural stimulation.

For example, 3 to 4 week old DBA/2J mice reliably exhibit severe, often fatal, seizures in response to an auditory stimulus of adequate intensity (Collins, 1972). The audiogenic seizure pattern of mice (Horton, Anlezark & Meldrum, 1980) and rats (Consroe, Picchioni & Chin, 1979) consists of a burst of wild running, which often develops into clonic spasms with loss of postural control. Another inbred strain of mice, El mice, has a convulsive disposition to postural stimulation (Suzuki, 1976). This strain of mice shows a violent tonic-clonic generalized convulsion when "thrown-up" 10 cm in the air several times at about 7 weeks of age. Cortical EEG recording during ictal and interictal periods showed bilateral and sporadic spikes (Suzuki, 1976).

Another well known genetic model of epilepsy is provided by the photosensitive Senegal baboon, Papio papio (Naquet & Meldrum, 1972). When these baboons are exposed to intermittent light stimulation (ILS), more than 60% of the total sample ( $N > 800$ ) show clonus of the facial and neck musculature. Some of them show self-sustained clonus continuing after the end of ILS, and about 10% of the total sample respond with generalized seizures (Naquet & Meldrum, 1972). The

excessive photosensitivity of Papio papio has been found to be characteristic of the baboon group in Casamance (the Southern part of Senegal), and not of a species or a genus. EEG recordings show the spread of the paroxysmal discharges from the fronto-rolandic region to deep structures including the thalamus, the reticular formation and certain hypothalamic structures (Fisher-Williams, Poncet, Riche & Naquet, 1968).

### C. KINDLING AS A MODEL OF EPILEPSY

#### 1. Phenomenon

Boast and McIntyre (1977) demonstrated that rats kindled bilaterally in the amygdalae subsequently had difficulty in learning a passive avoidance task. Pinel, Treit and Rovner (1977) examined the behavior of rats that had received many kindling stimulations in the amygdala or hippocampus (although they had not reached the spontaneous seizure stage), and they found that these rats reacted to tail tap and handling with increased aggression, compared to rats that were kindled in the caudate nucleus.

Some of these characteristics of limbic kindling in rats are similar to those seen in human patients with temporal lobe epilepsy (TLE). The initial facial seizure, the memory dysfunction, and the emotional responses are also seen in patients with TLE.

It has been reported that neurons within the kindled focus exhibit bursts of action potentials with a higher intraburst frequency and longer burst duration, compared to neurons of non-kindled brain (Racine & Zaide, 1978). Kindling has been demonstrated in the frog (Morrell & Tsuru, 1976), reptile (Rial & González, 1978), mouse (Leech &

McIntyre, 1976), rat (Goddard, McIntyre & Leech, 1969; Racine, 1972b), rabbit (Tanaka, 1972), cat (Wada & Sato, 1974), monkey (Wada, Mizoguchi & Osawa, 1978) and baboon (Wada & Osawa, 1976). Šramka, Sedlák & Nádvořník, (1977) described what appears to be a kindling effect in a human patient who received daily stimulation of the thalamus for 2 weeks (in order to relieve phantom pain). On the other hand, Blackwood, Cull, Freeman, Evans and Mawdsley (1980) claimed that repeated seizures produced during electroconvulsive therapy did not result in a kindling effect. The speculation among kindling researchers is that humans are likely to be susceptible to kindling if such a diversity of other species can be reliably kindled (Adamec, Stark-Adamec, Perrin & Livingston, 1981).

AD thresholds vary depending on structure and species. Low AD thresholds are typically found in hippocampus, septum, amygdala, and pyriform cortex, whereas neocortical and brainstem sites require higher intensity to evoke ADs. Many brainstem sites, in fact, do not appear to support AD activity. For amygdaloid kindling, AD thresholds are lowest in the rat, followed by cats and then primates (Racine, 1978).

The number of stimulations necessary to develop kindled seizures also varies depending on the structures and species. The amygdala, pyriform cortex and olfactory bulbs kindle most rapidly (require the smallest number of stimulations), followed by the entorhinal cortex, septal area, ventral hippocampus and dorsal hippocampus. Neocortical areas tend to kindle rather slowly. There seems to be some tendency for phylogenetically more advanced species to kindle slowly (Tsuru, 1981) although they may develop spontaneous foci more readily (Racine, 1978).

The intervals for kindling stimulation can be as short as 1 hour (Racine, Burnham, Gartner & Levitan, 1973), and as long as 7 days (Goddard, McIntyre & Leech, 1969).

## 2. Mechanisms

### a. Manifestation of bilateral seizures - Centrencephalic system:

Penfield introduced the term "centrencephalic system" in 1950, referring to "the neuron systems which are symmetrically connected with both cerebral hemispheres and which serve to coordinate their function" (Penfield & Jasper, 1954, p. 27). Although this hypothetical system has not been given precise anatomical identification, "it should occupy a central position between the two hemispheres" (Penfield & Jasper, 1954, p. 43), and so it is presumably located in the brain stem. Furthermore, the invariable lapse of consciousness at the onset of a primary generalized seizure seems to indicate the involvement of the ascending reticular formation, which is known to control arousal level (Lindsley, Schreiner, Knowles, and Magoun, 1950). Therefore, the term "centrencephalic system" is usually taken to include the reticular formation of the mesencephalon and upper pons, together with the nonspecific thalamus (Penfield, 1969). Since many of the structures that show a strong kindling effect are not directly involved in the control of motor functions, it is presumed that motor systems in the brainstem must be activated before a convulsion can be triggered. Whatever the relation between this brainstem system and the "centrencephalic" system proposed by Penfield, several attempts have been made to identify it as well as other structures significantly involved in the expression of behavioral seizures.

Using amygdaloid kindling in the cat, Wada and Sato (1974) noted that "a very active and entirely independent pattern of AD" emerges in the ipsilateral and then contralateral mesencephalic reticular formation (MRF) coincident with the appearance of head-nodding (comparable to stage 2 in rats). MRF AD frequency was 9-20 Hz compared to 3-8 Hz for the forebrain structures that were tested. Bilateral MRF ADs were typically followed by a further dissemination of ADs into contralateral structures, resulting in the final bisymmetrical discharge pattern, and the appearance of the tonic-clonic generalized convulsion. This pattern of MRF AD development was undisturbed even when total or partial forebrain bisection was performed prior to amygdaloid kindling (Wada & Sato, 1975a). Recently Baba, Ono and Mori (1980) used a computer program to analyse the EEG data through a multidimensional autoregressive model. They determined that a "reactive" (as opposed to a propagated or "independent") component of AD was augmented markedly in MRF (and the anteroventral thalamus) coincident with the development of amygdaloid kindled convulsions in cats.

Wada and Sato (1975b) performed unilateral lesions in the MRF, ipsilateral to the amygdaloid kindling site, after the cats had developed generalized kindled seizures. They found that the MRF lesion markedly elevated the stimulation intensity necessary to trigger the generalized seizure, and that it also reduced the susceptibility of these cats to convulsant effects of pentylenetetrazol. Similar lesions of globus pallidus produced no such effect.

Burnham, Albright, Schneiderman, Chiu and Ninchoji (1981) tested the effect of direct stimulation of the MRF. At a stimulation intensity



of about 1000  $\mu$ A peak to peak for 10 sec, first-trial, self-sustained bilateral convulsive seizures could be triggered that outlast the stimulation offset by 10-15 sec. The pattern of the convulsion, however, was primarily tonic, and did not resemble the standard stage 5 kindled seizure. Furthermore, there was complete absence of synchronized paroxysmal discharge at the stimulating site, or in forebrain sites, during this generalized convulsion.

It has been suggested that forebrain paroxysmal discharges gradually converge on to the MRF, which in turn initiates the spread of discharges down the brainstem to the spinal cord and the final output cells for the triggering of convulsive activity (Burnham et al., 1981).

In addition, the MRF may produce "synchronous" discharges (and clonic convulsions) when it is paced by forebrain discharges, but "asynchronous" discharges (and tonic convulsions) when triggered directly (Burnham et al., 1981).

Tanaka (1972) reported that rhythmic sharp waves in the frontal cortex of rabbits also evolve coincident with the manifestation of motor seizures during amygdaloid kindling. This observation was confirmed by Corcoran, Urstad, McCaughran and Wada (1975) who reported the appearance of "independent" AD in the motor cortex during stage 3 amygdaloid seizures in rats. On the other hand, lesions of various parts of the cortex in rats (Corcoran, Urstad, McCaughran & Wada, 1975) or in cats (Wada & Wake, 1977) had relatively weak effects, or no effect at all, on motor seizures triggered during amygdala kindling. Lesions of the nonspecific thalamus were also found to be essentially without effect on subsequent kindling (McCaughran, Corcoran & Wada, 1978a).

Wada and Sato (1975a) reported that forebrain bisection resulted in marked lateralization of AD propagation until the late stage of seizure development. Nevertheless, cats reached the stage of fully developed bilateral seizures at a considerably faster rate than control cats, although these generalized convulsions never became bilaterally synchronized. Similar experiments performed on rats (McIntyre, 1975; McCaughran, Corcoran & Wada, 1977; McCaughran, Corcoran & Wada, 1978b) also showed a marked lateralization of the AD propagation and asymmetrical motor seizures. Amygdaloid kindling rates, however, were not affected by the forebrain bisection, nor was 'transfer' kindling (i.e. the facilitated subsequent kindling of a secondary site, in this case the contralateral amygdala - McIntyre, 1975). In fact, McCaughran, Corcoran and Wada (1977) found transfer kindling to be more rapid after forebrain bisection.

The stria terminalis is one of the major pathways to and from the amygdala, and is known to contain afferent catecholaminergic fibers from the locus coeruleus (Lindvall & Bjorklund, 1974; Ungerstedt, 1971a), and efferent inhibitory fibers to the ventromedial nucleus of the hypothalamus (Dreifuss, 1972). Radio frequency lesions of the stria terminalis were found to facilitate amygdaloid kindling in rats (Engel & Katzman, 1977). The olfactory system is also known to be a source of input to the amygdaloid complex. Large aspiration lesions that included the olfactory penduncle were found to facilitate amygdaloid kindling in rats (Cain & Corcoran, 1978).

b. Development of AD propagation - Transsynaptic alterations:

Neurons within the kindling site may undergo some change which results

in a progressively stronger output to target sites. Alternatively, the strength of synaptic transmission may increase between the kindled site and its target sites, or the neurons in target sites may become more susceptible to discharge recruitment by a stable input from the kindling site. These hypothetical mechanisms are not mutually exclusive, and there have been studies claiming focal as well as extrafocal changes induced by kindling.

One of the kindling phenomena that suggests a focal change in association with kindling is the reduction of AD threshold at the stimulated site. Goddard, McIntyre and Leech (1969) originally interpreted their findings of progressive seizure development as evidence of progressive reduction in seizure threshold. Tress and Herberg (1972) examined the effect of repeated electrical stimulation in the septal area. They also reported long-lasting reduction in subsequent seizure threshold, which was localized to the stimulated site and specific to electrical stimulation. Racine (1972a) systematically investigated AD threshold in rat limbic kindling, and found that repeated electrical stimulation (at either above or below AD threshold intensity) reduced AD threshold significantly at the stimulated site. The AD threshold remained reduced for at least 100 days after kindling, and lowering the AD threshold in amygdala did not alter it in the contralateral amygdala, septal area or hippocampus, suggesting that this long lasting plastic change is localized. The long-term reduction of AD threshold due to kindling has now been reported in many studies (e.g. Pinel, Skelton & Mucha, 1976; Cain, 1977).

Racine (1972a) demonstrated that repeated amygdaloid stimulations at an intensity below AD threshold resulted in AD threshold reduction, but no savings on subsequent kindling. Also, Pinel, Skelton and Mucha (1976) demonstrated that administering high intensity stimulations (400  $\mu$ A) resulted in an increase rather than decrease in AD threshold while kindling proceeded normally. Other features, such as time course, also differ between the threshold alteration phenomena and the kindling phenomenon (defined as the increase in strength of electrographic and motor seizures). These results suggest that different mechanisms may be involved (Racine, 1972b; 1978).

Repeated injection of small doses of penicillin into rat motor cortex will eventually lead to recurrent bilateral clonus (Collins, 1978) comparable to that seen in rats injected with a single large dose of penicillin (Collins, Kennedy, Sokoloff & Plum, 1976). Collins (1978) examined the size and intensity of metabolic activity in the 'penicillin-kindled' focus, using the deoxyglucose autoradiography technique. This technique uses 2-deoxyglucose, or 2-DG, labelled with radioactive carbon ( $^{14}\text{C}$ ). 2-DG is an analogue of glucose and is taken up by active neurons that require immediate energy. 2-DG, however, is lacking an oxygen on the second carbon atom and cannot be metabolized. It remains in the neurons that were most active at the time of 2-DG administration, and can be detected radiographically (Kennedy, DesRosiers, Jehle, Reivich, Sharp & Sokoloff, 1975). When Collins (1978) examined the tissue from his penicillin-treated animals, he found that the most intensive activity was within the focus itself even after development of severe bilateral clonus. He suggested that the principal

alteration underlying the increased seizure susceptibility took place within the restricted region of the focus.

Engel, Wolfson and Brown (1978), on the other hand, ran a 2-DG study of rat amygdaloid kindling and found a shift in dominance of labelling as the seizures became stronger. The amygdala and its direct projection sites showed strong labelling during early stages of kindling, but other sites, such as the substantia nigra, became dominant (were heavily labelled) during the later phases of kindling.

There is other convincing evidence for changes in extrafocal mechanisms. Goddard, McIntyre and Leech (1969) reported the facilitatory effect of amygdaloid kindling on subsequent contralateral amygdaloid kindling. Racine (1972b) examined this "transfer" effect further, and demonstrated that destruction of the primary kindled amygdala did not abolish the facilitated rate of subsequent kindling of the contralateral amygdala. This finding was confirmed by McIntyre and Goddard (1973). McIntyre (1975) demonstrated that the total forebrain transection did not interfere with the transfer effect in forebrain sites.

Additional support for kindling-induced transsynaptic changes was presented by Messenheimer, Harris and Steward (1979) with kindling of the entorhinal cortex (EC) in rats. In intact rats, EC kindling requires an average of 23 stimulations to reach the maximal motor seizure stage, and subsequent kindling of the contralateral EC requires an average of 5 stimulations. Messenheimer, Harris and Steward (1979) kindled the EC of one hemisphere and then destroyed it by electrolytic lesions. It was known that a lesion of the EC in one hemisphere would

trigger massive deafferentation at its major target site, the dentate gyrus. This, in turn, would elicit sprouting of the surviving afferents to the dentate neurons including the sparse projection from the contralateral EC (Goldowitz, White, Steward, Cotman & Lynch, 1975; Goldschmidt & Steward, 1980). Two weeks later, when the sprouting of the contralateral EC afferents were presumed to have reinnervated the deafferented ipsilateral dentate gyrus, Messenheimer and colleagues kindled the contralateral EC. Nine out of ten rats responded to the first or second stimulation with fully developed motor seizures. This transfer rate was significantly higher than any of several control groups. The authors reasoned that the primary EC kindling had produced transsynaptic alterations either within the dentate gyrus or beyond. The strengthened projection from the contralateral EC now 'tapped into' the altered ipsilateral dentate and triggered fully developed kindled responses. Some caution, however, is called for in the interpretation of these results, since the electrolytic lesion itself has been shown to induce seizure activity (Dasheiff, Savage & McNamara, 1982). The question remains as to whether kindling-induced transsynaptic alterations are a necessary or sufficient condition for the development of AD propagation and convulsive activity.

Studies assessing evoked potentials, rather than epileptiform activities, have provided further evidence for kindling-induced transsynaptic alterations caused by kindling stimulation.

c. Long-term potentiation (LTP) effects: Racine, Gartner and Burnham (1972) measured the potentials (EPs) evoked within various limbic structures by test pulses applied to the kindling site, or other

structures, before and after ipsilateral amygdaloid kindling in rats. They found that the amplitude of EP components increased by as much as 80% (in hippocampus) due to kindling. This increased amplitude of EPs, or "potentiation effect", remained unchanged for at least 7 days after the completion of kindling (and discontinuation of stimulation). However, when the test pulse was applied to structures other than the kindled site, there was no potentiation effect seen, even at the kindled amygdala. This suggested to the authors that kindling results in a change in synaptic transmission specific to the pathways directly activated by the kindling stimulations.

The development of potentiation during kindling was further assessed by Douglas and Goddard (1975). They demonstrated that potentials evoked in the dentate gyrus by test pulses applied to the perforant path (PP, the major fiber system conveying monosynaptic inputs from the ipsilateral entorhinal cortex to granule cells in dentate gyrus) gradually increased in amplitude during PP kindling.

Racine, Newberry and Burnham (1975) pretreated rats with repeated non-AD-triggering stimulation trains, applied to the amygdala. The result was a potentiation of responses evoked in several target sites by pulse stimulation of the amygdala. This potentiating pretreatment significantly facilitated the subsequent amygdaloid kindling rate, indicating that potentiation effects and kindling effects may share some common mechanisms.

A similar potentiation effect was reported by Tanaka (1977) who measured EPs from temporal cortex (anterior sigmoid gyrus and anterior sylvian gyrus) of cats, before and after ipsilateral amygdaloid

kindling. The sigmoid gyrus is not known to have direct connections from the amygdala (Krettek & Prince, 1974; Prelevic, Burnham & Gloor, 1976), and no detectable EPs could be recorded prior to kindling. When the cats had developed the generalized kindled seizure, however, Tanaka (1977) observed an emergence of large EPs in the sigmoid gyrus with test pulses applied to the kindled amygdala.

Racine, Tuff and Zaide (1975) kindled the anterior neocortex in rats, and reported a potentiation effect in the transcallosal response (the response evoked in the contralateral homologous area by pulse stimulation of the callosum). In this case, however, potentiation in the reverse direction (back into the kindled site) was also observed.

Several recent studies have focused on the mechanisms of the potentiation effect itself. The long lasting potentiation effect in mammalian brains was first investigated by Bliss and his co-workers (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973). They measured EPs in the dentate gyrus while test pulses were applied to the ipsilateral perforant path. They reported that the application of non-epileptogenic stimulation trains (e.g. 10-20/sec for 10-15 sec) produced potentiation in dentate EPs lasting for hours or days.

Unit recordings from amygdaloid kindling sites (Racine & Zaide, 1978) revealed that the basic pattern of electrically evoked epileptiform discharge consisted of high frequency bursts of action potentials at about 300/sec for 50 msec, which is comparable in pattern to the pulse trains that produce strong LTP effects (Racine et al., 1975a).



McNaughton (1978) carefully measured the time course of the potentiation effects, and demonstrated at least two or more apparently independent types of postactivation potentiation in the perforant path to dentate system. One is short-term potentiation (STP), typically lasting for minutes (McNaughton, Douglas & Goddard, 1978), and another is long-term potentiation (LTP); usually lasting for hours or days (Bliss & Lomo, 1973; Barnes, 1979; Racine, Milgram and Hafner 1983).

A further challenge to the hypothetical causal relationship between LTP and kindling was the demonstration of reversed effects on the 2 phenomena by drugs which depress the catecholamine (CA) systems. Reserpine (which depletes CAs) and 6-hydroxydopamine (which destroys CA terminals) are known to facilitate kindling (Arnold, Racine & Wise, 1973; McIntyre, Saari & Pappas, 1979). If LTP is a primary underlying mechanism of kindling, then the same drugs should also facilitate LTP. On the contrary, Bliss, Goddard, and Riives (1983) found a 50% reduction of LTP by these drugs. Robinson and Racine (in preparation), however, failed to replicate this finding.

d. Involvement of neurochemical systems - Loss of tonic inhibition:  
 One of the most efficient treatments for the majority of epileptics has been drug therapy. Intra-venous injection of valium (a benzodiazepine), for example, is very effective for the arrest of status epilepticus (continuous grand mal seizure, fatal if uninterrupted - Gastaut & Broughton, 1972, pp. 165, 225), whereas oral administration of phenytoin (diphenylhydantoin) is generally the best choice for the prevention of generalized seizures or psychomotor seizures (Gastaut & Broughton, 1972, pp. 223-224).

Many anticonvulsants appear to exert their effects by depressing or enhancing activity in neurotransmitter systems. There are three major groups of putative neurotransmitters known in mammalian brains: acetylcholine (generally an excitatory neurotransmitter), monoamines (generally inhibitory neurotransmitters) and amino acids (some excitatory and some inhibitory).

(i) Acetylcholine: Several studies have indicated a role for acetylcholine (Ach) systems in kindling. Repeated injection of carbamylcholine (a cholinergic agonist), via a cannula implanted into the amygdala, produces a kindling-like seizure development (Vosu & Wise, 1975; Wasterlain, Jonec & Holm, 1978). These developments could be blocked by muscarinic blockers, but not by nicotinic blockers (Wasterlain & Jonec, 1981). Atropine, a cholinergic blocking agent, significantly retards subsequent amygdala kindling in Sprague-Dawley rats (Arnold, Racine & Wise, 1973). Corcoran, Wada, Wake and Urstad (1976) failed to find an atropine effect on Long Evans rats, but Albright, Burnham and Okazaki (1979) replicated the effect in both Sprague-Dawley and Long Evans rats. Girgis (1980) demonstrated that physostigmine (which elevates Ach activity by blocking the enzyme acetylcholinesterase) facilitated amygdala kindling in rabbits. Leech and McIntyre (1976) reported that the DBA/2 strain of mice, which is known to have a high level of brain Ach, kindled faster than C3H/He strain of mice, which have a lower brain Ach level. On the other hand, Fitz and McNamara (1979) demonstrated that the frequency of spontaneous inter-ictal spiking was increased by muscarinic antagonists (e.g.,

atropine, scopolamine) and decreased by muscarinic agonists (e.g., physostigmine, choline).

In any case, the brain level of Ach seems to have a modulatory effect on the kindling rates and the epileptic activities of neurons. It seems unlikely, however, that Ach systems are critically involved in the developing epileptogenesis.

Muscarinic cholinergic receptor binding was reported to be unaffected by kindling at an unspecified time after the 1st appearance of the generalized seizure (McNamara & Toomin, 1977). This negative finding was replicated with a 3 day interval between the final generalized seizure and sacrifice (McNamara, 1978a). The same author, however, later reported a significant reduction in muscarinic cholinergic receptor binding, in both the stimulated and contralateral amygdaloid regions, 15 hrs after the appearance of the generalized seizure. The kindling stimulations in this experiment had been applied once per hour (McNamara, 1978b). A similar decrease was reported by Byrne, Gottlieb and McNamara (1980), although repeated seizures induced by injections of pentylenetetrazol had no effect on muscarinic binding. Dasheiff, Byrne, Patrone and McNamara (1981) confirmed that the muscarinic receptor binding decrease found at 15 hrs is transient and disappears by the third day after the last kindling stimulation.

Dasheiff, Savage and McNamara (1982) found that a similar reduction in muscarinic receptor binding occurred with lesion-induced and electroconvulsive seizures, with a time course parallel to that seen during kindling. Reduction in muscarinic receptor binding appears to be a consequence of seizures in general. McNamara, Byrne, Dasheiff and

Fitz (1980) have proposed that such a reduction may provide a means of reducing seizure susceptibility in a system undergoing repeated seizures.

(ii) Monoamines: The facilitatory effect of monoamine depletion was demonstrated as early as 1954 by Chen, Ensor and Bohner, who injected mice with reserpine and then administered electroconvulsive shock. Reserpine is assumed to block reuptake of brain monoamines (noradrenaline, dopamine and serotonin) by synaptic vesicles in monoaminergic nerve terminals. Intraperitoneal injection of reserpine results in extensive depletion of brain monoamines with the peak effect at about 18 hours after the injection (Azzaro, Wenger, Craig & Stitzel, 1972). Chen et al. (1954) found that the electroconvulsive seizure was considerably intensified in reserpine-treated mice. The seizures could be evoked at a lower intensity, and the latency to onset was reduced. A similar facilitatory effect of reserpine on electroconvulsive seizures in mice was reported by Wenger, Stitzel and Craig (1973). The effect has also been found in rabbits (De Schaepdryver, Piette & Delaunois, 1962), and with pentylenetetrazol seizures (Lessin & Parkes, 1959).

Arnold, Racine and Wise (1973) tested the effect of catecholamine (CA) depletion on amygdala kindling. They injected rats with reserpine and applied kindling stimulations every 2 hours, so that the kindling would develop within the period of the reserpine effect. Reserpine-treated rats required significantly fewer amygdaloid stimulations to develop motor seizures compared to saline-injected rats. The effect of reserpine on cortical kindling was found to increase the strength of convulsions but not the electrographic responses (Racine,

Burnham & Livingston, 1979a). The rats in the latter study were injected with reserpine only three times during a session of 20 daily kindling stimulations. The prolonged CA-depleting effects of reserpine, however, and the relatively mild effect of reserpine on cortical kindling, led the authors to conclude that kindling is not likely due to a depletion or degeneration in brain monoamine systems. Sato, Nakashima, Mitsunobu and Otsuki (1976) reported a small reserpine-induced increase in the frequency of spontaneous inter-ictal spiking in amygdaloid- and hippocampal-kindled cats.

Intracerebral and intraventricular injections of 6-hydroxydopamine (6-OHDA) destroys both noradrenaline (NA) and dopamine (DA) nerve terminals, while largely sparing serotonin (5-HT) containing nerve terminals. The degeneration of catecholamine neurons is still evident two years later (Ungerstedt, 1971b). Arnold, Racine and Wise (1973) pretreated rats with intraventricular injection of 6-OHDA, which depleted NA and DA extensively (89.3% and 96.3% depletion, respectively). Subsequent amygdaloid kindling was significantly facilitated. Corcoran, Fibiger, McCaughran and Wada (1974) found a similar, but not significant, facilitatory effect of 6-OHDA. A significant effect was obtained only when rats were treated with combined injections of 6-OHDA and tranylcypromine sulfate (a monoamine oxidase inhibitor), which depleted DA extensively while not increasing NA depletion over that produced by 6-OHDA alone. These authors concluded that the destruction of dopaminergic neurons may be involved in kindling. Similarly, McIntyre, Saari and Pappas (1979) pretreated rats with intraventricular injections of 6-OHDA alone, or 6-OHDA plus

subcutaneous injection of desmethylimipramine (DMI). DMI protects noradrenergic neurons, again resulting in a relatively selective DA depletion (Peters, Pappas, Taub & Saari, 1977). Contrary to the results obtained by Corcoran, Fibiger, McCaughran and Wada (1974), McIntyre et al. (1979) found no facilitatory effect on kindling rate. In agreement with Arnold, Racine and Wise (1973), however, 6-OHDA treatment alone significantly facilitated the subsequent kindling rate. This finding has been replicated recently (McIntyre & Edson, 1981). Furthermore, McIntyre (1980) administered 6-OHDA directly into the amygdalae bilaterally. This produced a 62 to 69% depletion of NA, restricted to the amygdala-pyriform region, without significantly affecting the DA level (80 to 100% of its normal level). Subsequent amygdaloid kindling was significantly facilitated.

In order to produce a still more selective depletion of either NA or DA in forebrain, several attempts have been made to inject 6-OHDA directly into the ascending fibers of the ventral NA system or into the ascending fibers of the DA systems (e.g., the nigro striatal bundle). Using this approach, Corcoran and Mason (1980) selectively depleted either NA (90% depletion) or DA (60% depletion) in rat forebrain. They found that NA, but not DA, depletion significantly facilitated subsequent amygdaloid kindling. A similar facilitatory effect of selective NA depletion on amygdaloid kindling was also reported by Mohr and Corcoran (1981). Furthermore, Ehlers, Clifton and Sawyer (1980) surgically cut the ascending noradrenergic pathway, thereby selectively depleting NA in the amygdala and periamygdaloid cortical area. They demonstrated a significant positive correlation between the increasing

degrees of NA depletion in these areas and the increasing rate of amygdaloid kindling.

Serotonin (5-hydroxydopamine, or 5-HT), the 3rd major monoamine, has also been examined for its potential contribution to the kindling process. Kovacs and Zoll (1974) stimulated the median raphe nucleus (the major source of 5-HT fibers) at low intensity for an hour preceding, and for 5 min following, the kindling stimulation in pre-kindled animals. They found that rats with raphe stimulation were significantly protected against the seizure compared to those with similar stimulation of non-raphe midbrain areas.

The dorsal raphe nucleus (DRN) has been shown to have a marked inhibitory influence upon neurons in the amygdala, via the direct DRN-amygdala serotonergic pathway (Wang & Aghajanian, 1977). Racine and Coscina (1979) destroyed the midbrain dorsal and median raphe nuclei of rats, and then measured rates of cortical or amygdaloid kindling. The lesion pretreatment significantly increased the strength of the cortical convulsion, weakly facilitated amygdaloid kindling and lowered AD thresholds. Systemic injection of the 5-HT synthesis inhibitor, p-chlorophenylalanine, however, mildly strengthened the cortical convulsion, but significantly weakened the amygdaloid kindled convulsion.

Other studies have also demonstrated an inhibitory effect of increased 5-HT levels on subsequent amygdaloid kindling rates (Bowyer, 1982; Munkenbeck & Schwark, 1982), or on the amygdaloid AD threshold (Siegel & Murphy, 1979). The facilitatory effect of decreased 5-HT level on amygdaloid kindling rate has also been confirmed (Munkenbeck &

Schwark, 1982), but none of these effects are robust. It was demonstrated in one study that a 5-HT depletor and a 5-HT precursor both increased AD duration in fully kindled rats (Munkenbeck & Schwark, 1982).

