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THE DISCHARGE OF IMPULSES FROM GANGLION CELLS

By J. C. ECCLES

From the Physiological Laboratory, Oxford, and the Kanematsu Memorial Institute of Pathology, Sydney Hospital, Sydney

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Under normal physiological conditions the discharge of impulses from sympathetic ganglion cells appears to occur only when these cells are bombarded by impulses in preganglionic fibres [cf. Govaerts, 1935, 1936]. When an impulse in a preganglionic fibre reaches that part which establishes functional connexion (the synapse) with a ganglion cell, it acts on that cell, and may, after a brief interval (the synaptic delay), cause it to discharge an impulse along its axon (the postganglionic fibre). This paper gives an account of investigations into this action of the preganglionic impulse on the ganglion cell. A preliminary account of some of these investigations has already been given [Eccles, 1936*a*]. The methods employed in recording electrically from the ganglion [Eccles, 1935*a*, 1936*b*] and mechanically from the nictitating membrane [Eccles & Magladery, 1937*a*] have already been fully described.

A. The interaction between an antidromic and a preganglionic volley

In 1934 Brown studied this interaction for the S_1 ganglion cells which innervate the nictitating membrane. Single stimuli, adequate to set up maximal volleys, were applied to the preganglionic and postganglionic trunks of the superior cervical ganglion, and the resulting contractions of the nictitating membrane were recorded with an optical isometric myograph. When the preganglionic volley was set up between 9 and 5 msec. before the antidromic volley, the contraction of the nictitating membrane was no larger than for the antidromic volley alone, i.e. under such conditions the refractory period of the ganglion cells or postganglionic fibres

allowed only a single impulse to traverse every postganglionic fibre. With longer or shorter intervals, the increased contraction showed that there was more than one impulse in some of the postganglionic fibres, i.e. the preganglionic volley caused some ganglion cells to discharge impulses at such a long time earlier or later than the antidromic volley that blocking by refractory period no longer occurred.

In Fig. 1 the conditions of Brown's experiments are plotted, as explained in the legend, on time-distance co-ordinates [cf. Eccles & Sherrington, 1931, p. 520], using conduction velocities of 17 and 6 metres a second for the pre- and postganglionic fibres respectively

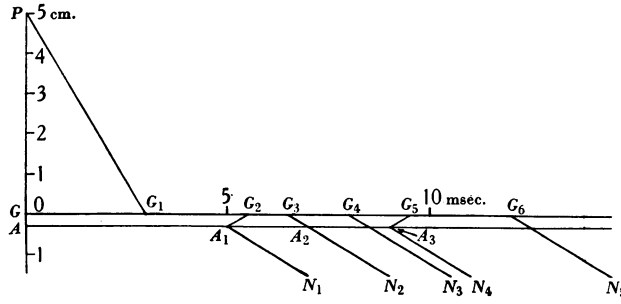

Fig. 1. The courses of impulses in the pre- and postganglionic pathways of the superior cervical ganglion are plotted on time (abscissæ) and distance co-ordinates, P, G and A being respectively the positions of the preganglionic electrodes, the ganglion, and the electrodes at which antidromic volleys are set up in the postganglionic trunk. The oblique lines represent the passage of impulses in the pre- and postganglionic fibres, the conduction velocities being assumed to be 17 and 6 metres per sec. respectively. An approximation is introduced by neglecting the length of the ganglion, but this does not significantly affect the argument developed in the text.

[cf. Eccles, 1935*a*]. PG_1 shows the time course of the fastest preganglionic impulses of a maximal preganglionic volley. This volley sets up the discharge of impulses along the postganglionic fibres and a consequent absolutely refractory period, from which recovery is first detectable at A_3 (9 msec. after P) when a testing stimulus is applied through electrodes placed at A on the postganglionic trunk. Since the absolutely refractory period of the postganglionic fibres is about 2 msec. [Brown, 1934; Eccles, 1936*b*], the earliest postganglionic impulses to be discharged in response to PG_1 must pass approximately through A_2 and so be represented by $G_3A_2N_2$. The shortest synaptic delay is thus G_1G_3, its duration 3·5 msec., being in close agreement with the value derived from direct electrical measurements [Eccles, 1935*a*].

Now, when the maximal antidromic volley is set up earlier than A_1, i.e. less than 5 msec. after P, the response of the nictitating membrane shows that PG_1 has set up a discharge of impulses from some ganglion cells, which of course must occur after recovery from the refractory period following the antidromic volley. As the antidromic volley is moved later, i.e. nearer to A_1, PG_1 sets up a discharge from progressively fewer ganglion cells, until, with an antidromic volley A_1G_2, limiting conditions are reached, any further retardation preventing the discharge from all ganglion cells. It appears from Fig. 1 that, under such limiting conditions, the antidromic volley reaches the ganglion cells even before the earliest discharge $G_3A_2N_2$ would be set up by PG_1, and certainly considerably before more delayed discharges. Moreover, it has been shown that the antidromic volley actually penetrates as far as the locus where the ganglion cell discharge arises and there gives rise to a refractory period [Eccles, 1936*b*, p. 11]. The interval after the limiting antidromic volley, A_1G_2, at which PG_1 must set up its minimal discharge, would be longer than the true absolutely refractory period of the ganglion cells (*ca.* 2 msec. [Eccles, 1936*b*]). Also, presumably, it would be shorter than their functional refractory period as determined by two maximal preganglionic volleys (*ca.* 3 msec. [Eccles, 1935*a*]), for under these latter conditions the second preganglionic volley is diminished in size by the preceding volley. Thus a value of about 2·5 msec. after G_2, as represented by the point G_4, may be taken as sufficiently accurate to illustrate the argument, G_4N_3 thus representing the minimal postganglionic discharge which PG_1 produces after the antidromic volley A_1G_2. Further, PG_1 is unable to set up a discharge later than G_4, for, as we have seen, any further retardation of the antidromic volley beyond A_1G_2 prevents all discharge in response to PG_1. Hence G_1G_4, 5 msec. in duration, represents the upper limiting value to the synaptic delay. Presumably the ganglion cells discharging with that long synaptic delay are likely to be amongst those cells which normally respond during the latter part of the temporally dispersed ganglionic discharge, hence the maximum lengthening of synaptic delay is probably less than 1·5 msec. which is the difference between the above values for the longest and shortest synaptic delays.

