THE ROLE OF NOREPINEPHRINE IN AMYGDALOID KINDLING: EFFECTS OF DSP-4 INDUCED DEPLETION AND INTRACEREBROVENTRICULAR NOREPINEPHRINE INDUCED REPLETION

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THE ROLE OF NOREPINEPHRINE IN AMYGDALOID KINDLING: EFFECTS OF DSP-4 INDUCED DEPLETION AND INTRACEREBROVENTRICULAR NOREPINEPHRINE INDUCED REPLETION

BY

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ABSTRACT

The role of norepinephrine in the development of amygdaloid kindling in rats was investigated. In the first experiment, pretreatment with the noradrenergic neurotoxin, DSP-4 (63 mg/kg, ip), whose action is reportedly specific to locus coeruleus neurons, markedly accelerated the rate of kindling. DSP-4 treated animals and saline controls required 3.8 and 7.5 after-discharges (ADs) to kindle respectively. Furthermore, DSP-4 treatment lengthened the latency of the onset to clonus during the first stage 5 seizure. Norepinephrine levels measured in the telencephalon were lowered by 45% with DSP-4 treatment, whereas dopamine and 5-hydroxytryptamine levels were unaffected.

In an attempt to test the hypothesis that a tonic presence of norepinephrine serves to inhibit kindling development, rats were treated with DSP-4, and chronically infused (icv) with either norepinephrine (5 ug/.5 ul/hr), or the vehicle, via an osmotic minipump. Telencephalic norepinephrine levels were not raised by the infusion of norepinephrine, and consequently there were no significant differences between the two groups on kindling development. Both infusion groups were combined and compared to those groups in experiment 1. The infused groups had lower norepinephrine levels and kindled faster than the saline group, indicating again that DSP-4 treatment accelerates kindling.

The third experiment represents a second attempt at ascertaining the effects of norepinephrine repletion following DSP-4 treatment on kindling development. Three groups were given DSP-4 treatment and 1 group saline. One DSP-4 group received chronic ventricular infusion of norepinephrine (10 ug/.5 ul/hr -double that in experiment 2) and another the vehicle alone. Once again the infusion of norepinephrine failed to raise norepinephrine levels above the vehicle control; however the infused groups had norepinephrine levels equivalent to the saline (non-infused) control. Despite a marked amount of within group variance, the DSP-4 alone group had a significant reduction of norepinephrine, and all three DSP-4 treated groups kindled significantly faster than the saline group. These results imply that: 1) initial abnormalities induced by DSP-4 were reversed during kindling, 2) the cannulation and/or vehicle infusion procedure itself accelerates kindling, or 3) norepinephrine depletion may not be necessary for kindling acceleration induced by DSP-4 treatment.

In conclusion, this study demonstrates that administration of the noradrenergic neurotoxin DSP-4 consistently accelerates kindling. The methods employed, however, failed to indicate whether this depletion-induced acceleration could be reversed by norepinephrine repletion.

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CHAPTER 1: INTRODUCTION

A central problem in neuroscience concerns the mechanisms underlying neuronal plasticity, the brain's ability to undergo lasting changes as a result of sensory stimulation or as a consequence of damage. Several studies have recently suggested that the widespread neuronal system of catecholaminergic fibers may play a critical role in the development of experimentally-induced plasticity. In a series of experiments Kasamatsu and Pettigrew employed the phenomenon of monocular deprivation in kittens as a model of plasticity to investigate possible noradrenergic modulation. Following monocular deprivation during the critical period (from time of eye opening to three months of age) in kittens, the majority of cells in the visual cortex lose their binocular responsiveness and can be driven only by stimulation of the non-deprived eye. However, animals with 6-hydroxydopamine (6-OHDA, a catecholaminergic neurotoxin) infused locally into the visual cortex no longer display the shift in ocular dominance produced by monocular deprivation (Kasamatsu and Pettigrew, 1979a), unless the area is chronically repleted by infusion with norepinephrine (Kasamatsu et al., 1979b). These findings have been extended to another form of visual deprivation, directional deprivation. If kittens are reared in an environment that continually moves in one direction, then a majority of directionally sensitive cells in the visual cortex will

respond to movement in the direction that the environment moved during rearing. 6-OHDA, infused locally into the cortex prior to the peak of the critical period, reduces the experience-induced shift in the proportion of directionally sensitive cells (Daw et al., 1983). Plastic changes induced by alterations of the normal visual input in the vestibuloocular reflex are also greatly diminished following intracisternally injected 6-OHDA (Kellar and Smith, 1983).

Further, the presence of catecholamines has been proven to be necessary for the acquisition of certain learned responses. Animals depleted of cerebellar norepinephrine by injection of 6-OHDA into the coeruleo-cerebellar pathway, are impaired in their aquisition of a novel locomotor task (Watson and McElligott, 1984), suggesting that cerebellar norepinephrine is strongly associated with the adaptive ability to coordinate and choreograph new motor tasks. Improved maze acquisition through environmentally enriched learning is blocked by pretreatment of newborn rat pups with 6-OHDA (O'Shea et al., 1983). Taken together, these studies suggest that the presence of catecholamines, and specifically, in some cases, norepinephrine, are essential to the alteration of brain function by experiential factors.

The cellular mechanisms underlying noradrenergic effects are unknown. Early studies emphasized the direct inhibitory effects of norepinephrine (see Moore and Bloom,

1979: Reader, 1983); however more recent experiments have shown that norepinephrine, or stimulation of norepinephrine-containing afferents, facilitate both inhibitory and/or excitatory synaptic responses while suppressing spontaneous activity (Woodward et al., 1979). Woodward and colleagues have demonstrated this type of action in the cerebellar cortex, showing that norepinephrine, at levels with little direct inhibitory effect on Purkinje cell spontaneous activity, acts to enhance both excitatory and inhibitory synaptic actions (Freedman et al., 1976, 1977; Moises et al., 1981, 1983). The combined effects of reducing spontaneous activity and enhancing both excitatory and inhibitory synaptically evoked activity result in an overall increase in the signal to noise ratio of neurons, acting as an enabling device which allows cells to respond more briskly to conventional input. Similar effects have been demonstrated in other areas of the CNS (McCall and Aghajanian, 1979; Waterhouse and Woodward, 1980). Either bath application of norepinephrine to hippocampal brain slices (Lacaille and Harley, Brain Research, in press: Stanton and Sarvey, Brain Research, in press), iontophoretic application of norepinephrine to the dentate gyrus (Neuman and Harley, 1983), or glutamate stimulation of the locus coeruleus in the intact animal (Harley and Milway, 1985), potentiates excitatory responses in the dentate gyrus to perforant path stimulation. This enhancement has been

observed to last more than 30 min, providing direct evidence for long-lasting changes induced by norepinephrine.

From these reports, the activation of CNS noradrenergic systems may be postulated to enhance the organism's sensitivity to environmental stimuli, and furthermore to facilitate long-term plastic changes in response to experiential factors. This hypothesis, however, is directly contradicted by the apparent role for norepinephrine in another form of neuronal plasticity called kindling. Kindling refers to the progressive and lasting increase in the ability of subconvulsive electrical stimulation to produce seizures. Continued kindling leads to generalized seizures and ultimately spontaneous seizures (Goddard et al., 1969; Pinel, 1981). Kindling has been proposed as a model of both the pathological process of epilepsy and of more adaptive forms of neural plasticity (e.g., Goddard, 1976). Paradoxically, previous studies have reported that catecholamines have an inhibitory influence on kindling development. Since these reports contradict a unitary role for norepinephrine in the facilitation of neuronal plasticity, they warrant further attention. Since the noradrenergic control of kindling is the focus of this thesis, a brief review of the kindling phenomenon and an overview of research directed at determining the role of norepinephrine in kindling will follow. A brief summary of studies concerning norepinephrine and other seizure models

will also be included.

1.1 The Kindling Model

The process of kindling (Goddard et al., 1969) provides an important animal model for the study of the role of neurotransmitters in the pathogenesis of epilepsy and in neuronal plasticity. Kindling refers to the progressive changes that result from repeated electrical stimulation of a particular anatomical site in the CNS. In the most common procedure for kindling a bipolar electrode is implanted into a susceptible brain region and, after a post-operative recovery period of several days, initially subconvulsive stimulations are applied once daily, for kindling can only be produced when stimulations are distributed over time. An appropriate stimulus would be a one second train of pulses at a frequency of 60 Hz and a current sufficient to evoke an afterdischarge (AD). The development is relatively slow, with seizures progressing from focal motor (partial complex) to generalized convulsive. Kindling stimulation does not produce the secondary characteristics common to other experimental models, such as gliosis, necrosis, denervation, or morphological distortion of cells in the kindled focus (Goddard et al., 1969; Racine et al., 1976).

In amygdaloid kindling, little behavioral response accompanies the first few stimulations, but with repeated stimulations, the animal responds to each stimulus by a

sequence of specific stereotyped motor responses, which outlast the stimulus and coincide with AD duration. These motor responses can be categorized into stages (Racine, 1972b). Movement arrest and repetitive chewing activity (Stage 1) begin to appear early in kindling, with convulsive responses following later. Stage 2 involves head clonus (rhythmic nodding of the head), Stage 3 involves fore-limb clonus, Stage 4 is seizure-driven clonic rearing, and Stage 5 the loss of postural control, or falling. Each stage usually includes those responses defining an earlier stage. Since any subsequent stimulation will produce a class 5 seizure, regardless of the time interval, the change is considered permanent. Once Stage 5 has been reached, the animal is said to be kindled. The behaviors of stages 1 and 2 are comparable to those found in human complex partial (limbic or temporal lobe) seizures, whereas the latter stages are representative of generalized motor seizures (Engel et al., 1978).

The three principal physiological effects of repeated kindling stimulation are a decrease in the threshold current necessary for the production of focal AD, a lengthening of the AD duration, and a spread of the seizure activity to extrafocal brain areas. Thresholds for kindling have not been well defined but it is thought that probably some minimal 'mass' of neural tissue must be activated over an appropriate duration on each trial

(Goddard, 1983).

If the stimulation is strong enough, kindling is possible from almost any area within the forebrain, although it is most readily obtained in the limbic system. The hierarchy of sensitivity (progressing from the least to the most number of AD's required to kindle the animal) of the various sites are as follows: amygdala, globus pallidus, pyriform cortex, olfactory area, anterior neocortex, entorhinal cortex, septal area, preoptic area, caudate putamen, and hippocampus (Goddard et al., 1969). No brainstem nuclei or areas of the cerebellum have shown kindling.

It is important to differentiate kindling, the kindled seizure, and the kindled state from one another. Kindling, the time period during which the kindled seizure is acquired, is a dynamic period when synaptic connectivity and associated mechanisms are presumably undergoing a series of changes. The kindled seizure is characterized by temporary biochemical, electrophysiological, and behavioral manifestations that are a direct result of the recent occurrence of a generalized seizure. The kindled state results from permanent alterations of brain function distinct from those temporary alterations produced by kindled seizures (Peterson and Albertson, 1982).

1.2 Catecholamines in Kindling

1.2.1 Kindling Process

1.2.1.1 Whole brain depletion studies

Initially, it was observed that depletion of catecholamines by 6-OHDA or reserpine treatment reduced the number of stimulations required to kindle rats. Systemic administration of reserpine, which disrupts monoamine uptake into neuronal vesicles, allowing the monoamines to be destroyed by monoamine oxidase (MAO), enhances the kindling rate of rats stimulated in the amygdala (Arnold et al., 1973: Wilkison and Halpern, 1979a), the hippocampus (Araki et al., 1983b), and in the neocortex (Racine et al., 1979). Arnold et al., (1973) found reserpine pretreated rats not only required fewer stimulations to produce a fully developed motor seizure (4.6 vs 10.7), but 50% of the reserpine-treated animals showed much stronger electrographic AD activity following the first stimulation than the control subjects. When pargyline, a monoamine oxidase inhibitor, was administered before reserpine to block monoaminergic depletion, the kindling rate was no longer enhanced (Wilkison and Halpern, 1979a)

Other short term depleters of catecholamines have been employed to study the roles of norepinephrine and dopamine in the kindling process. Alpha-methyl-p-tyrosine (150 mg/kg), which inhibits the synthesis of norepinephrine and dopamine, accelerates the rate of amygdaloid kindling

(Callaghan and Schwark, 1979); yet the same dosage has been shown to depress the development of AD duration (Wilkison and Halpern, 1979a). Pretreatment with alpha-methyl-p-tyrosine (200 mg/kg) did not significantly affect the rate of hippocampal kindling in rats (Araki et al., 1983b). Disulfiram, a catecholamine depleter reported to deplete norepinephrine more selectively than dopamine, also facilitated kindling, and, unlike alpha-methyl-p-tyrosine, accelerated the development of AD duration (Callaghan and Schwark, 1979). It is unclear how both drugs, having opposite effects on AD duration, could facilitate kindling, unless changes in AD duration are not necessary for the acquisition of stage 5 seizures.

Intraventricular administration of 6-OHDA, which produced a mean depletion of greater than 89% of both whole brain norepinephrine and dopamine, increased the kindling rate of rats stimulated in the amygdala (Arnold et al., 1973). These early studies could not specify which catecholamine, norepinephrine or dopamine, was responsible for the facilitation of kindling, and other studies were carried out to determine the relative contribution of each catecholamine. Corcoran et al., (1974) depleted dopamine to a greater degree than norepinephrine by using the monoamine oxidase inhibitor tranylcypromine in combination with intracerebroventricular (icv) 6-OHDA. 6-OHDA treatment alone produced whole brain norepinephrine levels that were

33% of controls and dopamine levels that were 60% of controls. Kindling was completed after a mean of 8.6 stimulations in the 6-OHDA group and 11.0 stimulations in the control group. 6-OHDA plus tranylcypromine treatment produced norepinephrine levels of 30% and dopamine levels of 6% when compared to controls, and the rate of kindling was significantly accelerated to a mean of 4.7 stimulations. Since a significant depletion of dopamine, and not norepinephrine, occurred in the tranylcypromine plus 6-OHDA group versus the 6-OHDA group alone, the authors suggested that the facilitatory effects of 6-OHDA depended on the depletion of both dopamine and norepinephrine, or dopamine alone.

