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Statistical evaluation of several aspects concerning the oscillation effect

JOHN GAITO, JOSÉ N. NOBREGA, and STEPHEN T. GAITO
York University, 4700 Keele Street, Downsview, M3J 1P3, Ontario, Canada

Previous experiments using a sequence of alternating unilateral stimulations of the amygdalae indicated an "oscillation effect," i.e., consistent low-latency values for convulsions elicited from one amygdala and consistent high-latency values for convulsions elicited by stimulation of the contralateral amygdala. The present study was concerned mainly with statistical evaluations of the reliability of oscillation events. Tests of the randomness of the observed primary and secondary oscillation patterns indicated that oscillation patterns were significant systematic ones in latency, criterion, and duration data, with the greatest frequency of oscillation occurring in the latency measure. Although there was no significant difference in the frequency of primary or secondary oscillation using chi-square methods, an analysis of variance trend analysis indicated that the primary oscillation pattern (low values on primary side) was the predominant one when considered over the total sample, 139 rats. Also, it was shown that the behavioral pattern (oscillation, nonoscillation) appears not to be related to the number of trials to reach the criterion of six convulsions. The exact basis for oscillatory behavior is unknown. However, for a number of reasons, it appears to be based probably on transfer and interference effects between the primary and secondary brain sites.

Repeated low-intensity electrical stimulation of any of a number of brain regions induces progressive changes which culminate in clonic convulsions; as stimulation trials proceed over days, the animal's behavior changes in a gradual and predictable way from an initial stage of no response to stimulation, except for exploratory behavior (Stage 1), to behavioral automatisms usually involving facial contractions, chewing movements, eye closure, and salivation (Stage 2), and finally to full-blown motor seizures (Stage 3). This developing sequence of events has been called the "kindling effect" (Goddard, McIntyre, & Leech, 1969).

Both "transfer" and "interference" effects are obtained when two homologous or nonhomologous brain sites are successively kindled. After kindled seizures have been obtained at one brain site (primary site), fewer stimulation trials are usually necessary to achieve the clonic convulsion (CC) stage on the contralateral site (secondary site), indicating the occurrence of facilitation or positive transfer from primary to secondary site; however, latencies for the secondary site are reported to be higher than those for the primary site (McIntyre & Goddard, 1973), indicating negative transfer. If stimulation is then reapplied at the primary site, a temporary suppression of convulsions is observed, and it takes a few trials (although less than originally) to evoke CCs from the primary site. This event would indicate the occurrence of interference or negative transfer from secondary to primary site (McIntyre & Goddard, 1973).

We became interested in the time course of the transfer/interference phenomena, i.e., in the question of what would happen if stimulation trials were extended to more than the primary-secondary-primary cycle of McIntyre and Goddard. Our initial expectation was that after a few alternations of stimulation between primary and secondary sites the positive and negative transfer effects would extinguish and convulsions obtained from two homologous sites would be indistinguishable, both in terms of number of trials necessary to elicit a given number of convulsions and in latencies to convulsion. The basic paradigm in our experiments has consisted of inducing a given number of clonic convulsions (usually six) from stimulation of one brain site, then stimulating the contralateral site until the same criterion is achieved; stimulation is then reapplied at the primary site, then at the secondary, and so on, in a schedule of sequential alternation of unilateral stimulation until 10 phases (each with six CCs) are completed. Using this paradigm, we have found that, while the number of trials to criterion usually does become stable after a few phases of alternations, latencies between adjacent phases do not stabilize at all. Instead, an oscillation pattern becomes established, such that stimulation of one site results in consistently low latencies and stimulation of the contralateral site results in consistently high latencies. We have referred to this result as the "oscillation effect" (Gaito, 1976a). This term is descriptive of the figure obtained in a line graph when mean latency is plotted over 10 phases of alternation between the primary and secondary sites; a saw-tooth,

oscillation-type curve occurs with many individual rats or for groups of rats (see Figure 2).

There are two types of oscillation which occur consistently in our research. Primary oscillation involves low values for the primary site and high values for the secondary site for at least 8 of 10 phases. Secondary oscillation is the opposite pattern. Over many experiments, primary oscillation has occurred most frequently.

As is the case with kindling itself, oscillation appears to be an extremely reliable phenomenon, occurring in latency data in approximately 75% of all rats tested so far. The effect occurs less frequently in criterion data (number of trials to six CC) and in the duration-of-convulsion measure.

The oscillation tendency in latency data has been remarkably resistant to a number of experimental manipulations. A summary of these results with integrating statistical analyses were reported by Gaito, Gaito, and Nobrega (1977).

