TRANSFER OF KINDLED SEIZURE SUSCEPTIBILITY BETWEEN ANTERIOR NEOCORTEX AND AMYGDALA

by

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ABSTRACT

Seizures induced by electrical stimulation (kindling) of the amygdala and the anterior neocortex have traditionally been viewed as or involving separate processes. However, when generalized the seizures from both sites appear very similar to each other.

This study was, therefore, designed to determine whether the kindling of generalized seizures in a primary site (amygdala or anterior neocortex) affected the subsequent development of generalized seizures in the secondary site (anterior neocortex or amygdala respectively).

The initial kindling of generalized seizures in the amygdala facilitated the generalization of subsequent seizures kindled in the anterior neocortex. Similarly, primary kindling of generalized seizures in the anterior neocortex expedited the development of generalized seizures induced in the amygdala. Partial kindling in the anterior neocortex had no effect on subsequent amygdaloid kindling. Another noteworthy result concerns the stability of the epileptiform afterdischarges (ADs) that result from the stimulations. Specifically, stimulations of the amygdala produced ADs 100% of the time whereas anterior neocortical stimulations did not always elicit ADs. Observations concerning postictal irritability and the propagation of AD to unstimulated sites are also discussed.

The present findings suggest the existence of a single seizure-generalization mechanism, most likely of midbrain or brainstem origin. Furthermore, they reveal the instability of ADs induced by stimulation of the anterior neocortex, which has not been discussed previously in the literature.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

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DEDICATION

This thesis is dedicated to my loving parents who made it all possible.

INTRODUCTION

"Kindling" refers to the production of epileptic-like activity, both behavioral and electrical, through repeated electrical or chemical stimulation of the brain. Ironically its discovery was not only accidental but undesired. In 1964 Goddard was researching the effects of low-intensity stimulation of the amygdala on learning in rats. In some of these rats, particularly those stimulated frequently, seizure-like behavior started to develop. Goddard was not the only researcher to come in contact with this "obtrusive" phenomenon (see Bogacz, St. Laurent, and Olds, 1965; Newman and Feldman, 1964; Wurtz and Olds, 1963). He was, however, the first to discern the significance of the development of these seizures and coined the term "Kindling" to describe the phenomenon (Goddard, 1967; Goddard, McIntyre, and Leech, 1969).

When stimulations produce epileptiform afterdischarges (ADs) that are spaced (in time) and repeated, alterations in the electrographic and behavioral responses occur (Goddard et al., 1969; Racine, 1972a,b). Specifically, these include increases in the duration, amplitude, and frequency of ADs accompanied by the development of clinical convulsions (Racine, 1972a,b). Propagation of the activity to other brain sites and decreases in the local threshold for AD have also been reported (Racine, 1972a,b). It appears that it is the elicitation of ADs rather than the stimulation intensity that is important since subthreshold stimulations do not facilitate subsequent kindling (Racine, 1972b). Furthermore, stimulation at both threshold and significantly above threshold intensities seem equally effective in kindling seizures (Corcoran, McCaughran, and Wada, 1973).

It is important to note that these behavioral and electrical changes have been shown not to be due to any detectable abnormality in or damage to tissue (Goddard et al., 1969; Goddard and Douglas, 1975; Racine, Tuff, and Zaide, 1975; Racine and Zaide, 1978). In fact small lesions made at the tip of the electrode have been shown to actually interfere with the kindling process rather than facilitate it (Goddard et al., 1969). The interval between stimulations is also important, with intervals less than one hour greatly increasing the number of stimulations necessary for producing seizures (Racine, Burnham, Gartner, and Levitan, 1973). Finally, the effects appear to be relatively permanent, with no evidence of reversal (Goddard et al., 1969; Racine, 1978; Wada, Sato, and Corcoran, 1974).

