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Formation of Synapses in the Adult Rat After Injury: Similarities and Differences Between a Peripheral and a Central Nervous Site

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Formation of synapses in the adult rat after injury: similarities and differences between a peripheral and a central nervous site

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[Plate 1]

This article reports two series of investigations into the formation of synapses after injury in the adult rat. A comparison is made between synapse formation in a peripheral nervous site – the superior cervical sympathetic ganglion after destroying almost all the afferent fibres by cutting the preganglionic chain, and a central nervous site – the septal nuclei, after selectively destroying afferent fibres from the hippocampus running in the fimbria. The method consists of a quantitative electron microscopic assessment of numbers of synapses in the neuropil of the deafferented regions at progressively increasing survival times after the injury.

The superior cervical ganglion has about 8.8×10^6 synapses and about 8000 preganglionic axons. After a freeze lesion of the preganglionic chain over 90 % of the synapses disappear, indicating that the preganglionic axons each form an average of about 1100 synaptic contacts. A proportion of the deafferented postsynaptic sites are recognizable by the presence of the vacated postsynaptic thickenings. As the preganglionic axons regenerate back across the lesion, the synapses gradually reappear, and the vacated thickenings disappear. If an insufficient number of axons regenerates, less than the normal complement of synapses is formed; the number of synapses bears a constant relation to the number of axons, and under these circumstances each axon forms about the same number of synapses as in the normal ganglion. This suggests that the number of synapses which an axon can form is limited, and that the preganglionic axons may be close to this limit in the normal ganglion. The ganglion can also be reinnervated by foreign nerves such as the vagus and hypoglossal. These nerves form less than normal numbers of synapses. In all cases of less than complete reinnervation, the numbers of vacated thickenings remaining are inversely proportional to the numbers of synapses which appear; this indicates that the synapses are formed at the originally denervated postsynaptic sites.

The septum receives inputs of hippocampal origin both through the ipsilateral and the contralateral fimbria. After section of one fimbria, the terminals of the cut axons are recognizable by the reaction of electron dense degeneration. About half the synapses on dendritic spines belong to the ipsilateral fimbria and about half this number to the contralateral fimbria. The degenerating terminals are progressively removed from the postsynaptic sites by the phagocytic action of astrocytes. However, unlike the ganglion, the sites only rarely appear as vacated thickenings. Instead, there is a progressive increase in the number of non-degenerating terminals as the degenerating terminals are removed. This spontaneous reinnervation process results in restoration of the original complement of synapses. However, the originally cut fimbrial axons do not regenerate: this process of synapse formation is due to formation of additional contacts by the other axons remaining in the deafferented region. If the second fimbria is now cut, it results in an amount of degeneration equal to the sum of that found after cutting both the ipsilateral and the contralateral fimbria. This indicates that the sites left vacant by section of one fimbria are reinnervated exclusively by the other fimbria. However, this preference is not absolute. After section of the second fimbria, the degeneration is still removed, and a normal complement of synapses once

again restored. Thus the septal neuropil has great powers of re-establishing normal synapse numbers even after major deafferentation.

The ganglion and the septum are similar to the extent that denervated postsynaptic sites are capable of being reinnervated – spontaneously in the case of the central nervous site. The major difference is that in the peripheral nervous site the originally cut axons can regenerate back to their former targets. It is not clear why the cut fimbria – behaving in a manner typical for central nervous tracts – does not regenerate. The proximal parts of the cut fimbrial axons and their cells of origin survive the injury. The denervated sites in the septum also survive, and are capable of being reinnervated. Further, this reinnervation process is selective for fimbrial axons from the opposite hippocampus. This also shows that fimbrial fibres are capable of forming new synapses. Thus the defect which prevents true regeneration of the originally cut axons back to their former targets does not appear to be due to failure in the mechanism of synapse formation or matching. This suggests that the defect may be an inability of the cut axons to regenerate across the site of the lesion.

Introduction

Injury to nerve fibres in the peripheral nervous system can be followed by regenerative repair leading to restoration of function. In the mammalian central nervous system, comparable axonal injuries do not appear to stimulate regeneration, and there is minimal restoration of function. We have attempted to examine possible reasons for this difference. The basic experimental design has been to make a precisely defined lesion of a fibre system afferent to a particular region of neuropil, and then use the electron microscope to follow quantitatively the synaptic changes occurring in the denervated area. The two areas selected were the superior cervical sympathetic ganglion (Östberg *et al.* 1976; Raisman *et al.* 1974) as an example of a peripheral nervous site, and the septal nuclei (Raisman & Field 1973; Raisman, Field & Coldham 1976) as an example of a central nervous site. All experiments were in adult rats.

Description of plate 1

Figure 1. A synapse from a normal, unoperated superior cervical sympathetic ganglion. The axon terminal (A) makes contact by means of a vesicle cluster directed towards a slight postsynaptic thickening (arrow) on a dendritic postsynaptic element (P). *, processes of non-neuronal elements. (Scale bars in all figures approximately 0.5 μm.)

Figure 2. Denervated superior cervical ganglion. A vacated synaptic thickening (arrow) on a postsynaptic element (P). In place of the axon terminal, the thickening is apposed by a non-synaptic part of a neuronal cell body (*).

Figure 4. A synapse from the neuropil of the lateral septal nucleus in a normal, unoperated rat. The axon terminal (A) makes synaptic contact by means of a vesicle cluster directed towards a marked postsynaptic thickening (arrow) on a dendritic spine (P). *, astroglial cell process.

Figure 5. Lateral septal nucleus. An axon terminal (A) showing electron dense degeneration 2 days after a lesion of the fimbria. Arrow, synaptic thickening; P, dendritic spine; *, phagocytic astroglial process beginning to invaginate the degenerating terminal and already containing isolated fragments of degenerating axoplasm (double arrow).

Figure 6. A vacated synaptic thickening (arrow) on a dendritic spine (P) in the lateral septal nucleus one month after a lesion of the fimbria. A thin astroglial process (*) is interposed between the thickening and a fragment of electron dense degenerating axoplasm (double arrow).

Figure 8. A 'double synapse' from the lateral septal nucleus one month after a lesion of the fimbria. The axon terminal (A) makes contacts with a transversely sectioned, tubule containing dendritic shaft (H) and a dendritic spine (P). Both contacts have marked synaptic thickenings (arrows). Astroglial processes (*) surround the contact sites but are not interposed between the pre- and postsynaptic neuronal elements.

