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THE NEUROANATOMY OF AN AMPHIBIAN EMBRYO SPINAL CORD

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[Plates 1 and 2]

CONTENTS

Horseradish peroxidase has been used to stain spinal cord neurons in late embryos of the clawed toad (*Xenopus laevis*). It has shown clearly the soma, dendrites and axonal projections of spinal sensory, motor and interneurons. On the basis of light microscopy we describe nine differentiated spinal cord neuron classes. These include the Rohon-Beard cells and extramedullary cells which are both primary sensory neurons, one class of motoneurons that innervate the segmental myotomes, two classes of interneurons with decussating axons, three classes of interneurons with ipsilateral axons and a previously undescribed class of ciliated ependymal cells with axons projecting ipsilaterally to the brain.

We believe that all differentiated neuron classes are described and that this anatomical account is the most complete for any vertebrate spinal cord.

INTRODUCTION

Our use of lower vertebrate embryos in the search for the nervous origins of behaviour was inspired by the studies of G. E. Coghill on *Ambystoma* (summarized in 1929). His idea was to correlate developing behaviour with the development of neruons in the nervous system. Though the idea was good, its execution has been repeatedly frustrated by the failure of specific stains, like the Golgi methods, to work on younger embryos. As a consequence, published work on the neuroanatomy of the early embryonic spinal cord in fishes and amphibians relies on silver

staining techniques that do not show dendritic anatomy and where the tracing of central and peripheral axons is difficult (teleosts (Leghissa 1942; Gideiri 1966), agnatha (Whiting 1948), dogfish (Harris 1962), urodeles (Coghill 1929; Youngstrom 1938, 1940), anurans (Hughes 1957, 1959; Gideiri 1971)). We have been studying the behaviour of *Xenopus* embryos (Roberts 1971), their sensory systems (Roberts & Smyth 1974; Roberts & Blight 1975; Roberts & Hayes 1977; Roberts 1978, 1980) and the way that the nervous system coordinates swimming locomotion (Kahn *et al*. 1982; Kahn & Roberts 1982; Roberts & Kahn 1982). To understand how sensory systems and neuronal activity relate to behaviour, we also need detailed information on the anatomy of neurons and their organization. This study presents a general account of the organization of differentiated neurons in the spinal cord of *Xenopus laevis* embryos in the period just before hatching. Our report is based on staining with horseradish peroxidase (HRP) applied extracellularly to damaged axons. This procedure results in a diffuse staining quite comparable in quality with that obtained by the Golgi technique. We have therefore been able to define the main neuron types in the spinal cord. Some of these, like the sensory Rohon-Beard cells or the motoneurons, are already documented. However, the interneurons or propriospinal neurons have not previously been described except in the most general terms. We can now distinguish anatomical types of interneuron on the basis of their soma position, dendritic fields and axonal projections. A new type of cell of unknown function has also been found. This is an ependymal cell with cilia in the spinal neurocoel and a ventral axon projecting rostrally to the brain. The small number of cell types and the general simplicity of organization make the description that we provide one of the most complete for any vertebrate, and encourage us to think that the *Xenopus* embryo is a very promising model system in which to study the nervous origins and bases for behaviour.

Materials and methods

Embryos of the clawed toad, *Xenopus laevis*, were obtained by induced breeding. At developmental stage 35/36 (Nieuwkoop & Faber 1956) embryos were removed from their egg membranes and anaesthetized in a dilute solution of MS222 in Ringer's solution. The central nervous system (c.n.s.) tissue was then exposed by dissection with fine pins. Neurons were labelled by crushing their axons with a pair of fine forceps whose tips were coated with dried, aqueous HRP (Boehringer grade I) solution.

In each embryo HRP was applied to one of four portions of a tissue.

(i) The intermyotomal clefts where motoneuron axons innervate the myotomes and where Rohon-Beard and extramedullary cell axons lie on their way to the skin.

(ii) The spinal cord in the tail region. Here the tissue was approached from the dorsal surface and the whole cord crushed between the forceps tips. This filled cells in the brain and rostral spinal cord whose axons projected caudally to the site of HRP application.

(iii) The left half of the hindbrain. This procedure filled cells in the spinal cord whose axons project to the hindbrain. As the application was unilateral it also separated cells with ipsilateral projections from those with contralateral projections.

(iv) One side of the rostral spinal cord. The dissection and rationale for this application were similar to those in the one-sided hindbrain crushes but it also filled cells in the spinal cord and hindbrain rostral to the HRP application. (Unilateral HRP applications to more caudal spinal cord were not possible due to the small diameter of the cord.)

After the HRP application the embryos were left to recover in Ringer's solution where the embryonic skin rapidly covered over the exposed tissues. During recovery the Ringer solution was diluted gradually. Approximately 6–8 h later the embryos were reanaesthetized and part of the belly yolk was removed to aid access of subsequent solutions (histochemicals) to the nervous tissues and then fixed for $1\frac{1}{2}$ h in a 5% (by mass) glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4. Fixative was removed by washing overnight in cold running tap water and then briefly in distilled water. The HRP was processed following the method of Lamb (1976). Tissue was first soaked in a 0.08% (by mass) solution of 3,3'-diaminobenzidine in 0.1 M phosphate buffer (pH 7.4) for 2 h. Hydrogen peroxide was then added to make a concentration of 0.01% (by volume) and the tissue was incubated, in the dark, at 20–22 °C, for a further 1 h. After washing in distilled water, the tissue was prepared either for serial sectioning or as a whole mount of the spinal cord and brain. For whole mounts the c.n.s. was dissected clear of remaining tissue including pigment cells with the aid of fine pins in buffer. For some preparations with labelled motoneurons the ventral two-thirds of the myotomes were left attached to the spinal cord so that we could examine ventral root formation. Once isolated, the c.n.s. was dehydrated in Lang's alcohols (Lang 1937), cleared in methyl benzoate and xylene and mounted in Canada balsam between two glass coverslips fixed to an aluminium slide. The specimen could then be viewed from either side by means of oil immersion lenses. Tissue to be serially sectioned was dehydrated in Lang's alcohols, cleared in methyl benzoate and toluene and embedded in paraffin wax. Sections were cut at 15 μm and mounted in a conventional manner.

Cells were drawn at a magnification of × 1250 by means of a camera lucida. Some of the cells labelled in whole mount preparations were reconstructed to appear as if in transverse section by means of a microscope attachment designed by McKenzie & Vogt (1976).

Over 100 embryos have been examined and more than 200 cells drawn.

Results

General organization of the spinal cord

At stage 37/38 (Nieuwkoop & Faber 1956) the spinal cord is a gently tapering tube about 100 μm in diameter rostrally (figures 1, 2). It lies over the notocord between the segmented myotomes and is covered loosely with pigment cells. Our account applies mainly to the part of the spinal cord in the mid-trunk region (5th to 12th postotic myotome).

The neural canal or neurocoel is lined by ependymal cells whose radial processes extend outwards and whose end feet expand and interdigitate to form the outer cord surface (plate 1). Dorsal to the ependymal cells are the large Rohon-Beard sensory cells whose outer membranes form the dorsal surface of the cord. They are the only class of neurons not protected by ependymal cell processes. More laterally the somas of differentiating and differentiated neurons lie outside the ependymal cell somas. Neuronal processes extend laterally towards the cord surface into the tracts of longitudinal axons on either side of the cord (plate 1). We make a distinction between the large lateral tract and a much smaller dorsal tract containing the axons of Rohon-Beard and extramedullary cells (see below). This dorsal tract lies just lateral to the somas of Rohon-Beard cells and is often separated from the main lateral tract by superficial neuron somas (figure 8c; plate 1). The lateral tracts contain ascending and descending axons from spinal cord cells all of which appear to have long axons projecting to distant sites. They also contain descending axons from cells in the ventrolateral hindbrain, which project to the spinal

cord. Neuron somas are not present on the ventral floor of the cord but the only connections between the two sides are found here forming a sparse ventral commissure.

All the cell classes reported here, with the possible exception of the extramedullary cells, can be reliably filled from appropriately placed applications of HRP (table 1). Motoneurons,
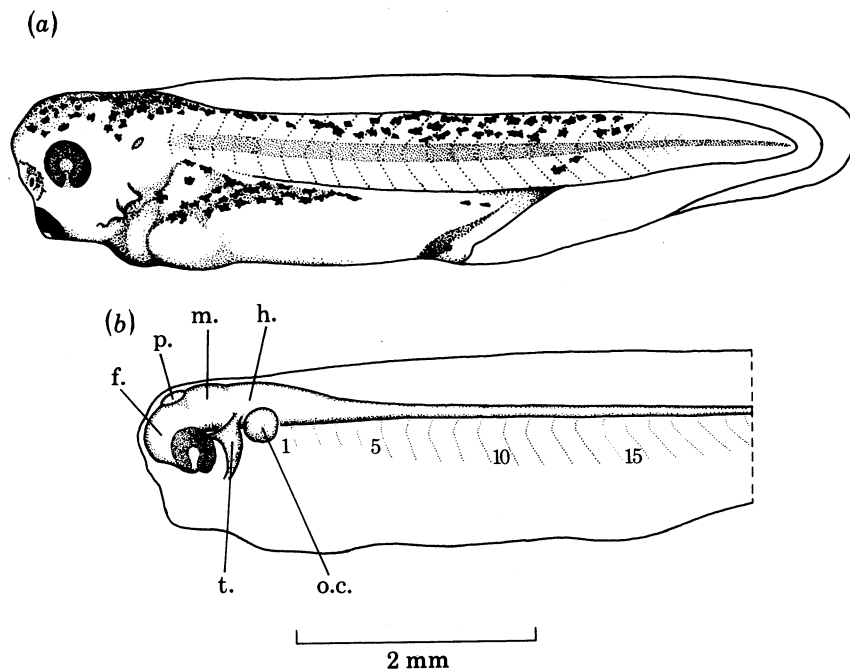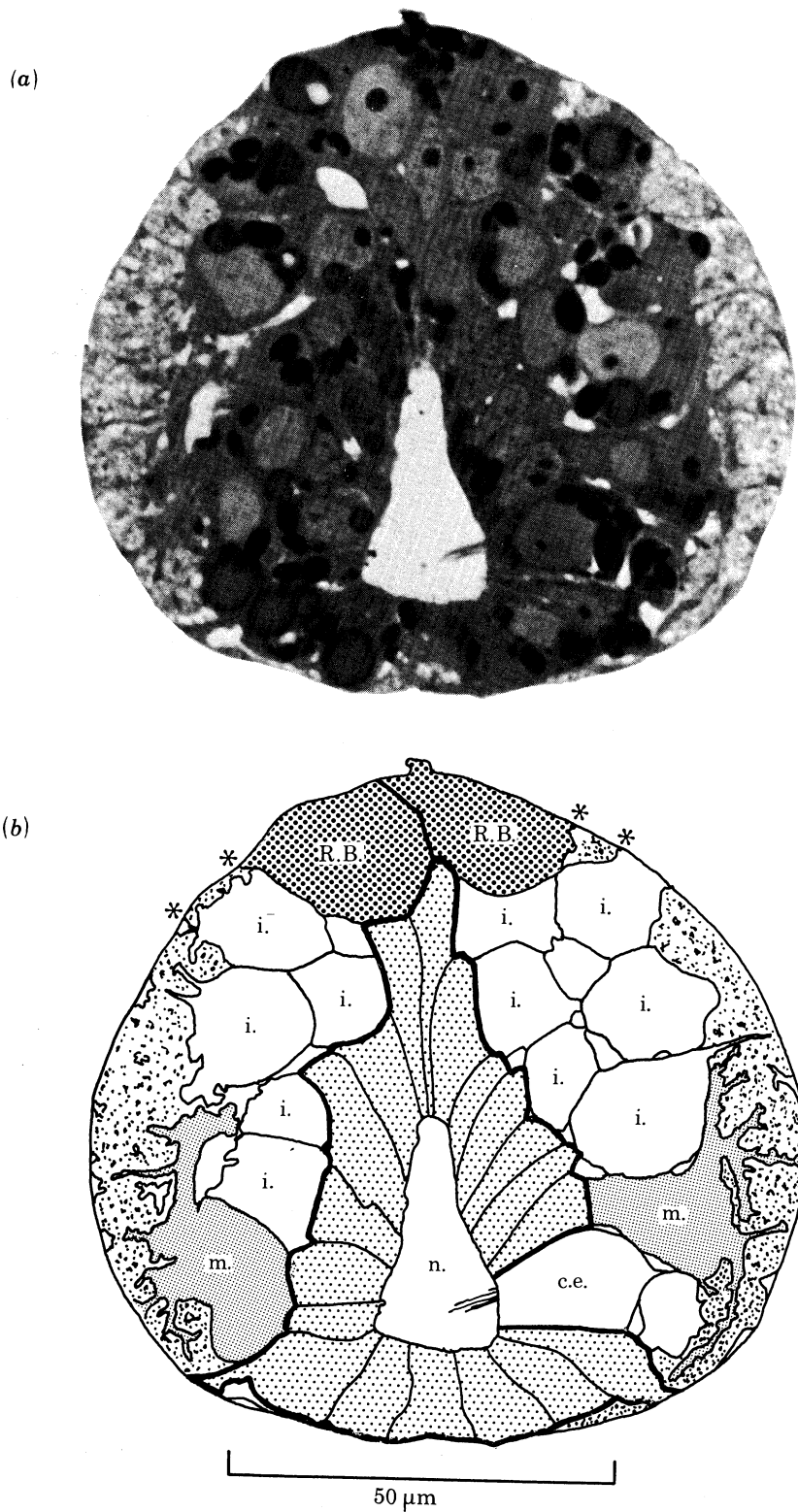

