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3-Hz brain stimulation interferes with various aspects of the kindling effect

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These exploratory experiments investigated the effect of 3-Hz brain stimulation on behavior induced by 60-Hz brain stimulation when the former was presented simultaneously with, or following, the latter. In the simultaneous case, 3-Hz stimulation to one amygdala and 60 Hz to the other produced a slower kindling rate than did bilateral stimulation with 60 Hz. When 3-Hz stimulation followed six convulsion trials of 60-Hz stimulation, there was no effect on the convulsive tendency; however, with rats in which the convulsive pattern was relatively stable and 48 or more convulsive trials were followed by 24 trials of 3-Hz stimulation at double intensity or 36 trials at the same intensity as previous 60-Hz stimulation, a reversal effect was observed, that is, a return to nonconvulsive behavior.

The "kindling effect" has been investigated in a number of laboratories (e.g., Gaito & Gaito, 1974; Goddard, McIntyre, & Leech, 1969). This effect involves a change from normal exploration (Stage 1: NE), to behavioral automatisms (Stage 2: BA, chewing, eye closure on ipsilateral side, salivation), and, finally, to clonic convulsions (Stage 3: CC) in response to electrical stimulation of a specific brain site (e.g., amygdala). Stage 3 behavior involves the rat standing on its hind paws and experiencing bilateral convulsions of the forelimbs. Behavioral, chemical, electrophysiological, and neurological aspects of this effect have been investigated by many researchers (Gaito, 1976b).

The frequency that has been used most frequently for the kindling process is 60 Hz. Other frequencies (e.g., 3 Hz) do not produce the convulsions (Fried & McIntyre, 1973; Goddard et al., 1969). Inasmuch as 3-Hz brain stimulation does not produce Stage 2 or 3 behavior, it seemed possible that brain stimulation with this frequency might reverse the effects of the 60-Hz brain stimulation and bring rats back to Stage 1 behavior. We conducted a preliminary experiment with five rats and found that not a single example of Stage 2 or 3 behavior occurred within 24 trials of 3-Hz stimulation for each rat. Therefore, we conducted some exploratory experiments to evaluate the possibility of simultaneous and successive competition of 3-Hz brain stimulation with that of 60-Hz brain stimulation. In the former case, rats were stimulated bilaterally with 60 Hz to one amygdala and 3 Hz to the other. In the latter case, unilateral stimulation occurred first with 60 Hz and then with 3 Hz; the effect of 3-Hz stimulation was determined by test trials with 60-Hz stimulation.

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EXPERIMENT 1

Method

This experiment was concerned with simultaneous competition. Twelve male Wistar rats (about 160 days of age) were implanted bilaterally with bipolar electrodes in each amygdala. The brain coordinates for electrode implantation were as in previous experiments: .5 mm posterior to bregma, 4.5 mm from midline, 8.5 mm from skull (Gaito & Gaito, 1974).

Stimulation trials began 1 week after electrode implantation. For one group of six rats, each trial consisted of passing a 60-Hz sine-wave current for 30 sec into both amygdalae using a Lafayette stimulator. A second group of six rats received 60-Hz stimulation to one amygdala and 3 Hz to the other one. The 3-Hz stimulator was constructed in the electronics shop of the psychology department at York University. There were 3 trials/day, about 1 h apart. Stimulation was at an intensity of 100 microA for 20 trials. Trials 21-25 and 26-30 used 150 and 200 microA, respectively, to insure that all rats reached the convulsion stage.

At the end of Experiment 1, histological analyses were performed on the 12 rats. The rats were sacrificed with an overdose of chloroform and perfused with saline and formalin. The brains were extracted and left for at least 10 days in a 10% formalin solution Each brain was frozen and 50-micron sections were mounted on glass slides; these slides were placed in a photographic enlarger and used as negatives to obtain prints of the desired brain sections. The location of the tip of each electrode was placed in one of four categories: A, in amygdala; B, intersection of amygdala and an adjacent structure; C, just outside amygdala, in adjacent structure; D, outside amygdala, in adjacent structure, but more remote. Previously, we found that most rats with electrodes in structures in Categories A-C convulse within about the same number of trials, usually 30. Thus rats with one or both electrodes in structures indicated by D were discarded. The data from one rat were rejected, from the 60-Hz/3-Hz group. The distribution of As, Bs, and Cs in the two groups was about the same. Histological analyses were completed prior to determining the group identification of each rat.

