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A FURTHER ANALYSIS OF THE EFFECTS OF HIGH-FREQUENCY EXCITATION OF NERVE.

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Cattell and Gerard [1934, 1935] have made the very interesting observation that, using separate high- and low-frequency stimulation, when the high frequency alone is giving far less than the maximum response (owing to the frequency) a similar stimulus of lower frequency applied simultaneously above, below, or at the same electrodes may produce a large increase. No block occurs at the electrodes to impulses started above them, provided that the shocks constituting the high frequency stimulus are not too prolonged; if they are, an electrotonic effect is produced [Bugnard and Hill, 1935] and impulses are unable to pass.

Prolonged condenser discharges of sufficient intensity (*e.g.* $RC = 5000$ μsec.; 4–6 volts; nerve at 5°; 10 shocks per sec.) will produce impulses running in one direction only along the nerve. An impulse started from the cathode is unable to penetrate the region between the electrodes. If the intensity is less (*e.g.* 2 volts), or if the discharge time is less (*e.g.* 250 μsec.), an impulse will travel both ways from the cathode. This, of course, is the same thing as Pflüger's law, applied to condenser discharges.

As regards simultaneous stimulation at the same electrodes by high and by low frequency, it was possible, as Cattell and Gerard suggest, that some physiological state is built up by regular high-frequency excitation which is "maintained with little fluctuation," so that the individual shocks do not excite. The high-frequency shocks might produce an effect like a weak constant current, and so fail to set up a regular rhythmic response. The low-frequency shocks might then excite in the normal way as they would in a weakly polarized nerve. There are two objections to this:

(1) The calculated electrotonic effect, for shocks of short duration, is too small at the frequencies considered.

(2) Discharges alternating in direction show a similar, though admittedly smaller diminution of response at high frequency.

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We believe that the effect found by Cattell and Gerard has a simple explanation, viz. (1) that the fibres are maintained by the high-frequency stimulus in a fluctuating relatively refractory state, responding only occasionally (see Fig. 1), and (2) that some of the shocks of the low-frequency series must necessarily fall so close to shocks of the high-frequency series that summation of stimuli occurs; the nerve, being for a large part of its time only just refractory to the high-frequency shocks, is excited by those which happen to be increased by the near coincidence of shocks of the low frequency. According to Erlanger and Blair [1931],

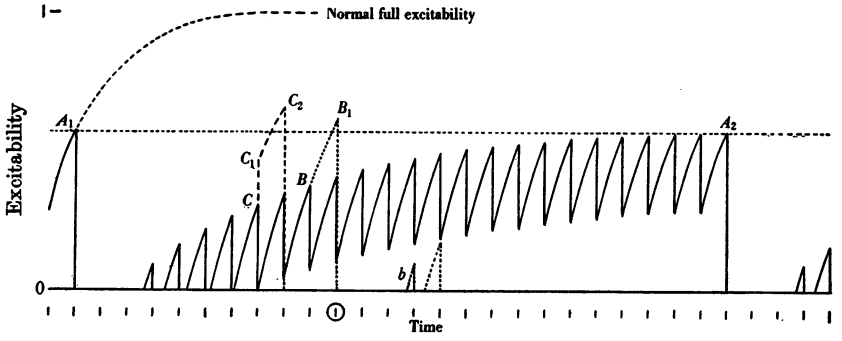

Fig. 1. Diagram to illustrate hypothesis of excitability changes during high frequency one-way stimulation with condenser discharges of short duration. Excitability of nerve vertically (as reciprocal of shock intensity required to excite). Time horizontally. Shocks shown by short vertical lines. The excitability falls to zero (A_1 and A_2) after an effective shock; then begins to rise again but is depressed by each succeeding ineffective shock, so only gradually reaching the level at which a shock is again effective. At B (dotted line) one shock is supposed omitted. The excitability continues to rise (instead of being depressed) and reaches the critical value within the next interval: at B_1 the nerve is excited and at b the process of recovery starts again. At C (broken line) one shock is supposed reversed in direction: the refractoriness is instantly diminished (excitability increased to C_1), recovery continues to C_2, when a shock is effective. The broken line upwards from A_1 represents the return of excitability as it would be if stimulation ended at the left of the figure.

following a shock of itself just sub-threshold, there is a phase during which another shock can sum with it to produce a greater response. If this summation phase (at 20° C.) be 150 μsec., and if one of the low frequency shocks fall within ± 150 μsec. of one of the high frequency, it will sum with it and excite. The interval of possible summation, therefore, is 300 μsec., the total interval between shocks of a 2000 per sec. series is 500 μsec., so that 60 p.c. of the low-frequency shocks should, on the average, be effective.

