

BRAIN PATHWAYS OF THE BRACHIAL NERVES OF *SEPIA* AND *LOLIGO*

By B. U. BUDELMANN¹ AND J. Z. YOUNG², F.R.S.

¹ Zoological Institute, University of Regensburg, D-8400 Regensburg, F.R.G.

² Department of Experimental Psychology, University of Oxford, South Parks Road,
Oxford OX1 3UD, U.K.

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Centripetal cobalt filling of the brachial nerves of *Sepia* and *Loligo* gave further information about the afferent and efferent connections of the arms. The supraoesophageal lobes of the brain contain direct motor pathways to the arms. Efferent fibres originate from somata of the inferior frontal (*Loligo*), and ipsilateral and contralateral (*Sepia*), precommissural lobes. In both genera, afferent fibres run to the inferior frontal lobe via ipsilateral and contralateral pathways. Some fibres continue backwards in the inferior frontal to palliovisceral tract. In *Loligo*, further afferent fibres run to the superior buccal, subvertical, precommissural and anterior basal lobes, and in *Sepia* to the superior frontal lobe. In the suboesophageal lobes, efferent fibres originate in both genera from somata in the anterior and posterior brachial, anterior chromatophore, and anterior pedal lobes. In *Sepia*, but not in *Loligo*, efferent fibres pass from the ventral magnocellular lobe direct to the arms. Many afferent fibres run from the arms to end in the brachial lobes, and some ipsilaterally, and a few also contralaterally, to the palliovisceral lobe. The brain pathways of *Sepia* and *Loligo* are thus very similar and the few differences are discussed. The magnocellular and palliovisceral lobes contribute less to the innervation of the arms in decapods than in octopods.

1. INTRODUCTION

The functional components of the nerves to the arms have recently been analysed in *Octopus* (Budelmann & Young 1985). A similar analysis has now been made by centripetal cobalt filling of the brachial nerves of the decapods *Sepia* and *Loligo*, allowing analysis of the directions of conduction in the pathways described by Boycott (1961) and Young (1976, 1979). In decapods and octopods the arms are used differently for the capture of prey (Wells 1962) and the inferior frontal system is less complex in decapods than in octopods (Young 1971, 1979); but there is some evidence of learning with afferents from the arms in *Sepia* (Sanders & Young 1940; Messenger 1973, 1977). It is therefore especially interesting to investigate the connections between the arms and the possible learning systems of the inferior frontal lobe, the superior frontal and vertical lobes and the precommissural lobe, which is the main output pathway for the vertical lobe complex (Young 1979).

2. MATERIALS AND METHODS

Four *Sepia officinalis* (75–85 mm mantle length) and two *Loligo vulgaris* (165 mm mantle length), obtained from the Gulf of Naples, Italy, were used in this study. For the centripetal cobalt filling of the brachial nerves, isolated head preparations were used with the body and arms removed. The experiments were done with the nerves of both the left and right side. In all preparations the nerve of the third arm was used, to allow comparison. There is no reason to believe that the results will differ for the four brachial nerves; differences may exist, however, for the pathways of the tentacle nerve. For details of the preparation and the subsequent treatment of the tissue after cobalt filling see Budelmann & Young (1984, 1985). Unlike the previous treatments, the brain was left in its cartilaginous capsule after the cobalt filling. Before the subsequent treatment, however, the cartilage was cut down to a thin layer for better visibility of the stained somata and fibres in the whole-mount preparations. The terminology of the *Sepia* and *Loligo* central nervous system is based on Young (1976).

3. RESULTS

After centripetal cobalt filling of a brachial nerve the pattern of distribution of filled fibres and somata is in general similar in *Sepia* and *Loligo* and they will be described together. Some differences in the supraoesophageal and the magnocellular lobes will be pointed out.

Some details can already be seen in the cleared brain before sectioning (plate 1). The brachial lobes are deeply stained as are the anterior chromatophore and anterior pedal lobes (figures 1, 3 and 4). In *Sepia*, but not in *Loligo*, a few large filled somata can be seen in the ventral magnocellular lobe at the antero-dorsal margin of the statocyst cavity (figures 1 and 3). Filled fibres are clearly seen in the cerebrobrachial tracts and the inferior frontal lobe. Some are seen running in the cerebral tract and others in the inferior frontal to palliovisceral tract (figure 2). Many filled fibres pass from the brachial lobe backwards through the ipsilateral, and some through the contralateral, brachiopalliovisceral connectives (figures 1, 3 and 4).

The more detailed distribution of the filled somata and fibres was followed on serial transverse and sagittal sections of the brain. After entry into the anterior brachial lobe, the nerve divides into four distinct bundles (I–IV) (figures 5 and 31).

Bundle I. The fibres of this bundle, shortly after entry, spread throughout the neuropil of the

anterior and posterior brachial lobes. Many cross to the other side (figures 6 and 7). Some filled fibres are seen in the roots of the ipsilateral, and a few in the roots of the contralateral, arm nerves, especially the third. Large, medium and small somata are filled dorsally and ventrally in the brachial lobes. These include some especially large somata dorsally at the posterior end of the posterior brachial lobe (figure 9) where many of the small somata are also filled. In the contralateral brachial lobes some large somata are filled medially, close to the midline, a few small somata at its lateral margin and many small ones at the very posterior end of the posterior brachial lobe (figures 6, 7 and 9).

Bundle II. The second bundle of filled fibres leaves the posterior brachial lobe through the ipsilateral cerebrobrachial tract and enters the inferior frontal lobe of the supraoesophageal mass of the brain. Another smaller bundle of fibres also enters this lobe through the contralateral cerebrobrachial tract; these latter fibres can already be seen dorsally in the posterior brachial lobe crossing as a bundle to the contralateral side (figure 8). In *Loligo* a few fibres pass to the superior buccal lobe through the cerebrobuccal connective (compare with Young (1979)) but no somata were seen filled in that lobe. In the inferior frontal lobe both crossed and uncrossed entering fibre bundles break up and spread as a meshwork of interweaving fibres throughout the neuropil. Some fibres of either bundle clearly cross the midline to all contralateral parts of the lobe (figure 10). Most of these fibres show the coarse swellings that distinguish them in silver preparations (figures 11 and 12; compare with Young (1971)). In *Loligo*, a few somata are filled anteriorly and posteriorly in the inferior frontal lobe (figure 13). No filled somata were seen, however, in the inferior frontal lobe of *Sepia*. Some fibres continue through the inferior frontal lobe and leave ventrally to run backwards through the ipsilateral inferior frontal to palliovisceral tract (figures 2 and 14).