Results

The 3-Hz stimulation produced a definite effect. The results of one-factor ANOVAs on each of the five dependent variables in Table 1 indicated the following: (1) The kindling process induced by 60-Hz stimulation

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Table 1
Means for the Two Groups in Various Measures (Experiment 1)

	Group	
	1	2
Number of trials to two consecutive BA*	4.7	22.2
Number of CCs in 20 trials (100 microA)**	5.2	.4
Number of CCs in 25 trials (150 microA)†	8.5	2.4
Number of CCs in 30 trials (200 microA)†	11.8	4.4
Composite score††	70.0	46.0

*Note—Group 1 = 60 Hz/60 Hz; Group 2 = 60 Hz/3 Hz. *Significant difference between the two groups [$F(1,10) = 17.19$, $p < .01$]. **Significant difference between the two groups [$F(1,10) = 6.33$, $p < .05$]. †No significant difference between the two groups ($p > .05$). ††Composite score maximum = 90, minimum = 30. Significant difference between the two groups [$F(1,10) = 11.37$, $p < .01$].*

was retarded overall by 3-Hz stimulation. This aspect is indicated by the composite score. Each rat received a value of 1 for Stage 1 behavior, 2 for Stage 2 behavior, and 3 for Stage 3 behavior for each of the 30 trials. The mean for Group 1 was 70, for Group 2, 46. (2) Movement from Stage 1 to Stage 2 was retarded by 3-Hz competition (BA data). Group 1 quickly attained Stage 2 behavior. (3) There was some indication that transition from Stage 2 to Stage 3 was retarded by 3-Hz stimulation (CC data), but the results were not as definite as were the BA data.

Four of the rats that had received 60-Hz stimulation to both amygdalae and convulsed by Trial 30 were stimulated with 3 Hz on one side and 60 Hz on the other for 4 trials. Convulsions occurred in one of the four rats on Trial 1, two on Trial 2, and three on Trial 3; all rats convulsed on Trial 4. These results might indicate that the 3-Hz stimulation appeared to temporarily suppress the convulsion tendency of some of the rats during the first three tirals.

Three other rats (of the 12 in Experiment 1) were stimulated unilaterally with 60 Hz until a convulsion was obtained. Then these rats were stimulated with 3 trials each at 30, 20, 15, 10, and 5 Hz, 3 trials/day. CCs were prominent from 30 Hz through 10 Hz. No convulsions were observed at 5 Hz, but behavioral automatisms did occur. In the preliminary experiment and another experiment described below, 3-Hz stimulation produced only Stage 1 behavior. No definite Stage 2 or 3 behavior was observed, except when high-intensity stimulation was used.

EXPERIMENT 2

Method

This experiment was concerned with the possibility of successive competition of 60- and 3-Hz brain stimulation. Twenty-three male Wistar rats, about 160 days of age, had bipolar electrodes implanted in one amygdala. Brain coordinates were the same as in previous experiments. All rats were stimulated with 60-Hz sine waves at 100-microA intensity, 3 trials/day, until 6 CCs resulted. For some rats, the intensity had to be increased to achieve these CCs. The rats were split into two groups. Group 1 (12 rats) received 24 more trials with 60-Hz sine waves. The other 11 rats (Group 2) were stimulated for 24 trials with 3-Hz sine waves at the same intensity as that used previously with 60-Hz stimulation. Then, both groups were stimulated with 60-Hz sine waves for six test trials.

Histological analyses were conducted on each of the rats at the end of all experiments. These analyses indicated no apparent lesion at the tips of electrodes or nearby. All electrodes were in structures indicated by Categories A-C.

Results

In the six trials, all rats in both groups had 1 CC on each trial. These results indicated that 3-Hz stimulation had no effect on the convulsion tendency produced by previous 60-Hz stimulation.

The mean latency to convulsion for both groups was less after the 24 trials of 3 or 60 Hz (Table 2). The pattern was present for 10 of the 12 rats in Group 1 and for all rats in Group 2. Thus, 21 of 23 rats showed this pattern. A consistent pattern in the opposite direction occurred for the duration-of-convulsion measure; the mean was greater after the interpolated treatment, with the greatest increase occurring for the 3-Hz group. Nine of the 12 rats in Group 1 and all rats in Group 2 (or 20 of 23 rats) had greater means in the test trials.

These results are consistent with previous research in our laboratory (Gaito, Gaito, & Nobrega, 1977); latency to convulsion decreases and duration values increase over trials during the early convulsion trials.

EXPERIMENT 3

Method

Six trials of convulsions as used in Experiment 3 do not produce stable behavior on the part of rats. The intensity of stimulation required to produce a convulsion decreases over trials quite drastically in early trials (Tress & Herberg, 1972).