This explanation was tested as follows. During a high frequency stimulus, (*A*) one out of every sixteen shocks was increased in intensity; (*B*) an extra shock was introduced exactly half-way between two (out of every eight) of the high-frequency shocks. On the hypothesis of summation (*A*) should produce an extra response, (*B*) should not. On the hypothesis of a uniform physiological state set up by the high-frequency stimulus, both should have the same positive effect. The answer was decisive: (*A*) always produced an increase in the response; with certain qualifications (see below) (*B*) did not. The physiological state, therefore, was not uniform, summation occurred when it should, an extra shock failed when summation was not to be expected: the fibres were being maintained in a fluctuating relatively refractory state by the high-frequency stimulus, with only occasional response.

The hypothesis on which the following experiments will be discussed is illustrated in Fig. 1. The excitability of a nerve fibre, as the reciprocal of the shock intensity required to excite it, is given as a function of the time during a high-frequency stimulus. The shocks are shown by the vertical lines below the axis. After an effective shock (A_1, A_2) there is an absolute refractory period followed by a relatively refractory period, the excitability gradually increasing but being depressed by each of the ineffective shocks of the stimulus. Finally another shock is effective. At *B* (Fig. 1) is the case of a single omitted shock. The excitability continues to rise of itself to B_1 and the next shock is effective, although, without the omission it would not have been. At *C* (Fig. 1) is the case of a single shock in the opposite electrical sense: this instantly raises, instead of depressing, the excitability, and a response occurs to the next shock.

One of the sixteen charging segments of a thirty-two segment commutator [see Hill, 1934, Fig. 1] was isolated from the ring to which the other fifteen were connected, and provided with a separate connection to a battery and key. In this way one in sixteen of the shocks of a high-frequency stimulus could be given any required intensity, positive or negative, or omitted altogether: or, by opening their key, the fifteen could be omitted and only the one used. The rest of the circuit was the same, for the one and the fifteen (cf. Fig. 2 below, left-hand side), so the intensity alone (not the discharge time, which was kept at $12\frac{1}{2}$ μsec.) could be varied. Using the one only, the shocks (at 125 per sec.) were normally effective, although, using all sixteen (at 2000 per sec.), with the commutator running at the same speed, the response was greatly diminished. This is a satisfactory confirmation of the conclusion from

direct tests that the commutator, even at the highest frequency, works correctly.

Let us call the fifteen shocks A and the one shock B, and denote by a suffix the voltage used. The following experiment may be quoted: nerve circuit about 40,000 ohms; shunt 250 ohms; 0·05 μF; 20° C.; 2000 per sec. for A and B together.

$A_{20}+B_{20}$	response 140 mm. diminishing rapidly
$A_{20}+B_{40}$	208 mm. not diminishing rapidly
B_{20}	200 mm.
B_{40}	208 mm.

