Kindling Antagonism: An Arrest of Epileptogenesis?

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Abstract

Concurrent alternating stimulation of two limbic sites culminates in typical kindling of generalized seizures from one site (dominant), whereas the other site (suppressed) supports only nongeneralized seizures for as long as stimulation of the dominant site continues (kindling antagonism). Burchfiel and Applegate (1989; 1990) claimed that antagonism reflects a frank arrest of kindling from the suppressed site at an intermediate stage. They argued, moreover, that the eventual generalization of seizures provoked from the suppressed site after the termination of stimulation of the dominant site reflects a resumption of kindling from its previous state of arrest.

Burchfiel and Applegate also claimed that the behaviorally stereotyped arrest of kindling from the suppressed site reveals critical transitions between sequentially expressed mechanisms that govern both antagonism and kindling. They therefore viewed kindling as a stepwise process that is mediated by qualitatively and temporally distinct mechanisms. This position hinges on the assumption that antagonism reflects a true arrest of kindling from the suppressed site rather than a transient inhibition of seizures. I conducted the following experiments to determine whether the assumption is justified.

In Experiment 1, I replicated and extended the observations of Burchfiel and Applegate concerning the expression of antagonism during alternating stimulation of limbic as well as nonlimbic sites. The results of Experiment 1 thus indicate that antagonism is indeed a robust phenomenon and therefore worthy of further study.

In Experiment 2, the imposition of a prolonged stimulation-free period (30 d) after the termination of stimulation of the dominant site (amygdala) did not significantly reduce the number of stimulations of the suppressed site (septal area) required to elicit a generalized seizure. Also, epileptiform afterdischarge provoked from the septal area increased during alternating stimulation, and the septal area supported generalized seizures after fewer stimulations in rats previously expressing antagonism as compared to control rats previously kindled from the amygdala. Collectively, these data are consistent with the view of Burchfiel and Applegate that kindling from the suppressed site progresses to an intermediate stage during alternating stimulation and resumes after the termination of stimulation of the dominant site.

The results of Experiment 2 also suggest the possibility that the development of seizures from the suppressed site after the termination of stimulation of the dominant site is dictated by the additive expression of: First, the well-documented facilitation of kindling from one site that reliably follows kindling from another (i.e., transfer between the amygdala, which

supported generalized seizures, and the septal area); second, (partial) kindling from the septal area, which previously supported nonconvulsive or partial seizures, during the Initial Phase. The results of Experiment 3 revealed that the facilitation of seizure development from the septal area observed in rats previously exposed to alternating stimulation, which perhaps is attributable to partial kindling from the suppressed site, was site-specific. Rats subjected to alternating stimulation of the left amygdala and right septal area and control rats previously stimulated only in the left amygdala subsequently demonstrated generalized seizures following similar numbers of stimulations of the previously unstimulated right amygdala.

Another plausible view is that antagonism reflects a long-lasting (> 30 d) form of inhibition that is perhaps uniquely invoked by alternating stimulation. While the results of Experiments 1 - 3 do not rule out this possibility, the results of Experiment 4 clearly indicate that the persistence of any such effects of alternating stimulation is not mediated by continuing influences of the dominant site: After the establishment of antagonism, radio-frequency lesions of the dominant site (amygdala) failed to alter the development of seizures provoked by stimulation of the suppressed site (septal area).

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I give special thanks to the Monki, with all my love and bubbles.

Dedication

To Ma with love from me and the Monki.

Introduction

KINDLING AND ALTERED BRAIN FUNCTION

With the repeated application of brief low-intensity high-frequency electrical stimulation to discrete regions of the forebrain, Goddard (1967) and Goddard, McIntyre, and Leech (1969) observed a progressive exacerbation of convulsive behaviors (kindling). Whereas initial stimulations elicited behavioral signs such as freezing and automatisms (e.g., grooming), later stimulations reliably provoked generalized convulsions. The accentuated behavioral responsivity that occurs as a consequence of repeated exposure to the fixed input (electrical stimulus) clearly indicates that kindling reflects profoundly altered brain function (Goddard et al., 1969). Even more striking are data indicating that the functional reorganization of the brain associated with kindling is long-lasting, perhaps even permanent. Several studies have shown that seizure sensitivity does not abate following prolonged stimulation-free periods (e.g., Goddard et al., 1969; Homan & Goodman, 1988).

Prior to the observations of Goddard and colleagues, Delgado and Sevillano (1961) noted a coupling of brain stimulation-induced behavioral convulsions and epileptiform afterdischarge (AD) recorded electroencephalographically (EEG) from the site of stimulation. Not only was AD necessary for the expression of convulsions, but it subsequently became apparent that duration, amplitude, frequency, and transynaptic propagation

of AD increased in conjunction with heightened behavioral responsivity (Racine, 1972a; Racine, Gartner, & Burnham, 1972). This suggests that AD rather than electrical stimulation initiates the functional reorganization of the central nervous system that subserves kindling. Indeed, repeated stimulation with low intensities of current that do not elicit focal AD does not kindle seizures (Pinel, Skelton, & Mucha, 1976; Racine, 1972a).

Although AD plays a critical role in kindling-related reorganization of the central nervous system, there remains debate as to the location(s) at which AD exerts its epileptogenic effects. Some data suggest that alterations to the site of stimulation dictate the course of kindling. Reductions in focal AD threshold (ADT: Minimum current intensity required to produce AD) are often evident during kindling (Racine, 1972a), suggesting that kindling arises as a consequence of aberrant function of cells directly affected by suprathreshold electrical stimuli. From another perspective, high-dose intracortical injections of penicillin induce convulsions that decrease in severity as a function of repeated treatment. Like electrical kindling, however, the intermittent application of lower concentrations of penicillin promotes intensification of seizures. With possible relevance to the role of stimulated cells in kindling, Collins (1978) found heightened metabolism of glucose at the site of low-dose application of the drug, suggesting that functional changes proximal to the site of infusion mediate the intensification of seizures. This may indicate that localized alterations in

brain function, as revealed by metabolic markers, are involved in electrical kindling.

Data implicating local mechanisms do not rule out the possibility that altered physiological processes distal to the site of stimulation are partially or even exclusively responsible for kindling. Reductions in ADT, like those seen during kindling, are also produced by repeated subthreshold stimulations, which do not kindle seizures (Pinel et al., 1976; Racine, 1972a). This indicates that local changes in neural function that mediate the reduction of ADT are not sufficient for kindling. Pharmacological data have further dissociated processes involved in the reduction of ADT and in kindling. Diazepam, for example, slows[1] kindling without necessarily affecting ADT (e.g., Wise & Chinerman, 1974). Also, cortical and subcortical structures differ with respect to initial ADT and its reduction during kindling. The differences, however, are not particularly predictive of kindling rates supported by the sites (Burnham, 1976; Racine, 1972a; Racine, 1975). Finally, with reference to the findings of Collins (1978), mentioned above, it is uncertain whether the metabolic alterations, associated with repeated infusion of penicillin and concomitant exacerbation of seizures, were restricted to the site of infusion.

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[1]Rates of kindling are reciprocally related to the number of ADs required to elicit generalized seizures. Thus, slow kindling rates indicate that seizures generalize following many ADs, whereas fast kindling rates indicate that seizures generalize following few ADs.

The investigation of transfer phenomena strongly implicates functional reorganization distal to the site of stimulation in kindling. Transfer refers to the facilitation of kindling obtained from one (secondary) site as a consequence of prior kindling from another (primary site; Burnham, 1976; Goddard et al., 1969; McIntyre, 1980; McIntyre & Goddard, 1973; Wada & Osawa, 1976). One plausible explanation of transfer is that AD propagated from the secondary site activates epileptic neurons at the primary site. The neurons then recruit distal circuits, the synchronous output of which drives convulsions. Tests of this hypothesis have revealed, however, that transfer does not to depend on altered function of the primary site. Lesions of the primary site following initial kindling had no effect on transfer (McIntyre & Goddard, 1973; Racine, 1972b). Likewise, transection of the forebrain commissures after primary site kindling, did not compromise transfer in rats (McIntyre & Edson, 1987). In fact, McCaughran, Corcoran, and Wada (1977), utilizing a similar preparation, actually reported facilitated transfer between homotopic amygdaloid sites. Clearly, transfer and hence kindling involve changes in brain function distal to the primary site.

HYPOTHESES OF KINDLING

Several hypotheses, which fall into two general categories, have emerged regarding the nature of the enduring transynaptic changes that contribute to kindling. Those in the first category (epileptic neuron

hypotheses) maintain that changes to the intrinsic properties (e.g., ionic balance mechanisms) of certain cells occur during kindling and persist thereafter. Those in the other category maintain that kindling involves altered synaptic efficacy throughout the central nervous system, producing net increases and decreases in excitatory and inhibitory neurotransmission, respectively. In subsequent pages, I shall conduct a broad albeit nonexhaustive review of research addressing the hypotheses concerning mechanisms of kindling, not so much to reveal what is known about kindling as to reveal that much is not known.

As a cautionary note, considerable research into the biological bases of kindling has employed correlational approaches. That is, kindling was often followed by some assay of behavior or brain structure or chemistry, which served as markers of some aspect of brain function (e.g., inhibition). It was frequently suggested, or even concluded, that an observed change in brain function evident after kindling (revealed by differences between kindled[2] and control subjects [typically rats] on assay) was responsible for kindling. There are, of course, alternatives to the assumption that "if it is evident following kindling, it caused kindling." One is that changes in markers of particular aspects of brain function may be secondary to kindling. It is also conceivable that some recurrent event (e.g., seizures)

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[2]Although I fully recognize that rats cannot, in principle, be kindled, I shall occasionally use this term in reference to rats that have expressed kindled seizures, as per typical usage within the literature.

independently causes both kindling and alterations to the functional markers. Moreover, kindling and the alterations to the markers may have different causes (e.g., seizures versus electrical stimulation).

In an attempt to resolve some of the interpretational morass associated with correlational research into mechanisms of kindling, Peterson and Albertson (1982) stated that alterations in brain function that subserve kindling must, like kindling, be enduring. That is, the changes must arise during the progressive intensification of seizures and persist for as long as the experimental subject demonstrates enhanced sensitivity to the epileptic effects of electrical stimulation. This implies that changes in brain function that are persistent (potential mediators of kindling) and those that are transient (evident only shortly following kindled seizures) are dissociable, in principle. In order to achieve such a dissociation, Peterson and Albertson (1982) suggested that assays be conducted long after the last kindled seizure. However, as will become evident (below), there is little consensus on the postseizure period required to fulfil this goal.

EPILEPTIC NEURON HYPOTHESES

Several investigators have observed synchronous burst discharges, occurring spontaneously or following afferent fiber activation, in hippocampal regions (Andersen, Gjerstad, & Langmoen, 1978; Dichter, Herman, & Selzer, 1973; Dingledine & Gjerstad, 1980; Lebovitz, 1979;

Schwartzkroin & Prince, 1978; Wong & Prince, 1979). It thus appears that hippocampal circuits may serve as generators, rather than mere conduits, of epileptiform discharge, and alterations to the characteristics of individual hippocampal cells may therefore be crucial to kindling (Mesher & Schwartzkroin, 1980; Wong & Traub, 1983). Some evidence suggests that altered hippocampal action potential-generating mechanisms are involved in kindling (McIntyre & Racine, 1986). On the other hand, resting membrane potential and resistance were unchanged after kindling (Kairiss, Racine, & Smith, 1984; McIntyre & Wong, 1985; Racine, Burnham, Gilbert, & Kairiss, 1986; Yamada & Bilkey, 1991). However, while Mody, Stanton, and Heinemann (1988) confirmed the findings of Racine and associates concerning resting membrane potential and observed no changes in amplitude or threshold of action potentials, dentate granule cell membrane resistance and slope conductance at resting potential were higher and lower, respectively, in rats kindled from either the amygdala or the ventral hippocampal commissure. Granule cells from kindled rats also demonstrated an anomalous increase in slope conductance with hyperpolarization, suggesting the emergence of an aberrant voltage-dependent conductance.

The observations concerning changes in hippocampal neuronal parameters and discharge patterns are consistent with the speculations of Oliver, Hoffer, and Wyatt (1980) that kindling depends upon modified hippocampal ionic transport. Following kindling, hippocampal cells

demonstrate heightened sensitivity to the epileptic effects of altered extracellular concentrations of K^+ and Ca^{++}, which synergistically increase the propensity of hippocampal cells to exhibit spontaneous bursts (Stringer and Lothman, 1988). Further implicating aberrant ionic kinetics in the capacity proposed by Oliver et al. (1980), electrically or synaptically elicited dendritic Ca^{++} conductances were enhanced after kindling from CA1 (Wadman, Heinemann, Konnerth, & Neuhaus, 1985). Also, whereas intracellularly recorded excitatory postsynaptic potentials (EPSPs) increased with hyperpolarization and decreased with depolarization of cells in control slices in vitro, the amplitude and width of EPSPs increased with either hyper- or depolarizing current injections in slices taken from kindled rats, reflecting increased N-methyl-D-aspartate (NMDA) receptor-dependent Ca^{++} conductance (Mody et al., 1988). These findings complement others indicating that kindling may involve altered Ca^{++} homeostatic mechanisms (Baimbridge & Miller, 1984; Baimbridge, Mody, & Miller, 1986; Miller & Baimbridge, 1983; Miller, Baimbridge, & Mody, 1986). In addition, Mody, Reynolds, Salter, Carlen, and MacDonald (1990) have found that both commissural and amygdaloid kindling lower the threshold of a transient Ca^{++} current and abolish a sustained Ca^{++} current in dentate granule cells. Because the sustained current returned following intracellular infusion of a Ca^{++} chelator, Ca^{++} channels themselves were not apparently compromised, and kindling may therefore involve reduced intracellular Ca^{++}

buffering capacity of the type described by Baimbridge and associates. This conclusion is consistent with the observation that kindled neurons also displayed an aberrant sustained outward current (possibly K^+; Mody et al., 1990).

The data implicating hippocampal burst generators in kindling are interesting in light of several observations concerning procpileptogenic properties of this structure. Attenuated rates of kindling from the amygdala or entorhinal cortex were evident following selective destruction of dentate granule cells (Dashieff & McNamara, 1982; Frush, Giacchino, & McNamara, 1986). Savage, Rigsbee, and McNamara (1985) claimed that knife-cuts to the perforant path had highly similar effects. Sutula, Harrison, and Steward (1986), however, failed to provide conclusive confirmation of the findings. Also, McIntyre and Racine (1986) reported that dorsal or ventral hippocampal lesions did not affect amygdaloid kindling. On the other hand, McIntyre and Racine (1986) observed that knife cuts that prevented propagation of AD from the amygdala to the hippocampus were actually facilitatory. Similar facilitations followed lesions of either CA3 or CA3c (Feldblum & Ackermann, 1987; Sutula, He, & Hurtenbach, 1987). This suggests that hippocampal circuits play an inhibitory role in kindling. In conclusion, while it is the case that bursting circuits within the hippocampus may undergo functional changes during kindling, it is unclear whether the burst generators or other hippocampal mechanisms contribute to kindling.

Empirical and theoretical concerns regarding the role of burst generators in kindling have shifted from those of the hippocampus to those of the amygdala-pyriform region for two principal reasons. First, following kindling-dependent status epilepticus, extensive cellular degeneration and gliosis in the amygdala-pyriform region are evident (McIntyre, Nathanson, & Edson, 1982b), suggesting that this region of the brain is an important participant in prolonged seizure discharge. Second, it is the case that the amygdala-pyriform region is typically the first to exhibit interictal spikes, regardless of which brain region receives stimulation. During kindling from the hippocampus, local interictal spikes appeared to have propagated from the amygdala-pyriform region. In vivo, therefore, the hippocampus may not autonomously generate such discharge patterns after kindling (Kairiss et al., 1984).

Further implicating burst generators of the amygdala-pyriform region in kindling, interictal spikes are thought, by some, to be markers of increased seizure susceptibility (Racine et al., 1986). Kairiss et al. (1984) observed increased interictal spike discharge in quiescent rats, which exhibit faster kindling than active rats (Grahnstedt & Ellertsen, 1984). Furthermore, Wada, Mizoguchi, and Osawa (1978) noted that baboons, which eventually developed generalized kindled seizures, exhibited greater interictal spike discharge than did monkeys, which were highly resistant to kindling. Also, spontaneous seizure activity induced by kindling from the hippocampus or

amygdala became evident following increased frequency of interictal spikes (Pinel & Rovner, 1978). The amygdala-pyriform region supports relatively rapid kindling (Goddard et al., 1969) and is generally the first site to express interictal spikes (Kairiss et al., 1984). Brain regions such as the dorsal hippocampus, by contrast, support relatively slow kindling and often fail to display interictal spikes (Kairiss et al., 1984).

Research concerned with the burst-generating circuits within the amygdala-pyriform region has produced some interesting results regarding the etiology of kindling. For example, in a study involving in vivo recording of single cell responses from the amygdaloid electrode site, Racine and Zaide (1978) found strong burst responses in kindled but not in nonkindled rats. In addition, McIntyre and Wong (1985) determined that stimulus-induced bursts in the pyriform cortex were longer in kindled rats. These researchers also found that stimulation of a variety of amygdaloid nuclei resulted in identical pyriform bursting. Thus, it seems that a number of exterior loci converge on a single pyriform burst generator via distinct neural pathways. Further supporting the claim is the finding that the pyriform region, isolated from the amygdala, remains capable of bursting (McIntyre & Wong, 1985). In addition to the above observations, McIntyre and Wong (1985) noted that amygdala-pyriform slices taken from kindled rats produced spontaneous bursts that were indistinguishable from those elicited by electrical stimulation.

Lesion studies have also implicated amygdala-pyriform circuitry in kindling. Briefly, either knife cuts that separated the amygdala from the pyriform cortex or extensive pyriform lesions retarded amygdaloid kindling (McIntyre & Racine, 1986, Racine, Paxinos, Mosher, & Kairiss, 1988). It is noteworthy, however, that kindling still occurred. This suggests that either the pyriform cortex is not necessary for kindling or pyriform tissue remaining after even extensive lesions was sufficient to fulfil some critical role. Unfortunately, these possibilities were not fully evaluated, as total destruction of the pyriform cortex proved lethal in all cases (McIntyre & Racine, 1986, Racine et al., 1988). Thus, it is unclear whether burst generators of the pyriform cortex are crucial to kindling.

The data concerning the role of the amygdala itself in kindling are also confusing. Goddard et al. (1969) observed that kindling rates related to the number of direct neural connections between the stimulated site and the amygdala, suggesting that the amygdala exerts a prokindling influence. In a more detailed examination, Le Gal La Salle (1979) observed that bilateral thermolytic lesions of the amygdala decreased the rate of kindling from the bed nucleus of the stria terminalis. Conversely, amygdaloid stimulation enhanced hippocampal kindling (Le Gal La Salle, 1983). Interestingly, this researcher failed to alter hippocampal kindling rates by either unilateral or bilateral amygdalectomy. Kaneko, Wada, and Kimura (1981), on the other hand, found that kainic acid-lesions of the amygdala actually facilitated later

kindling in rats. Furthermore, the facilitation was greater in rats with bilateral lesions. Similar results were obtained with hippocampal kindling, although bilateral and unilateral amygdalectomy facilitated kindling to the same degree (McIntyre, Stuckey, & Stokes, 1982c). Evidently, the role of the amygdala, and presumably other structures, in kindling is complex, involving both pro- and antiepileptogenic mechanisms.

SYNAPTIC REORGANIZATION

Long-lasting Potentiation

As mentioned previously, increases in duration, amplitude, and frequency of epileptiform discharge are readily observable at the primary site (Racine, 1972a). The changes are, however, more profound at distal sites, indicating that facilitation of excitatory synaptic contacts within extrafocal circuits (mono- or polysynaptically connected to the stimulated site) participates in kindling (Racine, 1972b; Racine et al., 1972). More systematic evaluation has revealed increased amplitude of potentials evoked in excitatory pathways as a function of kindling (kindling-induced potentiation; e.g., de Jonge & Racine, 1987; Douglas & Goddard, 1975; Leung & Shen, 1991; Maru & Goddard, 1987; Sutula et al., 1986). The results are reminiscent of the long-lasting potentiation of synaptic efficacy that follows high-frequency stimulation (with parameters adjusted such that seizures do not occur) of excitatory neuronal populations (long-term

potentiation; Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973). Because long-term potentiation and kindling apparently share a common outcome (i.e., heightened synaptic efficacy) and involve similar methods of induction (i.e., high-frequency trains), it is possible that kindling depends on long-term potentiation and its inherent mechanisms.

Pharmacological research has dissociated kindling-induced potentiation and long-term potentiation, lending little support to the hypothesis that long-term potentiation mediates kindling. For example, Cain, Boon, and Hargreaves (1989) have shown that while urethane anesthesia precludes the induction of kindling-induced potentiation but not long-term potentiation, the competitive NMDA receptor antagonist AP5 precludes long-term potentiation but not kindling-induced potentiation (but see Gilbert & Mack, 1990). A further dissociation between the two forms of neural plasticity is that long-term potentiation is susceptible to NMDA antagonists only during its induction, indicating that mechanisms mediating the expression of long-term potentiation are NMDA-independent (e.g., Abraham & Mason, 1988; Coan, Saywood, & Collingridge, 1987; Collingridge & Bliss, 1987; Harris, Ganong, & Cotman, 1984). By contrast, Jibiki, Fujimoto, Kubota, and Yamaguchi (1991) observed a remarkable attenuation of previously established kindling-induced potentiation by MK-801 in an acute preparation. This is consistent with the report of Mody et al. (1988), in which AP5 reduced potentials evoked in hippocampal slices prepared from kindled rats. Given

methodological limitations of the studies of Jibiki et al. (1991) and Mody et al. (1988), however, it is unclear whether the NMDA receptor antagonists reversed kindling-induced potentiation or merely masked its expression. In any event, it is apparent that kindling-induced potentiation and long-term potentiation are not unitary, a conclusion supported by an electrophysiological dissociation: Whereas long-term potentiation typically involves proportionately greater potentiation of the population spike than of the field EPSP (Abraham, Bliss, & Goddard, 1985), kindling-induced potentiation tends to involve enduring increases in the field EPSP and depression of the population spike (de Jonge & Racine, 1985; Douglas & Goddard, 1975).

Solely on the basis of the dissociation of kindling-induced potentiation and long-term potentiation, it remains uncertain whether either or both forms of potentiation contribute to kindling. Racine, Newberry, and Burnham (1975) observed facilitations of amygdaloid kindling by the prior induction of long-term potentiation. The enhancement of kindling occurred even though the long-term potentiation-eliciting trains of stimulation did not produce epileptiform discharge. However, more recent analyses indicate that kindling itself is dissociable from both long-term potentiation and kindling-induced potentiation. First, Cain (1989) has summarized numerous pharmacological distinctions between long-term potentiation and kindling. Furthermore, kindling is relatively permanent (Goddard, 1967; Goddard et al., 1969),

whereas long-term potentiation is a transitory phenomenon that typically decays in fewer than 14 days (Racine, Milgram, & Hafner, 1983). With respect to kindling-induced potentiation, Racine et al. (1983) also reported that potentiated evoked responses are not inevitably present after kindling. For example, Gilbert and Mack (1990) found that MK-801-treated rats exhibited full[3] albeit delayed kindling in the absence of kindling-induced potentiation. Finally, utilizing rapid kindling from the visual cortex of rabbits, Jibiki, Kubota, and Yamaguchi (1988) observed that changes in AD duration were not predictive of enhancement of evoked field EPSPs. Thus, it does not appear that mechanisms subserving either long-term potentiation or kindling-induced potentiation are crucial to kindling.

Morphological Alterations

A number of researchers have proposed that alterations to cellular morphology, perhaps involving dendrites or their synaptic components,

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[3]I shall occasionally use the term full kindling to indicate that kindling of stage 5 generalized seizures has occurred, although, as noted in text, I recognize that sustained application of high intensities of stimulation, over many days, can lead to further progression of symptoms, including multiple episodes of rearing and falling, running fits, and mild tonic episodes. Pinel and Rovner (1978) first described these more advanced stages of kindling, although I note that other investigators (e.g., Bertram & Lothman, 1993) do not describe similar signs in rats subjected to 1,500 stimulations of limbic sites. I also shall occasionally describe the kindling of nonconvulsive or partial seizures with the term partial kindling. This contrasts the term partial kindling as used by Adamec and Stark-Adamec (1983) in reference to the reduction of ADT via the repeated delivery of subthreshold stimuli.

mediate kindling. In a search for synaptic metamorphosis, Goddard and Douglas (1975) compared kindled and nonkindled tissue using electron microscopy. Although a great variety of measures were taken, no consistent physical differences were apparent, and attention subsequently turned to other regions of the brain. Racine, Tuff, and Zaide (1975) observed no changes in dendritic characteristics attributable to cortical kindling. Similarly, kindling from the hippocampus did not influence the dendritic constitution of CA1 or CA3 pyramidal cells or dentate granule cells (Crandall, Berstein, Boast, & Zornetzer, 1979).

Contrasting the negative findings, recent evidence supports the hypothesis that kindling involves altered neuronal morphology. Specifically, Sutula, He, Cavazos, and Scott (1988) reported dense Timm granule deposits in the supragranular layer of the dentate gyrus up to 5 months following 3 consecutive generalized seizures kindled by stimulation of the perforant path, olfactory bulb, or amygdala. Because positive Timm reactions, which reveal zinc-rich mossy fibers, are normally sparse in this region, kindling may involve increased associational innervation and hence feedback excitation of dentate granule cells via aberrant collateralization of mossy fibers.

Sutula et al. (1988) failed to observe overt cellular degeneration in hippocampal CA3/CA4, similar to researchers examining the effects of kindling on other cell populations (Crandall et al., 1979; Goddard & Douglas,

1975; Goddard et al., 1969; Racine et al., 1975; Represa, Le Gal La Salle, & Ben-Ari, 1989). The reports stand in marked contrast, however, to the results obtained through detailed postkindling cell counts. Cavazos and Sutula (1990) reported a dramatic rate of hilar cell loss in kindled rats (estimated at 1% per seizure). On the basis of this outcome, Sutula proposed that kindling involves the cyclical destruction of (inhibitory) hilar interneurons, which initiates synaptic reorganization and its associated functional increases in excitatory feedback to the granule cells of the dentate gyrus (Tauck & Nadler, 1985). This precipitates heightened sensitivity to seizure-eliciting stimuli, the ictal manifestations of which promote further neuronal mortality (Cavazos & Sutula, 1990; Sutula, 1990).

Although the hypothesis of Sutula is highly compelling, particularly given that lesions of CA3/CA4 produce "kindled" patterns of sprouting of mossy fibers (Laurberg & Zimmer, 1981; Nadler, Perry, & Cotman, 1980), its tenability may hinge on the observations of Racine, Burnham, Gartner, and Levitan (1973), concerning the time course of kindling with hourly stimulation. Generalized kindled seizures can develop over the course of a few hours, and it is uncertain whether this epoch is sufficient for cellular death and sprouting of the magnitude described by Cavazos and Sutula (1990). Moreover, it has not been conclusively determined whether the recurrent projections of mossy fibers, seen after kindling, form excitatory synapses with granule cells or with inhibitory interneurons.

Electrophysiological evidence favors the latter hypothesis, as Racine and colleagues have observed heightened recurrent inhibition of the population spike recorded in the granule cell layer after kindling (de Jonge & Racine, 1987; Tuff, Racine, & Adamec, 1983).

Of perhaps even greater detriment to the view of Sutula, concerning the mechanistic basis of sprouting of mossy fibers, is a recent report of Bertram and Lothman (1993), in which characteristics of the entire dentate gyrus were assessed. Following the induction of approximately 1500 seizures kindled via a rapid technique involving long stimulus trains, Bertram and Lothman (1993) ostensibly confirmed the findings of Cavazos and Sutula (1990) regarding reduced densities of hilar interneurons. However, Bertram and Lothman found that the decreases reflected, rather than reduced numbers of cells, increased area of the hilar neuropil (as well as that of the molecular layer of the dentate gyrus) in rats expressing seizures as compared to unstimulated control rats. Thus, it may be the case that the methodology of Cavazos and Sutula (1990) was not sensitive to interactions between kindling or kindled seizures and nonsomatic components of hilar cells. Although the findings of Bertram and Lothman (1993) suggest that death of hilar interneurons does not contribute to kindling, the implications of hilar enlargement for the mossy fiber sprouting hypothesis of kindling (Cavazos & Sutula, 1990) are unexplored.

NEUROCHEMICAL HYPOTHESES

The neurochemical hypotheses of kindling fall into two categories. Hypotheses in the first category presuppose that kindling arises as a consequence of increased activity of excitatory neurotransmitters and neuromodulators (e.g., glutamate and acetylcholine); those in the second posit that decreased activity of inhibitory neurotransmitters and neuromodulators (e.g., γ-aminobutyric acid and monoamines) mediates kindling. Some evidence also implicates endogenous opioid peptides in kindling, although the excitatory/disinhibitory actions of these substances in the brain remain conjectural. For example, opioid peptides have been reported to enhance excitatory transmission within the hippocampus by acting presynaptically, thereby promoting the release of excitatory transmitters (Haas & Ryall, 1980). Postsynaptically, the peptides enhanced the coupling of EPSPs and population spikes recorded near pyramidal cells, indicating direct excitatory effects (Lynch, Jensen, McGaugh, Davila, & Oliver, 1981), and reduced inhibitory input to the pyramidal cells, indicating disinhibitory effects (Masukawa & Prince, 1982; Zieglgansberger, French, Siggins, & Bloom, 1979). It is equally uncertain, therefore, whether increases or decreases in the output of opioidergic systems would be excitatory or disinhibitory with respect to kindling (Cain, 1989). Because the resolution of this debate is not within the scope of the present text, opioidergic mechanisms will receive consideration separately from

hypotheses in the two principal categories. Research has also implicated other neuropeptides (e.g., somatostatin) and biochemicals (e.g., polyamines) in kindling. However, given limited data, I shall not attend further to the potential roles of these substances in kindling.