In this paper, we are concerned with the authenticity of the oscillation effect; we evaluate statistically the possibilities that (1) oscillation patterns are actually random ones, and (2) primary oscillation patterns are the predominant pattern. A further objective is to evaluate a hypothesis concerning number of trials and behavioral responses.

RELIABILITY OF OSCILLATION EVENTS

Are the Primary and Secondary Oscillation Patterns Random Ones?

Table 1 indicates results with 139 rats which had been stimulated over 10 phases. The primary oscillation pattern (PO) is one in which low values occur for the primary site (first site stimulated) and high values for the secondary site (second site stimulated) in 8 or 10 of the 10 phases. Secondary oscillation (SO) involves the reverse pattern.

One might assume that these results are due to chance. The possibility that the oscillatory patterns are random ones can be assessed for each rat by the one sample runs test (Hoel, 1954). The requirement for oscillation has been the occurrence of a "low-high" or a "high-low" pattern over all 10 phases, or over 8 of the 10. The probability (p) that an oscillation pattern on all 10 phases would occur by chance is .0079 (10 runs). Such a pattern on 8 of 10 phases would involve 8 or 9 runs; the p for 8 runs is .1270; the p for 9 runs is .0317.[1] Then the p for 8, 9, or 10 runs is .0079 + .0317 + .1270 = .1666, or .17. The p that any rat will show one of the oscillatory patterns by chance is .17. Thus, of 139 rats (Table 1), one would expect 23.6 to show an oscillation pattern. The divergence of the observed values in Table 1 from the expected value of 23.6 can be assessed by the normal approximation to the binomial distribution[2] or by chi square (because $z^2 = \chi^2$). When the first procedure is used ($\mu = np = 139 \times .17 = 23.6$ and $\sigma = \sqrt{npq} = \sqrt{139 \times .17 \times .83} = 4.4$), the z values for latency, criterion, and duration data are 17.93, 6.11, and 8.61, respectively. Using a p of .01 for rejection of the null hypothesis requires a z value of 2.58. Thus, the null hypotheses that the observed patterns in each dependent variable are chance ones must be rejected for each of the three dependent variables; the p values for each of the three results are well below a p of .01 (z tables in most statistical textbooks do not go beyond a value of 3.00 or 4.00; the p associated with 4.00 is .00006—a two-tailed value).

Table 1
Frequency of Different Types of Behavior With Each Measure

	PO	SO	NO	Total
Latency	59	44	36	139
Criterion	28	23	88	139
Duration	37	25	77	139
Total	124	92	201	417

Note–PO = low values in primary site and high values in secondary site for at least 8 of 10 phases. SO = low values in secondary site and high values in primary site for at least 8 of 10 phases. NO = lack of systematic pattern.

These results clearly indicate that the primary and secondary oscillation patterns are not due to chance. Because oscillation patterns occur much more frequently in latency data, this measure would appear to be the most sensitive index of oscillation.

Is Primary Oscillation the Predominant Pattern?

In the first study, the side first stimulated tended to show the lower values (PO). However, in some later experiments, the secondary site had lower values (SO). Over all experiments, however, PO was most frequent. Table 1 indicates this aspect for the 139 rats that were stimulated over 10 phases.

The most obvious explanation for the differences in frequency of PO and SO patterns within each measure is that of chance. This aspect can be evaluated by the normal approximation to the binomial distribution, with p (probability of primary oscillation) = q (probability of secondary oscillation) = .5.

In latency data, 59 of the 103 rats oscillating showed a PO pattern. Thus, $\mu = np = 103 \times .5 = 51.5$, $\sigma = \sqrt{npq} = \sqrt{103 \times .5 \times .5} = 5.07$, and z = 1.38 (p = .17).

For criterion data, there were 28 POs in the 51 oscillating rats. Thus, $\mu = 51 \times .5 = 25.5$, $\sigma = \sqrt{51 \times .5 \times .5} = 3.57$, and z = .56 (p = .58).

Duration data produced 37 POs in 62 oscillating rats. Thus, $\mu = 62 \times .50 = 31$, $\sigma = \sqrt{62 \times .5 \times .5} = 3.94$, and p = 1.40 (p = .16).

