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Spinal Cord Neuron Classes in Embryos of the Smooth Newt Triturus vulgaris: A Horseradish Peroxidase and Immunocytochemical Study

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Spinal cord neuron classes in embryos of the smooth newt *Triturus vulgaris*: a horseradish peroxidase and immunocytochemical study

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SUMMARY

Spinal cord neurons were investigated in embryos of *Triturus vulgaris*, the smooth newt, just prior to hatching. These embryos can swim if freed from their egg membranes. Horseradish peroxidase (HRP) labelling, together with GABA and glycine immunocytochemistry (ICC), revealed nine distinct anatomical classes of neuron.

1. Ventrolateral motorneurons with mainly dorsal dendrites, sometimes a descending central axon and peripheral axon innervating the trunk muscles.
2. Dorsal primary sensory Rohon-Beard neurons innervating skin and with dorsal ascending and descending axons in spinal cord.
3. Commissural interneurons with mid-cord unipolar soma, glycine-like immunoreactivity, dendrites on initial segment of ventral axon which crosses cord to ascend or branch.
4. Dorsolateral commissural interneurons with multipolar soma in dorsolateral position with dorsal dendrites and ventral axon which crosses and ascends or branches.
5. Giant dorsolateral commissural interneurons with large dorsolateral somata widely spaced (130–250 μm spacing) with process projecting dorsally to other side, dorsolateral dendrites and ventral axon which crosses to ascend and branch.
6. Dorsolateral ascending interneurons in dorsolateral position with multipolar soma and ascending axon on same side.
7. Ascending interneurons with unipolar soma, GABA-like immunoreactivity and ascending axon on same side.
8. Descending interneurons with bi- or multi-polar soma, extensive dorsal and ventral dendrites, and descending axon on same side. They may also have ascending axons.
9. Kolmer-Agduhr cerebrospinal fluid contacting neurons with cilia and microvilli in lateral corners of neural canal, GABA-like immunoreactivity, no dendrites and ascending axon.

Eight of the nine cell classes were found to bear a marked resemblance to neurons previously described in zebrafish and *Xenopus* embryos in terms of their anatomy, distribution and immunoreactivity to GABA and glycine. Homologies and possible functions are discussed.

Giant dorsolateral commissural neurons, were not found in *Xenopus* or teleosts but were present in *Ambystoma mexicanum* and *Neoceratodus*. The regular, possibly segmental longitudinal distribution pattern of these cells within the cord is unusual amoung amphibian spinal neurons.

1. INTRODUCTION

To understand how the neural circuitry of the higher vertebrate spinal cord is constructed, develops and functions to produce behaviour we must be able to define its constituent neurons. This has proved to be a very difficult task as the adult spinal cord appears to contain a wider variety of neuron types than some higher brain areas like the cerebral cortex or cerebellum. Given this difficulty, it is likely that general principles of spinal cord neuron classification, organization and function will be more readily discovered in simpler vertebrate preparations, an approach first championed by Coghill (1929) in his studies of lower vertebrate embryos. After a period of relative neglect, Coghill's work on the neurons of the lower vertebrate embryo spinal cord has recently been followed up, often by those interested in questions of neural development. Studies of the interneurons present in the trunk spinal cord have been made in embryos of the Australian lungfish *Neoceratodus forsteri* (Whiting *et al.* 1992), the Japanese medaka *Oryzias latipes*

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(Kuwada 1986), the zebrafish *Brachydanio rerio* (Bernhardt *et al.* 1991), the bullfrog *Rana catesbiana* (Campbell *et al.* 1987) and the clawed toad *Xenopus laevis* (Roberts & Clarke 1982; Roberts & Alford 1986; Dale *et al.* 1986 1987*a*,*b*; Roberts *et al.* 1987 1988; Roberts & Sillar 1990). Most of these studies have used the current techniques of horseradish peroxidase (HRP) backfilling, intracellular dye injection or immunocytochemistry. An obvious omission is any study on a urodele amphibian which does not lose the tail, despite the fact that there has been work on the sensory systems and locomotor behaviour of *Triturus vulgaris* embryos (Roberts & Clarke 1983; Soffe *et al.* 1983).

