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FACILITATION AND INHIBITION OF CELL GROUPS WITHIN THE SUPERIOR CERVICAL GANGLION OF THE RABBIT

BY M. J. BRIMBLE, D. I. WALLIS AND B. WOODWARD
From the Department of Physiology, University College, Cardiff CF1 1XL

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SUMMARY

1. The patterns of facilitation and inhibition of the S_a and S_b components of the post-ganglionic compound action potential after a single conditioning stimulus were different and dependent on stimulus parameters.

2. With submaximal conditioning and test stimuli, the S_a component showed a phase of early facilitation (40–75 msec after the conditioning stimulus) followed by a prolonged tail of facilitation. With maximal stimuli, early facilitation and late facilitation (700–2000 msec after the conditioning stimulus) were separated by a phase of inhibition or relative inhibition, most pronounced 100–300 msec after the conditioning stimulus.

3. During early facilitation, a submaximal S_a response was facilitated by $33{\cdot}1 \pm 3{\cdot}9$ %, while a maximal S_a response was facilitated by $14{\cdot}5 \pm 2{\cdot}9$ %.

4. Providing preganglionic C fibres were excited, facilitation of the S_b component remained relatively constant for 40–500 msec after the conditioning stimulus, with no phase of inhibition.

5. Early facilitation of submaximal S_a responses was greatest when the conditioning stimulus excited about 50 % of the preganglionic B fibres, but that of maximal responses was greatest when the conditioning stimulus excited all the B fibres. The preganglionic C fibres modulated facilitation of the S_a component. Maximal facilitation of this component was associated with depression of the S_b component.

6. Submaximal S_a responses are more strongly inhibited than maximal S_a responses 200 msec after a conditioning stimulus. The C fibre pathway seems able to modulate the degree of inhibition of the S_a ganglion cells.

7. A neuronal model with divergent and convergent preganglionic B and C fibres supplying S_a ganglion cells is consistent with the results. The preganglionic input is able to vary the size of the subliminal fringe. The S_b component is in part due to the S_a ganglion cells firing to their C fibre input.

INTRODUCTION

In the rabbit, the post-ganglionic compound action potential has two major components; the first, S_a, is evoked by stimulating preganglionic B fibres, while the second, S_b, is evoked by stimulating preganglionic C fibres (Eccles, 1952). Since the work of Eccles (1935*a*), component waves of the action potential have generally been regarded as representing the activity of different ganglion cell pools, supplied by distinct groups of preganglionic fibres. The work described in this paper was started to see whether the S_a and S_b components displayed similar patterns of facilitation and inhibition. It was hoped such an analysis might help elucidate the processes, still poorly understood, which modulate ganglion cell excitability. However, the results suggested that the S_a and S_b components did not behave as if they represented independent cell pools and there was clear evidence of convergence of the preganglionic pathways.

Because initial results indicated that the patterns of facilitation and inhibition were complex, it was decided to avoid the further complications that trains of conditioning stimuli might produce. In all of the experiments reported here a single test stimulus was preceded by a single conditioning stimulus. The implications of the results for the anatomical and functional organization of the ganglion are discussed.

METHODS

Preparation

Rabbits (2–3 kg) were anaesthetized with urethane (1·5–2 g/kg I.P. as a 50 % w/v solution). Urethane was chosen because Larrabee & Posternak (1952) had shown that, in anaesthetic concentrations, it had no depressant action on ganglionic transmission. The superior cervical ganglion was removed together with its preganglionic and principal post-ganglionic nerves (cervical sympathetic and internal carotid, respectively). With the preparation in cold Krebs solution, the ganglion was desheathed under a microscope.

Recording methods

For recording, the preparation was mounted at 37° C in a moist chamber similar to that described by Eccles (1952). The chamber, which contained Krebs solution, was tilted to lift the preparation out of the solution 5 min before the start of recording. The arrangement of the platinum stimulating and recording electrodes is shown in Fig. 1. Conditioning and test stimuli were usually applied through the same pair of electrodes (S_2). In some experiments, a supramaximal test stimulus via the S_1 electrodes was used as an alternative to a submaximal one via the S_2 electrodes (Libet, 1964). Because of the partial transection of the preganglionic nerve only a portion of the preganglionic input was activated. However, the results obtained by this method were not identical with those obtained using submaximal stimuli via the S_2 electrodes (see Results).

Compound action potentials could be led off from the preganglionic nerve, the post-ganglionic nerve or from the ganglion, post-ganglionic action potentials being

led off from the internal carotid nerve at least 3 mm from the ganglion. RC amplification was used; the time constant of the channel recording the post-ganglionic signal was 1 sec. Action potentials were displayed on a Tektronix 502A oscilloscope and filmed or stored on a Racal–Thermionic T8000 instrumentation tape recorder. By recording at the fastest speed and replaying at the slowest, signals were time-expanded 128-fold. This allowed permanent records to be made on a pen recorder (Rikadenki B024, $\frac{1}{4}$ sec full-scale response).

In some experiments, a bath which allowed continuous perfusion of the ganglion with Krebs solution (37° C) was used. The preganglionic and internal carotid nerves were led through partitions sealed with stopcock grease (Scientific Industries, Inc., Springfield, Mass.) into chambers containing platinum electrodes and filled with liquid paraffin. The central perfusion chamber was earthed.

Measurement of facilitation and inhibition

The heights of the pre- and post-ganglionic responses to a conditioning or test stimulus were measured in the way indicated in Fig. 1. The heights of the S_b component and the preganglionic C component were measured from the trough of the subsequent diphasic artifact in the manner of Eccles (1935*b*). Changes in the amplitude of post-ganglionic compound action potentials were taken as an index of changes in the number of ganglion cells firing. Relative amplitude as a percentage of control values was used as the measure of facilitation or inhibition. Ganglionic records cannot be used to measure facilitation or inhibition, since the action potential evoked by the test stimulus appears superimposed on the ganglionic slow potentials set up by the conditioning stimulus (Lloyd, 1939*a*).

One difficulty in comparing the extent of early facilitation in different preparations was that facilitation was observed to increase with time after excision of the ganglion in certain of our experiments (see also Bronk, Tower, Solandt & Larrabee, 1938). This does not affect our major conclusions, since recording was usually completed within 3 hr of setting up the preparation and there was no sign of a decline in amplitude of the response, unlike the experiments reported by Bronk *et al.* (1938).

Components of the compound action potential

In the rabbit superior cervical ganglion, two major post-ganglionic deflexions were normally seen when the stimulus was applied 20 mm or less from the ganglion (Fig. 1*B*). We have confirmed that the first (S_a) is evoked by stimulating preganglionic B fibres; the conduction velocity of the B fibres, measured to the peak of the deflexion, was about 5 m/sec (Figs. 1*B*, 2*A*, 2*B*). The post-ganglionic S_a component was sometimes subdivided into two components, S_{a1} and S_{a2} (Fig. 2*A*). This subdivision is mainly a consequence of different conduction velocities in the post-ganglionic fibres (Kosterlitz & Wallis, 1966). The larger component was S_{a_2} and most of the analysis concerns this component.

The second post-ganglionic deflexion (S_b) was also subdivided in some preparations (Fig. 2*A*, 2*B*). In Fig. 2*A*, S_{b1} and S_{b_2} were evoked by stimulating preganglionic C fibres, whose conduction velocity, measured to the peak of the deflexion, was about 0·9 m/sec. In Fig. 2*B*, S_{b1} appeared to be evoked by a preganglionic fibre group whose threshold and conduction velocity (about 1·5 m/sec, measured to the peak of the deflexion) was intermediate between the main B and C fibre groups. S_{b_2} was evoked by stimulating the main C fibre group of fibres. Electrode placement in the experiments to be discussed usually resulted in an undifferentiated S_b component and our analysis relates to this.

