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# MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL DETERMINATION OF THE PROJECTIONS OF JAW-ELEVATOR MUSCLE SPINDLE AFFERENTS IN RATS

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SUMMARY

1. The fluorescent compound Lucifer Yellow was injected into the somata of nine identified jaw-elevator muscle spindle afferents, located in the V mesencephalic nucleus. Reconstructions of the central course of their axons were subsequently made from serial, transverse, sections to identify sites of projection.

2. Three sites of termination were identified on the basis of collaterals that ended in varicosities and/or boutons. All afferents projected to the V nucleus oralis and, all but one, also to the V motor nucleus. Two out of nine afferents had terminations in the supra-trigeminal nucleus, though a further four appeared to send collaterals to this area.

3. The relative density of projection, judged by the number of collaterals supplied to each area, decreased in the order: V nucleus oralis, V motor nucleus and supra-trigeminal nucleus. The central course of the afferent axons was such that impulses from the periphery would arrive first at the V motor nucleus, then the V nucleus oralis, the supra-trigeminal nucleus, and finally the afferent somata in the V mesencephalic nucleus.

4. In animals in which the masseter nerve was exposed in-continuity for electrical stimulation, electrophysiological recordings were made in the three areas described above to identify units that received a monoysnaptic input from spindles in the masseter muscle.

5. Criteria were formulated on the basis of the pattern of responses on stimulation of the masseter nerve, and the morphology of labelled neurones, for differentiating between afferents, interneurones, and motoneurones.

6. In the V motor nucleus, monosynaptic excitatory post-synaptic potentials (e.p.s.p.s) were obtained in both synergist and masseter motoneurones. These were assumed to arise from a masseter muscle spindle input as the thresholds for exciting such afferents and eliciting e.p.s.p.s were similar. Some interneurones, chiefly in the V nucleus oralis, were activated at thresholds close to that of muscle spindle afferents and could also fire in response to muscle stretch. As their latencies (measured

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extracellularly) were similar to that of e.p.s.p.s in motoneurones, they were assumed to receive a monosynaptic muscle spindle input. However, most interneurones were activated at longer latencies (up to 7 ms) and some also fired to muscle stretch. Arguments are advanced, based on the long rise time of e.p.s.p.s recorded in some, that the majority of these may also be candidates for monosynaptic activation.

7. Taken together, the results provide both morphological and electrophysiological evidence to support a monosynaptic projection of jaw-elevator muscle spindle afferents on to motoneurones and to interneurones in the areas outlined.

## INTRODUCTION

While it has been possible to record the patterns of activity of muscle spindle afferents during a variety of voluntary movements in alert animals (Cody, Harrison & Taylor, 1975: Prochazka, Westerman & Ziccone, 1976; Loeb & Duysens, 1979), only rather limited speculations have been possible as to the reflex consequences of these activity patterns. This is particularly so for the trigeminal motor system, where our knowledge of the central connexions of spindle afferents is more limited than for the lumbosacral motor system. In this context it is pertinent to note that the electro-physiological demonstration of a monosynaptic projection of jaw-elevator muscle spindle 'primaries' and 'secondaries' on to elevator motoneurones (Appenteng, O'Donovan, Somjen, Stephens & Taylor, 1978) succeeded the chronic afferent recordings (Cody *et al.* 1975). It is uncertain whether elevator spindle afferents can, in addition, influence motoneurone activity by multi-synaptic pathways, or even by what routes the afferent information reaches higher centres. Answers to these questions are of direct relevance in attempts to understand how spindle afferents contribute to the regulation of masticatory movements.

The morphological techniques now available for labelling single neurones (Jankowska, Rastad & Westman, 1976; Snow, Rose & Brown, 1976) provide one means of delineating the sites of projections of elevator spindle afferents. To date, this approach has not been adopted. In this paper, we report morphological observations on the central course of elevator spindle afferents following intracellular injections of Lucifer Yellow into single spindle afferents. Three areas of projection were noted and subsequent electrophysiological recordings provided evidence for a monosynaptic spindle afferent input on to neurones in those areas. With this information, it is now possible to examine specifically the projections of some of these neurones to determine if they form part of a multi-synaptic reflex pathway or project to higher centres. An abstract of some of the preliminary morphological data has been reported to the Physiological Society (Appenteng, 1984).

## METHODS

*General operative procedures*

Data was obtained from thirty-one rats weighing 0·22–0·27 kg. They were anaesthetized initially with a mixture of halothane in oxygen and, following insertion of a femoral venous catheter, they were transferred to urethane anaesthesia (initial dose of 1·5 g/kg I.V.). Supplementary doses of the latter were given as necessary to maintain a deep level of anaesthesia. A second femoral venous catheter was inserted for later administration of gallamine triethiodide and the trachea was

cannulated. A catheter placed in the right carotid artery allowed the monitoring of arterial blood pressure.

Animals were transferred to a stereotaxic holder and the head positioned so that a bar inserted under the upper incisors lay 5 mm above the ear-bars (Pellegrino, Pellegrino & Cushman, 1981). The cranium overlying part of the left cerebral cortex was removed. The dura was opened to allow insertion of glass micro-electrodes vertically into the brain stem at coordinates of 5–8 mm caudal and 1–3 mm lateral to bregma. Animals were then paralysed and artificially ventilated for the duration of the experiment. A bilateral pneumothorax was performed and the end-tidal carbon-dioxide levels continuously monitored and maintained around 4%.

*Preparation and stimulation of masseter nerve*

For the electrophysiological experiments, the left masseter nerve was exposed by removing part of the zygoma and prepared for stimulation before animals were transferred to the stereotaxic frame. The stimulating electrodes consisted of pairs of teflon-coated silver wires (bare diameter = 0·125 mm; Clark Electromedical Instruments) which were wrapped round the entire circumference of the in-continuity main nerve trunk. The electrodes were spaced by 1–1·5 mm, with the anode placed close to the first branch point of the nerve and the cathode more centrally. A small piece of plastic sheeting was placed under the electrodes to isolate them from the surrounding muscle tissue and a few drops of cyanoacrylate glue used to bond the wires to the nerve. The insulation provided by this was further supplemented by placing petroleum jelly over the above arrangement. The fascia of the masseter and temporalis muscles were sutured together and the skin wound closed.

The strength of electrical stimulation needed to elicit a just perceptible jaw-jerk was determined. The range of values obtained in different animals was 0·4–0·8 V when single stimuli of 0·05 ms duration were used. The value obtained in an individual animal varied by less than 10% over the course of an experiment. For centrally recorded units activated on stimulation of the masseter nerve, thresholds and latencies were determined. For the latter, stimulus strengths of twice the threshold for the unit were employed and no allowance was made for utilization time when calculating latencies. The same strength was used when assessing the ability of units to follow a standard train of ten stimuli.

*Recording*

Glass micro-electrodes were pulled from tubing of outside diameter 1·2 mm. These were either filled completely with a 5% Procion Yellow solution, or their tips filled with 3% Lucifer Yellow CH and the remainder with a 0·1% LiCl solution (Stewart, 1978). Electrodes were bevelled, by means of the slurry technique (Lederer, Spindler & Eisner, 1979), to resistances of some 15 MΩ for Procion Yellow filled electrodes and 20–25 MΩ for Lucifer Yellow CH filled electrodes. The tips of some electrodes were dipped briefly in a 2% solution of dimethyldichlorosilane (a hydrophobic compound) and this was found to improve the quality of intracellular recordings.

An electrometer amplifier (model M707: WPI) was used both for recording and current ejection from the electrodes. The bridge output, together with a high gain a.c. signal (bandwidth 0·2–3 kHz) derived from the amplifier output, was routinely recorded on an FM tape-recorder (Philips ANA-LOG7 or Racal Store 7: bandwidth 0–5 kHz). Also recorded on tape were the current monitor output of the electrometer amplifier and the stimuli applied to the nerve.