In *Sepia*, a few fine afferent fibres can be seen proceeding through the inferior frontal lobe to the superior frontal lobe via the interfrontal tract (figure 15). No such fibres were seen in *Loligo*. On the other hand, in *Loligo* some large and small fibres proceed into the cerebral tract (figures 13, 14 and 16). A few continue towards the subvertical lobe, but could not be followed into this lobe. Other relatively large fibres turn ventrally from the cerebral tract into the precommissural lobe, where some fine fibres are seen in its dorsal part and a few continue far ventrally (figure 16). A single fine fibre could clearly be followed into the precommissural to anterior basal lobe tract (figure 17). None of these fibres have been seen originating from somata of these lobes. In *Sepia*, no filled fibres were seen in the cerebral tract or entering the precommissural lobe from the dorsal end (but see bundle III).

Bundle III. The third bundle passes through the brachial lobes into the brachio pedal connective and probably contains only efferent fibres (figure 18). At the front end of the pedal lobes many of these fibres spread out and can be followed to somata in the anterior chromatophore lobe, both dorsally and ventrally (figures 18 and 19). They are mainly large and lie in the outer perikaryal layers, but medium sized somata could also be seen, especially ventrally in the lobe (figure 18). A few filled somata occur in the contralateral anterior chromatophore lobe, medially close to the midline. Many fibres continue to the anterior pedal lobe. There, they originate from large and medium, but not small, somata dorsally and ventrally in the outer perikaryal layers (figures 18 and 20). A few filled somata could be seen contralaterally, but mostly close to the midline (figure 20). The trunks of the somata of the anterior pedal lobe often show long collateral dendritic branches (figures 21–24). It is striking that in both the anterior chromatophore and the anterior pedal lobes there are no obvious

afferent fibres, compared with the brachial lobes. Proceeding backwards in the pedal lobe, the number of filled somata and fibres diminishes rapidly and no signs of filling are seen in the posterior pedal lobe (figures 1, 3 and 18), except of filled fibres in the brachiopalliovisceral connectives (see bundle IV).

At the posterior end of the pedal lobe of both genera a few fibres turn dorsally and proceed through the anterior pedal to precommissural tract into the ipsilateral precommissural lobe; there, ventrally in the lobe they were seen originating from somata lying close to the oesophagus (figure 25). Thus, the precommissural lobe in *Loligo* has an afferent supraoesophageal input from, and in both genera an efferent suboesophageal output to, the arms (compare with bundle I). In *Sepia*, there are filled somata also on the contralateral side. These belong to a distinct bundle of filled fibres that crosses the posterior end of the anterior pedal lobe in a small commissure and continues through the contralateral anterior pedal to precommissural lobe tract into the contralateral precommissural lobe (figure 20) where they enter somata ventrally close to the oesophagus (figure 25). No filled afferent fibres were seen entering the precommissural lobes ventrally on either side.

Bundle IV. The fourth bundle of filled fibres turns laterally in the brachial lobes and passes into the ipsilateral brachiopalliovisceral connective (figures 1, 3 and 4). Some of the fibres could be followed backwards into the palliovisceral lobe (figures 3 and 4); they are presumably afferent fibres, since no somata were found filled in that lobe. Similarly filled, presumably afferent, fibres could also clearly be seen passing backwards in the contralateral brachiopalliovisceral lobe connective (figures 3, 4 and 19). In *Sepia*, some efferent fibres separate from the connective to end in the magnocellular lobe where they originate from few large (up to 110 μm in diameter) and small somata in the ventral magnocellular lobe. Some of these somata are located at the antero-dorsal margin of the statocyst cavity and others more dorsally close to the oesophagus (figures 26–28). They can already be seen in the uncleared brain before sectioning (figures 1 and 3). The trunks of the somata show several very long (up to 200 μm) dendritic collaterals that often branch; their diameter is much smaller than that of the main trunk (figure 30). Some of the collaterals turn and run backwards towards the perikaryal layer (figure 29). No such cells were seen in *Loligo*, in spite of the fact that many fibres were filled in the brachiopalliovisceral connective (figure 4). The very fine and branching collaterals of these filled cells make it very difficult to decide whether some of the filled fine fibres in the magnocellular lobe are afferents.