It would seem that, if the internal carotid nerve allowed placement of recording electrodes 5 mm from the ganglion and if the stimulating electrodes were 2 or more

cm proximal to the ganglion, four components of the post-ganglionic compound action potential could often be seen. Four components were observed in post-ganglionic records from the cat superior cervical ganglion by Eccles (1935*a*).

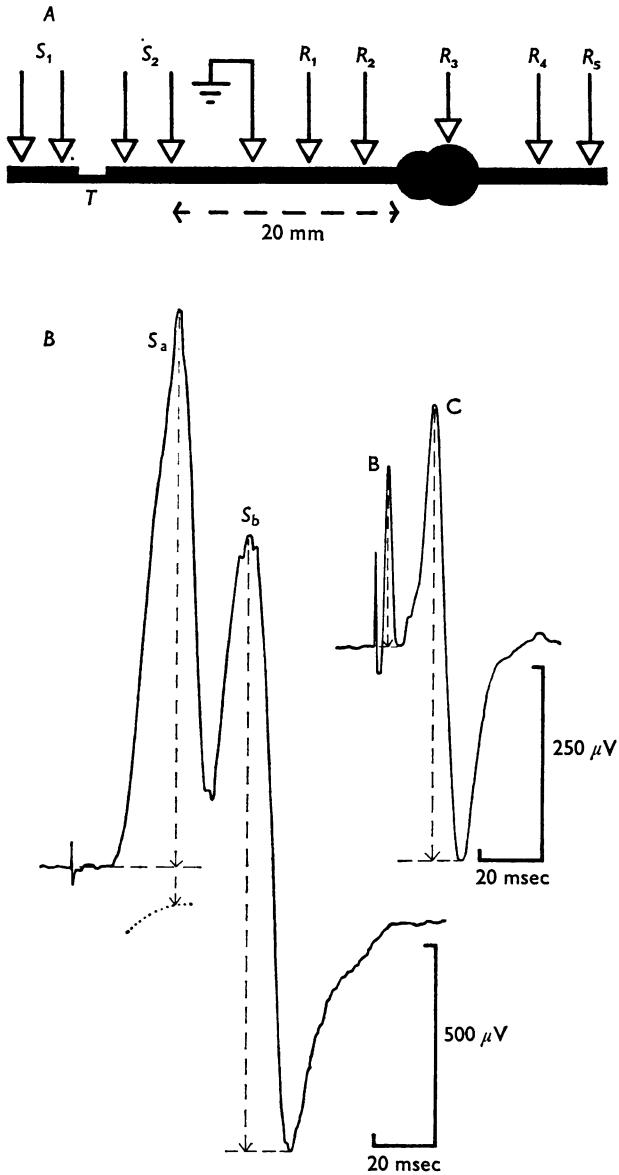

Fig. 1. For legend see facing page.

Solutions

Krebs solution was used, having the following composition (mM): NaCl 118, KCl 4·75, $CaCl_2$ 2·54, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 11; it was gassed with 5% carbon dioxide and 95% oxygen. Atropine sulphate (B.D.H.) was used in some experiments.

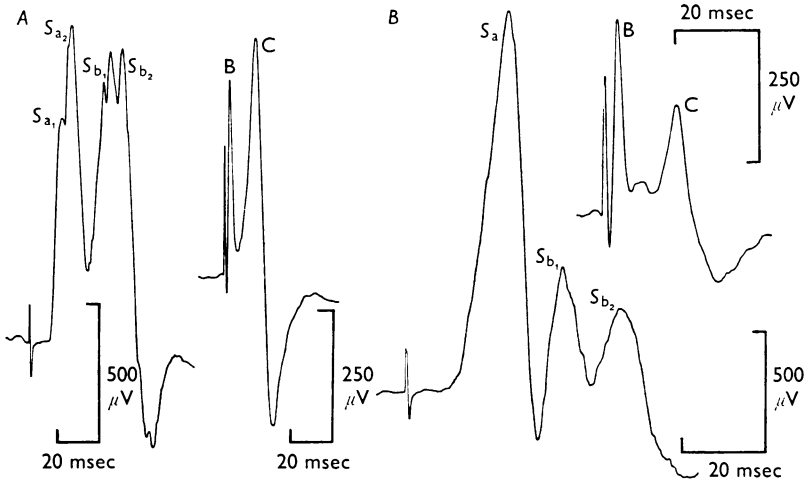

Fig. 2. Compound action potentials showing subdivisions of the two major deflexions in two ganglia.

A, left hand trace: post-ganglionic compound action potential with at least four components, S_{a1}, S_{a2}, S_{b1} and S_{b2}. The small component which immediately precedes S_{b1} was seen in only this experiment. Right-hand trace: preganglionic compound action potential.

B, post-ganglionic compound action potential with three components, S_a, S_{b1} and S_{b2}. Inset, preganglionic compound action potential.

Fig. 1. Electrode arrangement and compound action potentials recorded from the internal carotid nerve and the preganglionic nerve.

A, arrangement of stimulating (S_1 and S_2) and recording electrodes (R_1–R_5). The S_1 stimulating electrodes were positioned beyond a partial transection of the preganglionic nerve (T). The compound action potential from the preganglionic nerve was recorded between R_1 and R_2, that from the post-ganglionic nerve between R_4 and R_5. On occasion, ganglionic action potentials were recorded between R_3 and R_5.

B, pen recorder traces of typical action potentials to show component waves and the method of measuring their amplitude. Left-hand trace: post-ganglionic, S_a was measured from the projected base line (dashes) or, when preceded by an initial response, by subtraction from a tracing of that response (dots). S_b was measured by projection from the succeeding diphasic artifact. Right hand trace: preganglionic B and C components were measured in a similar manner to the post-ganglionic S_a and S_b, respectively.

RESULTS

Temporal patterns of facilitation and inhibition

The S_a ganglion cells showed a rather complex pattern of facilitation and inhibition, which varied with the stimulus parameters used (Fig. 3). If the conditioning and test stimuli evoked an S_a component 50% of its maximal size, a distinct phase of early facilitation was followed by a prolonged tail