*Intracellular labelling and histology*

Both Lucifer Yellow and Procion Yellow were ejected from pipettes by passing steady hyperpolarizing currents of intensities up to 5–8 nA. Lucifer Yellow was used for labelling spindle afferents, while both dyes were employed for other neurones. For the spindle afferents, a common finding was that after an intracellular penetration they would quickly lose their ability to generate action potentials. The criterion then used for determining if the electrode was still intracellular was a relatively constant d.c. potential recording. For other neurones the shape and amplitude of the spike evoked by stimulation of the masseter nerve were the criteria used.

Heparin (0·5 ml of 5000 u./ml) was given I.V. some 10 min prior to perfusion with a buffered saline solution (pH = 7·0, 100–200 ml). This was followed with 1 l of buffered formal-saline (pH = 7·0), both solutions being applied under pressures of 200 mmHg. The brains were immediately blocked and serial, transverse, frozen sections of 60 or 90 μm thickness, cut. These

were mounted onto gelatin-coated slides, air-dried and cover-slipped using DPX. They were subsequently viewed under incident ultra-violet illumination. Selected sections were photographed and both the neuronal morphology and the electrode tracks reconstructed.

In three animals, injections of True Blue were made into the left masseter muscle. The high viscosity of True Blue (a milky suspension) precludes it readily diffusing into other masticatory muscles. A total of 3 $\mu$l was injected in each case and attempts made to place injections along the course of the nerve. Following the injections, no True Blue was observed to leak out of the muscle at the injection site. At intervals of 4, 5 and 11 days afterwards, these animals were used in experiments aimed at labelling spindle afferents with Lucifer Yellow. They were subsequently perfused with a chilled Tyrode Buffer (200 ml) followed by (500 ml) freshly prepared 4% paraformaldehyde pH 7·4. Transverse frozen sections were cut, as above, and viewed either unmounted or cover slipped using 9:1 mixture of glycerol and phosphate buffered saline (pH 7·4). By using the appropriate U.V. filters the intracellularly labelled neurones (Filter System $I_2$) and True Blue containing cells labelled by retrograde transport (Filter System A), could be visualized in the same section.

## RESULTS

### *Intracellular labelling of spindle afferents*

The mesencephalic nucleus of the V nerve (me.n.) is known to contain somata of two afferent modalities: periodontal mechanoreceptor and jaw-elevator muscle spindle afferents (Cody, Lee & Taylor, 1972). During extracellular recordings in the me.n., units that showed an increased firing to jaw-opening (muscle-stretch) and to gentle muscle probing were classified as muscle spindle afferents. Nine of these units in six animals were then successfully impaled and labelled by electrophoretic injection of Lucifer Yellow. Six were from the masseter, two from medial ptyergoid and one from the temporalis muscle. A neurone was judged to be labelled successfully if its axon could be followed for at least one millimetre and varicosities and boutons observed on the terminal arborizations of collaterals. This required some 50 nA . min of charge.

#### *Central course of spindle afferent axons*

There was a striking uniformity in the central course of all spindle afferent axons and this was typified by the afferent from the medial ptyergoid muscle shown in Fig. 1. Starting at the most rostral point, the afferent axon was observed initially amongst fibres of the fifth nerve, just lateral to the lateral lemniscus. The axon assumed a ventromedial course till just caudal to the lemniscus, when it then headed dorsocaudally towards the motor nucleus of the fifth nerve. Fig. 1*A* shows a reconstruction at the level of the rostral half of the motor nucleus. Ventrally, the axon can be seen heading towards and just entering the motor nucleus. Dorsal to the motor nucleus, the afferent cell body and a length of axon can be seen. On entering the motor nucleus, the axon continued caudally and a reconstruction at the level of the caudal half of the motor nucleus is shown in Fig. 1*B*. One collateral was given off within the motor nucleus, while dorsal to the nucleus, another collateral was given off to an area over the dorsolateral margin of the nucleus (supra-trigeminal nucleus). However, a more prominent site of projection was to the area immediately caudal to the motor nucleus (Fig. 1*C*), the V nucleus oralis. Four collaterals were given off and these could be followed for some 800 $\mu$m laterally. At the level of the nucleus oralis, the axon gave off a branch that could be followed further caudally for some

1·2 mm to approximately the rostral border of the inferior olive. Over this distance, the axon assumed a slightly more medial course to that seen in Fig. 1*C*, and gradually headed ventrally. Three pairs of collaterals were given off, spaced by distances of 180, 666 and 900 $\mu$m, with respect to the most caudal section of Fig. 1*C*. Although these appeared to travel ventrolaterally initially, they could not be followed for more than some 100 $\mu$m and so their sites of termination could not be determined.

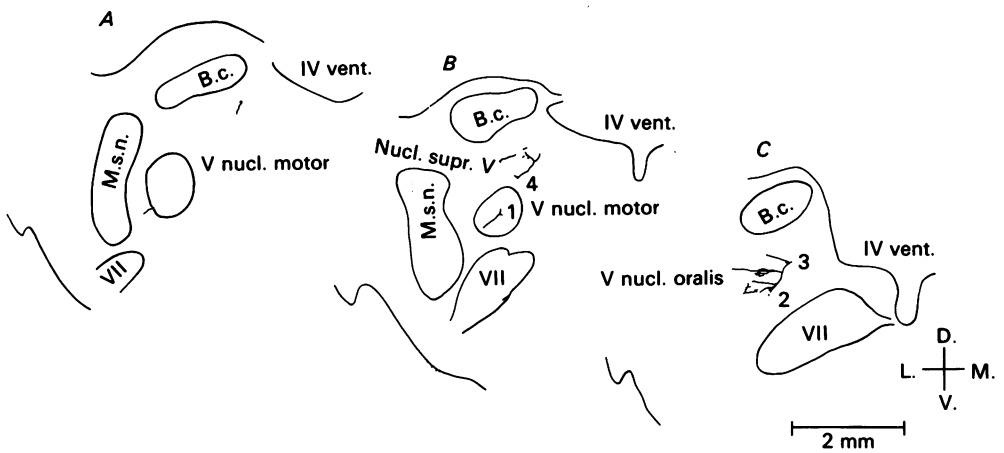


Fig. 1. Central course of a medial ptyergoid muscle spindle afferent reconstructed from transverse section at three levels, *A* being the most rostral and *C* the most caudal. Calibration and direction indicator in *C* apply throughout. *A*, reconstruction (over 180 $\mu$m) at level of anterior half of the trigeminal motor nucleus. *B*, reconstruction (over 300 $\mu$m) at level of caudal half of the motor nucleus and *C* (over 420 $\mu$m) caudal to the motor nucleus. The numerals in *B* and *C* indicate sequence of travel of axon (see text). Abbreviations: b.c. = brachium conjunctivum; IV vent. = fourth ventricle; m.s.n. = trigeminal main sensory nucleus; nucl. supr. V = supra-trigeminal nucleus; V nucl. oralis = trigeminal nucleus oralis; V nucl. motor = trigeminal motor nucleus; VII = seventh nerve; d. = dorsal; m. = medial; v. = ventral; l. = lateral.

The numerals in Fig. 1*B* and *C* underline the rather tortuous course taken by the afferent. From the motor nucleus (1, Fig. 1*B*), the afferent heads caudal to the nucleus oralis (2, Fig. 1*C*), where it branches. One branch then runs rostral (3, Fig. 1*C*) where it then emerges dorsal to the motor nucleus (4, Fig. 1*B*). From there, the axon runs rostrally to its cell body in the me.n. (Fig. 1*A*). All afferents adopted a similar central course, with the result that an action potential from the periphery will arrive at the motor nucleus first, then the V nucleus oralis, the supra-trigeminal nucleus and finally the me.n.

