

**PERSISTENT MODIFICATION OF SYNAPTIC  
INTERACTIONS BETWEEN SENSORY AND MOTOR NERVE  
CELLS FOLLOWING DISCRETE LESIONS IN THE  
CENTRAL NERVOUS SYSTEM OF THE LEECH**

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**SUMMARY**

We have examined changes that develop in the synaptic interactions of sensory and motor nerve cells following surgical lesions to the central nervous system of the leech. In one type of operation an individual ganglion was isolated from the rest of the nervous system by severing all the incoming and outgoing fibres. During the next few weeks, marked changes appeared in synaptic interactions.

1. In chronically isolated ganglia inhibitory potentials were recorded in the motoneurone which raises the skin into ridges (the AE cell) following impulses in sensory neurones that respond to pressure (P) or noxious (N) stimuli. In contrast the same AE cell in ganglia taken from normal animals shows excitatory synaptic potentials when the P or N sensory cells are stimulated.

2. Another altered synaptic interaction in ganglia isolated by lesions was that between sensory cells responding to touch and a motoneurone that supplies longitudinal muscles (L cell). Instead of the pure, electrical coupling potential seen normally, a large, additional chemically mediated excitatory potential was also apparent.

3. Some of the changes in synaptic interactions were not restricted to synapses within the isolated ganglion, but appeared gradually over the following year in successive ganglia along the length of the ventral nerve cord.

4. Indirect evidence suggests that the altered synaptic potentials that

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became conspicuous after operations are also present but smaller and obscured in normal animals.

5. It is concluded that some synapses in the leech nervous system are more readily changed than others by cutting the connectives. Furthermore, these changes influence in a predictable manner the way in which the animal behaves in response to mechanical stimuli.

#### INTRODUCTION

A feature of synapses in the central nervous system is that their properties can be modified by such circumstances as abnormal use or disuse or by lesions (see for example Wiesel & Hubel, 1965; Kandel & Spencer, 1968; Yoon, 1972). At present, there is little information about the mechanisms by which the interactions of neurones can be changed. Almost the only instance in which it is known how the properties of a post-synaptic cell can be altered for periods of weeks or months is the supersensitivity of skeletal muscles and certain autonomic neurones to acetylcholine (Miledi, 1960; Kuffler, Dennis & Harris, 1971), which persists until activity is resumed or new fibres grow back to form connexions (Miledi, 1970; Lømo & Rosenthal, 1972; Jansen, Lømo, Nicolaysen & Westgaard, 1973). It is natural to speculate whether similar processes might occur in neurones within the C.N.S. If so, partial denervation of a cell might modify transmission at the remaining synapses. In the vertebrate C.N.S., with its extreme complexity, direct experimental evidence is hard to come by for technical reasons.

An alternative approach is to use a simpler nervous system containing fewer synapses, where one could hope to determine in detail first the normal arrangement, then what changes can be produced and finally the underlying mechanisms. In many respects the nervous system of the leech offers advantages for studying normal and altered synaptic functions. A striking feature of the organization of the C.N.S. is its constancy from ganglion to ganglion and from animal to animal. This makes it possible to identify reliably an individual neurone and to ascertain (1) its function, (2) the pathways followed by the axons, (3) the fields it innervates in the periphery, (4) the pattern of synaptic connexions made with other neurones, (5) the properties of synapses, such as whether they are chemical or electrical, excitatory or inhibitory, monosynaptic or indirect (Nicholls & Baylor, 1968; Nicholls & Purves, 1970, 1972; Purves & McMahan, 1972). At the same time, the extreme stereotopy of normal animals provides a baseline for detecting abnormalities or changes in the properties of synapses (Jansen & Nicholls, 1973; D. Kuffler & Muller, 1974).

In the present series of experiments, we have examined physiological changes that occur in synapses after cutting discrete bundles of axons and

allowing the animals to recover. Our aim has been to establish which synapses change in their properties, and which lesions are most effective. The results to be described show that some synaptic interactions do become modified in a clear-cut and consistent manner after well defined groups of axons have been transected. A brief report of some of these observations has been published elsewhere (Jansen & Nicholls, 1972).

METHODS

*Brief description of the preparation.* The principal features of the leech nervous system and the positions of the sensory and motor nerve cells used for this study are shown in Fig. 1 (see also Coggeshall & Fawcett, 1964; Nicholls & Baylor, 1968). The ganglia are linked by two large bundles of axons, the connectives, and a smaller nerve between them, the nerve of Faivre. Each connective contains several thousand axons, many of which run for more than one segment. The ganglia supply the body

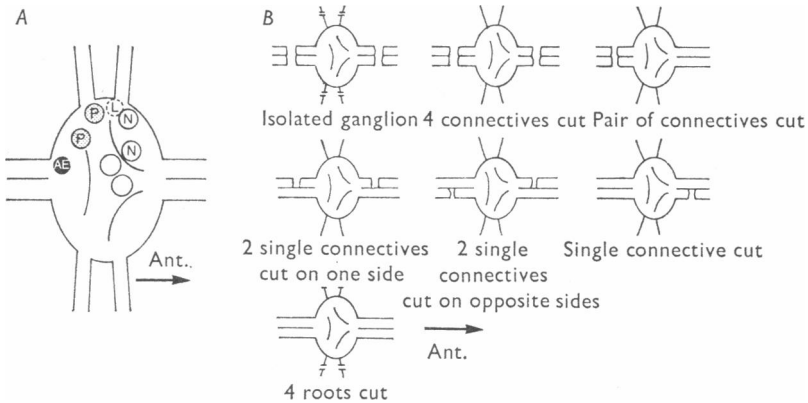


Fig. 1A. Diagram of the ventral aspect of a segmental ganglion in the leech, illustrating the positions of pressure (P) and nociceptive (N) sensory neurones and annulus erector (AE) and large longitudinal (L) motoneurones on one side of the ganglion. The ganglion is linked to neighbouring ganglia by paired axon bundles called *connectives* and communicates with the periphery via axons in the *roots*.

