The Effect of Low Frequency and Direct Current Stimulation on The Kindling Phenomenon in Rats

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SUMMARY: An experiment was conducted to evaluate the effect of 1-Hz or direct current brain stimulation on kindling behavior induced by 60-Hz sine wave stimulation. The effective threshold intensity to elicit a convulsion was determined on four separate occasions with 5 days of daily trials between determinations. On each day one group of experimental rats was stimulated with 1-Hz sine wave current before and after stimulation with 60-Hz sine wave current (1-60-1 group). Another group received direct current stimulation and 60-Hz current (D-60-D group). A third group received only 60-Hz stimulation. Suppression of kindling behavior usually induced by the 60-Hz stimulation occurred with 1-Hz stimulation; the mean threshold value increased on each successive determination. Suppression was pronounced for the direct current group; it appeared after a single trial and persisted for 32 days after the last threshold determination. In contrast, most of the rats in the 1-60-1 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 D-60-D group occurred after a single DC stimulation. These results are consistent with the hypothesis generated by previous research that suppression following 1-Hz stimulation is not due to tissue damage.

INTRODUCTION

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). In rats it involves a change from normal exploration (Stage 1) to behavioral automatons (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, viz., frog, reptile, mouse, rabbit, cat, monkey, and baboon (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 which might interfere with the production of convulsions by 60-Hz sine wave stimulation. In a series of experiments 3-Hz stimulation consistently produced an interference effect, i.e., suppression of convulsions (Gaito, 1979a,b; Gaito, et al., 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 second 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 severe effects. The greatest effect was with the 600 second stimulation period. The overall result was that of an increasing 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 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, Nobrega, and Gaito, 1980). Yet low frequency sine waves (1- and 3-Hz) are not too dissimilar from direct current, which does produce tissue damage. Thus the present experiment was designed to contrast the effect on the kindling phenomenon of both direct current and 1-Hz stimulation.

METHODS

Thirty male Wistar rats (approximately 150 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: .5mm posterior to bregma, 4.5mm from midline, 8.5mm from skull (Gaito, 1976b).

Stimulation was not imposed until at least 7 days after surgery. Then the 30 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 36 μA (root mean square, RMS). On the first trial of the second day the effective threshold intensity (ETI1) was determined. The 60-Hz current was increased until a Stage 2 or 3 response was elicited. Then 5 μA was added to allow for day-by-day threshold fluctuations. Two further trials of stimulation at this intensity were provided to check for consistency.

The 30 rats were separated into 10 sets of triplets based on the values of ETI1. Within each triplet the ETI1 value was approximately the same. Each of the rats in the triplets was randomly assigned to one of the three groups. 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 ETI1 value. A 60-Hz stimulation trial was provided on Trial 2 for 30 seconds at ETI1 (Group 1, 1-60-1). There was one hour between each trial. The second group of 10 rats was stimulated with direct current (DC) for 60 seconds at double the ETI1 value on Trials 1 and 3 and with 60-Hz sine wave current on Trial 2 for 30 seconds at ETI1 (Group 2: D-60-60-D). 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 3, X-60-X). All 60-Hz stimulation on Trial 2 was at ETI1 for 30 seconds, a duration which has been used routinely in our research. Stimulation on Trials 1 and 3 was for 60 seconds duration at two times ETI1; in previous experiments, this duration and intensity was found to produce a pronounced suppressive effect. The intensity of stimulation for any rat on these or later trials never exceeded 200 μA (RMS), an upper limit which has been used consistently in our laboratory.

Following this 5 day period, rats from all groups had ETI1 determined over six trials during two days. Then another 5 day block of stimulation occurred in which each group was treated in the same manner as during the 5 day block of trials prior to the ETI1 determination. This pattern of alternating ETI determinations and a 5 day block of trials was continued through the ETI4 determination. All rats were then rested for 32 days and ETI4 was determined on one trial.

