The Effect of Several Intertrial Intervals on the 1 Hz Interference Effect

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SUMMARY: *Experiments were conducted to evaluate the effect of two intertrial intervals of 1-Hz brain stimulation on kindling behavior induced by 60-Hz sine wave stimulation. In two experiments, 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 1-Hz current on the first and third trials for 120 seconds duration and with 60-Hz current for 30 seconds on the second trial (1-60-1 group). A second group was stimulated with 60-Hz current on each trial (60-60-60 group). A third group received no stimulation on Trials 1 and 3 and 60-Hz current on Trial 2 (X-60-X group). In Experiment 1, the intertrial interval was 3 hours; a 24 hour interval was used in Experiment 2. The results were similar in both experiments. For the 1-60-1 group, there was a steady increase in the intensity required to elicit a convulsion with 60-Hz current from ETI_1 to ETI_4. However, the 24 hour interval produced a lesser effect than did the 3 hour interval (or the 1 hour interval used in previous experiments). Rats in the other groups maintained relatively stable values from ETI_1 to ETI_4, with a slight decline occurring. Suppression of convulsive behaviour on daily trials was present with the 1-60-1 groups, and nonexistent with the other groups.*

RÉSUMÉ: *Nous avons fait des expériences afin d'évaluer deux intervalles interessais de stimulation cérébrale de 1-Hz sur le comportement kindling-induit par une stimulation sinusoidale de 60-Hz. Lors de deux expériences nous avons déterminé l'intensité-seuil capable (ETI) de provoquer une convulsion. Ceci fut fait à quatre occasions séparées par 5 jours d'essais journaliers entre chaque détermination. Chaque jour les rats expérimentaux furent stimulés avec un courant de 1-Hz au premier et au troisième essai pour une durée de 120 secondes et avec un courant de 60-Hz pour 30 secondes au deuxième essai (groupe 1-60-1). Un deuxième groupe fut stimulé avec un courant de 60-Hz au deuxième essai (groupe X-60-X). Dans la première expérience l'intervalle inter-essai fut de 3 heures; il fut de 24 heures dans l'expérience no 2. Les résultats furent semblables dans les 2 expériences. Pour le groupe 1-60-1, il y eut une augmentation constante de l'intensité requise pour causer une convulsion avec un courant de 60-Hz, de ETI, à ETI_4. Cependant l'intervalle de 24 heures produisit un effet moindre que l'intervalle de 3 heures (ou celui d'une heure préalablement utilisé). Les rats des autres groupes maintinrent des valeurs essentiellement stables de ETI à ETI_4, avec un léger déclin. Une suppression du comportement convulsif aux essais journaliers fut notée dans les groupes 1-60-1, mais non dans les autres groupes.*

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The kindling effect has been investigated in a number of laboratories (e.g., Gaito, 1976b; Goddard, McIntyre, and Leech, 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).

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 that of an increasing interference or suppression effect as duration of stimulation increased. Similar results occurred with 3-Hz stimulation (Gaito, 1980b).

In other experimentation it was found that interference or suppression varied 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).

All of these experiments showing an interference effect on the convulsive tendency of 60-Hz stimulation were conducted with three trials each day and one hour between trials. Mucha and Pinel (1977) have found that repeated stimulation with 60-Hz current over short intervals had a decremental or interference effect on both motor seizures and on after-discharges in the EEG. Thus, it is possible that the suppressive effect which we have found in our experiments occurs because of the closeness in time of the three trials, i.e., an intertrial interval of only one hour, and the effect might dissipate with longer intertrial intervals. This possibility is reinforced by results which indicate that the suppression effect is a relatively transient event; 1-Hz stimulated rats showed the suppression effect but convulsed again at previous low effective threshold intensity (ETI) levels following a 15 or 16 day rest at the end of the experiment (Gaito, 1980a).

This paper discusses results obtained when the suppression effect was evaluated over longer intertrial intervals (3 and 24 hours) than the 1 hour interval used in previous experiments. It was expected that the suppression effect would be diminished or lost with one or both of these longer time intervals.

METHODS

In Experiment 1, a 3 hour intertrial interval was used. Twenty-two male Wistar rats (approximately 130 days of age) had nichrome bipolar electrodes implanted unilaterally in the amygdala. The brain coordinates 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 22 rats were stimulated with 60-Hz sine waves for 30 seconds at an intensity of 100 μA during three trials on the first day with a Lafayette Stimulator. Three hours intervened between each trial. 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. Fifteen microamperes was added to allow for day-by-day threshold fluctuations. Two further trials of stimulation at this intensity were provided.

One group of eight rats received stimulation with 1-Hz sine waves for 120 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 were three hours between each trial. The second group of seven rats was stimulated with 60-Hz current for 120 seconds at double the ETI_1 values on Trials 1 and 3 and with 60-Hz on Trials 2 for 30 seconds at ETI_1 (Group 2: 60-60-60). Seven 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 3, X-60-X).

