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Prior treatment with 1-Hz stimulation retards the development of kindling induced by 60-Hz stimulation

JOHN GAITO and STEPHEN T. GAITO
York University, Downsview, Ontario M3J 1P3, Canada

Two experiments were conducted to evaluate the effect on kindling behavior of stimulation with 1-Hz current prior to 60-Hz sine-wave stimulation. In both experiments one group of rats had 30 trials of 1-Hz sine waves, 3 trials/day, 1 h between trials (1-60 group), and then received 30 kindling trials over 10 days. A second group had no stimulation on the initial trials (X-60 group) prior to 30 kindling trials. The 1-60 group showed retarded kindling behavior in both experiments.

The "kindling effect" has been investigated in a number of laboratories (e.g., Gaito, 1976b; Goddard, McIntyre, & Leech, 1969; Racine, 1972; Wada & Sato, 1975).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). During Stage 3 behavior, the rat stands on its hind paws and bilateral convulsions of the forelimbs occur. Behavioral, chemical, electrophysiological, and neurological aspects of this effect have been investigated by many researchers (Gaito, 1976a; Racine, 1978).

Goddard et al. (1969) indicated that there was a reduced probability of eliciting a convulsion at a given intensity for frequencies above and below 60 Hz. Thus, it might be possible to find some frequencies other than 60 Hz that could interfere with 60-Hz brain stimulation results. With a few rats some frequencies below and above 60 Hz were evaluated, those being 30, 20, 15, 10, 5, 3, and 1; 100, 150, 200, 300, 400, 500, 1000, 2000, and 4000. Stage 2 or 3 behavior was observed at all frequencies except 3 Hz and 1 Hz, although greater intensities were required to elicit these behaviors (Gaito, 1979b).

In this attempt to determine frequencies that might be used as potential interference agents, two criteria were used: (1) Stage 2 or 3 behavior should not usually be elicited with low or moderate intensities (e.g., up to 560 microA, (2) no consistent convulsion pattern should be elicited, even at higher intensities.

Only 3-Hz and 1-Hz stimulation met these criteria. Seldom did Stage 2 or 3 behavior occur below an intensity of 560 microA; Stage 1 behavior was the typical response in almost all cases. Furthermore, although convulsions did occur at intensities greater than 560 microA, stable convulsion patterns on successive trials of stimulation did not occur. Thus, 3 Hz, and later 1 Hz, were evaluated as potential "interference agents."

In a series of experiments, 3-Hz stimulation consistency produced an interference effect, that is, suppression (Gaito, 1979a, 1979b; Gaito, Nobrega, & Gaito, 1980). Another experiment evaluated the effect of varying duration of 1-Hz stimulation (viz., 0, 5, 15, 30, 60, 120, 180, and 600 sec) (Gaito, 1980b). The 5-sec condition gave the same results as the control condition (0-sec stimulation): There was no interference effect. With 15 sec of stimulation, there was a minor effect. The effect was more pronounced at 30 sec. The 60, 120, and 180 sec of stimulation produced drastic effects. However, the greatest effect was with the 600-sec stimulation period. The overall result was that of an increasing interference or suppression effect as duration of stimulation increased. Similar results occurred with 3-Hz stimulation (Gaito, 1980a).

All of these experiments introduced 1-Hz or 3-Hz stimulation after Stage 2 or 3 behavior had been attained. In the present experiment, 1-Hz stimulation was introduced before any kindling trials were attempted to determine if interference or "protection" was provided against 60-Hz induced convulsive tendencies.

METHOD

In Experiment 1, 20 male Wistar rats (approximately 125 days of age) were implanted unilaterally in the amygdala with nichrome bipolar electrodes. The brain coordinates for electode 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 (Gaito, 1976b).

Stimulation was not imposed until at least 7 days after surgery. Then one group of 10 rats was stimulated with 1-Hz sine waves for 60 sec in three trials per day for 10 days. Approximately 1 h intervened between each trial, and the intensity was 100 microA. A second group of 10 rats was placed individually in the stimulation apparatus but received no current for the 30 trials. Then 30 kindling trials over 10 days with 60-Hz sine waves were provided for all rats. The intensity was 100 microA for 30 sec. The threshold to precipitate a convulsion was determined for the 20 rats in three trials on Day 21.

At the end of the experiment, histological analyses were performed on all rats. The animals were sacrificed with an overdose of sodium pentobarbital and perfused with saline and formalin. The brains were extracted and placed in a 10% formalin solution. Each brain was frozen, and 50-micron sections were

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mounted on microscopic slides; these slides were placed in a photographic enlarger and were used to obtain information concerning electrode site and the presence or absence of lesions around electrode tips. The enlargement was approximately tenfold. A Luxo magnifier with a lens that provided approximately double enlargement was mounted over the microtome, so that the electrode tips and the electrode track could be observed during the obtaining of each tissue section. The use of this continuous viewing of the tissue greatly increased the efficiency of the histological analysis.

After identifying where the tip of each electrode was located, the result was placed in one of four categories: (1) in amygdala, (2) intersection of amygdala and adjacent structure, (3) just outside amygdala, in adjacent structure, or (4) outside amygdala, in adjacent structure, but more remote. In previous research we found that most rats with electrodes in structures in Categories 1-3 convulse within about 30 trials. Thus rats with an electrode in structures indicated by Category 4 usually were discarded.

A similar experiment was conducted with 20 more rats (10 rats/group) in Experiment 2; the ages were approximately 95 days.