B, chronic lesions as described in the text were made by severing roots and connectives in the patterns shown. An 'isolated' ganglion is one that has had all roots and connectives cut. Other ganglia are described as 'partially isolated'.

wall through pairs of roots. Three types of sensory cells have been identified, each with its specific modality: touch (T), pressure (P) and noxious (N) mechanical stimulation. These sensory cells make excitatory connexions upon motoneurones. Two of these were used for the present investigation, namely the L cell which shortens the segment by exciting the longitudinal muscles, and the AE cell which erects skin annuli into ridges (Stuart, 1970). The synaptic connexions are all made in the neuropile. There are no synapses on the cell bodies. These pathways between sensory and

motor cells have been shown to be monosynaptic by a number of criteria. The connexions of T cells to the motoneurons are electrical; the others are predominantly chemical (Nicholls & Purves, 1970; Muller & Nicholls, 1974). In normal ganglia one observes a depolarizing excitatory synaptic potential in an AE or L cell after a sensory cell has been stimulated. There are also known connexions of sensory cells in one ganglion with motor cells in the next, which will be mentioned later.

*Types of operation.* Leeches were anaesthetized with 8% alcohol and various axon bundles sectioned (Baylor & Nicholls, 1971). When both connectives were cut and the roots sectioned close to the ganglion, there was no regeneration. The types of cut made are shown in Fig. 1 B. When a single connective was transected, Faivre's nerve was also cut. To prevent regeneration of the connectives the cut was made directly at the site where the surrounding blood vessel had been opened. After recovery from anaesthesia the animals were kept at 2 °C in Ringer fluid for 1 or 2 weeks and then in water at 17–22 °C. Some were put back into the cold for periods of several months after changes had developed while others were kept at 17–22 °C. With these procedures the temperature appeared to make no difference to the final results we obtained. All experiments were made at 17–22 °C. Usually about 50% of the leeches died within the first 4 weeks, whereas others lived for as long as a year or more. They were fed on occasion by filling a natural sheepskin condom with serum or with L15 culture medium containing additional glucose, an effective if unaesthetic procedure (Galun & Kindler, 1966).

*Intracellular recording of synaptic potentials.* The methods have been described in detail elsewhere (Nicholls & Purves, 1970). Electrodes filled with 4 M-K acetate and having resistances of 80–100 M $\Omega$  were inserted into an identified T, P or N sensory cell and the L or AE motoneurone. By use of WPI preamplifiers the cells were stimulated by injecting current while recording their membrane potentials. Single shocks were routinely repeated once every 10 sec. In most experiments the Ringer fluid contained (in mM): NaCl, 115; KCl, 4; CaCl<sub>2</sub>, 7.5; Tris-maleate buffered to pH 7.4 with NaOH, 10; glucose, 8.6. The CaCl<sub>2</sub> in this fluid is more concentrated than the usual 1.8 mM in order to make the synaptic potentials larger and easier to record. When necessary, Ca and Mg concentrations were altered by exchanging with Na.

## RESULTS

### *Changes within isolated ganglia*

Isolated ganglia removed for experiment after the operation appeared normal under the dissecting microscope. Individual sensory and motor nerve cells could be readily identified and impaled with micro-electrodes. We did not make systematic measurements of their electrical properties, except to ascertain that the resting and action potentials were similar to those in normal ganglia. Each type of cell continued to give characteristic and distinctive impulses.

*Altered balance of excitation and inhibition.* In ganglia isolated for 30 days or more clear inhibitory synaptic potentials were recorded in the AE motoneurone following an impulse in a P or an N sensory cell. Normal ganglia typically showed excitatory potentials (in Fig. 2A, B). Fourteen annulus erector motoneurons were impaled in ganglia isolated for over 30 days and all showed pronounced inhibitory potentials after P and N cell

stimulation. In some instances a small excitatory potential could be discerned preceding the hyperpolarization.

The synaptic potentials recorded in the L motoneurons were also changed by isolating a ganglion, but more selectively. Stimulation of a P sensory cell gave rise to a new, added inhibitory response in the L cell, resembling that seen in the AE cell (Fig. 3). The initial excitatory potential was still present, but cut off by the hyperpolarization. Occasionally the inhibitory potential was apparent only when two shocks were given to the P cell.