Other experiments have attempted to show that the NA system (or the DA system) undergoes some functional changes in association with kindling. These experiments bear more directly upon the question of underlying mechanisms. Unfortunately, there is still a good deal of controversy in this area. Sato and Nakashima (1975) reported that, 2 weeks after the 10th generalized seizure had been triggered in cats kindled in the hippocampus, there was a significant reduction in the NA levels in the whole brain, and DA levels in the anterior half of the brain. This finding was indirectly supported by Farjo and Blackwood (1978), who measured tyrosine hydroxylase (the rate-limiting enzyme of catecholamine synthesis). They found that tyrosine hydroxylase was reduced in the stimulated compared to the non-stimulated amygdala "weeks" after the last amygdala stimulation. This may indicate that local catecholamine synthesis is suppressed. Kant, Meyerhoff and Corcoran (1980), however, measured the spontaneous and potassium-induced release of endogenous catecholamines in vitro and found no difference between kindled brains and non-kindled brains.

More specific measurements of NA or DA activities following kindling have not resolved the controversy. Ohshimo (1978) measured DA levels in 11 discrete brain structures up to 24 hours after evoking the last of 5 generalized seizures in amygdala kindled cats. His finding was unexpected in that there was a significant increase in DA level, but



only in hippocampus. Callaghan and Schwark (1979) measured both NA and DA 7 days after the establishment of "stable" generalized amygdaloid seizures in rats. They found a significant reduction of NA, but not DA, levels in hippocampus, midbrain, frontal cortex and limbic lobes.

Wilkison and Halpern (1979) measured the turnover rates of NA and DA 7 days after the 10th generalized seizure had been triggered in amygdala-kindled rats. In their study it was the DA, not NA, turnover rates that were significantly elevated, indicating increased DA activity. Engel and Sharpless (1977) measured NA and DA levels 1 month after 3 consecutive class 5 seizures had been evoked in rats and found a significant decrease in DA level in the stimulated, compared to the contralateral, amygdala.

McNamara (1978a) examined the  $\beta$ -adrenergic receptor binding in amygdala kindled rats. He demonstrated a significant reduction in binding sites 3 days after a single class 5 seizure (established by hourly stimulation of the amygdala). Gee, Hollinger, Bowyer and Killam (1979) reported significant but bidirectional alterations in affinity and density of dopaminergic binding sites in the amygdala and frontal cortex of rats 24 hours after 4 consecutive class 5 seizures (established by twice daily amygdaloid stimulation). They also measured the dopamine-sensitive adenylate cyclase (Gee, Killam, Hollinger & Giri, 1980). The two studies appeared to indicate that kindling results in an overall reduction in dopaminergic neuroreceptor functions. A series of experiments by Sato, Hikasa and Otsuki (1979), however, indicate that kindling enhances the sensitivity of cats to dopaminergic agonists, such as apomorphine.

Less work has been done on the 5-HT system but the results are still confusing. Ohshimo (1978) reported that levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, metabolic breakdown product of 5-HT) did not differ from control levels in any of 11 brain regions examined 7 days after the establishment of amygdaloid kindling, except for a small but significant increase of 5-HIAA levels in the stimulated amygdala. Recently, Munkenbäck and Schwark (1982) reported a significant reduction of 5-HT and 5-HIAA in the midbrain region, but not in the kindled amygdala.

Taking all this information together, it appears that kindling generally reduces the levels of brain monoamines, and more consistently NA levels. However, the data are often inconsistent and the precise time course or underlying mechanisms for these alterations are yet to be determined.

(iii) Amino acids: For the vertebrate CNS, there is evidence that at least three amino acids may each serve as inhibitory neurotransmitters. They are  $\gamma$ -aminobutyric acid (GABA, see Krnjević 1974 for review), taurine (see Mandel & Pasantes-Morales 1978 for review) and glycine (see De Feudis 1977 for review). Each of these three principal free monocarboxylic  $\omega$ -amino acids occurs in approximately equal concentrations in whole cat brain (Tallau, Moore & Stein, 1954). Glycine concentrations, however, have been shown to be almost four times higher in the spinal cord than in the cortex (Uhr & Sneddon, 1972), and is therefore considered to be a spinal cord inhibitory neurotransmitter.

Experimentally-induced epilepsy (penicillin or cobalt foci) is generally associated with decreased levels of several amino acids, including the 3 mentioned above. The extent of depletion in cats is proportional to the severity of the motor seizures (Mutani, Durelli, Mazzarino, Valentini, Monaco, Fumero & Mondino, 1977). A similar decline in GABA levels has been reported for patients with epilepsy (Manyam, Kats, Hare, Gerber & Grossman, 1980). It has also been demonstrated that manipulations which decrease brain GABA concentrations result in the occurrence of convulsions in animals (e.g., Wood & Abrahams, 1971). The failure of GABA systems has been suggested as a mechanism underlying the development of epilepsy (Meldrum, 1975).

Conversely, manipulations which increase GABA activity often have an anticonvulsant effect. Diazepam (the generic name for Valium) is one of 6 derivatives of the benzodiazepines (BZD), which are effective against several types of human epilepsy. Diazepam is known to occupy a group of receptors that are different from those occupied by GABA or other putative transmitters (Coupet, Rauh, Lipka & Beer, 1981). BZDs, however, have been repeatedly shown to facilitate the action of GABA systems (see Costa & Guidotti 1979 for review; also see Choi, Farb & Fischbach, 1977; McDonald & Barker, 1978).

Babington and Wedeking (1973) were the first to examine the effect of diazepam on kindling, and demonstrated that diazepam (1 mg/kg IP) completely blocked the motor seizures in fully kindled (amygdaloid) rats. This finding was confirmed by Wise and Chinerman (1974) and Racine, Livingston and Joaquin, (1975) in rats, and by Wauquier, Ashton and Melis (1979) in beagle dogs. Ashton and Wauquier (1979) estimated

the ED50 value of diazepam against forelimb clonus in kindled Wistar rats to be 0.28 mg/kg (IP); and Albertson, Peterson and Stark (1980) estimated that the ED 50 for reduction of AD duration in kindled Sprague-Dawley rats to be 1.2 mg/kg (IP).

Racine, Burnham and Livingston (1979b) reported that diazepam (0.5 or 1.0 mg/kg IP) was relatively ineffective in blocking the tonic component of the cortical kindled seizure, although it was effective against the clonic components (i.e. the limbic type seizure components).

Other drugs which affect the GABA system have also been tested on the kindling preparation. GABA inhibition can be potentiated by suppressing its degrading enzyme, GABA-transaminase. Valproic acid has such an effect and was found to retard the development of amygdaloid kindling and to block established seizures in kindled rats (Leviel & Naquet, 1977). A similar GABA-transaminase inhibitor,  $\gamma$ -vinyl GABA, decreased the severity of the amygdala kindled seizure in half of the rats tested (Myslobodsky, Ackermann & Engel, 1979). Similarly,  $\gamma$ -acetylenic GABA was reported to "noticeably diminish the motor seizure" in amygdaloid kindled rats (Myslobodsky et al., 1979; Myslobodsky & Valenstein, 1980).

Compared to the effects of diazepam, however, these inhibitory effects were quite mild. Furthermore, another GABA-transaminase inhibitor, aminooxyacetic acid, was found to lengthen AD duration in about 20% of the rats tested, although it did reduce the severity of convulsive activity in over half of the rats (Le Gal La Salle, 1980). Finally, Kalichman and Burnham (1980) demonstrated that not all GABA

agonists (e.g.  $\gamma$ -vinyl-GABA, imidazole acetic acid), or antagonists (e.g. picrotoxin) affected the development of amygdaloid kindling in rats.

Another putative inhibitory neurotransmitter, taurine, has been shown to have no suppressing effect on kindled seizures in rats (Osawa, Sato & Wake, 1977; Burnham, Albright & Racine, 1978), cats or baboons (Osawa et al., 1977).

The effect of kindling upon brain amino acids has been examined by only a few investigators. Liebowitz, Pedley & Cutler (1978) measured the release of amino acids in hippocampal slices obtained from rats kindled via entorhinal cortex electrodes. Twenty four hours after the 5th-7th stage 5 seizure, the slices were incubated in a medium containing either normal or high  $K^+$  concentration. The  $Ca^{2+}$ -dependent release of GABA, induced by high  $K^+$  medium (Redburn & Cotman, 1974), was 100% greater in the hippocampus from the kindled hemisphere compared to that from the contralateral hemisphere. No other amino acid release was found to be affected by kindling. It is not clear whether the increased GABA release simply reflects increased recurrent inhibitory activity, due to increased excitation, or whether GABA release mechanisms are directly altered. The release of glutamate (one of the major putative excitatory transmitters in the hippocampus), however, was not altered. In any case, these data run counter to the theory that epilepsy (or at least kindling) is associated with a failure in GABA systems (Meldrum, 1975).

Paul and Skolnick (1978) reported a transient (less than one hour) increase in BZD receptor binding following seizures induced by

electroconvulsive shock or pentylenetetrazol. McNamara, Peper and Patrone (1980) observed a significant increase in the number of hippocampal BZD receptor binding sites 24 hr after the last amygdaloid kindled seizure. These changes were not seen in the hippocampus from rats that were stimulated but not kindled, or from rats that were repeatedly made hypoxic. A significant increase in BZD binding was also induced by repeated electroconvulsive shocks.

Tuff, Racine and Mishra (1983) examined BZD receptor binding two weeks after the completion of amygdaloid or dentate kindling, and found a significant increase in the number of BZD receptor binding sites in the pyriform lobes and hippocampi in both hemispheres, and a decrease in binding in the caudate nuclei. These changes in BZD binding were consistent with the electrophysiological demonstration that kindling enhanced recurrent inhibition (presumed to be GABA mediated) in the dentate gyrus.

e. Cellular mechanisms - Ca<sup>2+</sup> influx theory: Racine and Zaide (1978) reported that, during an electrically evoked epileptiform discharge, cells in the kindled focus responded with bursts of action potentials with higher intraburst frequency and longer duration, compared to cells of non-kindled rat brains. The neurons in the vicinity of the kindling site appear to undergo some functional changes and generate stronger bursting responses. Racine, Burnham and Gartner (1973) demonstrated that high frequency pulse trains, similar in pattern to the bursts seen in such "kindled" neurons, can trigger first trial motor seizures. The cell bursting phenomenon in the focus, therefore, may be one of the key events for the development of kindling.

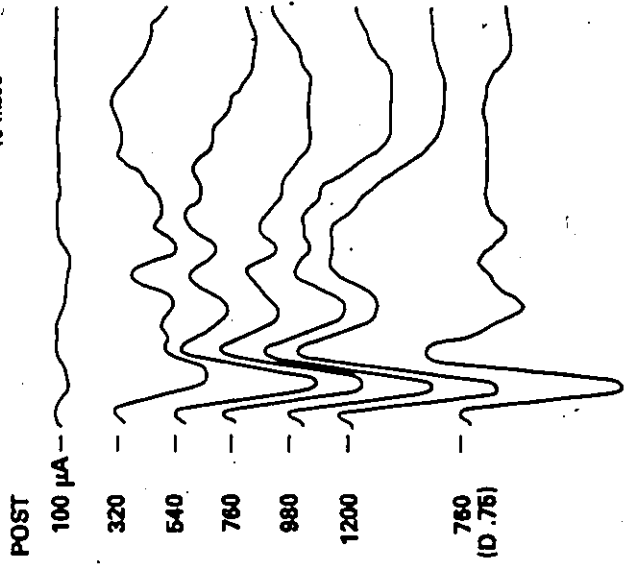
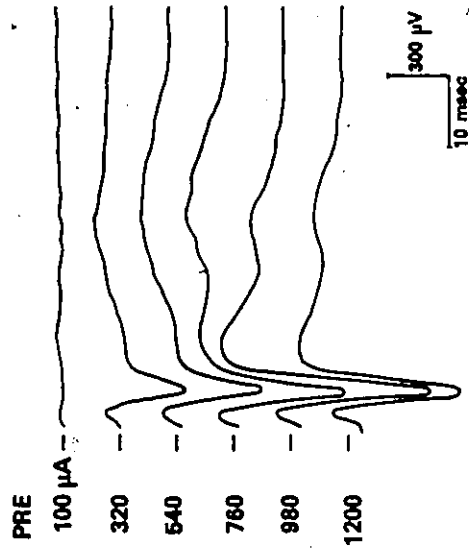
Recently, Racine, Kairiss and Smith (1981) examined interictal burst responses, which are characteristic of kindling as well as other epilepsy preparations. They suggested that the development of such burst responses is more likely due to "an alteration in the intrinsic response characteristics of the membrane itself", rather than "an increase in specific synaptic drive." Their argument is partly based on the following observations. They recorded evoked potentials from several secondary sites as test pulses were applied to the kindling site. During the course of kindling, they noted the evolution of new components in the evoked potentials. These components occurred with a somewhat longer latency than the potentiated primary component and tended to occur most frequently when the animal was showing inter-ictal spiking activity. These components were also similar in morphology to the spontaneous epileptiform bursts. Unlike synaptically driven responses, these burst-like components appeared in an "all-or-none" fashion in response to a series of graded intensity stimulations (Fig. 3). Furthermore, these components could be blocked by drugs, such as diazepam, while leaving the primary potentiated synaptic components intact. Finally, following the ictal discharges, when the potentiated primary responses are totally suppressed, these bursts occur more frequently.

Further analyses of the cell-bursting phenomena seen in kindling are yet to be done. A preliminary experiment by Kairiss, Racine and Smith (1981) found few differences between response properties of cells in the hippocampus of kindled, compared to control rats. In the same experiment, however, there was evidence that the amygdala was the

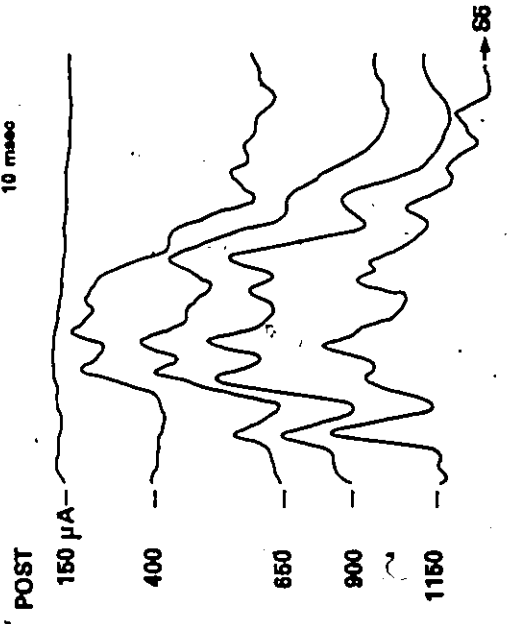
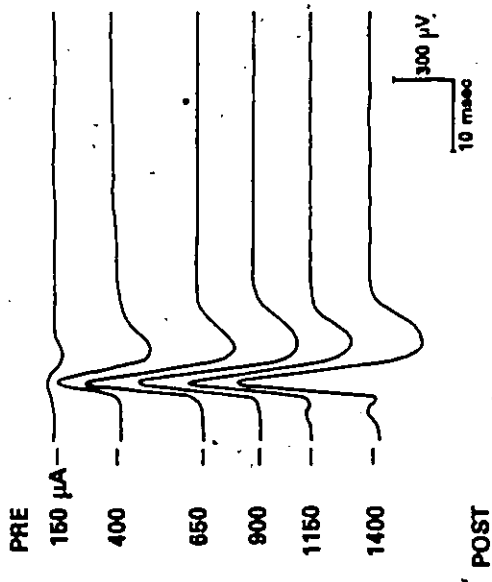
Figure 3 Left: An example of responses evoked during I/O tests before (PRE, above) and after (POST, below) kindling. EPs were recorded from the entorhinal cortex as test pulses were applied to the ipsilateral amygdala (kindling site). Note that diazepam (.75 mg/kg) diminished the late onset components (bottom). Each EP was based on an average of 20 sweeps. Right: An example of I/O curves for hippocampal EPs. The late onset, 'burst-like' response appeared with maximal amplitude, at an intensity of 400  $\mu$ A, after kindling had been established. These burst responses actually developed into ADs, accompanied by stage 5 seizure (S5), when evoked by several test pulses at 1150  $\mu$ A. Note that the primary responses were smaller after the kindling.



ANIMAL 624



ANIMAL 108



generator of inter-ictal discharge, even when another structure had been kindled. Inter-ictal spikes appeared first from the amygdala and usually with the largest amplitude as well. Work on amygdala cell properties is now underway.

Similar burst responses have been recorded intracellularly in many other models of epilepsy. These cells stereotypically elicit regular, all-or-none, high-amplitude (20-30 mV), long duration (50-100 msec) intracellular depolarization shifts on which appear high frequency bursts of action potentials (e.g. Dichter & Spencer, 1969a). Such paroxysmal depolarization shifts (DSs), coincident with epileptiform spikes in the EEG recording, are also found in neurons in human epileptogenic cortex (Calvin, Ojemann & Ward, 1973).

There are two major hypotheses that attempt to explain the mechanisms underlying DSs. One theory maintains that DSs are produced by an aggregate of normal neurons responding to an augmented hypersynchronous synaptic input (Ayala, Dichter, Gumnit, Matsumoto & Spencer, 1973). Another theory claims that they are generated by intrinsic properties of the cell, particularly the cell membrane, and that relatively normal synaptic events serve as a mechanism for synchronizing a population of these burst-prone neurons (Atkinson & Ward, 1964).

The first theory, postulating hypersynchronous synaptic inputs, is mainly based on evidence from studies with penicillin foci. DSs in these foci respond in much the same way as excitatory synaptic potentials when the membrane potential of the neuron is altered by application of intracellular current pulses (Dichter & Spencer, 1969b;

Matsumoto, 1964; Matsumoto, Ayala & Gumnit, 1969; Prince, 1968).

Furthermore, transitions between normal synaptic events and the large DSs have been observed, after the application of a convulsant drug (Prince, 1968; Matsumoto & Ajmone-Marsan, 1964). In immature neurons, where synaptic connectivity is not well developed, clear transitional forms between EPSPs and DSs have also been recorded (Prince & Gumnit, 1972).

As to the synaptic pathways supporting such DSs, Ayala and Vasconetto (1972) and Dichter and Spencer (1969b) postulated a role played by recurrent excitatory pathways. They transected inputs to cortical neurons and hippocampal pyramidal neurons, respectively, and activated these cells antidromically by stimulating the deafferented transcallosal fibers and deafferented fornix. Both groups recorded essentially the same intracellular characteristics of DSs as obtained from intact preparations. The authors, therefore, suggested that the collateral pathways (the axon-collateral-interneuron chain) in these systems are sufficient to produce DSs when evoked.

Based on these observations, Ayala, Dichter, Gumnit, Matsumoto and Spencer (1973) hypothesized that the DS and the subsequent hyperpolarization is a giant recurrent EPSP-IPSP sequence. According to their theory, the epileptogenic agents either increase EPSP (as recently demonstrated by Futamachi & Prince, 1974; Schwartzkroin & Prince, 1978) or decrease IPSP (as recently demonstrated by Wong & Prince, 1979; Schwartzkroin & Prince, 1980), thereby "enhancing the positive feedback operation of recurrent excitation (relative to inhibition) so that truly gigantic recurrent EPSPs were generated" (Ayala, Dichter, Gumnit,

Matsumoto & Spencer, 1973, p. 15). This dual recurrent hypothesis would predict that synchronous discharges cannot be generated in structures which lack a recurrent excitatory system. The cerebellar cortex is one such structure (Brookhart, Moruzzi & Snider, 1950), and the olfactory bulb, although it has a long latency excitatory recurrent system, appears to be dominated by a short latency inhibitory recurrent system (Frankenhaeuser, 1951). Both structures failed to show "strychnine spikes" when strychnine was applied topically.

The other theory, which postulates an inherent abnormality in the cell membrane, is also based on observations from the penicillin focus, as well as other models, including the alumina foci. DSs are known to appear abruptly at some threshold when evoked by stimulation of gradually increasing intensity (Matsumoto, 1964). When they are triggered, DSs are strikingly regular in form and amplitude, even when the latency for DS generation varies over a 100 msec period (Prince, 1966). After the generation of DSs, there are long periods of refractoriness (Matsumoto, 1964), beyond the length of normal IPSPs.

This regularity in form cannot be explained by the involvement of extensive interneuronal pathways, because these would have to be activated with an invariant pattern each time the DS was triggered. But a stereotyped response might be expected if DSs were generated as a result of intrinsic neuronal activities.

Speckmann and Caspers (1973) demonstrated that synaptically isolated snail cells were capable of responding to pentylenetetrazol, added to the bathing fluid, with DSs resembling those produced in intact cells. It appears, therefore, that cells are equipped to generate

large, long-lasting membrane depolarizations without being driven by synaptic inputs. Barker and Gainer (1974) also demonstrated that molluscan neurons can produce bursts of spikes that resemble the bursts seen in mammalian epileptiform cells. These bursts were triggered by the removal of most of the extracellular calcium and magnesium and in the absence of synaptic input.

Prince and his colleagues examined the possibility of an abnormal ion microenvironment as an underlying mechanism for the generation of cellular epileptiform activity (Prince, Lux & Neher, 1973; Futamachi, Mutani & Prince, 1974; Moody, Futamachi & Prince, 1974). They found a 2- to 3-fold increase in the extracellular potassium concentration ( $[K^+]_o$ ) 15-60 msec after the onset of epileptiform spikes. Such changes in  $[K^+]_o$ , however, did not appear to be a critical factor for the initiation or termination of the interictal or ictal discharges.

Neither the synaptic nor the non-synaptic theory has been conclusively shown to account for the generation and characteristics of paroxysmal DSs. A recent hypothesis proposed by Schwartzkroin and Wyler (1980), focuses on features common to epileptiform cellular activities across several different models. They proposed that "the proclivity for burst firing is present in many CNS neurons but is manifested only when normal stabilizing influences are removed" (Schwartzkroin & Wyler, 1980, p. 96). The mechanisms by which these stabilizing influences may be removed are as diverse as the number of different models. They further argued that "the hyperexcitability of epileptogenic neurons is based on disruption of the balance between depolarizing and hyperpolarizing

factors" (p. 104). According to their hypothesis, calcium influx serves as the most important depolarizing factor and potassium efflux as one of the more important hyperpolarizing factors (stabilizing influences). Their list of factors that can upset the balance between the two includes a change in local circuitry and impinging synaptic drive, a change in cell morphology, disruption of hyperpolarizing conductances, alteration of specific membrane properties, and a change in the extracellular milieu. Therefore, apparently conflicting findings from different models are not necessarily incompatible if the common end result is the removal of "stabilizing influences."

This concept of disturbed balance between excitatory and inhibitory influences has also been entertained by Racine, Kairiss and Smith (1981). According to their view, neurons can become "bursters" and/or show long-term potentiation, depending on several factors: the strength of tonic inhibition, the strength of recurrent inhibition, the susceptibility to failure of tonic and recurrent inhibition, the strength of plastic excitatory drive, the effectiveness of modulatory inputs, and the level of  $Ca^{2+}$ -conductance.

Clearly, the understanding of mechanisms underlying focal cellular activities must await further research.

### 3. Evaluation of kindling as a model of epilepsy

Kindling, kindled seizures, and related phenomena appear to "model" some of the characteristics of human epilepsy reasonably well. Kindling results in the development of chronic, spontaneously discharging epileptogenic foci with no apparent morphological abnormalities. Kindling is known to alter aggressiveness and other indices of emotional

reactivity in animals, and similar effects have been reported in some human patients with seizure disorders. The response of kindled seizures to pharmacological agents is similar to that of certain types of human seizures (see discussion by Racine and Burnham, 1983).

As an experimental preparation, kindling is relatively easy to develop and maintain, and provides a means of producing a relatively uncontaminated focus (unlike most chemical lesion models). The experimenters can control the location of the kindling sites and the extent of seizure severity.

There are certain disadvantages of kindling that are shared by other models as well. It is rarely clear if a given observation is attributable to kindling per se, or to seizures (ictal or inter-ictal) that result from kindling. This is a particular problem for biochemical assay studies.

#### D. KINDLING AS A MODEL OF NEURAL PLASTICITY

Although kindling would appear to be a useful experimental epilepsy model, many researchers have used it as a potential model of memory processes. One of the classic questions in physiological psychology concerns the mechanisms of 'engram' formation (the neural substrate of memory traces that must develop in the CNS when learning occurs). The prevailing view is that memory processes depend upon the strengthening of existing synaptic connections or perhaps the development of new connections. The apparent diffuseness of the engram precludes the easy identification, isolation and analysis of those connections (e.g. Lashley, 1950). Consequently, neuroscientists have

resorted to model phenomena that demonstrate forms of synaptic plasticity that are more amenable to experimental investigation.

Such models should fulfill certain criteria. First, the model should yield a relatively permanent change in neural function, resulting from some form of neural activation. Second, the process should not involve the degeneration of neurons or their surrounding milieu. Third, the changes should occur in a sufficient number of elements so that analysis of mechanism is possible.

As has been repeatedly suggested (Goddard, McIntyre & Leech, 1969; Goddard & Douglas, 1975; Racine, 1972a, Leech & McIntyre, 1976; Adamec, 1975, etc.), the kindling phenomenon appears to fulfill those criteria reasonably well. Kindling produces lasting changes in neural function and results in an increase in strength of both epileptiform activity (ADs) and non-epileptiform activity (measured by evoked potentials). Kindling produces no gross abnormalities in morphology of neurons in or around the focus, and the convenience of the model for various types of experiments has been demonstrated.

A serious criticism of kindling as a memory model is that its epileptogenic characteristics obviously have no parallel in normal learning mechanisms (Sato, 1975; 1976). Kindling, then, may be a better model for epilepsy than for learning and memory. On the other hand, it is possible that a pathological outcome can be produced by abnormal driving of otherwise normal synaptic mechanisms. In other words, although the activation of populations of neurons by kindling stimulations results in the development of an abnormal epileptogenesis,



the plastic changes taking place at each individual element (synapse?) may be based on otherwise normal physiological mechanisms.

If similar mechanisms are involved, rats that differ in kindling response should differ on some measures of learning and memory as well. Although this assumption, as stated, is debatable, there have been a few attempts to demonstrate correlates. Systematically inbred rats that were 'fast' learners on a maze task ('Tryon bright') had a significantly lower amygdaloid AD threshold than did slow learners ('Tryon dull'). The 'dull' strain, however, was found to kindle faster (i.e. required fewer stimulations to develop generalized convulsions) (Zaide, 1974). Racine (1969) chose the brightest and dullest rats on a visual discrimination task, and then measured their rate of AD threshold reduction produced by repeated electrical stimulation. He found that fast learners showed a greater reduction of cortical AD thresholds, compared to slow learners.

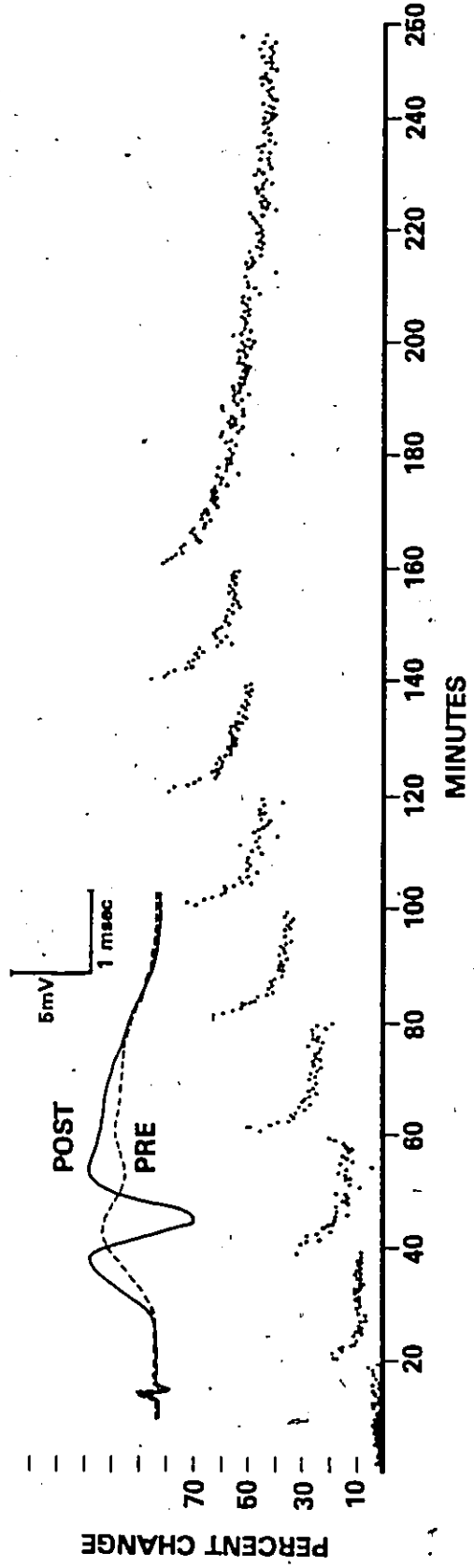
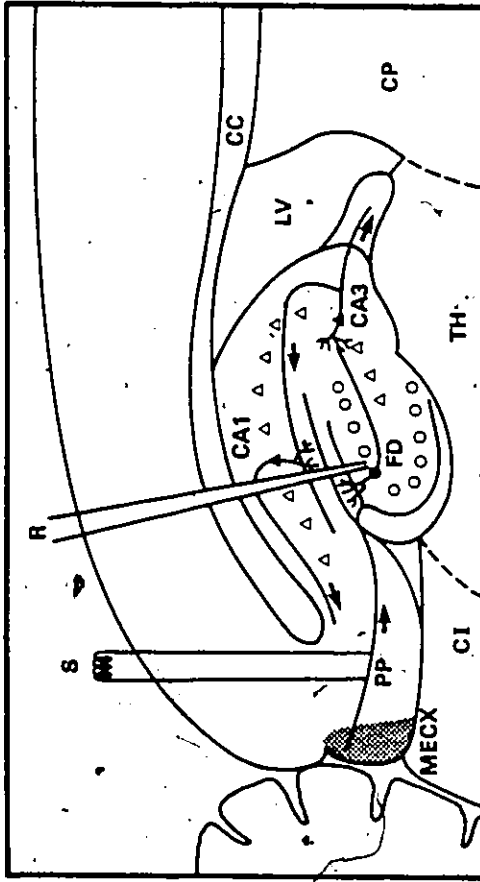
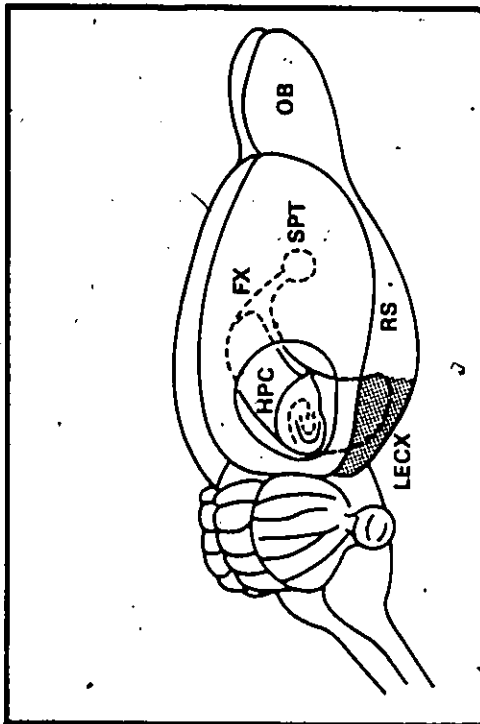
Such learning task parameters as trial schedule also appear to affect both kindling rates and learning. Leech and McIntyre (1976) kindled two strains of mice that differ in performance on a shuttle box avoidance task according to the trial distribution. DBA/2 mice are deficient in learning the task on a massed schedule, whereas C3H/He mice are deficient on a distributed schedule. Similarly, a distributed schedule of amygdala-pyriform kindling stimulations (one per day) resulted in a faster kindling rate for the DBA/2 strain. The DBA/2 strain still kindled faster on the massed schedule (50 per day, 60 sec interval), but after a 3 week rest it was the C3H/He strain that showed evidence of "savings" or retention of the kindling effect.