DESCRIPTION OF PLATE 1

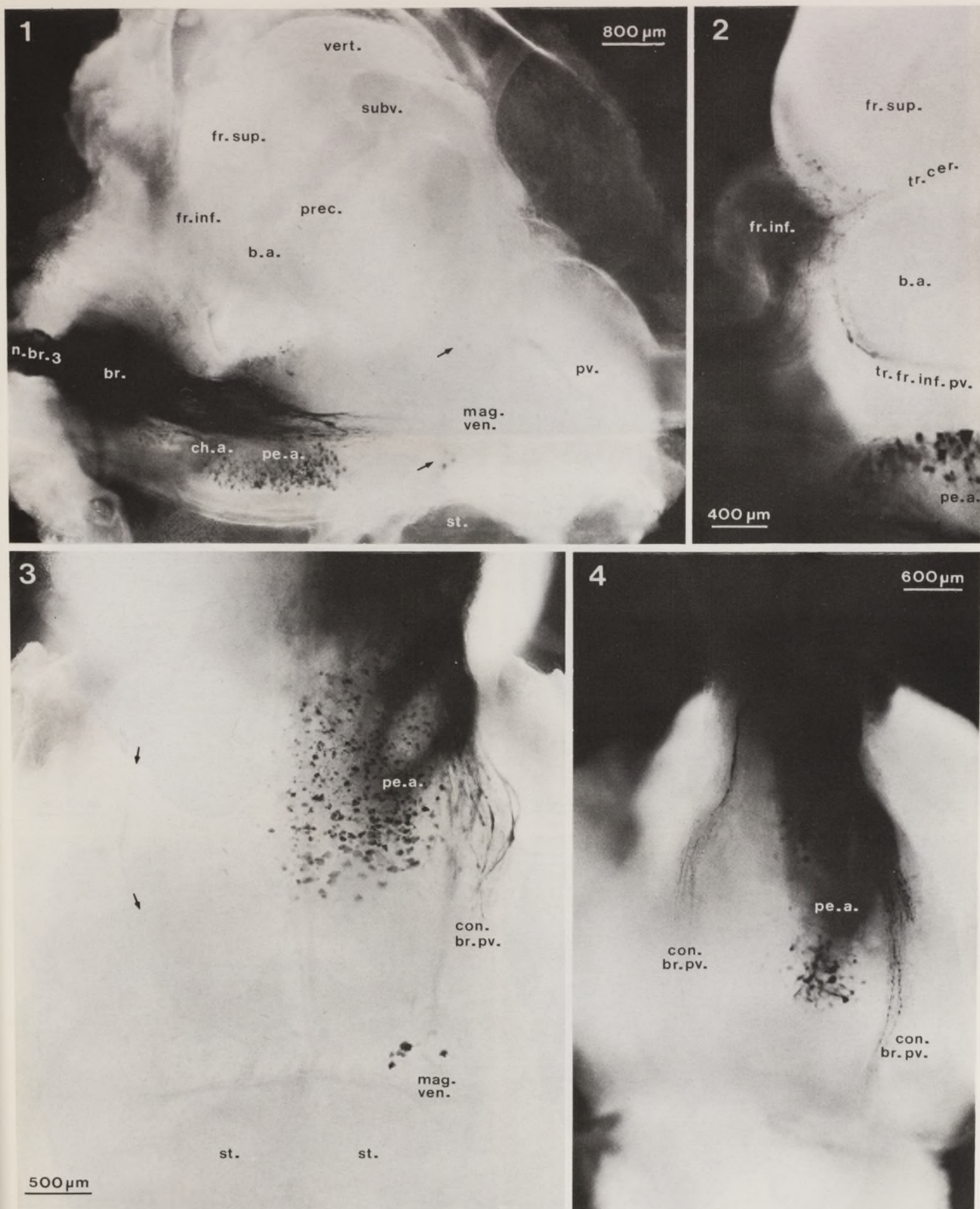
Whole mounts of the *Sepia* and *Loligo* brains after centripetal cobalt filling of the third brachial nerve.

FIGURE 1. Lateral view of the *Sepia* brain showing an intense staining in the brachial lobes, and somata in the anterior chromatophore and anterior pedal lobes. A few large somata (arrows) are seen in the ventral magnocellular lobe.

FIGURE 2. Lateral view of the anterior supraoesophageal lobes of the *Loligo* brain. Fibres enter the inferior frontal lobe through the cerebrobrachial lobe tracts. Some afferent fibres continue in the cerebral tract, and others in the inferior frontal to palliovisceral lobe tract.

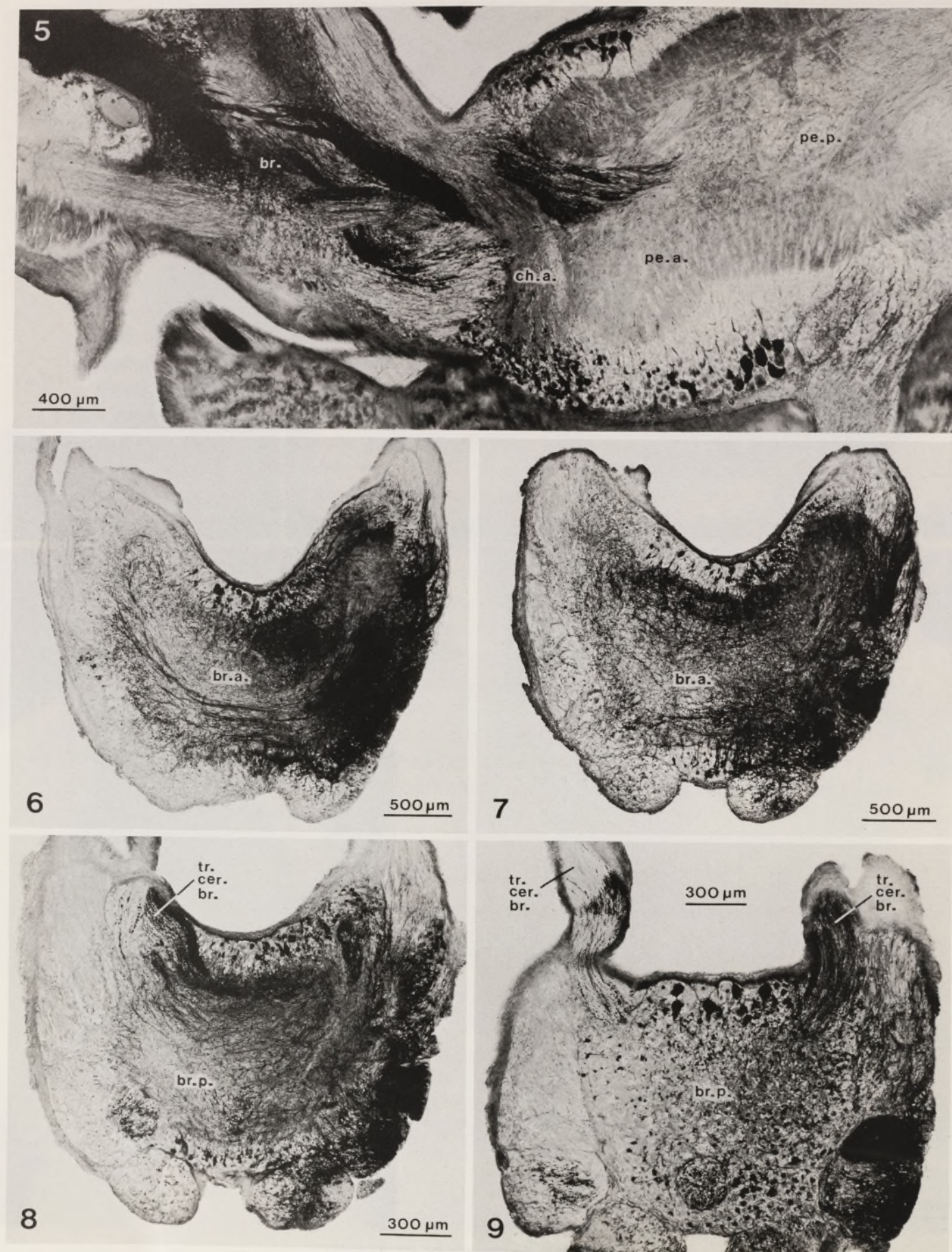
FIGURE 3. Ventral view of the *Sepia* brain showing many somata in the anterior pedal lobe and a few large somata in the ventral magnocellular lobe at the anterior margin of the statocyst cavity. Afferent fibres are seen in the ipsilateral, and a few (faintly stained; arrows) in the contralateral, brachiopalliovisceral lobe connectives.

