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COMPETITIVE AND NON-COMPETITIVE RE-INNERVATION OF MAMMALIAN SYMPATHETIC NEURONES BY NATIVE AND FOREIGN FIBRES

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SUMMARY

The ability of native (sympathetic preganglionic) and foreign (vagal) nerve fibres to re-innervate neurones of the guinea-pig superior cervical ganglion, either alone or in competition with each other, has been studied by means of intracellular recording and electron microscopy.

1. Native fibres make synaptic contacts with nearly all ganglion cells within one month of cervical trunk section; within 6 months the degree of innervation, judged by measurement of excitatory post-synaptic potential (e.p.s.p.) amplitude and electron microscopical synapse counts, approaches normal. However, even after 15 months innervation was weaker than in normal control ganglia.

2. Vagal fibres are less successful during re-innervation. Although a similar number of foreign fibres grow into denervated ganglia and make contact with nearly all ganglion cells within a month, after 6–12 months e.p.s.p. amplitudes in response to foreign nerve stimulation remain relatively small, and counts of synapses are only about 60% as great as in ganglia re-innervated with the native nerve.

3. When both native and foreign fibres are allowed to re-innervate ganglion cells simultaneously, about half the neurones in the ganglion receive synapses from both sources after 1 month. The proportion of dually innervated cells remains roughly constant for at least 14 months. Neither set of preganglionic fibres dominates or displaces the other, although neurones generally are re-innervated more effectively by native than foreign fibres, as is true during non-competitive re-innervation.

4. Thus during re-innervation of mammalian sympathetic neurones native fibres are preferred to foreign ones only in the sense that roughly

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the same number of native fibres form many more synapses on ganglion cells than do vagal axons. A foreign synapse, once formed, is as stable as a native one, and shows no tendency to be replaced by native terminals. These findings are discussed in relation to other evidence which has suggested specificity and selectivity during re-innervation of mammalian autonomic neurones.

INTRODUCTION

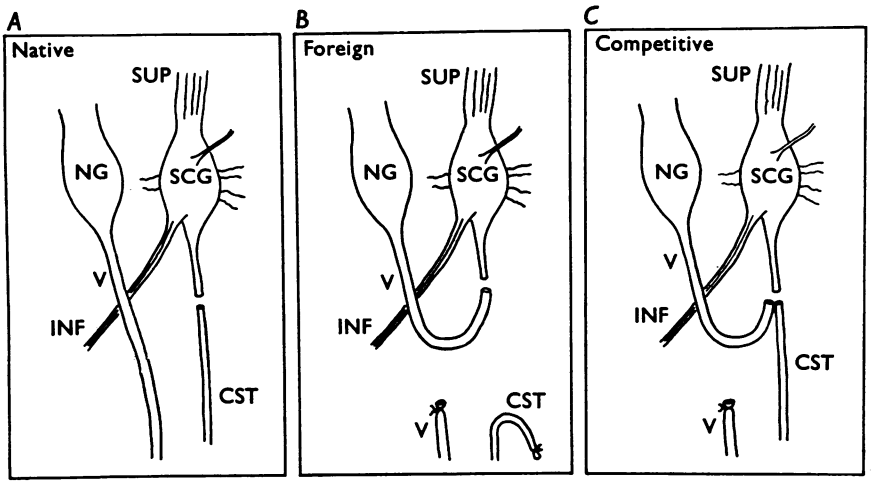
Denervated mammalian sympathetic ganglion cells can be re-innervated by a variety of foreign sources. Thus neurones of the superior cervical ganglion have been re-innervated with axons of the gastric vagus nerve (Langley, 1898; Guth, 1956; Cecarelli, Clementi & Mantegazza, 1971; Ostberg, Raisman, Field, Iversen & Zigmond, 1976), the afferent vagus proximal to the nodose ganglion (De Castro 1942, 1951; Matsumura & Koelle, 1961), and several different somatic motor nerves (Langley & Anderson, 1904*a*, *b*; De Castro, 1935; Hillarp, 1946; McLachlan, 1974; Ostberg *et al.* 1976). However, if these same neurones are re-innervated by sympathetic preganglionic fibres the process is specific, in the sense that particular groups of ganglion cells show a predilection for preganglionic fibres from particular segmental levels of the spinal cord (Langley, 1892, 1895, 1897; see also Landmesser & Pilar, 1970). Indeed, if preganglionic fibres from 'incorrect' spinal levels sprout to contact ganglion cells after partial denervation (Murray & Thompson, 1957; Guth & Bernstein, 1961), it appears that the arrival of the 'correct' preganglionic fibres can displace (or repress) the incorrect ones (Guth & Bernstein, 1961). This latter phenomenon is usually referred to as selectivity. Thus these nerve cells apparently have the ability to accept synaptic contacts from grossly inappropriate sources, and yet to select correctly between competing fibres whose only apparent difference is the level of the spinal cord at which the parent (preganglionic) neurones reside. The purpose of the present work was to re-investigate some aspects of these paradoxical properties with intracellular recording methods and electron microscopy.

The experiments reported here show that individual neurones of the denervated guinea-pig superior cervical ganglion (SCG) are more effectively re-innervated by native than foreign (vagal) fibres. In spite of this difference, when both native and foreign fibres re-innervate ganglion cells simultaneously, the majority of neurones accept synaptic contacts from both sources and remain dually innervated for at least 14 months. Thus although foreign fibres are less successful during re-innervation of ganglion cells, they are not at any disadvantage when competing with native fibres. Explanations that might account both for these results and those of

previous experiments demonstrating selectivity during re-innervation of mammalian ganglion cells (Guth & Bernstein, 1961) are considered. Some of these findings have been briefly reported (Purves, 1975*a*).

METHODS

Adult albino guinea-pigs (200–400 g) were anaesthetized with pentobarbitone (30–40 mg/kg I.P.). The right superior cervical ganglion was exposed under aseptic conditions and the cervical sympathetic trunk (CST) cut 2–4 mm below the caudal pole of the ganglion. Following section of the CST, the cut ends retracted 1–2 mm and no effort was made to approximate them. In a group of fifteen animals (native re-innervation) (Text-fig. 1*A*) nothing further was done, and the wound was closed.



Text-fig 1. Diagram of operative procedures. *A*, native re-innervation; *B*, foreign re-innervation; *C*, competitive re-innervation. SCG, superior cervical ganglion; CST, cervical sympathetic trunk; V, vagus nerve; NG, nodose ganglion; SUP and INF, superior and inferior post-ganglionic branches. Drawings are not strictly to scale.

In a second group of eight animals (foreign re-innervation) about a centimetre of the CST proximal to the point of section was resected and the stump ligated and turned back into the base of the neck. The vagus nerve was then transected 8–12 mm caudal to the nodose ganglion, and the proximal stump was freed and apposed to the cut end of the CST (Text-fig. 1*B*); the gap between the cut ends was similar to that after CST transection and no special effort was made to ensure nerve union. The distal stump of the vagus nerve was ligated. In a third group of twenty-two animals both the proximal end of the CST and the proximal end of the vagus nerve were placed in apposition to the stump of the distal CST so that native and foreign fibres could grow into the ganglion simultaneously (Text-fig. 1*C*). After intervals ranging from 1 to 15 months the animals were killed and re-innervation of ganglion cells was studied by means of intracellular recording and electron microscopy. The methods were the same as those used previously (Purves, 1975*b*), and

only a general outline of the procedures and a few points of difference are presented here.