The conclusions of this argument are not essentially affected by variations in the choice of the conduction times, refractory periods, etc., so long as these variations are kept within probable limits. Action potential records [Eccles, 1936*b*, pp. 9–13] indicate that an antidromic volley acts similarly on the discharge of impulses which a preganglionic volley sets up from S_2 ganglion cells, the maximum lengthening of synaptic

delay being probably not greater than 2·5 msec. *It may, therefore, be concluded that a preganglionic volley has lost its power of setting up a discharge of impulses from* S_1 *and* S_2 *ganglion cells, if that discharge is delayed by more than* 1·5 *and* 2·5 *msec. respectively by the refractory period following an antidromic volley.*

In most experiments Brown [1934] found that, as described above, a single antidromic volley, when suitably timed, blocked all the discharge which a single preganglionic volley sets up from ganglion cells, and hence concluded that a preganglionic volley sets up the discharge of no more than a single impulse from each ganglion cell [cf. Eccles & Sherrington, 1931, pp. 519–23]. Since an impulse discharged from a ganglion cell gives rise to a refractory period of that cell identical in all respects with that following an antidromic impulse [Eccles, 1935*a*, 1936*b*], the absence of a second discharge parallels the prevention of all discharge by a suitably timed antidromic volley, and again demonstrates the short duration of the excitatory effect produced by a preganglionic volley acting on the ganglion cells.

However, in a few experiments Brown found that an antidromic volley always failed to block completely the discharge set up by a single preganglionic volley, and in explanation he suggested that the discharge of single impulses was so asynchronous that a single antidromic volley was always ineffective in blocking some impulses. In Fig. 1 we have seen that A_3G_5 was the earliest time at which an antidromic volley could be set up after the earliest ganglionic discharge, $G_3A_2N_2$. According to Brown's suggestion some ganglion cells are capable of discharging impulses in response to PG_1 *after* the refractory period set up by A_3G_5, i.e. after G_6. The synaptic delay of such a discharge would be greater than 9 msec., hence a temporal dispersion of more than 5·5 msec. would have to occur. The only possible alternative explanation is that a preganglionic volley sets up the discharge of more than one impulse from some ganglion cells. However, in the present attempt to repeat these experiments, it was found that, only when it was not maximal, did an antidromic volley fail to block all the discharge evoked by a single preganglionic volley; hence there appears to be no necessity to postulate that, in some experiments, a single preganglionic volley sets up either a repetitive discharge from some ganglion cells or a discharge of single impulses having a large temporal dispersion.

Thus it may be concluded that, in so far as the setting up of a discharge of impulses is concerned, a single preganglionic volley exerts on ganglion cells an excitatory action which increases and decays so rapidly that only for a

very brief period (e.g. within extreme limits of 2·2–5 msec. for S_1 ganglion cells) is it above the liminal intensity at which a discharge is set up. The discharge of an impulse is a type of explosive act of a ganglion cell, and the brief action of preganglionic impulses in setting up this discharge resembles that of a detonator; hence it will be called the "detonator action".

B. The relationship of central excitatory state to the discharge of impulses by ganglion cells

It has already been shown that a single preganglionic volley produces in ganglion cells an increased excitability which decays gradually over a period as long as 200 msec. [Eccles, 1935*b*], and which has been called the central excitatory state (C.E.S.) on analogy with the similar prolonged excitatory condition exhibited by motoneurones of the spinal cord. Further, this excitatory state has been shown to be closely associated with a negativity (the *N* wave) of the soma of the ganglion cell relative to its axon. This *N* wave of the ganglion cell does not begin until about the end of the synaptic delay and does not reach a maximum until about 15–20 msec. after the arrival of a preganglionic volley at the synapses of these cells [Eccles, 1935*c*, pp. 473, 478; Eccles, 1936*b*, p. 9]. A similar late maximum (at about 10–15 msec.) for the C.E.S. set up by a preganglionic volley was suggested by experiments in which the excitability was tested by a second preganglionic volley, both volleys being set up by similar stimuli applied through the same electrodes. It was originally pointed out [Eccles, 1935*b*, p. 209] that such a late maximum of the facilitated response may be due to a diminution in size of the second preganglionic volley, for as long as 15 msec., by the refractory period following the first preganglionic volley. However, the similar late maximum for the closely associated *N* wave suggests that the C.E.S. which a preganglionic volley sets up in the ganglion cells also develops to a late maximum, and possibly also does not begin until about the end of the synaptic delay. This time course is confirmed by the experiments described in section C.

This late development establishes that the C.E.S. produced by a single preganglionic volley plays no significant part in relation to the detonator action of that volley, for this latter has set up the discharge of impulses before any appreciable C.E.S. has been produced. Moreover, by the time that the C.E.S. has attained its maximum intensity, there will have been almost complete recovery of the ganglion cells from the refractory period following the initial discharge, and yet there is no further discharge of impulses. A similar absence of discharge occurs during the summed C.E.S. set up by two or more volleys in quick succession. *Hence it may be concluded*

that ganglion cells are not excited to discharge impulses even by the largest intensities of C.E.S. *that normally can be produced by preganglionic impulses.*

The action of nicotine provides further evidence dissociating C.E.S. from the detonator action, for in small doses it greatly diminishes C.E.S., and at the same time even makes it easier for preganglionic impulses to set up the discharge of impulses from ganglion cells [Eccles, 1935*b*].

Thus the sole action of C.E.S. *appears to be a lowering of the threshold at which the detonator action of preganglionic impulses sets up a discharge from ganglion cells, i.e. it may be regarded as increasing the explosiveness of the ganglion cells.* The C.E.S. produced by a preganglionic volley is developed too late to have any significant action in lowering the detonator threshold for that volley. It must, however, be remembered that repetitive discharge along preganglionic fibres is usual under physiological conditions [Adrian *et al.* 1931], the C.E.S. set up by any impulse being thus normally of importance in lowering the detonator threshold for later impulses.