FIGURE 4. Ventral view of the *Loligo* brain showing somata in the anterior pedal lobe, but none in the ventral magnocellular lobe. Afferent fibres are seen in the ipsilateral and contralateral brachiopalliovisceral lobe connectives.



FIGURES 1-4. For description see opposite.

(Facing p. 348)



FIGURES 5-9. For description see opposite.

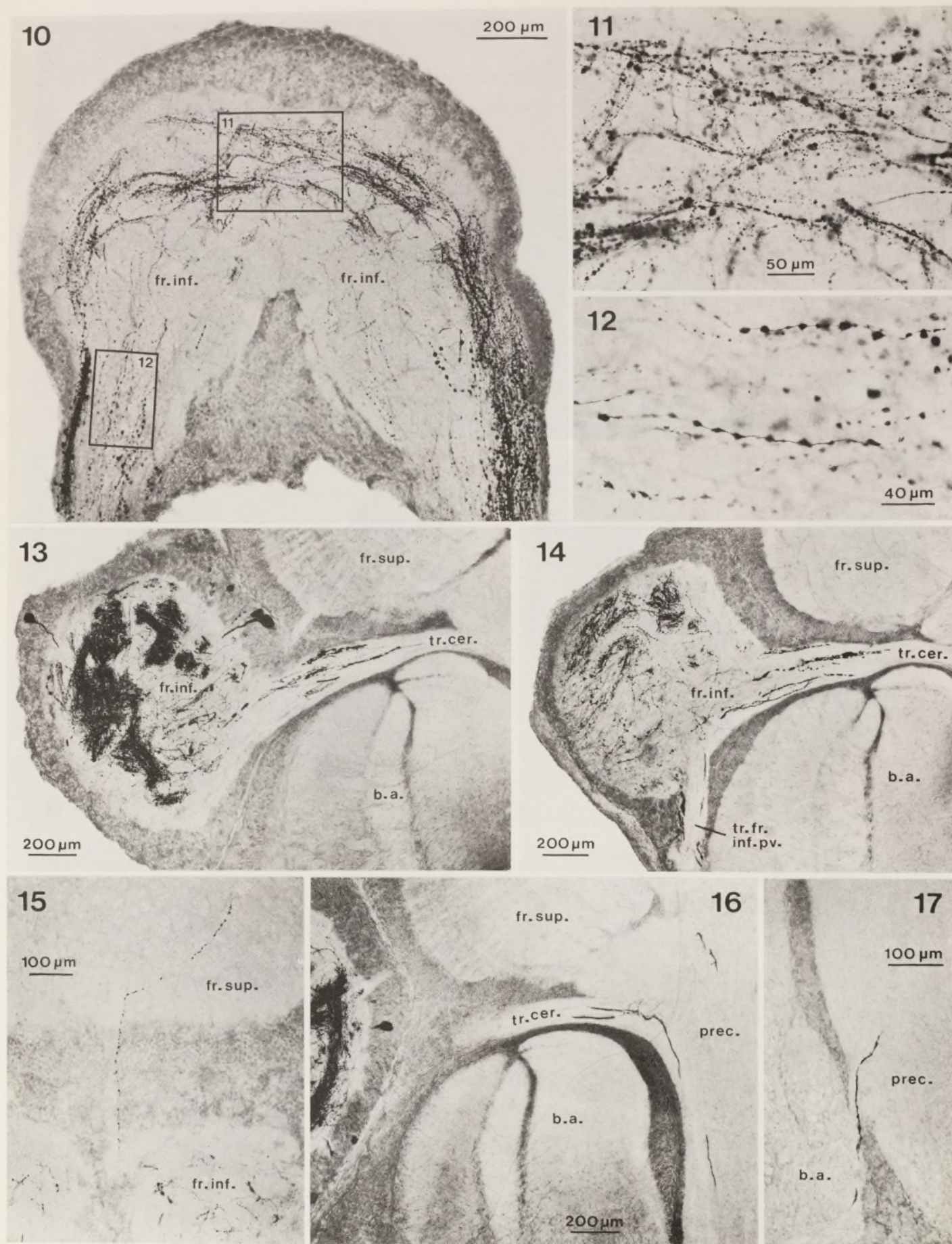
DESCRIPTION OF PLATE 2

Sagittal (figure 5) and transverse (figures 6-9) sections of the suboesophageal lobes of the *Sepia* brain after centripetal cobalt filling of the third brachial nerve.

FIGURE 5. Fibre bundles in the brachial lobes and somata in the anterior chromatophore and anterior pedal lobes.

FIGURES 6 AND 7. Fibres and somata in the anterior brachial lobe. Many fibres, and fibre bundles (figure 6), cross to the contralateral side of the lobe; there, some large somata are seen close to the midline, and a few smaller somata at its lateral margin.

FIGURES 8 AND 9. Fibres and somata in the posterior brachial lobe. Figure 8 shows a large fibre bundle crossing dorsally to the contralateral side and proceeding into the contralateral cerebrobrachial lobe tract. Figure 9 shows filled somata in the posterior end of the posterior brachial lobe, including some specially large somata dorsally close to the oesophagus. Many fibres are seen in the ipsilateral and contralateral cerebrobrachial lobe tracts.



FIGURES 10-17. For description see opposite.