This paper describes the neuronal anatomy of the embryonic trunk spinal cord of the *Triturus vulgaris* embryo at a developmental stage where swimming is possible if the animal is freed from its egg membranes. It is based on extracellular HRP applications and immunocytochemical staining for the inhibitory neurotransmitters GABA and glycine. We show that the *Triturus* embryo spinal cord contains a very limited complement of neuron types that are broadly similar to those defined in teleost and *Xenopus* embryos with one exception, the Giant dorsolateral commissural neurons. These large neurons with their unusual dorsal commissural processes are a prominent feature of the urodele embryo spinal cord but have also been described in the lungfish *Neoceratodus* (Whiting *et al.* 1992).

2. MATERIALS & METHODS

Triturus vulgaris embryos were obtained from a temporary captive breeding colony of eight pairs of smooth newts, or collected in small numbers from local ponds known to support a breeding stock of smooth newts, and where no other species of newt had been recorded in previous years. All embryos were collected prior to hatching and reared to the appropriate developmental stages in aerated pond water in the laboratory. *Ambystoma* (mexicanum) embryos were kindly supplied by Dr J. D. W. Clarke of King's College Anatomy Department, London.

(*a*) *Retrograde labelling with horseradish peroxidase (HRP)*

Embryos between developmental stages 32–48 (Gallien & Bidaud 1959) were removed from their egg membranes and anaesthetized in MS222 (Sandoz) in frog Ringer solution. The CNS was then exposed with fine mounted pins. Neurons were labelled extracellularly by crushing their axons between two pins, one of which was coated with dried aqueous HRP (Boehringer, grade 1). In each animal HRP was applied to one of three regions: the intermyotomal clefts, the hind brain or the spinal cord. In some hind brain and cord applications the HRP was applied to the left side only; in other cases the application was bilateral.

After HRP application the embryos were left to recover in frog Ringer solution diluted with distilled water. Recovery time was usually 4–8 h at room temperature. In a few cases the animals were kept at room temperature for 2 h, then at 6°C overnight, to allow the HRP to be transported further along the cord. The animals were then re-anaesthetized, killed and fixed in glutaraldehyde and processed with 3,3-diaminobenzidine (DAB) as described by Roberts & Clarke (1982). After processing, CNSs were dissected out and either mounted whole or embedded in wax (melting point 55°C) for sectioning. Whole mounts were dehydrated in Lang's alcohol series (Lang 1937), cleared and mounted in Canada balsam between glass coverslips; alternatively they were cleared in an aqueous solution of glycerol which was evaporated slowly to 100%, then mounted in fresh glycerol. The specimens could be viewed from both sides with an oil immersion lens. Material for sectioning was cut serially at 8–15 microns and mounted in Canada balsam or DPX.

Whole mount CNS preparations and serial wax sections were drawn using a camera lucida, or photographed. Results are based on observations made on a total of 76 *Triturus* embryos (including 6 prepared as serial transverse sections), and 8 *Ambystoma* embryos.

(*b*) *GABA and glycine immunocytochemistry (ICC)*

Specimens to be processed for ICC were fixed as for HRP specimens and the CNS was dissected out with fine pins. Purified polyclonal rabbit antisera (Ottersen & Storm-Mathisen 1984) were a gift from Professor J. Storm-Mathisen, Oslo University Anatomy Institute. Labelling was achieved using the three-layer peroxidase-anti-peroxidase (PAP) method of Sternberger (1979). Antibody penetration was first enhanced by brief immersion in absolute ethanol followed by distilled water. Tissue to be immunostained for glycine was further pre-treated by snap-freezing in isopentane chilled with liquid nitrogen, in a manner similar to that described by Dale *et al.* (1986). Non-specific staining was minimized by incubation for 1 h in a 3% normal goat serum in 0.1 M Tris buffer, pH 7.4, + 0.5% (by volume) Triton X-100 in 0.3 M NaCl.

