Bulletin of the Psychonomic Society
1982, Vol. 19(2), 108-110

The effect of prior treatment with 1-Hz stimulation on the kindling phenomenon in rats

JOHN GAITO
York University, Downsview, Ontario M3J 1P3, Canada

An experiment was conducted to evaluate the effect on kindling behavior of stimulation with 1-Hz current prior to 60-Hz sine-wave stimulation. In the first phase, 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. In a second phase, two other groups were treated in a similar fashion, but after a delay of 30 days. The results were the same in both phases: The 1-60 group showed retarded kindling behavior.

The "kindling effect" has been investigated in a number of laboratories (e.g., Gaito, 1976b; Goddard, McIntyre, & Leech, 1969; Racine, 1972; Wada & Sato, 1975). In rats, it involves a change from normal exploration (Stage 1) to behavioral automatisms (BA) (Stage 2: chewing, eye closure on ipsilateral side, salivation), and finally, to clonic convulsions (CCs) (Stage 3) in response to electrical stimulation of a specific brain site (e.g., amygdala). During Stage 3, the rat stands on its hind paws and bilateral convulsions of the forelimbs occur. A kindling progression occurs also in other animals, namely, frogs, reptiles, mice, rabbits, cats, monkeys, and baboons (Racine, 1978). A permanent change that does not damage tissue is assumed to occur in the brain during kindling (Goddard et al., 1969; Racine, 1978). Behavioral, chemical, electrophysiological, and neurological aspects of this effect have been investigated previously (Gaito, 1976a; Racine, 1978).

We attempted to determine sine-wave frequencies that might interfere with the production of convulsions by 60-Hz sine-wave stimulation. In a series of experiments, 1- and 3-Hz stimulation consistently produced an interference or suppression effect, that is, suppression of convulsions (Gaito, 1979a, 1979b, 1980a, 1980b; Gaito, Nobrega, & Gaito, 1980). When duration of stimulation was varied, the result was a suppression effect that increased as duration increased (Gaito, 1980a, 1980b).

In other experimentation, it was found that interference or suppression varies with remoteness from the kindling frequency. The least interference occurred with 60-Hz stimulation and the greatest, with 1-Hz current; 30-Hz, 10-Hz, and 5-Hz stimulation produced intermediate degrees of interference (Gaito, 1980c).

It is possible that suppression occurs as a result of tissue damage, although this seems unlikely, since the suppression effect disappears in most rats after 15 to 18 days without stimulation (Gaito, 1980a; Gaito et al., 1980). Yet low-frequency sine waves (1 and 3 Hz) are not too dissimilar from direct current, which does produce tissue damage.

An experiment was designed to contrast the effect on the kindling phenomenon of both direct current and 1-Hz stimulation. The behavior of rats stimulated with direct current (the DC group) was very different from that of those stimulated with 1-Hz sine waves (Gaito, 1981). Suppression was most pronounced for the DC group; it appeared after a single trial and persisted for 32 days (end of experiment) after the last threshold determination. In contrast, most of the rats in the 1-Hz-stimulated group had recovered from the suppression after the 32-day period of nonstimulation. A second phase of the experiment indicated that the increase in threshold values for the DC group occurred after a single stimulation. These results were interpreted as being consistent with the hypothesis generated by previous research that suppression following 1-Hz stimulation was not due to tissue damage.

All of these experiments introduced 1-Hz or 3-Hz stimulation after Stage 2 or 3 behavior had been attained. In preliminary experimentation, 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 (Gaito & Gaito, 1980). Some rats had 30 trials of 1-Hz stimulation in 10 days, followed by 30 trials of 60-Hz stimulation. A second group received only the 30 trials of 60-Hz stimulation. The 1-Hz-stimulated group showed a retarded kindling progression. In the present situation, the preliminary experiment was replicated. However, two similar groups were added that were not subjected to 60-Hz stimulation until 30 days after the initial stimulation phase. This part of the experiment was concerned with the possibility that the effects of 1-Hz stimulation would dissipate by 30 days.

