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THE TIME COURSES OF EXCITATORY AND INHIBITORY SYNAPTIC ACTIONS

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When recorded intracellularly the excitatory and inhibitory post-synaptic potentials (EPSP and IPSP) generated in motoneurones by virtually synchronous synaptic action (Brock, Coombs & Eccles, 1952; Coombs, Eccles & Fatt, 1955*b*, *c*) have time courses that resemble the end-plate potentials of curarized muscle in having a steep rising phase and a slower, approximately exponential, decay. With the end-plate potential it has been shown that the total displacement of charge, as distributed along the muscle fibre membrane, reaches a maximum in 1–2 msec and then decays in a strictly exponential manner (Fatt & Katz, 1951; Boyd & Martin, 1956). Since the observed rate of exponential decay is in close agreement with the rate to be expected from the measured electrical properties of the muscle fibre, it has been concluded that the neuromuscular transmitter acts for no longer than 2 msec in causing an active transfer of charge across the post-synaptic membrane (Fatt & Katz, 1951). Similarly, it was suggested that the approximately exponential decaying phases of EPSP and IPSP were entirely attributable to the passive decay of charge, and that the active transmitter action was no more than 2 msec in duration (Eccles, 1953; Coombs *et al.* 1955*b*, *c*).

Since, by means of compensatory circuits, it is now possible to measure directly the time constant of the motoneuronal membrane (Araki & Otani, 1955; Coombs, Curtis & Eccles, 1956, 1959), this suggestion can be tested experimentally. Furthermore, other methods give evidence relating to the time courses of active transfer of charge that is brought about by the excitatory and inhibitory synaptic transmitters. The necessity for this further investigation was emphasized when special precautions were taken to exclude distortions introduced by delayed synaptic actions. The IPSP was found always to decay faster than the EPSP; the postulated passive decay could not strictly obtain for both. A preliminary account of part of this investigation has already

been published (Coombs *et al.* 1956). Fessard & Tauc (1957) and Tauc (1958) have also reported that, with the giant ganglion cells of *Aplysia* and *Helix*, the EPSP's and IPSP's decay more slowly than would be predicted from the passive electrical properties of the membrane, and have likewise concluded that there is a long residual phase of excitatory synaptic transmitter action. Hagiwara & Saito (1957) have made comparable observations with the supramedullary neurones of the puffer fish.

METHODS

The methods of investigation have been described in the previous paper (Coombs *et al.* 1959). Special precautions were taken to ensure that the EPSP and IPSP were generated by virtually synchronous synaptic actions. The stimuli were kept as weak as possible so that the volleys would be largely in Group Ia fibres with a minimum of contamination by Ib impulses (Laporte & Bessou, 1957; Eccles, Eccles & Lundberg, 1957*a*). In all cases the afferent volley was recorded by means of an electrode appropriately placed on the dorsal root entry.

RESULTS

Analysis of EPSP's and IPSP's on the basis of the electrical properties of the post-synaptic membrane

In a considerable number of motoneurones it has been possible to measure the time course of the membrane potential change produced by rectangular current pulses and at the same time to record the EPSP's and IPSP's generated by Group Ia afferent volleys. For example, in Fig. 1 H–O, the difference between

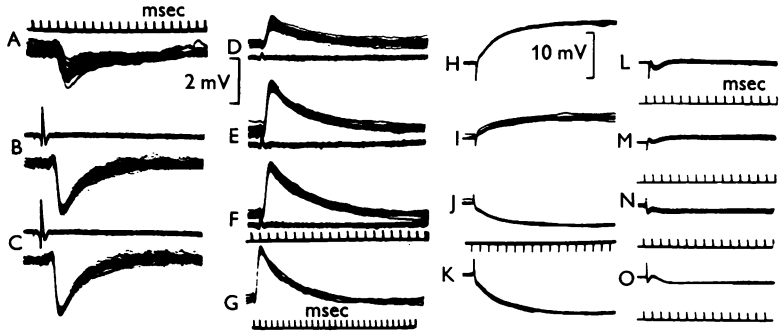

Fig. 1. Intracellular recording (double-barrelled K_2SO_4 micro-electrode) from a posterior biceps-semitendinosus motoneurone (resting membrane potential −66 mV) showing IPSP's produced by a Group Ia quadriceps afferent volley (A–C) and EPSP's produced by a Group Ia biceps-semitendinosus afferent volley (D–G). In addition in B–F the spike potentials of the afferent volleys, as recorded by an electrode on the appropriate dorsal root, reveal that there is minimal contamination by Group Ib impulses. All records formed by superposition of about 40 faint traces. H–K show intracellular potential changes for depolarizing pulses of 12 and 6×10^{-9} A and hyperpolarizing pulses of 6 and 12×10^{-9} A, the artifacts being largely balanced out by a compensatory circuit. L–O show just-extracellular potential changes for these same current pulses and the same setting of the compensatory circuit. Note separate potential scales for the synaptic potentials and the pulse responses. Time scales shown throughout in milliseconds, G being at a slower sweep than the other records.

the potential curves produced by a current pulse with intracellular or extracellular location of the micro-electrode gives the time course of the membrane potential change, and from this the time constant of the motoneuronal membrane can be calculated, as described in the preceding paper (Coombs *et al.* 1959). Fig. 1G was taken at a slower sweep speed in order to reveal the whole time course of decay of the EPSP produced by a Group Ia afferent volley; it follows an approximately exponential time course down to a slight hyperpolarization, as reported by Brock *et al.* (1952) and Coombs *et al.* (1955*c*). In contrast, the IPSP produced by a Group Ia volley from the antagonist muscle nerve declines exponentially to the initial base line (Fig. 1, B, C), which has been an invariable feature with IPSP's produced by Group Ia volleys.

