

An experimental evaluation of the differential electrode placement hypothesis as the basis for the oscillation effect

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Previous experiments with the kindling paradigm involved a sequence of alternating unilateral stimulations of the amygdalae, and an "oscillation effect" was observed, that is, consistent low-latency values for convulsions elicited from one amygdala and consistent high-latency values for convulsions elicited by stimulation of the contralateral amygdala. In the present study the effect of site of placement of electrode was investigated in two experiments to evaluate the possibility that the oscillation tendency is due to the differential placement of the two electrodes. In two groups of rats, one electrode was placed in the amygdala and the other in the dorsal caudate-putamen. In Group 1 stimulation of the caudate-putamen came first; in Group 2, stimulation of the amygdala occurred in the initial phase. In a third group both electrodes were placed in the amygdalae. Oscillation patterns occurred in the three groups in the two experiments but not in the pattern suggested by the differential placement hypothesis. These results reinforce previous findings indicating that site of electrode placement is not the basis for the oscillation effect. The behavioral pattern observed during the development of kindling with caudate-putamen stimulation was strikingly different from that found with the amygdala: a sharp pull of the head in the ipsilateral direction and spastic jerks of the forepaws upon the onset of stimulation. With termination of the current, this behavior ceased. During the development of kindling, this behavioral pattern was partially or completely displaced as kindling behavioral stages became prominent.

The "kindling effect" has been investigated in a number of laboratories as a model of learning, a model of epilepsy, or as an example of behavioral change of interest in its own right (Goddard, McIntyre, & Leech, 1969). Kindling involves a change from normal exploration to automatic behaviors, and finally, to clonic convulsions (CCs) in response to periodic low-intensity electrical stimulation of a specific brain site (e.g., amygdala).

In recent research in our laboratory, using a sequence of alternating unilateral stimulations of the amygdalae, an "oscillation effect" was observed (Gaito, 1976). The oscillation usually consisted of low values for the amygdala first stimulated (primary site) and high values for the contralateral amygdala (secondary site). The effect was most prominent in latency data, that is, the number of seconds between onset of stimulation and onset of convulsion. The oscillation tendency in latency data has been remarkably resistant to a number of experimental manipulations. The exact basis for the oscillation is not certain, but it is assumed to be due to transfer and interference effects between the two amygdalae (Gaito, Nobrega, & Gaito, 1978; McIntyre & Goddard, 1973).

There are two types of oscillation that occur consistently in our research. Primary oscillation (PO)

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involves low values for the primary site and high values for the secondary site for at least 8 of the 10 phases. Secondary oscillation (SO) is the opposite pattern.

Although the oscillation effect appears to be an authentic response of the amygdalae to sequential alternation of unilateral stimulation and is not based on chance, one possible artifactual basis for the results is differential placement of the two electrodes. The differential electrode placement explanation appears to be a reasonable one. However, there are a number of reasons why it does not seem to be a major contributor (Gaito, 1976). Furthermore, in the first study, and in several others since then, histological analyses were conducted to determine electrode placements. These analyses indicated no relationship between placement of electrodes and behavioral pattern, as long as one or both electrodes were in the amygdala or in nearby tissue.

Goddard et al. (1969) indicated that placement of electrodes in the amygdala produced faster rates of kindling than placement in other tissues. Thus, if one were to place one electrode in the amygdala and the other in another tissue (e.g., the caudate-putamen) and stimulate first the side in which the electrode is in the amygdala, the differential electrode placement hypothesis would predict a PO pattern. If the other side were stimulated first, a SO pattern would be expected. However, if both electrodes were in the amygdala, nonoscillation (NO) would be expected no matter which side was stimulated first. The present study was

concerned with investigating these possibilities for evaluation of the differential electrode placement hypothesis.

EXPERIMENT 1

Method

Twenty-six male Wistar rats (approximately 200 days of age) were implanted with bipolar electrodes in the amygdala and/or in the dorsal caudate-putamen. The brain coordinates used for implantation of bipolar electrodes into the amygdalae were the same as in many experiments in our laboratory: .5 mm posterior to bregma, 4.5 mm from midline, 8.5 mm from skull (Gaito, 1976). The coordinates for the caudate-putamen were the same except for depth; these electrodes were 5.5 mm from the skull.

The rats in Group 1 ($n=9$) had one electrode in the amygdala and the other in the dorsal caudate-putamen of the opposite side (Figure 1). The first side stimulated was that in which the electrode was in the amygdala.

Group 2 rats ($n=8$) had electrodes placed in the same fashion as those in Group 1, but the caudate-putamen side was stimulated first.