Isolated ganglia were pinned out in a bath perfused with oxygenated Ringer fluid at room temperature, and the preganglionic nerves (either the CST, vagus nerve, or both) were taken into close fitting suction electrodes for stimulation and recording. Preganglionic nerves were usually stimulated just caudal to the neuroma which formed at the site of nerve anastomosis (Text-fig. 1). In most experiments the distance from the stimulating suction electrode to the ganglion was 4–10 mm. One or both of the major post-ganglionic nerves (the superior and inferior post-ganglionic branches – see Text-fig. 1) were also taken into suction electrodes for recording and stimulation. Neurones on the dorsal surface of the ganglion were impaled with glass micropipettes. As in previous studies, only neurones capable of giving action potentials of 60 mV or more, in response to depolarizing current injected through the recording electrode, were considered sufficiently undamaged for inclusion in the results.

The degree of re-innervation of particular neurones was determined by measuring the amplitude of the excitatory post-synaptic potential (e.p.s.p.) elicited by supramaximal stimulation of either the native or the foreign preganglionic nerve. The maximum response to stimulation was determined by the amplitude of the compound action potential recorded in the superior post-ganglionic nerve. In order to compare responses above and below the threshold of the post-synaptic cell, e.p.s.p.s. were measured during the refractory period of an action potential initiated by injecting depolarizing current into the neurone under study (see Text-fig. 5 and Purves, 1975*b*, 1976*a*). In some cells the minimum number of innervating fibres was also determined by observing the number of discrete steps in the synaptic response as the strength of the preganglionic stimulation was gradually increased (see Methods, Purves, 1976*a*). A neurone was considered non-innervated if no synaptic response could be detected in five to ten successive trials of supramaximal preganglionic nerve stimulation.

Following intracellular recording, ganglia were fixed and prepared for light and electron microscopy and synapses were counted per unit area of electron microscopical section (Purves, 1975*b*). Some sacrifice in the quality of fixation resulted from prolonged physiologic study (see Pl. 1), but since occasional ganglia failed to become re-innervated it was essential to exclude these from anatomical study. The reason for such failures was usually evident on careful dissection of the neuroma where one or both of the preganglionic nerves had failed to join the distal CST. Four of forty-five animals fell into this category and were not considered further.

To study the effects of denervation alone, the CST was cut in an additional fourteen animals and the proximal end was resected and ligated (as in Text-fig 1*B*), but no nerve was apposed to the distal CST. In some of these animals the superior post-ganglionic branch was also crushed (see Results).

Axon counts were made from low power (× 3500) electron micrographic montages of the preganglionic nerves. Counts of ganglion cells and determinations of ganglion volume were made in the same way as previously described (Purves, 1975*b*).

RESULTS

Re-innervation by native fibres

One month after section of the cervical sympathetic trunk 2–4 mm below the ganglion, synaptic responses were detectable in fifty-eight of fifty-nine neurones impaled in five ganglia. However the synaptic response

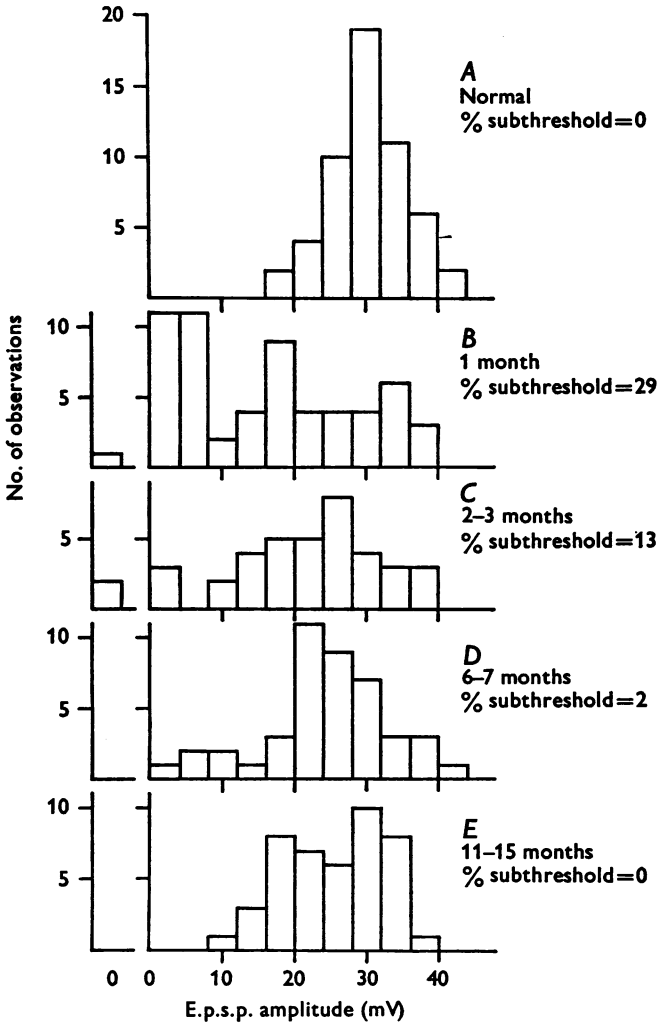
in the re-innervated neurones was generally weak. Normally innervated neurones are invariably brought to threshold by supramaximal preganglionic stimulation; when measured during the refractory period of a directly elicited action potential (Purves, 1975*b*) evoked e.p.s.p.s. range from 18–44 mV in amplitude (Text-fig. 2*A*) and, when the stimulus strength is gradually increased, are seen to be composed of about six discrete steps (Purves, 1975*b*, 1976*a*). In contrast, 1 month after CST section only about two thirds of the neurones were brought to threshold by maximal preganglionic stimulation and the amplitude distribution of e.p.s.p.s showed many subnormal responses (Text-fig. 2*B*). The number of steps in the synaptic response (corresponding to the minimum number of preganglionic fibres contacting a neurone) was reduced to 0–4 (mean ± S.E. of mean = 2·4 ± 0·2, n = 14). Over the succeeding months the strength of innervation gradually increased (Text-fig. 2*C–E*). After 6–7 months only one of forty-three neurones impaled in three ganglia gave a subthreshold response and most synaptic responses fell within the normal range (Text-figs. 2*D* and 5); the average number of synaptic steps had risen to 4·1 ± 0·4 (n = 35). However, even after 11–15 months, re-innervation was incomplete: all forty-five neurones impaled in two ganglia gave suprathreshold responses, yet the amplitude distribution of e.p.s.p.s. remained abnormal, with fewer large responses and an increased number of responses in the low normal or subnormal range (Text-fig. 2*E*). The number of synaptic steps also remained lower than normal (3·7 ± 0·2, n = 36). The input resistance of these neurones is not affected by denervation (McLachlan, 1974); in the present experiments input resistances measured during the course of re-innervation by either native or foreign nerves fell within the normal range (Purves, 1975*b*).

In parallel with these electrophysiologic studies, counts of synaptic profiles per unit area were made on thin sections from ganglia 6–15 months after CST section (Pl. 1 *A* and *B*). The number of synapses counted, as the e.p.s.p. distribution, remained somewhat below normal during this period (Text-fig. 3). Synapse counts at 11–15 months showed no increase over those after 6–7 months. Thus both e.p.s.p. measurements and synapse counts indicate that re-innervation reaches its final level within 6 months.

