Lack of interhemispheric transfer of the 1-Hz suppression effect in rats

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Three experiments were conducted to evaluate the effect of 1-Hz brain stimulation of the amygdala on kindling behavior induced by 60-Hz sine-wave stimulation in the contralateral amygdala. In Experiment 1, the effective threshold intensity (ETI) to elicit a kindled response with 60-Hz stimulation was determined on four separate occasions with 15 trials between determinations (three per day) for each of three groups. Experimental rats in Group 1 were stimulated with 1-Hz sine waves in one amygdala, then with 60-Hz current in the opposite amygdala, followed by 1-Hz stimulation of the first site (1-60-1, OA), with 1-h interstimulation intervals. Group 2 was treated with the 1-60-1 pattern, but with all stimulation in the same amygdala (1-60-1, SA). Group 3 received only the 60-Hz stimulation trials (X-60-X), on the second trial. Group 2 showed the typical suppression result, a gradual increase in threshold over the four ETI determinations. However, Group 1's responses were similar to those of Group 3: ETI values decreased gradually over the determinations. Later, when Group 1 received a set of trials in which 1- and 60-Hz current stimulation was to the same site, suppression responses occurred. Two further experiments were conducted with similar results. These results suggest that the suppression effect generated by 1-Hz stimulation appears to involve a local process, an intrahemispheric effect.

The kindling effect has been investigated in a number of laboratories (e.g., Goddard, McIntyre, & Leech, 1969; Racine, 1972; Wada & Sato, 1975). Repeated low-level electrical stimulation of any of a number of brain regions induces progressive changes which culminate in clonic convulsions; as stimulation trials proceed, the animal's behavior changes in a gradual and predictable way from an initial stage of exploratory behavior (Stage 1), to behavioral automatisms usually involving facial contractions, chewing movements, eye closure, and salivation (Stage 2-BA), and, finally, to a clonic convulsion (Stage 3-CC). Some researchers have extended this classification to more stages by expansion of Stages 2 and 3 (Racine, 1972).¹ Behavioral, chemical, electrophysiological, and neurological aspects of this effect have been investigated by many researchers (Gaito, 1976; Racine, 1978).

Once the motor seizure stage has been achieved, usually after 10-15 trials with rats, subsequent stimulation trials will induce the clonic convulsions, even if weeks or months elapse between stimulations. Thus, a permanent brain change is thought to result from kindling. Kindled seizures characteristically have a latency, measurable in seconds, between the onset of stimulation and the onset of the convulsion. They last for a number of seconds after the electrical stimulation is terminated. The effect is remarkably resistant to a number of experimental manipulations. Careful morphological analyses have indicated that the effect is not due to edema, ion deposition, or any form of damage to the stimulated tissue. Although the

amygdaloid complex appears to be particularly sensitive to kindling, the effect may be obtained from a number of different brain regions, especially those within the limbic system (Goddard et al., 1969; Racine, 1978).

In the course of investigating kindling, we have noted that kindling behavior could be altered or prevented by stimulation with low-frequency sine-wave stimulation (suppression effect). In a series of experiments, we found that 1- or 3-Hz sine-wave stimulation at moderate intensities before and/or after a 60-Hz stimulation trial suppressed the tendency of the 60-Hz current to produce kindling behavior (Gaito, 1979, 1980a, 1980b, 1980c; Gaito, Nobrega, & Gaito, 1980). The experiments with 3-Hz stimulation were conducted at an intertrial interval of 1 h between the imposition of the 3- and 60-Hz stimulation trials. Other intertrial intervals have been used with the 1-Hz agent. With 1- and 3-h intertrial intervals, the suppression effect was pronounced. The effect was present with a 24-h interval but was reduced greatly (Gaito & Gaito, 1981). When the interval was increased to 72-h, the effect was minimal, and it was lost completely, or almost so, with a 5- or 14-day interval (research underway).

These suppression results are one of three types of interference effects reported by individuals using the broad kindling paradigm (Gaito, 1980d). The other two are: that produced by repeated, periodic stimulation of the same site with 60-Hz current (Gaito, 1980d) and that involving successive stimulation of alternate homologous brain sites (Goddard et al., 1969; McIntyre & Goddard, 1973).

Positive and negative transfer effects occur during kindling procedures when stimulation is imposed in the opposite hemisphere (McIntyre & Goddard, 1973). It is pos-

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sible that the suppression event also could provide transfer results. Thus, we implemented research to investigate this possibility. In contrast to previous experiments, which involved 1-Hz and 60-Hz stimulation of the same site, the present experiments used 1-Hz sine-wave stimulation that was imposed to one amygdala and 60-Hz sine waves to the homologous amygdala.