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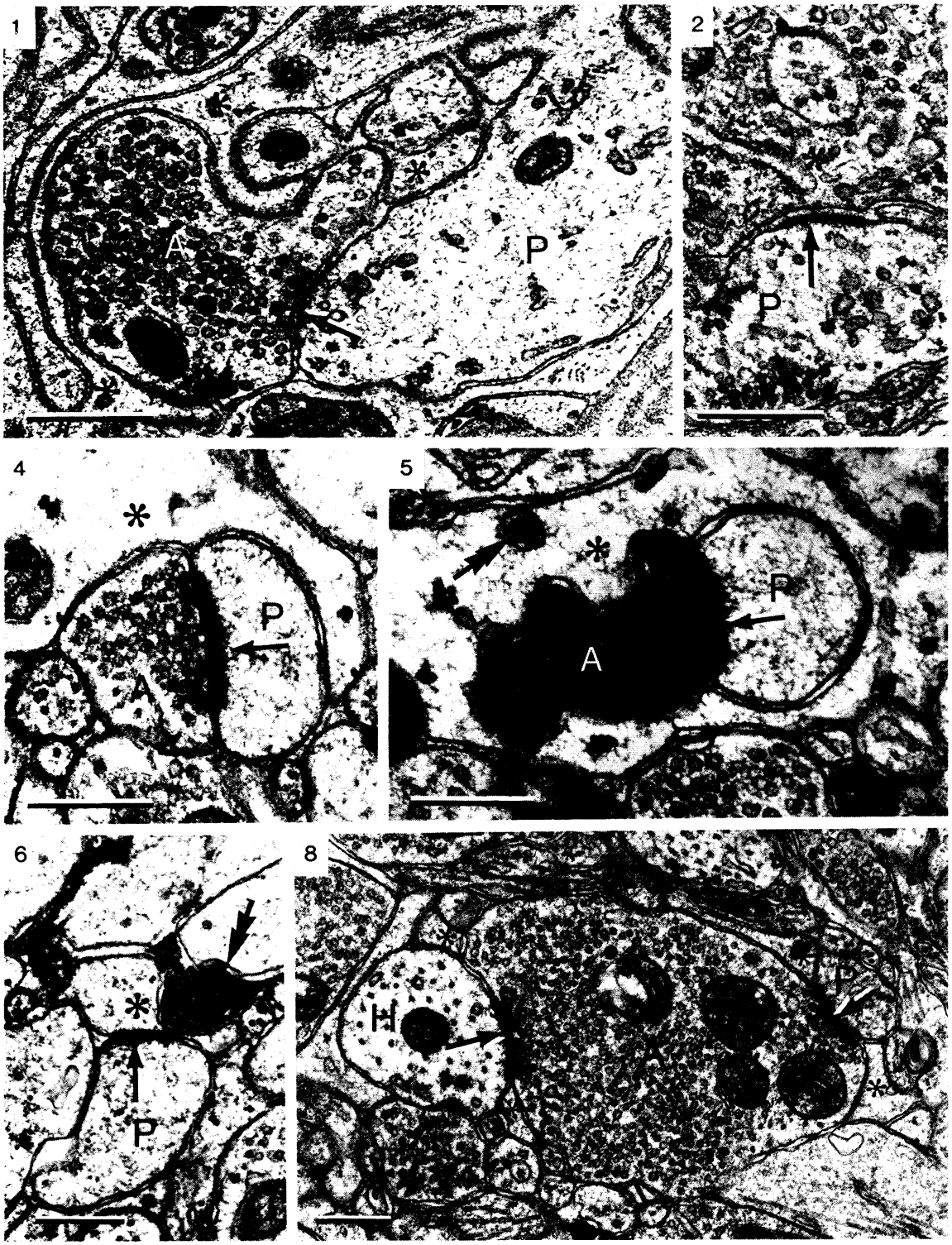

FIGURES 1, 2, 4–6 AND 8. For description see opposite.

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The superior cervical sympathetic ganglion

In the normal rat the superior cervical sympathetic gangli oncontains about 40000 neurons. These are innervated by some 8000 preganglionic afferent fibres ascending through the preganglionic nerve trunk. The ganglion contains about 8.8×10^6 synapses. More than 90% of the synapses are lost when the preganglionic chain is cut (see below), indicating that an average preganglionic axon forms about 1100 synapses. The synapses (figure 1, plate 1) are located principally on spine-like protrusions from dendrites or cell somata. In many of the synapses, the site of synaptic contact is marked on the postsynaptic element by a membrane-associated density (synaptic thickening).

The simplest injury we have studied (Raisman *et al.* 1974) was complete destruction of the preganglionic axons by direct application of a fragment of dry ice to the preganglionic trunk. The resultant injury destroyed the preganglionic axons at the site of application but left the physical continuity of the chain intact. This injury had a dramatic effect on the numbers of synapses in the ganglion. By 24 h after operation, synapse numbers had fallen to less than 10% of normal levels. In many cases the postsynaptic sites remained recognizable by means of the synaptic thickenings, which persisted in a deafferented form ('vacated thickenings') apposed by cytoplasmic processes belonging to the non-neuronal cells of the ganglion (satellite or Schwann cells) or sometimes by non-synaptic parts of neurons (figure 2, plate 1). The terminals of the preganglionic axons were represented by electron-dense degenerating fragments undergoing phagocytosis by non-neuronal cells. Despite a wide range of survival times, only very rarely, if ever were axon terminals showing degeneraive changes seen directly apposed to the postsynaptic thickenings. Presumably therefore, the terminals of the cut axons had become detached from the postsynaptic elements either before the terminals had begun to show recognizable degenerative changes or at the least immediately they had begun to show such changes.

Vacated synaptic thickenings were not seen in normal intact ganglia. The appearance of about 4.5×10^6 vacated thickenings in the denervated ganglia was correlated with the disappearance of about 8×10^6 presynaptic axon terminals. In considering this numerical discrepancy, it must be remembered that in the intact ganglia, the synapses did not all have thickenings recognizable in any one particular section. A proportion of the synapses were recognized by other features – such as the apposition of the pre- and postsynaptic membranes, the presynaptic dense projections or vesicle clusters, or the specialization of the synaptic cleft. These features could not be used to identify denervated sites. Thus the fact that a loss of some 8×10^6 synapses per ganglion was associated with the appearance of only 4.5×10^6 vacated thickenings is probably a reflexion of the fact that of the synapses in intact ganglia only about half had postsynaptic thickenings which were sufficiently well marked to permit identification independently of the presence of the presynaptic element.

When animals with freeze lesions of the preganglionic chain were left for longer survival periods, the proximal parts of the cut preganglionic axons underwent regenerative sprouting, and within a month after operation, further changes were found in the ganglion. These changes consisted of a gradual increase in the numbers of synapses and a concomitant fall in the numbers of vacated synaptic thickenings (figure 3). By 2–3 months after operation, synapse numbers had been restored to normal levels, and vacated synaptic thickenings had virtually disappeared. The fact that these synapses belonged to the regenerated preganglionic axons

was confirmed by showing that re-cutting the regenerated preganglionic chain caused a reversion of synapse numbers to the low levels found at short survivals after the initial operation, and a reappearance of equally large numbers of vacated synaptic thickenings. Thus, the spontaneous regenerative activity of the cut preganglionic axons was capable of restoring the normal complement of synapses in the ganglion. However, even at much longer survival periods, there was no increase in synapse numbers beyond (i.e. above) normal levels.