Re-innervation by foreign fibres

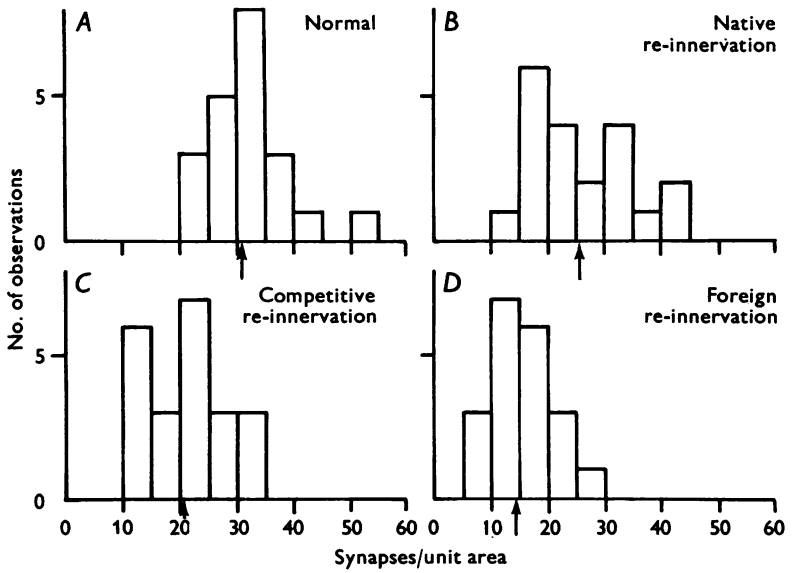
One month after the vagus nerve had been approximated to the distal stump of the cervical trunk, thirty-four of thirty-eight neurones impaled in two ganglia gave detectable e.p.s.p.s in response to supramaximal stimulation of the foreign nerve. The strength of the synaptic response was generally weaker than that elicited by native nerve fibres after one month, and relatively few re-innervated neurones were brought to threshold by

foreign nerve stimulation (about one quarter compared to two thirds after native re-innervation). The distribution of e.p.s.p. amplitudes (Text-fig. 4*A*) showed predominantly small responses compared to native



Text-fig 2. Distribution of e.p.s.p. amplitudes during the course of native re-innervation. All e.p.s.p.s. were measured during the refractory period of an action potential elicited by depolarizing current injection through the recording pipette. *A*, normal e.p.s.p. distribution (from ganglia contralateral to the re-innervated ones); *B*, after 1 month; *C*, after 2–3 months; *D*, after 6–7 months; *E*, after 11–15 months. The number of cells *not* innervated at each interval is shown in the bar graph to the left; the percent of neurones giving only a subthreshold response at each interval is also given.

re-innervation (compare with Text-fig. 2*B*), and the degree of multiple innervation was less than that following native re-innervation (mean number of steps ± S.E. = 1·6 ± 0·2, n = 31). Although there was some improvement in the efficacy of foreign innervation over the succeeding months (Text-fig. 4*B–D*), the synaptic responses remained smaller than those seen after native re-innervation (Text-fig. 5). Forty per cent of the neurones impaled after 8–11 months still gave subthreshold responses,

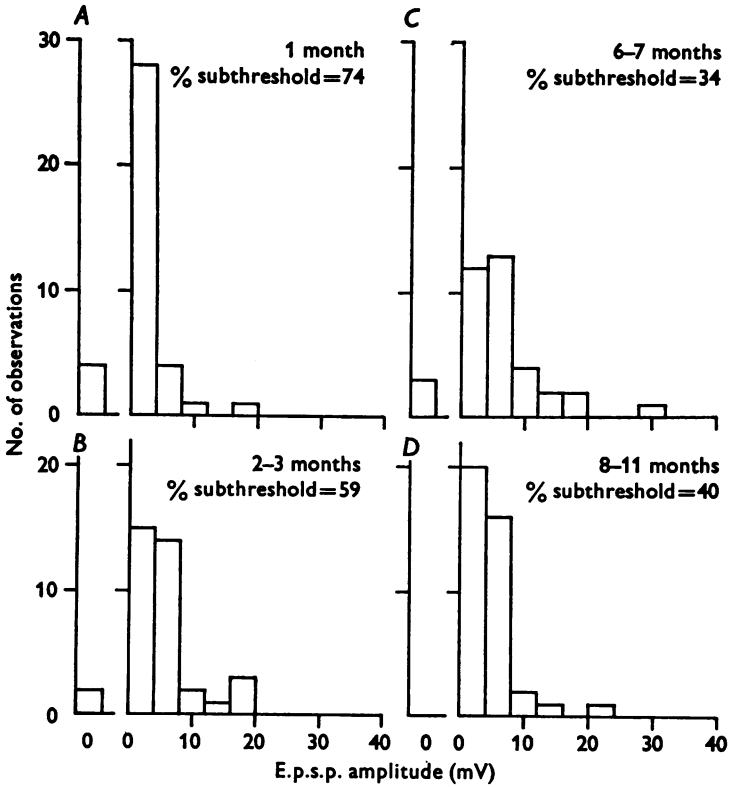


Text-fig. 3. Distribution of synapse counts per unit area 6–15 months after native, foreign or competitive re-innervation. *A*, distribution of counts in normal (contralateral) ganglia. Distribution agrees with distribution of synapse counts from normal ganglia in a previous study (Purves, 1975*b*) in which smaller animals were used (200–400 g; after 6–15 months, animals initially of this size weighed up to 1500 g). *B*, native re-innervation. *C*, competitive re-innervation. *D*, foreign re-innervation. Arrows indicate mean. Unit area = 10 − 400-mesh grid squares (approx. 8650 μm^2).

while synaptically evoked action potentials were elicited in nearly every cell re-innervated by native fibres after only 6–7 months. The distribution of foreign e.p.s.p. amplitudes was only marginally changed during the later course of re-innervation (Text-fig. 4*D*), and was greatly different from the distribution of e.p.s.p.s. during native re-innervation (Text-fig. 2*D* and *E*). The number of synaptic steps also remained low (mean ± S.E. = 1·9 ± 0·2, n = 31).

The reason for the relative weakness of foreign innervation does not

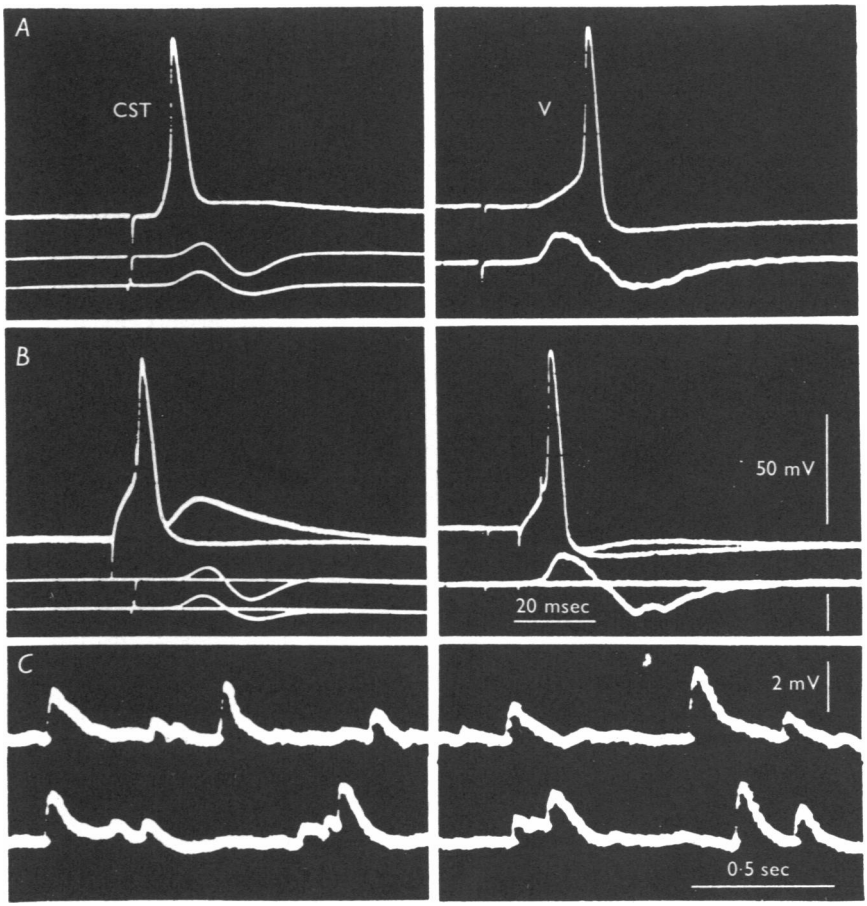
appear to be due to a difference in transmission at individual foreign contacts or the location of foreign synapses on re-innervated neurones. After both native and foreign re-innervation, synaptic contacts seen in electron microscopical sections occurred exclusively on the processes of ganglion cells (Pl. 1), mostly on dendrites, as is the case in normal ganglia (Purves, 1975*b*). Spontaneous synaptic activity could frequently be recorded in neurones re-innervated with vagal fibres and the amplitude and time