Increased Excitatory Transmission

Glutamate

As initially proposed by Crawford and Connor (1973), it appears that glutamate is the principal excitatory amino acid transmitter of the mossy fibers of the hippocampus[4]. Therefore, if the hypothesis of Sutula and coworkers (above) that kindling depends on seizure-induced death of cells and sprouting of recurrent axons is to remain tenable, it is essential that a positive role of glutamatergic transmission in kindling be established. Indeed, considerable research now indicates that the NMDA receptor participates in epileptogenesis. Specifically, NMDA receptor antagonists dose-dependently slow both amygdaloid and perforant path kindling (Bowyer, 1982; Cain, Desborough, & McKitrick, 1988; Callaghan & Schwark, 1980; Gilbert, 1988; Gilbert & Mack, 1990; McNamara, Russell, Rigsbee, & Bonhaus, 1988; Sato, Morimoto, & Okamoto, 1988). However, it is noteworthy that the studies do not directly implicate pathological

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[4]See Appendix A for summary of data implicating glutamate as the principal transmitter released by the mossy fibers in CA3.

release of glutamate from either the mossy fibers or any other discrete population of cells in kindling. Moreover, the involvement of the NMDA receptor in the cell death/sprouting cycle proposed by Sutula and associates has received no empirical attention. Additionally, whereas an NMDA-mediated Ca^{++} current sink, situated in the middle molecular layer approximately 150 μm from the granule cell layer, was evident following kindling (Mody et al., 1988), the emergent innervation reported by Sutula et al. (1988) appeared only in the inner molecular layer, closer to the somata of the granule cells. Thus, histological, electrophysiological, and pharmacological data do not provide a coherent view of the role of glutamate in kindling. It is equally unclear, therefore, whether kindling depends on aberrant glutamatergic transmission.

Acetylcholine

Mounting evidence indicates that kindling involves repeated activation of cholinergic systems. Goddard (1969) observed seizure development in rats following repeated intracerebral infusions of carbachol (cholinergic kindling). Vosu and Wise (1975) noted that not only were the amygdala, caudate, and hippocampus sensitive to cholinergic kindling, but also that electrical and chemical kindling rates supported by the structures were similar in relative terms. In addition to the anatomical parallels, cholinergic and electrical kindling show similar behavioral and electrographic patterns of

development (Vosu & Wise, 1975; Wasterlain & Jonec, 1980a; Wasterlain & Jonec, 1980b; Wasterlain, Morin, Jonec, & Billawala, 1979; Wasterlain, Masuoka, & Jonec, 1981), and, in both cases, heightened seizure sensitivity is enduring (Goddard, et al., 1969; Wasterlain & Jonec, 1980b).

There is evidence that stimulation of muscarinic rather than nicotinic receptors is crucial to cholinergic kindling. Briefly, epileptogenesis accompanies the repeated administration of muscarine, carbachol, or acetyl-B-methylcholine (Wasterlain & Jonec, 1980a; Wasterlain & Jonec, 1980b). Moreover, the muscarinic receptor antagonists atropine and quinuclidinyl benzylate block cholinergic kindling (Wasterlain & Jonec, 1980a; Wasterlain & Jonec, 1980b; Wasterlain & Jonec, 1983; Wasterlain, Jonec, & Holm, 1978; Wasterlain, Morin, & Jonec, 1982). In contrast, seizures did not develop with repeated infusions of nicotinic antagonists (Wasterlain & Jonec, 1980b), and blockage of nicotinic receptors with d-tubocurarine failed to inhibit kindling elicited by infusions of carbachol (Wasterlain et al., 1978).

Data concerning the effects of anticholinergic substances on electrical kindling provide a somewhat more complex picture. Although Racine and coworkers retarded limbic (Arnold, Racine, & Wise, 1973) and cortical kindling with atropine (Racine, Burnham, & Livingston, 1979), several attempts at replication failed (Blackwood, Martin, & Howe, 1982; Corcoran, Wada, Wake, & Urstad, 1976; Meyerhoff & Bates, 1985). Collectively, the

findings lend equivocal support to the hypothesis that muscarinic receptors participate in kindling. It appears, however, that the negative findings may have been dose-dependent, as scopolamine, a more potent muscarinic antagonist, exerts robust antikindling effects (Cain et al., 1988; Cain, McKitrick & Desborough, 1987; Kirkby & Kokkinidis, 1991; Lupica & Berman, 1988; Westerberg & Corcoran, 1987). Moreover, other results suggest that electrical kindling, unlike its cholinergic counterpart, may involve synergistic activation of muscarinic and nicotinic receptors. Specifically, Meyerhoff and Bates (1985) found that a dose of atropine, which was by itself ineffective, retarded kindling when administered in combination with mecamylamine, a nicotinic receptor blocker.

Also consistent with the position that cholinergic systems participate in kindling, Cain (1983) demonstrated significant bidirectional transfer[5] between electrical and carbachol kindling. Moreover, as evidence of a facilitative role for cholinergic systems, Burchfiel, Duchowny, and Duffy (1979) found that CA3 pyramidal cells became supersensitive to iontophoretically applied acetylcholine during the hour following AD elicited via stimulation of the fornix. Whereas restimulation prior to the development

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[5]In previous paragraphs, I used the term transfer in reference to facilitated electrical kindling obtained from one site as a consequence of prior electrical kindling from another. The term transfer may also refer to instances in which kindling by one means (e.g., repeated electrical stimulation) is facilitated by prior kindling by another means (e.g., repeated infusion of a drug). The precise meaning of the term transfer in this dissertation therefore depends on context.

of the hyperresponsive state resulted in shortened AD, prolonged AD was apparent when stimulation and cholinergic supersensitivity coincided. Unfortunately, the experimental preparation was acute, and it is therefore uncertain whether the effects of induction of AD on cholinergic sensitivity are permanent, as is kindling.

Disinhibition Hypotheses

Complementing the data indicating that heightened activity of excitatory neurotransmitter systems contributes to kindling, considerable research indicates that kindling depends on suppression of inhibitory neurotransmitters and neuromodulators. The evidence accumulated to date most strongly implicates γ-aminobutyric acid (GABA) and noradrenaline, whose efficacy as inhibitors of aberrant cellular discharge breaks down during kindling. There is also some evidence that dopamine and serotonin participate in a similar fashion.

GABA

Wasterlain et al. (1979) noted that the repeated intracerebral administration of bicuculline, a competitive antagonist of $GABA_A$ receptors, resulted in the progressive appearance of facial and forelimb twitches. A few of the subjects eventually exhibited generalized convulsions. Likewise, Sacks and Glaser (1941) observed a progressive decline in the convulsive

threshold dose of pentylenetetrazol, a noncompetitive $GABA_A$ receptor antagonist, with repeated administration (see also Pinel & Cheung, 1977). In addition to these observations, repeatedly administered subconvulsant doses of pentylenetetrazol or another noncompetitive $GABA_A$ receptor antagonist, picrotoxin, resulted in the gradual appearance of convulsive behaviors, the sensitivity to which persisted long after the last administration of the drug (Diehl, Smialowski, & Gotwo, 1984; Fabisiak & Schwark, 1982a; Nutt, Cowen, Batts, Grahame-Smith, & Green, 1982; Pinel & Van Oot, 1975). The data are consistent with the view that suppression of inhibitory neurotransmission contributes to electrical kindling.

Studies involving transfer phenomena also indicate that electrical and GABAergic kindling share common mechanisms. Cain (1981) observed accelerated electrical kindling following repeated systemic administration of pentylenetetrazol as well as bidirectional transfer between electrical kindling and kindling via repeated intracerebral administration of pentylenetetrazol (Cain, 1982). Similar transfer effects were observed between electrical kindling and kindling via repeated intracerebral or intraperitoneal infusion of picrotoxin (Cain, 1987).

Investigations evaluating the influence of other manipulations of GABAergic transmission also provide evidence that GABA plays an important role in kindling. First, Shin, Silver, Bonhaus, & McNamara (1987) slowed kindling with intranigral infusions of γ-vinyl GABA, which inhibits the

transamination of GABA, leading to increased concentrations of GABA. Also, Schwark and Haluska (1986) observed only nongeneralized seizures in rats treated with a GABA uptake inhibitor prior to electrical stimulation. In an earlier study, Wise and Chinerman (1974) similarly blocked kindling with either diazepam or phenobarbital, which enhance the coupling of the $GABA_A$ receptor to its chloride channel. Furthermore, rats that received either drug during a portion of the total number of kindling trials required more stimulations to reach criterion than did undrugged rats (Peterson, Albertson, Stark, Joy, & Gordon, 1981). The specific $GABA_A$ receptor agonist progabide also slowed kindling (Joy, Albertson, & Stark, 1984). Conversely, more rapid kindling was evident in conjunction with the administration of subconvulsant doses of either a $GABA_A$ receptor blocker or inhibitors of glutamic acid decarboxylase, which inhibit synthesis of GABA (Le Gal La Salle, 1980; Myslobodsky & Valenstein, 1980).

Several researchers have examined, via neurochemical assays, the hypothesis that kindling involves the progressive mitigation of GABA-mediated inhibitory mechanisms. As noted by Burnham (1989), most of the early investigations failed to reveal the hypothesized relations between kindling and chemical markers of GABAergic function. In brief, rates of GABA synthesis, as indicated by the activity of glutamic acid decarboxylase, were essentially normal in rats following kindling. Similarly, activity of GABA transaminase did not vary as a function of kindling, suggesting that

increased kindled seizure sensitivity does not interact with metabolism of GABA. Consistent with the negative observations concerning synthesis and breakdown of GABA after kindling, several research groups reported that concentrations of GABA were similar in kindled and control rats (Fabisiak & Schwark, 1982a; Fabisiak & Schwark, 1982b; Leach, Marden, Miller, O'Donnell, & Weston, 1985; Lerner-Natoli, Heaulme, Leyris, Biziere, & Rondouin, 1985; Liebowitz, Pedley, & Cutler, 1978). The kindling procedure also failed to alter either the release of or the cellular responsivity to GABA (Burchfiel et al., 1979; Liebowitz, et al., 1978). While changes in GABA receptor characteristics were not evident after kindling (McNamara, Peper, & Petrone, 1980; Tuff et al., 1983), measures of benzodiazepine binding decreased somewhat (Niznik, Kish, & Burnham, 1983).

It is noteworthy that the early investigations tended to take neurochemical measures shortly following the last of a series of kindled seizures. It is therefore possible that the inconsistent results obtained in these studies reflect transient effects of kindled seizures rather than the enduring changes that mediate kindling (e.g., Peterson & Albertson, 1982). Consistent with this speculation, recent investigations that employed longer intervals between the final kindled seizure and assay (e.g., 4 wk) have revealed persistent declines in some GABAergic parameters. Also, whereas neurochemical assays conducted in the early studies involved whole tissue homogenates, those conducted in more recent investigations utilized

synaptosomal fractions, which presumably provide a more accurate index of GABA-mediated inhibition. For example, Loscher and Schwark (1985; 1987) observed region-specific (i.e., amygdala and substantia nigra) reductions in activity of glutamic acid decarboxylase as well as concentrations of GABA in kindled rats. Itagaki and Kimura (1986) reported, after kindling, that resynthesis of GABA transaminase following the administration of gabaculine was impaired in the pyriform and parietal cortices, the amygdala, and the hippocampus. The finding may be consistent with the previously cited observations of Cavazos and Sutula (1990) concerning death of hilar interneurons during kindling. Specifically, impaired resynthesis of GABA transaminase observed in the brains of kindled rats may reflect smaller numbers of GABA-producing cells. On the other hand, this may indicate that brains of kindled rats, under certain circumstances, display reduced metabolism of GABA, a possibility that is not readily reconcilable with the disinhibition hypothesis of kindling.

Kamphuis, Huisman, Veerman, and Lopes de Silva (1991) observed increased K^+-stimulated Ca^{++}-dependent release of GABA from CA1 25-36 days after kindling. This may reflect a compensatory response to decreased postsynaptic efficacy of GABA (Gean, Shinnick-Gallagher, & Anderson, 1989; Hernandez, Rosen, & Gallagher, 1990). However, the increased release of GABA reported by Kamphuis et al. (1991) may also depend upon glutamatergic activity during spontaneous epileptiform bursting, often seen

in kindled tissue exposed to high concentrations of K^+ (e.g., King, Dingledine, Giacchino, & McNamara, 1985), although electrophysiological responses of the slices were not monitored during the assay of amino acids to determine whether bursting was occurring. Kamphuis et al. (1991) did not observe persistently increased release of glutamate in the slices, but this may reflect offsetting increases in glutamate uptake. It is thus unclear whether mechanisms participating in seizure-independent release of GABA are persistently altered as a function of kindling.

Even recent binding studies, despite their sensitivity to potentially confounding short-lived effects of kindled seizures, have not decisively implicated GABAergic pathology in kindling. Loscher and Schwark (1987) found decreased $GABA_A$ receptor binding in the substantia nigra of kindled rats; reductions in GABA and benzodiazepine receptor binding were transient and restricted to the dentate gyrus (Nobrega, Kish, Burnham, 1989; Nobrega, Kish, Burnham, 1990). Addressing the discrepant results, Nobrega et al. (1990) hypothesized that kindling does not depend on permanent alterations to basal GABAergic parameters. Rather, the temporary decreases in ligand binding that follow kindled seizures and perhaps the different rates of resynthesis of GABA transaminase in kindled and control rats (Itigaki & Kimura, 1986) indicate that kindling depends on persistently heightened reactivity of GABAergic mechanisms. The hyperreactive state could precipitate a sudden loss of GABA-mediated inhibition at the site of

stimulation during the initiation of seizures (Morimoto & Goddard, 1986) and perhaps periictally at distal sites, which would facilitate the propagation of AD and the development of convulsions. The mechanisms by which such hyperreactivity could arise and persist remain unknown.

The possibility that decreased GABA-mediated inhibition subserves kindling has also been assessed via electrophysiological techniques, which have revealed region-specific changes as a function of kindling. As noted previously, Racine and colleagues reported elevated recurrent inhibition in the granule cell layer of the dentate gyrus after kindling (de Jonge & Racine, 1987; Tuff et al., 1983). Complementing these findings, Maru and Goddard (1987) observed increased feedforward inhibition in the same region. By contrast, kindling appears to be associated with reduced levels of recurrent inhibition in hippocampal area CA1 (Adamec, 1991; King et al., 1985). Furthermore, inhibitory postsynaptic potentials evoked in the basolateral amygdala by stimulation of the stria terminalis (but not the lateral amygdala) were absent after kindling (Gean et al., 1989; Rainnie, Asprodini, & Shinnick-Gallagher, 1992). Also, Asprodini, Rainnie, and Shinnick-Gallagher (1992) found a diminished ability of the $GABA_B$ receptor agonist baclofen to suppress excitatory transmission in amygdaloid slices taken from kindled rats, implicating presynaptic mediation of disinhibitory processes in kindling. En masse, the electrophysiological as well as the neurochemical and pharmacological data suggest that while kindling does not involve a

ubiquitous loss of GABA-dependent inhibition, localized reductions may be important participants.

Noradrenaline

As in the case of GABA, research suggests that noradrenaline inhibits kindling, and kindling may therefore depend upon compromised central noradrenergic transmission. Generally speaking, experimental manipulations that antagonize forebrain noradrenaline facilitate kindling[6]. Thus, electrolytic, neurotoxic, and mechanical destruction of noradrenaline-producing cells accelerated kindling (Altman & Corcoran, 1983; Araki et al., 1983; Arnold et al., 1973; Bortolotto & Cavalhiero, 1986; Burchfiel, Applegate, & Konkol, 1986; Callaghan & Schwark, 1980; Carre & Harley, 1986; Corcoran, Fibiger, McCaughran, & Wada, 1974; Corcoran & Mason, 1980; Ehlers, Clifton, & Sawyer, 1980; McIntyre, 1980; McIntyre & Edson, 1981; McIntyre, Saari, & Pappas, 1979; Mohr & Corcoran, 1981). Conversely, the facilitation of kindling associated with 6-hydroxydopamine-induced (6-OHDA) lesions of the hippocampus was not evident after hippocampal grafting of fetal noradrenaline-rich cell suspensions (Barry et al., 1987). Presumably, this procedure reestablished the functional integrity of noradrenaline in the hippocampus.

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[6]Neither seizures nor epileptogenesis have been reported as a consequence of antagonism of central noradrenaline.

Complementing the lesion-related findings, acutely injected desmethylimipramine, an inhibitor of noradrenaline uptake, slowed kindling (McIntyre, Edson, Chao, & Knowles, 1982a). Also, electrical stimulation of the locus coeruleus prior to each kindling trial delayed the emergence of generalized seizures (Jimenez-Rivera, Voltura, & Weiss, 1987; Weiss, Lewis, Jimenez-Rivera, Vigil, & Corcoran, 1990). It is possible that the latter antikindling effect involves stimulus-induced efflux of noradrenaline from neurons of the locus coeruleus projecting to the forebrain. Consistent with this possibility, infusions of 6-OHDA into the dorsal noradrenergic bundle, the sole source of noradrenergic innervation of the forebrain, abolished the stimulation-related prophylaxis (Weiss et al., 1990).

Considerable research has focused on the relative contribution of specific noradrenergic receptors to the antikindling effects of noradrenaline. At present, it appears that the while α_1 receptors contribute little to kindling, α_2 and perhaps β receptors play an inhibitory role. Callaghan and Schwark (1979) and Gellman, Kallianos, and McNamara (1987) observed no changes in kindling as a function of administration of α_1 antagonists (i.e., phenoxybenzamine or corynanthine). Administration of a high-dose of propranolol facilitated kindling (Callaghan & Schwark, 1979), and McIntyre et al. (1982a) accelerated kindling by chronic treatment with desmethylimipramine, one of the effects of which is down-regulation of β receptors (e.g., Banerjee & Kung, 1977; Bergstrom & Kellar, 1979;

Meyerson, Ong, Martin, & Ellis, 1980); Gellman et al. (1987) failed to affect kindling with propranolol or specific antagonists of β_1 or β_2 receptors. On the other hand, marked enhancements of kindling were evident after treatment with the α_2 receptor antagonists idazoxan, yohimbine, or rauwolscine (Gellman et al., 1987); an agonist of the α_2 receptor, clonidine, slowed kindling, opposite to the effects of the antagonists (Gellman, et al., 1987; McIntyre & Guigno, 1988; Pelletier & Corcoran, 1993).

It appears that clonidine acts postsynaptically rather than presynaptically to delay kindling. Prophylactic effects of clonidine on kindling are evident only at doses above those reported to produce selective presynaptic binding (Freedman & Aghajanian, 1985; Svensson, Bunney, & Aghajanian, 1975). Additional evidence implicating postsynaptic α_2 receptors in kindling stems from a study in which clonidine slowed kindling following the destruction of presynaptic noradrenergic terminals by 6-OHDA administration (McIntyre & Guigno, 1986). This interpretation assumes, however, that pretreatment with 6-OHDA did not alter the functional characteristics of postsynaptic α_2 receptors, thus altering the actions of clonidine.

The neurochemical data obtained to date do not indicate that permanent reductions in central concentrations of noradrenaline are necessary to kindling. Although several early studies suggested that this may be the case (see Corcoran, 1981), they are not consistent with the

findings or more recent and systematic evaluations. Blackwood (1981) detected no persistent alterations to concentration or turnover of noradrenaline, whereas Lewis, Westerberg, and Corcoran (1987) observed only small inconsistent regional shifts in levels of noradrenaline up to 4 wk after kindling from the amygdala. The results compare favorably with those of Okazaki and associates, who found similarly unchanged levels, turnover, and metabolic disposition of noradrenaline (Okazaki, Warsh, & Burnham, 1988).

Several studies have revealed altered characteristics of specific populations of central receptors of noradrenaline as a function of either full or partial kindling. The investigations, summarized by Corcoran and Weiss (1990), suggest that transient increases in the number of α_2 receptors on locus coeruleus neurons are evident following partial but not full kindling (Jimenez-Rivera, Chen, Vigil, Savage, & Weiss, 1989). Because presynaptic receptors in the locus coeruleus regulate release of noradrenaline via feedback inhibition (Foote, Bloom, & Ashton-Jones, 1983), the observed changes in the distribution of receptors may contribute to kindling by decreasing synaptic concentrations of noradrenaline throughout the brain[7]. Contrasting the transient up-regulation of α_2 receptors, down-regulation of cortical β receptors is apparent several weeks after the termination of full

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[7]Note that the α_2 receptor agonist clonidine appears to act postsynaptically to inhibit kindling (e.g., McIntyre & Guigno, 1986).

kindling (McIntyre & Roberts, 1983; Stanford & Jefferys, 1985). Coupled with pharmacological data indicating that antagonism of β receptors facilitates kindling (Callaghan & Schwark, 1980; McIntyre et al., 1982a), the findings could be interpreted to indicate that kindling depends on enduring hypofunction of β receptors. However, the interaction between kindling and characteristics of β receptors varies with age, stimulation schedule, and severity of kindled seizures (Corcoran, 1988; Corcoran & Weiss, 1990; McIntyre & Roberts, 1983; Michelson & Buterbaugh, 1985; Stanford & Jefferys, 1985), and other evidence suggests that β receptors actually promote rather than suppress seizures (Mueller & Dunwiddie, 1983). Therefore, the precise role of β receptors in kindling remains to be established.

Dopamine

Very little evidence implicates dopamine as an important participant in kindling. In studies in which antagonism of catecholamines was found to facilitate kindling, the behavioral effects related better to changes in noradrenergic than in dopaminergic systems. Thus, for example, 6-OHDA-induced depletions of noradrenaline, both noradrenaline and dopamine, but not dopamine alone facilitated kindling in rats (Corcoran & Mason, 1980; McIntyre, 1980; McIntyre & Edson, 1981; McIntyre et al., 1979). In addition, kindling did not differ from control rates in rats treated with the

dopamine receptor agonist apomorphine or the dopamine receptor blockers haloperidol or pimozide (Callaghan & Schwark, 1979; Stock, Kummer, Stumpf, Zenner, & Sturm, 1983). Not all evaluations of dopamine have been negative, however. Sato, Hikasa, and Otsuki (1979) showed that treatment with apomorphine following chronic administration of cocaine inhibited kindling, whereas the administration of haloperidol facilitated kindling. Collectively, the results indicate that the proposition that dopamine importantly influences kindling is only marginally compelling due to rather inconsistent findings across experimental procedures. Hence, it appears unlikely that kindling arises as a consequence of progressive alterations to dopaminergic function.

Serotonin

Pharmacological studies have implicated serotonin as an endogenous inhibitor of kindling. Pretreatment with an inhibitor of synthesis of serotonin, para-chlorophenylalanine, accelerates both amygdaloid and cortical kindling (Munkenbeck & Schwark, 1982; Racine & Coscina, 1979). Facilitated kindling was also observed following lesions of the median raphe nucleus (Racine & Coscina, 1979). Conversely, Munkenbeck and Schwark (1982) found that the elevation of levels of serotonin by the administration of 5-hydroxytryptophan attenuated kindling. Some evidence indicates that serotonin may act within the olfactory bulb in its antikindling capacity.

Specifically, marked facilitations of kindling were evident following damage of the olfactory bulb (Cain & Corcoran, 1978; Watanabe, Nakanishi, Shibata, & Ueki, 1982). Similar facilitations of kindling followed selective destruction of serotonin-releasing terminals within the olfactory bulb (Lerner-Natoli et al., 1986).

Assessments of the effects of the kindling procedure on levels of serotonin within the brain also provide some support for the position that the monoamine is involved in kindling. Munkenbeck and Schwark (1982) found significant reductions in serotonin and 5-hydroxyindolacetic acid levels in midbrain but not forebrain tissue samples that were taken 7 d after amygdaloid kindling. Two wk following the last electrical stimulation, Lewis et al. (1987) observed depletions of serotonin in both the stimulated amygdala and the contralateral hippocampus. Although levels of amygdaloid serotonin recovered during 4 seizure-free wk, levels within the ipsilateral hippocampus actually decreased further. While the findings are suggestive of a role for serotonin in kindling, they should receive cautious consideration. In some of the experiments, depletions were observed shortly after kindled convulsions and may therefore not be directly related to persistent increases in seizure sensitivity. In any event, taken together with the pharmacological and neuroanatomical data, it appears that serotonin plays an inhibitory role in kindling. Kindling may therefore involve the progressive and persistent mitigation of serotonergic function.

Opioidergic Mechanisms

Some evidence indicates that specific opioid receptors participate in kindling. Repeated injection of opioid peptides into the posterior amygdala or ventral hippocampus kindles generalized seizures. Naloxone prevents seizure induction by β-endorphin, met-enkephalin, or morphiceptin, clearly implicating opioid receptors in the epileptic effects of the peptides (Cain & Corcoran, 1984; Cain & Corcoran, 1985; Cain, Boon, & Corcoran, 1990). The peptides demonstrate differential affinities for specific subtypes of opioid receptors, suggesting that a single subtype does not mediate opioidergic kindling. Consistent with this possibility, specific δ receptor antagonists failed to attenuate seizures induced by either β-endorphin or morphiceptin (Cain et al., 1990), which preferentially activate ϵ and μ receptors, respectively (Chang, Killian, Hazum, Cuatrecasas, & Chang, 1981; Schulz, Wuster, & Herz, 1981); Cain et al. (1990) found that the antagonists either prevented or attenuated seizures kindled by the repeated focal administration of the specific δ agonist DSLET.

Although opioid peptides can site-specifically kindle seizures, it is not clear that opioidergic mechanisms participate in electrical kindling. A few studies have evaluated the effects of systemically administered naloxone on kindling but have produced inconsistent results. Hardy, Pappas, Rossi, and Zolovick (1980) found facilitated kindling, whereas Corcoran and Wada (1979) observed no effect, and others have reported a retardation (Cain et

al., 1987; Post, Davenport, Pert, & Squillace, 1979). Collectively, the findings suggest that opioidergic mechanisms may play nonessential roles in kindling.

KINDLING AND HUMAN EPILEPSIES

There remains considerable interest in the possibility that the investigation of kindling and kindled seizures may contribute to the understanding of epileptogenesis and epilepsy in humans. Indeed, there are several interesting parallels between the development and expression of kindled seizures in nonhumans and aspects of temporal lobe epilepsy in humans. First, as summarized by Adamec, Stark-Adamec, Perrin, and Livingston (1981), limbic structures of the temporal lobe support rapid kindling, relative to other limbic sites. Also, comparative studies indicate an inverse relation between degree of encephalization and rate of kindling: In humans, generalized seizures often emerge only years after brain injury, a common precursor to temporal lobe epilepsy. Finally, spontaneous ictal activity has been observed after numerous kindling stimulations in several species (Pinel, 1981; Pinel & Rovner, 1978; Wada, 1978; Wada & Osawa, 1976; Wada, Sato, & Corcoran, 1974; Wauquier, Ashton, & Melis, 1979). Investigation of kindling-related spontaneous seizures may have important implications for the understanding of spontaneous ictal events associated with temporal lobe epilepsy (Schmutz, 1987).

The comparative findings suggest the possibility that endogenous kindling mediates epileptogenesis. If this is the case, then the human brain should be vulnerable to exogenous kindling. A particulary dramatic clinical example involves a patient receiving repeated thalamic stimulation for the treatment of a phantom limb pain. After a few weeks of therapy, the patient reported mild spontaneous movements of the face and hand. Later, Jacksonian seizures as well as epileptiform paroxysms emerged and became secondarily generalized (Sramka, Sedlak, & Nadvornik, 1977). The events strengthen the view that the human brain is vulnerable to kindling and hence that temporal lobe epileptogenesis may involve basic mechanisms of kindling.

As noted previously, connections of hippocampal mossy fibers may undergo progressive reorganization during kindling, perhaps in association with hilar cell death (Cavazos & Sutula, 1990; Sutula et al., 1988). CA3 and the transitional zone between CA1 and the subiculum are also reputedly susceptible to kindling-related reorganization (Sutula, 1990; Represa et al., 1989). Sutula (1990) has discussed these findings in light of several postmortem studies (summarized in Babb & Brown, 1987), in which profound neuronal loss in the dentate gyrus, subiculum, presubiculum as well as hippocampal regions CA1, CA3, and CA4 appeared as a common albeit nonuniversal manifestation of temporal lobe epilepsy. It is as yet uncertain whether the pattern of cellular degeneration (hippocampal

sclerosis) plays a causal role in temporal lobe epileptogenesis. However, on the basis of the kindling studies and data indicating that circumscribed neurotoxic lesions result in synaptic reorganization and persistently increased seizure susceptibility (Feldblum & Ackermann, 1987; Laurberg & Zimmer, 1981; Nadler et al., 1980; Sutula et al., 1987), Sutula (1990) has hypothesized that temporal lobe epileptogenesis can begin with events, such as hypoxia or febrile seizures, that kill specific central neurons. The death of the cells initiates a cycle involving synaptic reorganization, increased cellular excitability, and further neuron-destroying seizures. This elaborate hypothesis awaits further assessment.

McIntyre et al. (1982b) proposed that mechanisms of kindling may underlie aspects of partial status epilepticus in humans. Characteristically, status epilepticus involves extremely frequent and/or prolonged seizures, which may not remit in the absence of pharmacological intervention (Bleck, 1983). McIntyre et al. (1982b) kindled generalized convulsions in rats that were later exposed to continuous (60 min) sine-wave stimulation of the amygdala. With the offset of stimulation, convulsions persisted for between 10 and 24 hr. Subsequent histological analysis revealed brain damage similar to that seen in patients with status epilepticus. Rats that were not kindled prior to continuous stimulation exhibited neither prolonged seizure activity nor brain damage, suggesting an essential role for mechanisms of

kindling in both the behavioral and neurological manifestations of status epilepticus (McIntyre et al., 1982b).

