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THE IDENTIFICATION AND CHARACTERISTICS OF SACRAL PARASYMPATHETIC PREGANGLIONIC NEURONES

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SUMMARY

1. Sacral parasympathetic preganglionic neurones were identified by intracellular and extracellular micro-electrode recording of antidromic potentials in response to stimulation of the pelvic nerve or the second or third sacral ventral roots.

2. The segmental distribution of autonomic neurones varied in different cats. In some cats they were mainly in S2 segment, in others in S3 and in the remainder, in both S2 and S3.

3. The antidromic potentials showed initial segment-somadendritic (IS-SD) inflexions and delayed depolarizations and were slightly less prolonged than those of sympathetic neurones but more prolonged than those of spinal motoneurones. After-hyperpolarization was observed after the antidromic spike potential.

4. The conduction velocities for sacral parasympathetic preganglionic fibres were less than 12·5 m/sec and thus were similar to those of sympathetic preganglionic fibres.

5. Parasympathetic neurones were not excited by micro-electrophoretically applied 5-hydroxytryptamine, noradrenaline or acetylcholine.

INTRODUCTION

The assumption that the neurones giving rise to the preganglionic fibres of the sacral parasympathetic outflow lie in the intermedio-lateral grey matter of the sacral cord is based mainly on the location of the analogous preganglionic neurones in the thoraco-lumbar region (Elliott, 1907; Rexed, 1954). However, this view has never been convincingly established by retrograde degeneration. Sacral preganglionic axons leave the cord by

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several ventral roots, mainly the second and third roots (Elliott, 1907; Langley & Anderson, 1895, 1896), and pass into the viscera on either side as the pelvic nerves. In the pelvic plexus the preganglionic fibres synapse with post-ganglionic neurones which innervate the smooth muscle of the pelvic viscera.

The present paper describes the identification and location of parasympathetic neurones by neurophysiological techniques. The characteristics of the antidromic responses of parasympathetic neurones recorded either extracellularly or intracellularly are discussed. This identification of parasympathetic neurones serves as a basis for a study of their central synaptic connexions which is currently in preparation.

Preliminary accounts of these observations have been reported previously (de Groat & Ryall, 1967*a*; Ryall & de Groat, 1967).

METHODS

The experiments were performed on cats of either sex anaesthetized with pentobarbitone sodium (35 mg/kg, intraperitoneally) or with *α*-chloralose (50–70 mg/kg, intravenously) or made decerebrate under fluothane anaesthesia. The pelvic nerve and somatic branches of the sacral roots (i.e. the pudendal nerve, the posterior femoral cutaneous nerve, and a small branch to the great sciatic nerve) were dissected by a dorsal approach through the greater sciatic notch. The somatic branches, taken together, and the pelvic nerve were mounted for stimulation on bipolar platinum electrodes. Electrodes were positioned on the pelvic nerve central to its point of branching on the lateral surface of the rectum, so that it was possible to stimulate the entire population of preganglionic axons. The spinal cord was exposed by a laminectomy extending from the second sacral to the third lumbar vertebrae. The spinal cord was left intact in all but seven preparations in which the cord had been transected 7–42 days before the experiments. When units were to be identified by antidromic invasion in response to stimulation of the peripheral nerves (see above) all ipsilateral dorsal roots caudal to and including L7 were severed. On the other hand, in experiments in which neurones were identified by antidromic invasion in response to stimulation of the ventral roots, the appropriate ventral roots were sectioned and mounted for stimulation, and the dorsal roots were left intact. All exposed tissues were covered by a pool of warmed paraffin (B.P.). The cats were paralysed with gallamine triethiode and artificially respired. A bilateral pneumothorax was performed to reduce movements due to respiration. Blood pressure was monitored routinely with the aid of a Statham transducer.

Glass micropipettes filled with 4M-NaCl or 3M-KCl were employed for extracellular recording, whereas 3M-KCl or 1·4M potassium citrate (pH 7) was used for intracellular recording. Intracellular potentials were recorded with a cathode-follower having correction for input capacitance.

Monopolar recordings from the surface of the spinal cord or from nerve fibres were obtained by means of platinum electrodes. Nerves were stimulated with rectangular pulses of 0·1–0·5 msec (usually 0·1 msec) duration.

