FUSED NEURONS AND SYNAPTIC CONTACTS IN THE GIANT NERVE FIBRES OF CEPHALOPODS

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1. INTRODUCTION

The fact that there are two very large nerve cells in the central nervous system of the squid, *Loligo*, was discovered by Williams (1909), who also gave a brief description of their connexions. His account appears never to have been amplified, or indeed even mentioned, by any subsequent worker until these enormous nerve fibres were accidentally rediscovered in 1933 (see Young 1935*a*, 1936*a*, *b*, *c*). Williams considered

that the whole giant-fibre system on each side of the body consists of the processes of one of the two main giant cells. In fact the arrangement is much more complicated than this, and contains two curiously opposite features of the greatest interest for the neurologist (Young 1936*b*). First, the processes of the two main giant cells provide a clear case of the complete fusion of the axons of two nerve cells, thus infringing the strict canon of the neuron theory. Nevertheless, and this is the second point, there are also present, elsewhere in the system, discontinuous synapses which are perhaps more clear and easy to study than any yet described.

Investigation of the structure and functioning of these axons and synapses therefore throws considerable light on the fundamental problems of nerve physiology, and it is from this point of view that the present investigation of the anatomy and cytology of the system has been undertaken. The optical, physical and chemical properties of the axons have been described elsewhere (Young 1935*a*, 1936*c*, 1937; Bear, Schmitt and Young 1937). In other investigations it has been shown that the very large fibres in the stellar nerves, which are so useful for the study of the functioning of single nerve fibres, are the motor axons for the muscles of the mantle (Young 1938*a*; Prosser and Young 1937). The conduction rates of fibres of various sizes have also been investigated, and the significance of the presence of large axons discussed (Pumphrey and Young 1938).

The limits to be given to the term "giant-fibre system" are arbitrary, since in these animals axons of all diameters from 1 μ to nearly 1 mm. are found. The present account will be confined to the specially large fibres which operate the contractions of the retractor muscles of the head and funnel and of the muscles of the mantle, movements by means of which the animal shoots rapidly through the water. These large fibres occur only in decapod cephalopods, being absent from the octopods in which the mantle is no longer the main locomotor organ, and some of the cells of the giant-fibre system have apparently been converted into neuro-secretory cells (Young 1936*a*).

The arrangement of the giant fibres is essentially similar in sepioid and teuthoid decapods, but the individual fibres are larger, less numerous and, therefore, easier to follow in the teuthoids. The following account refers to *Loligo pealii* Lesueur, except where stated. The conditions in other forms are referred to only where they are of importance for study of the general anatomy of the system. A comparative study of the other decapods will be given in a later paper.

2. Material and methods

The methods used to provide a satisfactory description of the system have been gross dissection, and the examination of some 200 sets of serial sections through the central nervous system and stellate ganglia. The material consisted mainly of *Loligo pealii* Lesueur collected at Wood's Hole, and also of some *L. forbesi* Steenstrup, *L. vulgaris* Lamarck, and *Sepia officinalis* L. obtained at Naples and Plymouth. It is a pleasure

to thank the Directors and staffs of these Stations for their assistance in obtaining the animals in good condition.*

It is unnecessary to describe in detail the large number of histological techniques adopted, but attention to certain points is necessary for obtaining preparations suitable for critical study of axonic and synaptic structure. For many fixatives it is *essential* that sea water be used as a solvent (Young 1935*b*), and this is advisable for all. The best fixatives for preservation of the shapes of the giant cells and axons were found to be picric acid (saturated in sea water), formaldehyde (15 parts of 40 % formaldehyde mixed with 85 parts of sea water), or a mixture of these (15 parts of 40 % formaldehyde with 85 parts of saturated picric acid in sea water). Bouin's fluid sometimes gave good fixation, but the presence of acetic acid was often found to distort the fibres, especially in the peripheral nerves. A great variety of chrome- and osmium-containing fixatives, such as those of Flemming, Champy, Altmann and Regaud were tried, but found satisfactory, if at all, only for very small pieces of tissue. Mercuric chloride, alone or with acetic acid, was found to produce shrinkage, as also the mercuric, bichromate and formol mixture suggested by Bartelmez and Hoerr (1933) for the study of synapses.

Fixation was usually by immersion of small, freshly excised pieces. These were always taken immediately after death, since *post-mortem changes are very rapid in these animals*. Fixatives can readily be injected by a needle introduced through the ventricle into the cephalic aorta, either with or without previous washing of the blood vessels with sea water.

Washing of the fixative from the tissue, if an aqueous fluid is used, should be done with sea water.

Serious distortions are liable to appear during embedding. The best results were obtained by using celloidin, but for thin sections, and especially for series, double embedding in celloidin-paraffin, or with the methyl-benzoate-celloidin paraffin technique, produced satisfactory results. Direct embedding in paraffin through cedar-wood oil, benzene or xylene often produced serious distortion.

Of the many stains used Mallory's technique, its azan modification, and Masson's iron haematoxylin, ponceau de xylidine and light-green method were found to be especially valuable. It cannot be over-emphasized that the use of careful fixation and staining, such as one would employ in a delicate cytological investigation, is essential for proper appreciation of the structure of nervous tissues, and especially of synapses (see Bartelmez and Hoerr 1933; Bodian 1937). The silver methods, though they often give results which appear very clear, may involve serious distortion during both fixation and staining. A great many of these methods have been used, and they give beautiful results with cephalopod material. The most generally useful techniques were found to be the formol-Cajal and d'Ancona-Cajal methods, which have already

* Much of the work was done during tenure of a Fellowship of the Rockefeller Foundation, and I am greatly indebted to the Foundation and its Officers for their help.

been described (Sereni and Young 1932). However, for critical problems all appearances seen with silver methods need to be controlled by study of material treated in ways less likely to cause distortion.

3. General arrangement of the system

The rapid movement of a squid or cuttlefish through the water involves the expulsion of a jet of water through the funnel, a tube which can be directed either forwards or backwards. Three sets of muscles are involved in this movement, the circular muscle fibres of the mantle, the longitudinal fibres of the m. retractor (depressor) infundibuli, which holds the funnel, and the mm. retractor capitis posterior, by which the head is attached to the body (fig. 1).

These muscles are all innervated by giant nerve fibres, whose connexions are such that the whole system can be set in action by impulses arising in either one of a single pair of giant nerve cells lying in the central nervous system. These two cells will here be called the *first-order giant cells* (fig. 2). They lie close to the meeting point of the cerebral, optic, pedal and palliovisceral ganglia, and close also to the statocyst. In this central position they are able to receive impulses from a great variety of sources.

The axons arising from these cells are remarkable in that they are joined across the middle line by a complete protoplasmic bridge (p. 477), an arrangement which presumably ensures that an impulse set up in either one of them is always propagated to the other. Beyond this bridge the axons break up in the palliovisceral ganglion into several branches, each of which makes synaptic contact with one of the *second-order giant neurons* whose cell bodies lie in this ganglion (fig. 2 and p. 481). The second-order axons are numerous, there being about seven of them in *Loligo* and more still in *Sepia*. They run in the following nerves:†

Nerve	Destination
N. infundibuli posterior ...	Funnel
N. visceralis	M. retractor infundibuli (front part)
N. retractor capitis posterior	M. retractor infundibuli (hind part) M. retractor capitis posterior
N. pallialis	Stellate ganglion.

The fibres in the first three of these nerves run direct to motor endings in the muscles which they innervate (fig. 2), but those in the pallial nerve end in the stellate ganglion, where they make synapse with *third-order giant fibres* which arise in the ganglion and pass out through the stellar nerves to innervate the circular muscle fibres of the mantle.

The muscles effecting the quick movements can therefore all be reached by impulses arising in the first-order giant cells. The excitation must pass over one synapse to reach the retractor muscles, two to reach the muscles of the mantle. At these synapses

† The names of the nerves and muscles are almost entirely those adopted by Schkaff (1914).

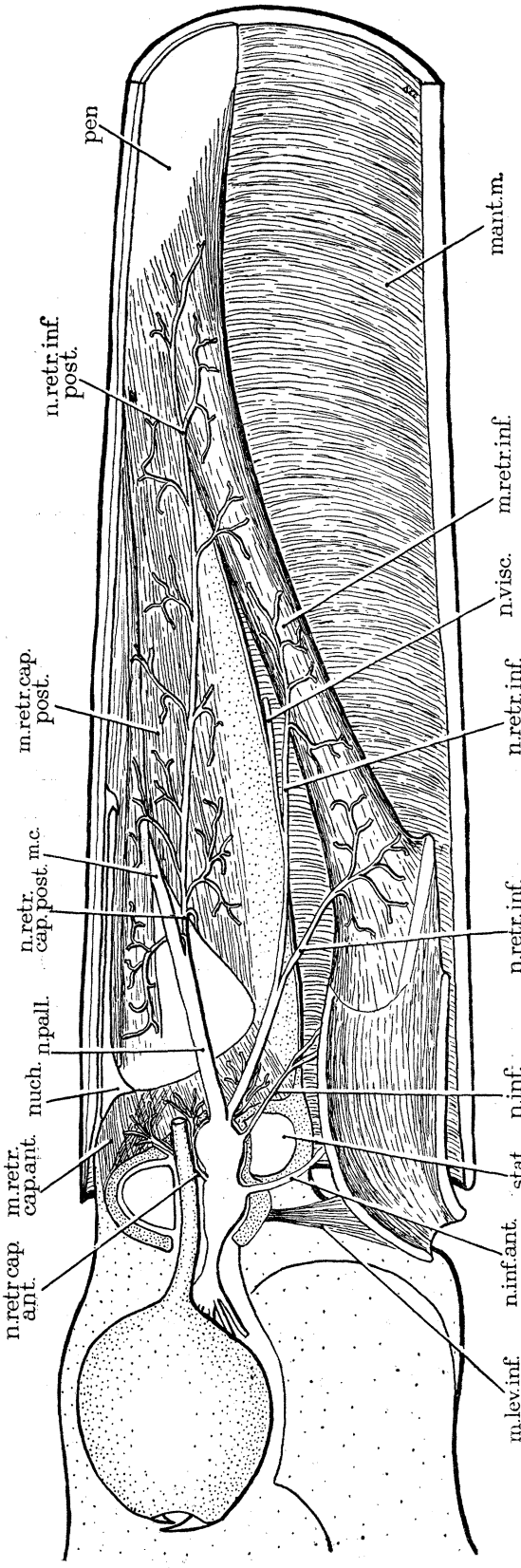

FIG. 1. Drawing of a dissection of *L. pealii* to show the arrangement of the retractor muscles and their nerves.

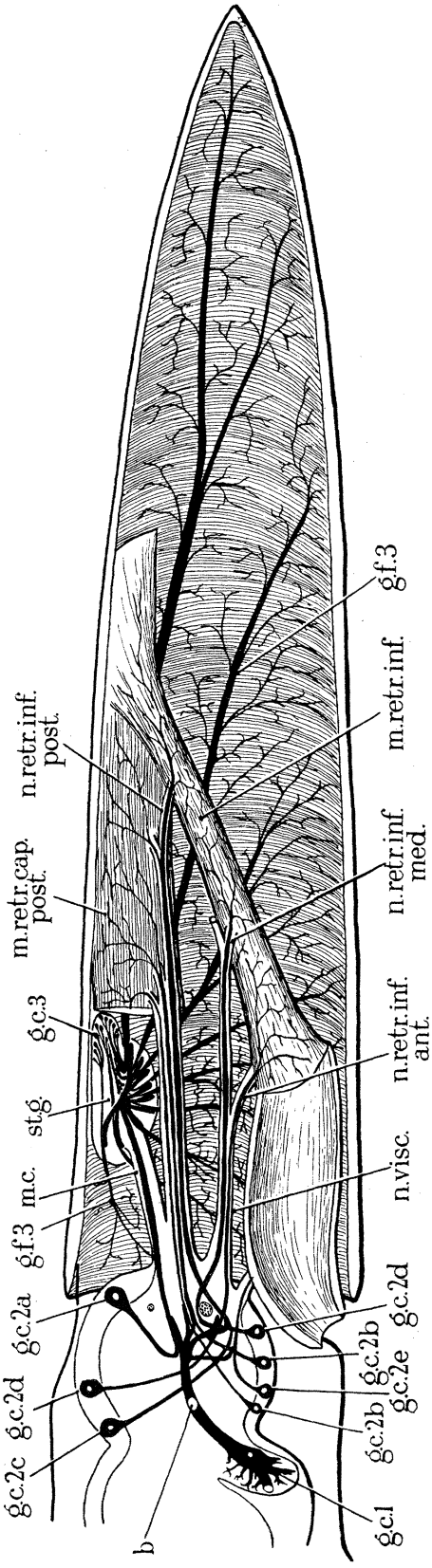

FIG. 2. Diagram of *L. pealii* to show the whole system of giant fibres which can be activated from the first-order giant cells. The ganglia and fibres are shown unduly large and the relative lengths of some of the nerves have been distorted.

(The abbreviations used for the lettering of all figures and plates are listed on p. 502).

there are also endings other than those of the giant-fibre system itself (p. 483), and it is possible that these points do not constitute mere relay links but are correlation points at which a balance of excitation and inhibition is struck.

The various parts of the system will now be described in detail.

4. The first-order giant cells

(*a*) *The lobus magnocellularis*

The two first-order giant cells, one on each side, are the largest of a set of large motor neurons lying in a clearly marked lobe, which will be called the *lobus magnocellularis* (Young 1937*b*). This lobe lies just above the statocyst and close to the meeting point of the optic, cerebral, pedal and palliovisceral ganglia, and is thus very conveniently placed for receiving and transmitting impulses. In sections it forms a conspicuous band of tissue, not very clearly marked externally, lying on each side laterally to all of the other ganglia except the optic (figs. 3–5 and figs. 15–18, Plate 42).

The morphological status of this lobe is not yet altogether clear, since its neuropil and cell layers are continuous with those of all of the adjoining ganglia mentioned above. A dorsal part leads to the lobus basalis posterior of the supra-oesophageal ganglia, a lateral part to the optic lobe and a posterior part extends back on either side of the palliovisceral ganglia (fig. 5). The ventral part, in which the giant cells lie, is very close to the statocyst and many of the fibres of the nerves of the maculae of the statocyst actually end within it, the n. cristae staticae running above it to its well-known commissure in the pedal ganglia (figs. 3, 4). Two large commissures connect the lobes of the opposite sides, the commissura magnocellularis anterior running below the hind end of the pedal ganglion (fig. 5 and fig. 17, Plate 42) and the commissura magnocellularis posterior through the ventral part of the palliovisceral ganglion (figs. 3, 9).

These relations suggest that the lobe represents the expanded lateral and ventral parts of the perioesophageal ring of the ancestral mollusc. Subcerebral commissures, having essentially this position below the pedal commissures, are known in Amphineura and various gastropods. Further discussion of the interesting morphological problems involved will be reserved for a later paper.

The axons arising from the walls of the lobe are mostly large and they run to many of the main motor centres of the animal. Those of the dorsal part pass backwards into the centre of the palliovisceral ganglia, and those of the lateral and ventral parts mostly to the hind end of the pedal ganglion, which is the region from which the motor axons of the arms and tentacles arise. The two giant cells themselves also lie in the ventral part of the lobe and their axons pass backwards into the palliovisceral ganglia. The lobus magnocellularis thus seems to be the motor centre controlling many of the muscle systems which are most important for the life of the animal. From it can be

initiated the movements by which the prey is captured, involving co-ordination of movements of the arms and tentacles with those of the retractor muscles and mantle by which the rapid dart through the water is produced. Such a motor centre might be expected to develop in the ventral part of the perioesophageal ring if this represented the central nervous system of the earliest molluscs.

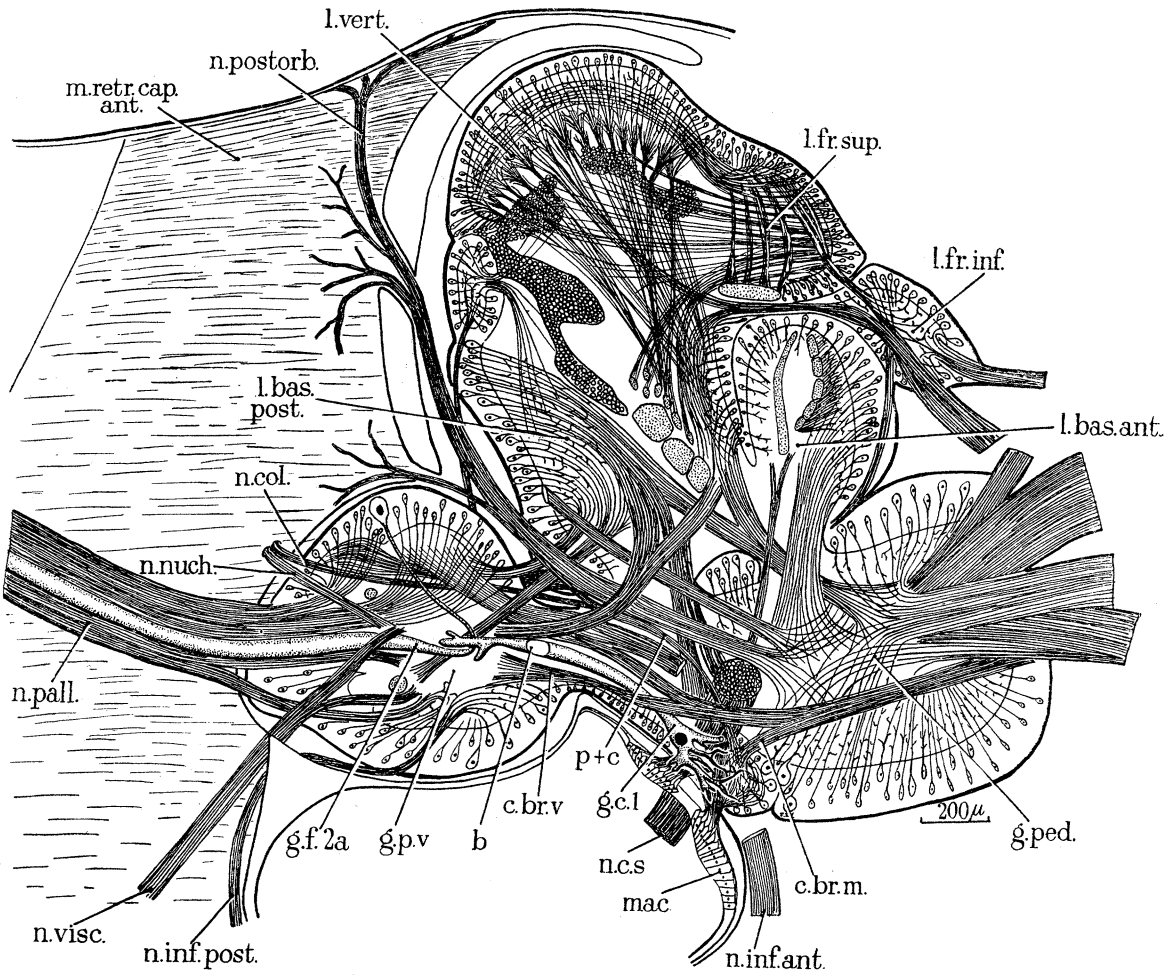

FIG. 3. Semi-diagrammatic view of a thick sagittal section through the lateral portion of the central nervous system of a young *Loligo*. Main outlines drawn with camera lucida from a single section stained with Cajal's method (HMb 6.1.4); details from neighbouring sections. The section lies to the left of the middle line and is seen from its inner side.

(*b*) *Position and cytology of the cells*

The giant cell on each side is the most ventral member of the single row of large cells which make up the posterior wall of the ventral part of the lobus magnocellularis (figs. 3, 4 and figs. 15, 18, Plate 42). Each cell lies with its long axis nearly vertical at the postero-median corner of the lobe. It differs from the ordinary cells of the ganglion in that it lies within the neuropil rather than in the outer cell layer. Correlated

with this position is the fact that the cell is not T-shaped, as are the ordinary neurons, but bipolar, with the nucleus lying between axon and dendrites. However, the internal structure of the cell shows that it is built essentially on a T-shaped plan,

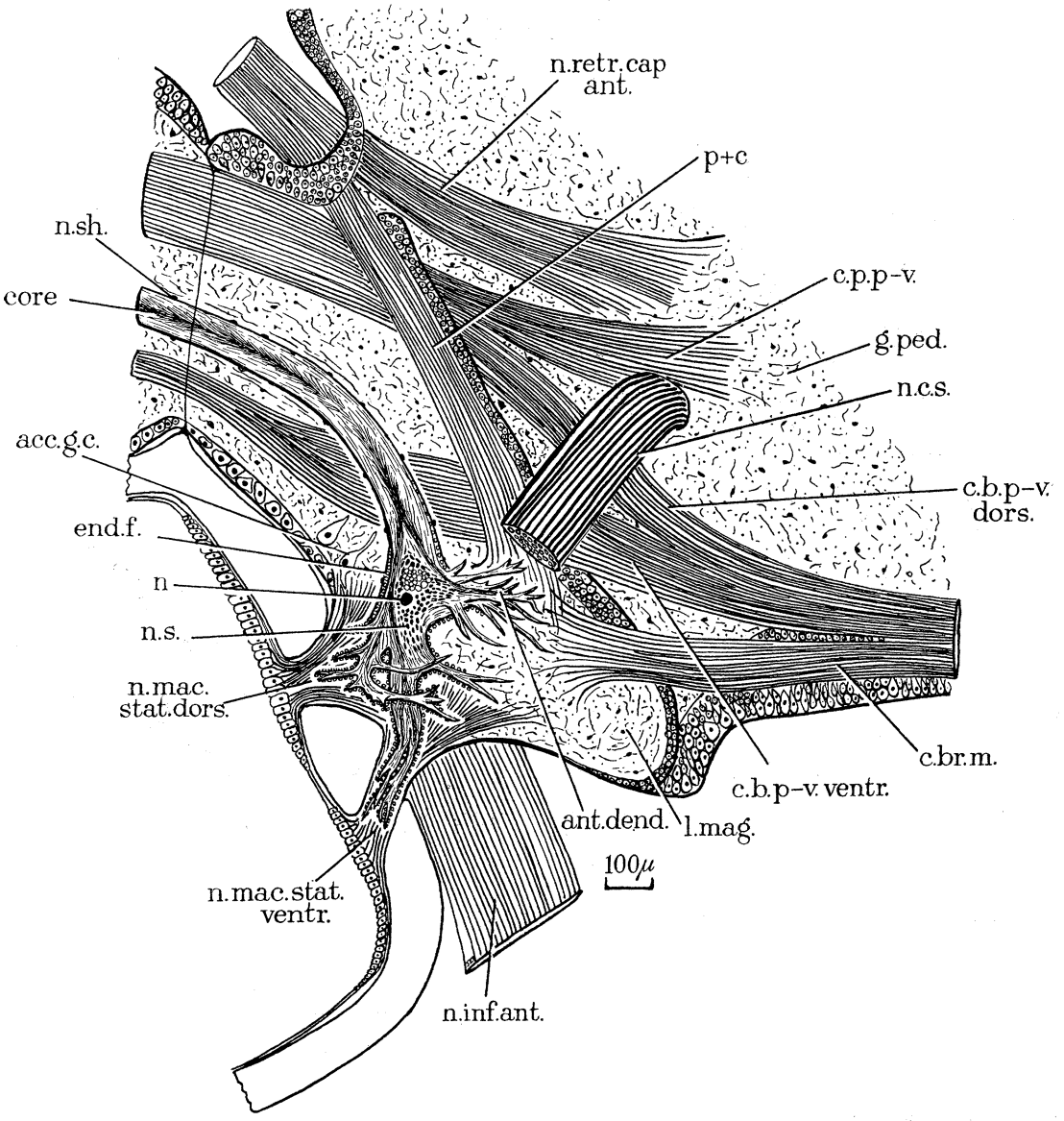

FIG. 4. Drawing to show the structure of the first-order giant cell in an adult *L. pealii*. Combined by projection from a series of sagittal sections (*Loligo* OB, slide 17). Fixed formaldehyde, stained haematoxylin and eosin. The cell is that of the right-hand side, seen with its lateral surface nearer to the observer.

though the vertical portion of the T is so shortened as to be nearly absent. The nucleus lies excentrically on the hinder (outer) side of the cell, and this is especially clear in young animals (figs. 18, 19, Plate 42). On the anterior (inner) side of the nucleus there is a curious pathway of axoplasm connecting the axon with the dendrites

(figs. 18, 19, Plate 42), this representing the horizontal arm of an ordinary T-shaped neuron. The bipolar shape of the cell is therefore a result of the fact that it has grown to such a size that the cell body can no longer be contained within the outer cell layer. The neighbouring neurons of the lobe, though never as large as the giant cell itself, often show a tendency to leave the cell layer (fig. 4). Evidently the morphogenetic

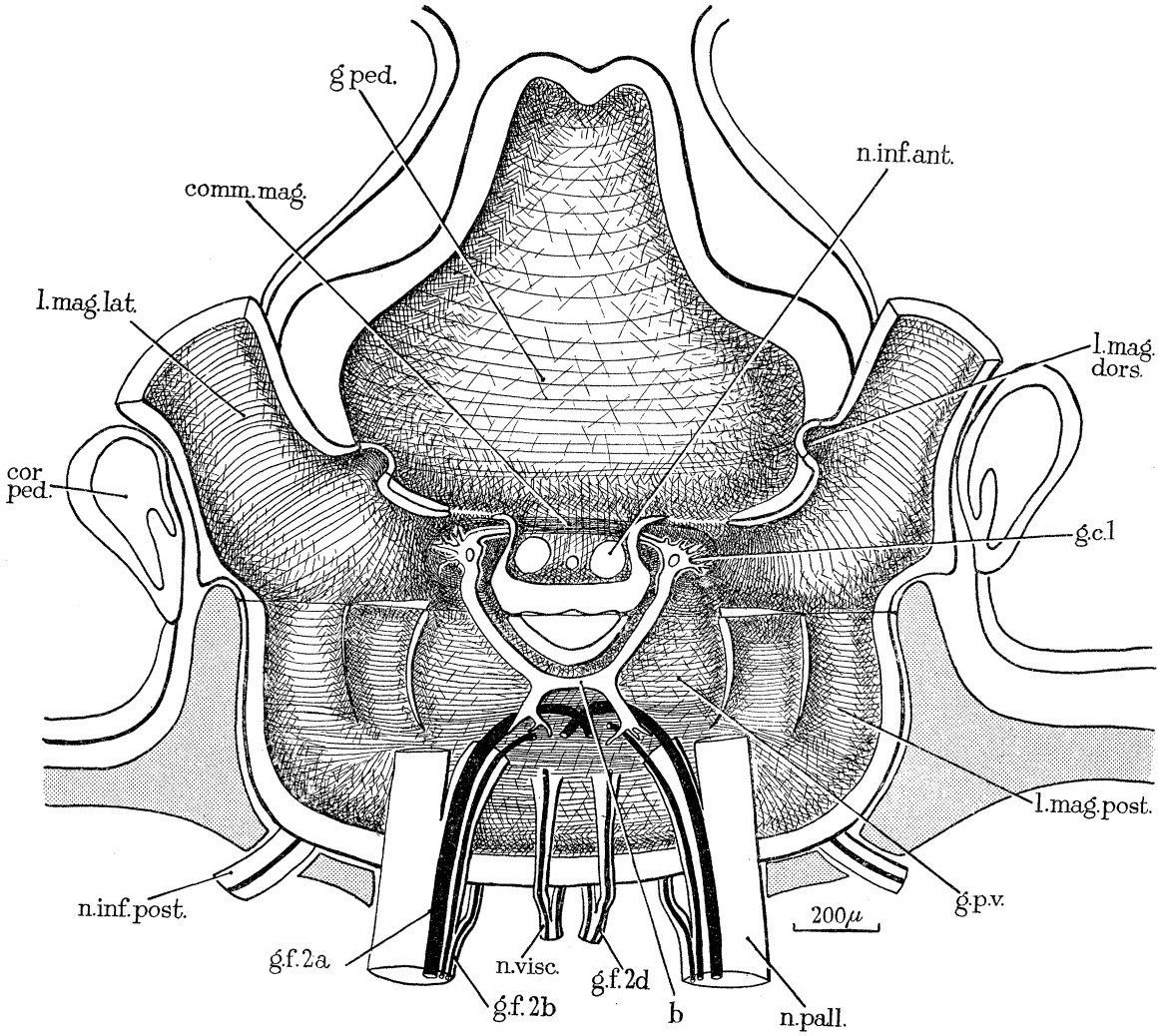

FIG. 5. Giant fibres in the central nervous system of a freshly hatched *L. pealii*, seen from above. Semi-diagrammatic; outlines projected from a single horizontal section (HGb 3.2.4) and supplemented from neighbouring sections.

processes tending to produce large neurons work on all the cells of the row, but most strongly on its most ventral member.