In another kindling-related form of status epilepticus, Buterbaugh, Michelson, and Keyser (1986) pretreated kindled rats with a low dose of the muscarinic receptor agonist pilocarpine. A subsequent kindling stimulation produced, in addition to its anticipated generalized convulsion, pronounced ictal activity that persisted for up to 4 hr. In contrast, control rats treated with pilocarpine without electrical stimulation did not display even transient ictal episodes. Moreover, pilocarpine-induced status epilepticus was not evident following stimulation in rats previously displaying only partial kindled seizures. The results are consistent with a pivotal role for kindling mechanisms in pilocarpine-induced status epilepticus. However, it is also possible that the effects of pilocarpine are not specific to either kindling or even generalized kindled seizures. It may be that generalized seizures provoked by methods other than focal electrical stimulation (e.g., maximal electroshock) would interact similarly with pilocarpine.

THE ARCHITECTURE OF KINDLING

It is quite evident from the preceding paragraphs that while several promising avenues of research regarding mechanisms of kindling have opened, a striking number of important questions remain unanswered. Burchfiel and Applegate (1989; 1990) have proposed that the state of

uncertainty regarding the mechanistic bases of kindling reflects a fundamental lack of understanding of the underlying structure or architecture of kindling[8], within which the epileptogenic mechanisms operate. In an attempt to elucidate the architecture, they have employed a variant of the kindling procedure that involves concurrent alternating stimulation of two limbic sites. That is, a stimulus train is delivered to site A. After a predetermined interval, a train is delivered to site B. Then site A is restimulated, etc. Under this condition, one (dominant) site supports typical progressive seizure development. By contrast, seizures provoked by stimulation of the other (suppressed) site fail to generalize, provided that stimulation of the dominant site continues.

Duchowny and Burchfiel (1981) have dichotomized the phenomenon, termed kindling antagonism, according to the most severe convulsive indicators produced by stimulation of the suppressed site. In one pattern of antagonism (absolute), stimulation of the suppressed site elicits only nonconvulsive seizures, the motoric manifestations of which include behavioral arrest (freezing) and automatisms (e.g., grooming). The behaviors

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[8]The underlying structure or architecture of kindling, as described by Burchfiel and Applegate, is determined by the number of qualitatively and temporally distinct mechanisms subserving kindling. Thus, should kindling reflect quantitative changes to a unitary mechanism, the architecture of kindling would be better represented by a single mathematical function relating behavioral state to time. On the other hand, should kindling reflect a series of qualitatively and temporally distinct mechanisms, then the architecture of kindling might be better represented by a series of functions.

and their electrographic correlates correspond to those seen during the earliest trials of single site kindling[9]. In the other pattern of antagonism (relative), stimulation of the suppressed site either sporadically or consistently elicits partial seizures, which can be classified according to the criteria of Racine (1972b). Initially, partial seizures involve twitching of the facial musculature (stage 1). With continued development of seizures, twitching of the neck muscles accompanies stage 1 behaviors, culminating in bobbing of the head (stage 2). In stage 3, clonic movements of the forelimb contralateral to the site of stimulation occur. In both absolute and relative antagonism, the suppressed site unlike the dominant site consistently fails to support generalized seizures, which typically involve bilateral forelimb clonus in addition to rearing (stage 4) and falling (stage 5).

Burchfiel and Applegate have proposed that antagonism reflects a frank arrest of kindling associated with stimulation of the suppressed site. Support for this proposal stems from observations made both during alternating stimulation and following the termination of stimulation of the dominant site. As noted above, Burchfiel and Applegate (1989; 1990) reported, during alternating stimulation, that seizures generated from the suppressed site are behaviorally and electrographically indistinguishable from

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[9]I shall occasionally use the terms single site kindling and primary site kindling, which are synonymous, when referring to kindling as initially described by Goddard et al. (1969; i.e., kindling obtained via the repeated application of electrical stimulation to a particular site via a single electrode).

those observed at early points during single site kindling. It also appears, following the cessation of stimulation of the dominant site, that the number of suppressed site ADs required to elicit generalized seizures is similar to that required during single site kindling in rats that have been matched on indicators of convulsive severity. Thus, for example, generalized seizures appear after a similar number of ADs in rats previously expressing absolute antagonism (subsequently stimulated only in the suppressed site) and in naive rats receiving stimulation of the corresponding site.

Burchfiel and associates have made considerable progress in elucidating of the mechanisms responsible for the expression of antagonism. Based on work conducted during the past decade, they have proposed metaphorically that neural gates (described below) open for the dominant site but remain closed for the suppressed site. The gates thus prevent the transynaptic reorganization that is necessary for kindling of generalized seizures from the suppressed site. In the case of absolute antagonism, the suppressed site supports only nonconvulsive seizures, because both the first and second gates remain functionally closed. However, in cases in which the first gate opens to the suppressed site, partial seizures (stages 1 - 3) and hence relative antagonism occur.

Burchfiel and Applegate (1989; 1990) provided some indirect evidence that the first gate involves circuitry within the amygdala-pyriform region and depends upon noradrenaline. As noted earlier, the amygdala-

pyriform region is the first to display interictal epileptiform discharge during kindling from sites thus far evaluated (Kairiss et al. 1984). Some view the discharge pattern as indicative of enhanced sensitivity to seizure-eliciting stimuli (e.g., Racine et al., 1986). Also, stimulation of either the amygdala or the pyriform cortex produces partial and subsequently generalized kindled seizures following relatively few nonconvulsive seizures (Goddard et al., 1969; McIntyre & Racine, 1986; McNamara et al., 1980; Racine, 1978). Moreover, the destruction of noradrenaline-releasing terminals of the entire brain, the forebrain, or the amygdala facilitates kindling from a variety of sites, including the amygdala-pyriform region (Corcoran et al., 1974; Corcoran & Mason, 1980; Ehlers, Clifton, & Sawyer, 1980; McIntyre et al., 1979; McIntyre & Edson, 1981). The facilitation reflects a reduction in the number of nonconvulsive seizures, like those provoked by stimulation of the suppressed site during absolute antagonism, occurring prior to the emergence of partial and generalized seizures (Corcoran, 1988; Michelson & Buterbaugh, 1985).

Given the forgoing, one might predict that depletion of noradrenaline from the forebrain would prevent the expression of absolute antagonism. The results of early investigations failed to support this prediction, however, as a small number of adult rats that received 6-OHDA as infants demonstrated absolute antagonism, even though the treatment obliterated noradrenaline of the forebrain (Applegate, Burchfiel, & Konkol, 1986;

Applegate, Konkol, & Burchfiel, 1987). On the other hand, Burchfiel and Applegate (1989) indicated that an unpublished study furnished the expected outcome. A potential source of the inconsistent results pertains to the infrequent occurrence of absolute antagonism. In the hands of Burchfiel and associates, absolute antagonism typically occurs in fewer than 25% of undrugged rats stimulated in the lateral septal area and the entorhinal cortex (e.g., Applegate et al., 1986; Applegate et al., 1987). In fact, absolute antagonism failed to occur in any of 7 control rats in a later study (Applegate & Burchfiel, 1990).

Data concerning the pharmacological and anatomical properties of the hypothesized second gate are considerably more consistent. Whereas the destruction of noradrenergic terminals throughout the brain reduced the incidence of relative antagonism, selective destruction of terminals in the forebrain was ineffective, indicating that the second gate is both noradrenaline-dependent and situated within the brainstem and/or cerebellum (Applegate et al., 1986; Applegate et al., 1987; Burchfiel et al., 1986). In an extension of these studies, Applegate and Burchfiel (1990) again treated infant rats with 6-OHDA, in order to deplete the forebrain of noradrenaline. In adulthood, the rats received an additional infusion of either vehicle or a low or high dose of 6-OHDA. Confirming their previous findings, rats treated with the neurotoxin only in infancy demonstrated both relative antagonism and marked reductions of noradrenaline levels in the forebrain.

This was also true of rats that received the low dose of 6-OHDA in adulthood. The treatment depleted the forebrain and the cerebellum of noradrenaline, while sparing terminals in the brainstem. In contrast, rats treated as adults with the high dose of 6-OHDA, which also destroyed noradrenergic terminals in the ponto-medullary region of the brainstem, were significantly less likely to express antagonism. Because the pattern of antagonism displayed by all control rats in this study was relative, it appears that the pons-medulla houses the second noradrenaline-dependent gate and thus subserves relative antagonism (Applegate & Burchfiel, 1990).

According to Burchfiel and Applegate (1989; 1990), at least some of the mechanisms that produce antagonism during concurrent alternating stimulation of two limbic sites are essential to the expression of single site kindling. Further, the stereotyped patterns of antagonism (absolute and relative) indicate that successive qualitatively distinct mechanisms subserve kindling, the architecture of which is therefore viewed as a noncontinuous (stepwise) process. Within this framework, kindling is divided into three phases, which overlap with yet are distinct from the five stages described by Racine (1972b). Note that the theory does not address the later stages of limbic kindling, after stage 5, that were described by Pinel and Rovner (1978): stage 6, multiple episodes of rearing an falling; stage 7, running fits; and stage 8, mild tonic components. Unfortunately no other

investigators have, to my knowledge, seen the more advanced stages of kindling described by Pinel (e.g., Bertram & Lothman, 1993).

In the first phase of kindling, as described by Burchfiel and Applegate (1989; 1990), behavioral and electrographic seizures are identical to those produced by stimulation of the suppressed site during the expression of absolute antagonism. AD is spatially restricted, involving only local neuronal recruitment. Alterations to the response characteristics of local circuits, which determine such parameters as ADT and duration of AD, may be somewhat dissociable from kindling, per se. Thus, Racine (1972a) observed that ADT declined with the repeated application of either sub- or suprathreshold stimulation. Kindling from a primary site facilitates subsequent kindling from a secondary site (transfer), without influencing the ADT of the latter (e.g., Burnham, 1976; Racine, 1972a). From the pharmacological perspective, intraamygdaloid infusions of GABA agonists attenuated kindled convulsions without influencing duration of AD (Applegate & Burchfiel, 1988). The first phase of kindling culminates in the opening of the first gate, which, as previously discussed, may be noradrenaline-dependent, situated within the amygdala-pyriform region, and fails to open for the suppressed site during absolute antagonism. With the opening of the first gate, kindling progresses to its second (middle) phase.

During the second phase, AD propagates extensively throughout the forebrain, reorganizing its neural pathways. The spatially extensive

reorganization is stereotyped and hence does not depend on the limbic site stimulated. That is, the second phase of kindling from any limbic site involves qualitatively similar reorganization of the forebrain. As this occurs, seizures progress through stages 1 - 3 of Racine (1972b). Supporting these claims are studies comparing patterns of seizure development from limbic sites that support fast and slow kindling. The factor that determines intersite differences in kindling rates appears to be the number of nonconvulsive seizures displayed prior to the exhibition of partial seizures (Duchowny & Burchfiel, 1981; Le Gal La Salle, 1981; Sato & Nakashima, 1975). Thus, once partial seizures have developed, generalized seizures occur following similar numbers of ADs, regardless of the site of stimulation. Ultimately, seizures propagated through the reorganized forebrain act to open the second gate, which, as noted above, appears to be noradrenaline-dependent, is situated in the ponto-medullary brainstem, and fails to open for the suppressed site during the expression of relative antagonism.

Some data suggest that, following the opening of the hypothesized second gate, extensive stereotyped reorganization of circuitry of the brainstem occurs, which allows the expression of stage 4 and ultimately stage 5 seizures. Specifically, lesions of the midbrain reticular formation, but not forebrain commissural fibers, inhibit the development of generalized kindled seizures (Wada & Sato, 1975a; Wada & Sato, 1975b). Also, electrographic activity within the midbrain reticular formation is predictive of

generalization of kindled seizures (Wada & Sato, 1974). Finally, the reticular formation is important for the expression of tonic and clonic components of seizures induced by a variety of methods, including kindling (e.g., Browning, 1985; Burnham, 1985; Burnham & Browning, 1987).

PURPOSE

The hypothesis that kindling antagonism furnishes a window on the architecture and hence the neural bases of kindling is compelling, albeit far from substantiated. The hypothesis hinges on the assumption that kindling antagonism reflects an actual arrest of kindling from the suppressed site and does not, therefore, reflect transient inhibition of seizures (Burchfiel & Applegate, 1989; Burchfiel & Applegate, 1990). In the present dissertation, I shall assess whether the assumption is justified. In order to accomplish this, I shall attempt to confirm that antagonism occurs reliably in response to alternating stimulation of limbic sites and extend my analysis to include nonlimbic sites. After the establishment of antagonism, I shall repeatedly deliver stimulation distally to the dominant site. The resulting patterns of seizure development may yield information regarding the functional state of the brain responsible for antagonism. I shall also propose mechanisms by which antagonism might arise during concurrent alternating stimulation (see General Discussion).

General Methods[10]

Subjects

Male Long-Evans rats (Charles River, Quebec), weighing 250 - 400 g, served as subjects. They were housed individually in standard stainless steel mesh cages with food and water available ad libitum. Ambient temperature was maintained at 20°C (± 1). All kindling trials occurred during the light portion of the 12-h light/dark cycle.

Surgery

I anesthetized rats with intraperitoneally administered sodium pentobarbital (65 mg/kg) and scopolamine methylbromide (1 mg/kg) and stereotaxically implanted pairs of bipolar stimulating/recording electrodes (3 such electrodes were implanted in Experiment 3). Electrodes consisted of twisted strands of Nichrome wire (76 μm), which were insulated except for the tips with enamel to a total diameter of 127 μm. A jeweller's screw, which was secured to the right frontal pole of the skull served as the ground/reference electrode. In Experiments 2 - 4, the following stereotaxic coordinates (mm) for the amygdala and septal area were used with anterior-posterior (AP) and lateral (L) coordinates given with respect to bregma and dorsal-ventral (DV) measurements given with respect to dural surface:

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[10]Deviations from the General Methods are noted in Methods for individual experiments as required.

Amygdala - AP -0.4, L +4.5, DV -7.5; septal area - AP +1.8, L +0.8; DV -5.0. The upper incisor bar was positioned 5.0 mm dorsal to interaural zero.

Kindling

Following a 1-wk postsurgical recovery period, the ADTs were determined. Rats were connected to the recording/stimulation lead and placed in a clear Plexiglas testing chamber (33 X 37 X 50 cm), and basal EEG was recorded for approximately 30 s. Electrical stimulation consisted of a 1-s train of constant current balanced biphasic square-wave pulses (1 ms duration; 60/sec) and was delivered to one of the sites (randomly determined) at an initial current intensity of 50 μA (base-to-peak), which increased in increments of 50 μA each min until AD[11] appeared in the EEG. The lowest intensity of stimulation that induced AD was arbitrarily designated as the ADT. The procedure was repeated on the next day, with stimulation delivered via the other stimulating/recording electrode.

Twenty-four hr later, rats began once-daily test sessions. In rats in which I attempted to establish antagonism, the site of stimulation alternated on a daily basis, and stimulation was delivered at an intensity 100 μA above the ADT. This procedure, termed the Initial Phase in Experiments 2 - 4,

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[11]AD was indicated by the presence of spikes of at least twice the amplitude of maximal voltage observed in the EEG prior to stimulation, occurring at a frequency of at least 1/s over a period of at least 2 s (i.e., at least 3 spikes).

continued until one of the sites supported stage 5 seizures on 6 consecutive trials and equal numbers of stimulus trains had been delivered to each site[12]. In Experiments 2 and 3, I employed control rats, which received daily exposure to the apparatus, but stimulation was delivered to only one of the sites, once per 48 hr. For these rats, the Initial Phase ended with the expression of 6 consecutive stage 5 seizures provoked from the stimulated site and an even number of test sessions. Also in Experiments 2 - 4, the Initial Phase was followed by a Final Phase, in which rats continued to receive daily exposure to the apparatus, but stimulation was delivered to only one site in 48-hr intervals[13]. The Final Phase ended with the expression of a single stage 5 seizure. In all experiments, assignment of subjects to treatment groups was random.

Histology

At the end of each experiment, rats were deeply anesthetized with sodium pentobarbital, and direct current (1 mA) was passed between the

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[12]I operationally defined antagonism as the occurrence of 6 consecutive stage 5 seizures provoked from one site (dominant) with the other site supporting only stage 0 - 3 seizures during alternating stimulation.

[13]In Experiments 2 - 4, I refer to the development of seizures observed during the Final Phase as Final Phase kindling. I do this out of convenience rather than to imply that such development of seizures reflects kindling, per se, and not a time-dependent decay of seizure inhibition. To do so would presuppose that kindling from the suppressed site indeed undergoes a frank arrest during alternating stimulation, as proposed by Burchfiel and Applegate (1989; 1990).

tips of each electrode for 10 s (an exception was Experiment 4), just prior to transcardial perfusion with physiological saline. After removal of the brain and its fixation in 10% formalin, tissue was sectioned (80 μm) in the coronal plane and stained with thionin for localization of electrode tips, as revealed by the electrolytic lesions, according to the atlas of Paxinos and Watson (1982).

Experiment 1

Haas, Sperber, and Moshe (1990; 1992) observed antagonism between the amygdala and dorsal hippocampus in adult rats but not in rat pups with electrodes implanted in either the amygdala and dorsal hippocampus or bilaterally in the amygdala. The results are suggestive of developmental changes in central antiepileptogenic mechanisms and provide independent confirmation of the results of Burchfiel and Applegate, concerning the occurrence of antagonism during alternating stimulation of limbic sites. Here I attempt to reproduce kindling antagonism using adult rats receiving stimulation of various other pairs of sites.

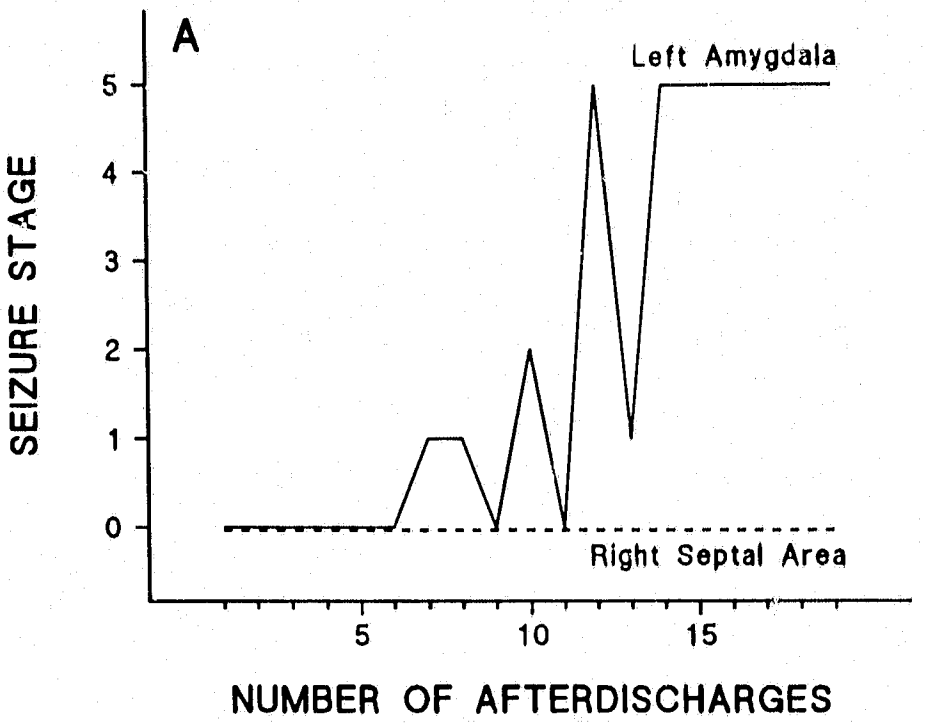
Methods

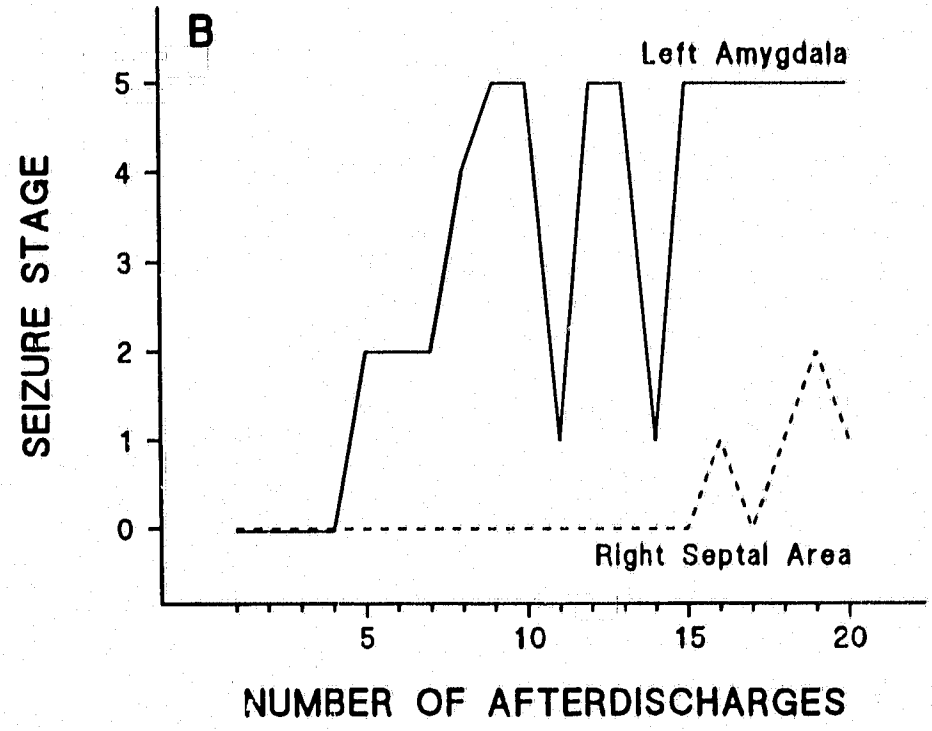
Eighty rats served as subjects in Experiment 1. Surgery, ADT determination, alternating stimulation, and histological procedures occurred as outlined in General Methods.

Results

Eighteen of 32 rats stimulated in the amygdala and septal area expressed antagonism, with 8 and 10 expressing absolute and relative antagonism, respectively. Examples of these patterns of antagonism are depicted in Figures 1A and 1B. In rats stimulated in the amygdala and septal area, septal electrodes were situated in either the left or right hemisphere,

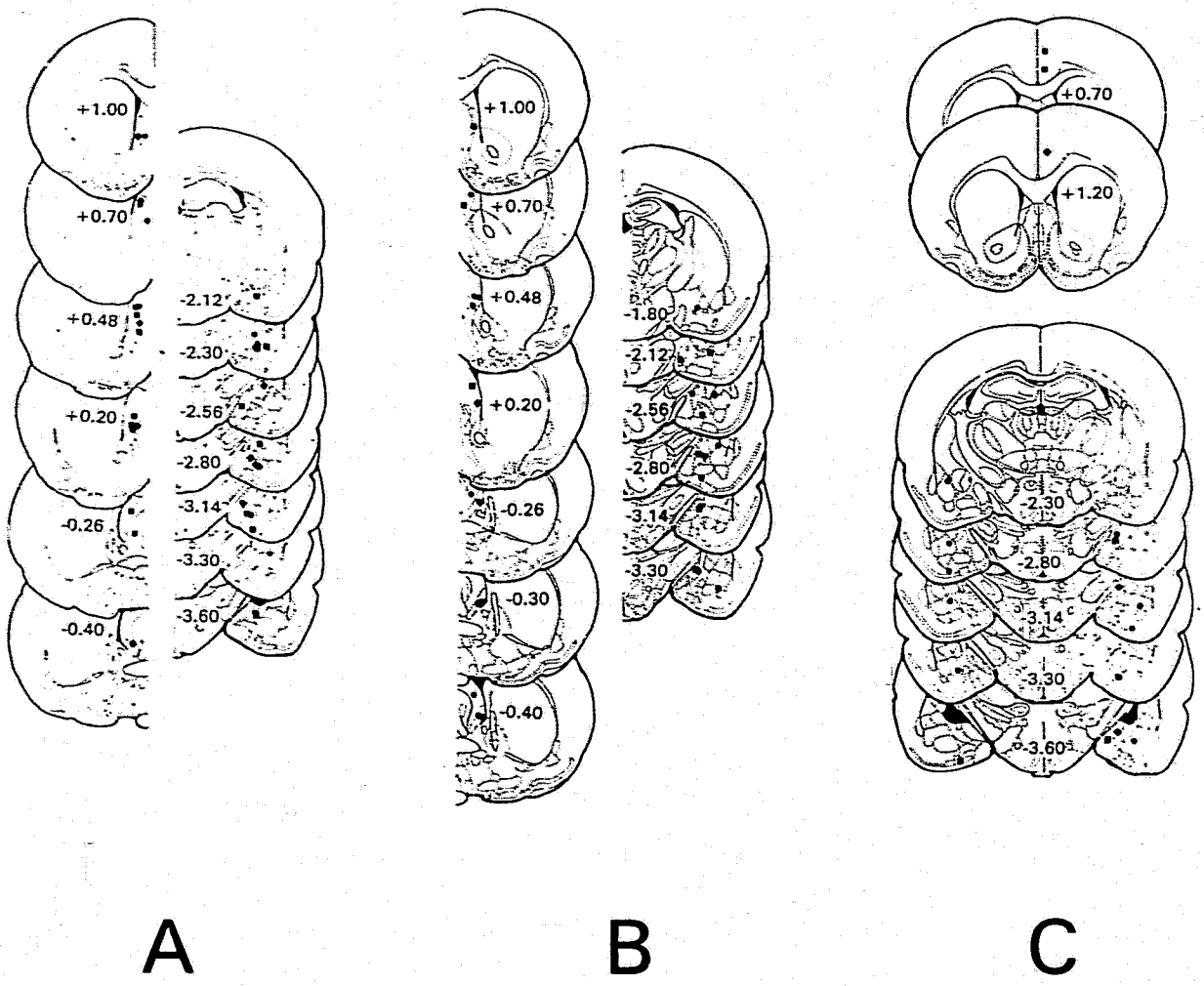
Figure 1. Patterns of antagonism expressed by individual rats receiving alternating stimulation of the left amygdala and right septal area (Experiment 1). A, absolute antagonism; B, relative antagonism.


A
Left Amygdala
Right Septal Area
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
NUMBER OF AFTERDISCHARGES


B
Left Amygdala
Right Septal Area
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
20
NUMBER OF AFTERDISCHARGES

ipsilateral or contralateral to the amygdaloid electrode, respectively (histological data for these rats are presented in Figures 2A and 2B). I also varied the site from which the ADT was first determined (see General Methods). Thus, treatment groups comprised rats receiving: Ipsilateral implants and initial stimulation of the amygdala (Ipsilateral-amygdala group) or septal area (Ipsilateral-septal area group); or contralateral implants and initial stimulation of the amygdala (Contralateral-amygdala group) or septal area (Contralateral-septal area group). Rats were classified according to whether they developed or did not develop kindling antagonism, and this was the dependent measure, John. A two-way ANOVA, utilizing a 2 (Hemispheric Relation) X 2 (Initial Site) design, revealed a significant Hemispheric Relation X Initial Site interaction for the expression of antagonism ($F(1,28) = 8.65$, $p < 0.01$), as well as a significant main effect for Hemispheric Relation ($F(1,28) = 17.80$, $p < 0.001$). The Initial Site main effect was nonsignificant. Post hoc analysis (Fisher's Exact Test) revealed that rats in the Ipsilateral-amygdala group were significantly less likely to exhibit antagonism (0 of 8) than were rats in either the Ipsilateral-septal area (4 of 7; $p = 0.03$) or Contralateral-amygdala groups (5 of 5; $p = 0.001$). The Ipsilateral-septal area and Contralateral-amygdala groups did not differ significantly, however, from the Contralateral-septal area group (9 of 12). The data indicate that the probability of obtaining antagonism in rats with ipsilateral amygdaloid and septal electrodes was significantly lower

Figure 2. Reconstructions of placements of electrodes (front view; Experiment 1). Rats expressed either no antagonism (circles), absolute antagonism (diamonds), or relative antagonism (squares) with electrode tips situated in the left amygdala and: A, the right septal area; B, the left septal area; C, the right amygdala or left cingulate cortex. Numbers indicate distance (mm) from bregma in the anterior-posterior plane.


+1.00
+0.70
+0.48
+0.20
-0.26
-0.40
-2.12
-2.30
-2.56
-2.80
-3.14
-3.30
-3.60
+1.00
+0.70
+0.48
+0.20
-0.26
-0.30
-0.40
-1.80
-2.12
-2.56
-2.80
-3.14
-3.30
+0.70
+1.20
-2.30
-2.80
-3.14
-3.30
-3.60

A B C

when initial stimulation was delivered to the amygdala. This is inconsistent with a previous report in which antagonism between the septal area and entorhinal cortex was not sensitive to the hemispheric relation of the electrodes (Burchfiel, Serpa, & Duffy, 1982b).

Unexpectedly, electrographic consequences of repeated amygdaloid stimulation were related to the expression of antagonism between the amygdala and septal area[14]. I regressed Hemispheric Relation of electrodes, Initial Site of stimulation, and the interaction between these variables on the duration of AD recorded from the amygdala during the first seizure (regardless of stage) and the first stage 2 and stage 5 seizures provoked by stimulation of the amygdala (criterion for forward [entry] and backward [removal] selection: $\alpha = 0.05$). These variables did not enter the regression equation. By contrast, the expression of Antagonism entered the equation ($\Delta R^2 = 0.09$, $F(1,30) = 9.49$, $p < 0.005$). Following the partialing of within subjects variability, the linear trend for Seizure entered the equation ($\Delta R^2 = 0.39$, $F(1,60) = 80.80$, $p < 0.001$), and the expression of Antagonism was removed. Whereas the Hemispheric Relation X Seizure and Initial Site X Seizure interactions did not reach statistical significance, the Antagonism X Seizure interaction entered the equation (ΔR^2

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[14]Dependent measures did not vary with the type of antagonism expressed (i.e., absolute vs relative). Results obtained from rats expressing either form of antagonism were therefore pooled for assessment of relations between the expression of antagonism and other dependent variables.