6-OHDA icv treatment, which facilitated the rate of amygdaloid kindling, produced significant norepinephrine depletions in several brain areas (hippocampus, striatum, neocortex, amygdala, and olfactory bulb), but significant dopamine depletions only in the striatum (McIntyre et al., 1979: McIntyre, 1981). If 6-OHDA treatment was preceded by a subcutaneous injection of desmethylimipramine (DMI), a noradrenergic uptake inhibitor, the number of areas depleted of norepinephrine decreased and kindling was no longer facilitated. DMI offered a significant protection for the norepinephrine fibers in all areas but the striatum, when compared to the 6-OHDA alone group; however there was a reliable reduction of norepinephrine in the DMI plus 6-OHDA

group compared to controls in the hippocampus and anterior neocortex (McIntyre et al., 1979). Olfactory bulb dopamine was significantly depleted from controls in the 6-OHDA plus DMI group but not in the 6-OHDA group alone. Finally, in both 6-OHDA groups, striatal dopamine had been significantly depleted when compared to control groups. These results suggest that a critical level of norepinephrine depletion must be present in order to facilitate kindling, but do not rule out a possible interaction between depletion of norepinephrine and depletion of dopamine. When neonatal animals were treated with the same combinations of 6-OHDA and DMI, and kindled as adults, the results were identical as those of adult treated rats (McIntyre et al., 1979). The same treatment applied before hippocampal kindling, also facilitated the rate of kindling development (McIntyre and Edson, 1982).

When Stage 5 convulsions were first provoked, the latency to onset of forelimb clonus (Stage 3) was increased in the 6-OHDA treatment groups over the control groups (McIntyre et al., 1979). A tendency to kindle quickly may necessitate the recapitulation of preclonus motor behavior during kindled seizures. However, the increased latency was still evident after 6 stage 5 seizures. The persistence of the delayed forelimb clonus would presumably rule out the recapitulation theory, since the necessity to recapitulate the early stages should diminish once the overall time spent

in stages 1 and 2 approaches that of untreated animals.

To summarize, reserpine studies first implicated monoamines as possible inhibitors of the kindling process. Corcoran et al., (1974) showed that facilitation of kindling did not occur with a significant depletion of norepinephrine alone, but required the depletion of dopamine to levels below 60% of controls, implying that dopamine was the critical catecholamine underlying the control of kindling rate, either in conjunction with norepinephrine or alone. McIntyre et al., (1979,1981) reported results that supported a different catecholamine hypothesis; that norepinephrine was the critical catecholamine having an inhibitory action on the kindling process, either alone, or in interaction with dopamine. Further studies were undertaken to isolate the respective roles of both catecholamines.

1.2.1.2 Region specific depletion studies

There are two main noradrenergic ascending fiber systems: the dorsal bundle, which arises from cells in the locus coeruleus innervating forebrain areas such as the cortex, hippocampus, amygdala, and septum; and the ventral bundle, a plexus of fiber groups ascending ventrally to innervate mainly the hypothalamus, basal ganglia, and septum (Lindvall and Bjorklund, 1983; O'Donohue et al., 1979). In attempts to define more precisely the role of norepinephrine in the development of kindling, transections of the

ascending noradrenergic pathways have been used to deplete forebrain norepinephrine. Less than half the stimulations required to kindle the amygdala in intact control or sham-operated rats were needed to kindle animals that received complete transections of the ascending noradrenergic pathway (Ehlers et al., 1980). The concentration of norepinephrine in the amygdala as a consequence of the complete transections was reduced by 70%, whereas those animals receiving sham transection had norepinephrine levels reduced by 50%. In the hypothalamus however, complete transections produced an 83% reduction whereas sham-cuts had no effect. This indicates that the sham-cuts probably partially disrupted the dorsal bundle but not the ventral bundle. The amount of depletion, or the destination of the noradrenergic fibers may be critical in the control of kindling development, since a 50% reduction of norepinephrine in the amygdala did not facilitate kindling (sham-cuts), yet an 83% depletion of NE in the hypothalamus and 70% in the amygdala facilitated kindling. Thus, noradrenergic terminals from the ventral bundle, or terminals from the ventral and dorsal bundle together appear to be critical in the control of the kindling process. No assays were taken to determine possible effects on other biogenic amines which also originate in the brain stem.

Electrolytic lesions of the dorsal noradrenergic bundle (DNB) facilitate both hippocampal and amygdaloid

kindling (Araki et al., 1983a). The number of trials required for the establishment of the kindled seizure was significantly shortened in hippocampal kindling following DNB lesions to 11.0 stimulations from 22.5 stimulations (sham operated rats). In amygdaloid kindling, the number of stimulations was significantly reduced to 9.0 stimulations from 15.5 stimulations (shams). The number of AD's required to produce the early stages of kindling (preclonus) were most significantly reduced by DNB lesions. Neither 5-hydroxytryptamine nor dopamine was significantly decreased following these DNB lesions, whereas norepinephrine levels were significantly decreased in the cortex (70%), and to a lesser extent in the amygdala (<50%) and hypothalamus (15%). Lesions of the raphe nuclei, the main source of forebrain 5-hydroxytryptamine, did not facilitate the kindling from either structure. According to these results, and contrary to conclusions drawn from transection of the noradrenergic pathways (Ehlers et al., 1980), norepinephrine originating from axons travelling in the ventral noradrenergic bundle (VNB) does not appear to be critical for the noradrenergic modulation of the kindling process. Unfortunately, one cannot rule out a possible contribution of non-noradrenergic fibers that also course through the area of the lesion.

In the effort to produce more specific lesions of forebrain norepinephrine and dopamine, 6-OHDA has been injected into the ascending noradrenergic or dopaminergic

fiber bundles. This method should spare non-catecholaminergic fibers of passage through the lesion site. Rats with selective depletion of norepinephrine kindled significantly faster than controls and animals with selective depletion of dopamine (Corcoran and Mason, 1980). Ascending noradrenergic bundle lesions produced 96% and 73% norepinephrine depletions in the hippocampus-cortex and hypothalamus respectively, and no significant dopamine depletions in the caudate putamen or the nucleus accumbens. Those animals receiving 6-OHDA into ascending dopaminergic fibers had significant decreases of dopamine in the caudate putamen (65%) and the nucleus accumbens (55%) but no significant depletions of norepinephrine in the hippocampus-cortex or the hypothalamus. Animals with selective norepinephrine depletions displayed generalized seizure activity significantly earlier than the other groups. This is the first instance where it has been clearly demonstrated that depletion of norepinephrine alone will accelerate the kindling process. Since the AD threshold and the first AD duration were not significantly different from controls, and the spread of discharge from the stimulated to the contralateral amygdala was earlier in norepinephrine-depleted animals, the authors concluded that disinhibition of the spread of discharge from the stimulated amygdala underlies the observed potentiation of kindling, measured as both the acceleration of the first clinical sign, and of the

fully generalized seizure. Since dopamine levels were only partially depleted following 6-OHDA treatment into the ascending dopaminergic fibers, a role for dopamine as an inhibitory agent in the kindling process, as suggested by the study of Corcoran et al., (1974), cannot be ruled out.

Facilitation of kindling by 6-OHDA injection into the ascending norepinephrine fibers was found to be independent of the intensity of stimulation (Mohr and Corcoran, 1981), and could be extended to neocortical kindling (Altman and Corcoran, 1983). In the latter study norepinephrine levels were reduced by more than 95% in the hippocampus and cortex.

The increase in the latency to onset of clonus observed by McIntyre et al. (1979), during the first stage 5 seizure, using icv 6-OHDA, was not replicated when 6-OHDA was injected into ascending noradrenergic fibers (Mohr and Corcoran, 1981). Since icv injections of 6-OHDA would be expected to deplete cerebellar and spinal norepinephrine as well as forebrain norepinephrine, it was suggested that the effect on the latency to clonus was due to depletion of norepinephrine at sites other than the forebrain.

It should be pointed out that the relative contribution of the ventral and dorsal noradrenergic bundles was not at issue in those studies employing 6-OHDA into the ascending noradrenergic pathways. Corcoran and Mason (1980) significantly depleted the hippocampus and cortex of

norepinephrine to 4% and the hypothalamus to 27% of controls. Since the majority of norepinephrine fibers in the hypothalamus course through the VNB, the VNB as well as the DNB were significantly lesioned by 6-OHDA treatment.

In an attempt to determine whether the depletion of norepinephrine critical to kindling facilitation lies in the site of stimulation (amygdala) or elsewhere, McIntyre (1980) infused 6-OHDA directly into the amygdala of rats before amygdaloid kindling was initiated. Intra-amygdalar infusion of 6-OHDA into the site to be kindled facilitated the kindling rate, and produced a reduction of norepinephrine by 35 to 40% in the amygdala-pyriform region as compared to vehicle controls. The concentrations of dopamine or 5-hydroxytryptamine were unaltered. Local events (AD threshold and duration) were not altered by norepinephrine depletion. Since the number of bilateral amygdala ADs necessary to elicit kindling was decreased as well, the author suggested that local amygdalar norepinephrine reduction does not affect the production of local seizure, but rather facilitates the development of motor seizures after the AD has propagated bilaterally. Since local norepinephrine depletion had no effect on the latency to clonus, it was suggested that extraamygdaloid norepinephrine was responsible for this effect. Whole brain assays, minus the amygdala-pyriform region, indicated no effect of intra-amygdaloid 6-OHDA injections on monoamines outside of the

amygdala-pyriform region, but do not preclude the possibility that 6-OHDA-induced denervation of noradrenergic fibers went beyond the amygdalar-pyriform region. Possible circum-amygdalar effects could be masked in whole brain assays.

In reviewing the depletion studies, it seems quite clear that catecholamine depletion leads to an acceleration of the kindling process. Most studies have been unable to disassociate the respective roles of dopamine and norepinephrine in kindling development. Corcoran and Mason (1980) were able to demonstrate that forebrain depletion of norepinephrine alone can dramatically accelerate the kindling process. It is yet to be shown that selective depletions of dopamine will produce the same effects. Although Ehlers et al., (1980) indicated that noradrenergic fibers coursing along the ventral noradrenergic bundle were critical to norepinephrine's role in kindling development, Araki et al. (1983a) has shown that electrical lesions of the DNB, which did not reduce hypothalamic norepinephrine, were able to accelerate the kindling process. McIntyre (1980) went further by demonstrating that norepinephrine located in the amygdala-pyriform area is at least partially responsible for norepinephrine's inhibitory action on amygdaloid kindling.

2.1.3 Pharmacological studies

Another approach to investigate noradrenergic function in the kindling process has been the use of receptor-specific adrenergic agonists or antagonists. Alpha-adrenergic agonists and antagonists have yielded somewhat inconsistent results. Phenoxybenzamine, an alpha-adrenergic receptor blocker, when given 12 hrs prior to amygdaloid stimulation had no effect on the rate of kindling (Callaghan and Schwark, 1979). Clonidine (5 mg/kg), an alpha-adrenergic agonist, was also without effect on the kindling rate (Callaghan and Schwark, 1979). When tested for its effects on the clonic duration of kindled seizures, clonidine (.15mg/kg) decreased the duration of forelimb clonus (Ashton et al., 1980). Xylazine, an alpha-adrenergic agonist, was found to have a dose dependent action on kindling acquisition (Joy et al., 1983). The group given 0.3 mg/kg/day kindled faster and had more severe seizures during acquisition than controls, whereas the group given 3 mg/kg/day required more stimulations to kindle than the control group. Xylazine is a more potent agonist for alpha-2 receptors than for alpha-I receptors. Stimulation of alpha-2 receptors has been implicated in decreasing the stimulation-induced release of norepinephrine. The authors proposed that at the low end of the dose response spectrum (.3 mg/kg), xylazine would produce a relatively pure alpha-2 receptor action, and thereby induce its proconvulsive action

via inhibition of norepinephrine release. At high doses (3mg/kg), a mixed receptor action would be responsible for the anticonvulsant effect observed.

Propranolol (20 mg/kg), a beta adrenergic receptor blocker given 30 min before amygdaloid stmulation, caused a 40% decrease in the number of AD's required to produce a fully kindled seizure (Callaghan and Schwark, 1979). In another study (Peterson et al., 1981), propranolol, either 10 mg/kg or 20 mg/kg given 30 min before amygdaloid stimulation, failed to enhance the rate of kindling. Clearly, the receptor mediated action of norepinephrine on kindling needs further clarification.

1.2.1.4 Enhancement Studies

Few attempts have been made to demonstrate that elevation of norepinephrine activity inhibits the kindling process. Pargyline, a monoamine oxidase inhibitor, when given 2 hours prior to amygdaloid stimulation, delayed the development of AD duration increases (Wilkison and Halpern, 1979a). Acute treatment with DMI, which increases norepinephrine availability, given one hour before amygdalpid kindling stimulations, retarded the rate of kindling compared with controls (McIntyre et al., 1982b). DMI treatment also led to shorter latencies to clonus when stage 5 convulsions were provoked. These results are consistent with the proposed inhibitory action of

norepinephrine on the kindling process in intact animals, and suggest further that experimentally exaggerated levels will provide a strengthened prophylactic action.

1.2.2 Kindled seizures and kindled state

A number of investigations have been devoted to the assay of catecholamines in the brains of kindled animals to infer seizure mechanisms by identifying changes associated with the kindled seizure or state. It has been proposed that amygdaloid kindling results from induced hypofunction of the noradrenergic system (McIntyre, 1981; Corcoran, 1981). In support, depressant effects of iontophoretically applied dopamine and norepinephrine on glutamate-induced neuronal firing in the amygdala of cats were significantly reduced 1 and 2 hours following elicitation of an epileptiform AD (Spehlmann and Norcross, 1984). A general change in neuronal excitability cannot account for these results since responses to glutamate and GABA were not significantly altered.

