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COMPARISON OF ANTIDROMIC AND ORTHODROMIC ACTION POTENTIALS OF IDENTIFIED MOTOR AXONS IN THE CAT'S BRAIN STEM

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SUMMARY

1. Recordings were made from identified central axons at a known distance from their somata, to compare the action potentials resulting from antidromic and synaptic excitation. By taking advantage of the anatomical configuration within the brain stem of the motoneurones innervating the retractor bulbi muscle in the orbit, their axons were penetrated in the VIth nucleus and labelled by electrophoretic injection of horseradish peroxidase.

2. Excitatory post-synaptic potentials recorded in the retractor bulbi axons at about 3 mm from the soma were six times smaller than in the soma. The space constant of the axonal segment between the retractor bulbi and the abducens nucleus was estimated to be 1·7 mm. When the axons propagated action potentials the attenuation was increased to eighteen times due to the nodes of Ranvier intercalated between the soma and the site of recording.

3. Antidromic action potentials displayed stepwise changes in amplitude and shape when stimuli were applied at intervals decreasing from 5 ms to 0·7 ms. The changes were related to the different lengths of refractoriness of the soma, initial segment and axon. Orthodromic action potentials evoked by synaptic excitation displayed similar changes in amplitude and shape.

4. These observations lead to the conclusion that the soma, initial segment and neighbouring nodes of Ranvier contribute significantly to the shape of the action potential.

5. Contrary to the generally accepted view, it appears that the efferent discharge along motor axons can be initiated without a simultaneous activation of the somato-dendritic or even the initial segment membrane, as revealed by the lack of somato-dendritic and/or initial segment contribution to the shape of the synaptically evoked action potentials.

INTRODUCTION

The problem of how a motoneurone generates propagated action potentials following synaptic excitation has been extensively studied in the spinal cord by intrasomatic recordings and recordings of efferent discharges from single motor axons.

In the case of cranial motoneurones the available data concern intrasomatic recordings but there is no study which has established relations with the corresponding axonal activities.

In this study we benefited from the anatomical configuration of the retractor bulbi system of the cat. Motoneurones innervating the retractor bulbi muscles have been identified by retrograde labelling with horseradish peroxidase in the accessory abducens nucleus (Guegan, Gueritaud & Horcholle-Bossavit, 1978; Grant, Gueritaud, Horcholle-Bossavit & Tyc-Dumont, 1979; Hutson, Glendenning & Masterton, 1979; Spencer, Baker & McCrea, 1980). The axons cross the reticular formation in a dorso-medial direction to pass through the principal abducens nucleus before turning to leave the brain stem in the VIth nerve.

The peculiar course of the retractor bulbi axons allows them to be penetrated by micro-electrodes within the principal abducens nucleus. Their electrophysiological identification may be combined with intra-axonal labelling with horseradish peroxidase thus providing records from identified axons at a known distance from their somata.

The present investigation shows that electrical events occurring in the initial segment and the somato-dendritic region of the neurone can be observed from this remote axonal post of recording. This experimental condition furnished data on central conduction velocity, axonal space constant and how the propagated action potentials are generated by synaptic excitation.

METHODS

Preparation. The experiments were performed on twenty cats weighing between 2·2 and 3·2 kg and anaesthetized with sodium pentobarbitone (Nembutal, 40 mg/kg I.P., supplemented with 2 mg/kg per hour). The trachea was cannulated. The animals were paralysed with gallamine triethiodide (Flaxedil) and artificial ventilation was adjusted to keep the end-tidal P_{CO_2} close to 4%. Femoral artery pressure was continuously monitored. The temperature of the animals was maintained between 36 and 37 °C (rectal temperature) by a heating pad. Precautions were taken continually throughout the experiment to ensure that the animals were anaesthetized to the depth required. These conditions were ascertained by the absence of clinical signs of suffering such as changes in blood pressure, heart rate and mydriasis. The head of the animal was fixed in a stereotaxic apparatus. After enucleation of the right eye, the branches of the VIth nerve innervating the lateral rectus muscle and the retractor bulbi muscle were dissected free and mounted for stimulation. Alternatively, only the lateral rectus muscle and the four slips of the retractor bulbi muscle were dissected to be stimulated directly. The nictitating membrane was retracted and used to form the boundary of a pool which was filled with warm paraffin oil. A small craniotomy on the dorsal portion of the occipital bone permitted introduction of the recording micro-electrode, the cerebellum being left intact to avoid damage to the brain stem. In some experiments, the nerve originating from the ampulla of the left horizontal semi-circular canal was prepared for stimulation as described in a previous paper (Grant, Gueritaud, Horcholle-Bossavit & Tyc-Dumont, 1976).

Recording and stimulation. Glass micro-electrodes were filled with 3 M-KCl, 2 M-K citrate solution or a 10% solution of horseradish peroxidase (Sigma type VI) in 0·5 M-KCl. The tips were broken to 1·5–2·0 μm or bevelled as described by Brown & Flaming (1975). Micropipettes had a resistance of 5–12 MΩ when filled with KCl or K citrate solution and of 12–25 MΩ when filled with peroxidase solution. Axons of the retractor bulbi motoneurones were sought within the principal abducens nucleus since previous studies (Guegan *et al.* 1978; Grant *et al.* 1979; Hutson *et al.* 1979; Spencer *et al.* 1980) had shown that they cross this nucleus before joining axons of the VIth nerve. The principal abducens nucleus was found by the presence of antidromic field potentials following electrical stimulation of the abducens nerve or muscle in the orbit (Fig. 1). When electrical