Text-fig. 4. Distribution of e.p.s.p. amplitudes during the course of foreign re-innervation: *A*, after 1 month; *B*, after 2–3 months; *C*, after 6–7 months; *D*, after 8–11 months. Number of cells not innervated (bar graph to left) and percent of neurones giving subthreshold response are also given.

course of spontaneous e.p.s.p.s (Text-fig. 5*C*) were similar to those that have been observed in normal neurones (Purves, 1975*b*) and in neurones re-innervated with native fibres (Text-fig. 5*C*).

To further assess the possibility that native or foreign contacts might be different in size (see Kuno, Turkanis & Weakly, 1971), or terminate on neuronal processes of generally different diameters, electron micrographs



Text-fig. 5. Comparison of typical synaptic response 6–7 months after native (left-hand column) and foreign (right-hand column) re-innervation. *A*, neurones re-innervated by native nerve (left) were generally brought rapidly to threshold and showed after-depolarization due to the continued action of acetylcholine. Neurones re-innervated by the foreign nerve (right) were usually brought to threshold more slowly, if at all, and showed less after-depolarization. Lower traces are the inferior (middle) and superior (lower) post-ganglionic nerve compound action potentials (left), and superior branch compound action potential (right column). Calibration mark for these traces is 2 mV for native re-innervation, 0·5 mV for vagal re-innervation. *B*, e.p.s.p.s in same neurones during the refractory period of a directly elicited spike. E.p.s.p. amplitudes were measured 10–20 msec after the peak of the action potential; the exact interval was adjusted to give a response of maximum amplitude without a regenerative component. *C*, spontaneous activity after repetitive preganglionic nerve stimulation (see Purves, 1975*b*) in another pair of cells. Input resistance of neurone re-innervated by vagal fibres was 67 MΩ, and 100 MΩ for neurone re-innervated by native axons. No consistent difference was observed in spontaneous activity in native and foreign re-innervated neurones.

were made of about fifty sequentially encountered synapses in blocks from four ganglia 6–15 months after re-innervation by native and foreign axons respectively. The mean length of the presynaptic membrane thickening (see Pl. 1) was $0{\cdot}86 \pm 0{\cdot}06$ μm ($\pm$ S.E., $n = 47$) for native synapses and $0{\cdot}79 \pm 0{\cdot}08$ μm ($n = 53$) for foreign ones. The average diameter of the post-synaptic processes contacted by the native terminals was $1{\cdot}29 \pm 0{\cdot}1$ μm ($\pm$ S.E., $n = 45$), and by foreign terminals $1{\cdot}26 \pm 0{\cdot}08$ ($n = 52$). The appearance of the two sets of terminals (Pl. 1) was not obviously different but vesicle size and morphology was not studied quantitatively.

TABLE 1. Number of axons in normal and regenerate preganglionic trunks after 6–12 months (normals are trunks contralateral to those examined in native re-innervation)

Normal			Native re-innervation			Foreign re-innervation		
Myelinated	Non-myelinated	Total	Myelinated	Non-myelinated	Total	Myelinated	Non-myelinated	Total
1087	3761	4848	442	2655	3097	265	2700	2965
1114	2392	3506	277	2651	2928	520	4500	5020
869	2656	3525	295	2298	2593	137	3608	3745

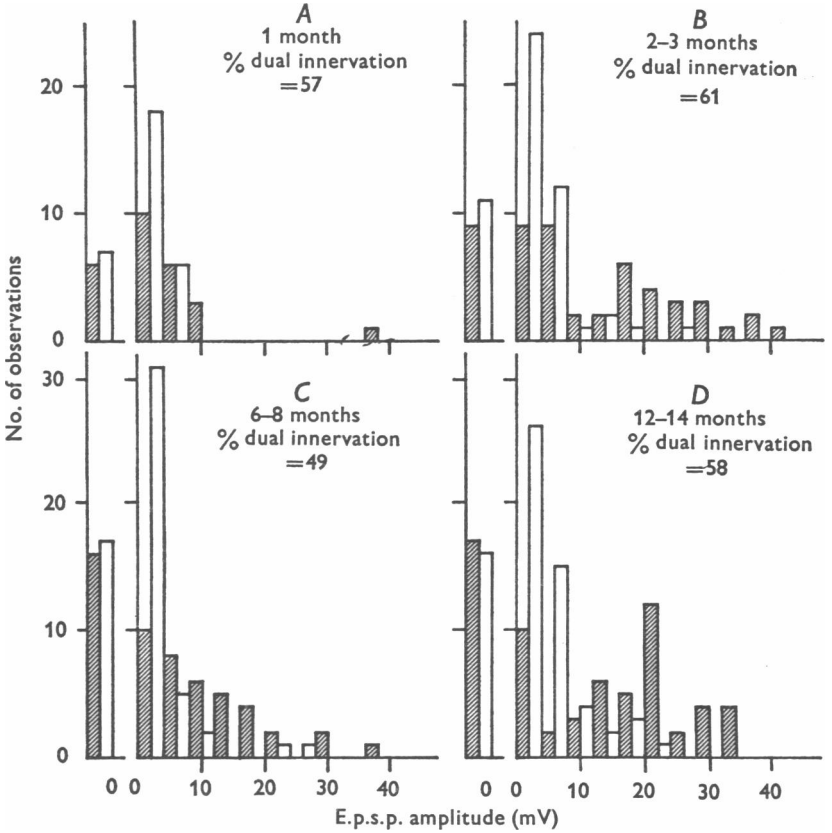
An additional point of interest was the number of axons growing into the superior cervical ganglion after native and foreign re-innervation. The results of axon counts from electron micrographs of native and foreign 'trunks' near the caudal pole of the ganglion are shown in Table 1. About three quarters of the normal number of axons returned to the ganglion 6–12 months after native re-innervation; at similar times after foreign re-innervation the number of axons was the same or somewhat greater. Axonal sprouts form during the initial stages or re-innervation (Bray & Aguayo, 1974); although these sprouts appear to atrophy within 6 months, it is uncertain whether the number of axons corresponds strictly to the number of preganglionic nerve cells.

