STIMULUS FREQUENCY AS A MEANS OF ANALYZING SYNAPTIC ACTIVITY

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CONSIDERING that many sense organs activate the centres by long trains of impulses it is surprising how little is known about the central effect of this mode of stimulation. In this laboratory Bernhard and Skoglund (2) have shown that a transient reflex pattern can be evoked in cats by restricting stimulation of certain nerves (e.g., the popliteal) to certain frequencies. It consists of late widespread extensor contractions in all limbs, rise in blood pressure, pupil dilatation, etc., is absent below frequencies of 50 per sec., maximal around 100 per sec., lasts some 20–30 sec. and then disappears despite continued stimulation, even at optimal frequency. Their result shows that stimulus frequency may be a means of selecting certain reflex patterns and is of interest in view of the fact that this frequency range is well within the limits of normal sense organ activity.

Gasser’s laboratory (9, 13, 10) has supplied us with important information about the effect of stimulus frequency upon peripheral nerve, and Lorente de Nó and Graham (20) have shown that the summation of subnormality known from peripheral nerve and augmented by repeated stimuli, is of significance in the recovery cycle of motoneurons. These studies have drawn attention to the depressing effects of frequency, analogous in peripheral nerve and centres, but there must also be slow facilitating effects to account, for instance, for slow “recruitment” (16).

Depression of synaptic activity, “recruitment” and selective effects of frequency are phenomena which also have turned up in this work, which is devoted to preliminary observations on the effect of stimulus frequency on direct and relayed waves caused by stimulation with electrodes buried in the spinal cord when the response is recorded from the sciatic nerve (22, 17). Prior to analyzing the effect of long trains of impulses under separately evoked facilitating and depressing influences it was deemed necessary to possess some information about the effects of frequency as such on direct and relayed waves.

METHOD

Stimulating electrodes have been a pair of needles, at a distance of 2 mm., insulated except at the tip, thrust into the spinal cord of decerebrate cats through the unopened dura in the lumbar region, generally in a ventro-lateral position, sometimes in the dorsal root region. The sweep circuit operating through an amplifier has been connected through a compensator bridge to these electrodes. A maximal rate of 850 per sec. could be obtained by these means. The strength of the stimuli was independent of rate.

In most experiments the leads were on the sciatic nerve, one of them on the crushed end, and connected to a balanced condenser-coupled amplifier.

The sweep circuit drove the beam of the cathode ray horizontally at each stimulus and the film was passed slowly at right angles to the sweep. Now and then the film was
stopped and several records superimposed, so as to give an average picture of the effect. Rather slow speeds had to be used to save film. As the sweep was linear no time marking was necessary and the total width of each picture, being inversely proportional to stimulus frequency, measured time directly. As a consequence the events recorded are expanded horizontally by an increase in stimulus frequency.

The cat was surrounded with vapour from a layer of water covering the bottom of the screened animal-box heated from below. The rectal temperature was around 37.5–38.5°. The animal was undenervated with the exception of the nerve to be recorded from, as our intention was to allow a background of free play of impulses from the natural sources.

**Results**

1. *General description of response.* Confirming Renshaw (22) and Lloyd (17) we find the direct spike wave caused by stimulation of the ventral horn cells or their axons, succeeded by one or several relayed waves. The direct wave is easily recognized by measuring its latent period which is practically equal to the conduction time at alpha velocity (around 80–100 per sec.). Simpler still is to diagnose it by stimulating with a high frequency. As is to be expected from the work of Gasser (9) direct waves generally follow the whole range available in the stimulator (350 per sec.) whereas relayed waves disappear far below this value. By subtracting the latent period of the direct wave from those of relayed waves the central reflex times of the latter are obtained. These, as we shall see, vary from case to case. The necessity of having a direct wave has restricted us to stimulation of places giving such waves (17) or to crossed stimuli symmetrically placed on the opposite side of a place for which the direct waves have been measured previously.