DL-Homocysteic acid (Na salt, pH 8, 0·2M), 5-hydroxytryptamine creatinine sulphate (saturated solution), noradrenaline bitartrate (1M) and acetylcholine bromide (1M) were administered electrophoretically from multibarrelled glass micropipettes (see Curtis, 1964). Appropriate current controls were obtained by the passage of electrophoretic currents through a barrel of the micro-electrode containing NaCl.

RESULTS

Identification and location

In order to differentiate adequately parasympathetic neurones from motoneurones, experiments were first performed to compare the distribution, threshold to stimulation and conduction velocities of preganglionic and motor axons in the spinal roots.

Ventral root volleys. Short latency ventral root volleys (Fig. 1*F*) evoked by stimulation of motor axons in the somatic nerve were encountered in each sacral root. On the other hand, the longer latency responses to stimulation of preganglionic fibres in the pelvic nerve (Fig. 1*E*) were recorded mainly in the second and third sacral ventral roots. The distribution of these pelvic nerve responses in sacral roots varied in different animals, large responses sometimes occurring in only one root and at other times occurring in both roots. The conduction velocities for impulses in the preganglionic axons were calculated from such records (Fig. 1*E*) to lie between 3·3 and 13 m/sec, whereas the slowest conducting axons in the somatic nerve had a velocity of approximately 15 m/sec (Fig. 1*F*). The threshold electrical stimulus for exciting the preganglionic fibres was about 10 times that for the somatic efferent fibres.

Focal antidromic potentials. Stimulation of ventral roots evoked potentials in the spinal cord which were recorded by means of platinum electrodes placed upon the dorsal surface. Some typical records obtained in the segments L7 to S3 on stimulation of the corresponding ventral roots are shown in Fig. 1*A–D*.

In all segments there was a short latency triphasic positive-negative-positive potential, corresponding to the antidromic volleys recorded in the somatic efferent fibres. In the more rostral segments (Fig. 1*C*, *D*) there were also small, undulating potentials caused by the discharge of Renshaw cells (Eccles, Fatt & Koketsu, 1954). In the second sacral segment only (Fig. 1*B*), there was a later positive potential with a latency of 2·5 msec, a peak at 4 msec and terminating at about 6·25 msec. The conduction velocities corresponding to these latencies were 10 m/sec, 6·3 m/sec and 4·5 m/sec. These values fall within the range for the conduction velocities of the preganglionic fibres in the pelvic nerve and the threshold stimulus intensity for evoking this response was about 10 times the strength required for activating the somatic efferent fibres.

In 50 % of the cats tested, the surface potential generated by the antidromic activation of parasympathetic neurones was only evident in the second sacral segment. In 25 % of the cats it was only present in the third sacral segment and in the remaining 25 % of the cats it was evoked by stimulation of either ventral root.

The positive antidromic potentials recorded on the surface of the sacral cord corresponded in latency and duration with positive-negative focal potentials recorded with micro-electrodes at various depths within the cord. Some typical records are shown in Fig. 2.

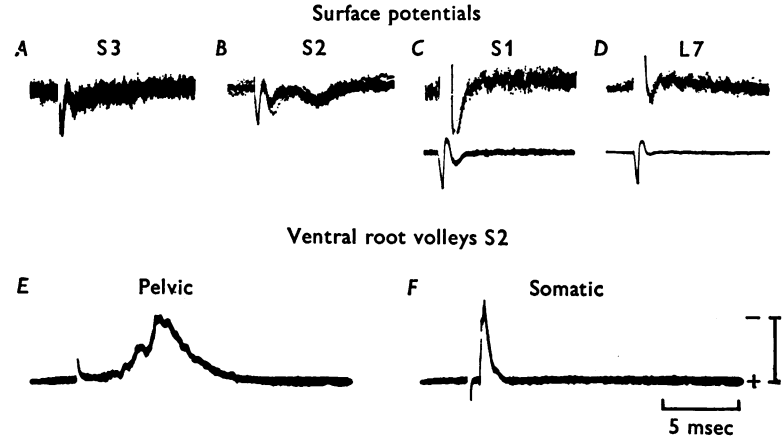

Fig. 1 *A–D*. Superimposed potentials recorded on the dorsal surface of the spinal cord in segments S 3 to L 7 on stimulation of ventral roots at a stimulus intensity approximately 6 × threshold for parasympathetic preganglionic fibres. In the lower records of *C* and *D*, the gain was reduced to ½ of that in the upper records. *E*, *F*; volleys recorded in the S 2 ventral root on stimulation of the pelvic nerve (*E*) or the sacral somatic nerve (*F*); stimulus intensity approximately 6 × threshold for parasympathetic fibres. Negativity upwards.