C. The detonator action of preganglionic impulses

In the first section it was shown that a preganglionic volley sets up a discharge of impulses from ganglion cells by means of a brief excitatory action (the detonator action) which it exerts on them. Now it has been shown [Eccles, 1935*b*, p. 216] that in most experiments a single impulse in a single preganglionic fibre fails to set up a discharge from any ganglion cells, i.e. its detonator action is subliminal. A liminal intensity is attained only when the ganglion cell is bombarded by more than one impulse of a single preganglionic volley. There must, therefore, be a summation of the detonator actions exerted by impulses reaching different synapses on a ganglion cell, and this summation must occur during the brief period of the synaptic delay. This fact suggests the following method by which the detonator action may be more directly investigated.

Single volleys are set up in two separate groups of preganglionic fibres, and the consequent discharge of impulses from ganglion cells is determined for each stimulus interval by analysing the spike action potentials led from the postganglionic fibres. Any effect which the first volley might exert on the second is measured by subtracting the action potential set up by the first volley from that set up by the combined volleys, the subtracted action potential being then compared with the action potential evoked by the second volley alone.

Fig. 2 shows a typical series of such action potentials (recorded from the postganglionic trunk of the superior cervical ganglion) from an experiment in which one volley is set up in one branch of the annulus Vieussens

and the other volley in the other branch. Both volleys are submaximal for the S_1 preganglionic fibres, the complex shape of the spike potential being due to the subsidiary S_1 waves which separate out on account of differences in the conduction velocities in different groups of the S_1 preganglionic fibres [cf. Eccles, 1935*a*, p. 191]. It is obvious that at all intervals except 4·5 msec. the first volley enables the second to set up a discharge from many ganglion cells in addition to those which respond to the second volley alone (spatial facilitation). These facilitated ganglion cells must be subliminally excited by each single volley, these two subliminal excitations, when summed, being adequate to set up a discharge. Preganglionic fibres from each branch of the annulus Vieussens must thus enter into synaptic relationship with such ganglion cells. The subtracted action potentials of Fig. 2 show that, when the volleys are simultaneous, this spatial facilitation is probably maximal, i.e. it is effective in producing a discharge from the largest number of ganglion cells; but this number is still large at an interval of 2·3 msec., while at 4·5 msec. effective summation occurs in very few, if any, ganglion cells. Temporal facilitation (shortening of synaptic delay) is also clearly present at intervals of 1·4 and 2·3 msec., but such facilitation is never large with simultaneous stimuli, and usually it progressively increases as the stimulus interval is lengthened to about 2 msec. At intervals longer than 4·5 msec. spatial and temporal facilitation again develop, the observation at 17·6 msec. interval (Fig. 2) showing practically maximal conditions. The time course of the decay of this second facilitation wave establishes its identity with the facilitation wave determined for two volleys in the same preganglionic fibres [Eccles, 1935*b*], i.e. it is due to the C.E.S. of the ganglion cells. The rising phase of the second facilitation wave thus gives the course of C.E.S. development when its determination is unaffected by

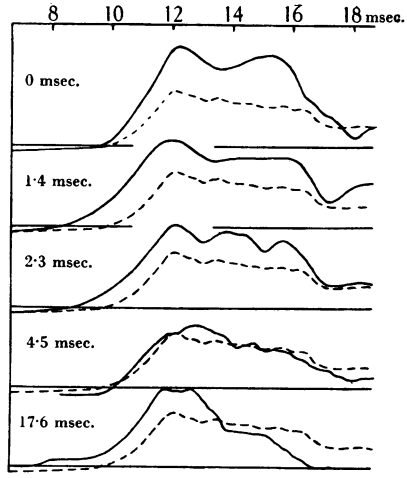

Fig. 2. Single submaximal stimuli have been applied to each of the branches of the annulus Vieussens at various intervals apart. The continuous lines show the ganglionic action potentials (S_1 spike responses only) produced by the second stimulus at the indicated intervals, as determined by subtracting the first action potential from the combined action potential. The broken lines show the action potential set up by the second stimulus alone, all stimuli being synchronized at the zero of the time scale.

the refractoriness of preganglionic fibres, and it shows that, in this part of its time course also, the C.E.S. set up by a preganglionic volley resembles the *N* wave, having a delayed rise to a maximum at about 15 msec. (cf. section B). On the other hand, there must be a very rapid decay of the excitatory effect responsible for the facilitation at short intervals, for it has practically disappeared when tested at a stimulus interval of 4·5 msec. The following evidence shows that this excitatory effect is directly related to the detonator action of preganglionic impulses.

(1) When the two volleys are simultaneous, summation of the subliminal excitatory effects produced in many ganglion cells by each volley results in the discharge of impulses from these cells after the normal synaptic delay, i.e. there is exactly the type of summation that occurs between the detonator actions of the individual impulses of a single preganglionic volley.

(2) The rapid decay of the subliminal excitatory effect responsible for the initial wave of facilitation in Fig. 2 corresponds closely to the short duration which was demonstrated for the detonator action by the antidromic experiments of section A.

Two volleys in separate groups of preganglionic fibres may also be set up by applying two stimuli to the cervical sympathetic, the first being submaximal and considerably weaker than the second [cf. Lorente de Nó, 1935*a*, *b*]. So long as the second volley is set up during the refractory period of the first volley, these volleys must traverse separate groups of preganglionic fibres. The disturbing effects of post-kathodal depression have been avoided by applying the stimuli through separate pairs of electrodes on the cervical sympathetic. Further, if the weaker stimulus is applied near the ganglion and the stronger to the cervical sympathetic at the root of the neck, the range of intervals over which the ganglion cells will be subjected to bombardment by two volleys in entirely separate groups of preganglionic fibres is extended by twice the conduction time between the two sets of electrodes. This procedure has, therefore, been adopted in most of the present experiments, and Fig. 3 shows a series of subtracted action potentials (determined as for Fig. 2) for the various stimulus intervals. On account of its longer preganglionic conduction time, the larger volley set up distally from the ganglion will reach the ganglion slightly later than the smaller volley even in the first observation of Fig. 3. There the subtracted curve shows an early spike considerably above the control response to the second volley alone. This early spike must have arisen either on account of a shortening of the synaptic delay of some ganglion cells which also respond to the second

volley alone (temporal facilitation), or on account of the response of additional ganglion cells (spatial facilitation), and is probably due to both these types of facilitation. The smaller spike with an interval of

