An evaluation of different methods of producing the 1-Hz suppression effect

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A number of exploratory experiments produced results that suggested a simplified method of producing suppression of kindled behavior by 1-Hz sine-wave stimulation. This method involved determining the effective threshold intensity to produce kindled behavior (ETI₁), followed 1 h later by stimulation with 1-Hz sine waves at the ETI₁ intensity for 5 min. Three hours later, a second 1-Hz trial occurred. Eighteen hours later, ETI₂ was determined. Four experiments with this method indicated clearly its effectiveness. However, when contrasted with the previous method used (a 1-60-1 sequence over a number of days with an intertrial interval of 1 h) both methods were equally effective.

The kindling effect has been investigated in a number of laboratories (e.g., Gaito, 1976; Goddard, McIntyre, & Leech, 1969; Wada & Sato, 1975). In rats, this effect involves a change from normal exploration (Stage 1) 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, 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, 1976; Racine, 1978).

In a series of experiments, it was found that 1- or 3-Hz sine-wave stimulation trials suppressed the tendency of the 60-Hz current to produce kindling behavior (Gaito, 1980a, 1980b, 1980c; 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- 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). When the interval was increased to 72 h, the effect was minimal (Gaito, 1982). With 5 or 14 days, the effect appears to be minimal also (Gaito, research in progress). Thus, there is a decay of the effect over time.

This procedure involves initially determining an effective threshold intensity (ETI₁) and then conducting a series of trials over days. For example, in many experiments, a trial sequence of 1-Hz, 60-Hz, and then 1-Hz

(with a 1-h interval) is used each day for 5 days. Then, a second threshold is determined. Two more blocks of 5-day trials occur, followed by ETI determinations.

This procedure requires 5 days within which suppression gradually develops. On the 1st day, most rats show the same behavioral response to 60-Hz stimulation as had occurred in the previous ETI trial. By the 3rd day, a number of rats show the suppression effect; most rats are affected by the 5th day.

This procedure is lengthy. It would be economical in time and effort if a procedure with a fewer number of trials could be developed. The variables of intensity, duration of 1-Hz stimulation, intertrial interval, and sequence of 1-Hz and 60-Hz trials are important in controlling the suppression of kindled behavior. Thus, a number of exploratory experiments involving these four variables were conducted to develop a simplified method of demonstrating the suppression effect.

EXPLORATORY EXPERIMENTS

Eight male Wistar rats (of approximately 400 days of age) that had been involved in a previous experiment were used in 10 experiments here. Surgical and stimulation conditions were as in many experiments in our laboratory (Gaito, 1980a).

A number of different procedures were used in an attempt to find an effective method to suppress convulsions, but one that was more simplified and could provide a quicker effect than the usual procedure used in our laboratory. There were four variables: (1) sequence (number of 1-Hz trials): 1, 1-1, 1-1-1, or 1-1-1-1; (2) intensity: 1 x ETI₁ or 2 x ETI₁; (3) duration: 2, 3, 4, or 5 min; (4) intertrial interval: 1 or 3 h.

In these experiments, the two methods that produced the most effective suppression were: 1-1-1-60, 3-min duration, 1 x ETI₁ intensity, 1-h intertrial interval; 1-1-60, 3-min duration, 2 x ETI₁ intensity, 3-h intertrial interval.

EXPERIMENTS 1-4

To achieve a method that would allow greater flexibility than either of the two most effective ones in the exploratory experiments, we combined the best parts of each method and increased

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slightly the duration of stimulation with Wistar males that were about 240 days of age. This flexibility was most apparent in the intensity aspect. For example, we used an upper limit of 200 microA (root mean square). Many rats reached this point and could not be used further in our experiments; the $1 \times \text{ETI}_1$ intensity allowed a greater number of trials before the upper limit was reached. To maximize the probability that suppression would occur, the duration was increased to 5 min. The interval between the two 1-Hz trials was set at 3 h; the interval between the second 1-Hz trial and the ETI_2 determination was 18 h (overnight); with the intensity and duration of stimulation used, these periods allowed more time for the brain events precipitated by 1-Hz stimulation to continue and possibly to increase the probability of behavioral-suppression tendencies. Thus, the sequence for these experiments was: ETI_1 ; 1-h rest; 1-Hz stimulation; 3-h rest; 1-Hz stimulation; 18-h rest; ETI_2 .