Fig. 2 shows in more detail the morphology of the collaterals from the same afferent to the motor nucleus (Fig. 2*A*) and the nucleus oralis (Fig. 2*B*). The single collateral given off within the motor nucleus arborized within a restricted area of some 300 $\mu$m. In contrast, the collaterals to the nucleus oralis extended for considerably greater distances (up to 800 $\mu$m). Characteristically, these travelled laterally and at intervals gave off branches which assumed a more ventral course. The evident disparity in the extent of collateral arborizations to the two areas suggests that while relatively

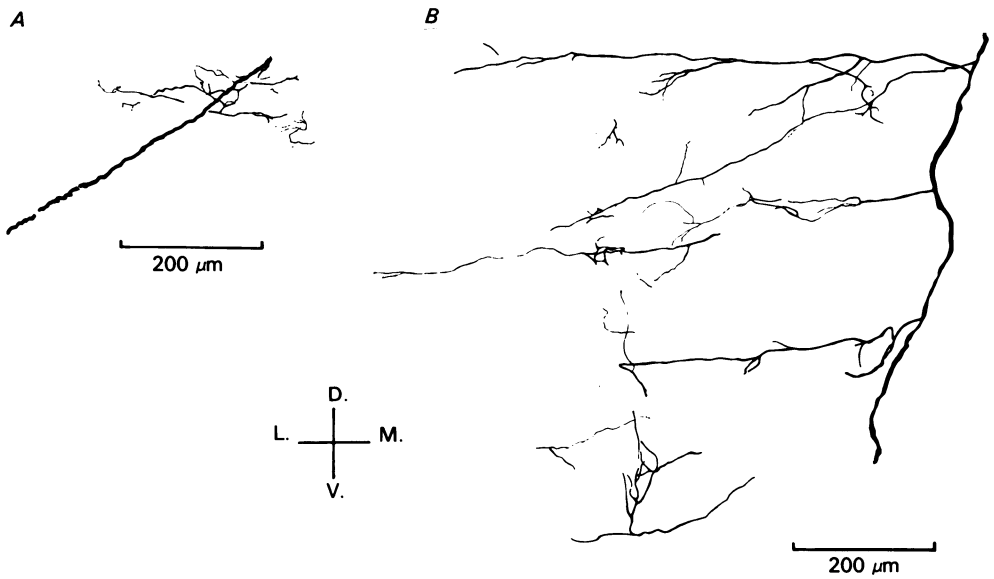


Fig. 2. Reconstructions of the projection of a medial ptyergoid spindle afferent (same as for Fig. 1) to the trigeminal motor nucleus (*A*) and nucleus oralis (*B*). The extent of the projection in *B* is far greater than in *A*. Note also the characteristic laterally directed collaterals in *B*. Direction indicator is common to *A* and *B*.

few motoneurones may receive a synaptic input from the afferent, a greater number of interneurones would receive an input. Some indication of the degree of innervation of the motoneurone pool was gained by concomitant labelling of two masseter afferents with Lucifer Yellow and masseter motoneurones with True Blue (Fig. 3).

The motoneurones were labelled by an intramuscular injection given four days prior to sacrifice. Some sixty labelled motoneurones were counted and their outlines (filled) are shown. Motoneurones were found almost throughout the entire rostro-caudal extent of the motor nucleus. They lay either in the dorsal or ventral margins of the motor nucleus and relatively few were found in the central portion. The two afferent axons adopted different courses through the motor nucleus. For the more dorsally situated axon, a single collateral was observed and this in turn arborized within the dorsal half of the motor nucleus in areas away from the majority of labelled motoneurones. In particular, note that no collateral arborizations are evident near the ventral group of motoneurones. The more ventrally situated afferent axon did not appear to give off a collateral within the motor nucleus, though both afferents gave collaterals to the nucleus oralis. In two other animals treated with True Blue (five and eleven days prior to sacrifice) the distribution of labelled motoneurones was essentially similar. The only exception was that relatively more labelled motoneurones could be found in the central portions of the motor nucleus and so giving the appearance of a continuous column of cells in the lateral half of the nucleus. The major effect of increasing the survival time was to improve the quality of labelling at the expense of the absolute number of motoneurones stained.

For the remaining four masseter afferents labelled, all gave a single collateral to the motor nucleus. In three cases, the arborizations were confined to the dorsal half

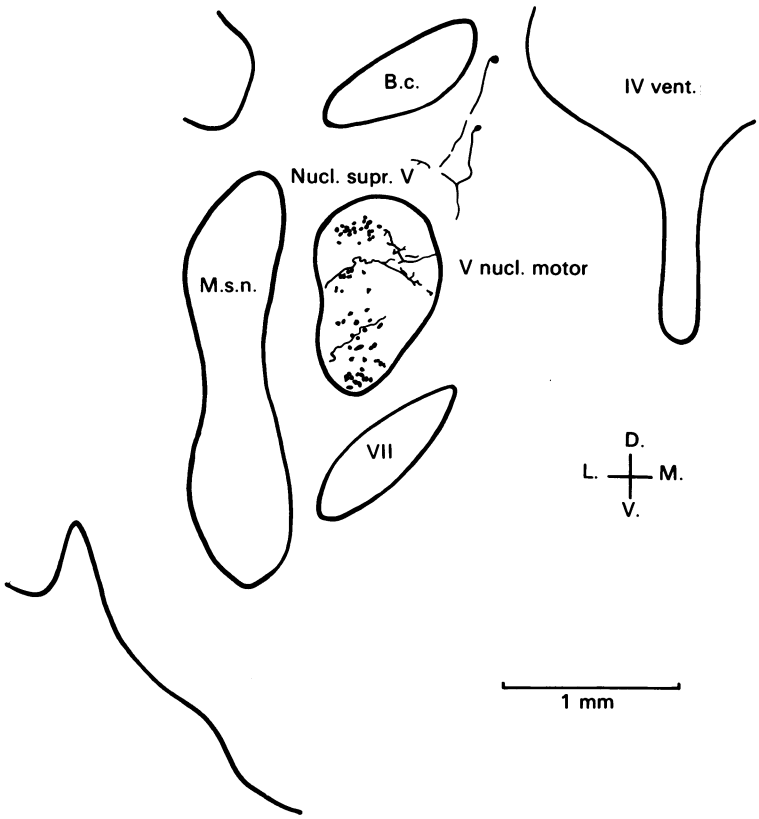


Fig. 3. Concomitant labelling of two masseter spindle afferents, with Lucifer Yellow, and masseter motoneurones (filled 'circles' in motonucleus) with True Blue. Only one afferent gave a collateral to the motor nucleus and this was directed away from the majority of labelled motoneurones. Reconstruction over 600 $\mu$m. Abbreviations as for Fig. 1 legend.

of the motor nucleus and in one case to the ventral half of the nucleus. However, for each of these afferents the area encompassed by the arborizations was similar to that shown in Fig. 3. For the two remaining afferents in the total sample, the collateral arborizations of the temporalis afferent were confined to the dorsal half of the motor nucleus while the arborizations of the medial ptyergoid afferent were restricted to the central portion.

In summary, eight (out of nine) afferents gave a single collateral to the motor nucleus and these invariably arborized within a restricted area. However, all afferents gave off collaterals to the nucleus oralis, the numbers ranging from two to five.

In both areas, varicosities and boutons were observed over the terminal 50 $\mu$m of the collateral branches. Examples of these are shown in Fig. 4 from six different afferents (Fig. 4*A–C* in the nucleus oralis; Fig. 4*D–F* in the motor nucleus). Both types of endings could be found on individual terminals, though occasionally a terminal would lack either varicosities (Fig. 4*A*) or a bouton (Fig. 4*B* and *D*). Also evident in the Figure is the fact that there was considerable variability in the number