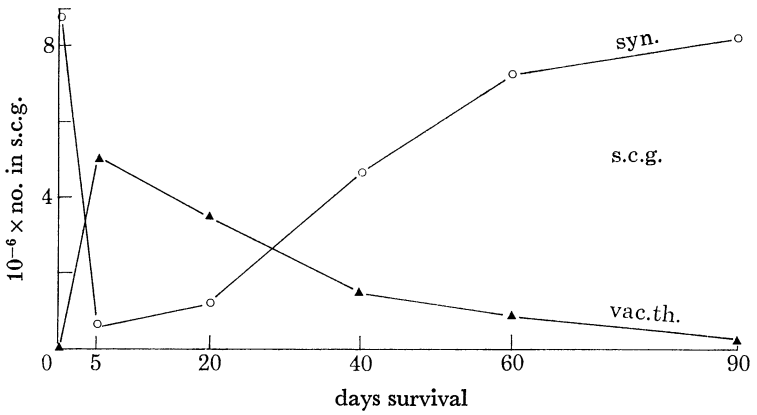

FIGURE 3. A schematic representation of the total numbers (in millions per ganglion, ordinate) of synapses (syn.) and vacated thickenings (vac.th.) in the superior cervical sympathetic ganglion (s.c.g.) at various survival times in days (abscissa) after a freeze lesion of the preganglionic chain.

These observations suggest that postsynaptic sites in the ganglion are capable of surviving after deafferentation, and this is supported by the further observation that if the preganglionic chain was surgically transected and tied down into the thoracic musculature (to prevent regrowth back to the ganglion), synapses did not reappear within the ganglion, but vacated thickenings remained recognizable for several months after operation. During reinnervation after the freeze lesion, the inverse relation between numbers of synapses and numbers of vacated thickenings indicates that the newly regenerated axon terminals had established synaptic contacts within the population of the original postsynaptic sites. The fact that the number of regenerated synapses did not exceed normal levels suggests that each neuron may have a limited number of postsynaptic sites, and this may act as an upper limit on the density of innervation which that cell can accept. However, this hypothesis requires it to be shown that the axons are potentially able to form more than the normal numbers of synapses, and the subsequent experiments show that this condition is not necessarily fulfilled in the superior cervical ganglion.

In a further series of experiments (Östberg *et al.* 1976), the preganglionic chain was surgically transected a few mm below the ganglion and the two ends joined in an end to end anastomosis by suture with fine monofilament nylon. For mechanical reasons, this anastomosis is difficult to perform, and the effectiveness of axon growth across the junction of the cut nerve was variable from one animal to another. Within the ganglion, a variable number of synapses formed. At its least effective, there was practically no recovery of synapse numbers (and no disappearance of vacated thickenings), while even at its most successful, only some 5×10^6 synapses formed, as compared to 7.5×10^6 after regeneration following a freeze lesion. Most animals were intermediate between these two extremes. This variability was largely due to local conditions at the suture. However, the variability in itself provided important correlative information. In the first

place, a comparison of the numbers of synapses and the numbers of vacated thickenings in different animals showed that there was an inverse relation between these two values. Taken together with the similar negative correlation observed in the animals with different survival times after a freeze lesion, this is further proof that reinnervation takes place at the originally denervated sites, so that the reappearance of synapses necessarily causes the disappearance of vacated thickenings. This receives further support from similar observations after anastomoses of foreign nerves (see below).

In seeking the cause of the variability in the results of preganglionic anastomoses, we counted the numbers of preganglionic axons which had regenerated across the suture. It was seen that this number of axons was also variable from one animal to another, but that the number of axons crossing the suture in any particular animal was positively correlated with the number of synapses found in the ganglion in that animal. The regression curve was a straight line whose slope gives an estimate of the average number of synapses formed by each axon. It was found that under these circumstances each axon formed on the average about 700 synapses. This figure is lower than the figure of 1100 synapses per axon found in the normal intact ganglia. However, it is known that during nerve regeneration each regenerating axon produces a large number of sprouts which explore the environment and grow back towards the target tissue. Once synaptic connections are re-established, the number of sprouts becomes drastically reduced, it being usually assumed that this is due to the loss by degeneration of those sprouts which fail to establish peripheral connections (although this is not the only possible explanation). Bray & Aguayo (1974) have shown that there is a transient, fourfold increase in axon numbers in the regenerating preganglionic chain, and this has subsided to normal at 6 months after operation. Since the experiments of the present series were taken at survival times of 3–4 months after operation (at a time when synapse numbers show no further increase), we have to take into account the possibility that the transient increase in axon numbers may not have had time to be completely reduced. With this in mind, therefore, we must allow that the figure of 700 synapses per axon formed after preganglionic anastomoses may be an underestimate of the number per axon which might be found at even longer survival periods, and therefore that the difference between this figure and the figure of 1100 synapses per axon for the normal intact ganglia may be exaggerated.

The fact that the preganglionic anastomoses did not result in the restoration of as many ganglionic synapses as are formed after the freeze lesions is itself interesting. It is clear from the freeze lesioned animals, that all the vacated sites are capable of accepting reinnervation. The preganglionic anastomoses resulted in the growth into the ganglion of numbers of preganglionic axons, axons which were capable of reinnervating denervated ganglionic sites. The fact that they did not reinnervate *all* the available sites, together with the fact that in individual animals the numbers of synapses were directly proportional to the numbers of axons, suggests that the number of synapses that each axon can form is limited. Thus, the reason that the anastomoses did not result in the reinnervation of all the ganglionic sites was simply that there were not enough axons available – i.e. that each axon had formed the maximum number of synapses of which it was capable.

If therefore, the number of 700 synapses per axon is a reflection of the maximum synaptogenic capacity of the anastomosed preganglionic axons (and taking into account the possible reasons why this may be an underestimate) then in the normal intact ganglion, it seems likely that the number of 1100 synapses per axon is also close to the limit of axonal synaptogenic capacity.

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This would have two important implications. First, the fact that after recovery from a freeze lesion of the preganglionic chain we never found hyperinnervation of the ganglion, can no longer be attributed solely to the fact that the ganglion cells have a limited number of synaptic sites, since it is also likely that even should the entire complement of 8000 preganglionic axons regenerate back to the ganglion, their total synaptogenic capacity may be insufficient to result in the formation of *more* than 8.8×10^6 synapses (i.e. 1100 synapses per axon).