The single axon of each giant cell passes dorso-medially and backwards into the palliovisceral ganglion, and its very numerous dendrites spread throughout the giant cell lobe. The greatest overall length from the tip of the most ventral dendrite to what may be considered as the origin of the axon was found to be about 750μ, after

fixation, in a specimen of *L. pealii* of mantle length 25 cm. The greatest diameter of the same cell was 150μ. The nucleus has diameter 35μ (with a nucleolus of 8μ) and is thus very small, in fact little larger than that of one of the much smaller neurons nearby.

Surrounding the nucleus there can be seen in preparations fixed in Flemming's fluid to be a mass of fuchsinophil granules (fig. 4 and fig. 25, Plate 43). Similar granules occur in all cephalopod neurons (Young 1932). They are dissolved away by procedures which do not preserve fatty substances, leaving large empty spaces (fig. 22, Plate 43).

Around these granules lies the Nissl substance, which is curiously small in amount, and stains less strongly than that of the neighbouring neurons. After fixation it appears as a finely granular substance, broken up, especially towards the periphery of the cell, into flakes or blocks, similar to the Nissl granules of a vertebrate ventral horn cell. From the main central mass of the substance tongues project both into the axon and dendrites (fig. 4 and figs. 19–21, Plate 42); there is therefore no true axon hillock.

It is interesting that only the Nissl substance which is near to or on the main track of conduction through neurons is broken up into the characteristic granules ("Schollen"). Thus in the dorsal root cells of vertebrates, or in the cell bodies of the T-shaped invertebrate neurons, the Nissl substance forms masses of substance not divided into the characteristic granules. In the first-order giant cells here described the substance becomes more and more divided up as it approaches the conduction path. It seems that the factor which produces the longitudinal organization of the axon, and of the conduction paths through the cell, breaks up the Nissl substance into elongated granules. It is even possible that the highly dispersed basophil material which is found in the axons (Bear, Schmitt and Young 1937) represents the same material as the Nissl substance under a different form. It is not unreasonable to suppose that the production of this substance would be a property of neuroplasm in general. But the nature of the difference in molecular organization between the central part of the cell on the one side, and the axon and conduction paths from dendrites to axon on the other remains obscure. The pathways from the various dendrites all meet above the nucleus, to form a central strand of more basophil substance, which extends into the axon (fig. 4) and often, though not always, runs throughout its whole length (fig. 8 and fig. 36, Plate 45).

(*c*) *Synaptic end feet on the giant cells*

The dendrites of the first-order giant cells can be divided for purposes of description into three sets (figs. 3, 4 and figs. 23–25, Plate 43). The lateral dendrites spread widely in the neuropil of the lobe; the ventral dendrites mingle with the fibres of the n. maculae staticae. The anterior dendrite, which is the largest, passes forward into the commissura magnocellularis anterior, in which it breaks up into numerous branches which are not easy to follow, and may perhaps extend to the opposite side.

The whole surface of the cell body and dendrites is covered with the very numerous

end feet (fig. 4 and figs. 24–27, Plate 43). As will be seen from the figures, these are similar in some ways to those of mammals, though mostly somewhat larger. Usually they do not have the ring structure or more lightly staining core which has been described for so many vertebrate end feet.

These end feet are pressed directly against the surface of the cell, and are not separated from it by any of the sheaths. The surface of the axon of the giant cell is smooth and is enclosed in a tight sheath of the type already described in the peripheral nerves of cephalopods (Young 1936; Bear, Schmitt and Young 1937), composed of collagenous fibres with, presumably, an inner myelin-like layer and, certainly, a "protoplasmic" sheath next to the axon. The sheath does not fit tightly on to the cell body and dendrites, however, but is separated from the cell surface by a space occupied by the closely packed end feet (fig. 4 and fig. 25, Plate 43). No sheath elements intervene between the surfaces of synaptic contact, the boutons are closely pressed against the cell surface. But there is no complete continuity between the two: the substances of bouton and dendrite often stain very differently, so that it is quite clear that they do not mingle. Moreover, in many preparations, a narrow space, a few microns in width, appears between the end foot and the cell surface. This space is probably an artefact, but the fact that the two members of the synapse can separate in this way indicates the absence of mingling of their substances. The full implications of this arrangements are discussed on p. 494. The most interesting feature is the presence of a discontinuity such that an impulse passing over the bouton would not be expected to have the same effect on the dendrite as it would have on a portion of neuroplasm with which it was fully continuous.

A further interesting point is the occurrence of fuchsinophil granules at the synapses. These are best seen after fixation in Flemming's fluid and staining with Mallory's stain. Numerous red granules can then be seen in the region occupied by the end feet (fig. 25, Plate 43). Many of the granules lie actually within the end feet, others appear to lie in the fluid between them, but may have been extruded during post-mortem changes. Similar granules have been seen in the end feet on the Mauthner's cell of fishes ("mitochondria", Bodian 1937), and they occur also at the synapses in the stellate ganglion of the squid (p. 492).

(*d*) *Afferent connexions of the giant cells*

The fibres bringing impulses to the giant-cell lobes, together with the dendrites of the cells of the lobe itself, give to the neuropil of this region a characteristically close, tangled structure. It often stands out sharply in black in a section of the central nervous system stained with Cajal's method, and even a superficial inspection shows the mass of end feet scattered through it, and especially concentrated in the commissure which joins the two sides.

In this tangle it is not easy to follow individual fibres, or even bundles, especially since there are numerous incoming and outgoing pathways. A large bundle of fibres

enters the lobus magnocellularis dorsally from the lobus basalis posterior of the cerebral ganglion (fig. 3). The fibres of this bundle mostly scatter in the dorsal part of the lobe and are difficult to trace to their ends, but some of them almost certainly pass down and reach the giant cells.

A very large bundle of optic tract fibres enters the lobe at the side, and reaches to all parts of it. These fibres are the processes of large cells of the optic lobes, which v. Lenhossek (1896) and Cajal (1917) have shown to be the fourth neurons in the optic paths. They arise from all parts of the optic lobes and are presumably activated to discharge by sudden changes in illumination, or movement in the visual field, possibly only when these events occur in certain shape, sequence or other "gestalt".

The ventral part of the lobus magnocellularis lies very close to the statocyst (figs. 3, 4 and fig. 18, Plate 42). It is usually supposed that the n. staticus gives no fibres to the suboesophageal ganglia, but passes straight through, after a chiasma in the pedal ganglion, to the cerebral centres. This is indeed the course taken by many of the large fibres of the *n. cristae staticae* which enters laterally, crosses dorsally to the ventral part of the lobus magnocellularis and enters the pedal ganglion (figs. 3, 4). However, some of the smaller fibres of the n. cristae staticae probably end in the hinder part of the pedal ganglion, some perhaps even in the l. magnocellularis. But it is the *n. maculae staticae* which is in the most close relationship with the giant-cell lobe, which it enters posteriorly and ventrally (figs. 3, 4). The fibres spread out in the lobe and many of them branch and presumably make synaptic contacts in the lobe, though it is not easy to follow individual fibres with certainty. It has not yet been determined whether all the fibres end in the lobe, or if some pass to the chiasma in the pedal ganglion.

Even more remarkable than the spreading out of these statocyst fibres in the lobe, is the way in which the ventral dendrites of the giant cell itself are entangled in the n. maculae staticae (figs. 3, 4). Some of the finer dendrites accompany the nerve beyond the margin of the ganglion proper, and in fact almost to the sensory epithelium itself. The connexion is so close that it is difficult to believe it to be without functional significance. It is possible that impulses in the fibres from the statocyst influence the dendrite by a sort of collateral synapse.

Two other sets of fibres enter the ventral part of the lobus magnocellularis and are probably tactile. The brachio-magnocellular tract (figs. 3, 4 and fig. 24, Plate 43) is a bundle which, besides efferent fibres, carries many afferents which undoubtedly end in the lobe, forming a dense network of fibres and terminal knobs around the dendrites of the giant cells. It is reasonable to suppose that these are either afferent fibres running through from the skin of the arms, or are processes of cells in the brachial ganglion which are connected with such afferents. Entering close to this tract are bundles from the n. postorbitalis and n. collaris (figs. 3, 4) which contain sensory fibres, presumably tactile, from the head and region around the mantle aperture (collar). They also end as knobs on the anterior dendrites of the giant cell.

(*e*) *Summary of influences controlling the giant cells*

The dendrites of the giant cell are therefore brought under the influence of nerve impulses arising from the eye, statocyst, skin, and probably from the supra-oesophageal ganglia. The static and tactile impulses are brought in the axons of the neuro-sensory cells themselves. The optic impulses arrive after passing three synapses in the optic lobes.

Further anatomical and physiological information will be necessary before it will be possible to say in detail how impulses are set up in the first-order giant axons as a result of their bombardment from these many sources. Presumably a single impulse arriving at a single synapse is not sufficient to fire off the giant cell, since this would lead to behaviour by the squid even more "nervous" than that for which the animals have a reputation. Probably impulses in a number of the afferent fibres, possibly in certain patterns of them, are necessary before the giant fibre is excited to produce the impulse which will set the whole mechanism for rapid movement into action.

5. Interaxonic bridge

The axons arising from the two first-order giant cells pass backwards into the neuropil of the palliovisceral ganglion. Here they approach one another in the middle line, and are joined by the interaxonic bridge, already briefly referred to in previous papers (Young 1936*a*, *b*). The interest of this remarkable structure is that in the adult it consists not of a chiasma or crossing of two distinct fibres, but of a true protoplasmic bridge, joining the whole substances of the two axons (figs. 5–8 and figs. 28–36, Plates 43–45). The implication of this arrangement is that the two axons are united into a continuous protoplasmic mass with a continuous surface, contrary to the strict interpretation of the neuron theory. From what we know of the mechanism of nervous conduction it is to be expected that impulses set up on one side of such a bridge will be conducted across to the other side as regularly as from one part of a nerve fibre to another. Such an arrangement is so strange that it requires careful description, especially since Williams, in his very brief account, refers to the crossing of these two fibres in a chiasma. However, his figure (16 D) shows clearly that what he took to be the chiasma does not lie in the middle line. Probably it was really the synapse between first- and second-order giant fibres in the stellate ganglion, in fact the structure shown in fig. 37, Plate 45.

Fortunately the axons are so large that in addition to the usual neurological methods, others less disruptive of the cytoplasm can be employed, and the arrangement of the axons demonstrated with great clarity. The clearest preparations can be obtained from material fixed in saturated picric acid or in picro-formol, sectioned in celloidin and stained with Ehrlich's haematoxylin and eosin, or by Mallory's or Masson's methods.

In such preparations the bridge between the two axons can be seen to consist of a homogeneous substance, having no divisions or membranes across it, and perfectly continuous with the axoplasm of the two main axons. The presence of membranes

within this substance would certainly be detectable in such preparations, as it is, for instance, at the synapses in the stellate ganglion (p. 491). The axoplasm takes the blue of Ehrlich's haematoxylin readily, and can be seen to consist of a homogeneous, faintly fibrillated mass.

Sections made in the three main planes have been most carefully studied for traces of separation between the axons. If the "bridge" were a chiasma, then in at least

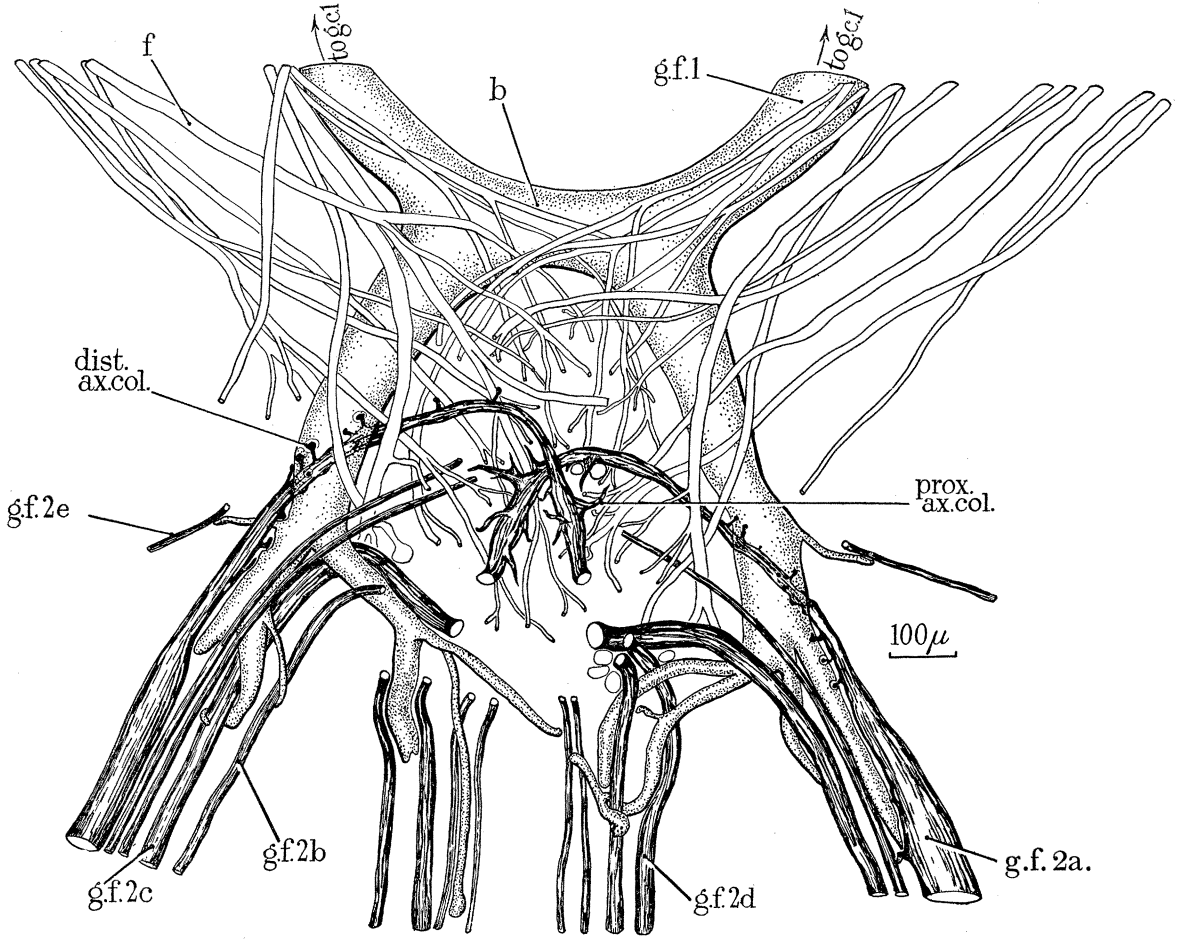

FIG. 6. Drawing of giant fibres in palliovisceral ganglion of small adult *L. pealii* (GS). Stained by Cajal's method, embedded celloidin and cut into horizontal sections at 50 μ.

one plane membranes should be visible dividing it. In transverse section it appears as a perfectly homogeneous strand across the middle line, separating into two "axons" in front and two behind (figs. 29, 30, Plate 44). In horizontal section there is, similarly, complete continuity from one side to the other, and the lines of fibrillation can be seen to run partly backwards on each side and partly across the bridge. Fortunate sections in this plane, though they are rare, show the general arrangement admirably (figs. 28, 31, Plates 43, 44).

But the most convincing evidence that the bridge truly unites the two axons is derived from sagittal sections (figs. 32, 33, Plate 44). If the affair were a chiasma then

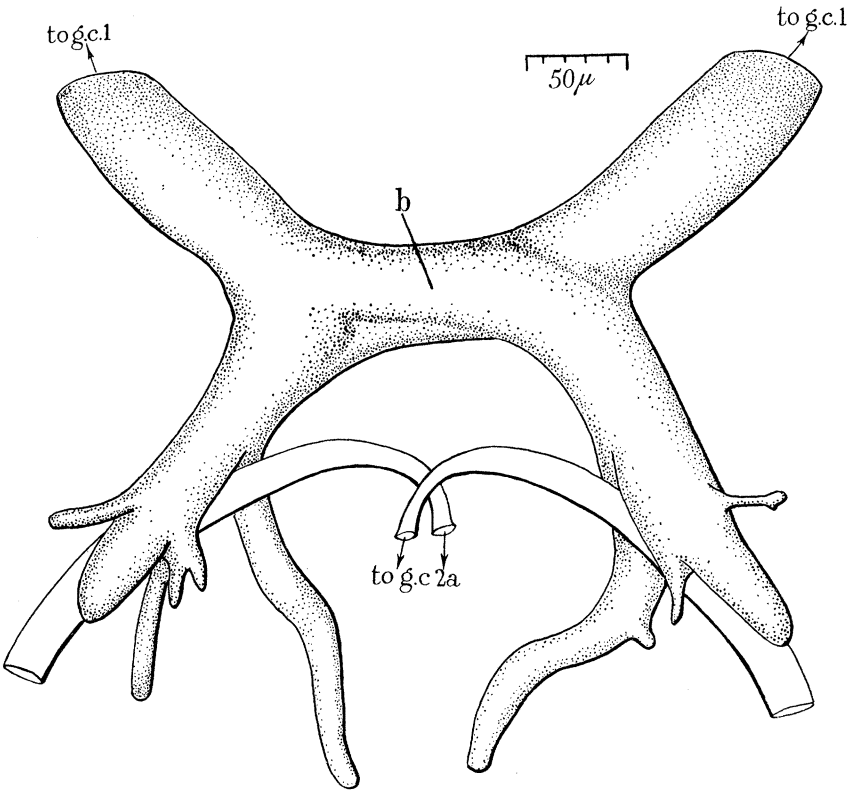

FIG. 7. Drawing of giant fibres in palliovisceral ganglion of freshly hatched *L. pealii* to show method of origin of interaxonic bridge. Outlines projected from a single section (HMc 4.2.8); some details from neighbouring sections.

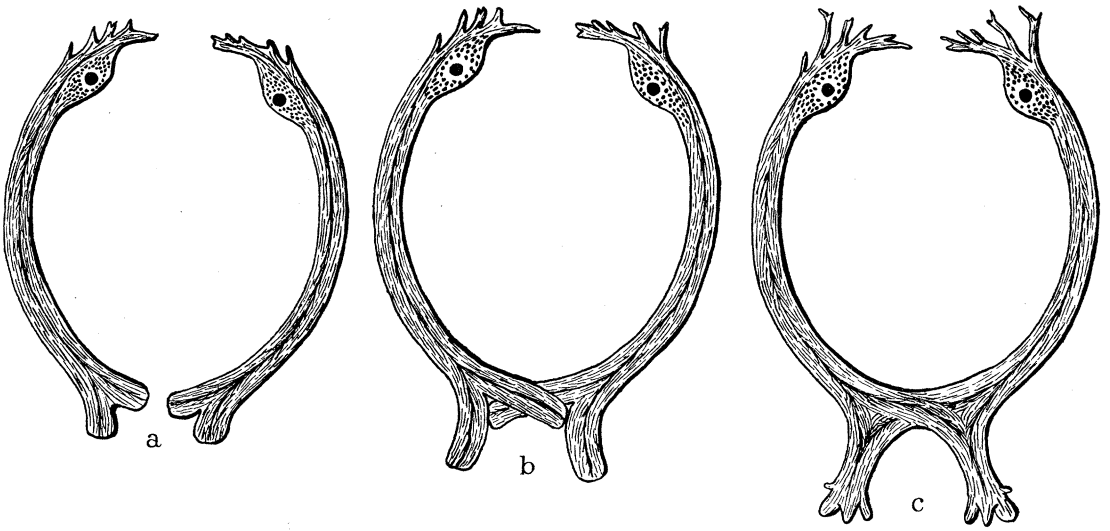

FIG. 8. Supposed embryological stages in fusion of giant axons to form the interaxonic bridge. Only the axons themselves are shown, but it is to be imagined that at about stage *b* the whole becomes covered with a continuous sheath. The axons are shown as having central dense strands, in order to show how the appearances of fig. 26 (Plate 43) can be explained, but such strands do not appear in all preparations.

a sagittal section, since it cuts the bridge itself transversely, must show any division which exists within it. No such division is seen. The bridge appears as a single mass of axoplasm, enclosed in a single sheath, and indistinguishable from one of the giant fibres in a peripheral nerve (Bear, Schmitt and Young 1937).

In a few of the preparations, especially those in which proper precautions were not taken during embedding, shrinkage of the axoplasm produced appearances which might, very superficially, be considered to be membranes. A class of artefact which is particularly liable to give rise to error is that in which the axoplasm becomes shrunken and creased along certain lines (fig. 35, Plate 44). Often the central part of the axons, including the bridge, stains more darkly than the periphery (see p. 474), this being a phenomenon which is also sometimes seen in a peripheral nerve (Young 1936*c*). In all of such cases, however, study of serial sections will show continuity of the substances of the axons. Often the more darkly staining central strand is itself continuous from axon to axon.

In one specimen of *L. pealii* the transverse bridge was found to be long and narrow (fig. 34, Plate 44). This abnormality is interesting since it approaches the condition normally found in the oegopsid teuthoids.

6. Embryological history of the interaxonic bridge

The full details of this subject have still to be worked out, but sufficient is already known to show that the bridge is formed during development by fusion of the originally separate outgrowths of two nerve cells. Little success has yet been obtained with study of these delicate structures in embryos, but good preparations were obtained from the post-embryonic *L. pealii* which are sometimes obtained at Wood's Hole. In such animals fusion of the substance of the two giant axons was found to be still incomplete. The outline of the sheaths is not so smoothly continuous as it is in the adult, but shows signs of being formed by the combination of two tubes (fig. 7 and fig. 36, Plate 45). Moreover, there are still traces of division of the substance of the axoplasm itself within the bridge. In fig. 36, Plate 45, the existence of two portions can be clearly seen. The membrane between them has broken down, so that continuity of axoplasm can clearly be traced from the one into the other; they have mingled but the central core of each axon is still distinct.

By study of the smaller axons of the magnocellular-palliovisceral tract, which run close to the giant fibres, it is possible to form a clear idea of the way in which the interaxonic bridge arises. These smaller axons divide as they approach the middle line, one branch continuing on the same side and the other crossing. Both branches pass backwards, and divide up in the neuropil of the palliovisceral ganglion (fig. 6, *f*). It appears likely that during development the same morphogenetic forces work upon the very large axons as on the smaller ones, making them branch in a similar manner. The giant fibres, however, lying at the very centre of the ganglion, become closely

pressed together, and enclosed in a common sheath, within which their axoplasms unite (fig. 8).

There is no physical or embryological reason against supposing that such a process takes place, and it is not difficult to see how the arrangement is of survival value to the squid, since it ensures that impulses set up on either side of the animal shall produce contraction of the whole mantle. Contraction of half of the sack alone could not be useful under any circumstances. The general neurological implications of this curious case of fusion are discussed on p. 497.

7. Second-order giant cells and their synapses

Behind the interaxonic bridge the two arms of the first-order giant fibres diverge slightly, and break up into a number of branches, which make synaptic contact with the second-order giant axons. These form a complex system, made up as follows in a *L. pealii*:*

Destination	Nerve	No. of fibres	Code name
Stellate ganglion	Pallialis	1	*g.f.2a*
M. retractor capitis posterior	Retractor capitis posterior	2	*g.f.2b*
M. retractor capitis posterior and M. retractor infundibuli	Retractor capitis posterior	1	*g.f.2c*
M. retractor infundibuli	Visceralis	2	*g.f.2d*
Intrinsic muscles of funnel	Infundibuli posterior	1	*g.f.2e*

There are, therefore, in this typical case, seven second-order giant fibres on each side, which are all activated by the first-order fibre, and which together operate the retractors of the head and funnel, some muscles in the funnel itself, and, through the stellate ganglion, the circular muscles of the mantle. Each of these fibres arises from a single cell in the palliovisceral ganglion. Unfortunately it is only rarely possible to trace the connexion of the fibres with their cells, since in the centre of the ganglion they become thin, stain poorly and are difficult to follow. Fig. 9 shows a fortunate case seen in a thick section (50μ) of material stained with Cajal's method. The axon of the large fibre which runs to the stellate ganglion (*g.f.2a*) can here be followed back to its cell, which lies in the dorsal wall of the palliovisceral ganglion, near to the mid line and above the dorsal palliovisceral commissure. On leaving its cell body the axon passes ventrally and forwards, giving off abundant dendrites. It crosses in the middle line, turns backwards and outwards below the first-order giant fibre, and passes out into the viscero-stellate connective. Fig. 6 confirms this course and shows further details of the collaterals which the fibre gives off at the centre of the ganglion.