By projecting and averaging several superimposed traces, the mean EPSP and IPSP produced by submaximal or just-maximal Group Ia volleys (Fig. 1B, C, E, F, G) have been obtained as in Fig. 2A and B respectively. It will be noted that for the EPSP curve the zero potential has been set at the level of the after-hyperpolarization, i.e. it is assumed that this low level of hyperpolarization is submerged beneath the whole decaying phase of the EPSP. It is immediately evident from Fig. 2 that throughout its whole decaying phase the EPSP is much slower than the IPSP. Both post-synaptic potentials have an approximately exponential decay with respective time constants of 5·0 and 3·7 msec, the time constant for the IPSP being very close to that derived from the current pulses, 3·6 msec (Coombs *et al.* 1959). This difference between the time constants of decay of the EPSP and IPSP was observed for every motoneurone of Table 1 (column 9). It was also observed for all other motoneurones that were in reasonably good condition and in which EPSP's and IPSP's were produced by Group Ia afferent volleys. For the twelve motoneurones in this additional series the time constant of the EPSP (mean 4·5 msec) was on the average 1·5 times the time constant for the IPSP (mean 2·9 msec).

The significance of this difference between the decays of the EPSP and the IPSP is best appreciated by employing the membrane time constant in analysing the respective curves into their active and passive components. Thus $\mathrm{d}V/\mathrm{d}t = I/C - V/\tau_m$, where $\mathrm{d}V/\mathrm{d}t$ is the slope of the potential curve at any instant t, C and I are respectively the capacity of the membrane and the current flowing through it and τ_m is the electric time constant of the membrane (cf. Hill, 1936). Such analyses give the time courses of the active currents which are required to produce the EPSP and the IPSP; provided that it is assumed that the generating foci of these currents are uniformly distributed over the soma and dendrites. As is shown in Fig. 2A, B, the rapid rising phases of both the EPSP and IPSP are produced by brief intense currents in the positive and negative directions respectively (cf. the broken lines). As would be expected, the relatively slow decay of the EPSP is shown by the analysis to be attributable to a prolonged low residuum of depolarizing current. On the

other hand, the current generating the IPSP does not continue to any appreciable extent after the initial brief peak and the brief phase of reversal. The values for the electric time constants of the membrane and the time constants of the decay of the EPSP and IPSP indicate that with other motoneurones the active currents generating the EPSP and IPSP have time courses resembling those of Fig. 2A, B. For example, analyses (Fig. 3) of the EPSP's produced in another motoneurone by two converging afferent volleys, as previously

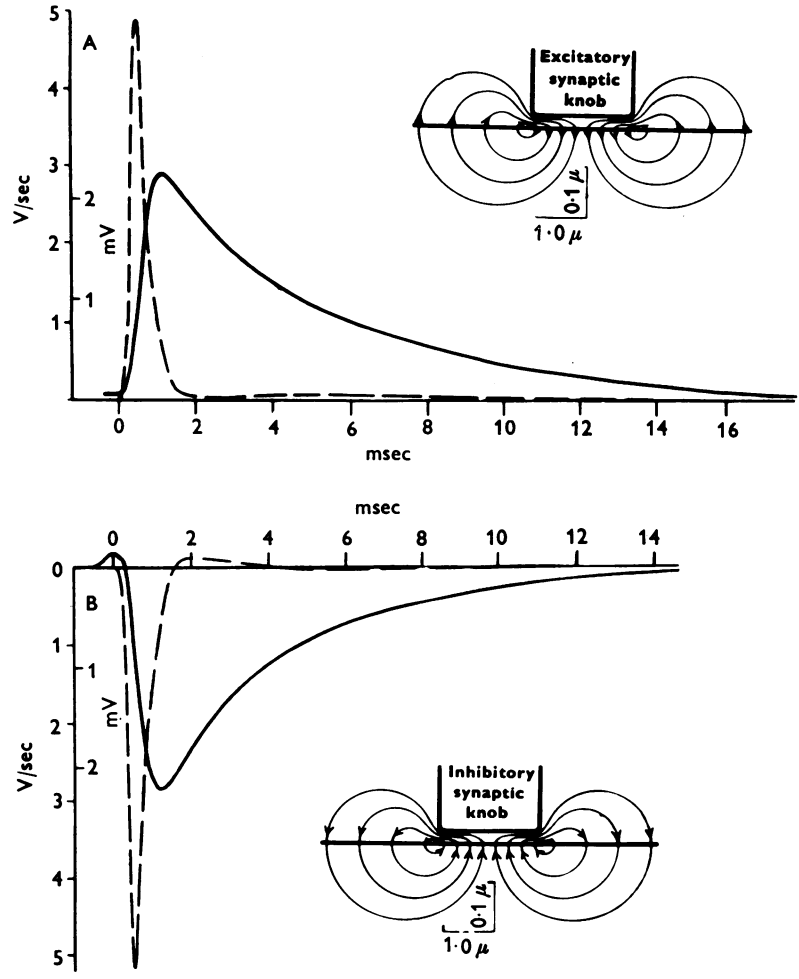

Fig. 2. Mean curves of the EPSP (A) and IPSP (B) are plotted from the records illustrated in Fig. 1E–G and B, C respectively. These curves are analysed as described in the text on the basis of the membrane time constant derived from Fig. 1H–O. The excitatory and inhibitory synaptic currents so derived are plotted as broken lines on the ordinate scales of V/sec. The inset curves show the respective directions of current flows for excitatory and inhibitory synapses.

illustrated (Coombs *et al.* 1959, Fig. 3), reveals low residual active depolarizing currents as in Fig. 2A. The directions of the depolarizing and hyperpolarizing currents produced by excitatory and inhibitory synaptic action are shown in Fig. 2 by the inset figures of schematic synapses.