Amygdaloid catecholamine levels were found to be significantly decreased in rats having received 3 stage 5 amygdaloid seizures one month earlier (Engel and Sharpless, 1977). The decline in norepinephrine however was not significantly different from those control rats which received an electrode implant alone. Catecholamine depletion was observed in whole cat brains one week

following hippocampal kindling (Sato and Nakashima, 1975) but not in the amygdala or other brain regions 24 hours following the last of 9 to 14 tonic-clonic amygdaloid kindled seizures (Stock et al., 1983). Wilkison and Halpern (1979b) found no change in catecholamine levels in the forebrain of rats 7 days after kindling but the turnover of dopamine and not norepinephrine was increased. Blackwood (1981) failed to demonstrate a change in the turnover rates of catecholamines in the amygdala and in the hippocampus four weeks after completion of amygdaloid kindling. Selective reductions in beta-adrenergic receptor binding of rats 3 days after the completion of amygdaloid kindling was observed in the amygdaloid regions, but no other brain regions (McNamara, 1978). Tyrosine hydroxylase activity, the rate-limiting step in catecholamine biosynthesis, was decreased in the stimulated amygdala in rats killed one month after completion of amygdaloid kindling (Farjo and Blackwood, 1978). Using fluorescence histochemistry, Kimura et al. (1981) were unable to detect any morphological differences in the catecholamine systems of the various brain regions examined, including the amygdala and cerebral cortices, subsequent to amygdaloid kindling. Taken together, reports on catecholamine content and turnover measured during the kindled state are inconsistent. This may be in part due to variation in the number of kindled seizures produced and time intervals between seizure and

tissue analysis.

Two studies have explored the role of catecholamines in the expression of kindled seizures already established. Westerberg et al., (1984) found that intracerebral 6-OHDA treatment into the DNB 3 days following 3 stage 5 seizures induced by daily stimulation of the amygdala in rats failed to affect the intensity or duration of seizures initiated 14 days later. The effects of xylazine, given to amygdaloid kindled rats prior to stimulation of the amygdala, had no effects on the expression of the kindled seizure, unless given in doses high enough to produce analgesia, ataxia, and sedation (Joy et al., 1983). These data suggest that while norepinephrine may affect the rate of acquisition, it does not modulate the permanent alterations that make up the actual substrate of the kindled state.

In summary, studies employing localized lesions of ascending catecholaminergic pathways, or specific catecholamine depletions in discrete brain regions, suggest that the noradrenergic neural system provides a significant inhibitory action on the kindling process, presumably by inhibiting the spread of epileptiform activity from the kindled focus. The use of selective agonists and antagonists has failed to yield a consistent picture regarding possible receptor sites mediating this effect. Pharmacologically raising norepinephrine levels before kindling does retard the rate of kindling. Variations in

catecholamine content and turnover have been reported during the kindled state but there are inconsistencies. There is no indication that these differences are a cause rather than an effect of the kindling processes. Depletion or pharmacological manipulation of catecholamine action has no effect on the expression of seizures once the kindled state has been produced.

1.3 Catecholamines and Other Models of Epilepsy

Kindling has also been shown to increase the incidence of, or decrease the threshold for, seizures produced by other methods (see Kalichman, 1982). Any neurotransmitter or neuromodulator that plays a role in one epilepsy model should be tested in other models to evaluate its specificity of action. Since the observation by Chen et al. (1954) that reserpine lowered the dose of pentylenetetrazol (PTZ) needed to produce tonic extension of hindlimbs in mice, there has been a great deal of research effort towards elucidating a role for catecholamines in the pathogenesis of a wide variety of epilepsy models. All models represent a class of disorders characterized by paroxysmal discharges (giant depolarizations of the neuronal membrane) which spread into surrounding brain areas to produce a clinical seizure.

1.3.1 Pentylenetetrazol Model

Systemic injection of PTZ sequentially produces myoclonic, clonic, and tonic seizures, the latter requiring higher doses. Corcoran et al., (1974) demonstrated that in animals given 6-OHDA (icv), the severity of PTZ-induced seizures was increased, and a tonic seizure component not found in controls was introduced. Reserpine treatment and neonatal 6-OHDA markedly shorten the latency period of tonic seizures after PTZ injection as well as increase their incidence (Gross and Ferrendelli, 1982). Propranolol (10 mg/kg) and yohimbine (4 and 10 mg/kg), beta- and alpha-2-adrenergic receptor antagonists respectively, produced qualitatively similar results. These results lead to the conclusion that diminished norepinephrine influence in the CNS enhances PTZ seizure activity, most notably the tonic component. Mason and Corcoran (1978) selectively depleted forebrain norepinephrine through 6-OHDA injections into the ascending noradrenergic fibers, with the result that 6-OHDA treated rats displayed PTZ- induced seizures of a significantly greater duration. Furthermore, 5 of the 9 6-OHDA treated animals displayed an episode of tonic extension of the forelimbs not found in the seizures of control animals. In a further study using injections into discrete areas of the brain, Mason and Corcoran (1979) selectively depleted cerebellar or spinal norepinephrine. Neither treatment affected the duration or number of PTZ-induced

convulsions. These studies clearly isolate norepinephrine's action on PTZ-induced seizures to forebrain sites, and emphasize a consistent facilitatory action of norepinephrine depletion on the tonic component. It has been suggested that the tonic component is an indicator of seizure spread (Gross and Ferrendelli, 1982: Racine and Burnham, 1984), as opposed to a measure of susceptibility to seizure initiation. Electrical stimulation of the locus coeruleus, the nucleus providing the major source of forebrain norepinephrine, markedly suppressed the appearance of cortical epileptiform activity induced by a subconvulsive dose of PTZ (Libet et al., 1977).

1.3.2 Electroconvulsive Shock

Another experimental model of epilepsy with characteristics similar to that of PTZ-induced seizures is the electro-convulsive shock (ECS) treatment. Electrical stimulation of the head through corneal, scalp, or other electrodes produces seizures consisting of sequential appearances of generalized brief tonic flexion, tonic extension, and clonic seizures. It has been more difficult to demonstrate an effect of norepinephrine depletion on ECS treatment, perhaps because of the rapidity of this seizure. Reserpine appears to increase the sensitivity of animals to ECS and the seizure is prolonged by either pretreatment with a tyrosine hydroxylase inhibitor or a dopamine beta-

hydroxylase inhibitor (Wenger et al., 1973). Intracerebroventricular (icv) treatment with RO 4-1284 (a drug with a brief reserpine-like action) significantly increased the intensity of the ECS-induced seizure in rats (Stull et al., 1977). This enhancement could be antagonized by icv infusion of either norepinephrine (208 ug) or dopamine (1200 ug), or by systemic administration of L-dopa, the precursor to catecholamine synthesis. However, in intact rats, icv infusion of dopamine (32 ug), norepinephrine (8 and 16 ug), or serotonin (8 ug) facilitated corneal ECS-induced seizures by decreasing the threshold for minimal seizures and increasing the tonic extension component (Browning and Maynert, 1978). These findings contradict the depletion studies but it was determined that the monoamine infusions produced hypothermia and when external heat was administered to the animal to restore body temperature to normal, the facilitatory effects were abolished. In one of the few attempts to measure the pathological consequences of ECS treatment with respect to norepinephrine Bergstrom and Keller (1979) reported a reduction in beta-receptors following 7 days of ECS treatment.

1.3.3 Chemical Convulsants

Given the wealth of literature on the cellular mechanisms of partial seizures induced by chemical convulsants such as penicillin, alumina gel, and cobalt,

there are few data on the role of catecholamines in these seizures. Lesions of the locus coeruleus, ipsilateral to the site of cortical cobalt application, reduce the latency to onset, prolong the duration and facilitate the spread of the epileptic syndrome (Kafiluddin et al., 1978). Stimulation of the locus coeruleus during cobalt-induced epileptiform activity produced a marked reduction of the spike-wave activity immediately after stimulation for up to 120 sec (Fischer et al., 1983). This suppression was partly antagonized by pretreatment with the beta-receptor antagonist propranolol. Reduced high affinity uptake of norepinephrine is coincident with cobalt-induced epilepsy (Trottier et al., 1983), most likely reflecting a degeneration of noradrenergic terminals.

In an elegant study involving double transplantation of locus coeruleus cell bodies and hippocampal tissue to the anterior chamber of the eye, Taylor et al., (1980) were able to demonstrate functional innervation of hippocampal tissue by locus coeruleus nerve fibers. Superfusion of penicillin markedly excited locus coeruleus neurons without producing epileptiform activity in the hippocampus. However, when gamma-aminobutyric acid (GABA) was administered by microiontophoresis into the locus coeruleus portion of the graft, penicillin administration generated epileptiform activity in the hippocampal tissue, suggesting that a functional inhibitory mechanism developed between locus

coeruleus fibers and epileptiform activity in the hippocampus. In further support, subsequent excitation of locus coeruleus neurons by iontophoresis of glutamate following penicillin-induced seizures in the hippocampal tissue terminated the hippocampal seizure. Pretreatment with reserpine disrupts the inhibitory influence of locus coeruleus innervation on hippocampal epileptiform activity.

1.3.4 Genetic Models

There are a wide variety of genetic convulsive disorders that occur in animals most of which are characterized by stimulus-evoked, generalized convulsive seizures. The oldest of these models is the audiogenic seizure (AGS) induced by sound stimulation in AGS-susceptible animals. Treatments which cause severe reduction of norepinephrine and dopamine (reserpine and Ro 4-1284) markedly intensify AGS in the genetically epilepsy-prone rat (GEPR) (Jobe et al., 1973). Only treatments which reduced whole-brain norepinephrine concentration to 33% or less of control values resulted in enhancement of AGS, regardless of the relative reduction of dopamine, suggesting that the major role for catecholaminergic inhibition of seizure development is performed by norepinephrine. Drug combinations which increase norepinephrine and dopamine levels produce a significant decrease in sound-induced seizure intensity, while those that increase dopamine alone

have no effect on seizure intensity in GEPRs (Ko et al., 1982). In fact, GEPRs have lower levels of norepinephrine, and lower turnover rates (Jobe et al., 1982; 1984). Taken together these results suggest that the genetically determined deficit in GEPRs may be a deficit in noradrenergic transmission. At doses high enough to stimulate alpha-1 receptors, clonidine (0.5 and 1.0 mg/kg) increases seizure latency and reduces seizure severity for AGS in rats (Tacke and Kolonen, 1984) suggesting an important alpha-1 receptor mechanism in the noradrenergic control of AGS. Mice of the DBA/2 strain are genetically prone to AGS at an early stage of development, and display an increased density of beta-adrenergic receptors in their midbrain during this seizure susceptible period (Lints and Nyquist-Battie, 1985). Two beta-receptor blocking agents, propranolol and pindolol, attenuated the incidence of sound-induced tonic and clonic convulsions in this strain of mice (Lints and Nyquist-Battie, 1985). In contrast to other audiogenic models, the latter study suggests that beta-adrenergic receptors play a facilitory role in the expression of audiogenic seizures.

Another genetic model of epilepsy is the epileptic baboon, Papio papio. This animal displays a paroxysmal electroencephalogram and motor activity in response to intermittent light stimulation. ICV norepinephrine and epinephrine in doses of 250 ug or more and 100 ug or more,

respectively, reduced seizure intensity induced photically in this baboon (Altshuler et al., 1976). Neither icv injections of dopamine (.5-1.5 mg) or 5-hydroxytryptamine (1.0 mg) affected seizure intensity.

The most recent genetic model of epilepsy is the mutant tottering mouse. In this model focal motor seizures occur spontaneously and feature intermittent focal myoclonus, and cortical spike-wave discharges accompanied by behavioral arrest. The only CNS abnormality as yet uncovered is a selective overgrowth of the noradrenergic locus coeruleus axons, raising norepinephrine level 100-200% within most locus coeruleus terminal fields (Noebels, 1984). Selective neonatal denervation by 6-OHDA treatment prevents the later appearance of spike-wave seizures in the adult animal (Noebals, 1984). Suprisingly, in this model, hyper-innervation by norepinephrine appears to underlie seizure susceptibilty.

1.4 Summary

Most studies concerning norepinephrine and various epilepsy models suggest an inhibitory action of norepinephrine on those processes responsible for the expression and development of seizures. In view of the fact that different neurochemical abnormalities and control systems could underlie different types of epilepsy, the results are surprisingly homogeneous. It is difficult to

propose a cohesive role for norepinephrine across the various seizure models because of the heterogeneity of seizure states as evidenced by the variety of seizure models and clinical states (Snead, 1983). It should be pointed out that catecholamines are not the only neurotransmitters implicated in the expression and development of seizures (see Kalichman, 1982; Peterson and Albertson, 1982; Snead, 1983).

Since kindling is essentially an elaboration of the convulsive response to an originally minimal or subconvulsive stimulus, progressing over stimulations through characterized stages of development, it is an ideal model to study the noradrenergic control of neuronal plasticity and generalized seizure development. As suggested by Racine and Burnham (1984), convincing support for the role of any biochemical mechanism involved in the suppression of kindling development requires evidence that (1) suppression of the hypothesized system accelerates kindling, and (2) correction of the abnormality antagonizes the kindling process. The purpose of this thesis is to provide this dual support for norepinephrine's role in kindling development.

In the first experiment, an attempt was made to reinforce previous studies, most notably those of Corcoran and Mason (1980) and McIntyre (1981), indicating that depletion of norepinephrine alone will accelerate the

kindling process. Furthermore, depletion of norepinephrine was restricted to that of locus coeruleus origin. In subsequent experiments, an attempt was made to restore norepinephrine in depleted animals to antagonize depletion-induced acceleration of the kindling process.

CHAPTER 2: EXPERIMENT 1

2.1 Introduction

A wide variety of compounds have been used to influence noradrenergic systems during kindling. Lack of precision in the selectiveness of their actions however, limits the inferences that may be drawn. The strongest support for a specific role for norepinephrine in seizure development comes from studies using the neurotoxin, 6-OHDA. Animals depleted of norepinephrine by 6-OHDA administration show an acceleration of kindling rate. However, depending on the site of administration, 6-OHDA produces several non-specific effects, that might affect kindling. 6-OHDA administered intracerebroventricularly (icv) reduces dopamine as well as norepinephrine (Uretsky and Iversen, 1970), and has recently been shown to deplete CNS epinephrine and 5-hydroxytryptamine (Reader and Gauthier, 1984). Neonatal treatment, which is more specific to noradrenergic terminals, retards proper development of the CNS (Brenner et al., 1983). Intracerebral punctate infusions, such as those aimed at the dorsal noradrenergic bundle, produce significant non-specific damage as evidenced in part by tissue cavitation and gliosis (Butcher, 1975). Intracerebral 6-OHDA also produces extensive damage to the blood-brain barrier, allowing extravasation of intravenously

administered horseradish peroxidase for up to 21 days following 6-OHDA (Cooper et al., 1982). Leakage of iodionated human serum albumin (HSA) across the blood-brain barrier occured following punctate 6-OHDA lesioning of the locus coeruleus when blood-brain barrier functions had been previously stressed by bicuculline-induced seizures (Harik and McGunigal, 1984). HSA leakage had not occurred as a result of 6-OHDA treatment alone.