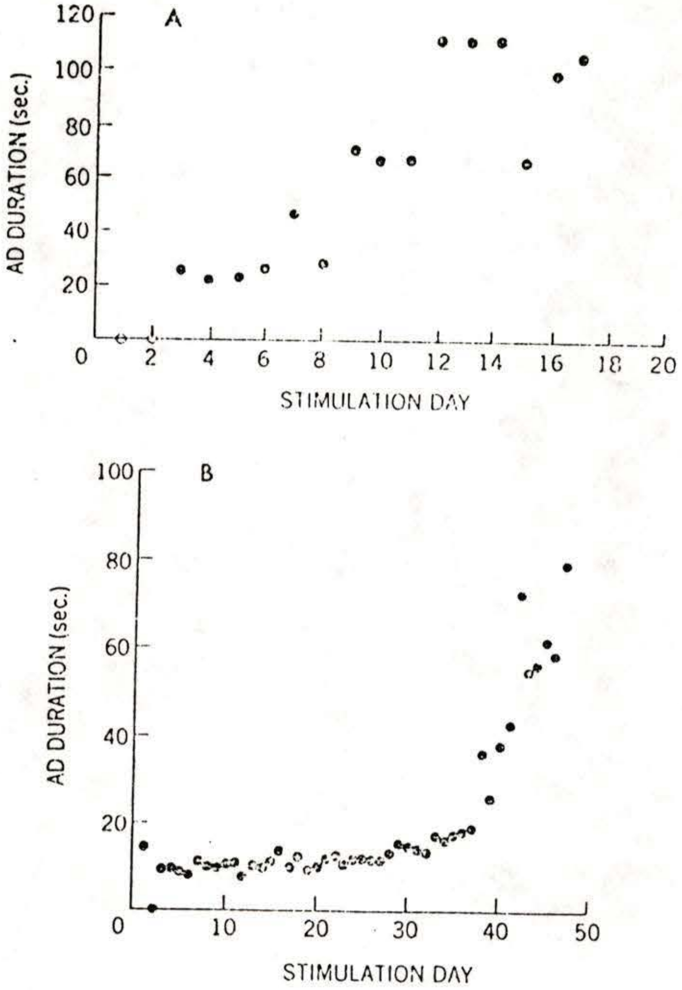
Kindling has been observed, although at different rates, in all forebrain and some brain stem sites (Goddard et al., 1969; Racine, 1972b; Racine, 1978). It has also been observed in all species reportedly tested (Goddard et al., 1969; Goddard and Morrell, 1971; Leech and McIntyre, 1976; Morrell and Tsuru, 1971; Rial and Gonzalez, 1976; Tanaka, 1972; Wada, Osawa, and Mizoguchi, 1975). The resulting seizure-like activity can eventually become spontaneous, that is, no longer needing an external stimulus trigger (Pinel, Mucha, and Phillips, 1975; Wada and Osawa, 1976; Wada, Osawa, Wake, and Corcoran, 1975; Wada et al., 1974). For many of these reasons kindling has been argued to be a good model of human epilepsy

(Wada, 1978).

Although different brain sites kindle at different rates there are some qualitative differences too. This is especially true when comparing anterior neocortical areas with limbic sites. The first obvious difference concerns the animals' response to the stimulation itself. Animals stimulated in neocortical areas display a "forced" motor movement (e.g. contralateral forelimb extension) that lasts for the duration of the stimulation and is similar to that reported for the head of the caudate nucleus (Corcoran and Wada, 1979). Animals stimulated in limbic sites, however, show no behavioral response to the stimulation itself (Goddard et al., 1969).

Another difference involves the intensity necessary to elicit an AD. Specifically, neocortical areas have been shown to have much higher thresholds for AD than limbic sites (Burnham, 1978). In addition, limbic sites initially show short and localized ADs, with no accompanying motor response (Racine, 1972a). On the other hand, the short ADs produced in the anterior neocortical areas are always accompanied by a motor seizure of equal duration (Racine, 1975). When stimulations are repeated in a limbic structure the short focal ADs get longer, more generalized, and are frequently accompanied by immobility (Racine, 1972b). Eventually, if yet more stimulations are administered, full motor seizures including rearing and falling develop, which is referred to as a stage-5 seizure (Racine, 1972b). These longer behavioral seizures grow in small increments and are accompanied by growing electrographic seizures (Burnham, 1975). Repeated stimulations in the anterior neocortex initially produce a short clonic seizure and eventually, with continued stimulations, a tonic part as well (Racine, 1975). With yet more stimulations another clonic part is added, resulting in the final clonic-tonic-clonic type of seizure (Racine, 1975). This resulting tri-partite form is commonly represented by <C1+T+C2> (Burnham, 1978). The growth of these seizures is more gradual than the limbic type except for a large increase in the C2 component at the end (see Figure 1). Although the neocortical seizures differ from limbic seizures in many ways, the final mature form of the seizure, and in particular the late <C2> component, is a rearing and falling seizure that is "identical" to the limbic type (Burnham, 1978). When mature, these cortical and limbic seizures are referred to as generalized (Burnham, 1978). After a generalized

FIGURE 1: AD growth comparison.



A: The pattern of AD growth typically seen in Amygdaloid kindling.

B: The pattern of AD growth typically seen in Cortical kindling (from Burnham, 1978).

seizure takes place a period of hyperirritability is typically seen (Burnham, 1978). Postictal spikes are also common when seizures are mature and seem to represent the first sign of spontaneous epileptogenesis in kindled organisms (Pinel and Van Oot, 1975).

Although generalized cortical and limbic seizures appear very similar, there are some important differences. Some rats stimulated in the anterior neocortex do not seem to develop mature seizures at all, whereas other rats stimulated in the same area develop generalized seizures in the first few days and subsequently drop back to normal kindling rates (Burnham, 1978). It has been argued that this is evidence that the pathways necessary for cortical generalization are present from the start and are inhibited by some unknown mechanism (Burnham, 1978). Cortical seizures also seem to be more unstable than limbic, with the <C2> component occasionally and temporarily dropping out (Wake and Wada, 1975; Burnham, 1978). Finally generalized cortical seizures have a brief tonic period <T> that is not seen in generalized limbic seizures (Burnham, 1978).

Another noteworthy property of kindling is the effect of a primary kindled site on secondary site kindling.