After completing this portion of the experiment, a second phase was instituted to evaluate the daily changes in ETI values during one block of trials. The 10 rats from the 1-60-1 and X-60-X groups were provided with three trials on one day to determine the threshold (ETI1). Based on this determination, 9 rats from each of the two groups were assigned to new D-60-
The tissue at the electrode tips was viewed for the presence or absence of lesion characteristics, specifically, a hole at the site.

RESULTS

The gross histological analyses for the 10 DC rats suggested a lesion or probable lesion in 9; 6 had a score of 1 (definite lesion), 3 had a 2 (probable lesion), and 1 had a 3 (probable nonlesion). The analyses for the 1-60-1 and X-60-X group showed a score of 4 (nonlesion appearance) for all rats evaluated. Analyses in previous experiments had indicated similar results with 1-60-1 and X-60-X rats.

Neither the 1-60-1 nor the D-60-D groups showed the usual kindling progression prominent in X-60-X rats. No Stage 2 or 3 behaviour occurred in response to either 1-Hz or DC current stimulation.

The usual dependent variables, ETI and Composite Score, were used in the present experiment. With the latter measure, a score of 1 denotes Stage 1 behavior, 2, a Stage 2 response, and 3, a clonic convulsion. Any rat showing a Stage 1 response at the upper limit of 200 μA was assigned a score of 208 for that and later ETI determinations and was not used for the remaining block of trials. The results are shown in Table 1. 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. The 1-60-1 group had the gradual increments over ETI determinations, typical of rats stimulated with 1-Hz or 3-Hz sine waves in previous experiments.

The suppression produced during Block 1 stimulation by DC current was quick and very severe. The mean ETI value was beyond the upper limit of 200 μA for the D-60-D group. On Trial 1, 9 of the 10 rats showed Stage 1 behavior, a 90% suppression rate. Suppression was complete for all rats on Trial 2 and for the remaining 3 days. During the ETI, determination, 9 rats had Stage 1 behavior at the upper limit of 200 μA and were not used in Block 2 trials. Thus, the mean value was beyond the upper limit of 200 μA. The tenth rat had Stage 1 responses during the ETI, determination. These suppression results are much stronger than any encountered previously in our research.

A few rats in the 1-60-1 group showed Stage 1 behavior for ETI, and later determinations at 200 μA and were not used thereafter until the ETI, determination; there was one rat for the ETI, determination, two others for the ETI, determination, and a fourth rat during the ETI, event. The ETI, value for the one rat which showed Stage 1 behavior on the ETI, determination was 170 μA, which was very close to the upper limit.

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 are 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 group had the usual kindling progression over the three blocks of trials with most rats showing Stage 3 behavior during Block 3. The 1-60-1 group had Mean Composite scores which were at the boundary of Stage 1 and 2 and did not show a kindling progression. The D-60-D group had a mean score indicating early Stage 1 behavior.

Table 3 shows the Mean Composite Score for the five days of trials within each block. The minimum and maximum scores, respectively, for each trial and each rat is 1 and 3. The X-60-X group shows the usual gradual increase over trials until most rats are showing a convulsion on Day 5 of Block 3. The 1-60-1 group shows the opposite pattern (as in previous experiments). There is a gradual decline over trials. On Day 5 of each block the mean response is below that of Stage 2. A severe suppression effect is present for the D-60-D group which goes from a beginning Stage 2 response to a beginning Stage 1 response on Trial 1. Nine of ten rats had a Stage 1 response. By Trial 2, all rats showed Stage 1 behavior. Thus a single trial of DC current (Day 1) produced a drastic effect, whereas 1-Hz stimulation required a number of trials to bring forth a pronounced effect.

