

## Gradient of Interference by Various Frequencies on 60 Hz Kindling Behavior

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**SUMMARY:** *An experiment was conducted to evaluate the effect of various frequencies of brain stimulation on kindling behavior induced by 60-Hz sine wave stimulation. The effective threshold intensity (ETI) to elicit a convulsion was determined on four separate occasions with 5 days of daily trials between determinations. On each day experimental rats were stimulated with current of a specific frequency on the first and third trials for 60 seconds duration and with 60-Hz current for 30 seconds on the second trial (one hour intertrial interval). There were five experimental groups, one each*

*for 1, 5, 10, 30, and 60-Hz stimulation. A sixth group received no stimulation on trials 1 and 3 and 60-Hz current on trial 2. Suppression of convulsive behavior induced by the 60-Hz stimulation trial was present for all ETI determinations with 1-Hz and 5-Hz stimulation; the mean ETI increased on each successive determination. Suppression was prominent also for the 10-Hz group until the ETI<sub>4</sub> determination. Suppression was moderate for the 30-Hz and 60-Hz groups. Overall, it appeared that the interference effect gradually increased with remoteness from the 60-Hz point.*

The "kindling effect" has been investigated in a number of laboratories (e.g., Gaito, 1976b; Goddard et al, 1969; Racine, 1972; Wada and Sato, 1975). This effect involves a change from normal exploration (Stage 1) to behavioral automatisms (Stage 2 — chewing, eye closure on ipsilateral side, salivation), and finally to clonic convulsions (Stage 3) 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).

**RÉSUMÉ:** *Nous avons évalué l'effet de plusieurs fréquences de stimulation cérébrale sur le comportement kindling induit par une stimulation sinusoïdale à 60 Hz. Le seuil d'intensité efficace (ETI) pour élucider des convulsions fut déterminé en 4 occasions séparées avec 5 jours d'essais quotidiens entre les déterminations. Chaque jour les rats expérimentaux furent stimulés par un courant de fréquence spécifique au premier et troisième essai pendant 60 secondes et ensuite avec un courant de 60 Hz pour 30 secondes au deuxième essai (intervalle inter-essai de 1 heure). Il y eut 5 groupes expérimentaux à des stimulations*

*de 1, 5, 10, 30 et 60 Hz. Un sixième groupe n'a reçu aucune stimulation au 1er et 3ième essai et 60 Hz au 2ième essai. Nous avons noté une suppression du comportement convulsif induit par l'essai à stimulation 60 Hz avec toutes les déterminations ETI de 1 et 5 Hz de stimulation. L'ETI moyen augmente à chaque détermination successive. La suppression fut aussi importante pour le groupe 10 Hz jusqu'à la détermination ETI<sub>4</sub>. La suppression fut modérée pour les groupes de 30 et 60 Hz. Donc il semble que l'effet d'interférence augmente graduellement avec la distance du point de 60 Hz.*

We attempted to determine frequencies which might interfere with the occurrence of convulsions by 60-Hz stimulation. In a series of experiments, 3-Hz stimulation consistently produced an interference effect, i.e., suppression of convulsions (Gaito, 1979a, b; Gaito, Nobrega and Gaito, 1980). Another experiment evaluated the effect of varying durations of 1-Hz stimulation, viz., 0, 5, 15, 30, 60, 120, 180, and 600 seconds (Gaito, 1980a). The 5 seconds condition gave the same results as the control condition (0 seconds stimulation) — there was no interference effect. With 15 seconds of stimulation there was a minor effect. The effect was more pronounced at 30 seconds. The 60, 120, and 180 seconds of stimulation produced drastic effects. However, the greatest effect was with the 600 seconds stimulation period. The overall result was increasing interference or suppression effect as duration of stimulation increased. Similar results occurred with 3-Hz stimulation (Gaito, 1980b).

Mucha and Pinel (1977) found that repeated, periodic stimulation with 60-

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Hz current had a decremental effect on both motor seizures and on after-discharges in the EEG. In some experiments we have found a slight decremental effect of 60-Hz current, much less than that produced by 1-Hz or 3-Hz brain stimulation. However, this decrement has not appeared consistently. In the present experiment, the possibility of interference or suppression produced by five frequencies (1, 5, 10, 30, and 60-Hz) was evaluated.

#### METHODS

Fifty nine male Wistar rats (approximately 140 days of age) were implanted unilaterally in the amygdala with 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 (Gaito, 1976b).

