

## SENSITIVITY OF NEURONES IN *APLYSIA* TO TEMPORAL PATTERN OF ARRIVING IMPULSES\*

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A complete description of the sequence of spikes in a neuron discharge consists of an ordered series of intervals  $t_1, t_2, \dots, t_n$ , extending over the entire observation time. Neurophysiologists commonly discuss ongoing activity in terms of mean frequency of spikes thus ignoring variability and sequence of intervals. Histograms of intervals between two consecutive spikes or histograms of time between three, four,  $\dots$ , or  $n$  consecutive spikes (Gerstein & Kiang, 1960; Rodieck, Kiang & Gerstein, 1962), while giving information about distribution, also ignore the precise succession of events. Two-dimensional histograms showing the relative occurrence of all ordered pairs of intervals between three successive spikes or, more generally, a display of the frequency of all ordered sequences of  $n$  intervals between  $n+1$  successive spikes can reveal a tendency for patterns of spikes.

Each method must be evaluated according to two criteria: first, the accuracy and extent with which it conveys information about the discharge; secondly, the degree in which it summarizes features that are functionally significant. From the latter viewpoint, the analysis of mean spike frequency has contributed much to our understanding of neuronal systems. It is necessary to pose the additional query of whether more sophisticated statistical descriptions are of value also. The present study was designed to investigate whether physiologically significant differences in output occur in neurons submitted to an input that has constant mean frequency but whose higher order statistics, namely interval histogram and pattern, are systematically varied. The only previous study of this question appears to be that of Wiersma and collaborators in neuromuscular preparations of crustacea, where changes of timing of the nerve stimulation led to remarkable changes in contractions (Ripley & Wiersma, 1953; Wiersma & Adams, 1950).

### MATERIAL AND METHODS

Experiments were performed on isolated visceral ganglia of *Aplysia californica* in natural sea water at 12-15° C. in one group of experiments or 18-22° C. in another. Electrical stimuli (0.1-5.0 msec.) were applied to the left visceropleural connective (*Lvp*), anal (*A*) and/or genital (*G*) nerves (Fig. 1c; see also Eales, 1921); for optimal preservation nerves were kept under water. The electrical activity

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of neurones in the visceral ganglion was recorded intracellularly with 3 M-KCl-filled glass pipettes.

Most observations were carried out in the largest pigmented cell, usually located close to the right side of the origin of the right visceropleural connective and occasionally displaced toward the origin of the branchial nerve; this neuron, usually referred to as 'giant nerve cell', was normally silent in our isolated ganglia and responded with excitatory post-synaptic potentials (EPSP) to stimulation of the left visceropleural connective or of the anal and genital nerves (Hughes & Tauc, 1961). Shocks were applied to left visceropleural, anal and/or genital trunks and the terms 'homologous' and 'heterologous' will indicate whether all shocks of one burst were applied to the same or to different trunks, respectively. Related observations, mainly concerned with inhibitory input to a spontaneously active neurone, have been reported (Moore, Perkel & Segundo, 1963; Perkel, Moore & Segundo, 1963; Schulman, 1963).

Two ranges of voltage of pre-synaptic stimulation were used separately; (a) values referred to as 'sub-threshold' produced an EPSP but no spike, when applied singly or in pairs; (b) values referred to as 'supra-threshold' were barely capable of consistently evoking one spike when applied singly.

The principal mode of stimulation was in a series of three shocks called  $S_1$ ,  $S_2$  and  $S_3$  or 'Trios' (Fig. 1*a*). When the interval  $S_1$ - $S_3$  was fixed and  $S_2$  was shifted, the mean frequency could be said to be constant and the most elementary test of sensitivity to interval distribution and sequence, i.e. to 'pattern' or 'timing' was provided. As an extension of this mode, 'prolonged bursts' of shocks lasting many seconds or minutes were used and different patterns at the same mean frequency were compared. Finally, bursts of two shocks or 'pairs' were also used.

The output of each stimulation was evaluated as follows. For the shorter bursts (of 2, 3 and up to 10 pulses) by applying two criteria, amplitude of synaptic potential and spike probability. (1) The amplitude of each synaptic potential was observed both as increment of depolarization due to each shock alone (partial value  $P$ ) and as accumulated depolarization due to all shocks up to the moment (total value  $T$ ) (Fig. 1*b*).  $P$  values constitute a measure of the responsiveness of the system at that instant.  $T$  values measure the overall effectiveness of the burst up to that instant, and obviously relate to the probability of it evoking a spike.  $P$  and  $T$  heights were measured and, for each shock and pattern, means, variances and standard deviations were computed; comparisons were made with a  $t$  test for significance of difference of means and estimates of the confidence limits for the differences were obtained using the  $t$  test. In order to obtain an estimate of each individual excitatory post-synaptic potential both in isolation and forming part of pairs or trios,  $T$  and  $P$  measurements were taken on the film every 10 msec.: subsequently, the  $T$  graph corresponding to the observed pair or trio was compared with the graph derived from linear summation with adequate phase shifts of two or three curves from isolated EPSP; in addition, the  $P$  graph corresponding to each shock in the trio was compared with that from the same shock in isolation. (2) The probability of evoking a spike was estimated for each burst by the ratio of 'applications followed by a spike' to 'total number of applications' or, for each component shock when supra-threshold shocks were used, by the ratio of the 'number of times it produced a spike' to the 'number of times it was presented'.

When prolonged bursts were used, the output was evaluated by (1) the mean number of spikes produced by a single input shock ('efficiency coefficient') and (2) the interspike interval histogram.

In all observations involving trios efficacy comparisons were performed between: (i) differently patterned trios (e.g.  $S_1-S_2-S_3$ ,  $S_1'-S_2'-S_3'$  . . .); (ii) trios and component pairs in isolation (e.g.  $S_1-S_2-S_3$ ,  $S_1-S_2$ ,  $S_2-S_3$ , . . .); (iii) trios and component single shocks in isolation (e.g.  $S_1-S_2-S_3$ ,  $S_1$ ,  $S_2$ ,  $S_3$ , . . .). Comparison (i) informed as to

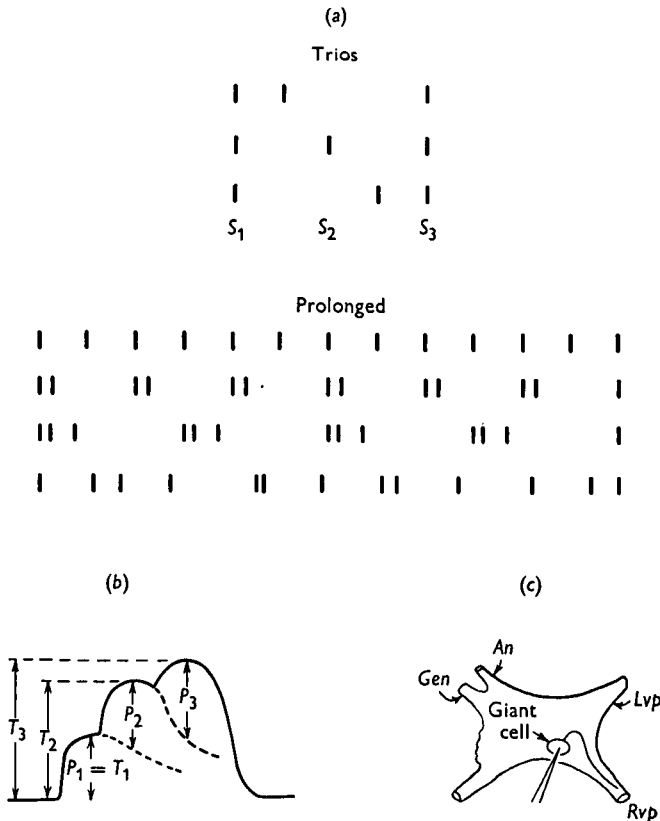


Fig. 1. Technique (see also text). (a) Stimuli for the study of timing: (i) trios. Given an  $S_1-S_3$  interval, i.e. a mean frequency, different  $S_1-S_2$  intervals, i.e. different timings, were tested; (ii) prolonged bursts. Stimuli having the same mean frequencies but different timings were compared. (b) Measurements (for sub-threshold stimuli). (c) Visceral ganglion (schematic). *An*, Anal nerve; *Gen*, genital nerve; *Lvp*, left visceropleural connective; *Rvp*, right visceropleural connective.

the main point, the relative importances of frequency and timing; comparison (ii) indicated whether dissimilarities were veritable trio effects or depended exclusively on differences between component pairs; comparison (iii) controlled differences between isolated and programmed stimuli and indicated the excitability of the system at various instants. Having estimated the efficacy of each given burst and of each component part as described above, results were plotted in the following fashion.

*Pairs.* The effectiveness of the second shock was expressed as a function of the

interval between  $S_1$  and  $S_2$ , i.e. of total duration or mean frequency; this conforms to a current 'conditioning shock-test shock' procedure for evaluating excitability.

*Trios.* The effectiveness of the third shock, measured in the same parameters, was plotted in two separate ways. (1) Given a  $S_1$ - $S_2$  interval, as a function of the interval between  $S_1$  and  $S_3$ , i.e. of total duration or mean frequency. This also conforms to a current 'conditioning-test' routine for excitability, only that the conditioning stimulation now consists of a pair of shocks ( $S_1$ - $S_2$ ) at a fixed interval; different separations provide a family of curves. (2) Given a  $S_1$ - $S_3$  interval (i.e. total duration or mean frequency), as a function of the interval between  $S_1$  and  $S_2$ , i.e. of timing.