TABLE 1. Time constants of the motoneuronal membrane (τ_m) and of the approximately exponential decays of Group Ia EPSP's and IPSP's (τ_{EPSP} and τ_{IPSP}). All motoneurones are BST

Experiment	Electrode type	Membrane potential (mV)	τ_m (msec)	τ_{EPSP} (msec)	τ_{IPSP} (msec)	$\frac{\tau_{EPSP}}{\tau_m}$	$\frac{\tau_{IPSP}}{\tau_m}$	$\frac{\tau_{EPSP}}{\tau_{IPSP}}$
1	2	3	4	5	6	7	8	9
5/3/56	*S* KCl	−60	2·4	4·9	2·8	2·0	1·2	1·7
5/3/56	*S* K_2SO_4	−58	3·3	4·3	3·3	1·3	1·0	1·3
20/6/56		−55	3·6	5·8	3·7	1·6	1·0	1·6
25/6/56		−60	2·3	4·6	2·7	2·0	1·2	1·7
25/6/56		−60	2·5	4·3	2·9	1·7	1·2	1·5
18/7/56		−70	2·4	6·1	3·4	2·5	1·4	1·8
18/7/56	*D* K_2SO_4	−56	2·3	4·8	3·6	2·1	1·6	1·3
18/7/56		−70	3·6	6·0	4·0	1·7	1·1	1·5
1/2/57		−56	2·0	3·5	2·5	1·7	1·2	1·4
1/2/57		−57	2·9	4·8	3·3	1·7	1·1	1·5
1/2/57		−56	3·6	4·7	3·8	1·3	1·1	1·2
5/2/57		−66	3·6	5·0	3·7	1·4	1·0	1·4
Means		−60	2·9	4·9	3·3	1·7	1·2	1·5

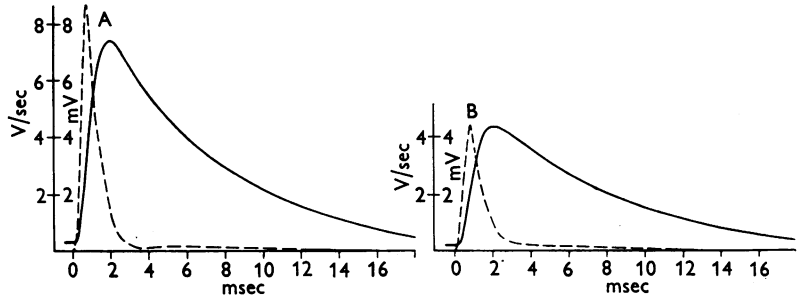

Fig. 3. Lateral gastrocnemius motoneurone with resting membrane potential of −69 mV. Mean curves for intracellularly recorded EPSP's (double K_2SO_4 electrode) are shown by the continuous lines in A and B for lateral and medial gastrocnemius afferent volleys respectively, the original records being illustrated in the preceding paper (Coombs *et al.* 1959, Fig. 3). The curves have been analysed on the basis of the measured membrane time constant and are plotted as broken lines as in Fig. 2A.

Frequently the analysis reveals that the peak of the IPSP current declines to a low transient phase of reversal, as in Fig. 2B at 2–4 msec. This does not necessarily imply that there is actually a reversal of the current flowing out at the synapses. It is more likely that the inhibitory synapses are not distributed uniformly over the soma and dendrites, as was assumed. If they were concentrated on the soma and proximal region of the dendrites, there would be a late electrotonic distribution of the hyperpolarization to more remote

regions, which would have the same effect on the potential recorded intrasomatically as a reversal of synaptic current. It seems likely that a similar electrotonic redistribution of depolarization occurs with the EPSP, but usually it appears merely as an abnormally low phase of residual current at about 2–4 msec, as in Figs. 2A, 3A, though exceptionally there may be a transient reversal (Fig. 9C).

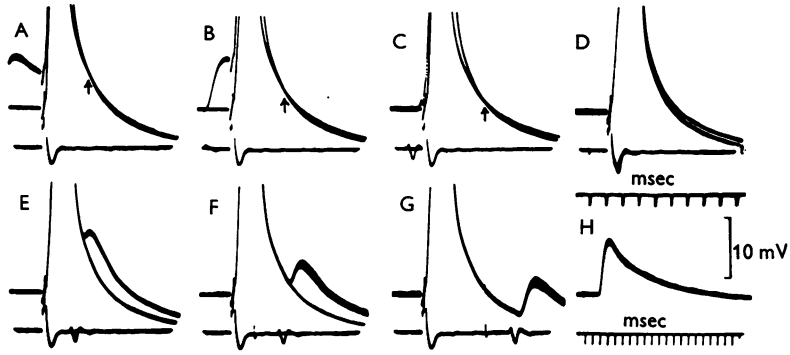

Fig. 4. Interaction of antidromic spike potential and the EPSP with intracellular recording from a gastrocnemius cell with a single K_2SO_4 electrode, the resting membrane potential being −70 mV. The EPSP was set up by a gastrocnemius afferent volley which in A–F was turned on only for about half the superimposed traces, so that the other half give the control antidromic spike potential. In G the afferent volley was not turned off. The lower traces of C–G show the dorsal root spike set up by the afferent volley (it preceded the trace in A and B). H shows the control EPSP at a slower sweep speed. Same potential scale applies to all records.

Analysis of EPSP's and IPSP's by interaction with an antidromic impulse, and by their interaction with each other

It should be possible to demonstrate the residual depolarizing current shown in Figs. 2A and 3 by recording the interaction of an EPSP and an antidromic impulse at various intervals. If an impulse is propagated over the surface of the soma and dendrites of a motoneurone after the peak phase of the current generating the EPSP, the intense membrane currents of the impulse will remove all the potential change produced by this peak phase. Any subsequent development of depolarizing potentials superimposed on the declining phase of the spike would be due to the low residual phase of active current depicted in Figs. 2A and 3.