= 0.10, $F(1,60) = 20.67$, $p < 0.001$). Subsequent pairwise comparisons with point-biserial correlations (df = 1,30) revealed that rats expressing antagonism demonstrated shorter durations of AD during the first stage 2 ($p < 0.01$) and stage 5 seizures ($p < 0.001$) provoked by stimulation of the amygdala. Because antagonism was not significantly correlated with either the duration of AD produced by the first stimulation of the amygdala or the number of amygdaloid stimulations required to elicit the first stage 2 and stage 5 seizures, it appears that antagonism was associated with reduced rates of growth of AD elicited by stimulation of the amygdala (dominant site) [Figure 3; illustrative EEG records are presented in Figure 4]. In essence, therefore, more rapid growth of amygdaloid AD was predictive of a diminished probability that the amygdala would exercise dominance over the septal area.

Unlike amygdaloid AD, durations of AD elicited by the first stimulation of the septal area and the septal stimulations corresponding to the first stage 2 and stage 5 seizures produced by stimulation of the amygdala[15] were not significantly correlated with the expression of antagonism. During multiple regression analysis, the Hemispheric Relation of electrodes, Initial Site of

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[15]Because I applied alternating stimulation, septal stimulations correspond in number to amygdaloid stimulations. Thus, for example, should the seventh and 15th stimulations of the amygdala in a given rat elicit stage 2 and stage 5 seizures, respectively, the seventh and 15th stimulations of the septal area would correspond to the first stage 2 and stage 5 seizures elicited by stimulation of the amygdala.

Figure 3. Duration of AD provoked from the amygdala in association with the first seizure and the first stage 2 and stage 5 seizures (Experiment 1). Note the more rapid growth of amygdaloid AD in rats failing to express antagonism (*, $p < 0.05$; **, $p < 0.001$).

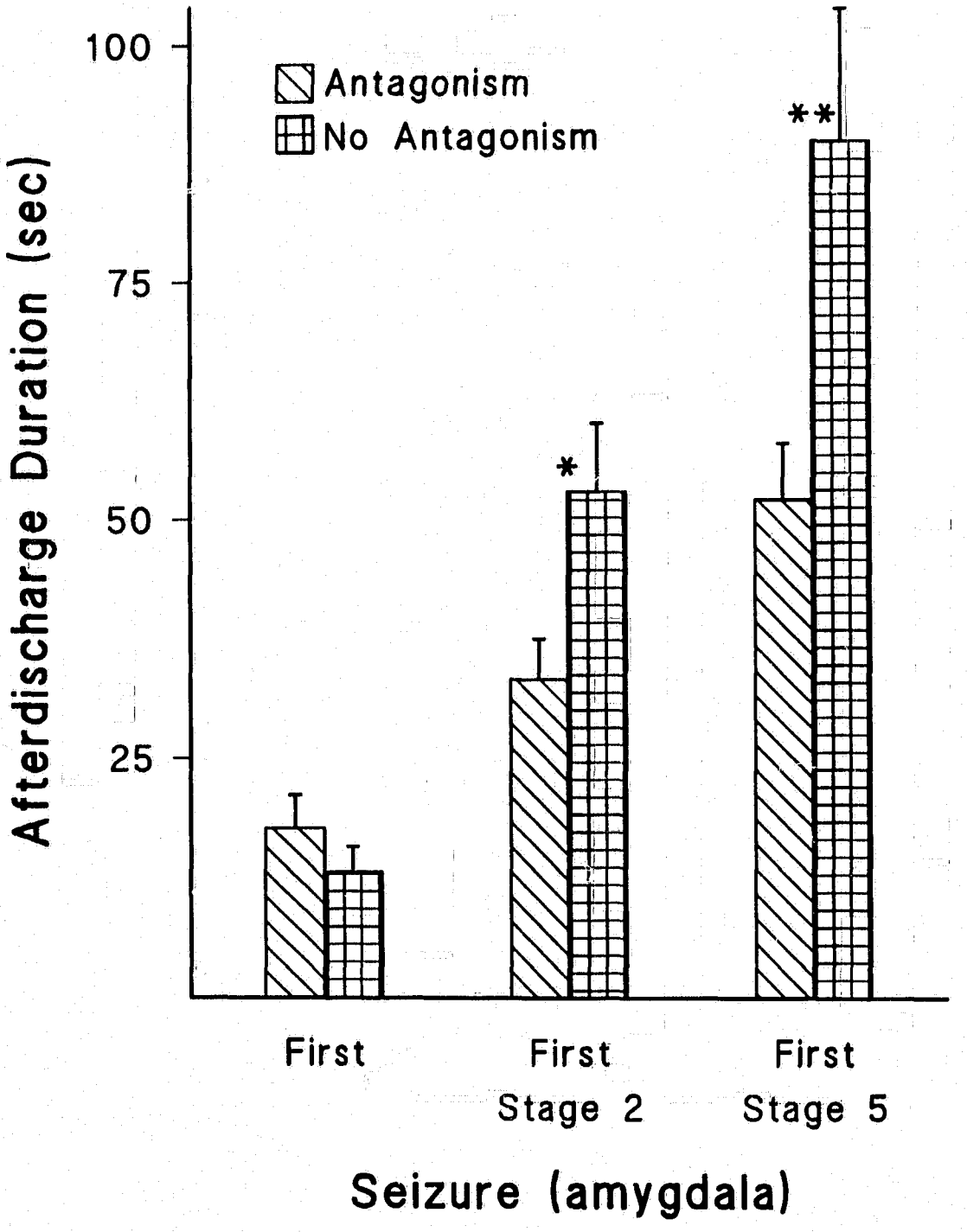
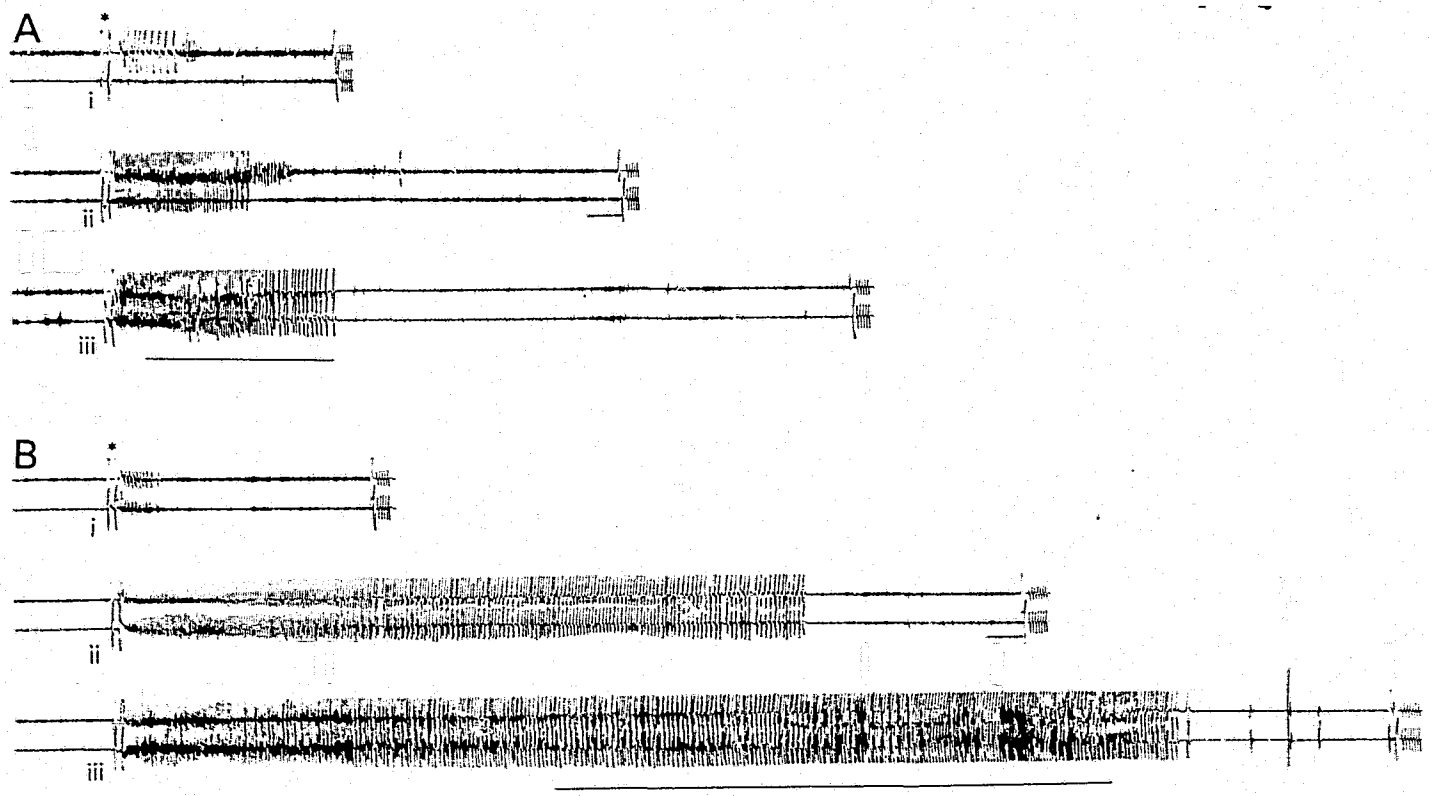

100
75
50
25
Afterdischarge Duration (sec)
Antagonism
No Antagonism
*
**
First
First
Stage 2
First
Stage 5
Seizure (amygdala)

Figure 4. EEG records obtained via electrodes situated in the amygdala and septal area in individual rats (Experiment 1). Rats either: A, displayed antagonism (contralateral electrodes); B, failed to display antagonism (ipsilateral electrodes). Records i, ii, and iii (amygdala - top traces; septal area - bottom traces) were obtained in association with the first AD, first stage 2 seizure, and first stage 5 seizure, respectively, elicited by stimulation of the amygdala. Stimulus trains are indicated by asterisks (records i); calibration pulses indicate 1mV (peak-to-peak); horizontal calibration bars indicate 5 sec (records ii). Note that the rat displaying antagonism (carrying contralateral electrodes) showed substantially shorter AD during the first stage 2 and the first stage 5 seizures and shorter latency to and duration of bilateral forelimb clonus during the first stage 5 seizure, as indicated by horizontal bars on records iii. In both rats, stage 2 and stage 5 seizures were observed after 6 and 13 AD-eliciting amygdaloid stimulations, respectively.


A
i
ii
iii
B
i
ii
iii

stimulation, Hemispheric Relation X Initial Site interaction, and the expression of Antagonism failed to account for significant proportions of the variance in duration of septal AD and thus did not enter the regression equation. After the partialing of within subjects variability, the linear trend for Seizure entered the equation ($\Delta R^2 = 0.07$, $F(1,60) = 86.08$, $p < 0.001$), whereas the Hemispheric Relation X Seizure, Initial Site X Seizure, and Antagonism X Seizure interactions did not reach significance. Therefore, AD from this site was not subject to the influence of my manipulations nor significantly related to the expression of antagonism (Figure 5).

Like electrographic aspects, behavioral consequences of the first stage 5 seizure generated from the amygdala were also subject to the influence of my manipulations. With respect to latency to bilateral forelimb clonus, Hemispheric Relation entered the multiple regression equation ($\Delta R^2 = .14$, $F(1,30) = 4.98$, $p < 0.05$), whereas the Hemispheric Relation X Initial Site interaction, and the expression of Antagonism failed to reach statistical significance. The results indicate that rats carrying electrodes contralaterally demonstrated significantly shorter latencies to clonus during the first stage 5 seizure elicited by stimulation of the amygdala (Figure 6). With respect to duration of clonus, Hemispheric Relation, Initial Site, and their interaction did not enter the equation. The expression of Antagonism, however, entered the equation, producing significant changes in the proportion of variance

Figure 5. Duration of AD provoked from the septal area in association with the first seizure and septal seizures corresponding to the first stage 2 and stage 5 seizures provoked from the amygdala (Experiment 1). Despite a pronounced tendency towards briefer AD in rats expressing antagonism, duration of septal AD did not differ significantly as a function of the expression of antagonism.

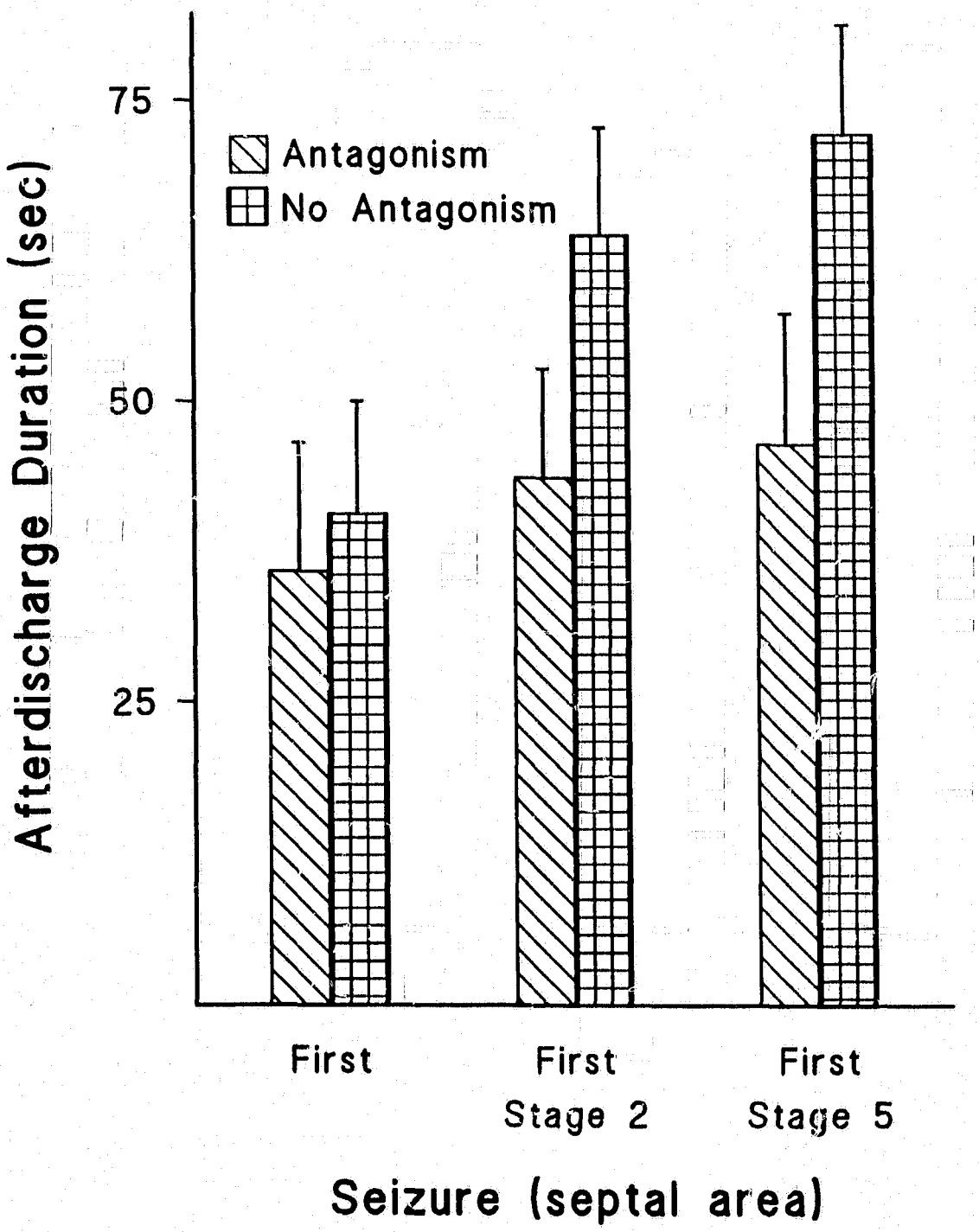
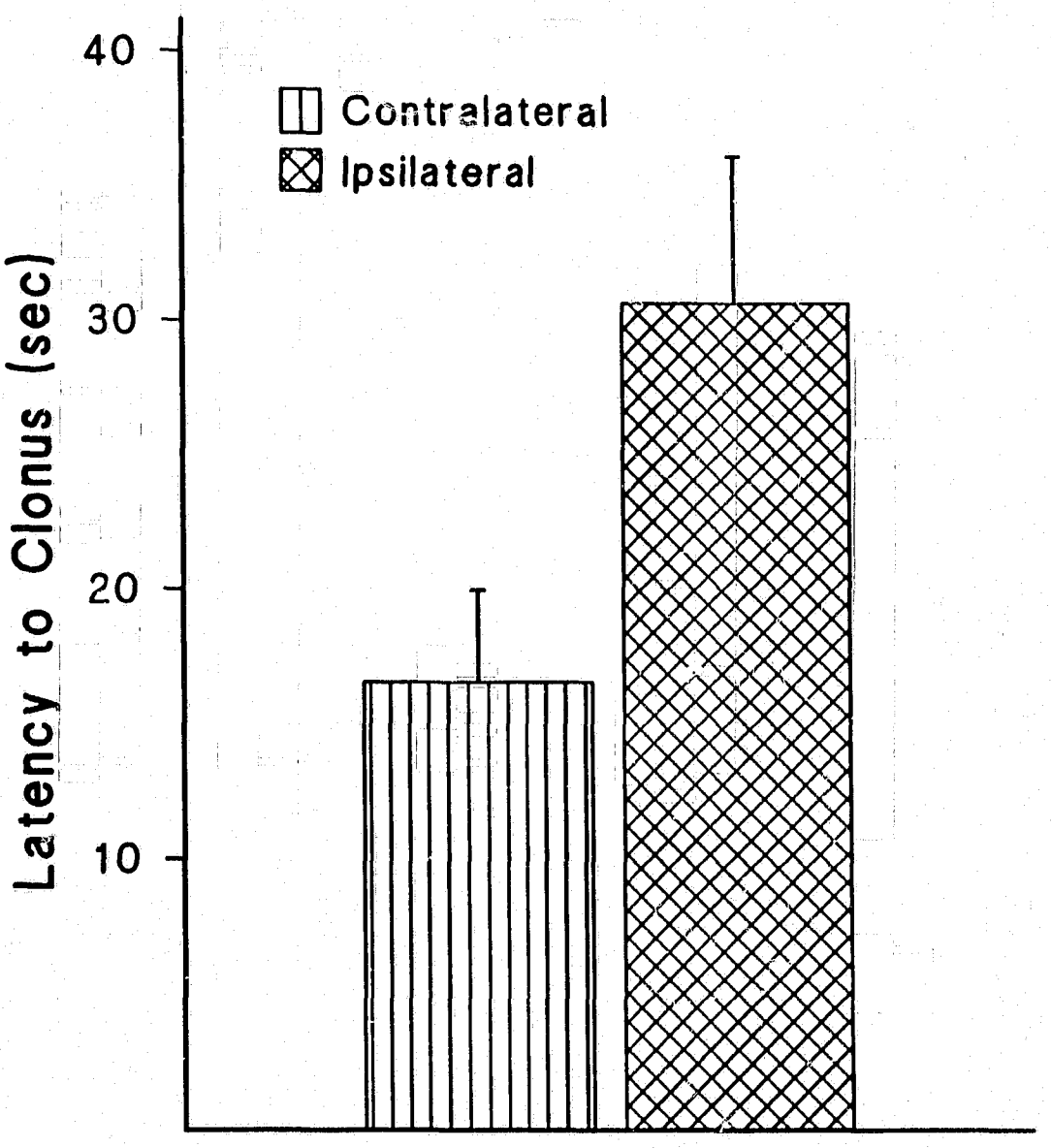

75
50
25
Afterdischarge Duration (sec)
Antagonism
No Antagonism
First
First
Stage 2
First
Stage 5
Seizure (septal area)

Figure 6. Latency to bilateral forelimb clonus during the first stage 5 seizure provoked by stimulation of the amygdala as a function of the hemispheric relation of electrodes (ipsilateral vs contralateral). Latency to clonus differed significantly ($p < 0.05$).


40
30
20
10
Latency to Clonus (sec)
Contralateral
Ipsilateral

accounted for (ΔR^2 = .31, $F(1,30) = 13.27$, $p < 0.001$). This indicates that the expression of antagonism is predictive of shorter duration of forelimb clonus during the first stage 5 seizure elicited by stimulation of the amygdala (Figure 7; see also Figure 4).

Only 1 of 6 rats stimulated bilaterally in the amygdala expressed (relative) antagonism, whereas 4 of the remaining rats inconsistently displayed stage 5 seizures from one or both sites. Burchfiel et al. (1982b) have reported that these patterns of seizure development are prevalent among rats receiving bilateral amygdaloid stimulation. Histological data obtained from these rats are presented in Figure 1C.

In addition to the amygdala and septal area, antagonism was evident with stimulation of the splenium of the corpus callosum and the septal area (6 of 8). The septal area was dominant in 5 rats, in 2 and 3 of which antagonism was absolute and relative, respectively. In 1 rat, absolute antagonism occurred with the splenium of the corpus callosum being dominant. Histological data obtained from these rats are presented in Figure 8A (see also Tables 1 and 2).

Antagonism also developed in a small number of rats receiving stimulation of other paired sites. Three of 3 rats stimulated in the cingulate cortex and the amygdala displayed antagonism, with the cingulate cortex being dominant in all cases (see Figure 2C for histological data). Similarly, in

Figure 7. Relation between expression of antagonism and duration of bilateral forelimb clonus during the first stage 5 seizure provoked by stimulation of the amygdala (Experiment 1). Duration of clonus differed significantly as a function of the expression of antagonism ($p < 0.005$).

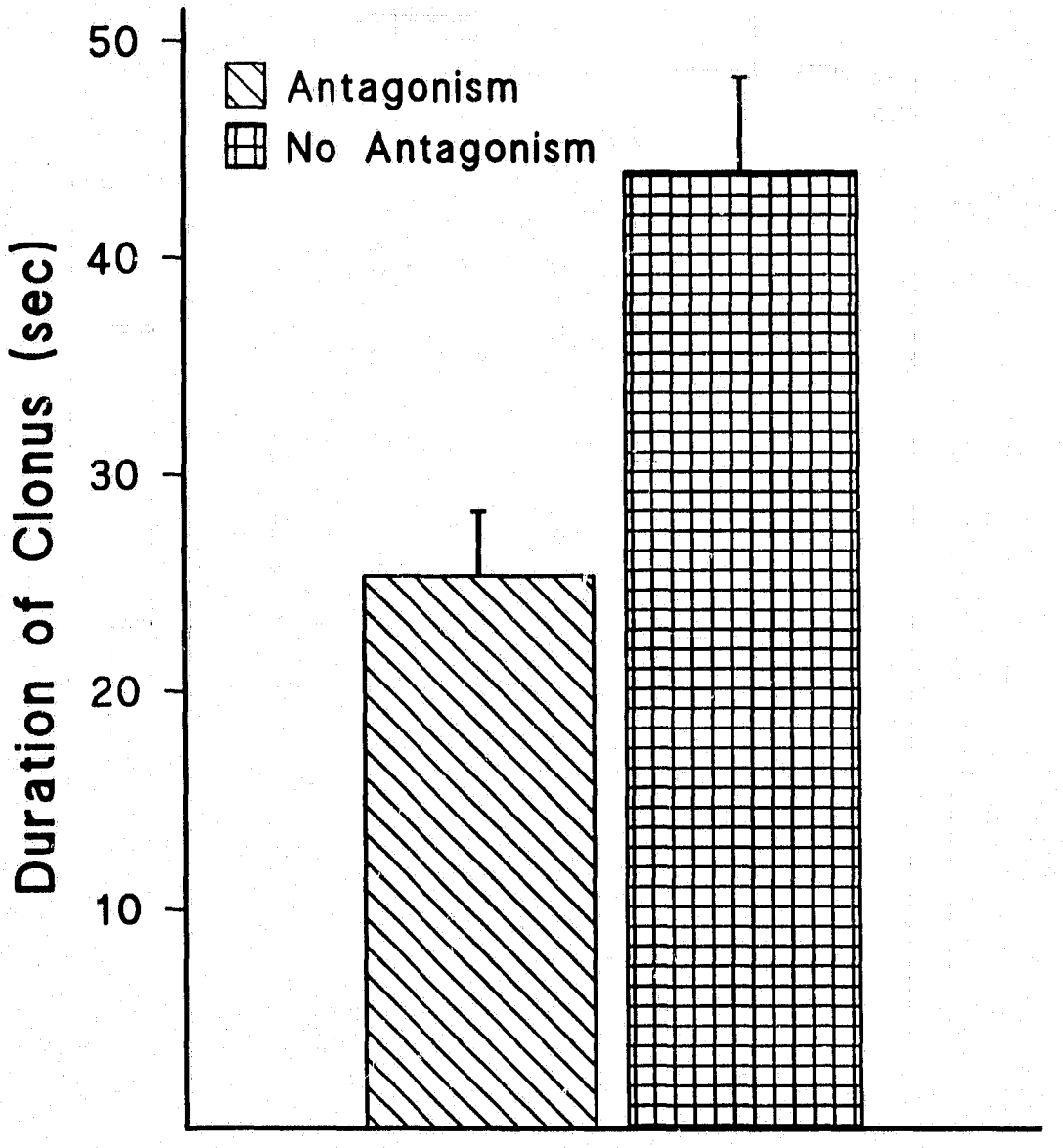

Antagonism
No Antagonism
Duration of Clonus (sec)
50
40
30
20
10

Figure 8. Reconstructions of placements of electrodes (front view; Experiment 1). Rats expressed either no antagonism (circles), absolute antagonism (diamonds), or relative antagonism (squares). Electrode tips were situated: A, in the septal area and either the splenium of the corpus callosum or occipital cortex; in the caudate nucleus and either the splenium of the corpus callosum or occipital cortex; B, in the septal area and either the entorhinal cortex, dorsal hippocampal commissure, subiculum, or presubiculum; in the fornix and either the entorhinal cortex or dorsal hippocampal commissure; C, bilaterally in the septal area. Numbers indicate distance (mm) from bregma in the anterior-posterior plane.

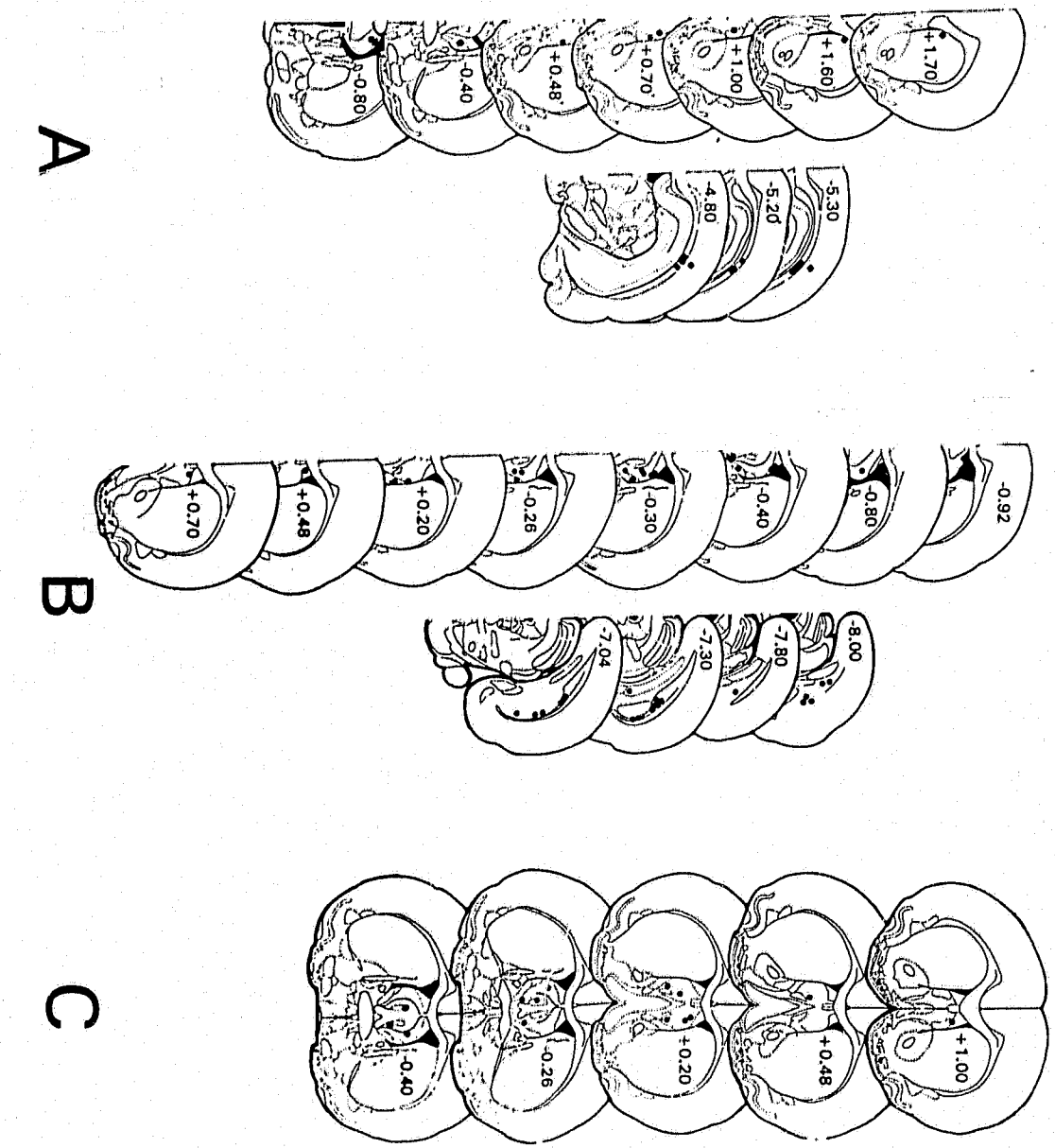

A
+1.70
+1.60
+1.00
+0.70
+0.48
-0.40
-0.80
-5.30
-5.20
-4.80
B
-0.92
-0.80
-0.40
-0.30
-0.26
+0.20
+0.48
+0.70
-8.00
-7.80
-7.30
-7.04
C
+1.00
+0.48
+0.20
-0.26
-0.40

Table 1. Mean (±SEM) ADT (μA) and duration (s) and number of ADs to first stage 5 seizure and first of 6 consecutive stage 5 seizures for rats expressing antagonism (Experiment 1).