Complementary precautions were taken in experiments with trios or pairs. (i) In order to avoid each test exerting a variable influence upon the following ones, and based upon excitability studies described below (see Results), test combinations were applied at regular intervals of 10 sec. when using 'sub-threshold' intensities or of 30-60 sec. when using 'supra-threshold' intensities; the sequence was determined 'randomly', except for the limitation of using different combinations a comparable number of times. (ii) In order to be sure that changes in excitability of the stimulated nerve were not an issue, and based on observations of Goldman showing that recovery of *Aplysia* nerve trunks is complete after 30-50 msec., separation between component shocks of each test combination was not smaller than 100 msec. (Goldman, 1963).

So as to anticipate the unavoidable limitations of some of our conclusions, we must state the characteristics of a hypothetical preparation, ideally suited for this project: anatomically, one or more separate pre-synaptic fibres converging directly upon a single post-synaptic cell in such a manner that it would be possible to stimulate and to record from each one individually; functionally, stability over periods long enough to obtain significant data. The preparation used here departs from this ideal in two respects: first, nerve trunks consist in more than one fibre and, consequently, each shock initiates a volley of spikes; secondly, connexions between excited fibres and monitored cells are not necessarily mono-synaptic or unique. In spite of these qualifications, the preparation is of interest and among the most suitable in the battery available to neurophysiologists. Since shocks were never approximated closer than 100 msec. and nerve recovery is complete at 30-50 msec., it can be accepted that all shocks to a given trunk triggered the same initial volley. (Nerve potentials were monitored in some experiments and confirmed that this was the case in terms of recognizable spikes and compound potentials; it is still conceivable however that certain fibres, whose activity could not be detected, did not respond uniformly.) Then, dissimilarities in post-synaptic responses associated with changes in timing should reflect the existence of an intercalated pattern-sensitive link. An additional shortcoming could result from the position of the recording tip placed in the soma of a unipolar neuron far from the synaptic surface and the spike trigger zone (Bullock & Horridge, 1963; Tauc, 1960, 1962): *a priori* it is therefore questionable that the recorded potentials are pertinent to the function of the impaled unit as an integral part of a neuronal chain. Their relevancy (accepted by workers in the field) is based on the defensible argument that soma electrodes record phenomena which are some function of activity in the more critical regions of the cell. The stability of the preparation is remarkable and commonly permits many hours of uninterrupted observations without significant changes in behaviour.

## RESULTS

Several authors (Tauc, 1957; Chalazonitis & Arvanitaki, 1961) have analysed the response of *Aplysia* nerve cells to single shocks. Responses to 'sub-threshold' stimuli producing an EPSP but not a spike show two kinds of modifications when the shock intensities are progressively increased. In certain cases, as when exciting the left visceropleural connective and recording from the giant cell, the EPSP shows a slight

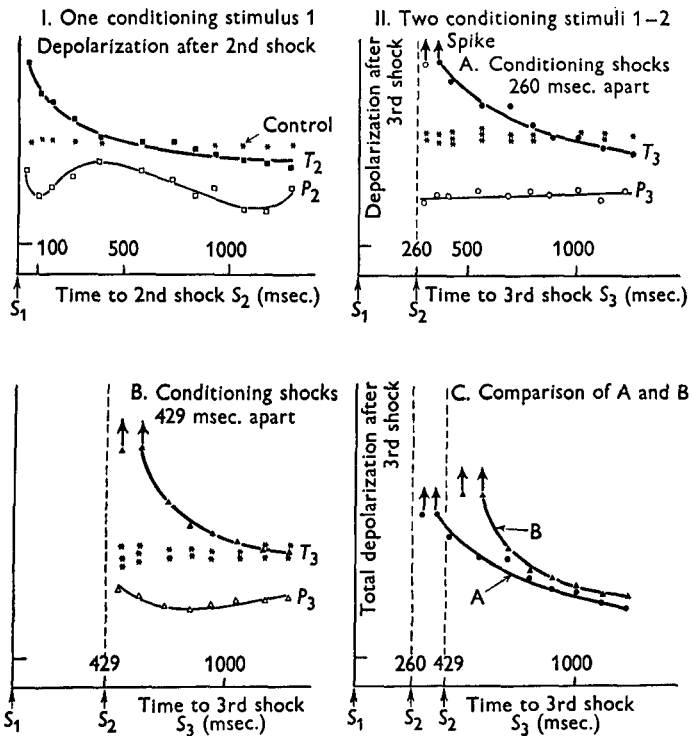


Fig. 2. Excitability and timing. Sub-threshold stimuli to the same nerve (homologous). On abscissae: interval (msec.) between conditioning and test stimuli. On ordinates (arbitrary units): depolarization after test shock, total  $T$  (black marks, heavy lines) and partial  $P$  (open marks, light lines); stars indicate control effects of test shocks applied alone. I. Pairs. 1-shock conditioning stimulus ( $S_1$ ); 1-shock test stimulus ( $S_2$ ). II. Trios. 2-shock conditioning stimuli ( $S_1$ - $S_2$ ) with separation of either 260 msec. (A) or 429 msec. (B); single-shock test stimulus ( $S_3$ ). On II, C, total value ( $T_3$ ) graphs corresponding to II, A (graph with circles) and II, B (graph with triangles) are superimposed and illustrate the influence of timing: at each  $S_1$ - $S_3$  interval, i.e. at each mean frequency (on abscissae), the effectiveness of the trio measured in terms of  $T_3$  (on ordinates) depended on the position of shock  $S_2$ , i.e. on the timing ( $S_2$  was placed at 429 msec. in curve with triangles or at 260 msec. in curve with circles). For definition of  $T$  and  $P$  values, see text and Fig. 1b.

amplitude increase for each small increase of the stimulus; this is interpreted as progressive activation of larger numbers of pre-synaptic fibres. In other cases, the EPSP shows a limited number of abrupt increments each at a certain intensity value; this is interpreted as staggered activation of a limited number of fibre groups. Higher ('supra-threshold') stimuli can produce a spike; in certain nerve-cell combinations, however, spikes cannot be produced in response to a single shock at any intensity.

## (I) Pairs of shocks

(a) 'Sub-threshold' stimuli. Let us first consider  $P_2$  values (that reflect upon the excitability of the system). With 'homologous' stimulation (shocks to the same nerve), the response to the conditioned second shock varies in  $P$  value from half to twice that of the response to a test shock. When interaction is present, each combination of stimulated nerve and monitored cell yields characteristic reduction or augmentation; in one group of experiments higher temperatures ( $18^\circ$ – $22^\circ$ ) favoured augmentation whereas lower temperatures ( $12^\circ$ – $16^\circ$ ) favoured reduction. Effects are maximal at the shorter intervals and decay progressively with occasional irregularities, through

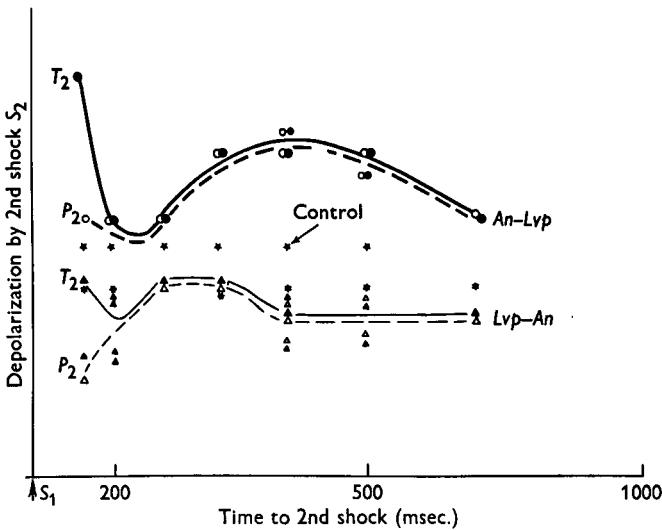


Fig. 3. Non-commutative heterologous summation. Pairs of shocks. On abscissae, interval between shocks  $S_1$  and  $S_2$  (msec.); on ordinates (arbitrary units), depolarization produced by second shock  $S_2$  in total  $T_2$  (continuous lines) and partial  $P_2$  (broken lines) values. Upper graphs (with circles) correspond to the sequence 'anal-left visceropleural' and control effects of shock  $S_2$  applied alone are marked as stars; note that the effect of  $S_2$  was augmented when preceded by  $S_1$ . Lower graphs (with triangles) correspond to the opposite sequence 'left visceropleural-anal' and control effects for  $S_2$  are marked as asterisks: note that the effect of  $S_2$  was reduced when preceded by  $S_1$ . At intervals depicted here, the sequence *An-Lvp* was more effective than *Lvp-An*.

periods of 5–10 sec. (Fig. 2, I). This protracted time course is confirmed by regular repetitive excitation; at frequencies above one every 20 sec. the amplitude decreases progressively after the first few shocks and then reaches a stable value, smaller than that of the control.

With 'heterologous' testing (shocks to different nerves) the magnitude and duration of effects, when present, are comparable to those with 'homologous' testing. Reduction was by far the most frequent change encountered (Fig. 3,  $P_2$  graph for *Lvp-An*; Fig. 4, A1, B1). Augmentation was observed occasionally as, for instance, when conditioning and test shocks were applied to the anal nerve and the left visceropleural connective respectively (Fig. 3, *An-Lvp*; Fig. 4, A2, B2). This may depend on certain fibre groups in the excited trunks since it is occasionally necessary to test

various relative positions of nerve and electrodes. 'Heterologous' interactions were not always reciprocal. The fact that one input, used for conditioning, had a given influence upon another, used for testing, did not imply the sign or even the existence of an effect with the opposite sequence. Figure 3 exemplifies a case in which the left visceropleural connective reduced the effect of the anal nerve (broken thin line with open triangles) but the latter augmented the effect of the former (broken thick line with open circles).