FIGURE 1.
(a) St. 37/38 *Xenopus laevis* embryo.
(b) Diagram to show the main parts of the nervous system and their relation to the myotomes which are numbered caudally from the otic capsule. Abbreviations: t., trigeminal ganglion; f., forebrain; h., hindbrain; m., midbrain; p., pineal; o.c., otic capsule.
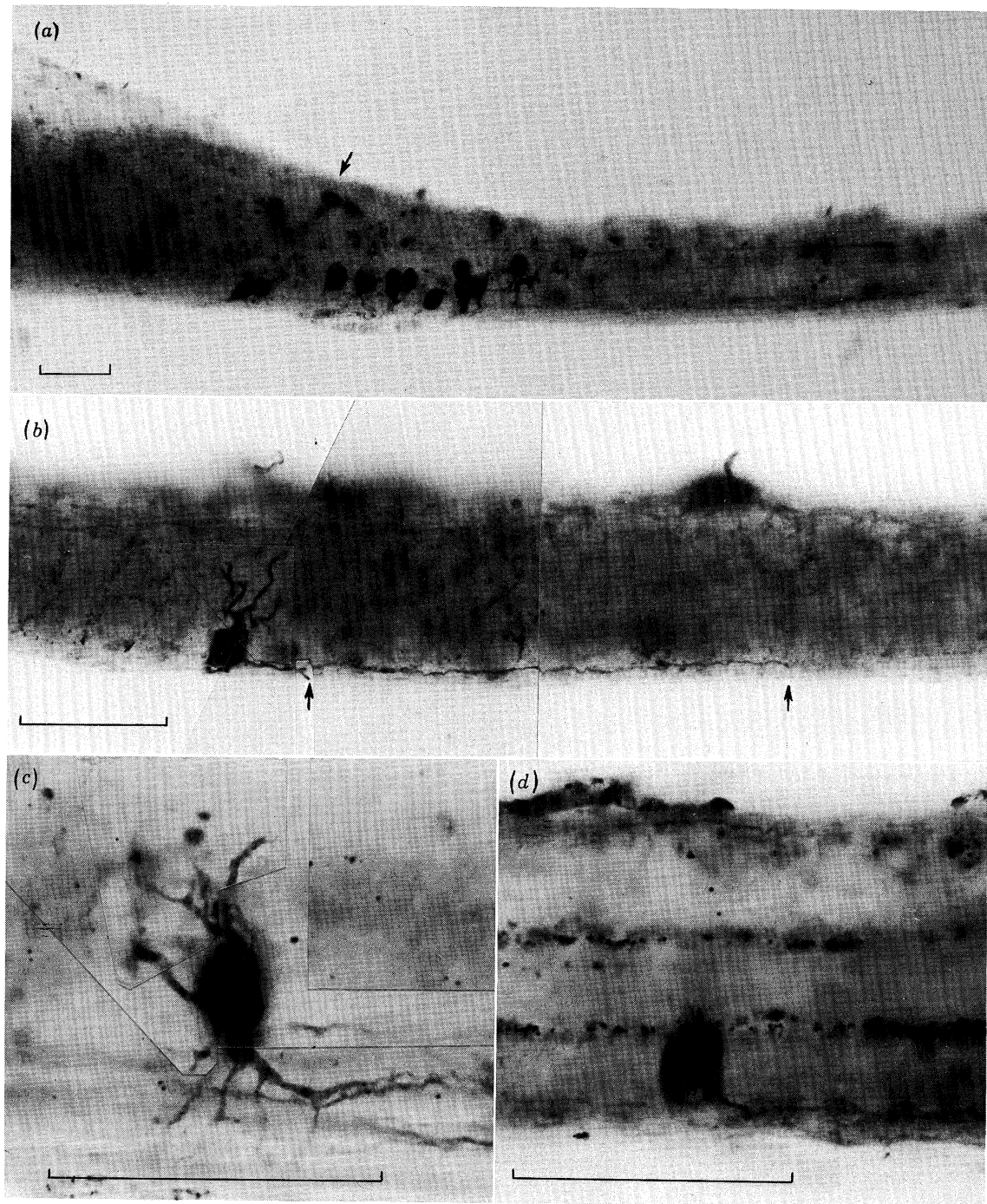
TABLE 1

cell class	number of embryos with clear examples of each cell class	number of cells drawn
Rohon-Beard cells	30	20
extramedullary cells	4	6
dorsolateral commissural interneurons	15	20
commissural interneurons	18	32
descending interneurons	9	19
ascending interneurons	8	17
dorsolateral ascending interneurons	8	12
motoneurons	32	60
ciliated ependymal cells	9	17

Rohon-Beard cells, dorsolateral commissural, commissural and descending interneurons are easily recognized in most preparations. Ascending, dorsolateral ascending and ciliated ependymal cells are filled ipsilateral to HRP applications and their recognition is thus often impaired by the overlying axon tracts which are also filled with HRP reaction product. The dissection necessary for wholemount preparations will have removed any extramedullary cells filled; however their low numbers in section material is puzzling.

(a)

(b)

50 μm

Cellular organization of the spinal cord at st. 35/36 as seen in transverse section. (a) Photomicrograph of 1 μm Araldite-embedded spinal cord stained with alkaline methylene blue. Cell types are identified in the diagram (b). Dark elliptical cell inclusions are yolk granules. (b) Scale diagram based on (a). R.B., Rohon-Beard cells (dark stipple); i., interneurons (white); m., motoneurons (light stipple); c.e., ciliated ependymal cell (white); ependymal cells are not marked but enclosed by a heavy outline and stippled; lateral tract is irregularly dotted; dorsal tract is similar but marked by asterisks; n., neurocoel. Dorsal up.

(a) Side view of caudal hindbrain and rostral spinal cord from a st. 35/36 embryo prepared as whole mount. Eleven motoneurons have been filled with HRP applied to myotomes 2 and 3. Some of these cells are illustrated in figure 9 b. The axon near the dorsal surface of the cord is the caudal axon from one of the three Rohon-Beard cells seen out of focus dorsal to the motoneurons (arrowed).

(b) Photomontage of a whole mount viewed from the left side, showing a motoneuron and a Rohon-Beard neuron both filled with HRP applied to myotomes 5 and 6. Motoneuron has two peripheral axons (arrowheads) and is illustrated in figure 8 d. St. 37/38.

(c) Photomontage of descending interneuron filled with HRP applied to the spinal cord. This side view of a whole-mounted cord from a st. 35/36 embryo is also illustrated in figure 5 a.

(d) Ventral view of whole mount spinal cord from a st. 37/38 embryo showing a ciliated ependymal cell and the initial part of its ascending axon. Cell was filled with HRP applied to the left hindbrain and is illustrated in figure 9.

Rostral to the left, dorsal up in (a), (b) and (c). Rostral to the right in (d). Scale bars are 50 μm.