Not a single rat from the D-60-D group recovered from the effect of the DC current after 32 days of nonstimulation. On the ETI, determination at the upper limit of 200 μA, nine of the ten rats had a Stage 1 response while the tenth showed Stage 2 behavior. The mean of 207 μA for this group was well above the 100 μA for the ETI, determination.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>ETI Determinations</th>
<th>Recovery Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-60-X</td>
<td>108 86 78 68 65</td>
<td>80</td>
</tr>
<tr>
<td>1-60-1</td>
<td>111 149 171 182 114</td>
<td>70</td>
</tr>
<tr>
<td>D-60-D</td>
<td>100 205 208 208 207</td>
<td>00</td>
</tr>
</tbody>
</table>

Some rats showed a Stage 1 response at 200 μA; a score of 208 was assigned to each of these rats. These were, 1-60-1 group: 1 rat, ETI, - 4 rats, ETI, - D-60-D group: 9 rats, ETI, - 10 rats, ETI, - 9 rats, ETI, - All ETI values are in microamperes, root mean square (RMS).

Recovery rate refers to the percentage of rats which had non-Stage 1 behavior during the ETI, determination at approximately the previous low ETI determination value.

### Table 2

<table>
<thead>
<tr>
<th>Blocks of Trials</th>
<th>Mean Composite Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>X-60-X</td>
</tr>
<tr>
<td>1</td>
<td>10.2</td>
</tr>
<tr>
<td>2</td>
<td>12.7</td>
</tr>
<tr>
<td>3</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Table 3 shows the Mean Composite Score for the five days of trials within each block. The minimum and maximum scores, respectively, for each trial and each rat is 1 and 3. The X-60-X group shows the usual gradual increase over trials until most rats are showing a convulsion on Day 5 of Block 3. The 1-60-1 group shows the opposite pattern (as in previous experiments). There is a gradual decline over trials. On Day 5 of each block the mean response is below that of Stage 2. A severe suppression effect is present for the D-60-D group which goes from a beginning Stage 2 response to a beginning Stage 1 response on Trial 1. Nine of ten rats had a Stage 1 response. By Trial 2, all rats showed Stage 1 behavior. Thus a single trial of DC current (Day 1) produced a drastic effect, whereas 1-Hz stimulation required a number of trials to bring forth a pronounced effect.
TABLE 3
Mean Composite Score Within Blocks

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>Blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>X-60-X</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>I-60-1</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>D-60-D</td>
<td>2.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

0. last trial of the previous ETI determination. M, mean score per trial in each block of 5 trials.
* One rat had Stage 1 behavior at 200 μA and was not used during Block 2 and 3 trials; thus, n=9 during Block 2 trials.
** Three rats had Stage 1 behavior at 200 μA and were not used during Block 3 trials; thus, n=6 during Block 3 trials.
* Only one rat showed non-Stage 1 behavior and was used during Block 2 trials. This rat had a convolution at 180 μA during the ETI determination and on Trials 1 to 3. On Trials 4 and 5 a Stage 1 response occurred. This rat also showed Stage 1 behavior during the ETI determination and was not used for Block 3 trials.

By contrast, and consistent with previous results, most of the 1-60-1 rats recovered from the suppression effect after the 32 days. Seven of the rats (70%) were at about the ETI1 value. The mean for ETI2 was approximately the same as that for the ETI1 determination.

As in previous experiments the X-60-X group had a mean ETI1 value at or slightly below that of the previous low point, ETI4. Eight of these rats (80%) had a lower value on ETI1 than on any earlier ETI determination.

During Phase 2 the DC group had a sharp increment on Day 1 of Block 1 (one DC trial) and by Day 2 (after three DC trials) was close to the upper limit. The 1-60-1 group showed a gradual increase, and the X-60-X group, a slight decrease. The ETI values for the preblock day, the five days of Block 1, and the one postblock determinations were:

D-60-D: 70:151, 199, 200, 200, 203; 205
1-60-1: 74: 79, 94, 96, 101, 109; 115
X-60-X: 100; 96, 95, 91, 93, 88; 89

DISCUSSION

The results obtained in this experiment with the 1-60-1 and X-60-X groups were similar to those obtained in previous experiments (e.g., Gaito, 1980a). There was a gradual increase for the former in mean ETI values (but with a sharp decrement on ETI2), and a gradual decrease for the latter. In earlier experiments the intensity was specified as microamperes from peak to peak (a complete cycle), a value 2.8 times greater than the RMS measure involved in this experiment. When the differences between each ETI determination are multiplied by 2.8, the values obtained are similar to those present in previous experiments. For example, the 38μA (RMS) difference between ETI1 and ETI2 determinations for the 1-60-1 group is equal to 106μA (peak-to-peak). This is approximately the increment noted for the 1-60-1 groups in all experiments conducted so far. The small decrements for the X-60-X group in this experiment are as expected from previous results.

Likewise, the mean composite score over the three blocks of trials, and within each block of trials, were similar for these two groups to those from previous experiments. The X-60-X group had mean values over the three blocks of trials, and within each block, which showed that kindling progression was proceeding; by Block 3 most rats were convulsing. The 1-60-1 group had an opposite pattern: The mean remained below the Stage 2 level (10) in mean composite score for all blocks of trials. Within each block of trials, the mean value on Day 5 was always well below that on Day 1.

Thus, the suppression pattern for the 1-60-1 group in this experiment was similar to that observed in previous experiments. Furthermore, most of these rats had recovered from the suppression effect following the 32 day rest and were convulsing at their previous low threshold value.

The results for the D-60-D group were much different from those obtained with the 1-60-1 group in all dependent variables of concern. The ETI values increased greatly on ETI2 and remained approximately the same through ETI5. Phase 2 results indicated that the major portion of the sharp increase during the first block of trials occurred on the first day after a single DC stimulation. The mean went from 70 μA to 151 μA. Little increase resulted beyond the second day (after three DC stimulations); the mean ETI for Day 2 was 199 μA. Furthermore, this group never progressed beyond early Stage 1 behavior.

Other significant results were as follows:
1. On Day 1 of the first block of trials (Phase 1), only one rat from the DC group had a non-Stage 1 response. This result occurred after a single DC trial. In contrast, on this same trial, most of the rats in the 1-60-1 group (80%) were showing the same response as observed on the previous day during the ETI determination.
2. During the ETI₂ determination, 9 of the 10 rats in the DC group had a Stage 1 response and were not used in Block 2 trials. Only 1 of the 10 rats in the 1-60-1 group showed this effect. Furthermore, the ETI₁ value for this rat was 170 μA, very close to the upper limit of 200 μA.

3. Most of the rats in the 1-60-1 group (70%) recovered from the suppression effect after 32 days of rest, as indicated by the low ETI₅ value; the mean for this group decreased by 68 μA. No rats in the D-60-D group had recovered. The mean value for this group remained at approximately the same level from the ETI₂ to ETI₅ determinations.

These results indicate that the DC effect is a sudden one of strong nature occurring after a single DC trial, whereas the 1-Hz stimulation produces a slower, cumulative effect of transient nature. It appears that at the moderate intensities used in our experiment, DC stimulation produces lesions whereas 1-Hz stimulation does not. There were definite indications of lesions for the DC group in histological analyses and in behavioral responses. The histological analyses and behavior for the 1-Hz group suggested a transient, non-lesion effect. Still one might argue that small lesions were present in this group but were undetectable by the analyses used. In this context, the behavioral recovery would be explained as due to tissue recovery. However, the decrease for the ETI₂ determination value (relative to ETI₁) was very great for most rats, rendering this explanation quite implausible. Thus, in the final analysis, the data obtained so far in our laboratory tend to indicate a high probability that an inhibitory process is set in motion by the 1-Hz stimulation (Gaito, 1980d). In contrast to the relatively permanent process produced during the 60-Hz stimulation (Goddard et al., 1969; Racine, 1978), this one appears to be transient, dissipating slowly over time and similar to the “after effect” reported by McIntyre and Goddard (1973).

REFERENCES