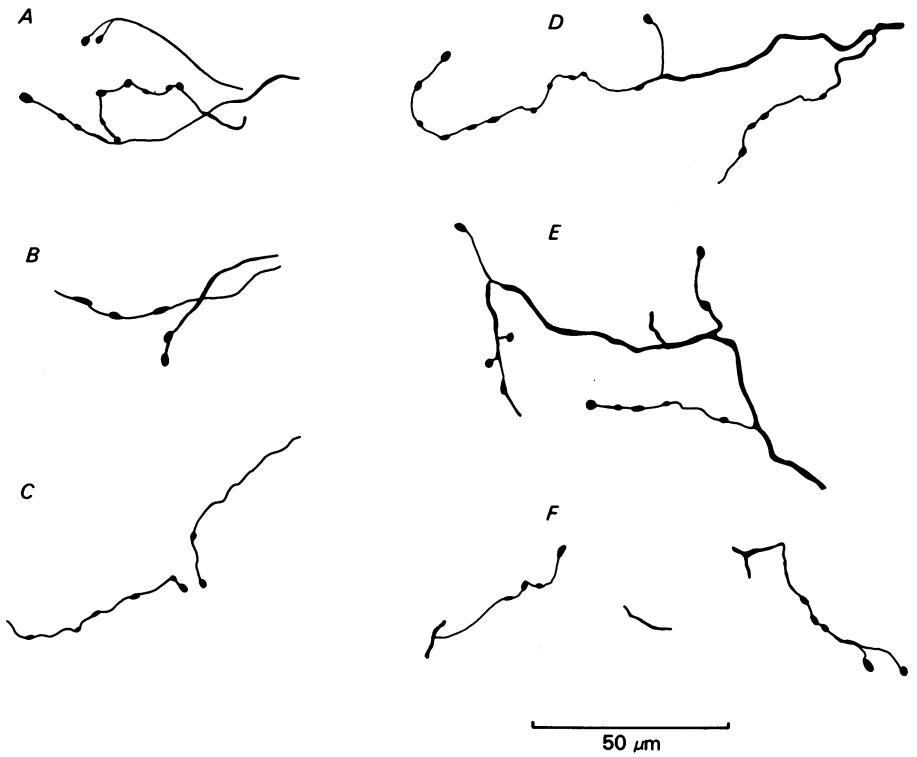


Fig. 4. Examples of the terminal arborizations of six spindle afferents to the trigeminal nucleus oralis (*A*, *B* and *C*) and the motor nucleus (*D*, *E* and *F*). Note that collaterals to each area commonly give off a number of varicosities (maximum of 10; *D*) before ending in a bouton. Occasionally, a terminal lacked varicosities (e.g. *A*). Terminals from: *A* and *B*, medial ptyergoid afferents; *C*, *E* and *F*, masseter afferents; *D*, temporalis afferent.

of varicosities encountered on a terminal. The ten varicosities seen in Fig. 4*D* (from a temporalis afferent) were the most encountered along a single terminal.

Only six of the nine afferents labelled gave a collateral to the supratrigeminal nucleus. For these a single collateral was observed to enter this area but varicosities and boutons were clearly observed in only two cases.

### *Unitary recordings*

The presence of varicosities and boutons in the three areas described above suggests that neurones receiving a monosynaptic muscle spindle afferent input may be found there. To determine if this was the case, recordings have been made from units excited by electrical stimulation of the masseter nerve. However, it was necessary to establish two points initially. The first was the strength of electrical stimulation needed to excite masseter muscle spindle afferents. The second concerned the formulation of clear criteria for differentiating the responses of afferents, motoneurones and interneurones.

*Electrical stimulation of muscle spindle afferents*

In four animals, twelve units that fired in response to both muscle stretch and gentle probing over the masseter muscle were recorded in areas outside the me.n. Their thresholds to electrical stimulation were close to that just needed to elicit a jaw-jerk in the unparalysed animal. Taking the latter response as an arbitrary threshold ($T$), the relative thresholds of the units were 0·64–1·2$T$, with one afferent having a value of 1·76$T$ (Fig. 9$A$). Overall, the mean threshold was 0·99$T$ (S.D. = 0·3). On masseter nerve stimulation they were activated at latencies of 0·5–0·78 ms (Fig. 9$A$). Seven units were recorded at the level of the nucleus oralis, while five were recorded just ventrolateral to the motor nucleus. There was no apparent difference in latencies for the two groups, the mean value being 0·6 ms (S.D. = 0·09). All could follow, one-to-one, stimulus trains applied at frequencies of at least 0·6 kHz, and often up to 1 kHz. An example from one unit is shown in Fig. 5$A$ and $C$. The threshold of the unit was 1·0$T$ and its latency 0·75 ms (Fig. 5$A$). It followed stimulus trains applied at 0·9 kHz (Fig. 5$C$). Both the responses to natural inputs and to electrical stimulation are consistent with these units being muscle spindle afferent axons.

*Identification of motoneurones*

Intracellular recordings were obtained from thirty-four units within the motor nucleus. In each case, there was an inflexion on the rising phase of the spike, indicating an intra-somatic penetration (Coombs, Curtis & Eccles, 1957). All units could follow stimulus trains of at least 0·33 kHz and sometimes up to 0·66 kHz but at these rates the full spike was not developed. An example of one such unit is shown in Fig. 5$B$ and $D$. On masseter nerve stimulation, the neurone was activated at latency 0·5 ms (Fig. 5$B$). The spike, of total amplitude 40 mV, was followed by an after-hyperpolarization of amplitude 4·5 mV and duration 29 ms. It followed stimulus frequencies of 0·3 kHz, but as is evident (Fig. 5$D$), only the initial-segement spike was elicited at these rates. At higher stimulus frequencies the neurone failed to respond to each stimulus pulse. The neurone was stained with Procion Yellow and subsequently identified as a multi-polar neurone, lying in the ventral half of the motor nucleus (Fig. 6). Six dendrites could be seen arising from the soma and the axon identified by virtue of showing a pronounced initial constriction (see Ramon-Moliner, 1968). Significantly, the axon could be traced into the bundle of fifth nerve fibres, ventro-lateral to the motor nucleus. This suggests the neurone may be a motoneurone, and the electro-physiological recordings are consistent with it being a masseter motoneurone. Ten other neurones were stained with Procion Yellow and identified as multi-polar neurones lying in the motor nucleus. Their locations within the nucleus were similar to that of the True Blue labelled motoneurones described earlier. The maximum and minimum diameters of the neurones ranged from 26 to 12 $\mu$m and up to seven dendrites observed arising from the soma. The axons could be clearly traced only for distances of some 100 $\mu$m and so a positive identification of the unit type could not be made solely on morphological grounds. No axon collaterals were observed.

The electrophysiological characteristics of all units were similar. Thus, the range of latencies was 0·4–0·7 ms, with the mean value being 0·56 ms (S.D. = 0·06). The

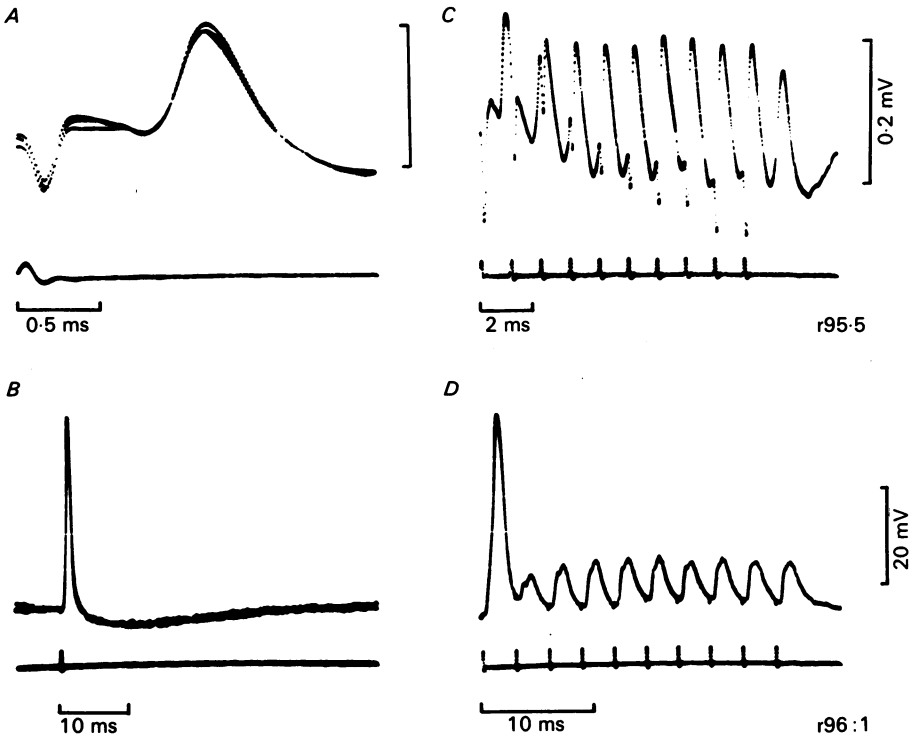


Fig. 5. Identification of a masseter spindle afferent (*A*, *C*), motoneurone (*B*, *D*: intracellular record). In each case, the left panel shows superimposed responses to a single shock of the masseter nerve, while the right shows responses to trains of ten stimuli. The lowest trace in each panel shows the stimulus marker. Stimulus intervals were 1·1 ms in *C* and 3 ms in *D*. Voltage calibration for each unit shown to the right.