By means of Fig. 1, a typical experiment will be surveyed. The recording electrodes are on the undivided sciatic nerve. Stimulation begins in A with frequency 70 per sec. After 20 impulses the film is stopped for a moment. Two large waves are then clearly seen; the first, the direct wave with an average latent period of 0.9 msec., the second a relayed wave with an average latency of 3.0 msec. The central reflex time of the relayed wave is thus 2.1 msec., assuming that it is conducted in the same fibres as the direct wave. But going back in the same record A upwards there can be seen a second relayed wave, best marked in the beginning and fading out as stimulation proceeds. Its latent period is 5.7 msec. Subtracting the direct wave makes the central reflex time of the second relayed wave 4.8 msec., still assuming it to be conducted by the fibres conducting the direct wave. This assumption is a necessary approximation.

Record B of the same figure corresponds to stimulation frequency 100 per sec. Both relayed waves are seen in the beginning, the first alone when somewhat later (bottom of record) the film is stopped for a moment in order to make a good print. This frequency is the limiting frequency for the second relayed wave. It is, of course, possible to push the limen somewhat higher. For practical reasons it is, however, easier to take as the limiting frequency a value for which in the beginning of each record there are still traces left of the wave to be analyzed, say, for the first three to
five stimuli. Using this criterion the relayed wave with the central reflex time 4.8 msec. possesses the limiting frequency 100.

In the next record C, at stimulus frequency 140 per sec., only the direct and the first relayed wave are seen, further apart now, while this frequency means that the sweep traverses its path in 7.15 msec. as against 14.3 msec. in record A. The width of the sweep path in msec. is obtained from the inverse value of the frequency. In record D the frequency is 198 per sec. The first relayed wave still persists though now pushed near the end of the picture. Its latent period is shorter in the beginning of the record and lengthens downwards. In record E, at frequency 230 per sec., the first relayed wave is present only in the beginning. In record F the frequency is 268 per sec. and this is the limiting frequency for the first relayed wave with central reflex time 2.1 msec. which is still seen in a few initial beats. In the last record G the frequency is 310 per sec. Only the direct wave is seen. At this frequency the greater part of the relayed wave would have passed over the shock artifact to reappear in the beginning of the sweep, if present. In records D, E, and F it is seen to have done so.

The experiment shows that the limiting frequency is reduced by a
lengthening of the central reflex time and that, as stimulus frequency increases, the relayed waves disappear, so to speak, in good order and not by getting lost in general desynchronization. An essentially constant response pattern (but see below) is kept up until the limiting frequency is reached. During continued stimulation at higher frequencies the waves rapidly flatten out and disappear.

Figure 2 is introduced to show another type of pattern in which the direct wave is large and the first and second relayed waves are small because the stimulus is placed low in the cord. At slow speeds, A and B, the fast direct wave merely appears as a gap in the path of the sweep, but its size is shown late in record B when the film has been stopped for a while.

![Image of waveforms](image)

**Fig. 2.** Leads on the popliteal nerve. Bipolar stimulating needle electrodes just above the crista. A, at 100 per sec.; B, 170 per sec.; C, 230 per sec.; D, 268 per sec.; E, 310 per sec. Limiting frequency between D and E, nearer to the latter. To be read downwards.

Figure 3A shows some portions of the course of the typical diminution of the two relayed waves of a response caused by repetition of the stimulus. The frequency is 140 per sec. This diminution which need not be noticeable for several seconds at lower frequency is also determined by the strength of the stimulus. Thus in Fig. 3, at 100 per sec., the stimulus is strong in record B and weaker in record C. The relayed wave decreases rapidly in C and not at all in B. The film is stopped after practically the same number of stimuli in both B and C, taken in successions with stimulating and recording electrodes in the same position. Record D of Fig. 3 illustrates the often relatively complex response of crossed stimuli to the spinal cord. It may, however, be much simpler. Records E and F in another experiment, taken at different rates of stimulation show the first wave to possess two peaks.
The first peak is the direct wave, as measurements show, but how about the second peak in this case? The frequency test showed it to be present at rates (600 per sec.) as high as to be above any values seen with other relayed waves. It is, at least at this stage of our work, safer to regard secondary waves of this character as direct waves delayed by slower conduction rates in the motoneurones concerned.