EXPERIMENT 1

Method

Thirty-three male Wistar rats (about 90 days of age) had electrodes placed bilaterally in the amygdala (nichrome bipolar electrodes). The brain coordinates for electrode implantation 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 (bregma as reference point; Gaito, 1979). With these coordinates, the electrode tips were aimed at the central part of the amygdala, approximately in the intercalated nucleus.

Stimulation was not imposed until at least 7 days after surgery. Then the 33 rats were stimulated with 60-Hz sine waves for 30 sec during three trials on the 1st day. One hour intervened between each trial. A Lafayette stimulator was used; the intensity on Trials 1 and 2 was 36 μ A (root mean square, RMS, equivalent to 100 μ A, peak to peak). On the third trial, the first effective threshold intensity (ETI₁) was determined; the intensity of the 60-Hz current was increased until a Stage 2 or Stage 3 response was elicited. Then 5 μ A was added to allow for day-by-day threshold fluctuations. A further trial of stimulation at this intensity was provided the following day to check the reliability of ETI₁. Then the rats were separated into three groups, with the rats matched in triplets so that the mean ETI of all groups would be approximately the same. However, there was one restriction. Previous research had indicated that during repeated 60-Hz stimulation trials the mean effective threshold intensity (ETI) dropped slightly, whereas with 1-Hz stimulation ETI increased. Therefore, rats whose ETI₁ value was close to the upper limit (200 μ A) were placed in the X-60-X group. This procedure usually results in a mean ETI₁ that is greater than that for the 1-60-1 group, as was the case in this experiment.

Then two groups of 11 rats received stimulation with 1-Hz sine waves for 120 sec on Trials 1 and 3, 4 and 6, 7 and 9, 10 and 12, and 13 and 15 at twice the ETI₁ value. A 60-Hz-stimulation trial was provided on Trials 2, 5, 8, 11, and 14 for 30 sec at ETI₁. This procedure involved a 60-Hz stimulation trial sandwiched between 1-Hz stimulation trials each day. There were three trials each day with 1 h between each trial. Group 1 rats were stimulated with 1-Hz sine waves in one amygdala and 60-Hz current in the opposite amygdala (1-60-1, OA). Group 2 rats had 1- and 60-Hz stimulation to the same amygdala (1-60-1, SA). Group 3, with 11 rats, received 60-Hz stimulation on the same trials as did the 1-60-1 groups; on the other trials, each of these rats was placed in the apparatus without stimulation (X-60-X).

All 60-Hz stimulation was at ETI₁ for 30 sec, a duration that has been used routinely in our research. Stimulation with 1-Hz current lasted 120 sec at two times ETI₁; this duration and intensity had been found to produce a drastic suppressive effect in previous experiments. Stimulation with 1- and 60-Hz current never exceeded 200 μ A (RMS), the upper limit used routinely in our research.

Following these 15 trials, rats from all groups had ETI₂ determined in a single trial on each of 2 days. Then another block of 15 trials of stimulation occurred, in which each group was treated in the same fashion as during the previous block of trials prior to the ETI₂ determination. This alternation of ETI determinations and a block of trials was continued through the ETI₄ determination.

A further block of 15 trials and ETI₅ determination were provided for the 11 rats in Groups 1 and 3. The 1- and 60-Hz stimulation was administered to the same site for Group 1 (rather than to the opposite site, as in previous trials). Group 3 rats were treated in the same fashion as previously.

Because histological analyses performed in previous experiments in which most electrode placements had been in the amygdala had detected

no lesions at intensities of 200 μ A and below, and because the tissues around the electrode tips were indistinguishable among rats that had been stimulated with 1 Hz, with 1 and 60 Hz, or only with 60 Hz, no histological analysis was conducted in the present experiments.²

Results

The dependent variable (ETI) that had been most sensitive to the effects of 1-Hz and 3-Hz stimulation in previous experiments was utilized in the present experiment. The results are given in Table 1.

The data for Group 2 show the typical suppression effect; ETI values increase over the ETI determinations. Rats stimulated with 1-Hz current on the opposite side to that receiving 60-Hz current (Group 1) showed the gradual decline in threshold values typical of X-60-X groups, as occurred with Group 3. However, when 1-Hz stimulation was provided on the same side for Group 1 during the fourth block of trials, the final threshold value increased prominently (ETI₅), producing a mean of 143.6 μ A. This was an increase of 89.9 μ A beyond the ETI₄ value of 54.7.