At each test interval of Fig. 4A–F about ten traces were photographed for the antidromic spike potential alone, and then about ten with the excitatory synaptic action as well. The control of the EPSP alone is seen in H at a slower sweep speed in order to show the full time course of its approximately exponential decay, the time constant being 4·8 msec.

When the afferent volley follows the antidromic impulse (E, F, G), it generates

an EPSP that is diminished in size and with an earlier summit and faster decay, as already reported (Eccles, 1952; Coombs *et al.* 1955*c*). Even when the afferent volley arrives at the spinal cord at the same time as the antidromic impulse, it is seen in D to add a small low depolarization to the decaying phase of the spike. When the afferent volley is earlier still (C, B, A), so that it generates the EPSP before the antidromic impulse invades the motoneurone, a complication is introduced by its action in shortening both the M–IS and IS–SD delays that occur in the antidromic invasion of a motoneurone (Coombs *et al.* 1955*a*; Frank & Fuortes, 1956; Coombs, Curtis & Eccles, 1957*a*, *b*). Thus, in making an accurate assessment of the depolarization added to the decaying phase of the spike, an allowance has to be made for the temporal discrepancy between the respective spikes. Even before this allowance is made, it is evident in B and C that the afferent volley has a delayed depolarizing action that is sufficiently large to cause the combined potential to cross the control at the points indicated by the arrows. Even in A the latter part of the combined trace is above the control, causing a distinct widening of the trace from the arrow onward.

In plotting the depolarizing potentials in Fig. 5, as determined by subtraction of the traces of Fig. 4, allowance has been made for the temporal discrepancy between the respective spikes. Subtraction is a very inaccurate procedure during the rapid declining phase of the spike. In any case the very short time constant of the spike decay shows that the post-synaptic membrane has an ionic conductance about ten times its resting level, which would correspondingly diminish the membrane potential change produced by currents flowing into activated synapses. Furthermore, even during the latter part of the spike, these currents are diminished because the motoneuronal membrane potential is closer to the equilibrium potential for the activated excitatory synapses, which is about zero potential (Coombs *et al.* 1955*c*). The action of excitatory synapses on the membrane potential has therefore been plotted in Fig. 5 only from the terminal phase of the spike potential onwards.

Even when the antidromic spike potential was superimposed so late that the EPSP had declined to about half (Fig. 4A), a small depolarizing potential was built up after the spike (Fig. 5B **A**). This residual depolarizing action was progressively increased as the antidromic spike was moved earlier relative to the EPSP (Fig. 5, **B** to **C** to **D**), and finally there was a rapid increase in this depolarization when the peak of the synaptic excitatory action (Fig. 4E, F, G) occurred late on the decline of the spike (Fig. 5B **E**, **F**, **G**).

In previous attempts at this type of experiment it was reported that residual depolarization could not be detected (Coombs *et al.* 1955*c*; Fatt, 1957), or when a small amount was observed it was attributed to stray synaptic action of long latency. The residual depolarizations illustrated in Figs. 4 and 5 are now regularly observed in all experiments (cf. Eccles, 1957, Fig. 12) in which

conditions are rendered specially favourable by the large size of the EPSP and the low noise level of the intracellular recording. Furthermore, special precautions have been taken to eliminate delayed synaptic action through polysynaptic pathways. The afferent volleys have been set up by stimuli either just submaximal or just-maximal for Group Ia, which would virtually eliminate complication by Group Ib afferent impulses (Laporte & Bessou, 1957; Eccles *et al.* 1957*a*). In any case the motoneurones principally employed have been

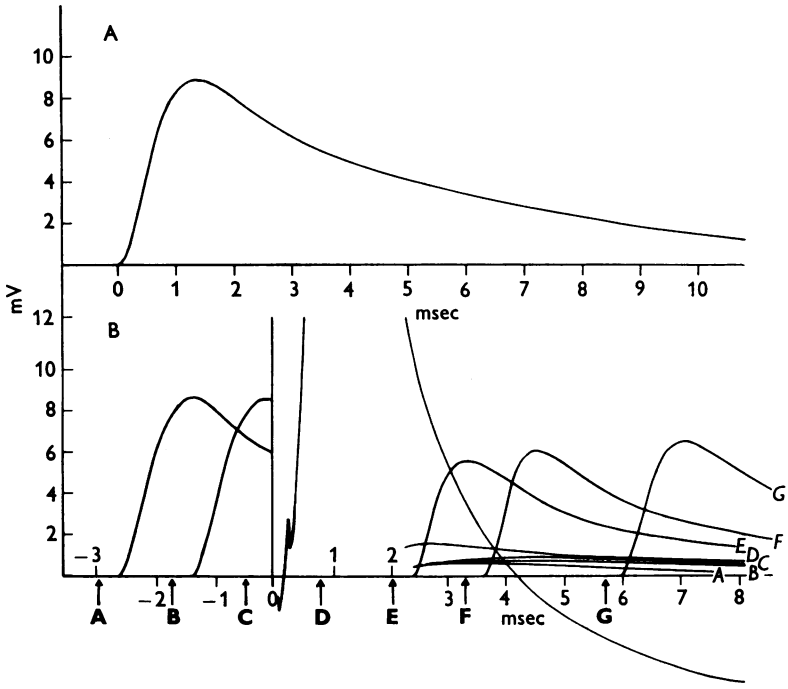

Fig. 5. Plotted curves derived from records shown in Fig. 4. A gives the control record for the EPSP, while B shows the potentials added by the excitatory afferent volley at the various times of Fig. 4 A–G relative to the antidromic spike potential, which is shown truncated and beginning just after the artifact produced by the antidromic stimulus at zero on the time scale. The arrows marked **A–G** give the times of arrival at the spinal cord of the afferent volleys for the series of Fig. 4 A–G. The respective potentials produced after the antidromic spike are determined by subtraction as described in the text and are labelled (*A–G*).