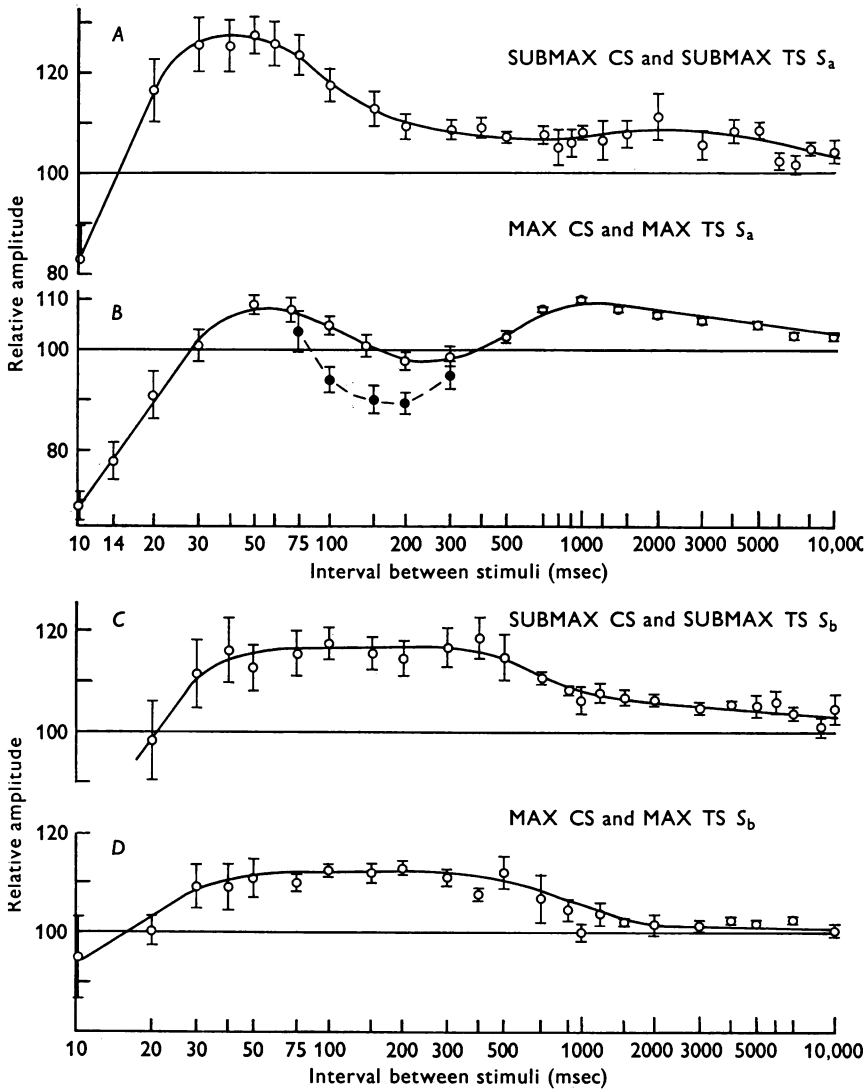

Fig. 3. For legend see facing page.

of facilitation (Fig. 3*A*). With conditioning and test stimuli which were maximal for the S_a component, i.e. just supramaximal for preganglionic B fibres, facilitation occurred more obviously in two distinct phases (Fig. 3*B*, open circles). This distinction was more pronounced if the stimuli were supramaximal for both B and C preganglionic fibres (Fig. 3*B*, filled circles). In this case, the two phases of S_a facilitation were separated by an inhibitory phase. We have termed the second phase of facilitation 'late facilitation'.

It can also be seen from Fig. 3*A* and *B* that the extent of the early S_a facilitation was greater when submaximal stimuli were used. The peak of this early facilitation occurred 40–75 msec after the conditioning stimulus, the peak varying in different experiments. If peak values for early facilitation are averaged for all experiments, a response to a submaximal test stimulus was facilitated by $33{\cdot}1 \pm 3{\cdot}9\,\%$ ($n = 27$) following a submaximal conditioning stimulus. A response to a maximal stimulus was facilitated by $14{\cdot}5 \pm 2{\cdot}9\,\%$ ($n = 19$) following a maximal conditioning stimulus ($P < 0{\cdot}01$). Facilitation must depend upon recruitment of cells from a subliminal fringe, but it is important to remember that facilitation is expressed as a percentage of control values and so is not synonymous with the number of cells recruited. Thus, 30 % facilitation of a half-maximal response (say due to $50x$ cells) correspondsto an increment of $15x$ cells, while 10 % or so facilitation of a maximal response ($100x$ cells) corresponds to an increment of $10x$ cells and a smaller subliminal fringe.

The extent of the early S_a facilitation for all intervals up to 75 msec, but particularly for the shorter intervals between stimuli, was subject to

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Fig. 3. Temporal patterns of facilitation of the S_a and S_b components of the compound action potential.

A, facilitation of an S_a response to a submaximal test stimulus. A TS (test stimulus), submaximal for the preganglionic B fibres and the S_a response, was preceded by a similar CS (conditioning stimulus).

B, facilitation and inhibition of S_a responses to maximal test stimuli. CS and TS are just supramaximal for the preganglionic B fibres and the S_a response (open circles). The effect of a CS, supramaximal for both B and C fibres, on the phase of inhibition of the S_a response to a similar TS is shown for comparison (filled circles).

C, facilitation of an S_b response to a submaximal test stimulus. A TS, submaximal for the preganglionic C fibres and the S_b response, was preceded by a similar CS.

D, facilitation of an S_b response to a maximal test stimulus. A TS, supramaximal for the preganglionic B and C fibres and the S_b response, was preceded by a similar CS.

Ordinates: relative amplitude as a percentage of control value. Abscissae: interval between CS and TS in msec (log scale). Bars show ± S.E. of the mean.

considerable variation because the preganglionic pathways showed signs of refractoriness at these intervals. This refractoriness varied from experiment to experiment and was seen as a reduction in amplitude of the B fibre component of the preganglionic compound action potential. To combat this difficulty, paired stimuli supramaximal for the intact B fibres were delivered via the S_1 electrodes. S_a responses were reduced in height by 50 % or so by partial transection of the preganglionic nerve. However, the resulting pattern of facilitation and inhibition resembled that for paired supramaximal stimuli (cf. Fig. 3*B*) and not that for paired submaximal stimuli (Fig. 3*A*).

The phase of inhibition of the S_a component was most pronounced between stimulus intervals of 100–300 msec. Clearly, the degree of inhibition was closely related to the stimulus parameters, for when the conditioning stimulus was supramaximal for both preganglionic B and C fibre pathways inhibition was considerably more pronounced (Fig. 3*B*, filled circles). Thus, the C fibre pathway, contrary to our expectation, was able to modulate the degree of inhibition of the S_a ganglion cells, a feature dealt with more extensively in the section on inhibition. The phase of late facilitation, whose peak lay at intervals between stimuli of 700–2000 msec, appeared little affected by the stimulus parameters used (Fig. 3*A* and *B*).

The pattern of facilitation of the S_b component was simpler than that of the S_a component. Facilitation of S_b responses did not occur until the conditioning stimulus excited preganglionic C fibres; facilitation using submaximal stimuli for the C fibre pathway was only marginally, if at all, greater than facilitation to supramaximal stimuli (Fig. 3*C* and *D*). Facilitation persisted for a long period after the conditioning stimulus, and, with intervals between stimuli of 40–500 msec, the amount of facilitation remained relatively constant. At intervals of 1 sec and longer, facilitation markedly declined. Note that no phase of inhibition is to be seen in either Fig. 3*C* or *D*.

Early facilitation

During early facilitation, the subliminal fringe can clearly vary in magnitude. To discover more precisely how it varies with conditioning stimulation of varying proportions of the preganglionic B and C fibres, a further series of experiments was carried out. The preganglionic nerve fibres branch extensively and it is probable that several may synapse with the same post-ganglionic neurone (Elfvin, 1963; Libet & Tosaka, 1969). A cell might be subliminally excited by activation of one or more of its B fibre presynaptic inputs and supraliminally excited by the full B fibre input. On this basis, a larger subliminal fringe might be expected when submaximal rather than supramaximal stimuli are employed. Two repre-

sentative experiments showing the effect of varying the voltage of the conditioning stimulus and so the number and type of preganglionic fibres activated are shown in Figs. 4 and 5. Early facilitation is maximal around

