EFFECT OF DEPLETION OF NORADRENALINE ON CORTICAL KINDLING

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IRWIN M. ALTMAN

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We accept this thesis as conforming
to the required standard

[redacted]

Dr. Michael E. Corcoran

[redacted]

Dr. Louis D. Costa

[redacted]

Dr. Terry W. Pearson

Supervisor: Dr. Michael E. Corcoran

ABSTRACT

The role of forebrain noradrenaline in seizures induced by electrical stimulation of the anterior neocortex (kindling) was investigated in control rats and rats pretreated with bilateral injections of 6-hydroxydopamine (6-OHDA) into the mesencephalic trajectory of the dorsal tegmental noradrenergic bundle. Extensive depletion of forebrain noradrenaline (arbitrarily defined as greater than 80%) significantly facilitated the rate of kindling of cortico-generalized seizures. In contrast, a group of 6-OHDA-treated rats that showed minimal depletion of noradrenaline (arbitrarily defined as 80% or less) appeared to develop generalized seizures at a slower rate than controls.

The present findings extend previous observations that 6-OHDA-induced depletion of noradrenaline facilitates amygdaloid kindling, by demonstrating that cortical kindling is similarly affected.

Dr. Michael E. Corcoran _____

Dr. Louis D. Costa _____

Dr. Terry W. Pearson _____

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DEDICATION

This thesis is dedicated to my loving parents who provided me with the opportunities that they never had.

INTRODUCTION

"Kindling" (Goddard, 1967; Goddard, McIntyre & Leech, 1969) refers to a model of epilepsy in which repeated electrical stimulation of certain areas of the brain may trigger epileptiform afterdischarges (ADs). Racine (1978) defined kindling as, " ... a progressive increase in neural responsivity produced by spaced and repeated epileptogenic stimulation of certain brain structures" (p. 234). When the ADs are triggered repeatedly and spaced in time, a number of changes occur in the electrographic and behavioural response (Goddard et al., 1969; Racine, 1972a,b). These changes include increases in the duration, spike amplitude, and spike frequency of the ADs and propogation of the activity to other brain sites coincident with the development of clinical convulsions. Decreases in the threshold of the ADs and an increases in the strength of the convulsion are also observed (Racine, 1972a,b).

A reversal of kindling has not been reported; rather it has been observed that its effects are long lasting (Goddard et al., 1969; Racine, 1978; Wada & Sato, 1974). Indeed, spontaneously evoked seizures in kindled animals have been reported (Pinel, Mucha & Phillips, 1975; Wada, Sato & Corcoran, 1974).

The appeal of kindling as a model of epilepsy to neuroscientists is twofold. First, unlike with other models such as chemical lesions, greater control can be exercised over site of stimulation, and the frequency and intensity of stimulation can be precisely graded. Second, it is one of the few models of neural plasticity that involves a permanent and stable change in brain function without any apparent tissue damage. A number of studies using a variety of techniques have supported the latter contention. For example, using the Nissl stain, Goddard et al. (1969) reported that the tissue surrounding the tip of the stimulating electrode was not damaged.

Attempts to find structural changes in kindled as compared to nonkindled tissue have either failed or have produced ambiguous results. Racine, Tuff and Zaide (1975) used the Golgi-Cox technique and found no changes in dendritic spine size or density in the cortex of cortically kindled rats. At the ultrastructural level, Goddard and Douglas (1975) reported increases in synaptic terminal size in the amygdala of amygdaloid kindled rats, but qualified these results by identifying a confounding variable (i.e., the control vs kindled groups differed in electrode placement). However, they found no gross abnormality in the synaptic morphology of stimulated neurons. Racine and Zaide (1978) found an increase in the size of synaptic terminals

in kindled as compared to nonkindled rats, but again no degeneration was reported.

For the most part, the early work that was carried out on kindling in the rat focused on limbic sites, in particular the amygdala (Goddard et al., 1969; Racine, 1972a,b). Goddard et al. (1969) noted, however, that a small area of the anterior dorsal cortex was very responsive. They referred to it as the "anterior limbic field." Racine (1975) showed that limbic forebrain and paleocortical sites responded similarly to kindling, but that neocortical sites responded differently.