stimulation of the retractor bulbi nerve was used, the penetrated axons were identified as retractor bulbi axons when they were antidromically activated from this nerve but not following stimulation of the lateral rectus nerve. When electrical stimulation of the slips of the retractor bulbi muscle was used, the penetrated axons were identified on the basis of the following properties: (1) a short-latency antidromic invasion (mean, 0·45 ms; $n = 10$; S.D. = 0·16) following stimulation of the retractor bulbi muscle in the orbit; (2) a short (< 0·9 ms) refractory period; (3) an orthodromic response to electrical stimulation of the ipsilateral vibrissal pad with latency < 3 ms, as described by Gogan, Gueritaud, Horcholle-Bossavit & Tyc-Dumont (1981); (4) a lack of response to the stimulation of the contralateral labyrinth nerve, since such responses have been described in lateral rectus but not in retractor bulbi motoneurones (Baker, McCrea & Spencer, 1980; Gogan *et al.* 1981; Guegan & Horcholle-Bossavit, 1981). The first two tests allowed identification of the penetrated axons as motor axons and not motoneuronal somata or interneurones, while tests (3) and (4) allowed their identification as retractor bulbi and not lateral rectus motor axons. Moreover, some records were made with horseradish-peroxidase-filled micro-electrodes during the passage of depolarizing current applied to eject horseradish peroxidase. Intracellular staining gave the direct evidence that such records were indeed from retractor bulbi axons (Grant *et al.* 1979). Following stimulation of the retractor bulbi muscle, antidromic action potentials in retractor bulbi axons were evoked by stimuli near threshold; orthodromic action potentials in the same axons could be evoked by higher stimulus intensities and were attributed to the stimulation of trigeminal afferents (Gogan *et al.* 1981). In some experiments intracellular records were, in addition, obtained from retractor bulbi motoneurones in the accessory abducens nucleus, as previously described by Grant *et al.* (1979). Only somata and axons with stable resting potentials above 60 mV were considered in this study.

Staining. Horseradish peroxidase was electrophoretically ejected from micro-electrodes using constant current of 2–10 nA with electrode tip positive. After allowing about 1–2 h for diffusion of the ejected enzyme, an additional dose of Nembutal was given and the cats were perfused through the thoracic aorta with 1 % paraformaldehyde and 1 % glutaraldehyde solution. After post-fixation for 24 h and washing in phosphate buffer with 30 % sucrose for the next 24 h, serial sections of the brain stem were made (60 μm) and the horseradish peroxidase reaction product was visualized by using diaminobenzidine or as described by Hanker, Yates, Metz & Rustioni (1977). Reconstructions of twelve stained axons were made using a light microscope with camera lucida attachment. The position of the penetration was estimated by finding the intersection between the reconstructed axon and the micro-electrode tract. For some axons, a photomontage reconstruction was made from photomicrographs of a few neighbouring sections (Fig. 1).

Data analysis. All records were photographed and, in addition, stored on magnetic tape. The analysis was performed 'off-line' using a Hewlett–Packard digitizer (type 9864A) and computer (type 9830A).

RESULTS

Electrotonic decrement of synaptic potentials

Electrical stimulation of the ipsilateral vibrissal pad evokes large-amplitude excitatory post-synaptic potentials (e.p.s.p.s) in the somata of retractor bulbi motoneurones (Gogan *et al.* 1981). The mean amplitude of these e.p.s.p.s is 11·00 mV (S.D. = 3·45, $n = 45$) at 3 times threshold, with a maximal value of 17·6 mV. After similar vibrissal stimulation, these e.p.s.p.s were also recorded from retractor bulbi axons within the principal abducens nucleus (Fig. 1*A*, *B*) and showed a mean amplitude of 1·86 mV (S.D. = 0·72, $n = 61$) with a maximal value of 3·5 mV. By increasing the strength of the vibrissal stimulation, action potentials were evoked and were seen to be superimposed on the rising phase of the e.p.s.p.s (Fig. 1*C*).

A typical recording of one e.p.s.p. evoked in a retractor bulbi soma by vibrissal stimulation at three times threshold is illustrated Fig. $2A_1$. It is superimposed on a corresponding e.p.s.p. recorded in the retractor bulbi axon of Fig. 1 within the principal VIth nucleus, at a distance of 3 mm from the soma (Fig. $2A_2$). After

