# DEPLETION OF NORADRENALINE

# AND

# THE KINDLING OF SEIZURES

by

ERICH MOHR

B.Sc., B.A., University of the Pacific, California

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DEAN

DATE Sept 1980

We accept this thesis as conforming
to the required standard

[redacted]

Dr. Michael Corcoran

[redacted]

Dr. Otfried Spreen

[redacted]

Dr. John Hayward



UNIVERSITY OF VICTORIA

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Supervisor: Dr. Michael E. Corcoran


ABSTRACT

Seizure development provoked by electrical stimulation of the amygdala (kindling) was examined in control rats and rats depleted of forebrain noradrenaline (NA) by bilateral injections of 6-hydroxydopamine into the mesencephalic trajectory of the dorsal tegmental noradrenergic bundle. Half the rats were kindled with stimulation delivered at the threshold intensity for amygdaloid afterdischarge (AD) and half with suprathreshold stimulation. Both groups of NA-depleted rats kindled significantly more rapidly than controls, and this facilitative effect of NA depletion did not vary as a function of the intensity of stimulation used.

The present findings replicate previous reports that depletion of NA facilitates the development of kindled seizures. Furthermore, they demonstrate that this effect occurs independent of the intensity of stimulation used for kindling, as long as the stimulation is sufficient to evoke AD.


Dr. Michael Corcoran _____

Dr. Otfried Spreen _____

Dr. John Hayward _____

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## Dedication

This work is gratefully dedicated to my father, Dr. Harald Mohr, who made it all possible.

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## Introduction

The technique of "kindling" (Goddard, 1967; Goddard, McIntyre, & Leech, 1969) has been used extensively in the last decade in the scientific investigation of epilepsy. Kindling involves a "progressive increase in the strength of epileptiform activity evoked by spaced (in time) and repeated electrical stimulation of certain brain structures" (Racine, 1978). The term kindling itself was proposed by the Canadian researcher Graham Goddard of Dalhousie University, who, together with his students, carried out many of the initial experiments in the field.

This model of epilepsy has its basis in the observation that epileptiform afterdischarges (ADs) can be triggered when low intensity current of brief duration is passed through the uninsulated tip of electrodes implanted in certain brain areas. The responsiveness of the area depends upon its anatomical location. The ADs are initially weak, and little or no behavioral signs are noted. Repetition of the stimulation at a fixed interval (e.g. every 24 hours) and a given magnitude of intensity (usually at or above the threshold for an AD, which is typically defined arbitrarily as the lowest intensity of stimulation that evokes an AD at the site of stimulation) will eventually lead to an increase in epileptiform activity, manifesting itself in typical behavioral responses and characteristic EEG patterns (Racine, 1978).

Kindling then can be defined as "the progressive increase in neural responsivity produced by spaced and repeated epileptogenic stimulation of certain brain structures" (Racine, 1978). No reversal of the kindling

treatment has been reported and many studies have shown that it is a long lasting effect (Racine, 1978). Indeed, seizures have been reported to occur spontaneously in kindled animals (Pinel, Mucha, & Phillips, 1975; Wada, Sato, & Corcoran, 1974).

The kindling model of epilepsy offers several advantages over previously employed models: The evolution and characteristics of electrophysiological patterns of seizures can be examined; alterations in stimulus parameters can be employed to modify the developmental sequence of the seizure state; and the epileptic focus can be precisely characterized anatomically, as well as neurophysiologically and neurochemically (Taber, McNamara, & Zornetzer, 1977).

One of the characteristics of the kindling phenomenon is that there appears to be little or no tissue damage produced in the stimulated site. Goddard et al. (1969) used the Nissl technique to examine tissue at the tip of the implanted electrode and found no significant difference between a kindled group and a similar group with implanted electrodes that had received no stimulation. Racine, Tuff, and Zaide (1975) failed to find morphological changes in cortical pyramidal cells in cortically kindled rats examined with the Golgi technique. Negative findings were also reported at the ultrastructural level by Goddard and Douglas (1975) in the amygdala and by Racine and Zaide (1978) in the cortex. Consistent with this, Goddard et al. (1969) reported that tissue damage might actually interfere with the kindling process.

They made lesions at the tip of stimulating electrodes by passing weak direct currents for short periods. The small lesions thus produced actually interfered with the kindling process rather than facilitating it, suggesting that tissue damage is not a mechanism of kindling.

If tissue damage is not involved, what are the causes for the occurrence of epileptiform behavior after repeated electrical stimulation? The first relevant question that comes to mind in this context is whether different stimulation sites differ in responsivity. Goddard et al. (1969) did a mapping study in order to assess the different responses toward kindling in the various brain areas. In order of decreasing rates of kindling the following sequence emerged: amygdala (with the fewest stimulations necessary), globus pallidus, pyriform cortex, olfactory area, frontal area, entorhinal cortex, olfactory bulb, septal area, preoptic area, caudate putamen and hippocampus. They also reported that most brainstem and several neocortical and caudate sites tested did not show any kindling effect, even if stimulated up to 200 times.

The amygdala is the area most frequently stimulated in the kindling paradigm, perhaps because it seems to display the fastest rate of kindling of any anatomical area tested. Many investigators have attempted to investigate the participation of other anatomical sites in amygdaloid kindling as this might help reveal some of the underlying mechanisms involved in the phenomenon. For example, McCaughran, Corcoran, and Wada (1976) examined the development of kindled amygdaloid seizures

after section of the forebrain commissures in rats and concluded that transhemispheric connections via the corpus callosum and related midline structures do not play an essential role in the development of convulsions. According to McCaughran et al. the integrity of extracommissural pathways is sufficient to support the development of generalized amygdaloid seizures. They further suggested that at least one of these pathways involves the rostral thalamus.

The effects of lesions of the frontal cortex on amygdaloid kindling were investigated by Corcoran, Urstad, McCaughran, and Wada (1975). Their results suggest that some areas of the frontal cortex participate in the generalization of amygdaloid seizures in the later stages of kindling; they hypothesized furthermore that other structures, presumably in the brainstem, participate in the development of clinical manifestations in the early stages of kindling. McIntyre (1975), who sectioned the commissures between hemispheres in the rat brain and subsequently kindled the amygdala, provided another strong argument for active involvement of the brainstem in amygdaloid kindling. He found that AD failed to propagate to the contralateral amygdala during kindling of generalized seizures in forebrain-bisected rats. When subsequently stimulated in the contralateral amygdala, however, these rats nonetheless showed a "transfer effect", in which secondary-site (contralateral) kindling occurs at a facilitated rate after the completion of primary-site kindling (Goddard et al., 1969). He concluded that since the primary-site discharge never reached the contralateral amygdala when the forebrain commissures were

transected, some brainstem sites must have been actively involved.