The soma positions for many of the neuron types with axons in the spinal cord are shown in figure 2b. In this preparation HRP was applied to the right side of the spinal cord and a large number of cells were filled. On the right (filled) side are cells with axons ipsilateral to their somas, including many hindbrain interneurons, motoneurons and, caudal to the application site, ependymal cells with rostrally projecting axons and Rohon-Beard cells (solid black). On the left side are cells whose axons cross to the right in the ventral commissure. These cells are distributed in the hindbrain and along the cord. The largest is the Mauthner cell in the hindbrain (solid black). This preparation gives a good overall view of the neurons with axons in the trunk spinal cord though dorsolateral ascending and ascending interneurons were not filled.
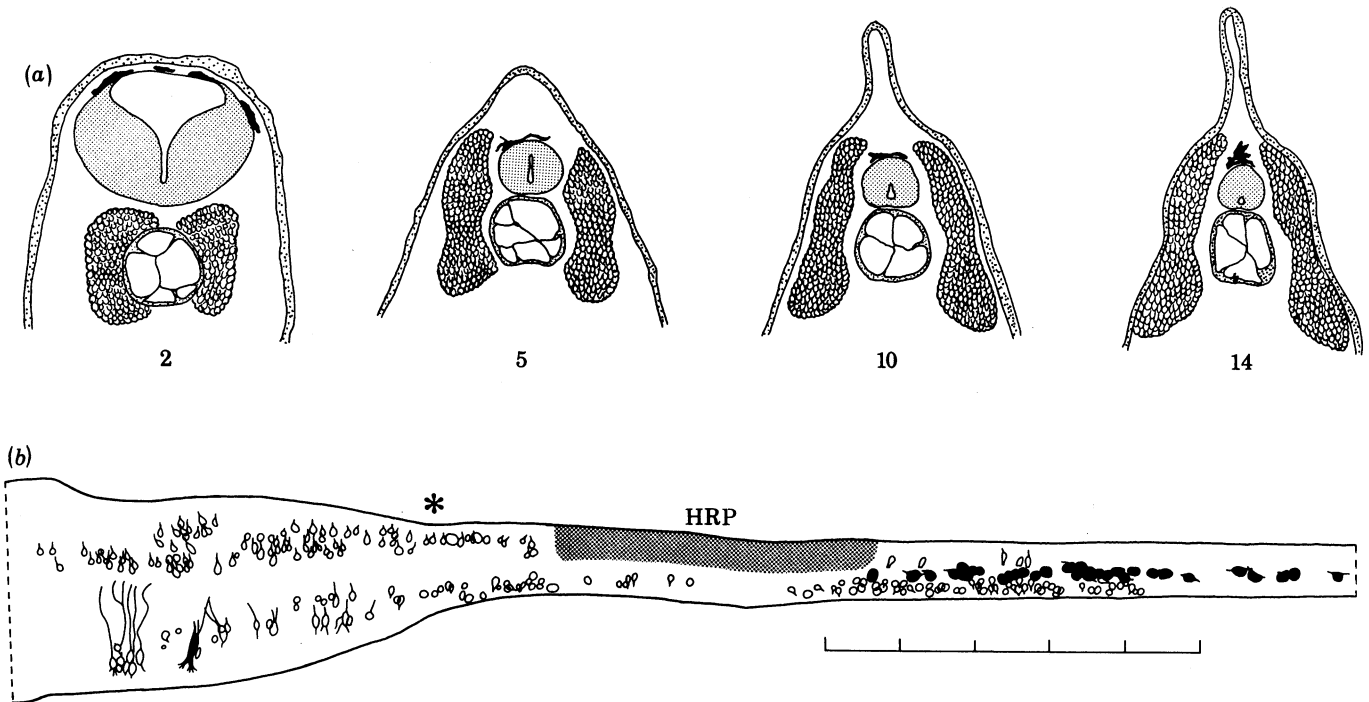

FIGURE 2.

(a) Typical transverse sections at the myotome levels indicated, showing arrangement of nervous system, notocord, myotomes and skin. The irregular black shapes dorsal to the nervous system are pigment cells.
(b) Camera lucida drawing of whole mount viewed ventrally. In this specimen HRP was applied to the right side of the cord in the stippled region and all clearly filled cell somas have been drawn. Mauthner neuron and Rohon-Beard cells are solid black, the hindbrain is to the left of the asterisk. Scale for (b) also applies to (a), 100 μm per division.

Sensory neurons

Rohon-Beard cells. These are primary mechanosensory cells with peripheral neurites innervating the skin with 'free' nerve endings (Hughes 1957; Roberts & Hayes 1977). Their somas are the largest in the cord and form a nearly continuous double row of cells along the medial dorsal surface of the cord (figure 2b). They contribute ascending and descending axons to the dorsal tract (figure 3). They have one peripheral neurite which can arise directly from the soma or from either of the central axons within a few tens of micrometres of the soma. Rohon-Beard cells are filled with HRP by application to their peripheral neurites in passage through the myotomes,

to their caudal axons in the spinal cord or to their rostral, ascending axons in the spinal cord or hindbrain. When Rohon-Beard cells are filled from the cord or the hindbrain, their peripheral neurites can be traced running between the cell layers in the skin. A number of cells filled from
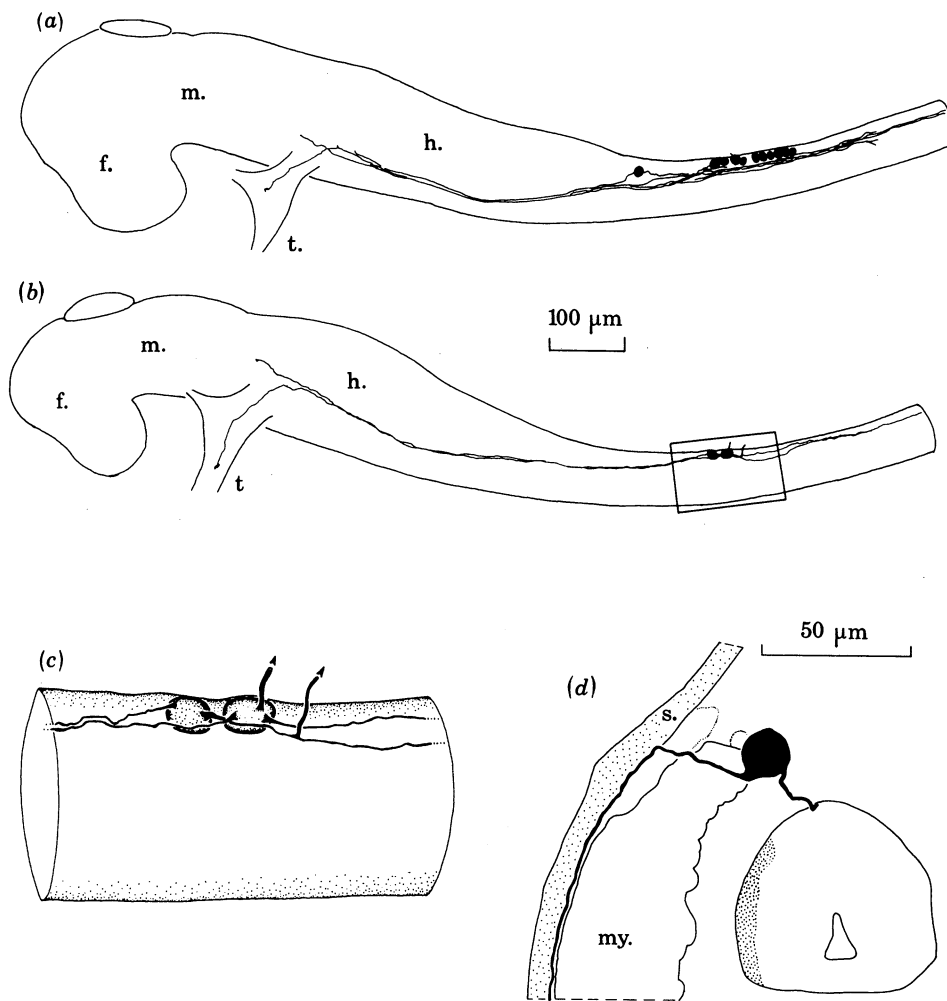

FIGURE 3. Sensory cells. (a), (b) Rohon-Beard cells filled by HRP applied to their peripheral neurites in the myotomes on the left side, seen in side view of whole mounts. Ascending and descending axons are clear in the dorsal tracts. In both preparations a single ascending axon turns into the trigeminal ganglion (t.). (a) St. 37/38. (b) St. 35/36. (c) The area enclosed in (b) at higher magnification, showing somas, central axons and peripheral neurites. Arrowheads in this and subsequent figures indicate that the axon continues to the site of HRP application. (d) Extramedullary cell filled by HRP applied to left hindbrain seen in transverse section at st. 37/38. Reconstruction from three serial sections.

In this and subsequent transverse sections the dorsal and lateral tracts are stippled only on the same side as the HRP application. Abbreviations: f., forebrain; m., midbrain; h., hindbrain; my., myotome; p., pineal; t., trigeminal ganglion; s., skin. Scale in (d) applies also to (c).

the spinal cord had ascending axons that passed through the hindbrain to the level of the trigeminal ganglion. Some of these axons turned to travel out into the trigeminal ganglion (figure 3a, b). The majority of central axons have periodic bulges but no branches. Rarely, short ventral branches from rostral axons and somas have been seen (figure 9c). Ascending axons in the hindbrain are always near the dorsal edge of the lateral tract, which becomes

located on the side of the brain. Descending axons from trigeminal ganglion cells also occupy this position.

Extramedullary cells, which lie outside and dorsal to the spinal cord, were described in *Xenopus* by Hughes (1957). We have examples filled by HRP applied to their rostral axons in the hindbrain (figure 3*d*). Such examples show clearly that these cells project to the hindbrain on the same side as the soma. The peripheral neurites pass between the myotomes with those of Rohon-Beard cells and then run down the undersurface of the skin. Other extramedullary cells have been filled by spinal cord HRP application. They often lie at the level of the intermyotomal cleft where groups of Rohon-Beard cell neurites leave the cord. Cell bodies of extramedullary cells would have been dissected away with the pigment cells in all our wholemount preparations, so we have only seen them in sections.

Interneurons

Interneurons have been stained after HRP application to spinal cord or to one side of the hindbrain. They have been divided into types on the basis of features that we hope have functional significance. These are:

(i) possible connections made by the axon, particularly whether the axon projected on the same side as the soma (ipsilateral) or on the opposite side (commissural) and whether it ran rostrally, caudally or both;

(ii) possible connections received by the neuron, as indicated by the dorsal, ventral and longitudinal extent of the dendrites in the lateral tracts;

(iii) whether the soma was unipolar or multipolar;

(iv) the location of the soma dorsoventrally, and whether it was superficial or deep to the cord surface.

Five categories of interneurons are described, based on major differences in these features. As in the adult vertebrate nervous system, there is variation in the detailed morphology of cells in any category. The kind of variation found among the interneurons in *Xenopus* embryo spinal cord was comparable to that found among the motoneurons (see below), where we feel confident that we are dealing with a single category of cell.