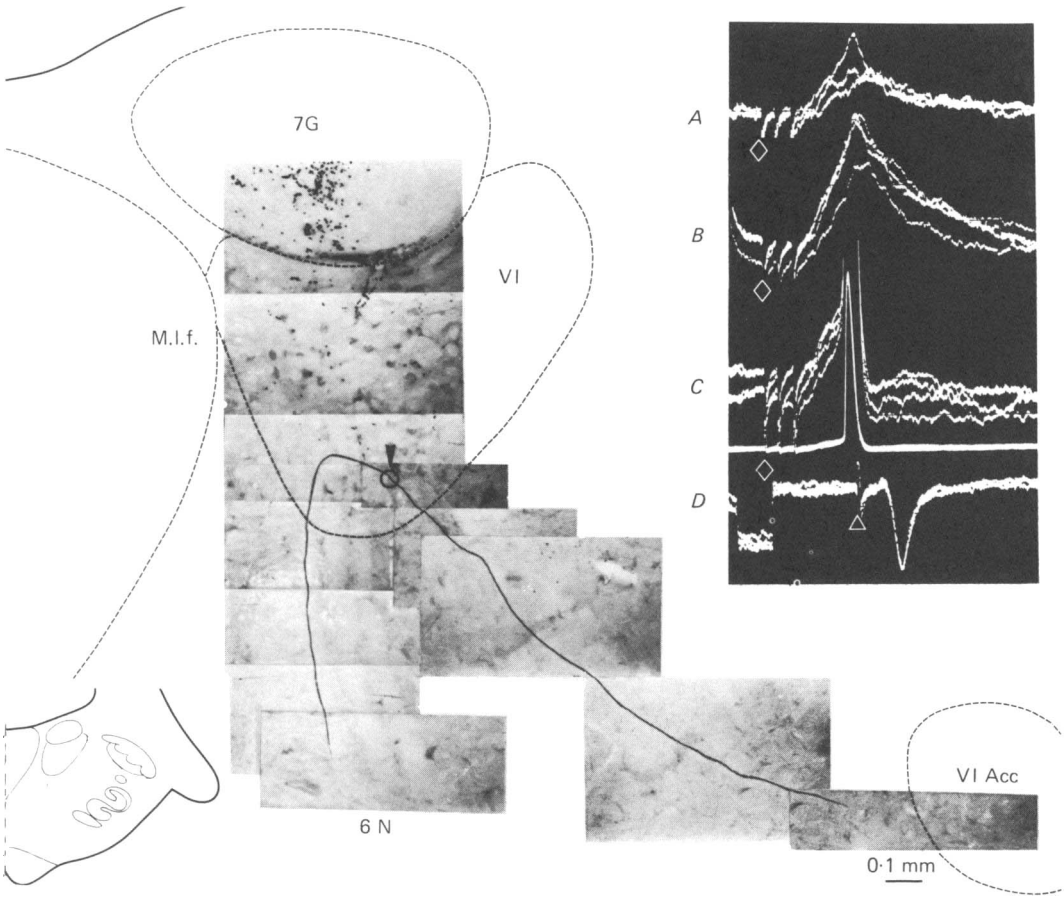

Fig. 1. Reconstruction of a retractor bulbi axon impaled (arrowhead) in the principal abducens nucleus (VIth) and injected with horseradish peroxidase. Electrophysiological recordings (*A*, *B*, *C*) obtained from this retractor bulbi axon illustrate e.p.s.p.s evoked by electrical stimulation (◇) of the ipsilateral vibrissal pad (*A*, *B*) and action potentials superimposed on those e.p.s.p.s (*C*). *D*, extracellular field potential at the site of recording following antidromic stimulation (△) of the VIth nerve. Square pulse in *D* gives time and voltage calibrations: 1 ms; 1 mV for *A*, *B*, *C* upper traces and *D*; 10 mV for *C* lower trace. 7G, genu of facial nerve; M.l.f., medial longitudinal fasciculus; VI; principal abducens nucleus; 6N, root of the VIth nerve; VI Acc., accessory abducens nucleus.

averaging e.p.s.p.s recorded from ten different retractor bulbi somata and ten different retractor bulbi axons in ten animals, the mean time course and the mean amplitude of these two phenomena were compared (Fig. 2B_1, B_2). The original (Fig. 2*A*) and computed (Fig. 2*B*) responses show remarkably similar time courses. The amplitude ratio of the two computed curves (Fig. 2B_1, B_2) is 6·4 when measured at the peak. The dotted line of Fig. 2*B* gives the shape of the mean axonal e.p.s.p.s of Fig. 2B_2 when multiplied 6·4 times. This curve and that of the somatic e.p.s.p.s of Fig. 2B_1 match well. Thus the value of 6·4 times can be taken as the attenuation of the vibrissal e.p.s.p.s recorded in the axons 3 mm away from the somata. When the

attenuation is computed from the mean amplitudes (11 mV and 1·86 mV) given above and obtained from a larger population of e.p.s.p.s, a similar ratio of 5·9 is obtained. From Fig. $2B_1$ it can be noted that the attenuation of 6·4 stands only for about 3 ms around the peaks of the curves. Thus, considering this lapse of time as an almost steady-state condition, the space constant of the retractor bulbi axon can be

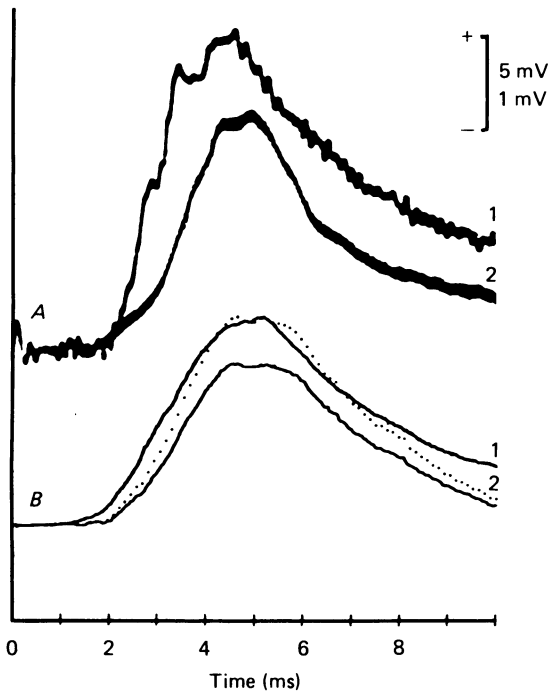

Fig. 2. A_1, e.p.s.p. recorded from the soma of a retractor bulbi motoneurone following electrical stimulation of the ipsilateral vibrissal pad. Voltage calibration: 5 mV. A_2, e.p.s.p. evoked by the same intensity of stimulation recorded from a retractor bulbi axon 3 mm away from the soma. Voltage calibration: 1 mV. B_1, average of e.p.s.p.s recorded from ten retractor bulbi somata. Voltage calibration: 5 mV. B_2, average of e.p.s.p.s recorded from ten different retractor bulbi axons at an average distance of 3 mm from the soma. Voltage calibration: 1 mV. When the amplitudes of curve B_2 are multiplied by 6·4, the dotted line is obtained; it fits well with curve B_1. Curve B_1 and the dotted line have the same voltage calibration.

estimated using the cable equation (Rall, 1977): $1/D = e^{-x/\lambda}$, where D is the decrement in amplitude, x is the distance along the fibre and λ is the length constant or space constant. Taking a value of 6 for D and a value of 3 mm for x, the space constant λ is 1·7 mm.

Antidromic action potentials

The results described in the preceding section indicate that electrical phenomena occurring in the soma, initial segment and neighbouring nodes of Ranvier must significantly contribute to the shape of the recorded axonal action potentials. An