The conclusion from these analyses is that neither PO nor SO patterns predominate. However, it should

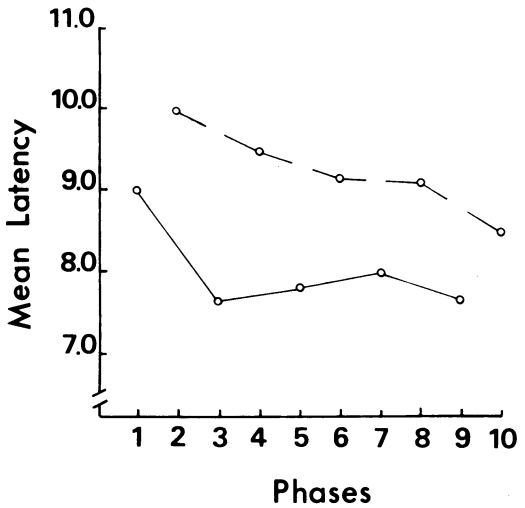

Figure 1. The two significantly different sets of phases in latency data. Lower curve, primary-site stimulation phases; upper curve, secondary-site stimulation phases.

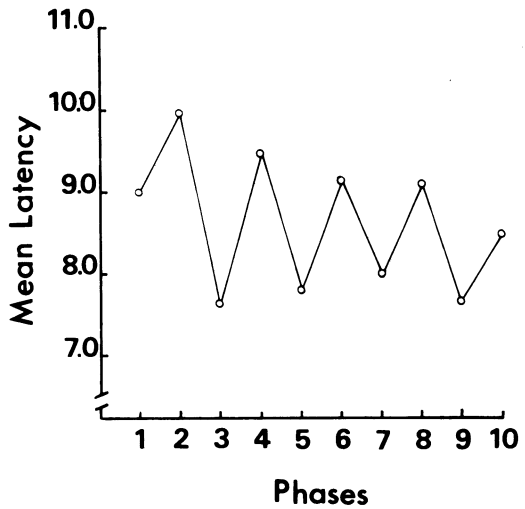

Figure 2. The trend in latency over the 10 phases showing a pronounced primary oscillation pattern.

be noted that POs predominate in actual numbers in *each* of the three dependent variables.

Frequency data do not consider the magnitude of differences between primary and secondary site values. Thus, a procedure such as analysis of variance which considers magnitude aspects would be more sensitive to any differences which might exist. Two separate sets of these analyses were used, and they indicated that the PO pattern was the predominant one.

(1) If the PO pattern were the predominant one, the mean values for the primary site stimulation phases (PS) should be lower than those for the secondary site stimulation phases (SS). Figure 1 shows this aspect clearly for the 139 subjects in latency data, with the curve for the former being below that for the latter over all phases. Because of the large size of the sample involved and the tendency for Type I errors to increase in a repeated measurements design with the usual analysis of variance F tests, in that the assumption of independence of errors is usually violated (Gaito, 1973), the α level for rejection of null hypotheses was set at a more stringent level, .01, than the usual .05 probability level. Significant differences between PS and SS were present ($F = 31.59$, $df = 1,1242$, $p < .01$). Significant differences between PS and SS did not occur in the criterion and duration measures. In the former, most of the variation between phases was concentrated in differences between the first two phases and later ones (see Figure 3). There was a sharp decrease in the mean number of trials to six CC after Phase 2. These results suggest clearly the tendency for the PO pattern to be the dominant one when all subjects are used in the analysis, but only in latency data.

(2) This conclusion was supported by a Subjects by Phases trend analysis over the 10 phases for latency, criterion, and duration data separately (Figures 2, 3, 4). If a PO pattern were a dominant

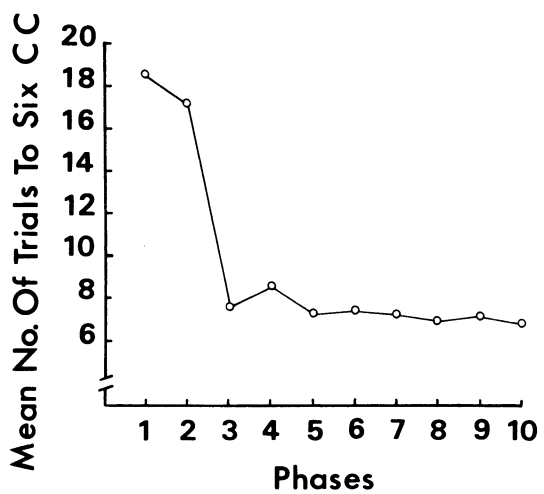

Figure 3. The trend in criterion data for the 10 phases.