Dorsolateral commissural interneurons (figure 4) were filled by HRP applied to the contralateral hindbrain or rostral spinal cord, to which their axons project ventrally in the lateral tract. Their somas lie at the cord surface near the dorsal limit of the lateral tract, which they often seem to interrupt, separating the small dorsal tract of Rohon-Beard cell axons from the main part of the lateral tract. Dendrites extend dorsally and longitudinally from the soma into the dorsal tract, where they could contact Rohon-Beard cell axons, and into the dorsal half of the lateral tract. A large tapering process from the soma runs ventrally on the inside edge of the lateral tract. In transverse sections this sometimes shows short lateral processes extending into all levels of the lateral tract dorsoventrally (figure 4*d*, *e*, *g*). Ventrally the main process narrows and crosses to the other side of the spinal cord, where the axon enlarges slightly as it joins the ventral half of the opposite lateral tract to ascend to the hindbrain (figure 4*c*). The range of form for this cell type is illustrated in figure 4. The longitudinal extent of the dorsal dendrites from the soma varies considerably. Most of the axon initial segments have small radial dendrites in the lateral tract but this is not always so (figure 4). Some cells with unipolar somas are included in this class if their dendrites extend far enough dorsally to contact the dorsal tract and their somas are fairly superficial (figure 4*a*, *f*). Occasionally the contralateral axon only
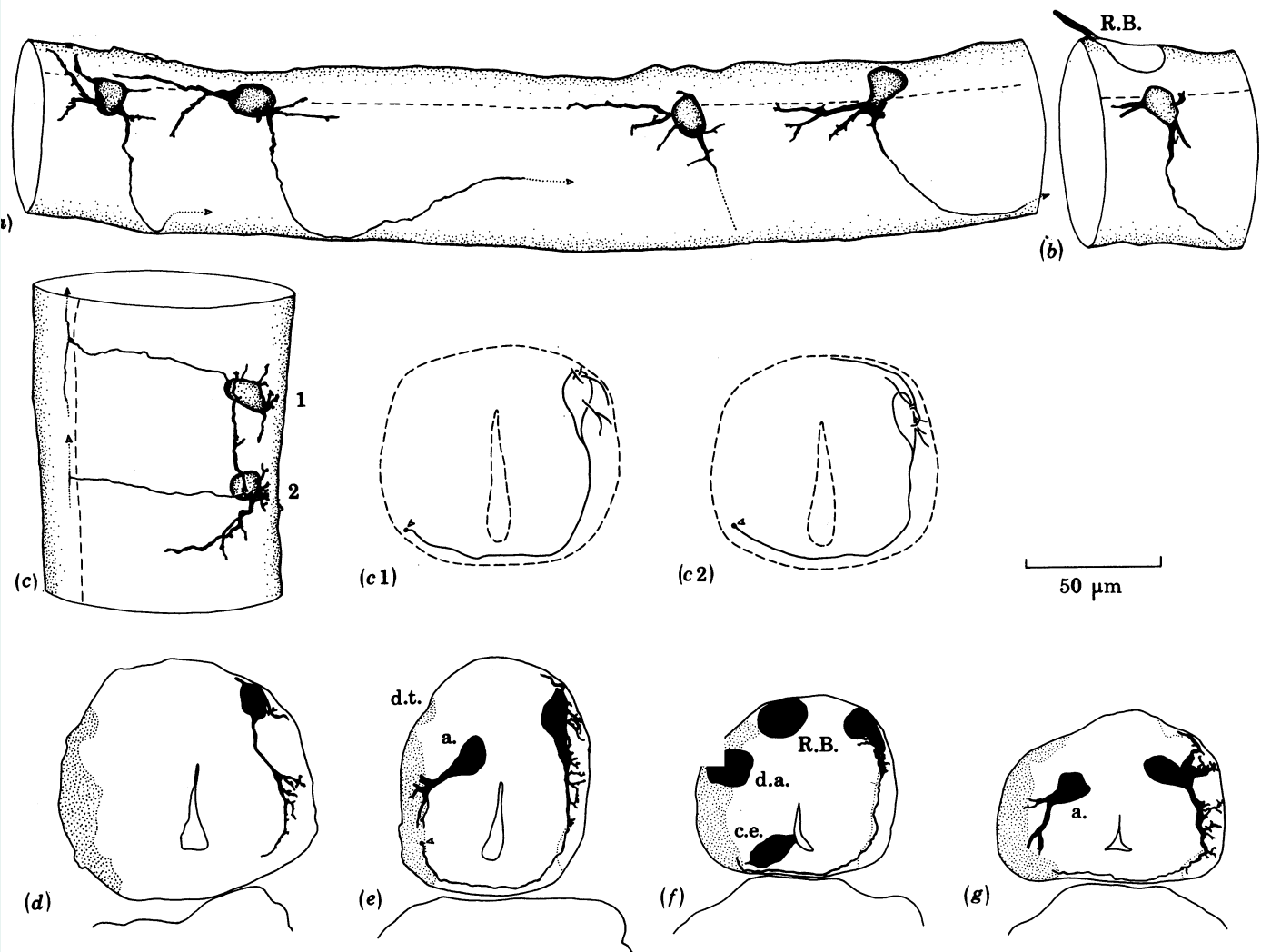
FIGURE 4. Dorsolateral commissural interneurons. (a) Lateral view of whole mount showing right-side cells at st. 37/38, filled by HRP applied to left hindbrain. Somas lie at dorsal limit of lateral tract (dashed line). Axons cross to left side ventrally and join left lateral tract (open arrowheads). Level of 10th myotome. (b) Similar preparation at st. 35/36 filled from rostral spinal cord. Cell with short dendrites at 13th myotome. R.B. is a Rohon-Beard cell with peripheral neurite. (c) Two right-side cells in a whole mount at st. 37/38 viewed ventrally to show commissural axons which T-branch in left tract and ascend to left hindbrain where HRP was applied. Dashed line is ventral limit of left lateral tract. (c1, 2) Transverse plane reconstructions of these cells. Level of 7th myotome. (d–g) The range of form in these interneurons seen in transverse sections: (d–e) at st. 37/38 were filled from left rostral spinal cord; (f–g) at st. 39 were filled from left hindbrain. Lateral and dorsal tract (d.t.) are shown stippled on the left, filled side. Other cell types are as follows: R.B., Rohon-Beard; d.a., dorsolateral ascending; a., ascending; c.e., ciliated ependymal.

travels rostrally (figure 4a), but more usually it forms a T-branch to ascend and descend. We have not filled these cells rostral to a spinal HRP application, which suggests that their caudally directed axons are short.

Commissural interneurons were filled from the hindbrain or spinal cord. They are distinguished from multipolar commissural interneurons mainly by their more limited dendritic field, which does not extend to the dorsal tract and consequently does not reach the longitudinal axons of
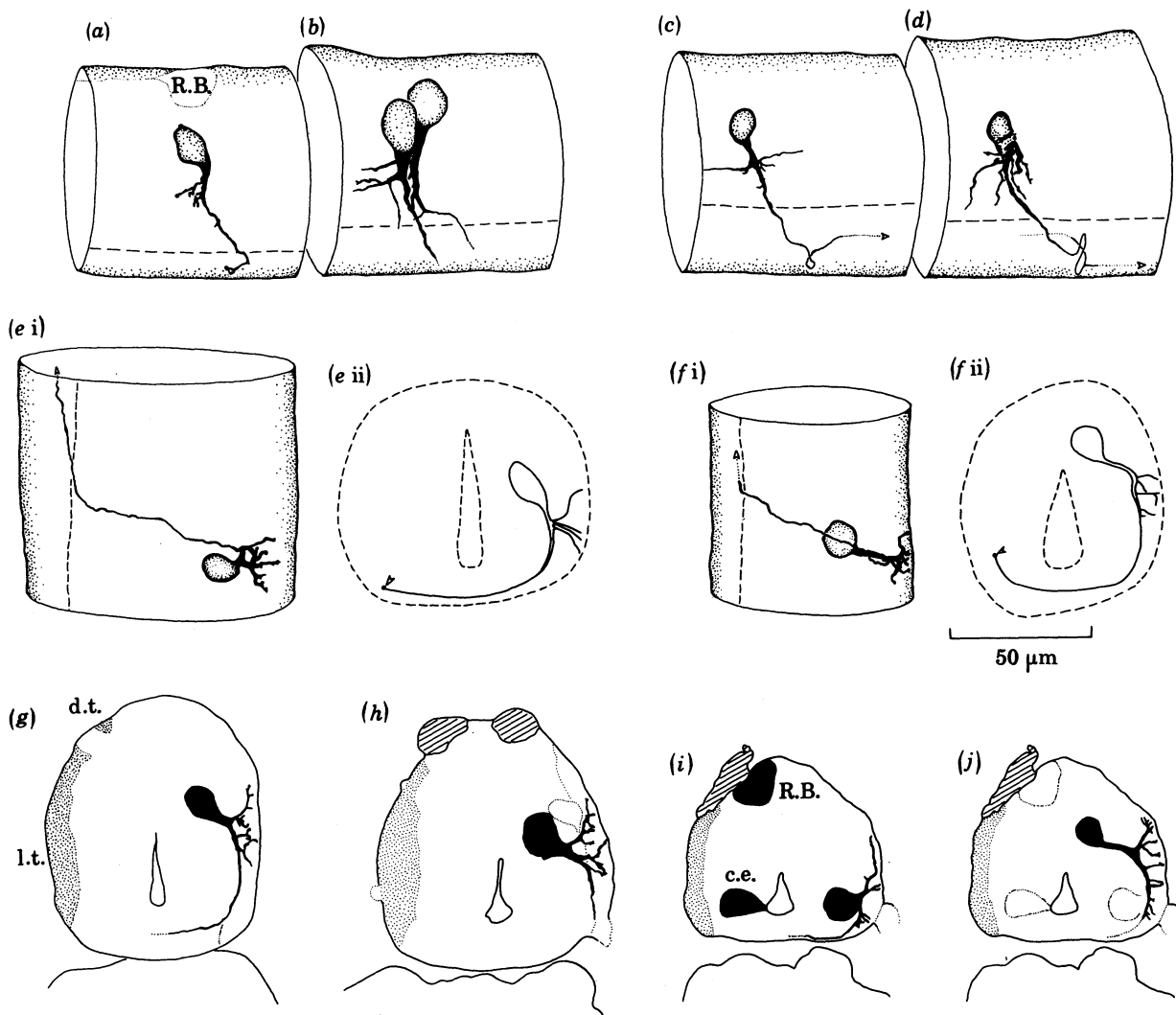
FIGURE 5. Commissural interneurons. (a, b) At st. 35/36 were filled rostral to spinal cord HRP application. They are drawn from the right side of whole mounts. The dashed line is the ventral edge of lateral tract. The axons pass ventrally to the left side. (a) At 14th myotome rostral to HRP application. R.B. is a Rohon-Beard cell. (b) At 10th myotome rostral to HRP application. (c, d) Ventrolateral view from the right side of two cells at 4th and 5th myotomes from one st. 37/38 embryo filled by HRP applied to left hindbrain. Axons cross ventrally to project (open arrowheads) to left hindbrain. (e i) Ventral view of cell, at level of 6th myotome, in whole mount at st. 37/38 filled by HRP applied to left hindbrain. Axon crosses ventrally to ascend (open arrowhead) near ventral edge of lateral tract (dashed). (e ii) Transverse plane reconstruction of this cell. (f i, ii) As for (e i, ii), but cell is at level of 8th myotome. (g–j) Cells in transverse section, filled via the left lateral (l.t.) and dorsal (d.t.) tracts following HRP application to left hindbrain. (g, h) At st. 39. (i, j) From a single section at st. 37/38. (i) Unusually ventral. (j) Unusually deep and dorsal. R.B., Rohon-Beard cell; c.e., ciliated ependymal cell. Pigment cells are hatched.