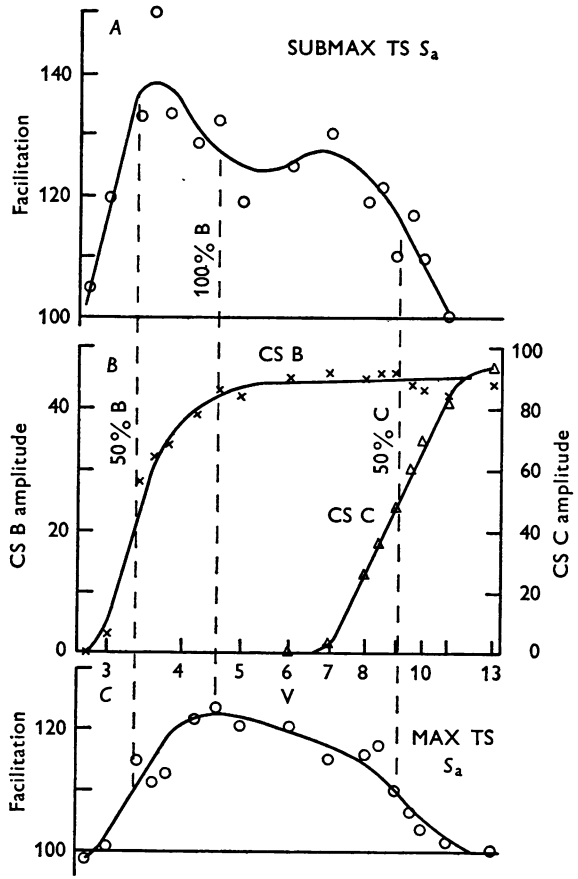

Fig. 4. The effect of varying the strength of the conditioning stimulus on early facilitation of submaximal and maximal S_a responses in the same preparation. A TS was preceded by a CS 50 msec earlier. All abscissae show voltage of CS.

A, facilitation of a submaximal S_a response (SUBMAX TS S_a). TS submaximal for preganglionic B fibres. Ordinate: facilitation expressed as relative amplitude, percentage of control value.

B, relative amplitude of B and C fibre components of the preganglionic compound action potential evoked by CS (CS B and CS C respectively). Ordinates (left and right): amplitude of B and C fibre responses, respectively, in arbitrary units. The dashed vertical lines indicate the stimulus strength at which approximately 50 % and 100 % of the preganglionic B fibres and 50 % of the preganglionic C fibres were excited by the CS.

C, facilitation of a maximal S_a response (MAX TS S_a). TS supramaximal for preganglionic B fibres. Ordinate as in *A*.

50 msec and this interval between conditioning and test stimuli was used. In experiments where submaximal test stimuli were involved, only those where the B fibre response was not depressed because of refractoriness were analysed. In no experiment was the B fibre response to the test stimulus facilitated by conditioning stimulation.

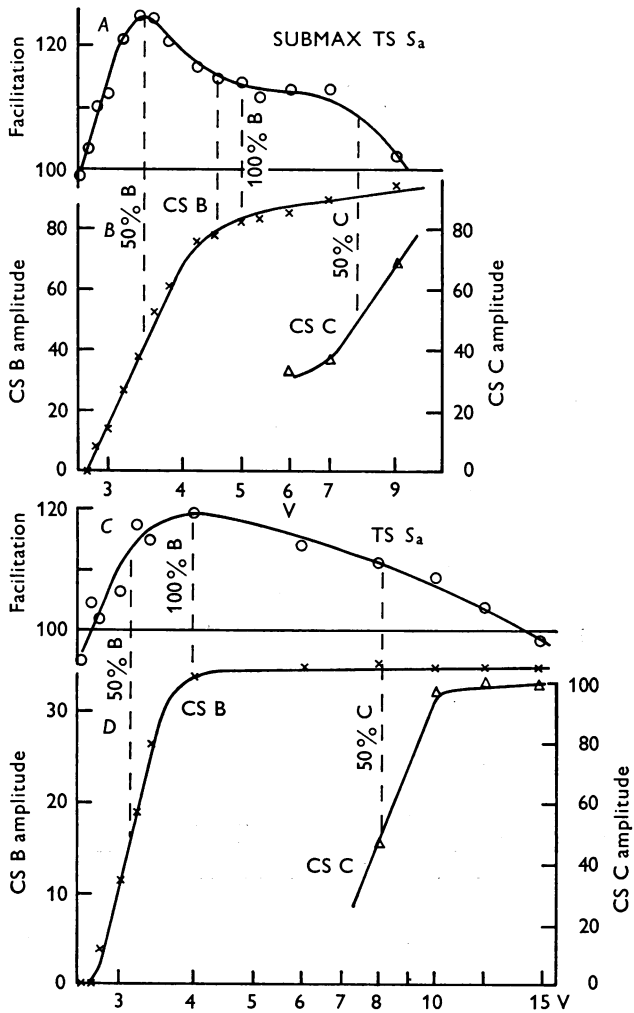

Fig. 5. For legend see facing page.

Fig. 4 shows the results of an experiment where facilitation of S_a responses to submaximal and maximal test stimuli were compared in the same preparation. Lesser facilitation of an S_a response to a submaximal test stimulus, but showing a similar pattern, can be seen also in Fig. 5*A*. Fig.

4*B* indicates the degree of involvement of preganglionic B and C fibres in response to the conditioning stimuli. It can be seen that greatest facilitation of a submaximal S_a response occurred when the conditioning stimulus was of sufficient strength to activate about 50 % of the B fibres. When all of the B fibres were activated, facilitation of the submaximal S_a response was still present but reduced in extent. Activation of the preganglionic C fibres resulted in a secondary reduction in facilitation; with the majority of C fibres activated, no facilitation could be seen (Figs. 4 and 5).

The pattern observed with S_a responses to a supramaximal test stimulus consistently showed two points of difference. First, the maximum extent of facilitation tended to be smaller and, secondly, peak facilitation coincided not with 50 % activation of the B fibre pathway by the conditioning stimulus but with full activation of the B fibre pathway (cf. Fig. 4*B* and *C*). As with a submaximal S_a response, the involvement of preganglionic C fibres by the conditioning stimulus decreased the amount of facilitation.

When the test stimulus was applied through the S_1 electrodes (see Fig. 1), a supramaximal stimulus excited only a portion of the B fibre pathway, but the refractoriness of these preganglionic fibres was overcome. However, an S_a response to this kind of partial excitation of the B fibres (Fig. 5*C* and *D*) consistently revealed a pattern of facilitation more like that for an S_a response to supramaximal stimuli (cf. Fig. 4*C*) than that to submaximal stimuli (Fig. 5*A*). The peak of facilitation occurred when the conditioning stimulus was maximal or near maximal for the B fibre pathway; with only

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Fig. 5. The effect of varying the strength of the conditioning stimulus on early facilitation of S_a responses to a submaximal TS and to a supramaximal TS distal to a partial transection of the preganglionic nerve.

A TS was preceded by a CS 50 msec earlier. All abscissae show voltage of CS.

A, facilitation of a submaximal S_a response (SUBMAX TS S_a). TS submaximal for preganglionic B fibres. Ordinate: facilitation expressed as relative amplitude, percentage of control value.

B, relative amplitude of B and C fibre components of the preganglionic compound action potential evoked by CS (CS B and CS C respectively). Ordinates (left and right): amplitude of B and C fibre responses, respectively, in arbitrary units. The dashed vertical lines indicate the stimulus strength at which approximately 50 % and 100 % of the preganglionic B fibres and 50 % of the preganglionic C fibres were excited by the CS.

C, facilitation of an S_a response rendered submaximal by partial transection of the preganglionic nerve (TS S_a). TS supramaximal for intact preganglionic B fibres (S_1 electrodes), CS via S_2 electrodes. Ordinate as in *A*.

D, relative amplitude of B and C fibre components of the preganglionic compound action potential evoked by CS for the experiment shown in *C*. Ordinate and dashed lines as in *B*.

50 % activation of the B fibres facilitation was distinctly below peak values (cf. Figs. 5*C* and 4*C*).