All 60-Hz stimulation on Trial 2 was at ETI_1 for 30 seconds, an intensity and duration which has been used routinely in our kindling research. Stimulation on Trials 1 and 3 was for 120 seconds duration and at an intensity of two times ETI_1. These values have been found to produce a drastic suppressive effect in previous experiments. However, no intensities were used above 560 μA with any frequencies, so as to reduce the possibility of lesions.

Following this 5 day period, rats from all groups had ETI_2 determined over six trials during two days (three trials each day). 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 (three blocks of trials and four ETI determinations). Then all rats were rested for 16 days and ETI_5 was determined on one trial.

Experiment 2 was similar to Experiment 1 except that a 24 hour intertrial interval was used. There were 13 Wistar rats and 11 Hooded rats (approximately 100 days of age). Our previous research indicated that Wistar and Hooded rats showed similar suppressive effects. The Wistar and Hooded rats were distributed in an approximately equal fashion between the three groups.

At the end of most previous experiments, histological analyses had been performed on all rats. However, no gross lesions had been detected at intensities of 560 μA and below. The tissue around the electrode tips of rats stimulated with 1-Hz and 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 either of the present experiments.

RESULTS

Two dependent variables, ETI and Composite Score, have been effective in previous experiments and were used in the present case. The latter measure involves a score of 1 for Stage 1 behavior, 2 for Stage 2 response, and 3 for a clonic convulsion. The results are shown in Table 1. These results with a 3 hour intertrial interval are similar to those with a 1 hour interval in the previous experiments. The Mean ETI increased gradually over the ETI determinations for the 1-60-1 group and the measure showed a gradual decline for the X-60-X group. The values for the 60-60-60 group are intermediate in that they remain approximately the same. Thus they differ in a modest way from the X-60-X group in that no decline occurs over ETI determinations.

In the 1-60-1 group, two rats did not convulse at 560 μA during the ETI_3 determination, and five did not convulse for ETI_4. (This intensity is the upper limit we impose to reduce the possibility of lesions.) In these cases, 25 μA was added to the upper limit to produce a score of 585 μA. Thus, in Table 1 a mean value greater than 560 μA occurs for ETI_4 in the 1-60-1 group. Convulsion at or below 560 μA occurred for the other groups at all ETI determinations.

The Mean Composite Score for the 1-60-6 group remained at a Stage 1 response; a mean value of 10 would be required to indicate a Stage 2 response over the five trials. Both the 60-60-60 and X-60-X groups showed kindling progression over the three blocks of trials.

Table 2 shows the results for a typical block of 5 day trials, Block 2. Behavior over the five trials for the 1-60-1 group went from a mean response well into Stage 2 to a beginning Stage 1 response on Trial 5; from Day 3 to Day 5 all rats had a Stage 1 response. However, both the 60-60-60 and X-60-X groups remained relatively stable throughout this series of trials. The mean response for these groups in the five trials was close to a convulsion whereas the 1-60-1 group showed a mean of 1.3, indicating a response near the beginning of Stage 1.

In previous experiments, most 1-Hz or 3-Hz stimulated rats which were

TABLE 1

Results of Stimulation in Experiment 1
(3 Hour Intertrial Interval)

	Mean ETI Values (in Microamperes) ETI Determinations					Mean Composite Score Blocks of 5 Day Trials		
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**
1-60-1 (8)*	112	252	483	575	217	7.0	6.4	7.0
60-60-60 (7)	122	118	116	130	136	10.9	13.4	14.1
**X-60-X (7)	116	94	84	86	104	10.6	13.3	14.7

*Size of sample in parentheses.
**X refers to nonstimulation in all tables.
ETI is effective threshold intensity in all tables.

TABLE 2

Mean Composite Score in Block 2 of Experiment 1
(3 Hour Intertrial Interval)

	Days						
Group	**0***	**1**	**2**	**3**	**4**	**5**	**M****
1-60-1	2.4	1.9	1.5	1.0	1.0	1.0	1.3
60-60-60	2.7	2.6	2.7	2.7	2.7	2.7	2.7
X-60-X	2.6	2.4	2.9	2.9	2.6	3.0	2.8

*Last trial of ETI_2 determination in Table 2 and 4.
**Mean of Composite Scores over Days 1 to 5 in Table 2 and 4.