The standard deviations tend to vary consistently with the means. Thus, the means and standard deviations gradually decrease over the ETI determinations for Groups 1 and 3. These decreases are in sharp contrast to the increased means and standard deviations for the 1-60-1 (SA) group. The one exception is the standard deviation for ETI₄. In that case, the standard deviation was artificially reduced because 6 of the 11 rats reached the upper limit of 200 μ A without a kindred response and were therefore given a score of 208 (a routine procedure in our research program).

Changes in thresholds are shown clearly in a relative manner by forming a ratio of each ETI value to ETI₁ for each group. These are shown in Table 1 and in Figure 1.

Table 1
Mean Thresholds, Standard Deviations, and ETI/ETI₁ Ratios for Four ETI Determination Points in Experiment 1 (in Microamperes, Root Mean Square)

	ETI Determinations			
	1	2	3	4
Group 1 (1-60-1, OA)				
Mean Thresholds	86.8	65.5	57.7	55.2
Standard Deviations	46.8	25.6	19.9	20.8
ETI/ETI ₁ Ratios	1.00	.75	.66	.64
Group 2 (1-60-1, SA)				
Mean Thresholds	79.5	120.0	164.0	195.3
Standard Deviations	39.2	56.3	54.3	19.2
ETI/ETI ₁ Ratios	1.00	1.51	2.06	2.46
Group 3 (X-60-X, X)				
Mean Thresholds	102.3	83.8	82.5	78.3
Standard Deviations	53.7	37.2	35.2	33.1
ETI/ETI ₁ Ratios	1.00	.82	.81	.77

Note—The OA group received 60-Hz stimulation to one amygdala and 1-Hz stimulation to the opposite amygdala. The SA group had 60- and 1-Hz stimulation to the same amygdala. Group 3 received 60-Hz stimulation only; these rats were placed in the apparatus during the 1-Hz stimulation trials for Groups 1 and 2, but the current was not activated. There were 11 rats in each group.

Figure 1. The ETI/ETI_1 ratios indicating the relative changes for the three conditions or groups in Experiment 1. Note the sharp discrepancy between the ratio for the same amygdala group and those for the X and the opposite-amygdala groups. — (OA) opposite amygdala stimulation; — — (SA) same amygdala stimulation; — (X) no stimulation with 1-Hz current.

The latter indicates clearly the suppression effect in the SA group and the nonsuppression result in the OA group. The decline in the latter group and in the X group is essentially the same, whereas the SA group has a substantial increase over successive ETI determinations (as does Group 1 for ETI_s). There was a drastic difference in ETI_s ratios between Groups 1 and 3 when 1-Hz stimulation was shifted from the opposite amygdala to the amygdala receiving 60-Hz stimulation, 1.65 and .75 for the two groups, respectively.

In previous research, the results have been so clear that there has been no need to resort to statistical tests of null hypotheses. This clarity was present in this experiment too. The trend of the 1-60-1 group (same-side stimulation) over the four ETI determinations was a consistent increase. That for the X-60-X group showed the typical small decrement over determinations. The group that received 1-Hz stimulation to the opposite amygdala showed a trend that was similar to that of the X-60-X group. However, the latter began the experiment with a slightly greater mean ETI than did this 1-60-1 group, and this difference persisted thereafter.

EXPERIMENT 2

It is possible that, in Experiment 1, an effect was present in the hemisphere opposite to that of 1-Hz stimulation, but that it was slight and difficult to detect. Therefore, in Experiment 2 three changes were introduced to increase the sensitivity of the methods: (1) The same-side-to-opposite-hemisphere comparison was made within the same subject, thus eliminating intersubject variability;

(2) an increase in the duration of stimulation, from 120 to 300 sec was used; and (3) the intensity for 1-Hz stimulation was decreased from $2 \times ETI$ to $1 \times ETI$; which had the advantage of maintaining rats in the 1-Hz stimulation groups for longer periods of the experiment, since $200 \mu A$ was our upper limit.