Near the surface of the spinal cord the antidromic response of the parasympathetic neurones was recorded as a positive potential. This positivity increased in magnitude down to a depth of about 1·5 mm (Fig. 2*B*) where the response became diphasic. Below this depth the negative phase of the response increased in magnitude and usually reached a maximum between 1·8 and 2·2 mm from the dorsal surface. Simultaneously, the positivity decreased in amplitude. Unitary parasympathetic potentials were commonly encountered in the region in which a diphasic complex was recorded. Below the region in which the negative potential was maximum, unitary potentials were rarely encountered, and the negativity decreased in amplitude without reversing in polarity. In four experiments, electrodes were left in place in the spinal cord at the end of the experiment and sections were made so as to locate the micro-electrode track. Invariably, it was found that parasympathetic units were only encountered over a distance of about 300 μ along the micro-electrode track and were situated in the intermediolateral part of the grey matter, as in the thoracic cord (Hongo & Ryall, 1966). However, an intermedio-lateral horn was not visible in cross-sections of

the sacral cord. The parasympathetic potentials were obtained only when the stimulus intensity was approximately 10 times greater than that required to excite motor axons. With stimuli of low intensity, negative potentials of a shorter latency, indicative of the antidromic invasion of motorneurones, were recorded in the ventral horn (Fig. 2*C*). In the experiment from which the records in Fig. 2 were obtained, the motoneurone

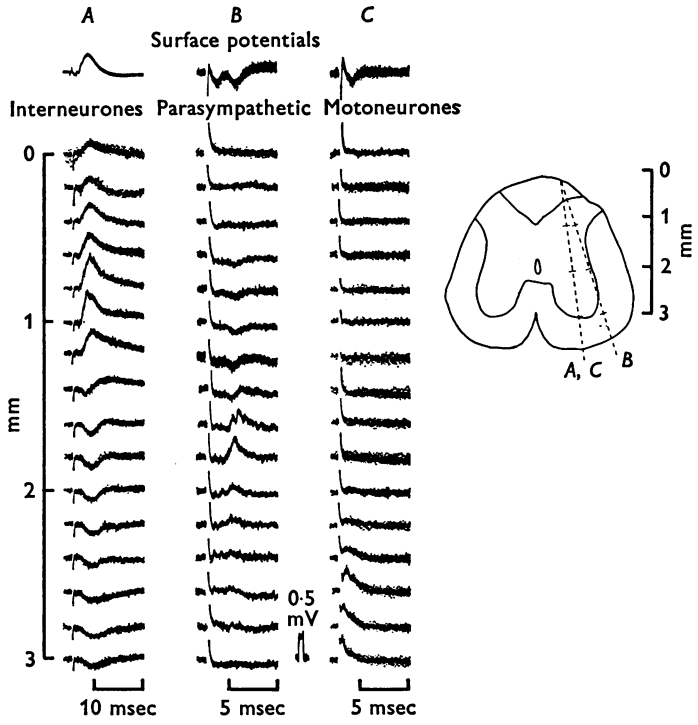

Fig. 2. Upper line of records; surface potentials recorded with a platinum electrode in S 2 segment on stimulation of (*A*) sacral somatic nerve, (*B*) S 2 ventral root at 6 × threshold for preganglionic fibres and (*C*) below threshold for preganglionic fibres but suprathreshold for motor axons. Lower records were obtained with capillary micro-electrodes at consecutive 0·2 mm depths, as indicated by the scale on the left. Voltage calibration (negativity upwards) refers only to micro-electrode recordings. The upper record in *A* is at a lower gain than those in *B* or *C*. The inset diagram shows the approximate position of the micro-electrode tracks in the spinal cord; the parasympathetic focal antidromic potentials were very small along track *C* (not shown), and the motoneurone potentials were very small along track *B*.

potentials were larger along a more medial track than that in which the parasympathetic neurones were located (compare Fig. 2*B* and *C*). However, in other experiments, large motoneurone potentials were often observed ventral to the parasympathetic potentials along the same micro-

electrode track (Fig. 3). The location of the parasympathetic nucleus was ventral to the region from which large negative focal synaptic potentials were generated in the dorsal horn on stimulation of somatic afferent fibres (Fig. 2*A*).