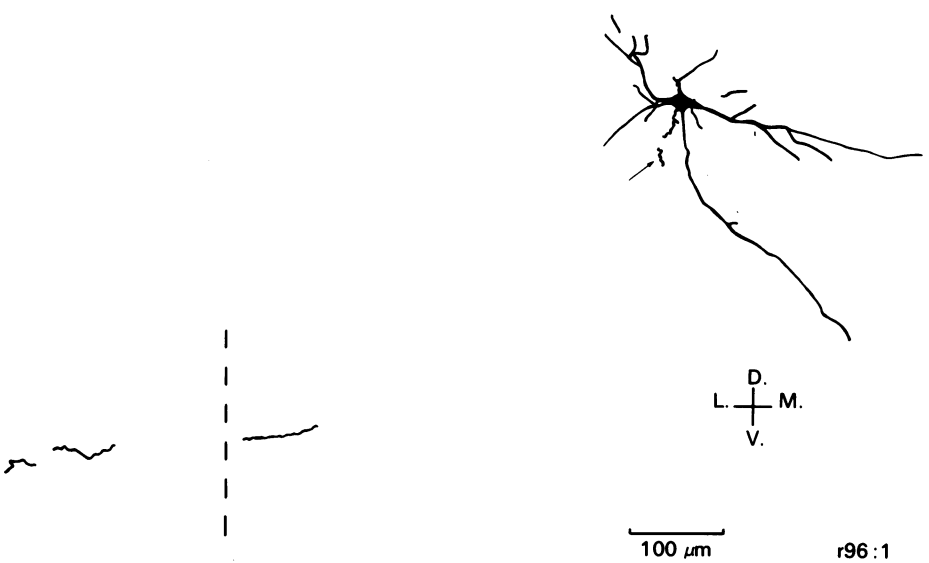


Fig. 6. Partial reconstruction of the morphology of a masseter motoneurone (same as for Fig. 5*B*, *D*). The axon, identified by arrow, enters the bundle of V nerve fibres (dashed line indicates medial border of fibres). Note the single, long, ventrally directed dendrite. This was a feature observed in all stained motoneurones. Reconstruction over 360 μm.

spikes were followed by after-hyperpolarizations of duration 20–30 ms and amplitude 0·6–8 mV. These thirty-four neurones were identified as antidromically activated masseter motoneurones.

*Identification of interneurones*

For the purposes of this study, an interneurone was defined as any neurone that was not either a first order afferent or a motoneurone. Two sets of criteria were adopted for identification of interneurones. The first was that the response of a unit should differ from those described for afferents or motoneurones. The second, reserved for cases where some similarities were evident, was that the recording should be from a cell body outside the motor nucleus or me.n. Recordings were obtained from fifty-four interneurones in the nucleus oralis, main sensory nucleus and supra-trigeminal nucleus.

Seven of the fifty-four units fired spontaneously. The discharge was invariably highy irregular, with spikes occurring in doublets or triplets. The responses to electrical stimulation of the masseter nerve could be broadly divided into two categories: units showing repetitive firing (i.e. two or more spikes) and those that did not. For the former category, twenty units fired repetitively to a single stimulus pulse applied to the masseter nerve while fourteen required either two or three closely spaced stimuli to be activated. In either case the number of spikes fired could range up to twelve but five was not uncommon (see Fig. 10*A* and *D*). The second category consisted of units that fired only a single spike in response to either single ($n = 4$) or multiple ($n = 16$) stimuli applied to the masseter nerve. Intracellular penetrations were obtained for the four units that fired only a single spike and the form of the response suggested an intra-somatic recording in each case. As seen in Fig. 7*A*, the recording consisted of an initial rapid phase of depolarization, latency 2 ms and amplitude 15 mV, followed by a prolonged but decrementing level of depolarization which persisted for some 8 ms. The unit, which was located in the nucleus oralis, was partially labelled with Procion Yellow and its morphology is shown in Fig. 7*B*. The response of Fig. 7*A* was virtually identical for all three units and bears a striking resemblance to that displayed by some lumbosacral Renshaw cells (e.g. see Eccles, Fatt & Koketsu, 1954; Eccles, Eccles, Iggo & Lundberg, 1961).

*Evidence for a muscle spindle input*

(*a*) *Motoneurones.* For fifteen of the sample of masseter motoneurones, the effects of electrical stimulation at strengths below that needed to elicit antidromic activation were studied. Two examples are shown in Fig. 8*A–C*. In Fig. 8*A*, masseter nerve stimulation at intensities greater than 1·5*T* evoked an antidromic spike of latency 0·55 ms. On reducing the strength of stimulation below 1·5*T*, there was an abrupt alteration in both the latency and form of the response (Fig. 8*B*). A depolarization (excitatory post-synaptic potential (e.p.s.p.)) of latency 1·1 ms and amplitude 3·8 mV was observed, and on some occasions, a single spike was fired off its rising phase. The abrupt transition between responses, coupled with the latency difference of 0·55 ms, suggests that they arose from different pathways. The longer latency response (Fig. 8*B*) is most simply ascribed to synaptic activation of the motoneurones (see Coombs *et al.* 1957). Fig. 8*C* shows another cell, previously

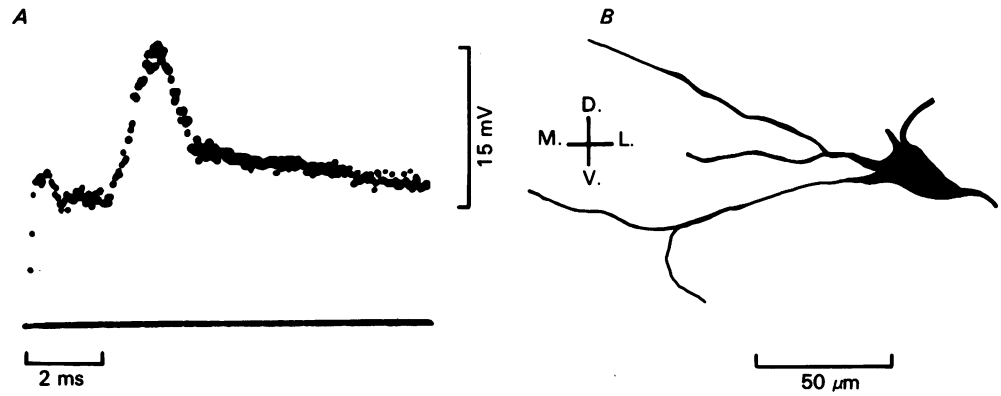


Fig. 7. Signal average (16 sweeps) of intracellular response obtained in an interneurone, to single shocks of the masseter nerve. Note the prolonged, decrementing, level of depolarization. The neurone was partially labelled (*B*) and its location identified as the nucleus oralis.