The second point is of a more general nature. If the ganglion is partially denervated (e.g. by cutting some but not all of the ventral roots containing the preganglionic fibres), it is not clear how the surviving axons could show collateral sprouting (Guth & Bailey 1961; Guth & Bernstein 1961), if they have already expressed their synaptogenic potential to the full.

There is, however, one difficulty in the interpretation of the incomplete reinnervation after preganglionic anastomoses. It is known that the preganglionic fibres in different ventral roots establish connections with different groups of ganglionic neurons (Langley 1892). Thus, stimulation of different preganglionic roots results in the activation of different sympathetically innervated peripheral structures (e.g. the upper thoracic roots activate the iris, and the lower roots the blood vessels of the ear). It also appears that this pattern of specific connections may be restored as a result of the regeneration of cut preganglionic fibres (Langley 1895). This raises the possibility that preganglionic fibres could be specified with regard to the ganglion cells which they can innervate, so that when only a limited number of preganglionic fibres regenerate to the ganglion, they will innervate only the properly specified ganglionic neurones and not extend to other ganglionic neurons even though these may be denervated.

This possibility cannot be excluded without further experimentation, and one of the experiments we have used for this purpose (Östberg *et al.* 1976) was the anastomosis of foreign nerves into the denervated ganglion. The results of these anastomoses were complex, and here reference will be made only to the relevant aspects. After anastomosis of the cut cranial end of either the hypoglossal or the cervical vagus nerve into the lower pole of the deafferented ganglion, axons grew readily across the suture, and penetrated the ganglionic neuropil in large numbers. In each case some ganglionic synapses reappeared and some vacated thickenings disappeared. The numbers of synapses were characteristic of the type of anastomosis. The vagus nerve formed about 4.4×10^6 synapses in the ganglion, and the hypoglossal about 1.5×10^6 synapses. Taking various groups at different survival times, it was found that in every group the numbers of synapses were inversely correlated with the numbers of vacated thickenings. Thus, these experiments further confirm that reinnervation had occurred at the originally denervated sites exactly as in the case of re-innervation by the proper preganglionic nerves. Now, the foreign nerves each formed only a limited number of synapses, and in each case there were denervated sites still left as vacated thickenings in the ganglion. However, the axons all belonged to completely foreign nerves, which do not normally form any synapses in the ganglion. Therefore, unlike the situation with the regenerating preganglionic axons, the ability of the foreign nerves to innervate particular ganglionic sites cannot be due to re-establishment of a specific pattern of connections. Conversely, the inability of the foreign nerves to innervate the remaining deafferented sites is less likely to be due to any specific synaptic incompatibility. This suggests that in this case too, the regenerating axons had reached an upper limit of synapse formation – i.e. they had expressed their synaptogenic capacity in full, and could not form further synapses despite the close proximity of available denervated synaptic sites.

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In the experiments so far described, the best explanation of the phenomena of synapse formation in the ganglion would include:

(1) Each ganglionic neuron has a fixed number of synaptic sites; these sites persist after denervation and are available for reinnervation.

(2) Each axon can form only a limited number of synapses.

(3) During innervation by any type of compatible axons, the number of synapses is determined by the maximum synaptogenic capacity of the axons up to a limit which is set by the fixed number of postsynaptic sites on the receptor neurons.

We will now proceed to examine to what extent hypotheses such as these may be extended to a site in the central nervous system.

The septal nuclei

The situation in the septal nuclei is more complicated than that in the sympathetic ganglion. There are more different types of synapses (e.g. figure 4, plate 1). Approximately two thirds of synapses are located on dendritic spines, one third on dendritic shafts, and only 1–2 % on cell somata. The axon terminals include those of intrinsic axons or collaterals as well as those of several different extrinsic afferent fibre pathways, not all of which are known. One clearly identified group of extrinsic afferents arises in the hippocampus. These axons travel through the fimbria and terminate in both the ipsilateral and the contralateral septal nuclei. The effect of destruction of these afferents can be studied conveniently because the fimbria is a compact bundle which can be totally transected at a point between its hippocampal origin and its septal termination; this transection destroys all fimbrial afferents from the hippocampus of that side without any direct damage to the septum itself.

We have examined the neuropil of the septal nuclei at successively longer times after lesions of the fimbria (Raisman & Field 1973). Two days after cutting the fimbria, electron microscopy of the septal neuropil showed that the terminals of the transected fimbrial axons had a marked increase in electron density and shrinkage, together with the general degradation of the contained organelles (figure 5, plate 1), characteristic of the reaction of orthograde degeneration found in many central pathways. Unlike the situation in the sympathetic ganglion, these degenerating terminals largely remained still in contact with the postsynaptic elements at the synaptic thickenings. This permitted direct identification of the synapses formed by the fimbrial axons and of their precise postsynaptic sites. After section of the fimbria about 30 % of the dendritic spine synapses showed electron dense degeneration in the dorsal part of the lateral septal nucleus of the same side, and about 15 % on the contralateral side. Vacated synaptic thickenings were not seen.

At longer survival times, the degenerating axon terminals became increasingly degraded and they were engulfed by phagocytic astroglial processes. By 2 weeks after operation fewer terminals remained in contact with the postsynaptic elements, and by 1 month after operation practically none remained in contact. None the less, at no survival time did we find more than very occasional vacated synaptic thickenings (figure 6, plate 1). When the number of non-degenerating spine synapses was counted, it was found that during the first week after operation, when there was a maximal number of degenerating synapses, there was a corresponding decrease in non-degenerating synapses. At progressively longer survival times, as the number of degenerating synapses fell, the number of non-degenerating spine synapses

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rose proportionately. By the time all the degenerating synapses had been removed, the non-degenerating synapses were once again as numerous as in the normal, unoperated septal neuropil (figure 7).

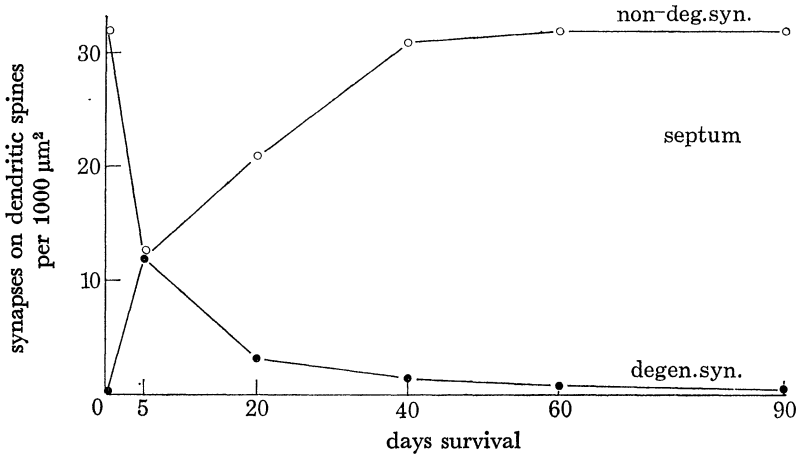

FIGURE 7. A schematic representation of the numbers per unit area of 1000 μm² (ordinate) of non-degenerating (non-deg. syn.) and degenerating (degen. syn.) synapses on dendritic spines in the septal neuropil at various survival times in days (abscissa) after transection of the ipsilateral fimbria.