The development of a new neurotoxin, N-(2-chloroethyl)-N-ethyl-bromobenzylamine (DSP-4), which is structurally different from 6-OHDA, and has been shown to be capable of producing selective degeneration of both central and peripheral noradrenergic nerve terminals (Ross, 1976; Jaim-Etchevarry and Zieher, 1980; Jonsson et al., 1981), allows for an additional and relatively specific test of norepinephrine's role in kindling development. Furthermore, unlike 6-OHDA, DSP-4 will cross the blood-brain barrier and hence can be administered peripherally, thereby precluding the possibility of local effects on CNS tissue through intracerebral injection. Having a different drug profile than 6-OHDA, DSP-4 treatment would further substantiate the hypothesis that norepinephrine suppresses kindling development, should DSP-4 treatment accelerate kindling.

DSP-4 treatment causes severe norepinephrine, but not dopamine depletion in the CNS, and a 20-30% decrease in 5-hydroxytryptamine which can be blocked by pretreatment

with a 5-hydroxytryptamine uptake inhibitor (Jonsson et al., 1981; Dooley et al., 1983a). Small decreases of dopamine in the hippocampus and cerebellum have been noted after high DSP-4 doses, and have been attributed to the loss of precursor dopamine localized in noradrenergic terminals (Archer et al., 1984; Jonsson et al., 1981). In further support of the drug's selectivity, there is an increased sensitivity to noradrenergic agonists following DSP-4 treatment (i.e., depletion-induced receptor supersensitivity) but no increased sensitivity to dopaminergic or serotoninergic agonists (Dooley et al., 1983a, 1983b).

A peripheral injection of DSP-4 causes a selective lesion of locus coeruleus neurons innervating the neocortex, hippocampal formation, cerebellum and spinal cord (Jonsson et al., 1981; Logue et al., 1985). Since areas innervated predominantly by the noradrenergic neurons of the lateral tegmental area are left intact, it is apparent that DSP-4 is selective towards locus coeruleus neurons. Neuronal degeneration presumably begins at the presynaptic terminals as characterized by histochemical fluorescence, norepinephrine concentrations, uptake of tritiated norepinephrine, and dopamine B-hydroxylase activity (Ross, 1976; Jonsson et al., 1981; Dooley et al., 1983a; Archer et al., 1984). The more distant from the locus coeruleus, the more pronounced is the norepinephrine depletion (Jonsson et al., 1982; Logue et al., 1985). Retrograde degeneration of locus coeruleus

neurons as a consequence of DSP-4 administration does not extend to cell bodies, which show a decreased firing rate (Olpe et al., 1983).

Many parameters have been studied to determine possible cytotoxic and other non-specific effects. Light microscopy has revealed no morphological abnormalities in a variety of cell types after DSP-4 treatment (Dudley et al., 1981; Olpe et al.,1983; Bickford et al., 1984). Functioning of the hypothalamo-pituitary-adrenocortical axis has also been assessed following DSP-4 treatment. Basal plasma corticosterone levels were unaltered compared to those of controls, under either stressed or non-stressed conditions (Dooley et al., 1984). The concentration of liver microsomal cytochrome P-450, an important enzyme in oxidative drug metabolism, was not altered by DSP-4 treatment thereby ruling out possible effects on drug metabolism (Dooley et al., 1983b). It has been reported as a personal communication that DSP-4 is also free of effects on amino acids and acetylcholine in the CNS (Delini-Stula et al., 1984).

In the peripheral nervous system, a transient depletion of norepinephrine follows DSP-4 treatment; however, heart rate, blood pressure, and atrial contractility are not significantly affected 10 days after DSP-4 treatment (Dooley et al., 1983b). The adrenergic nerve plexus in both the iris and atrium exhibits an almost

normal appearance one week after the DSP-4 administration, and norepinephrine levels in the ventricle of the heart are normal 10 days after treatment (Jonsson et al., 1981). A moderate but significant norepinephrine reduction was found in the iris 10 days after DSP-4 but not at 30 days (Jonsson et al., 1981). Hence a complete recovery of function follows an initial depletion of norepinephrine levels in the peripheral nervous system, whereas DSP-4 affects CNS norepinephrine levels and DMI binding maximally at 7 days and the effects remain essentially unchanged for at least 8 weeks (Swann, 1984).

The pharmacological action of DSP-4 is believed to be expressed only after the parent compound undergoes cyclization to form the aziridinium ion. Since the aziridinium ion will not cross the blood-brain barrier (Zieher and Jaim-Etcheverry, 1980), and DSP-4 will cyclize with a half life of about 7 minutes (Ross, 1976), care must be taken to inject immediately after preparation. Pretreatment with either desimipramine, a norepinephrine uptake inhibitor, or pargyline, a mohoamine oxidase inhibitor, almost completely blocks the action of DSP-4 (Hallman and Jonsson, 1984; Landa et al., 1984). It appears that DSP-4 must be actively taken up by noradrenergic terminals to produce its neurodegenerative results. How MAO inhibitors block DSP-4 actions is less clear. It has been suggested that MAO may transform DSP-4 to the active agent

(Hallman et al., 1984).

A pilot experiment involving 14 animals, which had been undertaken to explore the effect of DSP-4 treatment on the amygdaloid kindling rate in rats, indicated that DSP-4 treatment facilitated kindling. Saline controls required 8.9 ADs to kindle whereas DSP-4 animals required 5.8 ADs to kindle. The purpose of the present experiment was to replicate these preliminary results and characterize the action of DSP-4 on monoamine levels in the brain. Based on experiments where lesions of the ascending noradrenergic fibers (Corcoran and Mason, 1980), or lesions of the DNB (Araki et al., 1983a) accelerated kindling, it was anticipated that lesions of the noradrenergic terminals of the locus coeruleus by DSP-4 treatment will also accelerate kindling.

2.2 Methods

Norepinephrine Depletion:

Twenty-seven male albino Sprague Dawley rats obtained from the Canadian Breeding Laboratory and weighing between 370 and 470 g at the time of injection were used. The preparation of animals consisted of pretreatment with CGP 6085A (2.7 mg/kg,i.p.; CIBA Giegy, Switzerland), an inhibitor of 5-hydroxytryptamine (5-HT) uptake (Waldmeier et al.,1979), 30 min. before the injection of DSP-4 (63

mg/kg,i.p.; Astra Lakemedel AB, Sweden) or a control solution (0.9% saline). Intraperitoneal injections were given in the upper right quadrant of the abdomen, just below the rib cage. Care was taken to inject DSP-4 immediately after preparation. The injection volumes were 5 ml/kg.

Surgery:

Fifteen to twenty days following injection the animals were anesthetized with Avertin (10 ml/kg), and received stereotaxic implantation of electrodes unilaterally into the basolateral amygdala. The electrodes (Plastic Products) were bipolar consisting of two wires separated by 0.25 mm at the tip. The stereotaxic coordinates were 2.7 mm caudal and 4.7 mm lateral to bregma (skull flat) and 8.4 mm ventral to the skull's surface. The electrode assembly and anchor screws were held in place with dental acrylic applied to the exposed skull surface.

Kindling:

Following a recovery period of 20-24 days the animals were handled for 20 min a day for four days. At this point the experimenter was 'blind' to the animal's drug treatment. On the fifth day each animal was placed in the experimental chamber for 20 min. The next day, approximately 40 days following the drug injection, the afterdischarge (AD) threshold was determined. This time interval is more than ample for the depletion of norepinephrine levels and allows for a recovery of

norepinephrine loss in the periphery (Jonsson et al., 1981; Swann, 1984). Stimulation of the basolateral nucleus of the amygdala was accomplished using a Lafayette sine wave stimulator and consisted of a 1 sec. train at 60 Hz. Threshold for AD was arbitrarily defined as the lowest intensity of stimulation required to evoke AD. Stimulation was initially delivered at an intensity of 10 uA and was incremented by 10 uA, at one min intervals, until AD was evoked. Kindling began the following day (day 1), at a suprathreshold current of 200 uA. Animals were given a 1 sec train at 60 Hz, once every 24 hours until they displayed a stage 5 motor seizure. Amygdaloid EEG was recorded for one min before and several min after each stimulation. The development of behavioral seizure manifestations was videotaped and classified using a five point scale (Racine, 1972): stage 1,mouth movements and ipsilateral eye-blinking; stage 2, head-nodding; stage 3, clonus of the ipsilateral forelimb; stage 4, bilateral clonus with rearing; stage 5, rearing and falling.

The measurement of primary interest was the number of ADs until a stage 5 seizure. Other seizure parameters investigated were the AD threshold, duration of stage 5 motor and electrographic seizures, and the latency to exhibit clonus during the stage 5 seizure after the kindling stimulus.

Biochemistry and Histology:

Concentrations of dopamine, norepinephrine, and 5-HT were measured in the brains of all rats. The rats were decapitated one month after the stage 5 seizure, the brains removed and blocked sagittally down the midline over ice. The cortex on the side contralateral to the electrode placement was peeled back, and the telencephalon was isolated by a coronal section from the anterior thalamus to the anterior hypothalamus. The dissected telencephalon was immediately wrapped in aluminum foil, frozen in liquid nitrogen, briefly kept over liquid nitrogen, and stored at -70 degrees Centigrade pending chemical analysis. To determine electrode placement, the ipsilateral hemisphere was placed in a cryostat, and saggital slices were taken and reacted for acetylcholinesterase (Mesulam, 1976), and then counterstained with cresyl violet. The basolateral nucleus has a high acetylcholinesterase content, and is therefore highlighted when stained for acetylcholinesterase.

For reasons related to equipment availability, the degree and selectivity of norepinephrine depletion by DSP-4 was not ascertained until all experiments had been completed. The samples were weighed, homogenized and analyzed spectrofluorometrically for dopamine, 5-hydroxytryptamine, and norepinephrine levels according to Jacobowitz and Richardson (1978).

The data were analyzed statistically with a two-

tailed t-test between the saline and DSP-4 groups for each dependent variable.

2.3 Results and Discussion:

The results are summarized in table 1 (pg. 44).

Eight out of eighteen rats treated with DSP-4 died within a few days following DSP-4 treatment; the remaining animals recovered and appeared healthy. No weight difference between the two groups was found two weeks following drug treatment (i.e., at time of surgery). One animal died following injection of the anesthetic prior to surgery. Four animals from the saline and 1 animal from the DSP-4 group were eliminated from the study after their dental cement caps fell off before completion of the experiment, and one animal was removed when it had shown no ADs in response to the kindling stimulus following three days of testing.

As expected, DSP-4 treatment produced a significant depletion ($t(10)=3.92, p < .005$) of norepinephrine (45%) in the telencephalon with no significant alterations in the concentrations of dopamine or 5-hydroxytryptamine.

Histological verification of amygdaloid electrode placements aimed at the basolateral nucleus are illustrated in figure 1 (pg. 45). Generally, placements were in or dorsal to the basolateral nucleus. There was no locational bias between groups as the tips appeared to be randomly

TABLE 1: EXPERIMENT 1 SUMMARY TABLE

Dependent Variable		Experimental Group: Saline	Experimental Group: DSP-4
Weight (g)	Mean	446.3	435.3
	Standard Error	8.9	12.3
	N	6	6
AD Threshold (uA)	Mean	45.0	78.3
	Standard Error	8.9	20.4
	N	6	6
AD's to Stage 5	Mean	7.5	3.8
	Standard Error	.56	1.08
	N	6	6
Stage 5 Motor Seizure Dur (sec)	Mean	39.8	62.8
	Standard Error	6.4	7.1
	N	5	5
Stage 5 AD Dur (sec)	Mean	74.0	81.0
	Standard Error	49.0	10.1
	N	2	5
Stage 5 Latency to Clonus (sec)	Mean	4.0	29.2
	Standard Error	.45	5.4
	N	5	6
NE Concentration (ug/g)	Mean	.513	.287
	Standard Error	.025	.052
	N	6	6
DA Concentration (ug/g)	Mean	3.52	3.11
	Standard Error	.31	.56
	N	6	6
5-HT Concentration (ug/g)	Mean	1.08	1.34
	Standard Error	.09	.20
	N	6	6