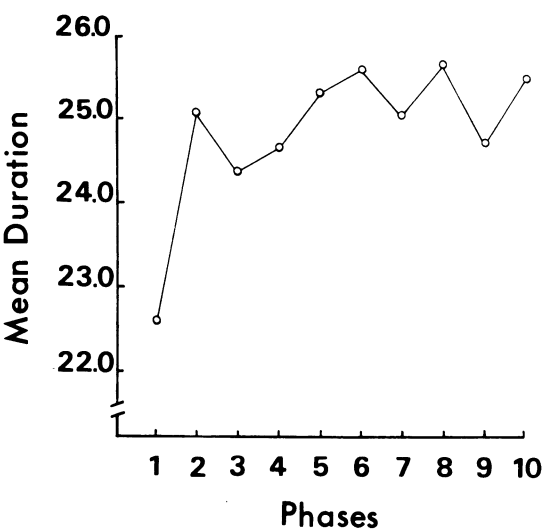

Figure 4. The trend for duration values for 10 phases.

tendency, the ninth regression component of the phases dimension would be a significant one. Only in Figure 2, for the latency data over the 10 phases, does this appear to be the case. When the SS associated with each of the nine components (linear, quadratic, etc.)[3] were determined and F tests performed relative to each of these components in the three dependent variables, the ninth component was significant only in latency data ($F = 24.27$, $df = 1,1242$, $p < .01$.[4]

NUMBER OF TRIALS RELATED TO BEHAVIORAL PATTERNS

In the conduct of these experiments, it was noted that a fast rate of kindling in Phase 1 (e.g., meeting the criterion of six CC by 10 trials) might preclude the occurrence of oscillation. This observation seemed to imply that a moderate number of trials were required for the process underlying the tendency to develop in both the primary and secondary sites. To evaluate this hypothesis, the data on 139 subjects from previous experiments were used. Each of these subjects was placed in a two-dimensional diagram based on the following characteristics: rate of kindling (10 and fewer trials to six CC, greater than 10 trials to six CC), and behavioral designation (nonoscillation, NO; oscillation, O). The number of rats in each category were: 10 and <, NO = 10; >10, NO = 26; 10 and <, O = 35; >10, O = 68. The chi-square and phi coefficient values concerned with independence between these two dimensions were .494 and −.058, respectively, indicating no significant relationship ($p > .05$). Thus, this hypothesis appeared to be a false one, i.e., the number of trials to six CC on Phase 1 did not affect the rat's inclination to oscillate or not to oscillate. Even a small number of trials to criterion (10 or less) appears to be sufficient for the development of the oscillation process.

DISCUSSION

These statistical evaluations indicate that the number of trials to six CC on Phase 1 (less than 10, greater than 10) did not influence the tendency of the rats to oscillate or not to oscillate, that oscillation patterns are nonrandom events, and that PO is the predominant oscillation pattern in latency data over all rats. Apparently there are a number of rats which show the PO tendency but do so on fewer than the 8 or 10 of the 10 phases, i.e., their tendency is less pronounced and does not meet our criterion for oscillation.

That the oscillation effect is an authentic phenomenon is suggested also by the data of other researchers. McIntyre (1975) and McIntyre and Goddard (1973) reported latency values of primary oscillation nature for groups of rats over three phases of sequential alternation, i.e., a "low-high-low" pattern. Individual data were not shown. Presumably, some secondary oscillators were present, but primary oscillation predominated overall, as in our research.

There are a number of possible bases for the oscillation effect. These are:

(1) *Chance.* The hypothesis that the observed oscillation patterns are due to chance aspects must be discarded, as indicated above. These statistical results are substantiated by empirical results. The same brain site was stimulated over 10 phases in three separate experiments. In each experiment, the number of oscillators observed was not significantly different from the number expected based on a p of .17. Over the three experiments, there were seven oscillators with 35 rats, a number not significantly different from the six expected (Gaito & Nobrega, 1978).

(2) *Differential thresholds for the two sides.* The previous experiments were conducted with the same intensity of stimulation for both amygdalae. It is possible that slight differences in the placement of electrodes in the amygdalae could result in differential intensities required to elicit the clonic convulsions. Thus, the same intensities in both amygdalae might result in the CC stage being reached for one amygdala in a fewer number of trials than the other one, and this aspect might be the basis for the oscillation effect in the latency measure. However, in a number of experiments in which intensity of stimulation was approximately 15 μA above threshold for each side, the oscillation results were the same as for the experiments in which the stimulation was the same for both sides, clearly eliminating this possibility as the basis for oscillation (Gaito, 1977b).

(3) *Differential placement of the two electrodes.* Differential placement of electrodes appears to be a reasonable exploration for oscillatory behavior. However, there are a number of reasons why it does not seem to be a major contributor; these have been cited previously (Gaito, 1976a). Furthermore, in the first study, and in several others since then, histological analyses were conducted to determine electrode placements. These analyses indicated no relationship between placement of electrodes and behavioral pattern, as long as one or both electrodes were in the amygdala or in nearby tissue (Gaito, 1976a, 1977c; Nobrega & Gaito, 1978).