gastrocnemius-soleus, flexor digitorum longus or plantaris, on all of which Group Ib action is inhibitory rather than excitatory (Eccles, Eccles & Lundberg, 1957*b*). Finally, it should be pointed out that synaptic excitatory action by Group Ib impulses is not more than 3 msec later than monosynaptic excitatory action, and hence could not account for the very late residual excitatory action seen in Figs. 4 and 5. It therefore seems probable that the initial Group Ia afferent volley has a delayed residuum of activity for many milliseconds after the brief initial peak of action. Thus independent evidence

has been obtained in support of the time course for the active phase of synaptic excitatory action that is shown in Figs. 2A and 3. An objection (cf. Fatt, 1957) to both the analytical procedures illustrated in Figs. 2, 3 and 5 will be considered in the Discussion, before a quantitative comparison is made between the results obtained by these two procedures, and before final conclusions are reached with respect to the time course of action of a single excitatory synapse.

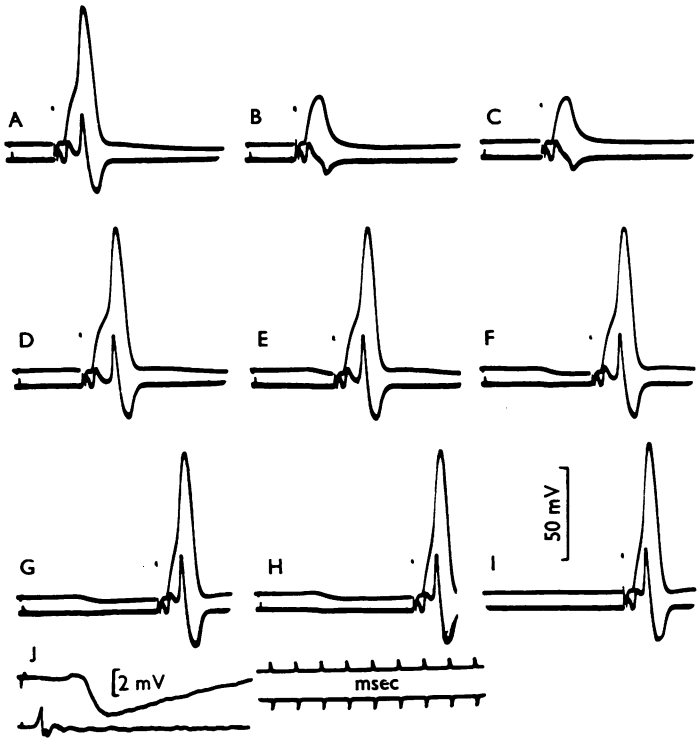

Fig. 6. Biceps-semitendinosus motoneurone with single K_2SO_4 electrode, the resting membrane potential being −70 mV. Interaction of antidromic spike potential and an IPSP produced by a Group Ia afferent volley in quadriceps nerve. The lower traces of A–I are electrically differentiated records. The IPSP produced by the quadriceps afferent volley is shown at higher amplification in J. All records are at same sweep speed and the artifact of the quadriceps stimulus is seen at the same position at the onset of each record except I, which is the control antidromic spike potential. The antidromic impulse is moved progressively later relative to the IPSP from A to H.

The effect of an IPSP in depressing the peak of an antidromic spike has already been illustrated (Coombs, Eccles & Fatt, 1955*d*, Fig. 6A). A significant depression was observed only during a brief period of about 2 msec that corresponded to the assumed phase of inhibitory transmitter action (cf. Fig. 2B). The IPSP also exerts a depressant action on the IS–SD transmission of an antidromic impulse. For example, in Fig. 6 the Group Ia IPSP, shown at

higher amplification in Fig. 6J, caused the IS–SD blockage over the range of intervals from Fig. 6B–C. When the antidromic impulse was slightly later (D), there was merely a delayed IS–SD transmission, though it was occurring at the maximum phase of hyperpolarization of the IPSP. With still later positions of the antidromic impulse (E–H) the differentiated records in particular reveal that the IS–SD delay was progressively shortened till it was the same as in the control antidromic response (I). It appears that, during the rising phase of the IPSP, antidromic IS–SD transmission is severely depressed by some factor other than hyperpolarization *per se*. This depression thus provides additional evidence for the existence of a brief intense phase of active hyperpolarizing current, such as is indicated by the analysis of the IPSP in Fig. 2B.

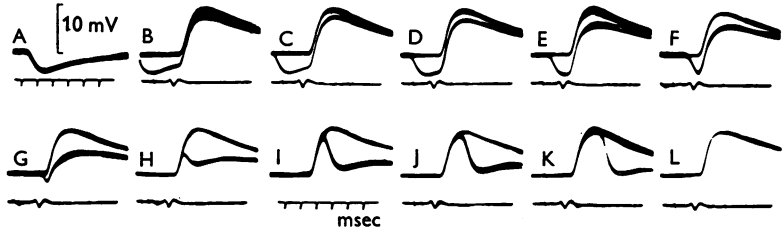

Fig. 7. Biceps-semitendinosus motoneurone with double-barrelled K_2SO_4 electrode, the resting membrane potential being −70 mV. IPSP's are set up by quadriceps Group Ia afferent volleys, and the EPSP's by biceps-semitendinosus afferent volleys. B–K show interaction of IPSP and EPSP at various intervals, the control responses being given in A and L respectively. All records were formed by the superposition of about 40 faint traces, but the quadriceps afferent volley was only turned on for about half the traces, so that the control EPSP was superimposed on all records from B to K. Lower traces show the record from the L 7 dorsal root, the quadriceps afferent volley consequently giving a very small spike potential. Same time and potential scales for all records.