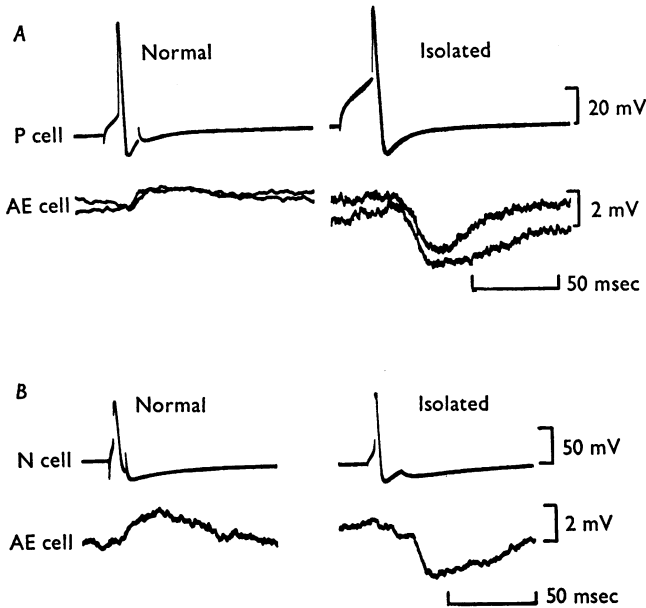


Fig. 2. Synaptic potentials in the annulus erector motoneurone (AE) following impulses in pressure (P) and nociceptive (N) sensory neurones in a normal ganglion and in one that had been isolated within the animal by cutting the roots and connectives 36 days (A) and 315 days (B) before the experiment. In the isolated ganglion, inhibitory synaptic potentials arose in the AE cell instead of the normal, monosynaptic excitatory potentials.

In contrast, however, the same L cell showed an excitatory synaptic potential, with no trace of inhibition, in response to stimulation of a nociceptive sensory cell. Hence, while both P and N cells could produce abnormal inhibitory responses in the AE cells of isolated ganglia, only the P cell inhibited the L cells.

An obvious feature of recordings made from cells in isolated ganglia was the prevalence of 'spontaneous' inhibitory potentials. Their frequency was higher than normal in the AE and L cells and they also appeared in neurones such as the N cells where they are not normally seen. In most

but not all isolated ganglia the synaptic interactions between two N cells were also altered from excitation to inhibition, as shown in Fig. 4 (see Baylor & Nicholls, 1969).

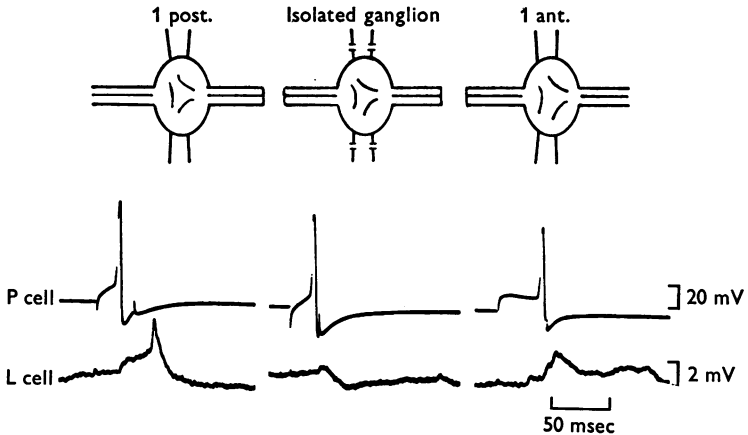


Fig. 3. Normal and changed synaptic effect of a P sensory cell on the L motoneurone. In a ganglion isolated for 38 days, an impulse in the P cell gave rise to an inhibitory potential in the L cell; a trace of the normal excitation can be discerned. In the ganglia next to the isolated one, in the same animal, impulses in P cells elicited normal excitatory synaptic potentials in the L cells.

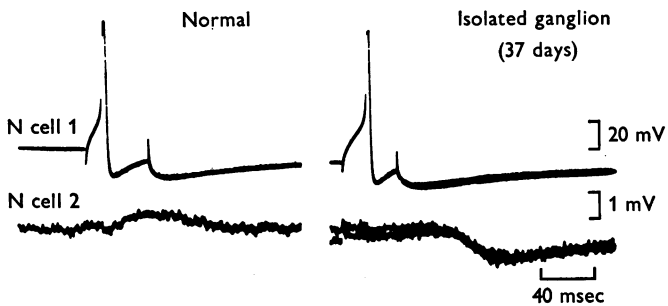


Fig. 4. Normal and changed synaptic interactions between N sensory neurones. Normally an impulse in one N cell gives rise to excitatory synaptic potentials in the three other N cells of the same ganglion. In the ganglion which had been completely isolated in the leech 37 days previously, inhibitory potentials arose in one N cell after stimulation of the other.

*Changes in the balance of electrical and chemical synaptic transmission.* In normal ganglia the sensory cells that respond to light touch (T cells) are electrically coupled to the L motoneurone (Nicholls & Purves, 1970). The synaptic potential that results in the L cell from T cell stimulation is small and has all the hallmarks of being transmitted electrically; an example is

shown in Fig. 5. In ganglia isolated for 30 days, a large chemically mediated synaptic potential appeared in the L cell after an impulse in the T cell (Fig. 5). This second component arose after a delay of about 5 msec, was abolished by Mg and was greatly amplified by hyperpolarizing the L cell, as expected for a chemical synapse.

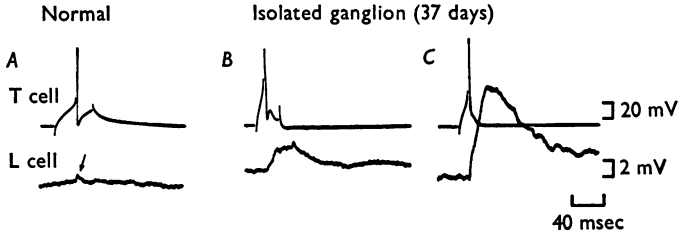


Fig. 5. Abnormal chemical synaptic potentials in the L motoneurone within an isolated ganglion following stimulation of a touch (T) sensory neurone.

A shows the normal response of the L motoneurone which is a small electrically mediated potential (indicated by arrow).

B shows the results obtained in a ganglion isolated 37 days previously by cutting roots and connectives; an impulse in the T cell produced a large, delayed excitatory potential characteristic of a chemical synapse.

