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A RECLASSIFICATION OF B AND C NEURONES IN THE NINTH AND TENTH PARAVERTEBRAL SYMPATHETIC GANGLIA OF THE BULLFROG

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SUMMARY

1. The cellular organization of the ninth and tenth paravertebral sympathetic ganglia in the bullfrog was studied with intracellular and extracellular recording methods. An isolated preparation was used in which anatomical details of individual cells could be resolved while making physiological measurements. This permitted the characterization of neurones in terms of their size, the segmental origin of their cholinergic innervation, and their orthodromic and antidromic conduction velocities. With these criteria, three classes of sympathetic neurones were identified.

2. As in previous studies, C cells were distinguished from B cells by the origin of their innervation. C cells are innervated by slowly conducting axons (0·4 m/sec) from spinal nerves 7 and 8 and B cells are innervated by rapidly conducting axons (2·4 m/sec) from the sympathetic chain above ganglion 7.

3. In earlier work it has been suggested that the conduction velocity of a preganglionic axon generally matches that of its target neurone. In this study we have characterized a large group of B cells for which this is not true. The axons of B cells fall into a rapidly conducting group (2·0 m/sec) and a slowly conducting group (0·6 m/sec). In contrast, C neurones, like their preganglionic inputs, have only slowly conducting axons (0·3 m/sec). Consequently, neurones have been classified as C type, fast B type, and slow B type. Fifty-nine percent of the B cells that we studied were slow B cells. These findings were corroborated by measurements of compound extracellular responses in post-ganglionic nerves.

4. Some neurones can be identified also by the size of their cell bodies. C cells are about 30 μm in diameter while B cells are about 50 μm in diameter. In our sample, 96% of the cells with radius $< 16\,\mu$m were C cells and 94% of the cells with radius $> 21\mu$m were B cells. However, fast B cells could not be distinguished from slow B cells by size.

INTRODUCTION

The integrative properties of autonomic ganglia arise from specific patterns of synaptic connexions, from interaction between synaptic mechanisms and from the intrinsic firing properties of ganglionic neurones. In the bullfrog the organization of the ninth and tenth paravertebral sympathetic ganglia makes it a useful model for

several aspects of ganglionic integration. This paper shows that an earlier scheme for cell classification in this preparation is inadequate and defines parameters for the identification of three major cell types. In the accompanying paper (Dodd & Horn, 1983) we examine interactions between three different synaptic potentials which occur together in only one of these cell types. The observed diversity of neuronal properties demonstrates that these ganglia could function as more than simple relays of nerve impulses from the spinal cord to peripheral targets.

In previous studies the sympathetic neurones of frogs and toads have been classified as either B-type or C-type cells, based on the origin and properties of their inputs, axonal conduction velocity, cell size and the expression of membrane conductances. In the paravertebral chain ganglia, the pre- and post-ganglionic nerves each have a population of rapidly conducting axons (about 2·5 m/sec) called B fibres and slowly conducting axons (less than 1 m/sec) called C fibres (Bishop & Heinbecker, 1930; Erlanger & Gasser, 1930; Bishop & O'Leary, 1938). The two preganglionic conduction velocities presumably arise from two separate populations of preganglionic neurones. Similarly, the ninth and tenth ganglia contain neurones with B axons and neurones with C axons (Nishi, Soeda & Koketsu, 1965). It has been suggested that the cells with B axons have larger somata than those with C axons (Nishi et al. 1965; Honma, 1970). The neurones in ganglia 9 and 10 are innervated by cholinergic fibres from spinal nerves 4–8. At the segment of origin, these preganglionic fibres enter the sympathetic chain by way of the rami communicantes and then travel caudally. In frogs, roots 4–6 supply the B fibres, and roots 7 and 8 supply the C fibres (Skok, 1965; Libet, Chichibu & Tosaka, 1968). Preganglionic B fibres are thought to project to neurones with B axons, and C fibres to neurones with C axons (Nishi *et al.* 1965; Tosaka, Chichibu & Libet, 1968; Weight & Padjen, 1973*b*; Jan, Jan & Kuffler, 1979; Jan, Jan & Brownfield, 1980; MacDermott, Connor, Dionne & Parsons, 1980; Feldman 1979, 1980; Kuffler, 1980; Marshall, 1981; Horn & Dodd, 1981).

In addition to fast cholinergic excitatory post-synaptic potentials (e.p.s.p.s), the ninth and tenth ganglia also exhibit two different slow muscarinic synaptic potentials, each of which can last for seconds. One response is excitatory and the other is inhibitory. However, only one of the two muscarinic responses is ever present in any given cell (Tosaka *et al.* 1968; Weight & Padjen, 1973*a*, *b*; Jan *et al.* 1979). The muscarinic inhibitory post-synaptic potential (i.p.s.p.) is found in cells innervated by spinal nerves 7 and 8, and the muscarinic excitatory e.p.s.p. is found in cells innervated by spinal nerves 4–6. In the course of analysing the muscarinic i.p.s.p., which occurs primarily in the smaller ganglion cells (Horn & Dodd, 1981), we found that i.p.s.p.s. were not always present in cells with slowly conducting axons. We therefore decided to re-examine the relations between innervation, axon conduction velocity, and cell size. The results show that, in addition to the previously described B and C cells, bullfrog sympathetic ganglia contain a third major population of cells: B cells with slowly conducting axons. The implications for the analysis of slow potentials are discussed.

METHODS

Preparation. Fourteen 4–6 inch bullfrogs (*R. catesbiana*) of both sexes were used for these experiments. After decapitation and pithing, animals were eviscerated except for the kidneys. Under a dissecting microscope the kidneys were then removed so that the connective tissue sheet, which

embeds the lumbar paravertebral sympathetic chain and the spinal nerves, remained intact. In order to isolate the chain, the dorsal aorta lying over the urostyle was bisected longitudinally, the sciatic nerve was dissected free of the pelvis, spinal nerves 6–10 were cut just distal to the dorsal root ganglia, and the sympathetic chain was cut just posterior to ganglion 6. Then the sheet of connective tissue containing the isolated caudal sympathetic chain was teased away from the dorsal wall of the body cavity to which it adheres. The preparation was then pinned out in a dish filled with a Ringer solution composed of (mM): 115, NaCl; 2, KCl; 1·8, $CaCl_2$ and 1, HEPES(*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) with pH adjusted to 7·2. It was then possible to remove several layers of connective tissue overlying ganglia 9 and 10 and to clean the cut ends of the sciatic nerve, of spinal nerves 7 and 8 and of the sympathetic chain rostral to ganglion 7. The