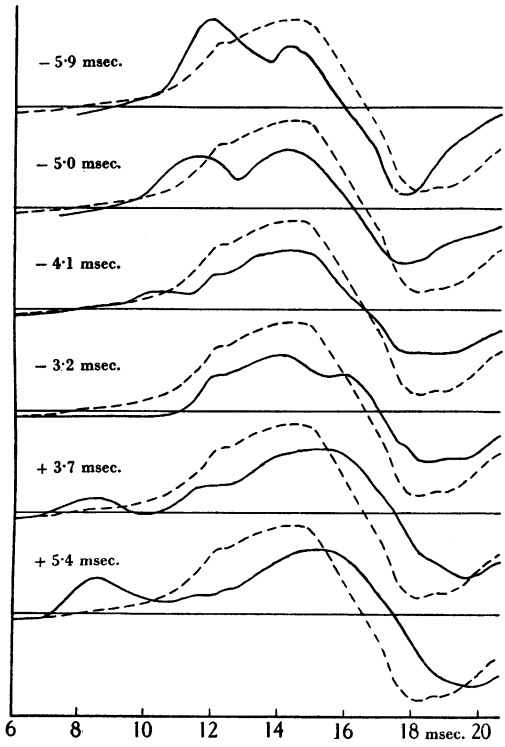

Fig. 3. A stimulus has been applied to the cervical sympathetic near the superior cervical ganglion at the indicated times after (observations 1, 2, 3 and 4) and before (observations 5 and 6) the application of a stronger stimulus through electrodes placed on it at the root of the neck, i.e. about 11 cm. from the ganglion. Both stimuli were submaximal for S_1 preganglionic fibres, and the stimulus intervals were chosen so that the volley set up near the ganglion always reached the ganglion first. At each stimulus interval the action potential evoked by the second volley (shown by the continuous lines) has been determined as in Fig. 2 by subtracting the first action potential from the combined action potential. The broken lines show the action potentials set up by the second volley alone, all stimuli being synchronized at the zero of the time scale as in Fig. 2.

5 msec. indicates facilitation of fewer ganglion cells, but the synaptic delay is still further shortened, and comparison of the observation at 4·1 msec. interval with that at 3·2 msec. shows that in the former there is still a little very early facilitated response. Thus Fig. 3 corresponds

closely with Fig. 2, lengthening of the interval between the times of arrival of the two volleys being accompanied in both by a rapid disappearance of spatial facilitation and by an initial increase in temporal facilitation. As the volley interval is still further lengthened, the smaller stimulus now being applied 3·7 and 5·4 msec. before the larger, the two lowest observations of Fig. 3 show a development of the facilitation due to C.E.S. (cf. the lowest observation of Fig. 2). The synaptic delay of this facilitated response is much shorter than with facilitation by summation of detonator actions. To a small extent this difference is detectable in Fig. 2, and it has also been a feature of other experiments.

Similar experiments suggest that summation of detonator actions also occurs for S_2 ganglion cells, but the complication introduced by the preceding S_1 spike prevents the application of the subtraction method in determining the response set up by the second of two successive volleys. In addition, temporal facilitation by summation of detonator actions has previously been described for S_2 ganglion cells in experiments on the interaction of two maximal volleys in the two branches of the annulus Vieussens [Eccles, 1935*a*, p. 201].

In eight of the present series of twenty experiments, the interaction of volleys in separate groups of preganglionic fibres did not reveal any spatial or temporal facilitation due to summation of detonator actions, and in several of the remaining twelve experiments these facilitations were only slightly developed. Occlusion experiments, such as those already described [Eccles, 1935*a*, section J], show that such experiments are not entirely explicable by an absence of ganglion cells on which there is an overlapping distribution of the two preganglionic volleys [cf. Eccles, 1935*b*, p. 211]. However, the absence of a detonator action of preganglionic impulses on ganglion cells must not then be assumed on account of this failure to demonstrate it by summation, for a preganglionic volley sets up a discharge which the investigations described in section A show to be produced by a normal brief detonator action on the ganglion cells. Presumably, in any such ganglion cell, there is little or no summation of the detonator actions exerted at its various synapses, the discharge set up from the ganglion by a single preganglionic volley being due to the supraliminal detonator actions of individual preganglionic impulses. A similar explanation has already been offered for those experiments in which diminution of a preganglionic volley was not accompanied by the relative increase usually observed in the subliminal fringe [Eccles, 1935*b*, p. 216]. In such experiments the existence of a small subliminal fringe shows that single preganglionic impulses do not all exert supraliminal detonator

actions at the synapses. There is merely no appreciable summation of any subliminal detonator actions which may be exerted at different synapses on a ganglion cell.

D. The relation of the detonator response to the synaptic delay

It has already been seen that, when it occurs, summation of the subliminal detonator actions of preganglionic impulses takes place during the synaptic delay, i.e. within about 3 msec., for the S_1 ganglion cells. Such a time would be too short for diffusion to allow a summation of the quanta of any hypothetical chemical mediator liberated at the different synapses; hence the subliminal detonator actions exerted by each preganglionic impulse would have to sum actually within the ganglion cell. On the other hand, if synaptic transmission is mediated by the action currents of the preganglionic impulses (see section E), there would, of course, be an instantaneous summation of the exciting effects of the different preganglionic impulses, i.e. of their action currents, but such a direct summation would be so diminished on account of the distance between the different synapses that it could hardly be responsible for the observed summation of detonator actions; thus on this hypothesis also it seems necessary to invoke a summation of subliminal responses of the ganglion cell. The existence of such a response of a nerve cell is also indicated by the experiments of Lorente de Nó [1935*c*, 1936], who showed that with oculomotor neurones the exciting effects of action currents acting across synapses are exerted during the synaptic delay, i.e. if such action currents are the synaptic transmitters, their delayed action in setting up the discharge of an impulse indicates the presence of an intermediate response of the nerve cell. Thus, on either hypothesis of synaptic transmission, detonator summation would involve summation of subliminal responses set up by preganglionic impulses at different synapses. This summation must be mediated by a rapid propagation through the cell of these subliminal responses, and, when these responses thus sum to a certain critical intensity, an impulse is set up in the cell and discharged along the axon. The response of the cell which thus mediates the detonator action of preganglionic impulses may be called the "detonator response". As this response is defined solely in terms of the discharge of an impulse by a cell, it is justifiable to assume that this discharge occurs immediately the detonator response attains a certain critical intensity. As we have seen, in some experiments the detonator response set up by a single im-

pulse may be above this critical intensity, but in most experiments it is subliminal.