C hyperpolarizing currents injected into the L cell increased the amplitude of the synaptic potential confirming that the newly appearing component was initiated chemically. In this record the L cell had been hyperpolarized by about 30 mV. (The current-voltage relation of the L cell is linear, and hyperpolarization has little or no effect on the electrically mediated potential produced by the T cell in normal ganglia. Nicholls & Purves, 1970.)

*The timing of changes in isolated ganglia.* All the changes in synaptic interactions described above were consistently seen by about 30 days after ganglia had been isolated. At earlier times the changes were less marked and harder to define. For at least two weeks after the operation the interactions between cells appeared normal. Next an inhibitory potential could often be seen in the AE or L motoneurone, but only after the second of two impulses in a sensory neurone following each other at short intervals (approximately 50 msec). At this stage some sensory cells were more effective than others in producing inhibition. The inhibitory potential appeared with progressively greater regularity and masked more of the original excitatory component, so that after about 30–40 days the final state had been achieved. The longest time after operation that we have examined an isolated ganglion is 315 days; it was not noticeably different from those tested after 30–40 days.

*Effects of partial isolation on synaptic interactions within a ganglion.* A number of different operations were made to see which nerve bundles had

to be cut for changes in synaptic interactions to be produced in a ganglion. In some animals all four of the roots were cut, leaving the connectives intact (Fig. 1*B*). After this procedure the synaptic potentials in AE and L motoneurons following impulses in sensory neurones were not noticeably changed. As in normal ganglia, excitatory synaptic potentials with no inhibition were produced by P and N cells, and the T cells gave rise to a purely electrical potential in the L cell. In contrast, when both pairs of connectives were completely severed, all the changes seen in completely isolated ganglia became apparent, even though the four roots were still intact.

If some but not all of the connectives were cut, the changes were less severe and their characteristics depended on the site of the lesion. Changes were most obvious when the nerve cord was completely transected (i.e. a cut through both of the connectives and Faivre's nerve linking a pair of ganglia). The ganglia on either side of such a cut correspond, of course, to those adjacent to an isolated ganglion. Their AE cells showed clear inhibitory synaptic potentials with P or N cell stimulation. The initial excitatory component was, however, more conspicuous than in ganglia that had been isolated completely or had had all four connectives cut. The inhibitory potentials were also evoked with less regularity. Sometimes only one or two of the sensory cells could evoke inhibitory potentials, while the other produced excitation. In these respects ganglia with one pair of connectives cut resembled isolated ganglia at an early stage, about 20 days after the operation, before changes had become fully established. There was little further progress and even after a year the changes were usually less pronounced than in an isolated ganglion. Section of a single connective, or of two single connectives, one at each end of a ganglion, (Fig. 1*B*) gave rise to little or no consistent changes in the synaptic potentials of the AE cell. This was true whether the cuts were on the same or opposite sides of the animal.

The synaptic potentials recorded in L cells were not changed in ganglia next to a complete transection of the cord (Fig. 3). Thus the L cells in ganglia adjacent to an isolated ganglion showed a normal, purely excitatory potential after P cell stimulation and only an electrically mediated potential after T cell stimulation. Occasionally we did observe suggestions of inhibition and delayed chemical excitatory synaptic potentials, but they were inconsistent. As expected from these observations, transection of a single connective or two single connectives had no discernible effect on the interactions of sensory cells with L cells within a ganglion. We shall see later, however, that such preparations do show characteristic changes in certain of the interactions of sensory cells in one ganglion with motor cells in the next.



*Progressive development of changes in synaptic interactions within ganglia at a distance from the site of cord transection.* The experiments described above have shown that the ganglia on each side of an isolated ganglion exhibit some, but not all of its characteristic changes. A natural question was whether similar changes would also appear in other ganglia, more distant from the site of cord transection. To test this, synaptic interactions were examined in fourteen or so of the ganglia of the ventral nerve cord at various times after isolating a ganglion or transecting the cord. In the first

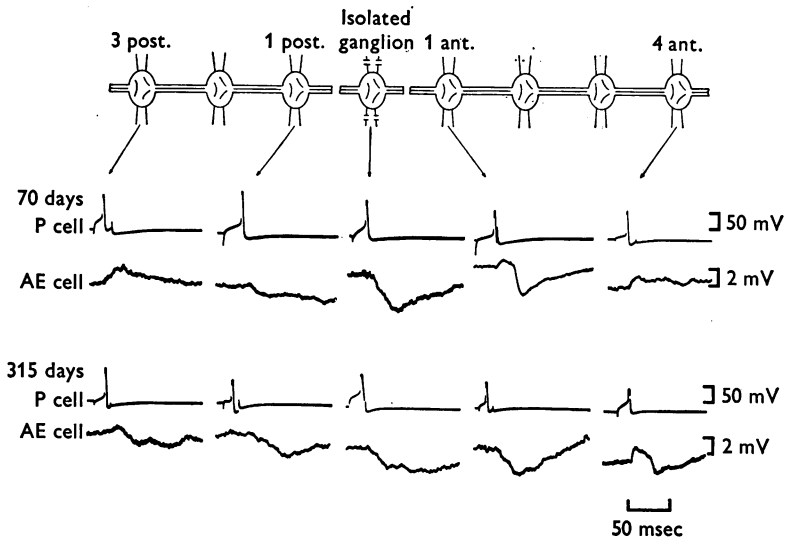


Fig. 6. With progressively longer times, synaptic potentials in the AE motoneurone became changed in ganglia along the length of the animal. Inhibitory synaptic potentials appeared first in the isolated ganglion after about one month, and after about 70 days in the neighbouring ganglia (1 post. and 1 ant.). The inhibitory synaptic potentials were often preceded by small excitatory potentials. In more distant ganglia 1 or 2 months after the operation, impulses in P sensory cells still produced normal excitatory synaptic potentials (3 post. and 4 ant., 70 days). Later (315 days) inhibitory synaptic potentials in the AE motoneurone appeared in progressively more distant ganglia. Even after a year, however, synaptic potentials in AE cells of ganglia far from the lesion were less changed than those within the isolated ganglion. A similar profile and time course of changes were seen in the effects of N sensory neurones upon AE motoneurones.