Method

Electrodes were placed in both amygdalae of 15 male Wistar rats (approximately 160 days of age). Nine of the rats were experimentals. On Trial 1, they were stimulated with 60-Hz sine waves in one amygdala. On Trial 2, stimulation was in the opposite amygdala. These stimulations allowed for a determination of thresholds of the two amygdalae. Trials 3 and 4 involved stimulation with 1-Hz current for 300 sec at $1 \times ETI$, of the amygdala used in Trial 1; 5 rats were stimulated in the left amygdala and 4 in the right. Thus, there was a 60L-60R-1L-1L sequence for stimulation with 60- and 1-Hz current for amygdalae 1 and 2 each day for 5 rats and a sequence of 60R-60L-1R-1R with 4 other rats. These sequences were continued for 8 days. On the 9th day, Trials 1 and 2 were conducted to obtain the final ETI_s . The other six rats received no stimulation on Trials 3 and 4 (controls, X group).

Results

The data for Trials 1 and 2 on Days 1, 5, and 9 are indicated in Table 2. The results are similar to those of Experiment 1; the mean ETI values and ETI ratios decrease when there is 1-Hz stimulation in the opposite amygdala or when there is no 1-Hz stimulation, and they increase with stimulation of the same amygdala. The standard deviations tend to be consistent with the means except in the X group, in which the standard deviations remain approximately the same over the 9 days.

EXPERIMENT 3

This experiment was similar to the previous one, except that it allowed for a comparison of the ETI values before and after 1-Hz stimulation on the same day. This procedure provides the chance to contrast the effect of

Table 2
Data in Experiment 2

	Days of ETI Determinations		
	1	5	9
Group 60-60-1-1, OA			
Mean Thresholds	77.8	67.2	64.0
Standard Deviations	44.4	45.7	38.2
ETI/ETI_1 Ratios	1.00	.86	.82
Group 60-60-1-1, SA			
Mean Thresholds	47.8	79.8	99.7
Standard Deviations	12.0	52.5	58.0
ETI/ETI_1 Ratios	1.00	1.67	2.09
Group 60-60-X-X			
Mean Thresholds	65.0	54.1	58.8
Standard Deviations	41.7	41.8	45.5
ETI/ETI_1 Ratios	1.00	.83	.90

Note—This table is similar to Table 1 except that the OA and SA groups were the same nine rats. There were six rats in the 60-60-X-X group. ETI determinations occurred daily for 9 days. Only the Day 1, Day 5, and Day 9 values are shown. There were nine observations for the OA and SA data and 12 for the X group (two stimulated amygdalae for each rat).

1-Hz stimulation 18 h after stimulation (i.e., the next day in Experiment 2) with the effect 1 h after stimulation (i.e., the same day in Experiment 3). These procedures involved a sequence of 60-60-1-1-60-60 each day instead of the 60-60-1-1 employed in Experiment 2.

Method

After a 14-day rest, the 15 rats of Experiment 2 plus 3 other rats that had been prepared for Experiment 2 but not used were employed in Experiment 3. The stimulation was for 10 days. There were 10 1-Hz-stimulated animals and 8 in the control group.

Results

The results were similar to those of Experiment 2: the means, standard deviations, and ratios for the same-side-stimulated rats increased, whereas those for the group with opposite-side stimulation and the nonstimulated group remained approximately the same (which happens occasionally) or decreased slightly over the determinations (Table 3).

GENERAL DISCUSSION

In three experiments, under three different sets of conditions, stimulation of the same amygdala with 1-Hz sine waves produced a suppression effect typical of that found in previous experiments. The results for the three experiments for the SA set were remarkably similar. Allowing Days 5 and 9 in Experiment 2 and Days 5 and 10 in Experiment 3 to be approximately ETI_2 and ETI_3 , the ETI/ETI_1 values for the SA groups in the three experiments were: 1.00, 1.51, 2.06; 1.00, 1.67, 2.09; and 1.00, 1.63, 1.94, respectively.

It had been expected that stimulation of the opposite amygdala with 1-Hz current also would bring about a suppressive result, but at a slower rate. However, no interference occurred for this case. Rats stimulated with 60- and 1-Hz current in opposite amygdalae showed kindling

responses similar to those of the control groups which did not have 1-Hz stimulation, with ETI values remaining about the same or showing a slight decline. The results for these two groups were very similar over the three experiments and to each other. The ratio values for the OA set were: 1.00, .75, .66; 1.00, .86, .82; and 1.00, 1.04, 1.01. The values for the X group were: 1.00, .82, .81; 1.00, .83, .90; and 1.00, 1.01, .89.

The results of the present experiments suggest that the suppression effect appears to involve a process that is localized to one hemisphere. However, these behavioral results are gross ones. More precise analyses (e.g., chemical or electrophysiological) might be able to detect differences if they are of a modest nature.