DESCRIPTION OF PLATE 3

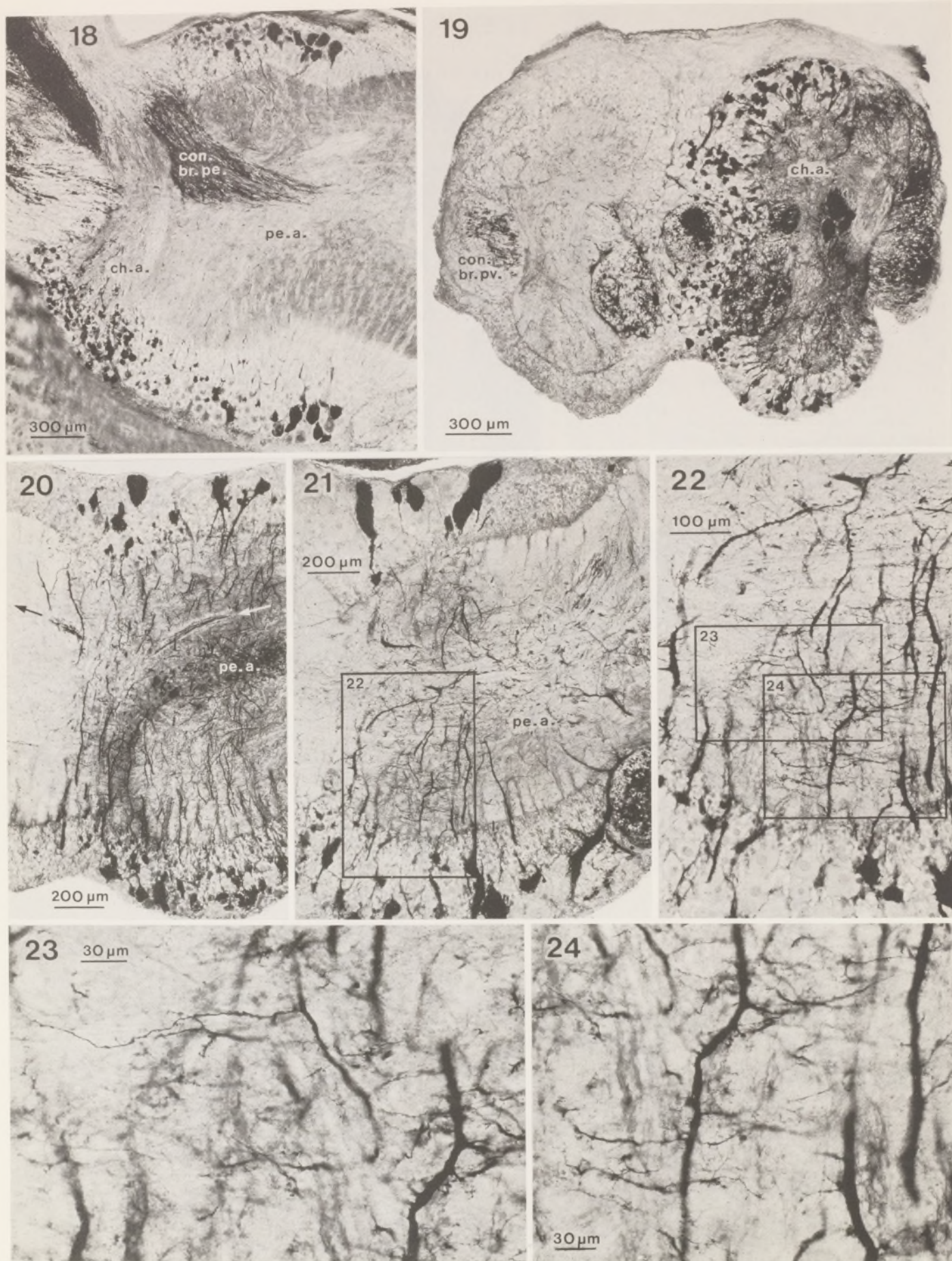
Transverse sections of the supraoesophageal lobes of the *Sepia* brain (figures 10–12 and 15) and sagittal sections of these lobes of the *Loligo* brain (figures 13, 14, 16 and 17) after centripetal cobalt filling of the third brachial nerve.

FIGURES 10–12. Afferent fibres, but no somata, in the inferior frontal lobe of *Sepia*. The fibres enter the lobe through the ipsilateral and contralateral cerebrobrachial lobe tracts.

FIGURES 13 AND 14. Afferent fibres, and a few somata anteriorly and posteriorly, in the inferior frontal lobe of *Loligo*. Some afferent fibres proceed into the cerebral tract, and others ventrally into the inferior frontal to palliovisceral lobe tract (figure 14).

FIGURE 15. A single afferent fibre proceeding from the inferior frontal lobe to the ipsilateral superior frontal lobe via the interfrontal tract.

FIGURES 16 AND 17. Fibres from the inferior frontal lobe proceeding through the cerebral tract into the precommissural lobe. Figure 17 shows a single afferent fibre entering the anterior basal lobe through the precommissural to anterior basal lobe tract.



FIGURES 18-24. For description see opposite.

