The oscillation effect during sequential alternation of amygdaloid stimulation with aged rats

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In two experiments, aged rats (approximately 420-475 days of age) were subjected to stimulation of each amygdala in an alternating sequence. Their behavioral response to this stimulation was similar to that found previously with younger rats, systematically going from normal exploration, to automatic behavior, to clonic convulsions. They also showed the "oscillation effect," i.e., low values in latency when stimulated on one side and high values when stimulated on the opposite side.

When certain brain areas are stimulated unilaterally by low-intensity electrical current at periodic intervals, the animal's overt behavior changes in an orderly fashion from normal exploration, to automatic behavior (e.g., chewing, eye closure), and finally to clonic convulsions (CCs). This event has been called the "kindling effect" (Goddard, McIntyre, & Leech, 1969). Once the CC stage is reached, subsequent stimulation will lead consistently to behavioral convulsions shortly after the onset of stimulation.

If one amygdala (primary site) is stimulated to a criterion (e.g., six CCs), using 24 h as the intertrial interval, and then the contralateral amygdala (secondary site) is stimulated, the criterion for the latter is reached within a fewer number of trials, but increased latency to convulsion results. Thus both positive and negative transfer occurs (Goddard et al., 1969; McIntyre & Goddard, 1973). If the animal is stimulated again at the primary site, it does not convulse on the first trial. A few trials are required to reach the CC stage, thus indicating negative transfer.

An interesting question is, "At what point in a sequence of alternations will a rat's behavior become stable, that is, show no positive or negative transfer effects?" In previous research in our laboratory (Gaito, 1976b), an unexpected result was obtained. When rats were stimulated three times per day over 15 to 19 phases of sequential alternation (six CCs within each phase), they tended to show an "oscillation effect" during these alternations, especially in the time between onset of stimulation and onset of convulsion (latency). They showed low values with stimulation of the primary site and high values when the secondary site was stimulated. Some rats persisted in this oscillation throughout the entire sequence of alternations. For example, a rat might show a mean latency of 5 sec over the six CC trials on the primary side. When stimulated in the contralateral amygdala, the mean latency would be approximately 15 sec. This oscillation effect occurred even if a single phase of bilateral stimulation was given prior to unilateral stimulation, or if two phases of bilateral stimulation were introduced after the

oscillation effect was pronounced. The effect was weaker in criterion data (number of trials to six CCs) and almost nonexistent in the duration of convulsion data.

These experiments had been conducted with rats approximately 135 days of age, young adults. Inasmuch as there are strong reasons for assuming that the development of kindling is based on neural plasticity (Goddard et al., 1969), and that older organisms may show reduced plasticity, we decided to determine: (a) if kindling would proceed efficiently with older rats and (b) if the oscillation effect would appear also in these rats.

METHOD

Wistar male rats were implanted bilaterally with bipolar electrodes in the amygdala (Nichrome wire with trimel coating, .127 mm in diam, dipped one time in epoxylite). The brain coordinates for electrode implantation were the same as in many experiments in our laboratory (e.g., Gaito & Gaito, 1974): .5 mm posterior to bregma, 4.5 mm from midline, 8.5 mm from skull. Seven or more days after surgery, the kindling procedures began.

In Experiment 1, nine rats were stimulated in one amygdala (side chosen randomly in each rat) for 30 sec with a 60-Hz sine wave of 100 microA (peak to peak), using a Lafayette stimulator. There were three trials per day, with 1 h between trials. On the day after the sixth CC, stimulation began at the secondary site and continued until six CCs occurred. Then stimulation at the primary site was instituted again, then at the secondary site, etc. This sequential alternation of unilateral stimulation was continued for 10 phases. These rats were approximately 420 days of age when stimulation began.

In Experiment 2, 18 rats were stimulated through 10 phases of unilateral stimulation as in Experiment 1. These rats were approximately 475 days of age at the beginning of stimulation. Four of these rats lost electrodes during the experiment and had to be discarded.