The interaction of an IPSP and an EPSP at various intervals has already led to the same inference (Coombs *et al.* 1955*d*, Figs. 4, 5). This interaction is more effectively displayed in Fig. 7, where at each interval the control course of the EPSP alone is shown for about half the superimposed traces. It will be seen that the hyperpolarizing action of the activated inhibitory synapses is greatly potentiated when it is superimposed upon the EPSP. This potentiation is satisfactorily explicable by the displacement of the membrane potential away from the equilibrium potential for the IPSP, which is at about 10 mV of hyperpolarization relative to the resting membrane potential (Coombs *et al.* 1955*b*, *d*; Eccles, 1957, p. 140). However, when the rising phase of the IPSP precedes the onset of the EPSP (Fig. 7B–F), there is no potentiation of the later decaying phase of the IPSP, which can be seen particularly well in the subtracted records of Fig. 8B. Again, this observation reveals that the active hyperpolarizing currents generating the IPSP cease within 2 msec of their onset, as is indicated by the analysis of Fig. 2B. A similar inference may be

drawn from the plotted points of Fig. 8C, where the potentiation of the IPSP is plotted against the interval relative to the EPSP for all the records of the series partly illustrated in Fig. 8B. The time course of the potentiation curve corresponds closely to the EPSP, but begins about 1 msec earlier. This is precisely the relationship that would be expected if the IPSP is generated by a brief hyperpolarizing current, as in Fig. 2B, which is potentiated by the depolarization of the EPSP. The large degree of potentiation (3 to 5·5 mV) would be expected with an equilibrium potential for the IPSP only about 10 mV greater than the resting membrane potential (Coombs *et al.* 1955*b*).

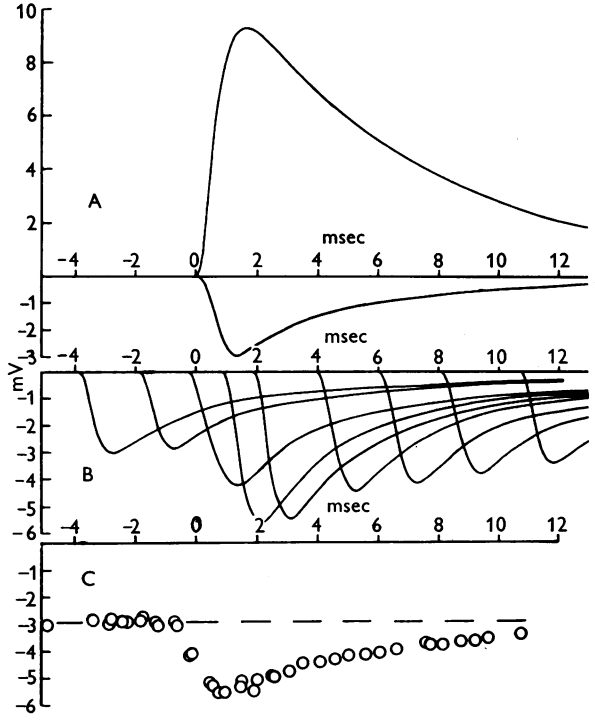

Fig. 8. EPSP'S and IPSP's for the same motoneurone and afferent volleys as in Fig. 7, but at a slower sweep speed. A shows the control EPSP and IPSP, while B shows an analysis of records like those of Fig. 7B–K, but at the slower sweep speed. It is assumed that the EPSP is unaltered by the superimposed IPSP's, which are themselves greatly potentiated as shown. The peak potentials of the IPSP's so determined are plotted in C against the interval between their onset and the onset of the interacting EPSP, part of the series being shown in B. Note that same time scale obtains for A, B and C, zero time being placed at the origin of the EPSP.

The EPSP during hyperpolarization

It has already been reported (Coombs *et al.* 1955*c*) that, when the motoneuronal membrane is hyperpolarized, the rising phase of the EPSP becomes steeper, the summit earlier, and the decay accelerated, as may be seen in the

EPSP illustrated in Fig. 9A, B. When the intensity of the hyperpolarizing current was increased to 3, 4·5 and 8×10^{-9} A, there were graded changes in the EPSP in all these three respects, which were reversed on cessation of the current (lowest records). Since hyperpolarization produces little or no change in the membrane resistance (Coombs *et al.* 1955*a*; Frank & Fuortes, 1956; Coombs *et al.* 1959), it is justifiable to assume constancy of the membrane time

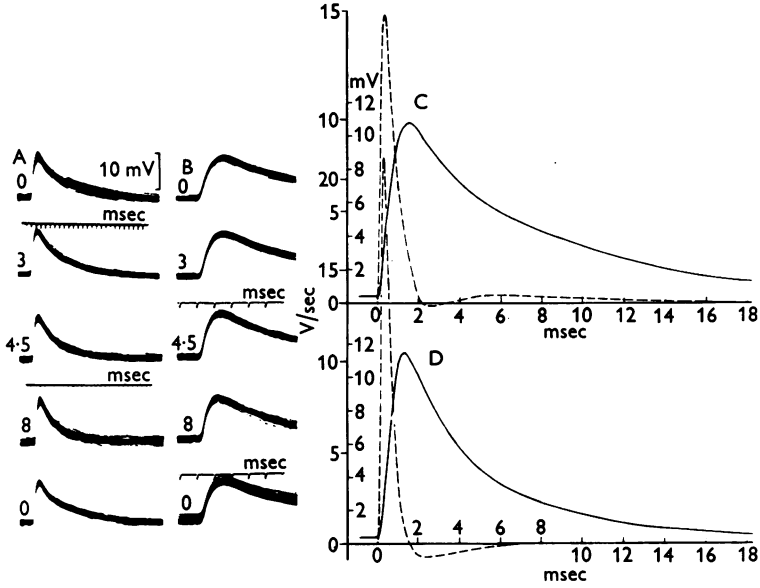

Fig. 9. Effect of hyperpolarizing current on the EPSP. Gastrocnemius motoneurone recorded by a single K_2SO_4 electrode, the resting membrane potential being −74 mV. A and B show EPSP produced by a maximal Group Ia afferent volley from gastrocnemius nerve, at slow and fast sweep speeds. The strength of the applied hyperpolarizing current is indicated on each record in units of 10^{-9} A. Each record is formed by the superposition of about 40 faint traces and the current was applied for the few seconds necessary for each record. The continuous line of C is the control EPSP, and is analysed on the basis of the measured membrane time constant in order to determine the time course of the synaptic current (broken line) as in Fig. 2A. Similarly, D gives the EPSP and calculated synaptic current during the action of 8×10^{-9} A hyperpolarizing current. Note the overlapping ordinate V/sec scales for the currents which are shown partly superimposed for reasons of economy in plotting.