stage, about 30–40 days after the operation, inhibitory potentials replaced the excitatory ones only in the isolated ganglion and its immediate neighbours. Gradually, the change spread to more distant ganglia. By about three months inhibitory potentials appeared in the AE cells of ganglia along the entire length of the cord. Examples of this spread are

shown in Fig. 6. Even after a year, however, the completely isolated ganglion usually showed more marked changes than the others. Furthermore, only in the isolated ganglion were the synaptic potentials of L cells altered (see above).

*Normal and altered signalling from one ganglion to the next*

Although section of a single connective did not modify synaptic interactions within a ganglion it did lead to certain obvious changes in signalling from sensory cells in one ganglion to motor cells in the next. One important feature of these observations was that they demonstrated that interneurons contributed to the development of abnormal inhibitory potentials.

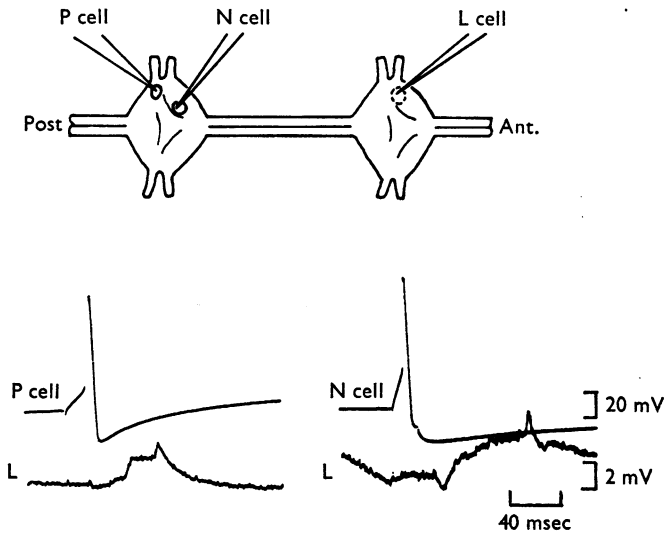


Fig. 7. In normal leeches the connexions of the P and N sensory neurones in one ganglion upon the L motoneurons in both of the adjacent ganglia are excitatory, as in the example shown on the left. However, the P and N cells in one ganglion can cause a small inhibitory potential to appear in the L cell of the next anterior ganglion. This is seen only rarely and the example shown on the right is exceptionally pronounced.

However, before the changes that follow section of a single connective are described, one needs to know how sensory P and N cells in one ganglion are connected to the L motor cells in the next in normal leeches.

*Normal connexions between P and N cells in one ganglion and the L cells of adjacent ganglia.* The axons of P and N sensory cells run to neighbouring ganglia by way of the ipsilateral connectives (see Baylor & Nicholls, 1969; Nicholls & Purves, 1970). In normal leeches an impulse in a P or N sensory cell evokes an excitatory synaptic potential in the L on the same

side of adjacent ganglia. Examples are shown in Fig. 7. Although direct evidence is lacking, the excitatory interganglionic connexions of sensory and motor cells in adjacent ganglia behaved as though they were monosynaptic. Thus, a unitary excitatory synaptic potential of constant latency was recorded in the L cells following each impulse in a P or N cell in the next ganglion. Moreover, the synaptic potentials, which were absent in fluid containing high Mg (20 mM), appeared when 15 mM-Ca was added to the Mg fluid. In this solution the threshold for firing is raised and polysynaptic pathways become blocked (Nicholls & Purves, 1970; see also below).

In addition to the direct excitatory connexions between ganglia, careful inspection revealed a second interaction that was inhibitory. Fig. 7 shows an example of the inhibitory potential in an L cell evoked by stimulation of an N cell in the next posterior ganglion. Unlike the excitation which travelled in both directions, inhibitory potentials were recorded in an L cell *only* when a P or N cell in the ganglion behind it was stimulated. Experiments to be described below indicate that inhibitory interneurons are activated by the P and N cells and their axons run forwards to the next ganglion towards the head. In normal animals the inhibitory component of the response was not usually as pronounced as that shown in Fig. 7 so that it could often be detected only with a signal averager. In summary, the normal effect of P or N cells on L cells in the next ganglion is to produce an excitatory potential; in one direction, from posterior sensory to anterior motor, there is a hint of inhibition.

*Altered synaptic interactions between ganglia after section of a connective.* Several weeks after a single connective had been cut, striking changes appeared along the length of the nerve cord. When a P or N cell was stimulated in one ganglion a large inhibitory potential followed in the L cell of the next most anterior ganglion. In the other direction of stimulation (anterior sensory to posterior motor) the L cell showed a normal excitatory potential with no trace of inhibition. An example of an abnormal inhibitory potential is shown in Fig. 8 (see also Jansen & Nicholls, 1972). Such potentials were usually present in L cells of ganglia along the length of the nerve cord on both sides of the animal, even though only a single connective had been cut. They were generally obvious by about 60 days after the operation and thereafter remained constant for at least 357 days, the longest time that we have waited. In many animals we saw that the changed synaptic interactions were still present after the cut connective had regenerated (see Jansen & Nicholls, 1972). The inhibitory potentials often consisted of several components occurring at 20 msec intervals as though they had been generated by the repetitive firing of an interneurone.