Sites[16]	ADT	AD Duration	ADs to First Stage 5	ADs to First of 6 Consecutive Stage 5's
SA[17]	137.50 (45.07)	84.08 (3.99)	20.25 (3.42)	20.25 (3.42)
SCC	306.25 (92.31)	48.31 (9.46)		(n = 6)
AM	83.33 (16.67)	24.04 (6.40)	8.33 (1.20)	10.33 (2.19)
CC	566.67 (233.33)	48.42 (16.91)		(n = 3)
SA	50	70.00	7	7
OC	450	96.75		(n = 1)
CN	200	33.79	14	19
SCC	250	2.96		(n = 1)
CN	900	77.21	4	5
OC	250	17.5		(n = 1)

[16]SA - septal area; SCC - splenium of the corpus callosum; AM - amygdala; CC - cingulate cortex; OC - occipital cortex; CN - caudate nucleus.

[17]Six of 8 rats stimulated alternately in the SA and SCC exhibited antagonism. The SA was dominant in 5 of the cases.

Table 2. Mean (±SEM) ADT (μA) and duration (s) and number of ADs to first stage 5 seizure and first of 6 consecutive stage 5 seizures for rats failing to express antagonism (Experiment 1).

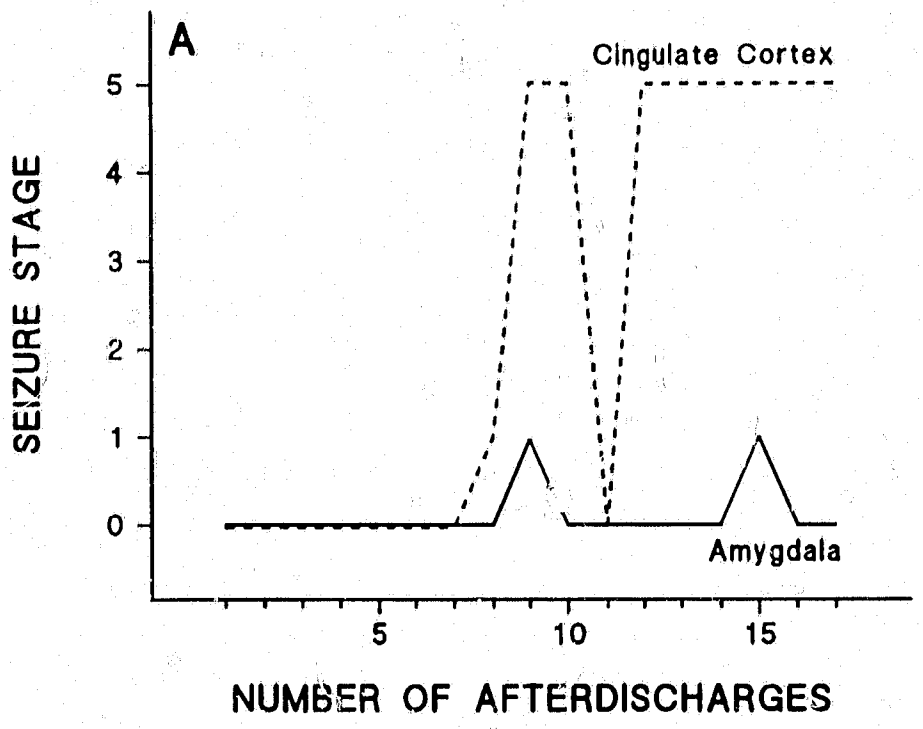
Sites[18]	ADT	AD Duration	Afterdischarges to First Stage 5	ADs to First of 6 Consecutive Stage 5's
SA	150.00 (100.00)	80.71 (1.46)	26.00 (4.00)	32.50 (2.50)
SCC	250.00 (50.00)	62.47 (5.93)		(n = 2)
SA	60.00 (10.00)	85.09 (4.62)	16.60 (4.81)	22.20 (4.74)
EC	440.00 (92.74)	81.01 (5.90)		(n = 5)
SA	170.00 (55.38)	87.53 (3.55)	13.20 (2.19)	18.40 (3.24)
dHC	435.00 (89.46)	81.87 (6.19)		(n = 10)
SA	137.50 (55.43)	89.11 (10.85)	7.50 (1.76)	11.75 (2.56)
SUB/p	250.00 (54.01)	78.35 (9.22)		(n = 4)
FX	350	93.81	11	11
EC	900	101.25		(n = 1)
FX	50	77.77	32	40
dHC	100	81.545		(n = 1)
l SA	128.57 (30.58)	89.42 (5.75)	13.29 (3.23)	16.71 (2.93)
r SA	235.71 (54.24)	87.85 (6.07)		(n = 7)

[18]SA - septal area; SCC - splenium of the corpus callosum; EC - entorhinal cortex; dHC - dorsal hippocampal commissure; SUB/p - subiculum/presubiculum; FX - fornix.

1 rat, the occipital cortex exerted dominance over the septal area in a pattern of relative antagonism. In other rats, the caudate nucleus was dominant over the splenium of the corpus callosum (1 of 1) and the occipital cortex (1 of 1). Histological data from these rats are presented in Figure 8A; patterns of antagonism expressed by some of these rats are presented in Figures 9 and 10 (see also Appendix B). Additional data from rats expressing antagonism are presented in Table 1.

By contrast, I observed that several paired limbic sites were remarkably resistant to the development of antagonism (histological data presented in Figures 8B and 8C). Stimulation of the septal area and the entorhinal cortex consistently failed to elicit antagonism (0 of 5). Moreover, I failed to observe antagonism in any of 10 rats stimulated in the septal area and the posterior subcallosal white matter (dorsal hippocampal commissure). Antagonism was also absent in rats receiving stimulation of the septal area and the subiculum/presubiculum (0 of 4) as well as the fornix and either the entorhinal cortex (0 of 1) or the dorsal hippocampal commissure (0 of 1). Finally, bilateral stimulation of the septal area was utterly ineffective (0 of 7). Patterns of seizure development expressed by some of these rats are presented in Figures 11 and 12. Additional data from rats not expressing antagonism are presented in Table 2.

Figure 9. Patterns of antagonism expressed by individual rats (Experiment 1). Alternating stimulation was delivered to the: A, cingulate cortex and amygdala; B, occipital cortex and septal area.


A
Cingulate Cortex
Amygdala
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
NUMBER OF AFTERDISCHARGES

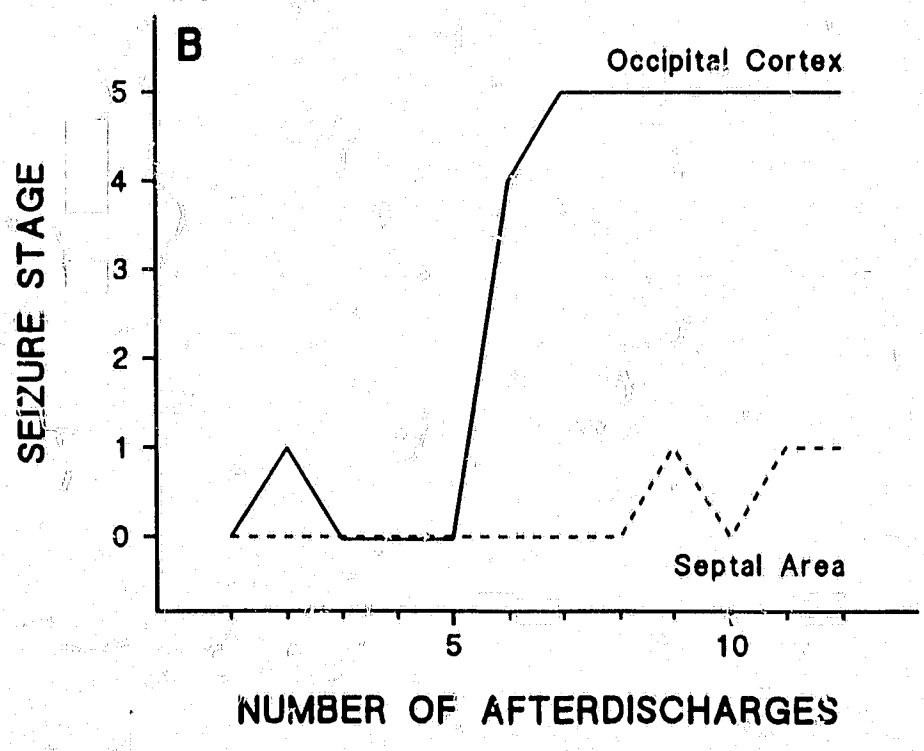
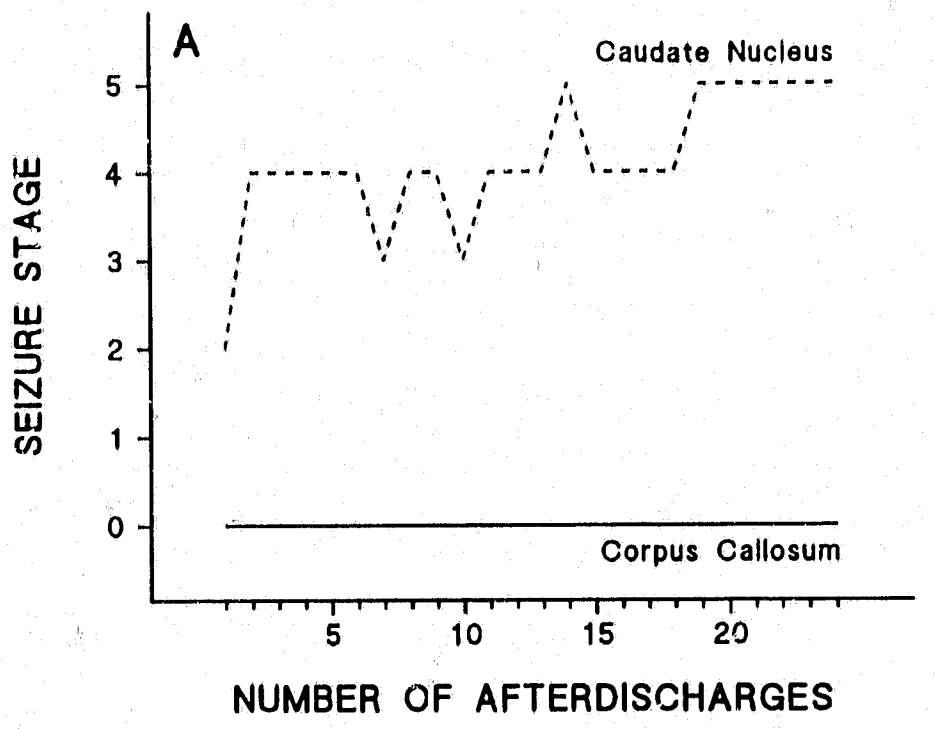

B
Occipital Cortex
Septal Area
SEIZURE STAGE
0
1
2
3
4
5
5
10
NUMBER OF AFTERDISCHARGES

Figure 10. Patterns of antagonism expressed by individual rats (Experiment 1). Alternating stimulation was delivered to the caudate nucleus and the: A, corpus callosum; B, occipital cortex.


A
Caudate Nucleus
Corpus Callosum
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
20
NUMBER OF AFTERDISCHARGES

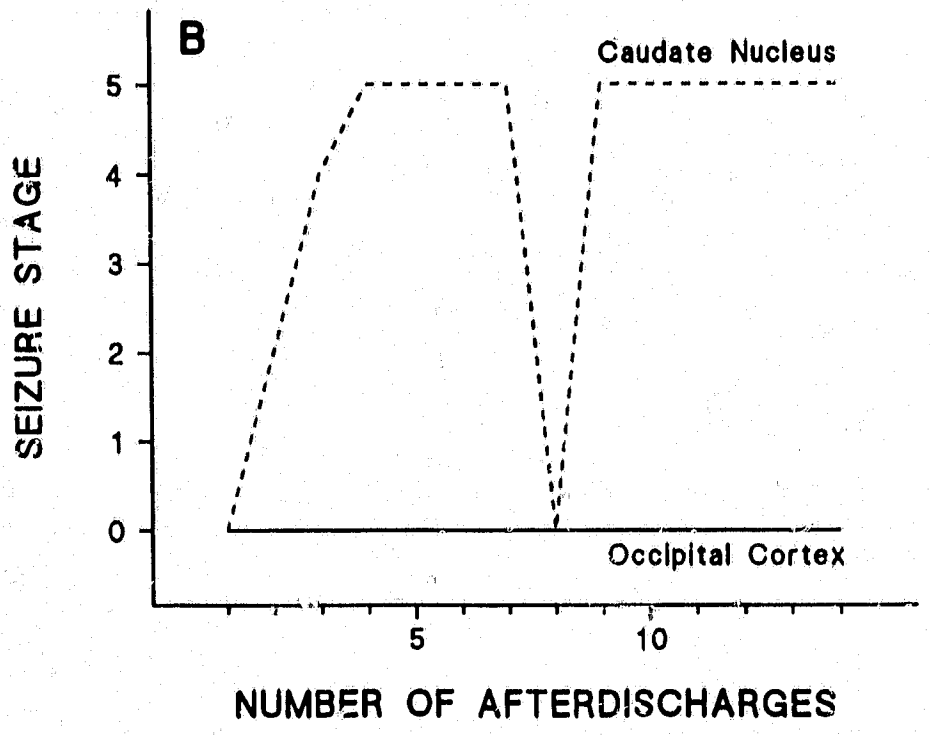
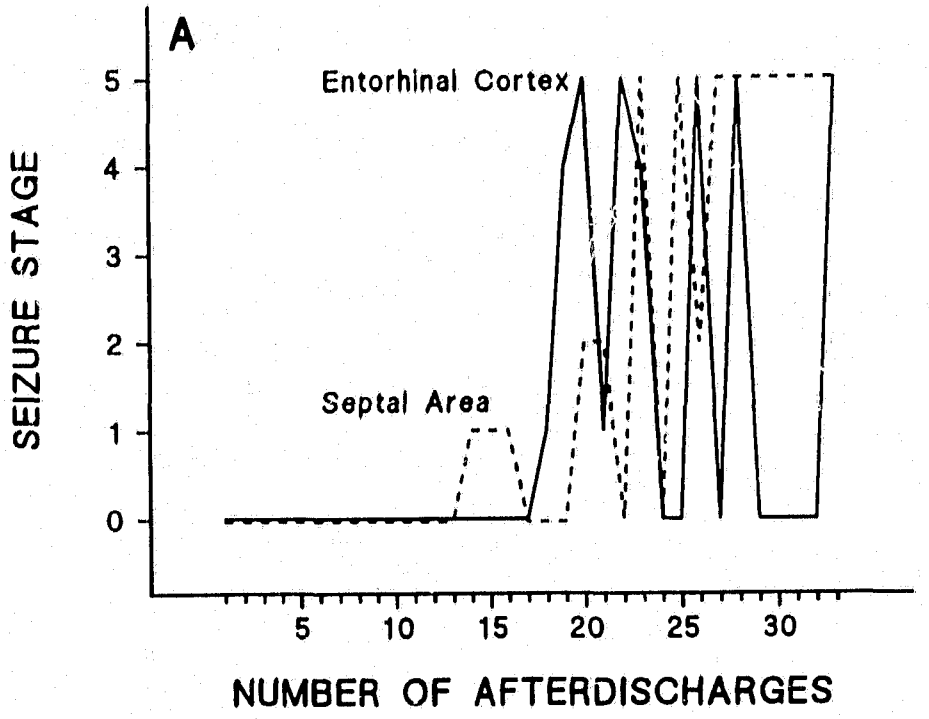

B
Caudate Nucleus
Occipital Cortex
SEIZURE STAGE
0
1
2
3
4
5
5
10
NUMBER OF AFTERDISCHARGES

Figure 11. Patterns of seizure development expressed by individual rats failing to express antagonism (Experiment 1). Alternating stimulation was delivered to the septal area and the: A, entorhinal cortex; B, dorsal hippocampal commissure.


A
Entorhinal Cortex
Septal Area
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
20
25
30
NUMBER OF AFTERDISCHARGES

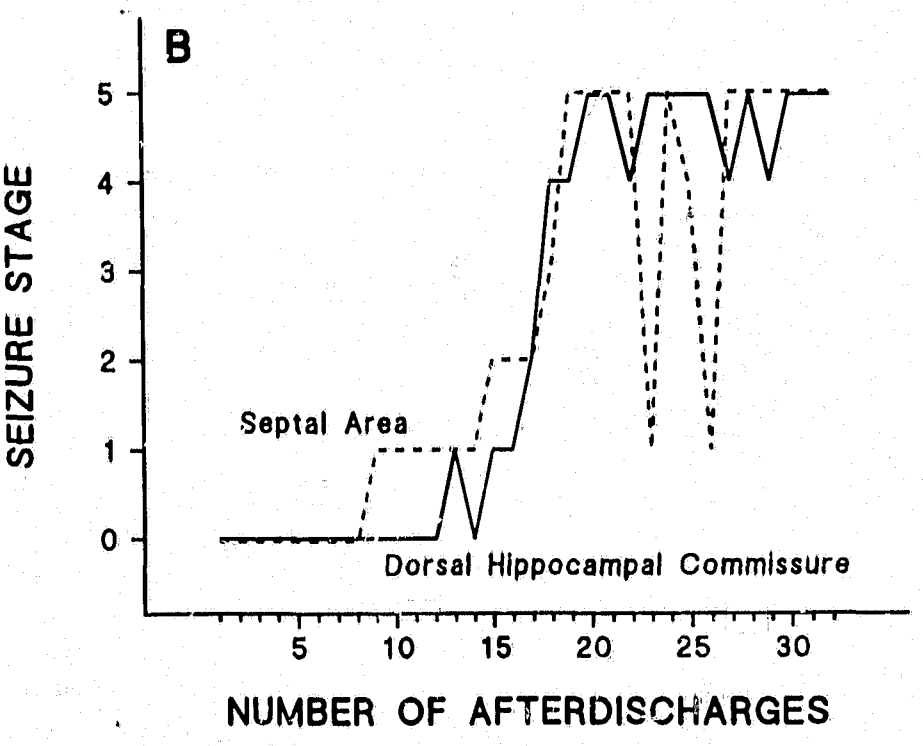
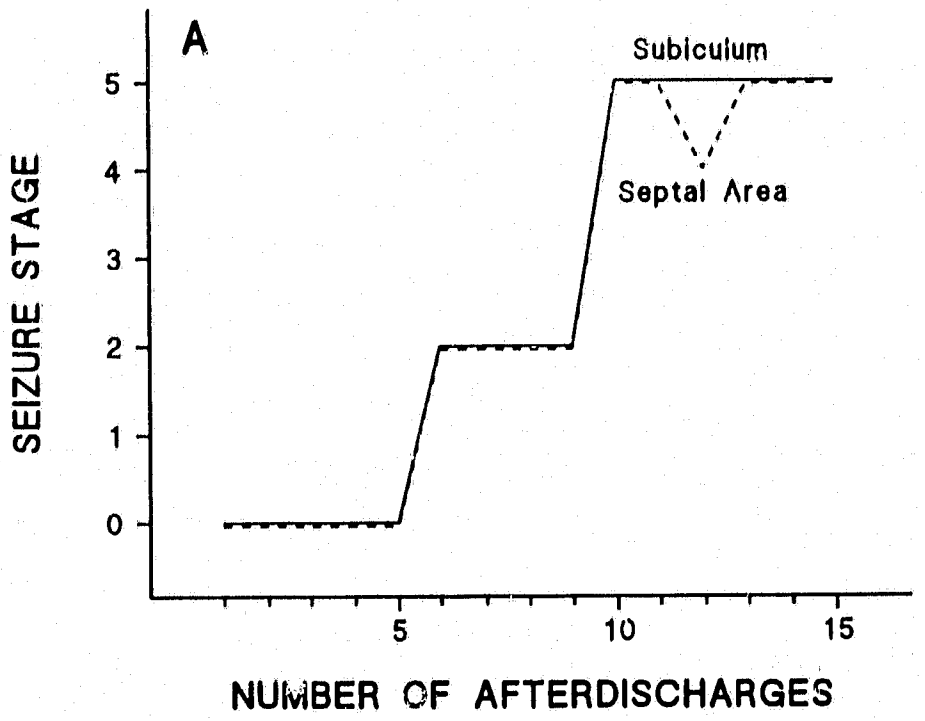
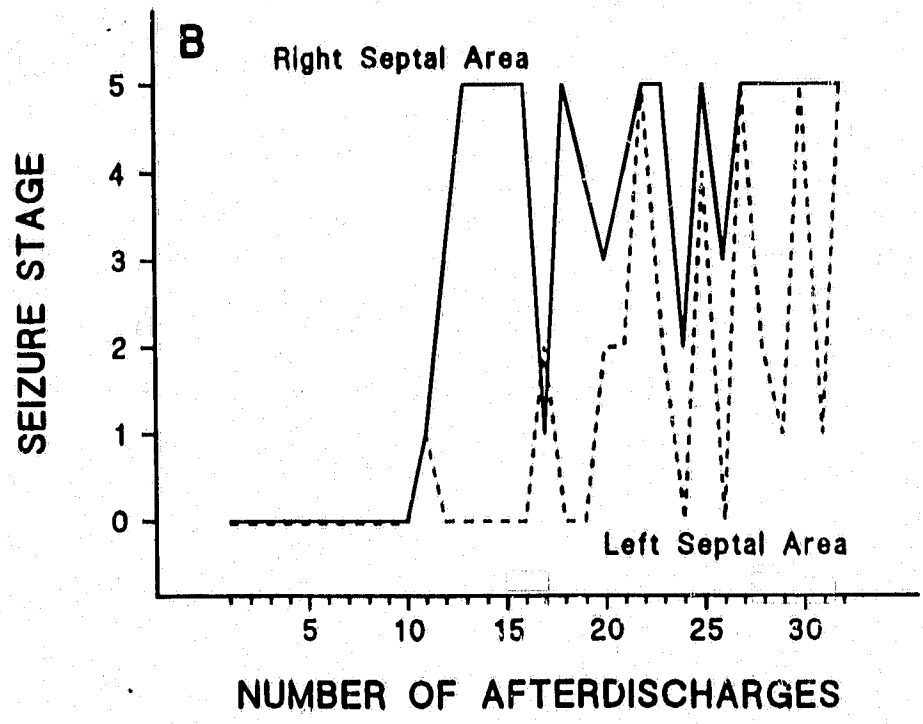

B
Septal Area
Dorsal Hippocampal Commissure
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
20
25
30
NUMBER OF AFTERDISCHARGES

Figure 12. Patterns of seizure development expressed by individual rats failing to express antagonism (Experiment 1). Alternating stimulation was delivered to the: A, septal area and subiculum; B, septal area (bilaterally).


A
Subiculum
Septal Area
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
NUMBER OF AFTERDISCHARGES


B
Right Septal Area
Left Septal Area
SEIZURE STAGE
0
1
2
3
4
5
5
10
15
20
25
30
NUMBER OF AFTERDISCHARGES

Discussion

I have confirmed the observations of Burchfiel and Applegate concerning the development of kindling antagonism from limbic sites during concurrent alternating stimulation. I have extended their observations, in that antagonism occurred in rats in which either or both electrodes were situated in nonlimbic structures. This suggests that antagonism is not unique to limbic circuits. On the other hand, antagonism failed to develop in conjunction with alternating stimulation of several paired limbic sites (e.g., septal area and entorhinal cortex). This runs contrary to numerous previous reports of Burchfiel and Applegate, in which stimulation of septal and entorhinal sites reliably produced antagonism. In sum, however, my results, like those of Haas et al. (1990; 1992), indicate that antagonism is a robust phenomenon and therefore worthy of further scrutiny.

Experiment 2

The results of Experiment 1 confirm previous statements of Burchfiel and Applegate that kindling antagonism can occur reliably. It does not appear, therefore, that antagonism depends entirely on variables peculiar to their laboratory. However, the results do not address the hypothesis of Burchfiel and Applegate that antagonism reflects a frank arrest of kindling. Burchfiel and Applegate (1989; 1990) have stated that the hypothesis hinges on the premise that kindling antagonism shares mechanisms in common with kindling itself and does not involve a transient form of seizure inhibition that is expressed during alternating stimulation. Thus, with the termination of alternating stimulation, stimulation of the suppressed site initiates typical kindling from the point at which it underwent arrest. Consistent with this view, the number of stimulations of the suppressed site required to kindle generalized seizures from the suppressed site is similar to that required by behaviorally matched rats subjected to stimulation of a single site. Further indirect evidence that antagonism does not reflect transient seizure inhibition stems from the statement of Burchfiel and Applegate that antagonism develops reliably even with interstimulus intervals as long as 4 d. Data to substantiate this claim, however, remain unpublished.

The above evidence, while suggestive, does not conclusively implicate arrested kindling as a mechanism of antagonism, as the temporal

parametrics of antagonism have not been well characterized. Thus, alternate interpretations of the above-cited data are plausible. It may simply be the case that the progression of seizures from the suppressed site, observed after the termination of stimulation of the dominant site, reflects the gradual time-dependent decay of inhibition of seizures. If this is the case, one might hypothesize, with the interposition of a prolonged stimulation-free period after the establishment of antagonism, that generalized seizures would emerge following very few stimulations of the suppressed site (i.e., 1 or 2). I test this hypothesis in Experiment 2.

Methods

Thirty-six rats received electrodes, which were aimed at the left amygdala and the right septal area. Following ADT determination, rats were assigned to 2 groups for the Initial Phase. Rats in the Dual Site group were subjected to alternating stimulation; rats in the Single Site group received stimulation of the amygdala only (see General Methods).

Twenty-four hr following the termination of the Initial Phase, Single Site and Dual Site rats were randomly assigned to 1 of 3 groups. Rats in the No Delay group were immediately subjected to the Final phase of the experiment, during which exposure to the apparatus occurred daily, but only the suppressed site (always the septal area) received stimulation once per 48 hr until a stage 5 seizure occurred (see General Methods). Rats in the

Delay group were placed in the apparatus but not stimulated on each of 30 consecutive d, at which point they entered the Final phase. Rats in the Stimulation group continued to receive their respective Initial phase treatments for 30 consecutive d prior to the Final phase. Thus, the Stimulation groups were exposed to the apparatus on 30 additional d, during which Dual Site rats received 30 alternating stimulations (1 per 24 hr) and Single Site rats received 15 amygdaloid stimulations (1 per 48 hr). Treatment groups are summarized in Table 3.

Results

All Dual Site rats demonstrated antagonism (Figure 13), with the amygdala and septal area being dominant and suppressed, respectively. This confirms the results of Experiment 1, which indicate that antagonism is a highly probable outcome of alternating stimulation of the left amygdala and right septal area. Moreover, the results of Experiment 2 indicate that alternating stimulation (Dual Site rats) actively prevented secondary generalization of septal seizures. None of the rats in the Stimulation group exhibited generalized seizures in response to any of the 15 septal stimulations delivered during the 30-d period of alternating stimulation interposed between Initial and Final Phases. At corresponding points in time, rats in the No Delay group were subjected to the Final Phase, during which stage 5 seizures occurred after fewer than 15 stimulations of the

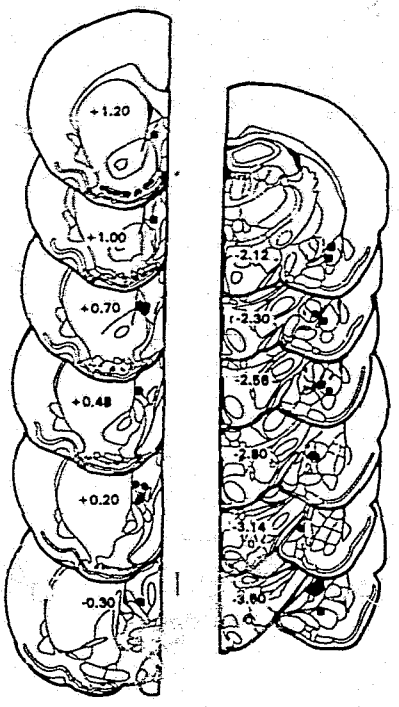
Table 3. Number of subjects (n) for treatment groups for Initial Phase and Final Phase (Experiment 2).

		Dual Site	Single Site
Initial Phase		n = 20	n = 16
Final Phase	No Delay	n = 6	n = 5
	Delay	n = 8	n = 6
	Stimulation[19]	n = 6	n = 5

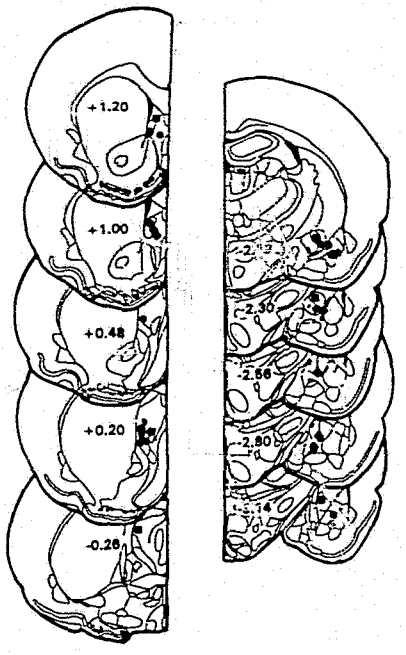
[19]Rats in the Stimulation group continued to receive their respective Initial Phase treatments for 30 d after the Initial Phase. The Final Phase commenced immediately thereafter.