Other experiments have shown that both kindling and memory are susceptible to protein synthesis blockers. Protein synthesis is considered to be necessary for the changes in neural function underlying long-term memory, and protein synthesis blockers have been shown to disrupt memory processes (Dunn, 1980 for review). Similarly, the protein synthesis blockers cycloheximide (Ogata, 1977) and anisomycin (Jones, Holm, Masuoka & Wasterlain, 1977) have been shown to retard kindling, an effect that would not be expected if kindling were based on purely degenerative mechanisms.

Other links with memory mechanisms have been established via the long-term potentiation (LTP) phenomena. This LTP effect has also been observed using non-epileptic preparations and has been intensively investigated in the hippocampal formation of rats. Although LTP appears to be a component phenomenon in the kindling model, its features have been most effectively studied in the non-kindled preparation.

If brief trains of pulses (e.g. 200-400 Hz) are applied to the perforant path (PP - the pathway between entorhinal cortex and dentate gyrus granule cells), one observes an increased amplitude of potentials evoked from granule cells by single test pulses applied to the PP (Fig. 4). The decay curve for this potentiation effect reveals at least two independent components. One decays within a few minutes (short term potentiation; STP), and the other remains potentiated for 20 minutes or more (LTP). With a step wise increment of train intensity, as is the case in Fig. 4, there is an eventual saturation of LTP, although STP does not show much change. The amount of LTP seems to be correlated with the number of synapses activated. By increasing the train

Figure 4 . Location of hippocampus in the rat brain is shown in the upper left. A parasagittal section of the rat brain (upper right) shows the relationship between the perforant path (PP) and the dentate gyrus (FD). In this diagram, only the median entorhinal cortex (MECX) is seen. The lateral entorhinal cortex can be seen in Fig. 1 bottom. Other areas shown in the figure are the corpus callosum (CC), the inferior colliculus (CI), caudate/putamen (CP), lateral ventricle (LV), and thalamus (TH). Two pyramidal cell regions of hippocampus (CA1 and CA3) are also indicated. An example of short-term and long-term potentiation effects in the PP-FD system is shown at the bottom. Averaged FD EPs before (PRE, dotted line) and after (POST, solid line) the application of potentiating trains are shown in the insert. Each point in the main figure indicates the averaged (N=10) amplitude of the population EPSP expressed as a percent of baseline. Eight trains were applied every 20 minutes at an increasing intensity. The rapidly decaying component (short-term potentiation) remains superimposed upon the long-lasting component (long-term potentiation) which reached a plateau after the 6th train application (data from Robinson, 1980).



intensity, more PP fibers and synapses are activated. The LTP (and STP) observed is specific to the synaptic pathway that was activated. A train applied to lateral PP, for example, produces LTP only in that pathway and not in the medial PP even though both sets of PP fibers transmit inputs to the same pool of granule cells. On the other hand, if both sets of PP fibers are activated concurrently, then an interaction effect can be observed. LTP at either PP circuit is then increased considerably, and this 'cooperativity' effect has been proposed as a model for associative memory mechanisms.

Barnes (1979) has demonstrated yet more direct parallels between LTP and learning ability. Since the hippocampal system has been shown to play an important role in spatial learning (O'Keefe & Nadel, 1978), she chose a spatial learning task to probe memory processes in young and old rats. She found a clear correlation between LTP decay time and learning ability on the maze task. The senescent rats (28-34 months old) required significantly more trials than mature adult rats (10-16 months old) to acquire the correct choice in the complex maze. They also showed significantly more rapid decay of LTP. The correlation between performance on the memory task, and LTP decay, was also true within each age group, with slow learners showing more decay of the LTP effect. If LTP was produced before the maze learning session, the senescent rats showed a significant impairment in the acquisition of learning, whereas the younger adult rats absorbed the LTP effect without any interference (see McIntyre and Molino, 1972, for a somewhat similar effect of bilateral amygdala kindling on conditioned emotional response learning).

Barnes' interpretation was that there are limited numbers of plastic synapses (capable of adapting to new environmental conditions) available in the hippocampal system. The old animals had utilized a large proportion of such synapses to the level of saturation. The LTP treatment resulted in a saturation of most of the remaining plastic synapses in senescent rats, leaving them further impaired compared to young rats.

A further possible link between memory and potentiation mechanisms was provided by Van Harreveld and Fifková (1975). She initially reported that the dendritic spines of dentate granule cells in mice were increased in size as a result of applying train stimulation to the entorhinal cortex. In a later study, a similar swelling was observed in the same area after classical conditioning, using tone and food pairings (Fifková & Van Harreveld, 1978).

There is good evidence that the LTP effect is a component of the kindling process, so the links that have been established between LTP and memory mechanisms apply to kindling as well. Although the kindling effect appears to be contaminated by additional mechanisms that relate more directly to epileptogenesis, the LTP effects during kindling are consistently strong and may serve as a good starting point for the study of LTP mechanisms.

#### E. RATIONALE FOR THE BREEDING STUDY

Nearly all kindling experiments have compared kindled with non-kindled groups. As previously mentioned, a major problem in such comparisons is that it is difficult to separate the primary phenomena from the secondary phenomena, such as transient reactions to ongoing

seizure activities. Such transient reactions have been reported by Dasheiff et al. 1981, for changes in muscarinic binding, and by Frenk and Yitzhaky (1981) for changes in pain threshold.

An alternative approach to the study of underlying mechanisms of kindling is based on the observation that the risks of epilepsy are somewhat higher for the siblings or offsprings of a proband with epilepsy (6-8%), compared to the general population (2-3%), (Ehrman & Parsons, 1981, p. 294). This means that the underlying mechanisms of epilepsy may be subject to genetic control. Furthermore, a number of studies involving selective breeding for seizure susceptible vs. seizure resistant rodents have been successfully performed with epileptogenic agents such as loud sound and postural stimuli. We attempted, therefore, to inbreed seizure-susceptible vs. seizure-resistant rats based on susceptibility to kindling stimulation. As a result of such breeding, two strains of rats were obtained which differed significantly in seizure susceptibility. This provided an opportunity to examine mechanisms that determine seizure susceptibility, without the necessity of triggering the contaminating seizure activity.

It seems likely that several genotypes may collectively create the predispositional susceptibility to the kindling treatment. It should be possible to observe differences in other phenotypic traits that are concomitantly expressed by all or some of these polygenes determining kindling rates. Such pleiotropic effects may involve morphological, physiological and behavioral traits. An examination of some of these traits may lead to the discovery of those mechanisms that most directly determine the development of epileptogenesis. The results

obtained from comparative studies on these inbred strains are to be interpreted with caution, but may provide additional data to test hypotheses based on other approaches.

As Schlesinger and Griek (1970) have rightly stated (with studies of audiogenic seizures in inbred mouse), such comparative studies become truly interdisciplinary, involving biochemistry, genetics, physiology, pharmacology, psychology and psychiatry. For the purpose of this project, it was necessary to restrict the realm of topics to those most currently advocated in the kindling literature.

One of the major hypotheses of the project was that kindling-prone rats would have a greater capacity for synaptic plasticity. This would result in greater potentiation effects, thereby facilitating the propagation of epileptic neural activity and the development of the final stage of seizure. Therefore, LTP experiments were undertaken with these strains using primarily non-epileptogenic stimulation trains.

Another hypothesis was that kindling-prone rats would be deficient in tonic or recurrent inhibition mediated by specific neurotransmitters. Therefore, several known convulsant drugs, that have been demonstrated to interfere with certain neurotransmitter systems, were tested on the two strains. In addition, electrophysiological indices of inhibition were measured in the 2 strains.

Other measures, including behavioral measures, were also taken to see if the change in function between these strains was reflected in behavior.



## CHAPTER II COMPARISON OF FAST vs. SLOW STRAINS: BASIC OBSERVATIONS

### INTRODUCTION

Selective breeding was pursued for eleven generations. Seizure susceptibility was measured in terms of amygdaloid kindling rate, the number of amygdaloid kindling stimulations required for the subject to develop the first generalized motor seizure (Stage 5, Racine, 1972b).

The amygdaloid kindling rates in the 2 parent strains (Wistar and Hooded) were previously found to range from 8 to 28 (Racine, Burnham, Gartner & Levitan, 1973). This range appeared to provide enough variability to allow for the development, by selective breeding, of 2 distributions of kindling rates. In this chapter, the breeding procedures and results will be described, as will some of the basic electrophysiological differences between the 2 resulting strains.

These basic electrophysiological variables include AD threshold, initial and final AD durations, AD spike amplitudes, spontaneous interictal spiking, secondary afterdischarge and several evoked potential measures. These measures were taken to determine the differences in the strength of synaptic propagation and epileptogenic activity in the non-kindled naive rat, as well as to track the development of these processes during kindling. These experiments were designed, in part, to determine if the seizure prone (FAST) animals showed stronger epileptogenic responses prior to kindling or if they showed a more rapid development of these responses, or both.

## METHODS

## KINDLING PHASE I (SELECTIVE BREEDING)

Animals: The foundation population consisted of 24 male and 24 female rats, obtained from Canadian Breeding Farms, St. Constant, Quebec. Half of the male and female rats were albino Wistar rats, while the rest were hooded Long Evans rats. The combination served to increase the variability of the initial gene pool. The rats were housed individually in wire cages, and were allowed free access to Purina rat chow and water. The animals weighed 250 - 350 g at the time of surgery.

Surgery and histology: The rats were anaesthetized with 65 mg Somnotal (sodium pentobarbital) per kilogram body weight. Bipolar stimulating and recording electrodes, made from isonel coated nichrome wires (0.25 mm), were implanted unilaterally into the basolateral amygdala. The co-ordinates were A.P. 1.0 mm posterior to Bregma, M.L. 5.0 mm lateral to midline, and D.V. 8.5 mm below the skull surface (Pellegrino & Cushman, 1967). A stainless steel 0.080 fillister head screw, with a male Amphenol pin attached, was inserted into the skull just posterior to lambda and used as the ground electrode. All three electrode pins were secured with dental cement and anchored by two additional screws inserted in the skull. All rats were given 15,000 units of penicillin (i.m.) at the end of surgery.

After the completion of the experiment, all rats were administered an overdose of somnotal. They were perfused through the heart with physiological saline, then with 10% formaline solution in physiological saline. The brains were removed and placed in formol-

saline for at least two weeks. Frozen sections of 50  $\mu$ m were taken and stained with thionine, and the electrode placements were determined.

Apparatus: A Grass stimulator (model S88) was used to generate the kindling stimulation, which consisted of biphasic 1.0 msec square-wave pulses at 60 c/sec for 1.0 sec at 400  $\mu$ A intensity. The stimulation was delivered through photoelectric stimulus isolation units (Grass, model PSIU 6B) to obtain constant current outputs and isolation from ground.

The EEG responses were led into a wide-band A.C. EEG pre-amplifier (Grass, model 7P5A) and polygraph D.C. driver amplifier (Grass, model 7DAC), and plotted on chart paper at a speed of 1.5 cm/sec. The amplification was adjusted for each rat so that the EEG response would not exceed the maximum pen excursion during the ictal seizure.

All recording and stimulation was performed while the rat was freely moving in an observation box (60 x 64 x 50 cm).

Kindling: Subjects were given one week postoperative recovery period, during which they were handled and introduced to the experimental environment. Each rat was then given one kindling stimulation each day. The stage of motor seizure as well as the duration of amygdaloid afterdischarges (ADs) were recorded. Kindling was continued for each animal until stage 5 of motor seizure development, which consisted of head and forelimb clonus, rearing and falling.

The 'fastest' 13 rats (5 males and 8 females) from the parent generation required a mean of 8.8 AD's and were selected as breeders for

the kindling prone strain (FAST). The 17 rats (8 males and 9 females) that required the greatest number of ADs (a mean of 15.0) were selected as breeders for the kindling resistant (SLOW) strain.

Breeding procedure: In each group of FAST and SLOW breeders, the male rats were randomly housed with one or more female rats in wire mating cages (40 x 24 x 18 cm). After 21 days, all females were then caged individually in 35 x 30 x 18 cm plastic cages with nesting material. The date of birth for each litter was noted. In order to keep comparable developmental conditions for both groups, the litter size was reduced to a maximum of eight (4 males and 4 females, if possible) within 2 days after the birth. Care was taken to keep the pups with the healthiest appearance and most vigorous movements, and an attempt was made to maintain the phenotype ratio for fur color (albino vs. hooded) unchanged as much as possible.

One month after birth, pups were weaned and caged together in plastic cages with siblings of the same sex. Each individual was labelled with an identification number which included the birth date and code for parental identification. Successful breeders were often used to produce more than one litter. These kindling and breeding procedures were repeated until the establishment of the F6 generation.

#### KINDLING PHASE II (ELECTROPHYSIOLOGICAL MEASURES)

Animals: A sample of 10 FAST and 10 SLOW male rats were randomly selected from the F6 generation and kindled to stage 5 seizures to confirm that the two strains had achieved significantly different kindling rates. A total of 142 male rats from FAST (74 rats) and SLOW

(68 rats) strains were subsequently randomly selected from the F7, F8, F9 and F10 generations to make up the subject pool for the electrophysiological experiments described below.

Surgery: All 142 rats were implanted with a unilateral amygdaloid electrode. 111 rats had additional electrodes implanted into one or more of the following areas: hippocampus (HPC), dentate gyrus (FD1 and FD2), the entorhinal cortex (ENT), and the perforant path (PP). These placements were based on coordinates from the Pellegrino and Cushman (1967) atlas, and were confirmed histologically (Fig. 5).

The maximum number of electrodes was 5, in the FD2 group, and included two monopolar recording electrodes bilaterally in FD2, two bipolar stimulating electrodes in bilateral PP and one bipolar amygdaloid (AMY) electrode. The stimulating/recording electrodes in the remaining cases were all standard bipolar electrodes, as previously described.

The surgical technique was identical to that of Phase I, except that all electrode pins in Phase II were collected into a 9 pin connector.

Kindling: Of these 142 rats, 51 were kindled prior to any additional manipulations being performed ("No pre K" group in Table I). Unlike the previous groups (up to the F6 generation), these rats were kindled at an intensity just above the AD threshold.

The remaining 91 rats were run in one of the following 2 tests prior to kindling: 1) post-activation potentiation (details in chapter IV) and 2) convulsant and anticonvulsant drug response (details in chapter V). Group 1 was then kindled at intensities just above AD

threshold, while group 2 was kindled at a fixed intensity of 400  $\mu$ A. It had been previously determined that the pre-kindling manipulations had no effect on subsequent kindling measures. It is the kindling measures that will be described in this chapter.

In order to obtain the AD threshold, the rat was first stimulated at an intensity of 25  $\mu$ A. If there was no afterdischarge, another stimulation at 50  $\mu$ A was applied 5 min. later. Failure at that stimulation led to a further doubling of the intensity to 100  $\mu$ A, then 200  $\mu$ A and so on, until ADs appeared that were longer than 3.5 sec in duration. The intensity which evoked the first AD in this manner was defined as the AD threshold, and constituted the kindling stimulation for that rat.

During the course of the kindling session, if the rat failed to respond with an AD on two consecutive daily trials, then its AD threshold was assumed to have risen and the following kindling stimulation intensity was increased in 25  $\mu$ A steps until reliable ADs reappeared.

Rats were kindled until they reached the first stage 5 seizure, and kindling rate was defined as the number of ADs required to reach that stage. Additional kindling stimulations were applied until five stage 5 seizures had been evoked, except for 27 rats that had bilateral amygdaloid electrodes. The latter group was kindled to the first stage 5 seizure in a randomly chosen hemisphere, after which the identical kindling procedure was repeated at the contralateral side until stage 5 seizures had again developed.

Evoked potential recordings during kindling: Twenty-three (13 FAST, 10 SLOW) rats were used to investigate possible differences between the strains in synaptic responses evoked in various amygdaloid target sites. These responses were evoked by pulse stimulation of the amygdala, before and after kindling, and were measured as field potentials in the target sites.

Prior to kindling, these rats were stimulated in the amygdala with single 0.1 msec biphasic square wave pulses at various intensities in order to assess the threshold and maximum intensities for eliciting evoked potentials (EPs) from the target areas. The intensity range was then divided into 5 steps, which evoked EPs at amplitudes near zero (threshold), 20%, 40%, 60% and 80% of the maximum (100%) amplitude. These input/output (I/O) measures were taken before the first AD and after the completion of the stage 5 seizures. The stimulation intensity that evoked 80% of the maximum amplitude was selected as the test pulse intensity, and was applied during the kindling session, typically on every other day, just before the application of the kindling stimulation.

EP amplitudes can vary due to changes in behavior, arousal level and other variables, so averaging techniques were used. Twenty biphasic 0.1 msec test pulses were delivered at a frequency of 0.5/sec. Also, an attempt was made to collect EPs while the rat was settled and relaxed. When this was not possible, the accompanying behavioral state was recorded for later interpretation.

The evoked potential responses were fed into a PDP8 computer and averaged. The averaged EP's were plotted by an X-Y recorder (Hewlett-Packard, model 7015A).

## RESULTS

Histology: The distribution of implanted electrodes was determined in 142 rats from the F7 to F10 generations. The amygdala electrodes were well placed in 112 rats (Fig. 5).

### KINDLING PHASE I (SELECTIVE BREEDING)

Kindling rates: Figure 6 shows the amygdaloid kindling rates of all rats tested from the parent generation to the F10 generation. The gradual separation of the two strains over the generations is clearly indicated. Table I summarizes several measures taken during kindling in the three major experimental subgroups.

Regardless of pre-kindling treatments, or intensity of the kindling stimulation, the kindling rates were significantly different between the FAST and SLOW strains. The difference in the kindling rate tended to be larger in groups that were kindled at 400  $\mu$ A than in the groups kindled near threshold.

Motor seizure development: Latency to the onset of forelimb clonus, recorded at the time of first stage 5 seizure (a mean of 9.5 A $\mu$ s for FAST rats and 18.2 for SLOW rats), was determined in 11 FAST rats and 14 SLOW rats from the F9 generation. The average latency for FAST rats was 24.3 sec, whereas SLOW rats had an average latency of 12.7 sec.



Figure 5 The electrode placements for amygdala (A,B,C, & D), hippocampus (E & F), dentate area (G, H, & I for FD<sup>1</sup>, J & K for FD<sup>2</sup>), entorhinal cortex (L, M, N & O) and perforant path (P). The atlas diagrams are from Pellegrino and Cushman (1967).

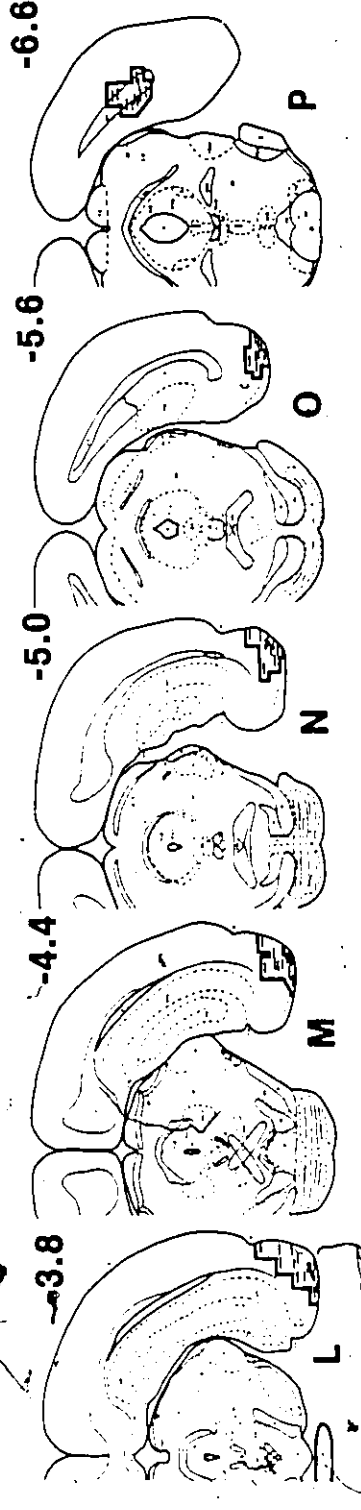
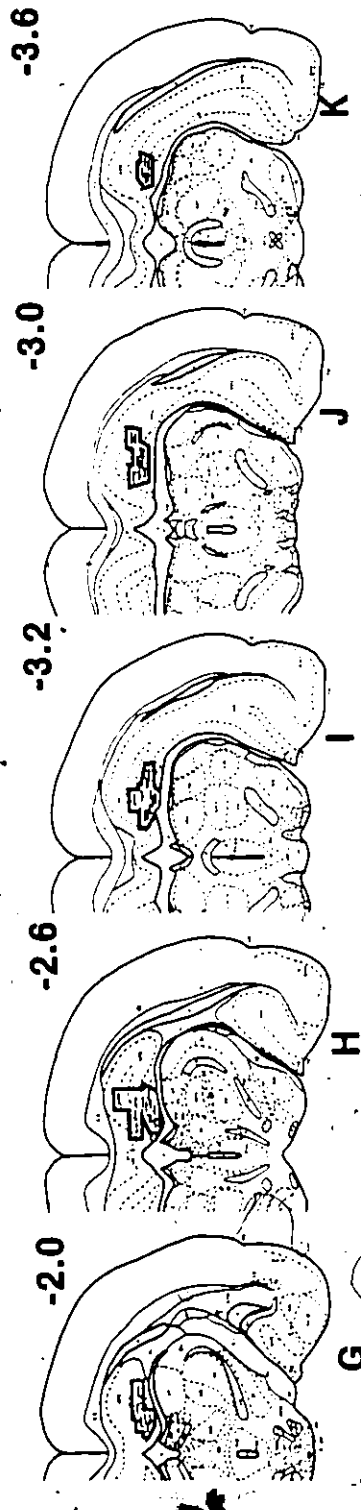
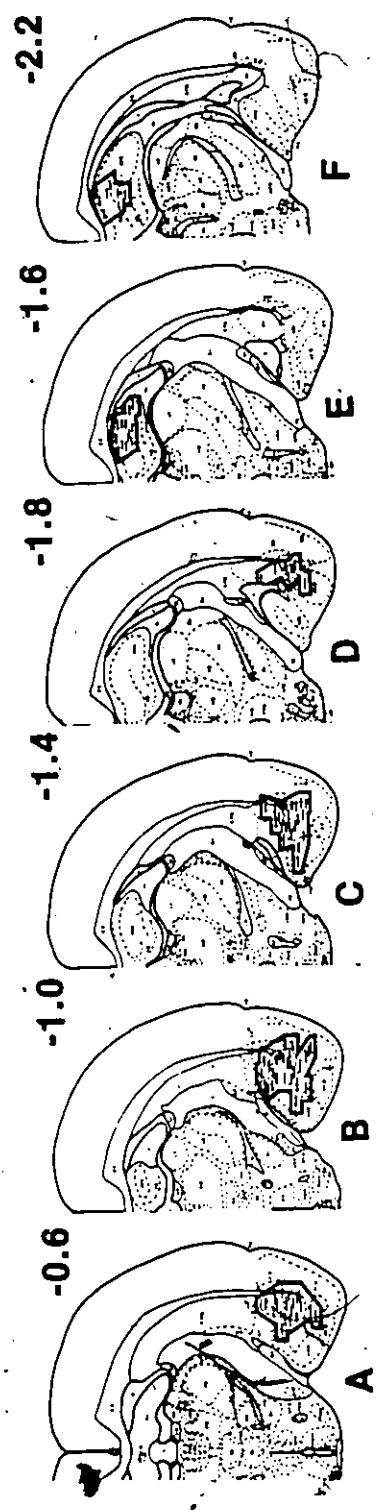


Figure 6 The distributions of amygdala kindling rates are shown for the parent generation (\*), and for FAST ( $\blacktriangle$ ,  $\bullet$ ) and SLOW ( $\triangle$ ,  $\circ$ ) rats for each generation. Rats were kindled at a fixed intensity of 400  $\mu$ A ( $\ast$   $\blacktriangle$   $\triangle$ ) or just above AD threshold level ( $\bullet$ ;  $\circ$ ). Some SLOW rats ( $\blacktriangle$ ,  $\circ$ ) had not yet developed stage 5 seizures when kindling was terminated, so the number of ADs to that point, though an underestimation, was used as their kindling rate. There was no overlap in the distributions for the F6 and F10 generations. The kindling rates of FAST and SLOW rats were significantly different for generations F6 (U=0), F7 (Z=3.612, p<.0002), F8 (Z=3.980, p<.0001), F9 (Z=7.559, p<.0001) and F10 (U=0).

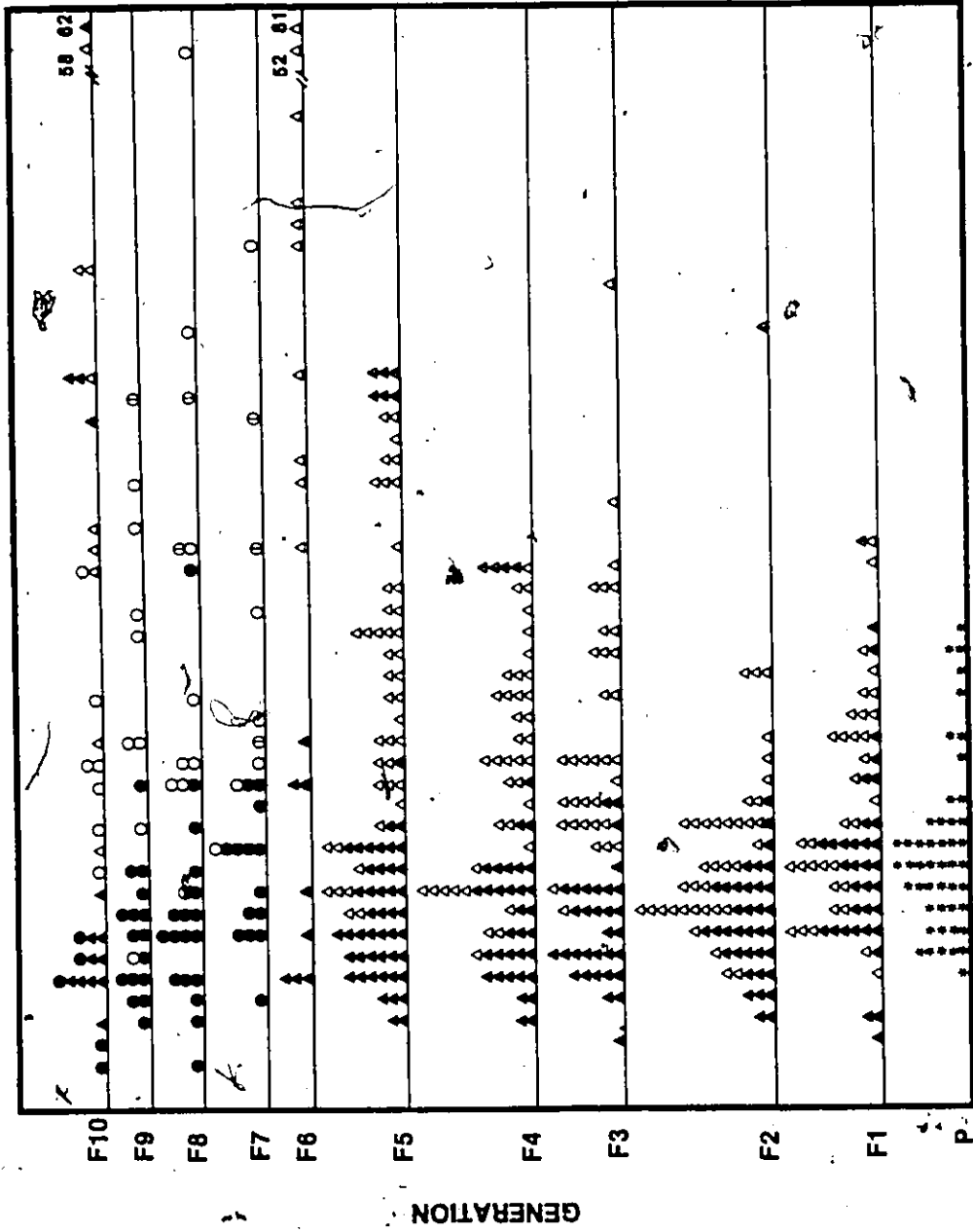


Table I A summary of the major dependent variables, examined during the kindling experiments, are listed for three experimental subgroups. The EEG measurements for all the rats in the potentiation (LTP) group (Chapter IV) and Propranolol group (Chapter V), and a further group with no additional pre-kindling tests ('No pre-K' group) were taken from the stimulated amygdala, or, for half the animals in the 'No pre-K' group, from the ipsilateral entorhinal cortex. Since these two recording sites showed nearly identical values for these measurements, the data were pooled.