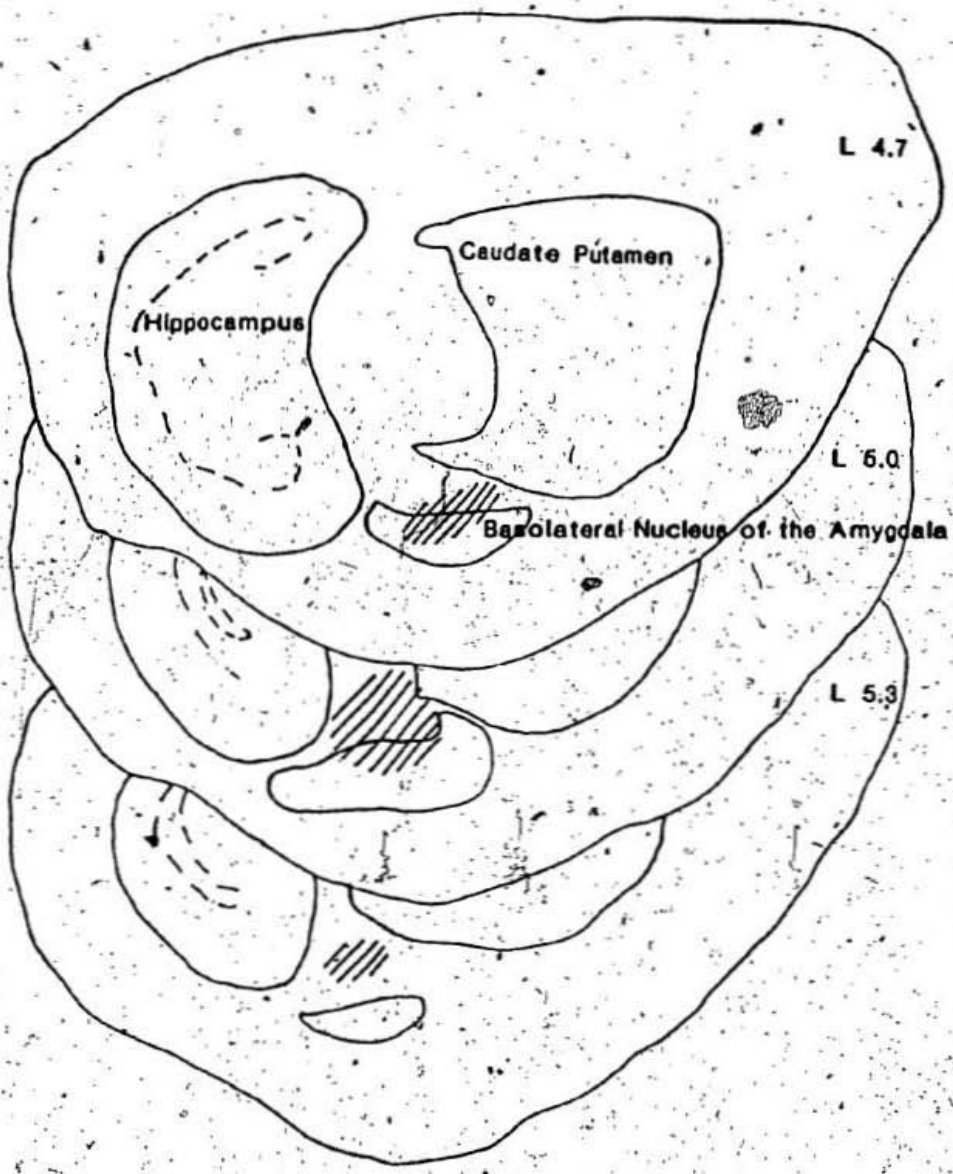

FIG. 1. DISTRIBUTION OF ELECTRODE PLACEMENTS.

Camera lucida drawings in the sagittal plane.

distributed within the area illustrated in fig. 1.

The afterdischarge thresholds determined before kindling were not affected by DSP-4 treatment ($t(10)=1.50$, $p= .18$), indicating that DSP-4, like 6-OHDA, treatment does not affect the production of local seizures (Corcoran and Mason, 1980).

Twice as many ADs were required to kindle saline treated rats as were needed to kindle DSP-4 treated animals. The number of ADs required to produce a stage-5 seizure was significantly reduced by DSP-4 treatment to 3.8 ADs for the DSP-4 group as compared with 7.5 ADs for the saline group ($t(10)=3.02$, $p<.02$). Only one animal from the DSP-4 group fell within the range of scores in the saline group. Although the saline treated rats kindled quite rapidly compared to controls of other amygdaloid kindling studies it has been reported previously that Sprague Dawley albino rats kindle faster than other rat strains, with a mean of 7.0 ADs to the first stage-5 seizure (Racine et al., 1973).

The magnitude of facilitation by DSP-4 depletion of norepinephrine on amygdaloid kindling rate falls within the range of those studies employing 6-OHDA or electrolytic lesions of the dorsal noradrenergic bundle. Mohr and Corcoran (1981), with a kindling rate of 16.7 ADs to stage 5 for controls, reduced the kindling rate to 30% of controls by intracerebral injection of 6-OHDA into the ascending noradrenergic fibers. McIntyre (1980), whose control

animals kindled after an average of 12.5 ADs, injected 6-OHDA locally into the amygdala and reduced the kindling rate by 40%. Araki et al. (1983a), whose control animals kindled after an average of 15.5 ADs, also reduced the kindling rate by less than 50% by electrolytic lesions of the DNB. All other studies reviewed produced a decrease to somewhere between 30% and 50% of control kindling rate. DSP-4 treatment here produced reductions in the lower range (50%); however a possible basement effect is suggested by the rapid kindling rate of this strain of rat.

Although treatment with DSP-4 facilitated the rate of kindling, it retarded the onset of forelimb clonus during the first stage five seizure ($t(9)=4.17, p<.005$), as has been reported elsewhere with icv 6-OHDA treatment (McIntyre et al., 1979; McIntyre, 1981). The overall length of the motor seizure was also longer for DSP-4 rats than saline treated animals ($t(8)=2.40, p<.05$) and was accounted for by the extra time spent in stages 1 and 2 (latency to forelimb clonus). The AD durations for the stage 5 seizure were similar between groups, but not reliable since data on this variable for 4 animals from the saline group were discarded due to masking of the electrographic record by noise.

While increased latency to clonus during the stage 5 seizure has been produced by 6-OHDA treatment given intraventricularly (McIntyre et al., 1979, 1981), it could not be replicated by 6-OHDA treatment into the ascending

noradrenergic fibers (Mohr and Corcoran, 1981) or into the amygdala (McIntyre, 1980). It was suggested that the depletion of spinal or cerebellar norepinephrine, spared by 6-OHDA-DNB treatment, was responsible for the latency effects (Mohr and Corcoran, 1981). This interpretation is consistent with the DSP-4 induced effects, which would have depleted cerebellar and spinal norepinephrine as well as forebrain norepinephrine.

CHAPTER 3: EXPERIMENT 2

3.1 Introduction

A variety of studies indicates that the presence of norepinephrine normally acts to retard the development of kindling, since its depletion facilitates the kindling rate. The previous experiment reaffirms the facilitating action of norepinephrine depletion on the development of kindled seizures, using the specific norepinephrine neurotoxin DSP-4. Important support for norepinephrine's apparent inhibitory role in kindling development would be reestablishment of the basal rate of kindling after norepinephrine depletion by restoring norepinephrine levels in DSP-4 treated animals.

Inherent in the question of repletion are questions about the critical area in which norepinephrine's presumed prophylactic action takes place, and the critical time of exposure to norepinephrine during the kindling process. With respect to the critical area, although local amygdaloid depletion of norepinephrine facilitated the rate of kindling (McIntyre, 1980), the facilitation was not of the same magnitude as reported by more general, widespread depletion studies. Secondly, a lack of clear effect of norepinephrine depletion on local seizure susceptibility as measured by AD threshold reinforces the concept that depletion of norepinephrine causes a disinhibition of the spread of

epileptiform activity (Corcoran and Mason, 1980). Presumably, forebrain norepinephrine outside the amygdala also contributes to its inhibitory role on kindling development. For these reasons, initial repletion attempts should be aimed at forebrain areas in general.

The critical time period at which norepinephrine may act to inhibit seizure spread cannot be identified by chronic depletion studies. The depletion however must be present prior to stage 5 seizures since depletion of norepinephrine following the first stage 5 seizure has been shown to have no effect on the expression of the electrically induced seizures (Westerberg et al., 1984). 6-OHDA treatment following 3 stage 5 seizures did not affect the ability to reproduce a stage 5 seizure on the first stimulus, or the duration of the AD and motor seizure produced. This suggests that norepinephrine acts to suppress the development of kindled epileptogenicity, and not the expression of established seizures. Therefore, repletion studies aimed at providing a chronic supply of norepinephrine throughout kindling, although not providing any information about the specific time course of norepinephrine action, will serve as a necessary test of the hypothesis that norepinephrine is tonically required to suppress seizure development.

Intracerebroventricular infusion of norepinephrine into the lateral ventricle, despite certain limitations,

appears to be the method of choice initially for the repletion of forebrain norepinephrine. The most obvious limitation to this technique is the restricted distribution to periventricular sites. The uptake of tritiated norepinephrine by any region of the brain is negatively correlated with the distance of the region from the injection site (Chalmers and Wurtman, 1971). Most exogenous norepinephrine is actively taken up by nerve endings and axons in the surrounding tissue, the remainder being localized in glial perikarya and blood vessels (Aghajanian and Bloom, 1967). Prior destruction of noradrenergic terminals may result in increased distances of diffusion due to the presumed loss of most of these specific uptake channels. On the other hand, degradation could be more rapid since exogenous norepinephrine would not be sequestered into vesicles to protect it from enzymatic destruction, although there is some evidence for extraneural spaces that are capable of accumulating exogenous norepinephrine (Koster et al., 1984).

One study has attempted to normalize norepinephrine, following depletion, through infusion of norepinephrine into the rat brain. Biswas and Jonsson (1981) chronically infused (icv) norepinephrine into 6-OHDA treated rats and found that 1 ug/hr and 5 ug/hr reversed the norepinephrine denervation induced increase of beta-adrenoreceptor binding in the rat neocortex. When the norepinephrine-depleted

animals were sacrificed those that received 1 ug/hr had endogenous norepinephrine levels in the cerebral cortex of 29% (when compared to non-depleted animals) and those receiving 5 ug/hr had norepinephrine levels of 141% of normals. They also noted that exactly the same results were obtained for the cannulated and the contralateral side, indicating that the intraventricular infusion technique employed was sufficient for the distribution of norepinephrine to both hemispheres. Following the continuous infusion of norepinephrine into the lateral ventricles, it is likely that the norepinephrine is immediately carried down with the normal flow of cerebrospinal fluid towards the fourth ventricle, via the third ventricle and the cerebral aqueduct, from which it gains access to the cerebellomedullary cistern and subarachnoid spaces (Nowaczyk et al., 1978: Milhorat and Hammock, 1983), the latter compartment probably supplying the greatest amount of norepinephrine to the cerebral cortices.

Kasamatsu and Pettigrew (1979b) and Biswas and Jonsson (1981) among others have used Alzet osmotic minipumps to provide continuous infusion of norepinephrine into the brain. The Alzet osmotic minipump consists of a semipermeable membrane, containing a collapsible reservoir of flexible, impermeable material, which is surrounded by a sealed layer containing an osmotic agent (hypertonic

saline). When the filled minipump is placed in an aqueous environment (e.g., subcutaneously) the osmotic agent imbibes water at a rate determined by the permeability of the outer membrane to water. Hydrostatic pressure is generated by the imbibed water on the flexible lining of the reservoir, gradually compressing it, producing a constant flow of its contents through a delivery port. The minipump can be implanted locally in the desired site of administration or attached to a catheter for delivery to sites distant from its location (i.e., the brain). The major advantages of these pumps are: their ability to deliver a constant flow of material at specific volumes, and the lack of movement restrictions inherent in pumps located away from the animal.

The present experiment employed osmotic minipumps to chronically infuse norepinephrine icv into animals pretreated with DSP-4, in an attempt to reestablish the prophylactic action on kindling development attributed to norepinephrine.

3.2 Method

The same general procedure of the previous experiment was maintained with the following exceptions.

In an attempt to reduce the high number of animals lost in the first experiment to DSP-4 treatment, all ip injections were given into the lower right quadrant of the abdomen rather than the upper right quadrant as previously.

Eighteen animals (345-447 g) were given the CGP 6085A/DSP-4 combination as in experiment 1. Twenty-five to 30 days following drug treatment all animals had stereotaxically implanted bipolar electrodes aimed at the basolateral amygdala and a guide cannula (Plastic Products, 22 gauge) positioned above the lateral cerebral ventricle ipsilateral to the electrode placement. Coordinates for the guide cannula were 1 mm posterior to bregma, 1.5 mm lateral to the midline, and 3.1 mm ventral from skull (skull flat). The cannula and the electrode were fixed to the skull by dental cement as indicated in experiment 1. A dummy cannula was inserted into the guide cannula, and fixed to the dental cement to prevent premature removal.

Following a recovery period of 20-23 days, and the five day handling period, an osmotic minipump (Alzet, model 2002) was implanted subcutaneously under Penthrane (methoxyflurane, Abbott Laboratories) with a connecting tube attached to an inner cannula (Plastic Products, 28 gauge) cut to protrude 1 mm from the guide cannula. The minipump and catheter were filled with a sterile saline (0.9%) solution containing 10 mg/ml of norepinephrine hydrochloride and 0.1% (w/v) ascorbic acid. Control animals received the vehicle (same solution without norepinephrine HCl) alone. The minipump delivered 5 ug of norepinephrine-HCl/.5 ul/hr for a minimum of 14 days. After incising the skin at the back of the neck, a hemostat was used to make a subcutaneous

pocket that extended from the caudal aspect of the incision. The minipump was passed into the pocket with the connecting tube and cannula protruding through the incision. The inner cannula was then placed down the guide cannula, following the clipping and removal of the dummy cannula, and cemented into place on the dental cement cap by a bead of epoxy glue. The skin was immediately sutured around the tubing between the pump and the cannula.

The animal was allowed to recover from the implant overnight, then habituated to the experimental chamber for a twenty minute period, and kindling started the second day after implantation.

Animals were decapitated two to three hours after the first stage five seizure, and histological and biochemical analysis followed as in experiment one.

3.3 Results and Discussion

The results are summarized in Table 2 (pg. 56). Histological verification of amygdaloid electrode implants revealed a distribution as indicated in experiment 1.

Two animals died 2-3 days following DSP-4 treatment and one animal died while under anesthesia during minipump surgery. Two animals lost their caps before completing the experiment, and one animal was removed when following three days of testing it had shown no AD s in response to the kindling stimulus.