0090-5054/82/020108-03$00.55/0

METHOD

Forty-three male Wistar rats (approximately 90 days of age) had nichrome bipolar electrodes implanted unilaterally in the amygdala. 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, and 8.5 mm from skull (Gaito, 1976b).

Stimulation was not imposed until at least 7 days after surgery. Then 21 rats were stimulated with 1-Hz sine waves for 120 sec during three trials on each of 10 days. The intensity was 100 microA (root mean square, RMS; equivalent to 280 microA peak to peak). (Stage 1 responses occur consistently with this intensity.) One hour intervened between each trial on each day. Then 10 of the rats received 10 days of 3 trials/day of 60-Hz stimulation at an intensity of 50 microA (RMS) for 30 sec (Group 1: 1-60, immediate). These durations and intensities are ones that have been effective in previous research. The other 11 rats received similar treatment, but after a 30-day delay (Group 2: 1-60, 30 days). The remaining 22 rats were placed in the apparatus, but 1-Hz current was not turned on. One group of 11 rats received the 60-Hz stimulation for 30 trials, beginning on the same day as Group 1 (Group 3: X-60, immediate). The other 11 rats were subjected to 30 trials of 60-Hz stimulation after a 30-day rest, beginning at the same time as Group 2 (Group 4: X-60, 30 days).

No histological analyses were conducted with any rats. Previous analyses had indicated that the electrode tips were in the amygdala or the adjacent structure in almost every case, and no lesions were detected. Stimulation of these sites with 60-Hz sine waves produced a kindling progression consistently.

Four dependent variables were used with 60-Hz stimulation data: cumulative composite score (after Trials 3, 12, 21, and 30), threshold value at completion of experiment, first trial of a BA response, first trial of a CC response. 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, 12, 21, and 30 trials, the minimum and maximum scores were 3 and 9, 12 and 36, 21 and 63, and 30 and 90, respectively. The threshold value was determined as the lowest intensity to produce a BA or CC response, plus 5 microA (RMS, to allow for day-by-day fluctuation). This is the measure that we have called the effective threshold intensity (ETI). For the last two dependent variables, an arbitrary value of 31 was used for any rat that had no Stage 2 responses and 32 for any that had not convulsed after 30 trials.

RESULTS

Table 1 shows the data for three of the dependent variables. Two-factor analyses of variance were used for each analysis (Gaito, 1973); the probability of rejecting the null hypothesis of no difference was set at .05 for all F tests.

No significant differences occurred between the immediate and 30-day periods within each dependent variable ($p > .05$ for each F test). There were significant differences between the 1-60 and X-60 groups in all variables, however. The interaction between groups and periods was a nonsignificant source of variation, suggesting a constant difference over the two periods, with the kindling progression of the 1-60 groups inferior to that of the X-60 groups.

Table 1
Results of Three Dependent Variables

Variable	Group 1	Group 2	Group 3	Group 4
Mean Threshold*	91.0	93.0	64.0	59.0
Mean First Trial of BA Response	22.8	18.3	7.2	10.0
Mean First Trial of CC Response	26.5	27.7	22.1	20.6

Note–Group 1 = 1-60, immediate; Group 2 = 1-60, 30 days; Group 3 = X-60, immediate; Group 4 = X-60, 30 days.
**At completion of experiment.*

Table 2
Means for Cumulative Composite Scores Over 30 Trials of 60-Hz Stimulation (3 Trials/Day)

	Group	N	Trials 3	12	21	30
Immediate	1-60	10	3.5	14.6	27.1	41.7
	X-60	11	4.9	20.3	37.1	56.8
30 Days	1-60	11	3.7	15.1	28.2	41.7
	X-60	11	4.5	19.1	37.2	56.6
Combined	1-60	21	3.6	14.9	27.7	41.7
	X-60	22	4.7	19.7	37.2	56.7

Table 2 gives the mean cumulative composite scores over the four trials. The analysis of variance design appropriate for these data is a partially "nested" design (Gaito, 1973). Two dimensions, groups and periods, were between-subjects sources and one dimension, trials, was a within-subjects or repeated-measures source. The mean differences between the immediate and 30-day periods were not significantly different; the interaction between groups and periods also was not significant. The mean differences between the 1-60 and X-60 groups, however, were significant. Because of nonsignificant differences between periods and the nonsignificant interaction source, the results for the two 1-60 and two X-60 groups are combined in Table 2.