Test responses could be altered even when they occurred after the conditioning EPSP was over and the base line had returned to its control level, indicating that the system can exhibit different degrees of reactivity at a given level of post-synaptic membrane potential.

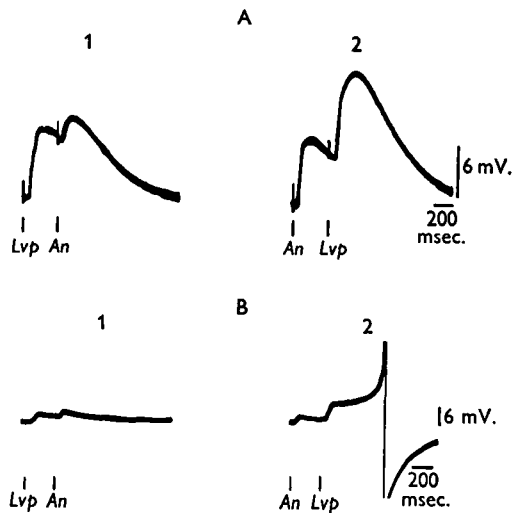


Fig. 4. Non-commutative heterologous summation. With pairs of shocks at a given interval the sequence 'anal-left visceropleural' (column 2) was more effective than the opposite one 'left visceropleural-anal' (column 1); it either produced better depolarization (A) or, with greater probability, evoked a spike (B).

For Figs. 4, 7, 8, 9, 10, 12 and 13: intracellular records (positive up) from giant cell in abdominal ganglion of *Aplysia californica*, unless otherwise indicated; vertical lines under tracings indicate single shocks of stimulation to left visceropleural nerve, unless otherwise indicated.

The total effect of the pair, as measured by the summed depolarization  $T_2$ , decreased as the separation between conditioning and test shocks became greater, indicating that higher frequencies were more effective (Fig. 2, I). With certain combinations, involving cells other than the giant cell, marked reductions of the test potential occurred at short intervals; in these rare cases the  $T$  curve increased, reached an early maximum and decreased so that the most effective separation (frequency) was one of an intermediate magnitude. When 'heterologous' summation was non-commutative, one sequence (e.g. *An-Lvp* in Figs. 3 and 4A) was able to produce significantly more depolarization than the opposite one (e.g. *Lvp-An*) and therefore at adequate intensities was associated with a higher percentage of post-synaptic spikes (Fig. 4B).

(b) *Supra-threshold stimuli*. The first (conditioning) shock was of sufficient intensity always to produce a spike. Studies of excitability, evaluated at each instant by the

percentage of second (test) shocks of the same intensity evoking a spike, show that the first spike is followed by an early subnormality that changes within 1–2 sec. into a transitory (2–5 sec.) supernormality. The assertion that this period is supernormal rests on complementary tests performed with sub-threshold shocks at intensities close to threshold. The supernormal period is followed by a late subnormality during which excitability reaches a minimum 5–10 sec. after the conditioning shock and then returns to the control level slowly (10–25 sec.) (Fig. 5, I). This curve, obtained also by hetero-synaptic testing, parallels closely the after-hyperpolarization (undershoot), after-depolarization and late-hyperpolarization phases of after-potentials which may follow each spike.

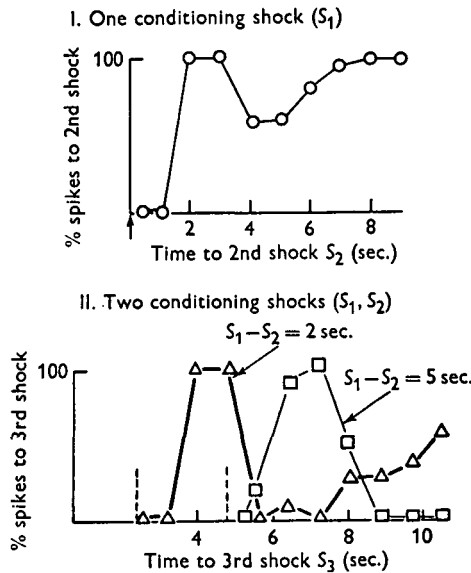


Fig. 5. Excitability and timing. Supra-threshold shocks. On abscissae: time (sec.) between conditioning and test stimuli. On ordinates: percentage of applications of the 'supra-threshold' test shock that evoked a spike. I. Pairs. Single-shock conditioning stimulus  $S_1$ , single-shock test stimulus  $S_2$ . II. Trios. 2-shock conditioning stimulus ( $S_1-S_2$ ) with separation of either 2 sec. (thick curve with triangles) or 5 sec. (thin curve with squares); single-test stimulus  $S_3$ . At each  $S_1-S_3$  interval, i.e. at each mean frequency (on abscissae), the effectiveness of shock  $S_3$  (on ordinates) depended on the position of shock  $S_2$ , i.e. on the timing. Note that with short durations (6–8 sec.) the optimal timing involved  $S_2$  close to  $S_3$  but that the opposite was true with long total duration (over 9 sec.),

Certain cells which exhibited after-potentials and excitability curves of this type were 'spontaneously' active; their inter-spike interval histograms closely paralleled the former curves and were therefore bimodal. A bimodal distribution could also reflect other basic mechanisms; for instance, cases in which a first mode is due to an endogenous pace-maker and a second mode is due to an occasional intercurrent PSP at a relatively fixed point of the cycle.

## (II) *Trios of shocks*

(a) *Sub-threshold stimuli.* The effectiveness of the third shock, measured in  $P$ ,  $T$  and spike-probability values, was plotted in two ways. (1) The first way consisted of grouping those corresponding to a given  $S_1-S_2$  interval and displaying them as a



Function of the  $S_1$ - $S_3$  interval, i.e. of the total duration or mean frequency of the burst (Fig. 2, II, A, B). When  $P$  values were graphed, veritable excitability curves were obtained and indicate that changes after two conditioning shocks (augmentation or depression) are qualitatively of the same kind as those after one (see § I); quantitatively, effects are greater and more durable. It is important to state that the efficacy of a third shock cannot always be predicted from recovery curves with only two shocks; that is, there may be additional effects after a second shock beyond those expected from mere repetition and this made the present study necessary. In all heterosynaptic cases studied, the individual influences of each conditioning shock upon the test potential were of the same sign, either augmenting or depressing. With respect to total values, comments made *a propos* of  $T$  values for pairs of shocks are applicable.

(2) The second method of displaying the data was by grouping those corresponding to a given  $S_1$ - $S_3$  interval, i.e. total duration and mean frequency, and plotting them as a function of the  $S_1$ - $S_2$  interval, i.e. of the timing. When  $P_3$  values (that reflect upon the excitability of the system) were graphed, timing became significant only for cases in which  $S_2$  was close to  $S_3$  and its influence was therefore strong; when  $S_2$  was not close to  $S_3$ , timing was not relevant and  $P_3$  depended exclusively on the fact that two shocks preceded  $S_3$  within a certain interval. This was true also using bursts of more shocks. Therefore, the size of the EPSP is exclusively a function of its serial number within the burst, unless the one preceding it is very close.

Total depolarization values relate to spike production and therefore are more relevant to the physiological efficacy of the burst. When  $T_3$  values were graphed it became obvious that timing is significant, under conditions defined by two additional parameters: size of EPSP and total duration (i.e. mean frequency) of trio.

Given a certain EPSP size, the relationship between the influence of timing and the total trio duration or mean frequency is best appreciated by inspecting Fig. 2, II, C, which indicates that three separate total duration or mean frequency ranges can be identified: (i) a 'late' range of long durations (e.g. 1000 msec.) or low frequencies, in which total depolarizations provoked by different patterns do not differ significantly; (ii) an 'intermediate' range of durations (e.g. 500 msec.) and frequencies, in which different positions of  $S_2$  are reflected by significantly different degrees of depolarization ( $T_3$ ) and, at higher frequencies, different spike probabilities (Figs. 6-8); (iii) an 'early' range, of short durations and high frequencies, in which all trios of sub-threshold shocks of moderate intensity evoke a spike, irrespective of the timing (this interval is not shown in Fig. 2). Consequently, it is justifiable to state that, under fixed conditions of nerve, cell and EPSP size, there exists an intermediate input frequency range within which output is critically dependent on timing.

Given next the total duration or mean frequency of the trio, the relationship between influence of timing and individual EPSP size is best appreciated by observing curves in which the effectiveness of  $S_3$  (expressed in  $T$  or spike-probability values) is plotted as a function of the  $S_1$ - $S_2$  interval, i.e. of the timing (Fig. 6). Three separate ranges can be identified. (i) With small EPSPs different timings do not produce different effects (this range is not depicted in Fig. 6). (ii) With 'intermediate' EPSPs, dissimilar timings are reflected by different efficacies in terms of significantly different degrees of total depolarization (Fig. 6, graphs 1-4; Fig. 7, I; Fig. 8; Fig. 10, II 1) and, with the larger potentials, significantly different probabilities of spike production

(Figs. 6, graphs 2-4; Fig. 7, II; Fig. 10, II<sub>3</sub>). Given an intensity within range (ii) the effectiveness of different patterns is always graded according to the same sequence. (iii) With large EPSPs, spikes are evoked consistently by all timings (Fig. 6, graph 5). Consequently it is justifiable to state that under fixed conditions of nerve, cell, and mean frequency, there exists an intermediate input magnitude range within which output is critically dependent on timing.