Figure 13. Reconstructions of placements of electrodes (front view; Experiment 2). Rats were stimulated in the amygdala (Single Site: Circles) or stimulated alternately in the amygdala and septal area (Dual Site: Absolute antagonism - diamonds; relative antagonism - squares). The treatment groups comprised rats subjected to the Final Phase: A, immediately following the Initial Phase (No Delay: Single Site n = 5; Dual Site n = 6); B, following 30 stimulation-free d (Delay; Single Site n = 6; Dual Site n = 8); C, following a 30-d continuation of stimulation delivered during the Initial Phase (Stimulation: Single Site n = 5; Dual Site n = 6). Numbers indicate distance (mm) from bregma in the anterior-posterior plane.

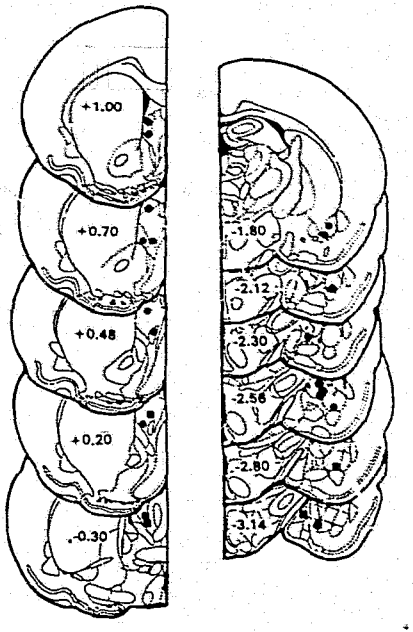
NO DELAY



DELAY



STIMULATION



septal area in 4 of 6 rats. Fisher's Exact test indicated that Dual Site rats in the No Delay group (4 of 6) were significantly more likely to express stage 5 seizures in response to septal stimulation shortly following the establishment of antagonism than were Dual Site rats in the Stimulation group (0 of 6; $p = 0.03$). It thus appears that alternating stimulation actively inhibited the generalization of seizures provoked from the suppressed site in Dual Site rats[20].

With respect to the number of septal ADs required to elicit a stage 5 seizure during the Final Phase, a two-way ANOVA, utilizing a 2 (Site) X 3 (Group) design, revealed that the interaction as well as the main effect of Group were not statistically significant. The main effect of Site was significant ($F(1,30) = 6.97$, $p < 0.05$), on the other hand. This indicates, for both Dual Site and Single Site rats, that the imposition of either a 30-d stimulation-free period or a 30-d continuation of treatments delivered during the Initial Phase did not significantly alter Final Phase kindling from the septal area, although Dual Site rats in the Delay group displayed a nonsignificant tendency toward more rapid Final Phase kindling (see Figure 14 and General Discussion). Specifically, Dual Site rats in the Delay group did not demonstrate significantly more rapid Final Phase kindling than did

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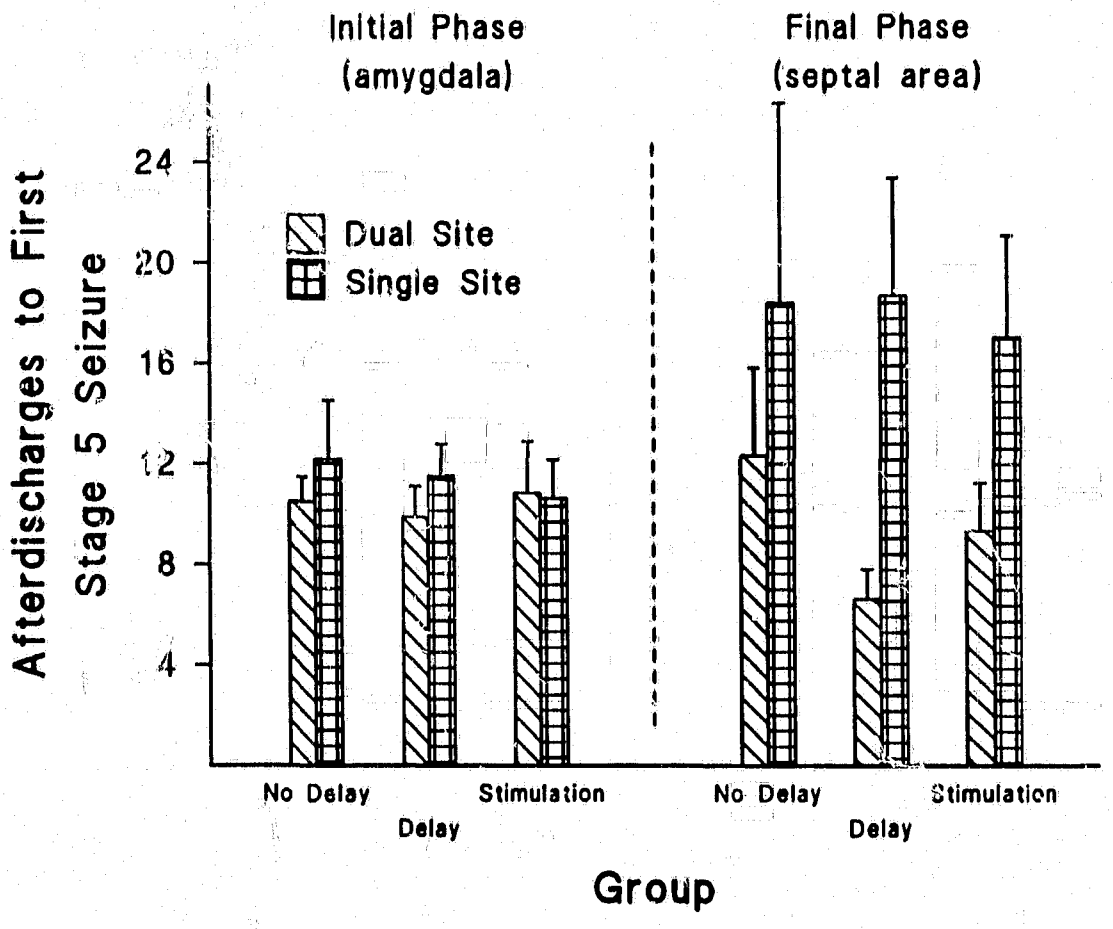
[20]Although these results suggest stongly that kindling antagonism was occurring in the Stimulation group, they do not establish the point conclusively. The more conclusive test would be to compare a Septal Kindling group stimulated in the septal area and yoked to Dual Site rats during the Initial Phase.

Figure 14. Number of ADs to first stage 5 seizures (Experiment 2). Seizures were provoked by stimulation of: The amygdala during the Initial Phase in Dual Site rats, which received alternating stimulation of the amygdala (dominant site) and septal area (suppressed site) and in control rats, which were stimulated only in the amygdala (Single Site); the septal area during the Final Phase. Rats in the No Delay group were subjected to the Final Phase immediately following the Initial Phase. For the remaining groups, the Final Phase proceeded after either 30 stimulation-free d (Delay) or a 30-d continuation of stimulation delivered during the Initial Phase (Stimulation). Final Phase kindling was significantly faster in Dual Site rats ($p < 0.05$).


Initial Phase
(amygdala)
Final Phase
(septal area)
Dual Site
Single Site
Afterdischarges to First
Stage 5 Seizure
24
20
16
12
8
4
No Delay
Stimulation
Delay
No Delay
Stimulation
Delay
Group

Dual Site rats in the No Delay group, with only 1 of 8 rats (Delay group) expressing stage 5 seizures after fewer than 4 AD-provoking stimulations (i.e., 2) of the suppressed site. For the remaining 7 rats, Final Phase kindling required 4 - 11 stimulations. The rates of Final Phase kindling between the groups were also not significantly different when analyzed with a 1-tailed t-test ($t(12) = 1.73$, $0.06 > p > 0.05$). The analyses also revealed that Dual Site rats exhibited more rapid Final Phase kindling than did Single Site rats (Figure 14).

Unlike Final Phase kindling, kindling rates observed during the Initial Phase of Experiment 2 did not differ significantly (Figure 14). ANOVA indicated that the interaction and main effects of Site and Group on the number of amygdaloid ADs required to elicit the first stage 5 seizure during the Initial Phase were not statistically significant. Groups of rats did not differ on several other electrographic and behavioral correlates of amygdaloid stimulation (e.g., durations of AD and clonus), measured during the Initial Phase (Tables 4 - 6). Data obtained from Stimulation rats during the 30-d period between Initial and Final Phases are presented in Table 7.

Duration of AD provoked from the septal area increased during the Initial Phase in Dual Site rats (Figure 15). Consistent with the results of Experiment 1, septal stimulation corresponding to the first stage 5 seizure provoked from the amygdala produced significantly longer AD than did the first stimulation of the septal area ($t(19) = -3.15$, $p < 0.005$). As a

Table 4. Mean (±SEM) ADT (μA) for left amygdala and right septal area (Experiment 2).

		Dual Site	Single Site
Left Amygdala	No Delay	125.00 (17.08)	80.00 (12.18)
	Delay	106.25 (11.33)	108.33 (8.33)
	Stimulation	100.00 (18.26)	100.00 (27.39)
Right Septal Area	No Delay	225.00 (38.19)	320.00 (131.91)
	Delay	200.00 (54.28)	308.33 (74.63)
	Stimulation	158.33 (15.37)	200.00 (35.36)

Table 5. Mean (±SEM) durations (s) of AD provoked from the amygdala and right septal area during the Initial Phase (Experiment 2).

		Dual Site	Single Site
Left Amygdala	No Delay	44.43 (9.49)	48.74 (7.93)
	Delay	47.49 (7.36)	33.49 (5.84)
	Stimulation	38.55 (7.33)	37.15 (5.98)
Right Septal Area	No Delay	49.07 (16.26)	--
	Delay	66.59 (11.68)	--
	Stimulation	40.68 (13.43)	--

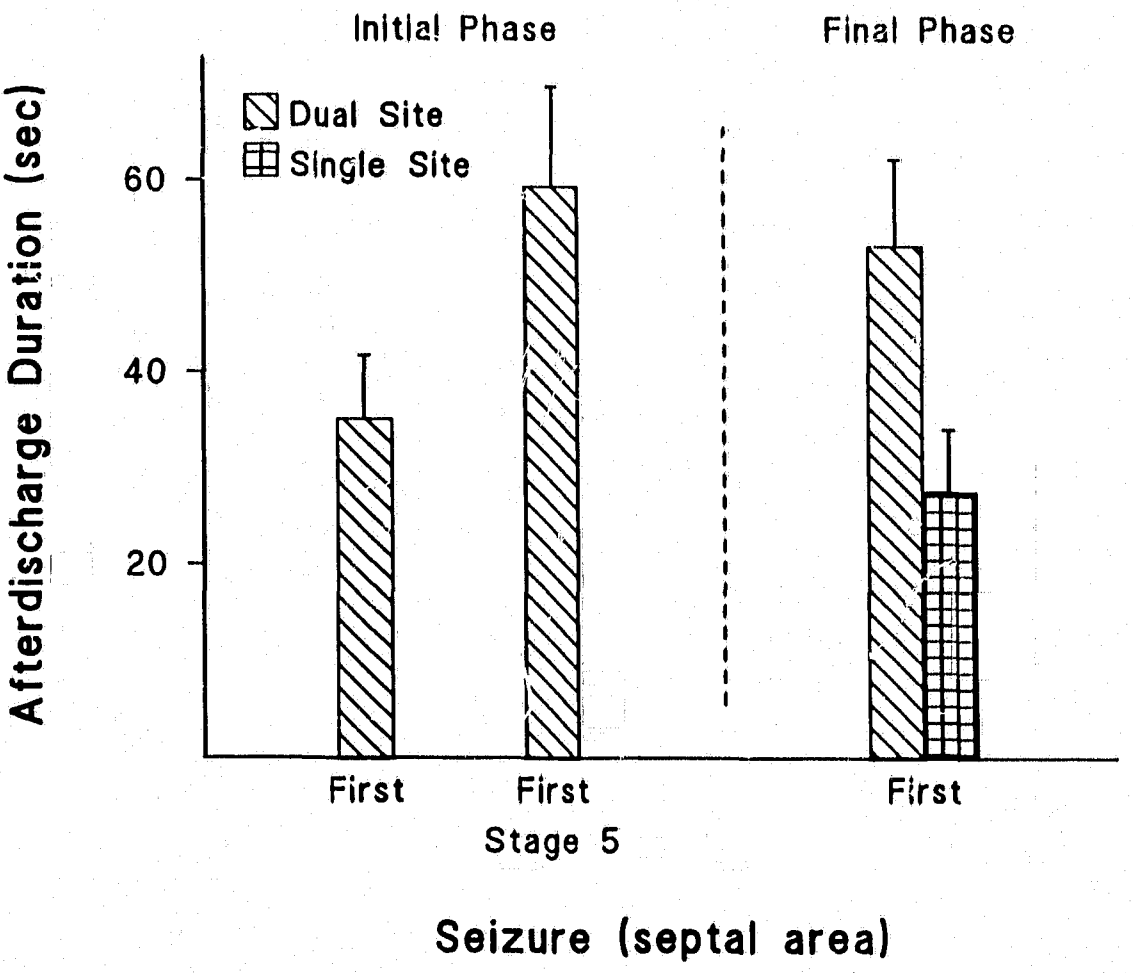
Table 6. Mean (±SEM) latency to and duration (s) of clonus during the first stage 5 seizure provoked from the left amygdala during the Initial Phase (Experiment 2).

		Dual Site	Single Site
Latency to Clonus	No Delay	9.83 (3.77)	12.00 (5.62)
	Delay	18.13 (5.75)	7.33 (2.87)
	Stimulation	18.50 (4.49)	27.00 (12.07)
Duration of Clonus	No Delay	26.00 (4.75)	29.80 (6.97)
	Delay	35.38 (5.95)	32.17 (7.53)
	Stimulation	16.00 (3.10)	40.80 (12.20)

Table 7. Mean (±SEM) number, latency (s), and durations (s) of ictal events observed in rats in the Stimulation groups during the 30-d period of stimulation interposed between Initial and Final Phases (Experiment 2)

	Dual Site	Single Site
Number of Stage 5 Seizures	13.33 (1.12)	14.00 (0.32)
AD Duration (amygdala)	90.25 (14.36)	93.65 (5.31)
AD Duration (septal area)	59.87 (17.44)	--
Latency to Clonus (stage 5)	11.33 (4.67)	5.57 (0.79)
Duration of Clonus (stage 5)	47.21 (3.27)	53.83 (4.73)

Figure 15. Duration of AD provoked from the septal area (Experiment 2). AD provoked during the Initial Phase by the septal stimulation corresponding to the first amygdaloid stage 5 seizure was significantly longer than that provoked by the first septal stimulation ($p < 0.005$). AD provoked by the first septal stimulation during the Final Phase was significantly longer in Dual Site rats ($p < 0.05$).


Initial Phase
Final Phase
Dual Site
Single Site
Afterdischarge Duration (sec)
60
40
20
First
First
First
Stage 5
Seizure (septal area)

probable consequence, AD elicited by the first septal stimulation during the Final Phase was longer in Dual Site rats than in Single Site rats (depicted in Figure 15; see also Table 8): ANOVA of this dependent measure revealed that the Site X Group interaction and Group main effect were not significant, in contrast to the significant main effect of Site ($F(1,30) = 5.69$, $p < 0.05$). Dual Site and Single Site rats did not differ significantly with respect to the behavioral correlates of the stage 5 seizure provoked during the Final Phase (Table 9).

Discussion

The view that antagonism does not merely involve transient inhibition of seizures provoked from the suppressed site leads to the prediction that the Delay and No Delay groups should require similar numbers of stimulations of the septal area for the expression of a generalized seizure during the Final Phase, for both Dual Site and Single Site rats. The results of the present experiment were somewhat ambiguous: Although the Delay group showed more rapid kindling than the No Delay group during the Final Phase, this difference did not prove to be statistically significant, perhaps due to the small size of the groups and resulting loss of statistical power. Given that there is little if any loss of seizure susceptibility following a prolonged period of nonstimulation after septal kindling (Goddard et al., 1969), another way to evaluate the hypothesis is to predict that septal

Table 8. Mean (±SEM) duration (s) of AD provoked from the right septal area during the Final Phase (Experiment 2).

		Dual Site	Single Site
AD Duration (septal area)	No Delay	70.57* (16.44)	41.69 (8.36)
	Delay	70.14* (6.24)	40.19 (10.49)
	Stimulation	95.94* (14.46)	61.93 (10.63)

* indicates that AD duration is significantly longer in Dual Site rats, $F_{(1,30)} = 10.72$, $p < 0.005$.

Table 9. Mean (±SEM) latency to and duration (s) of clonus during the first stage 5 seizure provoked from the right septal area during the Final Phase (Experiment 2).

		Dual Site	Single Site
Latency to Clonus	No Delay	44.50 (9.83)	26.60 (9.96)
	Delay	46.50 (8.04)	43.83 (6.62)
	Stimulation	63.67 (10.18)	60.60 (14.64)
Duration of Clonus	No Delay	47.67 (8.74)	30.20 (6.91)
	Delay	44.25 (7.58)	44.00 (8.25)
	Stimulation	38.83 (3.65)	47.80 (9.72)

kindling in the Delay group should proceed very quickly (i.e., within 1 or 2 ADs) if antagonism merely reflects a transient inhibition of seizures. However, the interposition of a prolonged stimulation-free period after the establishment of antagonism consistently failed to result in immediate generalization of seizures during the Final Phase. Thus, although far from conclusive, the results could be interpreted as being consistent with the hypothesis that antagonism reflects a genuine arrest of kindling from the suppressed site, which is held at an intermediate stage (i.e., partial kindling). Substantiating this view is the finding that Final Phase kindling was faster in Dual Site rats than in Single Site rats, presumably reflecting partial kindling from the septal area that occurred in Dual Site rats during the Initial Phase.

In summary, the absence of a *statistically significant* difference between the Delay and No Delay groups of Dual Site rats in rates of Final Phase kindling is generally consistent with Burchfiel's hypothesis of the nature of kindling antagonism, although admittedly other interpretations are possible and indeed reasonable. The results of the analysis thus force me, in the experiments that follow, to adopt the position that kindling antagonism is not due to a transient inhibition of seizures, although in the General Discussion I shall return to the question and offer an alternative explanation.

Experiment 3

The results of Experiment 2 indicate that the development of generalized seizures from the septal area (Final Phase kindling) after either alternating stimulation or single site kindling is stimulation-dependent rather than time-dependent and that Final Phase kindling in Dual Site and Single Site rats may therefore involve similar mechanisms. Several investigators have reported that kindling from one site is enhanced by prior kindling from another (transfer; e.g., Burnham, 1976; Goddard et al., 1969; McIntyre, 1980; McIntyre & Goddard, 1973; Racine, 1972b; Wada & Osawa, 1976). This suggests the possibility that mechanisms underlying transfer dictate the course of Final Phase kindling for Single Site as well as for Dual Site rats. However, as reported in Experiment 2, Dual Site rats displayed rates of Final Phase kindling that were actually faster than those exhibited by Single Site rats. It is therefore possible that qualitatively similar transfer mechanisms were involved, the effects of which differed in magnitude as a function of Site of stimulation during the Initial Phase of Experiment 2. On the other hand, it is possible that mechanisms involved in the Site-dependent enhancement of Final Phase kindling involve distinct mechanisms that act additively with mechanisms of transfer or even in their place. In order to determine whether antagonism is associated with a nonspecific enhancement of transfer or other mechanisms influencing Final Phase kindling, I subjected rats to either alternating stimulation or single site

kindling as described in Experiment 2. I subsequently assessed kindling from a third (naive) site during the Final Phase.

Method

I implanted electrodes bilaterally into the amygdala and into the right septal area of 12 rats. After ADT determination from the septal area and the left amygdala, rats were assigned to 2 groups for the Initial Phase. The Dual Site group (n = 7) received alternating stimulation of the septal area and left amygdala during the Initial Phase at a 24-hr interval; the Single Site group (n = 5) received stimulation of the left amygdala at a 48-hr interval. Twenty-four hr after the termination of the Initial Phase, the Final Phase occurred, during which I stimulated only the right amygdala at 200 μA, once per 48 hr, until a stage 5 seizure occurred.

Results

Experiment 3 confirmed several observations made during the Initial Phase of Experiment 2. All Dual Site rats expressed antagonism, with the left amygdala being dominant over the septal area in all instances[21]. Also

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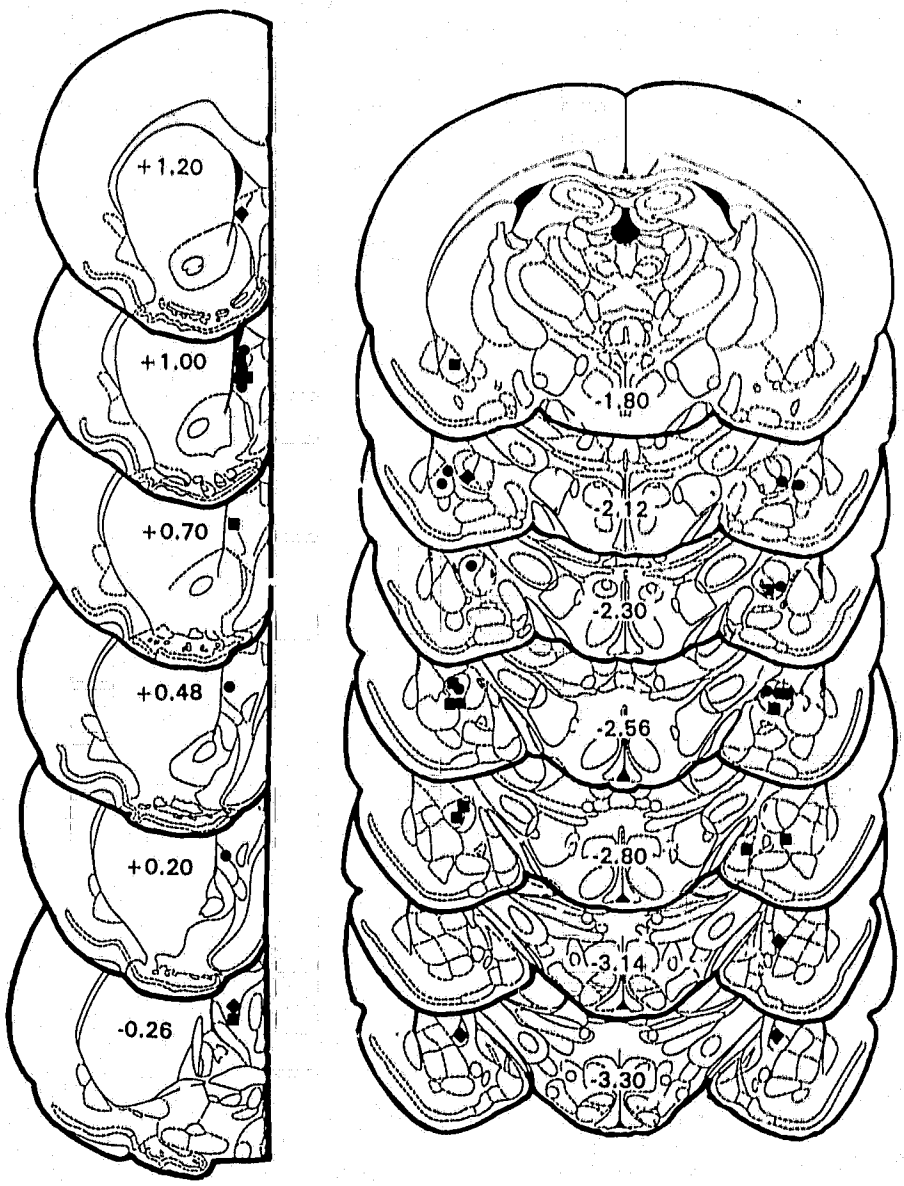
[21]Absolute and relative antagonism were expressed by 2 and 5 Dual Site rats, respectively, as depicted in Figure 16. Septal stimulation provoked seizures of a stage no higher than stage 1 in rats expressing relative antagonism.

Figure 16. Reconstructions of placements of electrodes (front view; Experiment 3). Rats were stimulated during the Initial Phase in the left amygdala only (Single Site [n = 5]: Circles) or were stimulated alternately in the amygdala and right septal area (Dual Site [n = 7]: Absolute antagonism - diamonds; relative antagonism - squares). Rats were stimulated in the right amygdala during the Final Phase. Numbers indicate distance (mm) from bregma in the anterior-posterior plane.


+1.20
+1.00
+0.70
+0.48
+0.20
-0.26
-1.80
-2.12
-2.30
-2.56
-2.80
-3.14
-3.30

in Dual Site rats, duration of septal AD increased during the Initial Phase. AD provoked by the septal stimulation corresponding to the first amygdaloid stage 5 seizure was significantly longer than that provoked by the first septal stimulation (dependent t-test; $t(6) = -2.80$, $p < 0.05$). During the Initial Phase, Dual Site and Single Site rats displayed stage 5 seizures after a similar number of stimulations of the left amygdala (Figure 17).

Unlike Experiment 2, Site of stimulation did not influence Final Phase kindling. Dual Site and Single Site rats exhibited stage 5 seizures after virtually identical numbers of ADs provoked from the right amygdala, which did not receive stimulation during the Initial Phase (Figure 17). The small differences that were observed between groups on this dependent measure were not statistically significant according to an independent t-test. Final Phase kindling was more rapid than Initial Phase kindling (as indicated by the number of amygdaloid stimulations required to elicit the first stage 5 seizure during the Initial Phase) according to a dependent t-test ($t(6) = 3.36$, $p < 0.01$), suggesting that transfer occurred in both groups.

Several other measures were also similar in Dual Site and Single Site rats (Tables 10 - 12). ADT for the left amygdala and right septal area did not vary as a function of Site, and all rats demonstrated AD after the first stimulation of the right amygdala at an intensity of 200 μA. Rates of AD growth and latencies to and durations of clonus during the first stage 5

Figure 17. Number of ADs to first stage 5 seizures (Experiment 3). Seizures were provoked by stimulation of: The amygdala during the Initial Phase in Dual Site rats, which received alternating stimulation of the amygdala (dominant site) and septal area (suppressed site), and in control rats, which were stimulated only in the amygdala (Single Site); the right amygdala, which was stimulated only during the Final Phase. Final Phase kindling was significantly faster than Initial Phase kindling for both Dual Site and Single Site rats ($p < 0.001$).

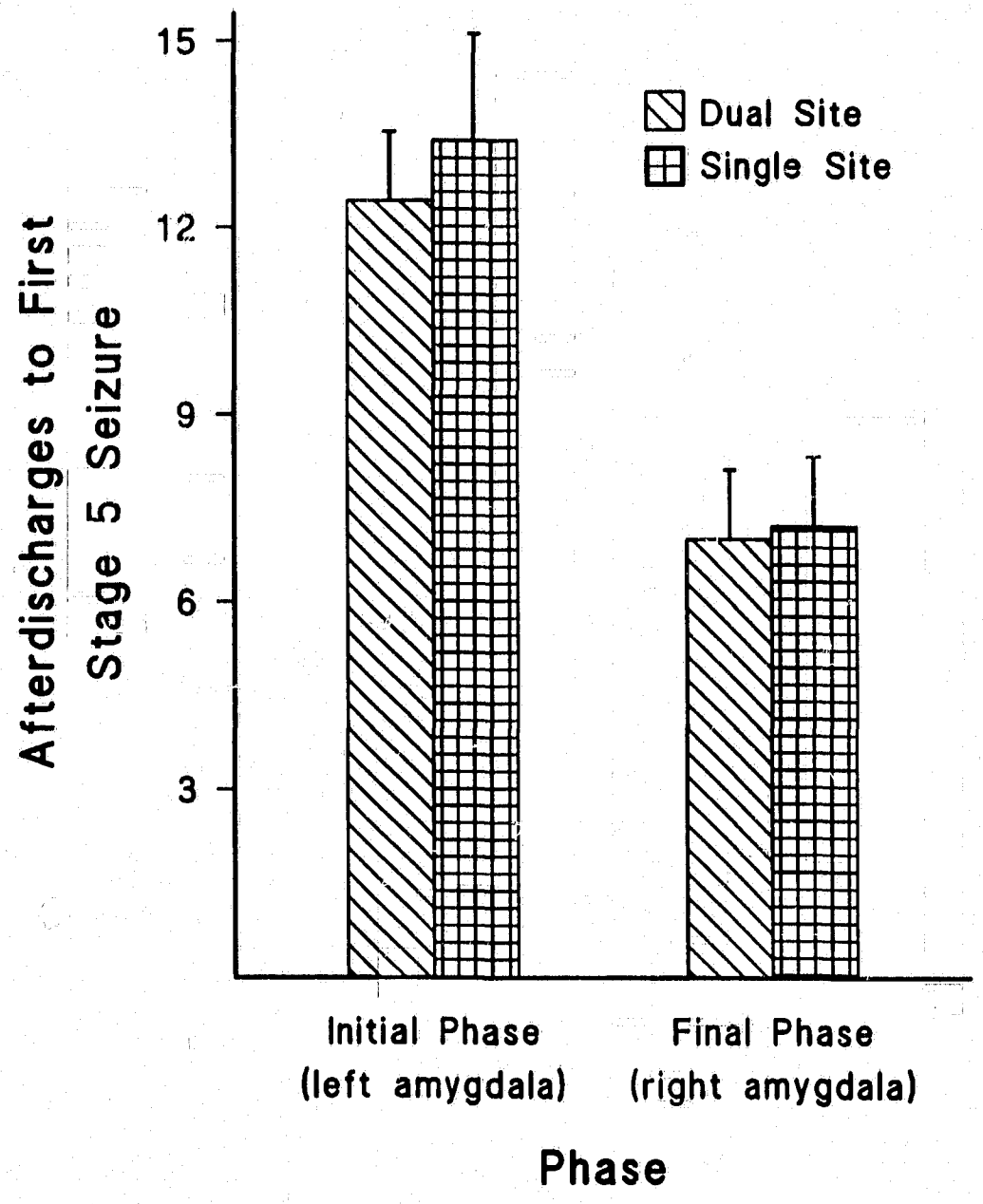

Afterdischarges to First Stage 5 Seizure
15
12
9
6
3
Dual Site
Single Site
Initial Phase (left amygdala)
Final Phase (right amygdala)
Phase

Table 10. Mean (±SEM) ADT (μA) left amygdala and right septal area (Experiment 3).