The synaptic connexions between the fibres of the first and second orders are made by special branches of the former, one of which makes contact with each second-order

* For convenience in reference a code of abbreviations has been adopted in which *g.f.*1 refers to the first-order giant fibre, *g.f.2a, b, c*, etc. to the fibres of the second order and *g.f.*3 to those of the third order.

fibre as it passes backwards (fig. 6 and fig. 37, Plate 45). The actual synaptic contact is made by collaterals of the second-order fibre, which can be seen clearly in fig. 6. These pass through the sheath of the first-order fibre and make contact with its axoplasm. This arrangement is similar to that of the synapses in the stellate ganglion, which, being larger, are more easily studied (p. 491).

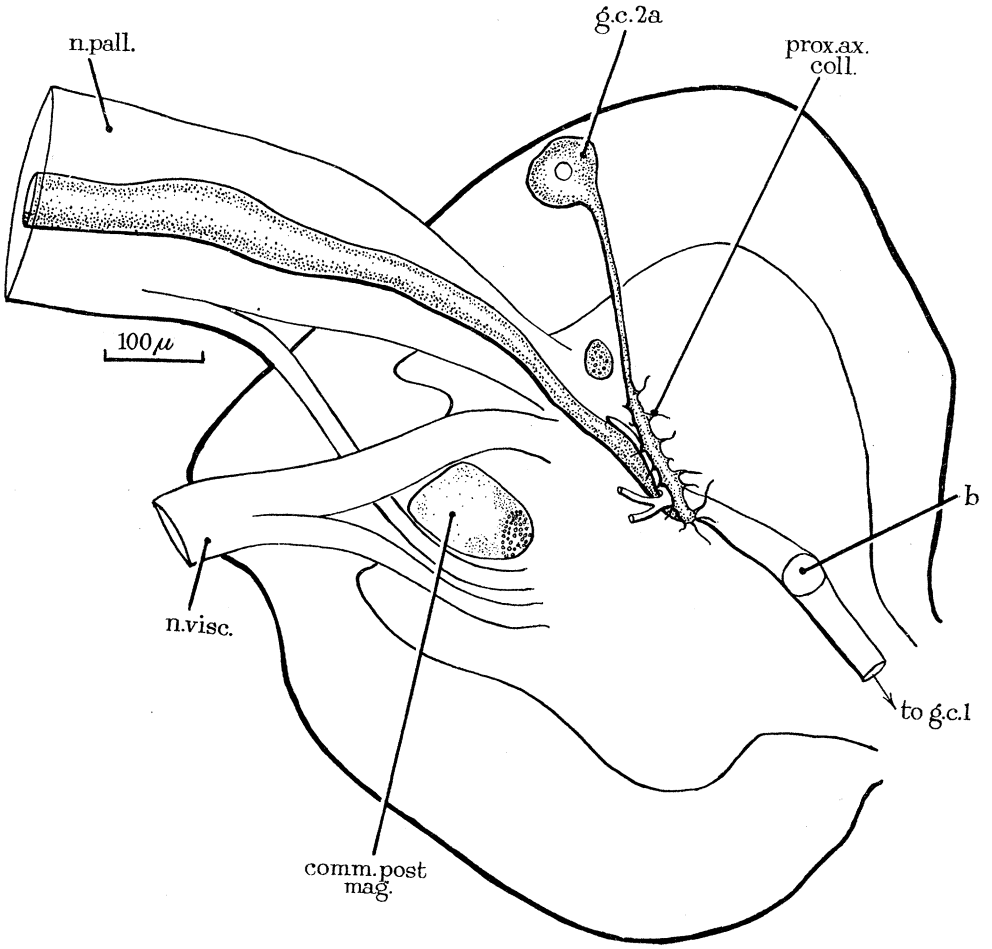

FIG. 9. Drawing of sagittal section of freshly hatched *L. pealii* to show second-order giant cell. Cajal's stain, celloidin section, 50μ thick (HGc 3.2.7).

Each second-order fibre comes under the influence not only of impulses arriving at its synapse with the first-order fibre, but also of those which affect the collaterals which lie closer to its cell body. The two sets of synapses may be distinguished as distal and proximal (Young 1936*b*). The fibres which make contact with the proximal collaterals include large bundles running from the brachio-visceral connective and others arising from cells in the dorsal part of the lobus magnocellularis. The former may perhaps be connected with tactile impulses from the arms and the latter are mainly under the influence of optic impulses, which dominate the dorsal part of the lobus magnocellularis.

The function of these proximal synapses and their relation to the distal ones remains obscure. It may be that they provide alternative paths for the activation of the muscles by optic or tactile impulses. It is also possible that they represent inhibitory connexions, since there may be situations in which one or more of the muscles controlled by the giant-fibre system is not required to contract with the rest. A similar mechanism of distal and proximal synapses is found in the stellate ganglion and the possible significance of the arrangement is discussed further on p. 493.

The positions of the cell bodies of the second-order axons other than of that which runs to the stellate ganglion have not been worked out in detail. The cells of some of them certainly lie in the dorsal wall of the palliovisceral ganglion, those of others probably in its ventral wall. Description of their courses is made especially difficult by the great variation which occurs between individuals. The n. retractor capitis posterior and n. visceralis arise each by two roots within the central nervous system, one root running dorsally and the other ventrally to the great commissura posterior magnocellularis (figs. 3, 5). The three giant fibres passing to the m. retractor capitis posterior sometimes all pass in the dorsal root, more rarely all three in the ventral root; usually there are some in one root, some in the other (fig. 1). The arrangement is often not even symmetrical on the two sides of the animal (e.g. in fig. 6). It is apparently a matter of embryological accident whether, during ontogeny, a fibre runs out in the dorsal or ventral root of these nerves.

The total number of fibres also varies. Characteristically in *L. pealii* there are three fibres in the n. retractor capitis posterior and two in the n. visceralis, but there may be more or less (fig. 6). In *L. forbesi* there are usually four or five fibres in the n. retractor capitis posterior, sometimes more (fig. 38, Plate 45). A further practical difficulty in the study of these axons is that there are other fibres in the nerves which approach the second-order giant axons in diameter.

An interesting feature is the constant differences in staining capacities shown by the various second-order fibres (Young 1937*a*). The fibres in the n. retractor capitis posterior are always strongly basophil, both within the visceral ganglion and at the periphery, whereas the fibre in the viscero-stellate connective stains very lightly along its whole length.

In addition to the fibre *g.f.*2*a* the viscero-stellate connective also contains a second or accessory giant fibre (to be called *g.f.a.*), which arises in the palliovisceral ganglion but does not make connexion with any branch of the first-order axon. This fibre runs to the stellate ganglion, where it makes connexion with the third-order giant fibres by means of a special set of proximal synapses (p. 492). In *L. forbesi* this fibre is always easily recognizable (fig. 38, Plate 45) being little smaller than *g.f.*2*a*. In *L. pealii* it is not easy to distinguish from some other rather large axons which run in this part of the mantle connective (fig. 39, Plate 45), and which may possibly also play some part in the activities of the giant-fibre system. At present it is impossible to say what the functions of this fibre *g.f.a.* may be (see p. 493).

8. Peripheral distribution of the second-order axons

The further courses of the fibres which leave the palliovisceral ganglion may now be described.

(*a*) *Nervus visceralis.* In each visceral nerve there are two or more large fibres which run to the m. retractor (depressor) infundibuli (figs. 1, 2, 5, 6). The two visceral nerves pass downwards together through the digestive gland, and then out through the body wall on to the ventral surface. At this point each gives off a branch laterally to the retractor muscle of the funnel. This branch may be called the n. retractor infundibuli anterior and contains one giant fibre, of diameter 61 μ in a *L. pealii* of mantle length 19 cm. (*Loligo* OL), as well as numerous smaller fibres.

A short way farther back the n. visceralis gives off a second twig, the n. retractor infundibuli medius, containing a giant fibre of diameter 81 μ in the above squid (*Loligo* OL). The rest of the visceral nerve, passing to the ink sack, viscera, gills, etc., contains several rather large fibres, up to 50 μ, but these are all distinctly smaller than those of the rest of the giant-fibre system, with which they probably have no connexion.

(*b*) *Nervus retractor capitis posterior.* The giant fibres in this nerve innervate the m. retractor capitis posterior and the hind part of m. retractor infundibuli (fig. 2). The nerve passes backwards with the viscero-stellate connective and fin nerve as far as the hind end of the nuchal cartilage, and then leaves them to run back across the inner face of the m. retractor capitis posterior (fig. 1). The arrangement of its branches, and of the giant fibres contained in them, shows considerable individual variation. The typical arrangement is that of figs. 1, 2, in which the nerve gives a branch immediately behind the nuchal cartilage to m. retractor capitis posterior dorsalis (see Williams (1909) for an account of the anatomy of these muscles). This branch contains the smallest of the three giant fibres which typically occur in the nerve, having diameter 70 μ, in its fresh state, at this point, in the squid taken for illustration (*Loligo* OL).

Farther back a second dorsal branch is given off, containing the medium-sized fibre (78 μ) which sends branches to the dorsal and lateral parts of the retractor muscle. The third giant fibre, considerably larger than the other two (170 μ), gives off branches to the hind part of the m. retractor capitis posterior lateralis, but its major branch runs still farther back, into a nerve which innervates the hind part of the m. retractor infundibuli, and may therefore be called the n. retractor infundibuli posterius. It has apparently not been observed by previous authors that a branch passes thus from the n. retractor capitis posterior to the retractor (depressor) of the funnel. The naming of these nerves and muscles becomes a matter of considerable difficulty, since two different muscles are here innervated by the same axon, and therefore presumably normally contract together. A further complication arises from the presence of the smaller fibres, which may activate either muscle separately.

(*c*) *Nervus infundibuli posterior.* This nerve contains a single giant fibre of diameter 70μ (*Loligo* OL), whose destination has not been traced in detail. The nerve runs to the intrinsic musculature of the funnel (figs. 1, 2) and the giant fibre presumably innervates some longitudinal muscles of this region.

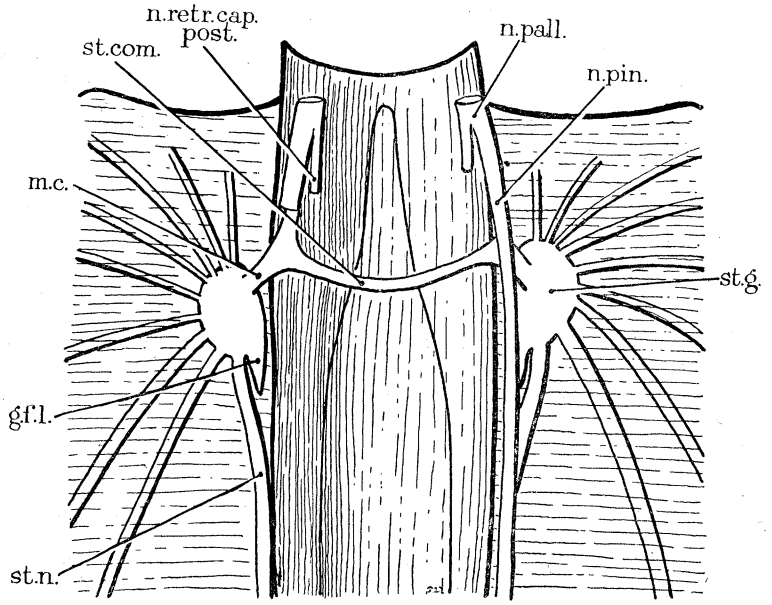

FIG. 10. Drawing to show the position and shape of the stellate ganglion in *L. pealii*. On the left side the fin nerve has been cut short to show the giant fibre lobe.

9. THIRD-ORDER GIANT FIBRES AND THEIR SYNAPSES

(*a*) *Origin from many cells*

On reaching the stellate ganglion the two giant fibres which run into it from the pallial nerve make synapse with the third-order giant axons, which, arising in that ganglion, pass to the circular muscle fibres of the mantle. There is one of these third-order giant fibres in each of the stellar nerves, making, in *L. pealii*, nine to eleven in all, which run through the centre of the ganglion into a special giant-fibre lobe (figs. 10, 11 and figs. 40–43, Plate 46) (Young 1936*a*). This lobe projects backwards for some distance alongside the hindermost stellar nerve and above the fin nerve. It can easily be recognized with the naked eye if the fin nerve is pulled away with needles and is rather larger and more conspicuous in *L. pealii* than in *L. forbesi* or *L. vulgaris*.

As the giant fibres pass into the lobe they break up into a very great number of branches (fig. 43, Plate 46), the finest of which are the processes of the small cells which make up the walls of the giant-fibre lobe. Each third-order axon is therefore a syncytium, formed by the fusion of many axons, several hundreds probably collaborating to make up the larger fibres (Young 1936*a*). It is important to recognize that

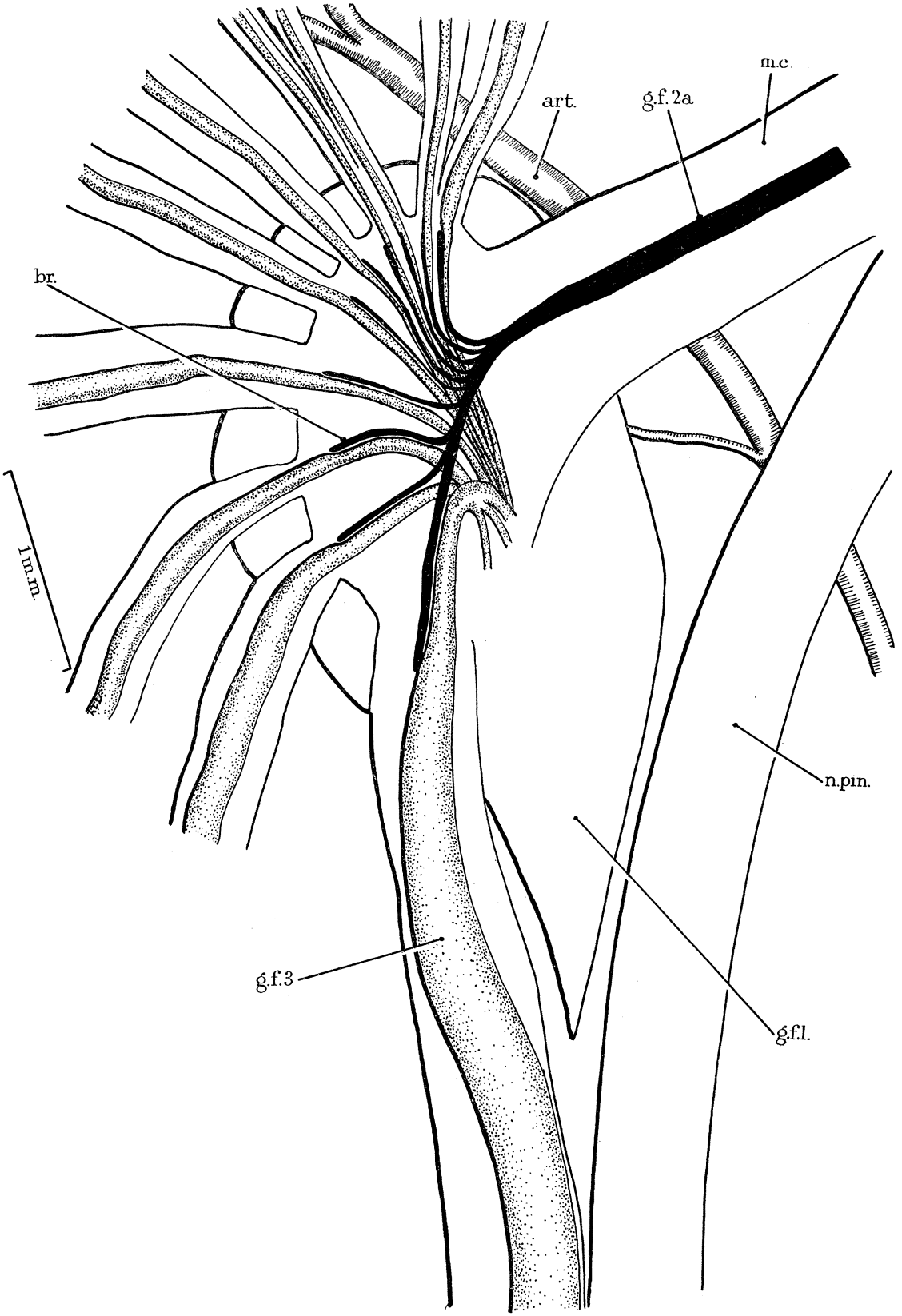

FIG. 11. Camera lucida drawing of the outlines of the giant fibres in the right stellate ganglion as seen from its ventral surface with a $\frac{2}{3}$ in. objective, by transmitted light, immediately after removal from the body of a small *L. pealii* in good health.

the fusion of these axons is complete, and that in no sense can the resulting axon be considered as a bundle of separate fibres or fibrils, each derived from one of the parent cells. This is shown by the following facts: (1) That the whole is enclosed in a single sheath (Young 1936*c*; Bear, Schmitt and Young 1937), of the sort which normally

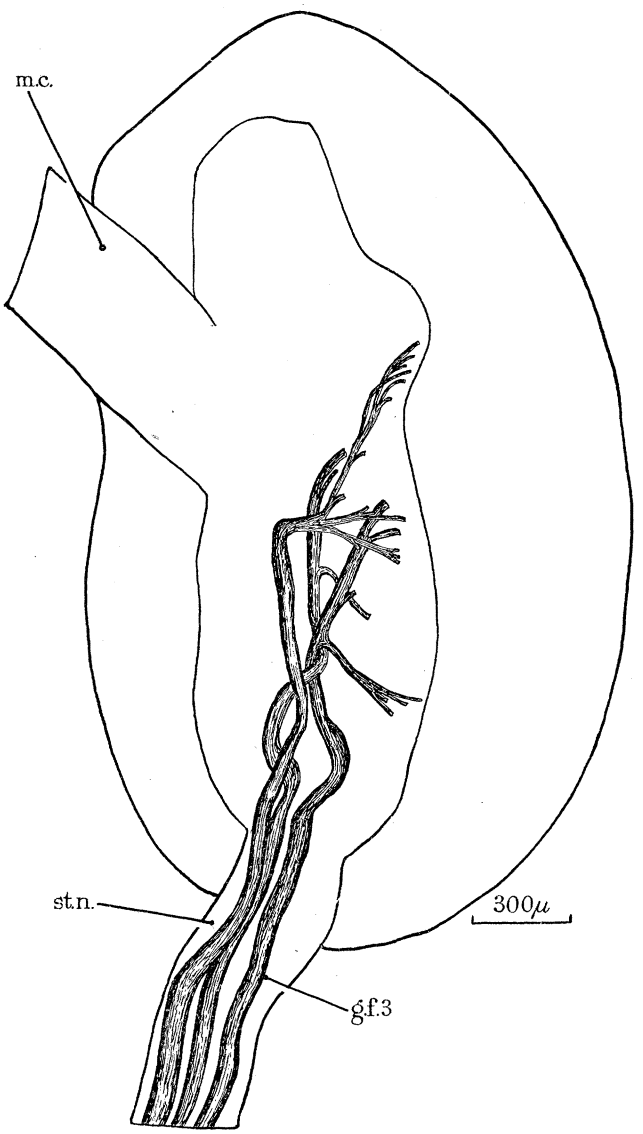

FIG. 12. Drawing, made by projection from several sections, of the giant fibres in the stellate ganglion of *Sepia officinalis*. Cajal's method (CEa).

encloses a single neural unit. (2) The axoplasm appears homogeneous in transverse and longitudinal section. The question of the status of the excessively fine fibrils which may under some circumstances be seen within it is discussed in the above papers. In no case do they give the appearance of subdividing the fibre into units. (3) On section of a fresh axon the axoplasm flows from the cut end of the sheath (Young 1935, 1936*c*). (4) The synapses described below (p. 489) are made by means

of collaterals which are given off at the surface, and could not provide connexions for separate fibrils within the fibre, but only for the whole as a unit. (5) By stimulating the axons with condenser discharges, or induction shocks, of graded intensity it can be shown that the fibre always fires impulses as a whole, it is not possible to find separate parts responding at different threshholds (Young 1938*a*). (6) The action

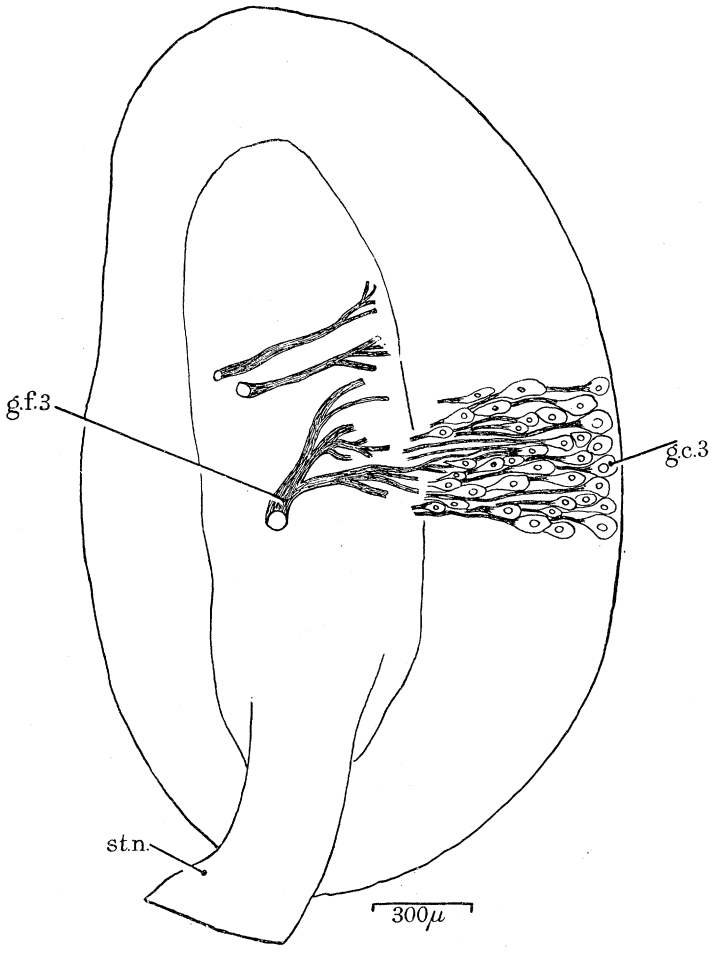

FIG. 13. Drawing of a single section (CEa 1.3.7) from the same series as fig. 12, to show the origin of the third-order giant fibres in *Sepia*.

potentials produced by the fibres are diphasic action potentials of the normal type (except where complicated by branching) (Pumphrey and Young 1938).

These points show decisively that the giant fibres in the stellar nerves conduct like other single nerve fibres as units, although they arise as the processes of a number of separate nerve cell bodies. In some cases, by following serial sections, the finest branches can be traced to the cells of the giant-fibre lobe. However, it is not usually possible in *Loligo*, even with good Cajal preparations, to follow them for the whole of their course, because although the original axons are only a few microns in diameter yet they may be nearly a millimetre long, and hence extremely difficult to follow from origin to fusion.

There can be no doubt, nevertheless, that the fusion takes place in the manner described. The axons undoubtedly break up into fine branches at the base of the giant-fibre lobe, and these branches are perfectly similar to the processes of the cells of the giant-fibre lobe. Moreover, there are no giant cells in the ganglion, or other possible cells of origin for the third-order fibres. Finally, in *Sepia* and *Ommatostrephes* there are no giant-fibre lobes and hence the fine fibres are shorter, so that it is possible to show definitely that the fusion of the processes of many cells go to make a single giant fibre (figs. 12, 13).

Although each fibre is a syncytium yet it remains anatomically distinct from its neighbours. This agrees with the conclusion reached from experiment that impulses set up peripherally in one giant fibre, though they can be conducted backwards along it and to all the muscle fibres with which it is connected, yet do not spread to neighbouring giant fibres (Young 1938*a*).

(*b*) *Giant synapses in the stellate ganglion*

The two giant fibres which enter the stellate ganglion from the mantle connective make different types of synapse with the third-order giant axons. The larger fibre, *g.f.*2*a*, which is activated by the first-order giant fibre in the central nervous system, makes the more distal synapses (Young 1936*b*), sending one branch to make contact with each third-order axon, shortly before the latter leaves the ganglion (fig. 11). The smaller fibre, *g.f.a.*, which is not connected with the first-order system in the central nervous system, makes the more proximal synapses, breaking up into a mass of terminal knobs in the giant-fibre lobe.

(i) *Structure of the distal synapses*

The actual synaptic contact between the fibres is very much more close and extensive than appears from fig. 11, since there are numerous collateral branches, not shown in that figure, which are given off from the outgoing fibre (fig. 14 and figs. 43*a*–55, Plates 46–48). The connective-tissue sheaths which enclose the two fibres are fused along the zone of contact between them, and the collaterals run through little holes in the common sheath which separates the two main fibres (figs. 44, 47, 50, Plate 47), ultimately penetrating through to establish contact with the surface of the second-order fibre. The latter does not give off collaterals, but ends abruptly, sometimes with a knob (figs. 44, 49, 50, 53, Plates 47, 48). The details of the shapes of the collateral branches of the third-order fibre, and their relation to the second-order fibre can be made out in great detail by careful study of sections.

The general shape and number of the collaterals of the third-order fibre are best seen in silver-stained material, with sections cut longitudinally to the main axis of the fibres. The finer details of the relationship of the partners at the synapse can only be studied in material in which there is a minimum of shrinkage, which is best ensured by fixation in picric acid or picro-formol.