TABLE 2: EXPERIMENT 2 SUMMARY TABLE

Dependent Variable		Group	
		Vehicle	NE
Weight (g)			
	Mean	496.0	489.4
	Standard Error	11.8	25.3
	N	5	5
AD Threshold (uA)			
	Mean	43.3	30.0
	Standard Error	8.0	6.8
	N	6	6
# of AD's to Stage-5			
	Mean	4.5	4.0
	Standard Error	.81	1.0
	N	6	6
Stage 5 Motor Seizure Dur (sec)			
	Mean	55.3	89.8
	Standard Error	2.5	27.6
	N	6	6
Stage 5 AD Duration (sec)			
	Mean	73.2	111.4
	Standard Error	2.7	24.76
	N	5	5
Stage 5 Latency to Clonus (sec)			
	Mean	11.7	26.8
	Standard Error	3.6	12.2
	N	6	6
NE Concentration (ug/g)			
	Mean	.352	.340
	Standard Error	.032	.039
	N	5	6
DA Concentration (ug/g)			
	Mean	2.98	3.31
	Standard Error	.19	.42
	N	5	6
5-HT Concentration (ug/g)			
	Mean	1.12	.97
	Standard Error	.11	.03
	N	5	6

There were no differences between groups on any of the measures: norepinephrine-infused rats did not differ from vehicle-infused (.1% ascorbic acid in .9% saline) rats in either AD threshold ($t(10)=1.26$, $p=0.24$) or the number of afterdischarges until the first stage 5 seizure ($t(10)=0.39$, $p=.705$). No differences were found between the vehicle-infused and norepinephrine-infused rats for the duration of the first stage 5 motor seizure ($t(10)=1.79$, $p=0.10$), the duration of the stage 5 electrographic seizure ($t(10)=1.53$, $p=0.16$), or the latency to clonus ($t(10)=1.19$, $p=.262$). Dopamine and 5-hydroxytryptamine levels did not differ between groups.

These results are not surprising considering the fact that norepinephrine-infusion did not raise norepinephrine levels measured in the telencephalon above those of vehicle infused animals ($t(9)=0.25$, $p=0.81$). However, considering the success that Biswas and Jonsson (1981) reported employing this method, these results are surprising. They treated newborn rats with 6-OHDA (2x100 mg/kg sc) or saline, and at 8-10 weeks of age used osmotic minipumps to chronically infuse either the vehicle (sterile saline and .1% ascorbic acid) or the vehicle with norepinephrine, for 9 days into the right lateral ventricle. All animals were sacrificed on the tenth day and an 8.9 mg tissue sample taken from either side of the cerebral cortex was assayed for norepinephrine levels. Animals with saline

treatment and vehicle infusion had an average norepinephrine concentration of 8.4 pmol/sample whereas those animals which were treated with 6-OHDA and vehicle infusion had an average of 2.5 pmol norepinephrine/sample representing a 70% decrease in norepinephrine levels. Animals treated with 6-OHDA and norepinephrine infusion (5 ug/ul/hr) had an average norepinephrine level of 11.8 pmol/sample, regardless of which hemisphere was sampled. Therefore chronic infusion of norepinephrine into animals pretreated with 6-OHDA was able to produce norepinephrine levels that were 41% higher than found in control animals with no 6-OHDA treatment.

Histological verification of the cannula placement indicated consistent placements of the inner cannula into the lateral ventricle, therefore it is unlikely that the infused norepinephrine was denied access to ventricular circulation.

The potential for auto-oxidation of norepinephrine was prevented by a 0.1% ascorbic acid vehicle. A 0.1% solution of ascorbic acid has been shown to protect 95% of the norepinephrine released by the minipump for at least 5 days (Kleinjans et al., 1981). Biswas and Jonsson (1981) measured norepinephrine levels which were above the level of non-depleted animals 9 days after initiation at the same infusion rate of 5 ug/hr. Clearly the inability to replete norepinephrine is not likely to be due to its deterioration in the minipump. Animals were infused for a range of 4 to

10 days before decapitation in this study.

Obstruction of the cannula is one possible source of failure, but difficult to isolate. One animal's histological record indicated a black mass at the tip of the cannula placement suggesting the possible obstruction of the cannula by coagulated blood. Another animal had an apparent infection at the site of the cannula tip. Data from these animals did not alter the outcome of the experiment.

Since the pump does not reach a steady flow rate until 4 hours after it has been implanted, the cannula would be most susceptible to blocking during the initial period of inactivity.

No untreated controls were run in this experiment. To determine whether DSP-4 treatment accelerated kindling as in experiment one, data from the two infusion groups were collapsed and all kindling and biochemical parameters were compared to the DSP-4 and saline groups of experiment 1. Animals from both experiments were treated in the same manner. Aside from the minipump surgery and vehicle infusion, the only procedural difference was a greater recovery time (10 days) in experiment 2 than experiment 1 between DSP-4 injection and stereotaxic surgery. The only significant difference distinguishing the collapsed infusion group from the DSP-4 group of experiment one was a lower AD threshold for the infusion group ($t(16)=2.61$, $p=.02$). It is unclear why the icv infusion procedure, including

implantation and anesthesia 48 hours before threshold testing, would lower the AD threshold.

Two kindling parameters were significantly different between the infusion group and the saline group from experiment one. Animals in the infusion group had lower levels of telencephalic norepinephrine ($t(15)=4.41$, $p=.001$) and kindled significantly faster ($t(16)=3.36$, $p=.002$) than the saline group. No significant difference was found on the latency to clonus measure ($t(15)=1.49$, $p=.16$), which was intermediate between the saline group and the DSP-4 group from experiment one.

In conclusion, the methods employed were unable to significantly replete telencephalic levels of norepinephrine following DSP-4 treatment. Further, no significant differences were found between the group receiving the vehicle and the group receiving norepinephrine on kindling development. When both infusion groups were combined and compared to those groups in experiment one, it was again apparent that DSP-4 treatment accelerated the rate of kindling in both studies, presumably by depleting norepinephrine in the central nervous system.

CHAPTER 4: EXPERIMENT 3

4.1 Introduction

The third experiment represents a further attempt to test the hypothesis that the tonic presence of norepinephrine underlies the inhibitory action of norepinephrine on kindling development proposed by norepinephrine depletion studies. As the 5 ug/ul infusion of norepinephrine proved insufficient to significantly elevate norepinephrine levels in the previous study, the concentration of norepinephrine to be infused by the osmotic minipumps was doubled. To reduce the probability of blocking, minipumps were loaded and incubated before implantation to ensure a steady flow rate at the time of placement. Finally, sample sizes were increased and DSP-4 and saline non-infusion groups were added as necessary within experiment controls.

4.2 Method

The methods were unchanged unless otherwise indicated. A summary follows.

Fifty-three animals (190-280 g at the time of injection) were randomly assigned to 1 of 4 groups; DSP-4 alone (N=13), saline alone (N=14), DSP-4 and norepinephrine infusion (N=13), and DSP-4 and vehicle infusion (N=13).

Surgery was performed 7 to 10 days following the injection of CGP-6085A (all groups), and DSP-4 (DSP-4 group and two infusion groups) or the saline vehicle (saline group). The first two groups were implanted with an electrode aimed at the basolateral nucleus of the amygdala, while the second two groups were implanted with both an amygdaloid electrode and a cannula placed into the lateral ventricle. Since electrode placements centered around the dorsal aspect, or just above, the basolateral nucleus in the previous two experiments, the electrode was lowered to 8.6 mm ventral from skull instead of 8.4 mm. All other coordinates were unchanged. A male Amphenol connector pin attached to one of the screws securing the dental cement to the head was added and connected to the cable shielding, already grounded, during each kindling session.

The animals started the 4 day handling and 1 day habituation session seven to ten days after surgery, prior to threshold testing or minipump implantation. The minipumps were filled with a Ringers solution of 20 ug/ul norepinephrine and 0.1% (w/v) ascorbic acid, or the Ringers/ascorbic acid solution alone. At a concentration of 20 ug/ul, the pump delivered 10 ug/0.5 ul/hr of norepinephrine icv. The pumps were incubated for 4 hours previous to implantation in a saline solution kept at 37 degrees centigrade. Kindling was performed until two stage five seizures were produced, and animals in all groups were

decapitated 1 to 4 hours after the last seizure. The duration of the first AD following threshold testing was added to the list of kindling parameters measured.

4.3 Results and Discussion

Seventy percent of the electrode placements were directly in the basolateral nucleus of the amygdala, while twenty percent were within 1 mm (fig. 2, pg 64). One electrode was deflected upon insertion outside the amygdala resulting in a tip location at the hippocampal-cortical boundary. Since no AD was elicited during threshold testing and three subsequent kindling attempts, the animal was rejected. Upon examination of electrode placements, there appeared to be no correlation between electrode location and the animals rate of kindling.

One animal died 7 days following DSP-4 treatment, and 4 animals died while under anesthesia during the stereotaxic surgery, 3 of them having had the DSP-4 treatment. Two animals died under anesthesia during minipump surgery, 1 of them having had the DSP-4 treatment. Three animals had faulty electrodes as indicated by flat recordings. Finally, 2 animals which had had DSP-4 treatment were not used as they were clearly underweight and fragile in appearance. All other animals appeared healthy and well groomed.

The results are summarized in table 3 (pg. 65).

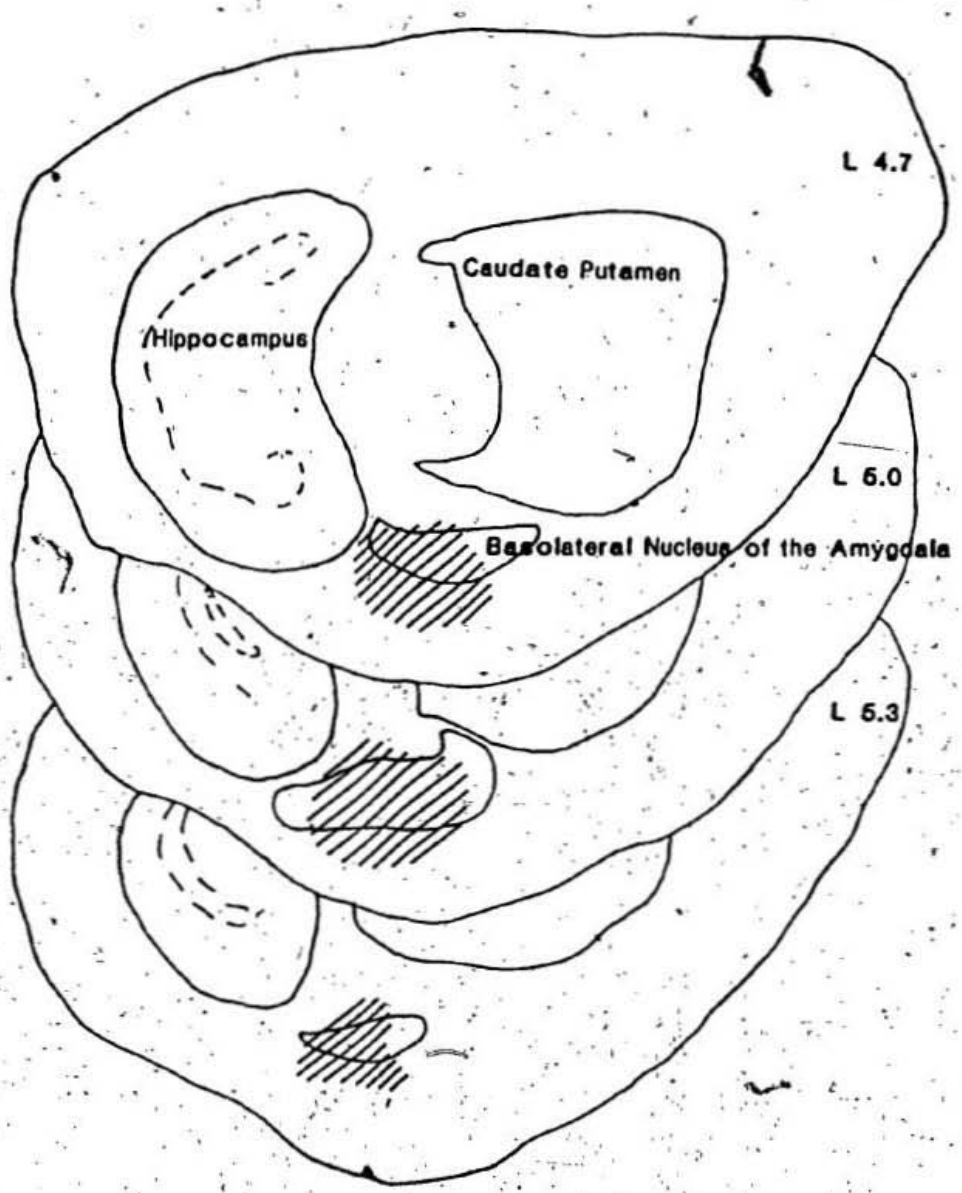

FIG. 2. DISTRIBUTION OF ELECTRODE PLACEMENTS.

Camera lucida drawings in the sagittal plane.

TABLE #3: EXPERIMENT 3 -SUMMARY TABLE

Dependent Variable	Experimental Group			
	DSP-4	Saline	NE	Vehicle
AD Threshold (uA)				
Mean	35.0	50.0	60.0	47.5
Standard Error	4.0	7.2	12.7	14.3
N	10	12	10	8
Length of First AD (sec)				
Mean	19.4	11.8	23.0	36.4
Standard Error	3.5	1.1	3.4	11.8
N	10	8	9	7
# of AD's to Stage-5				
Mean	5.5	7.3	5.7	4.9
Standard Error	.98	.70	.97	.97
N	10	12	10	8
Motor Seizure Duration(sec)				
Mean	32.2	37.5	52.6	52.4
Standard Error	3.2	4.2	6.7	9.6
N	9	12	10	7
AD Duration, Stage-5 (sec)				
Mean	27.5	38.1	55.6	66.0
Standard Error	3.1	3.9	6.0	11.7
N	6	11	10	6
Latency to Clonus (sec)				
Mean	7.8	10.3	23.7	17.8
Standard Error	1.8	4.8	6.5	4.4
N	9	12	10	8
NE Concentration (ug/g)				
Mean	.387	.463	.474	.465
Standard Error	.086	.059	.029	.042
N	9	10	10	8
DA Concentration (ug/g)				
Mean	3.08	3.05	4.61	4.28
Standard Error	.58	.41	.63	.42
N	9	10	10	8
5-HT Concentration (ug/g)				
Mean	1.37	1.15	1.67	1.32
Standard Error	.12	.11	.14	.19
N	9	10	10	8

Analysis of variance revealed a significant group effect on the first AD duration ($F(3,30)=2.97$, $<.05$). A post hoc Tukey test indicated that animals receiving the vehicle infusion displayed longer ADs than the saline treated group. There was a significant group effect on the AD duration of the first stage five seizure ($F(3,29)=6.27$, $p<.005$). Both infusion groups displayed longer stage five ADs than the DSP-4 group, and the vehicle infusion group displayed longer AD durations than the saline group as well.