For the within-subjects portion of the analysis, the trials dimension and the Trials by Groups interaction were the main sources of importance. The trials source was a significant one with increasing values over the four trials. This result shows the kindling progression for each group. The interaction of groups and trials is concerned with the question as to whether the progression over trials was the same for both groups. The F test of this source (involving an orthogonal polynomials trend analysis; Gaito, 1973) indicated that none of the three interaction regression components (linear, quadratic, cubic) was a significant source, suggesting that the kindling progression was a constant one for the 1-60 and X-60 groups.

Following this result, an orthogonal polynomials trend analysis was performed on the trials dimension; only the linear component was a significant source of variation. Thus, the regression equation for each group is:

$$1\text{-}60 : y = 22.0 + 6.35c_k$$

$$X\text{-}60 : y = 29.6 + 8.68c_k$$

(Each of these equations is of the form $y = \mu + \beta_L c_k$, which is estimated by $y = \bar{Y} + b_L c_k$, where μ and β_L are

the mean and linear regression coefficient in the population, $\overline{Y}$ and b_L are the same for the sample data, and y is the predicted score. The value of the regression coefficient indicates the magnitude of change in mean cumulative composite score from each trial point to the next one.)

The results of these analyses indicate that the means of 22.0 and 29.6 are different (based on the groups F test in the between-subjects portion of the analyses). However, the F test of the linear component for the interaction of groups and trials was nonsignificant. This result indicates that there is no evidence to show that the linear trends for the 1-60 and X-60 groups are different. Thus, one can conclude for practical purposes that the regression coefficients are the same within random error. In this case, an average of the two coefficients provides a more reliable estimate of the β_L. The final regression equations become:

$$1\text{-}60 : y = 22.0 + 7.52c_k$$

$$X\text{-}60 : y = 29.6 + 7.52c_k$$

DISCUSSION

The results of the first phase were in agreement with those of the preliminary study (Gaito & Gaito, 1980). Overall, the results with all dependent variables appeared to indicate clearly that the 1-Hz-stimulation trials prior to 60-Hz-stimulation trials produced a prominent suppression effect such that the 1-60 group was inferior to the X-60 group in kindling progression for both the immediate and 30-day periods. It appears that the massing of many trials of 1-Hz stimulation prior to 60-Hz stimulation does not allow the suppression or interference to dissipate. This result is in sharp contrast to that obtained when a 1-60-1 progression is used, that is, a 60-Hz trial sandwiched between two 1-Hz stimulation trials. In the latter case, a substantial suppression occurs when the intertrial interval is 1 or 3 h; with a 24-h interval, the effect is reduced (Gaito & Gaito, 1981). When the interval is 72 h, there is little suppression remaining (research under way).

These results, and others using the contrast between 1-60-1 and X-60-X groups, indicate clearly the unusual aspect of the 1-Hz stimulation acting as a suppression agent upon the 60-Hz kindling-producing potential. This suppression occurs with 1-Hz sine-wave stimulation at intensity levels of 200 microA and below (RMS). At these intensities, seldom does the 1-Hz stimulation produce a Stage 2 or 3 response.

Other individuals (Cain & Corcoran, 1979; Corcoran & Cain, 1980) have reported that kindling was achieved with 3-Hz and 2-Hz stimulation. These results may appear to be at variance with our results (especially our earlier results with 3-Hz stimulation). Their work differs from our research in two important aspects, however.

(1) They employed biphasic square-wave pulses of 1.0 msec duration in contrast to our sine-wave stimulation.