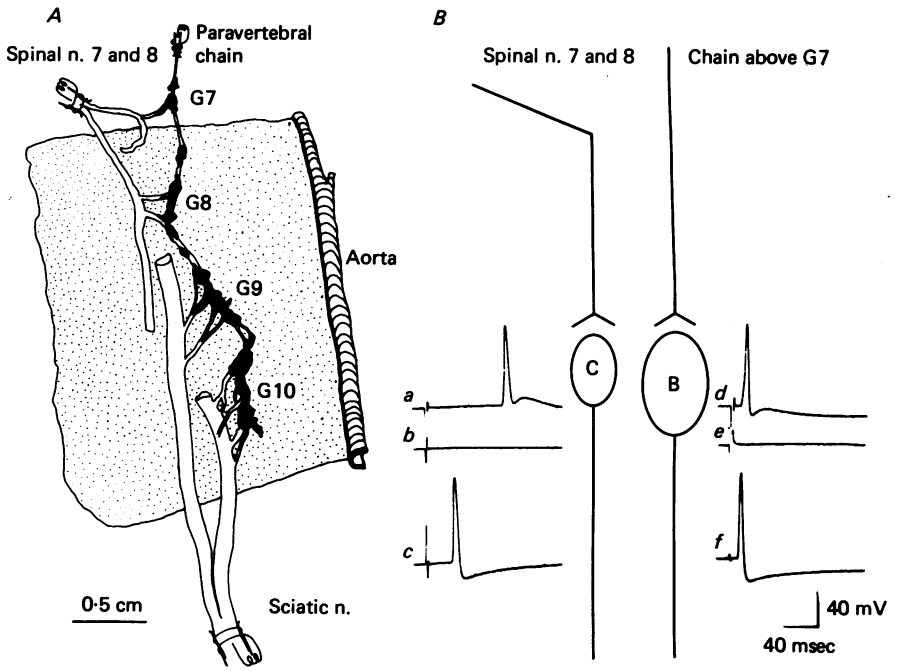

Fig. 1. Organization of the lumbar paravertebral chain ganglia. *A*, a camera lucida drawing of the preparation used in these experiments. It contains sympathetic ganglia 7–10, and spinal nerves 7–10. The connective tissue sheet embedding the ganglia was trimmed so that it provided a convenient means for handling and pinning out the preparation. The spinal end of nerves 7 and 8, the chain above ganglion 7 and the sciatic nerve were each fitted with suction electrodes thus permitting ortho- and antidromic stimulation of neurones in ganglia 9 and 10. *B*, a schematic representation of the anatomical separation of synaptic inputs to B and C neurones. *a–c*, intracellular responses of a C cell to stimulation of *a*: nerves 7 and 8, *b*: the chain above ganglion 7, and *c*: the sciatic nerve. *d–f*, intracellular responses of a B cell to stimulation of *d*: the chain above 7, *e*: nerves 7 and 8, and *f*: the sciatic nerve. Note the different latencies of the B and C responses (see text). The contribution of the fast nicotinic e.p.s.p.'s can be seen by comparing orthodromic with antidromic responses.

small eleventh spinal nerve, which sometimes exits from the urostyle but in other animals is absent, was not included in our preparations. The preparation, whose appearance is shown in Fig. 1 *A*, was transferred to a recording chamber (of 2 ml. volume) having a base made from a glass coverslip coated with a thin layer of Sylgard. The preparation was pinned so that ganglia 9 and 10 were spread flat for better visualization, and the cleaned nerves were fitted with stimulating suction electrodes (Fig. 1 *A*). For recording the chamber containing the preparation was moved to the fixed stage of

a compound microscope fitted with differential interference contrast optics (Zeiss 40× water immersion 'Nomarski' objective; total magnification = 400×). The temperature in the bath ranged from 21 to 26 °C.

Intracellular recording and nerve stimulation. Micro-electrodes were made from 1·0 or 1·2 mm o.d. thick wall glass ('Kwik-fil', WP Instruments) on a Narishige PN3 horizontal puller. When filled with 3 M-KCl, the electrodes had resistances of 70–150 MΩ. A standard commercial electrode amplifier was used. Nerves were stimulated with isolated 0·2–0·7 msec voltage pulses. Pulse polarity was chosen to give the minimum conduction delay.

Extracellular recording. The nerve of interest was sucked into a tight-fitting polyethylene tube containing a chlorided silver wire. A similar electrode filled with Ringer solution was the bath reference. The two leads were fed into a low-gain differential amplifier which had an input impedance of 10^{10} Ω (constructed by Mr R. B. Bosler).

Definition of cell parameters. Only neurones on the surface of the preparation were impaled. Before recording from a neurone, soma size was determined by measuring its major and minor axes (*A* and *B*) with an ocular micrometer. Since most neurones are ellipsoid in shape the radius of a circle of equal area would be equal to half the geometric mean of the axes.

$$Radius = 0{\cdot}5 \times (A \times B)^{0{\cdot}5}$$

After impalement, each cell was stimulated orthodromically and then antidromically. Although most neurones in this preparation receive only one suprathreshold input, in some cells there are additional subthreshold inputs (see Fig. 1 Dodd & Horn, 1983). Because the sub- and suprathreshold synaptic potentials were similar in their origins and latencies, the subthreshold inputs were not analysed. Only cells characterized in terms of size, innervation and antidromic action potential were included in the data.

Ortho- and antidromic conduction latencies were measured as the time between the end of a nerve stimulus and the start of an intracellular response. The approximate location of the cell was noted, and after the experiment the preparation was laid out so that the length of the pre- and postganglionic nerves could be measured. Conduction velocity was calculated as latency divided by nerve length. Synaptic delay was not subtracted from orthodromic latencies. Assuming a delay of 1 msec (Ginsborg, 1971), our estimates of the fastest and the slowest orthodromic conduction velocities would be low by about 20% and 3% respectively. This error would not alter the interpretation of the data.

RESULTS

Microscopy of living preparations

The gross morphology of the sympathetic chain varies from one animal to the next. In some cases the ganglia are ovoid and well defined, there being one ganglion associated with each spinal nerve. At the other extreme, ganglia are broken into diffuse islands which, in places, form a planar sheet of cells. A typical case of ganglionic fragmentation is illustrated in Fig. 1*A*. In the more diffusely organized ganglia, it was easier to dissect away the connective tissue, to distinguish individual neurones and to record systematically from whole fields of cells (Pls. 1 and 2, Fig. 2).

Bullfrog sympathetic ganglia are composed primarily of neurones, glia and small intensely fluorescent (s.i.f.) cells. In living preparations, viewed under 'Nomarski' optics, it is possible to distinguish between neurones and s.i.f. cells. The neurones have a simple monopolar shape with a large nucleus and well defined nucleoli (Pls. 1 and 2). Their somata range from 15 to 70 μm in diameter. Sometimes the preganglionic axon can be seen coiled around the initial axon segment (Pl. 2*B*). Other workers have shown that synaptic boutons are located on the cell bodies and initial axon segments of these cells (Taxi, 1976; Weitsen & Weight, 1977; Marshall, 1981). The s.i.f. cells are always small (10–20 μm) and can be identified by their granular nuclei which are surrounded by relatively little cytoplasm (Pl. 2*C*; also see Weight & Weitsen, 1977). Such cells were generally in sparsely distributed clusters which had the appearance

of being surrounded by a connective tissue sheath. The number of s.i.f. cell clusters varied greatly from one preparation to the next. In fact, in some preparations, s.i.f. cells were located outside ganglia, in the sheet of connective tissue containing the paravertebral chain.

Intracellular recordings

A sample of 188 neurones was characterized by size and physiological properties (see Methods). We did not record from s.i.f. cells. The neurones were identified as type B or C according to the origins of their nicotinic cholinergic input: B cells are innervated by the chain anterior to ganglion 7, and C cells are innervated by spinal nerves 7 and 8 (Fig. 1*B*, also see Tosaka *et al.* 1968). We did not observe any cells innervated by both the chain above 7 and nerves 7 and 8.