The most likely explanation of the recovery of synapse numbers is that, as in the ganglion, new synapses had been formed. This suggests that, as in the ganglion, deafferented postsynaptic sites persist, and can be reinnervated. However, they only rarely appear as vacated thickenings. This absence of vacated thickenings can be ascribed to two factors. First, in the septal neuropil, the degenerating axon terminals remained in contact with the postsynaptic sites for up to 1 month, whereas in the ganglion the degenerating terminals were all removed by 24 h after operation. Secondly, in the ganglion there was a long delay before the regenerating axons could grow back to the ganglion, permeate the ganglionic neuropil, and differentiate new terminals capable of reinnervating the ganglionic sites. In the septal neuropil, however, it seems that the reinnervation took place by means of local axon terminals which were already in place very close to the denervated sites and which did not, therefore, need time for elongation.

Structural changes occurring in the septal neuropil gave some clues as to the mechanism of this reinnervation. An examination of the septal neuropil at survival times long enough for complete restoration of the synapse numbers revealed a proportion of synapses with an unusual feature – the axon terminal made synaptic contact in the plane of section with more than one postsynaptic element. These structures (figure 8, plate 1), which we have called double synapses, were uncommon in the normal septal nuclei in unoperated animals. Their association with the postulated reinnervation process suggested the possibility that the new synapses were formed by existing local axon terminals sprouting in such a way as to extend into the region of the denervated postsynaptic site, and there develop an additional presynaptic specialization which innervated the denervated site, thus converting the formerly 'single' synapse into a 'double' synapse. The suggestion that it is local axon terminals which formed the new synapses in the denervated septum is also supported by the fact that the cut fimbrial axons showed no sign of regenerating back across the lesion. Unlike the ganglion, therefore, the originally cut axons could not be the cause of the recovery of synapse numbers.

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One way of examining the question of which local axon terminals reinnervate the denervated septal sites is to take advantage of the fact that the lateral septal nucleus receives an input both from the fimbria of the same and from that of the opposite side. Therefore, one fimbria was cut, and after allowing time for complete restoration of synapse numbers (i.e. at least 6 weeks), the opposite fimbria was also cut (Raisman *et al.* 1976). At a survival time of 2 days after the second lesion the occurrence of electron dense orthograde degeneration was used to identify and assess quantitatively the terminals belonging to the second fimbria. We found that the second fimbria had extended its territory so greatly that it had now formed a number of synapses equal to the sum of both the original ipsilateral and contralateral projections. This occurred regardless of whether we examined the ipsilateral fimbria extending into the territory of the contralateral fimbria or *vice versa*. This suggests that the sites left vacant by section of one fimbria had been exclusively reinnervated by the opposite fimbria. The extent to which this process was selective for the remaining fimbria can best be appreciated when one considers that after section of one fimbria, the remaining fimbrial terminals comprise no more than 20 % of the entire population of axon terminals in the septum; none the less they occupy all the denervated sites.

At first sight, this selectivity could be due to some kind of specific attraction between fimbrial axons and fimbrially innervated postsynaptic sites. However, there are two reasons to reject this hypothesis. The first arises from a consideration of the anatomical arrangement of the fimbrial axons. Cajal (1911) showed that at least a proportion of fimbrial axons branch before reaching the septum. One branch crosses the midline in the ventral hippocampal commissure and turns back into the opposite fimbria to reach the opposite hippocampus. This contralateral branch would be cut when a lesion is placed in the other fimbria. Thus, the terminal branches of the fimbrial axons which extend their synaptic distribution in the septum after the opposite fimbria has been cut, belong to parent axons which have already been deprived of a large part of their own synaptic territory (i.e. the opposite hippocampus). This deprivation may in itself be a factor in encouraging the remaining, septal branches of these axons to sprout. If axons have a limited synaptogenic capacity (as suggested by the findings in the ganglion), such a 'pruning' measure may be important for releasing sufficient synaptogenic potential for the formation of new synapses necessary for the axons to reinnervate denervated areas.

The second, and most conclusive evidence that the fimbrially denervated postsynaptic sites in the septum are not absolutely specified to accept only fimbrial axons comes from an additional series of experiments in which both fimbria were cut either simultaneously or sequentially (Raisman *et al.* 1976). Allowing sufficient time for the removal of the degeneration (which seems to take longer under these circumstances) it was found that once again the synapse numbers in the septum were restored to normal levels, and there was a further large increase in the numbers of double synapses. Since there were no fimbrial axons left in the septum at this time, we must conclude that the denervated postsynaptic sites had now been reinnervated by non-fimbrial axons. Thus, the apparently exclusive preference for fimbrial reinnervation by fimbrial axons after destruction of only one fimbria (i.e. when other fimbrial axons are available) is not due to an absolute type of specific compatibility between fimbrial axons and sites.

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Summary

The comparison of the reaction in the septal neuropil with that in the ganglion reveals some similarities and some differences (compare figure 3 with figure 7). In both areas, the postsynaptic sites survive after deafferentation. In both areas, the sites can be reinnervated, and the number of synapses can therefore be restored to normal levels. In the ganglion the original preganglionic axons are capable of regenerating back to the denervated sites. In the septum the cut fimbrial axons do not regenerate. The mechanism of septal reinnervation is that local, undamaged axon terminals form additional specializations which occupy the denervated sites. In the ganglion the degeneration is removed rapidly, and during the interval required for the regenerating axons to reach the denervated sites, the sites are recognizable as vacated thickenings. Vacated thickenings only occur occasionally in the septal neuropil because the degenerating terminals remain in contact with the postsynaptic sites for long periods, and there is no significant delay between the removal of the degenerating presynaptic elements and the occupation of the postsynaptic sites by the newly formed axon terminals.

The similarities between the regulation of synapse formation in the denervated sympathetic ganglion and the septal nuclei are so great as almost to obscure the vital difference: the septal nuclei are not reinnervated by the originally cut fimbrial axons. The fimbria – behaving in a manner typical of cut central nervous fibre tracts in mammals – does not regenerate. The present experiments serve to narrow the nature of the defect. Neither the cut fimbrial axons nor their cells of origin in the hippocampus undergo retrograde degenerative changes. Neither the denervated postsynaptic sites in the septum nor the neurones on which they are borne undergo transsynaptic (transneuronal) degeneration. On the contrary, the sites are spontaneously reinnervated. Further, the reinnervating terminals arise preferentially from the septal branches of axons belonging to the opposite fimbria. The septum shows an actual predilection for reinnervation of the sites by fimbrial axons. Why therefore does the originally cut fimbria not regenerate? At present it seems most likely that the defect resides in an inability of the cut axons to regenerate across the site of injury in a manner necessary for them to reach the denervated target tissue. The present studies do not offer any immediate explanation of this defect. I hope they show that the question of central nervous regeneration is still alive. Against such a positive background as is now accumulating, the further experimental definition of the nature of the defect may enable us to envisage some kind of positive interference, interference of a type which may one day permit us to intervene beneficially in the treatment of injury to the human brain and spinal cord.