Primary rabbit antiserum was diluted in 0.1 M Tris buffer with Triton X-100, NaCl and 1% normal goat serum added as above. Optimum serum dilutions were 1:300 (GABA) and 1:400 (glycine). Incubation was for 5–6 d at 4–6°C, with gentle agitation on an orbital shaker throughout. Specificity of primary serum varied from batch to batch and in some cases it was necessary to improve specificity by pre-absorption of non-specific antibody. This was achieved by adding complexes of glutaraldehyde with various amino acids to the primary serum before incubation with the tissue. Complexes were prepared according to Ottersen *et al.* (1984) and added to a concentration of 300 mM amino acid

After 5–6 d the tissue was rinsed in several changes of buffer during a 1 h period, then incubated for 1 h at room temperature in second-layer antibody (goat anti-rabbit IgG, ICN Biomedicals), diluted 1:100 in Tris buffer solution. Tissue was again rinsed in several changes of Tris buffer for 1 h, then incubated for 1 h at room temperature in PAP (ICN Biomedicals)

diluted 1:100 in Tris buffer. After several further rinses in Tris buffer, tissue was rinsed in three changes of 0.1 M phosphate buffer pH 7.4, then soaked in 3,3 diaminobenzidine (DAB)/phosphate buffer (0.25 mg ml^{-1}) for 5 min at 21°C. Freshly prepared aqueous hydrogen peroxide was then added to the DAB/buffer (125 ml of a 1:150 dilution of 30% peroxide per 10 ml DAB/buffer) for 10–13 min at 21°C. After several rinses in distilled water to remove DAB, tissue was transferred to 0.1 M phosphate buffer, pH 7.4, containing 0.04 g l^{-1} sodium azide to act as preservative.

(*c*) *Controls*

Control plates were prepared as described by Ottersen & Storm-Mathisen (1984). Amino acid was conjugated to *Xenopus* embryo tissue protein extract with glutaraldehyde. Null conjugates were tissue protein with glutaraldehyde but no amino acid. Conjugates were spotted onto nitrocellulose filter plates using a micropipette and allowed to dry overnight at room temperature. Plates were incubated and processed in the same vessels with tissue.

For negative controls, primary antibody was pre-absorbed from the serum before it was applied to the CNS tissue or control plates. Pre-absorption was achieved using amino acid glutaraldehyde complexes similar to those used to improve specificity of primary antiserum, but using 300 mM GABA to pre-absorb anti-GABA antibody, and 300 mM glycine to pre-absorb anti-glycine antibody. CNS tissue and plates were then incubated in the pre-absorbed antiserum, and processed as normal.

The immunostained CNSs were mounted whole, viewed and recorded as described for HRP whole mounts. Ten CNSs were stained for GABA and six for glycine.

3. RESULTS

The spinal cord in *Triturus vulgaris* at developmental stage 34 (Gallien & Bidaud 1959) forms a tapered tube with a rostral diameter of about 100 μm, lying dorsal to the notocord between the myotomes. The dorsal surface has scattered pigment cells.

Transverse sections show that the neural canal is surrounded by ependymal cells (figure 1*b*). The ventral floor of the canal is formed by a simple epithelium, or floor plate, through which run commissural axons. Some ventrolateral ependymal cells bear microvilli and cilia, which project into the neurocoel. These cells appear identical to the Kolmer-Agduhr cells described for *Xenopus* embryos (Dale *et al.* 1987*a*,*b*).

Dorsal and lateral to the neurocoel lie the somata of neurons in various stages of differentiation. At the dorsal surface of the cord two longitudinal rows of large neurons, one on either side of the dorsal midline, correspond to the mechanosensory Rohon-Beard neurons of the *Xenopus* embryo (Roberts & Clarke 1982; see discussion). A small, dorsal tract contains the axons of Rohon-Beard cells. It lies just lateral to the