Some rats did not convulse in the first 30 trials of Phase 1 and/or Phase 2 of the unilateral stimulations. Thus, for these animals, the current intensity was increased slowly over trials (to a maximum of 560 microA) until a CC resulted. This intensity was maintained for the remaining phases. On the basis of our previous results, only the latency and criterion data were analyzed in each experiment.

Four rats from Experiment 1 were stimulated for up to 32 phases to determine the stability of their performance. One of

Table 1
Number of Oscillators

	Criterion			Latency		
	Experiment 1	Experiment 2*	Combined	Experiment 1	Experiment 2*	Combined
PO	0	1	1	1	6	7
SO	2	2	4	6	4	10
NO	7	11	18	2	4	6

*Four rats lost electrodes

PO = primary oscillation, SO = secondary oscillation, NO = nonoscillation

these rats had shown oscillation in both latency and criterion measures in the initial 10 phases; one had not oscillated in either measure; the other two rats had oscillated in latency data but not in the criterion measure.

RESULTS

The mean number of trials to first CC was 13.4 in Experiment 1 and 10.4 in Experiment 2. These values are basically the same as the mean of 10.1 in the previous oscillation experiments (Gaito, 1976b) and 10.2 in other experiments (Gaito, 1976a). Thus kindling developed in a similar fashion with these aged rats as it did with younger rats.

The oscillation results for the two experiments are shown in Table 1. As in the previous work, the latency measure showed the stronger effect for oscillation, with 7 of 9 rats showing oscillation in Experiment 1, and 10 of 14 in Experiment 2. In the criterion measure, only 2 of 9 and 3 of 14 oscillated in the two experiments.

There are two types of oscillation observed in this experiment and in previous work (Gaito, 1976b). In one case, the low values appear for the side first stimulated (primary oscillation). In other cases, the low values may appear for the side stimulated second (secondary oscillation). In the earlier experiments, primary oscillation predominated. In the present experiments, primary oscil-

lation in latency also occurred most frequently in Experiment 2, but the reverse was true for Experiment 1. Furthermore, although few oscillators resulted for the criterion measure, most of these tended to secondary oscillation.

Typical data from each experiment are provided in Table 2. For each experiment, there is one example of primary oscillation, secondary oscillation, and non-oscillation.

The four rats in Experiment 1 which were stimulated beyond 10 phases tended to show more prolonged oscillation in latency than in the criterion data. The rat which had oscillated in both measures during the first 10 phases terminated oscillation in the criterion measure after 8 further phases, but continued to oscillate through Phase 32 in latency data. The rat which had not oscillated in either measure began to oscillate during the extra phases in both measures; however, in criterion data, the oscillation continued for 12 phases and then stopped for the remaining phases; in latency data, this rat began to show secondary oscillation for 10 phases and then reversed to primary oscillation during the last 14 phases. The other two rats which had not oscillated in criterion data continued nonoscillation during the extra phases; these rats continued with secondary oscillation in the latency measure during the extra phases.

Table 2
Typical Data Showing Primary Oscillation, Secondary Oscillation, or Nonoscillation for Latency (L) and Criterion (C) Measures

Rat Number	Measure	Phases									
		1	2	3	4	5	6	7	8	9	10
Experiment 1											
10	L	10.8	11.2	2.8	11.0	3.7	7.0	7.5	7.0	2.2	5.8
	C	21	62	6	9	6	6	7	6	6	6
3	L	10.0	4.2	12.2	4.8	12.3	5.3	14.0	5.2	12.8	6.2
	C	39	8	13	7	8	6	7	6	26	7
6	L	7.0	8.3	4.3	8.0	7.0	7.0	9.0	5.7	7.8	8.7
	C	19	7	8	8	16	8	8	8	7	6
Experiment 2											
18	L	5.3	13.8	6.3	11.2	4.8	10.3	4.7	13.5	4.5	14.3
	C	30	20	6	9	6	6	7	7	6	9
14	L	18.2	14.0	14.7	12.5	15.0	12.3	11.0	12.5	15.2	12.2
	C	15	13	24	7	8	6	16	7	20	7
19	L	11.2	9.8	6.8	7.0	6.3	7.0	14.5	7.3	11.0	8.2
	C	14	39	6	25	7	8	6	6	8	6