Rohon-Beard cells (figure 5). Typically the main neurite passes ventrally from the unipolar soma along the inside edge of the lateral tract. Short radial dendrites, with very limited longitudinal spread, extend from this neurite into the lateral tract. The axon crosses in the ventral commissure to reach the ventral part of the contralateral tract. Here it can turn rostrally to reach the hindbrain (figure 5c) or T-branch to ascend and descend in the cord. Some cells have
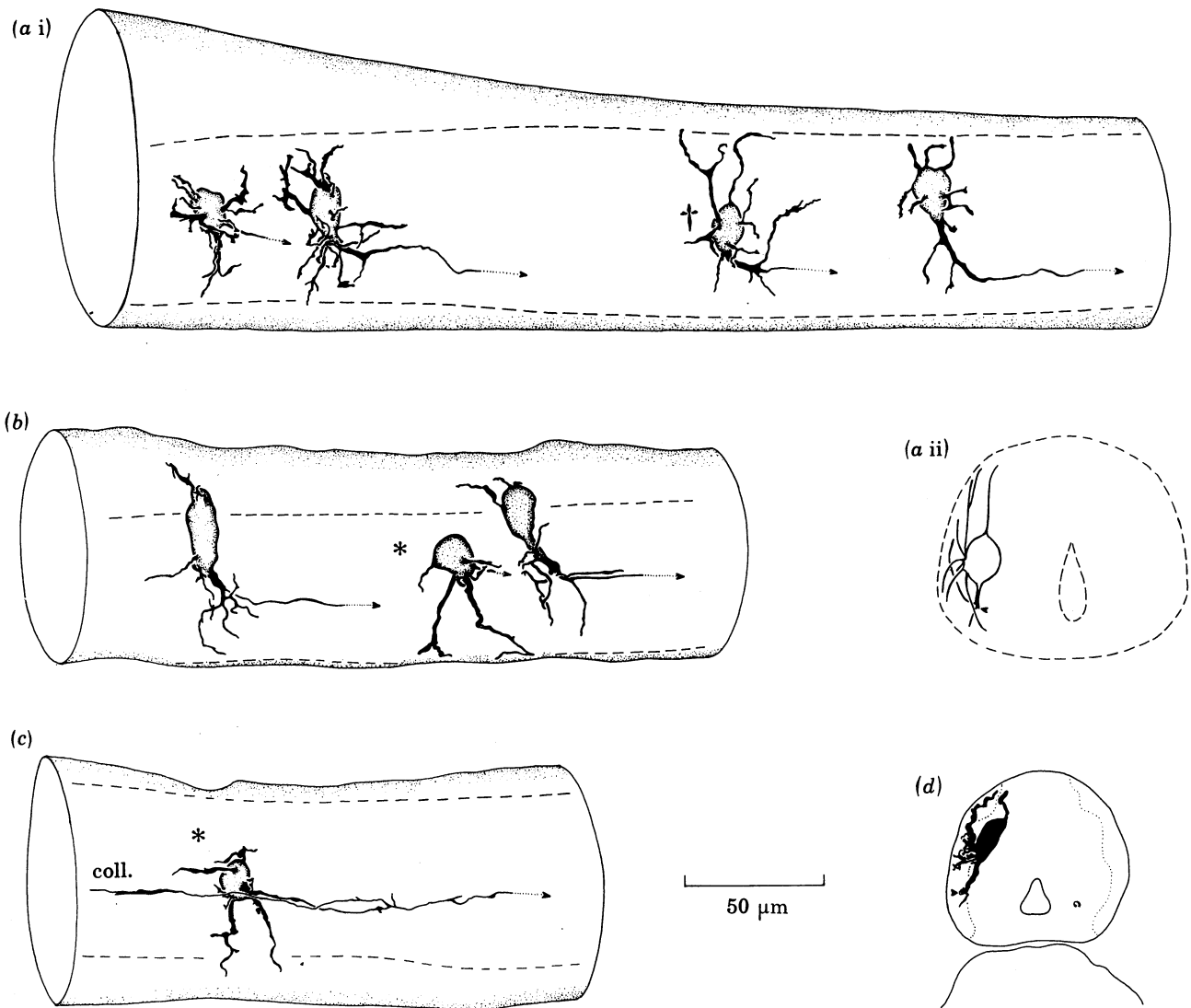
FIGURE 6. Descending interneurons, all filled with HRP applied to the spinal cord at level of myotomes 15–20. (a i, b, c) Side views of st. 35/36 spinal cords drawn from whole mounts. The upper and lower dashed lines indicate the dorsal and ventral limits of the tracts. Note that the dendrites from these cells usually spread into both dorsal and lateral tracts and the cells marked with asterisk in (b) and (c) are unusual as they have no dendrites in the dorsal tract. The axon in (c) also has a collateral that projects rostrally for a short distance (100 μm). (a) At level of myotomes 4–6; (b) at myotomes 4 and 5; (c) at myotome 7. (a ii) Reconstruction in transverse sectional plane of cell marked † in (a i). (d) Descending interneuron in the midtrunk region reconstructed from two adjacent transverse sections. From a st. 37/38 embryo. Abbreviation: coll. collateral.

been filled caudal to their soma. Patterns of dendrite branching differ according to the position of the cell soma, whose dorsoventral level and depth from the surface of the cord varies considerably (figure 5g–j). Deeper somas are usually more clearly unipolar (figure 5j). Somas are distributed more ventrally than those of dorsolateral commissural interneurons, usually in the middle third of the cord. Exceptionally they can lie ventrally, at the level usually occupied by motoneurons (figure 5i).

Descending interneurons are filled exclusively rostral to spinal cord HRP application. Their axons run caudally in the lateral tract just dorsal to the motoneuron axons. Often the axons arise from a curved tapering dendritic trunk. Plate 2*c* and figure 6*a* illustrate the typical appearance of these cells when drawn from wholemount preparations. The principal dendrites emerge from the multipolar soma and extend to all levels of the lateral and dorsal tracts. Transverse sections (for example, figure 6*d*) show the dendrites radiating out within these fibre tracts. The cell bodies are not found deep within the spinal cord but lie along the inside border of the lateral tract. They are usually situated in the middle third of the cord dorsoventrally.
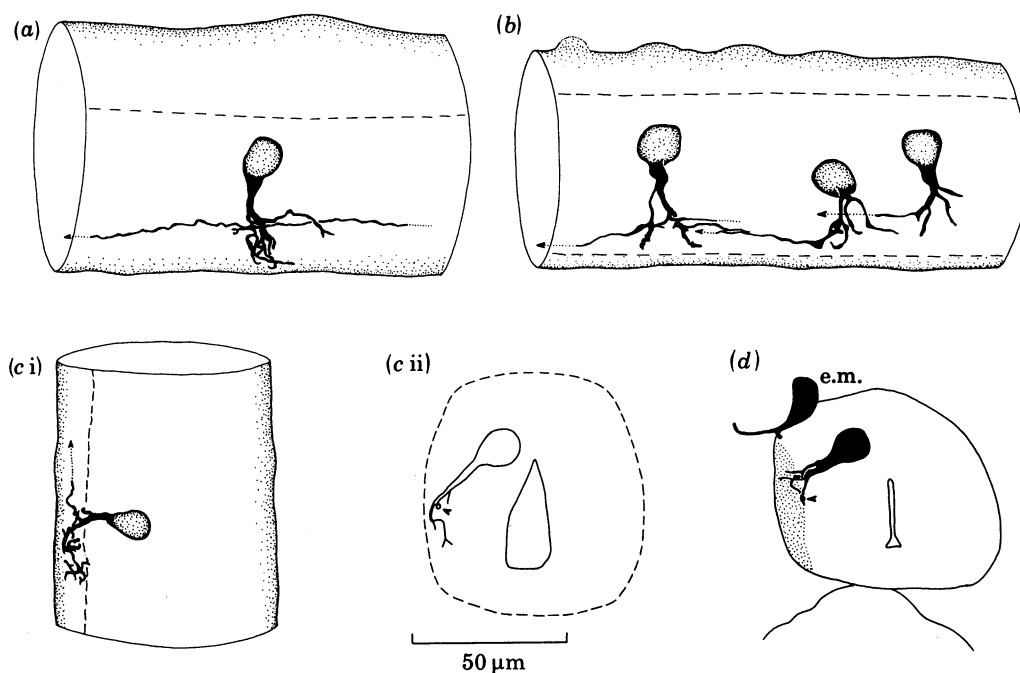

FIGURE 7. Ascending interneurons. (*a*) Slightly dorsal side view of whole mount at st. 37/38 filled from left hindbrain. Level of 4th myotome. Axon ascends and descends. (*b*) Three examples at st. 37/38 filled from left rostral spinal cord seen in whole mount side view. Level of 6th myotome. Left cell has ascending and descending axon. (*c* i) Ventral view at st. 37/38 of a 5th myotome cell filled from left hindbrain. (*c* ii) Transverse plane reconstruction of cell in (*c* i). (*d*) Cells filled from left hindbrain, 4th myotome level at st. 35/36. The interneuron is an example with a dorsal soma; e.m. is an extramedullary cell with peripheral neurite. Drawn from adjacent transverse sections.

Occasionally the dendrites of these cells do not reach into the dorsal tract (for example the cells marked with an asterisk in figure 6). Descending interneurons are also present within the tapering region of the hindbrain.

Ascending interneurons were filled by hindbrain and spinal cord HRP application. Their unipolar somas lie in the middle third of the cord dorsoventrally, and are deep relative to the lateral tract. In many cells the dendrites and axon were obscured by other filled axons. Generally the single process from the soma branches on reaching the inner margin of the lateral tract to give dendrites into the ventral half of the tract (figure 7). Dendrites extending to the dorsal limit of the lateral tract were not seen. The axon, when clear, lies in the inner part of the

lateral tract and either turns to ascend to the hindbrain or T-branches to run caudally for a short distance as well (figures 7 a, b).