If we assume that the basis of these rather complex patterns of facilitation might be the anatomical relationship between pre- and post-ganglionic elements, an arrangement of neurones can be drawn up which, on simple assumptions, yields approximately the same patterns of facilitation. This model, discussed in greater detail on page 647 (see Fig. 10) predicts, as did Lloyd (1937), that a cell in a subliminal fringe has fewer synaptic contacts with a particular presynaptic input than cells which are supraliminally excited by a single stimulus. Other presynaptic fibres may, however, synapse with such a cell and remove it from the subliminal fringe when they are activated as well, thus accounting for the reduced subliminal fringe seen with maximal stimuli. To account for the effect of preganglionic C fibres on S_a cell excitability, it is clear that they must converge, at least to some extent, on this ganglion cell pool. It is not necessary, however, to suppose that the C fibres are inhibitory. Perri, Sacchi & Casella (1970) found no evidence of preganglionic inhibitory fibres in either the rat or the guinea-pig superior cervical ganglion.

The results of Bishop & Heinbecker (1932) suggest that S_{a_1} cells could be functionally different from S_{a_2} cells, the former controlling structures in the orbit and the latter being mainly vasoconstrictor. For this reason it was of interest to analyse the patterns of facilitation of the S_{a_1} and S_{a_2} components (see Fig. 2). In one experiment where these were distinct, the patterns of facilitation of the responses were almost identical, except that S_{a_1} showed a lesser amount of facilitation than the larger S_{a_2} component.

The S_b component was also affected by a conditioning stimulus 50 msec previously, although there tended to be considerable variation from experiment to experiment. Facilitation of the S_b response was never seen until the conditioning stimulus activated a substantial fraction of the C fibres. However, when the conditioning stimulus was of insufficient strength to excite the C fibres, the S_b response to a test stimulus was not necessarily at control levels. When the conditioning stimulus was just subthreshold for B fibres, the S_b response was unaffected, being $100{\cdot}1 \pm 1{\cdot}4$ % ($n = 5$) of the controls; with conditioning stimuli which caused maximal facilitation of the S_a response, the S_b response to a test stimulus submaximal for the C fibres was depressed to $88{\cdot}5 \pm 2{\cdot}2$ % ($n = 5$) of the controls ($P < 0{\cdot}001$). This is the only situation in which we have seen apparent inhibition of the S_b component. In the neuronal model (Fig. 10) we suggest that the S_b component results in part from excitation by their C fibre input of the same cells as produce the S_a component. A similar conclusion, based on intracellular studies, was reached by Erulkar & Woodward (1968).

Late facilitation

It was clearly of interest to know whether the late facilitation of the S_a component, seen at intervals of 700–2000 msec, was affected by the presynaptic pathways in a manner similar to early facilitation. The results of Fig. 3 suggested this might not be the case. We tried to confirm this in

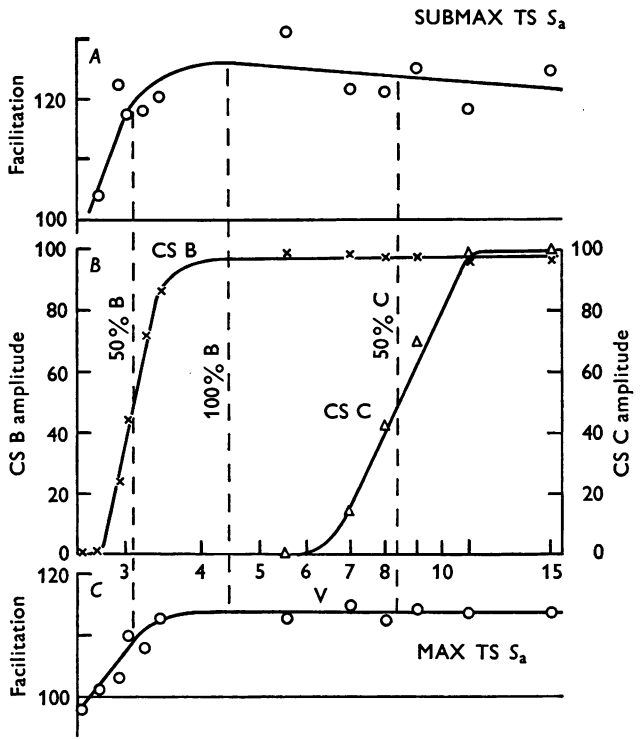

Fig. 6. The effect of varying the strength of the conditioning stimulus on late facilitation of submaximal and maximal S_a responses in the same preparation.

A TS was preceded by a CS 1500 msec earlier. All abscissae show voltage of CS.

A, facilitation of submaximal S_a response (SUBMAX TS S_a). TS submaximal for preganglionic B fibres. Ordinate: facilitation expressed as relative amplitude, percentage of control value.

B, relative amplitude of B and C fibre components of the preganglionic compound action potential evoked by CS (CS B and CS C respectively). Ordinates (left and right): amplitude of B and C fibre responses, respectively, in arbitrary units. The dashed vertical lines indicate the stimulus strength at which approximately 50 % and 100 % of the preganglionic B fibres and 50 % of the preganglionic C fibres were excited by the CS.

C, facilitation of a maximal S_a response (MAX TS S_a). TS supramaximal for preganglionic B fibres. Ordinate as in *A*.

experiments where the conditioning stimulus excited varying proportions of the preganglionic B and C fibres. An interval between stimuli of 1500 msec was chosen.

The facilitation of a submaximal S_a response is plotted against the voltage of the conditioning stimulus in Fig. 6*A* and that of a maximal response in Fig. 6*C*. The extent to which the preganglionic B and C fibres were activated by the conditioning stimulus is shown in Fig. 6*B*. With a submaximal test stimulus, an increase in the amount of facilitation was seen as the conditioning stimulus excited an increasing number of the B fibres (Fig. 6*A*), in contrast to the pattern in early facilitation. A similar pattern of facilitation occurred if the test stimulus was supramaximal (cf. Fig. 6*C* and 6*A*). From Fig. 6 it is clear that the main difference between facilitation of maximal and submaximal responses was in the amount of facilitation achieved. Although the pooled results of Fig. 3 did not show such a difference, it was usually observed that, when S_a responses to submaximal and maximal test stimuli were compared in the same preparation, a submaximal was facilitated more than a maximal response. In marked contrast to the pattern in early facilitation, preganglionic C fibre involvement did not reduce the amount of facilitation. As with early facilitation, S_{a_1} and S_{a_2} components showed very similar patterns of facilitation, although facilitation of S_{a_1} was smaller in magnitude. Thus, late facilitation possesses some features which distinguish it from early facilitation and which may suggest a difference in the underlying mechanism producing it. Uncertainty about the basis of late facilitation made it difficult to interpret the results in terms of the neuronal model (Fig. 10).

Late facilitation appears at approximately the same time as the depolarizing LN wave recorded from the surface of the ganglion, although the facilitation may outlast the LN wave. It has been shown many times (see Libet, 1964; Volle, 1969; and Libet, 1970 for references) that the LN potential results from the activation of muscarinic sites on ganglion cells and is readily blocked by concentrations of atropine of 1 μg/ml. or less. However, the LN wave and the facilitation of transmission it produces are more readily elicited by trains of conditioning stimuli. In our experiments, we could find no evidence that atropine in concentrations of 1, 10 or 100 μg/ml. (2·9, 29 and 290 μM respectively) had any effect in depressing the late facilitation. However, with short trains of conditioning stimuli (10 Hz for 1 sec), a proportion of the late S_a facilitation does appear to be atropine sensitive (M. J. Brimble, unpublished observations), a result in agreement with Libet's findings (1964).