Taken together these results indicate that either the process of cannulation (insertion, under anesthesia and subcutaneously, of a minipump attached to a cannula inserted into a lateral cerebral ventricle two days prior to kindling), or the infusion of the Ringers/ascorbic acid vehicle can lengthen the AD duration initiated by a kindling stimulus. AD duration does not appear to be related to the severity of the kindling induced seizure or to the susceptibility of the animal to the kindling process. Long AD may be indicative of a high degree of hippocampal activity for Engel et al., (1978) demonstrated an association of increased 2-deoxyglucose uptake in the hippocampus with AD duration and not seizure stage during amygdaloid kindling. They suggested that an alternate system exists for the generation of local AD that may be independent of both partial and generalized seizure-generating mechanisms. However, Le Gal La Salle (1981)

found that rats that initially respond to amygdaloid stimulation by long ADs tend to kindle faster than those that respond by short ADs. It is unclear how cannulation or the vehicle may account for these results, especially when the infused groups of experiment two did not differ from the non-infused groups of experiment one on AD duration.

No group effects were found on any of the other kindling parameters, or on neurotransmitter levels. This is somewhat surprising given the DSP-4 effects demonstrated in experiment one and two. The raw data indicate a clear dichotomy in the distribution of scores on the two variables of primary interest: the number of ADs to kindle, and the norepinephrine concentration in the DSP-4 group (see fig. 3 and 4, pg. 68 and 69). A Fisher exact test was performed on these two variables between groups using the lowest score in the saline group as the criterion cutoff point, on the basis that the saline group represents a range of scores representative of intact animals. This was a value of 5 for ADs to stage 5 and .29 ug/g for norepinephrine concentration. With respect to the former variable, the DSP-4 group ($p<.01$), norepinephrine infusion group ($p<.005$), and vehicle infusion group ($p<.005$) all differed significantly from the saline group, suggesting a significant acceleration of kindling due to DSP-4 treatment. Norepinephrine levels were significantly lower in the DSP-4 group ($p<.005$) but not in the two infusion groups when

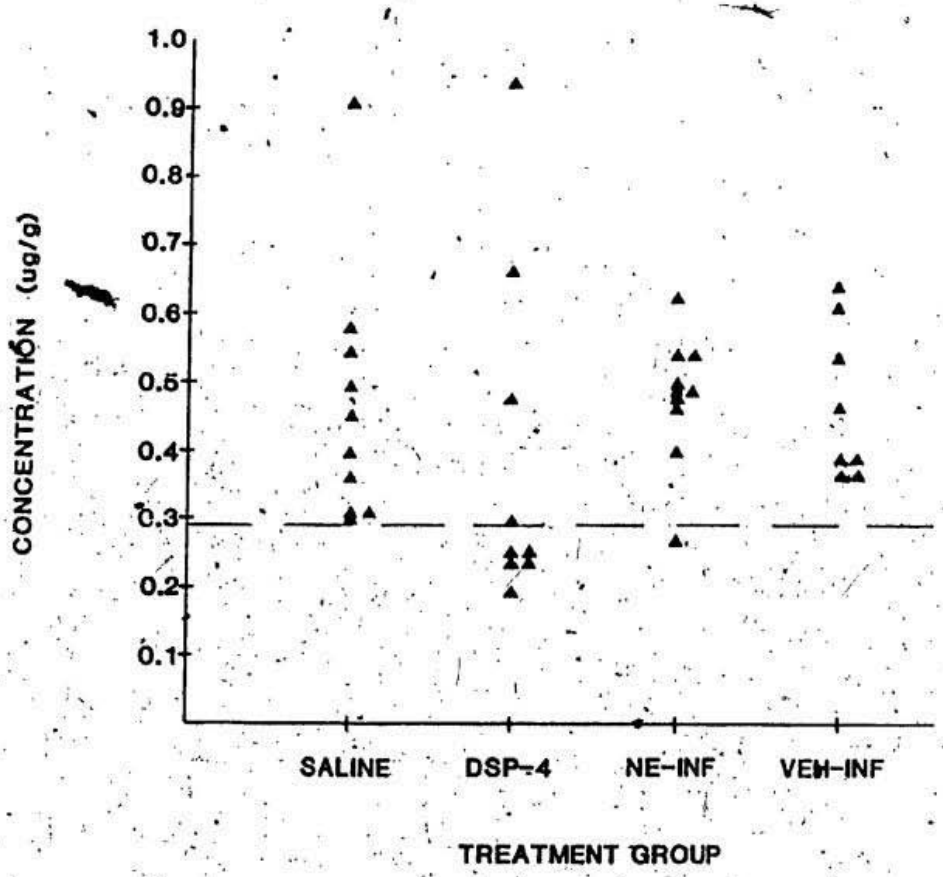

FIG. 3. DISTRIBUTION OF SCORES ON NOREPINEPHRINE LEVELS.

Dashed line indicates cutoff point for Fisher exact test.

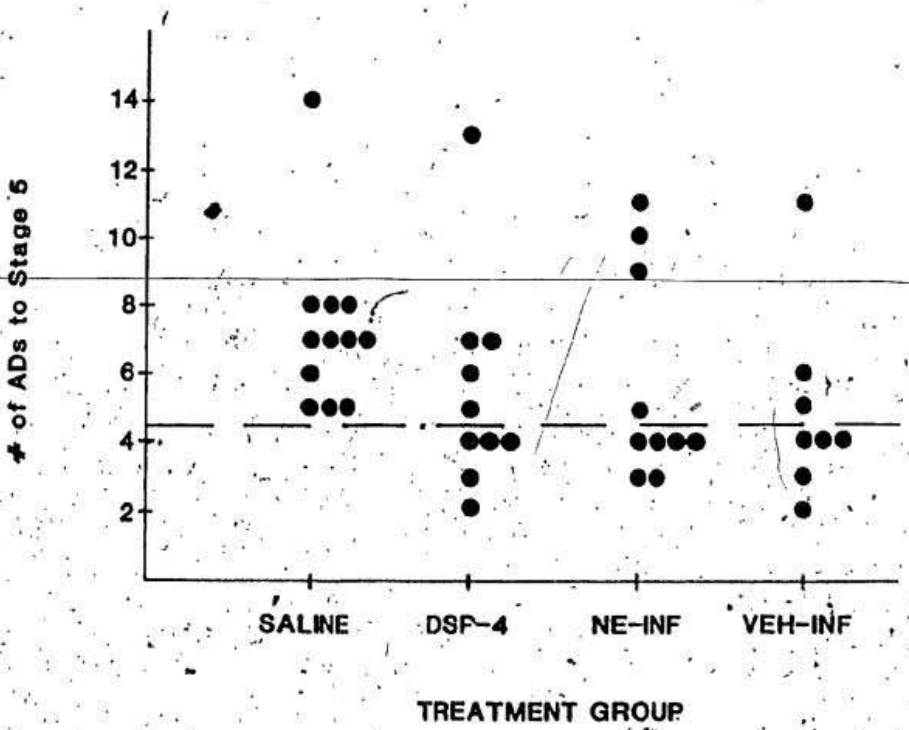

FIG. 4. DISTRIBUTION OF SCORES ON KINDLING RATE.

Dashed line indicates cutoff point for Fisher exact test.

compared to the saline group. Four of the five animals that kindled in less than 5 AD's had norepinephrine concentrations below the .29 ug/g cutoff point.

Norepinephrine levels were raised by minipump infusion of norepinephrine to levels equivalent to that of the saline group, but this result is confounded by the fact that norepinephrine levels in the vehicle group were also raised to that of the saline group. Norepinephrine levels after norepinephrine or vehicle infusion, .474 and .465 ug/g respectively, were indistinguishable from each other and the saline treated animals (.463 ug/g). This result is hard to reconcile considering the success of Biswas and Jonsson (1981) in raising norepinephrine levels in the cortex using half the concentration of norepinephrine infused. One possible explanation is that their choice of cerebral cortical tissue assayed was adjacent to the subarachnoid space at which the level of norepinephrine would be high. A large telencephalic block such as the one assayed here may mask possible increases in periventricular tissue.

An acceleration of kindling in both infusion groups without a concomitant decrease in norepinephrine levels at the time of sacrifice suggests at least three possibilities: 1) that norepinephrine levels were low initially but increased over the course of kindling, 2) that DSP-4 treatment may induce acceleration of kindling by a mechanism independent of norepinephrine depletion, and 3) that the

cannulation/infusion procedure may itself act to accelerate kindling regardless of norepinephrine levels.

It is postulated that norepinephrine levels were depleted at the time of kindling initiation, but increased by the time the animals were decapitated (2-3 hr following the second stage five seizure). This explanation would account for the accelerated kindling rate, given an initial depletion of norepinephrine, and yet satisfy both the hypothesis that norepinephrine serves to inhibit the kindling process, and the fact that norepinephrine levels were high at the time of animal sacrifice. Given the fact that the severe reduction of norepinephrine levels following DSP-4 treatment remains unchanged for at least 8 weeks (Swann, 1984), and that animals in experiment 3 were sacrificed on average within 30 days following DSP-4 treatment, this explanation seems unlikely. Since the postulated compensatory mechanisms did not occur in those animals receiving DSP-4 treatment alone, it must be assumed that these mechanisms were triggered by the cannulation/infusion procedure employed in the two infusion groups. Furthermore, since norepinephrine levels of the two infusion groups in experiment 2 were lower than saline controls, it must be assumed that procedural differences in experiment three accounted for the raise in norepinephrine levels. There were critical time and age differences between the two experiments. The rats in experiment three were younger at

the initiation of the experiment (235 g vs 400 g), and the time difference between injection and kindling was much shorter (22 days vs 52 days).

The sprouting of noradrenergic fibers is one mechanism that could be responsible for reinstating norepinephrine levels. One form of sprouting, the lesion-induced sympathetic sprouting of peripheral noradrenergic fibers to forebrain sites, occurs within a time frame consistent with that of experiment 3 (2 to 6 weeks; Madison and Davis, 1983), but seems to be induced by destruction of cholinergic neurons and not noradrenergic neurons (Crutcher, 1982). Another form of sprouting, the lesion-induced compensatory sprouting of homotypic central neurons, could also account for increased norepinephrine levels (see Cotman and Nieto-Sampedro, 1982). This response appears to have two distinct stages, a typically rapid onset (starting within 4-5 days after the lesion) and a fairly short duration (waning within a few weeks) characterizes the first stage, termed reactive synaptogenesis. A second stage, termed compensatory collateral sprouting, is characterized by a slow onset (1 to 3 months after lesion) and a long duration (for at least 6 months) (Gage et al., 1983a; 1983b). Considering the time frame, reactive synaptogenesis could have occurred in those younger animals of experiment 3, by locus coeruleus neurons spared of DSP-4 effects or by noradrenergic neurons originating in the lateral tegmental

area. Although it is possible that the cannulation/infusion procedure induced the sprouting response, this seems unlikely since it did not appear to occur in experiment 2. The younger animals of experiment three may have been more susceptible to cannulation/infusion-induced compensatory mechanisms. It has been reported that both sympathetic sprouting and reactive synaptogenesis declines with age (Scheff et al., 1978). Although ascorbic acid has been reported to affect dopaminergic neurotransmission (Hadjiconstantinou and Neff, 1983) there is no evidence that it may affect noradrenergic mechanisms, let alone produce a recovery of norepinephrine levels following DSP-4 treatment.

Another possible mechanism by which norepinephrine repletion could have occurred in the infused rats of experiment 3 is the phenomenon of accumulation that follows the destruction of noradrenergic nerve terminals. Central monoamine depletion is accompanied by a temporary, localized, amine accumulation in the degenerating axons proximal to the lesion site (see Willis and Smith, 1985). Accumulation of norepinephrine in degenerating locus coeruleus fibers has been observed within 1 to 4 days following 6-OHDA treatment or electrolytic lesion of noradrenergic fibers, and occurs from telencephalic sites to distances half way to their cell bodies (Chiba et al., 1979; Richardson and Jacobowitz, 1973). These swollen fibers have been observed as long as 70 days after 6-OHDA treatment, but

at reduced concentrations (Richardson and Jacobowitz, 1973). Since degenerating neurons are retracted from their terminal fields, biochemical assays restricted to terminal fields will not detect the amine buildup (Willis et al., 1984). Accumulation has been observed in the DNB when examined 1 week after DSP-4 treatment (Jonsson et al., 1981). However, there is no evidence to date that any aspect of the cannulation/infusion procedure (eg. surgical stress or ascorbic acid) would enhance or protract the lesion-induced accumulation of norepinephrine, in order to explain why it occurred in infused animals and not in animals treated with DSP-4 alone. Assuming that the cannulation/infusion procedure could enhance norepinephrine accumulation, a shorter time period between DSP-4 administration and animal sacrifice in experiment 3 may account for why these animals appeared to have normal norepinephrine levels, as opposed to those animals in experiment 2.

The second proposal states that DSP-4 accelerates kindling by a mechanism other than norepinephrine depletion; however it seems unlikely considering the specific action of DSP-4 on locus coeruleus neurons outlined in chapter 2. DSP-4 treatment could prekindle brain sites whose recruitment is necessary for the expression of a full motor seizure induced by amygdaloid kindling, thereby facilitating the kindling process. Had this occurred at the amygdala, a decrease in AD threshold should have been detected, since

repetitive stimulation, or kindling, lowers the threshold for evoking an AD (Racine, 1972a). There was no difference between the AD threshold of DSP-4 treated animals and their controls. DSP-4 treatment could still have caused prekindling of sites secondary to subsequent amygdaloid kindling, thereby facilitating the generation of full motor seizures. It would be necessary to record from these sites during DSP-4 administration to answer this question. There is no indication in the literature whether this has been done for 6-OHDA treatment.