The identity of the late potentials recorded when ventral roots were stimulated was also confirmed by recording the focal potentials generated by antidromic volleys in the pelvic and sacral somatic nerves. The records

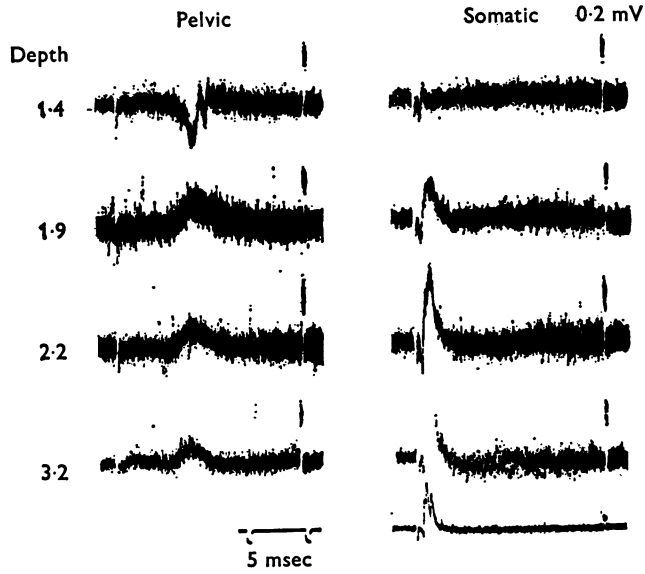

Fig. 3. Focal potentials recorded with a micro-electrode in the second sacral segment at the indicated depths in mm when either the pelvic or sacral somatic nerves were stimulated at approximately 6× threshold for preganglionic fibres. All ipsilateral dorsal roots caudal to L 7 were transected. The calibrations are 0·2 mV and 5 msec for all records. The lowermost record in the right-hand column was recorded at 3·2 mm at reduced gain. Negativity upwards.

in Fig. 3 show that the late negative potential recorded in the intermediate region of the grey matter was only observed when the pelvic nerve was stimulated and the early negative potential was only obtained when the somatic nerve was stimulated. The conduction velocities for the axons of the neurones giving rise to the late negative focal potential corresponded to those for preganglionic fibres.

Unitary antidromic potentials of parasympathetic neurones. Sixty-two units were positively identified as parasympathetic neurones by antidromic invasion on stimulation of preganglionic fibres in the pelvic nerve. The axonal conduction velocities for these units ranged from 3·9 to 12·5 m/sec, with a peak in the distribution between 5 and 11 m/sec (Fig. 4*A*). In contrast,

forty-seven somatic motoneurones, which were identified by antidromic invasion on stimulation of their axons in the sacral somatic nerves, had axonal conduction velocities between 17·5 and 105 m/sec (Fig. 4*A*).

Since there was such a clear separation of the conduction velocities of parasympathetic and motor axons, it was possible to differentiate between the responses of these two types of neurones, even when they were antidromically activated on stimulation of ventral roots. The histogram in

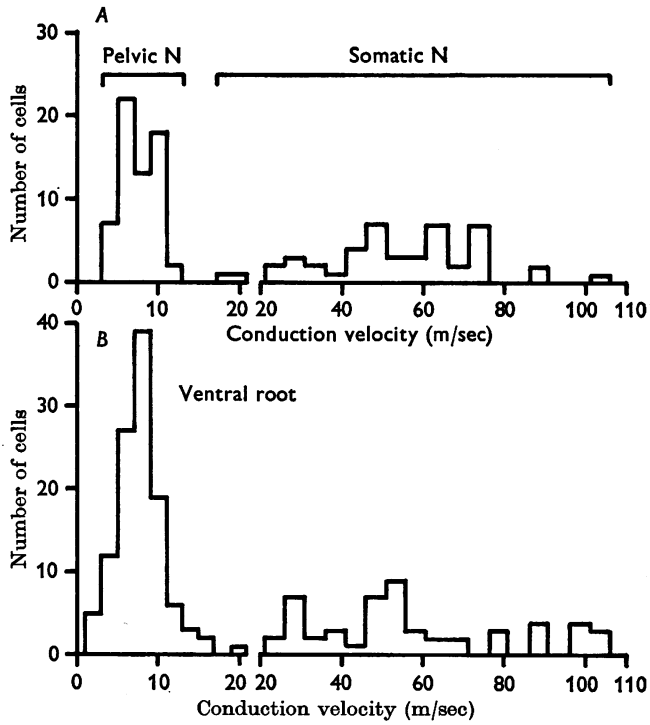

Fig. 4. Distribution of axonal conduction velocities for sixty-two units antidromically activated by stimulation of the pelvic nerve and for forty-seven units antidromically activated from the sacral somatic nerve (*A*) and for 165 units antidromically activated by stimulation of sacral ventral roots (*B*).