References (Raisman)

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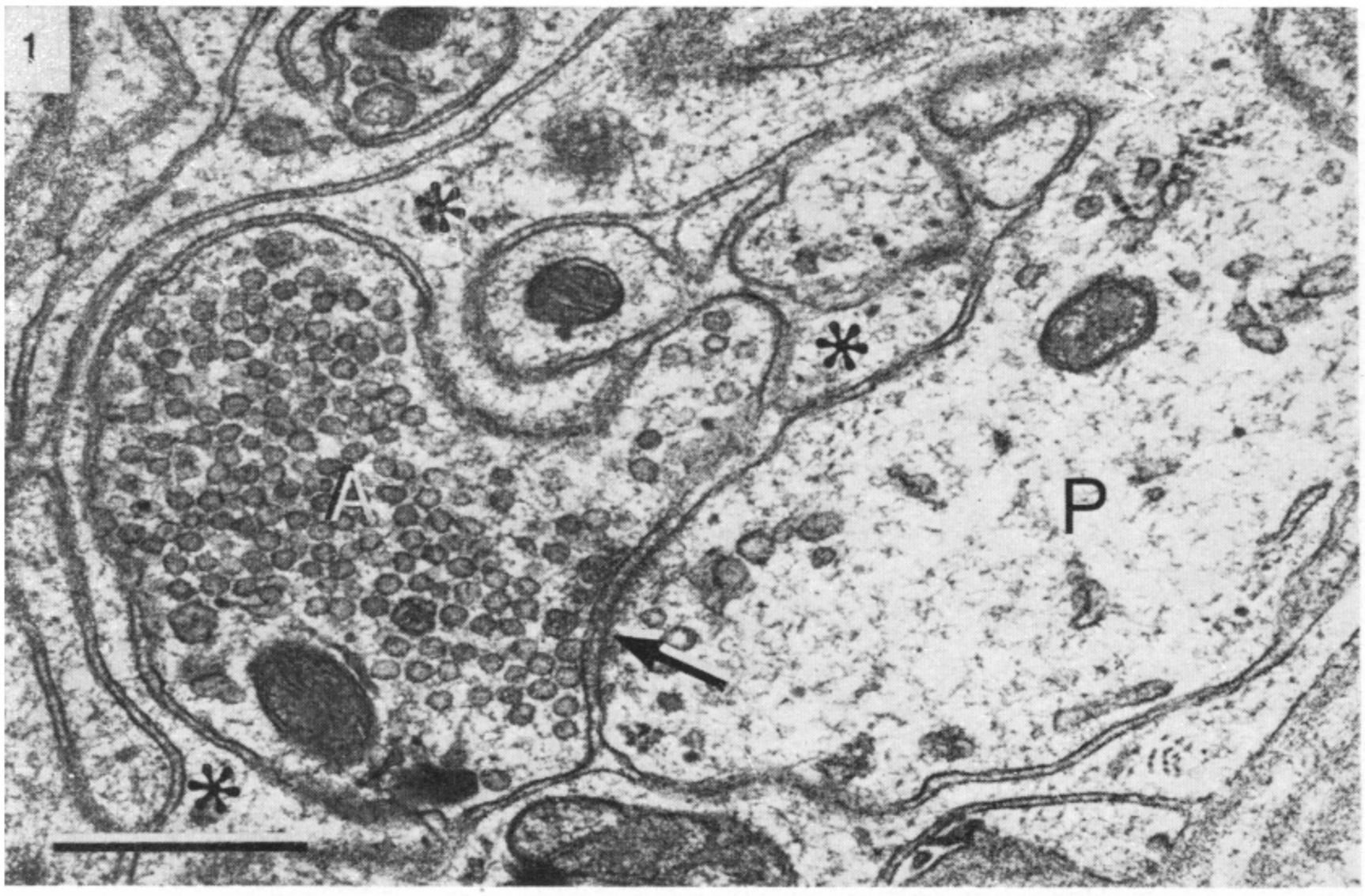