Stimulation was not imposed until at least 7 days after surgery. Then the 59 rats were stimulated with 60-Hz sine waves for 30 seconds during three trials on the first day. One hour intervened between each trial. A Lafayette Stimulator was used; the intensity was 100  $\mu$ A. On the first trial of the second day, the effective threshold intensity ( $ETI_1$ ) was determined. The 60-Hz current was increased until a Stage 2 or 3 response was elicited. Then 15  $\mu$ A was added to allow for day-by-day threshold fluctuations. Two further trials of stimulation at this intensity were provided.

Then one group of 10 rats received stimulation with 1-Hz sine waves for 60 seconds on trials 1 and 3 each day for 5 days at twice the  $ETI_1$  value. A 60-Hz stimulation trial was provided on trial 2 for 30 seconds at  $ETI_1$  (Group 1, 1-60-1). There was one hour between each trial. The second, third, fourth, and fifth groups of rats were stimulated with 5-Hz, 10-Hz, 30-Hz, and 60-Hz current, respectively, for 60 seconds at double the  $ETI_1$  values on trials 1 and 3 and with 60-Hz on trial 2 for 30 seconds at  $ETI_1$  (Group 2: 5-60-5; Group 3: 10-60-10; Group 4: 30-60-30; Group 5: 60-60-60). There were 10 rats in each group except for the last one, in

which there were nine. Ten other rats received 60-Hz stimulation on trial 2, but on trials 1 and 3 each rat was placed in the apparatus without stimulation (Group 6, X-60-X). All 60-Hz stimulation on trial 2 was at  $ETI_1$  for 30 seconds, a duration which has been used routinely in our research. Stimulation on trial 1 and 3 was for 60 seconds duration; this duration has been found to produce a drastic suppressive effect in previous experiments.

Following this 5 day period, rats from all groups had  $ETI_2$  determined over six trials during two days. Then another block of 5 days of stimulation occurred in which each group was treated in the same fashion as during the 5 day block of trials prior to the  $ETI_2$  determination. This alternation of  $ETI$  determinations and a 5 day block of trials was continued through the  $ETI_4$  determination. Then all rats were rested for 16 to 17 days and  $ETI_5$  was determined on one trial.

At the end of the previous experiments (Gaito, 1980a), histological analyses were performed on all rats. However, no gross lesions were detected at intensities of 560  $\mu$ A and below (intensities which are used routinely in our experiments). The tissue around the electrode tips of rats stimulated with 1-Hz or 1-Hz and 60-Hz current was indistinguishable from that of rats stimulated only with 60-Hz current. Thus, no histological analyses were conducted in the present experiment.

#### RESULTS

The rats subjected to 10, 30, and 60-Hz sine wave current showed the usual kindling progression. The 5-Hz stimulated rats also had stage 2 and 3 responses, but these responses later disappeared and stage 1 behavior was the typical reaction. As in the previous research, seldom did behavioral automatisms or clonic convulsions occur during 1-Hz stimulation.

Two dependent variables,  $ETI$  and composite score, have been sensitive to the effects of 1-Hz and 3-Hz effects in previous experiments. These variables were used in the present experiment. The latter measure involves a score of 1 for stage 1 behavior, 2 for a stage 2

response, and 3 for a clonic convulsion. The results are shown in Table 1. As in previous experiments the control rats, those subjected to no stimulation on trials 1 and 3 (X-60-X group), showed a gradual decrease over the four determinations with the greatest decrement occurring between  $ETI_1$  and  $ETI_2$ . Overall the mean  $ETI$  values decreased slightly for the 60-60-60 group, although a slight increment occurred on  $ETI_3$ . The 30-60-30 group had  $ETI$  scores which remained approximately the same over the  $ETI$  determinations. The 10-60-10 group showed  $ETI$  increases to the  $ETI_3$  determination, but remained approximately at that level for the  $ETI_4$  determination. The 1-60-1 and 5-60-5 groups had the gradual increments over  $ETI$  determinations which have been typical of rats stimulated with 1-Hz or 3-Hz sine waves in previous experiments. In the 1-60-1 group, one rat did not convulse at 560  $\mu$ A for the  $ETI_2$ ,  $ETI_3$ , and  $ETI_4$  determinations. A second rat did not convulse during the  $ETI_3$  and  $ETI_4$  determinations. One rat in the 10-60-10 group did not convulse for  $ETI_2$ ,  $ETI_3$ , and  $ETI_4$  determinations. In each case of non-convulsion, an  $ETI$  value of 585  $\mu$ A was assigned to that rat. (Twenty-five was arbitrarily added to the upper limit of 560  $\mu$ A.)