Fig. 4*B* represents the distribution of axonal conduction velocities for 165 units located by the latter technique. On the basis of these data, as well as data on the threshold for activation and location in the cord, cells with axonal conduction velocities below 15 m/sec were considered to be parasympathetic neurones (see Discussion).

Characteristics of antidromic potentials

When relatively large (5–7 MΩ tip resistance) micro-electrodes were used, the parasympathetic unitary potentials recorded extracellularly were small (less than 500 μV) and predominantly negative in polarity. Larger (up to 10 mV) and predominantly positive extracellular potentials (Figs. 5, 6*A*) were recorded with higher resistance micro-electrodes.

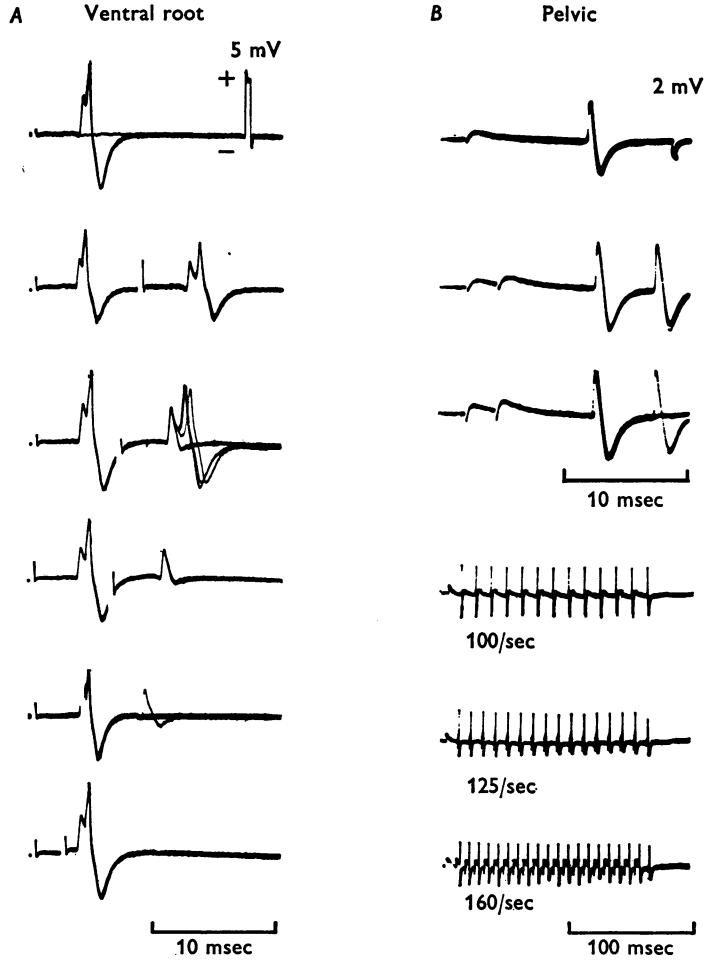

Fig. 5. Extracellular records of antidromic potentials recorded from two parasympathetic neurones, showing the effect of two shocks applied to the ventral root in *A* and to the pelvic nerve in *B*, and the effect of stimulation of the pelvic nerve at frequencies up to 160/sec in *B*. Voltage calibrations are shown in the upper records of *A* and *B*. Note the slower sweep speeds in the lower records in *B*. Positivity upwards.

The duration of the positive-negative complex recorded extracellularly was within the range 1·7–6·6 msec (average for 50 cells 3·64 ± 1·1 S.D. msec). Most of these potentials showed a clear inflexion on the positive phase of the spike (see Figs. 5*A*, 6), which corresponded with the initial segment-somadendritic inflexion (IS-SD; Eccles, 1957) recorded intracellularly. The IS-SD inflexion was accentuated when the cells were repetitively activated antidromically at high frequencies (Fig. 7*A*). At still higher frequencies there was a blockade of the SD component, and at this stage the late negative phase of the response was also blocked, showing that it was related to the SD component of the spike potential (see Fig. 5*A*).