Burnham (1978) described the extent to which "limbic-type" and "cortical-type" responses differ. When stimulation is initially applied to limbic sites, short localized ADs are produced. Convulsions do not accompany these ADs and often no change in the rat's behaviour is observed (Burnham 1978; Goddard et al., 1969; Racine, 1972a). Early stimulation of the anterior neocortex also produces brief, simple ADs. These ADs are somewhat similar to the early ADs triggered from limbic sites, although they are shorter in duration (Burnham, 1978). Every AD triggered by stimulation of the anterior neocortex is, however, accompanied by a convulsion that exactly matches it in duration (Burnham, 1978; Racine, 1975).

The patterns of development of generalized clinical convulsions also differ in these two types of kindling. Limbic kindling begins with brief focal ADs that later become longer and partially generalized convulsions that may be coupled with immobility and stereotyped facial movements. In time, fully generalized convulsions with bilateral clonic movements involving the whole body are observed. Finally, a characteristic rearing and falling pattern emerges typically referred to as a stage-5 convulsion (Burnham, 1978; Goddard et al., 1969; Racine, 1972a,b).

The very different pattern of seizure development in the anterior neocortex is best seen at near-threshold stimulation levels. As noted above, even the first AD is accompanied by a convulsion. Convulsions are usually mild and involve mouth movements and myoclonic contractions of the forelimbs (C1). If stimulation is repeated, the predominantly clonic convulsion (C1) takes on a clonic-tonic form (C1+T). This is seen as an immediate loss of postural control and extension of the forelimbs and head (episthotonus-like). The development then proceeds to include a late clonic stage, thus giving the overall convulsion a tripartite form (C1+T+C2). All of the above takes place in approximately the first 15 days of stimulation, before any growth in AD duration occurs (Burnham, 1978; Racine, 1975).

The pattern of growth of AD duration also seems to differ between the two types of kindling. Stimulation of limbic sites produces AD growth that tends to take place in sudden increments (see Fig. 1A; Burnham, 1975). The achievement of long "mature" electrographic seizures is roughly correlated with the onset of generalized "rearing-falling" convulsions (Burnham, 1975,1978). In cortico-generalized seizures, AD durations grow slowly and gradually except for one large step at the end (see Fig. 1B; Burnham, 1978). As AD growth progresses, the late clonic phase of the convulsion (C2) grows as well, keeping pace with the AD development. Thus, C2 lasts longer and longer, gradually developing into a full-scale stage-5 convulsion (Racine, 1972b) **identical** to those triggered from limbic sites. This growth of convulsive activity keeps pace with the growth of AD duration up to a length of 20- 30 seconds, at which point a final large growth in AD duration occurs unaccompanied by a change in the duration of the convulsion. Propogation of AD outside the cortex is increased at this stage, and Burnham (1975) referred to this later phase of cortical kindling as the "cortico-generalized response."

There are two other characteristics of cortical kindling that differentiate it from limbic kindling. First, some cortically stimulated rats do not seem to generalize at all and continue to exhibit only focal seizures (Burnham,

1978). Second, a few cortically stimulated rats display one generalized seizure in the first week, after which seizures regress to the focal level. To develop stable cortico-generalized seizures, a large number of subsequent daily stimulations are then needed (Burnham, 1978).

Mature cortico-generalized seizures are nearly identical to limbic generalized seizures. For example, the duration of the AD is approximately equal in the two groups, and the convulsions consist predominantly of rearing and falling in both. A period of postictal hyperarousability, initially reported by Racine (1972b) with limbic generalized seizures, is also observed after cortico-generalized seizures. In addition, postictal spikes are observed in the EEG.

There does however seem to be one distinct difference between the two types of seizure: the brief period of tonus observed at the start of the cortico- generalized seizure. Occasionally, some clonus (C1) is seen at the start of cortico-generalized seizures (limbic kindled subjects usually exhibit facial automatisms) but this is often concealed by the tonic phase, which has a very short latency in mature seizures.

As mentioned above, kindling does not result from edema, gliosis, poisoning or tissue damage at the tip of the stimulating electrode. It has been hypothesized that

kindling involves a permanent transsynaptic change in brain function, and a great deal of work has been done to identify the neurotransmitters involved. Much of the attention in this area has been paid to the catecholamines dopamine (DA) and noradrenaline (NA). The work that has been conducted has applied one or both of two experimental approaches: the correlative approach and the interventive approach. At this time, I shall review some of the relevant literature on this subject (for a more detailed review, see Corcoran, 1981).