Counts of synapses per unit area carried out on sections from ganglia re-innervated with vagal fibres were lower than counts after native re-innervation (Text-fig. 3). The mean number of synapses 6–11 months after foreign re-innervation was $14{\cdot}8 \pm 1{\cdot}2$ ($n = 20$) compared to $25{\cdot}4 \pm 2{\cdot}0$ ($n = 20$) 6–12 months after native re-innervation. Thus by both physiological and morphological criteria, the ability of vagus nerve axons to re-establish synapses on individual ganglion cells is generally less than that of the native axons.

Competitive re-innervation by native and foreign fibres

When both native and foreign fibres were allowed to grow into the denervated superior cervical ganglion, most neurones (thirty-five out of

thirty-seven) impaled in three ganglia a month after the operative procedure were re-innervated. Twenty-one of these cells (57 %) gave e.p.s.p.s in response to stimulation of both the vagus nerve and the proximal sym-



Text-fig. 6. Distribution of e.p.s.p. amplitudes elicited by native (cross-hatched bars) and foreign (open bars) nerve stimulation during the course of competitive re-innervation. *A*, after 1 month; *B*, after 2–3 months; *C*, after 6–8 months; *D*, after 12–14 months. Proportion of neurones dually innervated at each interval is also given. Smaller bar graphs to the left indicate that the number of neurones *not* innervated by native fibres (cross-hatched bars) is about the same as the number not innervated by foreign axons (open bars) throughout the period of study. Only thirteen of 253 of neurones impaled were not innervated by either source.

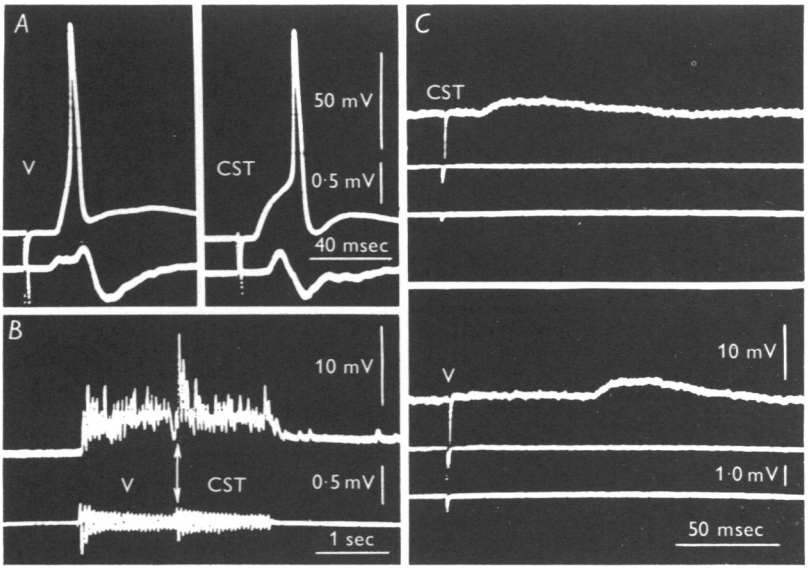
pathetic trunk and thus appeared to be dually innervated. The remaining cells were about equally divided into those innervated by the CST alone and those innervated by only the vagus nerve.

The amplitude distribution of e.p.s.p.s elicited in re-innervated neurones

by stimulation of the native and foreign nerves over a period of 1–14 months is shown in Text-fig. 6. After all intervals, most neurones (95 %) were innervated to some degree. However on average, neither source of innervation was able to attain the synaptic influence that developed when non-competitive re-innervation by either native or foreign fibres occurred (compare Text-figs. 2, 4 and 6). Perhaps the most obvious indication of this is that while re-innervation from a single source resulted in nearly every neurone receiving functional contacts from the ingrowing fibres, in the competitive situation many neurones (roughly 40 %) were contacted by one or the other, but not by both sources, a situation which remained relatively unchanged for up to 14 months. A corollary is the stability of dual innervation: of seventy-three neurones impaled 12–14 months after competitive re-innervation forty-two (58 %) remained innervated by both sources, a fraction nearly identical with that observed after 1 month (see Text-fig. 6). Furthermore, measurement of the synaptic influence of the native and foreign nerves on individual neurones gave no indication that one source of innervation tended to dominate the other, even after 14 months. The simplest index of this was the fraction of dually innervated neurones brought to threshold by both sources during the course of re-innervation (Text-fig. 7*A*). After 1 month 0·5 % of dually innervated neurones could be fired by stimulation of either the native or foreign nerve, 41 % after 2–3 months, 23 % after 6–7 months and 43 % after 12–14 months. Thus the synaptic influence of the native and foreign axons appeared to gain in parallel, rather than one axon type supplanting the other. In general, native nerve stimulation elicited larger e.p.s.p.s than foreign nerve stimulation (see Text-fig. 6), as in non-competitive re-innervation.

A number of experiments were undertaken to rule out the possibility that neurones which appeared to be dually innervated might in fact be innervated by a single source. For example, ingrowing fibres might branch in the region of the nerve anastomosis with one axon branch growing to the ganglion and one growing into the proximal portion of the other preganglionic nerve. Indeed, some fibres from one nerve often did extend into the proximal portion of the other since a small compound action potential could sometimes be recorded in one proximal nerve when the other was stimulated. However, it is unlikely that an axon reflex could account for the high degree of dual innervation. If one preganglionic nerve was repetitively stimulated, the post-synaptic response elicited in an impaled neurone was gradually depressed; yet when the stimulation was suddenly switched to the other preganglionic nerve, the depression was relieved, indicating that each nerve contributed a different set of terminals to the ganglion cell (Text-fig. 7*B*). The fact that a similar phenomenon

could be seen in the amplitude of the compound action potential recorded from a post-ganglionic nerve (Text-fig. 7*B*, lower trace) further supports the independence of the native and foreign contributions. Another



Text-fig. 7. Evidence for dual innervation. *A*, dually innervated neurone impaled 9 months after the initial operative procedure. Upper traces show that a suprathreshold response is elicited following stimulation of either the vagus (V) or the native (CST) nerve. Lower trace is an extracellular recording from the superior post-ganglionic nerve bundle. *B*, upper trace shows that repetitive stimulation (20/sec) of the vagus nerve causes a depression of transmission in another dually innervated neurone impaled nearly 10 months after initial operative procedure but does not affect the response to subsequent stimulation of the CST (arrow). The same result was observed when the sequence of stimulation was reversed; the amplitude of initial e.p.s.p. in the second train of the sequence was generally the same as a test e.p.s.p. elicited by itself. This effect can also be seen in the compound action potentials recorded from the superior post-ganglionic nerve bundle (lower trace). The intracellularly recorded effect was clearer when, as in this cell, the e.p.s.p.s elicited were subthreshold and thus not obscured by action potentials. Ganglia shown in both *A* and *B* were unusually well innervated by foreign nerve; typically the compound action potential elicited by native nerve stimulation was somewhat larger than that elicited by foreign nerve stimulation. *C*, dual innervation in the presence of 8 mM-Mg^{2+}, 0·5 mM-Ca^{2+} (after 1·5 hr); subthreshold e.p.s.p.s can still be evoked by both native (CST) and foreign (V) preganglionic nerve stimulation, even though ganglionic transmission is largely blocked. Lower traces are of inferior and superior post-ganglionic nerves which in normal Ringer fluid showed compound action potentials of about 0·1 mV.