Table I

Pre-kindling Treatments	No pre- kindling experiment	LTP then kindling	Propranolol (or NaCl) then kindling* <sup>1</sup>
Kindling Variables			
N (FAST/SLOW)	22 / 13	17 / 13	13 / 13
K-rate (number of ADs)	10.5/23.4	7.1/20.0	8.5/32.5
AD threshold ( $\mu$ A)	62.5/105.8	61.8/61.5	-----
Threshold increase (%)	18.2/15.4	11.8/69.2	-----
First AD duration (sec)	10.0/10.0	21.3/7.7	25.7/27.4* <sup>2</sup>
First #5 AD duration (sec)	49.6/56.9	71.6/64.9	49.3/52.6
Longest AD duration (sec)	86.2/70.2	102.5/86.9	63.9/101.5

\*<sup>1</sup> Kindling at 400  $\mu$ A

\*<sup>2</sup> Second (Non-drugged) AD duration

The difference was statistically significant (Mann-Whitney U-test,  $z = 2.148$ ,  $p < .04$ , two-tailed).

Seventy one percent of the FAST rats examined ( $N = 62$ ) advanced directly from head clonus (stage 2) to stage 4 or 5. A similar rapid progression of the motor seizure was seen in only 23.1% of SLOW rats ( $N = 39$ ). The difference in the proportions of the two strains was highly significant ( $z = 7.773$ ). In addition, 51.5% of the FAST rats showed an immediate loss of postural control, with no preceding rearing component, at least once during the five stage 5 seizures. These animals typically fell on their side immediately following the appearance of forelimb clonus and engaged in clonus of all four limbs. This phase of the convulsion was then often followed by a regaining of postural control, a resumption of forelimb clonus, and the appearance of the more typical rearing and falling sequence. A similar pattern was found in only 33.3% of the SLOW rats. The difference in the proportions of the two strains was significant ( $z = 2.052$ ,  $p < .05$ , two-tailed).

The latency to the falling component, at the time of the 1st stage 5 seizure, also differed between the strains. FAST rats showed an average latency of 46.5 sec, whereas SLOW rats had an average latency of 21.1 sec. The difference was statistically significant ( $z = 2.082$ ,  $p < .04$ , two-tailed test).

Among the rats that were run to a total of 5 stage 5 seizures (47 FAST, 33 SLOW), 70.2% of the FAST rats completed the five stage 5 seizures within five consecutive stimulations. Only 42.4% of the SLOW rats showed a similar sequence, and the difference in the proportions of the two strains was significant ( $z = 3.693$ ,  $p < .0003$ , two-tailed). The

remaining SLOW rats regressed to weaker stages one or more times during this last phase of kindling.

In conclusion, the FAST rats showed faster kindling rates, often advancing directly from stage 2 to stage 5 convulsions, and more reliable stage 5 convulsions during late phases of kindling.

#### KINDLING PHASE II (ELECTROPHYSIOLOGICAL MEASURES)

Primary afterdischarges: Measures of the electrographic responses are summarized in Table I. AD thresholds, measured via an entorhinal electrode in the "LTP group" or via amygdaloid electrodes in the remaining groups, did not differ between the two strains, although there was a tendency for the SLOW rats to have higher AD thresholds.

There was also a tendency for AD thresholds to increase, during kindling, in the SLOW group compared to the FAST group. This threshold rise was only significant in the potentiation group ( $z = 5.582, p < .0002$ ). AD durations were not significantly different either early or late in kindling, but the FAST group achieved their longest AD durations more rapidly than did the SLOW group.

AD amplitude, measured on the first kindling day and on the first stage 5 seizure day, also did not differ between the strains. Other variables such as epileptiform spike frequency and the complexity of the spike waveform appeared comparable in the 2 strains at given stages of seizure development.

Propagation of afterdischarges: Of 22 rats with bilateral amygdaloid electrodes, 17 (12 FAST and 5 SLOW) were found to have both electrodes accurately placed within the amygdaloid complex. These



animals provided information about AD propagation between the two amygdalae. All but two rats showed clear evidence of propagated ADs in the contralateral amygdala recordings within 8 kindling stimulations. The remaining 2 were from the SLOW group and required 13 and 23 stimulations before bilateral amygdaloid ADs became evident. Due to the limited data available from SLOW rats and the apparent overlap on these measures it remains inconclusive whether SLOW rats tend to develop bilateral discharges more slowly than FAST rats. Once primary site kindling was complete, transfer kindling in the contralateral site showed no differences between the two strains.

The propagated entorhinal (ipsilateral) discharge was evident on the first kindling trial in 100% of the rats (19 FAST, 15 SLOW). The propagated ipsilateral hippocampal discharge was clearly seen on the first kindling day in 59% of the rats (no difference between the 23 FAST and 16 SLOW), and there was no difference in the remaining rats in the number of ADs required before the appearance of propagated discharge in the hippocampus.

It appears that the rate of development of discharge duration, amplitude and propagation, as measured here, does not correlate well with kindling rates. It is possible, however, that the recruitment of the brain structures tested had little effect on the subsequent development of convulsive responses. Other sites (e.g., brainstem sites) may prove to be more critical. Also, it may not be possible to determine from EEG measures the extent to which a structure participating in the epileptogenic activity; the spikes may be large but the output may still be relatively weak.

Pre- and post-ictal spikes: Additional EEG recording was done for some of the rats (16 FAST, 9 SLOW) from the F9 generation to allow the observation of pre- and post-ictal spontaneous spikes. EEG recording was taken for 2 min prior to the delivery of the kindling stimulation. Similarly, 2 min of EEG was recorded postictally.

Four FAST rats (25%) showed pre-ictal spikes after 7 to 9 kindling stimulations, and developed stage 5 seizures after an average 8.5 ADs. On the other hand, 8 SLOW rats (89%) exhibited such spikes, after 8 to 16 kindling stimulations, and reached the stage 5 seizure with an average of 21.9 ADs. The remaining rats showed no pre-ictal spiking activity during the recording session. With these methods, pre-ictal spikes were more likely to be seen in SLOW rats ( $z = 5.972$ ), but the timing of their appearance was not associated with the development of convulsive activity and did not predict the kindling rates. All rats from both strains showed 2 or more post-ictal spikes after 2 to 8 kindling stimulations. The average latency to the first spike was 54 seconds from the offset of the ictal discharge. The time of appearance of post-ictal spikes did not correlate with kindling rates.

The development of pre- and post-ictal spiking appears to be determined by the number of AD's rather than the extent of development toward convulsive responses. The post-ictal spikes appear within a week of daily kindling, and pre-ictal spikes appear in the second week, regardless of kindling rates.

Secondary afterdischarges: Secondary ADs were measured in 52 FAST and 39 SLOW rats. 39 FAST (75.0%) and 20 SLOW (51.3%) rats were found to show secondary ADs emerging after the clear termination of the

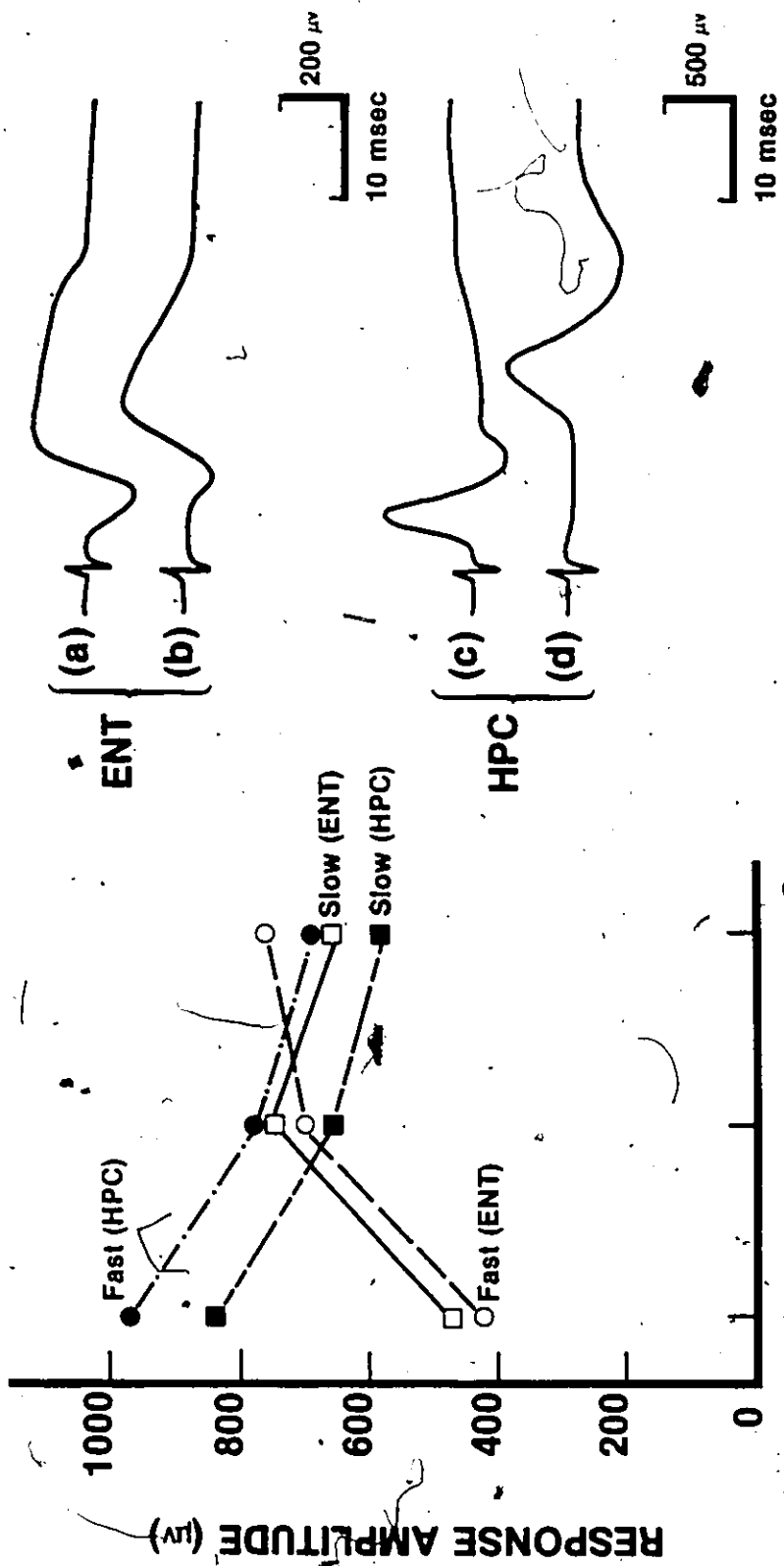
primary ADs. At least 3 sec separated the two discharges. The first incidence of secondary AD occurred on the 9th kindling day for the FAST group and the 15th kindling day for the SLOW group (median values). The difference was not statistically significant. Both groups showed comparable secondary AD duration as well (median values of 14.5 sec vs. 19.0 sec for FAST and SLOW respectively). The latency to the onset of the secondary AD, however, differed significantly between the groups. FAST rats took 17.0 sec whereas SLOW rats took 33.5 sec (both median values, U-test;  $z = 2.196$ ,  $p < .05$ , two-tailed), suggesting that FAST rats had a shorter or weaker post-ictal depression.

Evoked potentials: A typical response evoked in the entorhinal cortex by pulse stimulation of the ipsilateral amygdala is shown in Figure 7a. The amplitude of the first peak from the pre-artifact baseline was measured on pre-AD1, pre-stage 5 (the last average taken before appearance of stage 5 seizures), and post-stage 5 days. A second type of entorhinal response is shown in Figure 7b. Among 10 FAST and 7 SLOW rats, both types of EPs occurred with roughly equal frequency.

As is shown in Fig. 7 (broken lines), there is little difference between the strains with respect to either pre-kindled baseline amplitudes or to the degree of kindling-induced potentiation of the response. Regardless of kindling rates, or the number of ADs experienced by each rat, both strains reached comparable levels of EP potentiation (about 60% above baseline). This increase in EP amplitude was evident just prior to the first stage 5 seizure.

The responses evoked in the hippocampus by amygdala stimulation fell into 2 categories based on the morphology of the waveform. These

Figure 7 Average EP amplitudes before (Pre AD1), during (Pre S5) and after completion of kindling. The amplitude of entorhinal EPs (open symbols) increased for both FAST (N=10) and SLOW (N=7) rats. The two groups showed comparable increases, but the FAST group required only 9.0 ADs, whereas the SLOW group required 16.7 ADs, to reach the stage 5 seizure level. The amplitude of hippocampal EPs (closed symbols) showed a kindling-induced decrease in both FAST (N=7) and SLOW (N=7) rats. The FAST rats reached stage 5 seizures, and the accompanying change in EP amplitude, after 7.4 ADs compared to 19.7 ADs in SLOW rats. These data were obtained from rats showing stable EP responses that fell into 2 categories for both the entorhinal (a and b) hippocampal (c and d) placements.



PreAD1 PreS5 Post S5

SEIZURE STAGE

patterns are shown in Figures 7(c and d), and were found to occur roughly equally between the strains. Seven rats from each strain provided reliable EPs throughout the kindling session.

Both strains showed a decrement of about 20% in EP amplitude just prior to the stage 5 seizure, and a further decrement of 10% by the time the stage 5 seizure was established. Again, these effects did not correlate with kindling rates.

#### CONCLUSIONS AND DISCUSSION

The selective breeding procedure successfully produced two strains of rats that differed significantly from each other in terms of their amygdaloid kindling rates. FAST rats reached the full seizure stage within a week or so, while the SLOW rats typically reached the same stage only after 3 or more weeks of kindling. The 2 strains did not differ on most of the measures of the electrographic discharge either early or late in kindling. Nor did they differ on selected evoked response measures early or late in kindling. The fact that the strains did not differ in initial AD durations, AD thresholds, AD spike amplitudes or baseline evoked response amplitude, indicates that the FAST animals did not start out in a state that could be considered as 'partially kindled'. It appears more likely that the difference between the strains lies in one or more of the mechanisms that determine the rate at which kindling proceeds. The fact that spontaneous interictal spiking develops after the same number of discharges in the 2 strains, suggests that the difference in rate of kindling may not be due to focal

mechanisms but rather to the recruitment of distant structures. The apparently greater inter-ictal spike frequency seen in SLOW animals, once spiking appeared, may have been due to behavioral differences between the strains. FAST rats, for example, might have been more active and aroused during the kindling sessions compared to SLOW rats. This would have the effect of suppressing spontaneous spike activity, which is known to increase in frequency when the animals are relaxed and drowsy.

FAST rats did differ from SLOW rats in terms of motor seizure manifestation, latency to clonic seizures, frequency of spontaneous spiking, latency to the secondary ADs and seizure suppression during the last phase of kindling.

FAST rats were more likely to advance abruptly from stage 2 to stages 4 or 5, and often engaged in full body clonic seizures with hindlimb clonus. FAST rats showed a longer latency to the onset of forelimb clonus and falling compared to SLOW rats. FAST rats had a shorter latency to the onset of the secondary ADs. Finally, the majority of FAST rats completed five stage 5 seizures within five days without a single failure, whereas more than half of the SLOW rats showed seizure suppression (failure of full seizure) at least once. The longer latency to secondary discharge and the greater occurrence of failure of stage 5 seizures in the SLOW animals raises the possibility that certain inhibitory systems may be stronger, or less susceptible to failure, in the SLOW vs. the FAST animals.

It is known that kindling stimulation produces powerful potentiation effects in the neural pathways that are activated by the

stimulation. FAST rats may be more susceptible to such electrophysiological alterations. The previous experiments showed that comparable levels of kindling-induced potentiation were achieved in the 2 groups, although the rate at which these effects were produced differed between the strains.

These data then raise two hypotheses: 1) FAST animals are deficient, compared to SLOW animals, in the function of one or more inhibitory systems; 2) FAST animals show a more rapid potentiation as a result of driving certain excitatory synaptic systems (e.g. the monosynaptic pathway between the entorhinal cortex and dentate gyrus).

These two hypotheses do not exhaust the list of possible underlying mechanisms, but they are the 2 most common hypotheses expressed in the kindling literature and they are, at least to some extent, testable. Experiments designed to test these hypotheses are described in chapters IV and V.



## CHAPTER III BEHAVIORAL COMPARISONS

### INTRODUCTION

Three approaches have been taken to the study of behavioral correlates of kindling. One is to study the effect of kindling on behavior (e.g. Adamec, 1975; Boast & McIntyre, 1977; Bawden & Racine, 1979; Frenk & Yitzhaky, 1981; Pinel, Treit & Rovner, 1977, etc.), and another is to measure kindling rates in behaviorally different strains (e.g., Leech & McIntyre, 1976; Racine, Burnham, Gartner & Levitan, 1973; Zaide, 1974). The third approach is a correlational study of pre-kindling behavioral measures and subsequent kindling measures (Ossenkopp & Sanberg, 1979).

Ossenkopp and Sanberg (1979) found that rats which urinated more often in the open-field tests, prior to amygdaloid kindling, tended to show longer latencies to the onset of clonic convulsions. In a previous study (Sanberg and Ossenkopp, 1978), they reported a positive correlation between kindling rates and latency to onset of convulsion, so slow kindlers would presumably also urinate more often in their open field test.

Pinel et al. (1977) reported that rats kindled in amygdala or hippocampus were more reactive to certain stimuli and reacted more aggressively to handling. Caudate kindling did not have the same effect. Rat-killing behavior was found to be reduced in cats when amygdaloid AD threshold was lowered (Adamec, 1975), but muricide in rats was either not affected (Bawden & Racine, 1979), or slightly facilitated by kindling (McIntyre, 1978).

Pain threshold, measured by latency to tail-flick in response to radiant heat stimulation, was found to be elevated in amygdala kindled rats (Frenk & Yitzhaky, 1981). This effect was evident at 2 min, 24 hrs and 7 days after kindling and then disappeared. The early onset suggests that the effect may have been initiated by an immediate enkephalin secretion, triggered by the amygdaloid ADs, and then maintained by inter-ictal focal activity.

Boast and McIntyre (1977) reported that bilateral amygdaloid kindling impaired the subsequent acquisition of one-trial avoidance behavior in rats. This finding was in agreement with an earlier study (McIntyre & Molino, 1972) which demonstrated that the combination of a lesion in one amygdala and kindling of the contralateral amygdala impaired subsequent acquisition of conditioned emotional responses (CER). This "functional lesion" effect on CER learning was peculiar to amygdaloid kindling; cortical kindling did not produce such an effect unless the kindled seizure had reached the generalized 'limbic' seizure stage (McIntyre, 1970; 1979).

On the basis of these reported correlates, it is reasonable to suspect that rats that are different in their susceptibility to the kindling treatment may behave differently on selected behavioral tasks. Consequently, several behavioral measures were taken for the FAST and SLOW strains; including open-field behavior, muricide, hot plate pain sensitivity and passive avoidance behavior. Finally, both FAST and SLOW rats were tested for their sensitivity to audiogenic seizure stimulation.

## METHODS

OPEN-FIELD STUDY

Twenty-six male and female rats (9 FAST, 17 SLOW) were randomly selected from the F1 generation and tested individually in an open field (75 x 75 x 9 cm). Their reactions to the experimenter's attempt to capture them by hand were rated according to the categories of "no resistance", "squealing", and "biting".

Another 23 male rats (8 FAST, 15 SLOW) from the F1 generation were observed in an open field of 90 x 90 x 45 cm with black stripes dividing the field into a 4 by 4 grid. Each rat was placed in the corner of the field, and the number of moves across grid squares was recorded for 2 minutes.

From the F3 generation, 66 male and female rats (33 FAST, 33 SLOW) were randomly selected, and tested on the same open field. Each rat was placed in the corner of the field and observed for two minutes for 3 consecutive daily trials. The occurrence of vocalizations during removal from the cage was recorded. The number of boluses and the incidence of urination during the observation period were also recorded as was the number of grid squares where the rat placed at least both forelimb paws.

Finally, 64 male and female rats (20 FAST, 20 SLOW, 24 CONT) were randomly selected from the F6 generation, and tested for their reaction to handling, acquisition on a passive avoidance task, and mouse killing behavior.

All these rats were first taken out of their group cages and placed into individual wire cages. Twenty four hrs later, each rat was

taken out of the wire cage and placed in an open field (75 x 75 x 9 cm). At 5, 10, and 15 seconds after placing the rat in the field, the experimenter attempted to grasp the rat. The rat's reaction to the experimenter was scored on each of the three trials according to following scale: 0, little or no avoidance; 1, avoided, struggled and/or squealed while being grasped but ceased when in hand; 2, avoided, struggled and/or squealed for several seconds; 3, avoided, struggled and/or squealed continuously; and 4, same as 3 but also showed biting of the glove. In order to ensure reliable measures, scoring was done by two independent observers. These tests were repeated over the next two days, for a total of 9 trials.

#### MURICIDE TEST

The same 64 rats from the F6 generation that were subjected to the handling test were later tested for mouse-killing in their individual home cage. An experimentally naive mouse was placed in the cage for 15 minutes, and the latencies for the rat to approach (physically contact) and to kill the mouse were recorded together with the location of the bite(s) delivered. These measures were also taken by 2 independent observers.

#### HOT PLATE PAIN SENSITIVITY TEST

A total of 61 FAST rats (33 male and 28 female) and 60 SLOW rats (32 male and 28 female) were randomly selected from the F7 generation. Animals were coded to mask their strain identity. The weight of the rat and the number of siblings caged together were recorded for later analyses and interpretation of the results.

The hot plate apparatus was made of a rectangular copper container (19 x 19 x 15 cm) in which a 1200 ml mixture, consisting of equal parts by volume of acetone and ethyl formate, was kept boiling. A plastic cylinder (15 cm in diameter, 50 cm high) was placed on the surface of the vessel. The temperature at the surface of the copper container (now the floor of the cylindrical tube in which rat was placed) was constantly monitored and maintained at 54.5 °C. Pain sensitivity was assessed by placing the rat, inside the tube, on top of the vessel surface and recording the latency to either the first paw licking or the first jumping response.

The experiment was replicated as described with 110 FAST rats (33 male and 77 female) and 118 SLOW rats (32 male, 86 female) randomly selected from the F8 generation, together with 83 control rats (46 male, 37 female).

#### PASSIVE AVOIDANCE TASK

The same rats tested on the muricide experiment were subsequently tested on the passive avoidance task (19 FAST, 20 SLOW, 24 CONT).

Each rat was placed on a retractable wooden platform (15.5 x 11 x 5.5 cm) within a plywood box (34 x 34 x 29 cm). As soon as the rat stepped off of the platform with all 4 limbs onto the scrambled electric grids, a 1.6 mA shock was delivered for 3 sec, while the safe platform was retracted. Thus, all rats received shocks of the same intensity and duration. After receiving the shock, the rats were returned to their cages. On the following day, the rats were placed on the same wooden platform with no electric shock delivered. The latency for each rat to

step off of the platform and onto the grids with four limbs was measured. If no response was seen within 300 sec, the observation was terminated.

Another 30 male rats (15 FAST, 15 SLOW) were randomly selected from the F7 generation and coded to mask their strain identity. They were subjected to the same procedure, except that the shock intensity was increased to 3.5 mA. The latency for each rat to step off the platform was measured on each of the following 3 days. The experiment was partly replicated with another 14 male rats (7 FAST, 7 SLOW) randomly selected from the F9 generation, together with six male control rats. The procedure was the same except that the shock intensity was reduced to 1.0 mA for 2.0 sec, and the effect of the shock was measured only once on the following day.

#### AUDIOGENIC SEIZURE TESTS

Twenty-three FAST rats (9 male, 14 female) and 26 SLOW rats (11 male, 15 female) were randomly selected from the F7 and F8 generations. The rats were randomly coded and the measurements were done blind.

Rats were placed in a glass cylindrical container (30 cm in diameter, 30 cm high) with sawdust bedding. An electric door bell attached to the lid of the container generated a sound level of approximately 110 db measured at the bottom of the inside of the container. This stimulus was continued until the onset of convulsion or for 120 sec. The evaluation of convulsive behavior was made according to a standard audiogenic response scoring system (Jobe, Picchioni & Chin, 1973). Rats that failed to show convulsive behavior on the first day were tested three more times. In order to obtain objective scoring,

two assistants provided additional independent measurements during this test.

## RESULTS

### OPEN-FIELD STUDY

Of the 26 rats tested from the F1 generation, 77.8% of the FAST rats (7/9) reacted with biting when grasped by the experimenter. Only 41.2% of the SLOW rats (7/17) attempted to bite, and a test for significance of difference between two proportions indicated that the two groups were significantly different on this measure ( $z = 2.897$ ,  $p < .005$ , two-tailed).

The open-field observation of another group of F1 rats showed that eight FAST rats tested entered an average of 3.0 squares, significantly less than the 5.1 squares entered by 15 SLOW rats ( $z = 2.024$ ,  $p < .05$ , two-tailed U-test).

F3 rats ( $N = 68$ ), however, did not show any difference in the number of squares entered. They did show a difference in vocalization in response to the experimenter's handling. More FAST rats squealed on days 2 and 3 (61.8% and 55.9%, respectively), compared to SLOW rats (35.3% and 38.2%, respectively), and the test for significance of difference between two proportions confirmed the difference ( $z = 3.206$ ,  $p < .002$  and  $z = 2.101$ ,  $p < .04$ , two-tailed, respectively). Another striking difference between the groups was found in the incidence of defecation and urination (Fig. 8). There were fewer FAST rats that defecated in the open field during the three daily trials (31.4%, 20.0% and 37.1% respectively), compared to SLOW rats (61.8%, 70.6% and 82.4%

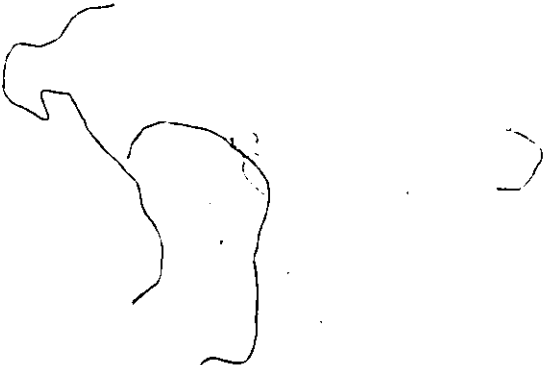
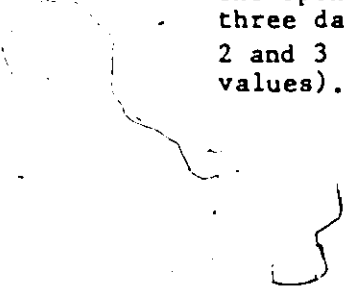
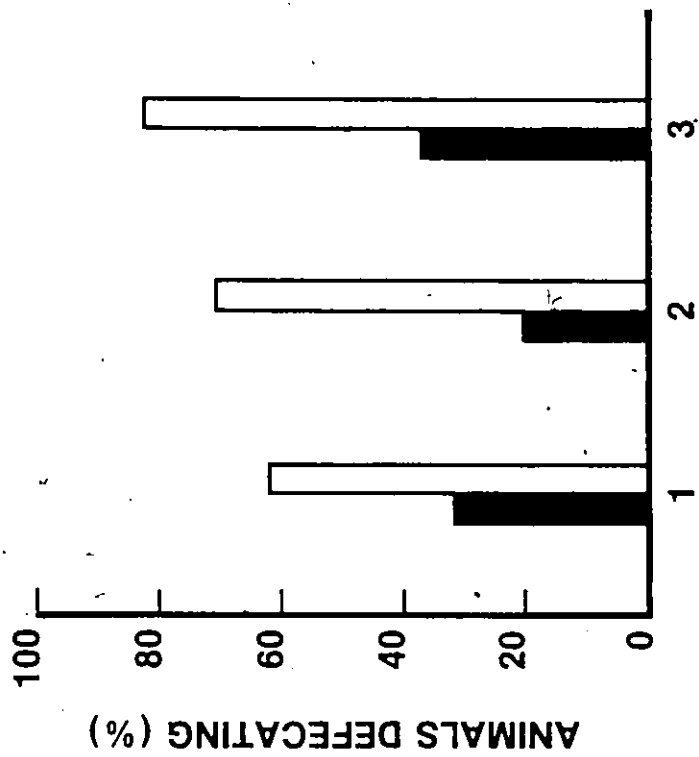
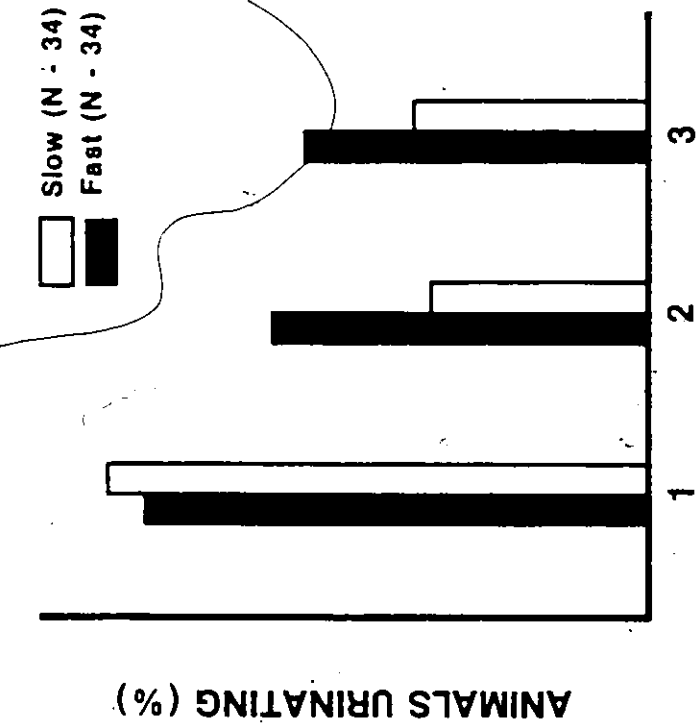


Figure 8 (a) The proportions of rats that defecated at least once during the open-field observation test (two minutes each day for three days). The differences seen between the groups on all three days were highly significant (see text for p values).