The findings of studies seeking catecholamine correlates of kindling have been inconsistent. Sato and Nakashima (1975) were the first to report that kindling may be correlated with changes in catecholamine concentrations. They found that hippocampal kindled cats showed a depletion of both DA and NA in cortical samples. Research of later investigators attempted to specify which catecholamine is critically involved. Callaghan and Schwark (1979) reported that amygdaloid kindling in rats was accompanied by a depletion of NA in the hippocampus, midbrain, limbic lobe, and frontal cortex, but no effect on DA concentrations were observed. In contrast, Engel and Sharpless (1977) found that amygdaloid kindling produced a significant depletion of DA in the stimulated amygdala, whereas the observed depletion of NA was not significantly greater than that produced merely by implantation of electrodes. Finally,

Wilkison and Halpern (1979) investigated turnover rates of catecholamines in the forebrain of amygdaloid kindled rats (measurements of the depletion of DA and NA after the administration of the tyrosine hydroxylase inhibitor alpha methyl-p-tyrosine methylester (AMPT) were used). They found a significant increase in the DA turnover rate constants in the hemisphere ipsilateral to electrical stimulation, whereas there was no effect of kindling on NA turnover rate constants.

The rate-limiting step in the biosynthetic pathway of the catecholamines is the conversion of tyrosine to L-DOPA by the action of the enzyme tyrosine hydroxylase (TH), and several investigators have attempted to describe the effects of kindling on TH activity. Farjo and Blackwood (1978) measured TH activity in the hippocampus, thalamus, hypothalamus, frontal cortex, striatum, and amygdala of amygdaloid kindled rats. A consistent and significant decrease in TH activity was found only in the stimulated amygdala. In addition, Reedy, McGeer, Staines and Corcoran (1978) found no change in TH activity in the olfactory bulbs and caudate nucleus of amygdaloid kindled rats.

The question of whether there is a catecholaminergic involvement in kindling is not adequately answered by the correlative work. More consistent findings have, however, been obtained in studies using the interventive approach.

The first indication of catecholamine involvement in kindling, using this approach, was reported by Arnold, Racine and Wise (1973). Their results showed that rats receiving intraventricular infusions of 6-hydroxydopamine (6-OHDA), a neurotoxin that is selectively taken up by axons and terminals of catecholaminergic neurons and damages or kills them, or intraperitoneal injections of reserpine, a drug that interferes with the uptake and storage mechanism of the amine granules, required significantly fewer amygdaloid stimulations than control rats to develop convulsions. Since both catecholamines were depleted, it was not possible to determine if either DA or NA alone was responsible for this marked facilitation. Similarly, Corcoran, Fibiger, McCaughran and Wada (1974) found a facilitation of amygdaloid kindling by an intraventricular injection of 6-OHDA. The experimental group that had a large depletion of both DA and NA showed the facilitation, whereas a group with approximately the same NA depletion but a much smaller DA depletion was not significantly different from controls. These results suggest that the facilitation is due to either a combined depletion of NA and DA or to a depletion of DA alone. McIntyre, Saari and Pappas (1979) also used intraventricular injections of 6-OHDA and tried to establish the catecholamine of importance in the production of the facilitation of amygdaloid kindling. They found that

a group with depletion of both DA and NA showed this facilitation, but that a depletion of DA alone was without effect. Depletion of DA alone was accomplished by first giving the latter group a subcutaneous injection of desmethylimiprimine (DMI), a drug that provides protection of NA neurons from 6-OHDA. These results suggest that a combined depletion of DA and NA or depletion of NA alone could produce the effect.