Size and distribution of identified neurones. There was a tendency for ganglionic neurones to segregate by size. Plate 1*A* shows a field containing many small neurones and several larger neurones. The latter are confined to the left side of the picture. Plate 1*B* is an example of a field with many large cells and only three or four small cells amongst them. After identifying neighbouring cells as type B or C it became apparent that they also tend to be distributed in clusters. Fig. 2 illustrates two fields of neurones that were systematically impaled and identified. In the upper example, which comes from the edge of a ganglion, there are open spaces that were filled with nerve fibres and a few cells that were too small to impale. The lower example was taken from an area packed with somata. Although Fig. 2 contains exceptions, results from this kind of experiment suggest that C cells are smaller than B cells and that clustering by size may correlate with clustering by type.

We were not able to distinguish B cells from C cells on the basis of their microscopic features (Pl. 2). However C cells, as a group, are smaller than B cells (Table 1). Although the size histograms of B and C cells (Fig. 3) do overlap, 96% of all cells with a radius less than 16 μm were C cells and 94% of all cells with a radius greater than 21 μm were B cells. More important, three quarters of the sample fell into either the 'radius < 16 μm C cell' category (24%) or the 'radius > 21 μm B cell' category (51%). Therefore, using the cues of cell size and clustering, it is possible to impale selectively either B or C cells on a routine basis.

Presynaptic conduction velocities. As noted earlier (Fig. 1 and see Skok, 1965; Tosaka *et al.* 1968), preganglionic fibres innervating C cells conduct at a slower rate (around 0·4 m/sec) than those innervating B cells (around 2·4 m/sec). Our data confirm this observation (Table 1). There was virtually no overlap between the distribution of values in the B and C groups (Fig. 4). This means that orthodromic conduction velocity indicates whether a fibre originates from nerves 7 and 8 or from the chain above ganglion 7.

Antidromic conduction velocities. It has been generally accepted that antidromic conduction velocity is a good criterion for identifying B and C cells (see Introduction). However, this was not true for a large percentage of the cells in our sample. An example is illustrated in Fig. 5 in which three adjacent cells were studied. On the left of the Figure is a tracing of the three cell bodies and to the right are the intracellular records of orthodromic and antidromic responses. Cells 1 and 2 conform to the well known scheme (Fig. 1*B*) of slowly conducting fibres from nerves 7 and 8 innervating

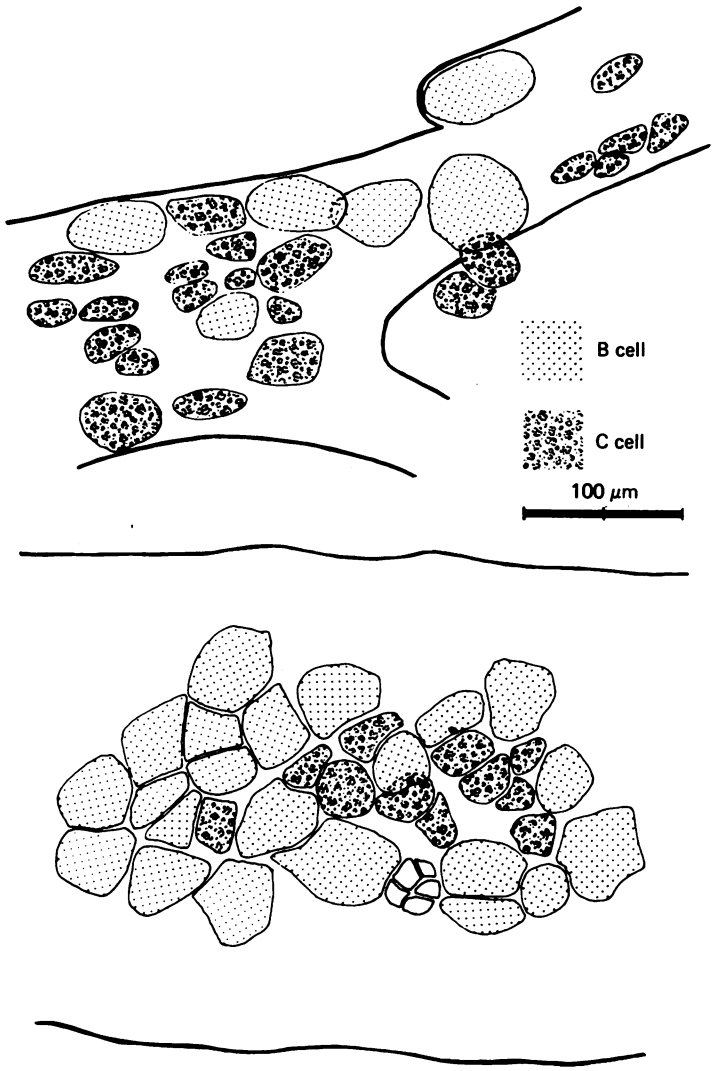

Fig. 2. The distribution of B and C cells in two ganglia in which neighbouring cells were systematically impaled and identified. The drawings were made by photographing the preparation during the experiment and then tracing the outlines of cell bodies. The loose packing of cells in the upper example was due to the presence of numerous nerve fibres and some cells which were too small to impale. In the lower field, a group of tightly packed cells was studied. The cluster of five small cells at the bottom were s.i.f. cells. The open space above the s.i.f. cells contained a blood vessel and several small neurones. The area surrounding the identified neurones contained additional neurones that were tightly packed.

C cells with slowly conducting axons, and of rapidly conducting fibres from the chain above ganglion 7 innervating B cells with rapidly conducting axons. Cell 3 was innervated by a rapidly conducting input from the chain above ganglion 7 and thus was a B cell. However, it had a slowly conducting antidromic action potential. We propose referring to such cells as slow B cells, and to B cells with rapidly conducting axons as fast B cells. The converse of a slow B cell, namely a C cell with a rapidly conducting axon, has not been observed.

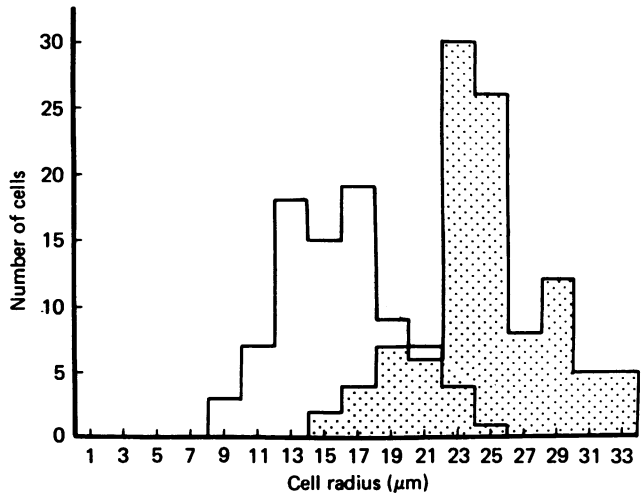

Fig. 3. Size histograms of B neurones (stippled) and C neurones (open). After measuring cell radii with an ocular micrometer (Methods), each cell was physiologically identified by the origin of its innervation (text).