condition that might result in the appearance of dual innervation would be the presence of extensive collateral innervation between ganglion cells. If such innervation were present, and foreign and native terminals generally contacted separate nerve cells, then individual neurones might appear dually innervated if the two sets of neurones made contacts with each other. The same argument would apply if interneurones linked ganglion cells. The possibility of an intervening neurone in the pathway was examined by stimulating the native and foreign preganglionic fibres in the presence of increased Mg^{2+} and lowered Ca^{2+} in the bathing fluid, which reduced the amplitudes of evoked responses to subthreshold levels. In three experiments in which normal Ringer fluid was replaced by perfusion fluid containing 0·5 mM-Ca^{2+} and 8·0 mM-Mg^{2+} (NaCl was reduced to maintain isotonicity), the post-ganglionic compound action potential was largely blocked within 15–20 min (the residual compound action is presumably due to the presence of some preganglionic fibres which pass through the ganglion – Perri, Sacchi & Casella, 1970; Purves, 1975*b*). However, in many neurones impaled under these conditions small synaptic responses one or a few millivolts in amplitude could still be elicited and often both native and foreign nerve stimulation evoked responses (Text-fig. 7*C*). This suggests that preganglionic connexions are indeed monosynaptic. Other lines of evidence also make unlikely an explanation involving collateral innervation, or interneurones (see below).

Electron microscopical synapse counts from ganglia re-innervated by both native and foreign fibres remained below normal levels for the duration of the experiments (Text-fig. 3*C*). The average number of synapses observed 6–14 months after competitive re-innervation ($20{\cdot}6 \pm 1{\cdot}4$, $n = 22$) was less than the number 6–12 months after native re-innervation alone ($25{\cdot}4 \pm 2{\cdot}0$, $n = 20$), indicating that somewhat fewer synapses formed during competitive re-innervation. This occurred despite a larger number of axons in the preganglionic trunk after competitive as opposed to non-competitive re-innervation. Re-innervating fibres in the pre-ganglionic trunk rostral to the three-way nerve anastomosis (see Text-fig. 1*C*) tended to run in a large number of small, often widely separated bundles (up to 151). The large area involved made direct axon counts from electron micrographs impractical. A rougher estimate of the total number of re-innervating fibres was made by comparing the total cross-sectional areas (determined with the aid of a computer assisted planimeter; Cowan & Wann, 1973) of the rostral preganglionic trunk after native, vagal, and competitive re-innervation. The cross-sectional area of trunks directly counted (Table 1) ranged from 8630 to 18,507 μm^2 for native re-innervation and 11,996 to 22,365 μm^2 for vagal re-innervation. The total cross-sectional area measured for three trunks 6–14 months after dual re-innervation was

20,259, 33,194 and 52,524 μm^2. Assuming a similar density of axons in nerve bundles after dual re-innervation (an assumption which appeared valid on electron microscopical examination of trunks after native, foreign and competitive re-innervation), this result indicates that the number of axons returning to ganglia during competitive re-innervation was approximately the sum of the numbers of axons which regenerated after native and vagal re-innervation.

Effects of chronic denervation

Since ganglion cells are, to a greater or lesser extent, denervated during the early stages of re-innervation, a further series of experiments was undertaken to examine the effects of denervation *per se* on ganglion cells. The following questions are especially relevant to the interpretation of the experiments reported in the preceding sections. (*a*) Does the number of neurones in the ganglion change after denervation? (*b*) What is the nature of the small number of ganglionic synapses which remain after denervation of the superior cervical ganglion (Raisman, Field, Ostberg, Iversen & Zigmond, 1974; Purves, 1975*b*; Ostberg *et al.* 1976)? (*c*) To what extent are the terminals which remain after CST section capable of sprouting and thus forming a further source of competition with re-innervating axons?

(*a*) *Number of neurones in ganglia after denervation*

In order to determine whether significant neuronal degeneration occurs following chronic denervation, counts of neurones and measurement of ganglionic volume were made in four pairs of ganglia 2–4·5 months after section and ligation of the CST to the right ganglion of each pair. While the average volume of denervated ganglia was 9 % less than the contralateral controls, the mean number of neurones in right and left ganglia differed by less than 1 %, and all values were within the normal range (Purves, 1975*b*). This indicates that although denervated ganglia undergo mild shrinkage, there is little or no loss of neurones for at least 4·5 months (see also Hamlyn, 1954). In electron microscopical sections, denervated ganglia appeared to contain more collagen than normal ganglia; this presumably fills most of the space usually occupied by the preganglionic fibres. Although the effects of denervation were not studied beyond 4·5 months, it seems unlikely that changes in cell number or ganglion volume affected synapse counts during re-innervation.

(*b*) *Residual ganglionic synapses after cervical trunk section and ligation*

The average number of synapses counted per unit area in eleven blocks taken from three ganglia denervated 2–8 days was 0·7 ± 0·3 (± S.E.). Since counts in normal ganglia are about thirty synapses per unit area (Text-fig. 3; see also Purves, 1975*b*, 1976*a*), more than 97 % of ganglionic synapses

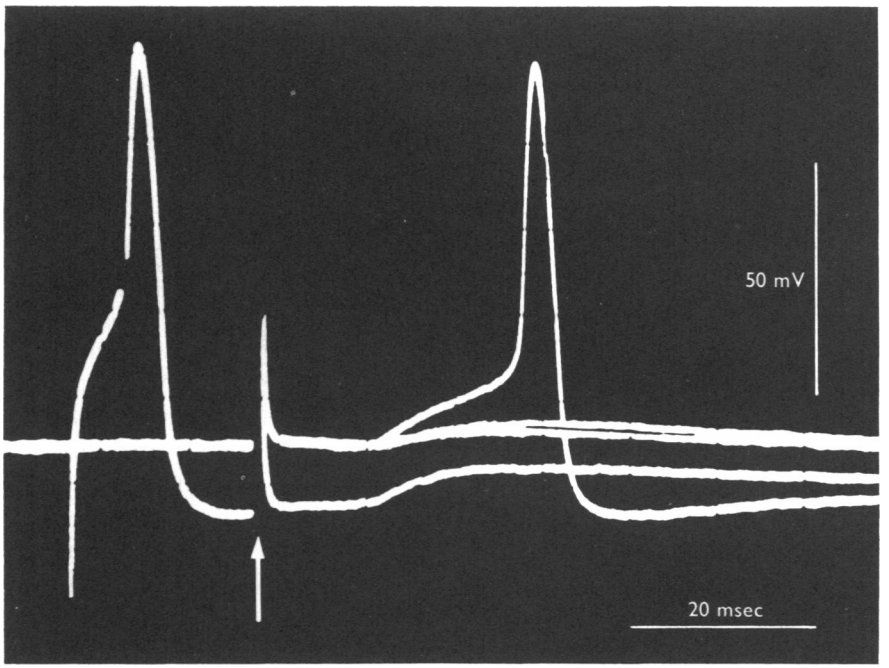
degenerate within a few days of preganglionic trunk section. Post-synaptic thickenings unapposed by a presynaptic profile were infrequent and were not studied quantitatively (see Raisman *et al.* 1974). The small number of synapses remaining are generally indistinguishable in electron microscopical sections from synapses in normal ganglia (Purves, 1975*b*), or ganglia following re-innervation (Pl. 1*A* and *B*).