Corcoran and Mason (1980) examined more precisely the role of NA by injecting 6-OHDA into the bilateral trajectories of the ascending NA fibres in the dorsal bundles in the mesencephalon. This produces a depletion of NA in the diencephalic and telencephalic terminals of the ascending noradrenergic axons that arise from the locus coeruleus, but spares DA and spinal and cerebellar NA. They reported that depletion of NA alone was sufficient to facilitate amygdaloid kindling. Ehlers, Clifton and Sawyer (1980) confirmed these results. They transected the bilateral ascending noradrenergic pathways and found a significant facilitation of amygdaloid kindling. In addition, they reported a significant correlation between the concentration of NA in the amygdala and periamygdaloid cortex and the number of ADs required for the development of fully kindled seizures. Further, Callaghan and Schwark (1979) reported that drugs that inhibit the synthesis of NA,

AMPT and disulfiram, enhanced the rate of amygdaloid kindling. Similarly, administration cf propranolal, a B-adrenergic receptor blocker, produced the same effect, thus suggesting that it is at this receptor that NA exerts an antikindling effect. In contrast, pimozide, a DA antagonist, and apomorphine, a DA agonist, had no effect. Finally, McIntyre (1980) depleted NA in the amygdala by a local injection of 6-OHDA and found a facilitation of kindling when stimulation was carried out in the NA-depleted amygdala, but not in the contralateral amygdala.

In summary, the interventive studies suggest that NA plays a specific role in kindling. Its proposed role is one of a central seizure-suppressant substance (Corcoran, 1979; Corcoran, 1981; Corcoran & Mason, 1980). The reports dealing with the role of DA are less consistent, but seem to indicate that it is not a catecholamine cf major importance in the kindling process.

The above results have, however, been found exclusively with limbic kindling. It would be of interest to know the effects of selective depletion of forebrain NA on cortical kindling (i.e., on focal cortical and cortico-generalized convulsions). The present study attempts to examine the role of NA in development of cortical kindling. Bearing in mind that the cortico- generalized seizure is almost identical to the limbic-generalized seizure, I hypothesized

that since depletion of NA facilitates the development of the limbic - generalized seizure, it will also facilitate the development of the cortico-generalized seizure (i.e., the rate at which the focal seizure generalizes into the cortico-generalized seizure will be increased). In addition, in limbic kindling depletion of NA fails to affect the development of the focal response, the threshold at which AD is elicited, the duration of the initial AD, and the duration of the first generalized seizure. Therefore I did not expect to find that depletion of NA would alter these measures with cortical kindling.

METHOD

Surgery

Injection of 6-OHDA

Male hooded rats (Canadian Breeding Farms, St. Constants, Quebec). weighing approximately 300 grams at the time of surgery were used, with 7 rats in each group completing the experiment. Under pentobarbital anesthesia one group of animals received stereotaxic injections of 6-OHDA via a 34-gauge cannula into the bilateral trajectories of the ascending NA fibers. The following coordinates were used to position the cannula, with interaural zero as base value: anterior 2.6 mm, lateral 1.1mm, and dorsal 3.7 mm. Four μg of 6-OHDA (6-OHDA HBr, weight expressed as free base) dissolved in 2 μl of 0.9% saline (containing 0.2mg/ml ascorbic acid as antioxidant) were infused bilaterally at the rate of 1 μl/min. To permit diffusion of the drug the cannula was left in place for an additional minute after the end of the injection. Rats in the control group received similar bilateral injections of an equal volume of a saline-ascorbic acid vehicle.

Electrode implantation

Two weeks after the injections of 6-OHDA or vehicle, bipolar electrodes were implanted bilaterally into the anterior neocortex using conventional stereotaxic techniques. The electrodes used were bipolar and consist of twisted nichrome wire 127 μm in diameter. The following coordinates were used: 3.5 mm anterior to bregma, 1.5 mm lateral, and 2.0 mm ventral from the superior surface of the skull, with the incisor bar at +5.0 mm.

Kindling

Kindling began approximately two weeks after electrode implantation and thus four weeks after the injections of 6-OHDA. This time interval, according to Ross and Reis (1974), is sufficient for the completion of anterograde degeneration of catecholaminergic neurons. Each rat received electrical stimulation for one sec daily. Stimulation was a constant- current, balanced biphasic square wave, with a pulse width of 1.0 ms and a frequency of 60 Hz.

Determination of threshold

Initially, stimulation was given at an intensity of 100 μA (base to peak) and was raised by 200μA daily until AD was evoked. Thus AD threshold is arbitrarily defined here as

the lowest intensity of stimulation that evoked AD. Pilot work indicated that 100μA was subthreshold for cortical AD.