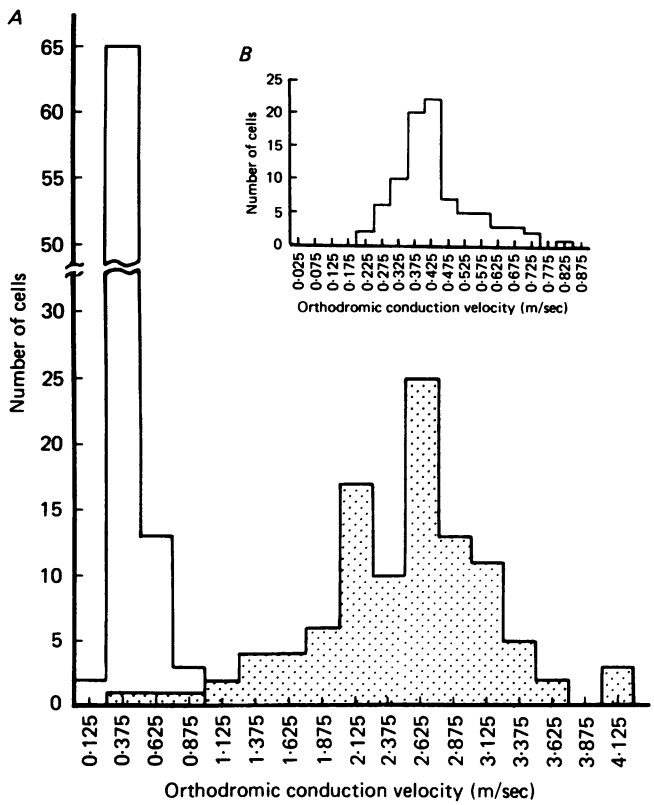

Fig. 4. Conduction velocity histograms of single presynaptic fibres supplied by the chain above ganglion 7 (stippled) and spinal nerves 7 and 8 (open). The inset *B* is an expanded histogram of the nerve 7 and 8 data in *A*. There is virtually no overlap between the two groups of cells, each of which are innervated by axons having symmetrically distributed conduction velocities.

It seems unlikely that the difference between the antidromic latencies of cells 2 and 3 (Fig. 5) arose from unequal conduction distances. Although the cells were adjacent, it might be argued that their axons were of different lengths, for example because one made a loop (see Lichtman, 1977; Purves & Hume, 1981) before entering the sciatic nerve. However, if we assume that cell 3 had an axonal conduction velocity of 2 m/sec, then its axon would have been 46 mm longer than that of cell 2. This seems implausible since the entire preparation is only about 5 cm long (Fig. 1*A*) and because looping in autonomic neurones occurs on a much smaller scale (Lichtman, 1977; Purves & Hume,1981).

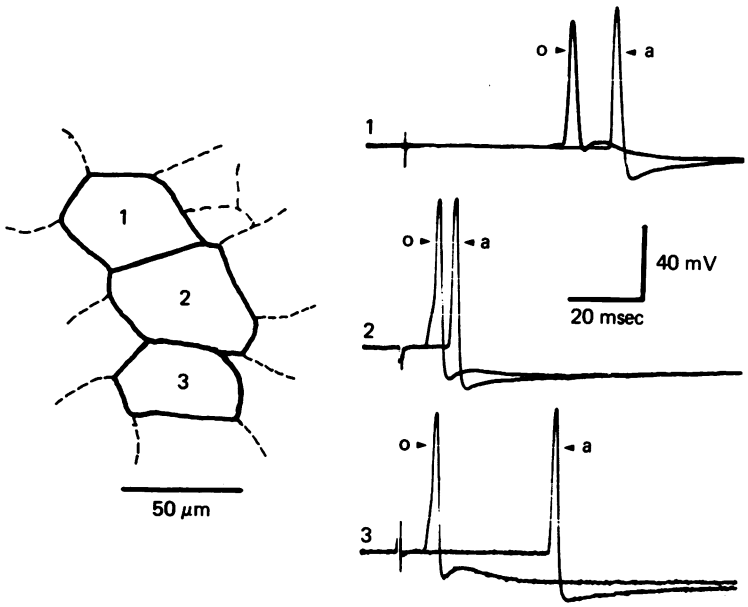

Fig. 5. The three neuronal cell types in the ninth and tenth ganglia. In this example, the adjacent cells were photographed and then identified. On the left are the outlines of the three somata. On the right are the intracellular records of orthodromic (o) and antidromic (a) responses of each cell. Cell 1 was innervated by nerves 7 and 8. Cells 2 and 3 were each innervated by the chain above ganglion 7. The cells are of the C, fast B and slow B types (see text for explanation).

The distribution of antidromic conduction velocities (Fig. 6) shows that all C cells had slow conduction velocities ($< 0{\cdot}75$ m/sec). By contrast the B cell velocities were widely distributed, with a large number in the C range. We interpret the skew in the B cell data as representative of two classes of cells: slow B cells and fast B cells. The suggestion that B cell axon conduction velocities are bimodally distributed was later confirmed by extracellular measurements (see below). In order to compare the two types of B cells, the intracellular data were divided into a slow group with conduction velocities < 1 m/sec and a fast group with conduction velocities > 1 m/sec. Using this criterion, 59 % of our sample of 105 B cells were slow B cells. The grouped data, presented in Table 1, show that fast and slow B cells cannot be distinguished on the basis of size or orthodromic conduction velocity. The distribution of these data describing slow B and fast B cells also were indistinguishable (not shown).

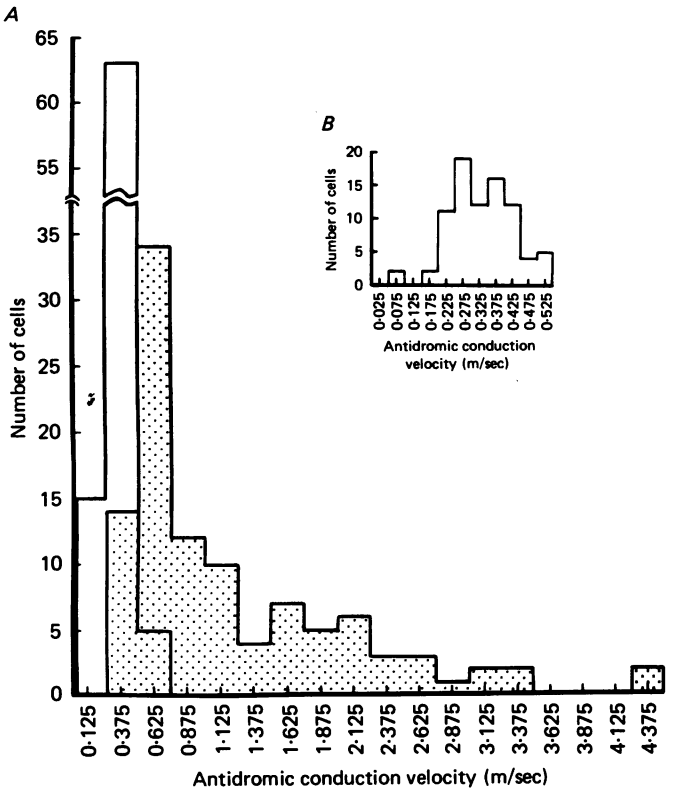

Fig. 6. Antidromic conduction velocity histograms of individual neurones innervated by the chain above 7 (stippled) and nerves 7 and 8 (open). The inset *B* is an expanded histogram of the data for cells innervated by nerves 7 and 8. Note the proximity of the groups of B cells (stippled) and C cells (open). The marked asymmetry of the B cell data is presumably due to the fact that it includes two distinct populations: slow B and fast B cells. See text for details.