The nine rats in Group 3 had both electrodes in the amygdalae. The expected behavioral pattern would be NO, irrespective of which side was stimulated first. These rats were similar to the rats used in all previous experiments in which oscillation occurred in latency data for approximately 75% of the rats. This aspect of the differential or misplaced electrode hypothesis had been rejected consistently in past research, and it was assumed that it would be rejected in these experiments as well. Thus, results with Groups 1 and 2 were of major concern.

Stimulation was not imposed until at least 7 days after surgery. Then, the 26 rats were stimulated with a 60-Hz sine wave over 10 phases of alternating unilateral sites for 30 sec, three trials per day, with a Lafayette stimulator until 6 CCs occurred for each phase. With some rats, the intensity had to be increased beyond the usual 100 microA to achieve convulsion

during Phases 1 and 2. The intensities used were 100-196 microA for most rats; one rat required 280 microA for one side, and two others had 420 microA on one side.

All rats were sacrificed with an overdose of sodium pentobarbitol and perfused with saline and formalin. The brains were removed and kept in a 10% formalin solution for at least 1 month. Tissue slides were prepared from 50-micron brain sections and placed in a photographic enlarger and used as negatives to provide permanent prints to identify the brain sites of electrode tips.

Results

Figure 1 shows a stereotaxic plate taken at bregma, with the electrode tip sites for one rat in each group indicated by the number of that group (i.e., 1, 2, or 3). The electrode tips tended to cluster at this anterior-posterior coordinate, with some anterior to and others posterior to bregma. Most rats in the three groups had both electrodes at or around these sites. A few rats had one of the two electrodes in an adjacent structure: two from Group 1, three in Group 2, and one in Group 3. Two rats from Group 2 had both electrodes in adjacent structures.

Although the sites of electrode placement were different for each of the three rats indicated in Figure 1, the behavioral pattern for the three in latency data was the same, SO. The rat in Group 2 showed a SO pattern in the criterion measure also; the other two had NO behavior. This result seems to suggest that behavioral pattern is somewhat independent of electrode placement.

The results were not consistent with the differential electrode placement hypothesis for any of the groups (Table 1). The number and pattern of oscillators in the

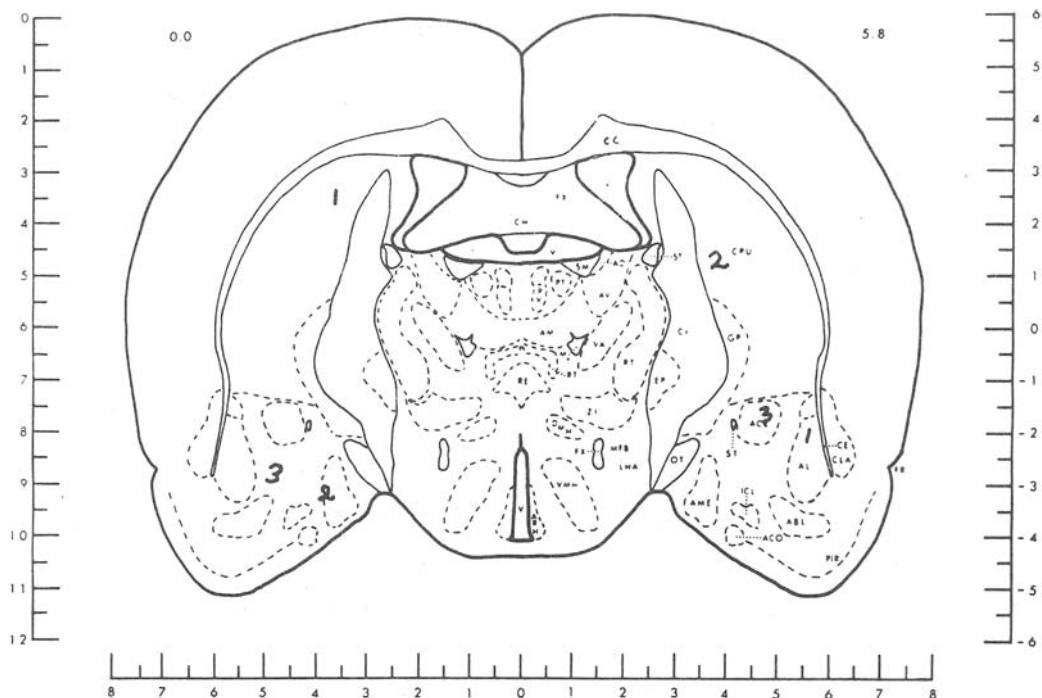


Figure 1. A stereotaxic plate of the rat brain, at bregma. The numbers 1, 2, and 3 indicate the site of electrode tips for one rat in Groups 1, 2, and 3, respectively. Most rats in both experiments had the electrode tips in or near these sites.