(2) They needed higher intensities to achieve kindling than those involved in our research, and they reported little or no kindling with lower intensities. They used 200 microA and greater, measured from base to peak. This type of measurement would be equivalent to the RMS intensity of sine-wave stimulation. Peak-to-peak intensity determinations with sine waves are 2.8 times as great as the RMS one. Our upper limit of stimulation was 200 microA (RMS; equivalent to 560 microA peak to peak), with most stimulation intensities well below this level. We thought that the higher intensities (of low frequencies or any frequencies) would have an increased probability of producing lesions. Thus, we set the 200-microA (RMS) level as our upper limit.

The results of the Corcoran-Cain studies are summarized as follows: "The threshold current intensity necessary was approximately 300 microA (peak pulse intensity measured baseline-to-peak). Intensities much below this were completely ineffective even when administered repeatedly. Pulses of 0.1 msec duration were also completely ineffective" (Cain & Corcoran, 1979, p. 623).

The intensity difference probably is the more important of the two. Thus, an unusual result occurs: Low intensities of stimulation produce suppression of kindling, whereas high intensities elicit kindling.

REFERENCES

Cain, D. P., & Corcoran, M. E. Rapid kindling induced by low-frequency stimulation. *Neuroscience Abstracts,* 1979, **5**, 623.

Corcoran, M. E., & Cain, D. P. Kindling of seizures with low-frequency stimulation. *Brain Research,* 1980, **196**, 262-265.

Gaito, J. *Introduction to analysis of variance procedures.* New York: MSS Information Corporation, 1973.

Gaito, J. The kindling effect as a model of epilepsy. *Psychological Bulletin,* 1976, **83**, 1097-1109. (a)

Gaito, J. An oscillation effect during sequential alternations of unilateral amygdaloid stimulations within the kindling paradigm. *Physiological Psychology,* 1976, **4**, 303-306. (b)

Gaito, J. Suppression of 60-Hz-induced convulsive behavior by 3-Hz brain stimulation. *Bulletin of the Psychonomic Society,* 1979, **13**, 223-226. (a)

Gaito, J. 3-Hz brain stimulation interferes with various aspects of the kindling effect. *Bulletin of the Psychonomic Society,* 1979, **13**, 67-70. (b)

Gaito, J. The effect of variable duration one hertz interference on kindling. *Canadian Journal of Neurological Sciences,* 1980, **7**, 59-64. (a)

Gaito, J. The effect of varying durations of stimulation on the 3-Hz interference effect. *Bulletin of the Psychonomic Society,* 1980, **15**, 211-214. (b)

Gaito, J. Gradient of interference by various frequencies on 60-Hz kindled behavior. *Canadian Journal of Neurological Sciences,* 1980, **7**, 223-226. (c)

Gaito, J. The effect of low frequency and direct current stimulation on the kindling phenomenon in rats. *Canadian Journal of Neurological Sciences,* 1981, **8**, 249-253.

Gaito, J., & Gaito, S. T. Prior treatment with 1-Hz stimulation retards the development of kindling induced by 60-Hz stimulation. *Bulletin of the Psychonomic Society,* 1980, **15**, 351-353.

Gaito, J., & Gaito, S. T. The effect of several inter-trial intervals on the 1-Hz interference effect. *Canadian Journal of Neurological Sciences,* 1981, **8**, 61-65.

Gaito, J., Nobrega, J. N., & Gaito, S. T. Interference effect of 3-Hz brain stimulation on kindling behavior induced by 60-Hz stimulation. *Epilepsia,* 1980, **21**, 73-84.

Goddard, G. V., McIntyre, D. C., & Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology,* 1969, **25**, 295-330.

Racine, R. J. Modification of seizure activity by electrical stimulation: I. After-discharge threshold. *Electroencephalography and Clinical Neurophysiology,* 1972, **32**, 269-279.

Racine, R. Kindling: The first decade. *Neurosurgery,* 1978, **3**, 234-252.

Wada, J. A., & Sato, M. The generalized convulsive seizure state induced by daily electrical stimulation of the amygdala in split brain cats. *Epilepsia,* 1975, **16**, 417-430.

(Received for publication November 10, 1981.)