DESCRIPTION OF PLATE 4

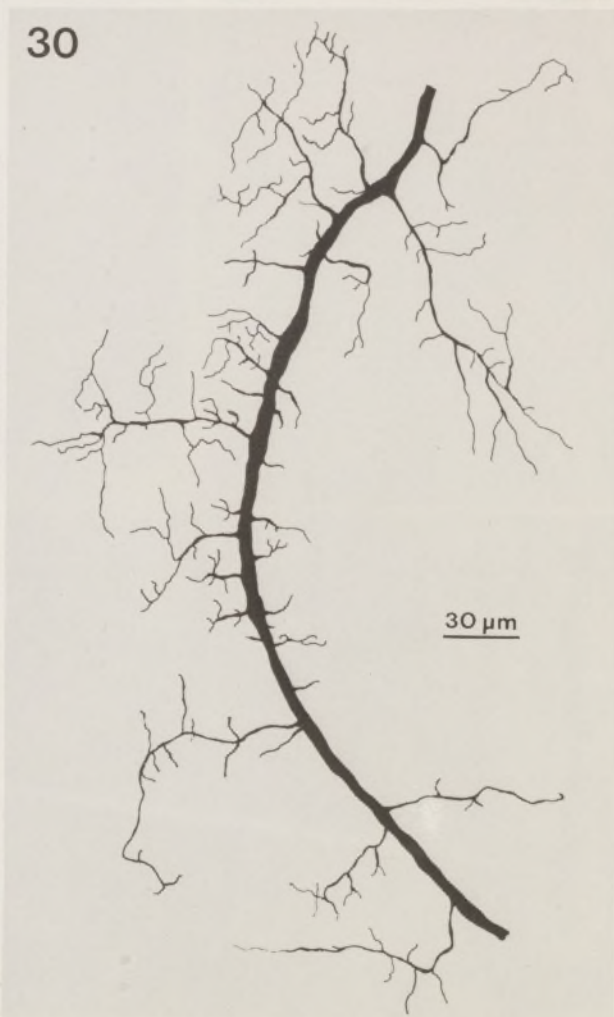
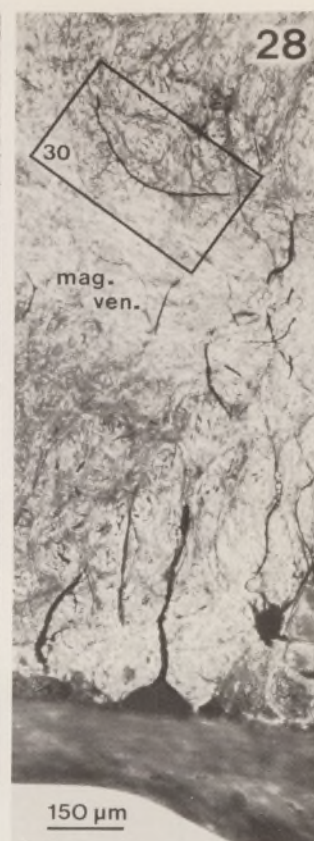
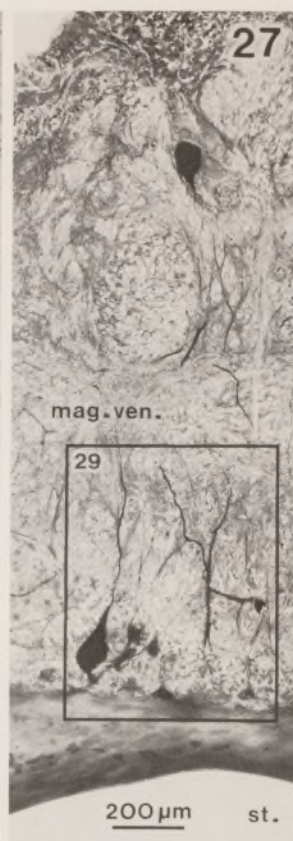
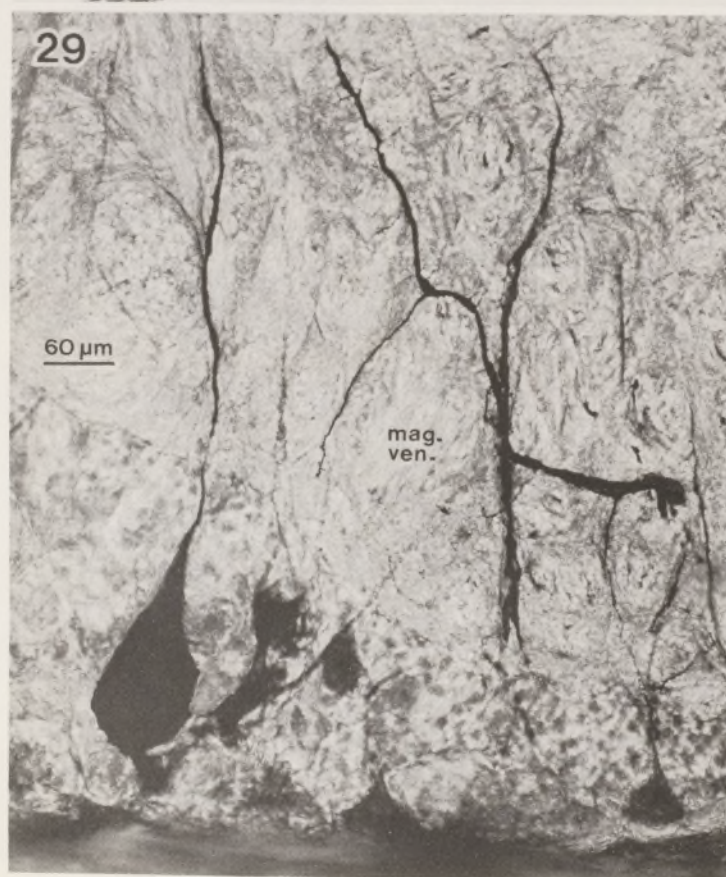
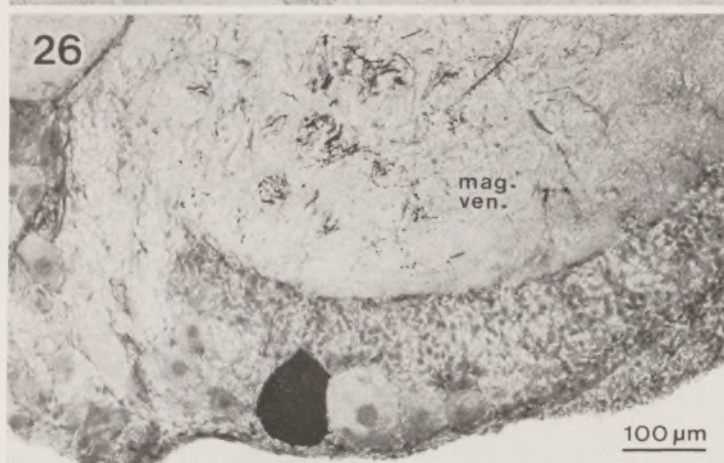
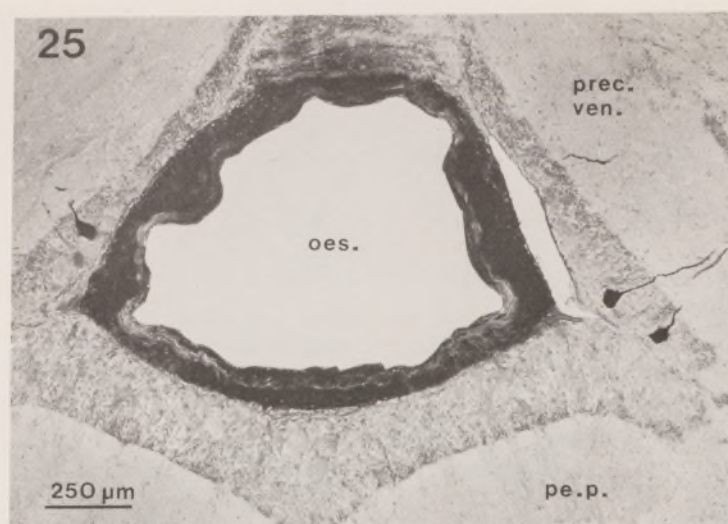
Sagittal (figure 18) and transverse sections (figures 19–24) of the suboesophageal lobes of the *Sepia* brain after centripetal cobalt filling of the third brachial nerve.