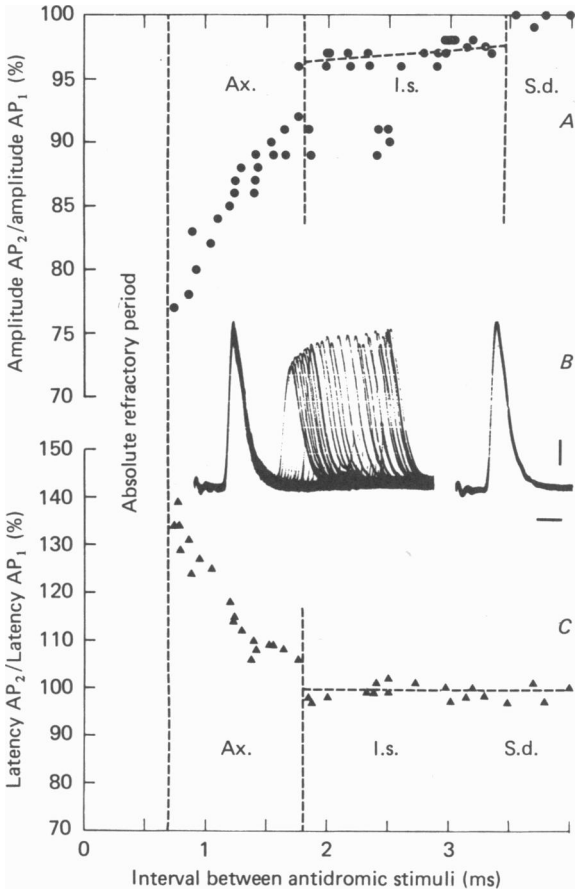

Fig. 3. *B*, the refractory period of a retractor bulbi axon is illustrated by superimposed traces of antidromic action potentials evoked by two successive stimuli delivered at intervals from 5 ms to 0·7 ms. The recordings were obtained in the principal abducens nucleus as in Fig. 1. The first potential at the beginning of the traces is the superposition of thirty responses to the first antidromic stimulus. The last potential on the right is evoked by a second stimulus 5 ms after the first one. Voltage calibration: 10 mV; time calibration: 0·5 ms. *A*, the relative amplitudes of antidromic action potentials (AP_1 and AP_2) of the same axon are plotted against the intervals between the two antidromic stimuli. The reference is the mean amplitude (62 mV) of antidromic action potentials evoked by single shock. Three amplitude populations (Ax, axon; I.s., initial segment; S.d., somata-dendritic region) are separated by jumps occurring at 3·4 ms and 1·85 ms (dashed lines). *C*, for the same axon, the relative latency of the second antidromic response is plotted against the intervals between the two antidromic stimuli. The reference is the latency of the first response. The dashed line divides two populations of latencies at an interval of 1·85 ms.

attempt has therefore been made to differentiate the different components making use of the well-known differences in refractoriness of the soma, initial segment and axon of the neurone (Coombs, Curtis & Eccles, 1957*a*; Fuortes, Frank & Becker, 1957). From intrasomatic recordings of retractor bulbi motoneurones, the mean refractory periods following antidromic activation have been found to be 3·15 ms (S.E. = ±0·4 ms) for the soma and 1·75 ms (S.E. = ±0·2 ms) for the initial segment.

Intra-axonic recordings have given a mean value of 0·83 ms (S.E. = ±0·2 ms) for the axon. The refractory period has been defined as the shortest interval allowing activation by the second of two antidromically conducted nerve impulses. Fig. 3 shows the results which have been obtained from the study of the refractory period of one retractor bulbi axon representative of a population of ten axons which have been similarly analysed. The recordings in *B* show a series of antidromic action potentials evoked by stimuli applied at decreasing intervals (from 5·00 to 0·7 ms). They exhibit the well-known phenomenon of the gradual decrease in amplitude of the action potential close to the end of the absolute refractory period of the axon. However, an analysis of such potentials has revealed that their amplitudes changed stepwise at two intervals between the successive antidromic stimuli. The plot of Fig. 3*A* shows the ratio between the amplitudes of the second and the first action potentials as a function of the intervals between stimuli. The group of data on the right (Fig. 3*A*, S.d.) is for the action potentials which have the same amplitudes (62 mV) as those evoked by the first stimulus. They have been recorded at intervals greater than 3·4 ms. At an interval close to 3·4 ms the amplitude of the second action potential dropped abruptly to 97 % of the reference (Fig. 3*A*). A new drop occurred at 1·85 ms, the amplitude of the second action potential being reduced to 92 % of the reference. Then, the amplitude decreased steadily to 77 % and responses to the second stimulus failed at a 0·74 ms interval. The three breaking points of these changes corresponded to the intervals given above for the refractory periods of the retractor bulbi soma (3·15 ms; S.E. = ±0·4 ms), of the initial segment (1·75 ms; S.E. = ±0·2 ms) and of the axons (0·83 ms; S.E. = ±0·2 ms).

These results show that the functioning of the initial segment and the somato-dendritic region of the retractor bulbi neurone significantly affects the amplitude of action potentials recorded from the axon.

This conclusion is further supported by the data of Fig. 3*C* which plots the ratio of the latencies of the second to the first antidromic potential as a function of the interstimulus interval. While the relation remains stable for intervals above 1·85 ms, a progressive increase is observed at 1·85 ms to reach a maximal value for an interval which corresponds to the absolute refractory period of the axon. This increase expresses the well-known increase of axonal conduction time during relative refractoriness (Tasaki, 1968). Thus, any potential variations occurring for intervals greater than 1·85 ms cannot be attributed to the refractoriness of the axon.

The functioning of the initial segment and somato-dendritic region also modifies the shape of the antidromic axonal action potential. This can be visualized by subtracting from each other the action potentials recorded at the critical intervals of the refractory period in the same single axon. The three antidromic potentials of Fig. 4*A* are those evoked by the first of two stimuli (A_1), and by the second stimulus delivered 3·00 ms (A_2) or 1·84 ms (A_3) after the first one, during the refractory period of the soma, and of both the soma and the initial segment, respectively. The difference between the first two potentials is shown in Fig. 4*B* and represents the somato-dendritic component. It corresponds partly to the hump on the falling phase of the first potential. The difference between the second and the third potentials is shown in Fig. 4*C* and represents the initial segment component. The time difference between the onset of the initial segment component and the onset of the antidromic action

potential was 0·1 ms. It provides a measure of the time needed for the antidromic action potential to travel the 3 mm between the recording site and the initial segment, and gives a conduction velocity of 30 m/s. Fig. 4*D* illustrates similar data for action potentials recorded during the relative refractory period of the axon. The potentials 2 to 7 were recorded at intervals of 1·84 ms to 0·73 ms as indicated. These potentials