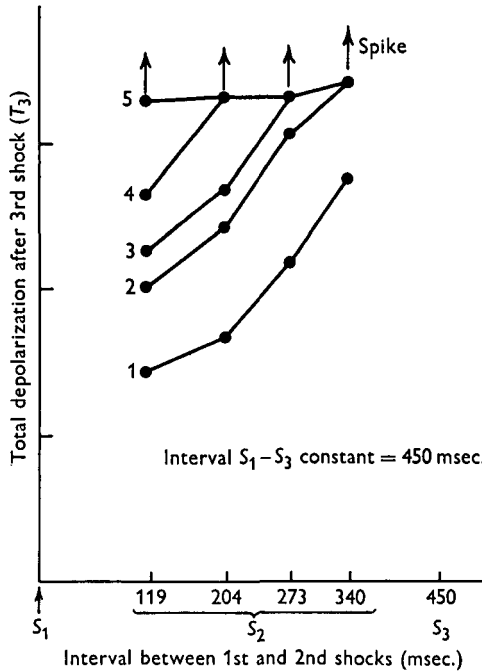


Fig. 6. Timing and size of EPSP. On abscissae: timing represented by the interval between the 1st and 2nd shocks ( $S_1-S_2 = 119, \dots, 340$  msec.) of trios with identical intervals between the 1st and 3rd shocks ( $S_1-S_3 = 450$  msec.), i.e. with identical mean frequencies. On ordinates (arbitrary units)  $T_3$ , total depolarization after  $S_3$ ; arrows indicate production of spikes. Each curve corresponds to a certain EPSP size: the latter increases progressively from graphs 1 to 5. Note that for EPSPs of sizes 1-4 (but not of size 5), effectiveness was a function of timing. See text.

Two points should be clarified: first, that there is a maximum total duration value above which even the largest and most prolonged EPSPs cannot summate or interact with each other and therefore timing becomes irrelevant; secondly, that even with small EPSP sizes there will exist a total duration that is short enough so that the influence of timing will become manifest.

The efficiency of a trio is therefore dependent on its timing; the following paragraphs describe this relation, valid within critical EPSP size and frequency ranges, in more detail. Two parameters of the effect were controlled preferentially: maximal depolarization  $T$  and spike probability. The first parameter appears to be a continuous function of timing, for small changes in  $S_1-S_2$  produce small changes in  $T_3$  (Figs. 6, 7). It usually is also a monotonically increasing function of  $S_1-S_2$ ; the optimal timing is that in which  $S_1-S_2$  is relatively long and  $S_2$  is close to  $S_3$  (Figs. 6-8). Figure 7 illustrates these points particularly well; in I, all responses are sub-threshold, but there is a

progressive augmentation of the  $T_3$  as the  $S_1$ - $S_2$  interval is lengthened; in II,  $D$  is of such significantly higher amplitude that a spike is produced by this, and only this, pattern. More rarely, the curve of  $T_3$  as a function of  $S_1$ - $S_2$  presents a relative minimum or maximum. In the former case (minimum) the optimal timing still corresponds to  $S_2$  close to  $S_3$ ; in the latter case (maximum) the optimal timing corresponds to an intermediate position of  $S_2$  (Fig. 9). With unfavourable timings  $T_3$  can be smaller than  $T_2$ ; in every case, however, some timings have the maximum peak at  $T_3$  and these produce optimal depolarization. The optimal pattern is a characteristic of each cell-nerve arrangement and is reproducible, either during the same experiment or in

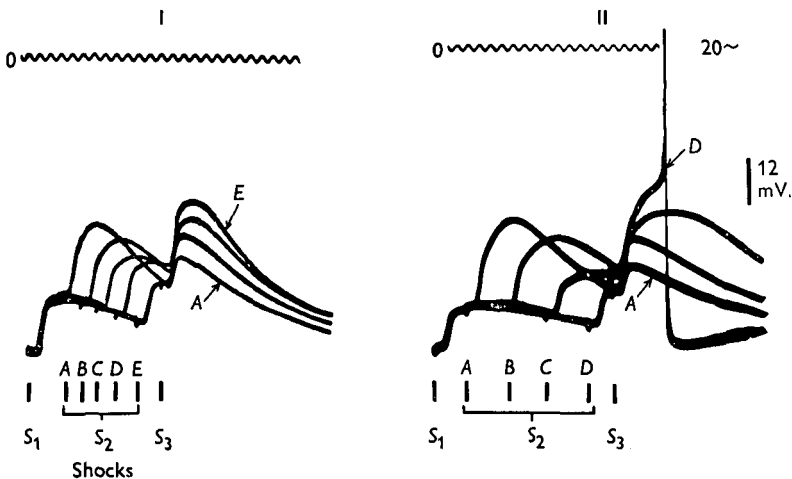


Fig. 7. Timing and efficiency of trios with identical mean frequencies. Figs. 7-10 show trios having identical separations between shocks  $S_1$  and  $S_3$ , i.e. having identical mean frequencies, in which the effectiveness depended on the separation between shocks  $S_1$  and  $S_2$ , i.e. on the timing. In this figure with superimposed sweeps, when  $S_2$  is moved farther from  $S_1$  and closer to  $S_3$  (positions  $A$  to  $E$  in I and  $A$  to  $D$  in II) total depolarization after  $S_3$  increases up to a maximum in the extreme position ( $E$  in I, and  $D$  in II where it reached firing level). In I, the third EPSP of pattern  $B$  and the third EPSP of pattern  $C$  are superimposed.

The 20 cyc./sec. sinusoid indicates  $DC$  zero level in this figure only. The overshoot of the spike is not shown complete in Figs. 7, 9, 10 and 13.

different preparations. When heterologous excitation is used, the number of times in which each nerve is shocked and the sequence in which they are activated are important; in certain cases, for instance, the advantage lies with ' $S_2$  close to  $S_1$ ' if sequence '*Lvp-Gen-Lvp*' is used and with ' $S_2$  close to  $S_3$ ' if '*Gen-Lvp-Gen*' used.

The degree of improvement in  $T_3$  height that can be obtained by shifting  $S_2$  to its optimal position is between  $\times 1.2$  and  $\times 2.0$ . Note that this order of magnitude is clearly smaller than that of the  $\times 40$  facilitation of contraction obtained by Wiersma & Adams (1949). Improvements are highly significant, however (e.g. beyond a 0.005 level), as shown by a  $t$  test and by estimations of the differences of the means using the same test. The second criterion for trio efficiency was spike production, quantified for each pattern by the quotient of the number of presentations producing a spike and the total number of presentations. In so far as this fraction constitutes an adequate estimate of the probability of evoking a spike, the latter is a function of timing. Different positions of  $S_2$  are associated with different probabilities and contrasting values

are of the order of 0 against 0.6, 0.09 against 0.92, 0.2 against 0.7, etc.; the highest probability value always corresponds to the timing that induces better depolarization when it does not evoke a spike. Figure 7, II; Fig. 9, I, IIB and Fig. 10 illustrate the preferential production of spikes by certain patterns as opposed to others. Occasionally, trios evoke a local response. These also are more frequent with patterns more effective in other respects. The situation is exemplified by Fig. 10, II showing that

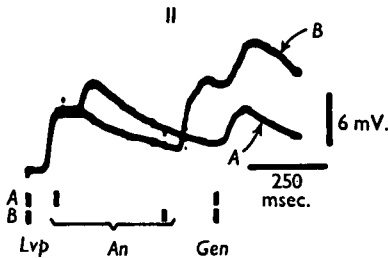
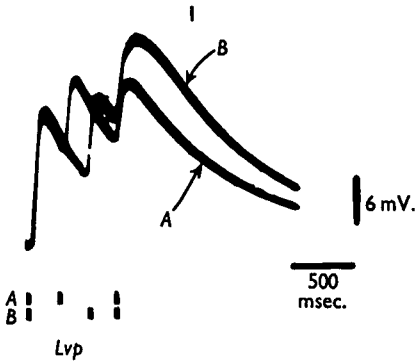


Fig. 8

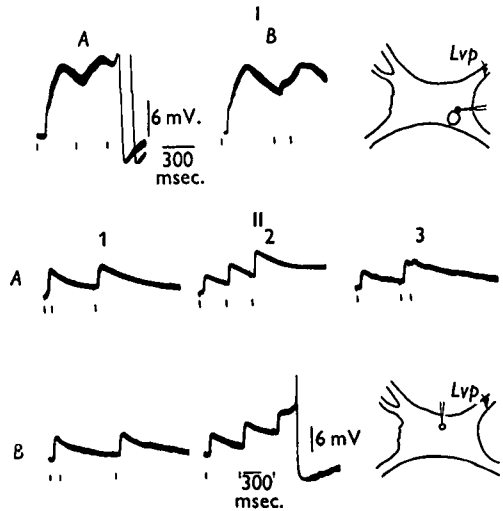


Fig. 9

Fig. 8. Homologous and heterologous stimulation. Superimposed sweeps. I. All shocks to *Lvp*; intervals between  $S_1$  and  $S_3$  uniformly 585 msec. Better depolarization was obtained when the 2nd shock  $S_2$  was in position *B* (at 429 msec. from  $S_1$ ) than when it was in *A* (at 260 msec.). II. Shocks  $S_1$ ,  $S_2$  and  $S_3$  to left visceropleural, anal and genital trunks respectively; interval between  $S_1$  and  $S_3$  uniformly 600 msec. Better depolarization with  $S_2$  at 475 msec. from  $S_1$  (position *B*) than at 118 msec. (position *A*).