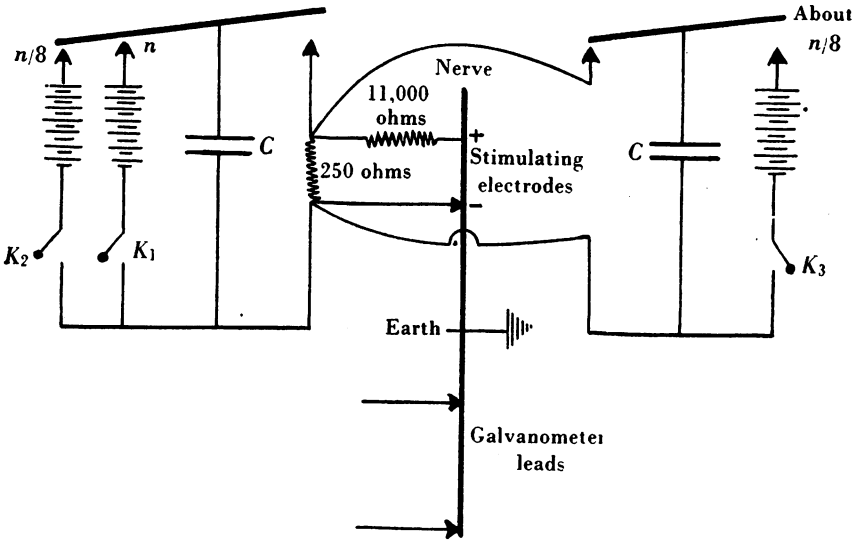

Fig. 2. Arrangement for stimulating with frequency n (key K_1), $n/8$ (key K_2), about $n/8$ (key K_3), separately or together. The shocks from K_2 fall exactly half-way between two (out of every eight) of the shocks from K_1: the shocks from K_3 fall independently of those from K_1. The two circuits to the right and left can interfere with one another, according to their phase, but only to the extent of sharing the charge instantaneously between the two condensers: this had no effect, since the discharge time chosen was so short that the quantity discharged was the sole effective agent in excitation.

The nerve, with $A_{20}+B_{40}$, was apparently responding to the B_{40} alone.

In other similar experiments the effect of varying the intensity of the B stimulus was studied:

$A_{20}+B_0$	response 225	$A_{20}+B_{30}$	response 275
A_0+B_{20}	260	$A_{20}+B_{26}$	230
$A_{20}+B_{20}$	40	$A_{20}+B_{22}$	140
$A_{20}+B_{40}$	275	$A_{20}+B_{20}$	50

and again:

B_{20}	response 265	$A_{20}+B_{20}$	response 30
B_{16}	250	$A_{20}+B_{16}$	90
B_{12}	175	$A_{20}+B_{12}$	100
B_{8}	125	$A_{20}+B_{8}$	175
B_{4}	0	$A_{20}+B_{4}$	180
B_{0}	0	$A_{20}+B_{0}$	185
B_{-6}	40	$A_{20}+B_{-6}$	260
B_{-12}	175	$A_{20}+B_{-12}$	290
B_{-16}	250	$A_{20}+B_{-16}$	290
B_{-20}	270	$A_{20}+B_{-20}$	300

These results precisely illustrate the hypotheses embodied in Fig. 1, viz. (1) that during the relatively refractory phase an ineffective shock produces at its cathode a proportional decrease of excitability, at its anode a proportional increase, and (2) that most of the shocks of a high-frequency stimulus are ineffective because the nerve is kept in a relatively refractory state. Increasing one in sixteen of the shocks, even a little, produces a large effect: increasing them all, however, has only a small effect, since a greater degree of refractoriness is produced immediately by greater ineffective shocks. The nerve, in fact, for a large part of its time, is kept, by high-frequency stimulation, just below the level at which it is excited by whatever intensity is employed.

If this view be correct, the results just described should not be found with a stimulus of lower frequency: this is the case. For example:

20° C.: 333 shocks per sec.

$A_{20}+B_{20}$	response 299
$A_{20}+B_{40}$	303
$A_{20}+B_{0}$	290
$A_{0}+B_{20}$	83

The result is quite different: all the shocks of A are effective: introducing B merely increases the response. An intermediate case was observed at 1200 shocks per sec.

$A_{20}+B_{20}$	response 260
$A_{20}+B_{40}$	290
$A_{20}+B_{0}$	280
$A_{0}+B_{20}$	158

The experiments were repeated at 5° C., with identical results, except of course that these occurred at a lower frequency. With a very high frequency, which is relatively higher for the cold nerve, the omission of one shock in sixteen had no effect—the interval was not long enough to allow a sufficient degree of recovery to occur.

Influence of the duration of stimulation at high frequency on the efficacy of a gap in the stimulus.

Experiments made (at 5° C.) with relatively low-frequency excitation had shown that the refractory period increases as stimulation proceeds. The omission of one in every sixteen of the shocks of a high-frequency

stimulus may produce a large increase in the response, by allowing time for recovery to above the threshold of excitation; but at very high frequencies—unattainable experimentally except at a low temperature—this increase does not occur. It was foreseen therefore that, with prolonged stimulation, leading to an increase in the refractory period and hence probably to a decrease in the speed of recovery, the efficacy of a gap in the regular series would be diminished. This is the case at both temperatures; for example at 20° C. and 2000 shocks per sec.