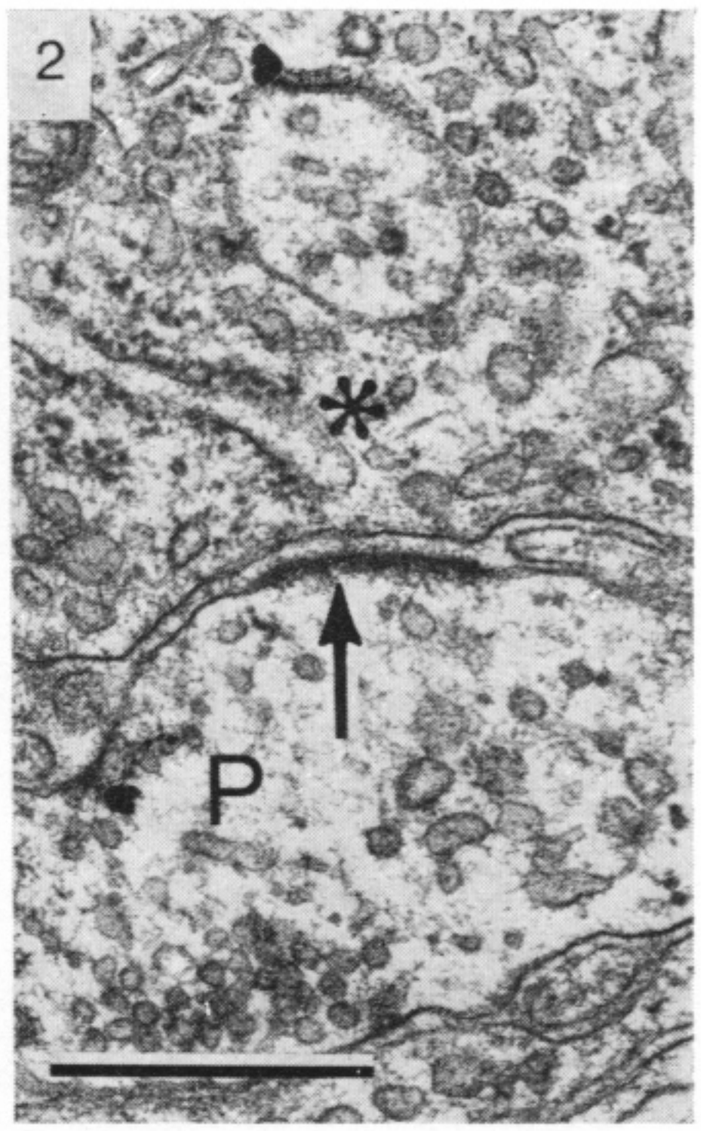

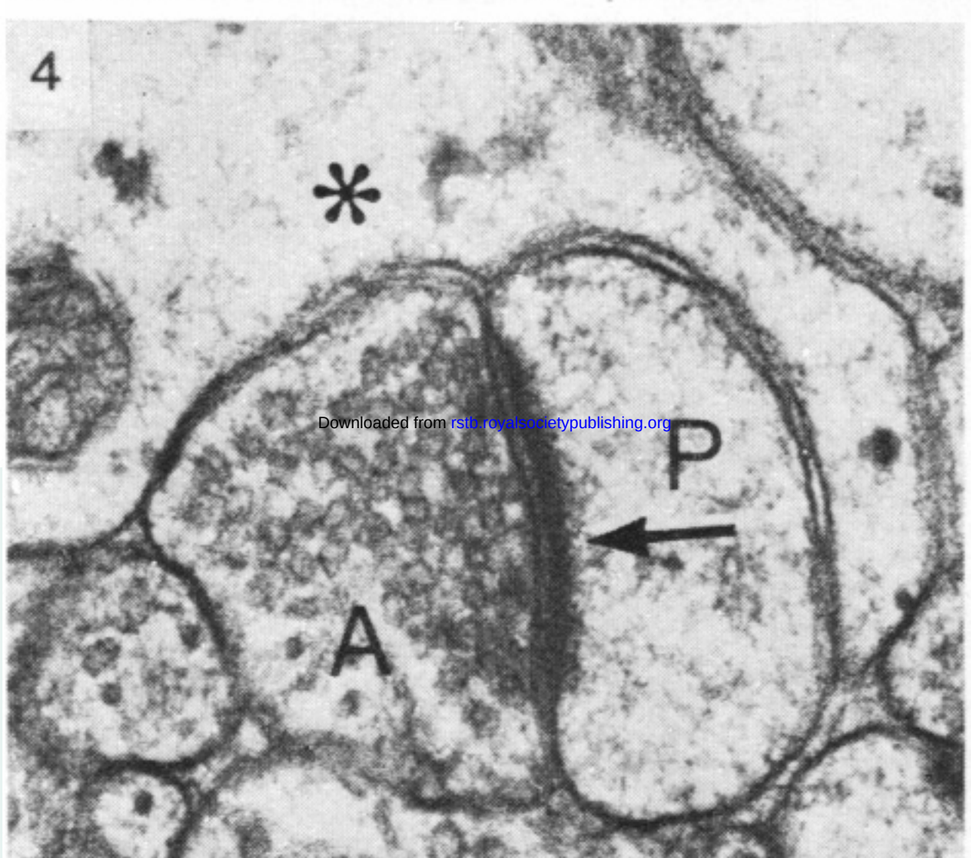

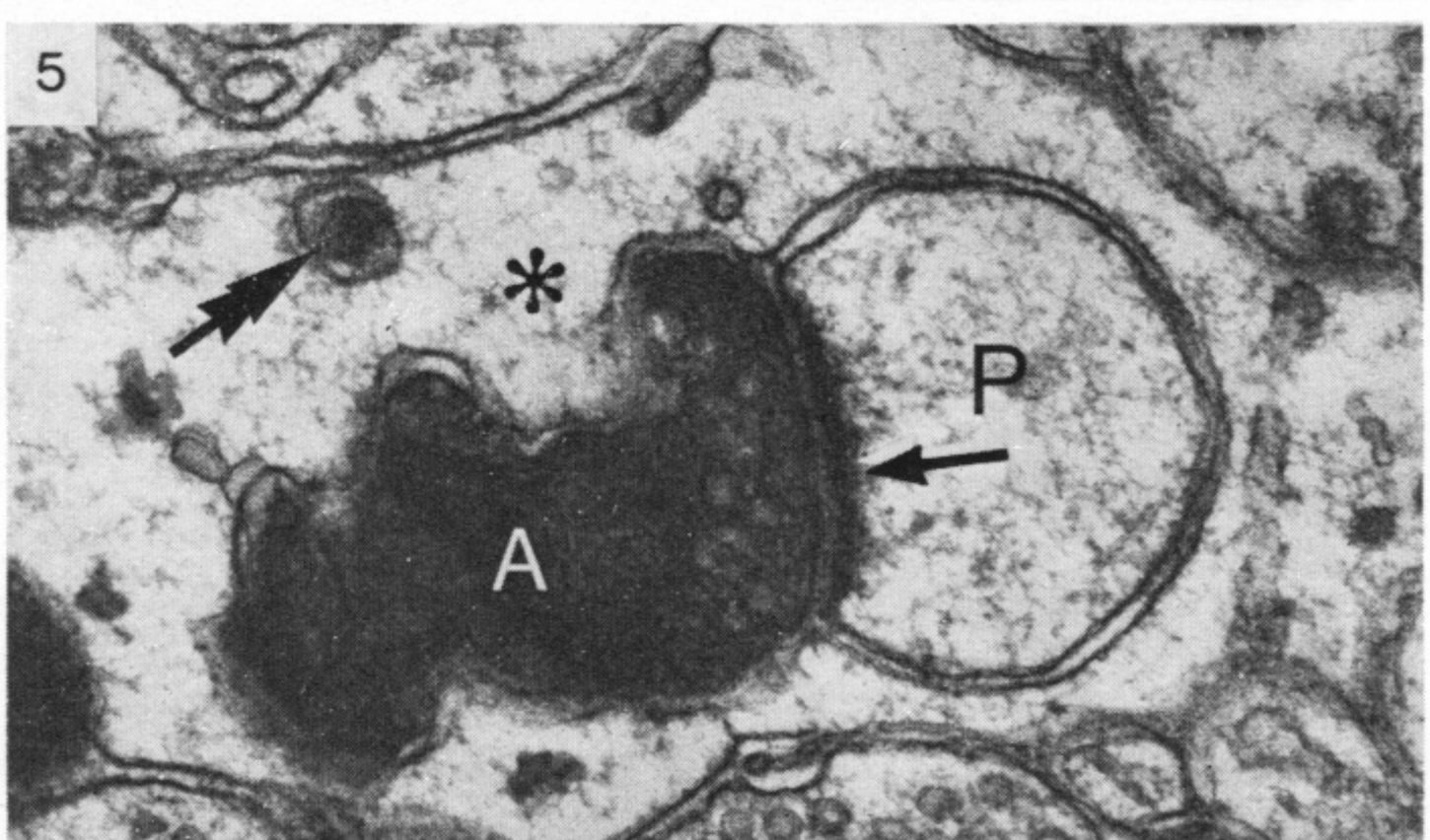