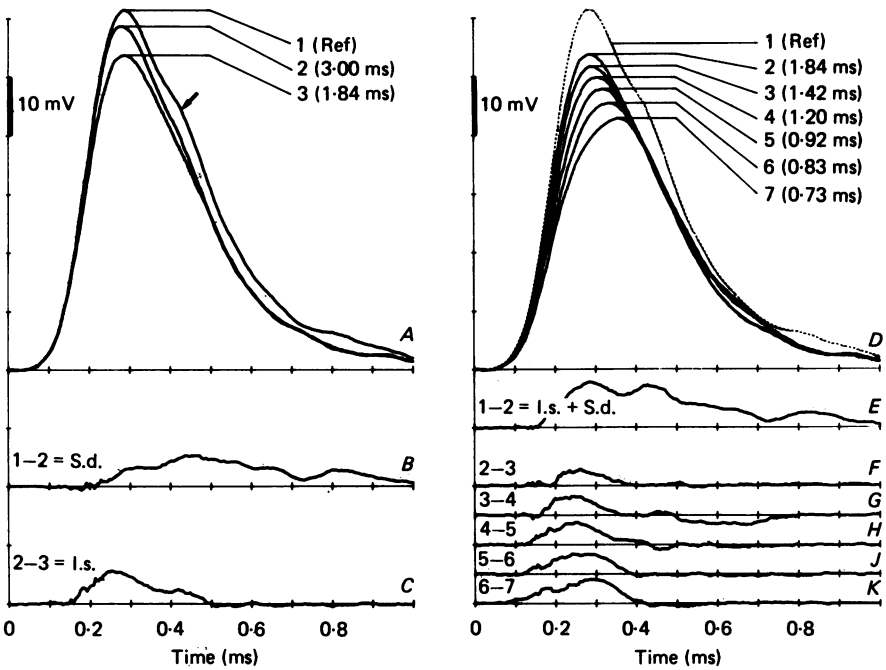

Fig. 4. Antidromic action potentials evoked in the same axon as in Fig. 3 are digitized. The curves in *A* represent the shape of the reference potential (1) and the potentials at two points of the curve of Fig. 3*A*, corresponding to intervals of 3·00 ms (2) and 1·84 ms (3). Their algebraic subtraction reveals the somato-dendritic (S.d.: *B*) and the initial segment (I.s.:*C*) components of the action potential of tracing A_1. Curves 2 to 7 in *D* represent the shape of action potentials at six points of the curve of Fig. 3*A* at indicated intervals. The algebraic subtraction of curve 2 from curve 1 (same as A_1) gives the shape of the initial segment plus somato-dendritic (I.s.+S.d.) component (*E*). The following differences (*F–K*) reveal changes due to axonal refractoriness. The arrow in *A* indicates the hump on the falling phase of the reference potential.

showed a gradual decrease in amplitude and a gradual increase in both their time-to-peak and the slope of their rising phase. The differences between the successive pairs of these potentials (Fig. 4*F–K*) show that the axonal potentials are delayed with intervals closer to the absolute refractory period. The delays can be related to the increase in the axonal conduction time illustrated in Fig. 3*C*. They also indicate that the activity of nodes of Ranvier between the recording site and the initial segment contributes to the shape of the potential actually recorded. The microscopic observation of labelled axons revealed the presence of at least two nodes of Ranvier between the soma and the recording site. The internodal distance was around 1 mm for the axonal segment running from the retractor bulbi to the abducens nucleus.

Orthodromic action potentials

To allow comparison between orthodromic and antidromic potentials, the orthodromic potentials to be described were recorded from the same retractor bulbi axon as was used to analyse the antidromic potentials. When two silver ball electrodes are placed, one over the slips of the retractor bulbi muscle and the other in the posterior part of the orbit, the electrical stimuli produce antidromic and

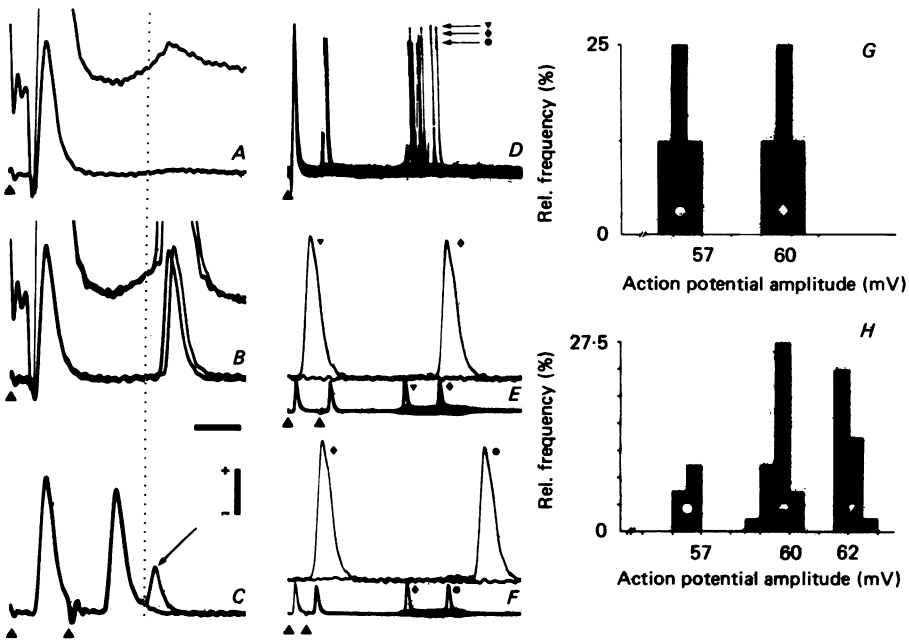

Fig. 5. Antidromic and orthodromic responses recorded from the same axon described in Figs. 3 and 4. The orbital stimulation (▲) evoked a first antidromic potential followed by orthodromic trigeminal e.p.s.p.s (*A*) and action potentials at short (*B* and *D*) and long latencies (*D*). The orthodromic potentials show variable amplitudes (grouped into three categories indicated by ▼, ◆, ●) in twenty superimposed recordings in *D* (slow sweep). The lower traces in *E* and *F* are recordings taken with the same sweep as in *D* and the enhanced brightness zone is shown at fast sweep and higher gain in the upper traces of *E* and *F* to exemplify details of potentials. The amplitude distributions of short- and long-latency orthodromic potentials are illustrated in *G* and *H* respectively. Double-shock orbital stimulation (*C*) at critical interval allowed selective excitation of the initial segment (arrow) of the axon (see Fig. 7). Time calibration: 1 ms for *A*, *B*, *C* and upper traces in *E* and *F*; 4 ms for *D* and lower traces in *E* and *F*. Voltage calibration: 20 mV for *A*, *B*, *C*, *D* and upper traces in *E* and *F*; 100 mV for lower traces in *E* and *F*; 2 mV for upper traces in *A* and *B*.