Time min.	Stimulus	Response	Stimulus	Response
0	$A_{20}+B_{20}$	80	A_{20}	175
2	,,	68	,,	126
4	,,	53	,,	93
6	,,	50	,,	85
6	$A_{20}+B_{40}$	240	B_{20}	240
20	$A_{20}+B_{20}$	15	A_{20}	25

After 6 min. excitation the response of the nerve to an effective stimulus was as great as ever, but the response to high frequency was considerably diminished. Judging from the last entry where, after 20 min., the response to high frequency was about zero, and with the gap scarcely more, prolonged stimulation with shocks not 1 p.c. of which were effective kept the rate of recovery at a greatly diminished level and the relative refractoriness much enhanced.

Let us turn next to the type (*B*) of experiment referred to on p. 418 above. A commutator was arranged to have (i) eight "charge" segments at regular intervals all connected to one ring, and (ii) one "charge" segment exactly half-way between two of the eight, separately connected to a battery and key. By this means an extra shock of any desired intensity could be introduced at the middle of one interval in eight. It was impossible to drive the commutator fast enough to get the frequencies required for a nerve at 20° C.; a temperature, therefore, of 5° C. was employed.

The frequency from the eight regular segments being n, that from the extra segment is $n/8$. Another commutator, separately driven, was arranged to give a frequency about $n/8$ of similar shocks at the same electrodes. It was possible for the two circuits (see Fig. 2) to interact, so that the charge of one condenser was instantly shared with the other before discharging through the nerve. Since, however, discharges of very short duration ($12\frac{1}{2}$ μsec.) were employed, the effective agent in excitation was the quantity of electricity, and this was unaffected by the sharing of the charge between two condensers and its rather slower discharge to the nerve. The second commutator was to provide shocks at a gradually

altering phase relatively to the first, instead of exactly between two out of every eight.

Let us call A, B and C the stimuli of frequency n, $n/8$ and about $n/8$ respectively, B being exactly half-way between two of A, C being independent of A and B. Sometimes C will coincide with units of A, in which case summation will occur; sometimes C will fall exactly between units of A, in which case there will be no summation. A beautiful phenomenon was seen at once, viz. that with A running at an appropriate frequency n, and C at nearly (but not quite) $n/8$, the response waxed and waned as C came in and out of phase with units of A, *i.e.* as summation occurred or failed. Other results were as follows:

(i) Over the plateau of the response-frequency curve, up to 150–200 shocks per sec., $A+B=A+C=A$: B and C can have no extra effect when superimposed on A, since the response is already at its maximum.

(ii) From 150–200 to 300 per sec.

$$A+B = \text{or is slightly} < A.$$
$$A+C > A.$$

The reason for the former is that the extra shocks of B fall when the nerve fibres are even more refractory than they are to the shocks of A: and the B shocks may make the nerve more refractory, acting indeed much as though the frequency were increased by $n/8$. The C shocks, however, can fall at any phase of the cycle, and many of them will sum with units of A as described above.

(iii) Above 300 per sec.

$$A+B \text{ is slightly} > A,$$
$$A+C \text{ is largely } > A.$$

In this range the B shocks also are beginning to come close enough to the A shocks to sum with them for some of the fibres. The average summation effect of C with A is greater the shorter the interval. With a higher frequency of A more of C will come within the summation interval. A typical experiment is as follows:

Frequency A	Response A		
99	142	$A+B=142$	$B=67$
		$A+C=142$	C (12·5 per sec.) $=68$
194	110	$A+B=110$	$B=95$
		$A+C=125$	C (27 per sec.) $=100$
349	60	$A+B=63$	$B=128$
		$A+C=95$	C (50 per sec.) $=135$
418	35	$A+B=45$	$B=138$
		$A+C=90$	C (50 per sec.) $=138$

If, at a high frequency, the shocks of B and C are reduced in intensity, no summation occurs between B and A, considerable summation between C and A. For example, with A 512 per sec., B 64 per sec., C 65 per sec., and denoting volts by suffixes, responses at 5° C. were as follows:

$A_{20}=30$	$A_{20}+B_{10}=30$	$A_{20}+C_{10}=80$
$A_{20}=30$	$A_{20}+B_{14}=35$	$A_{20}+C_{14}=90$
$A_{20}=25$	$A_{20}+B_{16}=35$	$A_{20}+C_{16}=90$
$A_{20}=15$	$A_{20}+B_{20}=25$	$A_{20}+C_{20}=85$

C at 10 volts is capable of producing a large effect when superimposed on A at 20 volts, B at 10 volts has no effect at all.