Fig. 9. Other optimal timings. Records from small cell close to giant cell in I or from small cell close to base of *Lvp* in II; stimulation of *Lvp*. In these two instances, the optimal timing was that in which the second shock ( $S_2$ ) was placed at approximately the same distance from the first ( $S_1$ ) and the third ( $S_3$ ). Note that in I, *A* and II, *B*2 the optimal pattern produced spikes. Two superimposed sweeps in I, *A*, *B*.

the optimal pattern with  $S_2$  close to  $S_3$  selectively produces a spike (*C*) or local response (*B*) or induces better depolarization (*A*). These are veritable trio effects and not differences due exclusively to the advantage of using a certain pair in one pattern and not in the others; this is demonstrated by the fact (revealed by the same statistical treatments as above) that different combinations (trios, pairs, singles) have different effects and that the greatest depolarization corresponds to the maximum of the 'optimal' trio (Fig. 10, 1).

Thus the effectiveness of any trio depended on (1) the amplitude and time course of each EPSP, (2) the mean frequency of the burst (interval between  $S_1$  and  $S_3$ ),

and (3) the timing of the burst (position of  $S_2$ ). The model corresponding to the simplest assumption, namely that EPSPs sum linearly and there is superposition without interaction, is represented in Fig. 11 where the total depolarization  $T_3$  is plotted as a function of frequency and timing; for reasons of clarity in the drawing it was found convenient to use  $S_1-S_2$  and  $S_2-S_3$  as the independent variables. On the plane determined by the  $S_2-S_3$  and  $S_1-S_2$  axes each point corresponds to a trio. In the upper drawing the line  $AB$  is the locus of all trios of constant  $S_1-S_3$  interval; the curve  $T'_3$  is generated by the vertical plane at  $AB$  and reflects the depolarization  $T_3$  that can be achieved by trios having this fixed duration. From geometrical considerations it can be inferred that for the linear case, long  $S_1-S_2$  values are more favourable than short

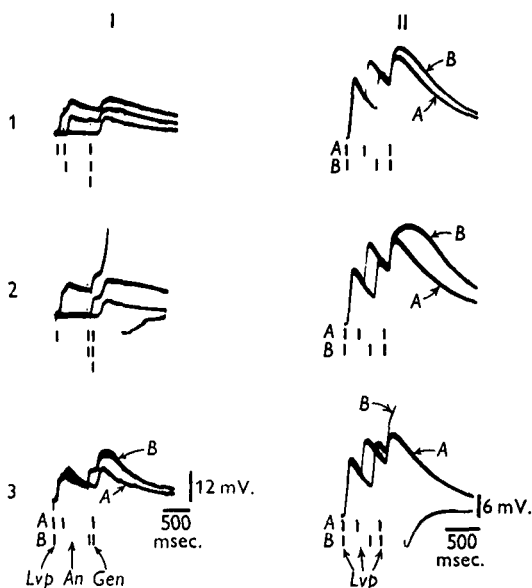


Fig. 10. I. Trios, pairs and singles. Heterologous stimulation to *Lvp* ( $S_1$ ), *An* ( $S_2$ ) and *Gen* ( $S_3$ ); Superimposed sweeps (1). A trio  $A$  (involving 190 msec. between  $S_1$  and  $S_2$ ) was compared with a component pair ( $S_2-S_3$ ) and a single ( $S_3$ ). (2) Another trio  $B$  of same duration but of different timing (involving 545 msec. between  $S_2$  and  $S_3$ ) produced a spike and was compared with a component pair ( $S_2-S_3$ ) and single ( $S_3$ ). (3) Both trios were compared with each other; note more depolarization was obtained with timing  $B$  in which  $S_2$  was closer to  $S_3$ .

II. Different possibilities. Homologous stimulation; superimposed sweeps. Trios of identical mean frequencies but different timings. The trio with timing  $A$  ( $S_1-S_3$ , 260 msec.) produced relatively little depolarization (1, 2, 3) and, occasionally, a local response or a spike. The trio with timing  $B$  ( $S_1-S_3$ , 429 msec.) produced greater depolarization (1) and, frequently, a local response (2) or a spike (3).

ones, when  $S_1-S_3$  is fixed: thus trio pattern  $P_1$  produces greater depolarization than pattern  $P_2$ . The physiological importance of a given depolarization can be determined most significantly by its relation with the spike threshold potential of the cell; there will always be a range of EPSP amplitude in which the achievement of the threshold potential will depend critically on the pattern of the arriving trio. This is illustrated in the lower drawing of Fig. 11 in which threshold is indicated by a horizontal plane. For the set  $AB$  of trios of fixed  $S_1-S_3$  duration, only the subset indicated by  $AC$  is capable of summing to reach threshold level. Graphs also show that the significance of timing

is reduced at  $S_1-S_3$  values that are extreme, relative to the time course of the EPSP. Indeed, if  $S_1-S_3$  is long, the effect of the trio will at best be identical with that of a closely spaced pair (when  $S_2-S_3$  is short) and, at its worst, will be no less than that of a single shock (when  $S_2-S_3$  is long). If the  $S_1-S_3$  interval is short relative to the time course of the EPSP (and falls within the shaded area of the trio plane) the pattern of the trios is less important because all timings surpass the threshold plane.

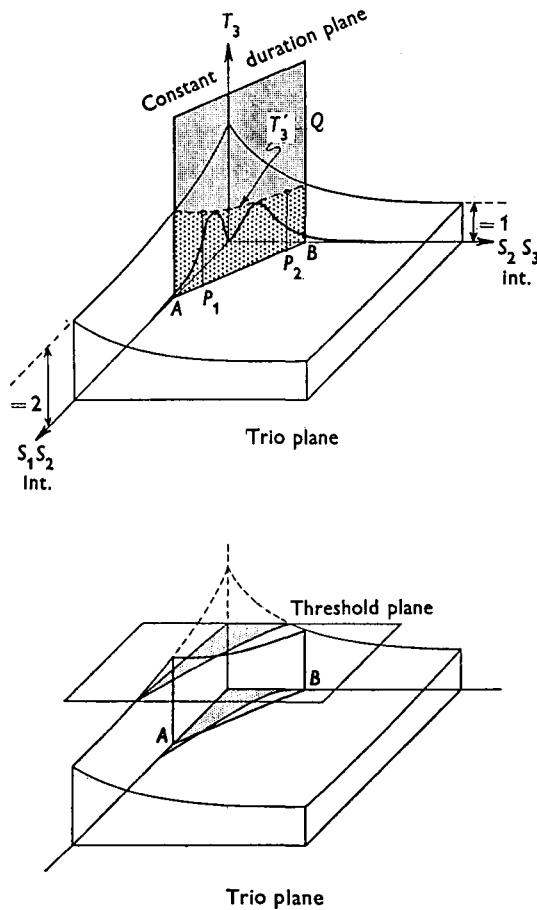


Fig. 11. Linear model. Schematic representation of the effect of EPSP trios. Each point on the horizontal plane corresponds to a particular trio, defined by its  $S_1-S_2$  and  $S_2-S_3$  values. The  $T_3$  axis measures the summed depolarization produced after the third shock.

Upper diagram. The line  $AB$  in the horizontal plane is the locus of all trios having a fixed  $S_1-S_3$  duration.  $P_1$  on  $AB$  represents a trio with relatively long  $S_1-S_2$ , compared to  $S_2-S_3$ ;  $P_2$  represents the converse pattern. The curved surface is the plot of total depolarization for a given EPSP size. The line  $AB$  projects on to this surface via the constant duration plane  $Q$  generating curve  $T'_3$ . The latter curve indicates that pattern  $P_1$  produces greater depolarization than  $P_2$ .

Lower diagram. The same co-ordinate system and curved surface have been drawn this time with an arbitrary horizontal plane which represents the spike threshold potential of the cell. All depolarizations above threshold will generate spikes (shaded area). For trios of a given total duration of intermediate value (line  $AB$ ) only part of curve  $T'_3$  lies above the threshold plane: therefore certain timings only will produce a spike (i.e. those of  $AC$ , with short  $S_2-S_3$  values. With long durations, no trios will surpass the threshold plane; with short durations, all trios will surpass the threshold plane. See text.

Invisceral ganglion cells of *Aplysia*, this simplified linear situation is closely approximated in certain cases. Departures from the linear model are frequent, however, and reflect the influence of each volley upon those following it. As mentioned above the most common deviation is quantitative; the short  $S_2$ - $S_3$  pattern is still the best but not in the measure expected on the basis of linearity. Less commonly deviation is qualitative and the optimal response is produced by evenly spaced shocks.

(b) '*Supra-threshold*' stimuli. (Note that stimulus voltages were adjusted so that  $S_1$  and  $S_2$  always produced spikes.) Results can again be observed from two points of view. (1) Given a constant separation between  $S_1$  and  $S_2$ , observation of each curve reveals responsiveness to  $S_3$  to be a function of the  $S_1$ - $S_3$  interval, i.e. total duration and mean frequency (Fig. 5, II). The curve showing the excitability after two conditioning stimuli exhibits the same general shape as after a single shock and again parallels after-potentials; late sub-normality is enhanced and lasts for a longer period. (2) Given a constant separation between  $S_1$  and  $S_3$  (constant duration and mean frequency), comparison of different curves reveals responsiveness to be a function of the position of  $S_2$ , i.e. of timing (Fig. 5, II). Three burst duration or frequency ranges can be identified. (i) A 'late' range of long durations (e.g. over 20 sec.) and low frequencies in which  $S_3$  is always effective and therefore any timing produces three spikes. (ii) An 'intermediate' range of intermediate durations (e.g. 7 or 10 sec.) and frequencies in which the effectiveness of  $S_3$  and therefore the ability of each trio to produce three spikes depends on the timing. This range is not uniform for within it the timing of the optimal pattern depends on the duration. (iii) An 'early' range of short durations (e.g. 5.5 sec.) and high frequencies in which  $S_3$  was consistently ineffective, and therefore each trio always produced only two spikes. Consequently, also for 'supra-threshold' shocks, the influence of timing is felt at intermediate frequency ranges. The influence of the intensity can be summarized by saying that pattern is significant close to threshold.