orthodromic responses in accessory abducens motoneurones at about the same stimulus intensity as described by Baker *et al.* (1980). The responses evoked by such stimuli are shown in Fig. 5*A* and *B*. Such stimuli, in our experimental conditions, also evoked long-latency trans-synaptic potentials as illustrated in Fig. 5*D*.

The first orthodromic responses appeared at a latency of 2·9–3·00 ms. In the axonal recording they were superimposed on the electrotonic image of the somatic e.p.s.p.

(Fig. 5*B*), which reached an amplitude of 2 mV (Fig. 5*A*). The latencies of the later orthodromic responses ranged between 9 and 12 ms (Fig. 5*D*). They were likewise superimposed on somatic e.p.s.p.s of about 2 mV. In contrast to the early ones, which are of trigeminal origin, their origin remains undefined. The striking feature of these orthodromic potentials was that they displayed variations in amplitude; the late ones showed three different amplitudes (Fig. 5*D–F*) while the early ones displayed two amplitudes (Fig. 5*B*). A similar grouping in amplitudes has been found in the nine other axons analysed, although their absolute amplitudes were different.

An analysis of these amplitudes in the neurone illustrated in Fig. 5 revealed that the long-latency potentials were grouped in three distinct populations with amplitudes of 62 mV, 59·5 mV and 56·5 mV respectively (Fig. 5*H*). The short-latency potentials could be grouped in two amplitude populations corresponding to the two lower ones of the long-latency responses (Fig. 5*G*).

The comparison of these amplitude populations with the amplitudes of the antidromic potentials of the same axon (Fig. 3*A*) revealed a close correspondence. The largest amplitudes (62 mV) were the same as the full-size antidromic potentials which include the axonal (A.x.), intial segment (I.s.) and somato-dendritic (S.d.) components. The medium amplitudes (59·5 mV) were the same as the amplitudes of the antidromic potentials when the somato-dendritic component was refractory. Finally, the smallest amplitudes were the same as the amplitudes of the antidromic potentials at the time when the initial segment had just become refractory.

Fig. 6 further shows that there are great similarities between the shapes of these orthodromic potentials and those antidromic ones which were concluded to be modified by the somato-dendritic and initial segment compartments. The differences between pairs of these potentials, labelled S.d. (Fig. 6*B* and *E*) and I.s. (Fig. 6*C* and *F*), appeared to be practically the same. However, it will be noted that the onsets of initial segment components are displaced in time. In the case of the antidromic invasion, the initial segment potential begins 0·1 ms after the onset of the antidromic potentials (Fig. 6*A*, *C*). The onsets of the initial segment and of the orthodromic action potentials (Fig. 6D_1, D_2) are, on the other hand, simultaneous as required for propagation of nerve impulses along the axon initiated by the initial segment (Fig. 6*F*). It will also be noted that both the full-size antidromic and orthodromic potentials (Fig. 6A_1, D_1) had marked humps (arrows) on their falling phases, reflecting the somato-dendritic activity. However, while this hump disappeared from the antidromic potential during somato-dendritic refractoriness, it remained to some extent in the records of the orthodromic potentials in which the somato-dendritic component was concluded to be missing (Fig. 6D_2, D_3). The origin of such smaller humps could not be analysed in this study.

These observations suggest that, contrary to the generally accepted view, the efferent discharges along the motor axons can be initiated with, as well as without, a simultaneous activation of the somato-dendritic or even the initial segment membrane.

Comparison of attenuation in active and inactive retractor bulbi axons

In the first paragraph of Results the attenuation in retractor bulbi axons was calculated from the somatic e.p.s.p.s recorded in them, giving the value for resting

conditions. In order to compare the attenuation in the resting and active states, the latter has also been estimated from the initial segment activity.

Axonal records of initial segment activity in active fibres during antidromic and orthodromic invasion, computed as illustrated in Figs. 4*C* and 6*C* and *F*, were used. Their amplitudes were then compared with the amplitudes of axonally recorded

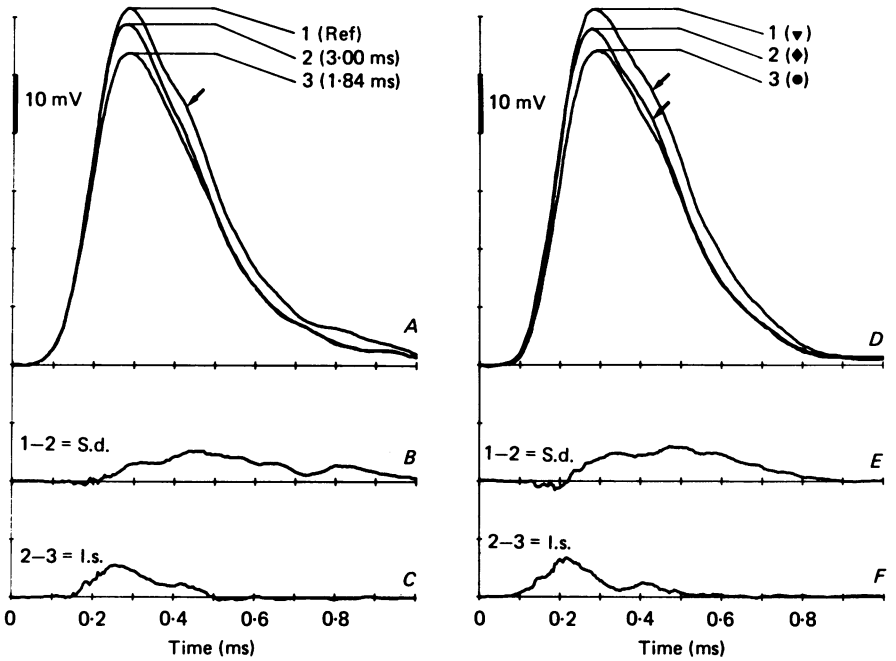

Fig. 6. The comparison between digitized antidromic (*A*; same as in Fig. 4*A*) and orthodromic (*D*) action potentials reveals a striking similarity in shape. $D_{1\text{-}2\text{-}3}$ are samples of action potentials at the three amplitudes (▼, ◆, ●) shown in Fig. 5. Moreover, in both cases the result of their differences gives the identical initial segment (I.s.) and somato-dendritic (S.d.) components in *B*, *E* and *C*, *F* respectively. Arrows indicate humps on the falling phase of the potentials.