Inhibition of the S_a response

It has already been suggested that inhibition of the S_a ganglion cells is partly a consequence of convergent B and C fibre inputs. Further, it is generally agreed that the inhibition is related to the generation in ganglion cells of the hyperpolarizing P wave (Eccles, 1935*c*; Eccles & Libet, 1961; Gebber, 1968). It seemed important to investigate more carefully the effect

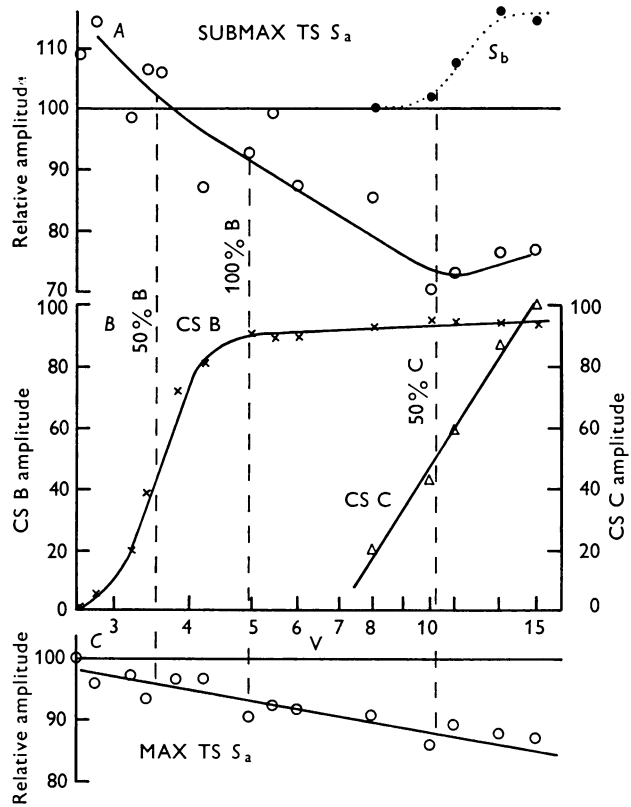

Fig. 7. The effect of varying the strength of the conditioning stimulus on the inhibition of submaximal and maximal S_a responses in the same preparation.

A TS was preceded by a CS 200 msec earlier. All abscissae show voltage of CS.

A, open circles and continuous line, inhibition of submaximal S_a response (SUBMAX TS S_a). TS submaximal for preganglionic B fibres. Ordinate: relative amplitude, expressed as percentage of control value. Filled circles (S_b) indicate facilitation of S_b responses to TS submaximal for preganglionic C fibres; no facilitation occurred when the CS was below threshold for the C fibres (points omitted).

B, relative amplitude of B and C fibre components of the preganglionic compound action potential evoked by CS (CS B and CS C respectively). Ordinates (left and right): amplitude of B and C fibre responses, respectively, in arbitrary units. The dashed vertical lines indicate the stimulus strength at which approximately 50% and 100% of the preganglionic *B* fibres and 50% of the preganglionic C fibres were excited by the CS.

C, inhibition of a maximal S_a response (MAX TS S_a). TS supramaximal for preganglionic B fibres. Ordinate as in *A*.

of the preganglionic pathways on inhibition of S_a, 200 msec after a conditioning stimulus.

The results of an experiment where S_a responses to a submaximal and a maximal test stimulus were compared in the same preparation are shown in Fig. 7. Also shown is the extent to which the preganglionic B and C fibre pathways were activated by the conditioning stimulus (Fig. 7*B*). The amount of inhibition of the submaximal response varied from one experiment to another and even within the same experiment, but inhibition of a submaximal response was always greater than that of a maximal response (see Fig. 7). At an interval of 200 msec, there was no sign of refractoriness in the preganglionic pathway. Less variation was observed when the S_a response was rendered submaximal by a partial transection of the preganglionic nerve (Fig. 8).

The greater inhibition of submaximal S_a responses was confirmed in other experiments where the conditioning stimulus was of insufficient strength to excite more than a small fraction of the preganglionic C fibres, but excited all the B fibres. Under these conditions, a submaximal S_a response was inhibited to $85{\cdot}1 \pm 3{\cdot}3\,\%$ ($n = 7$) of the control value, while a maximal S_a response was not significantly affected, being $98{\cdot}3 \pm 2{\cdot}3\,\%$ ($n = 6$, $P < 0{\cdot}01$) of control values (see also Fig. 3*B*).

As can be seen from Figs. 7 and 8, there was little or no inhibition when the conditioning stimulus excited only 50 % of the preganglionic B fibres. Indeed, the S_a response was often facilitated when 50 % or less of the B fibres were excited. Even with full activation of the B fibre pathway the inhibition was often small. With a further increase in the strength of the conditioning stimulus, inhibition increased (Figs. 7 and 8, see also Fig. 3*B*). This increased inhibition may be the result of recruitment of preganglionic C fibres; however, the rate of increase of the inhibition was not linearly related to the increase in amplitude of the preganglionic C fibre component evoked by the conditioning stimulus (Fig. 7).

In the experiment illustrated in Fig. 7, the P potential recorded from the surface of the ganglion reached its maximal amplitude when both B and C fibre pathways were fully excited by the conditioning stimulus. When all the B fibres but only 50 % of the C fibres were excited, it was already 96 % maximal. When the B fibres but no C fibres were excited, the P potential was 63 % of its maximum; excitation of 50 % of the B fibres alone produced a P potential which was 29 % maximal.

The relationship between C fibre activation and inhibition of the S_a response in a series of experiments is summarized in Fig. 9. For statistical analysis, experiments where the test stimulus was submaximal for S_a and maximal for S_a were lumped together. It is clear that there was more inhibition when all the B fibres and 50 % of the C fibres were excited

(column 3) than when only 50 % of the B fibres were excited (column 1, $P < 0{\cdot}01$). Further, there was more inhibition when all B and C fibres were excited (column 4) than when the B fibre pathway alone was fully activated (column 2, $P < 0{\cdot}02$). Full activation rather than 50 % activation of the C fibres did not significantly alter the degree of inhibition.

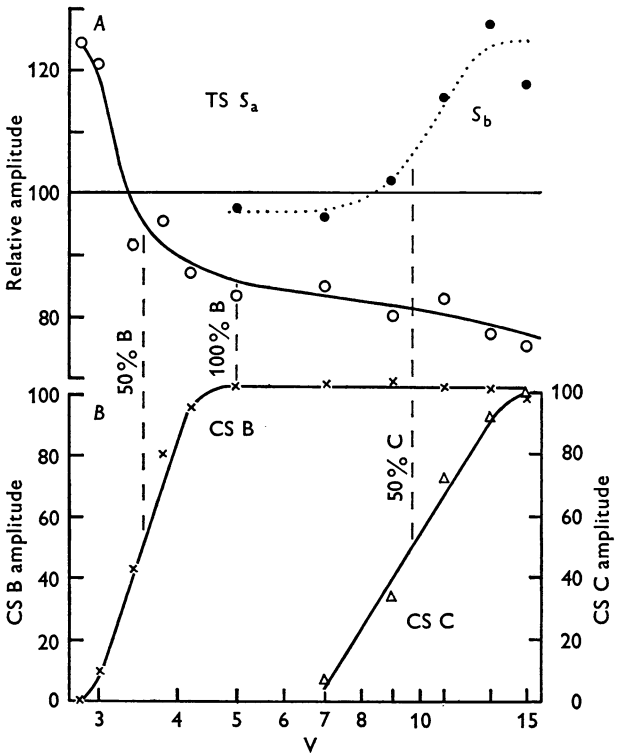

Fig. 8. The effect of varying the strength of the conditioning stimulus on inhibition of S_a responses rendered submaximal by partial transection of the preganglionic nerve.

A TS was preceded by a CS 200 msec earlier. All abscissae show voltage of CS.