The recovery cycle following a spike is the same when the latter is evoked by a trio of sub-threshold shocks as when it is evoked by a single supra-threshold shock; apparently the excitability after a spike does not depend on the number or timing of the sub-threshold stimuli which summated to evoke it.

### (III) *Prolonged bursts*

As more shocks are added to a trio that has not provoked a spike two types of response are seen. (1) The first is that after a variable number of shocks a 'plateau' is reached, meaning by this that depolarization, though oscillating, does not reach the firing level (for a similar phenomenon, see Eccles & MacFarlane, 1949 and R. M. Eccles, 1955). (2) The second possibility is that of an uninterrupted increase of  $T$  values until a spike is evoked. In this situation and with bursts of 4-10 shocks, it is still possible to demonstrate that when bursts with identical numbers of shocks and identical durations (i.e. mean frequencies) are compared different timings lead to significantly different degrees of depolarization and, if sufficient shocks are used, different spike probabilities (Fig. 12). The influence of timing becomes apparent also in another manner: when a certain mean frequency is given, less EPSPs are required to reach a certain depolarized level if a favourable pattern is used (Fig. 12).

If the considered level is that at which a spike will be triggered this can be expressed by saying that the waiting time for the first spike will be shorter.

Continuation of stimulation after occurrence of a first spike can itself have different outcomes. (a) On the one hand, when spikes are evoked frequently (one or more every 20–30 sec. for the giant cell), a limited number occurs and the cell then becomes non-responsive. Such ‘adaptation’ is observed frequently, and may be specific to a given input. (b) On the other hand, when spikes are evoked less frequently, cells continue to fire regularly.

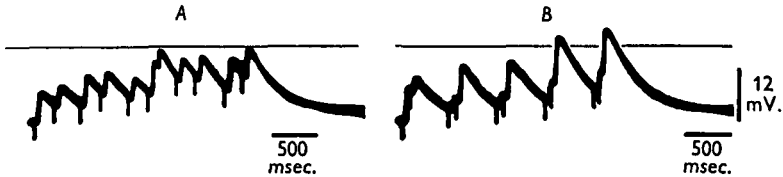


Fig. 12. Intermediate duration bursts. Bursts *A* and *B* have the same mean frequencies but different timings. Pattern *B* was more efficient. Given an arbitrary ‘depolarized’ membrane potential value indicated by the horizontal line, this pattern required less shocks to reach it; given a number of shocks, this pattern produced more depolarization. A spontaneous EPSP occurred in *A* between the 6th and 7th shocks.

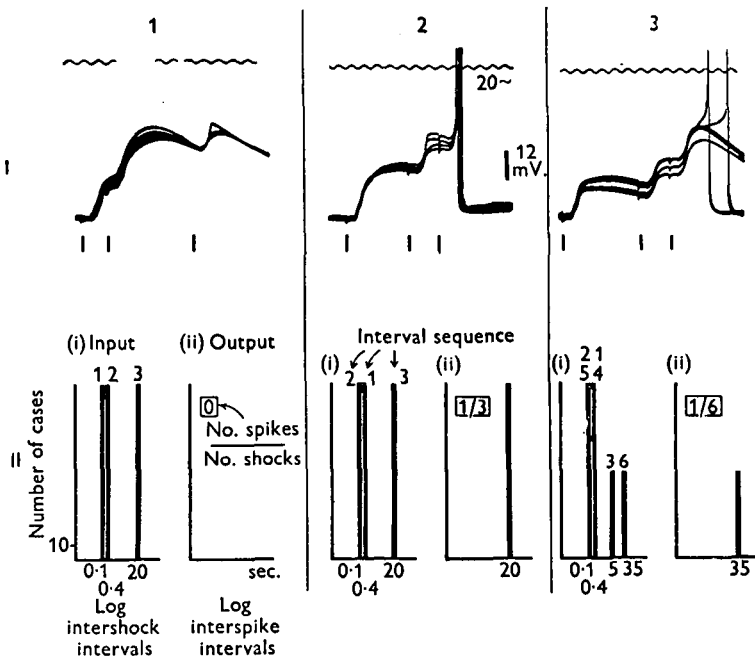


Fig. 13. Prolonged input. Timing and output. Homologous stimulation to *Lvp*. Three prolonged inputs (1, 2, and 3) with the same mean frequencies but different timings are compared. I, Superimposed sweeps triggered by every third shock. II, Histograms of each input in inter-shock intervals (i) and of the corresponding output in inter-spike intervals (ii) (logarithmic scale on abscissae). Burst 1 involved intervals of 0.1, 0.4 and 20 sec. generated in that order (the order is indicated by the number above each column of the histogram); no output was evoked (as indicated by an ‘efficiency coefficient’ of 0 in square). Burst 2 involved the same intervals but in a different order (0.4, 0.1 and 20 sec.); one output spike was evoked every three input shocks. Burst 3 involved intervals of 0.4, 0.1, 5, 0.4, 0.1 and 35 sec. generated in that order; one output spike was evoked every six input shocks.



Restricting this description to situation (2) (*b*), if the same number of shocks is delivered over the same period, they can produce significantly different outputs if dissimilar timings are used. This conclusion, which is self-evident when referring to clearly discordant designs (as for instance 'high frequency burst of  $(n-1)$  shocks-long pause-single shock' versus 'regularly spaced sequence of  $n$  shocks'), is valid for patterns that exhibit subtle differences. This is illustrated by Fig. 13 in which three inputs (1, 2, 3) with the same mean frequencies but different timings are compared. The contrast between inputs 1 and 2 is particularly interesting since they differ only in the sequence with which the same intervals 'emerge' from the generators, short-intermediate-long in 1, intermediate-short-long in 2. For comparisons involving a variety of patterns (evenly spaced; regular pairs or trios; randomly distributed, etc.) it became apparent that given a nerve-cell preparation, a mean input rate, a minimum inter-EPSP interval and an individual EPSP size, the output over long periods evaluated by the number of spikes and their distribution is a function of the timing. Moreover, under each set of conditions there exists a certain pattern, which depends on the elementary characteristics of the system, that determines optimal production of spikes. It should be pointed out that the constraints mentioned (nerve, cell, rate and interval bounds and EPSP size) are not artificial since they are present in any natural synapse.

#### DISCUSSION

It has long been a commonplace conclusion that timing is important in determining nervous function, but the available evidence with rare exceptions (Ripley & Wiersma, 1953; Wiersma & Adams, 1950) does not permit conclusions about the relative effectiveness of different micro-patterns of the same numbers of impulses in the same period of time. This investigation was designed to evaluate whether timing should be considered in an analysis of integrated nervous function; discussion will be framed around three questions that are separate but complementary.

(I) First, we may ask, is there demonstrable sensitivity to differences in spacing of a given number of shocks in a given time? Results answer in the affirmative; within intermediate input size and frequency ranges the timing of a pre-synaptic barrage can indeed influence the production and gradation of a post-synaptic response. This sensitivity to timing is a consequence of elementary neuronal characteristics inherent in the excitability curves and its genesis can be followed step by step with bursts that involve successively higher numbers of individually sub-threshold shocks.

(1) With trios, in the simplest situation, two factors must be distinguished: summation and interaction. The first, summation, would be expected to give a certain sensitivity to timing, even in the absence of 'interaction'; recognition of obvious characteristics of the time course of the EPSP (e.g. rapid ascent to a maximum, slower descent) leads by simple geometrical induction to forecasts concerning the results of linear summation, including the basic conclusion that, at intermediate frequency ranges, timing is significant and that the optimal depolarization is obtained with shock  $S_2$  close to shock  $S_3$ . Qualitatively, such predictions are frequently but not always confirmed by the experimental results; quantitatively, rarely so. Discrepancies can be assigned to the second factor, 'interaction', which also depends on timing and

can variously reinforce, detract from, cancel or even reverse the influence of linear summation; the total effect therefore issues from a summation that is commonly non-linear.

The question remains open as to whether interaction (i) derives from a complex interplay of excitatory and inhibitory influences occurring in series and parallel inter-neuron circuits and/or (ii) depends on post-activation changes occurring at the junctional level. The latter is the presumed basis for effects encountered in neuromuscular preparations (Del Castillo & Katz, 1954; Dudel & Kuffler, 1961; Eccles, Katz & Kuffler, 1941; Eccles & MacFarlane, 1949; Lundberg & Quilisch, 1953; Hoyle & Wiersma, 1958; Takeuchi & Takeuchi, 1959) moto-neurons (Eccles, Hubbard & Oscarsson, 1961), dorsals pino-cerebellar tract cells (Eccles, Oscarsson & Willis, 1961; Fadiga & Brookhart, 1962), sympathetic ganglia cells (R. M. Eccles, 1955; Job & Lundberg, 1953; Laporte & Lorente de No, 1950), giant squid synapse (Takeuchi & Takeuchi, 1962) and *Aplysia* neurons (Fessard & Tauc, 1958). The present experiments do not elucidate the relative contributions of mechanisms (i) and (ii). As discussed in Results, one can accept that sensitivity to timing did not depend on excitability changes at the preganglionic trunk.