When the preganglionic fibres were stimulated twice (Fig. 5), the configuration of the second antidromic spike was unaffected when the interval between stimuli was greater than 10 msec. At shorter intervals there was on most cells a progressive increase in the IS-SD inflexion, then a failure of the SD spike and finally a failure of the IS spike. With prolonged tetani, the IS-SD spikes followed frequencies of stimulation to 160/sec (Fig. 5*B*) but did not follow higher frequencies. Some units followed frequencies of stimulation to only 40/sec. In a few units it was not possible to block differentially the SD component of the spike without simultaneously abolishing the IS component (Fig. 5*B*).

Intracellular records of antidromic potentials were obtained from nineteen cells in which the size of the spike ranged from 20 to 90 mV and resting membrane potentials of up to 60 mV were recorded. The average duration of the intracellularly recorded potentials, measured to the point at which the potential returned to the base line, was 5·7 ± 2·3 (S.D) msec in ten neurones in which the spike amplitude was greater than 50 mV, and was similar in the remaining nine cells having smaller spikes (5·3 ± 1·1 msec). The correlation between the extracellularly and the intracellularly recorded potentials is shown in Fig. 6*A*. As reflected in the averages, the intracellularly recorded potential in Fig. 6*A* is more prolonged than the extracellular potential of the same cell.

On some neuronal responses there were pronounced humps on the repolarizing phase of the antidromic potentials, as shown in the extracellular record in Fig. 6*A* and the intracellular records in Fig. 6*B* and *C*. Such potentials have been observed both in chronic spinal cats and in cats with an intact spinal cord and in extracellular as well as intracellular records. They were present in cells with antidromic spikes as large as 90 mV, as well as in cells with much smaller potentials, and so it is unlikely that they were the result of cell damage by the micro-electrode. These delayed potentials were occasionally observed to be all-or-none in nature (see Nelson & Burke, 1967).

The antidromic spike potentials were followed by an after-hyperpolarization lasting for approximately 60 msec (Fig. 7*B–D*); the average duration in ten cells with antidromic spikes of 30–80 mV was 60 ± 12 msec. No hyperpolarizations were recorded when the stimulus strength was just below threshold for the axon of the parasympathetic neurone being studied (Fig. 7*D*). The hyperpolarization caused a reduction in the excitability of the cell as shown by the prolonged reduction in 'spontaneous' firing in

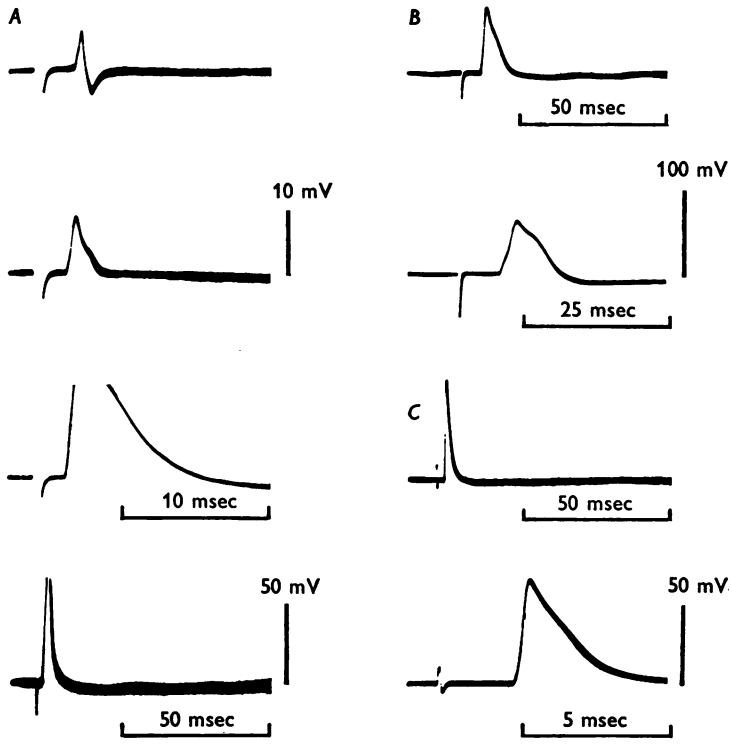

Fig. 6. Antidromic potentials of three parasympathetic neurones. *A*. Upper two records are extracellular antidromic potentials; the second record was obtained just before penetration of the cell membrane. Lower two records are intracellular potentials recorded from the same cell. *B*, *C*; intracellular antidromic potentials from two other units. All three units were located by stimulation of the sacral ventral roots above threshold for preganglionic fibres. The three units are from two experiments in cats with a chronic transection of the spinal cord in the upper lumbar region. Positivity upwards.