A, open circles and continuous line, inhibition of S_a response (TS S_a). TS supramaximal for preganglionic B fibres, but delivered distal to a partial transection of the preganglionic nerve. Ordinate: relative amplitude, expressed as a percentage of control value. Filled circles (S_b) indicate facilitation of S_b responses to TS supramaximal for preganglionic C fibres, but delivered distal to the partial transection; no facilitation occurred when the CS was below threshold for the preganglionic C fibres (points omitted).

B, relative amplitude of B and C fibre components of the preganglionic compound action potential evoked by CS (CS B and CS C respectively). Ordinates (left and right): amplitude of B and C fibre responses, respectively, in arbitrary units. The dashed vertical lines indicate the stimulus strength at which approximately 50 % and 100 % of the preganglionic B fibres and 50 % of the preganglionic C fibres were excited by the CS.

This inhibition is quite consistent with, and in the neuronal model (Fig. 10) is explained by, reduced excitability due to a hyperpolarizing after-potential in the ganglion cells subsequent to their discharge (cf. Lloyd, 1939*b*). This after-potential is probably the initial component of the P wave, for it is doubtful whether the P wave is a unitary phenomenon. The

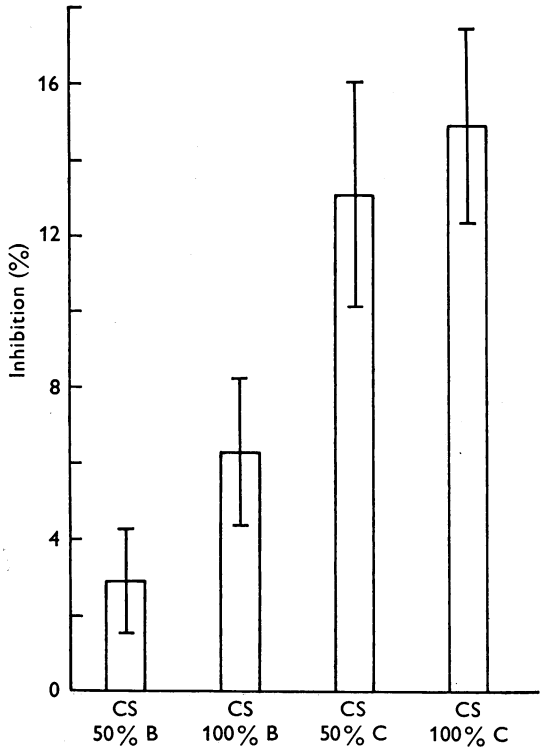

Fig. 9. Inhibition of S_a response to the test stimulus in relation to pre-ganglionic fibre groups activated by the conditioning stimulus 200 msec previously.

Inhibition expressed as a % of control value. Histograms show means ± S.E. of the mean (n = 10). The CS activated 50 % of the B fibres (CS 50 % B), all the B fibres (CS 100 % B), all the B fibres plus 50 % of the C fibres (CS 50 % C) or all the B and C fibres (CS 100 % C).

major and initial part of it, following a single action potential, is due to a phase of increased membrane permeability to potassium ions (Kosterlitz, Lees & Wallis, 1968). The later part is particularly enhanced by trains of stimuli and may be generated by a mechanism involving muscarinic receptors on a type of interneurone which releases a catecholamine (for references see Koketsu, 1969; Libet, 1970).

From Figs. 7 and 8 it is clear that S_b responses were not affected by the

conditioning stimulus until the latter excited the preganglionic C fibres. Then, facilitation of S_b was approximately proportional to the C fibre involvement.

DISCUSSION

A neuronal model of the superior cervical ganglion showing the relationship between pre- and post-ganglionic neurones must account for the following observations. First, reduced early facilitation of the S_a com-

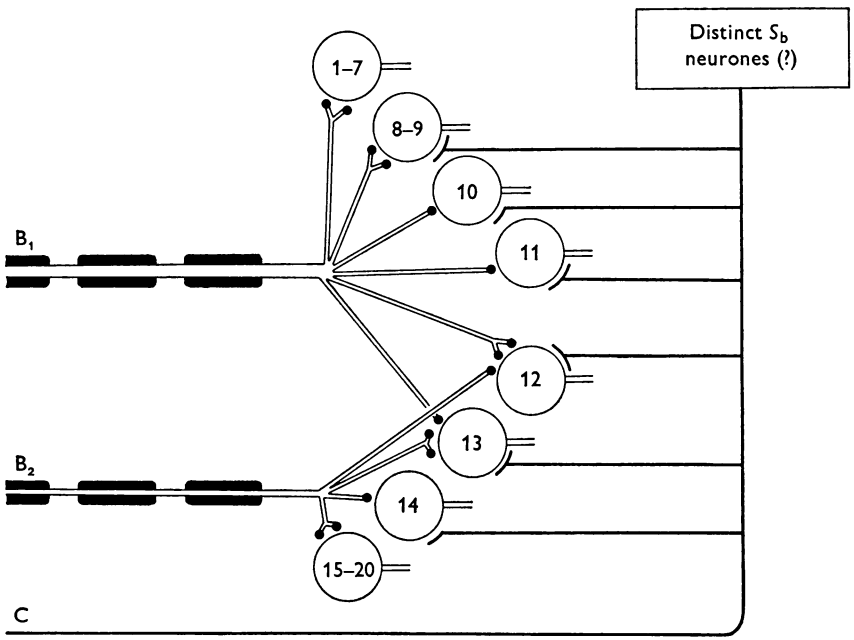

Fig. 10. Diagram of possible anatomical relationships between preganglionic and post-ganglionic neurones in the superior cervical ganglion, constructed on the basis of the experimental results.

In the proposed model: (1) the ratio between preganglionic B fibres and post-ganglionic S_a neurones is 1:10, but ratios of 1:20 or 1:30 could be simply accommodated. (2) It is possible to define S_a cells of eight different types, distinguished by their input; hence, eight distinct neurones are depicted. (3) The proportion of cells falling into each category varies, as indicated by the numbering of the cells.

B_1, low threshold myelinated preganglionic fibre; B_2, higher threshold myelinated preganglionic fibre; C, unmyelinated preganglionic fibre; circles, 20 post-ganglionic S_a neurones. The status of the S_b neurones is uncertain (see text).

When two or more terminals in synaptic contact with a cell are activated, it is assumed, arbitrarily, that the cell will discharge. Excitation of a single terminal results in subliminal excitation of the cell. Cells 10, 11 and 13 are in the subliminal fringe of pathway B_1; cells 14 (and 12) are in the subliminal fringe of pathway B_2. The double C fibre terminals only discharge cells after a delay, because of longer conduction times in the C pathway.

ponent and a smaller subliminal fringe are observed with maximal as opposed to submaximal conditioning stimuli. This suggests convergent B fibre pathways of different thresholds on to S_a ganglion cells. Secondly, a subliminal fringe of S_a cells exists under all conditions of B fibre excitation. Thirdly, as suggested by the results on early facilitation and inhibition of the S_a component, many S_a cells must be innervated by B and C fibres. Fourthly, an interdependence exists between the S_a and S_b components, for the depression of the S_b component when the S_a response showed greatest early facilitation suggests this.