The mean composite score over three blocks of trials also showed the suppression effect (Table 2). The minimum and maximum scores, respectively, for composite score over 5 trials were 5 and 15. Each rat received a score of 1 for stage 1 behavior, a score of 2 for stage 2 responses, and a value of 3 for each convulsion. The X-60-X, 60-60-60, and 30-60-30 groups had the greatest kindling progression over the three blocks of trials. The 10-60-10 and 5-60-5 groups had moderate increases in mean score. The least progression occurred for the 1-60-1 group; behavior during block 3 according to the mean composite score was at the boundary of stage 1 and 2.

The first block of trials usually showed the smallest interference effect, because behavior was not as stable at this early stage as it was later, and  $ETI_1$  was not as reliable as later  $ETI$  determinations were. However, by the second block of trials the effect was

TABLE 1  
Mean ETI Values (in Microamperes)\*\*\*

Groups (frequencies)	ETI Determinations				
	1	2	3	4	5+
1 (10)*	192	319	427	483	239
5 (10)	185	304	412	447	241
10 (10)	206	310	373	360	248
30 (10)	279	291	286	270	204
60 (9)	328	297	313	280	242
X (10)	309	213	195	170	149

\* Number of rats at beginning of the experiments in parentheses.

+ Determined after a 16 or 17 day rest following the previous ETI determination.

X, Rats not stimulated on Trials 1 and 3 each day.

\*\*\* Effective threshold intensity (ETI)

apparent. Table 3 shows the mean composite score for the five days of trials within the second block of trials. The minimum and maximum scores, respectively, for each trial and each rat were 1 and 3. The control group (X-60-X) was the only one which remained stable or showed an increase in mean composite score over the five trials. All other groups evidenced an interference effect, especially during the later trials. The effect was greatest for the 1-60-1 group and least with the 60-60-60 and 30-60-30 groups.

TABLE 2  
Mean Composite Score

Groups	Blocks of Trials		
	1	2	3
1	8.7	8.4	9.5
5	8.2	11.4	12.9
10	9.5	11.2	12.6
30	10.4	12.4	13.7
60	11.3	13.3	13.3
X	10.5	12.5	13.9

The mean value for the five trials was the lowest for the 1-60-1 group, with a value indicating behavior just below stage 2. The other groups had means which were approximately the same (approximately middle stage 2 behavior); however, each of these groups, except the X-60-X one, had a mean which was less than that for trial 1.

TABLE 3  
Mean Composite Score in Block 2

Group	Day						
	0+	1	2	3	4	5	M*
1	2.6	2.2	1.9	1.6	1.7	1.4	1.8
5	2.6	2.8	2.1	2.2	2.3	2.0	2.3
10	2.9	2.8	2.6	2.3	2.4	2.0	2.4
30	2.8	2.6	2.5	2.5	2.4	2.4	2.5
60	2.8	2.8	2.9	2.9	2.4	2.3	2.5
X	2.4	2.4	2.4	2.5	2.6	2.6	2.5

\* Mean score for last trial of ETI<sub>2</sub> determination.

\* Mean score per trial in Block 2.

The ETI<sub>5</sub> value was determined after 16 or 17 days of rest. The 1-60-1 and 5-60-5 results were similar — the ETI<sub>5</sub> determination was greater than the lowest threshold value (ETI<sub>1</sub>) but was well below ETI<sub>2</sub> and other determinations. The 10-60-10 group also had an ETI<sub>5</sub> value that was above ETI<sub>1</sub>. The ETI<sub>5</sub> for the other three groups showed a decline from ETI<sub>1</sub>, with ETI<sub>5</sub> being the lowest of the determinations. The X-60-X group had the greatest relative decrease; the 30-60-30 rats had the smallest relative decrement. The 1-60-1, 5-60-5, 10-60-10, 30-60-30, 60-60-60, and X-60-X groups had 4, 4, 7, 7, 7, and 10 rats, respectively, at or below the lowest ETI value, i.e., these rats apparently had recovered from the suppression effect.