initial segment activity initiated without a concomitant axonal invasion. This condition was fulfilled in three experiments, one of which is illustrated Fig. 5*A*, *B* and *C*, and explained in detail Fig. 7.

In this experiment the orbital stimulation initiated an antidromic potential followed by an orthodromic excitation (e.p.s.p. in Fig. 5*A* and action potentials in Fig. 5*B*). A second electric shock delivered 1·4 ms after the first evoked a second antidromic potential (Fig. 5*C*) of smaller amplitude since it did not invade the initial segment which was refractory at this time. This second response prevented the orthodromic response illustrated Fig. 5*B* from being evoked, but left a smaller potential (Fig. 5*C*, arrow). This experimental condition is fully explained in Fig. 7 in which the position of the orthodromic response to the first shock (S_1) is indicated (dotted line). The orthodromic response occurred just after the antidromic invasion of the axon following the second shock (S_2). At that time the axon was refractory,

the initial segment had just recovered from refractoriness, and the somato-dendritic region was still refractory. In consequence, the orthodromic excitation due to the first stimulus (S_1) resulted in a pure initial segment activation (ortho I.s.).

The axonal record of this initial segment activity (Fig. 5*C*, arrow) is reproduced in Fig. 8*A*, to be compared with those obtained from the active axon during

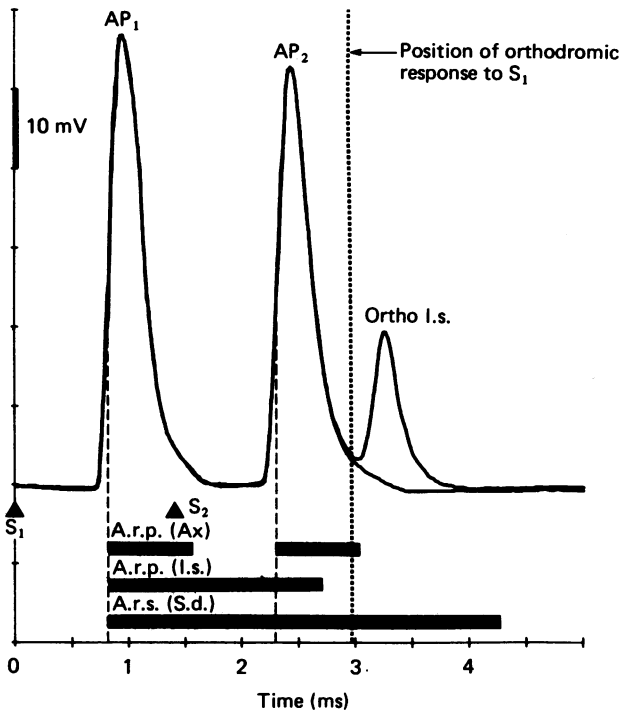

Fig. 7. Action potentials illustrated in Fig. 5*C* are shown, to explain how the initial segment of the axon can be excited selectively without invasion of axon and soma of the neurone. The orbital stimulation (S_1) provokes an antidromic potential (AP_1) followed by an orthodromic e.p.s.p. (as in Fig. 5*A*) reaching the firing level (as in Fig. 5*B*) at the time indicated by the dotted line. AP_1 reaches the initial segment 0·1 ms (dashed line) after passing through the recording site and is followed by absolute refractoriness (A.r.p., black bars) of the axon (Ax.), of the initial segment (I.s.) and of the somato-dendritic region (S.d.). A second orbital stimulation (S_2) delivered 1·4 ms after S_1 provokes an antidromic potential (AP_2) which reaches the I.s. at a time indicated by the dashed line and cannot invade the I.s. and S.d., since both are in absolute refractoriness. It produces a second absolute refractoriness in the axon. The e.p.s.p. provoked by S_1 reaches the firing level at a time (dotted line) when only I.s. is excitable. Thus, a pure I.s. action potential is evoked. Its attenuated electrotonic image is observed at the recording site (Ortho I.s.).

orthodromic (Fig. 8*B*) and antidromic (Fig. 8*C*) invasion. The comparison of amplitudes at the peaks gave ratios of 2·9 and 3·1 respectively. Similar values of 3·1, 2·8 and of 2·9, 3·2 were obtained for the two other axons analysed. Since we have shown a 6-fold attenuation in the resting axon, it is deduced that the axon at work attenuates by a factor 18. The larger attenuation results from the decrease in shunting resistance due to the functioning of the nodes of Ranvier intercalated between the soma and the recording site.

DISCUSSION

The present investigation is based on an axonal observing post which provides information on events originating in the soma, the initial segment and nodes of Ranvier of the neurone. Their differences in refractoriness and their sequential excitation allowed the contributions of the initial segment and somato-dendritic region to be separated by simple computation.