Table 1
Behavioral Patterns for the Three Groups

	Latency									Criterion								
	Group 1			Group 2			Group 3			Group 1			Group 2			Group 3		
	PO	SO	NO	PO	SO	NO	PO	SO	NO	PO	SO	NO	PO	SO	NO	PO	SO	NO
Experiment 1	6	2	1	1	4	3	4	2	3	0	1	8	1	1	6	0	1	8
Experiment 2	6	2	2	2	4	3	4	2	3	0	1	9	1	0	8	2	4	3
Combined	12	4	3	3	8	6	8	4	6	0	2	17	2	1	14	2	5	11

Note—The expected behavioral pattern based on the differential electrode placement hypothesis is SO, PO, and NO for Groups 1, 2, and 3, respectively.

three groups were consistent with results of previous experiments. In latency data, the eight out of nine, five out of eight, and six out of nine for number of oscillators in Groups 1, 2, and 3, respectively, were significantly different from the number expected using the binomial distribution with a p of oscillation equal to .17 ($p < .05$ in each case). Obviously, the finding of 19 oscillators out of the total 26 rats was a significant event.

In criterion data, the 1 out of 9, 2 out of 8, and 1 out of 9 were not significantly different from the number of oscillators expected by chance within each group ($p > .05$ in each case), nor was the finding of 4 oscillators over the 26 rats significantly different from 4.4, the number expected ($p > .05$). The criterion measure consistently has been a poor indicator of the oscillation process.

EXPERIMENT 2

During the conduct of Experiment 1, it was noted that the initial reaction to stimulation of the dorsal caudate-putamen in many rats was not the usual exploratory behavior but was one of sharp pulling of the head to the ipsilateral side, rolling in the ipsilateral direction one or more times, and paw jerks of a spastic nature. Thus, the experiment was repeated in order to verify the results of Experiment 1 and to provide an opportunity to describe in detail the progress of the caudate-putamen reaction pattern (CP) as kindling developed.

Method

The procedures were exactly the same as in Experiment 1, with 10, 9, and 9 male Wistar rats in Groups 1, 2, and 3, respectively (approximately 100 days of age).

Results

Histological analyses were similar to those in Experiment 1. Electrode tips were in the desired sites in most rats.

The results again were not consistent with the differential electrode placement hypothesis. In fact, as in Experiment 1, there appeared to be a slight trend favoring PO in Group 1 and SO in Group 2 in latency data, results exactly opposite from the hypothesis of concern. However, in Group 1 the number showing the

opposite pattern (PO) over the two experiments was not significantly different from the number showing the expected pattern (SO), using the normal approximation to the binomial distribution with $p = q = .5$ ($z = 1.75$, $p > .05$). In Group 2 similar results were obtained ($z = 1.20$, $p > .05$). In Group 3, the number of nonoscillators (six) was so close to the number showing PO and the number showing SO that it is obvious that they were not significantly different. The number of oscillators in each of the three groups, and over the three groups, was significantly different from the number expected using a p of .17 ($p < .05$ in each case).

The number of oscillators showing the opposite pattern was not significantly different from the number expected in latency data for Groups 1 and 2 in the two experiments; however, for Phases 1 and 2, a partially "nested" ANOVA design would be appropriate, in this case with the dimensions of phase (1, 2), a between-subjects effect, and site (amygdala, caudate-putamen), a within-subjects effect. The mean values indicate that there appears to be a significant interaction present between phase and site. In such a case, tests of main effects usually are of little value, and one must resort to other analyses. In the present case, the means for 17 subjects for the caudate-putamen and the amygdala were 8.6 for each when stimulation of the caudate-putamen occurred on Phase 2. However, when stimulation of the caudate-putamen was on the first phase, the means for 19 subjects for caudate-putamen and amygdala were 6.8 and 9.6, respectively. Thus, this difference can be tested simply by difference scores between the amygdala and the caudate-putamen electrode sites with a t test. The resulting $t(18)$ value was 2.47, which indicated that the caudate-putamen mean was significantly less than the amygdala mean ($p < .05$) for the first phase. Of the 19 difference scores, 5 were positive and 14 were negative.

In criterion data the number of oscillators was significantly different from the number expected only in Group 3. This result is probably a Type I error; in previous research, criterion values were rarely significant.

The mean number of trials to 6 CCs was essentially the same for caudate-putamen and amygdala electrode sites whether stimulation of the caudate-putamen occurred on Phase 1 or Phase 2. If stimulation of the caudate-putamen was on Phase 1, the means for caudate-

putamen and amygdala were 25.1 and 23.7, respectively. If stimulation of the caudate-putamen occurred on Phase 2, the resulting means were 21.5 and 22.9.