Evidence for dispersion of the relayed waves is found in most records (22, 17). They are nearly always wider than the direct waves meaning that the synapses themselves differ in kind or number, that the diameter and length of the pathways differ or that facilitation in the short proprio-

![Image](http://example.com/image.jpg)

Fig. 3. A, leads on the peroneal nerve. Continued stimulation of spinal cord at 140 per sec. to illustrate typical decrease of relayed waves. B and C, leads on the sciatic in another experiment. Stimulation at 100 per sec. Stimulus strong in B, weak in C. Record D from another similar experiment shows the complex response often obtained when the stimulating electrodes have been placed on the contralateral side of the spinal cord. E and F from another experiment with the usual homolateral stimulation of ventrolateral side of spinal cord at frequencies 100 (E) and 198 (F) per sec. See text. To be read downwards.

spinal neurones (17) comes on slowly. The potentials set up by the simultaneously active and by the anatomical situation best synchronized synapses probably also activate adjacent elements electrotonically thus contributing to the dispersion. "Grouped action" of this type is very characteristic for motor neurones (23) and probably means a great deal for the characteristic grouping seen here. Nevertheless it is clear that the direct wave does not leave room for the same amount of temporal spacing around the top and thus represents stimulation of a more uniform system.
2. Recruitment. A relayed wave which is fully developed from the beginning generally decreases in size during continued stimulation (cf. Fig. 3), provided that the frequency be sufficiently high. But quite often relayed waves are encountered which actually increase in size as a consequence of repetitive stimulation so that the total wave pattern is better developed after some time than in the beginning of a record. This result signifies that new neurones have been recruited into activity, partly perhaps also gradually improving synchronization. Lloyd (17) has studied the effects of facilitation to two or three shocks and also points out that the effect refers to grouped discharges with characteristic central reflex times.

Figure 4 illustrates a typical case. It begins with record A (to be read upwards) at frequency 70 per sec. In the beginning only the initial direct wave with latent period 1.0 m sec., followed by the second relayed wave with latent period 3.8 m sec., are clearly defined. Gradually a small wave becomes better visible between them. This is the first relayed wave with latent period 2.4 m sec. The direct wave is of practically constant size throughout the record, but both the first and the second relayed waves increase in size up to about the 9th stimulus. At this size they were seen to remain during stimulation for a couple of seconds. A late portion of the film is found in the second row (A). Record B is around the 60th stimulus at rate 100 per sec. All three waves are clearly visible. Record C, continued in the third row, shows the beginning of the film taken at stimulation rate 140 per sec. The second relayed wave is now quite well developed from the beginning, but the first relayed wave is broad and not definitely set off from the rest until the 9th stimulus has been reached. The same record, continued in the next row, illustrates almost regular variations in the size of the relayed waves of the kind quite often seen at high frequencies. The
interesting point here is that the first and second relayed waves always vary in parallel, indicating a common influence on both of them, reminiscent of the intermittent conduction described by Barron and Matthews (1), rather than of the alteration seen at high frequencies in peripheral nerve. The latent periods of the three waves are here: 1.4, 2.9, and 4.9 msec. Their average latencies in several series were 1.3, 2.6, and 4.4 msec. Thus the average central reflex times of the first and second relayed waves were respectively 1.3 and 3.1 msec. corresponding to measured limiting frequencies of 340 and 200.

3. Synaptic resonance. Figure 5 begins with record A at a frequency of 160 per sec. There is a large direct wave with latent period 1.1 msec. It is followed by a very small relayed wave with latent period 2.3 msec. and a second broad relayed wave at latent period 3.6 msec. The first relayed wave increases in size during stimulation in this and in the following record B at 198 per sec. In the next record C at stimulation rate 268 per sec. the first relayed wave is 4-5 times larger than in the beginning of the first record (A) and remains large for a considerable time during stimulation. Thus it has an optimum around this frequency range. The second relayed wave is still present in record D at 310 per sec. but on the verge of disappearing in record E at 360 which is the limiting frequency of this wave with an average central reflex time around 1 msec.