The ETI<sub>5</sub> results indicate that the 1-60-1 and 5-60-5 showed the greatest

interference effect in that 60% of the rats had not completely recovered from the effect of the interfering frequency. The ETI<sub>5</sub> value was at the greatest intensity for these two groups. The 10-60-10 rats seemed to be intermediate in degree of interference between the 1- and 5-Hz stimulated groups and the others. The interference effect was present up through the ETI<sub>3</sub> determination; by ETI<sub>4</sub> recovery appeared to have begun. At the ETI<sub>5</sub> determination, 7 of the 10 rats were back to the low threshold point. The 30-60-30 and 60-60-60 group showed the least degree of interference. The mean ETI<sub>5</sub> value was the lowest of the threshold determinations, and most of the rats had a threshold value which was at or below the previous lowest threshold point (ETI<sub>4</sub>). The X-60-X group, obviously, showed no interference effect — the lowest mean value occurred for ETI<sub>5</sub>, and all rats were at or below the previous lowest threshold point.

## DISCUSSION

The responses of the rats in the separate groups to the various frequencies and to 60-Hz stimulation for intensities at or below 560  $\mu$ A are shown in Table 4, under the conditions used in this experiment. Kindling progression occurred for the non-stimulated rats and for those stimulated with 60, 30, and 10-Hz. Rats stimulated with 5-Hz sine waves progressed up through stage 3, but this behavior quickly disappeared and stage 1 behavior was typical. The typical response to 1-Hz stimulation was stage 1 behavior. Occasionally stage 2 or 3 behavior occurred, but these responses did not persist.

In terms of the response to 60-Hz stimulation, as indicated in Tables 1-3, the groups tended to fall into four clusters relative to the ETI values. The first cluster consisted of the X-60-X group with a steady decline in ETI over these determinations; there was no evidence of suppression in any data. The second cluster consisted of the 60-60-60 and 30-60-30 groups for which the ETI values stayed approximately the same or decreased slightly. Any decreases were small compared to those occurring for the X-60-X group.

TABLE 4

*Responses of the Various Groups to Specific Frequencies*

Group	Response to Interference Frequency	Response to 60-Hz
1-60-1	Typical response is Stage 1 behavior. Rarely does Stage 2 or 3 behavior occur.	Suppression or interference is great.
5-60-5	Stage 2 or 3 behaviors develop but are not stable. Later, Stage 1 behavior is typical.	Suppression or interference is great.
10-60-10	Kindling progression occurs.	Intermediate degree of suppression.
30-60-30	Kindling progression occurs.	Minimal degree of suppression.
60-60-60	Kindling progression occurs.	Minimal degree of suppression.
X-60-X	Kindling progression occurs.	No suppression.

The 10-60-X group constituted the third cluster; ETI values increased (as for the 5-60-5 and 1-60-1 groups) up through the ETI<sub>3</sub> determination, but not for ETI<sub>4</sub>. Similarly, even though kindling proceeded, there was evidence of a modest suppression effect within the blocks of trials. The fourth cluster consisted of the 1-60-1 and 5-60-5 groups. Both showed steady increases over the four ETI determinations and the greatest suppression effect. However, the 5-60-5 group seemed to show a lesser degree of suppression than the 1-60-1 group.

Overall, the degree of suppression or interference tended to vary directly with remoteness from 60-Hz. This aspect could be indicated by the differences between each ETI determination. If these differences were obtained, the mean difference for the groups subjected to 1-Hz, 5-Hz, 10-Hz, 30-Hz, 60-Hz, and to nonstimulation were +97.0, +87.3, +51.3, -1.3, -16.0, and -46.3, respectively.

Thus, the present experiment indicates that the suppression effect uncovered in this laboratory with 1-Hz and 3-Hz stimulation could be obtained with other frequencies as well. Five-Hz stimulation tended to produce approximately the same degree of suppression as 1-Hz stimulation (and probably 3-

Hz current). The other frequencies produced lesser degrees of suppression or interference.

The exact basis for the suppression effect is not known. It probably involves an inhibitory process which is generated during the brain stimulation (McIntyre and Goddard, 1973; Mucha and Pinel, 1977; Nobrega and Gaito, 1978; Racine, 1978). It appeared to be a transient event inasmuch as many affected rats convulsed at previous low threshold levels after a 15 to 17 day rest (Gaito, 1980a) (as in the present experiment), and a 24 hour intertrial interval allowed for much of the effect to dissipate (Gaito and Gaito, 1980). This transient event may be similar to the transient interference ("after effect") reported by McIntyre and Goddard (1973). These transient aspects were in sharp contrast to the kindling process which was relatively permanent (Goddard et al., 1969; Racine, 1978).

These interfrequency interference effects ultimately 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 in some types of human epilepsy.

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