(2) With a prolonged pre-synaptic bombardment it appears that EPSPs continue to add according to the same principles of summation with interaction. Consequently the first post-synaptic spike will occur under either one or other of the following circumstances: first when the input achieves at least a minimum frequency and maintains it at least over a minimum period; alternatively, when a favourable pattern is formulated, even though the critical frequency and duration values are not quite reached. Since such episodic effective combinations of spacing and number of impulses have been designated 'word' patterns by Bullock (chapter 5 in Bullock & Horridge, 1963), this can be re-stated by saying that the first spike will be evoked the first time that a 'word' emerges from the sub-threshold background. Subsequent spikes will be contingent also upon post-spike excitability oscillations and will require an additional condition: they will occur not simply when 'words' are enunciated, but only when they are enunciated at opportune moments, i.e. when the cell is responsive. Optimal production of spikes will depend on adequate timing of 'words'.

Formal requirements for the existence of such a sensitivity to timing are summation of EPSPs of a certain shape, interaction between successive EPSPs and post-spike excitability cycles; they are encountered widely in neuronal, even monosynaptic, systems and therefore one can anticipate that sensitivity to patterns may be widespread. The preceding simple rules for relating input and output may be adequate for a first approximation only; it is probable that other factors are significant also, such as the influence of a spike upon subsequent EPSPs, accommodation, etc.

(II) The second question is that of the biological desirability of sensitivity to timing and its answer should be based upon two separate evaluations: (a) the possible limitations of a mechanism based exclusively on average frequencies; (b) the possible contributions of an additional mechanism based on timing. (a) Undoubtedly modulation of the 'mean frequency' offers considerable possibilities and usually constitutes the fundamental, and in certain systems or under certain circumstances, the exclusive mode of coding. Its versatility is limited, however. First, the frequency range within which nerve cells can fire is bounded, an assertion applicable to neurons generic-

ally and, especially so, to cells within a given class. Secondly, the usefulness of this range depends on the frequency-resolving power of the receiving neuron, a value which itself is limited by the minimum rate at which EPSPs can summate or interact and by the maximum rate at which spikes can be generated. In general the frequency change from input to output will be of the order of the ratio of 'EPSP amplitude' to 'difference between resting and firing levels'; this commonly is less than 1, and thus determines a frequency drop. (b) The possibility of distinguishing microstructure and sequence at the same average frequency would add another dimension to the range of

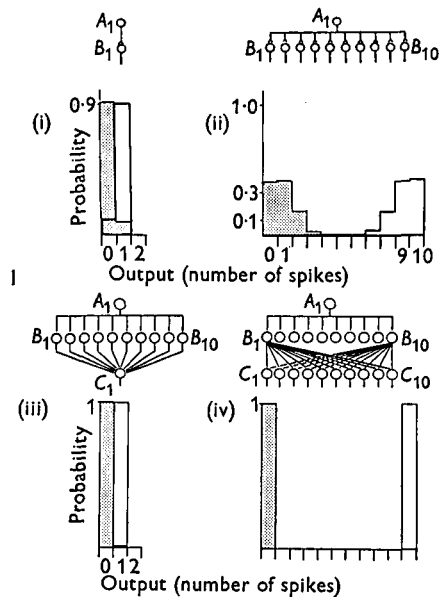


Fig. 14. Hypothetical models (first part). I. Anatomical arrangements drawn in (i), (ii), (iii) and (iv) exhibit increasing degrees of complexity. In diagram (iv) the postulated connexions that go from  $B$  cells to  $C$  cells (see text) are depicted only for  $B_1$  and  $B_{10}$ , so as to make the figure less intricate. Beneath each diagram is a histogram of the possible outputs in response to patterns 'u' (shaded histograms) and 'f' (unshaded histogram) fired by neuron  $A_1$ ; on abscissae, possible output in terms of number of spikes (0 or 1 in (i) and (iii); 0, 1, 2, ..., or 10 in (ii) and (iv)); on ordinates, respective probabilities. See discussion for further explanation.

signals carried in a single line. Results justify the general statement that it improves efficacy of transmission. This assertion can be made more specific with certain hypothetical but admissible conclusions as to how and to what extent sensitivity to timing combined with current structural arrangements and operational principles could improve the functional range of neuronal machinery. Three separate hypotheses may be considered.

(1) The first hypothesis claims that the effect derived from using one pattern and not another can be magnified far beyond the order of the relatively slender range ( $\times 1.2$ – $2.0$ ) observed when evoked depolarizations are compared. The point is critical to this argument, and in order to support it let us consider a first order neuron  $A$  shown in Fig. 14, I capable of firing two patterns 'u' and 'f' that involve the same number of spikes in a given time but differ in their timings, and examine the results

when  $A$  connects with neuronal arrangements of various degrees of structural complexity. (a) The first degree could involve a single order neuron  $B_1$  (diagram (i)) which responds to 'u' by a smaller depolarization (mean 16.0 mV.) involving a spike probability of 0.09 and to 'f' by a large depolarization (mean 19.5 mV.) involving a spike probability of 0.90. Consequently each pre-synaptic pattern 'u' or 'f' may have two different outcomes at the post-synaptic level  $B_1$ : 'no-spike' and 'one-spike', with the respective conditional probabilities of 0.91 and 0.09 given 'u' (shaded histogram), and of 0.10 and 0.90 given 'f' (un-shaded histogram). (b) The second degree of complexity could involve a number (e.g. 10) of post-synaptic elements in parallel ( $B_1, B_2, \dots, B_{10}$ ) (diagram (ii)), each with sensitivity similar to that of  $B_1$  of example (a) and exhibiting no interactions. With respect to each  $B$  cell the first example is reproduced. With respect to the set of ten  $B$  cells, each volley has eleven possible outcomes, 'no-spike', 'one-spike', ..., 'ten-spikes', and their conditional probabilities, given 'u' and given 'f' respectively, are depicted in histogram (ii) which shows that whereas 'u' (shaded histogram) will evoke multiple firing infrequently, and have one spike (in any one  $B$  cell) as its most probable consequence, 'f' (un-shaded histogram) will evoke multiple firing frequently, and have 10 spikes (one in each  $B$  cell) as its most probable consequence. The quantitative relationship between the most probable outcomes is now '1-10', of the order of the potentiation observed by Wiersma & Adams (1950). (c) The third degree of complexity could involve adding a third order neuron  $C$ , upon which all second order neurones ( $B_1, \dots, B_{10}$ ) converge (diagram (iii)) and which, in order to fire a spike, requires the practically simultaneous firing of at least a certain number (e.g. 5) of the  $B$  units. At  $C$  level we have only two possible outcomes, 'no-spike' and 'one-spike', with respective probabilities of 0.998 and 0.002 when  $A$  fires 'u' (shaded histogram), and 0.001 and 0.999 when  $A$  fires 'f' (un-shaded histogram). (d) The fourth degree of complexity (diagram (iv)) could involve a number (e.g. 10) of equivalent and non-interacting third order neurones in parallel ( $C_1, \dots, C_{10}$ ). The practically certain outcomes will be 'no-spike' for 'u' and '10 spikes' for 'f'.

In conclusion, it seems likely that, without undue complexity in structure, a small difference in depolarization at an initial pattern-sensitive stage can lead to remarkably dissimilar output at subsequent stages. The critical issue rests not so much on the extent of the initial dissimilarity, but on the degree of its statistical significance in the vicinity of the firing level. The situation is comparable to that of post-tetanic potentiation, where improvements of  $\times 1.5-2.0$  of the EPSP sampled from single units determine remarkable enhancements of the reflex sampled from a population of motoneurons (Lloyd, 1949; J. C. Eccles, 1961; Eccles & Rall, 1951).

It also appears reasonable to claim that, through exclusive participation of a pattern-sensitive link, a response may be graded in terms of both intensity and probability of occurrence. Small changes in pre-synaptic timing are reflected post-synaptically by small changes in induced depolarization and in spike probability (see Figs. 6, 7), and applying this finding to any of the models discussed above it can be easily shown that when timing is progressively changed (e.g. from pattern 'f' to pattern 'u') the concomitant shift in the probability of each possible outcome (e.g. 'no spike', ..., 'ten spikes') and in the identity of the most probable one (e.g. '10 spikes' for 'f', intermediate values for intermediate patterns, 'no spike' for 'u') is also progressive.

(2) The second hypothesis claims that firing of different patterns by a given cell may lead to qualitatively different responses. The first order neuron  $A$ , mentioned in paragraph (1) was capable of firing two patterns (' $u$ ', ' $f$ ') and a post-synaptic system  $S$  (composed of cells  $B_1, \dots, B_{10}, C_1, \dots, C_{10}$ ) reacted preferentially to ' $f$ '; conceivably,  $A$  could connect also with a system  $S'$  (composed of a similar organization of cells  $B'_1, \dots, B'_{10}, C'_1, \dots, C'_{10}$ ) that reacts selectively to ' $u$ '; as a consequence, different patterns ' $f$ ' or ' $u$ ' in  $A$  would energize separate neuronal pools  $S$  or  $S'$  and ultimately evoke dissimilar responses.