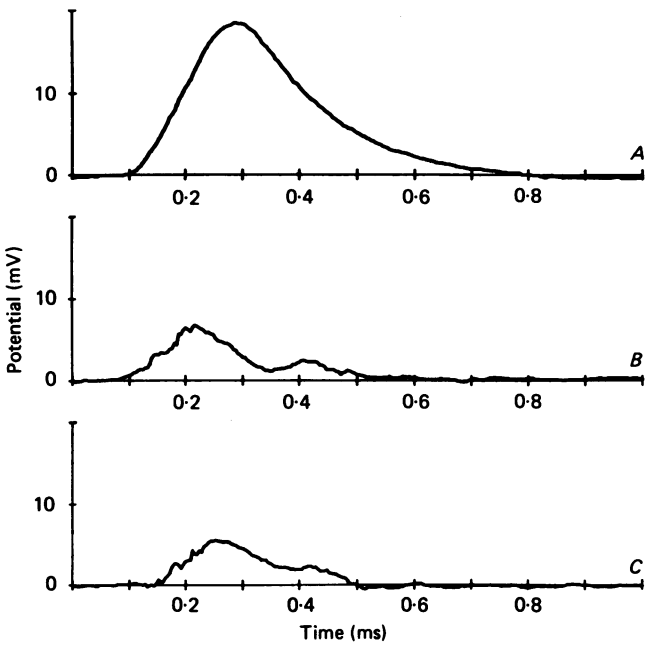

Fig. 8. The orthodromic initial segment potential illustrated in Fig. 5*C* is shown digitized and properly scaled in *A* to be compared with two extracted initial segment activities from Fig. 6*F* and 6*C* shown in *B* (orthodromic) and *C* (antidromic) respectively. Initial segment activity in the axon at rest (*A*) is three times larger than in the active axon (*B*, *C*).

The possibility of observing the remote initial segment activity together with the propagated axon potential allows calculation of the central conduction velocity within the 3 mm length between the initial segment and the recording site. The value of 30 m/s obtained in these conditions fits with the mean difference in latency of 0·1 ms found between intrasomatic (Grant *et al.* 1979; Baker *et al.* 1980) and intra-axonal recordings during antidromic activation. However, when the conduction velocity of retractor bulbi axons is measured from the length of the whole axon (around 45 mm) and the mean antidromic latency recorded in the soma (0·58 ms) following stimulation of the retractor bulbi muscle, a value of 77·5 m/s is obtained. Similarly a conduction velocity of 87·5 m/s is obtained for the distal part of the axon from the muscle to the abducens nucleus. These data indicate that conduction is faster in the distal than in the central segment running from abducens nucleus to the retractor bulbi somata. This difference could be explained by a non-uniformity of the axon. The internodal length for the 3 mm central segment is 1·0 mm. Light and

electron microscopic observations give a fibre diameter of 5 μm (J. Destombes, personal communication). The conduction velocity of 30 m/s in that part of the axon agrees with the results published for other axons of similar diameters and internodal lengths (Rushton, 1951; Coppin & Jack, 1972; Waxman, 1978). The relationships between internodal length, fibre diameter and conduction velocity predict a fibre diameter between 12 and 16 μm (Waxman, 1978) and an internodal distance of 1·0–1·4 mm (Coppin & Jack, 1972) for the conduction velocity of 87·5 m/s found in the distal part of the axon. An increase in the internodal length to 1·2 mm and in the fibre diameter to 7 μm was found in the distal segment within the brain stem. This is not enough to explain the 87 m/s. However, the change in conduction velocity could occur mainly within the axon outside the brain for which internodal length and fibre diameter are not available. An alternative explanation of the difference in conduction velocity could be the spread of the stimulating current applied to peripheral nerve to distant nodes of Ranvier. Neither explanation can be excluded but our results stress the problem of accuracy in measuring central conduction velocity when only one recording position is available.

Few data are available on the space constant of central axons. Barron & Matthews (1938) stated that the potentials recorded from cat ventral roots suffered a decrement to one half in 1·4 mm, which gives a space constant of 2 mm. Eccles (1946), also recording from ventral roots, gave a surprisingly long length of 6·5 mm. More recently, Richter, Schlue, Mauritz & Nacimiento (1974) published data showing that the somatic synaptic potentials were recorded with an attenuation of 1·8 when the micro-electrode was presumably situated in motor axons within the motoneurone pool. This attenuation increased to over 6 for motor axons recorded outside the motoneurone pool. But, in both cases, the distance from the somata was unknown and the space constant could not be evaluated. However, a close electrotonic coupling between the soma and distant parts of the axon is suggested in all these reports. Our results demonstrate that a close electrotonic coupling occurs also in central motor axons in the brain stem of the cat. The space constant of 1·7 mm for retractor bulbi axons is close to the 2 mm value obtained for the first time by Barron & Matthews in 1938.

Given the space constant of the first 3 mm of the axon, it is likely that the large synaptic potentials of the somato-dendritic region (Baker *et al.* 1980; Gogan *et al.* 1981) produce significant depolarization at the level of the first node of Ranvier. In consequence, it must be envisaged that the propagated action potential is sometimes initiated at the first node, as suggested by Coombs *et al.* (1957*b*). Our results clearly support this view in suggesting that the efferent discharge along the motor axon can be initiated without a simultaneous excitation of the somato-dendritic or even the initial segment membrane. The ability to excite the initial segment or the nodes of Ranvier directly, without firing in the somato-dendritic region, enhances the gain of the system, i.e. the neuronal mechanism governing the conversion of a synaptic input to an action potential output of a certain frequency. E.p.s.p.s may then influence the trigger zone for a longer duration without the limiting effects due to somatic action potentials and after-potentials.

The initial segment is considered to have virtually the same threshold as the first node (Coombs *et al.* 1957*b*). Therefore it is the most likely candidate for being excited

first, being closest to the somatic region. A problem then arises as to why the axonal action potential evoked by synaptic excitation can be observed with or without a sign of initial segment activity. Several suggestions can be made to explain this fact. It can be envisaged that the discharged impulse is initiated at the first node and secondarily invades the initial segment but with occasional failures of the retrograde invasion. This blockade might be due to the changes of the initial segment excitability induced by axo-axonic synapses which are observed at this level (Destombes, Durand, Grant, Gueritaud, Horcholle-Bossavit & Rouvière, 1982). An alternative explanation would admit that the initial segment is activated first but is blocked in some cases by the synapses which make contact at its level. In that case, the trigger zone will be displaced downstream in the axon. In both cases, the functioning of the synapses would provide a means of enhancing the gain of the system by preventing the retrograde invasion of the somato-dendritic region.

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