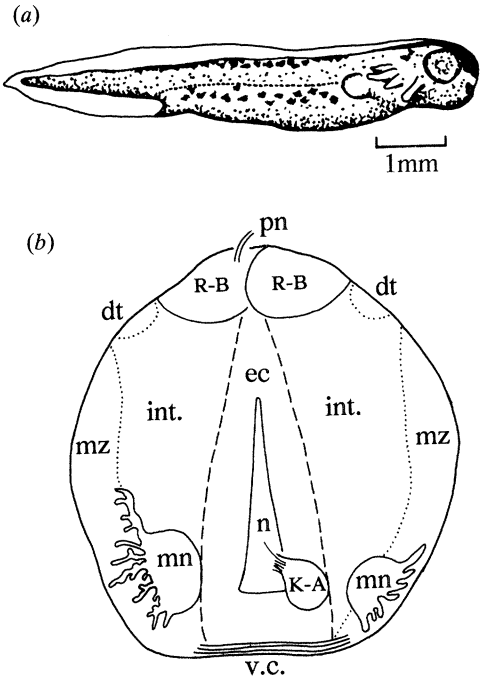

Figure 1. (*a*) Drawing of *Triturus vulgaris* embryo, stage 34, lateral view. (*b*) Schematic diagram of transverse section of stage 34 spinal cord. Abbreviations: c, caudal; d, dorsal; dt, dorsal tract; ec, ependymal cell layer; int, interneuron; K-A, Kolmer-Agduhr cell; mn, motorneuron; mz, marginal zone; n, neurocoel; P, pigment cell; pn, peripheral neurite; R-B, Rohon-Beard neuron; v, ventral; vr, ventral root; r, rostral. Broken lines mark dorsoventral midline; dotted lines mark dorsal and ventral edges of marginal zone in whole mounts, and its medial border in transverse sections; shaded or black areas mark approximate position of HRP fill sites on opposite or same side of CNS; asterisks show position of obex.

Rohon-Beard somata on either side, and its ventral edge is often defined by superficial dorsolaterally situated neuronal somata. Ventral to the dorsal tract lies a longitudinal axon tract which runs the length of the cord and will be referred to here as the marginal zone, as its outer border is just inside the edge of the cord. It contains both ascending and descending axons, including descending projections from the hindbrain to the spinal cord.

Within this general framework, it is possible to distinguish nine different cell classes, based on anatomical and positional criteria. They include motorneurons, and the putative sensory Rohon-Beard neurons and Kolmer-Agduhr cells. In addition, there are six different interneuron classes. Three of these classes have commissural axons: commissural interneurons, dorsolateral commissural interneurons, and Giant dorsolateral commissural interneurons. Three project axons on the same side of the cord: ascending interneurons, dorsolateral ascending interneurons and descending interneurons. A brief account of each of these cell classes as they are found in *Triturus* is given below. With the exception of the putative Rohon-Beard neurons and Kolmer-Agduhr cells, names of cell classes are based on position in the cord and projection of axon(s). Neuron classes are summarized diagrammatically in figure 1*b*.

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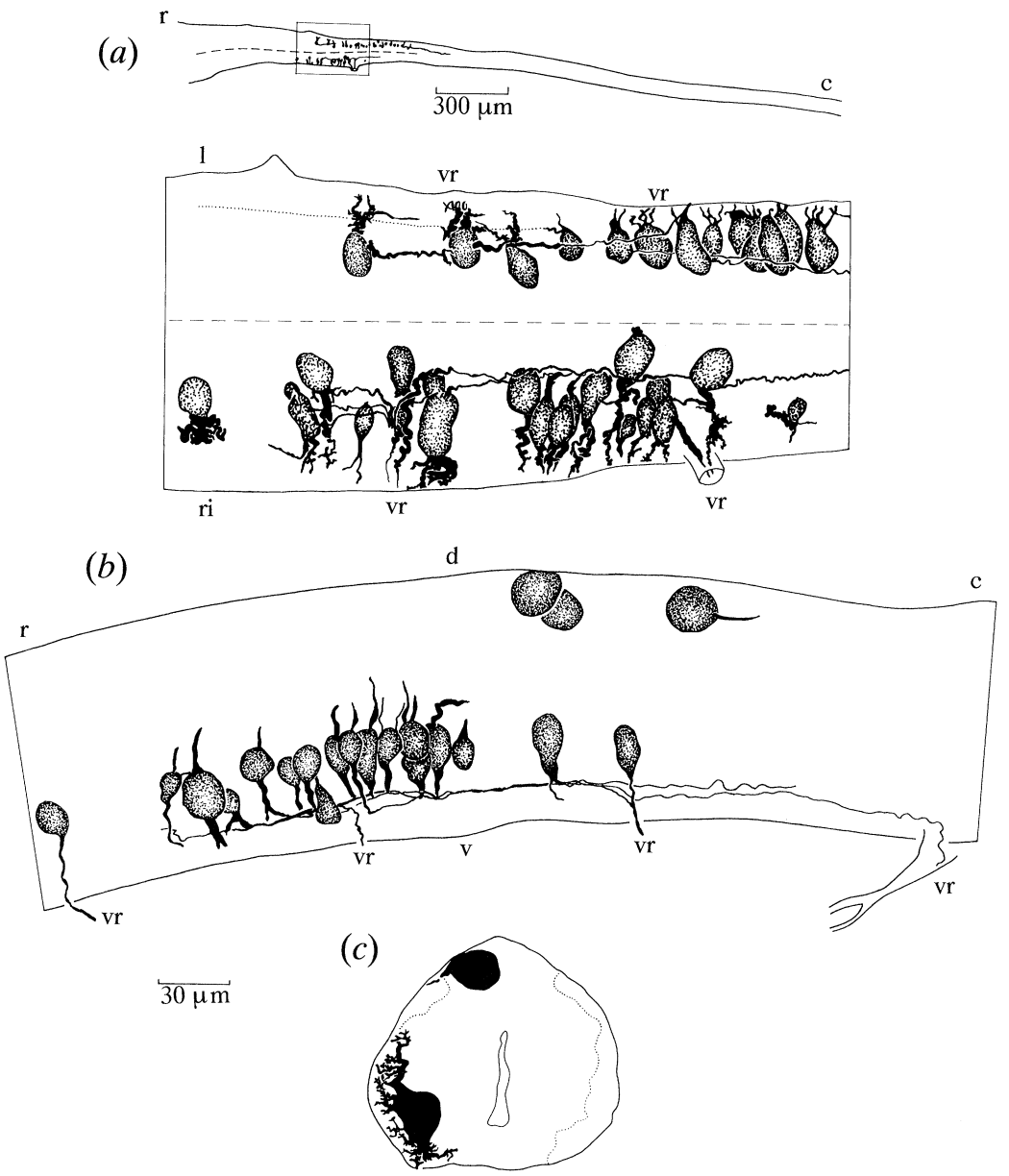