FIGURE 18. Fibres entering, and somata dorsally and ventrally in, the anterior chromatophore and anterior pedal lobes.

FIGURE 19. Somata dorsally and ventrally in the anterior chromatophore lobe.

FIGURES 20–24. Somata dorsally and ventrally in the anterior pedal lobe; their trunks show fine collateral fibres.

Figure 20 shows a small bundle of fibres that crosses the anterior pedal lobe in a small commissure (arrows) to continue in the contralateral anterior pedal to precommissural lobe tract.



FIGURES 25-30. For description see opposite.

4. DISCUSSION

4.1. *Comparison of decapods and octopods*

The fillings of the brachial nerve have added to an understanding of the significance of the tracts in the decapod brain already described by Boycott (1961) and Young (1976, 1979). They have shown a general similarity to the organization found by fillings of the brachial nerves of octopods, but with some differences (Budelmann & Young 1985). The composition of the tracts is remarkably similar in the two decapod genera, emphasizing the closeness of sepoids and teuthoids in spite of their long evolutionary separation. The afferent and efferent brain pathways are summarized diagrammatically in figure 31. The differences are shown in table 1. However it must be remembered that fibres in a nerve may be missed with this method. The fibres and cells filled in the supraoesophageal lobes are few and they are a long way from the source of the cobalt. There may well be more than we have found.

TABLE 1. DIFFERENCES IN THE ORGANIZATION OF THE CENTRAL PATHWAYS (COURSE AND TERMINATION OF AFFERENT FIBRES, LOCATION OF EFFERENT SOMATA) OF THE THIRD BRACHIAL NERVES OF *SEPIA* AND *LOLIGO*

(Notation: ' + ' indicates the presence of somata or fibres, and ' - ' their absence.)

		<i>Sepia</i>	<i>Loligo</i>
superior buccal lobe	afferents	-	+
inferior frontal lobe	somata	-	+
superior frontal lobe	afferents	+	-
subvertical lobe	afferents	-	+
anterior basal lobe	afferents	-	+
precommissural lobe	afferents	-	+
contralateral precommissural lobe	somata	+	-
ventral magnocellular lobe	somata	+	-

4.2. *Supraoesophageal lobes*

Of special interest is the question of how far the higher centres of the supraoesophageal lobes have direct control of the arms. Many afferents of the brachial nerve reach to the inferior frontal lobe and interweave there in a plexus that has some similarities to that in the median inferior frontal lobe of *Octopus* (Budelmann & Young 1985). A few of these afferents were seen proceeding to the vertical lobe complex, through the superior frontal lobe in *Sepia*, and to the subvertical and precommissural lobes in *Loligo*. However, the filled fibres in *Loligo* were so large as to raise the suspicion that they were efferents, but no filled somata were found connected with them. A few efferent somata were seen in the inferior frontal lobe in *Loligo*, none in *Sepia*.

DESCRIPTION OF PLATE 5

Transverse sections of the brain of *Sepia* after centripetal cobalt filling of the third brachial nerve.

FIGURE 25. Somata in the ipsilateral and contralateral ventral precommissural lobes, close to the oesophagus.

FIGURE 26. Large filled soma in the ventral magnocellular lobe, close to the antero-dorsal margin of the statocyst cavity.

FIGURES 27-30. Somata in the ventral magnocellular lobe, ventrally at the antero-dorsal margin of the statocyst cavity, and dorsally close to the oesophagus (figure 27). Some of their trunks show many fine collateral fibres (figure 30 is a camera lucida drawing).