Supplementing these quantitative data showing the "oscillation tendency" were qualitative behaviors. With some rats, stimulation of the side which showed high latency values caused the rat to rear on its hind paws in the usual way. However, instead of convulsing immediately, the animal would maintain this anticipatory posture as if the convulsive behavior was somehow restrained, and only after a few seconds would the CC ensue. For example, Rat 8 in Experiment 1 showed a CC within about 5 sec upon activating the stimulator for the secondary site. When the primary site was stimulated, this rat reared as quickly, but did not CC until approximately 10 sec had elapsed. The usual CC behavior appeared to be actively inhibited during this state of suspension. This behavior was exhibited by all animals on at least several trials. Most rats showed a greater frequency of this response on one of the two sides, and usually that side was the "high" side.

Other rats showed a different behavior during stimulation of the "high" side. For example, Rat 5 of Experiment 1 had the initial suspension of behavior. During this state, the forepaws would begin to show tremors which, however, would terminate within several seconds, with the animal reverting to normal exploratory behavior instead of showing a CC. This behavior was recorded as an "abortive CC" or an "incomplete CC." The criterion in our experiments has been that a complete CC must continue for at least 5 sec to be recorded as such. On other occasions, there was a pause of up to about 10 sec after the "abortive CC," and then a complete convulsion resulted.

DISCUSSION

The results indicate that kindling and oscillation proceed in a similar fashion in both aged and younger rats. These older rats kindled at the same rate as did younger ones in previous research. Their behavioral pattern leading to a CC was the same. The tendency to oscillate was similar also. In Experiment 1, 7 of 9 rats oscillated in latency data; in Experiment 2, oscillation resulted with 10 of 14 rats. These results (17 oscillators with 23 rats) are consistent with the previous work (18 oscillators in 19 rats) and suggest that oscillation is a genuine phenomenon.

One might think that oscillation is a chance effect. However, the likelihood of a number of rats showing a systematic change from low to high (or high to low) on successive phases is extremely low. The possibility also might be raised that oscillation is based on poor placement of one electrode in, or out of, the amygdala showing the high values. Although this latter explanation sounds reasonable, there are a number of observations which make it appear unlikely.

1. If the effect were due to differential electrode placement in the two hemispheres, oscillation should occur only over a few phases and then disappear, because the periodic stimulation lowers the threshold for seizure and both sides should become equally efficient (Tress & Herberg, 1972). However, although on a few occasions rats terminated oscillation after a few phases, most continued to oscillate throughout the entire series of phases, especially in the latency measure.

2. Most rats begin oscillation on Phase 1. However, in a few cases, oscillation does not occur until Phase 3 or later, e.g., Rats 25, 26, 28, and 29 of Experiment 2. Rat 7 began oscillation on Phase 9. Thus, by these phases, brain conditions should be sufficient to allow convulsions to ensue even though electrode placement may be slightly different for the two sides.

3. The behavior of Rat 7 in Experiment 1 was inconsistent with the misplaced electrode notion. In the latency measure, this animal showed nonoscillation for 8 phases and then secondary oscillation for the next 10 phases; in the final 14 phases, he was a primary oscillator. This type of oscillation, mixed oscillation, has been observed in other experiments in our laboratory.

4. Although the latency and criterion measures go in the same direction in many cases (especially during the early phases), they are relatively independent on most occasions, as indicated in these experiments and in the previous research. Oscillation occurs more frequently in latency data than in the criterion measure. Poor electrode placement would be expected to affect the criterion measure, e.g., by requiring more trials to achieve the six CCs. However, this effect need not be influencing the latency measure. In fact, in the previous research it was noted that the electrode did not have to be in the amygdala to achieve oscillation. A rat with a misplaced electrode (in the caudate putamen/globus pallidus area) provided primary oscillation in latency data. Even though 50 trials were required to reach criterion during stimulation of the primary site on the first phase (which is much more than usual), latencies were lower on this phase and on the third phase, showing excellent oscillation for the four phases on which this rat was run.