The exact details as to the anatomic mechanisms warrant further research.

In addition to the anatomic considerations, other phenomena, such as cytological, histological, and neurochemical evidence have to be evaluated to further investigate the question of the mechanisms involved in kindling.

As mentioned earlier, little or no tissue damage seems to take place in kindling. Presumably, the effect involves a permanent transsynaptic change in brain function. Since neurochemical phenomena are essential in this proposed transsynaptic change, a close investigation of possible neurotransmitter systems is warranted.

Most of the neurochemical work to date has dealt with the questions, "Which of the transmitter systems is involved?" and "What are their respective roles in kindling?" For example, Arnold, Racine, and Wise (1973) reported that treatment with atropine, a muscarinic acetylcholine antagonist, retarded the rate of amygdaloid kindling in rats. Corcoran, Wada, Wake, and Urstad (1976), on the other hand, failed to observe any effects of atropine on amygdaloid kindling in rats and cats and questioned the putative role of cholinoceptive neurons in kindling. To further complicate matters, McNamara (1978a) demonstrated a selective reduction in the number of muscarinic cholinergic receptor binding sites in both amygdaloid regions of rats sacrificed 15 hours following completion of kindling. McNamara (1978b) also demonstrated selective reductions in beta-adrenergic receptor binding in rats killed 3 days after kindling was complete. The reductions were observed in both the stimulated and

contralateral amygdala; no other brain area showed this effect, however. Since in each of McNamara's studies the kindling stimulations were delivered hourly, the validity of these results is questionable. This is because Mucha and Pinel (1977) have shown that kindling produces a gradually dissipating "inhibition" of subsequent seizure responses (lasting approximately 90 minutes) which may significantly alter the neurochemical response ordinarily encountered in the kindling phenomenon. Thus McNamara may have been measuring the neurochemical correlates of the inhibitory phenomenon and not of kindling itself.

5-Hydroxytryptamine (5-HT), a predominantly inhibitory transmitter, could also be a substance involved in the kindling phenomenon. Kovacs and Zoll (1974) reported that electrical stimulation of the raphe nuclei, a major source of 5-HT fibres, blocked kindled amygdaloid seizures. Raphe lesions, as reported by Racine (1978), significantly facilitated both amygdaloid and cortical kindling. Racine contended, however, that the overall effect on the kindling phenomenon of manipulating the 5-HT system is relatively weak.

The neurotransmitters that have received the widest interest in this context are the catecholamines dopamine (DA) and noradrenaline (NA). Unfortunately, however, the results of many of the studies are contradictory. Sato and Nakashima (1975) reported, for example, that hippocampal kindling resulted in a significant depletion of whole-brain concentrations of both DA and NA in cats. Engel and Sharpless (1977)

found a consistent and significant decrease in the concentrations of both catecholamines in the stimulated amygdala of amygdaloid kindled rats. They observed depletion of DA only in kindled animals, whereas significant depletion of NA was also seen in nonstimulated control rats, presumably as a consequence merely of damage produced by implantation of an electrode. Engel and Sharpless concluded that kindling produces either a persistent increase in DA release and metabolism, unaccompanied by increased synthesis, or a persistent decrease in DA production. In contrast, Callaghan and Schwark (1979) reported that amygdaloid kindling in rats is accompanied by depletion of NA in discrete regions of the brain without alterations in concentrations of DA. Finally, Wilkison and Halpern (1979) investigated the turnover kinetics of DA and NA in the forebrain of amygdaloid kindled rats. They reported that the turnover rate constants for NA were not affected by kindling whereas significantly higher rate constants for turnover of DA occurred in the hemisphere in which the rats were kindled.

The confusion produced by consideration of the conflicting results of the above neurochemical studies may be resolved by examining the effects on kindling of the neurotoxin 6-hydroxydopamine (3,4,6-trihydroxyphenylethylamine). When administered intravenously, 6-hydroxydopamine (6-OHDA) is rapidly and selectively taken up into the catecholaminergic nerve endings of the sympathetic nervous system. Due to its selective distribution and concentration and its extreme susceptibility to oxidation, 6-OHDA selectively destroys sympathetic neurons by causing acute degeneration of adrenergic terminals (Cooper, Bloom, & Roth, 1978). 6-OHDA greatly reduces tyrosine hydroxylase activity (the rate-limiting enzyme

Thus they suggested that depletion of NA is the critical mechanism underlying the potentiation of kindling produced by intraventricular injections of 6-OHDA.

The above emphasis on the role of NA in kindling was confirmed in the study of Corcoran and Mason (in press). They depleted NA and DA selectively via intracerebral injections of 6-OHDA and thus were able to discriminate between the influences of these two neurotransmitters. They concluded that depletion of NA in the forebrain terminals of the noradrenergic neurons is sufficient to facilitate kindling. Hence, the depletion of DA produced by intraventricular injection of 6-OHDA (Arnold et al., 1973; Corcoran et al., 1974; McIntyre et al., 1979) is not causally related to the facilitation of kindling that occurs; NA depletion alone seems to be sufficient for facilitation.

The present study was undertaken to further investigate the role of NA in kindling (for a detailed description of the anatomy of the noradrenergic neurons in the brain, see Appendix II). It is an extension of the study of Corcoran and Mason (in press), who described a remarkable facilitation in the rate of amygdaloid kindling in NA depleted rats kindled at an intensity of stimulation well above the threshold for amygdaloid AD. They chose a suprathreshold intensity of stimulation because previous facilitative effects of 6-OHDA on kindling were observed using suprathreshold stimulation (Arnold et al., 1973; Corcoran et al., 1974). In the present study I examined the effects of variation in the

intensity of stimulation (threshold vs. suprathreshold) on the facilitative effects of NA depletion on amygdaloid kindling, as a prelude to further research designed to characterize the effects of 6-OHDA on kindling with stimulation of regions other than the amygdala. For an experimental hypothesis I predicted that 6-OHDA-induced depletion of forebrain NA would result in facilitation in the rate of kindling in rats stimulated both at threshold intensity and at suprathreshold levels, in view of the findings of Goddard et al., (1969) and Racine (1972a) that variation in the intensity of the stimulation had no significant effect on the number of stimulations required to kindle the amygdala in rats without induced neurochemical alterations.