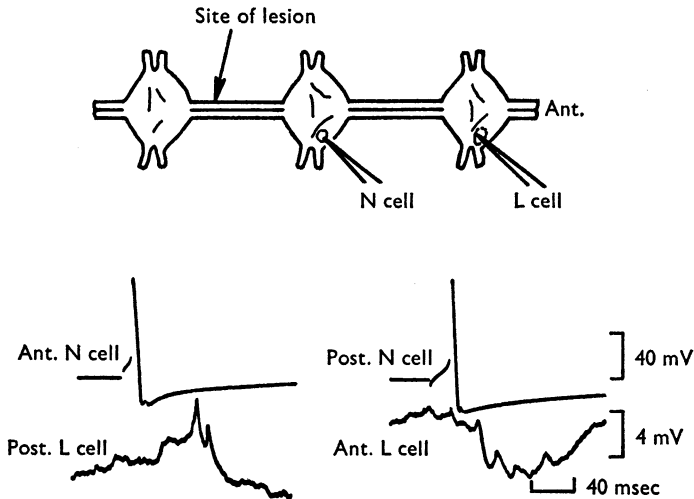


Fig. 8. Changed effect of impulses in N cells on the L cell of the next ganglion in an animal that had had one connective cut 125 days beforehand. Records were taken from cells in two ganglia one segment away from the site of the lesion. In one direction (anterior sensory N cell to posteriorly situated L cell) the synaptic potentials were normal and excitatory. In the other direction (with the N cell posterior) large inhibitory potentials were evoked. Asymmetric responses of this type in the L cell were seen consistently throughout the nerve cord between pairs of ganglia when sensory P and N cells in adjacent ganglia were stimulated.

Thus, section of one connective which had little or no effect on transmission within a ganglion produced marked changes in signalling between ganglia distant from the lesion.

*Are the abnormal inhibitory synaptic potentials  
mediated by interneurons?*

One striking change we have mentioned is that an impulse in a sensory cell inhibits a motor cell instead of exciting it. We shall examine two questions: (1) whether this novel inhibition is mediated by way of an interneurone? (2) are the inhibitory potentials merely an exaggeration of what is present normally in an occult form, or are they the result of new interactions that develop in response to injury of the C.N.S.? Several experiments described already indicate that traces of the abnormal inhibition can indeed be seen in normal animals. They were, however, always weak and inconsistent and were usually overlooked unless we specifically searched for them.

To determine whether interneurons were involved in producing altered interactions we made experiments using Mg and Ca in various concentrations. Both of these ions raise the threshold for firing of leech neurones; in addition the ions act antagonistically on transmitter release, raised Ca

enhancing and raised Mg reducing the amount liberated per impulse. Consequently, one would not expect an interneurone to be able to be fired synaptically in the presence of high Mg or in a combination of high Mg and Ca.

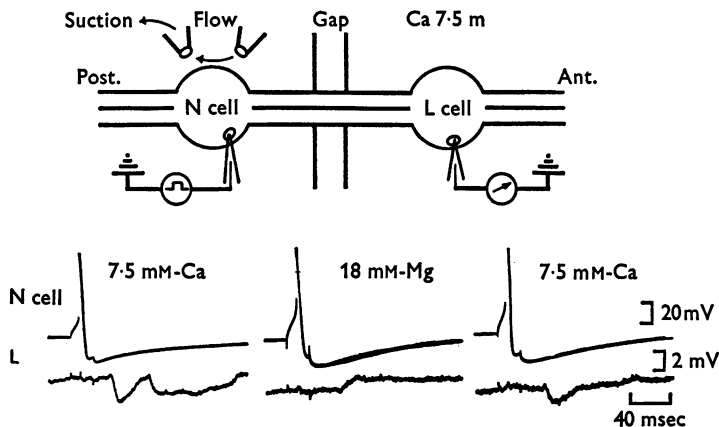


Fig. 9. Evidence that an interneurone mediates inhibitory synaptic potentials between sensory cells in one ganglion and the L motoneurone in the neighbouring anterior ganglion. The experimental arrangement is shown above. In the experiment shown below, the pair of ganglia were taken from an animal which had had one connective cut at a distance along the cord 196 days beforehand. Each impulse in a P or N cell evoked an inhibitory synaptic potential in the L cell in the next anterior ganglion when both ganglia were bathed in normal Ringer or Ringer containing 7.5 mM-Ca. When chemical synaptic transmission in the posterior compartment was blocked by bathing the left ganglion in leech Ringer containing 18 mM-Mg, only an excitatory synaptic potential remained. Returning the posterior ganglion to 7.5 mM-Ca restored the inhibitory synaptic potential. From this one can conclude that a rapidly conducting inhibitory interneurone located in the posterior ganglion is blocked in 18 mM-Mg, allowing the normal excitatory component to be revealed.