Four experiments were conducted with this method. In Experiments 1 and 2, most rats were at the BA stage (10 experimental and 6 control rats—Experiment 1; 9 experimental rats—Experiment 2). For Experiments 3 and 4, all rats had convulsed, most having had 12 to 15 convulsions. Other than this aspect, Experiment 3 was a replication of Experiments 1 and 2, with 11 experimental rats. Experiment 4 used 11 of the rats of Experiments 1-3. Seven were given the ETI_1 -1-1- ETI_2 treatment (experimental). Four control rats were placed in the stimulation box but received no current during the 1-Hz trial (ETI_1 -X-X- ETI_2). The rats were approximately 150 days of age at the beginning of the experiments.

EXPERIMENT 5

The 60-1-1-60 sequence proved to be an effective method. However, an important question was whether it would be more effective than the one used previously, the 1-60-1 sequence. Thus, Experiment 5 was conducted to contrast the effectiveness of these two procedures. Eighteen rats from another experiment were used so that there would be nine rats in each group, all having attained the clonic-convulsion stage. Two trials were used to provide the ETI_1 values. These values allowed the rats to be assigned to each group so that the mean ETIs were approximately equal. The 60-1-1-60 sequence was conducted within a 4-h period, with a 1-h intertrial interval, and thus was comparable to the routine 1-60-1 procedure used in our laboratory. The intensity was $1 \times \text{ETI}_1$ at a duration of 5 min. After 5 days of trials, ETI_2 was determined by two trials of 60-Hz stimulation. A second block of 5 days was followed by the ETI_2 determination on the 6th day.

RESULTS

In the exploratory experiments, the two most effective methods were: 1-1-1, 3-min duration, $1 \times \text{ETI}_1$ intensity, 1-h intertrial interval; 1-1, 3-min duration, $2 \times \text{ETI}_1$ intensity, 3-h intertrial interval. In these experiments there were four dependent variables of interest:

- (1) number of trials in which Stage 2 or Stage 3 behaviors occurred during or within a period of 1 h after 1-Hz stimulation;
- (2) type of behaviors during the 60-Hz trial (Stage 1, Stage 2, or Stage 3);
- (3) discrepancy between ETI_1 and ETI_2 values; and
- (4) $\text{ETI}_2/\text{ETI}_1$ ratio.

There were only two occurrences (with the same rat) in which Stage 2 or Stage 3 responses resulted in Experiments 1-4, with 1-Hz stimulation, thus reducing the importance of the first dependent variable. Also, the second dependent variable was dropped because it was considered unnecessary; it was included in the ETI_2 determination.

The results of the four experiments are indicated in Table 1 for the ETI_1 -1-1 (Day 1), ETI_2 (Day 2) sequence. In Experiment 1, which lasted only about 25 h over two days, the mean ETI value increased from the first to second determination, thus providing an $\text{ETI}_2/\text{ETI}_1$ ratio greater than 1.00 (1.285) and a discrepancy between ETI_1 and ETI_2 of +24.9 microA. The six control rats (with no 1-Hz stimulation) showed a mean decrease of 13.7 microA from ETI_1 to ETI_2 determination; the ratio was .846.

Nine of the 10 rats in the experimental group had an increased intensity for the ETI_2 point, a 90% suppression result (Experiment 1). The 10th rat had the same value for two determinations. However, all 6 rats for the control group had decreased intensities on the second determination, with a mean decrease of 13.7 microA. Thus, if the estimated drop on ETI_2 is considered, producing an expected ratio of .846, the value of 1.00 for the 10th rat would be indicative of a suppression response, giving a 100% suppression effect.

In Experiment 2 (a replication of Experiment 1), nine rats had an increased ETI_2 value: The mean increase was 19.6 microA; the $\text{ETI}_2/\text{ETI}_1$ ratio was 1.213. Thus, both experiments showed similar results and emphasized the presence of suppression of the kindling potential with the 25-h method. Over the two experiments, there were 18 examples of suppression in a possible 19 cases. The mean $\text{ETI}_2/\text{ETI}_1$ ratio and the ETI discrepancy were 1.249 and +22.1, respectively.

