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The effect of varying durations of stimulation of the 3-Hz interference effect

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Experiments were conducted to evaluate the effect of various durations of 3-Hz brain stimulation on kindling behavior induced by 60-Hz sine-wave stimulation of the amygdala. In two experiments the effective threshold intensity (ETI) to elicit a convulsion was determined on four separate occasions with 5 days of daily trials interspersed between determinations. On each day experimental rats were stimulated with 3-Hz current on the first and third trials for 5, 15, 30, 60, 120, or 300 sec duration and with 60-Hz current for 30 sec on the second trial. A steady increase in the intensity required to elicit a convulsion with 60-Hz current from ETI_1 to ETI_4 resulted for all rats with durations of 15 sec or greater. Rats stimulated only with 60-Hz sine waves and those in the 5-sec group maintained relatively stable values from ETI_1 to ETI_4, with a slight decline occurring. Suppression of the expected 60-Hz-induced convulsive behavior on daily trials was modest in the 15-sec group, pronounced with the 30-sec group, and drastic with the other groups. The 300-sec group had the greatest suppressive effect operating. The suppression effect appeared not to be due to tissues damage inasmuch as many of the experimental rats (except the 300-sec group) convulsed again at previous low ETI levels following a 16-day rest at the end of the experiment. This result suggests that the suppression effect is a relatively transient event.

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 (i.e., 30, 20, 15, 10, 5, 3, and 1; 100, 150, 200, 300, 400, 500, 1,000, 2,000, and 4,000). 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 which 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, and (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 consistently produced an interference effect, that is, suppression of convulsions induced by 60-Hz stimulation (Gaito, 1979a, 1979b; Gaito, Nobrega, & Gaito, in press). Another experiment evaluated the effect of varying durations of 1-Hz stimulation, namely, 0, 5, 15, 30, 60, 120, 180, and 600 sec (Gaito, in press). 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.

The suppression effect appeared not to be due to tissue damage inasmuch as most of the experimental rats convulsed again at previous low effective threshold intensity (ETI) levels following a 15- or 16-day rest at the end of the experiment. This result suggests that the suppression effect is a relatively transient event (Gaito, in press).

The present experiment was conducted to determine the effect of varying durations of 3-Hz stimulation. The same durations were used as in the 1-Hz study, except that 300 sec was substituted for 600 sec and the 180-sec duration was deleted.

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METHOD

Forty-seven male hooded rats (approximately 130 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 47 rats were stimulated with 60-Hz sine waves for 30 sec during three trials on Day 1. Approximately 1 h intervened between the trials. A Lafayette stimulator was used; the intensity was 100 microA. On the first trial of Day 2, ETI_1 was determined. The 60-Hz current was increased until a Stage 2 or 3 response was elicited. Then 15 microA was added to allow for day-by-day threshold fluctuations. Two further trials of stimulation at this intensity were provided.

Then one group of seven rats received stimulation with 1-Hz sine waves for 5 sec 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 at an ETI_1 value. There was approximately 1 h between trials. Other groups of seven rats were stimulated with 1-Hz current for 15, 30, 60, 120, and 300 sec at double the ETI_1 values on Trials 1 and 3, and with 60-Hz current on Trial 2. Five other rats received 60-Hz stimulation on Trial 2, but on Trials 1 and 3 they were placed in the apparatus without stimulation (0-sec group). All 60-Hz stimulation was at ETI_1 for 30 sec.

Following this 5-day period, rats from all groups had ETI_2 determined over six trials during 2 days. Then another block of 5 days of stimulations 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 continued through the ETI_4 determination. Then all rats were rested for 16 days, and ETI_5 was determined on one trial. This last determination allowed for an evaluation of the possibility that the rats had recovered from the suppressive effect.

At the end of all previous experiments, histological analyses were performed on all rats. However, no gross lesions had been detected at intensities of 560 microA and below (intensities that are used routinely in our research). The tissue around the electrode tips of rats stimulated with 3- 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 mean ETI values are indicated in Table 1. Two rats in the 60-sec group were discarded because behavior prior to the beginning of Block 1 trials did not show a clear BA or CC. As in previous experiments, the control rats, those subjected to no stimulation on Trials 1 and 3 (0-sec group), and the 5-sec group showed a gradual decrease over the four determinations. The other five groups showed an increase over the determinations, with the 15-sec group affected the least and the 300-sec rats affected most.

A number of rats in the 60-, 120-, and 300-sec groups did not convulse at or below 560 microA during ETI_2, ETI_3, or ETI_4. This intensity is the upper limit we use. No rats in the last group convulsed for ETI_3 or ETI_4 determinations.

The mean composite score over three blocks of trials also showed the suppression effect (Table 2). The minimum and maximum composite scores, respectively, over five 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 score of 3 for each convulsion. Within each block, there was a tendency toward a gradual decrement in the mean composite score, with increasing periods of stimulation with 3-Hz current.