A diagram (Fig. 10) of the possible anatomical arrangement in the rabbit superior cervical ganglion has been constructed on the basis of a ratio of preganglionic B fibres to post-ganglionic S_a neurones of 1:10, a ratio chosen in part because it made calculation simple. It is quite clear that there must be divergence of the preganglionic pathways, for ganglion cells outnumber preganglionic nerve fibres by a large factor. Billingsley & Ranson (1918) suggested the ratio was about 32:1 in the cat. Later estimates have suggested lower ratios because they have taken into account the preganglionic C fibres (Wolf, 1941; Samuel, 1953), although there is still considerable uncertainty because a variable number of unmyelinated fibres in the cervical sympathetic may be post-ganglionic (Foley & Dubois, 1940; Douglas & Ritchie, 1956; Douglas, Lywood & Straub, 1960). Wolf's (1941) figures for the cat superior cervical ganglion give a ratio of between 11:1 and 17:1 and Samuel (1953) concluded that similar ratios held for the rabbit.

On the basis of Fig. 10, a submaximal stimulus to the B fibres will excite B_1 alone, since this is of lower threshold. Cells 1–7, 8–9 and 12 (ten in all) will be excited to discharge and an e.p.s.p. will be set up in cells 10, 11 and 13, subthreshold for spike initiation. This e.p.s.p. may sum with a subsequent e.p.s.p. generated 50 msec later, so that a second stimulus to the B_1 pathway will caused these three cells, plus the original ten, to discharge. Predicted facilitation is 30%, compared with observed values for paired submaximal stimuli to the B fibre pathway of 27–33% (Fig. 3 and results in text). A conditioning stimulus which excites the B_1 and B_2 pathways will also cause facilitation if the test stimulus excites only the B_1 pathway. Cells 10 and 11 are subliminally excited and are recruited by a subsequent stimulus to the B_1 pathway. Cell 13 now discharges to the conditioning stimulus (as do cells 1–7, 8–9, 12 and 15–20); excitation of a single terminal on cell 13 by the test stimulus will not bring it to the threshold for spike initiation. The facilitation is therefore 20%. This reduction in the amount of facilitation was observed, facilitation being between 15 and 25% in Figs. 4*A* and 5*A*. A maximal stimulus to the B_1 and B_2 pathways discharges seventeen cells (see above). Subthreshold excitation of cells 10, 11

and 14 occurs, which may discharge if a second e.p.s.p. is generated in them before the first e.p.s.p. has fully decayed. Predicted facilitation for paired maximal stimuli to the B fibres is 17½%, compared with observed values which showed considerable variability (8–15%, Fig. 3 and text). However, when compared in the same preparation, facilitation was always greater than where the conditioning stimulus was submaximal for the B fibres. In Fig. 4*C* facilitation was about 10% when the conditioning stimulus excited 50% of the B fibres. In the model, a conditioning stimulus to B_1 alone excites cells 10, 11 and 13 subliminally; a test stimulus maximal for the B pathway normally discharges cell 13 (and sixteen other cells), so that only cells 10 and 11 are recruited to fire (total nineteen cells); predicted facilitation is 12%.

The model thus agrees reasonably well with the observed values for early facilitation on the assumption that the latter is the consequence of temporal summation of e.p.s.p.s arising in cells of a variable subliminal fringe. Eccles (1935*b*) also reported a smaller subliminal fringe as the conditioning stimulus increased in magnitude for the cat superior cervical ganglion, but with maximal stimuli the subliminal fringe had disappeared for one cell group at least. A different situation obtains in the rat superior cervical ganglion, for Dunant (1967) found that the size of the subliminal fringe increased as the number of preganglionic fibres excited increased.

Intracellular studies by Erulkar & Woodward (1968) in the rabbit superior cervical ganglion and by Perri *et al.* (1970) in the same ganglion of the rat and guinea-pig showed that some cells discharge two action potentials on stimulation of the preganglionic nerve, one in response to the B fibre input and the other in response to a higher threshold input, presumably a C fibre pathway. Further, Libet & Tosaka (1969) concluded that three to four, and even as many as seven, preganglionic neurones might converge on one ganglion cell in the rabbit superior cervical ganglion. Many cells received both B and C fibre inputs. Our results suggest that many S_a cells are innervated by both B and C fibres. When the C fibre pathway is excited, a delay is introduced due to its relatively low conduction velocity; the S_b response in part results from the activation of these same S_a cells by their C fibre input. In the diagram, activation of the C fibres would discharge cells 8–9, 11, 12 and 13; cells 10 and 14 would also fire, assuming the e.p.s.p. generated by the single C input can summate with the e.p.s.p. previously generated by the B input. Discharge in cells 10, 11, 14 (and 13) would result in dissipation of the e.p.s.p. evoked by a conditioning stimulus to the B fibres, so that subsequent excitation of single B fibre terminals would not evoke a discharge in these cells. Cells 8–9 and 12 are supplied by double B fibre terminals and so would still discharge (see below); however, no facilitation of S_a responses would occur.

When the conditioning stimulus excites B_1 and B_2, but not C, cells 10, 11 and 14 may be recruited from the subliminal fringe by a stimulus 50 msec later. If this test stimulus also excites the C pathway, the results indicated that the S_b response this evoked was depressed by $11\frac{1}{2}\%$. In the model, cells 10 and 14, recruited from the subliminal fringe, would now no longer fire to C fibre excitation, since firing of the cell as a consequence of B fibre excitation dissipates the e.p.s.p. This would explain why recruitment of S_a cells diminishes the size of the S_b response. However, it is not possible to predict the extent of the depression, since the number of distinct S_b neurones is unknown.

It is also more difficult to use the model to predict the consequences of partial transection of the preganglionic nerve. However, the results of Fig. 5 and those in the text are consistent with a non-selective effect. The test stimulus then excites some of the B_1 and B_2 fibres and gives a pattern of facilitation not unlike that for a maximal stimulus to the B fibre pathway.

The activation of both B and C fibres by the conditioning stimulus reduces a submaximal S_a response 200 msec later by 20–30% (Fig. 7) and a maximal S_a response by 10–15% (Figs. 3 and 7). How this might arise with the anatomical arrangement of Fig. 10 requires one further postulate about ganglion cell excitability subsequent to discharge. A small residuum of the e.p.s.p. may be rebuilt after the spike (Eccles, 1935*c*) and it is not until this declines and after-positivity is apparent, that inhibition is seen most intensely. 50 msec after the conditioning stimulus, cells 8–9 and 12 for instance would be relatively less inhibited than after 200 msec and fire to B fibre activation. Cells 10, 11, 14 (and 13 in some circumstances) do not normally fire to a second B fibre stimulus, unless a substantial residuum of an e.p.s.p. remains. At an interval of 200 msec, cells 8–9 and 12 could also be rendered inexcitable by the after-positivity generated in them by the B and C fibre-evoked responses. Then a test stimulus to the B_1 pathway would excite only cells 1–7; 30% inhibition as a control response involves ten cells. Since less inhibition is observed when the test stimulus is maximal for the B pathway, it must be supposed that B_2 fibres provide extra excitation to cells previously inhibited. Thus, cells 12 and 13 are shown with a B_2 input which lifts them out of the inhibited zone. Cells 8–9 remain inhibited, so that predicted S_a inhibition is 12% (two out of seventeen cells).

It remains possible that some cells exist with a purely C fibre input and which can be called S_b cells. Perri *et al.* (1970) in fact identified some neurones with a purely C fibre input in the guinea-pig superior cervical ganglion. In the experiments reported here, the fact that recruitment of cells occurs during facilitation of the S_b response (Figs. 3 and 7) means that there must be cells

in a subliminal fringe for the C fibre cell pool. These are not identified in Fig. 10 because we do not know whether they are separate S_b neurones or S_a neurones with single C fibre terminals. The exact status of the S_b cells, therefore, remains unresolved. However, in general we conclude that the notion of independent cell pools with separate functional connexions (Eccles, 1935*a*) needs revision. Either the electrophysiological methods discussed here cannot discriminate between the functional pools or the pools are less independent than was previously thought.

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