Seizure development

Daily application of electrical stimulation at threshold intensity proceeded until ten fullblown cortico-generalized seizures were evoked. A cortico-generalized seizure was defined by Burnham (1980) as "... a cortex-triggered seizure...(that) consisted of an electrographic discharge which lasted 30 or more seconds and was accompanied by a convulsion which involved an episode of 'late' (rearing-falling) clonus" (p. 163). Further stimulation was suspended for seven days so that I could investigate the persistence of the kindled seizures. That is, the number of stimulations required to rekindle cortico-generalized seizures after the 7-day respite was measured. Stimulation was terminated at an arbitrary cutoff of 40 days; therefore rats that had not developed generalized convulsions at that point were assigned a score of 41 but were not used in the statistical analysis.

The seizure data measured throughout kindling were the duration of the first AD (sec), the number of ADs to the first cortico-generalized seizure, and the duration of the first cortico-generalized clinical convulsion (sec).

Biochemistry and histology

Confirmation of NA depletion

After completion of the behavioural testing concentrations of NA were sampled in the brains of 6 experimental rats and 5 control rats. The rats were sacrificed by cervical fracture, and the brains were quickly disected on ice into hippocampi (left and right hemispheres pooled) and left and right cortices. Concentrations of NA in these regions were measured by using a spectrophotofluorometric technique (McGeer & McGeer, 1962). This assay involves measurement of a fluorescent derivative of NA (i.e., 3,5,6, trihydroxyindole; see Appendix I) and served to confirm the extent of the NA depletion in the experimental rats as compared to concentrations of NA in control rats. In addition, concentrations of NA were measured in the brains of 3 6-OHDA-treated rats that had failed to develop generalized seizures within 40 days.

Confirmation of electrode placement

The brains of the remaining 2 experimental rats and 2 control rats were used for histological confirmation of placement of the electrodes. The rats were perfused with 0.9% saline followed by 10% formalin. The brains were then removed and frozen sections in the area of the electrode tips were taken and stained with cresyl violet.

Statistics

A multivariate t-test with 3 dependent variables (initial AD duration, threshold, and duration of 1st generalized seizure) was used to analyze the data (Clyde, 1969; Harris, 1975). A multivariate analysis was used because of my a priori hypothesis that these 3 variables would not differentiate the groups in the whole test space. In addition, a one-tailed t-test was performed on the rate of generalization data (Winer, 1962) because I hypothesized that the groups would differ on this dependent variable in a specific direction.

RESULTS

Biochemistry and Histology

Confirmation of NA depletion

Based on the results of the spectrophotofluorometric assay (see Appendix I), the rats were divided into 3 groups. There were 5 rats in the control group, and inclusion of the 2 rats whose brains were reserved for histology brought the total n in this group to 7. Five 6-OHDA-treated rats constituted an experimental group with extensive depletion of NA, arbitrarily defined as greater than 80%, and in this group I arbitrarily included two 6-OHDA-treated-rats whose brains were reserved for histology. As shown in Table 1, the rats with extensive depletion of NA showed mean depletions of greater than 95% in the terminal regions sampled. The remaining group with minimal depletion of NA, arbitrarily defined as 80% or less, comprised four 6-OHDA-treated rats that displayed mean depletion of NA of less than 8% (i.e., NA concentrations of 92% of control) in the pooled hippocampi, a mean depletion of NA in the left cortex of less than 5%, and a mean depletion of NA in the right cortex of less than 5%. Because only 1 of the 4 rats

with minimal NA depletion developed generalized seizures within the 40-day period of the study, this group was not included in statistical analysis.

Confirmation of electrode placements

Histological confirmation of sample cortical electrode placements indicated that electrodes were accurately placed.

Kindling

Early manifestation of kindling

For the data of the control rats and experimental rats with extensive depletion of NA a multivariate t-test was performed on the early manifestations of kindling (i.e., threshold and duration of the 1st AD; Table 2) and the duration of the first generalized convulsion (Table 3). The analysis indicated that depletion of NA failed to significantly affect these variables ($p > 0.364$; Table 4).