Figure 2. Motorneurons and Rohon-Beard sensory neurons. (*a*) Drawing from whole mount stage 35, ventral view. Fill site was at level of myotomes 4/5 (right side) and 7/8 (left). (*b*) Whole mount stage 34–35 spinal cord, lateral view to show distribution of motorneuron somata and longitudinal extent of axons. (*c*) Transverse section of one motorneuron and one Rohon-Beard cell in mid spinal cord at stage 34. (*d*) Motorneurons and Rohon-Beard cells drawn in lateral view; top drawing shows rostral extent of Rohon-Beard axons. One motorneuron is unusual in extending a dorsal process which contacts a Rohon-Beard axon in dorsal tract (triangle). (*e*) Lateral view, stage 34–35 spinal cord. Arrowhead marks extent of long caudal motor axon, small arrows indicate positions of rostrally directed axons. (*f*) Rohon-Beard cells in lateral view, in a stage 34–35 spinal cord, showing stumps of peripheral neurites emerging from cord. Abbreviations as in figure 1.

(*a*) *Motorneurons*

Motorneurons were filled by HRP application to the myotomes or inter-myotomal clefts, or to the spinal cord caudal to their somata. They form compact rows on either side, extending from the hindbrain to caudal spinal cord.

The cell bodies lie in the ventral third of the cord, just inside the marginal zone (figures 2*b*,*c*) and are closely packed longitudinally in the cord and caudal hindbrain. There is some dorsoventral stacking of cell bodies (figure 2*b*). The shape and size of the soma is very variable (figures 2 and 3).

Motor neuron dendrites project dorsally and ventrolaterally within the ventral 70% of the marginal zone (figure 2). Very rarely, dorsally projecting dendrites overlapped axons of 'Rohon-Beard' neurons (figure 2*d*, triangle). The number of dendritic processes and the size of the dendritic field vary considerably, and the extent of lateral dendrites, clear in transverse sections (figure 2*c*), is underestimated in lateral views (e.g. figure 2*d*,*e*).