Dorsolateral ascending interneurons. These multipolar cells lie close to the dorsolateral surface of the cord at the same level as somas of dorsolateral commissural interneurons. They are labelled caudal to hindbrain and rostral cord HRP applications and their ipsilateral axons project rostrally in the dorsal part of the lateral tract. Often their dendrites are obscured by the overlying tract axons and we have only a limited number of examples that show clear dendritic processes. The dendritic branching is apparently very simple and restricted to within the dorsal tract and dorsal part of the lateral tract (figures 8 a, b). The main dendritic axis of these cells is longitudinal; so transverse sections have yielded little information other than the position of the cell body (figures 8 c).
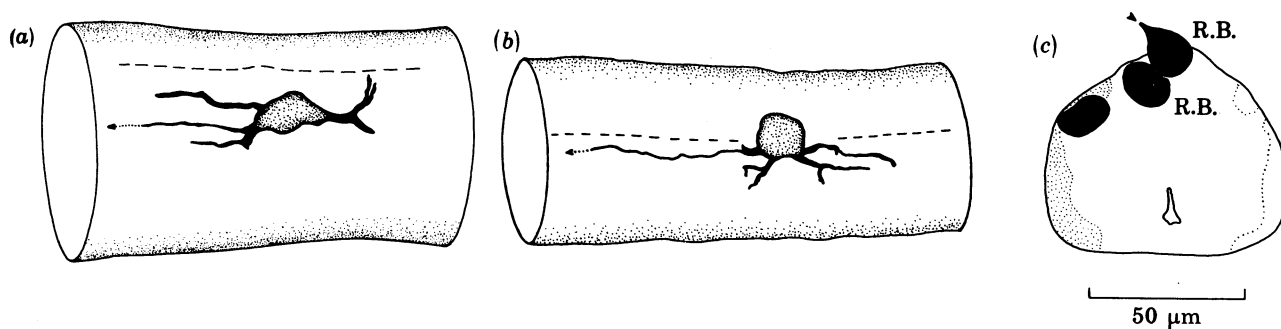

FIGURE 8. Dorsolateral ascending interneurons. (a) Left side view of st. 37/38 spinal cord at level of myotome 11. Cell filled with HRP applied to left hindbrain. (b) Slightly dorsal side view of st. 37/38 spinal cord at level of myotome 12. Both (a) and (b) show the mainly longitudinal orientation of dendrites characteristic of this interneuron class. The dashed line indicates the dorsal limit of the dorsal tract. (c) Transverse section of a st. 37/38 spinal cord from mid-trunk region, illustrating the superficial soma position of a dorsolateral ascending interneuron. Also shown are two Rohon-Beard cells (R.B.). All cells filled from the left rostral spinal cord.

FIGURE 9. Motoneurons filled with HRP applied to the myotomes. (a) Each outline in this side view of a st. 35/36 spinal cord indicates the position of a motoneuron cell body. Note the widespread distribution of soma positions within the ventral half of the cord and the dorsoventral stacking of some motoneurons. HRP was applied to myotomes 7 and 8. (b) Side view of caudal hindbrain and rostral spinal cord from a st. 35/36 embryo. These motoneurons, filled from myotomes 2 and 3, show rostral peripheral axons typical of cells in this region. Also two central motor axons are indicated that terminate within the rostral spinal cord. (c i) Side view of whole-mounted spinal cord from a st. 37/38 embryo. These cells, filled from myotomes 5 and 6, show the typical dendritic and axonal projections for motoneurons. The dashed line indicates the ventral limit of the lateral tract. Also shown is part of a central Rohon-Beard axon, which has short branches directed ventrally. (d i) As for (c i) but this motoneuron has two peripheral axons that contribute to adjacent ventral roots. (c ii, d ii) Reconstructions in transverse sectional plane of cell marked with asterisk in (c i) and cell in (d i) respectively. (e–g) Side views of spinal cords that illustrate a range of motoneuron shapes. The upper and lower dashed lines indicate the dorsal and ventral limits of the tracts. (e) This st. 37/38 motoneuron, filled from HRP applied to myotomes 11 and 12, has an unusually dorsal cell body. (f) This st. 35/36 cell, filled from HRP applied to myotomes 12 and 13, has no dendrites in the dorsal half of lateral tract. (g) Cells filled from myotomes 11 and 12. One motoneuron has two peripheral axons. At st. 39. (h) Transverse section of spinal cord illustrating a motoneuron filled with HRP applied to myotomes 5 and 6. St. 35/36.

Abbreviations: my., myotome; c.m.a., central motor axon; R.B.a., Rohon-Beard axon.
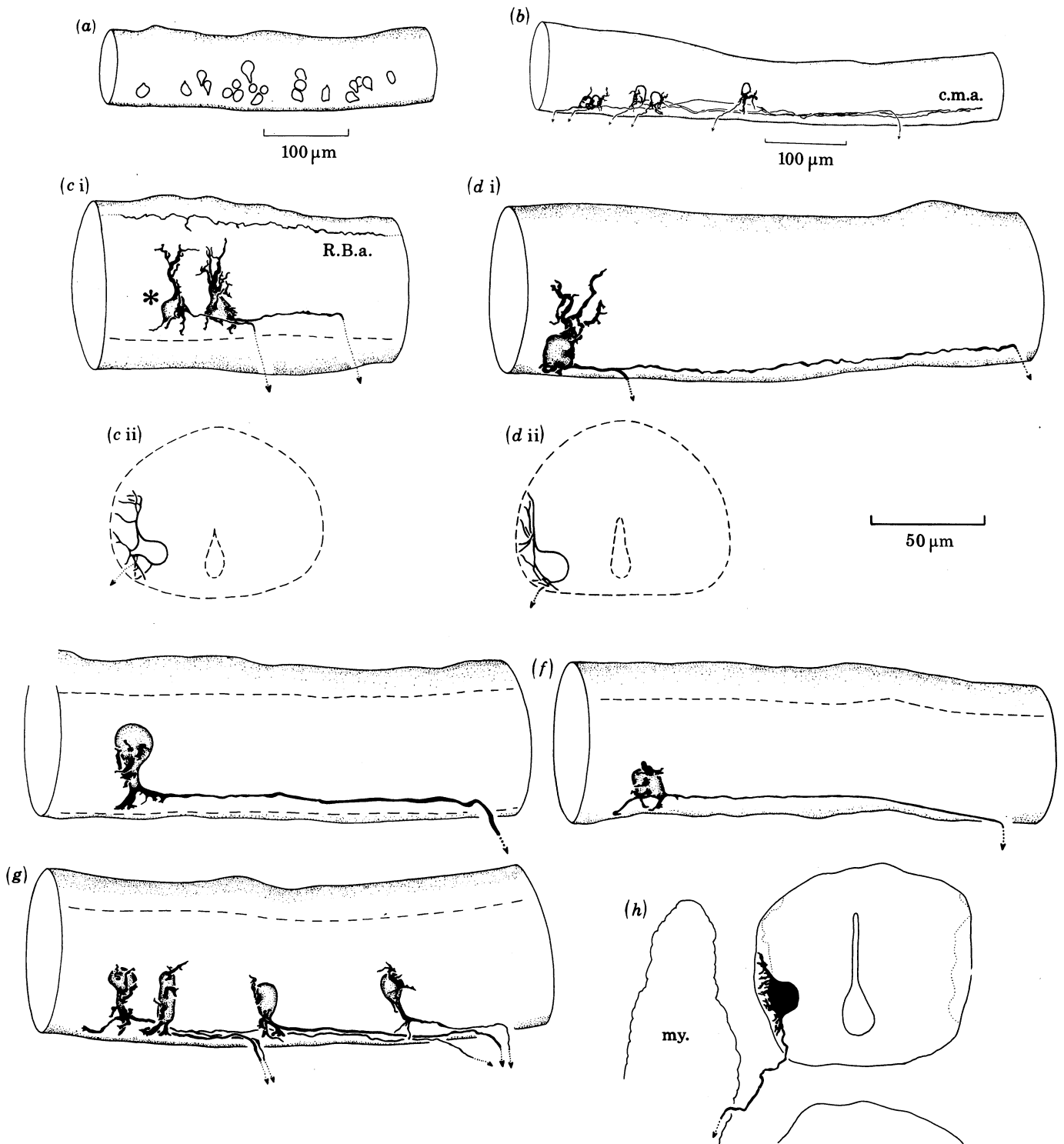
FIGURE 9. For description see opposite.

Motoneurons

Motoneurons were filled with HRP applied to the myotomes. The motoneuron cell bodies lie within the ventral quadrants of the cord and form an almost continuous longitudinal column of cells. Some dorsoventral stacking of motoneurons is present (figure 9*a*). The cell bodies lie along the inner edge of the lateral tract fibres and their dendrites spread out into this tract (figure 9*h*). The dendrites do not however reach into the dorsal tract, which is occupied by the central axons from Rohon-Beard and extramedullary cells. When seen in wholemount preparations the majority of motoneurons show the characteristic form illustrated in figure 9*c, d*
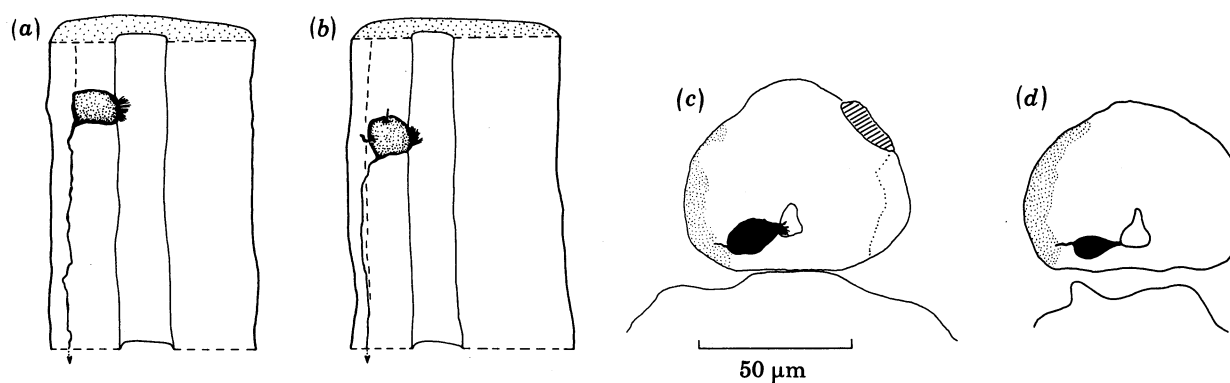

FIGURE 10. Ciliated ependymal cells in mid-trunk spinal cord of st. 37/38 embryos filled by HRP application to the left hindbrain. (*a, b*) Whole mounts viewed from dorsal side and drawn as if the dorsal part of the cord was removed. (*c, d*) Transverse sections. The cilia protruding into the neurocoel are clear in (*c*) where the hatched outline is a pigment cell.

and plate 2*b*. They have prominent dorsally directed dendrites while their lateral and ventro-lateral dendrites are less obvious when seen from the side of the cord. As described by Hughes (1959) the axons from nearly all motoneurons in the trunk region project caudally within the lateral tract before turning obliquely outwards to leave the cord. Within the tract, motoneuron axons pass close to the ventrolateral dendrites of more caudal motoneurons (figure 9*c, g*). Some motoneurons have two peripheral axons (figure 9*d*) that innervate adjacent intermyotome clefts. There is some variation in motoneuron morphology. Soma shapes and positions within the ventral quadrants of the cord may vary (figure 9*e, g*). The axonal and dendritic projections for these cells are however similar to those described previously. A few motoneurons were seen that lacked dendrites in the dorsal half of the lateral tract (figure 9*f*).

