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# The effect of a 72-h intertrial interval on the 1-Hz suppression effect in rats

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**An experiment was conducted to evaluate the effect of a 72-h intertrial interval of 1-Hz brain stimulation on kindling behavior induced by 60-Hz sine-wave stimulation. The effective threshold intensity (ETI) to elicit a kindled response with 60-Hz stimulation was determined on four separate occasions with 15 trials between determinations. Experimental rats were stimulated with 1-Hz sine waves before and after a 60-Hz brain stimulation trial with a 72-h interval between stimulation sessions (1-60-1 group). A second group received only the 60-Hz stimulation on the same trials as those on which the 1-60-1 group was stimulated with 60-Hz current (X-60-X group). In previous experiments with 1, 3, or 24 h between trials, the 1-60-1 group had a gradual increase in the intensity required to elicit a kindled response with 60-Hz current from $ETI_1$ to $ETI_4$ (the suppression effect). However, the 72-h interval produced a decline in ETI values similar to that of the X-60-X group, but not as great. Suppression of kindled behavior on daily trials was not present for the 1-60-1 group, in contrast to results of previous experiments. Apparently, the 72-h interval allowed much of the suppression effect to dissipate.**

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, 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, 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 by many researchers (Gaito, 1976a; Racine, 1978).

In a series of experiments, it was found that 1-Hz or 3-Hz sine-wave stimulation before and after a 60-Hz stimulation trial suppressed the tendency of the 60-Hz current to produce kindling behavior (Gaito, 1979a, 1979b; Gaito, 1980a, 1980b, 1980c, 1980d; Gaito, Nobrega, & Gaito, 1980). The experiments with 3-Hz stimulation were conducted at an intertrial interval of 1 h between the imposition of the 3-Hz and 60-Hz stimulation trials. Other intertrial intervals have been used with the 1-Hz agent. With 1- and 3-h intertrial intervals, the suppression effect was pronounced. The effect was present with a 24-h interval but was reduced greatly (Gaito & Gaito, 1981); these results indicate that the 1-Hz suppression process is time dependent and suggest the possibility that it may dissipate completely at longer time intervals. The present experiment was conducted with a longer time interval, 72 h, to evaluate this possibility.

## METHOD

Forty-three male Wistar rats (between 100 and 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 43 rats were stimulated with 60-Hz sine waves for 30 sec during three trials on the 1st day. One hour intervened between trials. A Lafayette stimulator was used; the intensity was 36 microA (root mean square, RMS; equivalent to 100 microA peak to peak). On the first trial of the 2nd day, the first effective threshold intensity ($ETI_1$) was determined. The 60-Hz current was increased until a Stage 2 or 3 response was elicited. Then, 5 microA was added to allow for day-to-day threshold fluctuations. Two further trials of stimulation at this intensity were provided. The 43 rats were separated into two groups, by pairing most rats so as to maintain approximately equal mean ETI values for the two groups.

Then, one group of 23 rats received stimulation with 1-Hz sine waves for 120 sec on Trials 1, 3, 4, 6, 7, 9, 10, 12, 13, and 15 at twice the $ETI_1$ value. A 60-Hz stimulation trial was provided on Trials 2, 5, 8, 11, and 14 for 30 sec at $ETI_1$ (Group 1, 1-60-1). This procedure involved a 60-Hz stimulation trial sandwiched between 1-Hz stimulation trials. There were 72 h between trials. A second group of 20 rats received 60-Hz stimulation on the same trials as the 1-60-1 group, but on the other trials each rat was placed in the apparatus without stimulation (Group 2, X-60-X). All 60-Hz stimulation was at $ETI_1$ for 30 sec, a duration that has been used routinely in our research. Stimulation with 1-Hz current was for 120 sec duration at two times $ETI_1$; this duration and intensity have been found to produce a drastic suppressive effect in previous experiments.

Following these 15 trials, rats from all groups had $ETI_2$ determined over 6 trials during 2 days (3 trials/day). Then another block of 15 trials of stimulation occurred in which each



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group was treated in the same fashion as during the previous block of trials prior to the $ETI_2$ determination. This alternation of ETI determinations and a block of trials was continued through the $ETI_4$ determination.

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 200 microA (RMS) and below, intensities that 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

With the 72-h interval between trials, the interval from one 60-Hz stimulation trial to the next one was 9 days. Previously, it had been found that intervals of 1 week or longer tended to produce a more severe convulsion (Gaito, 1978); the duration of convulsions increased and convulsions were more intense. The rats frequently would jump from the observation box when being placed back into the home cage; many rats were intermittently hyperactive in the home cage for 5 or 10 min thereafter. These results occurred also in the present experiment. Such hyperactivity probably was a contributing factor in the loss of the electrode assembly by six rats in each of the two groups.