Fig. 7*E*. Furthermore, when the ventral root was tetanized for 20–30 sec at a frequency of 20/sec, and at a stimulus intensity above threshold for antidromic invasion, the reduction in spontaneous firing lasted for periods up to 3 min and there was a similar reduction in the firing evoked by an

electrophoretic administration of an excitant amino acid, DL-homocysteic acid.

Micro-electrophoretic administration of drugs

Micro-electrophoretic administration of DL-homocysteic acid excited parasympathetic neurones. The maximum sustained frequency of firing was in the region of 15 impulses/sec. In contrast, no excitation was observed with 5-hydroxytryptamine, noradrenaline or acetylcholine administered by electrophoretic currents up to 200 nA on 14–20 cells tested with each substance.

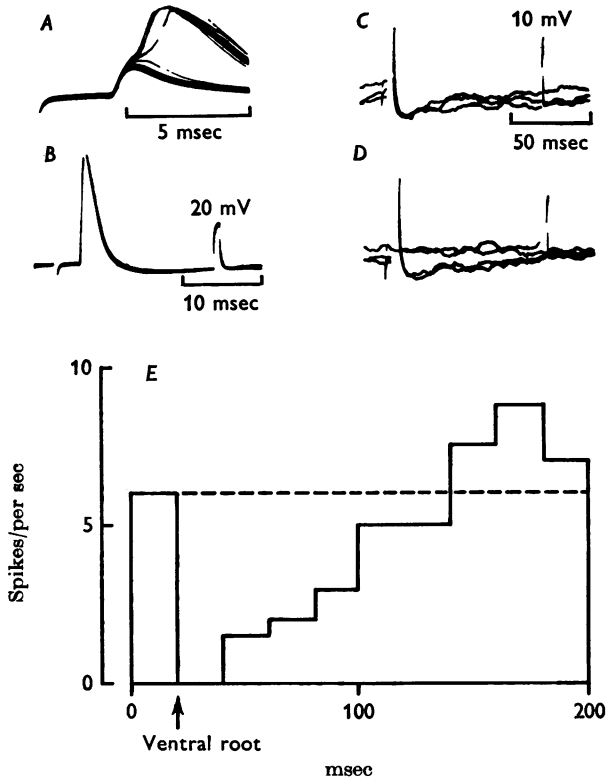

Fig. 7 *A–D*. Intracellular records of antidromic potentials in a parasympathetic neurone in a cat with an intact spinal cord. In *A*, the axon was stimulated at a frequency of 40/sec and in *D* the stimulus was on threshold. Positivity upwards. Voltage calibration for *A* is the same as in *B*. *E*: post-stimulus histogram of the frequency of 'spontaneous' firing of a parasympathetic neurone following single supramaximal shocks to the preganglionic axon in the ventra root. The dashed line shows the average frequency of firing in the absence of stimulation. Histogram computed from 140 sweeps.

DISCUSSION

Parasympathetic neurones in the sacral spinal cord were positively identified by antidromic invasion following stimulation of the preganglionic fibres in the pelvic nerve in experiments in which the ipsilateral dorsal roots were cut. In every instance the calculated axonal conduction velocity was less than 12·5 m/sec. In contrast, the conduction velocities of the axons of somatic motoneurones activated from the sacral somatic nerve in the same experiments were invariably greater than 17·5 m/sec. The threshold stimulus for activation of the somatic efferent fibres was about one tenth of that required for parasympathetic efferent fibres. The more slowly conducting motor fibres had conduction velocities within the range reported for small gamma efferents (Boyd, 1965; Appleberg, Bessou & Laporte, 1966).