TABLE 1. Characteristics of intracellularly identified sympathetic neurones (mean ±S.D.). B and C neurones were classified according to the segmental origin of their innervation. Fast and slow B cells were defined as those B cells with antidromic conduction velocities that were greater than, and less than, 1m/sec, respectively

Neuronal type	*n*	Antidromic conduction velocity (m/sec)	Orthodromic conduction velocity (m/sec)	Cell radius (μm)
All B cells	105	1·18±0·87	2·44±0·67	24·6±3·9
Fast B cells	43	2·01±0·79	2·55±0·52	24·9±3·7
Slow B cells	62	0·60±0·16	2·38±0·75	24·4±4·1
C cells	83	0·32±0·09	0·41±0·11	15·6±3·4

Extracellular recordings

Three populations of neurones. We were surprised to discover slow B cells and to learn that they were so numerous in our sample of intracellularly identified cells. To circumvent any sampling bias that was introduced by intracellular recording from cells on the surface of ganglia, we recorded extracellularly from cut post-ganglionic nerves with suction electrodes in three preparations, with similar results. The data

in Figs. 7, 8 and 9 are from one preparation. Extracellular conduction velocities were calculated as the latency, from the stimulus to the peak amplitude of the compound action potential, divided by the conduction distance. When a ramus communicans between a ganglion and spinal nerve 9 or 10 was cut near the spinal nerve, and the ganglionic end drawn into a suction electrode, it was possible to record the post-synaptic response to preganglionic stimulation. This revealed two populations

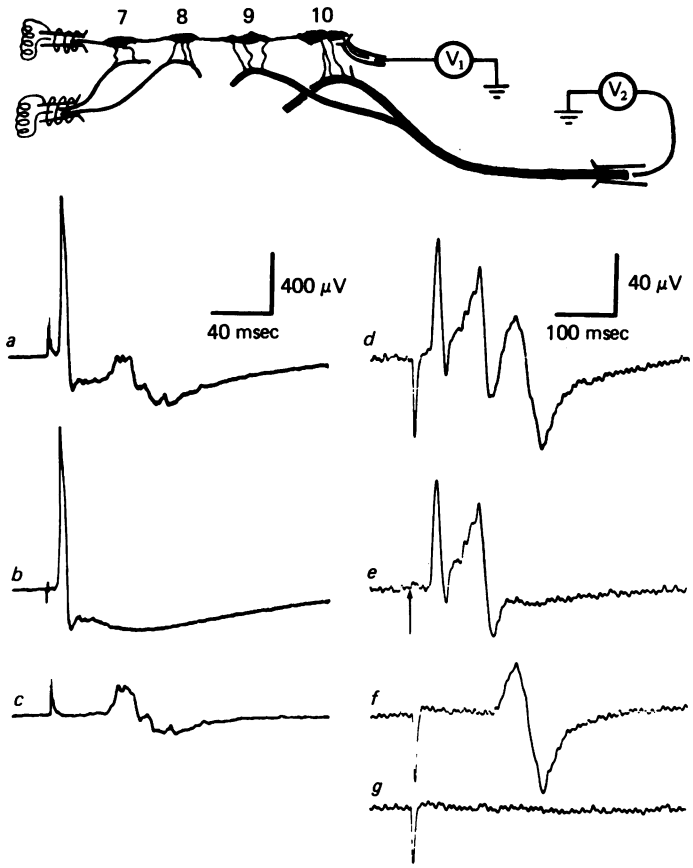

Fig. 7. Extracellular compound responses to stimulation of nerves 7 and 8 and the chain above ganglion 7. *a–c* were recorded from a post-ganglionic ramus at its point of exit from ganglion 10(V_1); *d–g* were recorded from the sciatic nerve several cm from ganglion 10(V_2). *a* and *d* are the responses to simultaneous stimulation of both presynaptic inputs. Note that in the record from the ganglion (*a*) there are two peaks, while in the sciatic nerve (*d*) the response to the same stimulus consists of three peaks. At the ganglion (V_1), the rapidly conducting response (*b*) was produced by stimulating the chain above 7, and the slowly conducting response (*c*) was produced by stimulating nerves 7 and 8. In a control experiment on the same preparation it was determined that nerve 7 and nerve 8 each contributed to the responses in records *c* and *f*. When recording from the sciatic nerve (V_2) the two faster peaks (*e*) were seen in response to stimulation of the chain above 7; they arise from fast and slow B cells respectively (see text). The slowest peak at V_2 (*f*) was the response to stimulation of nerves 7 and 8. The sciatic response (V_2) to stimulation of both preganglionic inputs, (*d*), was totally blocked by bathing the preparation in modified Ringer solution containing 0·18 mM-Ca^{2+} and 8 mM-Mg^{2+} for 20 min (*g*) and recovered after washing in normal Ringer solution for 30 min.

of ganglion cells: B cells, whose inputs conducted at about 2·75 m/sec and were activated by the chain above ganglion 7, and C cells, whose inputs conducted at about 0·36 m/sec and were activated by spinal nerves 7 and 8 (traces *a–c* in Fig. 7; also see Skok, 1965, Tosaka *et al.* 1968). This was consistent with the intracellular orthodromic data (Table 1). If B cells are divided into two populations on the basis of their axonal conduction velocities, then one would predict that at a site further down the post-ganglionic nerve the B wave would fractionate into two compound action potentials. This was indeed the case when we recorded from the sciatic nerve several centimetres from the tenth ganglion (traces *d–f* in Fig. 7). Two populations with conduction velocities of 2·2 and 0·77 m/sec were activated by the chain above ganglion 7 (Fig. 7*e*). A third population with a slower conduction velocity of 0·37 m/sec was activated by nerves 7 and 8 (Fig. 7*f*). These values are close to the intracellularly measured antidromic conducton velocities for fast B, slow B and C cells

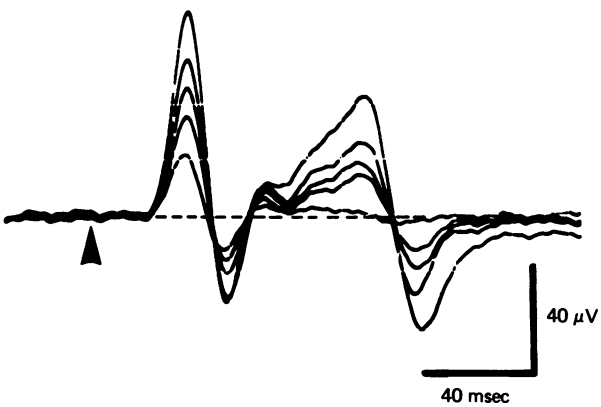

Fig. 8. Presynaptic stimulus thresholds of fast and slow B responses recorded from the sciatic nerve. This record was made in the same manner as *e* in Fig. 7. As the stimulus to the chain above ganglion 7 was increased, both the fast B and the slow B compound responses grew in a continuously graded manner. Responses to five stimuli that were increased from threshold to supramaximal are shown. Arrow denotes time of stimulation.