Early work demonstrated that significantly fewer stimulations were necessary to kindle the amygdala if the contralateral amygdala had previously been kindled (Goddard et al., 1969; Racine, 1972b). This transfer effect has also been shown to occur in other contralateral structures such as the hippocampi and between all ipsilateral limbic sites tested (Burnham, 1975; Racine, 1972b). Transfer is generally taken as evidence that kindling of different sites converges on a common mechanism at some level of the nervous system. One possibility is that transfer is the result of some change in the secondary structure since lesioning the primary site, after it was kindled, did not prevent transfer (Racine, 1972b). It is likely that secondary sites are at least partially kindled by the propagated discharge from the primary site during the initial kindling (Burnham, 1975). Another possibility is that some other seizure-generating structure is functionally connected to both the primary and secondary sites and is sensitized to input from the secondary site following primary-site kindling. This position is supported by the result that not even total forebrain sectioning prior to initial kindling, which removed all direct propagation, interfered with the facilitation in the secondary site (McCaughran, Corcoran, and Wada, 1976; McIntyre, 1975).

PURPOSE OF THIS STUDY

There are certain lines of evidence that suggest that kindling in the anterior neocortex and kindling in limbic sites are not entirely distinct phenomena. Although they develop differently, the seizures from both sites, when mature, are very similar in appearance. Also, depletion of forebrain noradrenaline, which has been shown to facilitate limbic kindling, differentially facilitates the development of the limbic-like generalized cortical seizures but has no effect on the nonlimbic-like early cortical seizures (Altman and Corcoran, 1983). Furthermore, both kindled amygdaloid convulsions and generalized anterior neocortical convulsions produce retrograde amnesia, whereas the initial focal seizures in the cortex do not (McIntyre, 1979). These data are at least suggestive that, although anterior neocortical seizures initially are focal and involve a possibly separate mechanism, the final generalized cortical seizure most likely involves the same mechanism as the limbic generalized seizure. If this is true one would expect good transfer between these sites after generalization. The present study, therefore, is designed to test transfer of kindling between the anterior neocortex and the amygdala. An important variable here will be whether generalized limbic-type seizures have developed during neocortical kindling. Transfer will also be measured from the kindled amygdala to the anterior neocortex. The effects will be assessed using measures of threshold, initial AD duration, final AD duration, and rate of generalization. I predict that, since the other variables most likely measure local changes, only the rate of generalization will be significantly different, and shorter, as a result of prior kindling.

METHOD

Subjects

Long Evans male hooded rats from Charles River Canada Inc. (St. Constant, Quebec) weighing approximately 300 grams at the time of surgery were used. They were housed individually in stainless steel cages with food and water continuously available except for 24 hours of food deprivation immediately before surgery. Light-dark cycles were maintained at 12 and 12 hours respectively.

Surgery

All instruments were sterilized prior to surgery to prevent infections. The rats were anesthetized with sodium pentobarbital (60 mg/kg) and received stereotaxic implantation of two bipolar electrodes consisting of twisted insulated nichrome wire 127um in diameter (see

Goddard et al., 1969). The electrodes were cut to length exposing uninsulated tips, checked for resistance, and affixed to the skull with stainless-steel screws and dental acrylic. One of the screws also served to ground and reference the animal during monopolar electrographic recording. The coordinates used for the right anterior neocortex were 2.5mm anterior to bregma, 1.7mm lateral to the midline, 1.7mm ventral from the dura, with the incisor bar at +5.0mm. The right amygdala coordinates were 0.8mm posterior to bregma, 4.5mm lateral to the midline, 8.5mm ventral form the surface of the skull, with the incisor bar at +5.0mm. A minimum of 7 days was allowed for recovery from surgery.

Threshold Determination

Few standard procedures for determining thresholds for kindling exist in the literature. Racine describes one of the most detailed procedures; however, it is only accurate to within 20% (Racine, 1972a). It was therefore decided to use a modified version of Kraepelin's "method of limits" procedure to determine the thresholds (see Coren, Porac, and Ward, 1979).

Electrical stimulation was applied in the form of a constant current balanced biphasic square wave, with a pulse width of 1.0 msec., a frequency of 60 Hz, and a train duration of 1 sec. All intensities are expressed in microamperes (ua), as base-to-peak. The initial intensity for stimulation in the amygdala was 30uA and was increased in steps of 10uA until an AD was elicited. At this point the intensity increment (in this case 10uA) was continually halved and subtracted from the intensity until no further AD was evoked. The intensity was then increased by the current increment value, which was continually halved and added until another AD was produced (see Figure 2). One exception to this procedure was that after the first AD was produced, only a maximum of three decrements were allowed, since it was felt that more AD elicitations would significantly alter the threshold (see Racine, 1972a). Another exception was that after the decreases in intensity failed to evoke an AD, increases were only permitted to a maximum of the last intensity to elicit an AD. Cortical thresholds were ascertained with the same procedure, but all values were increased by a factor of ten (e.g. initial increment = 100uA). Threshold was therefore defined as the lowest measured intensity

FIGURE 2

Example of the Threshold Determination procedure for the Amygdaloid site.