Rate of generalization

A one-tailed t-test was performed to examine the effects of depletion of NA on the number of ADs to first stage-5 seizure (Table 3). The analysis (Table 5) indicated that NA-depleted rats developed generalized seizures significantly more rapidly than the control group ($p < 0.003$). Indeed, the NA-depleted rats required less than

half as many ADs as control (means of 10.71 vs. 23.43) to develop stage-5 seizures. Finally, the rate of generalization of only 1 control rat (8 ADs) fell within the range (6-16 ADs) of the experimental group.

Persistence of seizures

The number of stimulations required to rekindle cortico-generalized seizures after a 7-day respite was measured in 10 rats, 6 experimentals and 4 controls. All rats showed stage-5 seizures on the first stimulation after the 7-day respite.

Rats with minimal depletion of NA

Four 6-OHDA-treated rats that had minimal depletion of NA (see Results: Confirmation of NA Depletion, above) were not included in the statistical analysis, but their data are of interest. Of the 4, the rat with the most extensive depletion (29% in the pooled hippocampi, 9% in the left cortex, and 14% in the right cortex) developed a generalized seizure on the twenty-third AD. The remaining 3 rats did not develop a generalized seizure within 40 ADs, at which point the experiment was terminated.

DISCUSSION

In the present experiment I examined the effects on cortical kindling of NA depletion produced by intracerebral injections of 6-OHDA. Depletion of forebrain NA (arbitrarily defined as greater than 80%) facilitated the rate of kindling but produced no effect on any of the other electrographic and behavioural variables. In contrast, a group of 6-OHDA-treated rats that showed minimal depletion of NA (arbitrarily defined as 80% or less) appeared to develop generalized convulsions at a rate different from both the experimental group and the control group. I shall discuss each of these findings separately.

Facilitation of kindling by extensive depletion of NA

The results reported here extend previous observations that 6-OHDA-induced depletion of NA facilitates amygdaloid kindling, by demonstrating that cortical kindling is similarly affected. There are several mechanisms by which depletion of NA could facilitate the rate of development of cortico-generalized seizures: by increasing the epileptogenicity at the site of stimulation, by facilitating the propagation of AD outside the area of stimulation, or

both. In that depletion of NA failed to affect the early manifestations of kindling such as threshold for cortical AD and the duration of the first cortical AD, it seems unlikely that the local epileptogenicity at the site of stimulaion was increased. Therefore, the observed facilitation is presumably a function of a disinhibition of the spread of epileptiform activity outside the area of stimulation. Noradrenergic mechanisms apparently control only the development of kindling, as measures taken at the completion of kindling, after cortico-generalized seizures had been established failed to indicate any differences between NA-depleted and control rats.

Effects of minimal depletion of NA

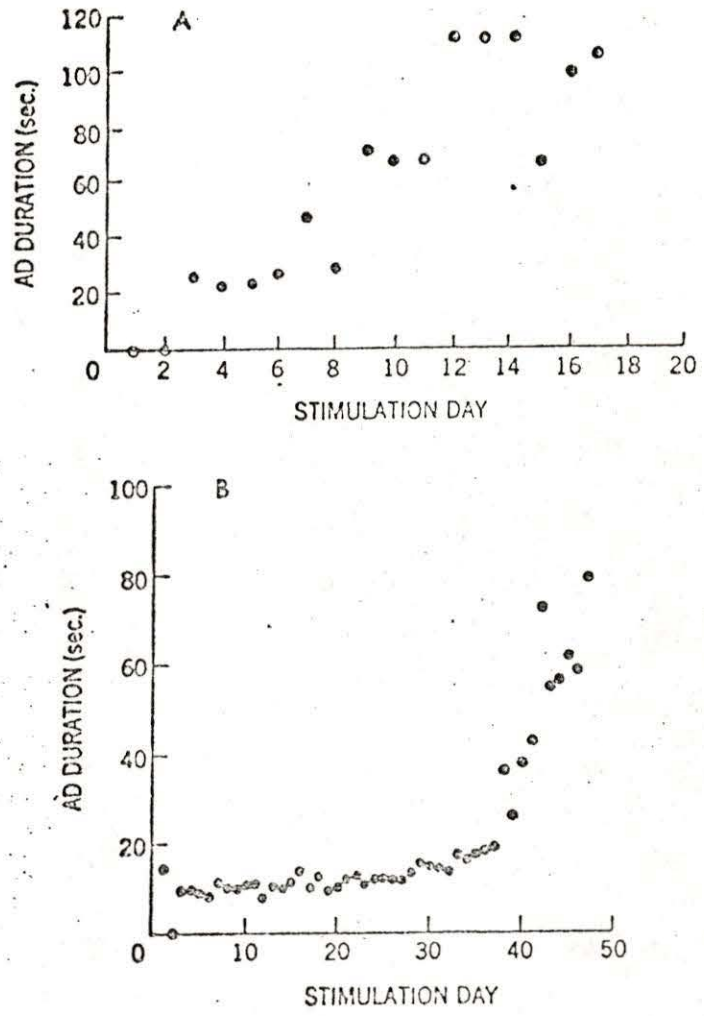
As mentioned above, four 6-OHDA-treated rats appeared to develop generalized seizures at a rate different from both the experimental and control group (experimental grcup $X=10.71$, control group $X=23.43$, minimal depletion group $X=36.5$). These results suggest that partial depletion of NA retarded the rate of development of generalized seizures. The partial depletion may have led to subsequent increases in turnover of NA in undamaged noradrenergic terminals (Zigmond & Stricker, 1974) or to postsynaptic supersensitivity to NA released from these terminals (U'Prichard, Reisine, Mason, Fibiger, & Yamamura, 1980).