Certain relayed waves are often absent or questionable at slow rates of stimulation below 70 per sec. and turn up at higher rates again to disappear when the frequency increases. There are thus at times optimal rates of stimulation for certain relayed waves and in this sense true frequency resonance (2).

4. Limiting frequency and central reflex time. In correlating limiting frequency and central reflex time it is necessary to realize at the outset that such measurements cannot claim great accuracy. Several factors contribute to make precision unattainable. Latent periods are easier to measure with precision than limiting frequencies but partial overlapping of the waves in some cases and slightly increased latencies at faster rates of stimulation in others may make decision difficult. The average latent periods, leaving out the first 4-5 stimuli, have been used.
The limiting frequencies often interfere with the stimulus artifact and then may be difficult to determine. This is the main source of error especially at high frequencies. The relayed waves may be small or badly defined and we have no means of appraising strength of stimulation for a relayed wave, except in a very general manner. The limiting frequency in peripheral nerve, theoretically determined by the refractory period, is also determined by strength of stimulus and conduction distance (9) as well as by temporal dispersion caused by slowed conduction and increased latency (3). At higher frequencies it is necessary to use a brief period of stimulation and long intervals (9). These precautions have been observed. But the difficulties mentioned, in determining limiting frequency and others not particularly enumerated, all serve to emphasize that our curve, shown in Fig. 6, cannot do more than indicate in a very general manner the relationship between limiting frequency and central reflex time. There are also physiological factors causing variations when in other respects optimal conditions for measuring have prevailed.

In Fig. 6 data from crossed stimulation are marked by broken lines. It is at once seen that these data tend to be grouped in the lower half of the curve, whereas those taken from experiments with ipsilateral stimuli are grouped around the upper half of the curve. There are exceptions from this rule. But in general the central reflex times are longer for delayed waves elicited by crossed stimuli.

The upper curve is put in to illustrate the theoretical limiting frequency given by the inverse values of the central reflex times. The vertical distance between the theoretical and the experimental curve is, of course, also a function of the definition of the limiting frequency. The experimental curve could be pushed upwards by requiring only two shocks to be effective at a given frequency of stimulation, but practical reasons have necessitated a different choice of index, as pointed out above. But, if the experimental curve is lifted upwards to coincide with the theoretical curve at the lower end it can be seen that the two curves diverge for short latencies, meaning that the corresponding high frequencies possess a depressing effect augmented by stimulus repetition (cf. Fig. 1). This no
doubt is identical with the effect described by Graham and Lorente de Nó (13) and by them identified with the subnormality accompanying positive afterpotentials in peripheral nerve (10). As is well known from Gasser's work development of subnormality is greatly favored by rapid stimulation rates.

The shortest reflex times found in these experiments have been between 0.7 and 1.0 msec. Values below 1 msec. must refer to a single synapse (22, 17, 6). The maximal limiting frequencies have been about 360 per sec. Considering the rapid onset of subnormality with repetition, the absolute limiting frequency, defined as the capacity to pass only two volleys, would probably be a great deal higher. Alternation and intermittent conduction may also come in and complicate such determinations.

**Discussion**

The curves of Fig. 6 are interesting from two points of view, one eminently practical, the other theoretical. From the practical point of view it means that it may become possible to use curves of this type for analyzing the degree of complexity of the path between two points anywhere in the central nervous system, provided that the relation found is confirmed for other systems and that definite grouped discharges occur. So far we have only analyzed the relayed waves found in the dorsal columns (14) which have limiting frequencies falling around our curve. In this system there is also a direct wave which, of course, is necessary for establishing the form of the standard curve. But once this curve has been established for a given index in measuring limiting frequencies, the central reflex time can be located on it from measurements of limiting frequency alone, while the curve eliminates an important unknown factor, the datum provided by the direct wave. This can, of course, only be obtained for a relatively limited number of combinations of stimulating and recording electrodes.