I.	increase until AD	30, 40, 50, 60-> AD
II.	decrease until no AD	55, 53-> no AD
III.	increase until AD	54, 55-> AD
IV.	threshold =	55

all values of .5 were rounded up due to equipment resolution limitations, and all numbers are in microamperes.

capable of eliciting an AD. After the threshold was determined the intensity was increased by the initial increment if three consecutive days of stimulation failed to produce an AD.

Kindling

All rats were kindled using the stimulation intensity that was individually determined by the threshold procedure. The animals were stimulated five days a week (monday - friday) with approximately a 24-hour rest period between weekdays and a 72-hour rest period over weekends. The rats were randomly assigned to one of three conditions or groups. Group one was initially kindled in the amygdala until three Stage-5 generalized seizures occurred (limbic seizures were classified according to Racine's (1972b) schema: Stage-1, mouth and facial movements; Stage-2, head nodding; Stage-3, forelimb clonus; Stage-4, rearing; Stage-5, rearing and falling). After a rest period the rats were then stimulated in the anterior neocortex until three generalized cortical seizures were produced. Group two was initially stimulated in the anterior neocortex until three generalized cortical seizures occurred and then, after a rest, switched to the amygdala until three Stage-5 seizures were elicited. The last group was partially kindled in the anterior neocortex (until 10 ADs were elicited), followed by a rest, and were then subjected to amygdaloid kindling until three Stage-5 seizures were produced. The criterion of 10 ADs is arbitrary, designed to lead to partial kindling, and 10 should be fewer than the number of ADs required for generalization based upon other research with similar sites (Burnham, 1978).

The rest period used between conditions was one week. This period was employed to eliminate the interference effects seen when the switch from a primary to a secondary site is immediate (McIntyre and Goddard, 1973).

The mature form of a cortical seizure is not as clearly defined as it is for the amygdala. Simply using a Stage-5 criterion is inadequate since rearing and falling are commonly observed early on during cortical kindling (Corcoran,M.E., personal communication). Some researchers have simply used a criterion of an increase in seizure duration to 30 seconds or more as sufficient for generalization (Burnham, Lychacz, Avila, Livingston, and Racine, 1980). However, based on pilot work this was also felt to be inadequate due to variability between rats. Therefore a multiple measure approach was used that was designed to pick up the late large increment in <C2> commonly called generalization. The three indicators used were an increase in the duration of AD greater than 40% over the previous level (3 prior consecutive days of AD), a rearing and falling seizure (Stage-5), and the postictal period of hyperirritability mentioned previously. The irritability of each rat was assessed tactually by tapping the base of the tail of the animal with a probe. Hyperirritability was considered to be present if the rat jumped (all four legs off the floor) repeatedly to this stimulation. A cortical seizure was called generalized if and only if at least two of these three indicators were present.

Histology

Following the conclusion of testing all animals were deeply anesthetized with sodium pentobarbital and perfused through the heart with 40ml of 0.9% saline followed by 40ml of 10% formalin. The brains were removed and frozen, and sections were taken from the area of the electrode track and tip. The sections were then mounted and stained with cresyl violet. Animals whose electrodes were positioned outside intended areas were discarded from the main analysis.

Statistics

Since my a priori hypothesis was that threshold, initial AD duration, and final AD duration would not differentiate the groups, a multivariate analysis of variance was used to analyze these data. In addition, since I predicted that the groups would differ in the rate of generalization, a one-way analysis of variance was used to test this hypothesis.

RESULTS

Histology

Only the data from animals whose electrodes were found to be in the intended areas (anterior neocortex and amygdala) were used in the analyses. This resulted in many animals being excluded due to their electrodes unwinding. Unwinding was attributed to the teflon insulation on the wire used and produced a gross uneveness at the electrode tip. Out of a total of 40 rats operated on, 13 completed the study successfully.

Cortical Kindling

Group 1: A multivariate analysis of variance was performed to examine the effects of prior kindling in the amygdala on threshold for AD, initial AD duration, and AD duration of the first generalized seizure during subsequent kindling in the anterior neocortex (see Tables 1 and 2). The analysis indicated that prior amygdaloid kindling did not significantly affect these variables (p=0.446; see Table 3). A one-way analysis of variance was then carried out on the rates of generalization between the control and amygdaloid kindled groups (see Table 4). This analysis revealed that prior amygdaloid kindling facilitated the rate of generalization in the anterior neocortex (p=0.053; see Table 5). This effect would most likely become more significant with larger groups of animals since it was quite strong with no group overlap (see Table 4).

Amygdaloid Kindling

Groups 2 and 3: A multivariate analysis of variance on threshold, initial AD duration, and first generalized Stage-5 seizure AD duration in the amygdala was performed comparing control animals, animals with 10 ADs previously elicited in the cortex (Group 3), and animals fully kindled in the cortex (Group 2) (see Tables 6 and 7). It should be noted that the large mean duration of the first AD in the group fully kindled in the cortex is accounted

TABLE 1

Effects of prior Amygdaloid kindling on the threshold and duration of ADs kindled in the Anterior Neocortex.