Table 2
Means for Latency (L) and Duration (D) Measures (in Seconds) for the Various Conditions

	Trials				Pretreatment		Posttreatment	
Group	CC	Treatment	Test	n	L	D	L	D
				Experiment 2				
2	6	24	6	11	9.7	21.9	6.3	32.3
1	6	24	6	12	9.2	22.0	5.8	26.7
				Experiment 3				
1	36	24	1	3	7.2	25.4	7.1	35.9
2	48	24	1	3	5.9	25.2	5.6	43.2
3	48	24	2	3	6.7	20.0	10.5	35.1
4	48	36	1	3	6.7	20.3	11.1	33.2
5	60	24	1	2	4.2	20.0	6.0	29.7
6	60	24	2	2	8.5	18.7	10.1	39.4
7	60	36	1	3	6.9	23.3	7.1	48.1
8*	36	24	1	4	8.6	25.0	8.8	18.7
	48	24	1	4	10.3	28.2	7.7	18.6
	48	36	1	4	10.3	28.2	6.4	21.2
	60	24	1	4	9.6	20.3	6.4	21.2
	60	36	1	4	9.6	20.3	8.1	16.3

*Note—CC trials refer to the number of CC trials with 60-Hz stimulation. Treatment trials refer to the number of treatment trials with 3-Hz stimulation (Experiment 3) or with 3 or 60 Hz (Experiment 2). Test trials refer to the number of test trials with 60-Hz stimulation (Experiment 2) or the intensity of the 3 Hz compared to the intensity of the previous 60 Hz used (Experiment 3). *Controls.*

Furthermore, latency to convulsion decreases and the duration of convulsion increases as stimulation trials progress. Previously (Gaito et al., 1977), we found that the threshold for convulsion and latency and duration values become relatively stable after 24 or more CC trials. Thus, the threshold for convulsion with 60-Hz stimulation was determined for each rat used in Experiment 2, and 15 microA was added to this value (to handle possible daily fluctuations) as the effective threshold intensity (ETI). These rats received 36, 48, or 60 CCs before being subjected to 3-Hz stimulation.

Three rats received 24 trials of 3-Hz stimulation at the same intensity as that used for 36-CC trials with 60-Hz stimulation. Then, they were given six test trials of 60-Hz stimulation (Group 1). There were seven other groups with the following characteristics before receiving the test trials: Group 2—three rats, 48 CCs, 24 trials of 3 Hz, same intensity; Group 3—three rats, 48 CCs, 24 trials of 3 Hz, double intensity; Group 4—three rats, 48 CCs, 36 trials of 3 Hz, same intensity; Group 5—two rats, 60 CCs, 24 trials of 3 Hz, same intensity; Group 6—two rats, 60 CCs, 24 trials of 3 Hz, double intensity; Group 7—three rats, 60 CCs, 36 trials of 3 Hz, same intensity; Group 8—four rats, every trial at 60 Hz, same intensity.

Group 8 was a control group with which to compare rats with 36, 48, or 60 CCs and 24 or 36 trials of 3-Hz stimulation. No control was required for the double-intensity conditions. Any rat convulsing at x intensity will convulse also at 2x intensity. Two of these four rats had 102 consecutive convulsions. Another one had 98 CCs, with consecutive CCs on the last 53 trials. The fourth rat convulsed on the last 45 trials and had 100 CCs overall.

Results

The results are shown in Table 2 for the 3-Hz groups. All rats convulsed on each of the six trials in the group that received 36 CC trials with 24 trials of 3-Hz stimulation and the group that involved 48 CCs with 24 trials of 3 Hz. However, in the other groups, the 3-Hz stimulation appeared to have some effect.

Each of the rats in Group 3 showed Stage 1 or 2 behavior in some of the test trials. One of these rats convulsed on each of the first three trials, then showed three trials of BA, before having six consecutive trials of CCs. The other two rats had early trials of Stage 1 behavior; the first rat did not convulse until Trial 8 (without an increase in the ETI); the second rat convulsed on Trial 10, when the ETI was increased by 70 microA.

In Group 5, one rat convulsed on each of the six trials. The other one showed Stage 1 behavior for the first 2 trials, then a BA on Trial 3; it began to convulse on Trial 4 and had eight convulsions in 12 test trials.

Both rats in Group 6 had nine test trials of Stage 1 behavior. Both rats required an ETI increase of 140 microA to show progression in the kindling process. One convulsed on Trial 10 and the other on Trial 13.