# Method

## Surgical

### NA depletion

Male hooded rats obtained from the Canadian Breeding Laboratory and weighing approximately 300 grams at the time of surgery were used. The number of rats that completed the experiment is shown in Tables II and III. The rats were anesthetized with pentobarbital. Two groups of animals then received stereotaxic injections of 6-OHDA via a 34-gauge cannula into the bilateral trajectories of the ascending NA fibres (see Appendix II). The cannula was positioned using the following coordinates, expressed with interaural zero as base value: anterior 2.6 mm, lateral 1.1 mm, and dorsal 3.7 mm. Four μg of 6-OHDA (6-OHDA HBr, weight expressed as a free base) dissolved in 2 microliters of 0.9% saline (with 0.2 mg/ml ascorbic acid as an antioxidant) were infused bilaterally at the rate of one microliter per min. To permit diffusion of the drug the cannula was left in place for an additional min after the end of each injection.

The rats in two control groups received bilateral injections via the same technique and with the same coordinates of an equal volume of the saline/ascorbic acid vehicle.

### Electrode implantation

Two weeks after the injections of 6-OHDA or vehicle, all rats in the four groups were again anesthetized with pentobarbital and stereotaxic implantations of electrodes into the amygdala were performed. Electrodes

were implanted bilaterally into the basolateral region of the amygdala. The following coordinates were used: -0.8 mm from bregma, 4.5 mm lateral, and 8.5 mm ventral from the superior surface of the skull, with the incisor bar at +5.0 mm. The electrodes used were bipolar and consisted of twisted nichrome wire 127 μm in diameter. A surgical screw driven into the frontal bone served as ground and two screws driven into the temporal bones bilaterally were used as anchor.

Kindling

Kindling did not begin until 10 days after electrode implantation, hence approximately 4 weeks after the injections of 6-OHDA. According to Ross and Reis (1974), this time interval is sufficient to allow for the completion of anterograde degeneration of the noradrenergic neurons.

Each rat received one second of electrical stimulation per day delivered via the electrode in the left hemisphere (primary site). Stimulation was in the form of a constant current, balanced biphasic square wave, with a pulse width of 1.0 msec and a frequency of 60 Hz. There were 2 phases for each of the four groups in this experiment (Corcoran & Mason, in press): determination of threshold for after-discharge (AD), and kindling of the primary site.

AD threshold in the primary site. The threshold for AD was arbitrarily defined as the lowest intensity of stimulation that evoked AD in the primary site of stimulation. Stimulation was initially administered at an intensity of 30 μA and was raised by 5μA daily until AD was evoked.

Primary-site kindling. Control rats and 6-OHDA-treated rats were assigned to threshold and suprathreshold conditions in such a manner that the groups were balanced for AD thresholds. Primary-site kindling then commenced, with the threshold groups being stimulated at threshold current and the suprathreshold at an arbitrarily chosen intensity of 200 μA.

The animals were stimulated every 24 hours between the hours of 9:00 a.m. and 12:30 p.m. The EEG was recorded from the primary site and from the contralateral amygdala (secondary site) for one minute before and several minutes after each stimulation. The development of epileptiform clinical activity was defined on a 5 point scale (Corcoran et al., 1975; Racine, 1972b): Stage 1, mouth movements and ipsilateral eyeblinking; stage 2, head nodding; stage 3, clonus of the contralateral forelimb; stage 4, bilateral clonus with rearing; stage 5, repeated rearing and falling. The following electrographic and behavioral variables were measured: Duration of first primary site AD; number of AD sessions until first bilateral AD was observed and number of AD sessions until a stage-5 seizure was evoked. Further, the following additional variables were measured in the first session in which a stage-5 seizure developed: Duration of AD in the primary site; duration of AD in the secondary site; latency from stimulation until behavioral epileptogenic signs appeared; latency from stimulation until stage-5 behavioral epileptogenic signs appeared; duration of behavioral epileptogenic signs. The animals were allowed to have three generalized (stage-5) seizures triggered by stimulation in the primary site.

Biochemistry and histology

To confirm the effectiveness of 6-OHDA in depleting NA, regional concentrations of NA were measured in the hippocampus-cortex of 12 6-OHDA-treated animals and 12 controls. The concentrations of NA were measured using a fluorometric technique (see Appendix I).

Electrode placements were examined in the 2 remaining 6-OHDA depleted animals and in 1 control. The animals were perfused with 0.9% saline followed by 10% formalin. After removal of their brains, frozen sections in the vicinity of the electrode tips and the cannula tracks were taken and stained with cresyl violet. The sections were then examined to confirm accurate placement of the electrodes in the amygdala.

Statistics

A 2-by-2 multivariate analysis of variance (MANOVA) with 9 dependent variables was used to analyze the data (Clyde, 1969; Cooley & Lohnes, 1971). A 2-by-2 analysis of variance (ANOVA) was used to analyze the biochemical data (Winer, 1962).

## Results

### Biochemistry and Histology

Table I shows the degree of depletion of NA caused by the injection of 6-OHDA. The NA-depleted rats showed a marked and widespread depletion of NA in the hippocampus-cortex region of the brain. In both the threshold and suprathreshold NA-depleted groups concentrations were significantly below those of their respective controls ($p < 0,000$), and there was no difference between the two NA-depleted groups.

Histological examination of sample electrode placements indicated that the electrodes were accurately placed in the basolateral region of the amygdala.

### AD threshold in the primary site

The mean thresholds for primary-site AD for NA-depleted and control animals are shown in Table II. The results indicate that NA depletion did not have any significant effect on the AD threshold. The duration of the first primary-site AD also failed to differ significantly between the groups, although the duration of NA-depleted rats kindled at threshold current did tend to exceed that of other groups. These results suggest that the amygdala's local susceptibility to epileptiform activity is not significantly influenced by NA (Table IV).

### Primary-site kindling

MANOVA revealed only one significant main effect, the 6-OHDA effect ($p < 0.008$). The effects of depletion of NA are therefore shown in Tables III and IV. The NA-depleted rats developed stage-5 seizures significantly more rapidly at both threshold and suprathreshold intensities

of stimulation than their respective control groups ($p < 0.001$). The NA-depleted rats that were kindled at threshold required only 1/3 as many AD sessions as their control counterparts (means of 5.71 vs. 16.71) to develop the first stage-5 seizure. Similarly, the NA-depleted rats that were kindled with suprathreshold stimulation required only a little more than half of the number of stimulations as their control counterparts (means of 8.14 vs. 14.50) to develop stage-5 seizures. Indeed, five of the seven NA-depleted rats in the threshold group developed their first stage-5 seizure during the first 4 AD sessions, with one animal exhibiting a stage-5 seizure in the first AD session, whereas the earliest stage-5 seizure developed in the threshold control group only after 6 AD sessions. Of the NA-depleted rats kindled at suprathreshold current, five of seven animals developed stage-5 seizures within the first 9 AD sessions, whereas the seizures did not appear in the control group until the tenth AD session. However, depletion of NA by treatment with 6-OHDA failed to exert a significant effect on any of the other dependent variables measured. These included the number of AD sessions until development of bilateral AD; and on the first AD session in which a stage-5 seizure developed, the duration of the primary and secondary-site AD, the duration of the seizure, and the latencies to the first clinical epileptogenic signs and to the stage-5 component of the clinical seizure.