Prior Condition	Threshold (uv)		Duration of 1st AD (sec)	
control	mean	340.0	mean	12.8
(n=5)	SD	316.8	SD	4.4
	range	130-900	range	8-20
kindled amygdala	mean	296.7	mean	15.3
(n=3)	SD	76.4	SD	11.0
	range	230-380	range	8-28

TABLE 2

Effect of prior Amygdaloid kindling on the duration of the first Generalized seizure AD (sec).

Prior Condition	Duration of 1st Gen. Seizure AD (sec)
control (n=5)	mean 100.6 SD 28.6 range 66-138
kindled amygdala (n=3)	mean 66.7 SD 13.0 Range 54-80

TABLE 3

Statistical analysis of the threshold and duration effects of prior Amygdaloid kindling on ADs kindled in the Anterior Neocortex.

Multivariate Test of Significance

Test of Roots	F	DF HYP	DF Error	Sig. of F
1 through 1	1.101	3.000	4.000	0.446

Univariate F-tests

Variable	F(1,6)	Mean Square	Sig. of F
Threshold	0.051	3520.833	0.829
Duration of First AD	0.225	12.033	0.652
Duration of First Gen. Seizure	3.588	2159.008	0.107

TABLE 4

Effect of prior Amygdaloid kindling on the rate of Generalized seizure development in the Anterior Neocortex.

Prior Condition	Rate of Generalization (ADs)
control (n=5)	mean 24.0 SD 11.9 range 14-42
kindled amygdala (n=3)	mean 6.7 SD 2.9 Range 5-10

TABLE 5

Statistical analysis of the effect of prior Amygdaloid kindling on the rate of Generalized seizure development in the Anterior Neocortex.

Analysis of Variance

source	DF	SS	MS	F	Sig. of F
between	1	563.333	563.333	5.801	0.0527
within	6	582.666	97.111		
total	7	1145.999			

TABLE 6

Effects of prior Cortical kindling on the threshold and duration of ADs kindled in the Amygdala.

Prior Condition	Threshold (uv)		Duration of 1st AD (sec)	
control	mean	44.6	mean	5.6
(n=5)	SD	31.8	SD	4.3
	range	23-100	range	2-12
10 ADs in cortex	mean	46.0	mean	4.7
(n=3)	SD	13.1	SD	3.8
	range	32-58	range	2-9
kindled cortex	mean	68.5	mean	50.8
(n=4)	SD	38.6	SD	62.8
	range	30-110	range	4-138

TABLE 7

Effect of prior Cortical kindling on the duration of the First STAGE-5 seizure AD (sec).

Prior Condition	Duration of 1st STAGE-5 Seizure (sec)
control (n=5)	mean 77.8 SD 62.8 range 18-179
10 ADs in cortex (n=3)	mean 47.3 SD 34.4 range 20-86
kindled cortex (n=4)	mean 133.5 SD 35.4 range 84-168

for by the fact that one animal in the group had a Stage-5 seizure on the first day of stimulation, which greatly inflated the mean for this group. The analysis indicated that there were still no significant differences between the three groups on any of these variables (p=0.398; see Table 8). The rate of generalization of seizures stimulated in the amygdala was then compared between the three groups (see Table 9) using a one-way analysis of variance. This analysis showed highly significant differences between the groups (p=0.002; see Table 10). T-tests demonstrated that control rats (Group 1) and animals with 10 ADs produced in the cortex (Group 3) were not significantly different from each other (p=0.669; see Table 10). Animals that were fully kindled in the cortex (Group 2), however, developed generalized seizures in the amygdala more rapidly than either control animals (p=0.001; see Table 10) or animals with 10 ADs elicited in the cortex (p=0.022; see Table 10).

Post Hoc Observations

The previously noted differences between kindling in cortical and limbic sites (i.e. forced motor movement,

TABLE 8

Statistical analysis of the threshold and duration effects of prior Cortical kindling on ADs kindled in the Amygdala.

Multivariate Test of Significance

Test of Roots	F	DF HYP	DF Error	Sig. of F
1 to 2	1.123	6.000	14.000	0.398
2 to 2	0.131	2.000	7.500	0.879

Univariate F-tests

Variable	F(2,9)	Mean Square	Sig. of F
Threshold	0.744	730.358	0.502
Duration of First AD	2.085	2761.150	0.180
Duration of First STAGE (5) Seizure	2.823	6877.892	0.112

TABLE 9

Effect of prior Cortical kindling on the rate of STAGE-5 seizure development in the Amygdala.

<u>Prior Condition</u>	<u>Rate of STAGE-5 Development (ADs)</u>	
control (n=5)	mean	16.2
	SD	1.3
	range	15-18
10 ADs in cortex (n=3)	mean	17.3
	SD	5.7
	range	11-22
kindled cortex (n=4)	mean	4.8
	SD	4.5
	Range	1-11

TABLE 10

Statistical analysis of the effect of prior Cortical kindling on the rate of STAGE-5 seizure development in the Amygdala.