Either of these changes might have overcompensated for any facilitatory effect of NA depletion. A much more extensive depletion of NA was found in the experimental group, however, and any compensatory changes occurring in response to degeneration of NA terminals were not sufficient to counteract the facilitation of kindling produced by the treatment. Future studies might investigate this hypothesis by systematically producing different degrees of NA depletion and observing the effects on kindling.

Implications

Burnham (1978) showed that kindling at a site in the anterior neocortex produces generalized seizures almost identical to those triggered from sites in the limbic system. The present study extends his findings by demonstrating that NA plays a role in the development of cortical kindled seizures similar to its role in development of amygdaloid kindled seizures. In both cortical and limbic kindled seizures, depletion of NA does not appear to affect the early manifestations of kindling. However, the development of generalized seizures is similarly affected, in that the rate of development of generalized seizures is facilitated. Since the seizures themselves and the effects of depletion of NA on cortical and limbic kindling are similar, one might speculate that development of generalized

seizures at both sites proceeds by involvement of the same mechanisms. In addition, the present study further supports the proposed role of NA as a seizure-suppressant substance that acts to restrict the spread of seizure discharge throughout the brain (Corcoran, 1979; Corcoran & Mason, 1980).



A: The pattern of AD growth typically seen in amygdaloid kindling. Note the sudden increments. The first generalized seizure was seen on day 9 of stimulation (from Burnham, 1975).
B: The pattern of AD growth typically seen in cortical kindling. Note that AD duration grows slowly and gradually. The first cortico-generalized seizure was seen on the 35th stimulation day. The "late step" started on stimulation day 38 (from Burnham, 1978).

Figure 1: Pattern of AD Growth in Amygdaloid and Cortical Kindling

TABLE 1

Effects of 6-OHDA on mean regional concentrations of NA in micrograms of amine per gram wet weight of tissue. Value in parentheses is the percentage depletion.

group		Hippocampus	L. Cortex	R. Cortex
control	Mean	0.3936	0.3226	0.3095
	SEM	0.0307	0.0291	0.0297
n=5	Range	0.320-0.488	0.260-0.398	0.227-0.380
experimental:	Mean	0.0101 (97.4%)	0.0115 (96.4%)	0.0047 (98.5%)
extensive	SEM	0.0050	0.0037	0.0017
depletion	Range	0.000-0.027	0.006-0.026	0.000-0.009
n=5				
experimental:	Mean	0.3646 (7.4%)	0.3094 (4.1%)	0.2949 (4.7%)
minimal	SEM	0.0445	0.0291	0.0546
depletion	Range	0.280-0.479	0.246-0.386	0.151-0.400
n=4				

TABLE 2

Effects of intracranial injections of 6-OHDA on early manifestations of kindling

		Threshold (μA)	Duration of first AD (sec)
control	Mean	1028.57	11.00
n=7	SD	670.11	3.00
	Range	100-1800	7-14
NA depleted	Mean	885.71	11.57
n=7	SD	307.83	4.08
	Range	500-1300	7-17