RESULTS

The histological analyses indicated that the electrodes were in structures indicated by Categories 1-3 for all rats in both groups in Experiment 1; no data were rejected. The 1-60 group had eight rats with the electrode in the amygdala and one rat with the electrode at the intersection of the amygdala and an adjacent structure, and the last rat had the electrode in an adjacent structure. The X-60 group had seven, two, and one, respectively, in Categories 1, 2, and 3.

In Experiment 2, no histological analyses were conducted because the electrode tips were in structures labeled 1-3 in Experiment 1, as in previous experiments. Seldom were the tips outside these structures.

Three dependent variables were used: composite score (after Trials 3, 6, and 30), threshold value, and first trial of clonic convulsion. For the first variable, each rat received a score of 1 for Stage 1 behavior, a 2 for Stage 2 responses, and a score of 3 for each convulsion. Over the 3, 6, and 30 trials, the minimum and maximum scores were 3 and 9, 6 and 18, and 30 and 90, respectively. The threshold value was determined as the lowest intensity to produce a BA or CC response, plus 15 microA (to allow for day-by-day fluctuation). This is the measure that we have called the effective threshold intensity (ETI). For the last dependent variable, an arbitrary value of 31 was used for any rat that had not convulsed after 30 trials.

The results for these variables are shown in Table 1. An ANOVA of the composite score data after 30 trials in Experiment 1 indicated a significantly greater mean score for the X-60 group [$F(1,18) = 4.68$, $p < .05$]. The results were in the same direction after three and six trials, but the differences were not tested for significance. Significant results occurred also with the threshold data; the mean threshold for the 1-60 group was much greater than that for the X-60 group [$F(1,18) = 5.51$, $p < .05$]. The mean score per trial for the 1-60 group was 1.5, a middle Stage 1 response. The X-60 group showed an early Stage 2 response, according to the mean, 2.2.

Table 1
Results of Experiments 1 and 2

Group	Mean Composite Score			Mean Score per Trial	Mean Threshold
	Day 1	Day 2	Day 10		
			Experiment 1		
1-60	3.3	7.2	45.8	1.5	175
X-60	4.3	9.6	64.8	2.2	104
			Experiment 2		
1-60	3.3	7.7	42.3	1.4	251
X-60	3.8	8.3	48.5	1.6	168
			Experiments 1 and 2 Combined		
1-60	3.3	7.5	44.1	1.5	223
X-60	4.1	9.0	56.7	1.9	136

Note–Mean thresholds are given in microamperes.

No statistical analysis was conducted with the first trial of clonic convulsion in Experiment 1 because six rats did not achieve a convulsion with the 1-60 group. Nine of the rats in the X-60 group convulsed within the 30 trials. The mean for this group was 18.0 trials. The mean for the 1-60 group was 24.7 trials. These results suggest a slower kindling rate for the 1-60 group. Furthermore, using the arbitrary value of 31 for rats that had not convulsed after 30 trials undoubtedly produced an underestimation of the mean for this group.

The results for Experiment 2 are indicated in Table 1. As in Experiment 1, the mean composite score for the X-60 group was greater than that for the 1-60 group after 3, 6, and 30 trials, and the mean threshold value for the former was lower than that for the latter. However, an ANOVA of the composite score after 30 trials and the threshold data indicated that these differences were not statistically significant.

The mean score per trial was 1.4 for the 1-60 group and 1.6 for the X-60 group; both groups had Stage 1 responses. No analysis of the first trial of clonic convulsion data was attempted because only three rats from each group achieved consistent convulsion trials.

When the data from the two experiments were combined, the results were similar to those in each experiment (Table 1).

DISCUSSION

The task of determining whether "protection" against 60-Hz stimulation occurs with 1-Hz stimulation is a difficult one. Other experiments have indicated that the suppression effect dissipates for many rats during trials of stimulation with 60 Hz or that it is lost after 15 or 16 days of nonstimulation (Gaito, 1980b; Gaito et al., 1980). With 10 days of 30 trials of stimulation with 60-Hz current, some of the suppressive or retarding tendency produced by the 1-Hz stimulation may be lost. Therefore, some degree of dissipation of the 1-Hz effect might have occurred within the 10 days of kindling trials during Experiments 1 and 2. However, one can note the effect during the early trials before potential dissipation occurs (Table 1). In both experiments, the

composite score after three trials (Day 1) and after six trials (Day 2), as well as after 30 days, was greater for the X-60 group than for the 1-60 group, suggesting that there was some "protection" against kindling afforded by the 1-Hz stimulation.

These results seem to be consistent with previous results involving stimulation with 1-Hz sine waves along with or after stimulation with 60-Hz current, in which the 1-Hz stimulation appears to suppress the convulsion producing capacity of 60-Hz stimulation (Gaito, 1980b). In Experiment 1 the 1-Hz current produced statistically significant results in the dependent variables of concern. The results were in the same direction in Experiment 2; however, the differences in the mean values were not statistically significant. The lack of definitive results in the second experiment may be based on the short-term duration of the suppression effect, as indicated above.

Our results showing what appears to be a short-term suppressive or interference effect in these and other experiments may be relevant to the results by McIntyre and Goddard (1973). They found a short-term "aftereffect" of interference nature in alternating stimulation of homologous amygdalae. Our "suppression effect" may be the same as their "aftereffect." Such transient events are in sharp contrast to the basic kindling process, which appears to be relatively permanent (Goddard et al., 1969; Racine, 1978).

Ultimately, the "suppression effect" might have important implications relative to the kindling effect and to brain function in general. Furthermore, a frequency that can suppress convulsions induced by another frequency might prove worthwhile as a potential anticonvulsant agent in some types of human epilepsy (e.g., focal epilepsy).

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