When using the limiting frequency in this manner as an instrument for determining the central reflex time one may well ask whether it is possible to assume any fixed relationship between number of synapses and limiting frequency. Probably there exists some general relationship and probably number of relays is a decisive datum in determining a given point on the curve. But the dispersion of the relayed waves and the great variations indicate that other factors also play a rôle. The "delay path" (Forbes) responsible for a central reflex time of 5 msec. may, for instance, be determined by a minimal number of five synapses but delayed conduction owing to decreased fibre diameter and lengthened conduction distance are factors which hardly can be neglected and suggest some caution in interpreting the data. Still, we are inclined to hold that a minimal average number of 1 synapse per msec. is a reasonable assumption. The number may be greater but hardly less. It should also be realized that the first synapse occupies a favorable position compared with synapses placed later in the path on account of the more perfect synchronization of the stimuli for this synapse. This may be the reason why, as we have found (unpublished data), the
motor end plate with a synaptic delay of 0.5 msec. (7) has much higher limiting frequencies than those found in the central nervous system, though in the latter the synaptic delay is of the same order (18, 19). A highly dispersed discharge as such would indicate complex pathways.

From the theoretical point of view the interest centers around the relation of these results to possible mechanisms of inhibition. Gasser’s view that subnormality may be one such mechanism is borne out by the frequency effect upon the relayed waves activated by high frequencies capable of piling up subnormality very quickly. The frequencies concerned are within the physiological limits of the sense organs. For this type of inhibition McCouch, Hughes and Stewart (21) very properly suggest the use of Dusser de Barenne’s term “extinction.” But a new possibility is introduced by the fact that the delayed discharge caused by every single repeated stimulus is cut down from the “tail” end by relatively moderate frequencies. This suggests that long-synapse paths are particularly inhabitable merely on account of the repetitive nature of the physiological stimulus. It also suggests that inasmuch as the natural stimuli for some reason or other set up high frequencies the immediate effects are transmitted with preference into the synaptic channels of least resistance, i.e., those with brief central reflex times. It is clear therefore that a frequency variation as such apart from how it may facilitate or block certain paths is a mechanism of significance in determining the central distribution of an effect entering a path containing synapses.

Late facilitation effects such as the recruitment and the synaptic resonance, described above, emphasize aspects of stimulus repetition which do not come out in the curve plotted as Fig. 6. Considering that in the superior cervical ganglion there is a late facilitation which has not required internuncial bombardment (4, 5); that this phase coincides with a slow negative potential change, and, further, that recruitment in peripheral nerve is favored by factors favoring the negative afterpotential (12, 8), it is probable that slow potential changes may serve to modify the central processes also in a positive direction. Synaptic resonance, demonstrated so clearly in the transient reflex discovered by Bernhard and Skoglund (2), is an interesting consequences of what different balancing influences may lead to.

**Summary**

When trains of electric stimuli are led through needle electrodes to the spinal cord certain wave patterns are set up which have been recorded from the sciatic nerve (decerebrate cats). These consist of a direct wave caused by stimulation of the ventral horn cells and by a number of relayed waves following after different latent periods.

By subtracting the latent period of the direct wave from those of relayed waves the central reflex times of the latter are obtained.

The direct waves follow frequencies of stimulation above 850 per sec.
The relayed waves follow frequencies which decrease with increasing central reflex times. A curve illustrating limiting frequency as a function of central reflex time is given in Fig. 6.

Some relayed waves gradually recruit neurones and thereby increase in size, others have definite frequency optima and thus demonstrate the existence of synaptic resonance.

The findings are discussed in relation to excitation and inhibition and also with a view to suggesting use of the limiting frequency as a method of studying the central paths.

Variations in frequency as such can serve as a means of distributing into special channels effects entering a central path containing synapses.

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