If, at high frequencies of A, the shocks of B and C are in the opposite electrical sense to A,

$$A_{20}+B_{-20}=A_{20}+C_{-20}>A_{20}$$

The shocks B and C cannot in this case be effective by summation, since summation between shocks of the opposite sign is impossible. They must act by destroying the refractory state left by preceding shocks, and allowing the next shock to be effective. For example, with the same frequencies as above:

$A_{20}=45$	$A_{20}+B_{20}=70$	$A_{20}+C_{20}=130$
$A_{20}=45$	$A_{20}+B_{-20}=185$	$A_{20}+C_{-20}=180$
	$B_{\pm 20}=C_{\pm 20}=185$	

It looks as though the negative interposed shock, whatever its phase, causes the next shock to be completely effective. The improvement in excitability—in recovery from refractoriness—effected by a shock in the opposite direction is the reason why, at high frequency, two-way stimulation at frequency $2n$ is more effective than one-way at frequency n. At low frequency the two-way is more effective than the one-way simply because it gives twice the number of actual responses.

Discussion.

All the facts described are in keeping with the simple hypothesis that excitation is associated with the removal, by the stimulating current, of some (probably charged) body X, from the neighbourhood of the cathode. After a shock, effective or otherwise, there is a period during which the excitability at the cathode is depressed: X has not yet returned to its full concentration. High-frequency one-way stimulation keeps the concentration of X low. A gap in the stimulus allows the concentration to be restored naturally; a shock in the reverse direction forces it up electrically. Fig. 1 can easily be expressed in these terms. No clue is given by the present experiments as to the nature of X; that might be decided, however, if there be any truth in the theory, by experiments with specific substances on the present lines.

SUMMARY.

1. Cattell and Gerard have found that a high frequency stimulus, itself producing a very small response, does not prevent a nerve from responding to a stimulus of lower frequency, applied above, at, or below the electrodes of the high frequency.

2. The effect of a low-frequency stimulus at the same electrodes is shown to be due to summation occurring between units of the two stimuli.

3. During high-frequency stimulation a nerve is kept in a relatively refractory state, each ineffective shock depressing the excitability, which only gradually rises to threshold. Response in each fibre is thus only occasional. Response can be made more frequent (*a*) by regularly allowing a double interval for recovery, (*b*) by regularly increasing the intensity of one of the shocks, (*c*) by periodically inserting a shock in the opposite electrical sense.

4. A diagram (Fig. 1) is given, illustrating the excitability changes during high-frequency stimulation. The hypothesis contained in it is in keeping with the results of Erlanger and Blair [1931], and with all the facts observed in the present research. The central point of it is that shocks which are "ineffective," owing to falling in the refractory period, nevertheless depress the excitability and extend the refractory state: or, if in the opposite electrical sense, raise the excitability and shorten the refractory state.

5. It is suggested that in the process of excitation some substance is removed by the current from the neighbourhood of the cathode, and recovery consists in its spontaneous return. The relatively refractory state is associated with its decreased concentration. One-way shocks keep its concentration low and the state refractory. A shock in the opposite electrical sense tends to force it back and so to restore normal excitability.

We are indebted to Dr R. W. Gerard for much suggestive discussion: and to him and Dr Cattell for the impetus supplied by the new facts described in their preceding paper.

REFERENCES.

Bugnard, L. and Hill, A. V. (1935). *J. Physiol.* **83**, 394.
Cattell, McK. and Gerard, R. W. (1934). *Ibid.* **83**, 26*P*.
Cattell, McK. and Gerard, R. W. (1935). *Ibid.* **83**, 407.
Erlanger, J. and Blair, E. A. (1931). *Amer. J. Physiol.* **99**, 108.
Hill, A. V. (1934). *J. Physiol.* **82**, 423.