The first block of trials usually shows the smallest interference effect, because behavior is not as stable at this early stage as it is later, and ETI_1 is not as reliable as later ETI determinations. However, by the second block of trials, the effect is apparent. Table 3 shows the

Table 2
Mean Composite Score

	Blocks of Trials		
Group	1	2	3
0	11.6	14.0	15.0
5	11.4	14.0	13.6
15	9.9	13.3	12.7
30	7.0	11.7	14.4
60	7.4	7.0	9.2
120	7.1	7.5	5.6
300	6.4	5.9	5.0

Table 1
Mean ETI Values (in Microamperes)

		ETI Determinations				
Group	N	1	2	3	4	5
0	5	370	260	179	168	151
5	7	316	282	264	244	235
15	7	209	224	232	286	234
30	7	178	328	414	416	188
60	5	160	312	482	*	297
120	7	166	478	**	†	258
300	7	186	557	†	†	†

**Only two rats convulsed at or below 560 microA. **Only one rat convulsed at 560 microA. †No rats convulsed at 560 microA.*

Table 3
Mean Composite Score in Block 2

	Day						
Group	0	1	2	3	4	5	M
0	2.8	2.8	2.8	2.8	2.8	2.8	2.8
5	2.6	2.9	2.7	2.9	2.9	2.7	2.8
15	2.9	3.0	2.9	2.6	2.4	2.4	2.7
30	2.9	2.3	2.6	2.6	2.1	2.1	2.3
60	2.5	2.5	1.3	1.5	1.0	1.0	1.8
120*	3.0	3.0	1.5	1.0	1.0	1.0	1.5
300*	2.8	2.5	1.0	1.0	1.0	1.0	1.3

*Note–Day 0 indicates the last trial of ETI_2 determination. M = mean score per trial in Block 2. *N = 4; three rats did not convulse at 560 microA during ETI_2 trials.*

mean composite score for the 5 days of trials within the second block. The minimum and maximum scores, respectively, for each trial and each rat were 1 and 3. Again, there was a gradual decrement, especially from Day 3 on, from the control group and 5-sec group to the rats stimulated for 300 sec. The 15-sec group showed a minimal suppressive effect. The 60-, 120-, and 300-sec groups had a mean response over the five trials of lower than 2.0 (i.e., below a Stage 2 response).

The ETI_5 determination was completed with most rats after a 16-day rest. Four rats in the 300-sec group had the ETI_5 determination after 30 days. All control rats and those in the 5-sec group convulsed at or below the lowest ETI value, which was ETI_4. The lowest ETI for the other rats was ETI_1. For the 15- and 30-sec groups, most rats had an ETI_5 value close to the ETI_1 result; 11 of the 14 rats were at or below ETI_1. In the 60- and 120-sec groups, 6 of the 12 rats attained a low ETI value on the ETI_5 determination.

DISCUSSION

The results with 3-Hz stimulation were essentially the same as in previous experiments with 1-Hz stimulation. Even though the number of rats in each duration group was small, the results were very clear. There was a gradual increase in interference effects as the duration of stimulation with 3 Hz increased (see Tables 1, 2, 3). The 5-sec condition had little or no effect and was almost equivalent to the control condition (0-sec stimulation). The effect at 15 sec was modest. By 30 sec the effect was more pronounced, especially in mean ETI. With 60, 120, and 300 sec of stimulation, the effect was drastic; one or more rats failed to convulse at an intensity of 560 microA during ETI_2, ETI_3, and ETI_4 determinations. No rats in the last group convulsed for ETI_3 or ETI_4 determinations.

The ETI determination after 16 days of no stimulation (ETI_5) indicated some interesting results. The mean ETI_5 values in Table 1 for the 0- and 5-sec groups are lower than those at any previous ETI. Thus, there was a steady decline for both groups. Although the mean ETI increased from ETI_1 to ETI_4 with the 30-sec group, the mean for ETI_5 is just above the mean for ETI_1. The mean ETI_5 for the 60- and 120-sec groups are well above the ETI_1 value, but far below the ETI_2. However, even though the mean ETI_5 is greater than the mean for ETI_1, six of the 12 rats in the two groups convulsed on the ETI_5 determination at approximately the ETI_1 level. If the 0- and 5-sec groups (which showed no suppression effect) and the 300-sec group (which showed a massive effect) are excluded, it can be seen that 17 of the 26 rats in the other groups showed complete recovery from the suppression effect by the ETI_5 determination. Thus, the suppressive effect had dissipated, or nearly so, in many rats within 16 days. However, the effect with the 300-sec group is drastic; massive increases occurred in mean ETI values over determinations, and no return to low ETI levels on the ETI_5 determination was present after 16 or 30 days.