(Table 1). All three responses were totally and reversibly blocked by superfusing the ganglion for 20 min in modified Ringer solution containing 0·18 mM-Ca^{2+} and 10 mM-Mg^{2+} (Fig. 7*g*). This indicates that these responses were synaptic in origin and not contaminated by through-conducting axons.

Presynaptic thresholds. The fast and slow B responses recorded from the sciatic nerve could not be distinguished by their presynaptic thresholds (Fig. 8). As the stimulus intensity applied to the chain above ganglion 7 was gradually increased above threshold, both responses grew in a graded manner until they saturated. Altering the stimulus polarity and duration failed to activate selectively either of the two responses. This supports the idea that a single population of preganglionic B fibres innervates the fast and slow B neurones.

Post-synaptic thresholds. The post-ganglionic components of the fast B, slow B and C conduction velocities were examined by recording from a length of sciatic nerve isolated from ganglion cells. A ramus communicans, cut free of ganglion 19, was drawn into a suction electrode and the distal end of the sciatic nerve was stimulated. This

arrangement enabled us to record exclusively from the sympathetic axons which travel in the sciatic nerve. As seen in Fig. 9, three populations of fibres with distinct thresholds and conduction velocities travel from the ramus communicans into the sciatic nerve. As expected, the thresholds of these fibres were inversely related to their conduction velocities.

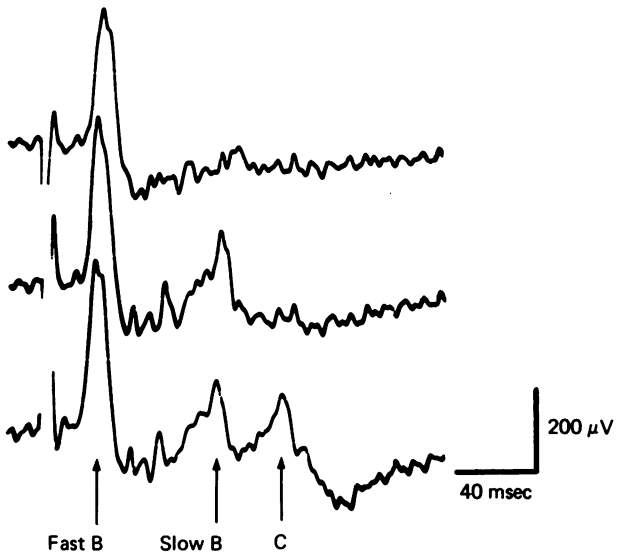

Fig. 9. Three populations of sympathetic axons travelling in the sciatic nerve. A ramus communicans connecting ganglion 10 to the tenth spinal nerve was cut and the distal end was sucked into a recording electrode. As the stimulus applied to the distal end of the sciatic nerve was increased, three populations with distinct conduction velocities were elicited. Stimulus strength was increased from the upper to the lower traces.

DISCUSSION

The major result of this study is the reclassification of B and C sympathetic neurones in the bullfrog as slow B, fast B and C neurones. C cells have a radius of about 15 μm, receive slowly conducting inputs from spinal nerves 7 and 8, and have axons that conduct at about 0·3 m/sec. B cells have a radius of about 25 μm, and receive rapidly conducting inputs from the chain above ganglion 7. B cells are subdivided into the fast and slow groups on the basis of antidromic conduction velocity. Slow B cell axons conduct at about 0·6 m/sec and fast B cell axons at about 2 m/sec. The relatively large size of the slow B response in extracellular recordings and the high percentage of slow B cells in our intracellular sample both indicate that these cells are common in this preparation. The results also indicate that antidromic conduction velocity is not a good criterion for classifying sympathetic neurones in this preparation. Although slow B cells and C cells have different axonal conduction velocities (Table 1 and Fig. 9), the distributions of their values overlap to such an extent (Fig. 6) that only fast B cells can be identified reliably on the basis of antidromic conduction velocity.

There are earlier observations in the literature that are consistent with our work on

slow B cells. In their study of sympathetic effects mediated by ganglia 9 and 10 in the frog, Hutter & Loewenstein (1955) observed that stimulating the sympathetic chain at ganglion 8 activates three populations of fibres in the sciatic nerve. They also found that these three populations have only two thresholds. At lower stimulus strengths, fibres conducting at 5 m/sec and 0·6 m/sec were activated simultaneously. As the stimulus was strengthened, a third population conducting at 0·2 m/sec was recruited (see their Fig. 2). These populations, which they labelled as B, C1 and C2, correspond to the axons which we have ascribed to fast B, slow B and C cells. D. H. Feldman (personal communication) has recently obtained similar results with intracellular recording in the frog.

Similarly, Nishi *et al.* (1965) found that sympathetic axons in the toad sciatic nerve have three distinct conduction velocities with values resembling our measurements and those of Hutter & Loewenstein (1955). Nishi *et al.* labelled the populations as B, C1 and C2 (see their Fig. 2). They classified neurones on the basis of antidromic conduction velocity and observed a strong correlation between the orthodromic and antidromic conduction velocities in both B and C cells. Their suggestion that C1 and C2 axons both belong to C cells is in contrast to our finding in the bullfrog where C1 axons belong to slow B cells and C2 axons belong to C cells. Furthermore, within each of the three neuronal types that we identified there was no significant correlation between pre- and post-ganglionic conduction velocities. In view of our findings in the bullfrog, the organization of toad ganglia should be re-examined.

There is little information on the size of physiologically identified sympathetic neurones in Amphibia. In the toad, Nishi *et al.* (1965) measured ten cells which had been classified on the basis of antidromic conduction velocity. Honma (1970) measured twenty-six B cells and sixteen C cells, also in the toad, which were identified by orthodromic or antidromic conduction velocity or both. In both studies B cells tended to be larger than C cells. These results should now be considered in the light of the difficulties associated with classifying cells on the basis of antidromic conduction velocity. Our data show that, in the bullfrog, B cells as a group are larger than C cells, and that at the extremes of the size distribution cells can be identified on the basis of size. However, note that fast B cells cannot be distinguished from slow B cells on the basis of size.

Previously it has been reported that the muscarinic i.p.s.p. occurs in C cells and not in B cells (Tosaka *et al.* 1968; Libet *et al.* 1968; Horn & Dodd, 1981). Our reclassification of B cells into fast and slow groups does not alter this characterization of the i.p.s.p. However, the discovery of slow B cells helps to explain why it has been difficult to record the i.p.s.p. with intracellular electrodes (see Introduction). Such experiments are often done in the presence of drugs which block nicotinic e.p.s.p.s. This makes it impossible to identify a cell by its innervation, unless there is a residual e.p.s.p. (for example, see Fig. 3*d* in Tosaka *et al.* 1968 or Fig. 3 in Dodd & Horn, 1983) or a muscarinic p.s.p. Consequently some workers have used antidromic conduction velocity as the criterion for cell identification (Weight & Padjen, 1973*a*). In our early experiments we found many putative C cells that had no i.p.s.p. and showed no signs of nicotinic innervation from nerves 7 and 8. The possibility now arises that they were slow B cells.