Finally, as noted above, MANOVA indicated that there was no significant effect of the intensity of current on the rate of kindling; nor

was the interaction between the 6-OHDA treatment and intensity significant. Thus the rate of kindling was greatly facilitated by treatment with 6-OHDA, and this facilitatory effect did not vary as a function of the intensity of stimulation used for kindling.

In the present experiment I investigated the effects on kindling of manipulation of two variables; 6-OHDA and the intensity of stimulation used for kindling. Depletion of forebrain NA with 6-OHDA considerably facilitated the rate of kindling but produced no effect on any of the other electrographic and behavioral variables measured. In contrast, variation of the intensity of current failed to produce any significant effects. I shall discuss each of these two main effects separately.

Facilitation of kindling by 6-OHDA

There are several likely mechanisms by which depletion of NA could facilitate kindling: by increasing the local epileptogenicity of the amygdala itself, by facilitating the spread of discharge from the amygdala, or both. Because depletion of NA failed to affect threshold for amygdaloid AD or the duration of the first AD in the primary site, the local epileptogenicity of the amygdala was apparently not increased. This, therefore, suggests that the facilitation of kindling produced by depletion of NA with 6-OHDA is a function of a disinhibition of the spread of epileptiform activity from the stimulated amygdala. Once stage-5 seizures are fully established, however, epileptogenicity apparently is no longer under the control of noradrenergic mechanisms, as the electrographic and behavioral measures taken at the completion of kindling failed to indicate any differences between NA-depleted and control animals, similar to the observations of Corcoran and Mason (in press) and McIntyre et al. (1979). The present findings differ from those of McIntyre et al.

(1979) in that I failed to find any significant effect of NA depletion on latency to onset of stage-5 seizures, in contrast to their observation that latencies were significantly increased in NA-depleted rats. The procedures of the two studies were quite different, however, and, for example, variation in the parameters of amygdaloid stimulation used (McIntyre: 5 sec of sinewave; Mohr: 1 sec of square wave) could account for the differences. Perhaps a more likely explanation can be found in the fact that McIntyre et al.'s intraventricular injections of 6-OHDA would be expected to deplete spinal and cerebellar NA as well as forebrain NA, whereas my intracerebral injections of 6-OHDA would be expected to deplete forebrain NA but to spare spinal and cerebellar NA (Mason & Corcoran, 1979). Further research might examine the effects of selective depletion of spinal or cerebellar NA on latencies to motor seizures in kindled rats.

The facilitation of kindling produced by depletion of NA could be taken to suggest that a breakdown of NA function is one of the mechanisms of kindling in nonlesioned rats. The wide distribution of NA in the brain (Lindvall & Bjoerklund, 1978) and the inhibitory effects of iontophoretically applied NA on single-cell activity in the cortex and subcortical structures (Dillier, Laszlo, Muller, Koella, & Olpe, 1978; Phillis & Kostopulos, 1977; Reader, Ferron, Descarries, & Jasper, 1979) clearly support the notion that NA neurons function as a seizure suppressant system throughout the brain. Hypotheses have been offered to explain how NA function might progressively decline as animals are

kindled (Corcoran & Mason, in press). For example, high-frequency cellular discharge observed in the AD episodes during kindling (Racine, Newberry, & Burnham, 1975; Racine, et al., 1975b) could progressively destroy NA terminals in the entire forebrain. This destruction could conceivably lead to a disinhibition of activity in the target neurons and produce the behavioral effects observed during kindling. Another hypothesis attempting to explain the role of NA in the kindling phenomenon is based on evidence that kindling is due to long-lasting potentiation of excitatory synapses (Douglas & Goddard, 1975; Goddard & Douglas, 1975; Racine et al., 1975a, 1975b; Racine & Zaide, 1978). If some of these excitatory terminals synapse on NA terminals, repeated activation could conceivably lead to a lasting decline in NA release through potentiated presynaptic inhibition (Eccles, 1969). Finally, noradrenergic neurons might receive synapses from inhibitory cells that in turn receive projections from excitatory cells. Repeated activation of the excitatory synapse could, through potentiation, lead to a lasting inhibition of activity in noradrenergic neurons. The decline in NA activity would then ultimately lead to progressive disinhibition of seizure discharge. I should emphasize, however, that these hypotheses are entirely speculative.

Lack of effect of intensity

The facilitatory effects of 6-OHDA occurred at threshold levels as well as suprathreshold levels of stimulation. My experiment replicates Racine's (1972a) findings in nonlesioned rats in that as long as AD is evoked by stimulation, kindling occurs; application

of stimulation of higher intensity seems to have no further effect. Similar to an action potentional (AP), this seems to be an "all or none" phenomenon; once the necessary threshold is reached, an AP takes place with a standard magnitude and intensity, regardless how far above threshold the initial stimulation may be (Carlson, 1977). Similarly, once triggered, the AD spread seems to be independent of the original stimulation intensity. Furthermore, my results extend Racine's (1972a) findings to NA-depleted animals in that they also displayed similar rates of kindling in response to threshold and suprathreshold intensities of stimulation.

The implications of my findings for future studies of kindling with stimulation of other anatomical areas are that there is evidently no necessity to stimulate animals at suprathreshold levels of stimulation in order to investigate possible facilitative effects of NA depletion.

Table I

Effects of 6-OHDA on regional concentrations of noradrenaline expressed as means in micrograms of amine per gram wet weight of tissue. Value in parentheses is the percentage depletion as compared to control groups.

| | | Hippocampus-Cortex | | |
|---|---|---|---|---|
| THRESHOLD | Control n = 6 | Mean | 0.3315 | |
| | | SD | 0.0149 | |
| | | Range | 0.324 - 0.362 | |
| | NA depleted n = 6 | Mean | 0.0300 | (90.9%) |
| | | SD | 0.0371 | |
| | | Range | 0 - 0.103 | |
| SUPRATHRESHOLD | Control n = 6 | Mean | 0.3201 | |
| | | SD | 0.0353 | |
| | | Range | 0.253 - 0.359 | |
| | NA depleted n = 6 | Mean | 0.0163 | (94.9%) |
| | | SD | 0.0170 | |
| | | Range | 0 - 0.040 | |

Table I, cont'd.