Within the trunk region the motoneuron axons leave the cord in groups at approximately mid-myotome level. There the axons are bundled together to form a motor nerve, which reaches caudally, usually to the next intermyotome cleft where it innervates the myotomes.

Post-otic myotomes 1–3 are innervated by motoneurons that lie within the rostral spinal cord and caudal hindbrain (figure 9*b*; plate 2*a*). Interestingly these cells often have long axons, which can project up to 700 μm caudally within the lateral tract and do not leave the spinal cord. These central motoneuron axons are in addition to the usual peripheral axons of those cells, which generally project slightly rostrally before leaving the cord.

Ciliated ependymal cells. Ciliated ependymal cells with ascending axons have been filled from

the hindbrain and spinal cord. Their ependymal surface is in the ventrolateral corner of the neurocoel covered with a tuft of short cilia (figure 10; plate 2d). The cell expands from the neck bearing its cilia to form a pear-shaped soma. Occasionally some short processes are given off from the soma (figure 10b). The axon emerges laterally from the soma, then turns rostrally to ascend to the hindbrain in the most ventral part of the lateral tract. Some variation in soma shape occurs, some cells being nearly spherical, while others can be quite elongated with cilia on a distinct neck.

Discussion

Anatomy

The aim of this study was to define anatomically all of the differentiated neuron classes in the spinal cord of the late embryo of *Xenopus laevis*. Previous work on lower vertebrate embryos (cited in the introduction) suggested that there could be a very small number of neuron classes in the cord, which would make these animals very promising for attempts to understand how the spinal cord works. We have therefore been looking for broad differences between cell classes, in the belief that the rules governing the development of neurons at this stage are not very precise, and will have to allow for considerable variation in the form and connections of the neurons in any one class. Our criteria have, therefore, been rather gross. For example we assumed that the most important feature of the dendritic fields of spinal interneurons is whether they extend far enough dorsally to contact sensory axons in the dorsal tract. Another simplification has been not to comment on size variation in dendrites and somas which will certainly influence their responses. Despite simplifications like these we have distinguished more neuron classes than have previous workers. These classes are shown diagrammatically in figure 11, and the defining features for interneurons are summarized in table 2. Examination of whole mounts and transverse sections (e.g. plate 1) has revealed the very simple overall arrangement and small number of cells present in the embryonic spinal cord. When compared with these, our HRP preparations have filled neuron classes that occupy all appropriate positions within the cord where differentiated neurons would be expected. Furthermore, when large numbers of cells were filled in single preparations, it was clear that neurons within one cell class could be arranged in closely packed longitudinal columns. This was especially obvious for Rohon-Beard cells, motoneurons, and the two classes of commissural interneurons. Some preparations even gave the impression that we had filled virtually all the cells of a given class for some short length of the spinal cord. While our staining technique is not so suited to the display of short-axon cells, we have looked, in many preparations, near the sites of HRP application for signs of dendritic branching patterns different from those of our classes of long-axon neurons. We have not seen signs of such differences close to application sites and, at present, have no evidence for short-axon interneurons in the spinal cord. For these reasons, we believe that we have defined, anatomically, all the differentiated neuron classes in the spinal cord of stage 37/38 *Xenopus* embryos.

The presence of growth cones on some axons and more rarely on dendrites has indicated that neurons are still differentiating. However, it is not clear at present how much variation in dendrite form or soma position can be attributed to differences in the stage of differentiation of the neurons. We are beginning to evaluate this by looking at the spinal neurons of younger embryos. It is possible that unipolar neurons are at an earlier stage of differentiation than are multipolars. For this reason we regard the soma position, dendritic extent and axonal

projections as more important features. At later stages of development in other lower vertebrates (in amphibians (Athais 1897; van Gehuchten 1898; Blight 1978), in elasmobranchs (Lenhossek 1892), in teleosts (Retzius 1893; Martin 1894; van Gehuchten 1895)) some of our neuron classes can be recognized in Golgi-stained material. There are also clear omissions in our earlier embryos. For example, the secondary motoneurons described by Blight (1978) in *Triturus* are not yet present.
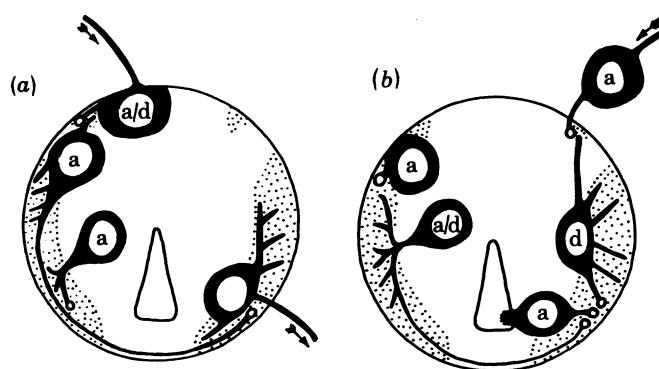

FIGURE 11. Summary diagram of neuron classes seen in transverse section. The classes are as follows (listed anticlockwise from top): (a) Rohon-Beard sensory cell, dorso-lateral commissural interneuron, ascending interneuron, motoneuron; (b) dorsolateral ascending interneuron, commissural interneuron, ciliated ependymal cell, descending interneuron, extramedullary sensory cell. Letters in cell somas indicate whether cell's axon ascends (a) or descends (d) or does both (a/d).

TABLE 2. INTERNEURONS

cell class	defining anatomical features		
	soma	dendrites	axon
commissural	unipolar, deep, middle	mainly radial in lateral tract	ventral commissural, ventral in lateral tract
dorsolateral commissural	multipolar, superficial, dorsal	radial and longitudinal in dorsal and lateral tract	ventral commissural, ventral in lateral tract
descending	multipolar, not deep, middle	mainly radial in lateral and dorsal tracts	midlateral tract, descend
ascending	unipolar, deep, middle	mainly radial in lateral tract	mid- to ventral lateral tract, ascend or T-branch
dorsolateral	multipolar, superficial, dorsal	longitudinal in dorsal tract and dorsal lateral tract	dorsal lateral tract, ascend

Development

There has been considerable revival of interest recently in the way that substrate pathways may guide neurites growing within central nervous tissue (see, for example: Egar & Singer 1972; Nordlander & Singer 1978; Silver & Sidman 1980). In *Xenopus* embryos, Katz & Lasek (1979) have shown how ganglion cell axons from eyes transplanted to the tail reach the brain by travelling in sensory tracts in the spinal cord. They could reach these tracts initially by

growing along the peripheral neurites of Rohon-Beard cells, which have contacted the skin by stage 26, well before retinal ganglion cells grow out their axons (Roberts 1978). By continuing to follow the Rohon-Beard axons in the dorsal tract, ganglion cell axons would be guided to the brain. The problem then becomes centred on how Rohon-Beard axons could be guided by substrates in forming the dorsal tract. Two observations from the present study could be relevant. First, the dorsal tract appears to be limited both dorsally and ventrally by rows of superficial neuron somas at the spinal cord surface. Dorsally these are the Rohon-Beard cells and ventral to the dorsal tract the dorsolateral commissural and dorsolateral ascending inter-neurons (plate 1). These rows of neurons could form barriers to confine the tract and separate the dorsal tract from the main lateral tract. Secondly, the apparently aberrant pathways followed by some Rohon-Beard axons, which grow out into the trigeminal ganglion, suggest that they are simply growing along other axons, following existing substrates (figure 3a, b). The early origin of the dorsal tract has been studied by means of the scanning electron micro-scope and is discussed in more detail elsewhere (Taylor & Roberts 1982).