Analysis of Variance

source	DF	SS	MS	F	Sig. of F
between	2	378.450	189.225	12.881	0.002
within	9	132.216	14.691		
total	11	510.666			

T-TESTS

Comparison	T	DF	2 Tail Probability
group 1 vs group 2	5.49	7	0.001
group 1 vs group 3	0.45	6	0.669
group 2 vs group 3	3.29	5	0.022

group 1 - control
group 2 - kindled in cortex
group 3 - 10 ADs in cortex

threshold, and AD development) were observed in this study. Another difference that has received little attention is the stability of the ADs produced. During kindling in the amygdala all animals displayed an amygdaloid AD to each and every stimulation. These animals, in other words, displayed ADs on 100% of the stimulations. The anterior neocortical site, on the other hand, was much less reliable, with much inter-animal variation (x=72.15%; see Table 11). The latter result was not significantly different (p=0.547) even if the animals were previously kindled in the amygdala (x=81.94%; see Table 11).

Another noteworthy observation concerns the occurrence of the hyperirritability mentioned previously. It appears that the probability of an animal being hyperirritable (by my definition) is related to the length of his preceding seizure (see Table 12). Specifically, seizures that are accompanied by a postictal period of hyperirritability are of significantly longer duration than seizures in which no period of hyperreactivity occurs (p=0.000).

Finally, AD propagation from amygdaloid stimulations was not initially present at the neocortical site. However, when noticeable behavioral changes (i.e. Stage-1:

TABLE 11

Effect of prior Amygdaloid kindling on the Stability of kindled seizures in the Anterior Neocortex.

Prior Condition	% of Stimulation days AD present	
control (n=5)	mean	72.15
	SD	22.07
	range	37.25-93.33
kindled amygdala (n=3)	mean	81.94
	SD	18.79
	Range	62.50-100.00

TABLE 12

Relation between postictal irritability and duration of ADs kindled in the Anterior Neocortex and the Amygdala.

Area	Postictal Irritability	Duration of AD (sec)	
neocortex	not irritable (118)	mean	22.43
		SD	17.61
		range	7-106
	irritable (18)	mean	99.94
		SD	22.01
		range	66-155
amygdala	not irritable (82)	mean	20.56
		SD	21.60
		range	1-120
	irritable (11)	mean	109.73
		SD	46.73
		range	30-179

numbers in parenthesis refer to the number of AD periods in the respective groups mouth and facial movements) started to develop, as a result of repeated stimulations, propagation at the neocortical site also developed. On the other hand, AD propagation was always present at the amgdaloid site from stimulations of the anterior neocortex. In other words, AD was present at both electrodes when the animal displayed a behavioral seizure no matter which site was stimulated.

DISCUSSION

The present results must be considered preliminary due to the small number of animals completing the study. This study is therefore considered a pilot, and a follow up study is currently under way (with the electrode wire insulated with enamel).

The present experiment investigated the effects of primary kindling in the amygdala or anterior neocortex on subsequent kindling in the anterior neocortex or amygdala, respectively. Prior kindling in the amygdala facilitated the rate of generalization of seizures kindled in the anterior neocortex, but had no significant effects on any of the other electrographic variables (threshold and duration measures). Similarly, primary kindling in the anterior neocortex facilitated the development of Stage-5 seizures in the amygdala. Partial kindling in the anterior neocortex, however, had no effect on later amygdaloid kindling. Prior kindling in the cortex regardless if full or partial had no effects on the other electrographic variables measured. These results will be discussed individually.

Facilitation of Anterior Neocortical Kindling

Facilitation of secondary anterior neocortical kindling through primary amygdaloid kindling links these two "distinct" processes together. One might argue that the effect is due simply to the direct propagation of ADs from the stimulated amygdaloid site to the secondary cortical site and that this results in a kindling of both sites. This is supported by the observed propagation of AD at the neocortical site from amygdaloid stimulations. Propagated ADs probably are different from ADs induced by direct stimulation, however, since direct stimulation decreases the threshold and increases the AD duration whereas propagated AD had no effect on these measures (as indicated by the absence of differences between the groups on these measures). This suggests that propagation of AD to secondary sites is not the mechanism of transfer of seizure susceptibility. Consistent with this idea, transfer occurs even when propagation of AD between primary and secondary sites is blocked by surgical transection of pathways connecting them anatomically (McCaughran, Corcoran, and Wada, 1976; McIntyre, 1975).

An alternative possibility is that both amygdala and anterior neocortex are functionally linked to some motor mechanism that develops or is released during primary site kindling. The sites most likely involved are midbrain and or brainstem structures, since these areas remain intact in "split-brain" rats, which demonstrate transfer without direct propagation of AD between primary and secondary sites (McCaughran, Corcoran, and Wada, 1976; McIntyre 1975). Additionally, other research suggests that "activation" of these areas is important for the involvement of the contralateral hemisphere in bilateral seizures (Wada and Sato 1974). This hypothesized mechanism of contralateral transfer effects could also be the process responsible for the ipsilateral transfer effects between the amygdala and anterior neocortex.