2-by-2 Analysis of Variance - Depletion of Noradrenaline

| Source | DF | SS | MS | F | p less than |
|---|---|---|---|---|---|
| Drug | 1 | 0.5495 | 0.5495 | 700.1310 | 0.000 |
| Intensity | 1 | 0.0009 | 0.0009 | 1.2072 | 0.285 |
| Drug X Intensity | 1 | 0.0000 | 0.0000 | 0.0092 | 0.924 |
| Error | 20 | 0.0157 | 0.0008 | | |
| Total | 23 | 0.5662 | | | |

Table II

Effects of depletion of noradrenaline on early manifestations of kindling

| | | | Threshold (μA) | Duration of first AD at primary site (sec) |
|---|---|---|---|---|
| THRESHOLD | Control | Mean | 70.71 | 8.42 |
| | n = 7 | SD | 29.35 | 5.50 |
| | | Range | 35 - 130 | 4 - 20 |
| | NA depleted | Mean | 46.42 | 38.57 |
| | n = 7 | SD | 25.93 | 43.13 |
| | | Range | 30 - 100 | 3 - 120 |
| SUPRATHRESHOLD | Control | Mean | 53.33 | 8.83 |
| | n = 6 | SD | 24.01 | 6.08 |
| | | Range | 30 - 90 | 3 - 20 |
| | NA depleted | Mean | 49.28 | 8.28 |
| | n = 7 | SD | 24.90 | 3.86 |
| | | Range | 30 - 90 | 3 - 12 |

Table III

Effects of depletion of noradrenaline on kindling of generalized (stage-5) seizures

| | | | # of AD sessions to first stage-5 seizure | Duration of AD at the prim. site (sec) | Duration of AD at the sec. site (sec) | # of AD sessions to the first bi-lateral AD |
|---|---|---|---|---|---|---|
| THRESHOLD | Control | Mean | 16.71 | 90.14 | 62.71 | 6.28 |
| | n = 7 | SD | 6.96 | 64.88 | 69.22 | 2.98 |
| | | Range | 6 - 24 | 38 - 225 | 0 - 207 | 3 - 12 |
| | NA depleted | Mean | 5.71 | 83.71 | 55.00 | 4.71 |
| | n = 7 | SD | 5.40 | 37.65 | 39.02 | 4.71 |
| | | Range | 1 - 14 | 30 - 120 | 0 - 95 | 1 - 14 |
| SUPRATHRESHOLD | Control | Mean | 14.50 | 59.33 | 48.83 | 5.66 |
| | n = 6 | SD | 3.01 | 35.95 | 38.36 | 2.33 |
| | | Range | 10 - 19 | 20 - 105 | 15 - 112 | 3 - 8 |
| | NA depleted | Mean | 8.14 | 98.85 | 75.14 | 5.14 |
| | n = 7 | SD | 3.53 | 50.63 | 61.61 | 1.67 |
| | | Range | 4 - 14 | 60 - 205 | 20 - 184 | 3 - 8 |

Table III, cont'd.

| | | | Duration of first clin. stage-5 (sec) | *Latency to first clin. seizure signs (sec) | *Latency to stage-5 of clinical seizure (sec) |
|---|---|---|---|---|---|
| THRESHOLD | Control | Mean | 88.85 | 5.71 | 29.28 |
| | n = 7 | SD | 52.35 | 6.47 | 34.67 |
| | | Range | 27 - 181 | 2 - 20 | 5 - 99 |
| | NA depleted | Mean | 77.85 | 5.71 | 33.28 |
| | n = 7 | SD | 27.93 | 2.21 | 16.43 |
| | | Range | 42 - 125 | 3 - 10 | 20 - 63 |
| SUPRATHRESHOLD | Control | Mean | 63.16 | 1.83 | 35.16 |
| | n = 6 | SD | 33.57 | 0.75 | 30.82 |
| | | Range | 30 - 114 | 1 - 3 | 7 - 72 |
| | NA depleted | Mean | 77.00 | 6.57 | 24.14 |
| | n = 7 | SD | 42.92 | 8.81 | 23.98 |
| | | Range | 44 - 168 | 1 - 25 | 1 - 28 |

* Latency from onset of stimulation

Table IV

Multivariate Analysis of Variance

A - Test of Drug Effect

| Test of Roots | F | DF HYP | DF ERROR | p less than |
|---|---|---|---|---|
| 1 through 1 | 4.149 | 9.000 | 15.000 | 0.008 |

B - Test of Intensity Effect

| | | | | |
|---|---|---|---|---|
| 1 through 1 | 0.804 | 9.000 | 15.000 | 0.619 |

C - Test of Drug-by-Intensity Effect

| | | | | |
|---|---|---|---|---|
| 1 through 1 | 0.818 | 9.000 | 15.000 | 0.141 |

Table IV, cont'd.

Univariate F Tests of the 6-OHDA treatment effect

| Variable | F(1,23) | Mean Square | P less than |
|---|---|---|---|
| # of AD sessions | 20.278 | 517.709 | 0.001* |
| Duration of AD primary site | 0.658 | 1590.885 | 0.426 |
| Duration of AD secondary site | 0.175 | 517.709 | 0.680 |
| Duration of behavioral epileptogenic signs | 0.001 | 1.238 | 0.978 |
| Latency to behavioral epileptogenic signs | 1.019 | 33.215 | 0.323 |
| Latency to stage-5 behavioral epileptogenic signs | 0.098 | 72.772 | 0.757 |
| # of AD sessions until bilateral AD | 0.770 | 7.738 | 0.389 |
| Threshold in the respective groups | 2.158 | 1483.512 | 0.155 |
| Duration of first AD on the primary site | 2.928 | 1479.124 | 0.101 |

*significant - the percentage variance accounted for by the treatment is $R^2 = \frac{SS_B}{TSS} = 0.469$, 46.9%

References

Arnold, P. S., Racine, R. J., & Wise, R. A. Effects of atropine, reserpine, 6-hydroxydopamine, and handling on seizure development in the rat. Experimental Neurology, 1973, 40, 457-470.

Callaghan, D. A., & Schwark, W. S. Involvement of catecholamines in kindled amygdaloid convulsions in the rat. Neuropharmacology, 1979, 18, 541-545.

Carlson, N. R. Physiology of behavior. Boston, Allyn and Bacon Inc., 1977, 37-42.

Clyde, D. J. Multivariate analysis of variance on large computers. Miami: University of Florida, Biometric Laboratory, 1969, 1-26.