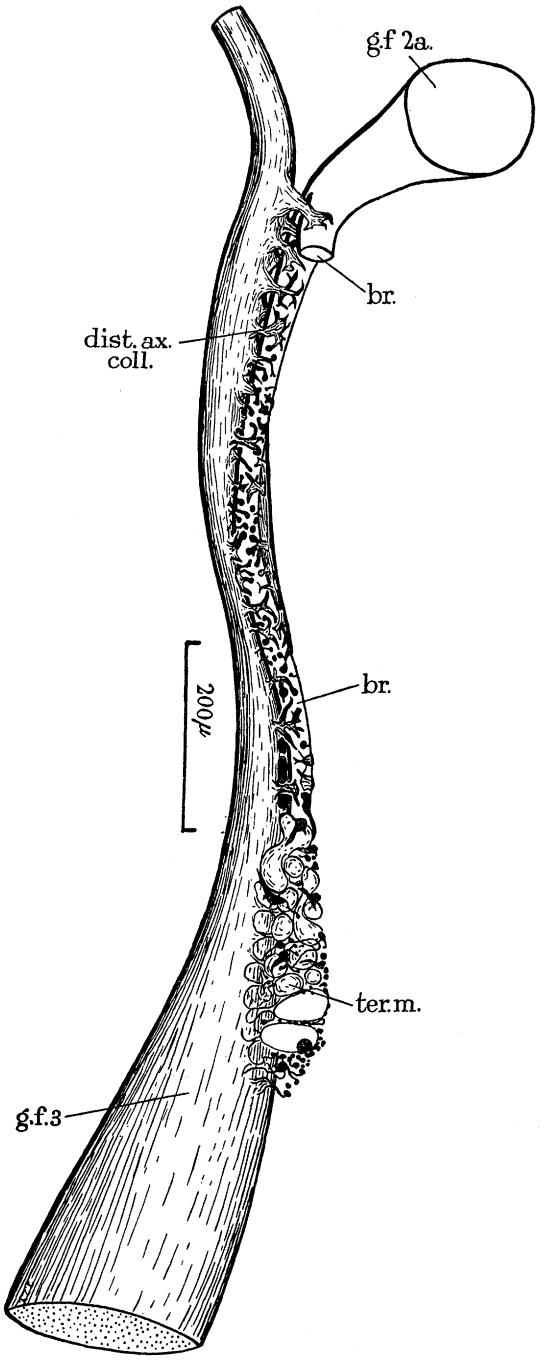

FIG. 14. Drawing, made by projection from several sections, to show collaterals of the third-order fibre in the stellate ganglion, wrapping around the end of the second-order fibre with which they make synaptic contact. Sagittal sections, stained with Cajal's method and sectioned in paraffin (*L. forbesi* AYa, slide 2).

The collaterals occur in very great abundance along the whole length of contact of the second- and third-order fibres, that is to say, over a distance of about 1 mm. Almost the whole surface of the second-order fibre is covered by the collaterals for this distance. It is difficult to give a clear picture to show the abundance of these branches, but figs. 14 and fig. 44, Plate 47 attempt to do this.

The endings of the collaterals against the second-order fibre are of the most various shapes. In the more proximal part of the surface of contact they do not usually indent the surface of the second-order fibre, but end as flat sheets just inside its sheath (figs. 45, 46, Plate 47). More distally, however, towards the tip of the second-order fibre, the collaterals, besides becoming more abundantly branched, swell out at their ends into knobs, often quite large, which lie within the sheath of the second-order fibre and indent its surface (figs. 47, 48, 55, Plates 47, 48). At the end of the synaptic zone, therefore, the second-order fibre is constricted by a great number of knobs of various sizes, some of them so large as almost to divide it into two channels.

In some, but not all, cases the collaterals continue to be given off by the third-order fibre for a short distance beyond the end of the second-order fibre (figs. 51–53, Plate 48). These most distal of the collaterals are often very large and rounded. It seems probable that during morphogenesis a space for collaterals is formed beyond the end of the incoming fibre. The collaterals flow into this space through the holes in the sheath, but, not meeting any second-order axon, they then become very large and fill the whole space. There are usually fewer of these extra collaterals in *L. pealii* than in *L. forbesi*. In the latter species they are sometimes so numerous as to produce a very large tangled mass of knobs and fibres of various sizes (fig. 51, Plate 48). At one stage of the investigation it was suspected that some of these knobs represented branches of the incoming second-order fibre, and that the whole mass forms an extra region of synaptic contact. The complexity of the tangle is such that it is difficult to disprove this view conclusively, but the indications are that the terminal mass is the result of an accident of morphogenesis and of no functional significance.

(ii) *Nature of contact at the synapses*

Since the outer connective tissue and myelin-containing sheaths of these fibres stain darkly with Mallory's or Masson's techniques it is possible to follow their courses very accurately, and to be certain that *these sheaths do not intervene between the surfaces of the two axons at the points of synaptic contact.* It is less easy to be sure whether the inner protoplasmic sheath intervenes, since it is difficult to stain (Bear, Schmitt and Young 1937). Nevertheless, it is very unlikely that this sheath runs between the two axons, since nuclei have never been seen between the two surfaces, though they are so common elsewhere around the fibres as to be readily found in every section. Fig. 54, Plate 48, shows a nucleus which partly separates the two members of the synapse, but this was the only case where such a degree of separation was seen, and even here the protoplasmic sheath does not separate the surfaces along their whole length.

In the best preparations the surfaces of the fibres appear to be pressed closely against each other, with no visible space between (fig. 43*a*, Plate 46 and figs. 48–50, Plate 47). In many cases, however, a narrow shrinkage space appears (fig. 47, Plate 47), and the separation of the two fibres in this way shows most clearly that they remain distinct, and do not mingle with each other at their place of contact.

The fact that there is not complete mingling between the substances of the two members of the synapse is also most strikingly shown by the difference in their staining capacities. The axoplasm of the outgoing, third-order, axon is deeply basophil, and after fixation with a protein precipitant, such as picric acid, contains a great number of small granules (figs. 47–50, 55, Plates 47, 48). The substance of the incoming fibre, however, hardly takes basic stains at all, and even after precipitation of the proteins contains only a few relatively large granules. In fact the incoming fibre appears to have a very much smaller protein content than the outgoing fibre, and much less of the acidic material. Some comment has already been made upon the gradation in amount of acidic material (and perhaps of total protein) which occurs along the length of the giant fibres (Young 1937). Whatever may be the functional significance of this gradation, it shows decisively that the substances of the two axons do not mingle at the synapse. They are kept apart, either because they are not mixable, or, more probably, because each is enclosed completely by its retaining membrane, the axiolemma. The significance of this separation is further discussed on p. 498.

Another interesting feature of these distal synapses is the presence, in some preparations, of oxyphil granules at and near the synaptic surfaces. These granules are found elsewhere in both incoming and outgoing axons, but in a few of the preparations they are certainly especially abundant at the synapses. They lie either just inside the axiolemma of one or both members, or in some cases actually in the region between the two (fig. 55, Plate 48). Nothing further can be said of the chemical nature of these granules than that they stain as if they were basic. Their significance remains entirely problematical, and of course it must not lightly be assumed that they represent any form of chemical synaptic transmitter.

(iii) *Proximal synapses*

The synapses of the smaller of the two giant fibres which run into the stellate ganglion (*g.f.a.*) are all made close to the cell bodies of the third-order giant fibres (figs. 41–43, Plate 46). The main fibre breaks up at the base of the giant-fibre lobe into a great number of branches, which finally end as knobs of various shapes, interspersed among the axons of origin of the third-order fibres (fig. 56, Plate 48). Although these knobs can be very beautifully stained with silver stains, the details of their relationship to the fibres with which they make contact cannot be so clearly determined as in the case of the distal synapses. It is not easy to see them in preparations other than those stained with silver, but it is probable that they end inside the sheaths of the third-order axons, the two being kept separate only by their axiolemmas. The

branching of the fibre *g.f.a.* is so extensive, and the knobs so abundant, that a very large surface of contact is provided. It is hardly profitable to speculate at present as to the possible modes of action of such synapses, which influence the component parts of the giant fibre *before they fuse* to make a single axon. Possibly they provide an alternative path for activation of the third-order giant fibres and their muscles, independently of the rest of the system which is worked by the first-order giant cells. It is even possible that this fibre *g.f.a.* exerts some inhibitory influence.

(*c*) *Peripheral distribution and endings of the third-order giant fibres*

The general arrangement of the stellar nerves has been sufficiently described in a previous paper (Young 1938*a*). When one of the nerves is examined alive the giant fibre can be seen very easily among the smaller nerve fibres as a clear channel, readily available for study of its optical, chemical and physical properties (Bear, Schmitt and Young 1937) and not difficult to isolate completely. The diameter of the fibres increases progressively, passing backwards (see Table 1) in the series of stellar nerves, and details of this variation, and of its possible significance, have been discussed in the paper by Pumphrey and Young (1938), in which also figures of the action potentials and of the rates of conduction of these giant fibres are given, and details of the way in which they taper. The fibres are somewhat larger in *L. forbesi* than in *L. pealii*, the largest observed being about 940μ in diameter in the former, 750μ in the latter. No attempt to define the upper size limits has been made. The animals continue to grow throughout life, and in very large individuals fibres over 1 mm. could probably be found.

It remains to discuss the branching and method of termination of the fibres. Small branches are given off at intervals of a few millimetres along the whole length of each of the fibres, including the most posterior. Each axon therefore innervates a roughly triangular segment of the mantle, the sharp apex of the triangle lying near the stellate ganglion (Young 1938*a*). Towards the periphery the fibres often divide into larger branches, and this usually occurs at similar points in all individuals. For instance, the hindermost axon nearly always bifurcates into two equal branches close to the widest part of the pen. There are, however, considerable individual variations. For instance, in one case the hindermost axon divided into two large branches close to the stellate ganglion, the two running side by side in the nerve until their separation at the usual place where the main trunk of the nerve divides.

The details of the peripheral endings of the third-order giant fibres have not yet been made out. They branch repeatedly among the muscles, and the finer fibres seem to run transversely across the circular muscle fibres of the mantle, to which they have been shown by experiment to send branches.

10. Significance of the giant fibres in the life of cephalopods

Experiments in which the stellar nerves have been stimulated electrically have shown that the giant fibres in them are motor axons which cause contraction of the circular muscle fibres of the mantle (Young 1938*a*). It may safely be assumed that, as the anatomical connexions suggest, the whole giant-fibre system is a motor system, operating the muscles by which rapid movement is produced.

Nerve fibres large enough to be classed as "giant fibres" occur in the nemertines, annelids, crustaceans and vertebrates, and in the cases in which their function has been investigated (earthworms, Stough 1926; crayfish, Johnson 1926) they have been found, as in cephalopods, to operate mechanisms for the performance of rapid movements of escape or attack. Since it is known that in vertebrates large nerve fibres conduct faster than smaller (see Erlanger and Gasser 1937), it is often assumed that the animals which possess exceptionally large fibres effect thereby a significant saving of reaction time. In no case, however, has it hitherto been shown that the time so saved is a significant fraction of the total reaction time, and there are at least two other possible advantages which may accrue from the possession of giant fibres, namely, ease of excitation of many muscle fibres together and, where fibres of different sizes are present, insurance that they all contract simultaneously or at appropriate intervals. These various possible advantages of the presence of giant fibres will now be considered separately.

(*a*) *The giant-fibre system as a final motor path*

The cephalopods show very strikingly the advantages and limitations of the operation of many muscles from few neurons. The two giant cells in the lobus magnocellularis can be activated by cerebral, optic, static and tactile impulses, and by their connexions with the second-order giant cells can produce contraction of the retractor muscles of the head and funnel and of the circular muscle fibres of the mantle. Moreover, it is probable that a *single impulse* set up in either of the giant cells is sufficient to produce this very widespread contraction. For it has already been shown that a single impulse in one of the stellar nerves can activate all of the muscle fibres reached by the giant axon (Young 1938*a*); no summation of the effects of several impulses is necessary. Further, since single condenser discharges (which set up single impulses) applied to the mantle connective of a squid will cause contraction of all of the mantle of that side, it seems that excitation of the third-order fibres can also be effected by single impulses arriving in the second-order axons (Young 1938*b*). There is every reason to suppose that the same applies to the synapses between the first- and second-order fibres in the palliovisceral ganglion, since these are similar in type to the synapses in the stellate ganglion.

However it is most unlikely that the first-order axons can be activated by single impulses from the statocyst, skin or eyes, since the animal would then hardly ever be at rest. Probably the collaboration of several impulses in time or space is necessary

to fire off the giant cell. It is significant that the end feet which produce this result resemble the synaptic boutons on a vertebrate ventral horn cell, which has a similar function, and that this arrangement differs considerably from the much-branched apparatus by which a single impulse is passed across the synapses in the stellate ganglion.

The giant-fibre system is arranged, then, for the production of movements in which a large number of muscle fibres act maximally and together. This is ensured by the presence of a small number of neurons which produce their effects by means of single impulses. The limitation of such a plan is that it provides little opportunity for the production of graded contractions, or of the holding of the muscles in a state of tonic contraction. It is the type of nervous organization most suited to a muscular system of the pure "movement" as opposed to the "tonic" type (Riesser 1936). The rapid movement of the decapod cephalopods is indeed a most clear example of such a mechanism, not only in the type of nervous control, but also in that the muscle fibres are activated by single impulses, in that they contract and relax quickly and show no wave summation of contraction when thrown into a tetanus (Prosser and Young 1937). Indeed the mantle can only contract and then relax again before it is ready for another active stroke, and a tonic mechanism would be of no value to the animal.

It may be concluded that the presence of few nerve fibres controlling many muscle fibres is an asset for an action system of the type of that of the squid, which depends on movement without holding (tonus) or precise grading. The reduction of the number of the nerve fibres in such a system makes it possible for them also to be very large, and hence to gain the advantages which follow from rapid conduction (see Pumphrey and Young 1938, and next section). It would hardly be possible for an animal with the type of organization found in the squid to produce actions which were both very rapid and well graded. It could not have *numerous* very large fibres in each nerve. A combination of speed with gradation of movement is practicable, however, in the animals in which increase in conduction rate is obtained by special structure or increased thickness of the myelin sheath, particularly in the vertebrates.

(*b*) *Saving of time effected by the giant-fibre system*

Study of the conduction rates of fibres of various diameters in the stellar nerves of *Sepia* and *Loligo* has shown that a significant saving of time is effected by the rapid conduction of the large fibres (Pumphrey and Young 1938). The animal probably begins to move in about half the time which would be taken by an animal having no fibres larger than, say, 50μ. However, *Sepia* and *Ommatostrephes* have smaller fibres than a *Loligo* of similar length. This must mean either that the last named is a quicker mover than the others or that saving of time is not the only reason for the presence of the large fibres.

(*c*) *Significance of the presence of fibres of various diameters*

It has been calculated that the presence of fibres of graded diameter in the stellar nerves of *Loligo* causes the muscles of the mantle to contract more nearly together

than they would do if the fibres were either all small or all large (Pumphrey and Young 1938). However, the difference between the condition with graded fibres and the hypothetical one with all large fibres was found to be slight, and in order to examine further this question of the significance of the presence of fibres of graded diameters, careful measurement was made of the lengths and diameters, not only of the third-order giant fibres of various diameters, but also of the second-order fibres which innervate the retractors of the head and funnel. The *L. pealii* used (OL) was of mantle length 19 cm. and all the measurements were made in the fresh state. Using the data of Pumphrey and Young (1938) for the relationship of conduction rate to fibre diameter, the times of arrival at the various muscles, of impulses starting together from the palliovisceral ganglion have been calculated, a synaptic delay of 2 m.sec. being assumed in the stellate ganglion. For comparison, the times which the impulses would take if all the fibres conducted at 5 m./sec. and 20 m./sec. have also been calculated.

It will be seen from Table 1 that the results of such calculations are similar to those arrived at by Pumphrey and Young. Considering either the stellar nerves alone, or the whole system together, it is found that, in spite of the gradation in the sizes of the

TABLE 1

Nerve	Diameter (μ)	Mass of muscle (g.)	Rate (m./sec.)	Length (mm.)	Conduction time m.sec.	Conduction time at 5 m./sec.	Conduction time at 20 m./sec.
N.inf.post.	70	Unknown	5·9	25	4·2	5·0	1·3
N.ret.inf.ant.	61	0·4	5·5	30	5·5	6·0	1·5
N.ret.inf.med.	81	0·5	6·4	39	6·1	7·8	2·0
N.ret.cap.post.	70	Unknown	5·9	15	2·5	3·0	0·7
	78	Unknown	6·3	20	3·2	4·0	1·0
	147	Unknown	9·0	65	7·2	13·0	3·3
Visc.-stell. conn.	88	None	6·7	26	3·9	5·2	1·3
(Synaptic delay)	—	—	—	—	2·0	2·0	2·0
(Times added to figures in rest of table)			—	—	5·9	7·2	3·3
Stellar nerves	131	0·4	8·4	23	8·6	11·8	4·4
	90	0·3	6·8	22	9·1	11·6	4·4
	86	0·3	6·6	22	9·2	11·6	4·4
	148	0·8	9·0	24	8·6	12·0	4·5
	227	1·6	11·7	43	9·6	15·8	5·4
	291	3·1	13·6	48	9·4	16·8	5·7
	291	4·2	13·6	76	11·5	22·4	7·1
	317	4·5	14·3	91	12·3	25·4	7·8
	447	10·2	17·5	142	14·0	35·6	10·4

fibres, impulses arrive sooner at the nearer muscles than at those farther away, but the difference is much less than it would be if the animal had no fibres conducting faster than 5 m./sec. Thus the extreme calculated difference in the actual animal is between 2·5 m.sec. to reach the front part of the m. retractor capitis posterior and 14·0 m.sec. to reach the hind end of the mantle, whereas if there were no giant fibres the difference would be between 3·0 and 35·6 m.sec. If all of the fibres were large the absolute time differences would be slightly less than with graded fibres (0·7 and

10·4 m.sec.), but the relative differences, if these have any significance, would be greater.

One further possible significance of the variation in size of the fibres is that they innervate varying numbers of muscle fibres, and therefore that some require to be larger in order to provide the necessary branches. It is indeed the case that the larger fibres innervate the greater numbers of muscle fibres, and to give some idea of these differences the pieces of muscle innervated by each giant fibre were separated as accurately as possible, and weighed. As will be seen from the second column of Table 1, the smallest fibre present (61 μ) was estimated to innervate 0·4 g. of muscle (including connective tissue, etc.) and the largest fibre (447 μ), 10·2 g. In view of the various uncertainties it has not been thought profitable to try to define the relationship more closely. On general grounds it does not seem likely that increase in the number of muscle fibres innervated would necessitate increase in the size of the nerve fibres. The initial part of the axons of the second-order fibres is very much thinner than the main trunk, a fact which by itself suggests that the significance of the large diameter is that it ensures rapid conduction, and not that it provides for the maintenance of the large mass of tissue which the fibre must innervate. In any case the large fibre *g.f.2a* does not have to control large numbers of muscle fibres but only the relatively few third-order giant-nerve fibres.

11. Clarifications of the neuron theory

The peculiar opportunities offered by the giant-fibre system of cephalopods enable us to formulate a number of important propositions about the organization of the nervous system, amplifying and clarifying the neuron theory. The main postulates of that theory, emphasizing the anatomical, embryological, trophic and functional independence of neurons, are usually true, but may be qualified and expanded by the following further propositions.

(1) *Complete fusion between the processes of two nerve cells occurs in some animals.* The evidence presented on pp. 480 and 488 shows that during development the axons growing out from two separate nerve cells may fuse, in exactly the manner which is held by strict adherents of the neuron theory never to take place. There is some evidence that a similar fusion takes place in other cases, for instance, in Crustacea (Johnson 1924) and Annelida (Stough 1926). It is not necessary to delay over the question of whether we should save the letter of the neuron theory by saying that such nerve cells are, by definition, not neurons (see Maximow and Bloom 1930).

(2) *When axons are fully fused they always work together, impulses set up anywhere in them spreading over the whole continuous neuroplasm.* It is most important to recognize that the occurrence of such fusion in any one case *does not invalidate the neuron theory in general.* Paradoxically, it serves rather to emphasize the true significance of the synapses, by showing clearly the manner in which complete fusion differs from the contact of

discontinuous neuroplasms at a synapse. In every case where the complete fusion occurs the axons are ones which *must always work together during the life of the animal.* This applies to the interaxonic bridge, to the axons of the stellar nerves and to those giant fibres of annelids and crustaceans which have been held to show complete fusion. In all of these cases it is biologically desirable for the survival of the animal that every impulse set up in one of the axons should be conducted to the other with which it is in continuity.* It is exactly this which it is alleged by adherents of the neuron theory is likely to happen after such fusions. This leads to a further point, emphasizing the significance of the synapse.

(3) *The indefinite spread of impulses in the nervous system is prevented by the presence of regions, the synapses, in which the neuroplasms of two neurons are in contact but do not mingle with each other.* It would be impossible here to review the whole controversy as to the exact relationship of the members of a synapse (see Cajal 1934; Boeke 1938). In spite of the difficulty which some histologists find in adjusting the terms of an old controversy to contemporary ways of thought, the important issue stands out quite clearly, and essentially in its original form (see Sherrington 1906, p. 15). It may be expressed by the question: "Does study of the structure of the nervous system provide any clue as to why nerve impulses do not spread throughout the whole central nervous system as they do over a continuous mass of neuroplasm in a peripheral nerve fibre?" The answer is clearly: "Yes. Histological studies show that at the regions known as synapses one mass of neuroplasm touches but does not freely mingle with another. Here is a region, then, in which the parts are not continuous in the same sense as they are in the various parts of a single neuron, or in the interaxonic bridge of *Loligo*. Therefore, whatever view is taken as to the nature of the propagated nerve impulse, we should not expect impulses to be forwarded across such synapses in quite the same way as they are over a continuous mass of neuroplasm."

It is tempting to go farther and to speculate about the nature of the barrier thus imposed and the means by which it is overcome. But study of the morphology of the synapse cannot by itself settle any of the questions about synaptic excitation. What is known of the relationship of the fibres at such junctions is not inconsistent either with the view that the excitation of one fibre by another is humoral, or that it is accomplished by a process similar to that by which one part of a nerve fibre excites another part of the same fibre, namely, by the "local electric circuits produced by the activity of adjacent parts" (Hodgkin 1937*a*).

An impulse arriving at the afferent side of a synapse is presumably not always able to propagate further, as it would do over a continuous mass of neuroplasm, because of the special electrical conditions set up by the fact that the two fibres do not freely mingle. It is not possible to specify at present exactly how the current would flow in such a system, but certainly the absence of a continuous internal connexion between two

* This may also be true of cases such as that described recently by P. Glees *Proc. Konin. Ned. Akad. Weten.* **41**, 3, 1938) in which nerve cells are joined by short "neuroplasmatic junctions".

regions makes the relations of the various portions of neuroplasm very different at a synapse from those in a peripheral nerve. Possibly the effect of an impulse passing over a portion of axoplasm, say a terminal end foot, is to produce a local response of the neuroplasm with which it makes synaptic contact, just as the activity of one part of a nerve fibre produces local responses in neighbouring parts of the same fibre (Hodgkin 1937*a*, *b*, 1938; Katz 1937). With a continuous mass of neuroplasm the response induced by the activity of each part is nearly always great enough to propagate further, and has probably a margin of safety of several times (Hodgkin 1937*a*). Perhaps, because of the discontinuity of the neuroplasms at the synapse, the local response induced in the efferent member by the afferent is usually not sufficient to propagate unless impulses arrive at several suitably placed end feet nearly simultaneously, so that the local response is produced over a sufficient area.

Thus the stimulation of the first-order giant cell, by which the whole motor system is activated, is effected by means of small end feet, scattered over its dendrites (see p. 475). Presumably propagation of an impulse in the giant cell only occurs when a sufficient number of suitably arranged knobs discharge together. On the other hand a single impulse in the fibre *g.f.*2*a* which enters the stellate ganglion can excite the fibres *g.f.*3 (see Young 1938*b*), and in this case a conspicuously large area of contact between the two fibres is provided (see p. 489).

If this view is correct the irreversibility of conduction across synapses does not mean that dendrites cannot excite end feet, but that there is no means by which the non-propagated local responses produced in the latter can summate, as can those set up by neighbouring boutons on a dendrite, to produce a sufficient area of activation for propagation to occur. This would not apply to symmetrical synaptic junctions, and, as has already been pointed out (Eccles, Granit and Young 1932; Young 1936*b*), conduction at symmetrical synapses such as those of the giant fibres of earthworms is reversible.

However, it must again be stressed that we know very little of the conditions of current flow which would be necessary for the excitation of one mass of neuroplasm by a separate one. It is not impossible that the shape of the zone of contact will prove to be significant as well as its area. Or it may be that the mechanism of transmission across synapses is quite unlike that of peripheral nerve. But, in any case, absence of mingling of the substances of the two fibres at a synapse must be important in determining the characteristics of conduction in this region.

12. Summary

A. *Structure of the giant fibres and their synapses*

1. The rapid movement of *Loligo* through the water is produced by impulses set up in either one of a single pair of giant nerve cells (first-order giant cells) situated in the central nervous system.

2. These cells lie in a special lobus magnocellularis, which possibly represents the ventral portion of the perioesophageal ring (sub-cerebral commissure) of the ancestral molluscan nervous system (p. 470).

3. In addition to the giant cells the lobus magnocellularis contains other large motor cells and probably constitutes the motor centre for the performance of the movements of the arms, mantle, etc. by which the prey is caught with a sudden dash (p. 470).

4. The first-order giant cells are activated by end feet (boutons terminaux) which are placed all over their surface and bring impulses from optic, static, tactile and probably also from various central sources (p. 475).

5. The giant cells lie in the neuropil and are bipolar. They have very small nuclei and relatively little Nissl substance, the possible relationship of which to the basophil substance of the axon is discussed (p. 474).

6. Each first-order giant cell has a single axon which passes backwards into the palliovisceral ganglion and there fuses with the axon of the opposite side, forming an interaxonic bridge (p. 477).

7. Series of sections in three planes show that the substances of the two axons are fully mingled with each other in this bridge, in a manner inconsistent with the orthodox neuron theory (p. 497).

8. Behind the interaxonic bridge the first-order giant fibres make synaptic contact with several second-order giant fibres, whose cell bodies lie in the palliovisceral ganglion and whose axons pass to the funnel, retractor muscles of the head and funnel and stellate ganglion.

9. Arising in the stellate ganglia are third-order giant fibres, which are syncytia, formed by fusion of the processes of numerous neurons collected, in *Loligo*, to form a giant-fibre lobe (p. 488).

10. The second-order fibre excites those of the third order by means of numerous collaterals of the latter. At these synaptic junctions the neuroplasms of the two fibres can be shown not to mingle with each other.