	Dual Site	Single Site
Left Amygdala	121.43 (24.05)	140.83 (18.71)
Right Septal Area	342.86 (101.44)	270.00 (64.42)

Table 11. Mean (±SEM) latency to and duration (s) of electrographic and behavioral responses provoked by stimulation of the left amygdala and right septal area during the Initial Phase (Experiment 3).

	Dual Site	Single Site
AD Duration (amygdala)	73.53 (12.11)	41.67 (13.22)
AD Duration (septal area)	70.15 (10.31)	--
Latency to Clonus (amygdala)	34.86 (14.30)	26.80 (17.92)
Duration of Clonus (amygdala)	42.57 (7.89)	28.00 (6.25)

Table 12. Mean (±SEM) latency to and duration (s) of electrographic and behavioral responses provoked by stimulation of the right amygdala during the Final Phase (Experiment 3).

	Dual Site	Single Site
AD Duration for First Stimulation	27.57 (10.50)	12.40 (2.02)
AD Duration for First Stage 5	135.43 (21.91)	74.20 (25.63)
Latency to Clonus (amygdala)	31.14 (12.19)	8.80 (3.29)
Duration of Clonus (amygdala)	53.29 (6.09)	28.00 (6.25)

seizure provoked from the amygdala were similar in Dual Site and Single Site rats during both Initial and Final Phases.

Discussion

Final Phase kindling from the right amygdala did not vary as a function of Site of stimulation during the Initial Phase. This finding contrasts the results of Experiment 2, which indicated that stage 5 seizures occur after fewer septal ADs during the Final Phase in Dual Site rats. The facilitating effect of septal area stimulation (Initial Phase) on Final Phase kindling (Experiment 2), therefore, shows some degree of site-specificity. Hence, it does not appear that the facilitation of Final Phase kindling observed in Dual Site rats in Experiment 2 involves a nonspecific enhancement of transfer or other mechanisms affecting Final Phase kindling.

Experiment 4

The nomenclature of Burchfiel and Applegate (1989; 1990) may yield hypotheses regarding the mechanism by which suppressed site seizures fail to generalize during alternating stimulation: The mere use of the term dominant site implies that intrinsic or extrinsic connections of the site participate in either the failure or the masking of epileptogenesis associated with stimulation of the suppressed site. In order to determine whether this is the case, I have assessed the development of Final Phase kindling from the septal area (suppressed site) after the establishment of antagonism and the subsequent destruction of cells of the dominant site (amygdala). If circuitry of the dominant site is critical to the expression of antagonism, then destruction of cells of the dominant site should lead to the rapid generalization of seizures provoked later from the suppressed site.

Method

Twenty-four rats received electrodes aimed at the left amygdala and right septal area. After ADT determination, rats were assigned to 2 groups (n = 12) for the Initial Phase. Rats in the Stimulation group underwent alternating stimulation as described for Initial Phases of Experiments 2 and 3; rats in the No Stimulation group were randomly yoked to rats in the Stimulation group in terms of the number of exposures to the testing apparatus during the Initial Phase but were not stimulated.

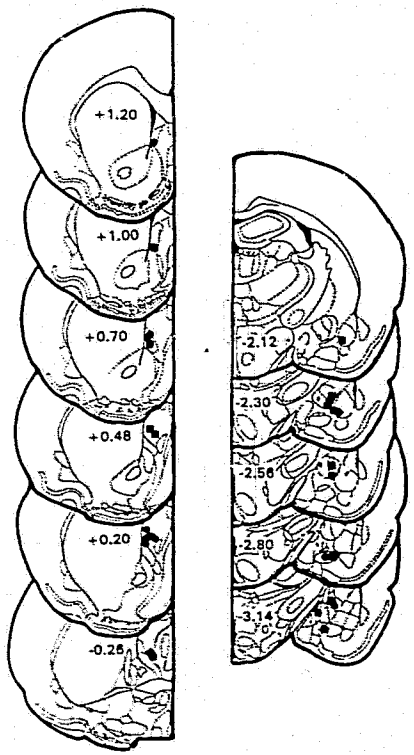
Twenty-four hr after the completion of the initial Phase, yoked pairs of rats were assigned to 2 groups: After monitoring of EEG for 30 s, radio-frequency current (5 mA; Radionics, Model RFG-4A, Burlington, Mass.) was delivered across the poles of the amygdaloid electrode for 75 s (Lesion groups; n = 7), after which I resumed the monitoring of EEG for an additional 30 s; control rats were treated identically, except that radio-frequency current was not delivered (No Lesion groups; n = 5). All rats subsequently underwent a 7-d stimulation-free period, during which daily exposure to the apparatus occurred, but no stimuli were delivered. I stimulated the septal area in all rats at a 48-hr interval during the Final Phase. Twenty-four hr after the Final Phase, I stimulated the amygdala in all rats at an intensity of 500 μA to determine whether the application of radio-frequency current destroyed cells involved in the elicitation of amygdaloid AD. Prior to perfusion of the rats, direct current (1 mA; 10 s) was passed between the tips of the septal electrode only, so that damage to amygdaloid tissue induced by radio-frequency current could be assessed histologically.

Results

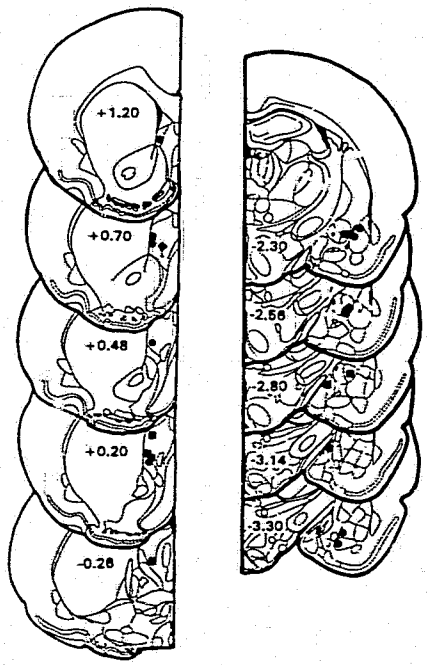
As in Experiments 2 and 3, alternating stimulation of the left amygdala and right septal area always resulted in the expression of antagonism (see Figure 18), and the amygdala was always dominant. With respect to rates of Final Phase kindling, a two-way ANOVA, utilizing a 2

Figure 18. Reconstructions of placements of electrodes (front view; Experiment 4). Rats received alternating stimulation of the amygdala and septal area (Stimulation: Absolute antagonism - diamonds; relative antagonism - squares) or were yoked (No Stimulation) to Stimulation rats during the Initial Phase but were not stimulated. Treatment groups comprised control rats (No Lesion: Stimulation n = 7; No Stimulation n = 5) and rats exposed to radio-frequency current delivered to the amygdala prior to the Initial Phase (Lesion: Stimulation n = 7; No Stimulation n = 5). Numbers indicate distance (mm) from bregma in the anterior-posterior plane.

NO LESION



LESION



(Stimulation) X 2 (Lesion) design, revealed that the Lesion main effect and the Stimulation X Lesion interaction were not statistically significant. The Stimulation main effect was statistically significant, $F(1,20) = 13.65$, $p < 0.001$, however, indicating that rats that were alternately stimulated during the Initial Phase exhibited more rapid Final Phase kindling from the septal area than did yoked unstimulated rats, a finding that is depicted in Figure 19. The failure of amygdaloid lesions to influence Final Phase kindling is not attributable to differential rates of seizure development observed in Stimulation rats during the Initial Phase, as the Lesion and No Lesion groups developed stage 5 seizures after a similar number of amygdaloid ADs (also shown in Figure 19). Moreover, ANOVA indicated that Stimulation and Lesion were without influence on several other measures of behavioral and electrographic seizures taken during both Initial and Final Phases (Tables 13 - 15).

Both electrophysiological and histological data indicate that the application of radio-frequency current after the Initial Phase destroyed neurons proximal to the amygdaloid electrode in rats in the Lesion groups. After the Final Phase, stimulation of the amygdala, at an intensity 1.6 - 10 times greater than amygdaloid ADTs measured during Experiments 1 - 4, failed to provoke AD in all rats in the Lesion groups; rats in the No Lesion groups demonstrated AD in all cases. In the Stimulation No Lesion group, AD triggered stage 5 seizures in 4 of 5 rats. The remaining rat in this group

Figure 19. Number of ADs to first stage 5 seizures (Experiment 4). Seizures were provoked by stimulation of: The amygdala during the Initial Phase in rats subsequently exposed to radio-frequency current delivered via the amygdaloid electrode (Lesion) and in unlesioned control rats (No Lesion); the septal area during the Final Phase. Rats in the Stimulation groups received alternating stimulation of the amygdala (dominant site) and septal area (suppressed site) during the Initial Phase. Final Phase kindling was significantly faster in Stimulation rats ($p < 0.001$).

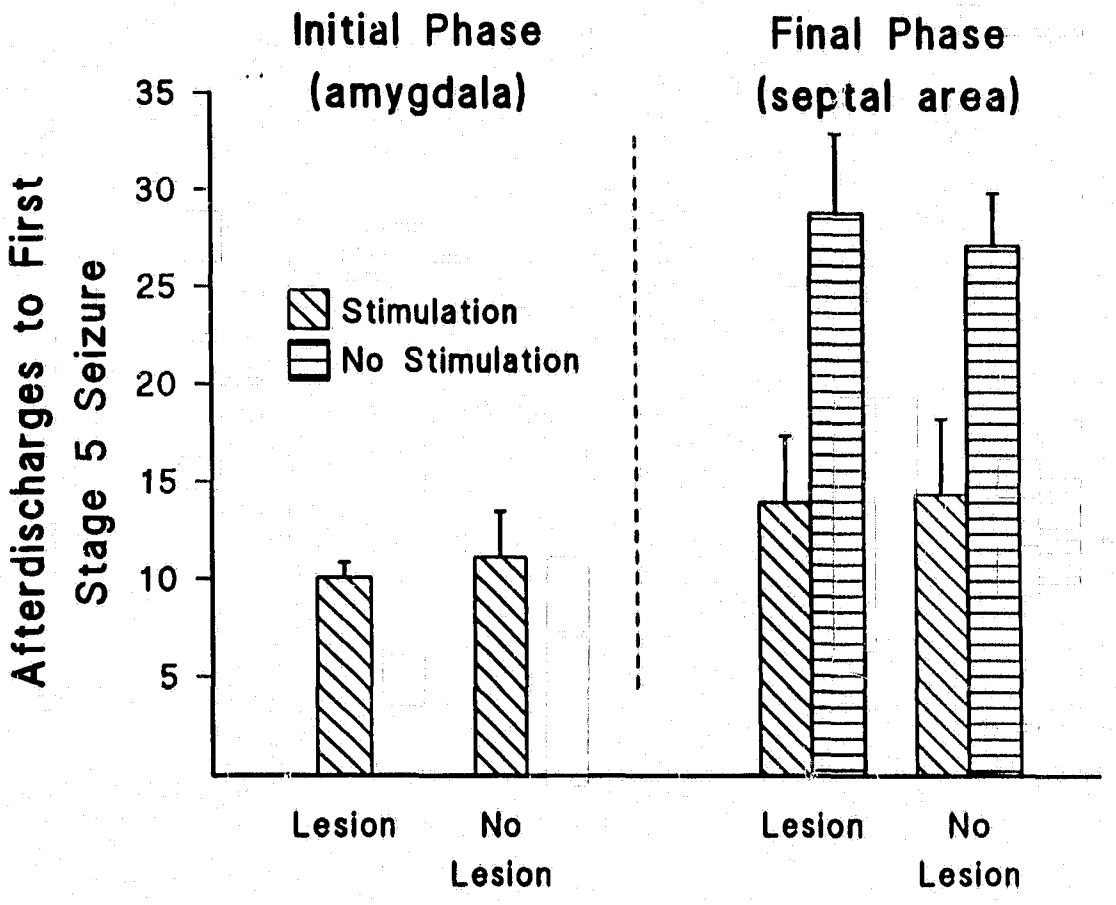

Initial Phase
(amygdala)
Final Phase
(septal area)
Afterdischarges to First
Stage 5 Seizure
35
30
25
20
15
10
5
Stimulation
No Stimulation
Lesion
No
Lesion
Lesion
No
Lesion

Table 13. Mean (±SEM) ADT (μA) for left amygdala and right septal area (Experiment 4).

		No Lesion	Lesion
Left Amygdala	No Stimulation	140.00 (33.17)	178.57 (35.95)
	Stimulation	200.00 (35.36)	121.43 (14.87)
Right Septal Area	No Stimulation	310.00 (73.14)	471.43 (130.41)
	Stimulation	370.00 (104.40)	178.57 (23.89)

Table 14. Mean (±SEM) latency to and duration (s) of electrographic and behavioral responses provoked by stimulation of the left amygdala and the right septal area during the Initial Phase (Experiment 4).

	Lesion	No Lesion
AD Duration (amygdala)	43.98 (8.04)	31.67 (8.27)
AD Duration (septal area)	51.05 (13.88)	42.08 (11.41)
Latency to Clonus for First Stage 5 (amygdala)	8.86 (3.33)	14.94 (4.52)
Duration of Clonus for First Stage 5 (amygdala)	25.29 (4.08)	34.13 (5.01)

Table 15. Mean (±SEM) latency to and duration (s) of electrographic and behavioral responses provoked by stimulation of the right septal area during the Final Phase (Experiment 4).

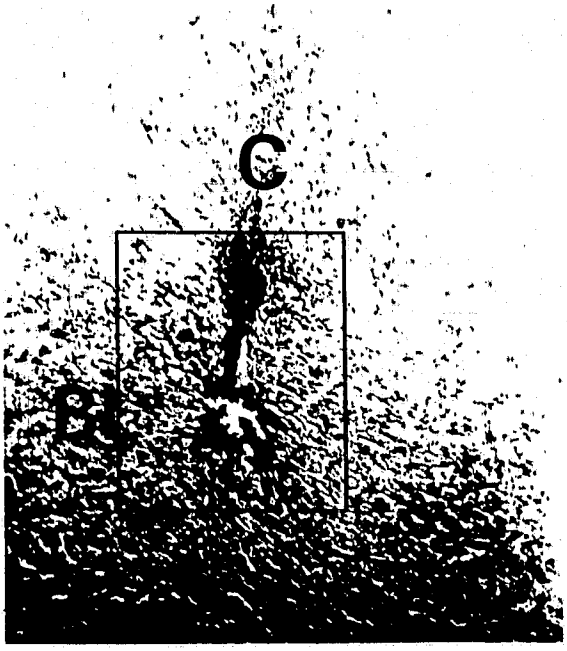
		No Lesion	Lesion
Duration of AD	No Stimulation	57.79 (10.85)	51.00 (5.81)
	Stimulation	47.63 (10.75)	70.37 (13.96)
Latency to Clonus	No Stimulation	38.00 (8.06)	44.29 (5.28)
	Stimulation	40.20 (9.48)	42.27 (8.60)
Duration of Clonus	No Stimulation	32.60 (8.16)	40.71 (5.87)
	Stimulation	48.40 (8.38)	49.57 (7.62)

exhibited a stage 2 seizure. In histological sections, thionin stain was visibly diminished in the immediate vicinity of the tips of amygdaloid electrodes (see Figures 20 and 21). These data indicate that radio-frequency current destroyed cells involved in the induction of AD from the amygdala at intensities of current employed during Experiments 1 - 4.

Discussion

After the establishment of antagonism, the destruction of cells necessary for the induction of AD from the dominant site failed to facilitate Final Phase kindling from the suppressed site. This suggests that the failure of suppressed site seizures to generalize early during Final Phase kindling and perhaps during alternating stimulation does not directly depend on the functional state of the dominant site and is therefore mediated distally.

Figure 20. Photomicrographs (14X) of the left amygdala of individual rats (Experiment 4). Rats were either not exposed to radio-frequency current (top; No Lesion group) or exposed to lesion-producing radio-frequency current delivered between the tips of the amygdaloid electrodes (bottom; Lesion group). Mechanical damage indicates, for both rats, that the tips of the electrodes were situated in the basolateral nucleus (BL), proximal to the central nucleus (C). For views of the boxed areas at higher magnification, see Figure 21.


C
B

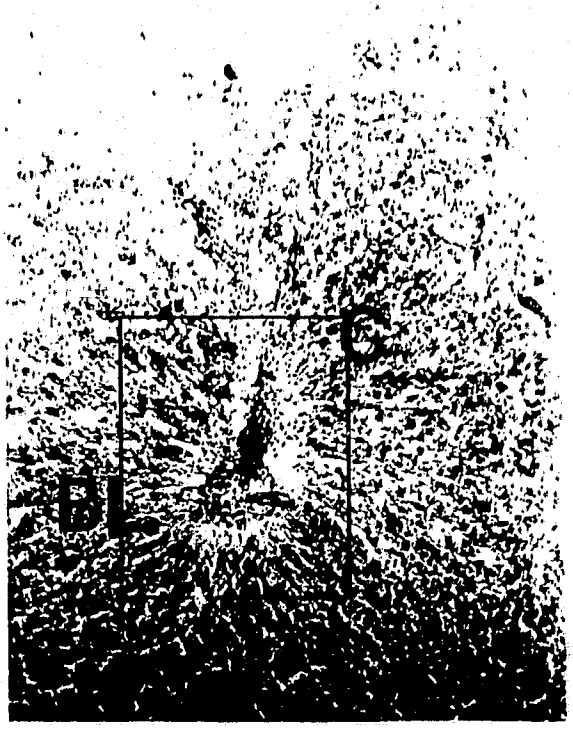
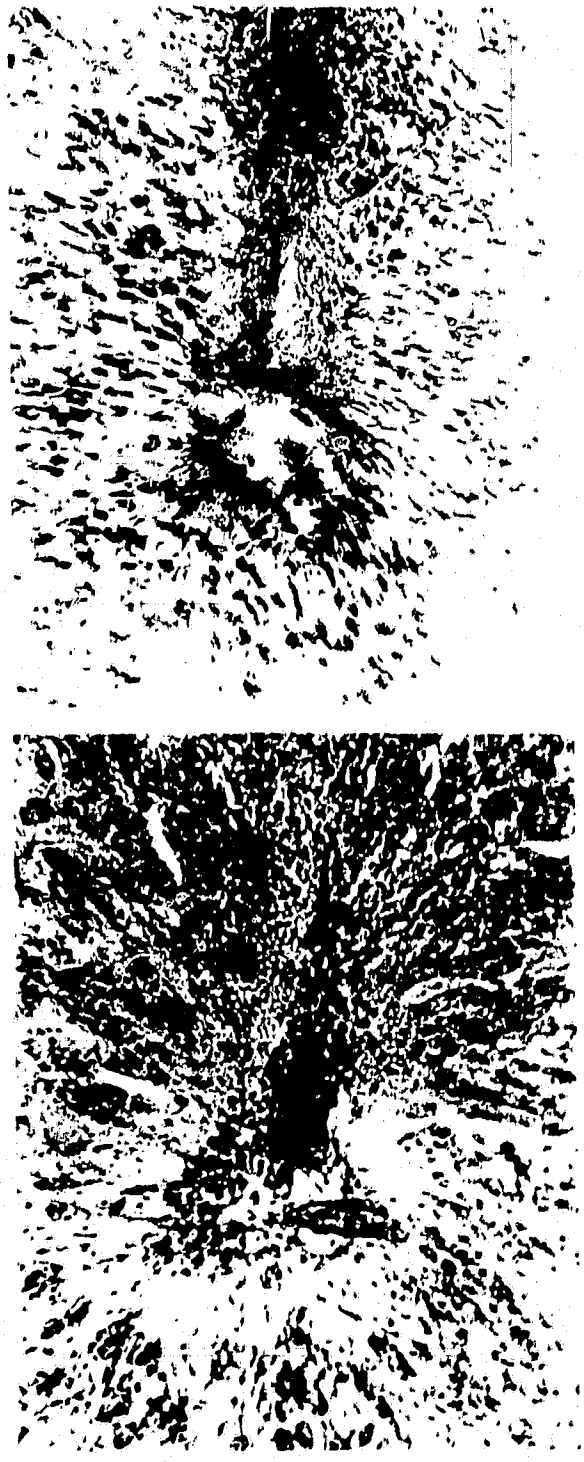

B

Figure 21. Photomicrographs (35X) of amygdaloid tissue surrounding the tips of the amygdaloid electrodes (boxed areas of Figure 20) of individual rats (Experiment 4). Rats were either not exposed to radio-frequency current (top; No Lesion) or exposed to lesion-producing radio-frequency current delivered between the tips of the amygdaloid electrodes (bottom; Lesion). Note the relatively stain-free and roughly circular area surrounding the tips of the electrode in the Lesion rat, indicating a loss of cells. The weakly stained area, which was always evident in brains of Lesion rats but not of No Lesion rats, extended 200 - 400 μm from the tips of the amygdaloid electrodes in all planes.



General Discussion

SITE-SPECIFIC OCCURRENCE OF KINDLING ANTAGONISM

In Experiment 1, antagonism was evident in 18 of 32 rats stimulated in the amygdala and septal area. Absolute and relative antagonism were expressed by approximately equal numbers of these rats. I found that the probability of observing antagonism between the amygdala and septal area diminished when electrodes were implanted ipsilaterally and the amygdala was the first site stimulated; all rats in Experiments 2 - 4 that were stimulated alternately in the left amygdala and right septal area demonstrated antagonism. This suggests that antagonism depends, under certain circumstances, on the hemispheric relation of the implanted electrodes. The observed hemispheric influence on the expression of antagonism between the amygdala and septal area is also interesting in light of the recent report of Haas et al. (1992), in which antagonism was present in 7 of 10 rats stimulated in the amygdala and ipsilateral dorsal hippocampus. It is unfortunate, however, that Haas et al. did not indicate whether the expression of antagonism by these rats was dependent upon the order in which the sites received initial stimulations.

In rats with amygdaloid and septal electrodes implanted in the same hemisphere, I observed antagonism only when initial stimulation was delivered to the septal area. In Experiment 1, I varied the hemisphere into which the septal electrodes were implanted, but held constant the

amygdaloid site in the left hemisphere. It is therefore possible that the critical interaction between the hemispheric relation of electrodes and the site of initial stimulation occurs only when the amygdaloid electrode is situated in the left hemisphere. Other hemisphere-specific behavioral effects related to kindling, such as state-dependent learning, have been reported (Stokes & McIntyre, 1981; Stokes & McIntyre, 1985). In any event, my data indicate that consequences of initial stimulations may contribute to the ultimate behavioral outcome of the entire stimulation procedure. The consequences could take the form of altered synaptic function, as it is well documented that high-frequency stimulation of limbic structures can produce persistent increases or decreases in the functional efficacy of homosynaptic or heterosynaptic connections (e.g., Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Bradler & Barrionuevo, 1989). Moreover, the changes can arise in either the absence (e.g., long-term potentiation) or presence (e.g., kindling-induced potentiation) of AD. In either case, changes in synaptic function that were perhaps incompatible with the expression of antagonism may in fact have occurred during determination of ADT, at which point trains of stimulation were delivered both below and at the ADT. Relations between the development of antagonism and phenomena such as long-term potentiation and kindling-induced potentiation remain to be evaluated systematically.

Also in Experiment 1, the expression of antagonism between the amygdala and septal area (see Appendix D for an additional discussion of the evidence for antagonism between these two sites) was related to rates of growth in duration of AD associated with repeated stimulation of the amygdala: Rats expressing antagonism demonstrated slower growth of amygdaloid AD than did rats failing to express antagonism. The finding is perhaps counterintuitive in that slower growth of amygdaloid AD was associated with a heightened probability that the amygdala would establish dominance over the septal area. However, a causal role of mechanisms responsible for growth of amygdaloid AD in the expression of antagonism has not been established, and it is possible that distinct mechanisms are involved. Rather, altered growth of AD from the amygdala may be secondary to changes in neuronal function responsible for antagonism. This could occur, for example, as increased AD-limiting inhibition within the amygdala, arising as a compensatory response to enhanced excitatory connections between the amygdala and the brainstem. Because circuitry of the brainstem has been implicated in the expression of generalized kindled seizures (Browning, 1985; Burnham, 1978; Burnham, 1985; Burnham & Browning, 1987; Wada & Sato, 1974; Wada & Sato, 1975) as well as in the development of antagonism (Applegate & Burchfiel, 1990; Applegate et al., 1986; Applegate et al., 1987; Burchfiel et al., 1986), the potentially offsetting effects could account for my failure to observe differing rates of

development of clinical indicators as a function of antagonism: Both stage 2 and stage 5 seizures occurred after similar numbers of amygdaloid ADs in rats expressing antagonism and in rats failing to do so. Such a view is also consistent with my finding that antagonism was predicted by shorter durations of bilateral forelimb clonus during the first amygdala-generated stage 5 seizure.

On the other hand, the hypothesis that antagonism involves increased intraamygdaloid inhibition and excitatory amygdalofugal coupling implies that rats displaying antagonism would tend to show a net reduction of growth of AD from the amygdala. It is equally plausible, based on the results of Experiment 1, that the observed disparity in growth of amygdaloid AD reflected a net increase in rats failing to express antagonism. The results of Experiments 2 and 3 are consistent with this view. Growth rates of amygdaloid AD were similar in Single Site and Dual Site rats, the latter of which expressed antagonism in all cases. This suggests that aspects of brain function that influence the development of electrographic and behavioral seizures produced by stimulation of the amygdala are similar in Single Site and Dual Site (amygdala and septal area) rats, provided that antagonism is expressed by the latter rats.

Antagonism was also displayed by rats in which one or both electrodes were situated in nonlimbic structures. Specifically, the septal area typically exerted dominance over the splenium of the corpus callosum.

Rats with electrodes implanted in the amygdala and the cingulate cortex also displayed antagonism, with the cingulate cortex being dominant in all cases. In other rats, the caudate nucleus was dominant over both the occipital cortex and the septal area, and the occipital cortex was dominant over the septal area.

To my knowledge, this constitutes the first report in which an extraamygdaloid site (cingulate cortex) suppressed the amygdala. Burchfiel et al. (1982b) have speculated that sites that more readily establish "privileged" connections with a central convulsive substrate will become dominant. Within this framework, my results, along with those of Burchfiel et al. (1982b), who observed consistent dominance of the amygdala over the entorhinal cortex, suggest that the amygdala, among limbic sites, establishes the epileptogenic connections with relative rapidity. Support for this contention stems from the single site kindling procedure, which reveals that sites within the amygdala-pyriform region support rapid kindling relative to many other limbic sites (e.g., Goddard et al., 1969; McNamara, Byrne, Dashieff, & Fitz, 1980; Racine, 1978). My demonstration of dominance of cortical and striatal sites over callosal and limbic sites, including the amygdala, is therefore consistent with data indicating that convulsive seizures can be observed following very few AD-provoking stimulations of either the cortex or the striatum (e.g., Burnham, 1978; Seidel & Corcoran, 1986; Saucier & Corcoran, 1992; see also Figures 9 and 10). It is also

possible that the suppression of other sites by the striatum and perhaps the cortex involves seizure-independent anticonvulsant effects associated with electrical stimulation. Considerable evidence indicates that activation of the caudate nucleus, for example, inhibits seizures induced by a variety of methods, including the application of electrical stimulation to the amygdala (Cavalheiro, Bortolotto, & Turski, 1987; Cavalheiro & Turski, 1986) and alumina to the cortex (Oakley & Ojemann, 1982). The latter of these findings may be particularly relevant to the observed suppression of the occipital cortex by the caudate nucleus. These speculations must be met with some degree of scepticism, however, as they are based on isolated observations made on relatively few rats (i.e., 1 - 3).

Burchfiel and Applegate have typically reported high probabilities (approximately 80%) of obtaining antagonism with electrodes implanted in the septal area and the entorhinal cortex. It is somewhat surprising, therefore, that I failed to obtain antagonism from rats stimulated in the septal area and either the entorhinal cortex or the dorsal hippocampal commissure, through which the principal efferents of the entorhinal cortex pass as the perforant path (Hjorth-Simonsen, 1972). My failure to observe antagonism between these limbic sites may be related to patterns of seizure development obtained from the amygdala and septal area. I found that the probability of observing antagonism between the amygdala and septal area diminished when electrodes were implanted ipsilaterally and the amygdala

was the first site stimulated, suggesting that antagonism depends, under certain circumstances, on the hemispheric relation of the implanted electrodes. This may be relevant to my failure to obtain antagonism between the septal area and the entorhinal cortex, for example, because all such electrode pairs were situated in the left hemisphere, although I varied the initial site of stimulation. On the other hand, it is possible that some specific properties of the left septal area or its connections with other regions preclude or limit its involvement in antagonism. The latter explanation is compatible with my findings that bilateral septal stimulation invariably failed to elicit antagonism. However, antagonism was displayed by a substantial number of rats stimulated in the left septal area and ipsilaterally in the splenium of the corpus callosum, which argues against a selective inability of the left septal area to participate in antagonism, at least as the dominant site.