Cooley, W. W., & Lohnes, P. R. Multivariate data analysis. New York: John Wiley and Sons, Inc., 1971, 223-241.

Cooper, J. R., Bloom, F. E., & Roth, R. R. The biochemical basis of neuropharmacology (3rd ed.). New York: Oxford University Press, 1978, 102-158.

Corcoran, M. E., Fibiger, H. C., McCaughran, J. A. & Wada, J. A. Potentiation of amygdaloid kindling and metrazol induced seizures by 6-hydroxydopamine in rats. Experimental Neurology, 1974, 45, 118-133.

Corcoran, M. E., & Mason, S. T. Role of the forebrain catecholamines in amygdaloid kindling. Brain Research, in press.

Corcoran, M. E., Urstad, H., McCaughran, J. A., & Wada, J. A. Frontal lobe and kindling in the rat. The Canadian Journal of Neurological Sciences, 1975, 2, 501-508.

Corcoran, M. E., Wada, J. A., Wake, A., & Urstad, H. Failure of atropine to retard amygdaloid kindling. Experimental Neurology, 1976, 51, 271-275.

Dillier, N., Laszlo, J., Muller, B., Koella, W. P., & Olpe, H. R. Activation of an inhibitory noradrenergic pathway projecting from the locus coeruleus to the cingulate cortex of the rat, Brain Research, 1978, 154, 61-68.

Douglas, R. M., & Goddard, G. V. Long-term potentiation of the perforant path - granule cell synapse in the rat hippocampus, Brain Research, 1975, 86, 205-215.

Eccles, J. C. Excitatory and inhibitory mechanisms in the brain. In H. H. Jasper, A. A. Ward, & A. Pole (Eds.), Basic Mechanisms of Epilepsies, Boston: Little, Brown, and Company, 1969, 229-252.

Engel, J., & Sharpless, N. S. Long-lasting depletion of dopamine in the rat amygdala induced by kindling stimulation. Brain Research, 1977, 136, 381-386.

Goddard, G. V. Development of epileptic seizures through brain stimulation of low intensity. Nature, 1967, 214, 1020-1021.

Goddard, G. V., & Douglas, R. Does the engram of kindling model the engram of long term memory? Canadian Journal of Neurological Sciences, 1975, 2, 385-395.

Goddard, G. V., McIntyre, D. C., & Leech, C. K. A permanent change in brain functioning resulting from daily electrical stimulation. Experimental Neurology, 1969, 25, 295-330.

Kovacs, D. A., & Zoll, J. G. Seizure inhibition by median raphe nucleus stimulation in rat. Brain Research, 1974, 70, 165-169.

Lindvall, O., & Bjoerklund, A. The organization of the ascending catecholamine neuron system in the rat brain. Acta Physiologica Scandinavica, 1974, 412, 2-48.

Mason, S. T., & Corcoran, M. E. Seizure susceptibility after depletion of spinal or cerebellar noradrenaline with 6-OHDA. Brain Research, 1979, 166, 418-421.

McCaughran, J. A., Corcoran, M. E., & Wada, J. A. Development of kindled seizures after section of the forebrain commissures in rats. Folia Psychiatrica et Neurologica Japonica, 1976, 30, 65-71.

McIntyre, D. C. Split brain rat: Transfer and interference of kindled amygdala convulsions. Canadian Journal of Neurological Sciences, 1975, 2, 429-437.

McIntyre, D. C., Saari, M., & Pappas, B. A. Potentiation of amygdala kindling in adult or infant rats by injection of 6-hydroxydopamine, Experimental Neurology, 1979, 63, 527-544.

McNamara, J. O. Muscarinic cholinergic receptors participate in the kindling model of epilepsy. Brain Research, 1978a, 154, 415-420.

McNamara, J. O. Selective alterations of regional beta-adrenergic receptor binding in the kindling model of epilepsy. Experimental Neurology, 1978b, 61, 582-591.

Mucha, R. F., & Pinel, J. P. Post seizure inhibition of kindled seizures. Experimental Neurology, 1977, 54, 266-282.

Phillis, J. W., & Kostopoulos, G. K., Activation of a noradrenergic pathway from the brain stem to rat cerebral cortex, Gen. Pharmac., 1977, 8, 207-211.

Pinel, J. P., Mucha, R. F., & Phillips, A. G. Spontaneous seizures generated in rats by kindling: A preliminary report. Physiological Psychology, 1975, 3, 127-129.

Racine, R. J. Kindling: The first decade. Neurosurgery, 1978, 2, 234-252.

Racine, R. J. Modification of seizure activity by electrical stimulation: I. Afterdischarge threshold. Electroenceph. Clinical Neurophysiology, 1972a, 32, 269-279.

Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor Seizure. Electroenceph. Clinical Neurophysiology, 1972b, 32, 281-294.

Racine, R. J., Newberry, F., & Burnham, W. M. Post-activation potentiation and the kindling phenomenon, Electroenceph. Clinical Neurophysiology, 1975a, 39, 261-271.

Racine, R. J., Tuff, L., & Zaide, J. Kindling, unit discharge patterns and neural plasticity. Canadian Journal of Neurological Sciences, 1975b, 2, 395-405.

Racine, R. J., & Zaide, J. A further investigation into the mechanism underlying the kindling phenomenon. In K. E. Livingston, O. Hornykiewicz (Eds.), Limbic Mechanisms, Plenum Publishing Corporation, 1978, 457-493.

Reader, T. A., Ferron, A., Descarries, L., & Jasper, H. H. Modulatory role for biogenic amines in the cerebral cortex. Microiontophoretic studies, Brain Research, 1979, 160, 217-229.

Ross, R. A., & Reiss, D. J. Effects of lesions of locus coeruleus and regional distribution of dopamine-beta-hydroxylase activity in rat brains. Brain Research, 1974, 73, 161-166.

Sato, M., & Nakashima, T. Kindling: Secondary epileptogenesis, sleep and catecholamines, Canadian Journal of Neurological Sciences, 1975, 2, 439-446.

Taber, K. H., McNamara, J. J., & Zornetzer, S. F. Status epilepticus: A new rodent model. Electroencephalogr. Clinical Neurophysiology, 1977, 43, 707-724.

Wada, J. A., Sato, M., & Corcoran, M. E. Persistent seizure susceptibility and recurrent spontaneous seizures in kindled cats. Epilepsia, 1974, 15, 465-478.

Wilkison, D. M., & Halpern, L. M. Turnover kinetics of dopamine and norepinephrine in the forebrain after kindling in rats. Neuropharmacology, 1979, 18, 219-222.

Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill, 1962, 105-162.