B. *Significance of the giant fibres in the life of cephalopods*

11. The mechanism for rapid locomotion in the squid shows the extreme development of a system for producing movement rather than tonus. Contractions are initiated by the action of the minimum possible number of motor units, namely, one pair of fused nerve cells. A single impulse set up in this unit can produce a quick twitch of the retractors and expulsion of a jet of water from the mantle. Such a system, though not well suited for the production of finely graded movements, allows the development of few, large, and hence rapidly conducting fibres. The combination of speed with fine gradation of movement is only possible in animals, such as Vertebrates, in which rapid conduction is obtained by thickness or special structure of the myelin sheath, rather than, as in the cephalopods, by increased diameter of the fibres (p. 495).

C. *Clarifications of the neuron theory*

12. As a result of these investigations three propositions clarifying the orthodox neuron theory are advanced (p. 497), and it is shown that the infringement of the neuron theory found in *Loligo* is an exception which most clearly proves the rule, since it shows the contrast between complete fusion of two axons and the condition of contact which occurs at synapses.

13. That a single impulse does not usually pass a synapse is presumably due to the electrical barriers imposed by the absence of mingling of the neuroplasms. These barriers make the effect which an impulse passing over the afferent member of a synapse has in producing a local response in a neighbouring dendrite different from that which it would have on a continuous neighbouring neuroplasm. Synapses, such as those in the stellate ganglion, which are normally passed by single impulses, provide for large areas of contact between the two fibres (p. 489).

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List of Abbreviations for all Figures and Plates

acc.g.c	accessory giant cell.
ant.dend. or *ant.dendr.*	anterior dendrite of giant cell.
art.	artery.
ax.	axon of *g.c.*1.
b.	interaxonic bridge.
br.	branch of *g.f.*2*a*, making synapse with *g.f.*3.
*c.*1	intact axonic collateral of *g.f.*3, making contact with *g.f.*2*a*.
*c.*2	autolysed axonic collateral of *g.f.*3.
*c.*3	axonic collateral of *g.f.*3 with darkly staining core.
c.?	large bulb in terminal mass, probably collateral of *g.f.*3.
c.br.m.	brachio-magnocellular connective.
c.br.v.	brachio-palliovisceral connective.
c.b.p-v.dors.	dorsal division of *c.br.v.*
c.b.p-v.ventr.	ventral division of *c.br.v.*
c.p.v. or *c.p.p-v.*	pedo-palliovisceral connective.
com.ant.m. or *comm.mag.*	commissura anterior magnocellularis.
com.post.mag.	commissura posterior magnocellularis.
co. or *core*	central 'core' of *g.f.*1.
cor.ped.	corpus pedunculatum.
dist.ax.col.	axonic collaterals of *g.f.*2*a* by which distal synapses are made.
e.f. or *end f.*	end feet on *g.c.*1.
e.f.'	endings of *g.f.a.* on *g.f.*3 in neuropil of giant-fibre lobe of stellate ganglion.
f.	fibres crossing in centre of palliovisceral ganglion.
g.	fuchsinophil granules (or region in which they occur).
*g.c.*1	first-order giant cell.
*g.c.*2*a.*	second-order giant cell whose axon runs to stellate ganglion and there makes the distal synapses.
*g.c.*2*b.*	second-order giant cell whose axon runs to *m.retr.cap.post.*
*g.c.*2*c.*	second-order giant cell whose axon runs to *m.retr.cap.post.* and *m.retr.inf.*
*g.c.*2*d.*	second-order giant cell whose axon runs to *m.retr.inf.*
*g.c.*2*e.*	second-order giant cell whose axon runs to funnel.
*g.c.*3.	cells of origin of third-order giant fibres.
*g.f.*1.	axon of first-order giant cell.
*g.f.*2*a.*	second-order axon running to stellate ganglion and making distal synapses.
*g f.*2*b.*	second-order axon running to *m.retr.cap.post.*
*g.f.*2*c.*	second-order axon running to *m.retr.cap.post* and *m.retr.inf.*
*g.f.*2*d.*	second-order axon running to *m.retr.inf.*
*g.f.*2*e.*	second-order axon running to funnel.
*g.f.*3	third order axon
g.f.a.	axon arising in palliovisceral ganglion and running to make proximal synapses with *g.f.*3 in stellate ganglion.

g.f.l. giant-fibre lobe of stellate ganglion.
g.ped. pedal ganglion.
g.p.v. palliovisceral ganglion.
k. knob at end of *g.f.*2*a*.
l.bas.ant. lobus basalis anterior.
l.bas.post. lobus basalis posterior.
l.fr.inf. lobus frontalis inferior.
l.fr.sup. lobus frontalis superior.
l.mag. or *l.mag.v.* ventral portion of *l.mag.*, containing the giant cell.
l.mag.dors. dorsal extension of *l.mag.*, leading to *l.bas.post.*
l.mag.lat. lateral extension of *l.mag.*, leading to *l.opt.*
l.mag.post. posterior extension of *l.mag.*
l.opt. optic lobe.
l.vert. lobus verticalis.
lat.dend. lateral dendrites of *g.c.*1.
m.c. mantle connective (viscero-stellate connective).
m.lev.inf. musculus levator infundibuli.
m.retr.cap.ant. musculus retractor capitis anterior.
m.retr.cap.post. musculus retractor capitis posterior.
m.retr.inf. musculus retractor (depressor) infundibuli.
mac. macula of the statocyst.
mant.m. mantle muscles.
n. nucleus.
n.sh. nucleus of the axonic sheath.
n.col. nervus collaris.
n.c.s. nervus cristae staticae.
n.inf.ant. nervus infundibuli anterior.
n.inf.post. nervus infundibuli posterior.
n.mac.stat.dors. nervus maculae staticae dorsalis.
n.mac.stat.ventr. nervus maculae staticae ventralis.
n.nuch. not nervus nuchalis, but a part n. collaris
n.pall. nervus palliallis.
n.pin. or *n.pinn.* fin nerve.
n.postorb. nervus postorbitalis
n.retr.cap.ant. nervus retractor capitis anterior.
n.retr.cap.post. nervus retractor capitis posterior.
n.retr.inf.ant. (*med.* and *post.*). nervus retractor infundibuli anterior (medius and posterior).
n.s. Nissl substance.
n.s.^{1}. central irregular granules of Nissl substance.
n.s.^{2}. longitudinally arranged peripheral granules of Nissl substance.
n.visc. nervus visceralis.
np. neuropil.
nuch. nuchal cartilage.
p. pathway from axon to dendrites in *g.c.*1.
p.+c. branches of n. postorbitalis and n. collaris leading to l. magnocellularis.
prox.ax.coll. or *col.* axonic collaterals of *g.f.*2*a* by means of which proximal synaptic contacts are made.
sh. axonic sheath.
sh.' sheath of end of *g.f.*2*a* pierced by numerous holes for collaterals of *g.f.*3.
st.com. interstellate commissure.
st.g. stellate ganglion.
st.n. stellar nerve.
stat. statocyst.
ter.m. terminal mass of collaterals of *g.f.*3, beyond the end of *g.f.*2*a*.
v.dend. ventral dendrites of *g.c.*1.

Description of Plates

Plate 42

Fig. 15. Transverse section of central nervous system of freshly hatched *L. pealii* at the level of *g.c.*1. Bouin, azan (HFe 12.2.2).

Fig. 16. Horizontal section of central nervous system of freshly hatched *L. pealii* at the level of *g.c.*1. Bouin, haematoxylin, eosin (HFb 5.2.1).

Fig. 17. From same series as fig. 16 but rather farther ventrally (HFb 5.2.7) to show the commissure which connects the lobi magnocellulares.

Fig. 18. Sagittal section of lobus magnocellularis of freshly hatched *L. pealii.* Bouin, haematoxylin, eosin (HFc 6.2.1).

Fig. 19. The giant cell of fig. 18 photographed with oil-immersion objective to show the arrangement of the neurofibrils, Nissl substance and pathway from dendrites to axon.

Fig. 20. The giant cell of fig. 15 photographed with oil-immersion objective to show the neurofibrils and Nissl substance.

Fig. 21. The giant cell of fig. 16 photographed with oil-immersion objective to show the arrangement of the Nissl substance and the pathways from dendrites to axon.

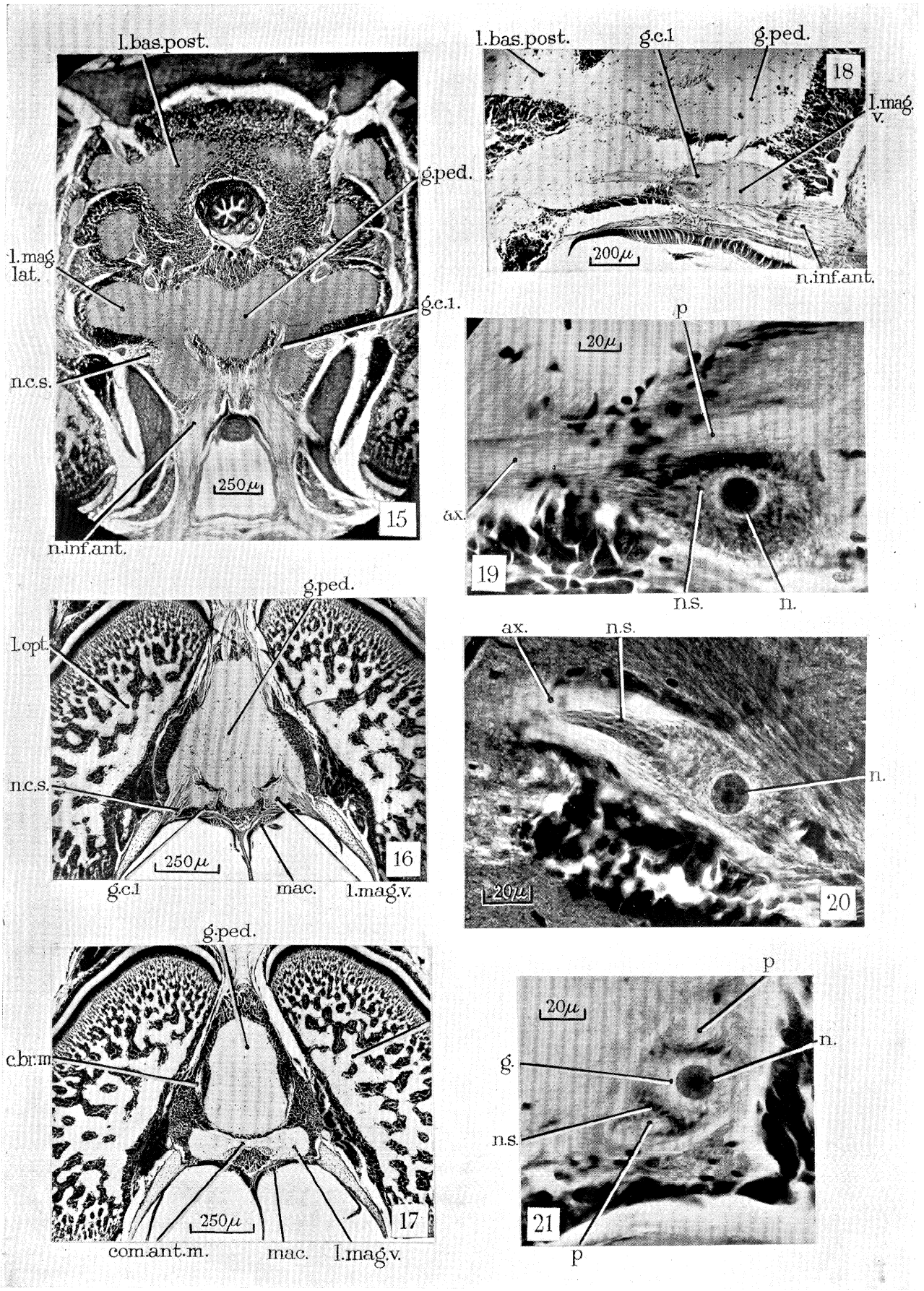

l.bas.post.
g.ped.
l.mag lat.
g.c.1.
n.c.s.
250μ
15
n.inf.ant.
l.bas.post.
g.c.1
g.ped.
18
l.mag. v.
200μ
n.inf.ant.
p
20μ
ax.
19
n.s.
n.
g.ped.
l.opt.
n.c.s.
250μ
16
g.c.1
mac.
l.mag.v.
ax.
n.s.
n.
20μ
20
g.ped.
c.br.m.
250μ
17
com.ant.m.
mac.
l.mag.v.
p
20μ
n.
g.
n.s.
21
p

PLATE 43

FIG. 22. Horizontal section of first-order giant cell of adult *L. pealii* through level of the nucleus and anterior dendrite. Picroformol, paraffin, haematoxylin and eosin (DTc 12.1.4).

FIG. 23. The same cell as fig. 22 but farther dorsally and hence nearer to the axon.

FIG. 24. Right first-order giant cell of small adult *L. pealii* reconstructed by superposed projection from a series of sections (GS) stained with Cajal's method, and cut 50μ thick in celloidin. ERRATUM. *For* b *read* e.f.

FIG. 25. Camera lucida drawing of horizontal section through upper part of first-order giant cell of left side, to show the sheath, end feet and fuchsinophil granules. Flemming, paraffin, Mallory (*L. pealii* FAc 22.2). The granules shown dead black in the drawing are red in the preparation.

FIGS. 26, 27. End feet on the anterior dendrite of the first-order giant cell of *L. forbesi*. Cajal's method, paraffin (BIc 5.1.8 and 5.2.3).

FIG. 28. Horizontal section of interaxonic bridge of freshly hatched *L. pealii*. Formalin-Cajal, sections cut 50μ in celloidin. The axoplasm of the giant axons is unstained (HG 3.2.4).

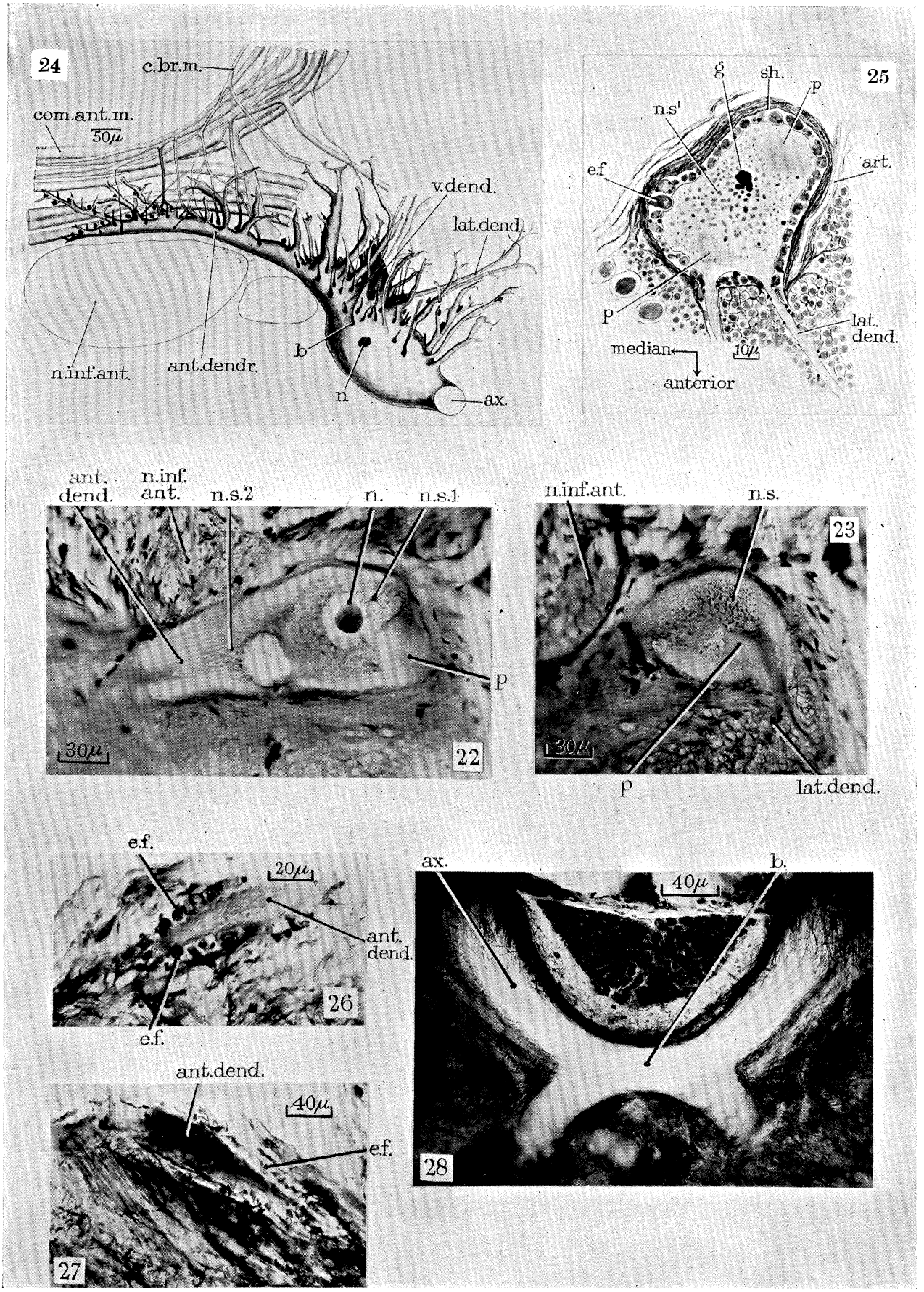

24
c.br.m.
com.ant.m.
50μ
v.dend.
lat.dend.
b
n.inf.ant.
ant.dendr.
n
ax.
25
g
sh.
p
n.s¹
e.f
art.
p
lat.
dend.
median
10μ
anterior
ant.
dend.
n.inf.
ant.
n.s.2
n.
n.s.1
p
30μ
22
n.inf.ant.
n.s.
23
30μ
p
lat.dend.
e.f.
20μ
ant.
dend.
26
e.f.
ant.dend.
40μ
e.f.
27
ax.
b.
40μ
28

Plate 44

Fig. 29. Transverse section of first-order giant axons immediately anterior to interaxonic bridge. Bouin, paraffin, haematoxylin and eosin (*L. forbesi* BGc 8.1.1).

Fig. 30. Transverse section of interaxonic bridge, taken a short way behind fig. 29 (BGc 8.1.6). Note complete continuity across the middle line, leaving no trace of the separate identity of the two axons.

Fig. 31. Horizontal section of part of interaxonic bridge of adult *L. pealii*. Picroformol (injected), paraffin section, 15μ, haematoxylin, eosin (PA 12.1.2).

Fig. 32. Sagittal section through interaxonic bridge of adult *L. pealii*. Bouin, celloidin-paraffin, haematoxylin, eosin. Note that there is no trace of the double nature of the fibre, one axoplasm and one sheath alone are discernible (KMa 18.1.3).

Fig. 33. Section made exactly as fig. 32 except that fixation was by injection of formalin (*L. pealii* OA 7.3.5).

Fig. 34. Transverse section to show abnormally flattened interaxonic bridge of *L. pealii*. Picroformol (injected), celloidin-paraffin, haematoxylin, eosin (OZ 25.1.3).

Fig. 35. Continuous series of transverse sections of interaxonic bridge of small *L. pealii* (mantle length 12 cm.). Picroformol (injected), celloidin-paraffin, azan, sections 15μ thick. The section at the top is the most anterior, showing the axons of the two first-order giant cells, one of them with a well-marked central core. In the bridge slight traces of the double origin still persist, but the axoplasms of the two fibres are undoubtedly continuous (OYc, slide 23).

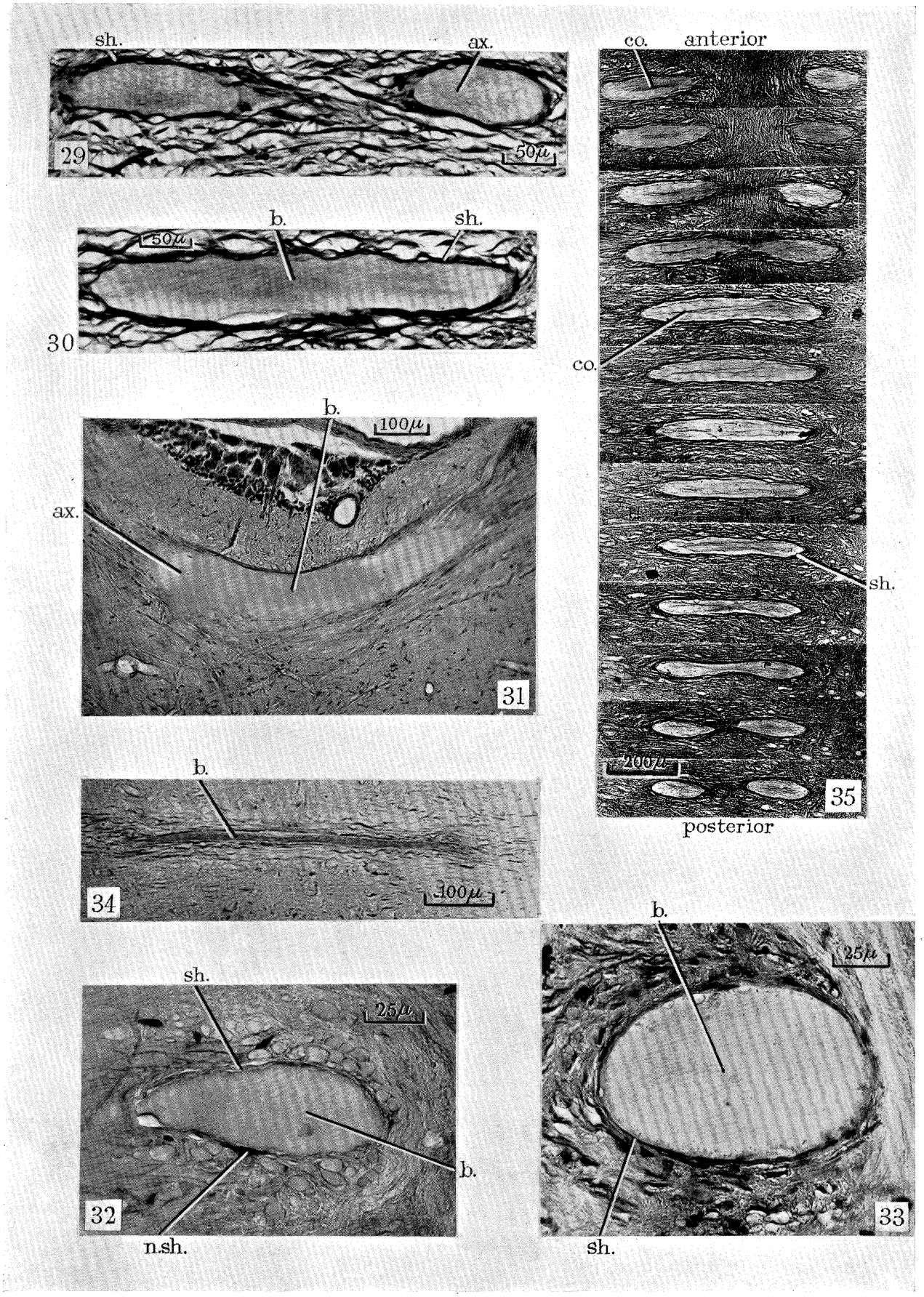

sh.
ax.
29
50μ
b.
sh.
50μ
30
b.
100μ
ax.
31
co.
anterior
co.
sh.
200μ
35
posterior
b.
100μ
34
sh.
25μ
b.
32
n.sh.
b.
25μ
33
sh.

Plate 45

Fig. 36. Continuous series of transverse sections of interaxonic bridge of freshly hatched *L. pealii* (mantle length 2 cm.). The section at the top is the most anterior, showing the axons of the two first-order giant cells with double central cores. In the bridge the two axoplasms are undoubtedly mingled, but the double origin is still evident from the shape of the outline of the bridge and from the cores. Formalin, methyl benzoate-celloidin-paraffin, Masson, sections 15μ thick (HMg, slide 18).

Fig. 37. First- and second-order giant fibres in the palliovisceral ganglion of *L. pealii*. Picroformol, paraffin, haematoxylin, eosin. The fibres are separated by a connective tissue sheath, the actual synaptic contact being by collaterals of *g.f.2a* which are not seen here (DTc 12.1.6).

Fig. 38. Transverse section of whole pallial nerve of *L. forbesi*. Flemming, paraffin, Mallory (BHb 2.6.11).

Fig. 39. Transverse section of viscerostellate connective and fin nerve of *L. pealii*. Picroformol, celloidin-paraffin, Masson (OYa 25.2.5).