Another factor that may have contributed to my inability to obtain antagonism from particular paired sites is the strictness of my operational definition of antagonism: The expression of 6 consecutive stage 5 seizures generated from one site, with the other site supporting seizures of a severity no greater than stage 3 at any point during the stimulation procedure. This differs somewhat from the criteria used by Burchfiel and Applegate, who apparently allowed for the sporadic expression of stage 4 or 5 seizures from the suppressed site prior to the 6 consecutive stage 5 seizures provoked

from the dominant site (see Burchfiel, Serpa, & Duffy, 1982a). The relaxation of my criteria to this degree, however, does not markedly elevate the incidence of antagonism obtained from any of the paired sites, particularly those from which antagonism, by my original criteria, was never obtained: Amygdala and ipsilateral septal area from 4/15 to 5/15, septal area and splenium of the corpus callosum from 6/8 to 8/8, septal area and entorhinal cortex from 0/5 to 1/5, septal area and dorsal hippocampal commissure from 0/10 to 1/10, and septal area and subiculum/presubiculum from 0/4 to 1/4.

Methodological factors may also account for my site-specific failure to observe antagonism. Burchfiel and Applegate used Sprague-Dawley rats, as opposed to my use of hooded rats: Thus, antagonism may be sensitive to strain differences. Moreover, they delivered monophasic pulses at a rate of 100/sec, as opposed to my use of biphasic pulses at 60/sec: Thus, properties of the stimulation trains unrelated to epileptogenesis may have been involved. Finally, Burchfiel and Applegate applied trains of stimulation many times daily (e.g., Burchfiel et al., 1982a), as opposed to my use of once-daily stimulation. In light of ample evidence that seizure sensitivity decreases shortly after the induction of kindled seizures (Goddard et al., 1969; Mucha & Pinel, 1977; Racine et al., 1973), it is possible that postictal refractoriness, associated with successive short-interval stimulations, contributed to the high incidence of antagonism typically reported by

Burchfiel and Applegate (see below). Note that Burchfiel and Applegate (1990) have stated that antagonism develops with somewhat protracted interstimulus intervals (e.g., 4 days), suggesting that this explanation is incorrect, although data to substantiate this statement have not been published. Finally, Burchfiel et al. (1982a) have observed that antagonism develops between the lateral septal area and the dorsal but not the ventral entorhinal cortices. This prompted the speculation that antagonism arises when the hippocampal terminal fields of the stimulated septal and entorhinal neurons do not overlap. Within this framework, it is possible that septal and entorhinal cells directly activated by my electrodes converged within the hippocampus.

STIMULUS-DEPENDENCY OF FINAL PHASE KINDLING

Burchfiel and Applegate believe that the progressive exacerbation of seizures provoked by stimulation of the suppressed site, following the termination of alternating stimulation, reflects the resumption of kindling from the point at which it was previously arrested. The expression of generalized seizures therefore depends on the number of ADs provoked from the suppressed site rather than on a time-locked decay of seizure inhibition. Perhaps consistent with this view, I observed that Final Phase kindling from the septal area (suppressed site) was accelerated, but not significantly, by a prolonged (30-d) stimulation-free period, which followed the Initial Phase

(Experiment 2). This supports the view of Burchfiel and Applegate regarding the temporally independent generalization of seizures provoked from the suppressed site following the termination of alternating stimulation.

The results of Experiment 2 concerning the temporal characteristics of Final Phase kindling are not completely unambiguous, however. Although not revealed statistically, Dual Site rats in the Delay group demonstrated a pronounced tendency towards facilitated Final Phase kindling, requiring only half the number of septal ADs required by Dual Site rats in the No Delay group for the development of a generalized seizure. Final Phase kindling may therefore be temporally dependent, but statistical power was not sufficient for the demonstration of the time-dependency. Burchfiel and Applegate (1990[22]) have also commented, based on unpublished data, that slight facilitation of postantagonism (Final Phase) kindling from the suppressed site occurs with the interposition of a 2 - 3-wk stimulation-free period. In Experiment 4, a 1-wk stimulation-free period occurred between Initial and Final Phases, whereafter Final Phase kindling of Dual Site control rats (not lesioned) progressed at rates comparable to those displayed by Dual Site rats in the No Delay group in Experiment 2 (compare Figures 14 and 19). This weakly suggests that a 1-wk stimulation-free period is not sufficient to reveal any time-dependence of Final Phase kindling. On the basis of these

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[22]See transcript of Discussion of Dr. Burchfiel's presentation (pp. 109 - 110).

findings, one might predict that time-dependent facilitations of Final Phase kindling, if reliable, would become evident between 1 and 2 wk following the termination of the Initial Phase. Should this be the case, it is noteworthy that only 1 of 8 Dual Site rats in the Delay group exhibited a stage 5 seizure after fewer than 4 (i.e., 2) suppressed site ADs during the Final Phase. The remainder required a substantial number of ADs (range = 4 - 11), indicating that a 30-d stimulation-free period following the Initial Phase was not sufficient to completely abolish the apparent stimulus-dependency of Final Phase kindling. It remains to be determined whether such a facilitation of Final Phase kindling would be maximal with 30 stimulation-free days.

The continuing possibility that antagonism and Final Phase kindling involve a temporally dependent component requires further scrutiny, the outcome of which may bear important implications for the hypotheses of Burchfiel and Applegate. As noted previously, Burchfiel and Applegate (1989; 1990) have referred to unpublished data indicating that antagonism develops with interstimulus intervals as long as 4 d. However, given modest evidence that 1 to 2 wk without stimulation facilitates Final Phase kindling from the suppressed site, one might argue that antagonism would fail to develop with interstimulus intervals longer than 1 wk. Thus, the use of a 4-d interstimulus interval by Burchfiel and Applegate may not have been sufficient to demonstrate a time-dependent failure of antagonism, assuming

that time-dependent mechanisms involved in the establishment and maintenance of antagonism are unitary.

TRANSFER AND FINAL PHASE KINDLING

Also in Experiment 2, Dual Site rats demonstrated more rapid Final Phase kindling than did Single Site rats. This raises important questions concerning the potential contribution of mechanisms of transfer to Final Phase kindling after alternating stimulation[23]. As noted in the Introduction, transfer is the facilitation of kindling from one (secondary) site as a consequence of prior kindling from another (primary site; Burnham, 1976; Goddard et al., 1969; McIntyre, 1980; McIntyre & Goddard, 1973; Wada & Osawa, 1976). One possibility, apparently favored by Burchfiel and Applegate (1989; 1990), is that transfer mechanisms do not contribute to Final Phase kindling from the suppressed site. They have presented data indicating that rats develop stage 5 seizures after similar numbers of stimulations, whether delivered to the suppressed site (during Final Phase kindling) or the corresponding site in behaviorally matched controls. Thus,

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[23]I base my interpretations of the results of Experiment 2 on the assumption that transfer influenced Final Phase kindling in Single Site rats, although I did not run further control rats that were not stimulated during the Initial Phase. However, it is noteworthy that whereas Final Phase kindling in Single Site rats required fewer than 19 septal ADs in Experiment 2, approximately 28 ADs were required by rats in Experiment 4 that were not stimulated during the Initial Phase. This suggests that Single Site rats indeed displayed transfer.

they claim that Final Phase kindling in rats previously expressing absolute antagonism is identical to single site kindling in naive rats. I failed to confirm these findings in Experiment 4. Rats in the No Stimulation group (not stimulated during the Initial Phase) required approximately 28 septal ADs for the expression of a generalized seizure during the Final Phase; rats in the Dual Site group that expressed absolute antagonism required approximately 17 ADs[24]. It is therefore possible in Dual Site rats that: Transfer mechanisms do not contribute to Final Phase kindling from the septal area (suppressed site) but other more effective mechanisms do; transfer mechanisms contribute and are enhanced by some aspect of alternating stimulation; transfer mechanisms contribute additively with other distinct mechanisms.

My data do not clearly substantiate any of the foregoing explanations of the mechanisms contributing to the development of seizures observed in Dual Site rats during Final Phase kindling from the suppressed site. It is noteworthy that septal AD in Dual Site rats increased during the Initial Phase of all experiments. At the end of the Initial Phase and at the onset of the Final Phase, Dual Site rats demonstrated considerably longer AD than at the

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[24]Note, however, that only 2 Dual Site rats demonstrated absolute antagonism in Experiment 4, with Final Phase kindling in these rats requiring 7 and 26 ADs. Experiment 4 therefore may not constitute an adequate assessment of the observations of Burchfiel and Applegate that similar rates of kindling are displayed by naive rats and rats expressing absolute antagonism.

onset of the Initial Phase. Single Site rats (Experiment 2), by contrast, demonstrated septal AD at the onset of the Final Phase that was similar in duration to that exhibited by Dual Site rats at the onset of the Initial Phase. Thus, the discrepant rates of Final Phase kindling attributable to the Site of stimulation employed during the Initial Phase (single site vs dual site) may reflect facilitating actions of either growing septal AD or mechanisms mediating the growth. Insofar as the growth of septal AD reflects mechanisms of kindling, the results of Experiment 2 are consistent with the view of Burchfiel and Applegate that suppressed site kindling progresses to an intermediate stage during alternating stimulation.

In Experiment 3, rates of Final Phase kindling from the right amygdala, which was not stimulated during the Initial Phase, did not differ between Single Site and Dual Site rats. Final Phase kindling progressed significantly faster than Initial Phase kindling, however, suggesting that equivalent transfer occurred in Dual Site and Single Site rats. On the assumption that transfer mechanisms acted in both groups of rats to an identical degree, it appears that the facilitation of Final Phase kindling induced by alternating stimulation (Experiment 2), beyond that attributable to transfer, is site-specific. The septal area, perhaps by virtue of its role as the suppressed site, may by uniquely vulnerable to the additional facilitation; the right amygdala, perhaps by virtue of its commissural connections with the dominant site, may be uniquely invulnerable. In any case, the results do not

definitively indicate that the quantitatively similar facilitations of Final Phase kindling from the naive right amygdala produced by alternating stimulation and single site kindling (Initial Phase) involve identical mechanisms.

THE NATURE OF KINDLING ANTAGONISM

CRITICAL SITES

The sites at which the inhibition that is crucial to antagonism acts are also unknown. Consistent with previous reports of Burchfiel and Applegate (1989; 1990), Experiment 1 revealed that antagonism does not reflect inhibition of AD at the suppressed site. In rats stimulated in the amygdala and septal area, initial septal AD was not predictive of later expression of antagonism. Moreover, the growth of septal AD was similar in rats expressing or failing to express antagonism (Experiment 1). This indicates that the failure of septal AD to secondarily generalize during alternating stimulation is mediated distally to the suppressed site.

Burchfiel and Applegate (1989; 1990) have suggested that the propagation of AD from the site of stimulation to the amygdala-pyriform region is crucial to kindling and hence to generalized kindled seizures. Specifically, the amygdala-pyriform region mediates the transition between the first and second phases of kindling and thus allows the expression of partial and ultimately generalized seizures (see Introduction). Although sophisticated objective measures were not taken (e.g., coherence analysis),

inspection of the raw EEG suggested that propagation of AD from the septal area to the amygdala did not vary as a function of either the expression of antagonism (Experiment 1) or the type of antagonism expressed (absolute vs relative; Experiments 1 - 4). This indicates that antagonism does not involve a failure of propagation of AD from the suppressed site to at least some portion of the amygdala-pyriform region, corresponding to the dominant site. Because periictal EEG was not sampled from multiple amygdala-pyriform sites in individual rats[25], it is not clear whether propagation of septal AD to amygdala-pyriform circuitry distal to the dominant site differed among rats expressing absolute, relative, or no antagonism, as might be predicted by Burchfiel and Applegate.

In principle, it may be impossible to determine whether the dominant site, beyond being the point of initiation of secondarily generalizing AD, contributes to the development of antagonism. Experiment 4 revealed, however, that Final Phase kindling from the suppressed site (septal area) was not influenced by radio-frequency lesions of the dominant site (amygdala) produced at the end of the Initial Phase in Dual Site rats and in yoked unstimulated controls. Thus, my failure to alter Final Phase kindling by lesioning of the dominant site is not attributable to opposing and

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[25]In Experiment 3, EEG was recorded bilaterally in the amygdala. In Dual Site rats, AD propagation from the right septal area to the left and right amygdalae did not vary as a function of the type of antagonism expressed (absolute vs relative). Note that only 2 of 7 Dual Site rats expressed absolute antagonism.

offsetting effects of damage to the dominant site and additional amygdaloid tissue. It appears, therefore, that Final Phase kindling from the suppressed site is not influenced by either tonic or periictal output from the dominant site after the termination of the Initial Phase: This is similar to transfer kindling from a secondary site, which is not affected by primary site lesions (Racine, 1972b). This interpretation also assumes, however, that my application of radio-frequency current after the Initial Phase did not spare some dominant site neurons that, while necessary for the both the expression of antagonism and Final Phase kindling, were not sufficient for the elicitation of AD from the dominant site. The complete resolution of this issue may require the use of lesions considerably larger than those employed in Experiment 4.

Based on the foregoing, it appears that the nomenclature of kindling antagonism may promote misconceptions regarding the anatomy of neural mechanisms responsible for the development and maintenance of antagonism. The classification of sites as dominant and suppressed may imply that one site directly or indirectly inhibits intrinsic mechanisms of another. However, as suggested above, it may be that dominant and suppressed sites are merely permissive conduits through which inhibition, acting at some unknown locus, is revealed behaviorally.

MECHANISMS UNDERLYING KINDLING ANTAGONISM

Several results of this study are consistent with the hypothesis of Burchfiel and Applegate that antagonism reflects the arrest of kindling from the suppressed site. First, I have confirmed that the pattern of seizure development, operationally defined as antagonism, arises during and is maintained by alternating stimulation delivered to paired limbic and nonlimbic forebrain sites. This indicates, along with previous reports of Haas et al. (1990; 1992), that antagonism occurs reliably and does not depend on idiosyncratic procedural variables unique to the laboratory of Burchfiel and Applegate. Second, I found that Final Phase kindling from the suppressed site remained substantially stimulus-dependent even following a prolonged stimulation-free period (30 d). This suggests that the progressive exacerbation of seizures provoked from the suppressed site during the Final Phase reflects kindling rather than the spontaneous decay of short-lived (< 30 d) inhibition. Third, the septal area (suppressed site) supported more rapid Final Phase kindling in Dual Site rats than in Single Site rats. This suggests the possibility that septal stimulation, during the establishment of antagonism, results in some kindling. Congruent with this suggestion is the observation that AD of the suppressed site grew during alternating stimulation in Dual Site rats. Last, Final Phase kindling from the septal area was not altered by the destruction of cells of the dominant site. Final Phase kindling is therefore not influenced by either tonic or periictal output from

the dominant site (amygdala) during the Final Phase, just as rates of transfer kindling from a secondary site are not dependent on such output from a primary site (Racine, 1972b).

Mechanisms critical to both dominant site kindling during alternating stimulation and single site kindling may be responsible for the arrest of kindling that appears to be associated with antagonism. This possibility may be somewhat remote, however, as kindling reflects a permanent increase in seizure sensitivity (Goddard et al., 1969; Homan & Goodman, 1988). Therefore, if antagonism depends on mechanisms inherent to kindling, one might predict that the suppressed site should remain so for as long as the dominant site remains sensitive to the epileptic effects of electrical stimulation (i.e., indefinitely). As demonstrated in Experiments 1 and 2 and also by Burchfiel and colleagues, the suppressed site supports generalized seizures after alternating stimulation has been terminated. This suggests either that antagonism does not depend on mechanisms responsible for the persistent increase in seizure sensitivity associated with kindling from the dominant site, or that generalization of seizures provoked from the suppressed site reflects a loss of sensitivity to the epileptic effects of stimulation of the dominant site (i.e., a reversal of kindling from the dominant site). The results of Experiment 4 suggest that the latter explanation is not likely: After the Final Phase, almost all Dual Site rats in

the No Lesion group exhibited stage 5 seizures in response to stimulation of the dominant site.

It is clear that the course of Final Phase kindling from the suppressed site is largely independent of the decay of transient inhibition of seizures. Final Phase kindling is therefore stimulus-dependent in two respects. Secondary generalization of suppressed site seizures requires, first, the termination of stimulation of the dominant site and, second, the continuation of stimulation of the suppressed site. This suggests the possibility that antagonism involves both transient effects of seizures provoked from the dominant site and persistent effects of seizures provoked from the suppressed site. More specifically, recurrent transient consequences of seizures provoked from the dominant site could prevent AD propagated from the suppressed site from effecting the changes necessary to kindling. By this model, the arrest of kindling from the suppressed site would persist for as long as stimulation of the dominant site continues. With the termination of stimulation of the dominant site, however, AD provoked from the suppressed site would be permitted to effect critical alterations to previously unchanged circuits, thus allowing full kindling from the suppressed site. This model does not necessitate that circuits of the dominant site or elsewhere directly inhibit of AD at the suppressed site. Rather, I propose that transient events prevent the transynaptic expression of specific

enduring functional consequences of seizures provoked from the suppressed site.

The transient events that might subserve an arrest of kindling from the suppressed site during alternating stimulation may be related to inhibitory effects of seizures revealed by other patterns of stimulation. Consistent with this view, Haas et al. (1990; 1992) observed antagonism in adult rats stimulated alternately in the amygdala and dorsal hippocampus but not in infants, which do not demonstrate prolonged postictal refractoriness during single site kindling (Moshe, Albala, Ackermann, & Engel, 1983). With the repeated application of suprathreshold stimulation to a single site at short intervals (e.g., minutes), seizure generalization eventually fails (Mucha & Pinel, 1977). Mucha and Pinel (1977) also found, following the induction of several generalized seizures provoked at 1.5-h intervals, that subsequent suprathreshold stimulations delivered at 24-h intervals produced only partial seizures, suggesting that a longer-lasting postictal inhibitory process might also have occurred. Although the methods of revealing these forms of inhibition involve short-interval stimulations delivered via a single electrode, it is possible that related forms of inhibition emerge during alternating stimulation at longer intervals and thus contribute to the arrest of suppressed site kindling and hence antagonism. A further form of transient seizure-dependent inhibition has also been demonstrated between sites of stimulation. Goddard et al. (1969) and McIntyre and Goddard (1973)

reported that, following secondary site kindling, several stimulations of the primary site were required to elicit generalized seizures (kindling interference). The imposition of a 2-wk stimulation-free period following secondary site kindling abolished interference, indicating that the inhibitory influence of secondary site seizures on primary site seizures was transient (McIntyre & Goddard, 1973).

Much of the foregoing implies that the transient events hypothesized to participate in the arrest of suppressed site kindling are invoked exclusively by seizures provoked from the dominant site. As noted above, however, seizures generated from one site can inhibit later seizures provoked from the same site. This suggests the possibility that a particular site contributes, to some degree, to its own suppression (autoinhibition). It is clear that suppressed site seizures, if acting in this capacity, must interact with process related to the induction of seizures from the dominant site. Otherwise, one could expect that septal stimulation should fail to produce generalized seizures, even in the absence of seizures provoked from the dominant site. Experiment 4 confirms previous reports stating that this is not the case, as all rats stimulated only in the septal area displayed generalized seizures. The autoinhibitory influence of septal seizures could thus be manifest as the relatively slow rates of single site kindling typically supported by this site (e.g., Goddard et al., 1969). Interacting with inhibitory effects of generalized or nongeneralized seizures provoked from

the amygdala, autoinhibition may prevent full septal kindling and thus establish antagonism. A further possibility that must be entertained is that seizure-independent effects of stimulation contribute to transient inhibitory events that may be crucial to antagonism.

CONCLUSIONS

I have demonstrated that patterns of seizure development defined as antagonism can develop as a consequence of alternating stimulation of paired limbic and nonlimbic forebrain sites, confirming and extending numerous previous reports of Burchfiel and Applegate. Utilizing alternating stimulation of the left amygdala and right septal area, I also provided evidence consistent with the view that antagonism involves an arrest of kindling from the suppressed site. Antagonism does not, therefore, merely reflect the transient inhibition of suppressed site seizures, although I have proposed that transient consequences of alternating stimulation may block distal epileptogenic effects of seizures provoked from the suppressed site, thus arresting kindling from that site.

What is not at all clear from the data obtained in Experiments 1 - 4 is whether the behavioral stages at which suppressed site kindling appears to undergo arrest, reveal the architecture of single site kindling, as proposed by Burchfiel and Applegate (1989; 1990). Other data indeed suggest that neural mechanisms that prevent secondary generalization of suppressed site

AD and those that inhibit single site kindling are unitary: Both kindling and the expression of antagonism are influenced by central noradrenaline. 6-OHDA, which destroys noradrenergic neurons, and α_2 receptor antagonists such as idazoxan facilitate kindling (e.g., Corcoran & Mason, 1980; Gellman et al., 1987). Burchfiel and colleagues have observed that antagonism does not occur in rats previously receiving 6-OHDA (e.g., Applegate et al., 1986; Applegate et al., 1987; Burchfiel et al., 1986). T.H. Gilbert, Corcoran, and I (unpublished) recently observed that the administration of idazoxan (1 mg/kg) prior to amygdaloid and septal stimulation prevents the induction of antagonism and abolishes established antagonism. On the other hand, it is possible that qualitatively distinct noradrenergic mechanisms mediate kindling and antagonism, suggesting that antagonism and Final Phase kindling from the suppressed site, respectively, involve the establishment and decay of a long-lasting (> 30 d) form of seizure inhibition that is perhaps uniquely invoked by alternating stimulation but is not apparently dependent on the dominant site. This being the case, the investigation of antagonism may reveal little about kindling, per se. In any event, further research into antagonism is warranted, as it may yet yield valuable insights into mechanisms that promote and oppose secondary generalization of seizures.

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APPENDIX A

Glutamate: Principal Transmitter of Mossy Fibers

Although the terminals of hippocampal mossy fibers contain an exocytotic pool of glutamate (Terrian et al., 1988), the cells release other amino acids (e.g., aspartate) that may also contribute to excitatory neural transmission in this region (de Montigny, Weiss, & Ouellette, 1987). However, immunohistochemical techniques have revealed substantially greater glutamate- and glutaminase- than aspartate- and aspartaminase-like immunoreactivity (Altschuler et al., 1985; Liu, Grandes, Matute, Cu'enod, & Streit, 1989). More recently, Terrian, Gannon, and Rea (1990) demonstrated that among 18 amino acids, including aspartate, measurably released by the mossy fibers, the application of K^+ selectively increased Ca^{++}-dependent release of glutamate. Collectively, these data indicate that glutamate is probably the principal excitatory amino acid transmitter of the hippocampal mossy fibers.

APPENDIX B

Limbic-type vs nonlimbic-type kindled seizures

It is noteworthy that considerable research indicates that early electrographic and motoric seizures provoked during kindling from nonlimbic sites differ from those associated with kindling from limbic sites (e.g., Burnham, 1978; Cain, 1982; Racine, 1975; Saucier & Corcoran, 1992, Seidel & Corcoran, 1991). An interesting feature of nonlimbic kindling, however, is the eventual expression of limbic-type clonic components, which correspond to rearing/falling episodes typical of stage 5 seizures, as described by Racine (1972b). I have taken the liberty of classifying nonlimbic-type seizures according to stages 1 - 4 of Racine (1972b; e.g., Figures 9 and 10) in order to maintain the architecture of kindling outlined by Burchfiel and Applegate (1989; 1990). I fully recognize that more detailed investigations of alternating stimulation of nonlimbic sites should allow for the potentially different architectures of kindling involving limbic and nonlimbic sites.

APPENDIX C

Regional factors and kindling antagonism

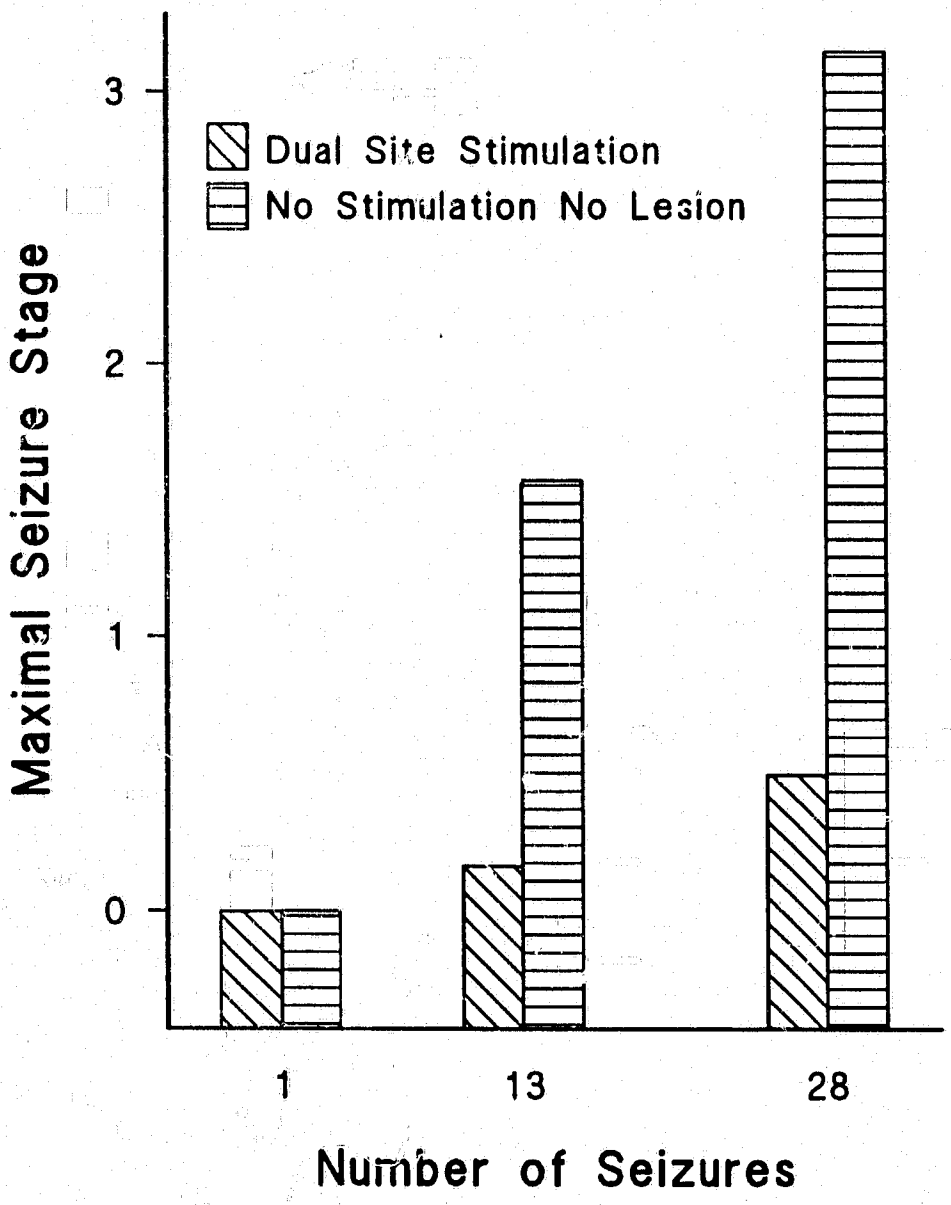
Le Gal La Salle (1981) reported that regional factors influence kindling rates supported by the amygdala. Specifically, the central nucleus supports somewhat more rapid kindling than does either the basolateral nucleus or the medial nucleus. This observation may be relevant to the results presented in Experiments 1 - 4, as Figures 2, 13, 16, and 18 indicate considerable variation in placements of amygdaloid as well as septal stimulating electrodes. The expression of antagonism, however, was chiefly influenced by the gross distribution of the septal electrode (left vs right hemisphere) rather than by subtle shifts of the septal or amygdaloid electrodes within hemispheres (Experiment 1). Moreover, the type of antagonism expressed (absolute vs relative) did not appear to vary as a function of rates of amygdaloid kindling during the initial phases of Experiments 1 - 4, and differences in the rostrocaudal, dorsoventral, mediolateral distributions of amygdaloid electrodes as a function of treatment group are not readily apparent. This also appears to be true for septal electrodes, although region-dependent variations in kindling rates supported by this structure have not been assessed. In any event, I advise those interested in the study of antagonism to remain sensitive to potential influences of regional variation, particularly when antagonism occurs less reliably than is desired.

APPENDIX D

Evidence for antagonism of septal kindling

The demonstration that kindling antagonism occurs between the amygdala and septal area is complicated by the fact that single-site kindling from the septal area is very slow; thus septal kindling provides a somewhat inappropriate baseline against which to demonstrate antagonism. In Experiment 2 I provided some data to substantiate the claim that the amygdala does establish dominance over the septal area. Figure 22 shows additional evidence for the establishment of kindling antagonism between the amygdala and septal area: Here I have plotted the profiles of septal kindling in the No Lesion No Stimulation group from Experiment 4 and in the Dual Site Stimulation group from Experiment 2. Note the divergence in rate of kindling between the two groups, with kindling proceeding as expected in the No Lesion No Stimulation group and kindling antagonism being evident in the Dual Site Stimulation group.

Figure 22. Maximal seizure stages expressed either during or prior to the first, 13th, and 28th seizures provoked from the septal area. Groups of rats were the No Lesion No Stimulation group of Experiment 4 (kindled from the septal area: Final Phase) and the Dual Site Stimulation group of Experiment 2 (during alternating stimulation).


Maximal Seizure Stage
3
2
1
0
Dual Site Stimulation
No Stimulation No Lesion
1
13
28
Number of Seizures