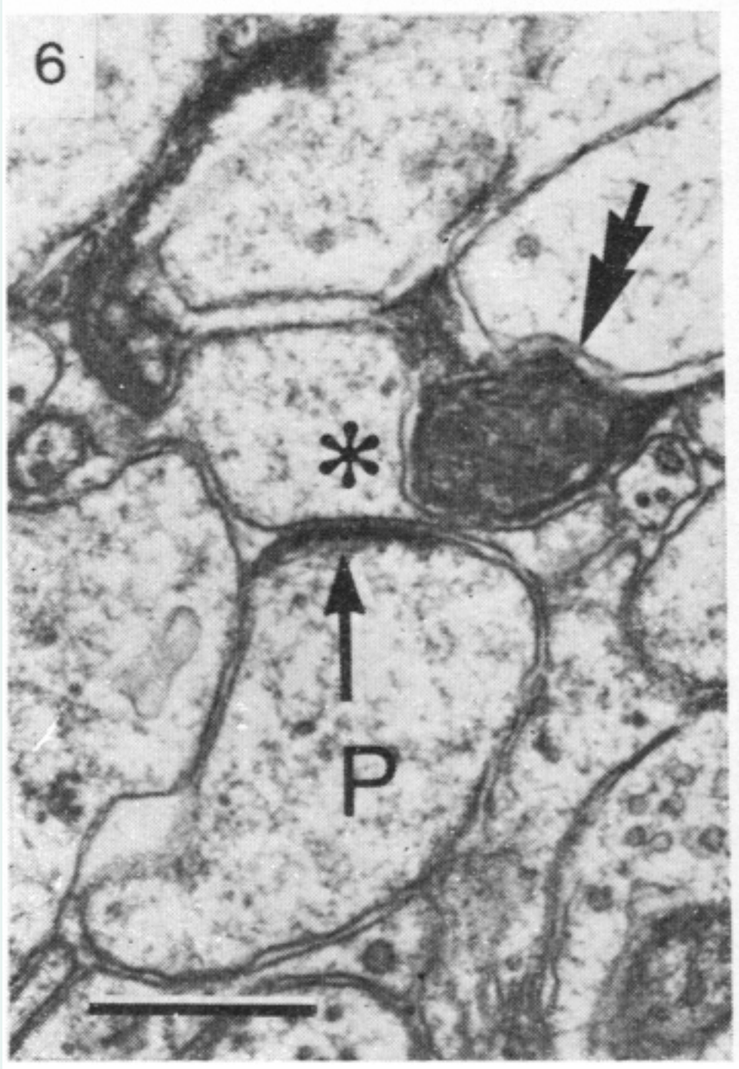

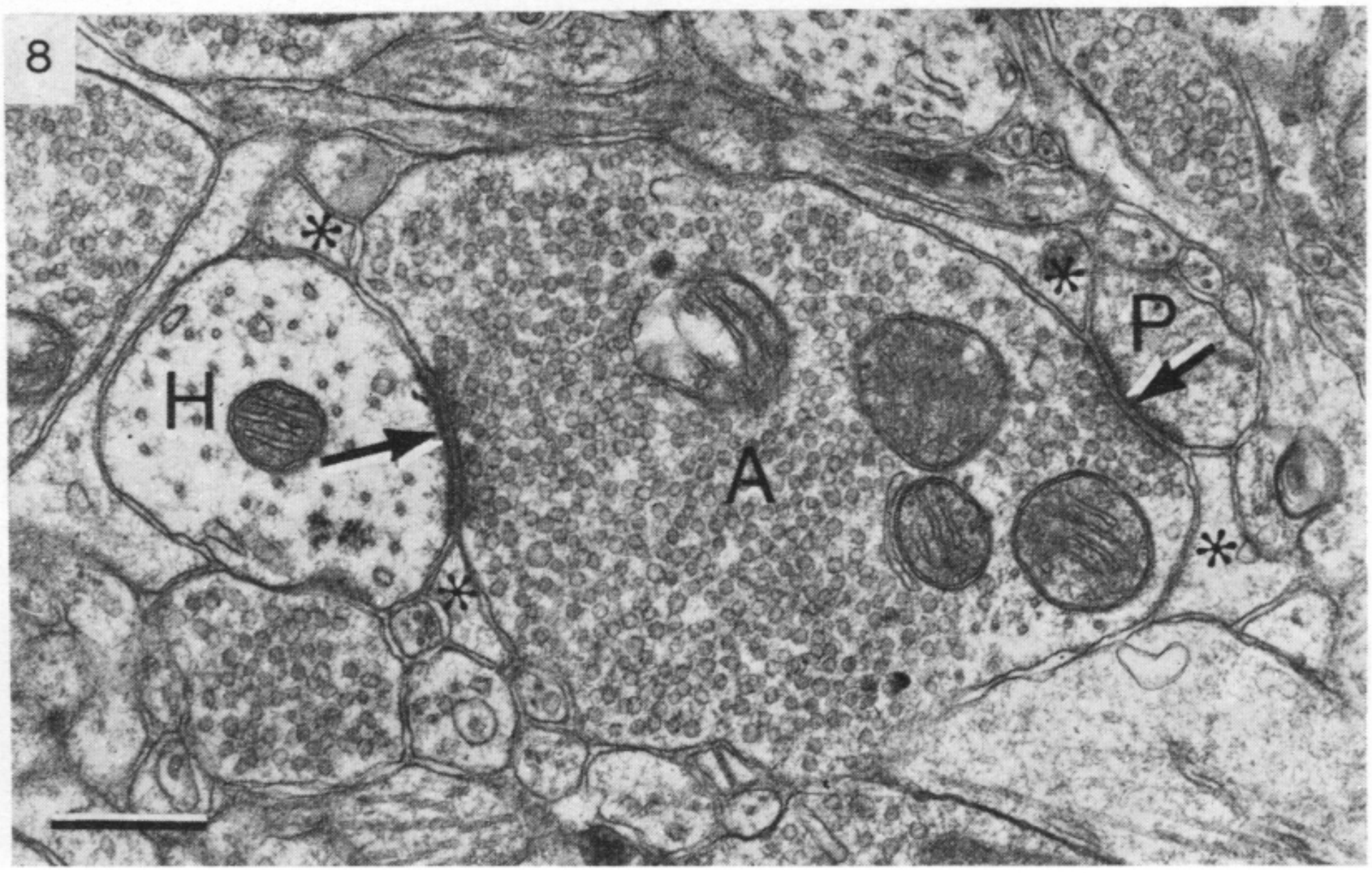

FIGURES 1, 2, 4-6 AND 8. For description see opposite.