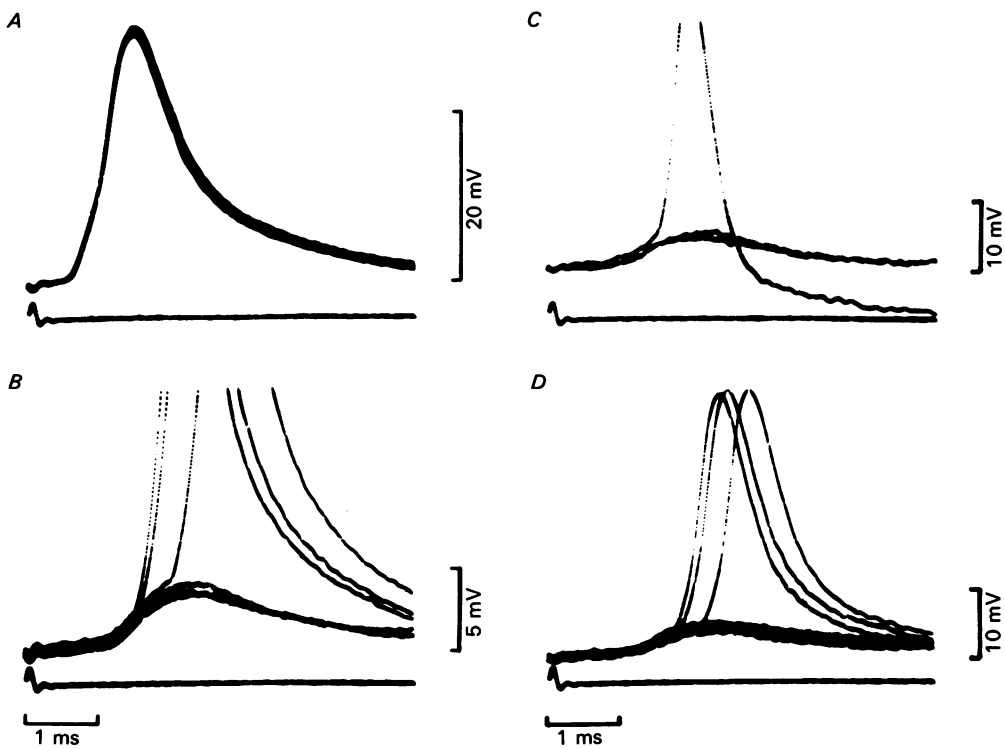


Fig. 8. Intracellular records (superimposed traces) from two masseter (*A–C*) and one synergist (*D*) motoneurone to show monosynaptic activation. *A*, shows the antidromic and *B*, the synaptic response for a masseter motoneurone. *C*, synaptic response in another masseter motoneurone (same as for Fig. 6) and *D* for a motoneurone that could not be antidromically activated by masseter nerve stimulation. Lower trace in each panel shows stimulus marker.

illustrated in Fig. 5*B*, which displayed similar behaviour. In this case, the threshold for antidromic activation was 1·3*T* and the latency was 0·5 ms. The threshold for synaptic activation was 0·9*T* and the latency was 0·8 ms. Increases in stimulus strength up to 1·1*T* produced a slight increase in the e.p.s.p. amplitude, and above this value a single spike was fired off the rising phase of the e.p.s.p. (Fig. 8*C*). The latency difference between the onset of the e.p.s.p. and the antidromic spike was 0·3 ms.

Only two of fifteen motoneurones tested failed to show a synaptic response. For the remainder, the amplitudes of the e.p.s.p.s ranged from 0·93 to 4·8 mV (mean = 2·8 mV: S.D. = 1·2) and the rise times ranged from 0·50 to 1·2 ms (mean = 0·91 ms: S.D. = 0·2). The latencies ranged from 0·80 to 1·38 ms (mean = 1·13 ms: S.D. = 0·19) and the thresholds from 0·55 to 1·28*T* (mean = 0·96*T*: S.D. = 0·18). The difference of only 0·57 ms between the mean latency of the e.p.s.p.s and that of the antidromic spikes, clearly suggests a monosynaptic origin for the e.p.s.p.s. An involvement of muscle spindle afferents is suggested by the virtually identical mean thresholds for excitation of such afferents and for evoking e.p.s.p.s (0·99*T* and 0·96*T* respectively).

Intracellular penetrations were also obtained in the motor nucleus from five other neurones, which were not antidromically excited by masseter nerve stimulation. They were, however, synaptically activated and so were presumed to be synergistic motoneurones. The neurone shown in Fig. 8*D* fired a single spike at latency 1·5 ms when stimulated at 1·6*T*. Below this strength, an e.p.s.p. of amplitude 4·0 mV and latency 1·0 ms was elicited. For these units, the e.p.s.p. latencies ranged from 1·0 to 1·4 ms, amplitudes from 1·5 to 4·0 mV, and thresholds from 0·85 to 1·4*T*. These values are sufficiently close to those reported above for masseter motoneurones to suggest that the e.p.s.p.s can be ascribed to a monosynaptic muscle spindle input. Six other units recorded within the motor nucleus could not be classified because of inadequate testing. Nevertheless, they all discharged tonically at rates up to 25 impulses/s and were activated at latencies of 1·4–1·8 ms. They could follow trains of stimuli at frequencies of 0·3 kHz, and for one unit, 0·6 kHz.

(*b*) *Interneurones: thresholds and response to muscle stretch.* The thresholds for activating interneurones, recorded extracellularly in the nucleus oralis, and for one unit, the supra-trigeminal nucleus, are shown in Fig. 9*B*. The caveat is that there was a bias towards identifying interneurones activated at stimulus strengths below some 3·0*T* as the search stimulus did not generally exceed this level. Half the sample was activated at strengths below 1·5*T*, and for those with thresholds below 1·2*T* ($n = 19$), i.e. within the range for eleven of twelve spindle afferents (see Fig. 9*A*), the possibility exists that these may receive a muscle spindle input. This would be strengthened if the units could, in addition, be shown to respond to muscle stretch.

This was examined for thirteen units, of which only one was spontaneously active. The stretches were applied manually and nine units fired, while four did not. Only rapid stretches were effective in eliciting firing and this consisted of a single burst of two, or three, spikes at instantaneous frequencies of some 600 impulses/s. Five had thresholds below 1·2*T*, while three had values clearly above this range (Fig. 9*B*, filled bars). The four units which did not respond had, in most cases, thresholds either within or close to 1·2*T* (Fig. 9*B*, hatched bars).

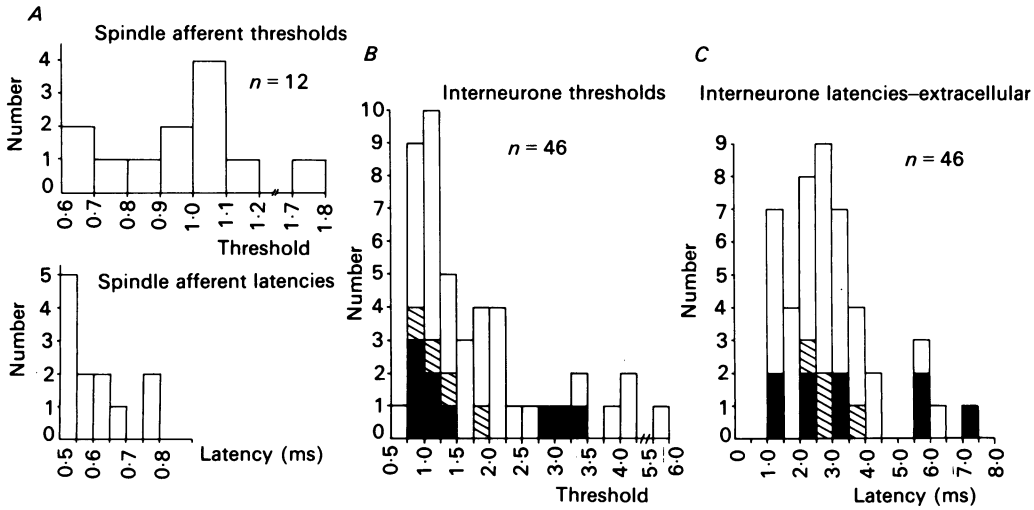


Fig. 9. Distribution of thresholds and latencies for spindle afferent axons (*A*) and interneurones (*B*, *C*). Only interneurones recorded in the supra-trigeminal nucleus and nucleus oralis are shown. Thresholds in all cases represent relative threshold with respect to that needed to elicit jaw-jerk in the unparalysed animal. Filled bars in *B* and *C* show units that responded to muscle stretch and hatched bars units that did not respond.