Since the synaptic delay of the discharge set up by a preganglionic volley is shortened either by a pre-existent C.E.S. [Eccles, 1935*b*; and Figs. 2 and 3] or by a pre-existent detonator response (cf. Figs. 2 and 3), and conversely since it is lengthened by the refractory period following an antidromic volley [Eccles, 1936*b*], the detonator response must have a

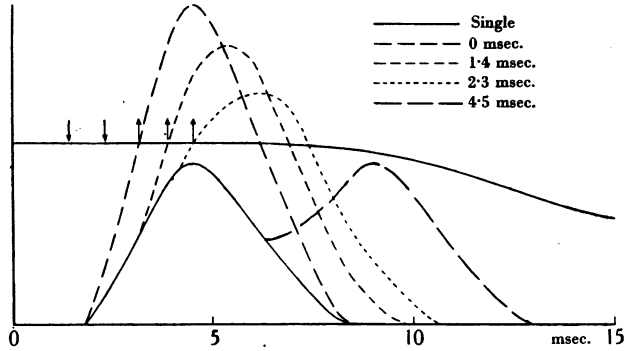

Fig. 4. Diagram showing for a single ganglion cell the presumed course of summation of the detonator responses at the various stimulus intervals of Fig. 2. The zero of the time scale (abscissæ) is the calculated time of arrival of the fastest preganglionic impulses at the synapses. The threshold intensity at which the detonator response sets up an impulse is shown by the horizontal line, the decline of this line beyond 6 msec. showing the diminution of the threshold by the developing C.E.S. This diminution would reach a maximum at about 15–20 msec., and return to normal would occur at about 200 msec. When the rising detonator response crosses the threshold line, an impulse is set up (indicated by the arrow pointing upwards), and the consequent refractory period prevents any further determination of the course of the detonator response. Its subsequent course is thus purely hypothetical. The arrows pointing downwards indicate the calculated time of arrival of the fastest impulses of the second preganglionic volley at intervals of 1·4 and 2·3 msec. (for zero interval it is at zero time on the graph), the synaptic delay being the interval between such an arrow and the corresponding arrow pointing upwards.

phase of progressive increase to a maximum which is attained not later than the end of the longest synaptic delay, i.e. with S_1 ganglion cells at about 4–5 msec. after the arrival of the earliest preganglionic impulses at the synapses. From the following argument it will be seen that the variations which occur in the detonator summation of two preganglionic volleys at various intervals, e.g. in Fig. 2, suggest that the detonator response in a single ganglion cell has a time course which is approximately shown by the continuous line in Fig. 4. The line initially running horizontally

represents the threshold intensity at which the detonator response sets up an impulse, hence the single detonator response is subliminal in that particular ganglion cell, which is thus in the "subliminal fringe". Other ganglion cells would be excited by a single preganglionic volley to varying degrees of subliminality or supraliminality, and the conditions for these various cells may be inferred from the special case depicted in Fig. 4. In constructing the curves (Fig. 4) for the summed detonator responses at the various stimulus intervals of Fig. 2, the detonator responses for each volley are assumed to be identical and to sum arithmetically. These curves illustrate the experimental finding that the second volley sets up a response with the shortest synaptic delay when there is an interval of about 2 msec. between the two volleys (cf. Figs. 2 and 3). With shorter stimulus intervals, the increased height of the summed detonator response explains the observed larger spatial facilitation, and the simultaneously observed decrease in temporal facilitation is also illustrated in Fig. 4. Again, with simultaneous volleys, Fig. 4 shows that there would merely be an increase in steepness of the already steep rising phase of the detonator response; hence an explanation is provided for the small shortening then observed in the synaptic delay, an exactly similar effect being produced by increasing the size of a single preganglionic volley. On the other hand, the rapid decline shown for the detonator response in Fig. 4 must be assumed in order to explain the observed absence of detonator facilitation (cf. Fig. 2) that is illustrated in Fig. 4 for an interval of 4·5 msec. Finally, the considerable synaptic delay (about 2 msec.) which occurs even under optimal conditions suggests that, as shown in Fig. 4, the detonator response only attains a significant intensity at about this interval after the calculated time of arrival of the preganglionic impulses at the synapses.

The curve shown in Fig. 4 for the detonator response also forms the basis of the following explanations which may be offered for other experiments on synaptic delay and facilitation in sympathetic ganglia.

(1) The late beginning for the detonator response is also suggested by the temporal facilitation produced by pre-existent C.E.S. [Eccles, 1935*b*]. Just as with detonator summation, there is a limiting value of about 2 msec. below which the synaptic delay cannot be shortened. Fig. 5 illustrates the experimentally observed effect of C.E.S. in producing both temporal and spatial facilitation by lowering the threshold at which the detonator response sets up the discharge of an impulse (cf. section B), the temporal facilitation being larger with those ganglion cells which normally have the longest synaptic delays. Thus in Fig. 5 the ganglion cell normally

excited just to threshold intensity has its synaptic delay shortened by 1·5 msec., while the cell excited to twice normal threshold has its delay shortened by only 0·6 msec.

(2) Fig. 6 illustrates the observed action of antidromic impulses in lengthening the synaptic delay [cf. Eccles, 1936*b*, p. 13, section A]. The continuous curve shows the course of the detonator response which normally would set up an impulse at R_1, which corresponds to G_3 in Fig. 1. After an antidromic volley the raised threshold of the relatively

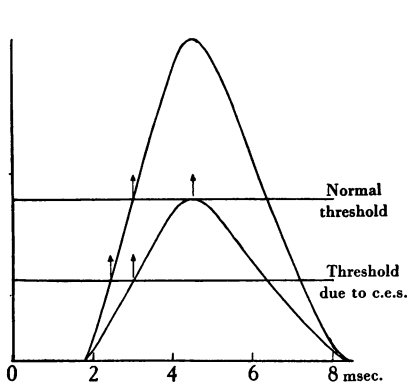

Fig. 5.