constant and to analyse the various EPSP's on this basis. For example, the time constant was measured for the motoneurone of Fig. 9 by the square pulse technique, as in Fig. 1 (cf. Coombs *et al.* 1959), and the calculated time course of the active depolarizing current is shown by the broken lines in Fig. 9C. The control analysis (C) reveals a prolonged residual current as in Figs. 2A and 3, though there was a brief reversed phase. On the other hand, during a hyperpolarizing current of 8×10^{-9} A (D), there was a considerable accentuation of this reversed phase, but no subsequent residual current. There was also

a considerable potentiation of the initial depolarizing current, its effectiveness being increased from 15 V/sec to 21 V/sec, which may be satisfactorily explained by the increased voltage driving that current (Coombs *et al.* 1955*c*). Thus, hyperpolarization appears to have eliminated the residual phase of synaptic excitatory action and so to have produced the earlier summit and faster decay of the EPSP, which, hitherto, have been inexplicable (cf. Coombs *et al.* 1955*c*). It should be noted that the same action is also effected by after-hyperpolarization, as may be seen by comparing the **E**, **F** and **G** records of Fig. 5B with the control EPSP in Fig. 5A, but there is then the additional complication of increased membrane conductance (cf. Coombs *et al.* 1955*c*).

DISCUSSION

Fatt (1957) has suggested that, if the synapses were mostly located peripherally on the dendrites, the electrotonic spread of current would produce the observed decay of the EPSP at a rate slower than would be expected from the membrane time constant. Thus he envisages a situation analogous to that occurring when the end-plate potential is recorded intracellularly at some distance from the end-plate (cf. Fatt & Katz, 1951). However, when the EPSP is analysed in order to determine the approximate time course of the generating current, it is found (cf. Figs. 2A and 3) that the initial peak is separated from the low residuum by a trough which may even indicate a reversal of current (Fig. 9C), such as is regularly observed with the IPSP (Fig. 2B). A trough of this time course would be expected if the activated synapses were preponderantly distributed to the soma and proximal dendritic regions, so that electrotonic spread of the EPSP peripherally to the dendrites would give effectively a current opposing the low residuum of synaptic depolarizing current. With the IPSP the residual synaptic hyperpolarizing current is negligible, the trough being correspondingly more prominent (Fig. 2B).

This interpretation of the analysed curves accords well with the observed location of the monosynaptic excitatory synapses on motoneurones (Szentagothai, 1958). By the bouton-degeneration method he has shown that the monosynaptic excitatory synapses are exclusively formed by the few large presynaptic terminals that make contact with the bases of the large dendrites, and then continue to terminate in knobs on the surface of the soma (cf. Cajal, 1934, Fig. 28). Because an interneurone is interpolated in the pathway for Group Ia inhibition (Eccles, Fatt & Landgren, 1956) Szentagothai has not been able to locate the site of the inhibitory synapses. However, a similar location on the soma and dendritic stumps is indicated by the reversal of current flow that follows the peak of current as determined by analysis of the IPSP (Fig. 2B).

In the preceding paper the electric time constant of the motoneuronal membrane was determined from the time course of potential change produced

by application of a rectangular current pulse (Coombs *et al.* 1959). In view of the considerable error that could be introduced by the allowance for electrotonic spread from soma to dendrites, the values so derived for the time constant can be regarded as no more than rough approximations. Nevertheless, it will appear that such time constants form the basis of reasonable explanations for several observations that are otherwise perplexing.

(1) The time course of decay of the Group Ia IPSP is invariably faster than the EPSP (Table 1, columns 6 and 5), which suggests either a difference in the location of the respective synapses, or a difference in duration of the respective synaptic transmitter actions. There is now strong evidence against the first alternative. When the time constant of the membrane (τ_m) is derived as described in the preceding paper (Coombs *et al.* 1959), it is little if any longer than the time constant of decay of the IPSP (τ_{IPSP}), as is seen in the ratios of column 8, Table 1. If τ_m is derived in the manner suggested by Rall (1957), it would be considerably longer than τ_{IPSP}. Throughout its whole course the IPSP would be decaying much faster than a change in membrane potential uniformly distributed over the whole soma-dendritic membrane. In contrast, on the present analysis there is merely a brief phase of such faster decay following the peak of the inhibitory synaptic current (Fig. 2B), an effect which would be expected if there is an electrotonic redistribution of charge to the more remote dendritic areas.

(2) The rebuilding of EPSP following its destruction by an intercurrent antidromic impulse (cf. Figs. 4, 5) might conceivably be explicable by electrotonic spread from remote dendritic areas that are not invaded antidromically, and in which the EPSP is primarily generated (cf. Fatt, 1957). However, this explanation is in conflict not only with the recent anatomical findings of Szentagothai (1958), but also with the derived time course for the excitatory synaptic current. This, as we have seen, shows a brief dip after the peak, which can be taken to mean that the EPSP is largely generated by synapses in proximity to the soma. There is approximate agreement on the one hand between the magnitude and time course of the residual depolarizing current, as calculated on the basis of the derived value for τ_m (cf. Figs. 2A, 3, 9C), and, on the other, the amount of rebuilt EPSP following its destruction by an intercurrent antidromic impulse (cf. Figs. 4, 5).