A source of these residual terminals might be endings from small interneurones containing dense core vesicles (Pl. 1*C*) which have been described in many sympathetic ganglia (see, for example, Grillo, 1966; Matthews & Raisman, 1969). However, terminals containing dense core vesicles (Pl. 1*F*) were very rarely encountered and were easily distinguished from the majority of residual synapses. Moreover, stimulation of the superior post-ganglionic branch (but generally not of the inferior branch) elicited small e.p.s.p.s (2–12 mV in amplitude) in eight of fifty-eight (14%) ganglion cells impaled 2–11 days after denervation. This finding suggests that residual synapses derive either from axon collaterals of ganglion cells or from preganglionic fibres entering the ganglion via the superior post-ganglionic branch. To distinguish these possibilities, in three ganglia the CST was sectioned and at the same time the superior post-ganglionic nerve was cut about 2 mm above the rostral pole. If the residual synapses derived from preganglionic fibres entering the ganglion via the superior nerve, then the number of residual synapses counted in sections from these ganglia should fall. However, counts of synapses per unit area carried out on twelve blocks from these ganglia after 2–7 days showed little change ($0{\cdot}6 \pm 0{\cdot}3$ compared to $0{\cdot}7 \pm 0{\cdot}3$ in ganglia denervated 2–8 days). This suggests that e.p.s.p.s which can be elicited in denervated ganglia by stimulation of the superior post-ganglionic nerve are intrinsic to the ganglion and probably result from collaterals of ganglion cell axons. Collateral innervation has been described in a lower vertebrate autonomic ganglion (McMahan & Purves, 1976; Roper, 1976) and in cultures of dissociated mammalian sympathetic neurones (O'Lague, Obata, Claude, Furshpan & Potter, 1974; Rees & Bunge, 1974). The pharmacology of transmission at residual synapses was not studied.

(*c*) *Ability of residual terminals to sprout*

To examine the extent to which residual terminals might be able to sprout and increase their field of innervation, in three animals ganglia were chronically denervated by means of CST section, and the effect of stimulating the superior post-ganglionic branch was tested after an interval of 4–5 months. Whereas only about 14% of acutely denervated ganglion cells impaled gave an e.p.s.p. in response to superior branch stimulation, after several months of denervation 40% gave detectable

synaptic responses (n = 75), sometimes large enough to elicit an action potential (Text-fig. 8). Thus the sidualre nerve terminals are indeed capable of considerable sprouting. To confirm that sprouting did occur (as opposed, for example, to an enhancement of synaptic responses by virtue of denervation supersensitivity), synapses were counted in electron



Text-fig. 8. Synaptic response in a neurone in a chronically denervated ganglion (after 4·5 months) elicited by stimulation of the superior postganglionic branch (arrow). Responses were usually subthreshold, variable in amplitude and fatigued easily. Four traces are superimposed; on one sweep the synaptic response is preceded by an action potential directly elicited by current injection through the recording electrode to rule out that the response might be due to an antidromic spike which failed to invade the cell soma.

microscopical sections after chronic denervation. The average number of synapses rose nearly tenfold from $0{\cdot}7 \pm 0{\cdot}3$ following acute denervation to $6{\cdot}4 \pm 0{\cdot}8$ per unit area after 2–5 months (n = 10). The stimulus to sprout would presumably be much less in the presence of prompt re-innervation. To test this, two ganglia which had been re-innervated by both native and foreign nerves 10 and 18 months previously were denervated at a second operation; of twenty-eight neurones impaled only three showed e.p.s.p.s in response to stimulation of the superior nerve. This is about the proportion

of cells showing a synaptic response to post-ganglionic stimulation in acutely denervated ganglia and suggests that, even after prolonged periods, little sprouting of axons which form residual synapses occurs when ganglia are promptly re-innervated. However it seems likely that synapse counts following vagal re-innervation alone, which is less complete (see Text-figs. 3 and 4), include some synapses from this additional source.

DISCUSSION

Re-innervation of ganglion cells by foreign axons

In his original experiments on vagal re-innervation of the superior cervical ganglion Langley (1898) noted that stimulation of the foreign nerve was less effective in causing peripheral sympathetic effects than stimulation of the cervical sympathetic trunk following native re-innervation. In the present experiments vagal re-innervation has been found to be less effective than native re-innervation at the level of the synaptic response recorded in individual ganglion cells. Thus although a similar number of foreign axons enter the ganglion and make some contact with the vast majority of ganglionic neurones, the distribution of e.p.s.p. amplitudes is shifted to lower values, and synapse counts per unit area are less than those in normal ganglia or ganglia that have been re-innervated with fibres of the cervical sympathetic trunk.

The vagus nerve is composed of visceral and somatic afferent fibres and somatic efferent axons, as well as preganglionic parasympathetic fibres (see, for example, Evans & Murray, 1954). This heterogeneity makes it difficult to compare vagal with native re-innervation. It is likely that several classes of foreign axons grow into the ganglion, and that these different classes re-innervate ganglion cells differently. However, this uncertainty should not obscure the fact that vagal fibres, as a class, are much less effective during re-innervation than native axons. More homogeneous nerves composed primarily of somatic motor axons, such as the nerve to the sternohyoid muscle in the guinea-pig (McLachlan, 1974) or the hypoglossal nerve in the rat (Ostberg *et al.* 1976), appear even less capable of re-innervating neurones of the superior cervical ganglion.

Why foreign nerves are generally less effective than native axons in forming synaptic contacts on sympathetic neurones during re-innervation is not known. It is unlikely that various classes of foreign axons are inherently less competent during re-innervation than sympathetic axons, since many studies have shown that mammalian peripheral nerves are capable of re-innervating their normal target tissues, although this usually occurs with little functional specificity (see Harris, 1974, and Purves, 1976*b*, for recent reviews). In spite of successful re-innervation of their

own targets, foreign axons re-innervating the superior cervical ganglion might be less effective than native fibres if individual foreign neurones were unable to support the same number of synapses as individual native preganglionic neurones. Alternatively, foreign preganglionic axons might respond less well to a trophic mechanism which acts normally to maintain native ganglionic synapses. Synapses on guinea-pigs SCG neurones depend on some aspect of the post-synaptic cell's axonal extension to the periphery; most synaptic contacts are lost within a few days of axon interruption or colchicine application to post-ganglionic nerves (Purves, 1975*b*, 1976*a*; see also Pilar & Landmesser, 1971) but are fully recovered coincident with post-ganglionic nerve regeneration. Chronic ligation of post-ganglionic nerves, however, prevents neurones from regaining their synapses (Purves, 1975*b*). If some classes of foreign axons were less responsive to the trophic effect implied by these findings then fewer synaptic contacts between the two cell types would be maintained (or perhaps formed). This possibility seems more plausible in the light of recent evidence which suggests that the protein nerve growth factor may play a role in synaptic maintenance in guinea-pig sympathetic ganglia (Purves & Njå, 1976). Since the effects of nerve growth factor are limited to the sympathetic and sensory systems, as far as is known (see, for example, Levi-Montalcini & Angeletti, 1968), it would not be surprising to find that some classes of foreign axons are less responsive to a trophic effect mediated by this agent.