(b) The proportions of rats that urinated at least once during the open-field observation test (two minutes each day for three days). The differences seen between the groups on days 2 and 3 were statistically significant (see text for p values).







TEST DAY

respectively). The difference was highly significant ( $z = 3.758, 6.933$  and  $6.117$ , respectively).

On the other hand, there were more FAST rats that urinated on days 2 and 3 (61.8% and 55.9%, respectively), compared to SLOW rats (35.3% and 38.2%, respectively). This difference was also significant ( $z = 3.206$  and  $2.101$ , respectively).

Finally, 40 rats from the F6 generation, together with 24 control rats, provided further information on behavioral differences between the FAST and SLOW groups. The reaction to the experimenter's handling again proved to be different in FAST compared to SLOW rats. More FAST rats reacted with biting (7/20) than SLOW rats (1/20) on the first test trial. The difference was statistically significant ( $z = 3.618, p < .0004$ ), but disappeared on the second trial 5 seconds later (FAST; 1/20 vs. SLOW; 0/20). The control rats (1/24) were similar to SLOW rats, suggesting that FAST rats were unusually reactive to handling on the first trial.

#### MURICIDE TEST

Among 64 rats tested, 13 showed mouse-killing behavior. FAST rats were more likely to kill mice (7/20) than SLOW or control rats (3/20 and 3/24, respectively). The differences between FAST and SLOW or control rats were statistically significant ( $z = 2.123, p < .03$  and  $z = 2.571, p < .02$ , respectively, two-tailed test). The latency to approach the mouse did not differ between FAST rats ( $\bar{X} = 12.2$  sec) and control rats ( $\bar{X} = 17.4$  sec), but SLOW rats showed a significantly longer latency to approach the mouse ( $\bar{X} = 95.3$  sec,  $z = 2.789, p < .006$  against control).

SLOW rats, then, approached the mouse with a longer latency but killed as readily as control rats, whereas FAST rats approached the mouse somewhat more rapidly than controls (though the difference did not reach significance) and were more likely to kill the mouse.

#### HOT PLATE PAIN SENSITIVITY TEST

The results are shown in Table II. FAST animals had a significantly shorter latency before licking of the paws or jumping, compared to control animals ( $t_{252} = 5.24$ ,  $p < .001$ , two-tailed), and control rats had, in turn, shorter latencies than SLOW rats ( $t_{259} = 2.24$ ,  $p < .05$ ). Body weights did not differ between the strains.

#### PASSIVE AVOIDANCE TASK

FAST rats from the F6 generation were less likely to step down on the test day (8/19), compared to SLOW or control rats (13/20 and 14/24, respectively). The difference between FAST and SLOW rats was statistically significant ( $z = 2.083$ ,  $p < .04$ , two-tailed test).

There were no significant differences, however, when F7 rats ( $N=30$ ) were tested with a higher intensity shock (3.5 mA for 3.0 sec), or when F9 rats ( $N = 20$ ) were tested with a lower intensity shock (1.0 mA for 2.0 sec) (Fig. 9).

#### AUDIOGENIC SEIZURE

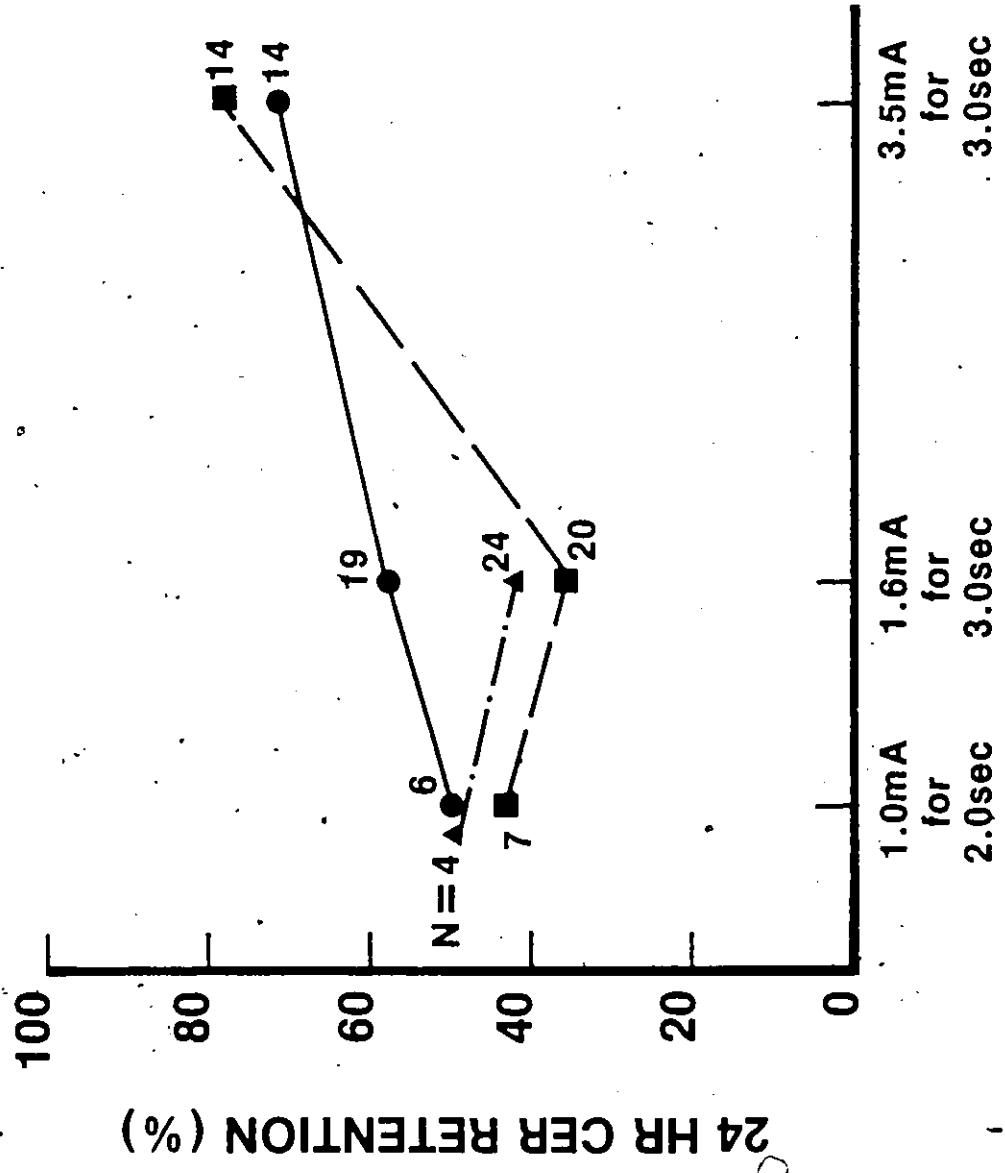
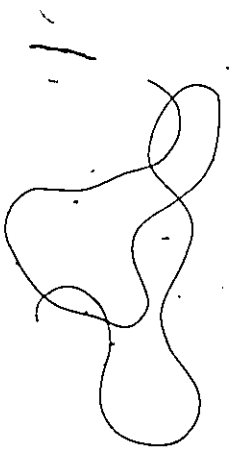
In response to a high intensity sound, applied for four daily trials, both strains showed a comparable ratio of seizure incidence (Table III). The differences in seizure susceptibility of the 2 strains, then, did not generalize to seizures that are presumed to be of brainstem origin.

Table II A summary of the response latencies (paw-licking/jumping) to thermic pain. Latencies for FAST rats were significantly shorter than for control rats ( $p < .001$ ) whose latencies were significantly shorter than SLOW rats ( $p < .05$ ).

Table II

Strain Sex	FAST		SLOW		CONT	
	M	F	M	F	M	F
Gen.	SEC (N)					
F7	9.1(33)	10.7(28)	12.9(32)	14.1(28)	12.8(46)	10.4(37)
F8	9.6(33)	8.4(77)	11.0(32)	13.6(86)		
Total	9.2(171)		13.1(178)		11.7(83)	

Figure 9 A summary of the data from the passive avoidance test is shown opposite. At the intermediate shock intensity level (1.6 mA for 3.0 sec), more FAST rats (●) showed the avoidance response (in the step down task) than SLOW rats (■). The difference in proportions was significant ( $Z=2.083$ ,  $p<.04$ , two-tailed). At lower and higher intensities, however, the two groups behaved comparably).



SHOCK INTENSITY

Table III The effect of administration of an epileptogenic auditory stimulus. Although SLOW rats were more than twice as likely to show epileptic effects than FAST rats on this test, the difference was not statistically significant.



Table III

Seizure	Tonic (a)	Running (b)	No Effect	(%) (a) + (b)
Strain				
FAST	1	0	8	8.7
	0	1	13	
SLOW	0	1	10	19.2
	1	3	11	

## CONCLUSIONS AND DISCUSSION

One of the most consistent findings was that FAST rats were excessively reactive. They squealed when grasped by an experimenter, and their tendency to bite the glove was demonstrated in two experiments. FAST rats also responded to thermal pain with a shorter latency, suggesting that they may be less tolerant to stress in general, compared to SLOW and control rats. The biting reaction of FAST rats may also be related to their increased aggression. They were more than twice as likely to kill mice than were SLOW or control rats. These findings were in agreement with the verbal reports of all of the people who have dealt with these two strains, that FAST rats are "jumpy", "hyperreactive" and "aggressive."

FAST rats were less likely to defecate on all three consecutive daily trials in an open field test, and yet they were more likely to urinate on days 2 and 3. Although defecation and urination have been used as correlated measures of emotionality, these observations indicate that the two behaviors may reflect quite different underlying processes. In support of this conclusion is the fact that both groups showed a steady 3 day increase in the number of rats showing defecation and a decrease in the number showing urination. The SLOW group showed the changes more rapidly than did the FAST animals. The 2 strains may, then, differ in the rate of habituation, with the FAST rats remaining hyperreactive to the stress of being placed in a novel environment.

Previous studies have reported that rats that urinate and defecate more frequently (e.g. Tryon maze brights - Searle, 1949; Powell, Martin & Kamano, 1967) tend to be more resistant to seizures

(Zaide, 1974). This correlation was also found in other strains by Martin and Hall (1941). Our defecation measures were consistent with these findings, with the seizure resistant SLOW rats showing more defecation in the open field measures. Our other measures (e.g. urination, response to handling and pain, etc) however, indicated that FAST rats were more emotionally reactive than SLOW rats.

Finally, FAST rats did not differ in susceptibility to audiogenic seizures, suggesting that the difference in seizure susceptibility did not extend to those brainstem systems activated by high intensity auditory stimulation.

The amygdala is known to contain relatively high concentrations of opiate binding sites and endogenous opioids (Hökfelt, Elde, Johansson, Terenius & Stein, 1977; Simantov, Kuhar, Uhl & Snyder, 1977). One study with rats (Uhl, Kuhar & Snyder, 1978) indicated that there is an enkephalin-containing fiber system that arises from the central nucleus of the amygdala, and sends output via the stria terminalis. Abott and Melzack (1978) demonstrated that ADs evoked in the lateral septal nucleus and hippocampus (but not in amygdala or cortex) produced a transient (15 min) analgesic effect. The effect was not seen if the rats had previously been kindled in the lateral septum. Frenk and Yitzhaky (1981), however, found that kindling stimulations applied to the amygdala also produced a transient analgesia. Furthermore, there is some evidence that amygdaloid kindling produces tolerance to the analgesic effects of morphine (Yitzhaky, Frenk & Urca, 1982), and reduces the severity of the morphine withdrawal syndrome (Le Gal La Salle & Lagowska, 1980). Other studies (Hong, Wood, Gillin, Yang &

Costa, 1979; Hong, Gillin, Yang & Costa, 1979) indicate that seizures increase opioid levels (up to 300% of normal level) for as long as 14 days.

The opioids may be involved in secondary, rather than primary, effects of kindling, as the manipulation of opioid systems by naloxone did not alter the rate of amygdaloid or caudate kindling itself (Corcoran & Wada, 1979). The difference in pain sensitivity between the strains, then, may reflect mechanisms secondary to epileptogenesis and may be due to a difference in the reactive secretion of opioids in stressful situations.

## CHAPTER IV ELECTROPHYSIOLOGICAL STUDIES

### INTRODUCTION

#### Rationale for electrophysiological studies

One of the hypotheses proposed to explain the physiological mechanisms of kindling is that kindling stimulation produces long-term potentiation (LTP) of synaptic transmission in excitatory pathways leading from the kindled site.

It is now certain that kindling does produce powerful LTP effects (Oshima, 1981). A causal relationship between the LTP effect and kindling has not, however, been proven. Recent evidence, in fact, suggests that changes in the response characteristics of cells in kindled foci may be more important (Racine et al., 1981). Nevertheless, kindling does produce LTP and prior development of LTP in forebrain pathways has been shown to facilitate subsequent kindling (Racine, Newberry & Burnham, 1975).

In the preceding chapters, it was shown that FAST rats kindled significantly faster than SLOW rats. It was also noted that FAST rats showed alterations in the amplitudes of evoked responses faster than SLOW rats. These alterations in EP amplitude could reflect an underlying difference in potentiation mechanisms or of some other correlates of the developing epileptogenesis (e.g. a change in output from stimulated cells). In order to examine the role of potentiation effects in seizure susceptibility, the two strains of rats were tested on several potentiation paradigms involving non-epileptogenic stimulation.

### Selection of pathways for potentiation studies

Most forebrain potentiation experiments have focused on pathways into the hippocampal formation, particularly the perforant path fibers from the entorhinal cortex to the dentate gyrus.

When a train of high frequency stimulation is applied to the entorhinal cortex or perforant path, subsequent responses evoked by single pulse stimulation of that pathway are temporarily increased in amplitude (Fig. 4). These field potential responses have been shown to reflect the intracellular events of granule cells fairly accurately. The rising phase of the first component has been shown to reflect collective EPSPs, whereas the sharp negative component (Fig. 4) depends upon the number of cells in which action potentials have been triggered (Lomo, 1971).

For the purpose of the present project, potentiation effects were examined in 2 pathways, the monosynaptic pathway from amygdala to entorhinal cortex (Experiment 1) and the monosynaptic pathway from entorhinal cortex to dentate gyrus (Experiment 2). Both short-term potentiation (STP) and long-term potentiation (LTP) phenomena were examined and compared between the strains.

#### EXPERIMENT 1: AMYGDALA TO ENTORHINAL POTENTIATION

One of the output pathways from the amygdala arises from the lateral and basolateral nuclei and projects to layer III of the ventral part of the lateral entorhinal cortex (Krettek & Price, 1977). This latter neural circuit was chosen for the electrophysiological studies because it is the primary route via which the amygdala communicates with

the hippocampus and because it has been shown to support long-term potentiation (Racine et al., 1983).

Several questions were addressed in these experiments. One was whether the amygdala of FAST rats would require lower intensity stimulations to evoke EPs in the entorhinal area compared to SLOW rats. If so, then kindling stimulations might be expected to affect target sites more strongly, or to affect a larger target area, in FAST rats. In order to test these baseline responses, and to determine thresholds and asymptotic response levels, input/output curves were determined. Such curves were obtained for entorhinal EPs by stimulating the amygdala at various intensities ranging from just above threshold to that required to evoke the maximum amplitude response. The threshold and maximal intensities, as well as the slope of the I/O curves, were used as indices of baseline excitability.

The next set of experiments were designed to test the responsivity of the system (i.e. how the system responds to repeated stimulation). The first experiment was designed to test paired pulse effects. If 2 identical pulses are applied to a pathway, the response evoked by the second pulse will often be smaller or larger than the response to the first pulse. In most pathways the second response will initially be depressed (at interpulse intervals of 5 to 30 msec) after which a period of facilitation is evident. The facilitation can last for several hundred msec after which the responses may again be depressed (for up to several sec). The first period of depression is likely due to recurrent inhibition (Adamec, McNaughton, Racine & Livingston, 1981), while the facilitation may be due to an increase in

transmitter release (Wigström, McNaughton & Barnes, 1982). It is possible that the FAST rats are capable of producing more facilitation and/or less recurrent inhibition effects. The paired pulse measure provides us with an initial test of this hypothesis.

The next experiment was designed to measure differences in response to high frequency trains of stimulation. In particular, differences in long-term potentiation (LTP) were examined in the two strains. The hypothesis was that FAST rats would show a greater amount of LTP, reach asymptotic levels sooner or show longer retention of the effect than SLOW rats. This would result in a more effective transmission and propagation of epileptiform activity. In order to test this hypothesis, brief trains of stimulation pulses were applied to the amygdala, and the entorhinal EPs (evoked by single pulses applied to the amygdala) were tested before and after application of the trains.

#### METHODS

Animals: Fifty-two male hooded rats (28 FAST, 24 SLOW), weighing 300.0 g to 450.0 g were randomly selected from the F7 to F9 generations. Bipolar electrodes were implanted into the basolateral amygdala, the lateral entorhinal cortex, hippocampus, and the dentate gyrus (FD1). These placements were confirmed histologically and are shown in Fig. 5.

All electrodes, together with one ground screw implanted in the skull just posterior to lambda, were connected to male amphenol pins and collected into a 9 pin head cap. Subjects were given a two week



recovery period during which they were handled regularly and introduced to the experimental environment.

Apparatus: Two stimulators (Grass S 88) were used to generate stimulation pulses and trains. One of the stimulators also triggered a dual beam oscilloscope (Tektronix, model D 13) for the purpose of monitoring the responses, and a PDP 8 computer for the purpose of data acquisition and averaging of the responses. The stimulation was delivered through photoelectric stimulus isolation units (Grass, model PSIV 6B) to obtain isolation from ground as well as constant current outputs.

The EEG responses were delivered to a wide band A.C. EEG pre-amplifier (Grass 7P5A). After amplification and computer averaging, a display unit (Tektronix 602) was used to monitor the currently averaged responses. The final averaged outputs were plotted on graph paper by an X-Y recorder (Hewlett-Packard 7015A).

I/O curves: Immediately after the recovery period, two sets of input/output curves for each rat were assessed by stimulating first the amygdala and then the entorhinal cortex. Measurement of the amplitudes of the EPs recorded from the target sites provided the data from which the I/O curves were constructed.

The test responses were evoked by single biphasic square wave pulses, (0.1 msec pulse duration), and 20 pulses were delivered, at a frequency of 0.2/sec, at each intensity. The threshold intensity inducing a just detectable response from the immediate target site (entorhinal cortex for amygdaloid I/O curves, hippocampus for entorhinal I/O curves), and the intensity inducing the maximum response amplitude,

were first assessed visually on the oscilloscope by a trial-and-error method. Then the range between the two intensities was divided into 5 steps, which provided 6 points for the I/O curves: threshold, 20%, 40%, 60%, 80% and 100% of the maximal intensity. Twenty EPs were collected and averaged at each of the six intensities. The behavioral status of the animal was also recorded for each EP average.

The test pulse intensity for the facilitation and potentiation experiments was determined according to the I/O curves. The intensity that induced 80% of the maximal EP amplitude was chosen for the test pulses, because it was strong enough to evoke reliable EPs while allowing for another 20% increase before the pretrain maximal response was reached. It was not known whether the potentiating trains could increase EP amplitudes above this pre-train asymptote. The train pulse intensity was also set at this 80% level.

Paired-pulses: Those rats that demonstrated stable EPs were stimulated in the amygdala with two biphasic square-wave pulses. The intervals between the two pulses (interpulse intervals - IPIs) were 1, 5, 10, 20, 30, 50, 70, 100, 150 and 200 msec. The intensity was set at the 80% level, as described, for both pulses. The pulse pairs were repeated 8 times at each IPI, at a frequency of 0.2/sec. The resulting EPs were averaged and recorded. The facilitation and depression effects were calculated by dividing the amplitude of the second EPs by the amplitude of the baseline (first) EPs. For comparison purposes, SLOW rats were paired with FAST rats on the basis of initial response characteristics (including morphology, threshold, maximum amplitudes and intensities required to trigger maximum amplitudes).

Long-term potentiation: Prior to the tests for LTP, the animals were subjected to a 2 week period during which EP averages were continually measured. These measures provided a control baseline against which to compare the effects of subsequent tetanic stimulation. During the control run, the test pulses were delivered on the same time schedule as in the subsequent train sessions but no trains were delivered. During the first three days in both the control and experimental sessions, EPs were measured at eight different times per day. They were measured (1) pre-trains, (2) immediately after each train, (3) immediately after the end of 20 trains, (4) 15 min, (5) 30 min, (6) 60 min, (7) 4 hours and (8) 8 hours after the end of the trains. Again, each set of EPs was obtained by averaging 20 EPs at a frequency of 0.2/sec, except for category (2) where a test pulse followed each train. The test pulse in this case was applied at the same frequency as the trains, which was 1/30 sec. On the fourth and the fifth days, as well as one week and two weeks later, additional EPs were measured. The behavioral state during each EP test was labelled as "asleep", "very relaxed", "relaxed", "restless", "motionless", "grooming/feeding" or "minor/vigorous movement". When it was possible, test pulses were selectively delivered when the rat was judged to be in a particular state, usually relaxed, in order to collect homogeneous EPs and to minimize extraneous factors.

After the completion of the control sessions, those rats that showed consistently reliable EPs were finally subjected to the actual train session. In this session, twenty tetanic trains, consisting of biphasic 0.1 msec square-wave pulses at 400 p/sec were delivered to the

amygdala, at a frequency of one train per 30 sec. The train duration was 20 msec and the pulse intensity was the same as for test pulses.

For the analysis of data, the pre-train EPs on the first train day was used as the baseline for all EPs of that session.

## RESULTS

I/O curves: A total of 48 rats (24 FAST, 24 SLOW) provided reliable evoked potential data for the construction of I/O curves. The average amplitudes of the threshold and maximal EPs from the entorhinal and dentate recording sites are shown in Table IV. The EPs in both sites were comparable between the strains, confirming the observation reported in Chapter II. Although there was a tendency for the FAST animals to show slightly smaller dentate EPs, the difference was not significant.

Paired-pulses: Of the 52 rats tested, the entorhinal EP data of 28 rats were matched (to produce 14 pairs) according to the response morphology, amplitude and stimulation intensity. Fig. 10 shows the percent increase or decrease of the responses evoked by the 2nd pulse across the range of interpulse intervals. There was no difference between the strains in the amount of facilitation or depression seen in this particular neural circuit.

The amygdala to hippocampal EP data of 10 rats were matched and the results are shown in Fig. 11. There was a tendency for FAST rats to show a larger facilitation effect. The average intensity of the paired-pulses, however, was also slightly higher for FAST rats (by 72  $\mu$ A). Neither difference, however, was significant.

Table IV A summary table showing the threshold and maximal amplitudes of entorhinal (top) and dentate (bottom) EPs, together with the stimulation intensities that evoked those responses. SLOW rats tended to produce larger EPs at higher intensity than FAST rats, but the differences were not significant.

Table IV

SITE	STRAIN (N)	THRESHOLD		MAXIMUM	
		INTENSITY $\mu$ A (S.D.)	AMPLITUDE $\mu$ V (S.D.)	INTENSITY $\mu$ A (S.D.)	AMPLITUDE $\mu$ V (S.D.)
ENT	FAST (15)	195.0 (163.4)	54.9 (32.0)	1012.0 (209.1)	363.7 (173.0)
	SLOW (15)	272.0 (157.4)	48.2 (23.1)	1176.0 (147.0)	494.0 (352.6)
FD	FAST (9)	247.8 (106.0)	73.1 (40.1)	840.0 (465.9)	655.7 (505.6)
	SLOW (9)	306.7 (166.8)	56.3 (41.1)	1204.4 (141.3)	1023.6 (820.1)

Figure 10 The amplitude of the second of a pair of entorhinal EPs, expressed as a proportion of the first EP, is shown for interpulse intervals between 1 and 200 msec. The test pulses were applied at the ipsilateral amygdala at a stimulus intensity producing about 80% of the maximal EP amplitude. Each data point is the average of 8 sweeps for 14 animals. There was a small paired-pulse depression at 10 msec. The remaining intervals yielded a consistent paired-pulse facilitation effect which peaked at 30 msec. FAST (●) and SLOW rats (■) showed similar depression/facilitation effects.

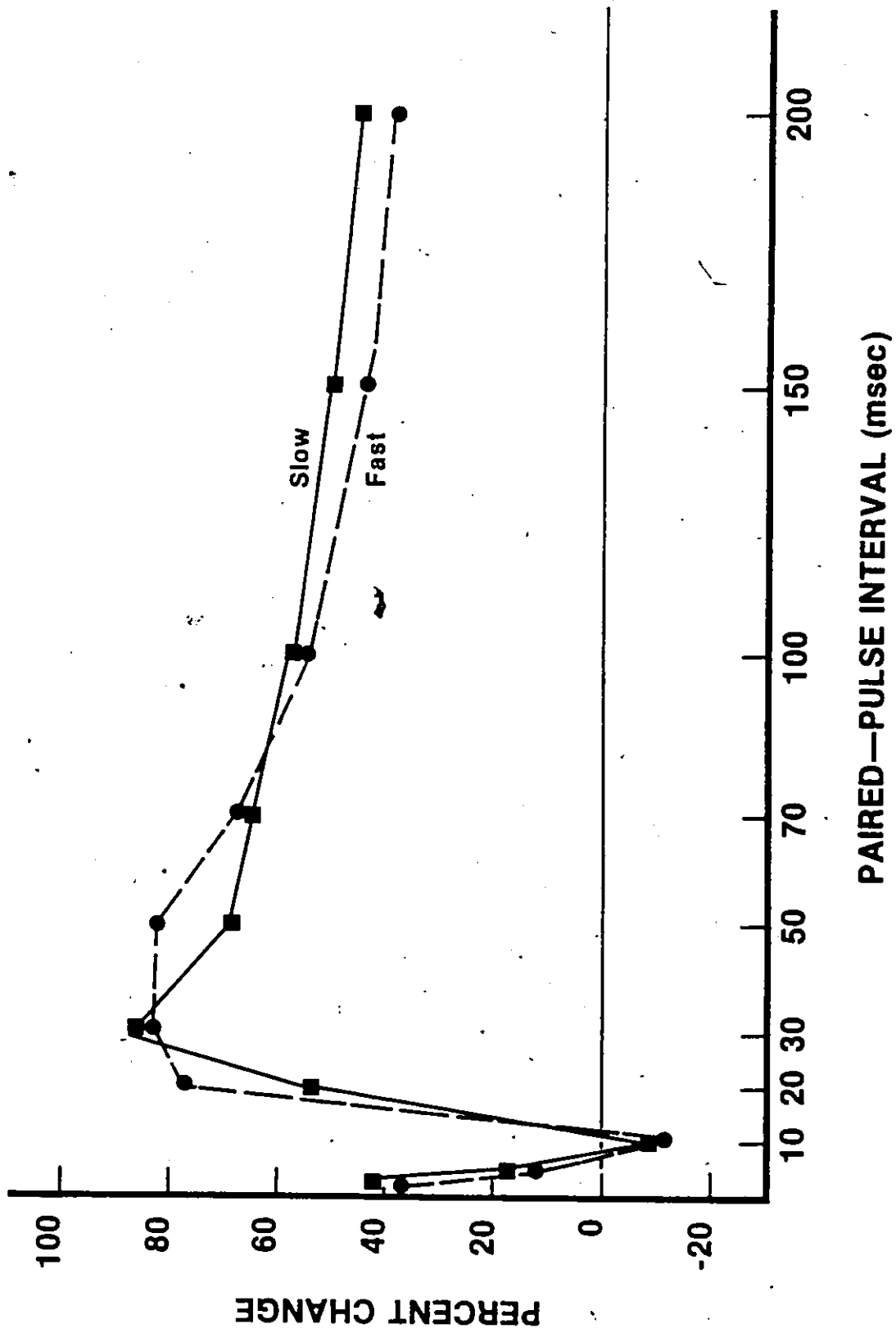
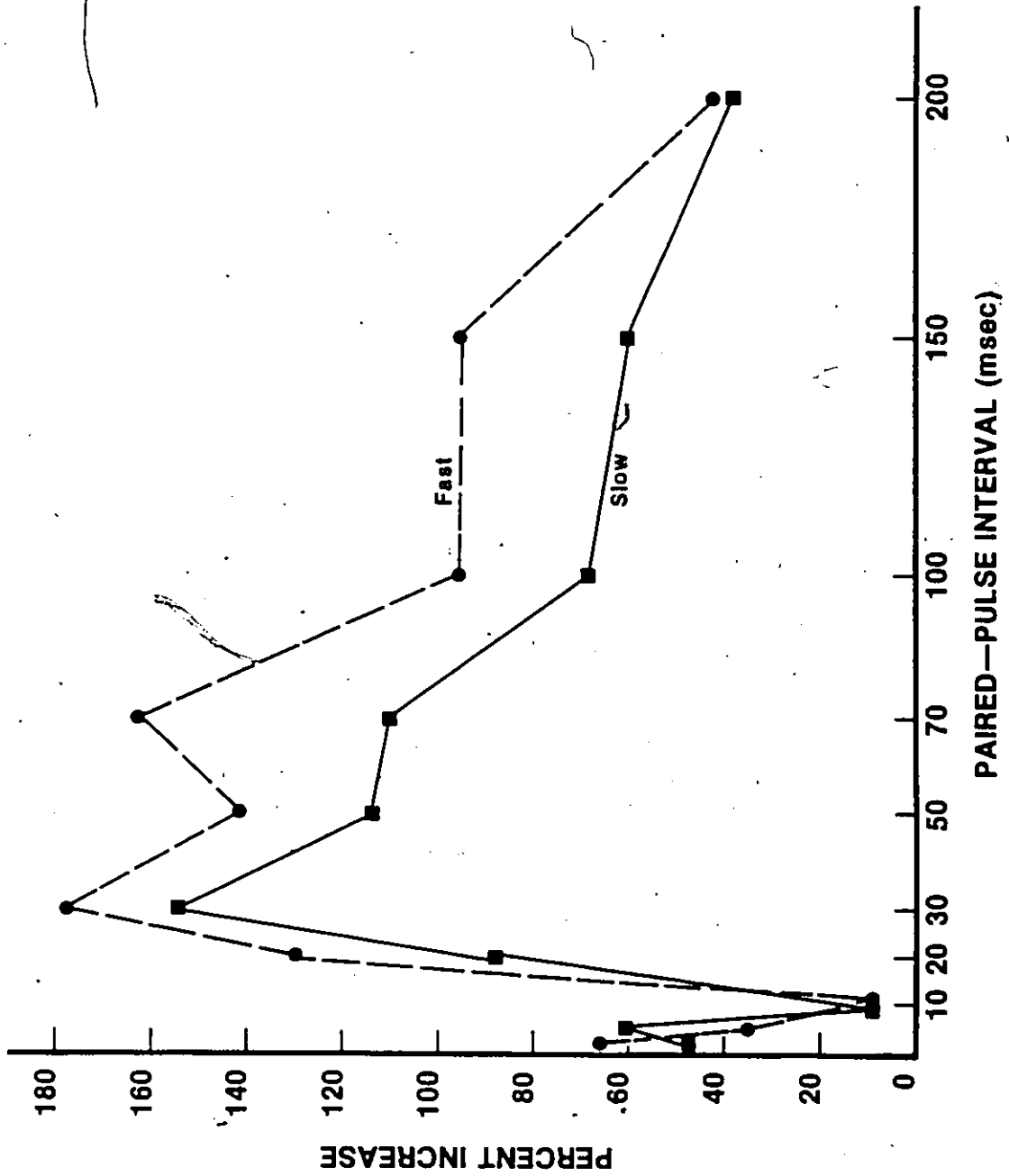




Figure 11 Paired pulse effects for hippocampal EPs are shown for interpulse intervals between 1 and 200 msec. The test pulses were applied to the ipsilateral amygdala at a stimulus intensity producing 80% of the maximal EP amplitude. Each data point is the average of 8 sweeps for 5 rats. There was a consistent facilitation effect which peaked at 30 msec. Although there was a tendency for FAST rats (●) to show stronger paired-pulse facilitation than SLOW rats (■), the difference was not statistically significant.