Fig. 9 shows how the presence of an inhibitory interneurone was revealed. A pair of ganglia from an animal that had had one connective cut 196 days earlier was set up in a bath containing a partition. The anterior ganglion lay in a pool of normal Ringer fluid with normal or raised Ca throughout. The fluid bathing the other, posterior, ganglion could be changed in a continuous flow system. With both ganglia in normal fluid or high Ca, an inhibitory potential arose in the L cell of the anterior ganglion when a P or N cell was stimulated in the posterior ganglion. This was the result expected in an animal with this type of lesion. Next the fluid bathing the posterior (i.e. presynaptic) ganglion was bathed in high Mg fluid, which effectively blocks chemical transmission but not axonal conduction (Stuart, 1970; Nicholls & Purves, 1970). Under these conditions a normal excitatory

potential appeared in the anterior L cell. Subsequently, when Mg-free fluid again flowed past the posterior ganglion, the inhibitory potential reappeared, virtually obliterating the excitatory potential. This result suggested that P and N cells excited interneurons situated within the same ganglion and that their axons gave rise to short-latency inhibitory potentials in the L cell of the next anterior ganglion.

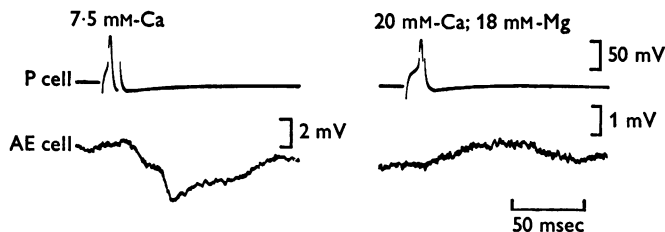


Fig. 10. Evidence that interneurons mediate inhibitory connexions of P sensory neurones upon the AE motoneurons within the same ganglion. In this experiment from a ganglion that had had both pairs of connective cut 354 days beforehand, polysynaptic pathways were blocked by solutions containing 20 mM-Ca and 18 mM-Mg. Such solutions only slightly reduce the amplitude of monosynaptic potentials measured in normal Ringer fluid because the effects of the two ions on transmitter release are antagonistic. In the partially isolated ganglion an impulse in a P cell evoked an inhibitory synaptic potential in Ringer fluid with 7.5 mM-Ca. Changing to Ringer fluid with 20 mM-Ca and 18 mM-Mg left only the normal excitatory synaptic potential.

Similar experiments showed that an interneurone was responsible for the inhibitory potential in the AE cell of a chronically isolated ganglion after P cell stimulation. Fig. 10 shows an example in Ringer fluid containing 7.5 mM-Ca. The predominant response of the AE cell was plainly inhibitory. When the isolated ganglion was bathed in fluid containing 18 mM-Mg and 20 mM-Ca; there appeared only the usual excitatory synaptic potential observed in normal animals. In the same solution, the inhibitory responses of L cells in isolated ganglia after P cell stimulation were also abolished, leaving pure excitatory potentials resembling those of Fig. 2.

A simple interpretation of these results is that after lesions to the nervous system the normal direct pathway may remain virtually unchanged, but a great enhancement of effectiveness occurs in pathways mediated by interneurons.

#### *Behavioural responses of animals after lesions to the C.N.S.*

It seemed likely that some of the changes we have observed in the performance of synapses after lesions might also be reflected in the response of the animal to sensory stimuli. In normal leeches pressing or squeezing the skin activates P and N sensory cells. These excite the annulus erector

motoneurons and ridges are raised in the skin. Animals in which a ganglion had been isolated or the cord transected behaved differently. In the first weeks after the operation their responses were normal. But, subsequently after 2-3 months the same stimuli applied to the skin did not cause annuli to be raised. Instead, if annuli were already raised for some reason, pressing or squeezing the skin caused them to become flattened. This is the response one would expect, knowing that the connexions in the AE cells are now predominantly inhibitory along the length of the animal.

#### DISCUSSION

At first glance the qualitative changes in synaptic interactions of the type described here appear somewhat surprising. There was no *a priori* reason to assume that discrete lesions made in the central nervous system would cause one neurone, which normally excites another, to give rise to inhibition. Characteristically, we observed that such changes developed slowly over weeks or months; once established, they appeared to be permanent. Furthermore, with progressively longer times, the effects could spread to segments far from the site of the lesion, until ganglia along the length of the entire nerve cord were modified. Aspects of the behaviour of these animals became changed, as one would expect from the altered interactions of the individual sensory and motoneurons. Pressing or pinching the skin caused annuli to be flattened instead of raised. At present we cannot say whether these alterations are an incidental effect or represent an adaptive, compensatory response to injury, since it is not even known what purpose is served by erection of the annuli in normal animals.

An additional, clear-cut and consistent observation was that certain synaptic interactions were modified while others were not; thus, cutting a single connective caused the appearance of inhibition in the pathway from sensory cells in one ganglion to the L motor cell in the next most anterior ganglion. But the same operation had no discernible effect on the interactions of the same cells within a ganglion. Another example of differential effects is provided by animals with both connectives cut; here the responses of AE cells in ganglia all along the nerve cord were changed, but only in the isolated ganglion did the L cells show modified responses. Each type of lesion, therefore, produced a characteristic and specific pattern of changes, which ranged from undetectable, in the case of root section, to the full variety seen after complete isolation of a ganglion. One can conclude that the changes observed in operated animals are indeed due to actual lesions of the appropriate bundles of axons, and not simply the result of non-specific damage or systemic factors.