The third proposal explaining kindling acceleration without a concomitant decrease in norepinephrine levels is that the cannulation/infusion procedure may accelerate kindling regardless of norepinephrine levels. This proposal suggests multiple mechanisms involved in the facilitation of the kindling process, which includes, but not necessarily, norepinephrine depletion. Ascorbic acid has antagonized the proconvulsant and anticonvulsant effects of apomorphine administration depending on the time and dosage of apomorphine administration (Wilcox et al., 1984). Given the complex pattern of action in conjunction with apomorphine administration it is difficult to extrapolate to the results found here. More to the point, McIntyre et al. (1979) used vehicle controls when studying the effects of icv 6-OHDA on amygdaloid kindling. Either the vehicle (0.5% ascorbic acid and saline) or 6-OHDA were injected one week prior to

kindling. 6-OHDA pretreatment, but not that of the vehicle alone, accelerated amygdaloid kindling. After 6 stage 5 convulsions activated in the 'primary' amygdala, all animals were kindled in the secondary amygdala on the other side. 6-OHDA treated animals kindled faster than all control groups except that of the vehicle control. Although there was no indication that the vehicle group kindled faster than the other control groups, it implies that ascorbic acid may have accelerated secondary kindling. Given that 5 ul of a 0.5% solution was given 1 week prior to primary kindling, the significance of such an effect is all the more relevant considering that 0.5 ul/hr of a 0.1% ascorbic acid solution was infused continuously throughout the kindling procedure in this experiment.

It remains unclear why ascorbic acid would affect kindling. A 4 ul acute injection of 1% ascorbic acid into the medial forebrain bundle has produced a significant depletion of striatal dopamine but did not effect cortical norepinephrine levels (Waddington and Crow, 1979). Dopamine measures were not affected by the ascorbic acid vehicle in this experiment. Ascorbic acid, a cofactor in dopamine metabolism to norepinephrine, can differentially modulate dopamine agonist and antagonist binding sites in the striatum (Hadjiconstantinou and Neff, 1983), and increase the unit activity recorded in the striatum (Ewing et. al., 1983). Although it is clear that ascorbic acid is not an

inert substance in terms of brain function, it is unclear how it may act on kindling. Additional control groups (i.e., norepinephrine/vehicle infused non DSP-4 treated animals) could have resolved the issue.

The use of methoxyflurane as the anesthetic for minipump surgery 2 days prior to kindling is another possible mechanism for kindling acceleration. Although there is no evidence in the literature regarding a proconvulsant action of fluorinated hydrocarbons, it cannot be ruled out. Fluorinated anesthetics, or its metabolites have been observed in the intact rabbit brain at least 98 hr after administration (Wyrwicz et al., 1983), indicating that the methoxyflurane or its metabolites would have been present at the time kindling was initiated.

Another mechanism by which kindling could have been accelerated is the added stress inherent in the minipump surgery applied 2 days before kindling started, and the stress inherent in the continuous presence of the minipump and the dental cement cap. Furthermore, Archer et al., (1984b) indicated that DSP-4 treatment produces a rat that is maladaptive and more susceptible to stress-induced effects on behavior. However, stress of another form has inhibited the development of the kindled seizure. Arnold et al., (1973) demonstrated stressed rats (unhandled prior to kindling) kindled slower than unstressed rats (handled for two weeks prior to kindling). However, stress induced by

footshock prior to each kindling stimulus was found not to affect the rate of amygdaloid kindling acquisition (Grahnstedt and Ellertsen, 1984). Footshock stress has had different effects on the expression of the kindled seizure. Footshock prior to stimulation of the kindled amygdaloid focus has inhibited the occurrence of kindled seizures (Shavit et al., 1984), or enhanced seizure duration and intensity (Grahnstedt and Ellertsen, 1984). It appears that the form of footshock-induced stress may affect the expression of the kindled seizure in different ways. Stress has been observed to potentiate the epileptiform effects of chemically-induced kindling. Epileptiform spiking and convulsions induced by infusion of morphine or beta-endorphin can be potentiated by handling, immobilization, or conspecific threat (Cain and Corcoran, 1984). Since various forms of stress to date have produced an inhibition, or had no effect at all on the rate of acquisition of the kindled seizure, it seems unlikely that the stress inherent in the cannulation/infusion procedure could have accelerated kindling.

In conclusion, parametric statistics were insensitive to the DSP-4 effects on kindling rate and norepinephrine levels in experiment 3 due to marked within group variability in DSP-4 treated rats. The Fisher exact test however indicated that all DSP-4 treated groups had significant proportions of animals which rapidly kindled.

Furthermore, the group receiving DSP-4 alone had a significant proportion of animals having low norepinephrine levels. This was not true for the infusion groups. Neither infusion group differed from the saline group in norepinephrine levels, more specifically the norepinephrine-infusion group had levels equivalent to, not higher than, vehicle-infused rats. These results imply that initial abnormalities induced by the noradrenergic neurotoxin were reversed during kindling, that cannulation and/or ascorbic acid infusion may accelerate kindling independently of norepinephrine levels, or that norepinephrine depletion may not be necessary for kindling acceleration induced by the noradrenergic neurotoxin, DSP-4.

CHAPTER 5: GENERAL DISCUSSION

The principal objectives of this thesis were to demonstrate: 1) that norepinephrine depletion through DSP-4 treatment accelerates amygdaloid kindling, and 2) that repletion of norepinephrine antagonizes the depletion-induced acceleration. The results of experiment 1 confirm those of previous studies regarding the proconvulsant effects of norepinephrine depletion in rats on electrical stimulation of the amygdala. DSP-4 treatment accelerated kindling by 50 % while significantly depleting telencephalic norepinephrine by 45 %. Dopamine and 5-hydroxytryptamine levels were unaffected. Furthermore, the facilitation induced by DSP-4 treatment was not a function of an enhanced local seizure susceptibility, as saline and DSP-4 treated rats did not differ in the threshold for afterdischarge. These results are consistent with earlier studies employing 6-OHDA or lesions of ascending noradrenergic bundles on amygdaloid kindling (Araki et al., 1983a: Arnold et al., 1973; Corcoran et al., 1974, 1980: McIntyre, 1980: McIntyre et al., 1979: Mohr and Corcoran, 1981), hippocampal kindling (Araki et al., 1983a: McIntyre and Edson, 1982) and neocortical kindling (Altman and Corcoran, 1983).

DSP-4 treatment increased the latency of onset to clonus during the stage 5 motor seizure in experiment 1. An

increased latency to onset has been produced by icv 6-OHDA pretreatment in both adults and infants during stage 5 amygdaloid kindled seizures (McIntyre et al., 1979). Acute DMI treatment, which inhibits kindling development by prolonging norepinephrine availability at the synapse, produces short latencies to clonus during the stage 5 seizure (McIntyre et al., 1982). Along with an inhibitory action on the kindling process, both studies suggest that norepinephrine acts to suppress the expression of preclonus motor signs during fully generalized seizures. Sanberg and Ossenkopp (1978) found that fast kindlers characteristically showed shorter latencies to clonic convulsions. Paradoxically, in experiment 1, norepinephrine depletion produced faster kindling and longer latencies. However, the onset of latency to clonus during amygdaloid kindling was not affected by intracerebral injections of 6-OHDA into ascending noradrenergic fibers (Mohr and Corcoran, 1981) or by local 6-OHDA-induced norepinephrine depletion in the amygdala (McIntyre, 1980), suggesting that depletion of norepinephrine at sites other than the forebrain may be responsible for the latency effects. This interpretation is consistent with DSP-4 induced depletion in non-forebrain sites, such as the cerebellum and spinal cord (Jonsson et al., 1981; Dooley et al., 1983). Furthermore, icv 6-OHDA treatment did not affect the latency to onset of clonus in generalized motor seizures produced by hippocampal kindling

(McIntyre and Edson, 1982). Together, these studies indicate the labile nature of this phenomenon, and suggest that it may be restricted to amygdaloid kindling and depletion of norepinephrine at non-forebrain sites.

Interpretation of these results could be strengthened if more was known about the selectivity and action of DSP-4. Since the amount of norepinephrine depletion in areas innervated by noradrenergic neurons is positively correlated to the percentage of norepinephrine originating from the locus coeruleus, then DSP-4 action in the CNS is presumably directed at locus coeruleus neurons, and not at noradrenergic neurons in general (Jonsson et al., 1981). The results presented here, along with those of Araki et al. (1983a), who accelerated amygdaloid kindling through selective lesioning of the DNB, indicate that full or partial lesions of noradrenergic neurons originating in the lateral tegmental area are not necessary towards enhancing the kindling process.

The selectivity of DSP-4 to locus coeruleus neurons may also account for the relatively small reduction of norepinephrine (45%) measured in the telencephalon. The lateral tegmental area provides a major source of norepinephrine to the septal area (Moore, 1978), and a minor source to the amygdala, suprarhinal cortex, entorhinal cortex, and piriform cortex (Fallon et al., 1978; Fallon and Moore, 1978), all of which would have been included in the

telencephalic block of tissue. In comparison, lesions of the locus coeruleus have been shown to lower telencephalic norepinephrine by only 37% (Sessions et al., 1976).

Intraventricular infusion of norepinephrine (5 ug/hr) failed to raise telencephalic norepinephrine levels above that in vehicle controls following DSP-4 treatment (experiment 2). Although histological verification confirmed placement of cannula tips into the ventricles, this method of verification may be unreliable (Myers, 1977); therefore cannula placements may not have been as accurate as originally indicated. Furthermore, intraventricular norepinephrine is accumulated and stored by catecholaminergic neurons (Aghajanian and Bloom, 1967: Glowinski et al., 1965: Reivich and Glowinski, 1967), protecting norepinephrine from extracellular enzymatic degradation (Glowinski and Iversen, 1966). Destruction of locus coeruleus terminals through DSP-4 treatment would presumably reduce the amount of icv norepinephrine accumulated and hence, facilitate degradation. The restricted spread of diffusion from ventricular sites is another possible explanation for the inability of the chronic infusion of norepinephrine to raise norepinephrine levels. ICV norepinephrine has been found to penetrate periventricular tissue no further than 500 um (Schubert and Ladisich, 1969). Possible increases in norepinephrine levels in periventricular tissue could have been masked by the large tissue

block assayed.

The inability to raise norepinephrine levels was reflected in the lack of a difference between the two infusion groups on any kindling variable measured, including kindling rate. However, their combined kindling rate was faster than that of the saline group (experiment 1). This replicates the observation that kindling is accelerated by DSP-4 induced norepinephrine depletion. The slightly higher norepinephrine levels in the two infusion groups (as opposed to the DSP-4 non-infused group) may account for the fact that the latency of the onset to clonus during the first stage 5 seizure was not significantly longer than that of the saline group of experiment 1.

Experiment 3 represented a further attempt to inhibit DSP-4 induced kindling acceleration through chronic icv norepinephrine infusion. The concentration of norepinephrine was doubled from experiment 2 and the saline and DSP-4 groups were replicated from experiment 1. Despite a marked amount of within group variance, DSP-4 treatment reduced norepinephrine levels and increased the kindling rate from saline controls. Contrary to results from experiment 1, the latency to onset of clonus during the first stage 5 seizure was not increased by DSP-4 treatment. Taken together DSP-4 treatment consistently accelerates kindling; however, the tendency to display long onset times for clonic convulsions is far from robust.

Clearly the infusion of norepinephrine failed to elevate norepinephrine levels in the telencephalon above vehicle controls; however, both infusion groups had norepinephrine levels indistinguishable from saline treated animals (despite DSP-4 pretreatment). Futhermore, the infused groups displayed faster kindling rates than the saline treated animals, despite normal norepinephrine levels. This implies that: 1) initial abnormalities induced by the noradrenergic neurotoxin were reversed during kindling, 2) the cannulation/infusion procedure may accelerate kindling regardless of norepinephrine levels, or 3) norepinephrine depletion may not be necessary for kindling acceleration induced by the noradrenergic neurotoxin, DSP-4.

In summary, pretreatment with DSP-4, a noradrenergic neurotoxin whose action is reportedly specific to locus coeruleus terminals, consistently accelerated the acquisition of amygdaloid kindled seizures. The method of chronic infusion of norepinephrine through osmotic minipumps was employed in an attempt to provide evidence that correction of DSP-4-induced depletion of norepinephrine inhibits the depletion-induced kindling acceleration. This method failed to replete norepinephrine levels when compared to the vehicle controls. In one experiment, however, the infusion of the norepinephrine solution or the vehicle alone, following DSP-4 treatment, produced norepinephrine

levels equivalent to the saline control, without affecting kindling rate. The lack of non-DSP-4 treated infused controls made interpretation of this result difficult and inconclusive. Therefore, the question of repletion-induced effects on kindling rate was left unanswered. This question needs to be answered in order to determine conclusively whether the hypothesis that CNS norepinephrine acts to suppress kindling development, is correct. Otherwise, the effects of the neurotoxic or electrolytic lesions could be attributed to non-specific actions (e.g., actions on neural activity mediated by other neurotransmitters). For example, it has been recently determined that two related neuropeptides, vasopressin and neurophysin, coexist with norepinephrine in locus coeruleus cell bodies (Caffe and van Leeuwen, 1983; Caffe et al., 1985). Consequently, lesions of locus coeruleus fibers would erase not only norepinephrine action, but also the possible neuroactive action of these peptides as well. These peptides may act alone or in concert with norepinephrine to inhibit kindling development.

The transplantation of fetal locus coeruleus cells into the adult CNS may provide an alternative method (Bjorklund et al., 1979). Suspensions of these cells could be injected into multiple forebrain sites of norepinephrine depleted animals (Bjorklund et al., 1984). After a period of 2-3 months, allowing for these cells to functionally

innervate surrounding tissue, the kindling rate of these animals could be tested (against sham controls). Should this method prolong the acquisition of a kindled seizure, noradrenergic antagonists, and the relevant neuropeptide antibodies, could be used to isolate the key agents in kindling control. Although this method would entail many experimental hours (i.e., a large number of animals would have to be run in order to produce groups of animals displaying the same fiber distribution), isolation of the demonstrated inhibitory mechanism of kindling development would provide valuable information. Such information regarding neurotransmitter modulation and control of seizure development may lead to novel forms of treatment for epilepsy.

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