# APPENDIX I

## Estimation of catecholamines

Following the completion of the behavioral measures the animals were killed by cervical fracture and their brains were quickly removed. The hippocampus and cerebral cortex were dissected out on ice and combined. The tissue was weighed and then homogenized in 5 ml perchloric acid. The homogenizing tube was rinsed with a further ml of acid, which was added to the homogenate. This was allowed to stand in the cold for ½ hour and was then centrifuged and the supernatant decanted. To the supernatant was added 0.5 ml of 0.1 M ethylenediaminetetracetic acid (EDTA).

An alumina column was prepared as follows: A glass tube 20 cm in length was fashioned so that the upper portion of the tube consisted of a 2 cm diameter reservoir of approximately 15 ml capacity. The lower portion was a 4 mm diameter shaft, drawn out to a fine tip and plugged with glass wool. To the tissue extract was added 1 ml EDTA and 1.5 ml potassium phospate (0.35M), and the pH was adjusted to 9.2-9.4. A consistent measure of alumina (about 0.5 gm) was added and stirred for 3 min. This slurry was poured through the glass tube, the flow through which was adjusted by application of gentle suction. The alumina was washed with 25-30 mls of distilled water. The catecholamines were then eluted with 0.5 ml acetic acid (0.5N).

Noradrenaline assay

To the 0.5 ml of eluant was added 0.5 ml of 1 M sodium acetate buffer (pH 6) and the pH was adjusted to 6.0 with 0.5N NaOH. Into each test tube was placed 0.5 ml of the sample solution, and 0.5 ml of 0.5M sodium acetate buffer (pH 5.4) was added, followed by 0.5 ml iodine solution (0.254 gm I + 5.0 gm KI/227 ml $H_2O$). The mixture was shaken and allowed to stand. After 15 min 0.25 ml sodium thiosulphate (0.5M) was mixed. in. To each tube was added 0.5 ml of a combination of ascorbic acid (5 mg/ml) and 5N NaOH in a ratio of 3:7. The samples were allowed to stand at room temperature under fluorescent lighting for 90 minutes. A "faded blank" was prepared with all reagents except the ascorbic acid, which was not added until immediately prior to fluorometric reading. All samples were read in a spectrophotofluorometer. The activation peak was set at 395 nm and the excitation peak was 505 nm. Both column and assay standards were employed to determine the percent recovery of amines of the column, and the relation of fluorometric reading to amount of noradrenaline per sample. Once the readings of all samples (assay standards, column standards and samples) were recorded, the following calculations were carried out:

1 Subtraction of blank reading from all other readings (assay standards, column standards and samples).

2 Calculation of percent recovery of standard (and sample).

$$\text{Recovery: } \frac{X}{\text{reading of column standard}} = \frac{\text{Amount of assay standard}}{\text{reading of assay standard}}$$

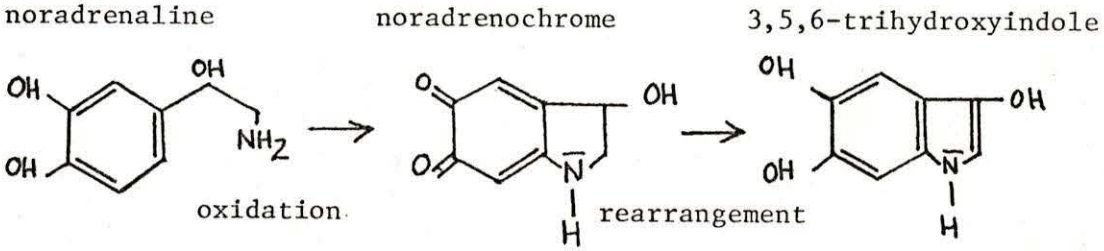
3 Calculate constant F = $\frac{\text{Amount recovered of Column std.}}{\text{Reading of Column std.}}$

4 Multiply this value by final sample reading

5 Divide by weight of wet tissue to find μg of NA/g of tissue

Principle of the noradrenaline assay

Although catecholamines will fluoresce (excitation peak, 285 nm; emission, 325 nm), this is no aid to their determination in small amounts. The reason lies in the fact that this fluorescence is a nonspecific property due to the phenol ring present in the compound. The task then is to convert the NA, and only the NA, into substances that can be specifically identified fluorometrically. The assay method is based on the determination of a derivative of NA, and not NA itself. The derivative employed in the presently described assay technique is 3,5,6-trihydroxyindole. The reactions required in the formation of this derivative are shown below (from Nagatsu, 1973).



The oxidation is carried out by iodine solution for 5 min after which time this reaction is stopped by the sodium thiosulphate. Rearrangement occurs in alkaline solution during exposure to light.

## References

Cooper, J. R., Bloom, F. E., & Roth, R. H. The biochemical basis of neuropharmacology (3rd ed.) New York: Oxford University Press, 1978, 104-105.

Nagatsu, T., Biochemistry of catecholamines, University Park Press, Tokyo, 1973.

# APPENDIX II

## Organization of the Ascending Catecholamine Neuron Systems in the Rat Brain

The rostrally projecting fibres of the ascending catecholamine (CA) systems from the mesencephalic, pontine, and medullary CA cell groups are mainly associated with four conduction pathways (Cooper et al., 1978; Lindvall & Bjoerklund, 1974): The noradrenergic central tegmental tract (CTT), within which a separate fibre system, the dorsal tegmental bundle can be found; the periventricular NA fibre system; the medial forebrain bundle containing both DA and NA; and the dopaminergic nigrostriatal pathway. The pathway of interest here is the noradrenergic dorsal tegmental bundle (DTB) in the central tegmental tract. 6-OHDA was injected into the DTB at the level of the mesencephalic trajectories of this fibre system. Arising from the medulla oblongata, the noradrenergic fibres in the CTT are located along myelinated tegmental fascicles from their place of origin to the caudal diencephalon. The ascending medullary NA axons assemble largely into a bundle situated just ventrolateral to the hypoglossal nucleus and ventral to the dorsal vagal nucleus. Going rostrally this medullary bundle passes just ventral to the genu of the facial nerve. Once having reached the pons, the number of NA axons and size of NA fibre systems increases considerably, due to

incoming pontine fibres from 3 different sites of origin: from the so-called A5 cell group, from the subcoeruleus cell group, and from the locus coeruleus. The most substantial projection from the locus coeruleus is confined to a separate bundle, the DTB.