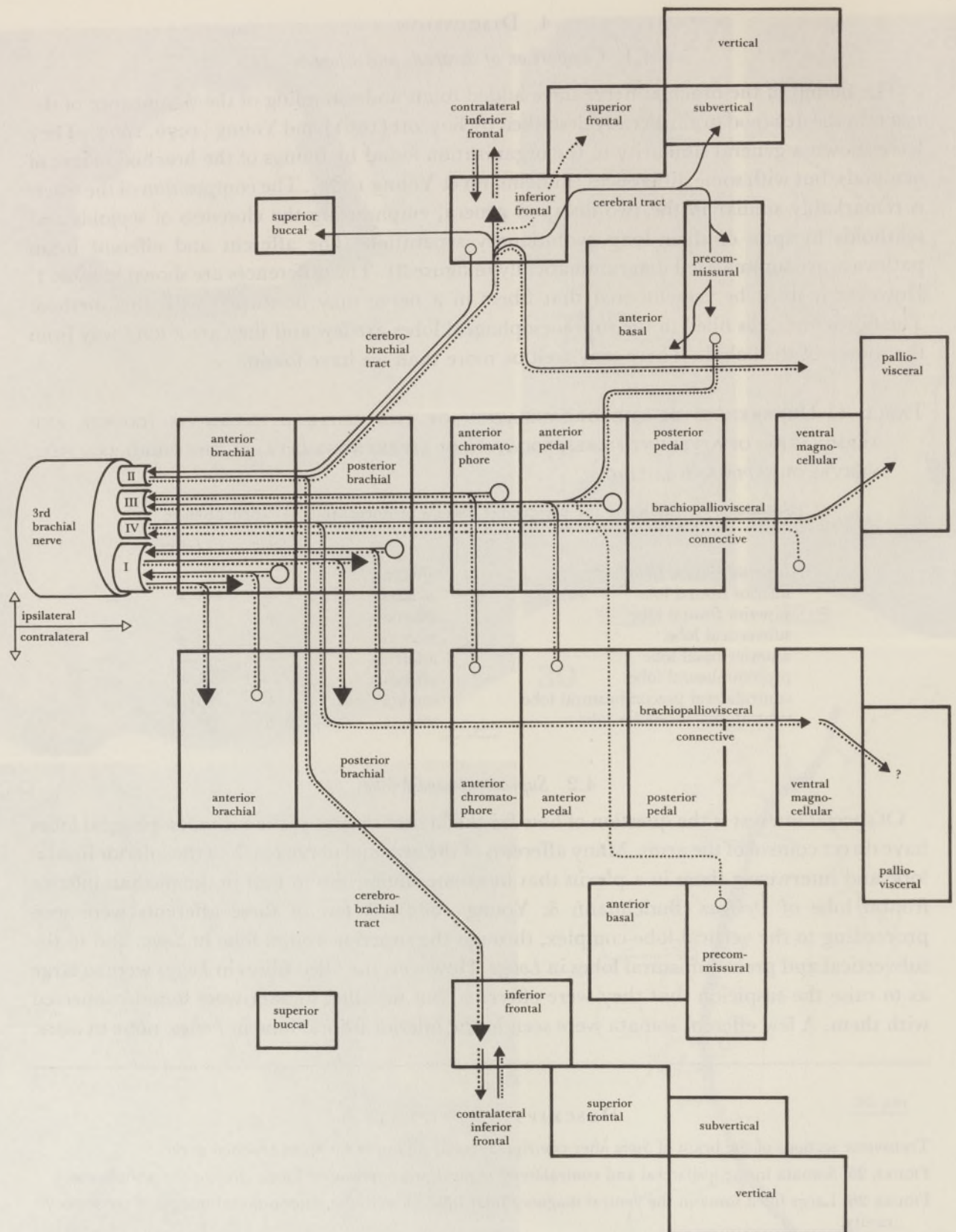


FIGURE 31. Schematic presentation of the afferent and efferent brain pathways of the third brachial nerve of *Loligo vulgaris* (solid lines) and *Sepia officinalis* (dotted lines), as obtained by centripetal cobalt fillings. The rectangles represent the ipsilateral and contralateral brain lobes as indicated. I-IV are the fibre bundles described in the text. Open circles indicate the origin (that is, the somata) of the pathways and arrowheads their termination. The sizes of the open circles or arrowheads provide an approximate indication of the numbers of cells or fibres filled. Large open circles indicate many filled somata, small open circles few. Large arrowheads indicate the termination of many filled afferents, small arrowheads of few. When branched lines are shown they indicate continuation of the pathway, not necessarily of individual fibres.

This contrasts with the many efferent cells of the posterior buccal lobe of *Octopus*, with axons proceeding directly to the arms (Budelmann & Young 1985). This posterior buccal lobe is presumably a development of the efferent part of the inferior frontal lobe of the ancestral coleoid stock (Young 1979). On the other hand, filled somata were found in the precommissural lobe of both *Sepia* and *Loligo*, but none in *Octopus*. These cells may perhaps correspond functionally to those in the median basal lobe of *Octopus* which were not seen in decapods. It is clear that the vertical lobe system has some pathways for direct control of the arms in decapods, presumably as a part of learned behaviour.

The finding of filled fibres in the inferior frontal to palliovisceral tract adds a further complexity to this puzzling pathway. In *Octopus*, it has been shown to include fibres running in both directions ('palliovisceral to supraoesophageal tract' (Young 1971)). Unfortunately, the posterior endings of these fibres have never been traced and our preparations also do not show them.

4.3. *Brachial and anterior pedal lobes*

The efferent cells that send their axons into the brachial nerves arise from all parts of the brachial lobe. This agrees with the evidence of Boycott (1961) that electrical stimulation of any part of this lobe produces movements of all arms on both sides. Cells were also filled in many parts of the anterior pedal lobe, from which Boycott (1961) elicited movements of individual arms. The filled somata in this lobe end abruptly halfway along the pedal lobe, as also in *Octopus* (Budelmann & Young 1985). There were no filled cells in the anterior or posterior lateral pedal lobes, although the neuropils of all parts of the pedal lobe are continuous, with no lines of separation.

4.4. *Chromatophore lobes*

As in *Octopus* (Budelmann & Young 1985), no afferent fibres from the arms enter the chromatophore lobes. Unlike *Octopus*, however, the filled somata were all in the anterior, and not in the posterior, chromatophore lobe. The functional significance of this difference remains unclear.

4.5. *Magnocellular and palliovisceral lobes*

A further difference from *Octopus* is that relatively few somata were filled in the magnocellular lobes in *Sepia* and none in *Loligo*. In *Sepia* the cells that were filled were in dorsal and ventral positions similar to the cells seen in *Octopus* after centripetal filling of the brachial nerve (Budelmann & Young 1985). Evidently cells in this region have direct motor control of the arms, perhaps when these are involved in escape reactions including other parts of the body. No cells were filled in the palliovisceral lobe in either decapod genus and only a few afferent fibres reach here from the arms. There is thus evidence that the system for the control of the arms in decapods is mainly concentrated at the anterior end of the suboesophageal mass of the brain, whereas in *Octopus* it is partly dispersed. This corresponds to the greater concentration of the whole nervous system in the octopods.

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ABBREVIATIONS USED ON THE FIGURES

b.a.	anterior basal lobe	pe.a.	anterior pedal lobe
br.	brachial lobe	pe.p.	posterior pedal lobe
br.a.	anterior brachial lobe	prec.	precommissural lobe
br.p.	posterior brachial lobe	prec.v.	ventral precommissural lobe
ch.a.	anterior chromatophore lobe	pv.	palliovisceral lobe
con.br.pe.	brachio pedal lobe connective	st.	statocyst cavity
con.br.pv.	brachio palliovisceral lobe connective	subv.	subvertical lobe
fr.inf.	inferior frontal lobe	tr.cer.	cerebral tract
fr.sup.	superior frontal lobe	tr.cer.br.	cerebrobrachial lobe tract
mag.v.	ventral magnocellular lobe	tr.fr.inf.pv.	inferior frontal to palliovisceral lobe tract
n.br.3	third brachial nerve	vert.	vertical lobe
oes.	oesophagus		