TABLE 3

Effects of intracranial injections of 6-OHDA on kindling of generalized (stage-5) seizures

		# of ADs to first stage-5 seizure	Duration of the first stage-5 clinical convulsion (sec)
control	Mean	23.43	41.86
n=7	SD	8.14	11.96
	Range	8-31	25-62
NA depleted	Mean	10.71	53.57
n=7	SD	4.31	10.75
	Range	6-16	38-68

TABLE 4

Statistical analysis of the effect of the intracranial injections of 6-OHDA on cortical kindling

Multivariate t-test of Drug Effect

Test of Roots	F	DF HYP	DF ERROR	p less than
1 through 1	1.185	3.000	10.000	0.364

Univariate F Tests of the 6-OHDA treatment effect

Variable	F(1, 12)	Mean Square	p less than
threshold	0.263	71427.625	0.618
Duration of first AD	0.089	1.143	0.770
Duration of first gen. convulsion	3.712	480.287	0.078

TABLE 5

Statistical analysis of the effects of intracranial injections of 6-OHDA on the rate of generalization

Source	DF	SS	MS	F	p less than
groups	1	565.784	565.784	13.335	0.003*
within	12	509.143	42.429		
totals	13	1074.927			

* significant - the percentage of the variance accounted for by the treatment is $R^2 = .526$, 52.6%

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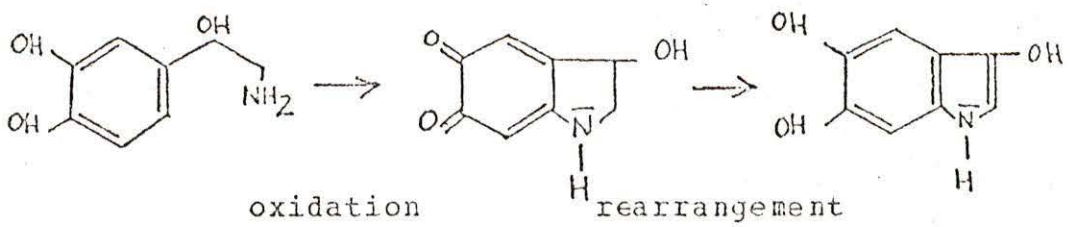
APPENDIX I

Principle of the noradrenaline assay

Catecholamines will fluoresce (excitation peak, 285 nm; emission, 325 nm), but this is no aid to their determination in very small amounts. The problem lies in the fact that this fluorescence is a nonspecific property due to the phenol ring present in the compound. One must therefore convert the NA, and only the NA, into substances that can be specifically identified fluorometrically. The assay involves the determination of a derivative of NA, and not NA itself. The derivative used in the present technique is 3,5,6-trihydroxyindole. The reactions in the formation of this derivative are shown below (from Nagatsu, 1973).

noradrenaline noradrenochrome 3,5,6-trihydroxyindole



oxidation rearrangement

The oxidation is carried out by iodine solution for 5 minutes whereupon the reaction is stopped by sodium thiosulphate. Rearrangement occurs in alkaline solution during exposure to light.

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VITA

Surname: Altman Given Names: Irwin Morris

Place of Birth: Montreal, Quebec.

Date of Birth: 4 September, 1959

Educational Institutions Attended, with Dates of Entering and Leaving:

Bar Ilan University, Israel	1976 to 1977
McGill University, Montreal	1977 to 1980
University of Victoria, B.C.	1980 to 1982

Degrees, Diplomas, Etc., Awarded, with Dates and Names of Institutions:

B.Sc. (Psychology)	1980	McGill University

Honors and Awards:

Graduation "with Distinction" McGill University	1980
Canadian Friends of Bar Ilan University Scholarship	1976/77
Research Assistantship-Summer, McGill University	1979
Research Assistantship-Summer, McGill University	1980
Research Assistantship-Summer, University of Victoria	1981
Research Assistantship, University of Victoria	1981/82
Teaching Assistantship, University of Victoria	1980/81
Teaching Assistantship, University of Victoria	1981/82

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Author [redacted]

Irwin M. Altman
Name

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