The detailed observation of the reaction to stimulation of the caudate-putamen indicated the following behaviors.

In most rats, a unique reaction pattern (CP) occurred on the first trial, and thereafter with the onset of stimulation, and ceased with the termination of stimulation. This pattern consisted of three components: (1) a sharp pull of the head to the side of stimulation (ipsilateral), (2) a half-roll in the ipsilateral direction or a complete roll one or more times, (3) spastic jerks of the forepaws. Reactions 1 and 3 were the most pronounced in all rats. Reaction 2 was absent or in reduced form in almost one-half of the rats.

The CP tended to decrease in duration and intensity as the kindling process developed. It appeared that the kindling process was partially or completely displacing or modifying the natural reactions to the electrical stimulation. This aspect is reflected in the fact that in later phases involving stimulation of the caudate-putamen, the reaction pattern of each rat was one or the other of the following: (1) If Reaction 2 was prominent (roll in the ipsilateral direction), the CP blended into the CC pattern. For these rats, the CC pattern was accompanied always by the CP. (2) If Reaction 2 was absent or in reduced form, the CP blended into the CC pattern and then was completely displaced in later phases when the CC pattern occurred alone.

GENERAL DISCUSSION

The results in both experiments were quite similar, and thus slight differences in placement of electrodes in the two experiments could not be a basis for the results.

The results of the two experiments clearly were inconsistent with the misplaced electrode or differential electrode placement hypothesis, but they were partially consistent with previous results that suggested that placement of electrodes (in amygdala or nearby tissue) is not the basis for the oscillation effect.

As in previous research, the latency measure was the most sensitive indicator of oscillation. The number of oscillators over the three groups in the two experiments (39 to 54 rats) was significantly different from the number expected on the basis of $p = .17$ (i.e., 9.2).

Also as in previous research, the criterion measure showed few examples of oscillation. Over the two experiments and three groups, the finding of 14 oscillators (out of 54 rats) was significantly different from the 9.2 expected. However, this event was based on Group 3 in Experiment 2, which had six oscillators in nine rats, a result that is rare in criterion data.

An unexpected finding in this study was the tendency for latency values to be lower for stimulation of the caudate-putamen site than for stimulation of the amygdala. Thus, either a PO or a SO pattern occurred, depending on the side that was

stimulated first. These results are different from those of previous research in which electrodes in the amygdala, in the junction of the amygdala and adjacent structures, or in nearby tissue were unrelated to the type of oscillation pattern (or nonoscillation) that occurred. Both electrodes could be in the amygdalae, at the junction between the structures, or in nearby tissue, or one electrode could be in one of these and the other in any of the other sites, without tending in the direction of either type of oscillation. Thus, the results of this study and the earlier ones indicate that there appears to be no relationship between placement of electrodes in the amygdala or in nearby tissues, but the placement of one electrode well above the junction of the amygdala and the caudate-putamen, in the dorsal part of the latter structure, does predispose the rat to a specific type of oscillation. It should be noted that the electrode aimed for the amygdala was 8.5 mm in length, whereas the one for the caudate-putamen was 5.5 mm. The difference of 3 mm is a gross one relative to brain dimensions. Thus, seldom would an electrode 8.5 mm in length be misdirected such that the tips would be in the dorsal caudate-putamen.

An interesting result was the finding that the reaction pattern to stimulation of the dorsal caudate-putamen was different from that for stimulation of the amygdala. The three stages for stimulation of the latter are: exploratory behavior, behavioral automatism, and CCs. The CP was different in each of the three stages, but especially in Stage 1. For most rats the immediate reaction to stimulation was a sharp pull of the head to the ipsilateral side, slight or extreme roll in the ipsilateral direction, and spastic jerks of the forepaws. These behaviors terminated with the offset of stimulation. The CP appeared to decrease in duration and intensity as kindling proceeded during the first phase. This pattern, in full or in modified form, also was present during later phases of stimulation. In many cases the CP blended into the onset of the CC. Thus, in each of the three stages, there might be a part or full CP with the onset of current; this behavior was partially or completely displaced by the behavioral automatism or CCs during Stages 2 and 3, respectively. It appears that as kindling proceeds with associated underlying brain changes, these changes bring about a partial displacement or modification of the brain events that are responsible for the CP. We see that an acquired reaction (kindling) is displacing or modifying a naturally involuntary reaction (CP). This result is another aspect of kindling that is similar to that seen during the learning process.

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