This study has other implications. In B cells one can record a slow muscarinic

e.p.s.p. and a very slow peptidergic e.p.s.p. (Jan *et al.* 1979). These two responses share a similar voltage sensitivity which varies from cell to cell (Kuba & Koketsu, 1976; Kuffler, 1980). It would be interesting to learn whether this variability in the behaviour of slow e.p.s.p.s. is related to the two classes of B cell. More generally, the evidence clearly shows a high degree of specificity in the connexions and properties of bullfrog sympathetic neurones. The functional significance of this organization and the mechanisms which control its formation and maintenance await future study.

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REFERENCES

Bishop, G. H. & Heinbecker, P. (1930). Differentiation of axon types in visceral nerves by means of the potential record. *Am. J. Physiol.* **94**, 170–200.

Bishop, G. H. & O'Leary, J. (1938). Pathways through the sympathetic nervous system in the bullfrog. *J. Neurophysiol.* **1**, 442–454.

Dodd, J. & Horn, J. P. (1983). Muscarinic inhibition of sympathetic C neurones in the bullfrog. *J. Physiol.* **334**, 271–291.

Erlanger, J. & Gasser, H. S. (1930). The action potential in fibers of slow conduction in spinal roots and somatic nerves. *Am. J. Physiol.* **92**, 43–82.

Feldman, D. H. (1979). Specificity of reinnervation of frog sympathetic ganglia. *Neurosci. Abstr.* **5**, 625.

Feldman, D. H. (1980). Synaptic competition and sprouting during reinnervation of frog sympathetic ganglia. *Neurosci Abstr.* **6**, 110.

Ginsborg, B. L. (1971). On the presynaptic acetylcholine receptors in sympathetic ganglia of the frog. *J. Physiol.* **216**, 237–246.

Honma, S. (1970). Functional differentiation in sB and sC neurons of toad sympathetic ganglia. *Jap. J. Physiol.* **20**, 281–295.

Horn, J. P. & Dodd, J. (1981). Monosynaptic muscarinic activation of K^+ conductance underlies the slow inhibitory postsynaptic potential in sympathetic ganglia. *Nature, Lond.* **292**, 625–627.

Hutter, O. F. & Loewenstein, W. R. (1955). Nature of neuromuscular facilitation by sympathetic stimulation in the frog. *J. Physiol.* **130**, 559–571.

Jan, L. Y., Jan, Y. N. & Brownfield, M. S. (1980). Peptidergic transmitters in synaptic boutons of sympathetic ganglia. *Nature, Lond.* **288**, 380–382.

Jan, Y. N., Jan, L. Y. & Kuffler, S. W. (1979). A peptide as a possible transmitter in sympathetic ganglia of the frog. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1501–1505.

Kuba, K. & Koketsu, K. (1976). Analysis of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cells. *Jap. J. Physiol.* **26**, 651–669.

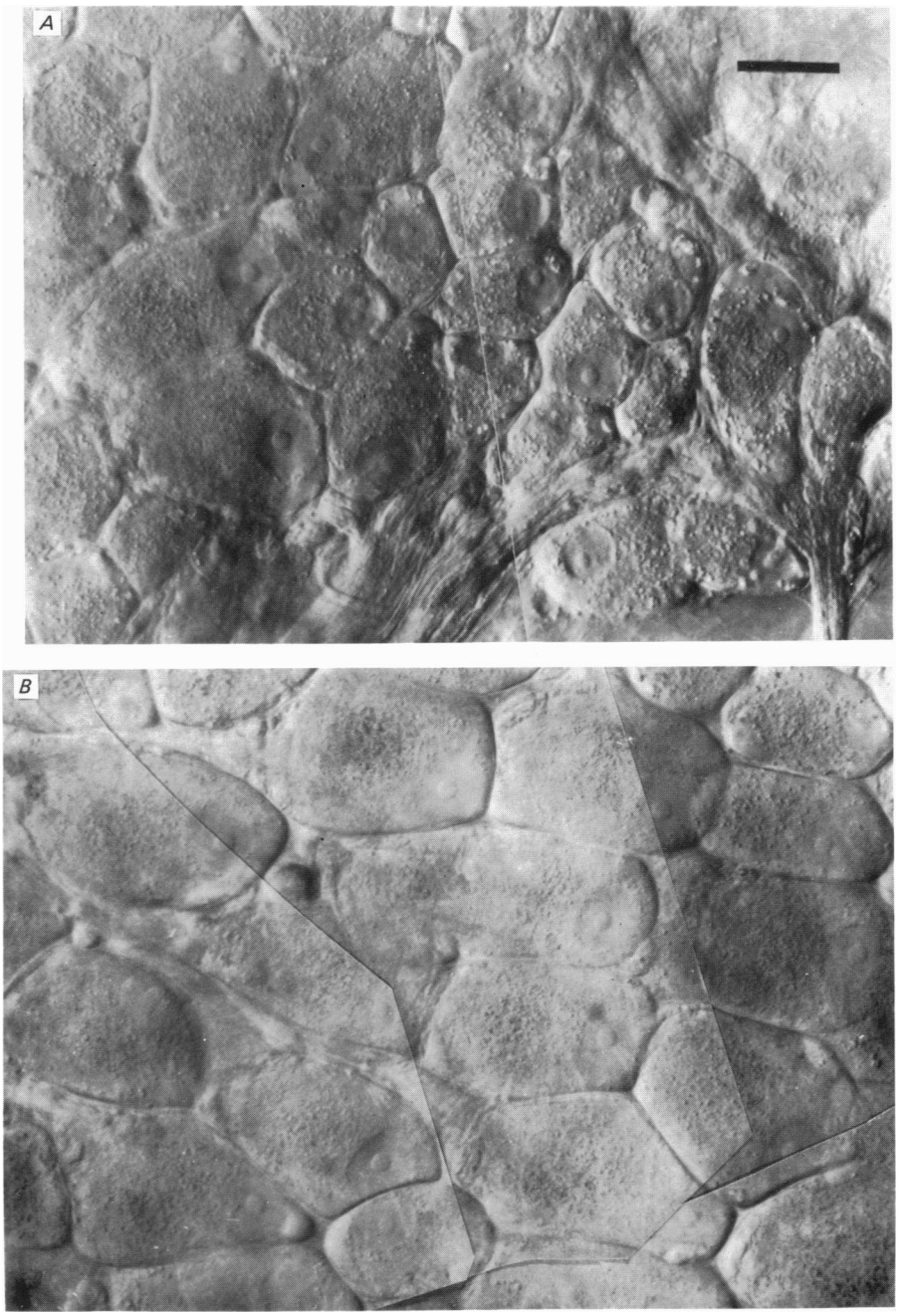
Kuffler, S. W. (1980). Slow synaptic responses in autonomic ganglia and the pursuit of a peptidergic transmitter. *J. exp. Biol.* **69**, 257–286.

Libet, B., Chichibu, S. & Tosaka, T. (1968). Slow synaptic responses and excitability in sympathetic ganglia of the bullfrog. *J. Neurophysiol.* **31**, 383–395.