The dentate EP data from 26 matched rats, on the other hand, revealed the same difference between the strains (Fig. 12). The difference was significant at the 70 and 200 msec intervals. In this case, the average intensity for SLOW rats turned out to be 100  $\mu$ A higher than for FAST rats. Although it is possible that the higher intensities produced a stronger recurrent inhibition for SLOW rats, resulting in a correspondingly smaller facilitation, Tuff, Racine and Adamec (1983b) found that increasing intensity produced about the same amount of measurable peak facilitation.

Long-term potentiation: There were 16 rats (8 FAST, 8 SLOW) that completed the LTP experiment. As can be seen in Figures 13 and 14, the 2 strains did not appear to differ on any of the LTP measures.

#### CONCLUSIONS AND DISCUSSION

The electrophysiological measures utilized here did not reveal any differences between the strains except for the paired pulse facilitation in the dentate, which was larger in FAST animals (or, conversely, a concurrent paired pulse depression may have been larger in SLOW animals). Otherwise, baseline responses, I/O curves, paired pulse responses and potentiation phenomena did not differ between FAST<sup>m</sup> and SLOW animals.

The relevance of the differences in the short-term potentiation effect in the dentate area to differences in kindling rates is uncertain. The hippocampal formation, however, is believed to be involved in temporal lobe epilepsy (Falconer, Serafetinides & Corsellis,

Figure 12 Paired pulse effects for the dentate gyrus are shown for interpulse intervals between 10 and 200 msec. The test pulses were applied to the ipsilateral amygdala at a stimulus intensity producing 80% of the maximal EP amplitude. Each data point is the average of 8 sweeps for 13 rats. There was a consistent paired-pulse facilitation effect, which peaked at 30 to 50 msec. At the 50 and 200 msec intervals, FAST rats (●) showed a significantly larger facilitation effect than SLOW rats (■) ( $p < .025$  for both cases, U-test, one-tailed).

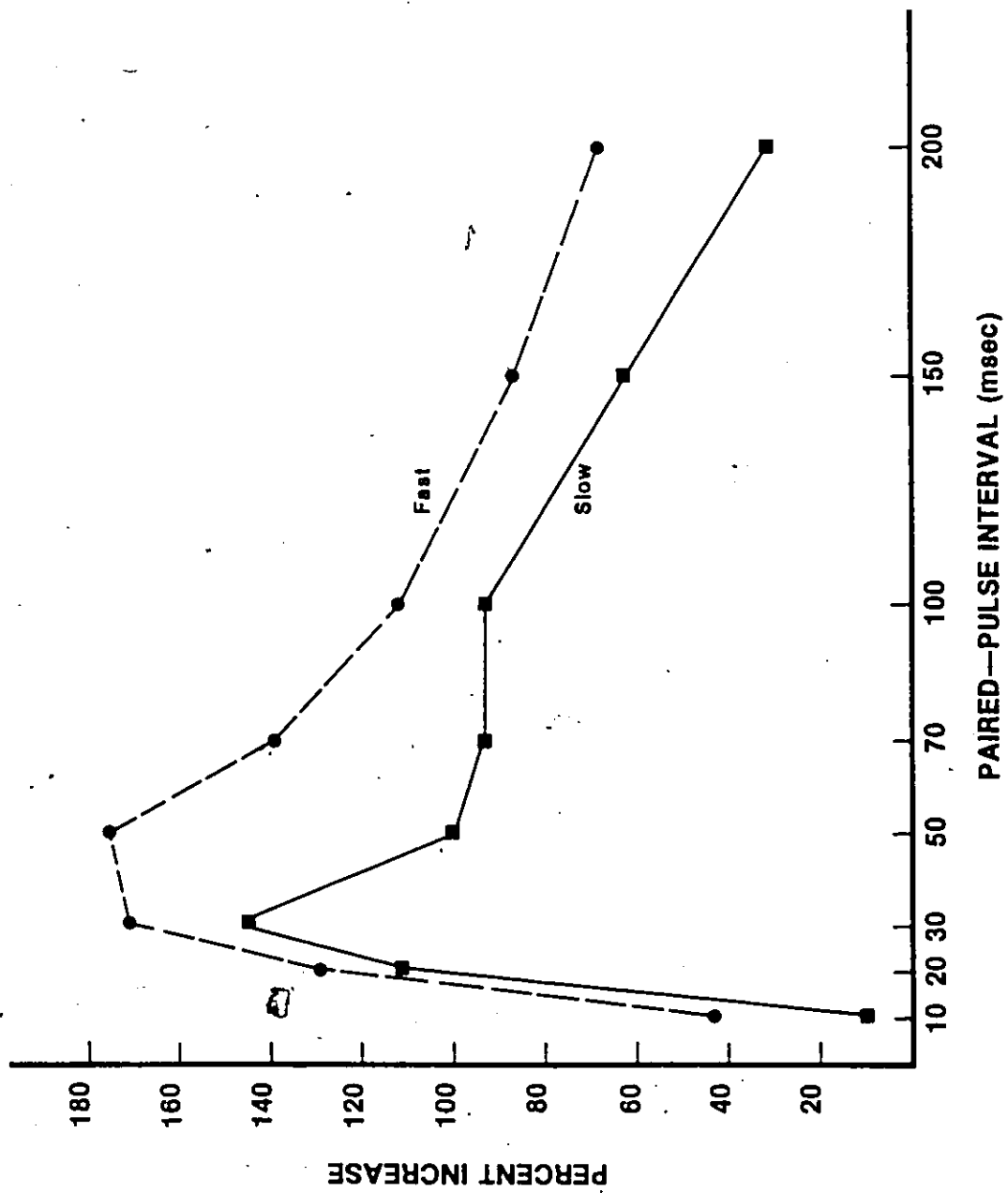


Figure 13 The amplitudes of entorhinal EPs (expressed as a percentage of the pre-train baseline amplitude) are shown for a 24 hr period following the application of stimulation trains (†). The trains were applied to the ipsilateral amygdala. The pulse intensity was that which produced 80% of the maximal pretrain EP amplitude. There were no significant differences between FAST (●, n=7) and SLOW (■, n=8) groups in the amount of potentiation produced by the trains. The data for days 2 and 3 (not shown) were essentially the same.

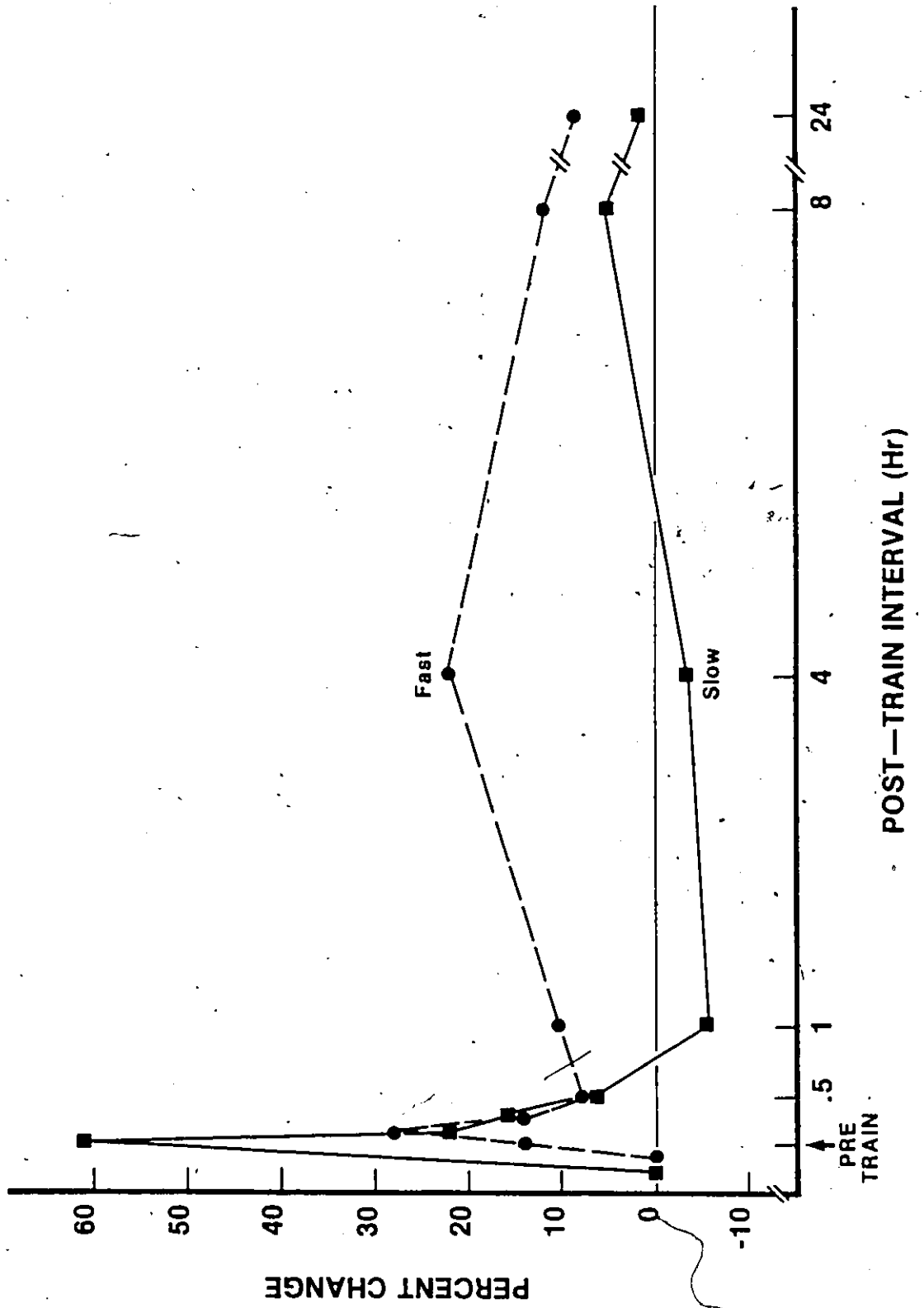
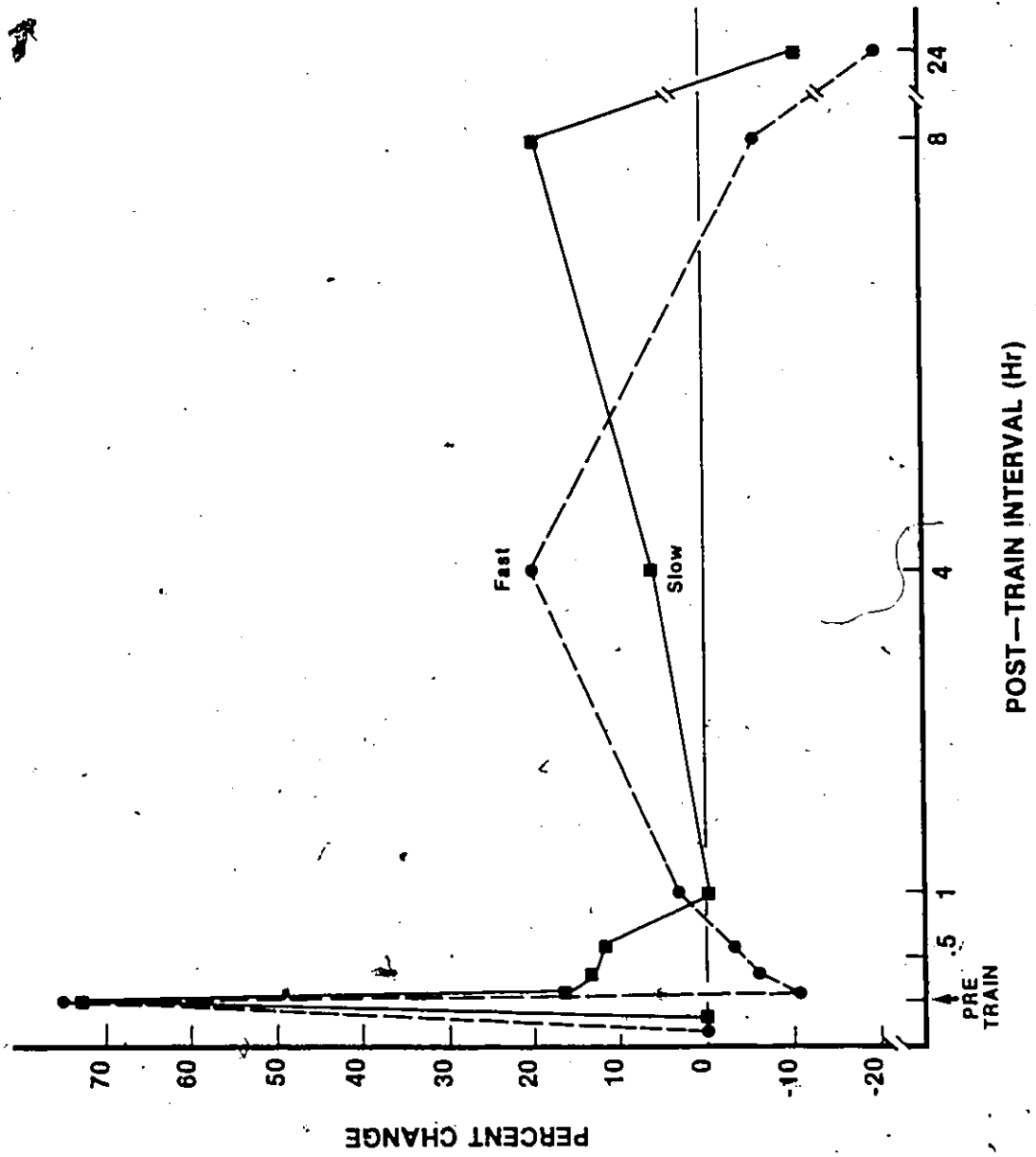


Figure 14 The amplitudes of hippocampal EPs (expressed as a percentage of the pretrain baseline amplitude) are shown for a 24 hr period following the application of stimulation trains ( $\dagger$ ). The trains were applied to the ipsilateral amygdala. The pulse intensity was that which produced 80% of the maximal pretrain EP amplitude. There were no significant differences between FAST ( $\bullet$ , n=6) and SLOW ( $\blacksquare$ , n=8) rats on any of the 3 test days.





1964), and differences within that structure might account for the increased epileptogenesis of the FAST animals.

In order to extend the findings pertaining to the differences in dentate responsivity, a series of experiments was designed to test the monosynaptic circuit from the entorhinal cortex to the dentate area.

#### EXPERIMENT 2: PERFORANT PATH TO DENTATE POTENTIATION

The monosynaptic pathway from the entorhinal cortex to the dentate area has proved useful for the study of neural plasticity. The most recent studies have examined the mechanisms of long-term potentiation of the response of dentate granule cells following high frequency stimulation of the perforant path.

There is some evidence that both pre- and postsynaptic mechanisms may underly the LTP effect. It has been suggested, for example, that an increased amount of neurotransmitter is released following the administration of a high frequency train (Dolphin, Errington & Bliss, 1982; Skjerve & Malthe-Sørensen, 1982). The increase in output of transmitter may, in turn, be caused by increased amounts of free calcium ions within the presynaptic terminals (Baimbridge & Miller, 1981; Turner, Baimbridge & Miller, 1982; Browning, Dunwiddie, Bennett, Gispen & Lynch, 1979; Browning, Bennett, Kelly & Lynch, 1981).

There are other findings that point to a postsynaptic mechanism, such as enlarged postsynaptic dendritic spines (Fifková & van Harreveld, 1978) and increased numbers of neurotransmitter binding sites (Baudry, Oliver, Creager, Wieraszko & Lynch, 1980). Furthermore, heterosynaptic

interactions between two distinct inputs to common target sites have been shown to significantly affect the resultant LTP effects (Douglas, Goddard & Riivers, 1982; Robinson & Racine, 1982). These interactions seem more likely to occur post- rather than pre-synaptically.

On the basis of these data, Bliss and Dolphin (1982) have suggested that both pre- and post-synaptic mechanisms are involved in the LTP effects. Whatever the mechanisms, it remains to be seen whether they can be genetically manipulated. The larger paired pulse facilitation in the amygdala to dentate response, reported in the previous section, suggests that such a possibility exists for short term potentiation effects. The following experiments were designed as a further test of the hypothesis that the FAST strain may possess circuitry that is more 'plastic' than in the SLOW strain. For these experiments, the focus was on the perforant path to dentate response. Input/output curves were measured to compare the baseline excitability of the system, and paired-pulse depression/facilitation was compared for short-term effects. High frequency tetanic stimulation was then applied to the pathway and EP responses to single test pulses were sampled and LTP effects were measured.

#### METHODS

Animals and surgical procedures: The experiments were performed on adult male rats, weighing from 350 to 500 gm. These were randomly obtained from the F9 and F10 generations of the FAST and SLOW strains (7 FAST, 8 SLOW).

The basic surgical procedure was as previously described. Monopolar recording electrodes, made of single strands of isonel insulated 0.25 mm nichrome wire, were implanted bilaterally in the dentate gyrus. Bipolar stimulating electrodes were implanted bilaterally into the perforant paths and unilaterally into the basolateral amygdala. The placements were monitored electrophysiologically during surgery. Small holes were drilled bilaterally in the skull for the dentate recording sites (4.0 mm posterior to and 2.5 mm lateral from bregma) and for the perforant path stimulating sites (8.0 mm posterior to and 4.9 mm lateral from bregma), with the skull plane being oriented horizontally on a stereotaxic frame.

The dura membrane beneath the skull hole was slit with a sharp needle just prior to the lowering of the electrodes. The remaining holes were covered with cotton balls soaked with normal saline. The wound edges were covered with cotton soaked with mineral oil. The stimulating and recording electrodes were lowered slowly, while monitoring EEG background activity. This activity provided some clues as to which cell layers the recording electrode was penetrating. When the electrodes were near the destination (3.8 mm and 4.0 mm below skull surface for dentate and perforant path, respectively), single test pulses were applied to evoke dentate responses. Both electrodes were then lowered, in small steps, until the optimal placements were found.

EEG and EPs were amplified by a Grass P15 AC preamplifier, with the high and low frequency cutoffs set at 3K Hz and 1 Hz respectively. The signal was further amplified by Grass polygraph EEG amplifiers and

monitored by a Tektronix 5114 storage oscilloscope. When necessary, the amplified signals were fed to an LSI 11 digital computer. The data acquisition and analysis program for the computer was provided by Dr. R.M. Douglas (McGill University) and modified by Dr. R.E. Adams (Scott Laboratory, Wellesley Hospital, Toronto).

When all electrodes were placed properly, they were secured to the skull with dental cement, and the Amphenol pins were collected into a 9 pin connector. All animals were injected with 15,000 units of penicillin intramuscularly.

At the conclusion of the chronic experiment, the brains were fixed and extracted from the rats by the standard perfusion method, and a histological examination was carried out in order to verify the electrode placements.

Electrical stimulation: All three types of stimulation, single pulse, paired pulse, and trains, consisted of biphasic rectangular waves, and were delivered to the animal through photoelectric stimulus isolation units.

The test pulses were 0.1 msec duration biphasic pulses with a 0.1 msec inter-pulse interval, delivered at a frequency of 0.1 Hz. The paired pulse stimulation consisted of two of these biphasic pulses with inter-pulse intervals ranging from 20 msec to 1000 msec. Finally, the train stimulation consisted of 8 of the above biphasic pulses delivered at a frequency of 400 Hz, for a train duration of 20 msec.

The stimulation intensity was based on the input/output curves obtained from each rat, and varied between 10 $\mu$ A to 1400 $\mu$ A, depending on the purpose of the experiment.

Two weeks after electrode implantation, the perforant path to dentate EP was tested in each hemisphere. The hemisphere showing the largest amplitude and most stable responses was chosen for further testing. The selected pathway was then stimulated at varying pulse intensities to determine threshold and maximal amplitude dentate EPs. These were initially determined by visual inspection on the oscilloscope. The arbitrary maximum current intensity (1400 $\mu$ A) was tested first and then reduced by 100 $\mu$ A until the EP amplitude started to decline (maximal intensity). The minimum intensity that produced detectable EPs was estimated by applying the lowest intensity (10 $\mu$ A) and increasing it in steps of 10 $\mu$ A.

Pre-train fixed-interval paired-pulse I/O curves: A series of paired-pulse stimulations, with a 20 msec fixed interpulse interval, was applied to the perforant path at ten graded intensities, covering the range between the minimum and maximum intensities as previously determined. In order to obtain reliable measures, the stimulation at each intensity was repeated five times at a frequency of 0.1 Hz. The averages of 5 pairs of EPs at each intensity were calculated by the computer and plotted on graph paper. The data from the first EPs in each pair were used to create baseline input/output curves, whereas the data from the second EPs were used to measure the facilitation and depression effects.

Pre-train variable-interval paired-pulse test: On the basis of these I/O curves, two intensities were selected for each rat for the examination of paired-pulse depression/facilitation effects across various interpulse intervals.

The lower intensity was selected to be high enough to produce reliable population EPSP responses but low enough to avoid the appearance of the population spike. This was done in an attempt to measure paired-pulse facilitation without evoking the cell discharge which would produce recurrent inhibition.

The higher intensity was set above the population spike threshold, in order to examine both the paired-pulse depression effect and the facilitation effect at higher stimulation intensities.

The interpulse intervals tested were 20, 30, 50, 70, 100, 150, 200, 300, 500 and 1000 msec. Again, the stimulation at each interval was repeated five times at 0.1 Hz, and the averages of 5 pairs of EPs for each interval were calculated and plotted.

Post-activation potentiation (PAP) experiment: Based on the I/O curves, a test pulse stimulation intensity was selected so that the evoked responses would be just above the threshold of the population spike. We had previously determined that potentiation effects would be detected most clearly at this intensity.

The test pulses were applied at 0.1 Hz for the 10 minutes prior to the application of the first of 9 pairs of high frequency trains. This provided a total of 60 pre-train baseline EP's. After the 60th test pulse, the first pair of trains was applied to the perforant path. The first train of the pair was applied at 4 sec and the second at 2 sec prior to the 61st test pulse. The dentate EPs continued to be evoked by the test pulses at 0.1 Hz for the next 10 minutes. The second pair of trains were given at 4 sec and 2 sec prior to the 121st test pulse. This cycle of train-test pulse application was repeated 9 times.

The last pair of trains was given before the 541st test pulse. These trains were also followed by 60 additional test pulses, providing a total of 600 EPs.

The intensity of the pulses within the first pair of trains was the same as for the test pulses (just above spike threshold). The next four train pairs were given at gradually higher intensities. The last four train pairs were given at the maximal intensities. This procedure allowed us to estimate the threshold intensity for PAP effects, and also to drive potentiation to asymptotic levels by applying several high intensity trains.

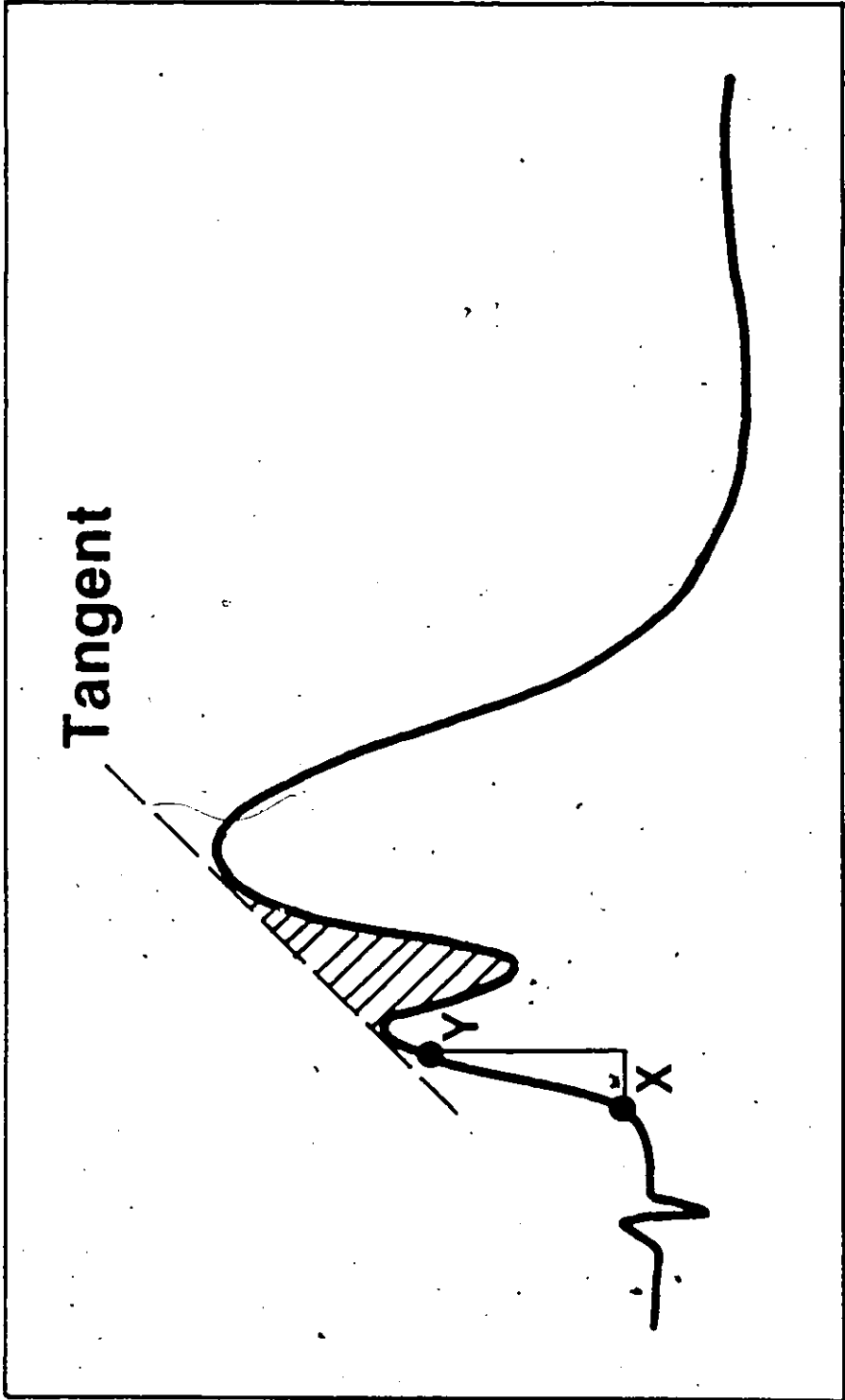
Post-train I/O curves: Immediately after the conclusion of the train experiment, the paired pulse I/O procedures were repeated to see how the train application affected subsequent measures of paired-pulse depression/facilitation effects.