All rats in Group 7 had Stage 1 behavior for a number of test trials. The first rat convulsed on Trial 8, with an ETI increase of 56 microA. The second rat had 3 trials of exploratory behavior followed by 2 trials of mild Stage 2 behavior; convulsions did not result until Trial 10, with an increase of 56 microA in the ETI. The last rat had 9 trials of exploration and began to convulse on Trial 10, with an increase of 140 microA.

The 3-Hz conditions also affected the latency measure with a tendency to greater values after the 3-Hz stimulation. Of the 19 rats in the 36-, 48-, and 60-CC conditions, 14 had greater mean latencies for the post-treatment trials. Of the 14 rats in the 48- and 60-CC conditions, 13 showed this pattern. Five of seven rats in the 60-CC conditions also had this pattern.

The 4 controls convulsed on every trial for the post-treatment equivalent of the various conditions for the other 19 rats. Furthermore, there was no tendency of increased latencies with these rats (Table 2). Thus, the controls showed neither of the two tendencies that a number of the rats demonstrated under some of the conditions, that is, nonconvulsion on early trials and increased latencies. Each of the 19 rats had a greater mean duration value after the 3-Hz stimulation than before.

EXPERIMENT 4

The results of Experiment 3 suggested that the conditions using 48 or more CCs coupled with 36 trials of 3-Hz stimulation at double intensity provided the most favorable condition for an effect of 3-Hz brain stimulation on behavior induced by 60-Hz stimulation. Thus, the four rats in Group 8 were used to evaluate this possibility. Each rat had convulsed 98 to 102 times. Each was given 36 trials of 3-Hz stimulation at double the ETI for the previous 60-Hz stimulation. None of these rats convulsed on the first three trials (Table 3, bottom). The first CC occurred on Trials 4-12. However, only one rat required an increase in the ETI. Three of the four rats also had an increased mean latency, and all showed a greater duration value.

A rest group might have been used in Experiment 3, but previous research had indicated no effect of a rest period of up to 30 days on the convulsive tendency. To check on this aspect, four of the rats from Experiment 3

Table 3
Aspects of 14 Rats That Showed Reversal Effect During Test Trials (Experiments 3 and 4)

	Trials*									
Rat	CC	Treat-ment	Test	CC 1	N	ETI	LP	LT	DP	DT
15	48	24	d	8	4/12	0	2.5	9.0	21.2	34.8
20	48	24	d	10	6/15	70	5.7	8.7	16.0	27.2
24	48	36	s	10	6/15	84	14.7	15.2	24.5	37.2
16	48	36	s	10	6/15	70	1.8	9.7	18.8	34.3
13	60	24	s	4	8/12	0	10.3	12.3	16.3	31.5
9	60	24	d	10	7/18	140	8.2	14.3	23.0	44.8
14	60	24	d	13	6/18	140	8.7	5.8	14.3	34.0
4	60	36	s	8	6/13	56	2.8	3.6	24.0	55.4
23	60	36	s	10	6/15	56	3.7	6.5	21.3	35.3
1	60	36	s	10	6/15	140	14.2	11.2	24.5	53.7
3	98	36	d	7	6/12	0	6.0	10.3	17.0	35.3
21	102	36	d	12	6/17	84	10.3	6.0	13.5	28.3
8	102	36	d	6	6/11	0	4.5	11.8	18.8	30.3
18	100	36	d	4	6/ 9	0	11.7	15.3	16.0	20.3

*Note—LP and DP refer to the mean latency and duration values (in seconds) of the last 6 CCs prior to stimulation with 3 Hz. LT and DT refer to the mean latency and duration values for the CC during the test trials. CC1 = test trial of 1st CC; N = number of CCs; ETI = increase in ETI (in microA); d = double; s = same. *See note, Table 2.*

were rested for various periods. Two rested for 12 days (equal to 36 trials with 3-Hz stimulation), one for 20 days, and the fourth for 22 days. Then, each was stimulated for six trials at the same intensity as had been used on the last 60-Hz stimulation trial. All rats convulsed on each of the six trials. This result confirmed our previous conclusion that a rest group was not necessary.

Two of these rats were used then to determine the intensity required with 3-Hz stimulation to produce a convulsion. Each rat was stimulated 3 times/day at four times the ETI, six times ETI, and so on. Three to six trials were provided for each intensity. One rat showed Stage 3 behavior only when the intensity was increased to the maximum of the instrument, 2.8 mA, which was approximately 15 times the ETI; it did not proceed to stable Stage 3 behavior with nine trials at this intensity. The second rat convulsed once when the intensity was 4 times as great as the ETI and once again when the intensity was 6 times the ETI, but a stable pattern of consecutive convulsions could not be obtained, even when 2.8 mA was used, an intensity approximately 25 times the ETI.