Facilitation of Amygdaloid kindling

Facilitation of secondary-site amygdaloid kindling through primary-site cortical kindling further implies a relation between these two sites. Interestingly, not only was every cortical AD accompanied by a seizure, but also propagation of AD to the amygdala was always seen. This makes the direct propagation argument highly unlikely since animals with 10 ADs elicited in the cortex, and therefore with 10 ADs propagated to the amygdala, did not differ from controls in subsequent amygdaloid kindling. This also suggests that the late large clonic step <C2>, present when cortical seizures become generalized, is critical for transfer to occur. This late step could represent the activation of the hypothesized general seizure mechanism.

Observations

An important incidental observation made in the course of the present study concerns the instability of the cortical seizures. This instability did not just involve the <C2> component, as reported by Burnham (1978), but it also included the periodic absence of ADs in spite of stimulations. These periods without AD, or "days off" as I shall refer to them, came unpredictably with much interanimal variation. The animals were still receiving the stimulations as indicated by the presence of the forced motor movement during stimulation. This phenomenon does not appear to be directly related to intensity, as increasing the intensity of the stimulation after the required three consecutive "days off" did not always produce a seizure. The effect did not seem to be directly related to the rest period as it would if it were simply a refractory period. "Days off" were still commonly observed on mondays, which followed a 72-hour rest since the last stimulation. Not even when both increased intensity of stimulation and large rest periods were combined did a seizure inevitably occur. Furthermore, even after the rats had been fully kindled in the amygdala, "days off" were still observed when stimulations were switched to the cortex. This phenomenon could be due to some local cortical effect induced specifically by stimulation, since the AD propagated there after amygdaloid stimulation did not have "days off". The phenomenon is therefore likely to be an effect inhibitory to the relevant process necessary to trigger seizures stimulated from the anterior neocortex.

Lastly, the hyperirritability observed is a general increase in reactivity that became apparent through tactile, auditory, and visual stimulation. It was related to the duration of AD and probably to the maturity of ADs also. This idea could be tested by continuing to kindle animals past generalization to see if they all eventually become Hyperreactive to external stimuli.

Concluding Remarks

Some of the major results and observations to come out of this study follow: First and foremost, a transfer of seizure susceptibility, in terms of the number of ADs necessary to produce a generalized seizure, was observed between anterior neocortical and amygdaloid kindling. Second, stimulation of the cortical site above threshold did not produce an AD (or seizure) every time. Third, a period of hyperreactivity to external stimulation was more likely to follow a long seizure than a short one. Finally, ADs were observed at both sites, anterior neocortex and amygdala, when a detectable behavioral seizure was produced by stimulation of either site.

There are two important implications from these results. First, the data support the possibility that there is a single seizure mechanism in the midbrain or brainstem responsible for the generalized seizure seen regardless of kindling site. Current theories about clinical epilepsies also implicate midline structures in the diencephalon and mesencephalon as the site of the neurophysiological system or systems involved in generalization (Schmidt and Wilder 1968). Attempts to learn more about this mechanism and its location will have to be made in future work. Second, there is an apparently localized influence on the cortex that is responsible for the "days off" seen in cortical kindling. Stimulation of limbic structures fails to recruit a similar phenomenon. To investigate the "days off" phenomenon further, future work could look at stimulation parameters in more detail, such as intertrial interval and intensity. The importance of finding out more about these effects on the triggering of seizures and their possible mechanisms is of obvious relevance for our understanding of the clinical epilepsies.

REFERENCES

Altman, I.M., and Corcoran, M.E. Facilitation of neocortical kindling by depletion of forebrain noradrenaline. *Brain Research*, 1983, *270*, 174-177.

Bogacz, J., St. Laurent, J., and Olds, J. Dissociation of self-stimulation and epileptiform activity. *Electroencephalography and Clinical Neurophysiology*, 1965, *19*, 75-87.

Burnham, W.M. Primary and "transfer" seizure development in the kindled rat. *Canadian Journal of Neurological Sciences*, 1975, *2*, 417-428.

Burnham, W.M. Cortical and limbic kindling: similarities and differences. In K.E. Livingston and O. Hornykiewicz (Eds.), *Limbic Mechanisms*. New York: Plenum Press, 1978, 507-519.

Burnham, W.M., Lychacz, B., Avila, J., Livingston, K.E., and Racine, R.J. Differential pharmacological response of cortex- and amygdala-kindled seizures. In M. Girgis and L.G. Kiloh (Eds.), *Limbic Epilepsy and the Dyscontrol Syndrome*. Elsevier: North Holland Biomedical Press, 1980, 161-170.

Corcoran, M.E., McCaughran, J.A., Jr., and Wada, J.A. Acute antiepileptic effects of delta-9 tetrahydrocannabinol in rats with kindled seizures. *Experimental Neurology*, 1973, *40*, 471-483.

Corcoran, M.E., and Wada, J.A. Nalaxone and the kindling of seizures. *Life Sciences*, 1979, *24*, 791-796.

Coren, S., Porac, C., Ward, L.M. *Sensation and Perception*. Academic Press, New York, 1979.

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

Goddard, G.V., and Douglas, R.M. Does the engram of kindling model the engram of long-term memory? *Canadian Journal of Neurological Science*, 1975, *2*, 385-394.