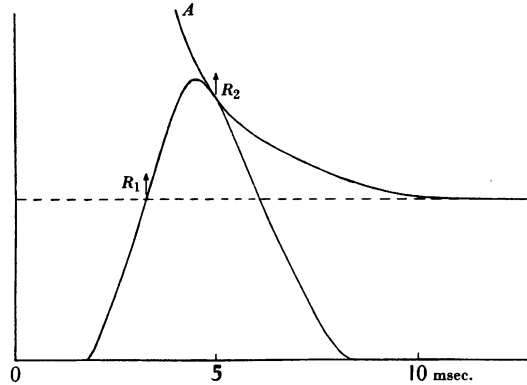

Fig. 6.

Fig. 5. As in Fig. 4, but to show the effect of the lowered threshold (due to C.E.S.) on the synaptic delay of two ganglion cells, one whose detonator response just attains threshold intensity, and the other with double this intensity.

Fig. 6. As in Fig. 4, but to show how the raised threshold following an antidromic impulse delays the time at which an impulse is set up by the detonator response.

refractory period [cf. Brown, 1934; Eccles, 1936*b*] recovers approximately along the line A, the antidromic volley in the figure being critically timed so that this line just touches the detonator response at R_2. Any further retardation of the antidromic volley prevents the detonator response from ever attaining threshold, hence R_2 represents the latest time at which the detonator response can still set up an impulse from that ganglion cell, i.e. R_2 corresponds to G_4 of Fig. 1. Fig. 6 in addition shows how the antidromic experiments of section A give evidence of the rapid decline of the detonator response.

(3) It has been shown that the synaptic delay of S_2 ganglion cells is not diminished, when, during its latter part, there is a further excitation of these cells by preganglionic impulses incident at synapses different from those primarily excited [Eccles, 1935*a*, p. 201]. Hence, after the arrival

of this secondary group of impulses, there must be a latent period of several milliseconds before any appreciable detonator response is produced. The conditions are illustrated in Fig. 7 in terms of the postulated detonator response curve which has, however, been modified in order to accord with the longer synaptic delays observed for S_2 ganglion cells.

Thus the time course suggested for the detonator response in Fig. 5 *provides a satisfactory explanation of all experiments on facilitation and synaptic delay, and on no other basis does a satisfactory explanation seem possible.*

The experiments of Lorente de Nó [1935*a*, *b*, 1936] on the oculomotor neurones provide an exact parallel to these observations on the ganglion cells of the superior cervical ganglion [cf. Eccles, 1936*c*,

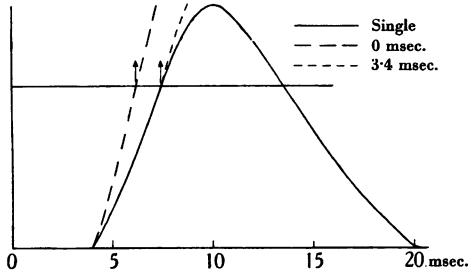

Fig. 7. As in Fig. 4, but to illustrate, with S_2 ganglion cells, the shortening of synaptic delay which is produced by a second preganglionic volley at the indicated intervals after the first. With simultaneous volleys the synaptic delay (measured from zero of the time scale) is shortened by about 1·2 msec., while, with a stimulus interval of 3·4 msec., the detonator response of the second volley is too late to affect the synaptic delay.

pp. 374–82], except that the detonator response runs a time course of about one-fifth the duration, the synaptic delay for these neurones being correspondingly about one-fifth of that for the S_1 ganglion cells. Again, with motoneurones of the spinal cord, it has recently been shown [Eccles & Pritchard, 1937] that there is an initial quick detonator response setting up the discharge of impulses with a synaptic delay of about 0·7–1·0 msec., the first development of C.E.S. again not being detectable until just after this discharge. The time course of the detonator response and the synaptic delay are here, too, almost five times shorter than with S_1 ganglion cells. *Thus it would seem likely that excitatory impulses incident at the synapses of all neurones set up a detonator response having approximately the course shown in Fig.* 4, *variations in the temporal scaling corresponding to the synaptic delays characteristic of these various types of neurone.*

Moreover, both in oculomotor neurones and in motoneurones of the spinal cord there is summation of the detonator responses produced at different synapses on a neurone. A detonator response must, therefore, be propagated rapidly from the region of the synapse where it is produced. Such a propagation of a subliminal excitatory condition can have no relation to the propagation of all-or-nothing impulses; nor would it seem, itself, to be all-or-nothing in character, for, in those ganglion cells where no detonator summation is demonstrable, the detonator response presumably disappears before reaching the regions of other synapses. Again, during an experiment lasting for several hours, detonator summation often is observed to diminish gradually (cf. Figs. 3 and 9*a*], an effect presumably resulting from the experimental interference. *Thus we have the picture of the detonator response as a brief excitatory state produced in a neurone at the region of a synapse and spreading rapidly through that neurone in a decremental fashion.*

According to the time course shown in Fig. 5, the detonator response increases in intensity during a considerable part (as long as 2 msec.) of the latter part of the synaptic delay. During this part of the synaptic delay no electrical potential can be detected with the usual leads from the ganglion cells, the first potential change being propagated directly along the postganglionic fibres, and hence presumably due to impulses discharged from the ganglion cells. Thus it would seem that the detonator response does not give rise to any detectable electrical change between the soma and the axon of a ganglion cell. In this respect the detonator response resembles the local excitatory state of peripheral nerve [Blair & Erlanger, 1936]; and in other respects also they appear to be closely related excitatory conditions [cf. Eccles, 1936*c*, p. 360].

The presumed time course of the detonator response in Fig. 5 shows that only the early part of the synaptic delay precedes the beginning of the detonator response, the latter part being occupied by the rising phase of this response. This initial part, which may be as long as 2 msec., undoubtedly is partly due to slowed conduction velocity in the very fine terminal branches of the preganglionic fibres, no allowance being made for this in calculation of the time of arrival of the preganglionic impulses at the synapses. The remainder would be due to the time which preganglionic impulses take to set up a detonator response after they have reached the synapses, and so may be occupied partly in the rising phases of the preganglionic impulses themselves, partly in transmission of their excitatory action to the ganglion cell, and partly by the delay experienced by such an action in setting up the detonator response. Thus the synaptic

delay is compounded of the times occupied in the following events [cf. Eccles, 1936*c*, p. 383].

(1) Delayed conduction of impulses in the fine terminal branches of the preganglionic fibres.

(2) Duration of the rising phases of the preganglionic impulses themselves.