DISCUSSION

These results indicated that 3-Hz stimulation was not as effective as 60-Hz brain stimulation when the two were presented simultaneously in the development of the kindling process. The greatest effect appeared to be in the early stages.

Likewise, successive presentation of 3-Hz stimulation after 60-Hz brain stimulation produced an effect under some conditions. The effect was most apparent when 48 or more CCs were linked with either 36 trials of 3 Hz following the 60-Hz stimulation or 24 trials of 3 Hz at double the intensity of the previous 60-Hz brain stimulation.

The most dramatic effect of the 3-Hz brain stimulation was the reversal from Stage 3 to an earlier stage in the kindling process. usually Stage 1. Table 3 shows 14 rats that seem to show the reversal effect; these rats were ones that had no convulsion in early trials. Some of the other rats might have shown the reversal effect if more optimum conditions had been used.

Of the 14 rats in the various conditions that showed the reversal effect, an increase in the ETI was required for all but 5 cases. These five rats required between four and eight trials of stimulation at the previous ETI before a convulsion occurred. The other nine rats required increases of 56 to 140 microA in the ETI to elicit the first CC. The average increase over the 14 rats was 60 microA. These magnitudes are well beyond what one might expect as day-by-day fluctuations, especially for rats that had achieved 48 or more trials of convulsions. Presumably, 3-Hz stimulation increases the ETI. Thus, the intensity has to be increased to elicit a convulsion, or else the ETI decreases over the early test trials with 60-Hz stimulation, as in the beginning trials (Tress & Herberg, 1972).

There were increases in duration and latency means following the 3-Hz stimulation. The duration increases in the 3-Hz groups were similar to those in 60-Hz rested rats in previous research, and thus are not unique to the interpolated 3-Hz condition. The longest convulsion observed in kindling research in our laboratory was approximately 5 min for a rat that had rested 30 days (Gaito, 1976a).

The tendencies for latency increases, the occurrence of Stage 1 or 2 behavior in early test trials, and increases of the ETI during test trials seem to be unique to the 3-Hz stimulation condition. Although all of these tendencies did not result with each rat, there seemed to be a predisposition for one or more of the tendencies to occur. It appears that 3-Hz stimulation has the greatest probability of producing these tendencies if 48 or more CC trials are used along with either 24 trials of 3 Hz at double intensity or 36 trials at the same intensity as previous 60-Hz stimulation.

Thus, it appears that there are three aspects that are associated with the reversal effect. They are: (1) no convulsions on early test trials, usually the first 3-10 trials (this aspect is the most important); (2) an increase in the ETI; (3) an increase in latency values.

One might suggest that the reversal effect is a direct result of lesions produced by 3-Hz stimulation. However, there are a number of points that tend to negate this possibility and suggest that the effect is based on 3-Hz stimulation producing an increase in ETI. (1) Five of the 14 rats that showed reversal behavior began to convulse on Test Trials 4-8 at the same intensity as they had with 60-Hz stimulation. If lesions had occurred, affected rats would have required greater intensities of stimulation for a convulsion to occur (Goddard et al., 1969). (2) A number of rats convulsed on each test trial, nine in Experiment 3. (3) Three rats from Experiment 3 required a greater ETI for the first convulsion, but with continued stimulation, they convulsed at the same intensity as each had with 60-Hz stimulation. These were the only rats for which this procedure was attempted. (4) Histological analyses showed no apparent lesions. Tissue at the electrode tips of both 60-Hz and 3-Hz rats were similar (Experiment 1). The 3-Hz tissue appeared similar also to those of rats stimulated with 60 Hz in previous research for which histological photos were available.

One might use the word "competition" in describing the effect of 3-Hz stimulation on 60-Hz stimulation results. But are the results really indicating a competition effect? The bilateral stimulation results in the simultaneous case may be seen as the 60-Hz/60-Hz stimulation providing a more efficient method of kindling than the 60-Hz/3-Hz condition; the kindling rate is faster for bilateral than for unilateral stimulation (Racine, Okujava, & Chipashvile, 1972). Thus, it might be inappropriate to assert that 3 Hz competes with 60 Hz in Experiment 1. However, in Experiments 3 and 4, the word "competition" seems more appropriate from the results that were obtained with the reversals for a number of rats. These results also suggest that it is possible that 3 Hz was competing with 60 Hz in Experiment 1 as well. Obviously, further research is required with 3-Hz stimulation used both in the simultaneous paradigm and in the successive one.

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