*Latency*

For the interneurones in Fig. 9*B*, the distribution of their latencies is shown in Fig. 9*C*. The range of values was from 1·1 ms to 7·2 ms. There was no apparent relationship between latency, recorded extracellularly, and threshold for activation of a unit. Initially, the criteria adopted for monosynaptic activation was a latency of less than 1·4 ms, this representing the maximum e.p.s.p. latency obtained in masseter and synergistic motoneurones. On this rather arbitrary basis, seven of the forty-six interneurones would appear to be monosynaptically activated, with two of these responding to stretch. However, a weakness of this argument is that no maximum limit can confidently be set on values still compatible with monosynaptic activation. This is because the e.p.s.p. time course and the excitability of the cell, both factors governing latencies measured extracellularly, are not taken into account.

To obtain such information, intracellular penetrations were made in ten interneurones. Two observations bearing on the above were made. The first was that following penetration, all units not only fired more spikes in response to the same parameters of stimulation, but there was also often a marked reduction in latency. This is illustrated in Fig. 10*A–C* for a unit that responded to muscle stretch. The threshold for evoking a spike on nerve stimulation was 0·9*T* and the latency at this strength was 4·7–6·2 ms (Fig. 10*A*).

Increasing the stimulus strength to 1·1*T* resulted in a shortening of the latency to 3·3 ms and two spikes being consistently evoked. On intracellular penetration (Fig. 10*B*), an e.p.s.p. of latency 1·4 ms was obtained and superimposed on this were three, sometimes four, spike-like responses of 5 mV amplitude. The latency of the

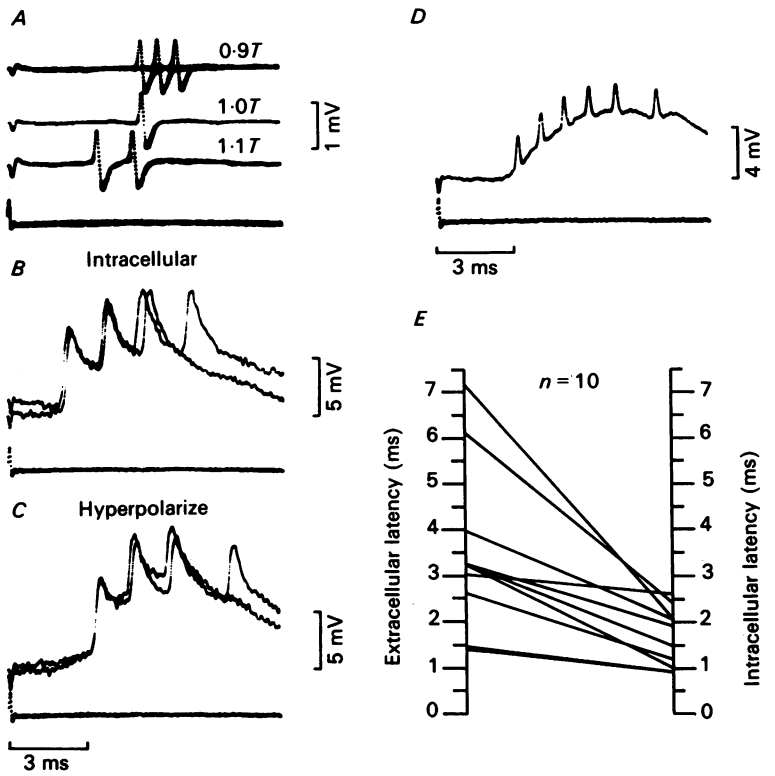


Fig. 10. Records from interneurones to show time course of e.p.s.p.s and differences between extracellular and intracellular latencies. *A–C* same unit. *A*, extracellular firings in response to graded stimulation of the masseter nerve (intensities shown with each trace). Top trace shows three superimposed sweeps and the lower two, single sweeps. *B* and *C*, intracellular records (two superimposed sweeps) in response to 1·1*T* stimulation. Note reduction of latency in *B* and the increase caused by hyperpolarization of the cell in *C* (2 nA current injected). *D*, intracellular record for another interneurone. Note long rise time of e.p.s.p. Lowermost trace in each panel shows stimulus marker. The units in *A–D* both responded to muscle stretch. *E*, differences between extracellular and intracellular latencies for ten interneurones. Note that this can be up to 5 ms.

initial spike-like response was 1·9 ms. On hyperpolarizing the cell (Fig. 10*C*), this latency increased to 3·3 ms – a value identical to that of the extracellularly recorded response. The shorter latency of the intracellularly recorded response in Fig. 10*B* may be ascribed to a depolarization caused by damage to the neurone by the electrode. Nevertheless, the observation indicates that a depressed excitability of the interneurones may significantly increase the latency recorded extracellularly. A further point suggesting a depressed excitability of interneurones in these preparations was the fact that only seven of the units shown in Fig. 9*B* and *C* fired spontaneously.

The small amplitude of the intracellular spike-like responses gave some cause for concern as these could represent either partial axonal spikes or spikes actively propagated down the dendrites to the soma. The latter possibility seems unlikely in view of the effects of hyperpolarization on the spike latency (Fig. 10*B* and *C*).

Spikes of comparable amplitude have been obtained in lumbosacral Renshaw cells by Eccles *et al.* (1961) and more recently by Walmsley & Tracey (1981) in depolarized Renshaw cells.

In Fig. 10*B*, the underlying e.p.s.p. appears to have a rise time of some 3·5 ms and an amplitude of 6 mV. A more precise determination of the e.p.s.p. rise time was possible for the unit shown in Fig. 10*D*, which also responded to stretch. In this case, the rise time was 4·4 ms and the e.p.s.p. amplitude 5 mV. Again note the spike-like responses, of amplitude 3 mV, superimposed on the e.p.s.p. For two other units in which an estimate of e.p.s.p. rise time could be derived, the values obtained were 1·6 ms and 5·2 ms. This compares with a range of values of 0·50–1·2 ms for synaptically activated motoneurones. For all ten interneurones penetrated, the latency difference between the extracellular spikes and e.p.s.p.s are shown in Fig. 10*E*. In each case, a spike-like response occurred within 0·5 ms after the onset of the e.p.s.p. Note that the latency difference can range up to 5 ms, a figure that could be accounted for by an e.p.s.p. of long rise time. Taking the above points into consideration, latency values up to 1·4 ms are clearly compatible with monosynaptic activation but values recorded extracellularly of 3·0 or up to 6·6 ms (1·4 ms e.p.s.p. latency plus 1·6 or 5·2 ms e.p.s.p. rise time) may still be equally compatible. On this basis, the majority of interneurones shown in Fig. 9*C* may be candidates for monosynaptic activation and this includes eight (out of nine) units which responded to muscle stretch.

## DISCUSSION

From both unitary morphological and electrophysiological techniques, evidence has been obtained for an elevator spindle afferent projection to motoneurones and interneurones. The morphological data further revealed that the weighting of the projection was greatest to the V nucleus oralis and that the V motor nucleus received a relatively restricted projection.

### *Morphological data*

The introduction of the horseradish peroxidase (HRP) technique for labelling single identified peripheral afferents (Jankowska *et al.* 1976; Snow *et al.* 1976) has resulted in unambiguous morphological descriptions of the central course of their axons. Thus the morphology of lumbosacral spindle primary (Brown & Fyffe, 1978; Ishizuka, Mannen, Hongo & Sasaki, 1979) and secondary (Fyffe, 1979) afferents has been described and this has provided a secure basis for interpreting electrophysiological data. Similar morphological data was not available for elevator spindle afferents and so the present study represents the first such description. We did not attempt to label separately elevator spindle primary-like and secondary-like afferents and so no comment can be made regarding the relative extent of their projections. This was because, in initial trials, the amplitude of units recorded extracellularly commonly decreased following infusions of suxamethonium chloride and it proved difficult to penetrate them subsequently. Presumably this was due to a movement to the brain stem relative to the electrode, perhaps caused by an alteration in blood pressure. The suxamethonium-chloride test is the only positive means of distinguishing between the two afferent types as their conduction velocities do not differ

apparently (Cody *et al.* 1972; Inoue, Morimoto & Kawamura, 1981). It may be mentioned in passing that one consequence of the tortuous central course described for elevator spindle afferents is that the conduction velocities obtained by these authors (maximum values of some 70 m/s) may represent significant underestimates. A second limitation of the data concerns the relatively short lengths over which afferents labelled with Lucifer Yellow could be followed (3–5 mm). Brown & Fyffe (1978) obtained HRP labelling of lumbosacral spindle afferents over distances up to 11 mm, indicating a superiority of HRP in this respect. Offsetting this is the fact that there is no danger of inadvertent labelling of other neurones, because of leakage of the dye into the extracellular medium, and the histological processing needed with Lucifer Yellow is far simpler. These limitations define the context within which the data from this study can be interpreted.