(3) Synaptic transmission from the preganglionic fibre to the ganglion cell.

(4) Delay incurred by the synaptic transmitter in setting up the detonator response.

(5) Time occupied by the rising phase of the detonator response in attaining threshold intensity for setting up an impulse. Presumably this rising phase is conditioned partly by the asynchronism in the time of arrival of preganglionic impulses, and partly by the conduction time (and hence summation time) of the detonator responses generated at the different synapses of a ganglion cell.

E. The synaptic transmitter

In section A the term "detonator action" was applied to that brief excitatory action by which preganglionic impulses set up a discharge of impulses from ganglion cells. Thus it includes the "detonator response" of the ganglion cell, but in addition it must also include the means by which impulses in the preganglionic fibres exert their excitatory action across the intercellular region of the synapse, i.e. the "synaptic transmitter". According to one hypothesis this transmitter is the acetylcholine which is secreted by the synaptic regions of the preganglionic fibres and which diffuses to, and excites, the ganglion cells. According to the alternative hypothesis the action currents of the preganglionic impulses directly excite the ganglion cells at their synaptic regions.

Now, on either hypothesis, the detonator response of the ganglion cell is produced by the action of the synaptic transmitter, which, consequently, must have a duration at least as brief as that demonstrated for the detonator response, i.e. about 6 msec. Such a brief duration presents no difficulty to the action-current hypothesis, for, according to this hypothesis, the synaptic transmitter, i.e. the action currents, cannot have a duration of more than a few milliseconds. With the acetylcholine hypothesis, however, such an extremely rapid inactivation of the secreted acetylcholine could conceivably occur only by the hydrolysing action of cholinesterase. Now the cholinesterase of the ganglion is inactivated by eserine, the acetylcholine secreted from the preganglionic terminals then

accumulating in the ganglion and appearing in the perfusate [Feldberg & Gaddum, 1934; Feldberg & Vartiainen, 1934; Brown & Feldberg, 1936]. If the synaptic transmitter is acetylcholine, it would therefore be expected to have a much more prolonged action after eserinization of the ganglion, but the following experiments show that this does not happen.

(1) Action potentials from the ganglion and from the postganglionic trunk show no alteration in the time courses either of the spike potentials or of the late potential waves (the N and P waves). An increase in the spike height of submaximal responses is produced by small concentrations of eserine, an effect which is due to the increased excitability and the consequent discharge of impulses by many ganglion cells previously in the subliminal fringe [Feldberg & Vartiainen, 1934; Eccles, 1935*b*, p. 222]; but the important points are, firstly, that the time course of the spike is not altered, i.e. there is no increase in the temporal dispersion of the discharge, and secondly, that there is no detectable repetitive discharge of impulses [Eccles, 1934; Bronk *et al.* 1935].

(2) This absence of a repetitive discharge has been confirmed by recording from the nictitating membrane, and repeating the experiments of section A after eserinization of the ganglion. Fig. 8 is typical of such experiments (five in all) and shows that a suitably timed antidromic volley, e.g. in Fig. 8 one set up 8 msec. after the preganglionic volley, still completely blocks all the impulses which are discharged from the ganglion cells in response to a single preganglionic volley. This latter volley, therefore, does not set up more than one impulse from any ganglion cell.

(3) The plotted points of Fig. 8 provide a more delicate test for any effect that eserine might have on the synaptic transmitter. As the antidromic volley is set up at progressively shorter intervals after the preganglionic volley, i.e. as A_1 is moved progressively further to the left in Fig. 1, the preganglionic volley finds that more and more ganglion cells have so far recovered from their refractoriness that a discharge of impulses can be set up from them. Fig. 8 shows that eserinization of the ganglion (0·4 mg. per kg. intravenously) makes no significant difference in the time course of this recovery curve. Now eserine also does not appreciably alter the refractory period of the ganglion cells (as observed by direct electrical recording from them), hence, by applying the argument of section A, it may be concluded from Fig. 8 that eserine also has no appreciable effect on the time course of the detonator response set up by the synaptic transmitter of the preganglionic volley. Therefore, presumably, the duration of the synaptic transmitter itself is also not significantly lengthened by eserine.

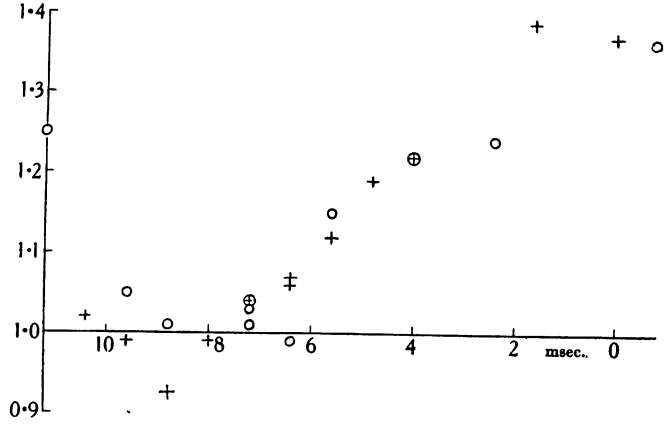

Fig. 8. A maximal antidromic volley is set up at various intervals *after* a maximal preganglionic volley, and the contraction tensions of the nictitating membrane are plotted as ordinates (measured as a fraction of the average tension produced by the antidromic volley alone) against the corresponding stimulus intervals as abscissæ. At intervals of 7–10 msec. the preganglionic volley produces no significant change in the contraction. The crosses show observations before and the circles after the intravenous injection of 0·4 mg. eserine per kg.

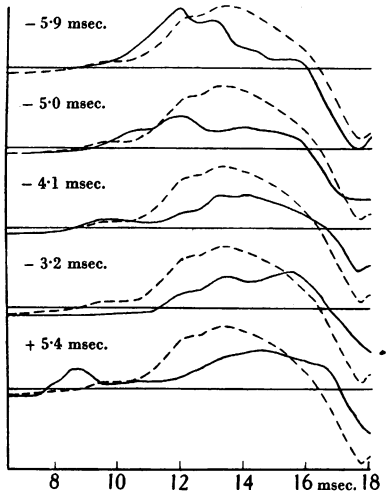

Fig. 9 *a*.