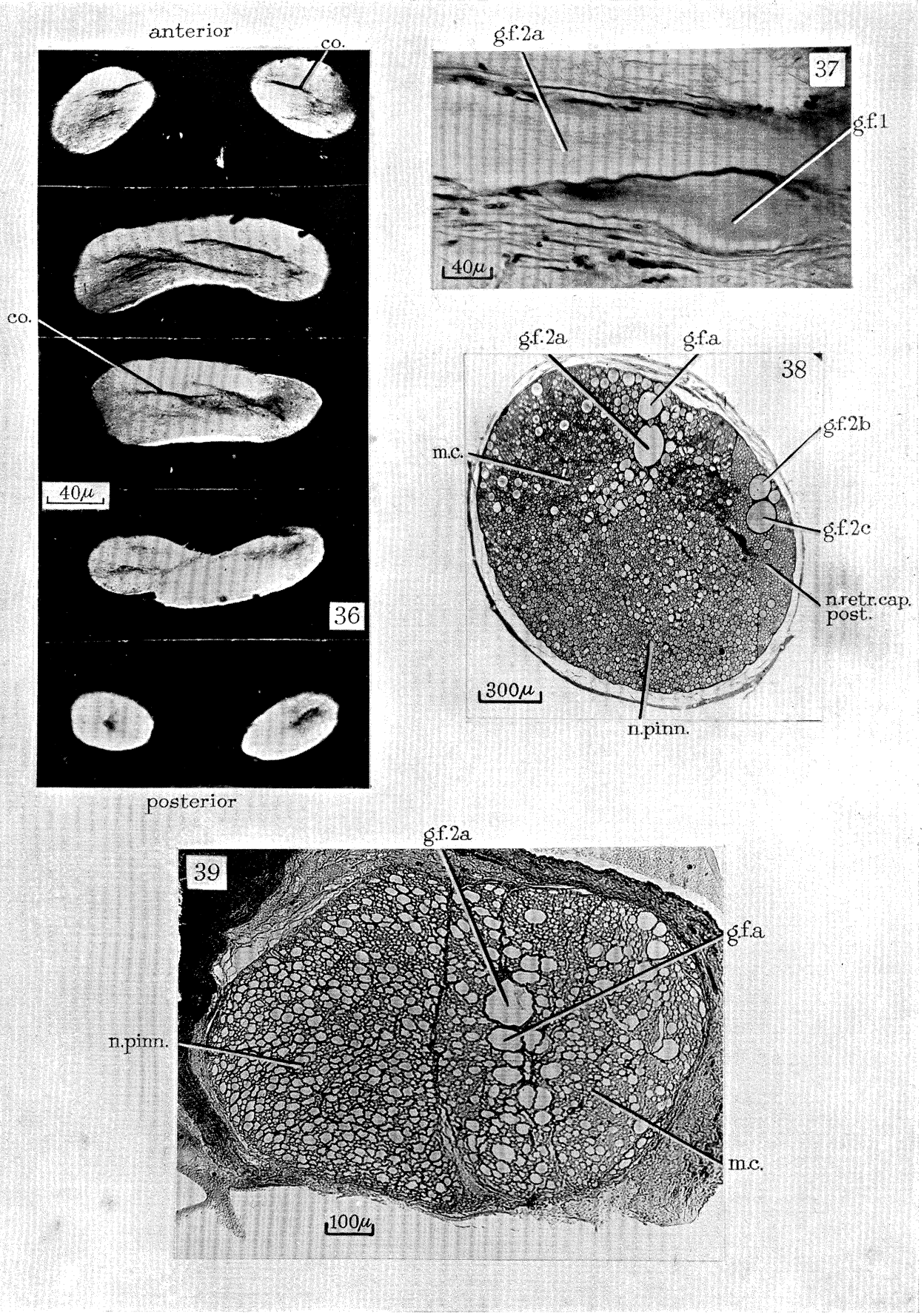

anterior
co.
co.
40μ
36
posterior
g.f.2a
37
g.f.1
40μ
g.f.2a
g.f.a
38
g.f.2b
m.c.
g.f.2c
n.retr.cap.
post.
300μ
n.pinn.
g.f.2a
39
g.f.a
n.pinn.
m.c.
100μ

PLATE 46

FIG. 40. Sagittal section of whole stellate ganglion of *L. pealii*. Picroformol, paraffin, azan (DTa 3.2.8).

FIGS. 41, 42. Two aspects of a model of the giant fibres in the stellate ganglion of *L. forbesi*. The model was constructed in wax from drawings of a series of transverse sections stained with Cajal's method. The cells of origin of the third-order neurons are shown diagrammatically (*Loligo* BCa).

FIG. 43. Sagittal section of portion of giant-fibre lobe of *L. pealii* (DTb 7.1.4) to show the fusion of the axons of the cells of the lobe to form the third-order giant fibres. Picroformol, Mallory.

FIG. 43*a*. Section transverse to the second- and third-order fibres in the stellate ganglion, to show the synaptic relationship of a collateral of *g.f.*3 to *g.f.*2*a*. Picroformol, Masson (*L. pealii* OYb 17.2.4).

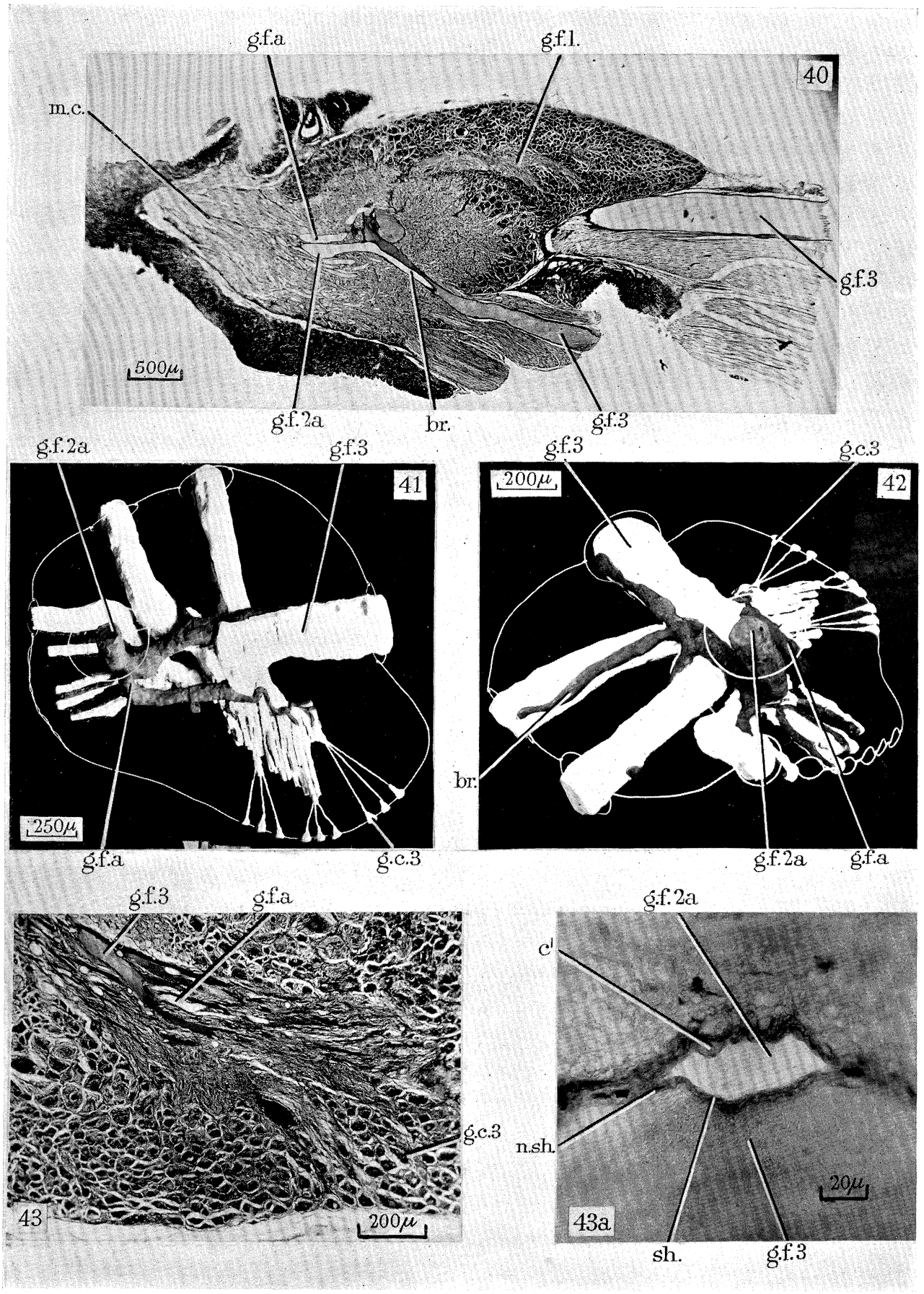

g.f.a
g.f.l.
40
m.c.
g.f.3
500μ
g.f.2a
br.
g.f.3
g.f.2a
g.f.3
41
250μ
g.f.a
g.c.3
g.f.3
g.c.3
200μ
42
br.
g.f.2a
g.f.a
g.f.3
g.f.a
g.c.3
43
200μ
g.f.2a
c
n.sh.
20μ
43a
sh.
g.f.3

Plate 47

Fig. 44. End of second-order fibre (*g.f.2a*) in stellate ganglion, and its synapse with third-order fibre (*g.f.3*). Sections cut transverse to the main body axis, cutting the fibres as they run out laterally. This synapse is also shown in the model of figs. 41 and 42. Cajal's stain, sectioned in paraffin. Note the knob at the end of *g.f.2a*, and the spaces between its surface and the sheath, which are occupied by axonic collaterals of *g.f.3*, these being imperfectly preserved in this preparation (*L. forbesi* BC*a* 2.3.12).

Fig. 45. Axonic collaterals of the third-order giant fibre, seen in a section cut parallel to the length of the fibre. The second-order fibre with which the collateral makes synapse (*g.f.2a*) is just above the plane of the photograph. Cajal's stain, sectioned in paraffin (*L. forbesi* BIa 5.1.6).

Fig. 46. Axonic collaterals of third-order fibre as in fig. 45. Cajal's stain, sectioned in paraffin (*L. forbesi* AYa 2.1.4).

Fig. 47. Section transverse to synapse of second- and third-order fibres in stellate ganglion. Picric acid, sectioned in paraffin, azan. A collateral of *g.f.3* can be seen on the right, expanding into a complex mass beneath the sheath of *g.f.2a*. A shrinkage space has developed between the surfaces of synaptic contact. On the left a second collateral (the connexion of which with *g.f.3* is not shown in this section) makes close contact with *g.f.2a* (*L. pealii* FBb 5.3.11).

Fig. 48. Another portion of the same series as fig. 47. The collateral of *g.f.3* is seen to make very close contact with *g.f.2a*, though the axoplasms clearly do not mingle.

Fig. 49. Synaptic contacts between collaterals of *g.f.3* and the end of *g.f.2a*, seen in a section longitudinal to the latter (the main trunk of *g.f.3* lies out of the plane of the section). Picro-formol, paraffin, azan (*L. pealii* DTb 6.1.4).

Fig. 50. Another synapse from the same ganglion as fig. 49. One of the collaterals (*c.* 1) is well preserved, but others (*c.* 2) have autolysed (*L. pealii* DTb 6.1.6).

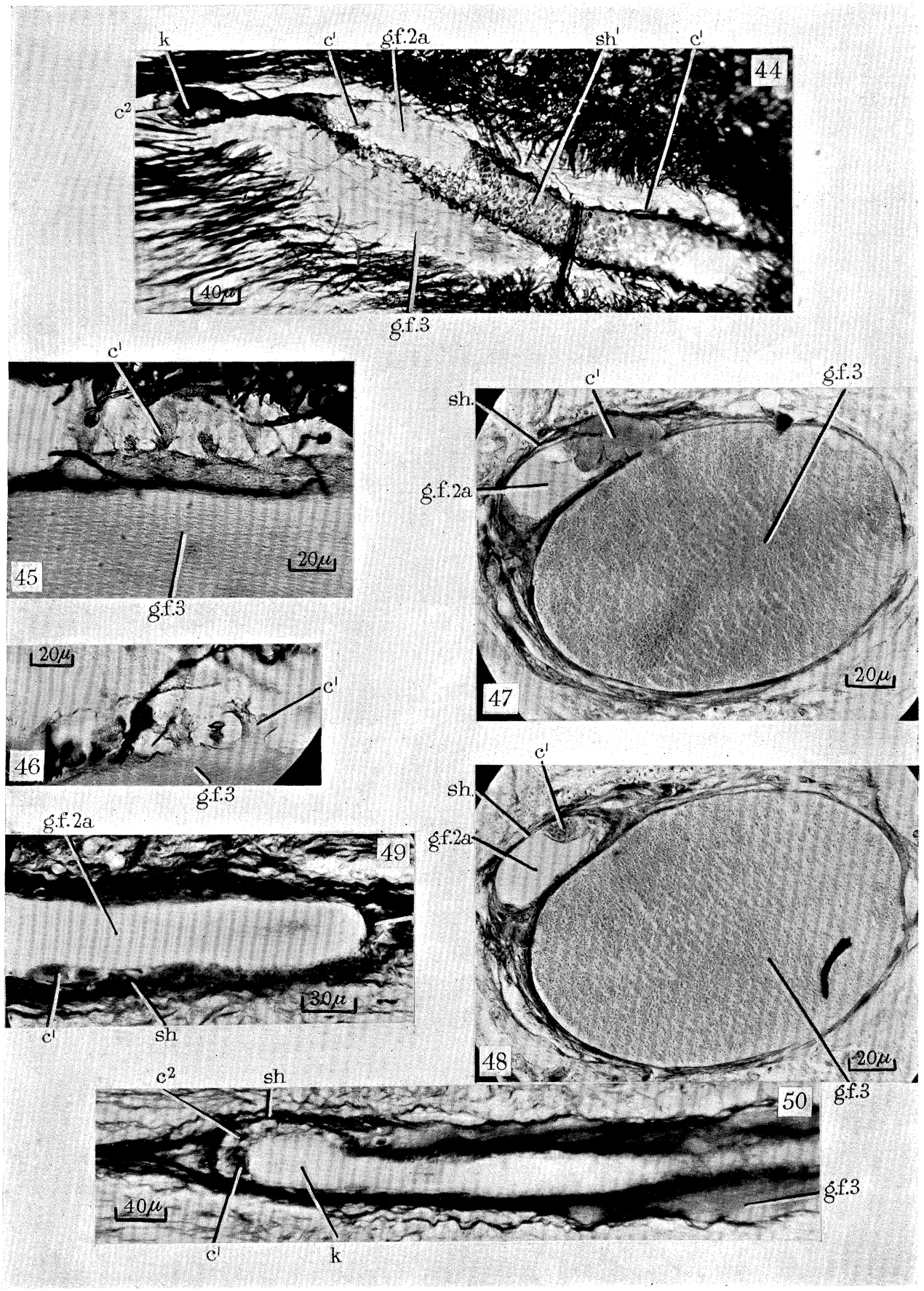

k
c¹
g.f.2a
sh¹
c¹
c²
44
40μ
g.f.3
c¹
45
20μ
g.f.3
c¹
g.f.3
sh.
g.f.2a
47
20μ
20μ
c¹
46
g.f.3
c¹
sh.
g.f.2a
48
20μ
g.f.3
g.f.2a
49
30μ
c¹
sh
c²
sh
50
40μ
g.f.3
c¹
k

Plate 48

Fig. 51. Terminal mass consisting mainly of collaterals of two third-order fibres beyond the end of a branch of *g.f.2a* with which they both make synapse. Cajal's stain, paraffin (*L. forbesi* BIb 4.1.12).

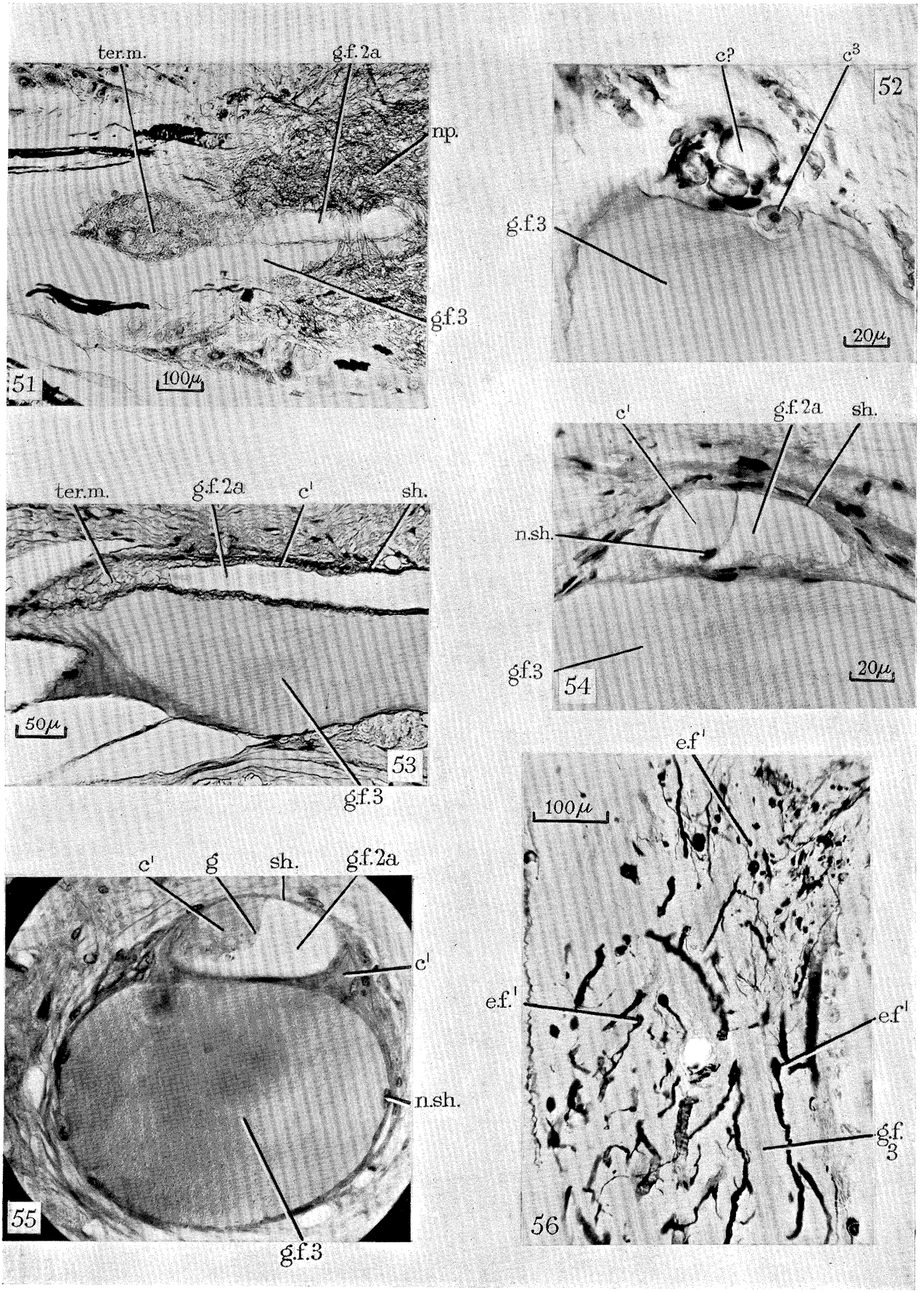
Fig. 52. Section transverse to a third-order giant fibre, showing the collaterals forming a terminal mass beyond the end of *g.f.2a*. Cajal's stain, paraffin (*L. forbesi* AYb 3.2.6).

Fig. 53. Section longitudinal to the second- and third-order giant fibres, showing the end of the former surrounded by collaterals of the latter, some of which form a terminal mass. Formol-bichromate, celloidin-paraffin, Masson's stain (*L. pealii* NTa 7.4.6).

Fig. 54. Section transverse to synapse between second- and third-order giant fibres, to show nucleus of inner sheath partly separating the two fibres. Picric acid, celloidin-paraffin, Masson's stain (*L. pealii* FBb 5.3.11).

Fig. 55. Section transverse to synapse between second- and third-order fibres. Picric acid, celloidin-paraffin, Masson's stain (*L. pealii* FBa 2.4.9). The granules showing black are red in the preparation.

Fig. 56. Part of sagittal section through the neuropil at the centre of the giant-fibre lobe of the stellate ganglion, to show the end feet which are branches of *g.f.a.*, making contact (proximal synapses) with the third-order giant fibres. Cajal's stain, paraffin (*L. forbesi* AYc 1.3.4).


ter.m.
g.f.2a
np.
g.f.3
51
100μ
c?
c³
52
g.f.3
20μ
c'
g.f.2a
sh.
n.sh.
g.f.3
54
20μ
ter.m.
g.f.2a
c'
sh.
50μ
53
g.f.3
c'
g
sh.
g.f.2a
c'
n.sh.
55
g.f.3
e.f'
100μ
e.f'
e.f'
g.f.3
56

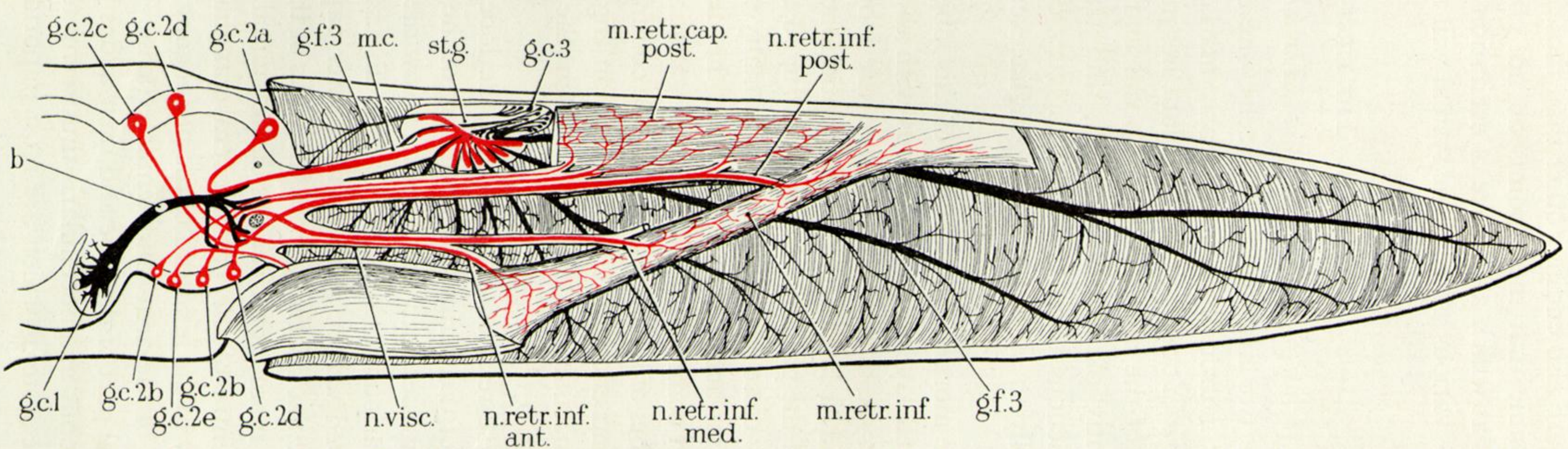


FIG. 2. Diagram of *L. pealii* to show the whole system of giant fibres which can be activated from the first-order giant cells. The ganglia and fibres are shown unduly large and the relative lengths of some of the nerves have been distorted.

(The abbreviations used for the lettering of all figures and plates are listed on p. 502).

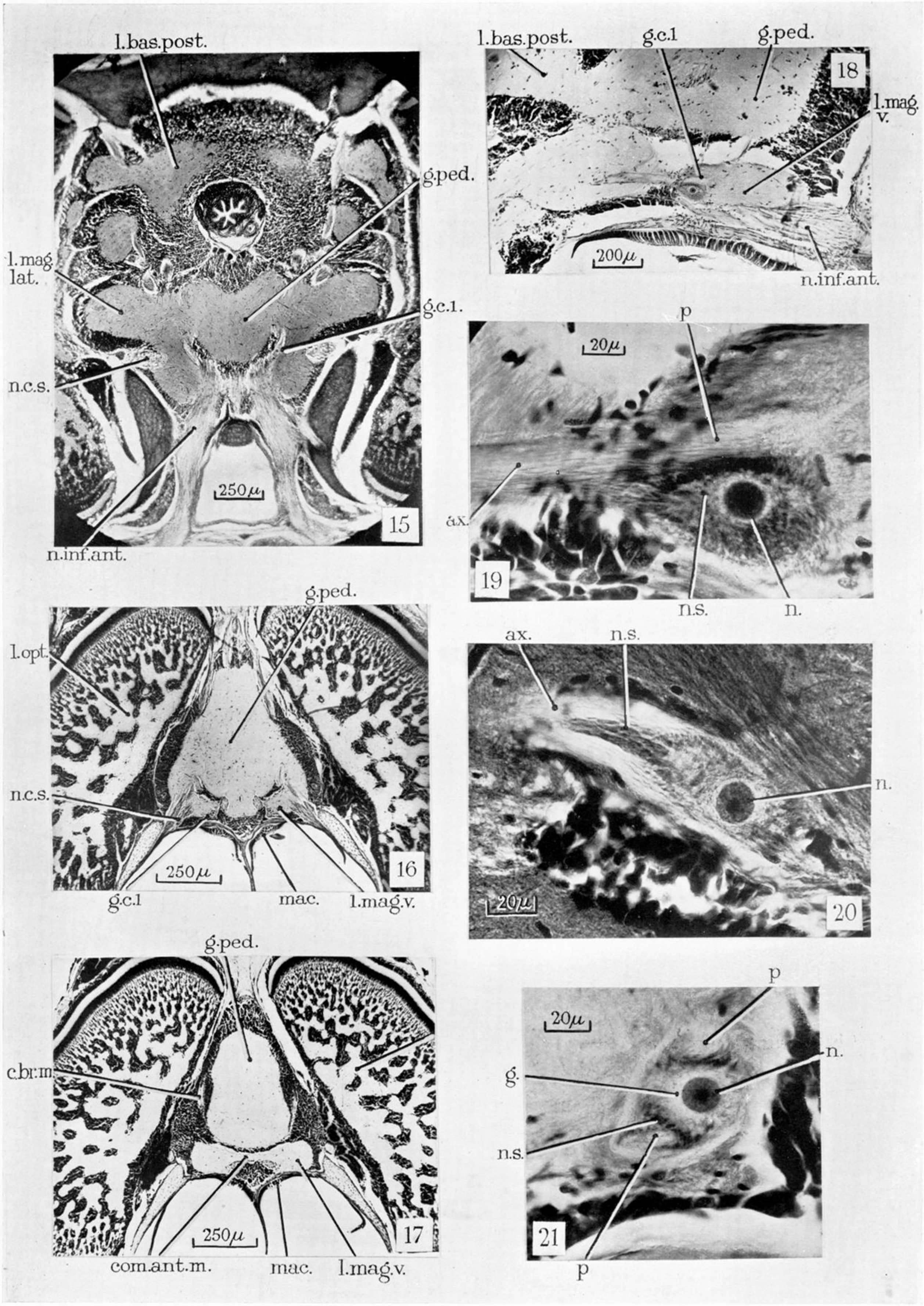


PLATE 42

FIG. 15. Transverse section of central nervous system of freshly hatched *L. pealii* at the level of *g.c.*1. Bouin, azan (HFe 12.2.2).

FIG. 16. Horizontal section of central nervous system of freshly hatched *L. pealii* at the level of *g.c.*1. Bouin, haematoxylin, eosin (HFb 5.2.1).