Stability of foreign synapses

In spite of the relative inefficiency of re-innervation of SCG neurones by vagal axons, the foreign synapses which form appear to be stable, even in the presence of large numbers of competing native axons. The major indication of the inability of native terminals to displace foreign ones is that the proportion of dually innervated cells (50–60 %) remains unchanged for over a year. Moreover, the strength of both sets of synapses increases roughly in parallel during the first few months following re-innervation (Text-fig. 6). These findings also suggest that the phenomenon of synaptic repression during competitive re-innervation of some lower vertebrate muscles (Marotte & Mark, 1970*a*,*b*, 1972; Mark, Marotte & Mart, 1972; Cass, Sutton & Mark, 1973; Yip & Dennis, 1976; although see Scott, 1975, and Frank & Jansen, 1976) is poorly developed in the mammalian sympathetic system, at least with the classes of competing axons used in these experiments. In this regard, sympathetic ganglion cells are like mammalian muscle cells on which foreign synapses also appear to persist indefinitely after dual re-innervation (Frank, Jansen, Lømo & Westgaard, 1975).

The finding that foreign synapses are stable even in the face of native competition is difficult to reconcile with earlier work of Guth & Bernstein (1961) which was interpreted as demonstrating selectivity in the course of re-innervation of sympathetic ganglion cells in the cat. Based on the end organ-response to ventral root stimulation, Guth & Bernstein suggested that terminals formed by collateral sprouting after partial denervation (Murray & Thompson, 1957) could be replaced by the ultimate arrival of the initially interrupted axons. At least two explanations might account for the apparent discrepancy between these results and the findings reported here. The experiments of Guth & Bernstein (1961) are different from the present experiments in that the 'foreign' terminals are sprouts from neurones which are presumably already making their usual number of synapses with target cells. Thus the terminals newly formed by sprouting represent an increase in the number of synapses that the preganglionic neurone would ordinarily support. In contrast, in the experiments reported here both native and foreign re-innervating fibres initially support no synapses. From this point of view, the critical distinction during competition might not be between 'native' and 'foreign' axons but between neurones supporting a larger or smaller number of synapses than normal. If terminals made by neurones maintaining more than their normal complement of synapses tended to give way to axons supporting fewer terminals than normal, then both the present results and those of Guth & Bernstein (1961) would be expected. Alternatively, the two sets of results might be explained if the specificity of sympathetic innervation (and re-innervation) originally demonstrated by Langley (1892, 1895, 1897) were not very strict at the level of individual nerve cells. If particular neurones had only a relative preference for preganglionic fibres from different spinal levels, then one might observe apparent selectivity measured by the end-organ response, and yet have widespread if weaker innervation of neurones by axons from 'incorrect' spinal segments. The persistence of foreign innervation in the face of native competition observed in the present experiments suggests that the specificity and selectivity of re-innervation found in previous experiments (Langley, 1895, 1897; Murray & Thompson, 1957; Guth & Bernstein, 1961) are unlikely to be based on an absolute preference of individual ganglion cells for a particular class of preganglionic axons, or on a mechanism of synaptic displacement based on 'foreignness' *per se*.

Since the way in which mammalian neurones strike a balance between competing sources of innervation is of fundamental importance, it would be of considerable interest to extend the original experiments of Langley and others to the level of individual neurones using intracellular recording methods.

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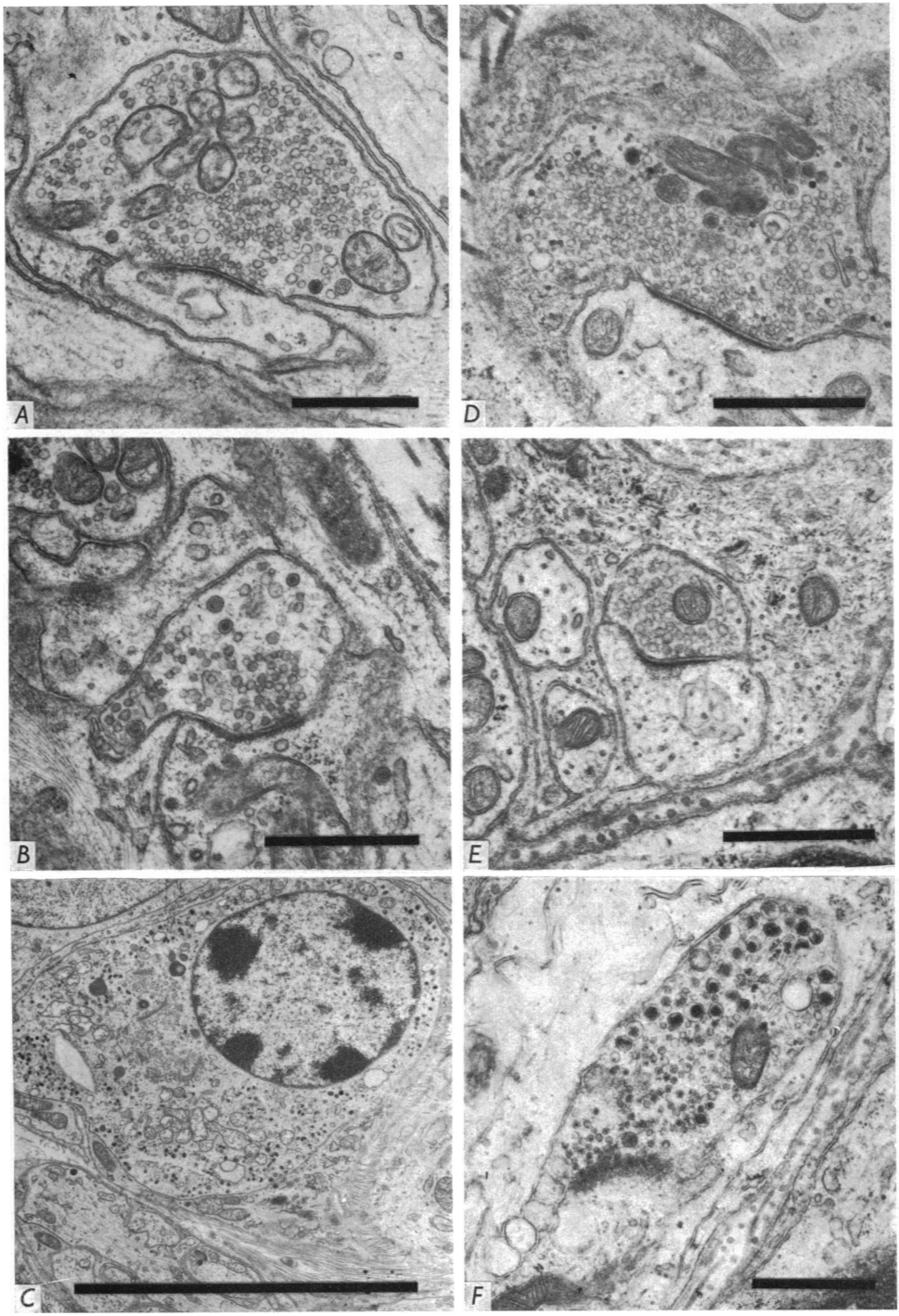

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EXPLANATION OF PLATE

Appearance of synapses after re-innervation by native and foreign (vagal) fibres. In each case the tissue was in the recording bath for several hours before fixation.

Calibration bars correspond to 1 μm except that in *C*, which corresponds to 10 μm. *A*, *B*, typical synapses observed 6–7 months after native re-innervation.

C, example of small neurone containing numerous dense core vesicles in its cytoplasm.

D, *E*, typical synapses observed 6–7 months after vagal re-innervation. Appearance is generally similar to contacts seen after native re-innervation.

F, synapse, presumably from the type of neurone shown in *C*, with numerous large and small vesicles, most of which contain a dense core. Such synapses were very rarely seen and easily distinguished from synapses typically observed after either native or vagal re-innervation.