Two dependent variables, ETI and cumulative composite score, have been sensitive to the effects of 1-Hz and 3-Hz stimulation 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. These ETI results with a 72-h intertrial interval are different from those for 1- and 3-h intervals in the previous experiments, for the 1-60-1 group. The mean ETI value decreased gradually over the ETI determinations for the 1-60-1 group (both for the rats that completed the experiment and those that lost the electrode assembly), rather than showing substantial increases. The results for the X-60-X group showed the typical decline over ETI determinations, a decline that appeared to be greater than that for the 1-60-1 group.

The data for the cumulative composite score for the three blocks of trials are shown in Table 2. The kindling progression appears to be similar for both groups, for all rats and for those that completed the experiment. There appear to be no differences beyond random variability between the two groups. However, there was an increase in the mean value over the determinations, indicating the usual kindling progression.

**Table 1**
**Mean Threshold Data for the Four ETI Determination Points (in Microamperes, Root Mean Square)**

| Group | ETI Determinations 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | | All Rats | | |
| 1-60-1 | 101.3 (23) | 96.8 (21) | 85.2 (18) | 79.7 (17) |
| X-60-X | 98.3 (20) | 73.3 (19) | 57.1 (16) | 45.6 (14) |
| | | Completed Experiment | | |
| 1-60-1 (17) | 108.5 | 100.0 | 87.9 | 79.7 |
| X-60-X (14) | 95.4 | 75.9 | 54.9 | 45.6 |

*Note–Numbers in parentheses indicate number of rats.*

**Table 2**
**Mean Cumulative Composite Scores for the Three Blocks of 15 Trials**

| Group | Block 1 | Block 2 | Block 3 |
|---|---|---|---|
| | | All Rats | |
| 1-60-1 | 11.2 (23) | 13.7 (19) | 14.4 (17) |
| X-60-X | 10.3 (20) | 12.8 (18) | 13.4 (14) |
| | | Completed Experiment | |
| 1-60-1 (17) | 11.0 | 13.6 | 14.4 |
| X-60-X (14) | 10.4 | 12.7 | 13.4 |

*Note–Numbers in parentheses indicate number of rats.*

## DISCUSSION

The ETI results of previous experiments with 1-, 3-, and 24-h intervals are provided in Table 3. The results with a 72-h intertrial interval were different from those with 1-, 3-, and 24-h intervals. Although the kindling progression for the 1-60-1 group proceeded in the same manner as that for the X-60-X group for the 72-h interval, there were differences in the ETI determination values (Table 1). The means for both groups showed a gradual decline; however, the decline for the X-60-X group was sharper, indicating a greater decline at each ETI determination.

The differential results between 1-60-1 and X-60-X groups for the four intertrial intervals is illustrated in Table 4 by the ratio of each ETI value to the $ETI_1$ mean. This ratio indicates the relative ETI values. Table 4 shows a number of points. (1) The discrepancy between the 1-60-1 and X-60-X groups begins at zero and increases over ETI determinations, indicating the cumulative effects of the 1-Hz brain stimulation. (2) The ratios for the 1-60-1 groups during the 1- and 3-h intervals increase sharply. The ratios for the 24-h interval with the 1-60-1 group show minimal increases, which are obviously different from those in the same groups for the 1- and 3-h intervals. These ratios for the 72-h interval show a gradual decrease, but not as great as that for the X-60-X group. (3) The ratios for the X-60-X groups are similar for all intervals, showing a gradual decline over ETI determinations.

The differential results also are highlighted in Table 5 by an orthogonal polynomials trend analysis (Gaito, 1977). For each group, there appears to be only a linear component present (a straight-line function). The regression curve for each of the two groups appeared to be of the following form: $y = \mu + \beta_L c_k$, where $\mu$ is the mean in the population of concern, $\beta_L$ is the linear regression coefficient, $c_k$ is the orthogonal polynomial coefficient for the kth ETI determination, and y is the predicted score. (In sample data, this equation is estimated by $y = \bar{Y} + b_1 c_k$.) Table 5 basically shows the same aspects as does Table 4 (but from a regression analysis viewpoint); however, it also provides the means for the two groups for each interval. Table 5 indicates the following aspects. (1) The greatest difference in means between the 1-60-1 and X-60-X groups is present in the 1- and 3-h intertrial intervals case. (2) The means are greatest for the 1-60-1 groups for the 1- and 3-h intervals. (3) The means for the X-60-X groups appear to be similar (within random variability) for all intervals except the 3-h one (see below). (4) The regression coefficient is positive (i.e., showing an increment) for the 1-60-1 groups during all intervals except for the 72-h one, for which it becomes negative. (The regerssion coefficient indicates the magnitude of change in ETI values with each ETI determination.) (5) The regression coef-