In most of our experiments we wished to study the effects evoked by stimulation of afferent fibres in the pelvic nerve (W. C. de Groat & R. W. Ryall, in preparation) and it was necessary to leave the dorsal roots intact. In these experiments the parasympathetic neurones were located and identified by their antidromic responses to stimulation of the central ends of transected sacral ventral roots. With this technique two criteria were available for identification of the parasympathetic units. These were, first, latency of antidromic invasion and, secondly, threshold strength of stimulation for activation. These criteria were amply justified by the evidence presented in this paper, and no further distinction is made between units identified either by stimulation of the pelvic nerve or by stimulation of the ventral root.

In many respects the characteristics of the preganglionic parasympathetic neurones were similar to those of sympathetic neurones. For instance, the conduction velocities for the preganglionic parasympathetic axons in the sacral outflow lie within the range 2·1–15 m/sec, with a peak in the distribution between 6 and 10 m/sec. This range is similar to that reported for other preganglionic fibres (Bishop & Heinbecker, 1932; Eccles, 1935; Hongo & Ryall, 1966; Fernandez de Molina, Kuno & Perl, 1965; Iggo, 1958; Widdicombe, 1966).

Most of the parasympathetic neurones are located in either the second or third sacral segments with individual differences in different cats, thus confirming the earlier histological observations (Langley & Anderson, 1895, 1896; Rexed, 1954). The micro-electrode recordings showed that they were confined to a region of the grey matter lying intermediate between the dorsal and ventral horns and situated laterally. The size of the nucleus was similar to that of the sympathetic nucleus in the thoracic cord (Hongo & Ryall, 1966). However, there is no indication in histological sections of

a laterally projecting intermedio-lateral horn in the sacral cord (see Rexed, 1954; Mitchell, 1956), as is present in the thoracic spinal cord.

The antidromic potentials recorded extracellularly from the parasympathetic cells were slightly briefer in duration (1·7–6·6 msec) than those recorded with intracellular micro-electrodes (2–10 msec). These values are somewhat longer than those reported for somatic motoneurones (Brock, Coombs & Eccles, 1953; Fatt, 1957; Frank & Fuortes, 1955) but are slightly less than those reported for sympathetic preganglionic neurones (Fernandez de Molina *et al.* 1965).

The hump occurring on the repolarizing phase of the antidromic potential of some parasympathetic neurones was also found by Fernandez de Molina *et al.* (1965) in the sympathetic neurones. Coombs, Eccles & Fatt (1955) have observed a similar phenomenon after the intracellular injection of choline ions to spinal motoneurones and Granit, Kernell & Smith (1963) and Nelson & Burke (1967) have observed delayed depolarizations in spinal motoneurones. The delayed depolarization has been attributed to the propagation of spikes into the dendrites, and to electronic spread into the soma (Granit *et al.* 1963; Fernandez de Molina *et al.* 1965; Nelson & Burke, 1967).

As in spinal motoneurones and sympathetic preganglionic neurones (Fernandez de Molina *et al.* 1965), the antidromic spike potential is followed by an after-hyperpolerization. The duration of the hyperpolarization was in the region of 60 msec and during this period 'spontaneous' firing and the firing to the micro-electrophoretic application of an excitant amino acid was depressed. The hyperpolarization and the depression of excitability was only observed when the stimulus was above threshold for antidromic invasion, and is thus unlikely to be caused by recurrent inhibition (Eccles *et al.* 1954). Therefore, the hyperpolarization is not the explanation of the recurrent inhibition of these cells revealed by other techniques (Ryall & de Groat, 1967, and in preparation). It is of interest that there was also a prolonged depression of the parasympathetic neurones following repetitive antidromic invasion. There is a similar depression of sympathetic ganglion cells and autonomic fibres (Grundfest & Gasser, 1938; Gasser, Richards & Grundfest, 1938; Grundfest, 1939; Bronk, 1939), which has been attributed to a prolonged hyperpolarization.

The ability of parasympathetic neurones to follow two stimuli applied to their axons was similar to that of sympathetic preganglionic neurones (Fernandez de Molina *et al.* 1965).

In contrast with results obtained on sympathetic neurones in the thoracic cord (de Groat & Ryall, 1967*b*; Ryall, 1967), 5-hydroxytryptamine, administered micro-electrophoretically, did not excite the parasympathetic neurones. However, preliminary experiments (unpublished observations)

have indicated that the parasympathetic neurones are depressed by noradrenaline and in this respect the parasympathetic neurones appear to resemble some sympathetic neurones.

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