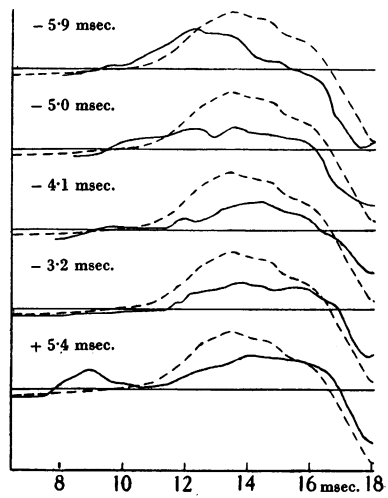

Fig. 9 *b*.

Fig. 9*a*. As in Fig. 3, and later in the same experiment.

Fig. 9*b*. A series of observations just after those of Fig. 9*a*, 0·4 mg. eserine per kg. being given between the two series of observations.

(4) The detonator summation between two preganglionic volleys has been examined in six experiments in which this summation was normally present. Fig. 9 shows that eserine has no appreciable action on the relationship of detonator summation to volley interval. Hence again the conclusion may be drawn that eserine produces no significant lengthening of the detonator response, and therefore of the synaptic transmitter by which a preganglionic volley sets up this response. The decline in the size of the detonator summation after eserine administration in Fig. 9 is probably no more than the progressive decline that was often observed in many of our experiments, e.g. the observations of Fig. 3 were recorded about an hour earlier in this experiment.

The foregoing experiments would seem to establish beyond reasonable doubt that the cholinesterase which is inactivated by eserine plays no significant part in bringing about the rapid decay of the synaptic transmitter, and therefore provide a very grave difficulty for the hypothesis which regards this transmitter as acetylcholine. The alternative action-current hypothesis thus receives indirect support. Two recent reviews [Eccles, 1936*c*, pp. 366–70; Eccles, 1937] may be referred to for a discussion of these results in relation to the acetylcholine hypothesis.

F. Conclusions

There are two possible mechanisms by which a preganglionic impulse exerts its excitatory action on a ganglion cell—the action currents associated with this impulse, and the acetylcholine which it causes to be secreted from the terminal branches of the preganglionic fibre—possibly from the *boutons* themselves. The available evidence [cf. Eccles, 1937] suggests that the former mechanism is responsible for the brief excitatory actions (the detonator response and C.E.S.) which are discussed in this paper, while the latter mechanism possibly has a more prolonged "tonic" excitatory effect, such as is observed, for example, in the action of adrenaline secreted by the motor fibres innervating smooth muscle [Eccles & Magladery, 1937*b*].

However this may be, the next detectable event is the detonator response, an excitatory state of the ganglion cell which appears directly to set up the discharge of impulses by the cell, and which is defined in terms of this action. Summation experiments indicate that the detonator response is propagated decrementally and rapidly through the cell, summation of the responses produced at different synapses thus being produced. These experiments also show that detonator responses of various degrees of subliminality may be produced in ganglion cells (the subliminal fringe). When thus too weak to set up the discharge of an impulse, the

detonator response disappears within a very few milliseconds. If, on the other hand, the detonator response sets up an impulse, its subsequent course is submerged by the consequent refractory period. The brief duration of the detonator response similarly precludes the demonstration of detonator summation by successive volleys in the same preganglionic fibres.

The more prolonged excitatory state, C.E.S., must be sharply distinguished from the detonator response. In ganglion cells its sole action appears to be a lowering of the threshold at which the detonator response sets up the discharge of an impulse. It does not begin until about the end of the detonator response, its relation to this response being illustrated in Fig. 4. However, such a delayed and prolonged course must not be regarded as evidence for the existence of a more prolonged type of synaptic transmitter, for the C.E.S. set up by an antidromic impulse, i.e. in the absence of a transmitter, runs an identical time course [Eccles, 1936*b*, section D]. Since C.E.S. always follows the detonator response, and since summation between different synapses has never been observed for C.E.S. when not preceded by an initial facilitation wave due to detonator summation (cf. Figs. 2 and 3), it seems likely that C.E.S. is not set up directly by a synaptic transmitter, but is produced secondarily to the detonator response. The setting up of the detonator response would then be the only primary action of the synaptic transmitter, but such a conclusion must be regarded as merely provisional, pending further experimental evidence.

Summary

By analysing experiments on the interaction of preganglionic and antidromic volleys, it is shown that a preganglionic volley sets up the discharge of an impulse from a ganglion cell (an explosive act) by exerting on it a very brief excitatory action, called the "detonator action". The "detonator action" must include the synaptic transmitter which mediates the transmission across the intercellular region of the synapse, but it also includes the response which this transmitter sets up in the ganglion cell, the "detonator response", which may be of varying intensity, the discharge of an impulse being, by definition, instantaneously produced when a certain critical intensity is attained.

Experiments on summation of the excitatory actions of two separate preganglionic volleys at different intervals show that this detonator response is a brief excitatory event which is rapidly propagated (apparently decrementally) from the site of its production to sum with the detonator responses produced on neighbouring synapses of a ganglion cell. Analysis of such summation indicates that the detonator response reaches a sig-

nificant intensity about 2 msec. after the calculated time of arrival of the preganglionic impulses at the synapses, attains a maximum about 2 msec. later, and then rapidly decays. This time course is shown to be in agreement with other experiments on facilitation and synaptic delay, and synaptic delay is discussed in relation to the detonator response.

The brief duration of the detonator response, and hence of the synaptic transmitter causally related to this response, is not significantly lengthened when eserine inactivates the cholinesterase of the ganglion. A grave difficulty is thus presented to the hypothesis which regards acetylcholine as the synaptic transmitter. On the other hand, the alternative "action-current" hypothesis accords well with the experimental observations of this paper, for according to it the synaptic transmitter is the action current of each preganglionic impulse and so necessarily would be of short duration and unaffected by cholinesterase inactivation.

The C.E.S. set up by a preganglionic volley runs a much slower time course than the detonator response. Facilitation experiments show that it does not begin until after the end of the synaptic delay, and it increases to a maximum at about 10–15 msec. later. C.E.S., therefore, can have no *direct* effect in setting up the discharge of impulses, its sole action being a lowering of the threshold at which the detonator action of later preganglionic impulses sets up such a discharge, i.e. it produces an increase in the explosiveness of the ganglion cells.

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