Table 3
Mean ETI Data for 1-, 3-, and 24-h Intervals

| Group | n | ETI Determination 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| | | 1-h Interval | | | |
| 1-60-1 | 6 | 77.2 | 141.0 | 188.7 | 198.3 |
| X-60-X | 6 | 90.5 | 65.8 | 55.8 | 46.7 |
| | | 3-h Interval | | | |
| 1-60-1 | 8 | 40.0 | 90.0 | 172.6 | 206.0 |
| X-60-X | 7 | 41.4 | 33.6 | 30.0 | 30.7 |
| | | 24-h Interval | | | |
| 1-60-1 | 9 | 78.3 | 82.2 | 93.3 | 117.8 |
| X-60-X | 7 | 105.0 | 83.6 | 70.0 | 61.4 |

Table 4
Mean ETI Values Relative to the $ETI_1$ Mean for the 1-60-1 and X-60-X Groups and Four Intertrial Intervals

| Group | ETI Determination 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | 1-h Interval | | | |
| 1-60-1 | 1.00 | 1.83 | 2.44 | 2.57 |
| X-60-X | 1.00 | .73 | .62 | .52 |
| | 3-h Interval | | | |
| 1-60-1 | 1.00 | 2.25 | 4.32 | 5.15 |
| X-60-X | 1.00 | .81 | .72 | .74 |
| | 24-h Interval | | | |
| 1-60-1 | 1.00 | 1.05 | 1.19 | 1.50 |
| X-60-X | 1.00 | .80 | .67 | .58 |
| | 72-h Interval | | | |
| 1-60-1 | 1.00 | .92 | .81 | .73 |
| X-60-X | 1.00 | .80 | .58 | .48 |

*Note–Values indicate ratio of $ETI_k$ to $ETI_1$, where k refers to each of the four ETI determinations.*

Table 5
Regression Equations for 1-, 3-, 24-, and 72-h Intertrial Intervals With ETI Data

| Interval | Group 1-60-1 | Group X-60-X |
|---|---|---|
| 1 | $153.4 + 21.0c_k$ | $64.7 - 7.1c_k$ |
| 3 | $127.2 + 29.0c_k$ | $33.9 - 1.8c_k$ |
| 24 | $92.9 + 6.5c_k$ | $80.0 - 7.2c_k$ |
| 72 | $94.0 - 4.9c_k$ | $67.9 - 8.5c_k$ |

*Note–Each regression equation is of the form $y = \mu + \beta_1 c_k$, which is estimated by $y = \bar{Y} + bc_k$ in sample data. $\mu$ and $\beta_1$ are the mean ETI and linear regression coefficient in the population, and $\bar{Y}$ and b are the corresponding sample values; y is the predicted value; $c_k$ is the orthogonal polynomial coefficient for each of the ETI determinations.*

ficient is negative (showing a decrement in ETI values) for all X-60-X groups. (6) The smallest difference between the X-60-X and 1-60-1 groups in the regression coefficients is for the 72-h interval.

The data for the X-60-X group for the 3-h interval appear unusual. Both the mean (Table 3) and $b_L$ (Table 5) have lower values than they have for the other X-60-X groups. This aspect is due to the low ETI value on $ETI_1$, a mean of 41.4 microA. The mean decreased over ETI determinations, as it usually does for X-60-X groups. However, the possible decrease from the initial low values was less than it is in the usual case with greater $ETI_1$ values. Thus, a small value occurs (−1.8) for the linear regression coefficient.

The results of the present experiment, along with the previous ones, suggest that the suppression effect involves a time-dependent process and that the effect dissipates almost completely with a 72-h interval. Furthermore, this indication of a time-dependent process tends to invalidate the possibility that the suppression effect is based on tissue damage.

With the suggestion that the suppression effect is almost nonexistent with a 72-h intertrial interval, one question becomes important: Will the effect dissipate completely at a longer time interval, or will there remain a slight residual suppression effect? For example, will a 14-day (or longer) intertrial interval bring about complete dissipation of the effect, as has been the case for the transient "aftereffect" or interhemispheric interference in the usual kindling procedure (McIntrye & Goddard, 1973), or will a slight effect remain? Such possibilities will be investigated in further research efforts.

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