5. In these and previous experiments, oscillation resulted when the same intensity of stimulation was used for both amygdalae. However, oscillation occurs also if intensities are just slightly above threshold; thus different intensities may be involved for the two sides (research underway). In this case, no oscillation occurs in the criterion measure, because most rats achieve the criterion of 6 CCs in the minimum number of trials on most phases. However, oscillation consistently occurs in the latency measure.

6. If a misplaced electrode in each pair were the basis for the oscillation, one would expect that if the number of trials to the criterion of 6 CCs were greater during Phase 1 than during Phase 2, the oscillation should be of secondary nature. If the values were the same in both phases, one would expect nonoscillation. If the value in Phase 2 was greater than that in Phase 1, primary oscillation should occur. These expectations would be for the criterion measure, but not necessarily for latency data.

To consider this aspect, the difference in number of trials to 6 CCs in Phases 1 and 2 was related to the type of behavior occurring (Table 3). A χ^2 test indicated that there was no basis for rejecting the null hypothesis of independence between the two dimensions for the criterion measure ($\chi^2 = 4.63$; $df = 4$; p , between .25 and .50), but the null hypothesis was rejected for the latency data ($\chi^2 = 12.76$; $df = 4$; $p < .05$).

These results suggest that differential placement of electrodes is not the main basis for oscillation. However, it might be inferred that the greater number of trials to attain criterion in one side may provide the opportunity for the inhibitory factor exhibited in the latency measure to gain strength.

It is important to note that even if a significant relationship had been indicated for the criterion measure, the differential

Table 3
Relationship Between Discrepancy in Number of Trials to Six CC for Phases 1 and 2 and Behavior

	Criterion Measure			Latency Measure		
	SO	NO	PO	SO	NO	PO
1 > 2*	3	5	0	6	1	1
1 = 2**	1	5	0	3	3	0
1 < 2***	0	8	1	1	2	6

SO = secondary oscillation, NO = nonoscillation, PO = primary oscillation

*1 > 2 by 5 or more trials

**1 = 2 (± 4 trials)

***1 < 2 by 5 or more trials

electrode placement interpretation could not be considered as the sole basis for the oscillation effect. Possibly it would suggest that the difference was important in *initiating* the oscillation behavior; however, there would be the need for determining the mechanism which maintains the oscillation tendency over many phases.

7. The qualitative behavior discussed above might be another basis for rejecting the poor electrode-placement interpretation for oscillation. Although a rat may rear quickly with stimulation of either side, the convulsion occurs immediately for one side but appears to be actively inhibited with stimulation of the other side. This appearance of *active inhibition* of potential behavior seems to argue against the notion that the longer time involved is due to poor electrode placement.

Thus it appears that oscillation is an authentic phenomenon developed by unilateral stimulation in both young adult rats and in older ones. Oscillation has been reported also by McIntyre (1975) with normal and split-brain rats; he found primary oscillation over three sequential alternation phases, the maximum number of phases which was utilized.

Apparently an inhibitory process is set up which affects the same and/or the contralateral brain site. This inhibitory process may involve the same chemical which produced the interanimal retardation effect in the development of kindling when naive recipient rats were injected with brain material from kindled rats (Gaito, 1976a; Gaito & Gaito, 1974).

The nature of the presumed inhibitory influences between the two amygdalae in the production of the oscillation effect remains unclear. In previous research, the inhibition appeared to be from the primary to the secondary site (primary oscillation). In Experiment 2, the same result occurred. However, in Experiment 1 secondary oscillation predominated. The reason for this discrepancy is obscure at this time. Further research is underway to elucidate the nature of the oscillation effect.

The unilateral sequential alternation procedure appears to be useful for obtaining information on some aspects of underlying mechanisms of kindling via the oscillation effect. By determining variables which minimize or maximize this effect, one could gain clues concerning brain mechanisms which are responsible for the

kindling effect. Because kindling shows many parallels to learning and to epilepsy, and can be considered as a model of consistent behavioral changes in response to an invariant stimulus (Gaito, 1974, 1976c), experimentation of the type reported in this paper has the potential to provide information relevant to brain function in general.

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