Previous research has indicated that, during the kindling process, stimulation of a specific site results in specific changes in the brain-wave pattern in that site; later similar electrical patterns occur in the contralateral site. Likewise, stimulation of one site facilitates the development of the kindling progression in the contralateral area (Goddard, et al., 1969; McIntyre & Goddard, 1973). Thus, stimulation in one hemisphere affects the contralateral hemisphere. In the present experiments, however, stimulation of the contralateral site with 1-Hz sine waves appeared to have no effect on behavioral responses elicited by 60-Hz stimulation of the ipsilateral side. These and previous results suggest that the suppression effect operates via a mechanism that is different from that of the kindling effect, and that the two differ in at least two important ways: (1) The kindling effect is a relatively permanent event, whereas the suppression effect appears to be transient and dissipates almost completely with time. The latter probably involves an inhibitory process (Gaito, 1980d) similar to the "aftereffect" of McIntyre and Goddard (1973) and Mucha and Pinel (1977). (2) The suppression effect seems to be localized to the hemisphere stimulated and produces no behavioral transfer to the opposite hemisphere. This aspect is in sharp contrast to the kindling effect, wherein transfer occurs from the primary to the secondary site.

The exact mechanism involved in the suppression effect is not clear at this time. Presumably, it involves some modification of the brain process responsible for kindling, or else another process is developed that is antagonistic to the brain-kindling process.

One possible basis for the suppression effect is that lesions are produced by the low-frequency stimulation, and the damage raises the threshold for kindled responses. However, this explanation does not seem to be appropriate. Our histological analyses in the earlier research indicated that the tissue around the electrode tips inserted into rats subjected to intensities of 200 μ A or lower with 1- or 3-Hz current appeared similar to that of rats subjected only to 60-Hz stimulation. Obviously, such analyses are of a gross nature and could miss subtle lesions.

A more important consideration is the apparently transient nature of the suppression effect, which decays un-

Table 3
Data in Experiment 3

	Days of ETI Value		
	1	5	10
Group 60-60-1-1-60-60, OA			
Mean Thresholds	39.8	41.2	40.0
Standard Deviations	19.6	19.2	22.4
ETI/ETI_1 Ratios	1.00	1.04	1.01
Group 60-60-1-1-60-60, SA			
Mean Thresholds	49.3	80.4	95.6
Standard Deviations	29.9	58.7	69.2
ETI/ETI_1 Ratios	1.00	1.63	1.94
Group 60-60-X-X-60-60			
Mean Thresholds	87.4	88.5	77.6
Standard Deviations	43.9	50.8	47.5
ETI/ETI_1 Ratios	1.00	1.01	.89

Note—The description is similar to that for Table 2. ETI values on Day 1 were those for Trials 1 and 2; those for Days 5 and 10 were for Trials 5 and 6. There were 10 rats for the OA and SA values and 8 rats (with two observations per rat) in the X group.

der certain conditions. This decay is suggested by data that indicates that most experimental rats convulse at, or near, the ETI₁ point after 15 to 32 days of nonstimulation following the ETI₁ determination (Gaito, 1981). Furthermore, results with intertrial interval variations indicate a time-dependent process, with the interference reduced in 24 h (Gaito & Gaito, 1981) and lost (or nearly) after longer intervals (Gaito, research underway).

A strong indication that tissue damage is not the basis for the suppression process was presented by Gaito (1981). Some rats were lesioned in the amygdala by direct current stimulation. These rats developed high ETI values on one trial and these thresholds did not change over time, in contrast to the 1-Hz-stimulated group, which had sizable increases in ETI values but returned to, or near, ETI₁ levels after 32 days of nonstimulation.

The results with the suppression paradigm suggest that this technique may be a useful supplement within the broad kindling methodology, and that the two methods might provide important information relative to how the brain functions during these automatic behaviors.

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NOTES

1. Although more stages provide an increased precision that is useful for many purposes, such a procedure reduces the reliability of stage determinations. Therefore, we have preferred the three-stage procedure and it has proved sufficient for our purposes.

2. The aim of our histological analyses in the early work was twofold: (1) to determine the site of the electrode tips, and (2) to evaluate the possibility of tissue damage at this site. The amygdala is a large complex of many nuclei (which is difficult to miss), and the tips usually have been in the amygdala. We have noted that kindling and suppression results occur efficiently when the electrode is anywhere in the amygdala, as well as in nearby tissue. Furthermore, we have noted that observable tissue damage appears not to occur with 60-Hz stimulation or with 1- and 60-Hz stimulation with the intensities that we have used.

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