The results in Experiment 3 (with rats at the CC stage) were similar to those of Experiments 1 and 2 (with rats at the BA stage). Ten of 11 rats had a greater value for ETI_2 than for ETI_1 ; the other rat showed no change. The ETI_2 mean value was 22.7 microA greater than the mean for ETI_1 ; the $\text{ETI}_2/\text{ETI}_1$ ratio was 1.230.

In Experiment 4, the experimental rats showed an

Table 1
Mean Values for ETI Determinations During Experiments 1-4

Experiment	Experimental Group				Control Group*			
	ETI_1	N	ETI_2	$\text{ETI}_2/\text{ETI}_1$	ETI_1	N	ETI_2	$\text{ETI}_2/\text{ETI}_1$
1	85.5	10	109.9	1.285	88.7	6	75.0	.846
2	92.2	9	111.8	1.213				
3	98.5	11	121.2	1.230				
4	82.6	7	105.0	1.271	81.3	4	77.5	.953
Combined	90.5	37	112.8	1.246	85.7	10	76.0	.887

*Control rats in Experiment 1 were later used as experimental rats.

Table 2
Results of Experiment 5

Group	ETI ₁	ETI ₁ /ETI ₁	ETI ₂	ETI ₂ /ETI ₁	ETI ₃	ETI ₃ /ETI ₁
1-60-1	47.7	1.00	79.2	1.66	103.0	2.16
60-1-1-60	47.3	1.00	83.6	1.77	130.4	2.76

increased mean ETI₂ value of 22.4 microA. Six of the seven rats had an increment. The seventh rat had a decrease of 5 microA. The four control rats had a slight mean decrement in the ETI₂ value (−3.8 microA).

Over the four experiments, there were 34 examples of suppression in 37 cases; that is, ETI₂ was greater than ETI₁. In 2 cases, there was no change, and in 1 case, a decrement of 5 microA occurred. The overall mean change was a decrease of 22.3 microA, and the ETI₂/ETI₁ ratio was 1.246. These results show clearly the effectiveness of the simplified method over the short time period (25 h).

The results of Experiment 5 are shown in Table 2. The ETI₁ values over the three determinations were approximately equal. The two procedures were the same also in terms of the number of rats that were suppressed after ETI₃; all nine rats in each group had a ETI₃ value greater than that of ETI₁.

DISCUSSION

For a method to be considered effective, it should allow for the following conditions to hold: (1) no, or at least very infrequent, BA or CC during 1-Hz trials; (2) most rats showing Stage 1 responses on 60-Hz test trials at the ETI value (the suppression effect); (3) increased ETI₂ for most rats, with a value greater than 1.00 for the ETI₂/ETI₁ ratio.

All procedures, except the ones in Experiments 1-5 using a sequence of ETI₁-1-1 (Day 1)-ETI₂ (Day 2) at 1 × ETI₁ intensity and a duration of 5 min and the routine 1-60-1 sequence in Experiment 5, failed to meet these requirements. Some procedures failed all three. Most met one or two of the requirements.

The method developed during these experiments that had a sequence of 60-1-1-60 with a 1 × ETI intensity and 5-min durations of stimulation produced results similar to those produced by the previously used method, when 5-day blocks of trials were incorporated (Experiment 5). With 1-Hz stimulation trials, the ETI value tended to remain the same or to increase. The latter was the more common result. Without 1-Hz stimulation interspersed among the 60-Hz ETI trials (as with control rats), the ETI value tended to stay the same or to decrease slightly. The latter is the usual event.

Although the 60-1-1-60 procedure clearly was superior to many of the other procedures, it was not more effective than the one used routinely in our laboratory (1-60-1), when con-

trasted over 5-day blocks of trials. Both of these procedures were very effective in eliciting suppression of kindled behavior. In one respect, the 1-60-1 procedure was simpler to handle because no ETI value was determined until after 5 days (or as many days as desired) had passed. This lack of an ETI on each day can be a disadvantage for the 1-60-1 method, however, and an advantage for the 60-1-1-60 procedure. Furthermore, the 60-1-1-60 sequence can be completed in a shorter period of time, even as short as about 4 h if a 1-h intertrial interval is used. Each of the two procedures can be useful for different purposes of the investigator.

Another result of this study is a partial validation of the 1-60-1 procedure. It had been used previously without comparing it with other possible methods, because it was effective in producing suppression of the kindled response. Thus, these results should provide greater confidence in the previous results.

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