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

Goddard, G.V., and Morrell, F. Chronic progressive epileptogensis induced by focal electrical stimulation of brain. *Neurology*, 1971, *21*, 393.

Leech, C.K., and McIntyre, D.C. Kindling rates in inbred mice: An analogy to learning? *Behavioral Biology*, 1976, *16*, 439-452.

McCaughran, J.A., Jr., Corcoran, M.E., and Wada, J.A. Development of kindled amygdaloid 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 Science*, 1975, *2*, 429-437.

McIntyre, D.C. Effects of Focal vs Generalized kindled convulsions from anterior neocortex or amygdala on CER acquisition in rats. *Physiology and Behavior*, 1979, *23*, 855-859.

McIntyre, D.C., and Goddard, G.V. Transfer, interference and spontaneous recovery of convulsions kindled from the rat amygdala. *Electroencephalography and Clinical Neurophysiology*, 1973, *35*, 533-543.

Morrell, F., and Tsuru, N. Kindling in the frog: Development of spontaneous epileptiform activity. Electroencephalography and Clinical Neurophysiology, 1976, 40, 1-11.

Newman, B.L., and Feldman, S.M. Electrophysiological activity accompanying intracranial self-stimulation. Journal of Comparative Physiological Psychology, 1964, 57, 244-247.

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

Pinel, J.P.J., and Van Oot, P.H. Generality of the kindling phenomenon: some clinical implications. Canadian Journal of Neurological Sciences 1975, 2, 467-475.

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

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

Racine, R.J. Modification of seizure activity by electrical stimulation: Cortical areas. Electroencephalography and Clinical Neurophysiology, 1975, 38, 1-12.

Racine, R.J. Kindling: The first decade. Neurosurgery, 1978, 3, 234-252.

Racine, R.J., Burnham, W.M., Gartner, J.G., and Levitan, D. Rates of motor seizure development in rats subjected to electrical brain stimulation: Strain and interstimulation interval effects. Electroencephalography and Clinical Neurophysiology, 1973, 35, 553-556.

Racine, R.J., Tuff, L., and Zaide, J. Kindling, unit discharge patterns and neural plasticity. Canadian Journal of Neurological Sciences, 1975, 2, 395-406.

Racine, R.J., and Zaide, J. A further investigation into the mechanisms underlying the kindling phenomenon. In K.E. Livingston and O. Hornykiewicz (Eds.), Limbic Mechanisms. New York: Plenum Press, 1978, 457-493.

Rial, R.V., and Gonzalez, J. The effect of diphenylhydantoin in the prevention of the threshold descent in the electroshock on the reptilian telencephalon. Presented at the First European Regional Conference on Epilepsy, Warsaw, 1976.

Schmidt, R.P., and Wilder, B.J. Epilepsy. F.A. Davis Company, Philadelphia, 1968.

Tanaka, A. Prgressive changes of behavioral and electrographic responses to daily amygdaloid stimulations in rabbits. Fukuoka Acta Medica, 1972, 63, 152-163.

Wada, J.A. The clinical relevance of kindling: Species, brain sites, and seizure susceptibility. In K.E. Livingston adn O. Hornykiewicz (Eds.), Limbic Mechanisms. New York: Plenum Press, 1978, 369-388.

Wada, J.A., and Osawa, T. Spontaneous recurrent seizure state induced by daily electric amygdaloid stimulation in Senegalese baboons (Papio papio). Neurology, 1976, 26, 273-286.

Wada, J.A., Osawa, T., and Mizoguchi, T. Recurrent spontaneous seizure state induced by prefrontal kindling in Senegalese baboons, Papio papio. Canadian Journal of Neurological Sciences, 1975, 2, 477-492.

Wada, J.A., Osawa, T., Wake, A., and Corcoran, M.E. Effects of taurine on kindled amygdaloid seizures in rats, cats, and photosensitive baboons. Epilepsia, 1975, 16, 229-234.

Wada, J.A., and Sato, M. Generalized convulsive seizures induced by daily electrical stimulation of the amygdala in cats. *Neurology*, 1974, *24*, 565-574.

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

Wake, A., and Wada, J. Frontal cortical kindling in cats. *Canadian Journal of Neurological Sciences*, 1975, *2*, 493-499.

Wurtz, R.H., and Olds, J. Amygdaloid stimulation and operant reinforcement in the rat. *Journal of Comparative Physiological Psychology*, 1963, *56*, 941-949.

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Coleman, D., Adams, H.E., & Seidel, W.T. Headache in a college population: Normative data. Paper presented at the meeting of the Association for Advancement of Behavior Therapy, San Francisco, California, December, 1979.

Coleman, D., Epstein, L.H., & Seidel, W.T. EMG discrimination using a magnitude production procedure. Paper presented at the meeting of the Society for Psychophysiological Research, Cincinnati, Ohio, October, 1979.

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Title of Thesis/Dissertation

TRANSFER OF KINDLED SEIZURE SUSCEPTIBILITY BETWEEN ANTERIOR NEOCORTEX AND AMYGDALA

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