The results with 1-Hz stimulation (Gaito, in press) and the present results with 3-Hz stimulation indicate that stimulation for 120, 180, 300, and 600 sec are not greatly different. Drastic suppressive effects are produced with 120 sec of stimulation. Increasing the duration beyond this point does deepen the suppressive effect somewhat, as shown in the mean ETI, the mean composite score, and the mean composite score per trial in Block 2. However, the suppressive effect is not proportional to the increased duration of stimulation. Thus, there appears to be no need to go beyond 120 sec in order to obtain a deep suppressive effect.

The interference effects we have observed of 1-Hz and 3-Hz stimulation on kindled behavior produced by 60-Hz stimulation are one class of interference effects noted within the kindling paradigm. There are two other classes of interference effects that can be considered to be similar, namely, the effect of 60-Hz stimulation of one amygdala on the stimulation of the contralateral amygdala (McIntyre & Goddard, 1973) and the effect of 60-Hz stimulation of the amygdala on subsequent stimulation of the same site (Mucha & Pinel, 1977).

It appears that there are both interfrequency and intrafrequency interference effects. The former is the suppression effect. The latter type consists of unilateral and alternating events. With unilateral stimulation of a brain site, interference to later stimulation develops as a result of each stimulation event. In the alternating stimulation case, stimulation of one brain site sets up interference effects to later stimulation of the homologous brain site. This type of interference effect is assumed to result because of inhibitory effects of the one brain site on the other (McIntyre & Goddard, 1973; Nobrega & Gaito, 1978). Thus, stimulation at the primary site may set up an inhibitory process relative to later stimulation of the homologous site in the opposite hemisphere, as well as an inhibitory process at the primary site that would interfere with later stimulation of the primary site. The McIntyre and Goddard and Mucha and Pinel results are consistent with this interpretation.

Presumably, the interference pattern set up in the case of 1-Hz and 3-Hz stimulation is similar to that observed in the other two cases. Postseizure inhibition processes may develop during stimulation and persist for a period of time. If stimulation occurs within the period that this inhibition process is functional, there is a reduced probability for the occurrence of a convulsion at its usual strength and duration (Goddard et al., 1969; Mucha & Pinel, 1977). Our research results suggest that this type of interference may be the stronger of the three cases.

The exact basis for the 1-Hz and 3-Hz suppression effect is not clear at this time. Presumably, it involves some modification of the brain process responsible for kindling, or else another process is developed that is antagonistic to the brain kindling process.

One possible basis for the suppression effect is that lesions are produced by the 1-Hz and 3-Hz stimulation, and the damage raises the threshold for Stage 2 or 3 responses. However, this explanation does not seem to be appropriate. Our histological analyses in the previous research indicated that the tissue around the electrode tips of rats subjected to intensities of 560 microA or lower with 1-Hz or 3-Hz current appeared similar to that of rats subjected only to 60-Hz stimulation. Obviously, such analyses are of gross nature and could miss subtle lesions.

A more important consideration is the apparently transient nature of the suppression effect, in that a time decay of the suppression event is indicated under certain conditions. This decay is suggested by the ETI_5 determinations in which many experimental rats (except those in the 300-sec group) convulse near the previous low threshold intensity. Similar results were obtained with 1-Hz stimulation. Furthermore, some of our recent results (research underway) indicate that the intertrial interval is important for the effect. With 1- or 3-h intervals between trials and a duration of 120 sec, the effect is present but very modest. For example, increases from one ETI determination to the next average more than 100 microA with 1- or 3-h intervals (see Table 1, 120-sec group). However, the average increment with a 24-h interval is about 30 microA. Furthermore, all rats convulse at or below 560 microA (the upper limit used) during ETI determinations for the 24-h interval, but some rats in the experiments using 1- and 3-h intervals do not convulse at this limit. In Table 1, only 1 of the 7 rats in the 120-sec group convulsed during the ETI_3 determination, and no rats convulsed on ETI_4. Further research with intervals of 24 h and longer are anticipated to gain information concerning the possible time decay of the suppression effect.

Thus, even though tissue damage is a possible explanation for the suppression results, the above evidence indicating a time

decay of the supression effect seems to suggest that the effect is not due to lesions. If lesions were responsible for the suppression events, one would expect the ETI values to increase (Goddard et al., 1969; Racine, 1978). However, the increasing ETI should be more permanent in nature, with little or no decrease over time (i.e., tissue damage would not be considered of such short-term nature as the time periods observed in our experiments).

These data indicating the transient nature of the suppression effect suggest that this effect may be similar to the "aftereffect" of McIntyre and Goddard (1973). They found an interference effect with stimulation of the primary site a second time if secondary site stimulation intervened between the two stimulations of the primary site. The interference was not present if an interval of 14 days or more occurred prior to the second time of stimulating the primary site.

Ultimately, these interfrequency interference effects 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 (e.g., focal epilepsy).

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