(4) *Differential effectiveness of the two electrodes.* It is possible that one electrode may be electrically more effective in one brain site than is the one in the contralateral site. However, this possibility could not be responsible for the oscillation effect, because the differential threshold method (Section 2, above) (Gaito, 1977b) should bring both to the same degree of effectiveness in the respective brain sites.

(5) *Differential natural reactivity of the two brain*

sites. It is possible that one of the two brain sites stimulated naturally has a lower reactivity than does the contralateral one, thus resulting in consistently lower latency values. This possibility seems to be excluded by specific behaviors observed in previous research (Gaito, 1976b, 1976c, 1977a, 1977b; Gaito & Nobrega, 1977) which suggest the operation of an active inhibitory process. Although a rat may rear upon its hind paws immediately with stimulation of either side, the convulsion tends to occur quickly for one side but appears to be actively inhibited with stimulation of the other side. On this latter side, the rat's forepaws may begin to move slightly (incomplete CC) but then will stop for 5 to 10 sec before a complete CC occurs. These incomplete CCs tended to be more frequent on the "high"-latency side.

This suggestion of an inhibitory process is consistent with the results obtained in previous research concerned with *interanimal* transfer effects (Gaito, 1976d; Gaito & Gaito, 1974). When naive recipient rats were injected with brain material from kindled rats, the development of kindling was retarded significantly.

Although the suggestion of an active inhibitory process during stimulation of one site appears to exclude differential natural reactivity as a basis for the oscillation effect, this aspect may be a contributor to the process. Its contributions should be evaluated in future research, possibly by electrophysiological methods.

(6) *Interaction between the two brain sites*. Transfer and interference effects between primary and secondary sites are presumed to be responsible for the results by McIntyre (1975) and McIntyre and Goddard (1973) over three phases of unilateral stimulation. Thus, we assume that some type of interaction between the two brain sites is occurring over the 10 or more phases involved in our sequential alternation research.

Presumably there are some inhibitory and/or facilitatory effects from the primary site to the secondary site, and vice versa, to produce the oscillation effect. However, it is not clear what the exact basis for the effect is. A pattern appears to be set up, either primary oscillation or secondary oscillation, during Phases 1 and 2, or by Phases 3 and 4, and most rats continue with this pattern for the remainder of the 10 phases. In one experiment in which rats were stimulated through 50 phases, some rats showed a consistent pattern of oscillation for the 50 phases (Gaito, 1978).

Irrespective of the basis for the oscillation effect, the unilateral sequential alternation procedure and the resulting oscillation effect appear to be useful for obtaining information on some aspects of the events underlying the kindling effect, e.g., possible differential electrical synaptic patterns related to the difference in latency values for the primary and secondary sites. Because kindling shows many parallels to learning and to epilepsy, and can be considered as a model of consistent behavioral changes in response to an invariant stimulus, experimentation with the oscillation effect may have the potential for providing information relevant to brain function in general.

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NOTES

1. If we let L = low and H = high for the latency values on each phase within each set of primary and secondary site stimulations, then the number of runs for a sequence of 10 phases involv-

ing 5 lows and 5 highs would be the number of letters that are different from the adjacent one. If oscillation occurred over the entire 10 phases, the sequence would be LHLHLHLHLH, or the opposite pattern, to give 10 runs. If oscillation resulted over 8 of the 10 phases, the number of runs would be 9 if the two phases out of harmony with the other 8 were at either the beginning or the end of the sequence (e.g., HLLHLHLHLH). If the two phases were somewhere else in the sequence, the number of runs would be 8 (e.g., HLHLLHHLHL). Thus, with our criterion of a consistent pattern of LH or HL over 8 or 10 of the 10 phases, the number of runs would be 8, 9, or 10.

2. Even though the p of oscillation is .17, and thus quite removed from a p of .50, the normal distribution provides a good approximation to the binomial distribution when five or more standard deviations can be obtained around the mean (as in the present situation) (Hoel, 1954).

3. Statistical books do not include orthogonal polynomial coefficients for regression components beyond the fifth one for data of this nature. Thus, these coefficients for the sixth to ninth components were derived using the equations of Lewis (1960, p. 405) in an IBM 370 computer at York University.

4. This component accounted for the major portion of the SS for Between Phases, 45.7%. The first component (Linear) was also significant in latency data.

In criterion data, the linear and quadratic components were significant ones which accounted for 83.3% of the SS for Between Phases.

The duration data produced significant linear and quadratic components, which accounted for 62.7% of the SS for Between Phases.

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