(3) The calculated time courses of synaptic current flow in Fig. 9C, D suggest that a hyperpolarizing current diminishes or abolishes the low residuum, and hence the advance of the summit and the accelerated decay of the monosynaptic EPSP (Fig. 9A, B) is satisfactorily accounted for. No explanation seems to be available for these results if, as suggested by Rall (1957), τ_m is as long as τ_{EPSP} and there is no residual transmitter action (cf. Coombs *et al.* 1955*c*). The similar effects produced during the after-hyperpolarization following a spike (cf. Figs. 4, 5) can also be explained by the action of

hyperpolarizing current. If the after-hyperpolarization is generated only by that part of the post-synaptic membrane invaded by the spike, and hence probably unrelated to synapses (cf. Grundfest, 1957; Eccles, 1957), it will cause an inward current to flow through the subsynaptic areas of the membrane, just as shown in the inset of Fig. 2A, i.e. in the same direction as with the applied hyperpolarizing current in Fig. 9.

We may postulate that the hyperpolarizing current has been either accelerating the process whereby transmitter substance is removed from the proximity of the subsynaptic membrane, i.e. from the synaptic cleft, or loosening the actual attachment of the transmitter substance to the subsynaptic membrane. Either of these alternatives suggests that the transmitter substance is negatively charged.

Group Ia volleys produce the EPSP and IPSP by an almost synchronous synaptic action (Coombs *et al.* 1955*c*; Eccles, Fatt & Landgren, 1956); hence the time courses of the observed EPSP's and IPSP's give approximately the time courses of potentials generated by single synapses, and this also would obtain for the derived synaptic currents. The present analyses are in agreement with investigations on single synapses of the giant ganglion cells of *Aplysia* and *Helix* (Fessard & Tauc, 1957; Tauc, 1958), where there is convincing evidence that the initial intense phase of synaptic action is followed by a residuum prolonged even over hundreds of milliseconds. The decay of the EPSP is much slower than that of the potential produced by a current pulse, while an intercurrent antidromic impulse is largely ineffective in destroying the EPSP. Hagiwara & Saito (1957) find that the excitatory synaptic potentials of the supramedullary neurones of the puffer fish have a half-time of decay of 20–30 msec, in contrast to values of 3–5 msec for potentials generated by applied current pulses; hence likewise it may be concluded that there is a prolonged action of the synaptic transmitter. A similar conclusion has been arrived at for the cholinergic activation of Renshaw cells in the spinal cord (Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956; Curtis & R. M. Eccles, 1958).

Since the synaptic cleft between the presynaptic and subsynaptic membranes is now known to be only about 200 Å across (Palay, 1956; de Robertis, 1956), it might be supposed that a considerable time would be occupied in the clearing of this cleft by the outward diffusion of transmitter substance, and hence that the prolonged residuum of synaptic transmitter is satisfactorily accounted for. However, if the synaptic cleft is reasonably clear of diffusional barriers, and the transmitter molecules have a diffusion coefficient approximating to that of acetylcholine, it can be shown that synaptic clefts under synaptic knobs one or two microns in diameter are cleared by diffusion within a fraction of a millisecond (Eccles & Jaeger, 1958). The calculated rate of transmitter depletion due to diffusion out of the synaptic cleft corresponds

well with the initial rapid decline of the excitatory and inhibitory transmitter actions as given by the derived currents in Figs. 2, 3, 9C, D. The prolonged residual action suggests that in part at least diffusion is obstructed by barriers. which may become remarkably effective with Renshaw cells, where, in the presence of an anticholinesterase, the transmitter (acetylcholine) liberated by a single volley may continue to excite for several seconds (Eccles *et al.* 1954). This diffusional barrier also obstructs the movement of many other molecules and ions (Eccles, Eccles & Fatt, 1956; Curtis & R. M. Eccles, 1958).

SUMMARY

1. Excitatory and inhibitory post-synaptic potentials (EPSP and IPSP) have been evoked in motoneurones by the virtually synchronous synaptic bombardment produced by Group Ia afferent volleys from appropriate muscle nerves. Both the IPSP and EPSP decay approximately exponentially, but the former always has a briefer time constant, the mean values being 3·1 and 4·7 msec.

2. By the application of rectangular current pulses and the balancing of artifacts with a compensatory circuit, the membrane time constant (τ_m) has been derived as described in the preceding paper and usually has been found to be approximately the same as the time constant of decay of the IPSP.

3. The values for τ_m have been employed in determining the time course of the currents generating the EPSP and IPSP. In both, the intense initial peak of current is followed by a brief dip, and, as expected with the EPSP, there is a later low residual current which is negligible with the IPSP.

4. The intercurrent dip is attributed to the electrotonic spread of potential to the remote dendritic areas, which presupposes a predominant distribution of the excitatory and inhibitory synapses on the soma and dendritic stumps in accordance with Szentagothai's histological findings for excitatory synapses.

5. The residual excitatory synaptic current is attributed to prolonged low transmitter action, which is independently indicated also by interaction of the EPSP with an antidromic spike potential, and by the effect of a hyperpolarization in shortening the time to summit and accelerating the decay of the EPSP.

6. The interactions of an antidromic impulse with the IPSP and of an EPSP with an IPSP are consistent with the conclusion that inhibitory synaptic action produces a brief intense hyperpolarizing current with no detectable residuum.

7. The results are discussed in relationship to the diffusion of transmitter substances out of the synaptic cleft and to evidence indicating that single synaptic bombardments of other types of nerve cell are followed by prolonged transmitter actions.

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