Data analysis: The slope of the population EPSPs, as well as the areas under the population spikes, were calculated by the computer. The EPSP slope was measured between two points on the initial rising component of dentate EPs. These points remained fixed throughout the experiment for each rat. The spike area was measured within the boundary formed by the spike and a tangent line joining the spike onset and offset (Fig. 15). Paired-pulse depression/facilitation effects were expressed as the ratio of the second to the first EPSP slopes times 100.

The EPSP slope and population spike areas were measured for all 600 EPs obtained in the train experiment. The average rate of decay of potentiation was determined across the 9 train runs. The 9 sets of 60



Figure 15 A typical field potential, evoked in the dentate gyrus by paired pulse stimulation of the perforant path, is shown opposite. The amplitude of the population EPSP was determined by measuring the slope (Y/X) between 2 points on the leading edge of the response. The amplitude of the population spike was determined by measuring the area within the boundary formed by the spike and a tangent line joining the spike onset and offset.



EP values following the trains were added and the average calculated for each of the 60 points. The resulting data were displayed and plotted and an attempt was made to fit the decay curves with exponential functions. The time constants for the best fit curves were used as a measure of decay rate.

### RESULTS

Baseline EP's were stable in all but one SLOW rat. This rat also showed an immediate population spike (no measurable population EPSP), so its data were excluded from further analysis.

Pre-train fixed-interval paired-pulse I/O curves: The EP thresholds varied between 10 $\mu$ A to 150 $\mu$ A and were of similar levels in both strains. The thresholds for the population spike also did not differ significantly between the groups. These thresholds varied between 40 $\mu$ A to 500 $\mu$ A. Similarly, the intensities required to evoke maximal EPs were comparable for the 2 strains, varying between 500 $\mu$ A to 1400 $\mu$ A, and the slopes of the I/O curves were also comparable (Fig. 16).

There was a tendency for FAST rats to attain maximal EP amplitude with lower intensities, and to show higher thresholds for the population spike. Neither of these differences, however, reached statistical significance.

Five pairs of FAST and SLOW rats were matched for EP waveform and spike size and subjected to further investigation of the strength of recurrent inhibition. In Table V, the EPSP slope of the 2nd EPs are expressed as a percentage of the slope of the 1st EPs at 10 graded

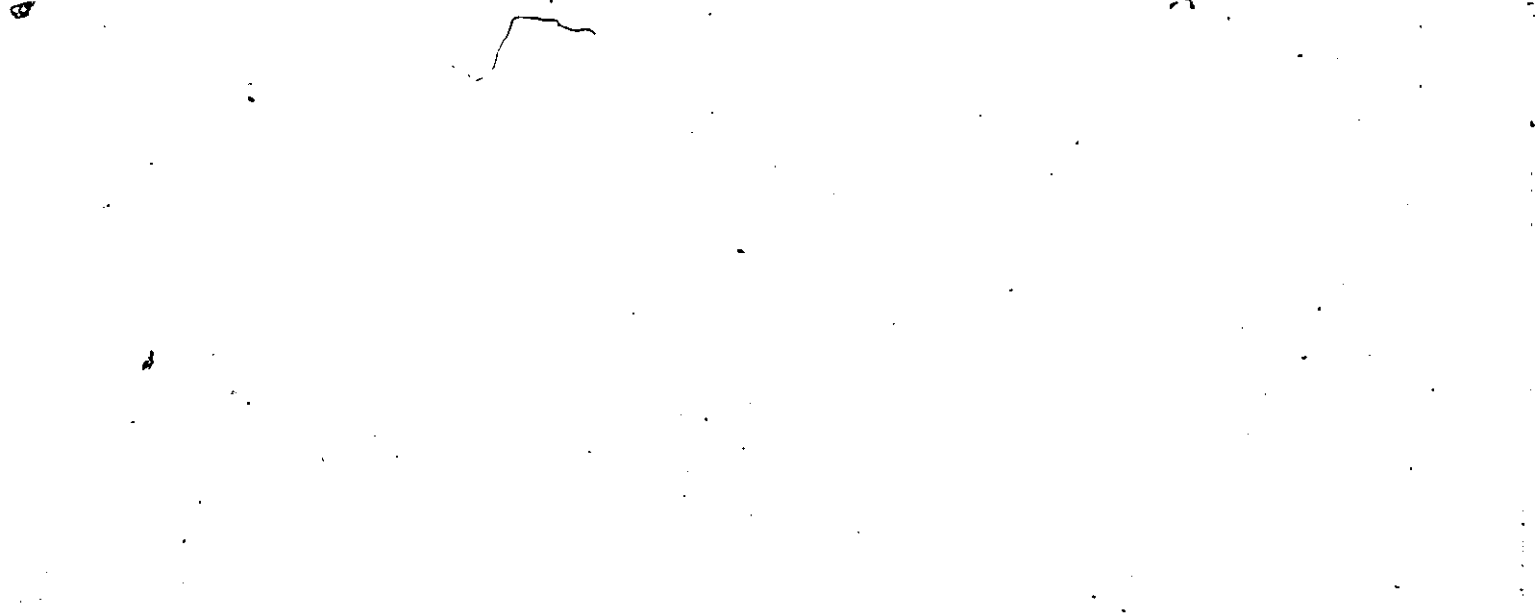


Figure 16 The pre-train I/O curves for the dentate population EPSP slopes are shown for FAST (●, n=7), and SLOW (■, n=7)-rats. Graded increases in the intensities of the stimulation pulses, applied to the perforant path produced graded increases in the EPSP slopes. Similar curves were generated for the 2 strains. The insert shows typical responses evoked by low and high intensity stimulation pulses.

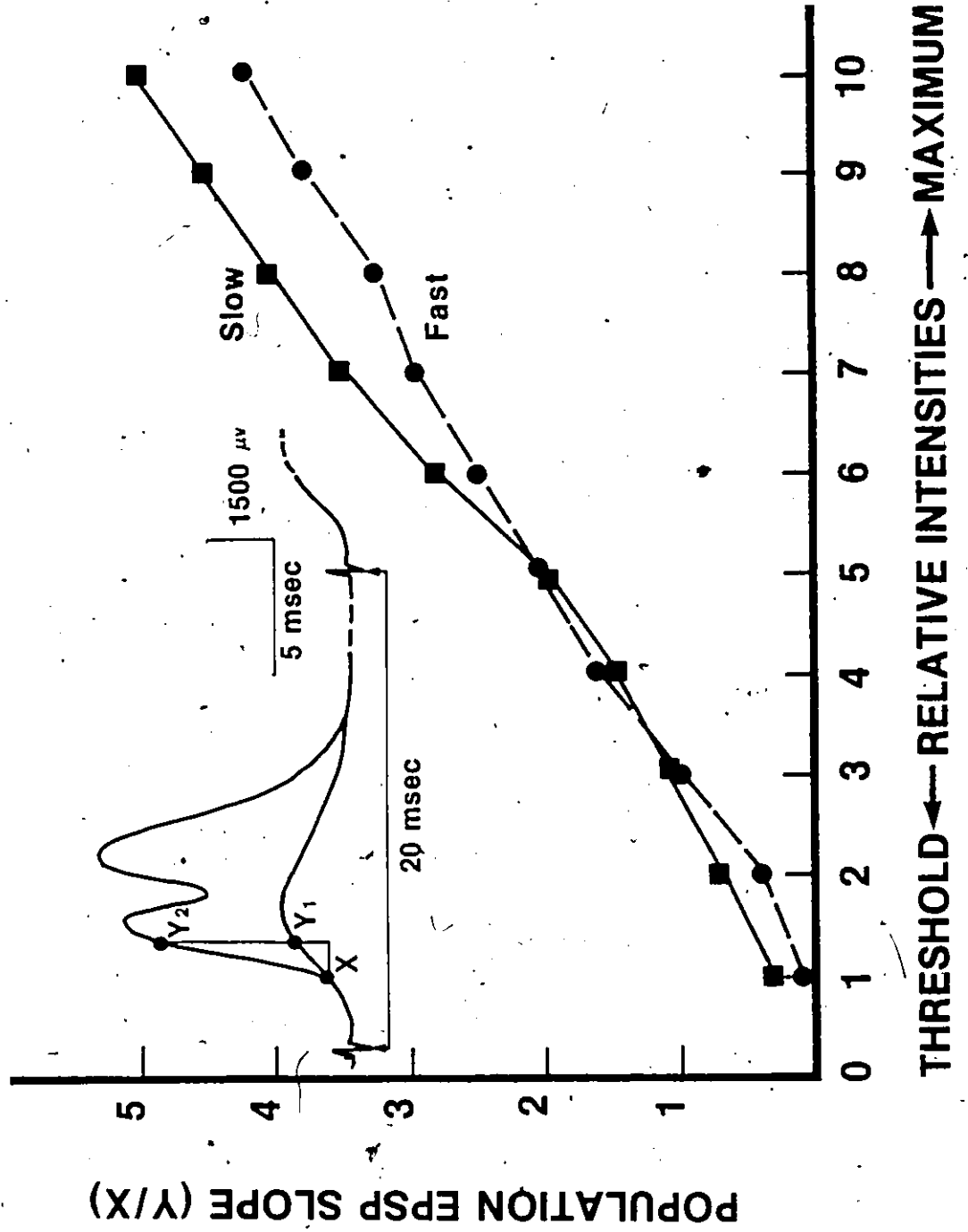


Table V The pre-train paired-pulse depression in dentate responses in five matched pairs of FAST (top) and SLOW (bottom) rats. The amplitude of the second EP, evoked at a 20 msec interpulse interval, is expressed as a percentage of the amplitude of the first EP. As the stimulation intensity increased in a step-wise manner, paired pulse depression increased. SLOW rats demonstrated a stronger paired-pulse depression than FAST rats.

EP

Table V

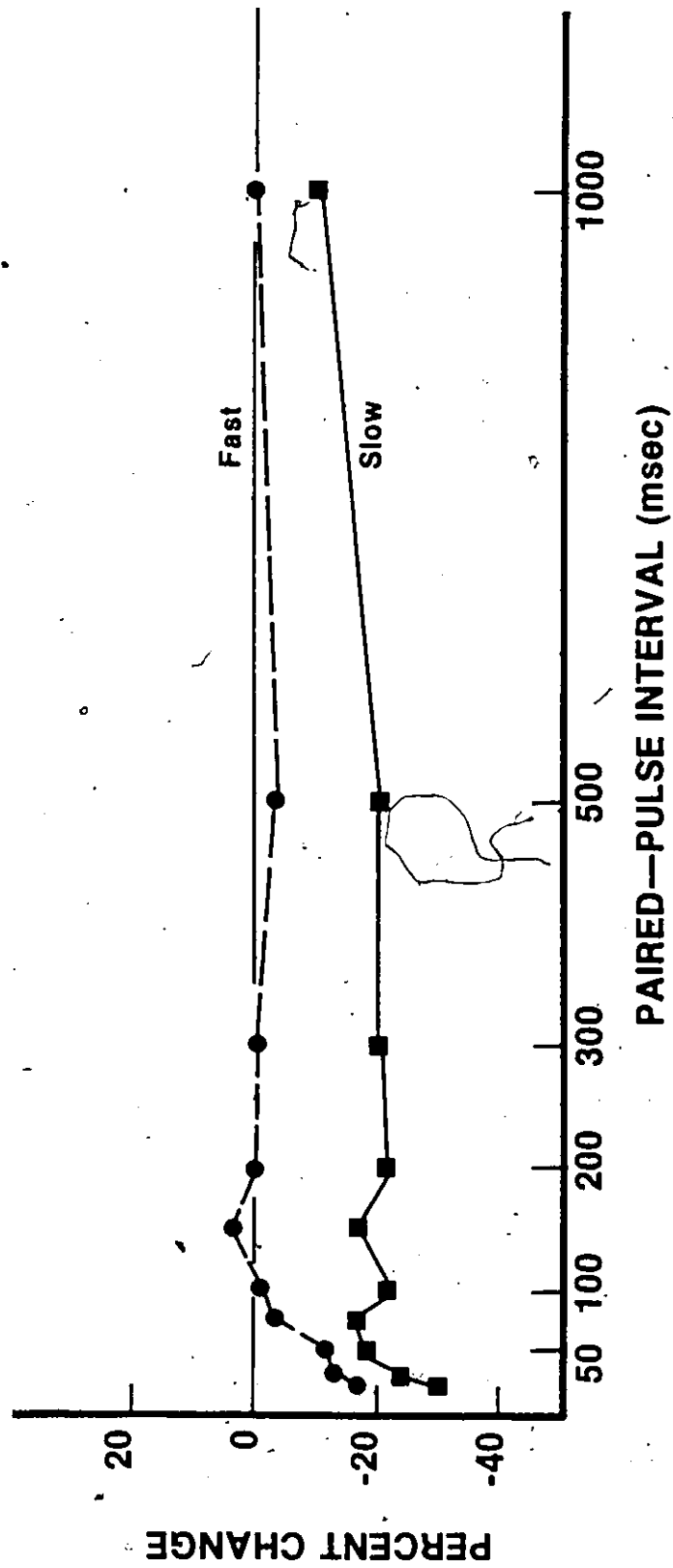
Animal #	MIN.	2	3	4	5	6	7	8	9	MAX.
F9 191	-----	100.0	95.8	90.3	79.1	76.9	72.9	75.6	70.0	41.8
192	100.0	100.0	75.0	111.1	127.3	76.9	77.8	94.7	43.4	36.7
F10 106	233.3	123.1	107.7	99.1	93.2	81.7	54.5	60.0	54.8	53.3
113	85.7	96.9	79.6	75.8	72.0	70.3	76.9	66.1	68.5	55.8
120	125.0	41.7	87.5	85.3	52.2	44.9	54.1	33.3	54.4	50.9
F10 304	23.1	74.4	74.5	76.4	79.1	54.0	60.1	46.6	36.9	35.5
305	60.0	57.1	52.0	46.8	41.2	34.0	33.6	29.3	31.3	33.9
307	-----	69.4	75.0	98.4	72.2	61.3	69.2	53.0	51.0	53.3
309	83.3	81.5	72.4	70.5	64.3	56.7	49.7	43.6	38.2	36.5
323	144.4	69.2	94.3	71.4	64.3	43.8	45.4	42.9	36.0	42.8

intensities for each rat. Although the groups were matched on the basis of wave forms, there was still a tendency for SLOW rats to require higher intensities to achieve maximal responses. Consequently, the data were analyzed both with and without matching for stimulation intensity. This was done to insure that any difference in levels of recurrent inhibition were not merely due to stimulation intensities. Seventeen out of 45 SLOW averages showed a reduction of the 2nd EP slope to less than 50% of the 1st EP slope. Only 7 out of 50 FAST (12%) averages showed comparable reductions. Spike thresholds were about the same between the 2 groups and the spike shapes were roughly matched, so this result may indicate that FAST rats have a lower baseline level of recurrent inhibition than SLOW rats.

Pre-train variable-interval paired-pulse test: Data from six pairs of FAST and SLOW rats were chosen for the analysis after matching of the EPs. The average spike areas for the 1st EPs for the SLOW rats was  $35.4 \text{ mm}^2$ , and for FAST rats it was  $31.4 \text{ mm}^2$  measured at  $1000 \mu\text{v}/\text{cm}$  calibration. As mentioned previously, however, the FAST rats required a somewhat higher intensity stimulation (and a correspondingly larger EPSP slope) in order to evoke these spikes. Although the differences were not significant, they do indicate that the matches were not ideal. Attempts were made to match primarily on the basis of population spikes, because our primary interest in this phase of the experiment was in the recurrent inhibition which is evoked by granule cell discharge. Fig. 17 shows that the SLOW rats showed a consistently stronger paired-pulse depression, particularly between 70 msec and 1000 msec. Another paired



Figure 17 Paired pulse effects for the dentate gyrus, over interpulse intervals between 10 and 1000 msec, for six matched pairs of FAST (●) and SLOW (■) rats. The test pulses were applied to the perforant path at an intensity that evoked dentate EPs with comparable population spike areas in both FAST and SLOW animals. The data points represent the amplitude of the second population EPSP as a percentage of the first. Values higher than 100% indicate paired-pulse facilitation, and values less than 100% indicate paired-pulse depression. Fisher randomization two-sample t-test revealed that SLOW rats showed significantly stronger paired-pulse depression effects at 30 msec and at all intervals between 70 msec and 1000 msec ( $p < .05$  or less).



pulse series was run on these animals with the stimulation intensity (SLOW  $\bar{X}$  = 201.7  $\mu$ A vs FAST  $\bar{X}$  = 216.7  $\mu$ A) and the 1st EPSP slopes (SLOW  $\bar{X}$  = 1.24 vs FAST  $\bar{X}$  = 1.32) matched, rather than the population spike. The results were similar to the previous run. The 2nd EPSP slope of the FAST rats varied between 87.9% and 97.0% of the 1st EPSP slope, while that of SLOW rats varied between 61.4% and 90.1%. The differences were statistically significant at the interpulse intervals of 20, 70, 100, 200 and 1000 msec.

Post-activation potentiation (PAP) experiment: Six rats from each strain were matched according to EP waveform, amplitude and test pulse stimulation intensity. The averaged decay curves were subjected to exponential curve fitting procedures. Based on previous experiments, it was assumed that short term effects would decay to near baseline levels within 10 minutes. The values at 10 min post-train were consequently subtracted from the raw averages. The curve fitting procedures were then applied.

The STP effect in FAST rats decayed steadily over the entire 10 minute period following the train. The average time constant of decay was estimated to be 225 sec. The STP effect for SLOW rats appeared to decay more rapidly than for FAST rats within the first third of the period following the train. Decay then proceeded more slowly than in FAST rats. Attempts to fit these data with single exponentials yielded consistently poor fits. The slowly decaying final 2 thirds of the SLOW curve yielded an average time constant of about 585 sec. Although the

decay process appeared to differ between the 2 strains, the peak measurable potentiation did not differ significantly.

Post-train I/O curves: Paired pulse I/O curves were determined at the completion of the train series. Three out of five FAST rats showed a residual potentiation of the EPSP slope, whereas none of the five matched SLOW rats showed EPSP potentiation. In fact, 35 out of 50 (70%) of the samples from the SLOW rats during the I/O tests showed a decrease in EPSP slope measures. Examination of the waveform showed that the post-tetanic "depression" of EPSP slope was not due to contamination of the EPSP slope with an earlier spike onset.

#### CONCLUSIONS AND DISCUSSION

The hypothesis that FAST rats have a greater plasticity in excitatory systems was not supported by these data. The baseline response levels also did not differ significantly between the strains, although there was a tendency for FAST rats to require slightly lower intensities to induce maximal amplitude EPs (but with smaller population spikes). There was, however, a significantly larger paired pulse depression effect in SLOW vs FAST animals.

There appeared to be some differences between the groups in the way in which the short-term potentiation effects decayed. FAST rats consistently showed a smooth exponential decay, whereas SLOW rats showed an initial rapid decay followed by a slower decay. The peak measurable effects, however, were not different between the strains. The long-term potentiation effects also did not differ between the strains, although

there was a tendency for SLOW animals to show a greater incidence of post-train depression of the EPSP slope.

The stronger paired-pulse depression seen in SLOW animals may reflect stronger inhibitory mechanisms. It seems unlikely that the differences are entirely due to recurrent inhibition, however, as they were still present at intervals of 1000 msec.

The differences in seizure sensitivity between these strains, then, may be due to an increased strength of inhibitory mechanisms in SLOW animals rather than an increased plasticity in FAST animals. The question remains as to the nature of the inhibitory process. The increased paired pulse depression that we observed in SLOW animals exceeded the normal time duration for simple recurrent collateral inhibition, at least for granule cells of the dentate area (220 msec, Matthews, McCafferty & Setler, 1981).

After-hyperpolarizations (AHPs) lasting as long as 2-5 seconds, have been recorded from pyramidal cells of CA1 (Hotson & Prince, 1980; Gustafsson & Wigström, 1981) and CA3 (Dingledine & Langmoen, 1980; Hablitz, 1981), as well as in the granule cells of the dentate area (Thalmann & Ayala, 1982). These long-lasting AHPs were found to be activated by  $Ca^{4+}$ -influx and mediated by a long-lasting conductance increase to potassium (Nicoll & Alger, 1981). The full AHP, then, is made up of at least 2 components. The first, primarily recurrent inhibition, lasts about 100-200 msec, with a peak at 10-15 msec after the stimulus. The long duration AHP has a half-decay time of about 250 msec and a peak at about 150 msec after the stimulation (Thalmann & Ayala, 1982). These two processes are distinguishable, not only for

their durations, but also for their mechanisms. The long-lasting AHP, but not the short-lasting AHP, can be eliminated by  $Mn^{2+}$  (manganese, a  $Ca^{2+}$  channel blocker) (Hotson & Prince, 1980). The short-lasting, but not the long-lasting, AHP was reduced by antagonists of GABA-mediated  $Cl^{-}$  conductances, such as picrotoxin (Thalman & Ayala, 1982).

The fact that SLOW animals showed a stronger paired pulse depression suggests that one or more of these inhibitory processes may differ between the 2 strains. This could be due to differences in synaptic mechanisms (e.g. in GABA systems) or to differences in ion channels in the neuron membrane (particularly for  $Cl^{-}$ ,  $Ca^{2+}$  and/or  $K^{+}$ ).

## CHAPTER V NEUROPHARMACOLOGICAL STUDIES

### INTRODUCTION

Epileptiform responses can be triggered by alterations in the function of specific neurotransmitter systems. For example, seizures can be triggered or facilitated by drugs that interfere with GABA (penicillin, picrotoxin, bicuculline, etc.); glycine (strychnine); or catecholamines (reserpine, 6-hydroxydopamine). Seizures can also be triggered by activation of excitatory transmitter systems (e.g., Ach, glutamate).

In addition, the development of epilepsy in some chronic epilepsy models is accompanied by permanent alterations in the function of certain transmitter systems. The alumina focus preparation shows reduced GABA levels in and around the focus (Ribak, Harris, Vaughn & Roberts, 1979) and the amygdala-kindled preparation shows alterations in catecholamine systems (McNamara, 1978a; Engel & Sharpless, 1977).

In order to further test the generality of the epileptogenic differences between the FAST and SLOW strains, and to provide information about the relative responsivity of the various transmitter systems between these strains, various convulsant drugs were applied in a series of dose response tests.

The convulsant drugs that were tested were pentylenetetrazol (PTZ), strychnine, picrotoxin, bicuculline and isoniazid. The first 2 drugs, PTZ and strychnine, are the 2 most commonly used forebrain (PTZ) and brainstem/spinal cord (strychnine) convulsants.

Strychnine has been shown to exert a specific blocking action against glycine receptors. Since glycine is known to be the dominant inhibitory neurotransmitter in the spinal cord, and strychnine seizures are restricted to brainstem and spinal cord, the test for strychnine seizures was included to determine if the neural differences between the 2 strains extended into the spinal cord or brainstem. PTZ, on the other hand, is believed to specifically affect the  $\text{Cl}^-$  channel and consequently should reduce the effects of GABA. Picrotoxin and bicuculline were used to further test the hypothesis that FAST rats were deficient in GABA-mediated inhibition (e.g., most types of recurrent inhibition in the forebrain). This hypothesis was based on our electrophysiological experiments which showed that paired pulse depression in the dentate gyrus (in part a reflection of GABA-mediated recurrent inhibition) was weaker in FAST than in SLOW animals. Finally, isoniazid was administered to see if the blockade of GABA synthesis would affect the strains differently.

The effects of diazepam (a benzodiazepine) and propranolol (a  $\beta$ -adrenergic receptor blocker) were also tested on the two strains. Since diazepam has an anticonvulsant effect, it was necessary to test the drug on kindled seizures. Propranolol, on the other hand, does not appear to affect convulsions, so its effect on the electrographic discharge was determined.



## METHODS

## CONVULSANT EFFECTS ON NAIVE ANIMALS

Animals: The animals were randomly selected from generations F7 through F11. They were kept food deprived for 24 hours prior to the test. The two strains of rats were tested in pairs, either against each other or against control rats.

Dose-response tests: Before a formal series of dose-response tests were begun, a pilot experiment was run for each drug with a wide range of dose levels. The seizure incidence rates and the onset latencies were recorded for each dose level. On the basis of these pilot tests, a maximum observation time (beyond which first seizures did not appear) was determined. In addition, an estimation of the ED50 for the generalized convulsion was determined for each drug. All subsequent dose-response tests were started at this 'key' dose level. The rats were coded so that the experimenter was unaware of the strain to which the rats belonged. At the conclusion of the "blind" test on the key dose level, a statistical test of difference in proportions was carried out. Additional data were then obtained on lower and higher dose levels in order to create the dose/response curves and to confirm the findings at the selected key dose level.

The number of dose levels and rats tested was based on the requirements for the creation of reliable dose/response curves, and on the availability of rats. Most drug tests were completed across two generations. Although it was unlikely that the neurochemical characteristics of one generation should be significantly altered in the next generation, the PTZ test was repeated, at the key dose level,

across four generations. This provided a confirmation of the stability of our measures.

The females had not been kindled after the 5th generation, raising the possibility that the seizure susceptibility might have become specific to males. Therefore, female rats were also examined with each convulsant drug.

All drugs were freshly prepared on the test day, except PTZ which was purchased in sterile solution (100 mg/cc) from Knoll Pharmaceutical Company, Toronto. The rest of the four drugs were obtained from Sigma Chemical Company in powder form. The key dose level for PTZ was 25 mg/kg for females and 30 mg/kg for males, and the observation time was 5 minutes.

Strychnine sulphate was dissolved in sterile water at a concentration of 1 mg/cc. The key dose level for strychnine was 1.6 mg/kg and the observation time was 15 minutes.

Picrotoxin was also prepared in sterile water at a concentration of 1 mg/cc and was kept in the dark throughout the experiment. The key dose levels were 1.0 mg/kg for females and 2.5 mg/kg for males, and the observation time was 30 minutes.

Bicuculline was first dissolved in sterile water. A small amount of 0.1 N hydrochloric acid was added to facilitate solubilization. Sodium hydroxide (0.1 N) was added to reduce the acidity of the solution to a pH of 4.8. The final solution was adjusted to a concentration of 2 mg/cc, and was kept on ice during the experiment. The key dose levels were 6.5 mg/kg for females and 5.0 mg/kg for males, and the observation time was 5 minutes.

Finally, isoniazid was dissolved in sterile water to a concentration of 100 mg/cc. The key dose level was 200 mg/kg for females and 175 mg/kg for males, and the observation time was 90 minutes.

The experiments were performed in a quiet room. This was particularly important for the strychnine tests, as seizures can be triggered in strychnine-treated animals by sensory stimulation. Each rat was weighed and promptly injected with the randomly assigned drug. For the PTZ and bicuculline tests, each rat was tested individually in a cylindrical glass container (30 cm diameter, 30 cm high). For picrotoxin and isoniazid, three to five rats were tested concurrently. Each rat was placed in a separate plywood observation box (30 x 40 x 30 cm) with a meshed wire front and a mirror on the back wall. For strychnine, the same box was used but only one rat was tested each time.

If the injection was incomplete, or misplaced, the data were discarded. The first sign of clonus (usually involving the head or forelimbs) was noted with its onset latency. The range and maximum severity of the convulsive responses were also noted together with the time of occurrence. In the case of strychnine, the act of picking up the animals at the end of the observation frequently triggered tonic convulsions and death. These convulsions were noted accordingly but separately from the within-session measures.

## EFFECTS OF DIAZEPAM AND PROPRANOLOL ON KINDLING

Twenty-four FAST and 14 SLOW rats that completed unilateral amygdaloid kindling with five consecutive stage 5 seizures were obtained from the F7 to F10 generations. They were considered to be "stable kindlers" suitable for the evaluation of anticonvulsant drug effects. These rats were administered with a fixed dose of diazepam (.75 mg/kg I.P.) on the day following the fifth stage 5 seizure. Ten minutes after the drug administration, the kindling stimulation was delivered. The effect of the drug on the electrographic and motor responses was measured. An additional non-drugged control AD was evoked on the following day. Twenty four hrs following the control AD, the diazepam dose was reduced to .50 mg/kg. If the anticonvulsant effect of diazepam was still clear (< stage 3), the same procedure was repeated with a dose level of .25 mg/kg. If, on the other hand, the rats responded to the .75 mg/kg dose of diazepam with a full seizure, they were administered a 1.00 mg/kg dose on the following test day.

Propranolol was prepared in NaCl solution for I.P. injection at a concentration of 10 mg/cc. Twelve FAST and twelve SLOW rats were randomly selected and were implanted with unilateral amygdaloid electrodes. Six animals in each strain were administered propranolol (30 mg/kg) and 6 with NaCl (1.5 cc/kg), on the first day of kindling. The kindling stimulation was applied 30 min after the injection, when rats were typically ataxic and unresponsive. The effect of the drug on the electrographic response (particularly AD duration) was measured.

## RESULTS

For the five convulsant drugs, a total of 28 dose levels were tested on 535 male rats (176 FAST, 156 SLOW, 203 CONT), and a total of 39 dose levels were tested on 712 female rats (244 FAST, 254 SLOW, 214 CONT).