DTB fibres, similar to other CA fibres in the CTT, can be traced along the surface of the individual myelinated tegmental fascicles. Before leaving the pons on its course from the locus coeruleus, the DTB can be found between the medial longitudinal fasciculus and the superior cerebellar peduncle. Going rostrally in the caudal mesencephalon it lies ventral to the root of the trochlear nerve. Along its course through the mesencephalon the DTB gives off fibres in several directions; the main system, however, can be traced going towards the mesodiencephalic junction. Here the DTB is situated ventrolateral to the periventricular gray of the third ventricle. From here the main bundles bend ventrally and somewhat laterally to pass in between fasciculus retroflexus and medial lemniscus, and then just lateral to the mamillothalamic tract. Along its course through the middle hypothalamus, the DTB merges with the medial forebrain bundle (MFB).

In the rostral hypothalamus, the MFB axons give off abundant collaterals. Some MFB fibres further rostrally leave the MFB dorsally to pass into the ventral part of the interstitial nucleus of the stria terminalis; within the stria terminalis the axons can be traced all the way to the amygdala. The MFB itself continues up to the level of the

rostral septum. Some fibres continue rostrally to the lateral part of the anterior olfactory nucleus. The major portion of the fibres turns dorsomedio-rostrally though. Ventral to the genu of the corpus callosum, the bundle divides into a caudal and rostral branch. The caudal branch continues underneath the corpus callosum in the fornix superior all the way back to the hippocampus. The rostral branch runs caudally above the corpus callosum within the cingulum. This branch gives off fibres along its course to large areas of the neocortex, and caudally it comes around the splenium of the corpus callosum and enters the hippocampus from the caudal site.

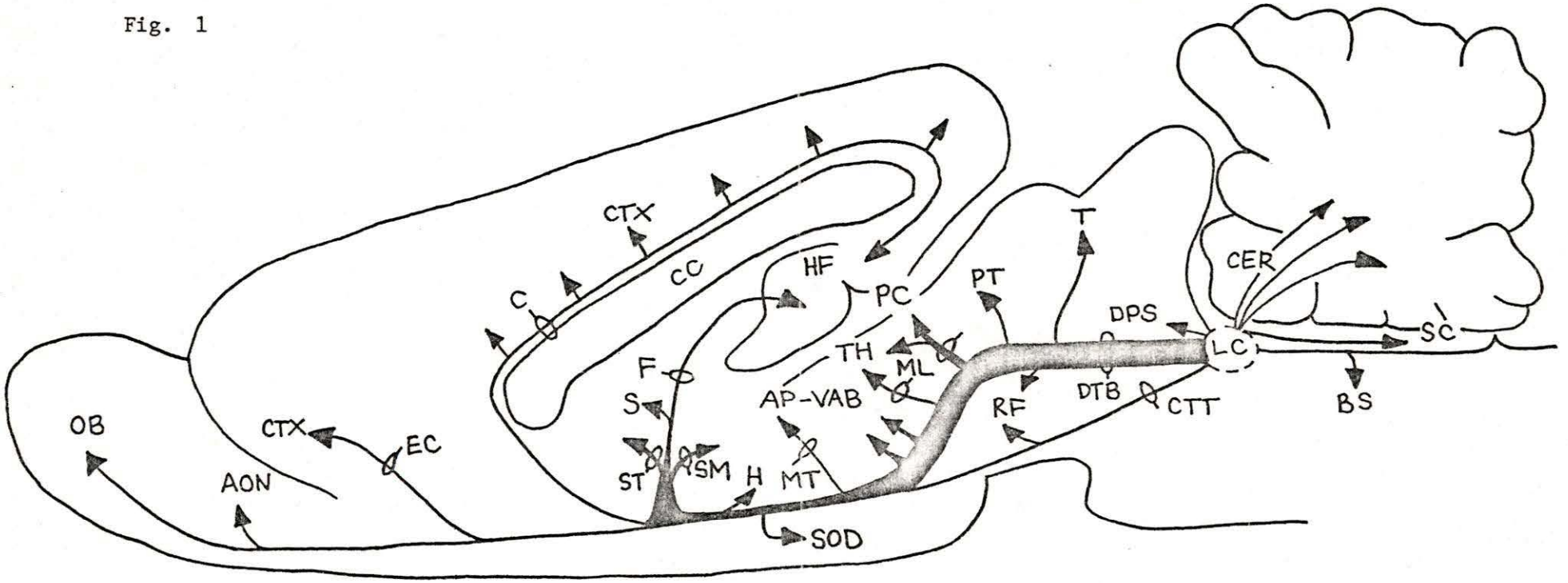
## References

Cooper, J. R., Bloom, F. E., & Roth, R. H. The biochemical basis of neuropharmacology (3rd ed.) New York: Oxford University Press, 1978, 102-193.

Lindvall, O., & Bjoerklund, A. The organization of the ascending catecholamine neuron system in the rat brain. Acta Physiologica Scandinavica, 1974, 412, 2-48.

Diagram of the projections of the locus coeruleus viewed in the sagittal plane

Fig. 1




Abbreviations: AON, anterior olfactory nucleus; AP-VAB, ansa peduncularis-ventral amygdaloid bundle system; BS, brainstem nuclei; C, cingulum; CC, corpus callosum; CER, cerebellum; CTT, central tegmental tract; CTX, cerebral neocortex; DPS, dorsal periventricular system; DTB, dorsal tegmental bundle; EC, external capsule; F, fornix; H, hypothalamus; HF, hippocampal formation; LC, locus coeruleus; ML, medial lemniscus; MT, mammillothalamic tract; OB, olfactory bulb; PC, posterior commissure; PT, pretectal area; RF, reticular formation; S, septal area; SC spinal cord; ST, stria terminalis; T, tectum; TH, thalamus. (Diagram compiled by R. S. Moore, from Cooper, Bloom and Roth, 1978).

# VITA

Surname: Mohr Given Name: Erich

Place of Birth: Tuebingen, Federal Republic of Germany

Date of Birth: 7 November 1954

Educational Institutions Attended with Dates of Entering and Leaving:

Technische Universitaet Muenchen, Federal Republic of Germany 1974 to 1975

University of the Pacific, Stockton, California, U. S. A. 1975 to 1978

University of Victoria, Victoria, B. C., Canada 1978 to 1980

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

B.Sc. (Chemistry/Biology) 1977 University of the Pacific

B. A. (Psychology) 1978 University of the Pacific

Honors and Awards:

Dean's List for Outstanding Academic Achievement, University of the Pacific 1977

Dean's List for Outstanding Academic Achievement, University of the Pacific 1978

Graduation "with Honors" University of the Pacific 1978

Fellowship, University of Victoria 1978/79

Fellowship, University of Victoria 1979/80

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Author [redacted]

*Signature*

Erich Mohr

*Name*

April 14, 1980

*Date*