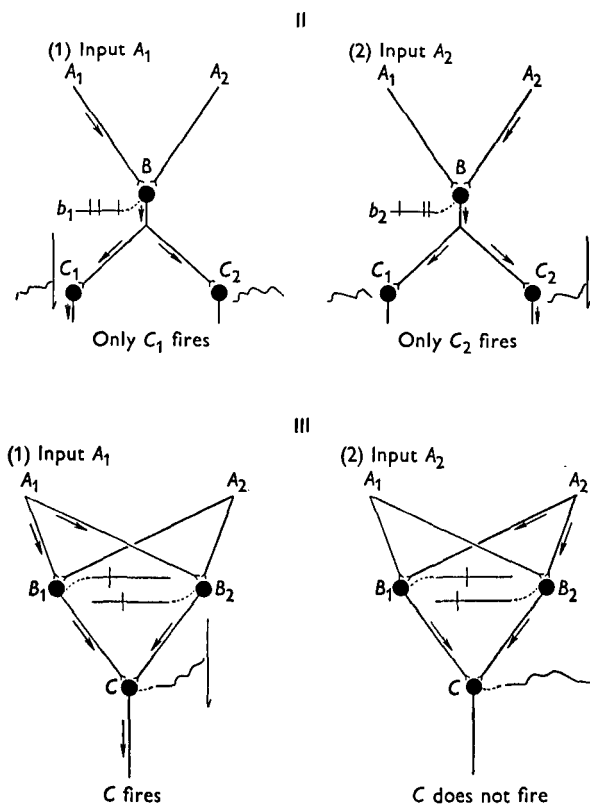


Fig. 15. Hypothetical models (*cont.*). II. Convergence, timing and divergence. 1 and 2 left and right represent the same anatomical arrangement. Both inputs  $A_1$  and  $A_2$  trigger different patterns ( $b_1$  and  $b_2$ , respectively) at cell  $B$ ; each of the cells  $C_1$  and  $C_2$  responds specifically to one pattern ( $b_1$  and  $b_2$ , respectively) but not to the other. (1) Input  $A_1$  evokes pattern  $b_1$  and therefore triggers cell  $C_1$  only. (2)  $A_2$  evokes  $b_2$  and triggers  $C_2$  only. III. Latency and non-commutative summation. 1 and 2 represent the same anatomical arrangement. Both inputs  $A_1$  and  $A_2$  trigger cells  $B_1$  and  $B_2$  but present contrasting latency patterns (' $B_1$  short- $B_2$  long' for  $A_1$  and ' $B_1$  long- $B_2$  short' for  $A_2$ );  $C$  responds specifically to sequence  $B_1$ - $B_2$  but not to  $B_2$ - $B_1$ . (1) Input  $A$  evoked  $B_1$ - $B_2$  and therefore a spike at  $C$ . (2) Input  $A_2$  evokes  $B_2$ - $B_1$  and no spike at  $C$ . See discussion for further explanation.

Such mechanisms would be useful in the so-called 'poly-sensory' systems (e.g., cat mesencephalic reticular formation, crayfish cord, etc.) where each unit is driven by a variety of sensory inputs (Amassian & Waller, 1958; Wiersma & Hughes, 1961). Thus defined these systems pose two problems. One concerns the afferent side and

questions whether inputs reaching an individual poly-sensory neuron  $B$  by way of different sets of neurons ( $A_1, A_2$ ) can selectively evoke differently patterned responses ( $b_1$  and  $b_2$ ) (Fig. 15, II); on the basis of the report by Amassian and collaborators, this can be answered affirmatively (Amassian, Macy & Waller, 1961; Amassian & Waller, 1958). Another concerns the efferent side and questions whether differently patterned bursts ( $b_1, b_2$ ) fired by cell  $B$  can selectively energize different sets of neurons ( $C_1$  and  $C_2$ , respectively); on the basis of the preceding results this also can be answered in the affirmative. This leads to the idea that funnelling of separate incoming volleys down to a common neuronal link  $B$  (convergence) does not necessarily mean that all will determine the same effect; the potential capacity of single cells to use specific patterns to reflect different inputs on the one hand and to evoke different outputs on the other hand could enable each neuron to operate as a veritable 'convergence-divergence filter' that could selectively channel each sensory volley to an appropriate effector mechanism (e.g.  $A_1$  through to  $C_1$  but not  $C_2$ ,  $A_2$  through to  $C_2$  but not  $C_1$ ).

(3) The third hypothesis claims that the parameter 'latency' may have more physiological significance than is currently attributed to it. This possibility issues from two separate experimental findings. One is the fact that heterologous summation is not necessarily commutative, a fact which may be due to asymmetric anatomical arrangements at the level of intervening circuits and/or of the terminals on the monitored cell. (Asymmetric synaptic facilitation has been noted in crayfish by Wiersma, 1949). The other finding is that in certain structures (e.g. cat mesencephalic reticular formation) represented by cells  $B_1$  and  $B_2$  in Fig. 15, III, different inputs represented by  $A_1$  and  $A_2$  evoke responses which, though intrinsically similar, have characteristic latencies for each stimulus and cell, e.g. at  $B_1$  short latency for  $A_1$  and long for  $A_2$ ; on passing to neighbouring units the relation can be reversed, e.g. at  $B_2$  long for  $A_1$  and short for  $A_2$  (Amassian & Waller, 1958). If cells  $B_1$  and  $B_2$  with contrasting latency patterns converge upon a third cell  $C$  where summation is non-commutative, it is conceivable that, on the basis of latency differences at level  $B$ , input  $A_1$  provoking the sequence  $B_1-B_2$  by which  $C$  is triggered would be 'differentiated' from input  $A_2$  provoking the sequence  $B_2-B_1$ , by which  $C$  is not triggered.

It should be made clear that each one of these models except (3) could operate exclusively on the basis of a sensitivity to frequency modulation. The point is, however, that they could also operate exclusively on the basis of a sensitivity to patterns and that this would add a new dimension to their versatility.

(III) The third question is that of the normal significance of sensitivity to timing, that is, whether it actually participates in the natural operation of some or all neuronal networks. In 1950, after demonstrating the significance of timing in crustacean neuromuscular junctions, Wiersma & Adams made the suggestion that 'impulse patterns do play a role in transmission in central nervous systems' (Wiersma & Adams, 1950). In spite of the interest of this proposal and of awakening concern with informational aspects of neuronal function, no direct consideration of the question has appeared since; from a factual point of view, experimental evidence for or against has been meagre (chapter 5 in Bullock & Horridge, 1963); from a theoretical point of view the possibility has been omitted from hypothetical models.

One is tempted to answer this third question by inference from the answer to

the first. Sensitivity to timing exists, and issues that determine it under experimental conditions participate in the natural operation of most systems; the natural relevance of distribution and sequence is therefore an inescapable conclusion, one subject to the sole constraint of the degree to which these parameters normally fluctuate. Remaining at the experimental level, an ultimate answer can issue only from a crucial experiment involving monitoring pre- and post-synaptic activity in a monosynaptic junction and observing whether, under physiological circumstances, changes in timing within the same mean frequency in the input are reflected by changes in the output. This would be difficult technically and it is doubtful that it will ever be performed in an unequivocal manner; therefore we must rely upon suggestive evidence. Relevant in this respect are the observations that changes in the parameters of an interspike interval distribution or in the distribution itself are associated with certain physiological events (responses to stimuli, motor reactions, etc.) and that certain micro-patterns are formulated preferentially, continuously or sporadically, by single neurones or neuronal groups (Gerstein & Kiang, 1960; Horridge, 1961; Rodieck, Kiang & Gerstein, 1962, etc.). Such findings would be meaningless and their analysis without functional value unless subsequent units are able to react differentially to dissimilar patterns. If they continue to be encountered with sufficient consistency and are taken in conjunction with those reported here, one can hardly reject the notion that not only the mean frequency but also other parameters of the spike discharge are representative variables of normal neural function. More specifically stated, it is by means of fluctuations in several parameters of the interspike interval distribution, and not exclusively in its mean, that a nerve cell serves the dual role required of any  $n$ th order link; namely, that of spanning simultaneously the range of an afferent relation that matches activity in the  $(n-1)$ th order neurones with activity in this cell and the domain of an efferent counterpart that matches activity in this cell with activity in the  $(n+1)$ th order neurones.

## SUMMARY

1. Experiments were carried out on the isolated ganglia of *Aplysia californica* to discover whether the precise timing (i.e. the interspike interval distribution and sequence) of a group of spikes entering a neuronal system at a given mean frequency constitutes a variable that is significant in the control of its output.

2. The input consisted of stimulation bursts with different timings but identical mean frequencies applied to one or more afferent trunks. The output (EPSPs or spikes) was monitored with an intracellular micro-electrode placed in the giant cell.

3. Changes in the timing of the input produce definite changes in the magnitude of the output, both in terms of depolarization induced and of spikes evoked. Because of the critical nature of the spike firing threshold, a consistent small increment in depolarization due to favourable timing can be functionally important in the vicinity of the firing level. The following properties contribute to timing-dependence: (i) *Temporal summation of successive EPSPs*. (ii) *Interaction*. Each EPSP is characteristically augmented or, more frequently, decreased by those preceding it; linear summation is a special case. The modification in general cannot be predicted from the excitability cycle after one EPSP; interactions between EPSPs of different origins are often irreciprocal; sequence of heterologous inputs is influential. (iii) *Post-spike excitability*.

Responsiveness changes which follow a post-synaptic spike depend on whether it is preceded by others, on their number and timing.

4. Nerve cells were thus found to exhibit a sensitivity to timing that issued from elementary functional attributes. Such sensitivity to timing would be biologically advantageous, especially in areas of sensory convergence, for it provides an additional coding parameter complementing mean frequency modulation. It is still too early to decide whether or not this mechanism is actually important in natural operation, though it appears likely.

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