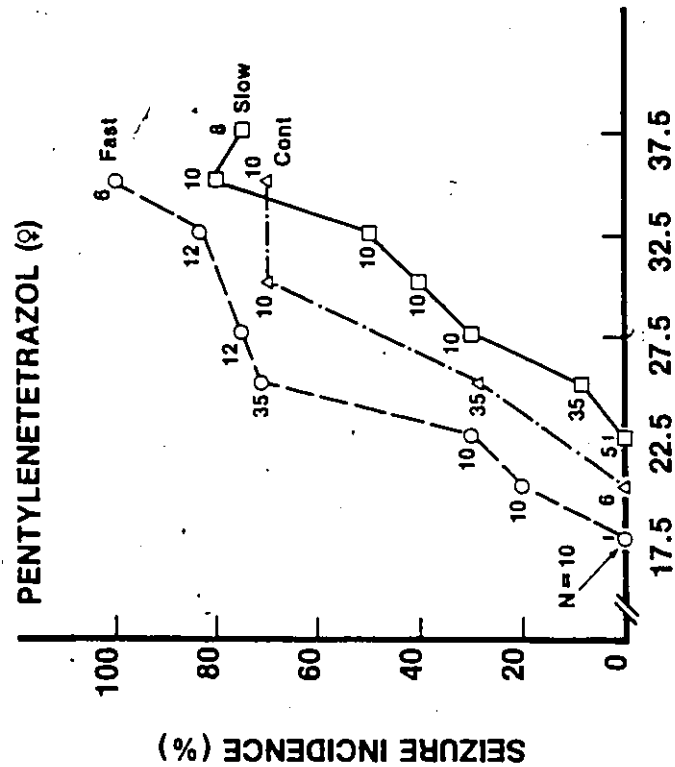
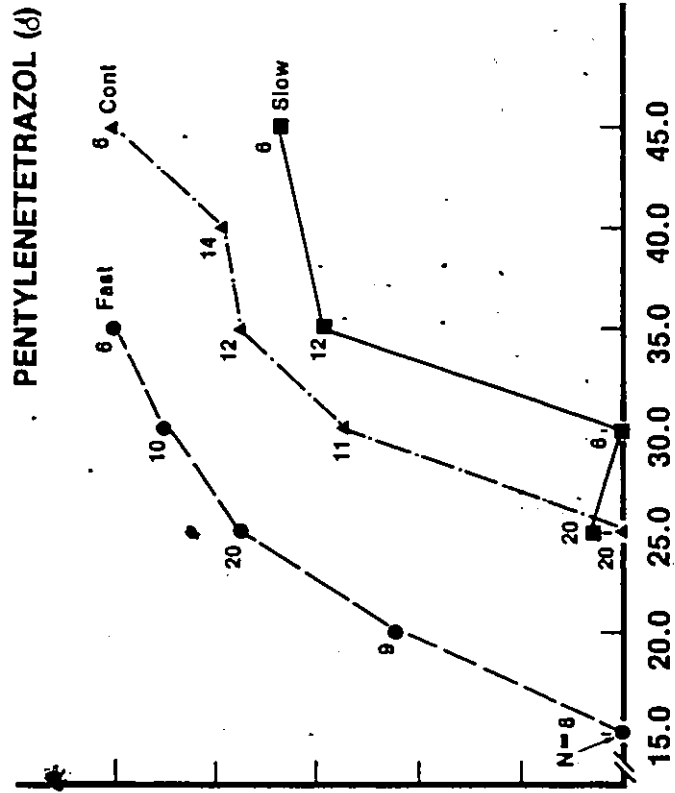
Pentylentetrazol: Shortly after the injection (within 2 minutes), slight head twitching was commonly seen. This gradually developed into clonic movements involving head, fore- and hind-limbs. A generalized convulsion was typically observed within three minutes. The results are shown in Fig. 18. At a dose level of 25 mg/kg, a significantly higher proportion of animals in the FAST group showed the fully generalized seizure ( $p < .001$ ). This was true for both males and females.

Strychnine: Between 5 and 12 minutes after the injection, animals started to show clonic movements. These typically started as mouth twitches, head jerks or whole body jerks. The frequency and the intensity of these jerks increased until the sudden transition to tonic-extension. A few animals died during these tonic extensions, probably due to respiratory arrest.

The results are shown in Fig. 19. Unlike the PTZ test, the strychnine tests failed to show a significant difference between the two groups.

Picrotoxin: Within 5 to 10 minutes after the injection, the rats settled into a stereotypical posture (prone on the ventral surface). During the next several minutes, the rats showed bursts of sniffing-like movements and weak body jerks. The body jerks

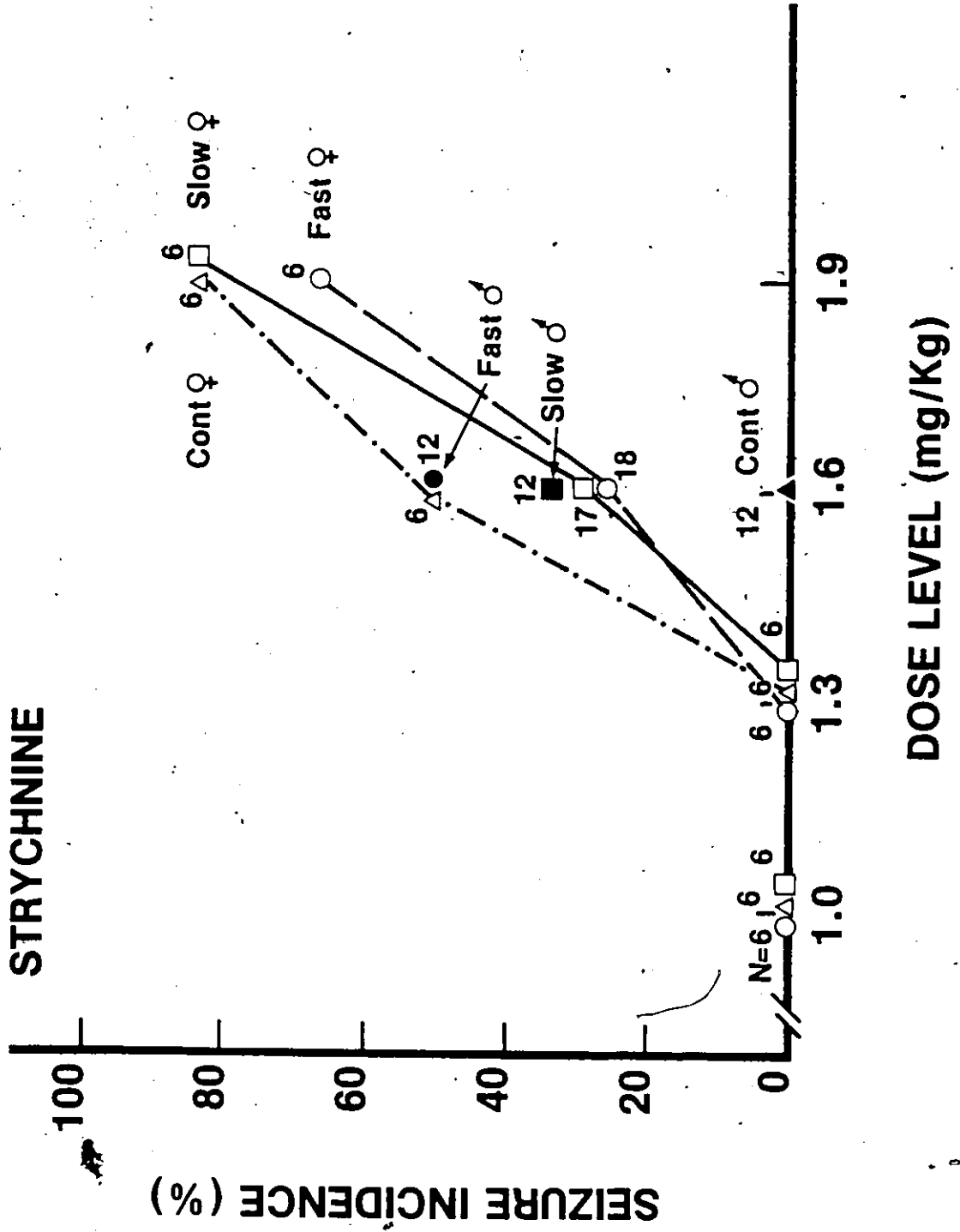
Figure 18 Dose-response curves for pentylenetetrazol. The key dose level for male rats (right) was 30.0 mg/kg, at which a significantly larger proportion of FAST rats (●) showed seizure responses compared to SLOW rats (■,  $Z=12.000$ ,  $p<.0001$ , one-tail) or control rats ( $\Delta$ ,  $Z=2.761$ ,  $p<.003$ , one-tail). A similar difference was found for female rats (left) at their key dose level of 25.0 mg/kg (FAST vs SLOW:  $Z=9.669$ ,  $p<.0001$ , one-tail; FAST vs Control:  $Z=5.476$ ,  $p<.00001$ , one-tail; Control vs SLOW:  $Z=3.119$ ,  $p<.005$ , one-tail).



DOSE LEVEL (mg/Kg)

Figure 19 Dose-response curves for strychnine. The key dose level for both male (single closed data points) and female (broken lines) rats was 1.6 mg/kg, at which there was no difference between FAST and SLOW groups for either males or females. There was, however, a significantly smaller proportion of male control rats showing seizures compared to FAST ( $z = 4.899$ ,  $p < .0001$ , one-tail) or SLOW rats ( $z = 3.438$ ,  $p < .001$ , two-tailed).





intensified, and occasionally developed into bursts of forelimb clonus. After some delay (20-25 min post-injection) the whole body started to show continuous clonic jerks after which the rat would suddenly fall on its side with extension of the limbs followed by forelimb clonus and head nodding.

The results are shown in Fig. 20. The FAST and SLOW strains showed dramatic differences in seizure incidence at nearly all dose levels. There were also substantial sex differences.

Bicuculline: Within one minute following the injection, rats showed the first incidence of clonus (typically a body jerk). At higher doses, this led directly to a full tonic extension. Lower doses affected rats gradually, and the body jerks continued and intensified over a period of about 3 minutes after which tonic extension appeared. These rats often showed a second tonic extension which was nearly always fatal. The results are shown in Fig. 21. Males showed a tendency to respond with full seizures at lower dose levels than females. In both males and females, FAST rats showed higher seizure susceptibility to bicuculline.

Isoniazid: Within 30 minutes following the injections, the rats began showing sudden bursts of sniffing movements. Although not clearly epileptic, this behavior was reliably triggered and preceded the onset of weak body jerks. These body jerks were quite mild, so that the subsequent generalized convulsion appeared to occur rather suddenly during a post-injection period ranging between 27 minutes to 90 minutes. The full convulsive pattern was characterized by myoclonus of the forelimb, flexion of the head and falling on the side with continuing

Figure 20 Dose-response curves for picrotoxin. The key dose level for male rats (right) was 2.5 mg/kg, at which a significantly larger proportion of FAST rats (●) responded with seizures compared to SLOW rats (■) or control (▲) rats ( $z=7.654$ ,  $p<.00001$ , one-tail). The key dose level for female rats (left) was 1.0 mg/kg, at which the same significance level ( $p<.00001$ ) was found between FAST and SLOW or control rats.

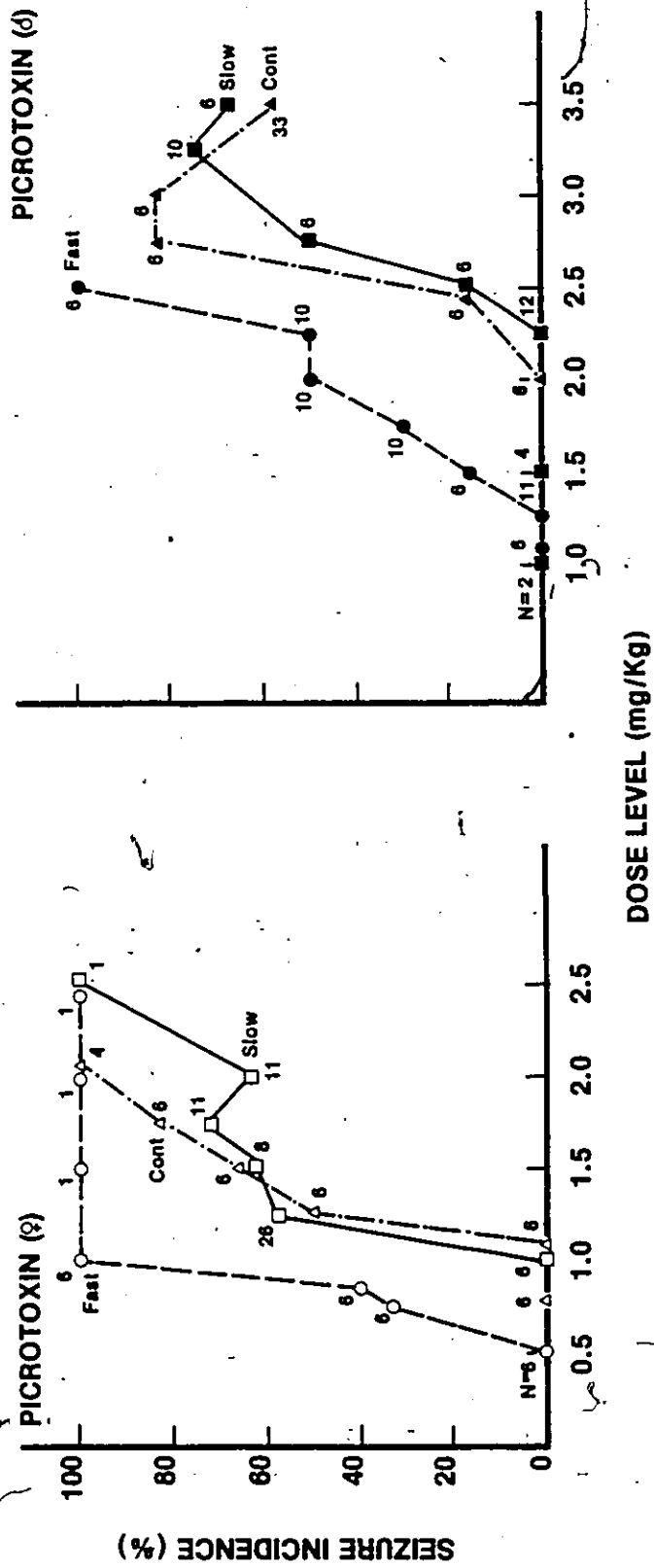
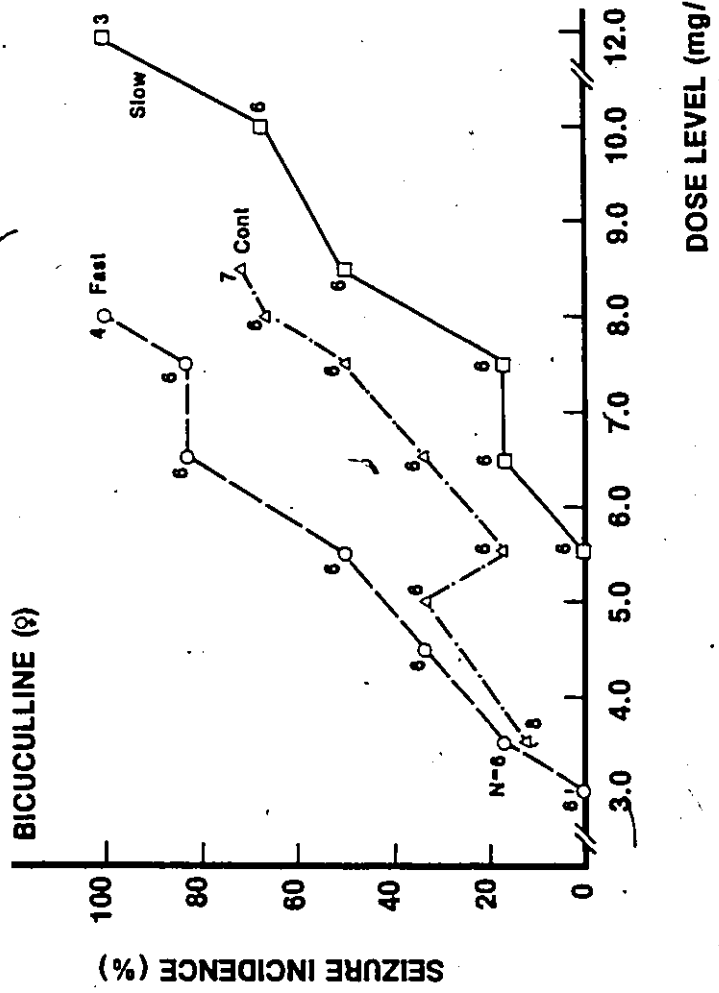
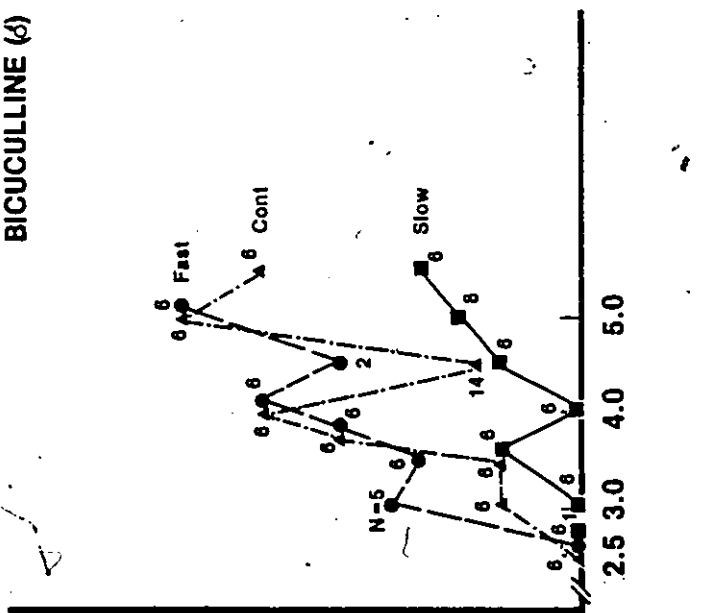


Figure 21 Dose-response curves for bicuculline. The key dose level for male rats (right) was 5.0 mg/kg, at which a significantly smaller proportion of SLOW rats (■) showed seizures compared to FAST rats (●) or control (▲) rats ( $z=3.786$ ,  $p<.0002$ , one-tail). The key dose level for female rats (left) was 6.5 mg/kg, at which a significantly larger proportion of FAST rats responded with seizures compared to SLOW rats ( $z=4.304$ ,  $p<.0001$ , one-tail) or control rats ( $z=2.878$ ,  $p<.005$ , one-tail).



DOSE LEVEL (mg/Kg)

clonic limb movements. The results are shown in Fig. 22. FAST rats were consistently more likely to show seizure activity than SLOW rats at all dose levels.

Diazepam and propranolol: The anticonvulsant threshold dose level of diazepam, operationally defined as the minimum dose required to reduce the seizure severity to at least stage 3, was found to be almost identical for FAST and SLOW rats (.65 mg/kg vs. .64 mg/kg, respectively).

The administration of propranolol on the first day of kindling indicated that the two strains might differ in their responses to drugs which affect catecholamine systems. The results are shown in Table VI. SLOW rats tended to show shorter AD durations under the influence of propranolol, compared to FAST rats ( $p < .064$ ). This effect appeared to be due primarily to suppression of discharge activity in the SLOW rats.

#### CONCLUSIONS AND DISCUSSION

The results of these tests confirmed that the FAST rats are more susceptible than SLOW rats to a range of epileptogenic agents. The results of the strychnine tests, however, indicated that the susceptibility seen in FAST rats is probably not due to spinal cord or lower brain-stem mechanisms. This was in accordance with the results of the audiogenic seizure tests, in which no differences were found between FAST and SLOW rats.

All of the drugs that differentially affected the SLOW and FAST strains are believed to exert their effects via the GABA system. Together with the apparent differences on a presumed measure of GABA-

Figure 22 Dose-response curves for isoniazid. The key dose level for male rats (single closed data points) was 175.0 mg/kg, at which a significantly larger proportion of FAST rats ( $\bullet$ ) showed seizure responses compared to SLOW rats ( $\blacksquare$ ;  $Z=5.256$ ,  $p<.0001$ , one-tail) or control rats ( $\blacktriangle$ ;  $Z=2.734$ ,  $p<.005$ , one-tail). The key dose level for female rats (broken lines) was 200.0 mg/kg, at which a significantly smaller proportion of SLOW ( $\square$ ) rats had seizures compared to FAST ( $\circ$ ) rats ( $Z=2.878$ ,  $p<.005$ , one-tail) or control rats ( $\triangle$ ;  $Z=3.098$ ,  $p<.001$ , one-tail).



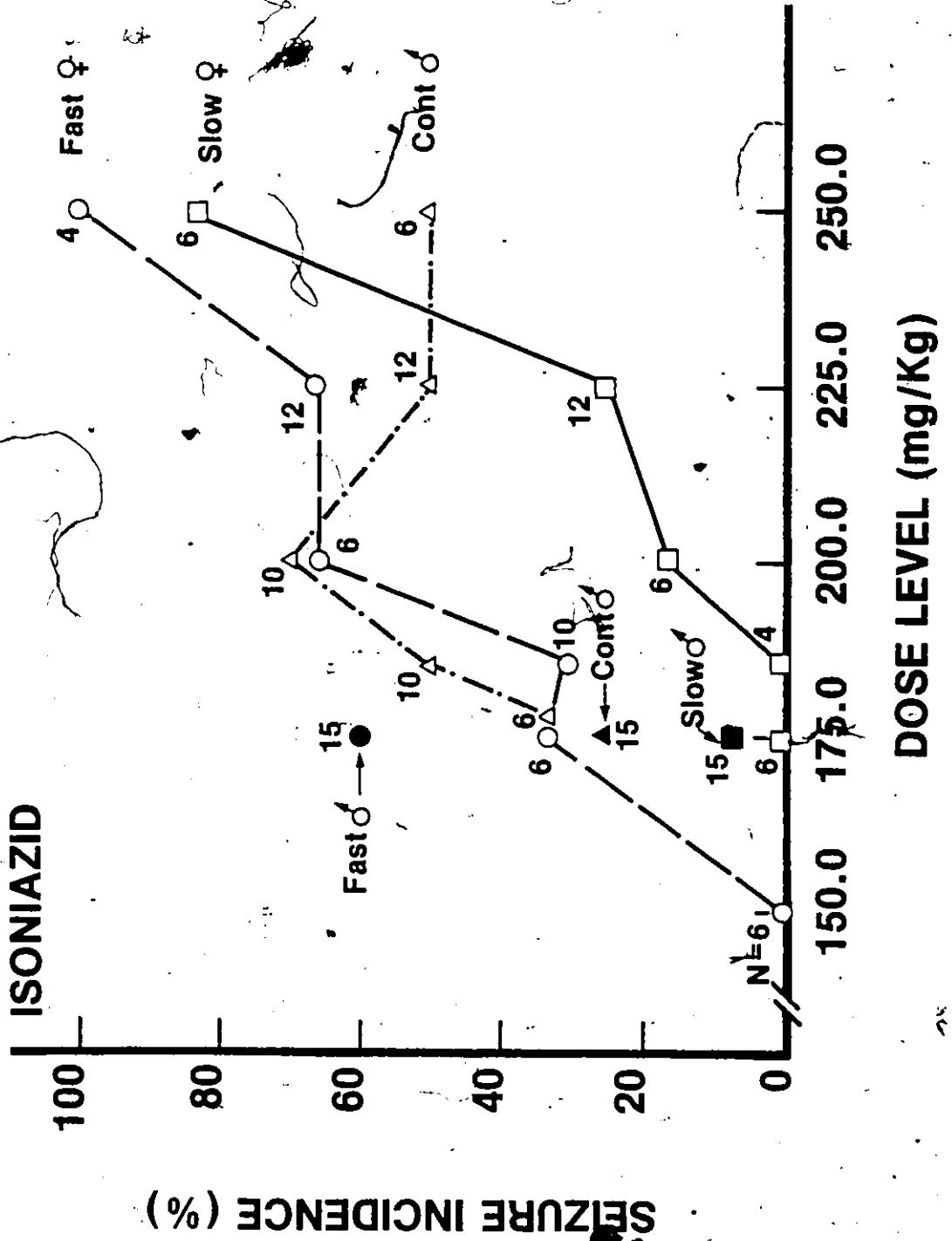


Table VI The effect of propranolol administration on the response to the first kindling train (at 400  $\mu$ A). AD's tended to be shorter in drug-treated SLOW rats compared to SLOW rats treated with NaCl (U=6,  $p < .064$ , two-tailed).

Table VI

Treatment	Propranolol		NaCl	
Strain	FAST	SLOW	FAST	SLOW
	41.0	15.0	31.5	43.5
AD	28.0	13.0	21.0	25.0
duration	17.0	7.5	14.5	16.5
(sec)	13.5	7.5	11.0	12.5
	11.5	0.0	9.5	11.0
	0.0	0.0	0.0	11.0
$\bar{X}$	18.5	7.2	14.6	19.9
SD	14.3	6.3	10.8	12.7
M	15.3	7.5	12.8	14.5

mediated recurrent inhibition (Chapter IV), these results raise the possibility that the differences between the 2 strains might be due to differences in levels of activity in GABA systems. The response of the 2 strains did not differ to diazepam (also believed to affect GABA systems), but these responses were measured in fully kindled animals.

Assay techniques may be required to determine differences in transmitter systems between the 2 strains. A preliminary study, with blind-procedures, indicated that [<sup>3</sup>H]-muscimol binding to the GABA-receptor did not differ between FAST and SLOW animals (Oshima, Tuff and Mishra, unpublished data). It is still possible that a deficiency in GABA function becomes apparent only when the system is driven to high levels of activity.

The effect of propranolol on the first evoked AD was unexpected. We reasoned that FAST animals, if deficient in catecholamines, would show greatly exaggerated seizure responses under propranolol. Instead, we found that SLOW animals showed depressed ADs under propranolol. Nevertheless, we measured NA levels in the pyriform lobes of the 2 strains (Oshima, Racine, Burnham and Kish, unpublished data) and found significantly higher levels in SLOW compared to FAST animals ( $\bar{X} = 0.45$  ng/mg tissue, SE = 0.03, vs 0.36 ng/mg, SE = 0.02, respectively). Clearly, further work must be done in these and other (e.g. benzodiazepine and peptide) transmitter systems.

## CHAPTER VI GENERAL DISCUSSION

AD thresholds, AD durations, AD spike amplitudes, 'transfer' kindling, and EP's evoked in limbic pathways did not differ between the strains. It seems likely, therefore, that the difference between FAST and SLOW animals cannot be attributed to differences in local, baseline excitability.

One of the possible mechanisms underlying an increased rate of kindling is an increased rate of potentiation in excitatory systems. This hypothesis was rejected when FAST and SLOW rats were found to show comparable levels and rates of potentiation. There were, however, differences in paired pulse facilitation/depression effects. FAST rats showed less depression at interpulse intervals up to 1000 msec.

These results suggested that a weakness in inhibitory mechanisms may underly the increased rate of seizure development in FAST rats. Consistent with this hypothesis were the observations that FAST rats showed fewer failures of stage 5 seizures, a tendency to show fewer AD threshold rises, and a shorter latency to secondary AD onset. It has been proposed that deficiencies in GABA-mediated inhibition may underly some human and experimental epilepsies (Meldrum, 1975). Also, GABA agonists are the most effective anticonvulsants, and GABA is believed to mediate the recurrent inhibition in hippocampal complex, where the paired pulse depression was found to be stronger in SLOW rats. Consequently, it was further hypothesized that FAST rats may be specifically deficient in GABA-mediated inhibition.

The data from the pharmacological studies were consistent with this hypothesis. The 2 strains reacted quite differently to drugs that affected the GABA system. A preliminary GABA binding study, however, revealed no differences between the strains. It is now known that kindling actually enhances GABA-mediated recurrent inhibition (Goddard, 1981; Maru, Tatsuno, Okamoto & Ashida, 1982; Tuff, Racine & Adamec, in press). Another possibility is that baseline GABA-mediated inhibition is normal but more prone to failure when driven to high levels of activation. Tuff, Racine and Adamec (in press), however, found that kindling results in a decrease in failure of inhibition. There is also some evidence that the number of benzodiazepine receptor sites (associated with the GABA system) is increased by kindling (Tuff, Racine & Mishra, in press).

Another mechanism is suggested by a further look at the paired pulse depression data. It was found that the greater depression, seen in SLOW rats, extended as far as 1000 msec. This is well beyond the duration generally assumed for recurrent inhibition. The late after-hypolarization, which presumably underlies the late depression in the paired pulse test, is believed to be a  $Ca^{2+}$ -mediated  $K^+$  efflux. This raises the possibility that ionic mechanisms in the neuron membrane may differ between the strains. There may, for example, be differences in the number of  $Ca^{2+}$  and/or  $K^+$  channels. Another possibility is that  $Ca^{2+}$  buffering mechanisms may differ between the 2 strains. Baimbridge and Miller (1982) have recently reported that  $Ca^{2+}$ -binding protein levels are decreased in dentate gyrus granule cells as a result of kindling. Further research on membrane characteristics, and on the  $Ca^{2+}$  buffering

properties of neural cells, might reveal the critical differences between the FAST and SLOW strains.

In summary, two strains of rats with different seizure susceptibilities were successfully bred out of the same original parent strains. Kindled seizures appear sensitive to some genetic predisposition, as are some types of human epilepsy (Gastaut & Broughton, 1972). The selective breeding of these strains has provided preparations that are ideally suited for the testing of hypotheses that cannot be tested in other preparations. The present experiments have paralleled the general direction followed by research on epileptogenesis in the last decade. It was generally believed that kindling was due to potentiation of excitatory function, then to loss of tonic or recurrent inhibition, and finally to alterations in the membrane properties of certain cell types (e.g. Racine et al., 1981). The data from the present experiments would appear to exclude differences in potentiation or recurrent inhibition, at least in the systems tested, as mechanisms underlying the strain differences. The preliminary finding of lower NA levels in FAST animals, however, leaves open the possibility that levels of some types of synaptic inhibition may differ between the two strains.

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