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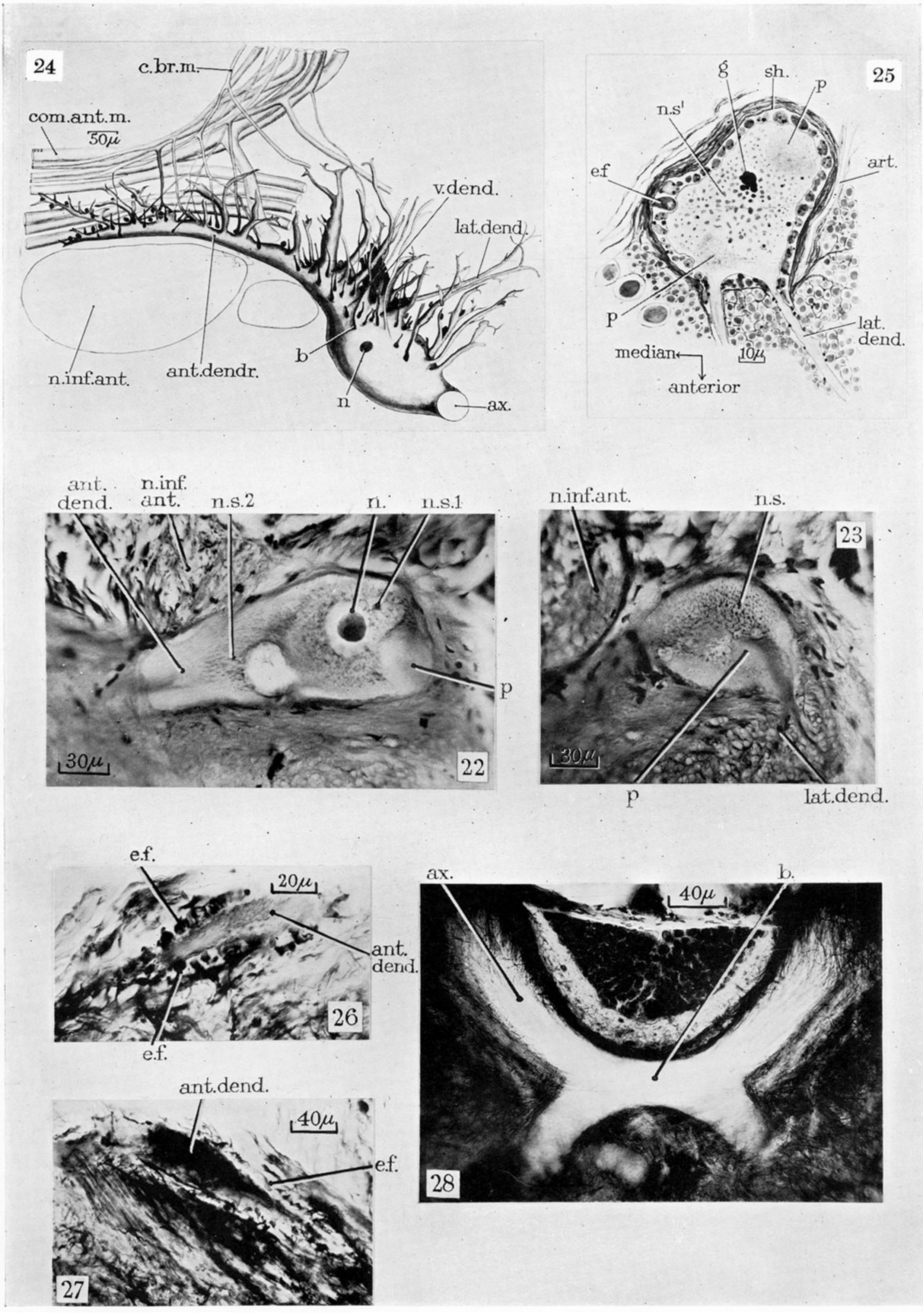

Plate 43

Fig. 22. Horizontal section of first-order giant cell of adult *L. pealii* through level of the nucleus and anterior dendrite. Picroformol, paraffin, haematoxylin and eosin (DTc 12.1.4).

Fig. 23. The same cell as fig. 22 but farther dorsally and hence nearer to the axon.

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Figs. 26, 27. End feet on the anterior dendrite of the first-order giant cell of *L. forbesi*. Cajal's method, paraffin (BIc 5.1.8 and 5.2.3).

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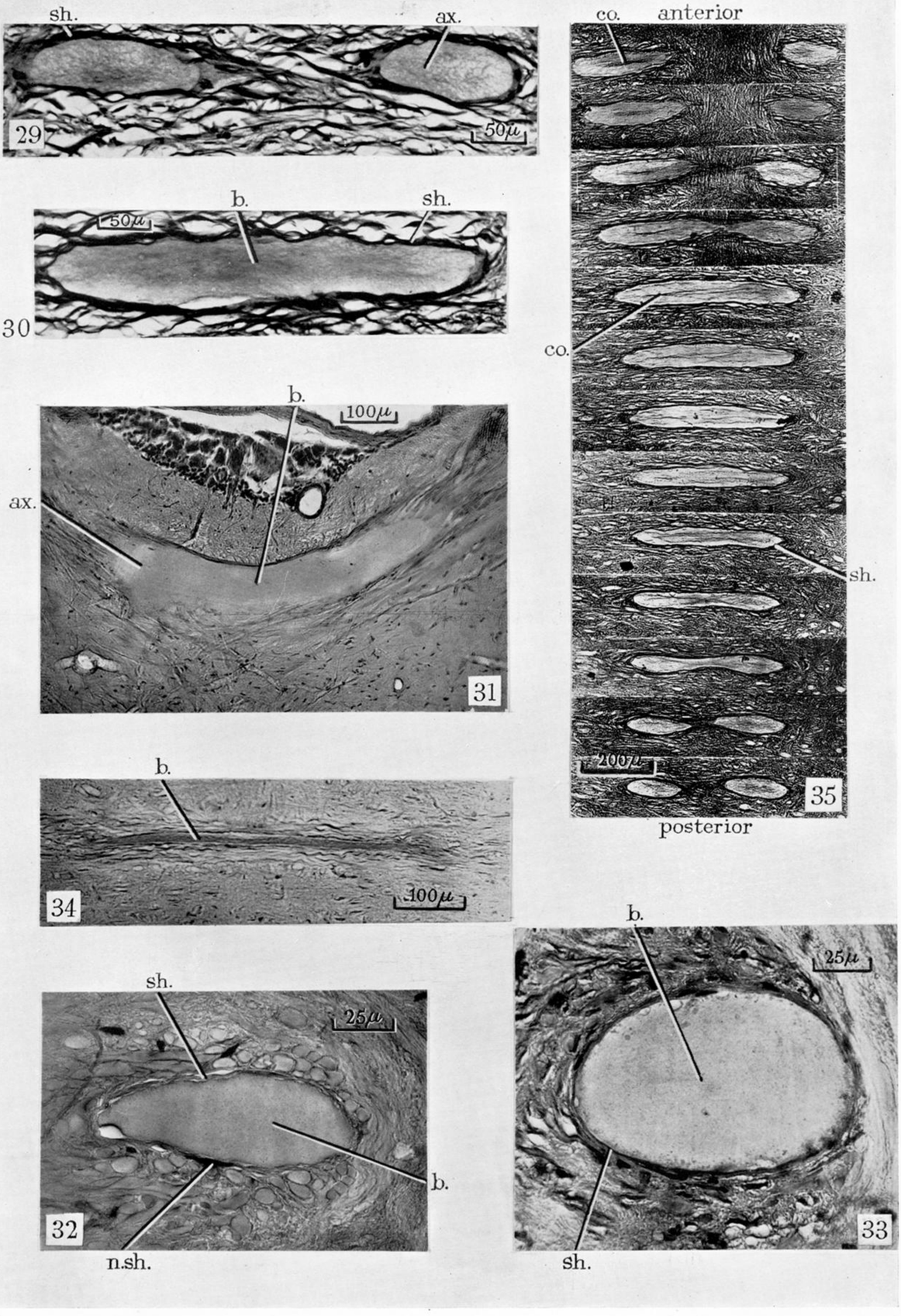

PLATE 44

FIG. 29. Transverse section of first-order giant axons immediately anterior to interaxonic bridge. Bouin, paraffin, haematoxylin and eosin (*L. forbesi* BGc 8.1.1).

FIG. 30. Transverse section of interaxonic bridge, taken a short way behind fig. 29 (BGc 8.1.6). Note complete continuity across the middle line, leaving no trace of the separate identity of the two axons.

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FIG. 33. Section made exactly as fig. 32 except that fixation was by injection of formalin (*L. pealii* OA 7.3.5).

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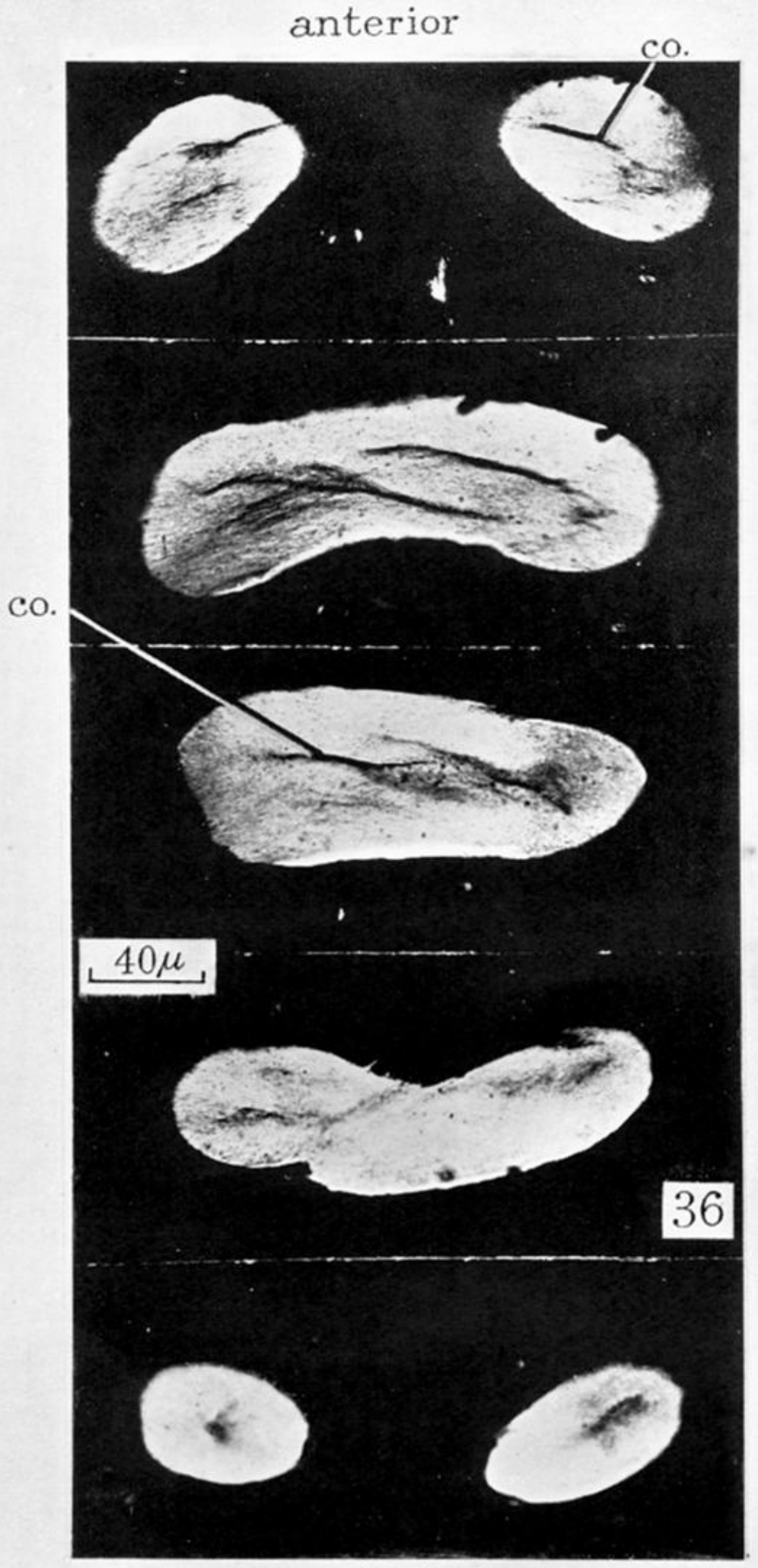
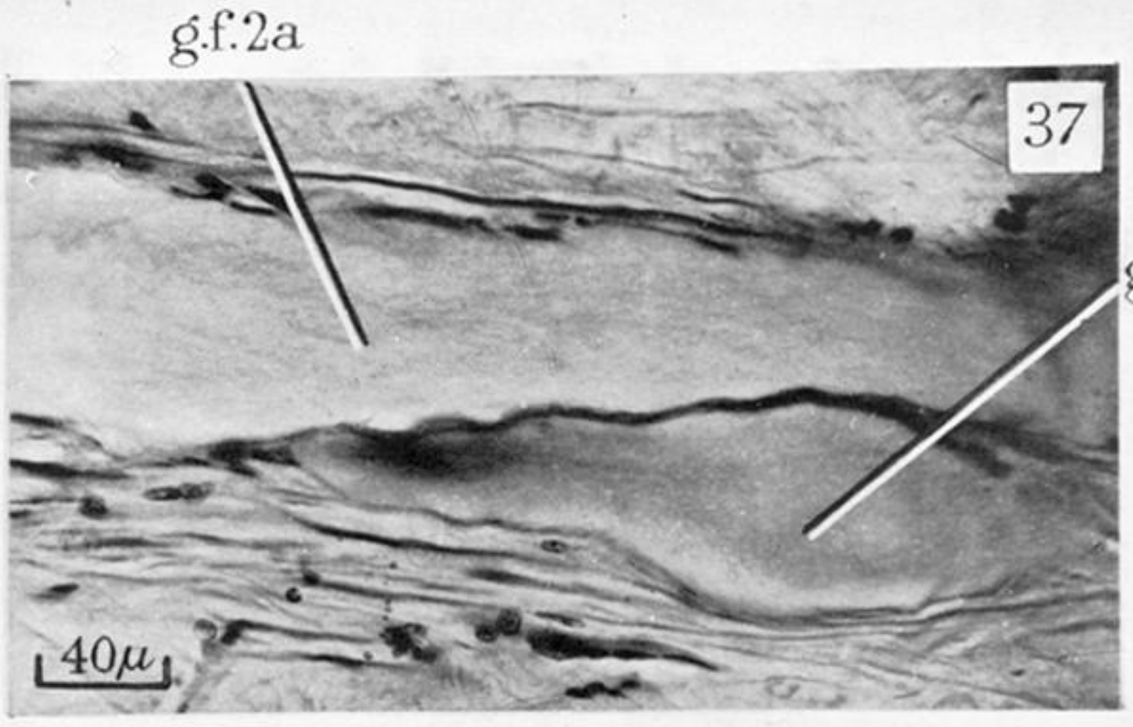
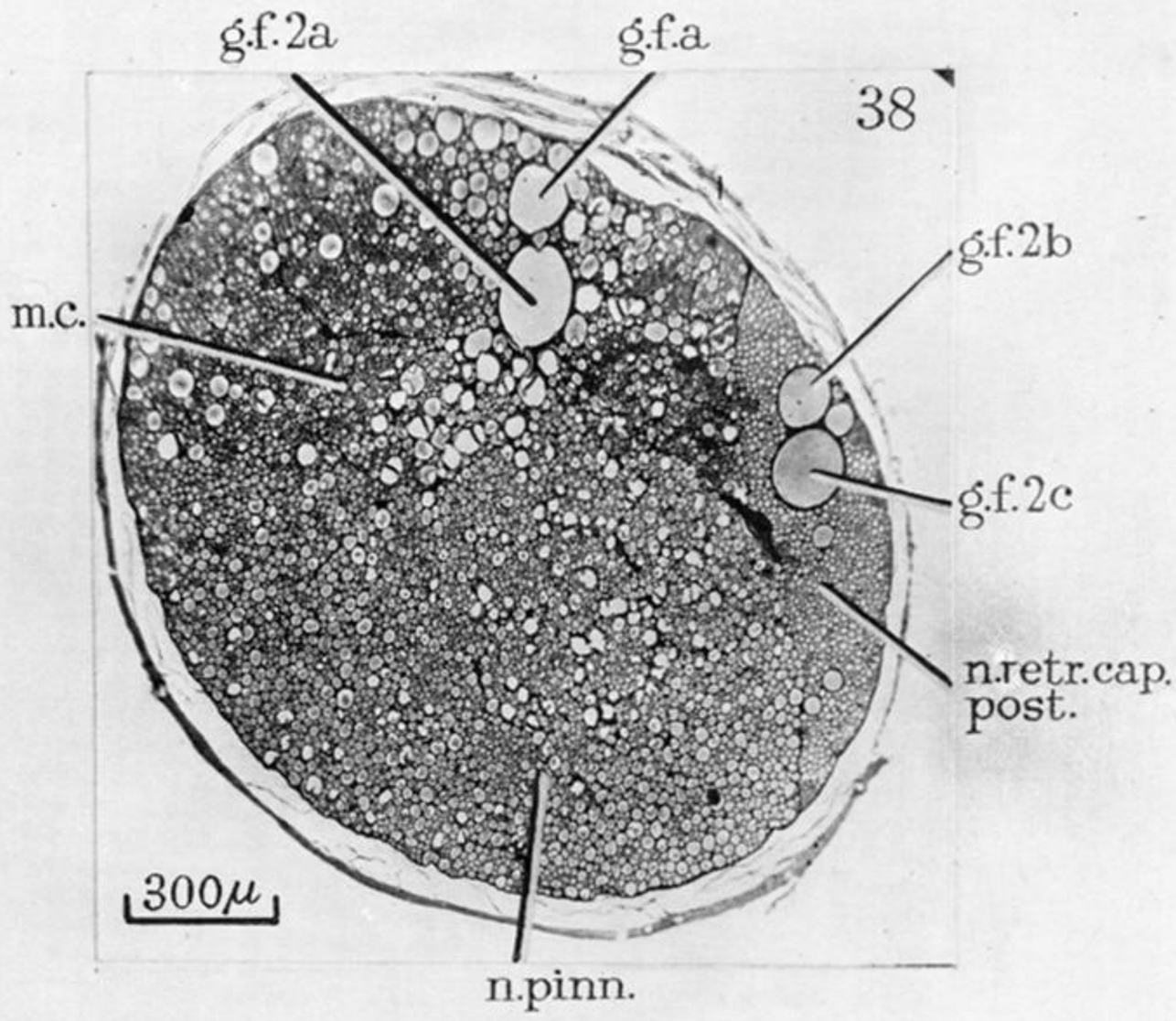
FIG. 35. Continuous series of transverse sections of interaxonic bridge of small *L. pealii* (mantle length 12 cm.). Picroformol (injected), celloidin-paraffin, azan, sections 15μ thick. The section at the top is the most anterior, showing the axons of the two first-order giant cells, one of them with a well-marked central core. In the bridge slight traces of the double origin still persist, but the axoplasms of the two fibres are undoubtedly continuous (OYc, slide 23).







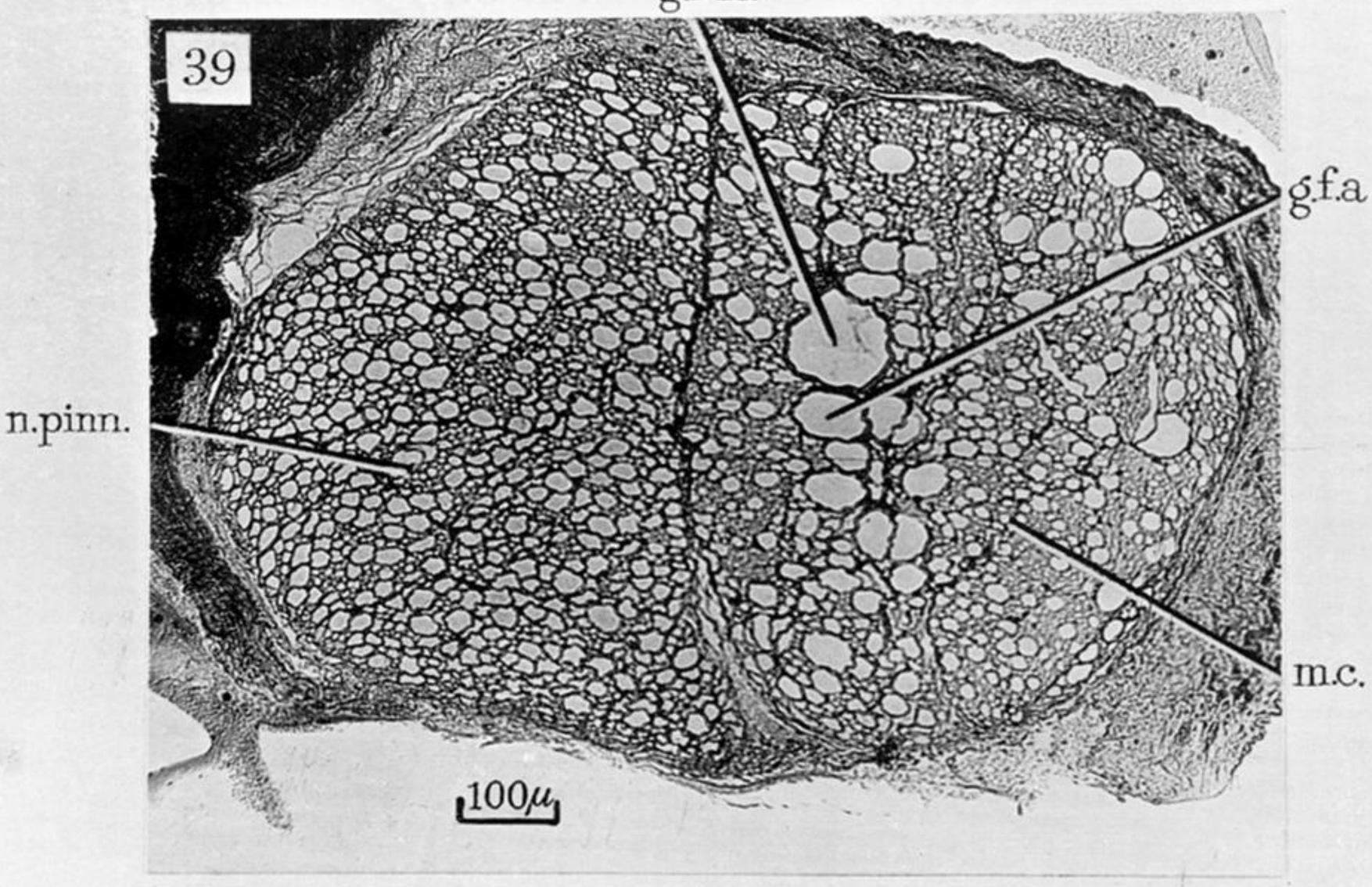


Plate 45

Fig. 36. Continuous series of transverse sections of interaxonic bridge of freshly hatched *L. pealii* (mantle length 2 cm.). The section at the top is the most anterior, showing the axons of the two first-order giant cells with double central cores. In the bridge the two axoplasms are undoubtedly mingled, but the double origin is still evident from the shape of the outline of the bridge and from the cores. Formalin, methyl benzoate-celloidin-paraffin, Masson, sections 15μ thick (HMg, slide 18).