In the rat some information on the central course of trigeminal afferents has been obtained by the use of the cobaltic–lysine method (Matesz, 1981) and application of HRP crystals to the 'whole mandibular ganglion and motor root' (Jacquin, Semba, Egger & Rhoades, 1983). Matesz (1981) identified terminals of fibres of V mesencephalic nucleus in: the supra-trigeminal nucleus; V motor nucleus; an area lying between the V and VII motor nuclei; the lateral part of the reticular formation at the level of the XII motor nucleus; and the medial part of laminae five and six of the second cervical segment. A broadly similar pattern of termination was noted by Jacquin *et al.* (1983), with the exceptions that terminals were also observed in the V main sensory nucleus and the V nucleus caudalis. The extent to which the above represent areas of projection of muscle spindle afferents cannot be assessed as included amongst fibres of the V mesencephalic nucleus are periodontal mechanoreceptor afferent axons. Nevertheless, results from our study supplement these findings in that collaterals of spindle afferents terminated in the first three of the areas described. One potentially significant difference concerns the projection to the supra-trigeminal nucleus. Matesz (1981) reported a dense meshwork of collateral terminations in this area whereas only a relatively sparse projection was obtained in this study. One possibility is that the nucleus may represent a relay principally for periodontal mechanoreceptor afferents. For the cat, there is clear electrophysiological evidence to support a periodontal mechanoreceptor input onto cells in the supra-trigeminal nucleus (Kidokoro, Kubota, Shuto & Sumino, 1968) but no specific evidence for a muscle spindle afferent input (see Jerge, 1963).

An unexpected feature of our data concerned the weighting of the afferent input to the nucleus oralis. As each afferent gave more collaterals to this area than to either the supra-trigeminal or the motor nucleus, the implication is that it may represent an important relay for spindle afferent input. Cells here are known to project to the thalamus, cerebellum and parts of the brain stem reticular formation (for review see Darian-Smith, 1973), and some have recently been shown to project to the trigeminal motor nucleus in the cat (Mizuno, Yasui, Nomura, Itoh, Konishi, Takada & Kudo, 1983; Durand, Gogan, Gueritaud, Horcholle-Bassavit & Tyc-Dumont, 1983). Mizuno *et al.* (1983) observed retrogradely labelled cells in the nucleus after making small extracellular injections of HRP into the motor nucleus. Durand *et al.* (1983) labelled single neurones that were activated by electrical stimulation of the vibrissal pad and long ciliary nerve and traced collaterals of these into the trigeminal motor nucleus,

in addition to the motor nuclei of the facial and abducens nerves. This finding holds forth the possibility that neurones in nucleus oralis that receive a muscle spindle input may also project to the trigeminal motor nucleus and so form part of a multi-synaptic 'segmental', reflex pathway. Some preliminary evidence suggesting that muscle spindle afferents have excitatory multi-synaptic connexions on to elevator motoneurones has been reported by Appenteng, Morimoto & Taylor (1979).

The projection to the motor nucleus was less extensive than that to the nucleus oralis. Some evidence that individual afferents may innervate only a portion of the homonymous motoneurones was obtained by concomitant labelling of motoneurones and two masseter afferents. It may be significant that one of the two afferents did not give a collateral to the motor nucleus, even though it gave collaterals to the nucleus oralis and the axon could be traced rostral to the motor nucleus. The other masseter afferent did give a collateral to the motor nucleus. Some of the collateral arborizations came near labelled motoneurones but no collaterals were observed in the ventral half of the motor nucleus where a large body of motoneurones lay. Four other masseter afferents showed a similarly restricted projection and based on the distribution of labelled motoneurones, it seems likely that they also innervated only a portion of the motoneurone pool. Central to this conclusion is the question as to whether a representative sample of masseter motoneurones were labelled. The distribution of masseter motoneurones in the rat, following intramuscular injections of HRP, has been reported by three groups (Mizuno, Konishi & Sato, 1975; DeSantis, Limwongse & Rigamonti, 1978; Lynch, 1985). Labelled motoneurones were observed only in the lateral portion of the motor nucleus (Mizuno *et al.* 1975; Lynch, 1985) and they lay throughout the dorsoventral extent of this region (Mizuno *et al.* 1975; DeSantis *et al.* 1978; Lynch, 1985). This is a similar distribution to that obtained in our study and so supports the validity of the above conclusion.

*Electrophysiological data*

An assumption made with regard to the strength of electrical stimulation needed to excite masseter spindle afferents, is that these have generally lower thresholds than other afferent modalities. Though the distribution of conduction velocities for all afferent modalities in the mandibular nerve have not been determined, the available evidence indicates that muscle spindle afferents may be amongst the largest diameter fibres in the nerves to elevator muscles (Karlsen, 1969; Morimoto, Inoue & Kawamura, 1982). In the present study the fact that the threshold for eliciting e.p.s.p.s in motoneurones was lower than the threshold for antidromic activation of these cells, suggests that spindle afferents have faster conduction velocities than motor fibres.

A monosynaptic spindle projection could be demonstrated in most motoneurones. Taken together with the morphological observations, these findings imply that, while each afferent may project to a limited number of elevator motoneurones, virtually all such motoneurones receive an input from some afferents. This conclusion is similar to that reached by Appenteng *et al.* (1978) who employed both the intracellular and extracellular spike-triggered averaging techniques to study the projections of elevator spindle 'primaries' and 'secondaries' on to elevator motoneurones in the cat. No specific morphological evidence has been available hitherto to support their

conclusion. The significance of the two masseter motoneurones for which e.p.s.p.s were not obtained is difficult to determine. Signal averaging was not employed and so no distinction can be drawn between whether the motoneurones received a particularly weak spindle input or no input at all. Both these possibilities raise the question as to whether there may be some weighting of the afferent input on to particular motoneurones.

A more difficult issue to resolve concerned the number of interneurones that also received a monosynaptic spindle input. At the heart of this lay uncertainties as to what reference value of latency to adopt and the maximum latency still compatible with monosynaptic activation. One feature of the morphological data that is relevant here is the long length of the collaterals to the nucleus oralis. A consequence of this is that interneurones located laterally in the nucleus may have longer latencies than those lying more medially, simply because of the longer conduction distance over gradually tapering collaterals (see Fig. 4*B*). An additional consequence is that the afferent volley may be more dispersed in the lateral regions of the nucleus, so perhaps contributing to a more prolonged e.p.s.p. rise time. Neither of these features was tested specifically in this study and so a distinction cannot be drawn between them. However, they do suggest that a broad range of extracellular latency values may be compatible with monosynaptic activation. In the extreme case, if we assume an equal potency of input onto all interneurones and similar levels of excitability, the latency range may reflect that of the e.p.s.p. rise times. Faced with these uncertainties, the conservative approach adopted initially of taking the motoneurone e.p.s.p. latency as the reference point, though probably underestimating the number of monosynaptically activated interneurones, seems the most prudent course of action. However, on the basis of the observations presented, we conclude tentatively that interneurone latencies of up to 6·6 ms may be compatible with monosynaptic activation. As this includes eight out of nine units that respond to muscle stretch, we suggest that this provides electrophysiological confirmation for a monosynaptic spindle afferent input on to interneurones. The difficulties encountered in resolving this issue emphasize the usefulness of the spike-triggered averaging technique, especially when applied in deeply anaesthetized preparations. Criteria exist for differentiating between unitary e.p.s.p.s elicited by mono- or multi-synaptic pathways on to intercostal or lumbosacral motoneurones (Kirkwood & Sears, 1980, 1982). There is as yet little information on the amplitude and time course of unitary e.p.s.p.s evoked in 'segmental interneurones' and clearly such information is a pre-requisite for resolving the above issue.

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