Function

All the neuron classes that we describe are arranged in longitudinal columns within the spinal cord, which would make mutual synaptic interaction easy. This is unlikely to occur between the sensory cells (see, for example, Spitzer 1976) but could be important for interneurons and motoneurons. During swimming activity motor discharges in a ventral root can be very closely synchronized (Roberts & Kahn 1982) and electrical coupling between neighbouring moto-neurons could help to synchronize their firing. In adult toad, weak electrical coupling between motoneurons has been proposed (Grinnell 1966). It seems likely that the spinal interneurons of any given class involved in coordinating swimming will also fire synchronously in any one segment; so, again, electrical coupling might be expected. The present anatomical results have confirmed the projections of Rohon-Beard cells, peripherally to innervate the skin (Roberts & Hayes 1977). The presence of extramedullary cells is confirmed (Hughes 1957). They could be involved in detection of skin impulses (Roberts 1971); however evidence is lacking on this and on possible sensory roles for the new ciliated ependymal cell class.
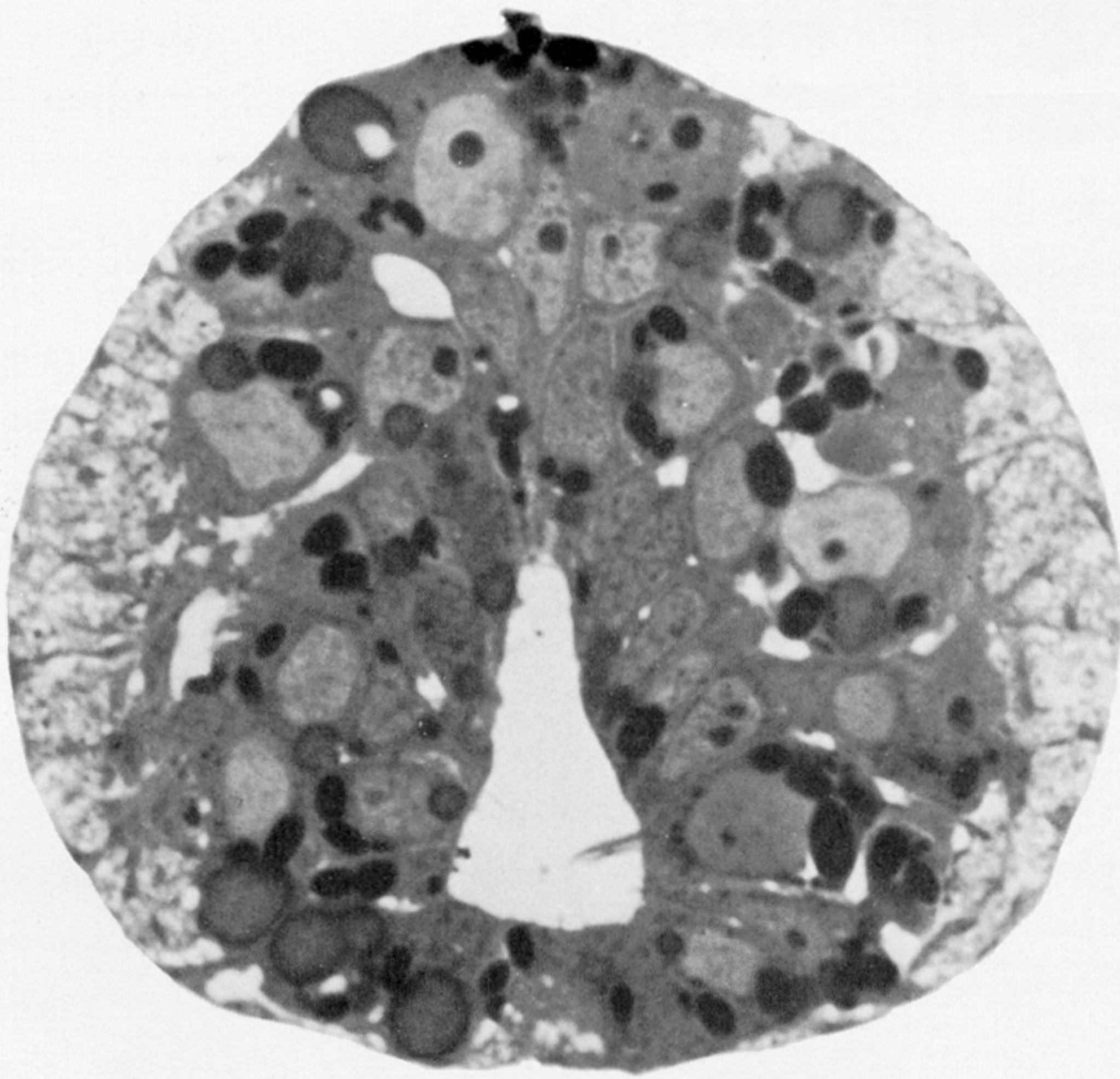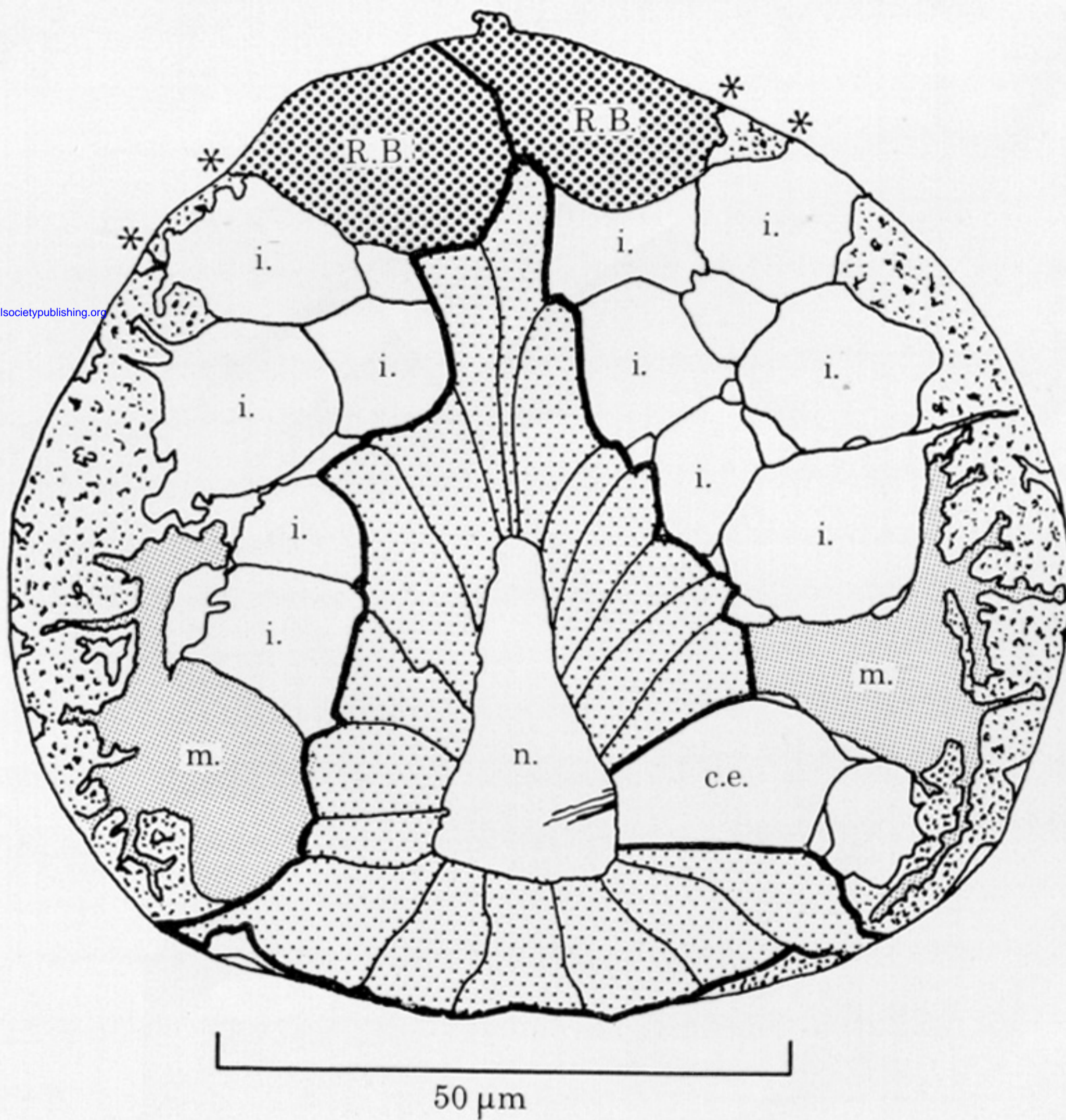
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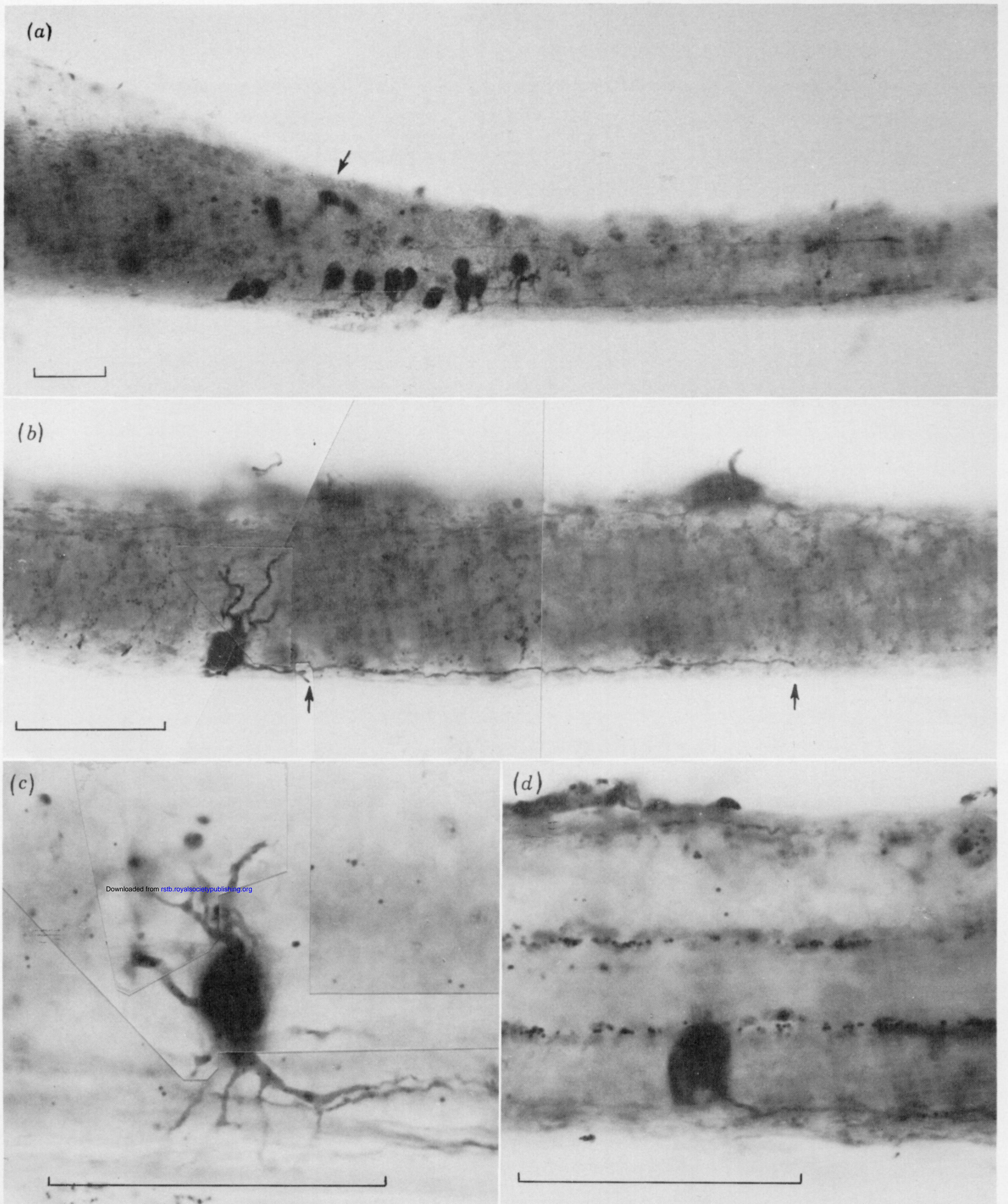
50 μm

Cellular organization of the spinal cord at st. 35/36 as seen in transverse section. (a) Photomicrograph of 1 μm Araldite-embedded spinal cord stained with alkaline methylene blue. Cell types are identified in the diagram (b). Dark elliptical cell inclusions are yolk granules. (b) Scale diagram based on (a). R.B., Rohon-Beard cells (dark stipple); i., interneurons (white); m., motoneurons (light stipple); c.e., ciliated ependymal cell (white); ependymal cells are not marked but enclosed by a heavy outline and stippled; lateral tract is irregularly dotted; dorsal tract is similar but marked by asterisks; n., neurocoel. Dorsal up.

(a) Side view of caudal hindbrain and rostral spinal cord from a st. 35/36 embryo prepared as whole mount. Eleven motoneurons have been filled with HRP applied to myotomes 2 and 3. Some of these cells are illustrated in figure 9 b. The axon near the dorsal surface of the cord is the caudal axon from one of the three Rohon-Beard cells seen out of focus dorsal to the motoneurons (arrowed).

(b) Photomontage of a whole mount viewed from the left side, showing a motoneuron and a Rohon-Beard neuron both filled with HRP applied to myotomes 5 and 6. Motoneuron has two peripheral axons (arrowheads) and is illustrated in figure 8 d. St. 37/38.

(c) Photomontage of descending interneuron filled with HRP applied to the spinal cord. This side view of a whole-mounted cord from a st. 35/36 embryo is also illustrated in figure 5 a.

(d) Ventral view of whole mount spinal cord from a st. 37/38 embryo showing a ciliated ependymal cell and the initial part of its ascending axon. Cell was filled with HRP applied to the left hindbrain and is illustrated in figure 9.

Rostral to the left, dorsal up in (a), (b) and (c). Rostral to the right in (d). Scale bars are 50 μm.