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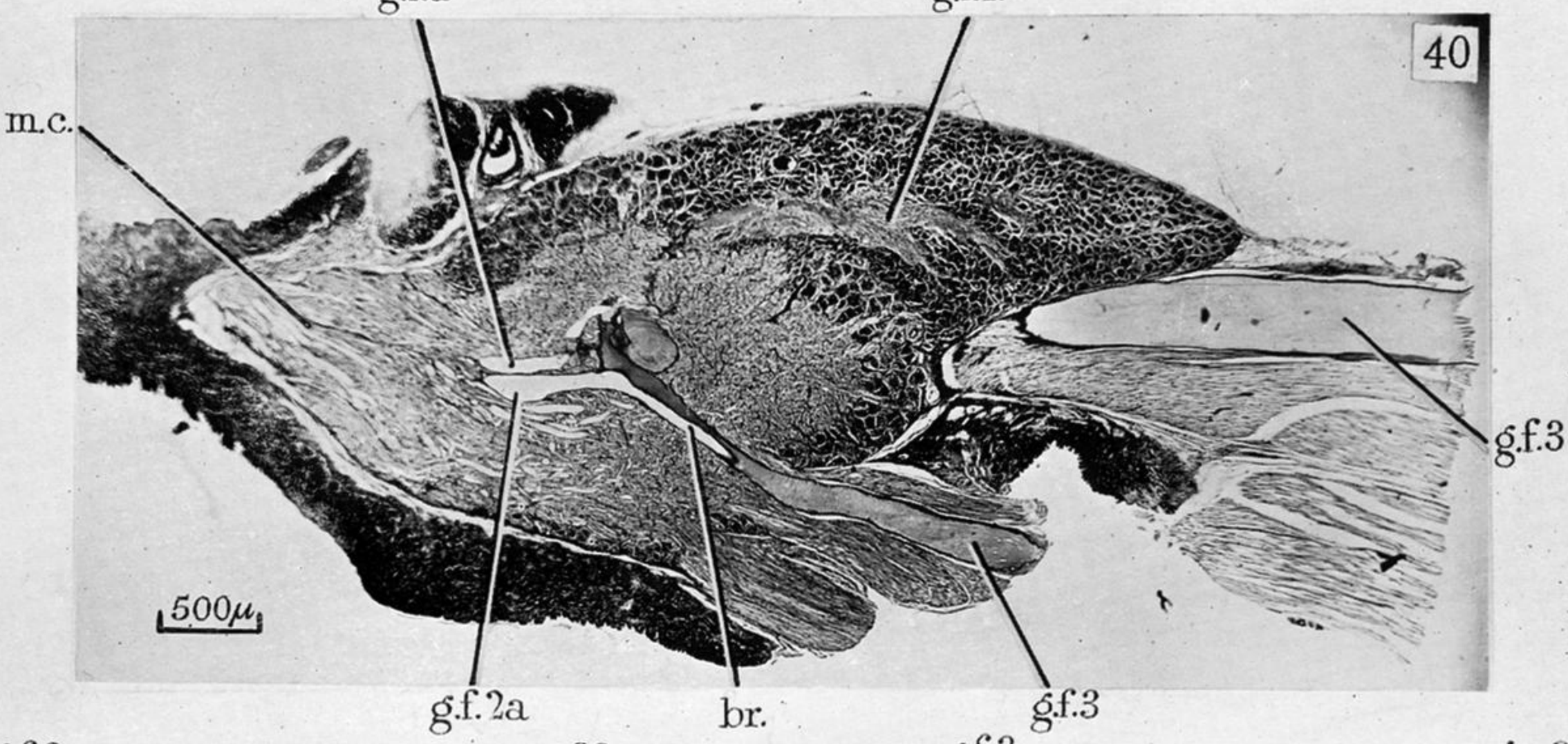
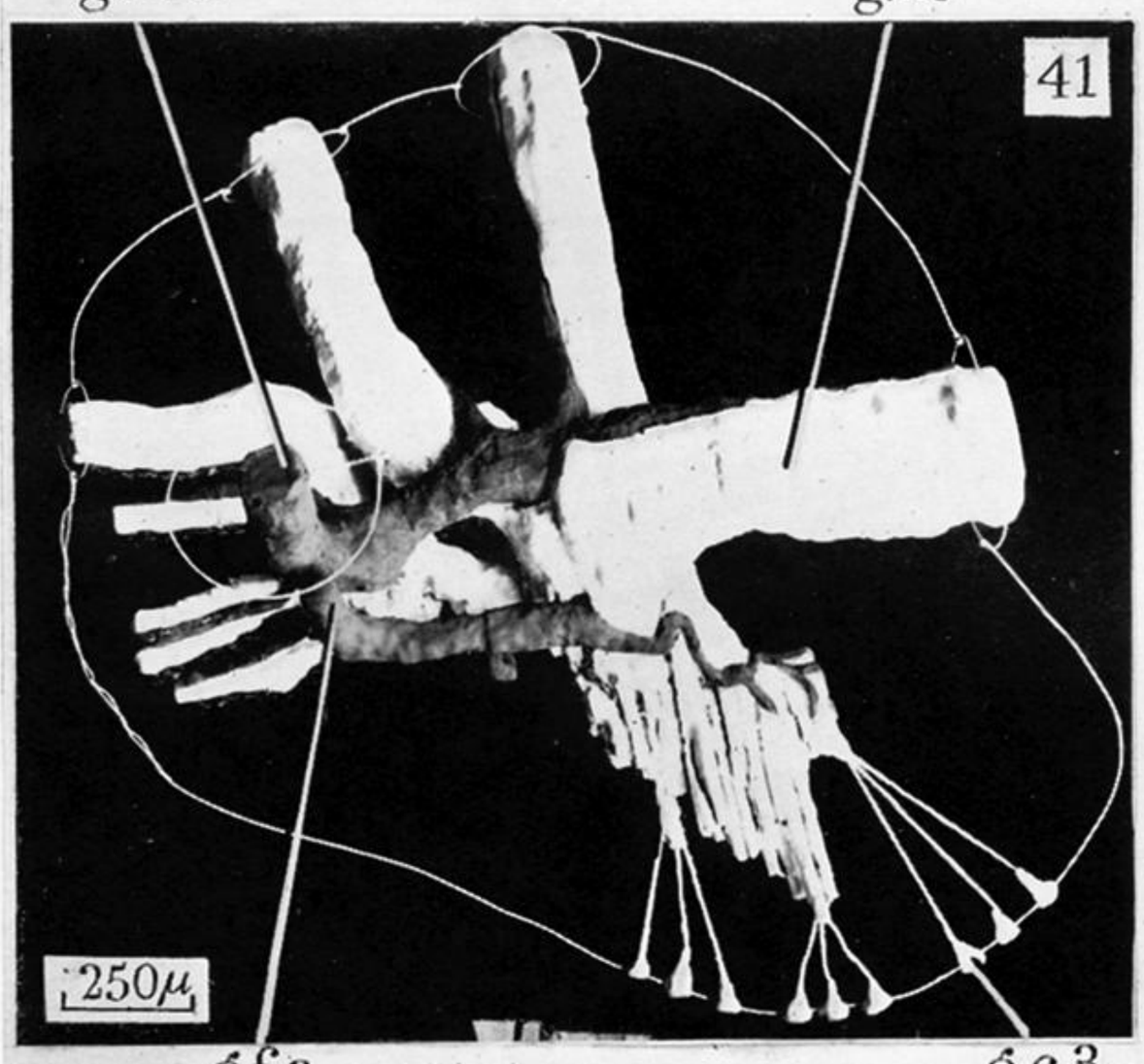
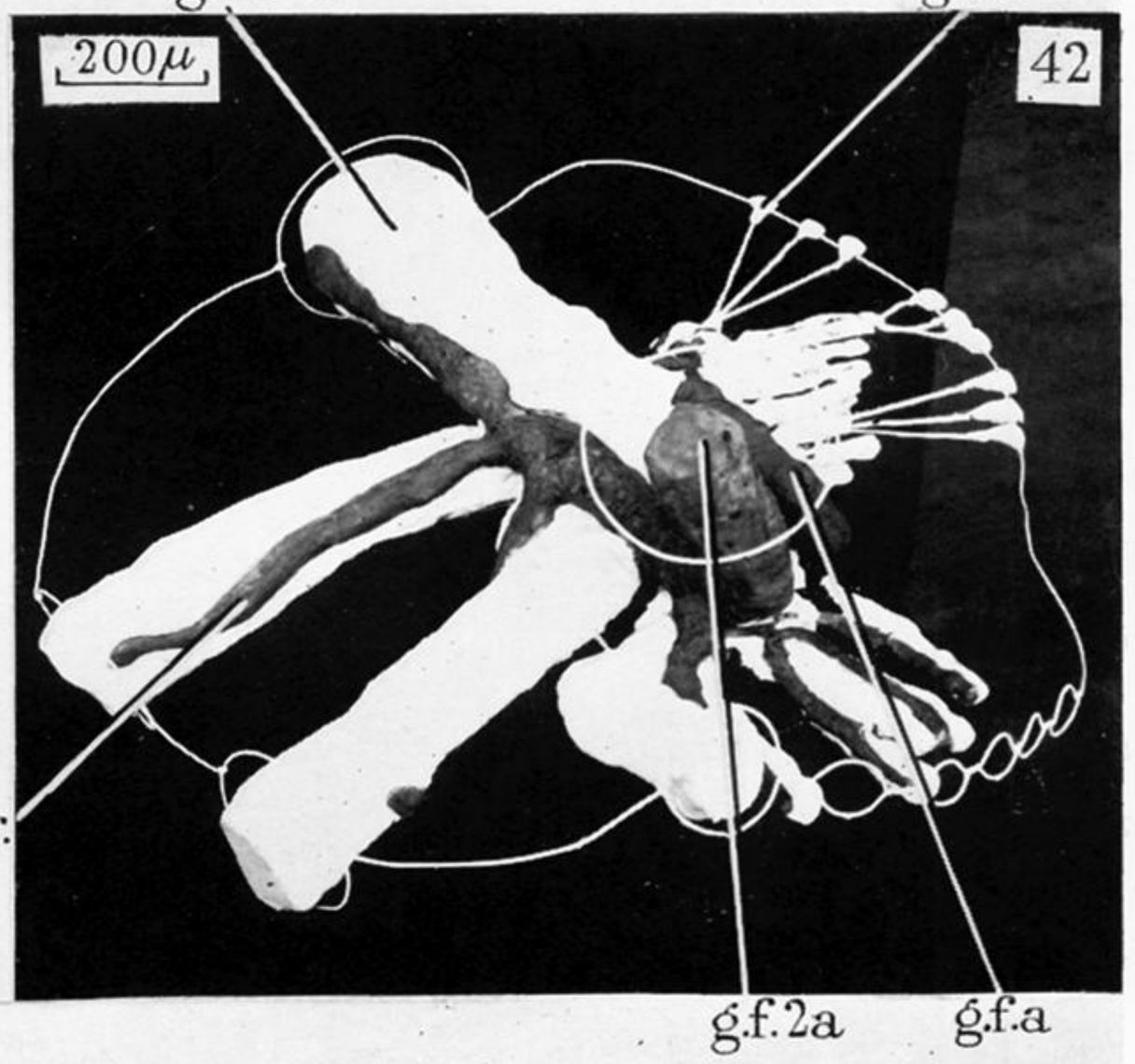
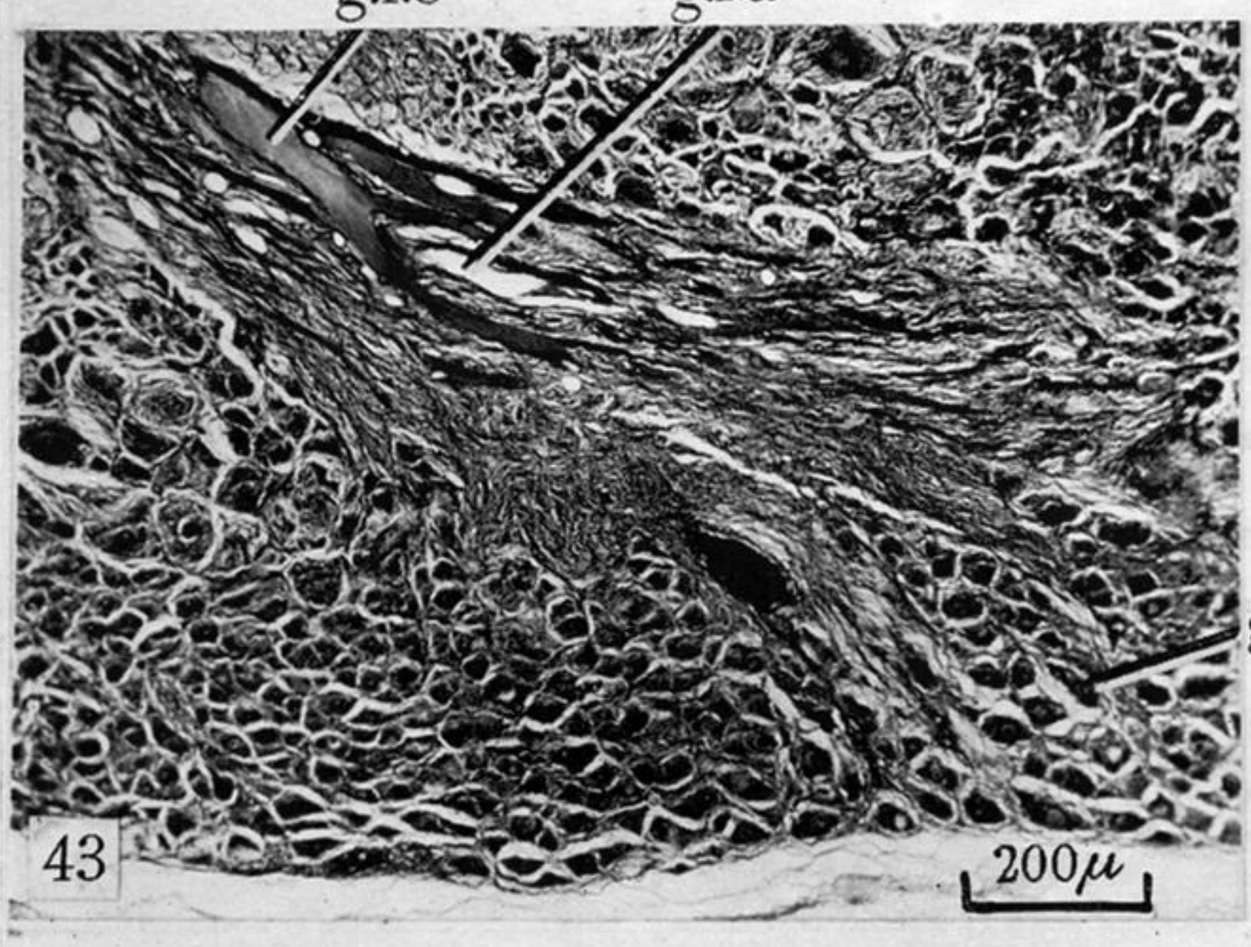
Fig. 39. Transverse section of viscerostellate connective and fin nerve of *L. pealii*. Picroformol, celloidin-paraffin, Masson (OYa 25.2.5).









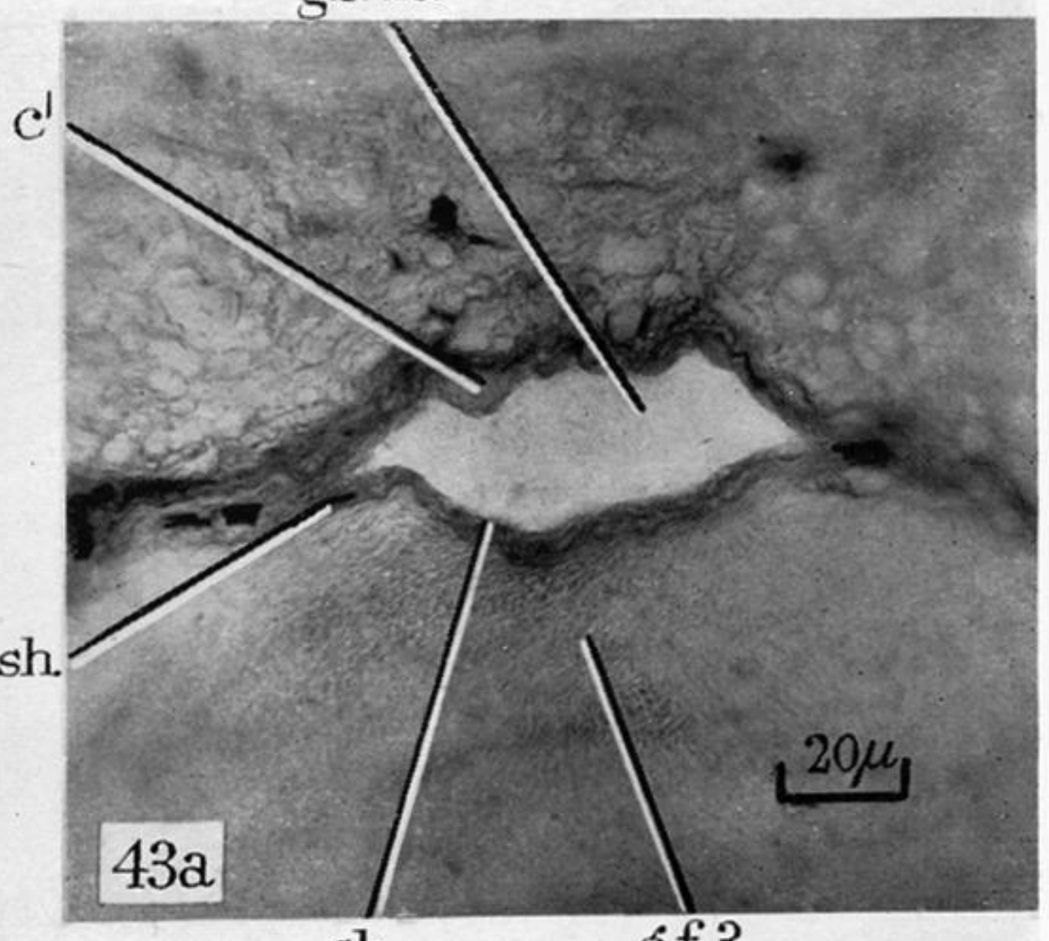


Plate 46

Fig. 40. Sagittal section of whole stellate ganglion of *L. pealii*. Picroformol, paraffin, azan (DTa 3.2.8).

Figs. 41, 42. Two aspects of a model of the giant fibres in the stellate ganglion of *L. forbesi*. The model was constructed in wax from drawings of a series of transverse sections stained with Cajal's method. The cells of origin of the third-order neurons are shown diagrammatically (*Loligo* BCa).

Fig. 43. Sagittal section of portion of giant-fibre lobe of *L. pealii* (DTb 7.1.4) to show the fusion of the axons of the cells of the lobe to form the third-order giant fibres. Picroformol, Mallory.

Fig. 43*a*. Section transverse to the second- and third-order fibres in the stellate ganglion, to show the synaptic relationship of a collateral of *g.f.*3 to *g.f.*2*a*. Picroformol, Masson (*L. pealii* OYb 17.2.4).

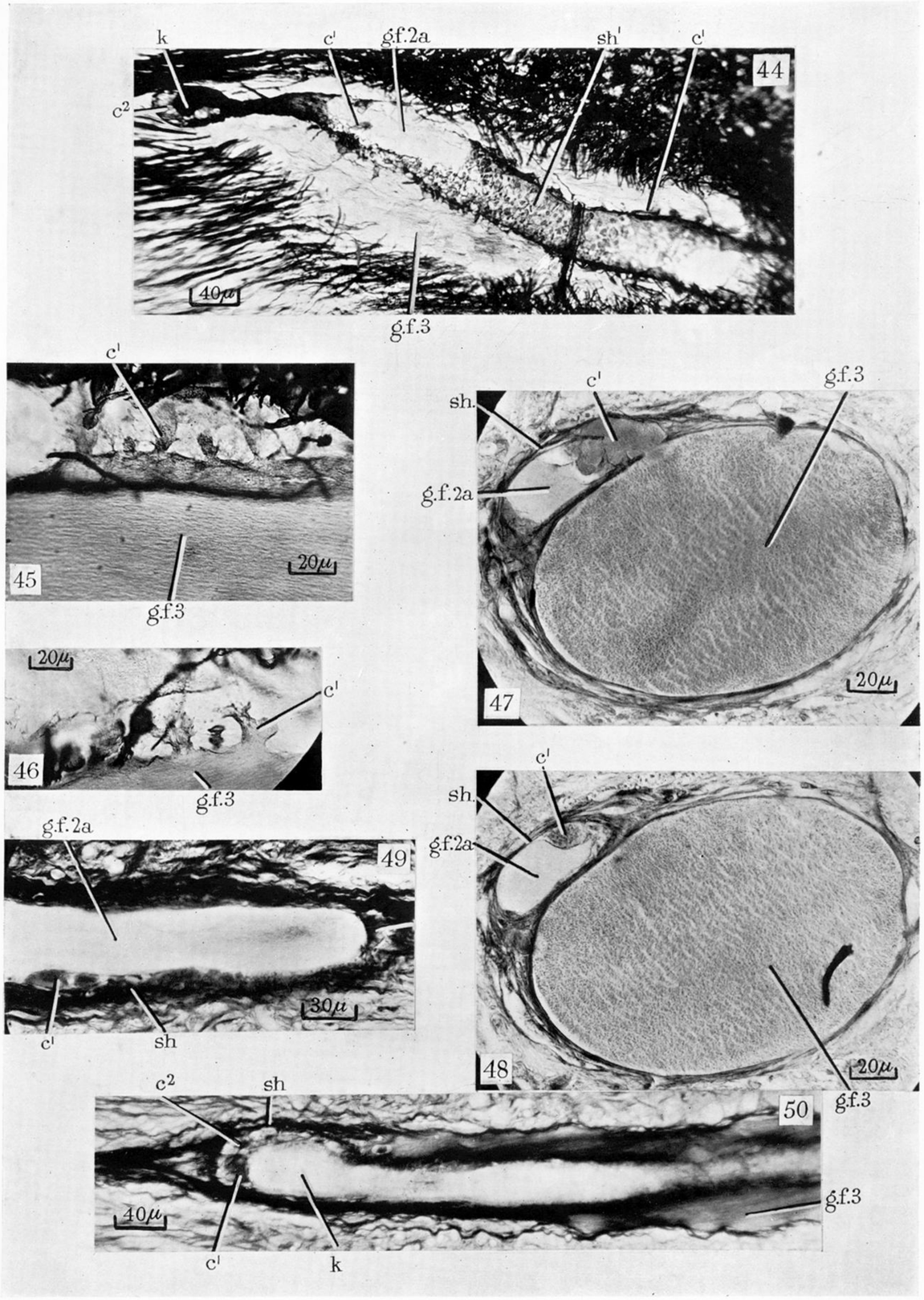


PLATE 47

FIG. 44. End of second-order fibre (*g.f.2a*) in stellate ganglion, and its synapse with third-order fibre (*g.f.3*). Sections cut transverse to the main body axis, cutting the fibres as they run out laterally. This synapse is also shown in the model of figs. 41 and 42. Cajal's stain, sectioned in paraffin. Note the knob at the end of *g.f.2a*, and the spaces between its surface and the sheath, which are occupied by axonic collaterals of *g.f.3*, these being imperfectly preserved in this preparation (*L. forbesi* BC*a* 2.3.12).

FIG. 45. Axonic collaterals of the third-order giant fibre, seen in a section cut parallel to the length of the fibre. The second-order fibre with which the collateral makes synapse (*g.f.2a*) is just above the plane of the photograph. Cajal's stain, sectioned in paraffin (*L. forbesi* BIa 5.1.6).

FIG. 46. Axonic collaterals of third-order fibre as in fig. 45. Cajal's stain, sectioned in paraffin (*L. forbesi* AYa 2.1.4).

FIG. 47. Section transverse to synapse of second- and third-order fibres in stellate ganglion. Picric acid, sectioned in paraffin, azan. A collateral of *g.f.3* can be seen on the right, expanding into a complex mass beneath the sheath of *g.f.2a*. A shrinkage space has developed between the surfaces of synaptic contact. On the left a second collateral (the connexion of which with *g.f.3* is not shown in this section) makes close contact with *g.f.2a* (*L. pealii* FBb 5.3.11).

FIG. 48. Another portion of the same series as fig. 47. The collateral of *g.f.3* is seen to make very close contact with *g.f.2a*, though the axoplasms clearly do not mingle.

FIG. 49. Synaptic contacts between collaterals of *g.f.3* and the end of *g.f.2a*, seen in a section longitudinal to the latter (the main trunk of *g.f.3* lies out of the plane of the section). Picro-formol, paraffin, azan (*L. pealii* DTb 6.1.4).

FIG. 50. Another synapse from the same ganglion as fig. 49. One of the collaterals (*c.* 1) is well preserved, but others (*c.* 2) have autolysed (*L. pealii* DTb 6.1.6).

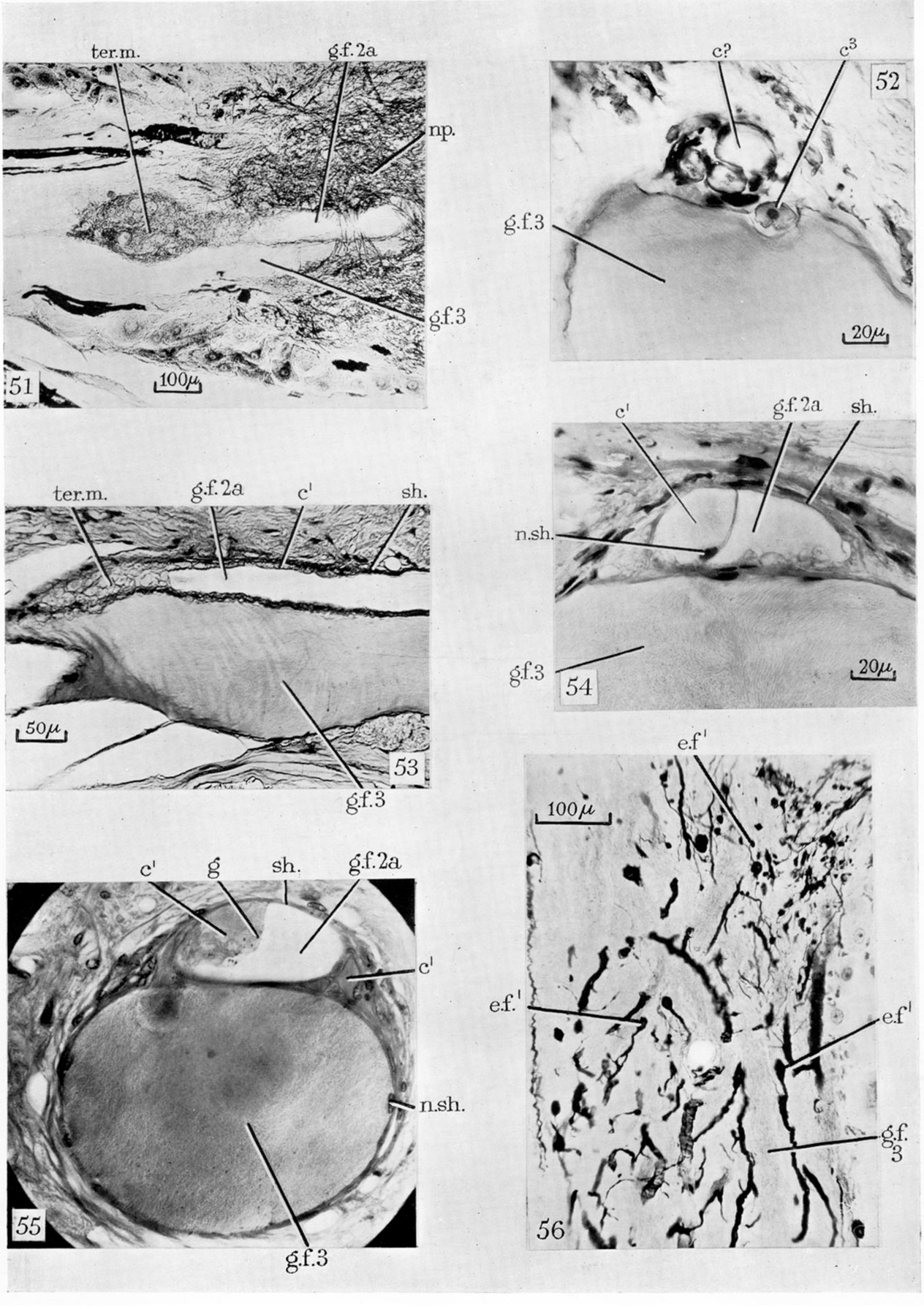


PLATE 48

FIG. 51. Terminal mass consisting mainly of collaterals of two third-order fibres beyond the end of a branch of *g.f.2a* with which they both make synapse. Cajal's stain, paraffin (*L. forbesi* BIb 4.1.12).

FIG. 52. Section transverse to a third-order giant fibre, showing the collaterals forming a terminal mass beyond the end of *g.f.2a*. Cajal's stain, paraffin (*L. forbesi* AYb 3.2.6).

FIG. 53. Section longitudinal to the second- and third-order giant fibres, showing the end of the former surrounded by collaterals of the latter, some of which form a terminal mass. Formol-bichromate, celloidin-paraffin, Masson's stain (*L. pealii* NTa 7.4.6).

FIG. 54. Section transverse to synapse between second- and third-order giant fibres, to show nucleus of inner sheath partly separating the two fibres. Picric acid, celloidin-paraffin, Masson's stain (*L. pealii* FBb 5.3.11).

FIG. 55. Section transverse to synapse between second- and third-order fibres. Picric acid, celloidin-paraffin, Masson's stain (*L. pealii* FBa 2.4.9). The granules showing black are red in the preparation.

FIG. 56. Part of sagittal section through the neuropil at the centre of the giant-fibre lobe of the stellate ganglion, to show the end feet which are branches of *g.f.a.*, making contact (proximal synapses) with the third-order giant fibres. Cajal's stain, paraffin (*L. forbesi* AYc 1.3.4).