Unfortunately, we have no information about how section of axons leads to modifications of synaptic interactions. The first question is whether

the abnormal inhibition is merely an exaggeration of normally undetectable synaptic effects, or whether it is the result of new connections between cells that develop in response to injury of the C.N.S. Several experiments indicated that traces of the inhibition, although weak and inconsistent, could indeed be seen in normal animals. It appeared, therefore, as though normal, weak pathways involving interneurons became overemphasized and more potent. Additional evidence supporting this conclusion is that we observed a marked increase in the amplitude and duration of certain excitatory potentials in AE, L and Retzius cells in isolated ganglia. These changes, which were quite obvious, are now being investigated further. One can speculate that several alternative mechanisms might play a part. For example, neurons might become supersensitive to chemical transmitters after section of presynaptic axons (Kuffler *et al.* 1971). It is known that each connective in the leech contains several thousand fibres (Coggeshall & Fawcett, 1963); some usually extend over only two segments (e.g. the axons of T, P and N sensory cells, Baylor & Nicholls, 1969), but others run farther, presumably to and from the head and tail ganglia. Furthermore, Purves & McMahan (1972) have shown that a cell such as the L motoneurone has at least 1000 presynaptic terminals impinging upon its dendrites. Hence, if some of these terminals degenerated or became silent, remaining inputs might become more effective. This might be due to new terminals that grow to occupy sites that have become vacant or perhaps to spread of receptors after partial denervation. Thus, entirely new connexions might be formed, or weak synaptic interactions enhanced. Yet another possibility is that through the maintenance of an abnormal pattern of signalling after the lesion some neurons now release more transmitter with each impulse. It should, however, be emphasized again that we do not know whether it is connexions or efficiency that have changed.

It would be of interest to search for comparable changes at synapses within the mammalian brain as a result of injury to cell groups or pathways. Synaptic interactions along the visual pathway are known to become altered with sensory deprivation (Wiesel & Hubel, 1965), again some being characteristically more vulnerable than others (for example, those in the cortex compared with those in the retina). Our results may also have some bearing on the properties of synapses between neurons maintained in tissue or organ culture, where many out of the usual complement of incoming fibres are presumably silent or have degenerated.

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## REFERENCES

- BAYLOR, D. A. & NICHOLLS, J. G. (1969). Chemical and electrical synaptic connections between cutaneous mechanoreceptor neurones in the central nervous system of the leech. *J. Physiol.* **203**, 591-609.
- BAYLOR, D. A. & NICHOLLS, J. G. (1971). Patterns of regeneration between individual nerve cells in the central nervous system of the leech. *Nature, Lond.* **232**, 268-270.
- COGGESHALL, R. E. & FAWCETT, D. W. (1964). The fine structure of the central nervous system of the leech. *J. Neurophysiol.* **27**, 229-289.
- GALUN, R. & KINDLER, S. H. (1966). Chemical specificity of the feeding response in *Hirudo Medicinalis* (L). *Comp. Biochem. Physiol.* **17**, 69-73.
- JANSEN, J. K. S., LOMO, T., NICOLAYSEN, K. & WESTGAARD, R. H. (1973). Hyperinnervation of skeletal muscle fibres: dependence on muscle activity. *Science, N.Y.* **181**, 559-561.
- JANSEN, J. K. S. & NICHOLLS, J. G. (1972). Regeneration and changes in synaptic connections between individual nerve cells in the central nervous system of the leech. *Proc. Natn. Acad. Sci. U.S.A.* **69**, 636-639.
- KANDEL, E. R. & SPENCER, A. L. (1968). Cellular Neurophysiological Approaches in the study of learning. *Physiol. Rev.* **48**, 65-134.
- KUFFLER, D. & MULLER, K. J. (1974). The properties and connections of supernumerary sensory and motor nerve cells in the central nervous system of an abnormal leech. *J. Neurobiol.* (in the Press).
- KUFFLER, S. W., DENNIS, M. J. & HARRIS, A. J. (1971). The development of chemosensitivity in extrasynaptic areas of the neuronal surface after denervation of parasympathetic ganglion cells in the heart of the frog. *Proc. R. Soc. B* **177**, 555-563.
- LOMO, T. & ROSENTHAL, J. (1972). Control of ACh sensitivity by muscle activity in the rat. *J. Physiol.* **221**, 493-513.
- MILEDI, R. (1970). The acetylcholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol.* **151**, 1-23.
- MULLER, K. J. & NICHOLLS, J. G. (1974). Different properties of synapses between a single sensory neurone and two different motor cells in the leech C.N.S. *J. Physiol.* **338**, 357-369.
- NICHOLLS, J. G. & BAYLOR, D. A. (1968). Specific modalities and receptive fields of sensory neurons in the C.N.S. of the leech. *J. Neurophysiol.* **31**, 740-756.
- NICHOLLS, J. G. & PURVES, D. (1970). Monosynaptic chemical and electrical connections between sensory and motor cells in the central nervous system of the leech. *J. Physiol.* **209**, 647-667.
- NICHOLLS, J. G. & PURVES, D. (1972). A comparison of chemical and electrical synaptic transmission between single sensory cells and a motoneurone in the central nervous system of the leech. *J. Physiol.* **225**, 637-656.
- PURVES, D. & McMAHAN, U. J. (1972). The distribution of synapses in a physiologically identified motor neuron in the central nervous system of the leech. *J. Cell. Biol.* **55**, 205-220.
- STUART, A. E. (1970). Physiological and morphological properties of motoneurones in the central nervous system of the leech. *J. Physiol.* **209**, 627-646.
- WIESEL, T. N. & HUBEL, D. H. (1965). Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *J. Neurophysiol.* **28**, 1029-1040.
- YOON, M. G. (1972). Reversibility of the reorganization of retinotectal projection in goldfish. *Expl Neurol.* **35**, 565-577.