Lichtman, J. W. (1977). The reorganization of synaptic connexions in the rat submandibular ganglion during post-natal development. *J. Physiol.* **273**, 155–177.

MacDermott, A. B., Connor, E. A., Dionne, V. E. & Parsons, R. L. (1980). Voltage clamp study of fast excitatory synaptic currents in bullfrog sympathetic ganglion cells. *J. gen. Physiol.* **75**, 39–60.

Marshall, L. M. (1981). Synaptic localization of alpha-bungarotoxin binding which blocks nicotinic transmission at frog sympathetic neurons. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1948–1952.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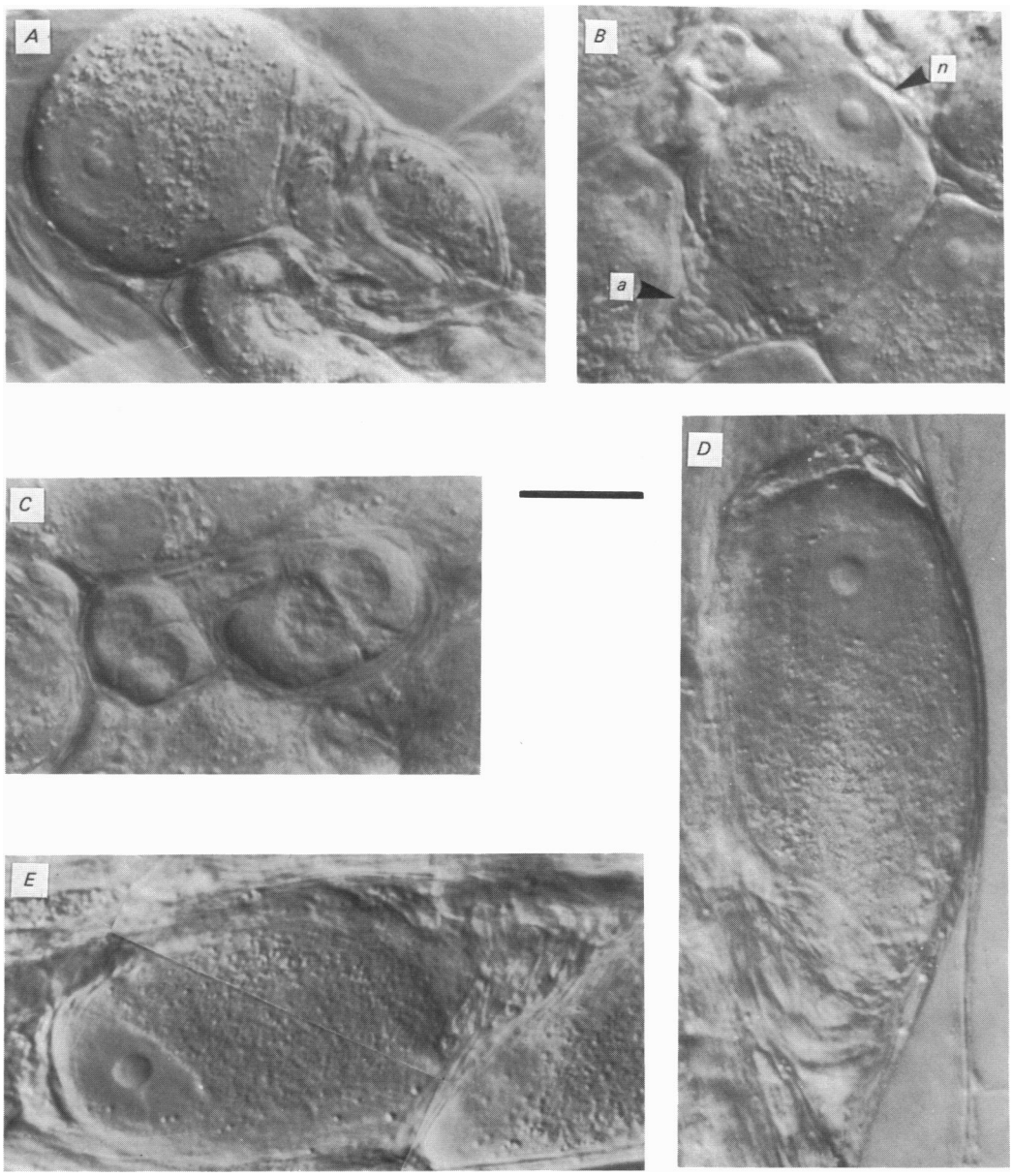
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NISHI, S., SOEDA, H. & KOKETSU, K. (1965). Studies on sympathetic B and C neurons and patterns of preganglionic innervation. *J. cell. comp. Physiol.* **66**, 19–32.

PURVES, D. & HUME, R. I. (1981). The relation of postsynaptic geometry to the number of presynaptic axons that innervate autonomic ganglion cells. *J. Neurosci.* **1**, 441–452.

SKOK, V. I. (1965). Conduction in tenth ganglion of the frog sympathetic trunk. *Fedn Proc. Translation Suppl.* **24**, T363–367.

TAXI, J. (1976). Morphology of the autonomic nervous system. In *Frog Neurobiology*, ed. LLINAS, R. & PRECHT, W., pp. 93–150. Berlin: Springer-Verlag.

TOSAKA, T., CHICHIBU, S. & LIBET, B. (1968). Intracellular analysis of slow inhibitory and excitatory postsynaptic potentials in sympathetic ganglia of the frog. *J. Neurophysiol.* **31**, 396–409.

WEIGHT, F. F. & PADJEN, A. (1973*a*). Slow synaptic inhibition: evidence for synaptic inactivation of sodium conductance in sympathetic ganglion cells. *Brain Res.* **55**, 219–224.

WEIGHT, F. F. & PADJEN, A. (1973*b*). Acetylcholine and slow synaptic inhibition in frog sympathetic ganglion cells. *Brain Res.* **55**, 225–228.

WEIGHT, F. F. & WEITSEN, H. A. (1977). Identification of small intensely fluorescent (SIF) cells as chromaffin cells in bullfrog sympathetic ganglia. *Brain Res.* **128**, 213–226.

WEITSEN, H. A. & WEIGHT, F. F. (1977). Synaptic innervation of sympathetic ganglion cells in the bullfrog. *Brain Res.* **128**, 197–211.

EXPLANATION OF PLATES

PLATE 1

Photomicrographs of living ganglion cells. Bar, 30 μm. *A* is a field of cells in which there were many small neurones and only a few large neurones. The larger cells are mainly towards the left of the picture. By contrast, *B* is a field that contains mainly large cells. In addition to the well defined outlines of neuronal somata, some cellular details such as nuclei and axons can be seen. At the bottom of *A* are two nerve bundles leaving the ganglion. Physiological experiments were done under similar optical conditions.

PLATE 2

Photomicrographs of identified cells. Bar, 20 μm. *A* and *B* are C neurones. In B the preganglionic axon (a) can be seen clearly as it coils around the axon hillock region of the cell. The nucleus (n) with its associated nucleolus is also easy to see in this and other neurones in the Plate. *C* is a cluster of four s.i.f. cells that can be recognized by their characteristically invaginated nuclei and small somata. *D* and *E* are B neurones whose microscopic features are indistinguishable from those of C neurones.