TABLE 3

Results of Stimulation in Experiment 2
(24 Hour Intertrial Interval)

	Mean ETI Values ETI Determinations					Mean Composite Score Blocks of 5 Day Trials		
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**
1-60-1 (9)	219	230	261	330	282	8.3	8.2	6.8
60-60-60 (7)	318	286	230	220	237	12.3	13.7	14.1
X-60-X (7)	294	234	296	172	167	10.7	13.7	14.3

TABLE 4

Mean Composite Score in Block 2 of Experiment 2
(24 Hour Intertrial Interval)

	Days						
Group	**0**	**1**	**2**	**3**	**4**	**5**	**M**
1-60-1	2.8	2.4	1.3	1.6	1.6	1.3	1.6
60-60-60	3.0	3.0	3.0	2.4	2.7	3.0	2.8
X-60-X	2.6	2.6	2.6	2.6	2.6	2.9	2.7

rested for 16 days after the ETI_4 determination showed an ETI_5 value below, at, or just above the lowest ETI, usually ETI_1, except for durations of 2 minutes or greater. At the 2 minutes duration a few rats had a low ETI but most had values greater than ETI_1. In the present experiment, two of the eight rats were well above the ETI_1 value; the other six rats were at or slightly above the ETI_2 value. The overall mean for ETI_5 (Table 1) falls well above ETI_1, but below ETI_2; thus some recovery from suppression had occurred.

Six of the seven rats in the 60-60-60 group had a low ETI value for ETI_5. The seventh rat showed a higher ETI_5 value than for any of the other ETI determinations. Likewise, the mean for ETI_5 was slightly above the ETI_4 determination, the lowest ETI point.

Three of the seven X-60-X rats were at or below the lowest ETI; the other four were just above a low ETI point. The mean ETI_5 was below ETI_1, but just above the other mean ETI determinations.

In Experiment 2 with a 24 hour interval, suppression was present for the 1-60-1 group as indicated by both dependent variables, ETI and Composite Score (Table 3). In the latter measure the response of the 1-60-1 group remained at a middle Stage 1 level. Although the Mean ETI indicated an increase over each determination, the increase was minimal: 11 μA from ETI_1 to ETI_2, 31 μA from ETI_2 to ETI_3, and 69 μA from ETI_3 to ETI_4. The increases for 1 and 3 hours intertrial intervals tended to be 100 μA or greater. Furthermore, if one compares ETI_4 with ETI_1 for the 1-60-1 groups in Experiments 1 and 2, the former was approximately five times as great as the latter with a three hour interval (575 vs. 112), but was about one and a half times as great when the interval was 24 hours (330 vs. 219). Thus, the 24 hour interval apparently allowed some of the suppression or interference effect to dissipate.

The ETI values of the two control groups decreased gradually over the four determinations, but the decline was less for the 60-60-60 group than for the X-60-X group. Such results are consistent with those of previous experiments. Likewise, these groups

showed Mean Composite Scores which indicated that kindling was progressing effectively.

During Block 2 the 1-60-1 group showed the suppression effect in a pronounced fashion (Table 4), going from a mean value of 2.8 during the last trial of the ETI_2 determination to a score of 1.3 on Day 5. The mean score over the five trials was 1.6. The Mean Composite Score for the 60-60-60 and X-60-X groups remained relatively close to the score on the last trial of the ETI_2 determinations.

After the 16 days of nonstimulation, 5 of the 9 rats in the 1-60-1 group had an ETI_5 value at or just above the low ETI point (ETI_1) and the other 4 animals were above the low ETI score. The overall mean for ETI_5 was greater than that for ETI_1 but less than that for ETI_5.

All rats in the 60-60-60 and the X-60-X groups had ETI_5 values below, at, or just above the low ETI point (ETI_4). The mean for the former group was just above the mean for the ETI_4 determination whereas that for the latter group was approximately equal to the mean for the ETI_4 determination.

DISCUSSION

The results in these experiments with 3 and 24 hour intertrial intervals were similar to those in previous experiments with 1 hour intertrial intervals (Table 5). In the two dependent variables, there were differences between the 1-60-1 and X-60-X groups for 1, 3, and 24 hour intervals. For the 1-60-1 group the mean ETI increased over determinations and the Mean Composite Score was less. Likewise, in Block 2 there was a gradual decrease in Mean Composite Score on each day for the 1-60-1 group. With the 1, 3 and 24 hour intervals the 1-60-1 rats showed a mean response of Stage 1 or early Stage 2 behavior during Block 2. However, the suppressive effect was less with the 24 hour interval than was the case for the 3 hour and 1 hour intervals, as indicated by the small increase in ETI. Furthermore, in Experiment 2 all 1-60-1 rats convulsed on each ETI determination, suggesting a lesser suppression effect than with the 1 and 3 hour intervals experiments in which one or more rats did not convulse at 560 μA for ETI_2, ETI_3, or ETI_4. For the 1 hour interval experiment, the number of rats (of 6) which did not convulse was 2, 4, and 5 on ETI_2, ETI_3, and ETI_4, respectively. The number of rats which did not convulse (of 8) with the 3 hour interval was 2 and 5 for ETI_3 and ETI_4, respectively.

Previously, it was found that 60-Hz stimulation (for the 60-60-60 group) produced a mild degree of interference (Gaito, 1980c), a result consistent with those of Mucha and Pinel (1977). The results of the present experiments again suggested a mild interference effect for the 60-60-60 group in the ETI variable. The ETI values for this group did not decline to the same degree over the determinations as was the case with the X-60-X group.

These results, along with those in previous experiments which indicated that many rats will convulse at previous low ETI values after 15 or 16 days of no stimulation, suggest that the suppression effect is a transient event; this event is in sharp contrast to the kindling effect which may persist for months or longer (Goddard et al., 1969; Racine, 1978). Such results also seem similar to those of McIntyre and Goddard (1973) who reported a transient interference effect ("after-effect"), during alternation of stimulation of homologous amygdalae, which dissipated within 14 days.

The results indicating some dissipation of the suppression effect with a 24 hour interval also seem to be evidence against the hypothesis that the suppression effect is due to lesions. For example, if the effect were due to tissue damage, one would expect that the Mean ETI increase over determinations should be approximately the same with 1, 3, and 24 hour intervals. However, the mean increases from ETI_1 to ETI_4 (ETI_1-ETI_2, ETI_2-ETI_3, ETI_3-ETI_4) for intervals of 1, 3, and 24 hours, respectively, were: 113, 209, and undetermined (because no rats convulsed at 560 μA); 140, 231, and 92 (an underestimation because only several rats convulsed at 560 μA); 11, 31, 69. The increases seemed to be similar for the 1 and 3 hour intervals, but the 24 hour interval appears to have allowed some of the effect to dissipate. If lesions were involved, one might expect some dissipation by 24 hours (recovery of tissue). However, one

TABLE 5

Results of Previous Experiment for 1-60-1 Group (n = 6) with 1 Hour Intertrial Interval

Mean ETI Values

ETI Determinations

1	2	3	4	5
216	329	538	____*	229

Mean Composite Scores

Blocks of 5 Day Trials

1	2	3
9.2	9.8	11.0

Mean Composite Score in Block 2

Days

0	1	2	3	4	5	M
3.0	2.8	2.0	2.0	1.5	1.5	2.0

*Only 1 rat convulsed at 560 μA.

would expect less recovery than was indicated by the differences present between the 24 hour interval means and those for the other groups.

The suppression effect has relevance to theoretical aspects of brain functions. The exact functioning of the brain during the kindling event is not clear. Goddard et al. (1969) and McIntyre and Goddard (1973) suggested the operation of two factors. The first was a positive transfer one (from one site to the homologous one) of a long term nature, involving brain circuitry changes in both sites; an "aftereffect" (second factor) was a less durable event of a negative transfer nature. Our suppression effect appears to be of transient nature and may be the "aftereffect" postulated by these individuals. Thus, the use of low frequency brain stimulation along with kindling frequencies may aid in the determination of brain events underway during kindling phenomena.

Another feature of the suppression effect is its obvious relevance to epilepsy. Many individuals have suggested that the kindling effect could be a useful model of epilepsy. The discovery of the "suppression effect" merely reinforces this possible analogue.

One use of experimental models of epilepsy has been that of evaluating potential anticonvulsant chemicals. However, because brain activities are of electrochemical nature, it is quite possible that not only chemical input, but electrical input as well, might produce a stabilizing effect on brain aspects and lead to a moderation of seizure conditions. We suggest the use of electrical brain stimulation to produce a seizure condition in which the behavior is similar to that observed in epilepsy. Then other frequencies of brain stimulation can be employed in an attempt to partially or completely eradicate the seizure conditions. Electrical brain stimulation via implanted electrodes has been used to locate seizure sites in humans (Valenstein, 1973), but it appears not to have been investigated as a possible means of attenuating the seizure state until recently.

A method of chronic cerebellar stimulation has been used by Cooper and his associates (Cooper et al., 1976). A number of patients who had intractable seizures were maintained seizure-free or with modified seizures for periods up to three years when subjected to chronic stimulation of the anterior lobe of the cerebellum. However, these results have been questioned by other individuals (Lockard, et al., 1979). They suggested that cerebellar stimulation was not generally efficacious with clinical seizures and that the favorable results of some epileptic patients were probably a function of placebo or psychological factors. Also tissue damage was another possible contributor in the case of non-seizure or modified seizure states. This method is still being evaluated by these investigators and others. The present method using kindling procedures might produce another worthwhile approach to epileptic problems.

Thus, the use of kindling and suppression methodologies, supplemented by other sophisticated techniques, might provide important information relative to how the brain functions during these automatic behaviors. However, practical results may occur also. A "brain stimulation therapy" may be developed for cases of focal epilepsy where surgery is inadvisable and chemical therapy ineffective.

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