The axon runs caudally in the ventral marginal zone before turning obliquely to exit in a ventral root. The extent of the central axon varies with the distance

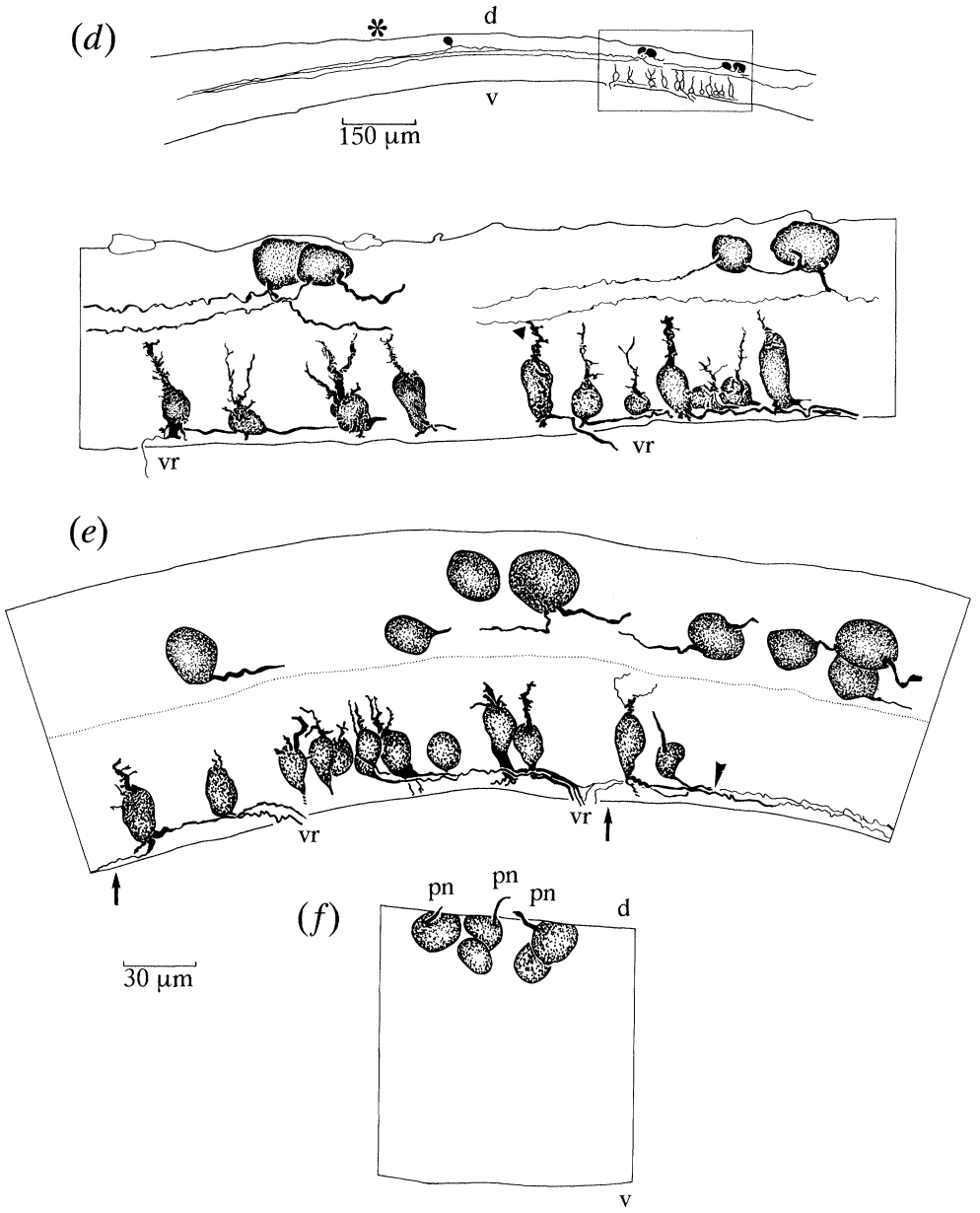

Figure 2. (*Continued*).

of the soma from the ventral root (figure 2*b*). The longest axon measured was 260 μm and ended in a growth cone without leaving the cord. In a few cases, a second, ascending axon has been identified (figure 2*d*,*e*, small arrows).

(*b*) *Rohon-Beard neurons*

These cells can be filled with HRP : via their peripheral neurites, by application to the myotomes; via their ascending axons, by application to the hindbrain or spinal cord rostral to the soma; or via their descending axons, by application to the spinal cord caudal to the soma.

The somata are typically large (20–30 μm in diameter), and form an almost continuous double row of cells running along the dorsal surface of the cord (figures 2*b*,*c* and 6*b*). A single peripheral neurite usually emerges from the soma (figure 2*f*) which also contributes both an ascending and a descending axon to the dorsal tract (figure 2*d*). Axons usually possess small varicosities at intervals along their length, but no branches. Ascending axons can sometimes be traced into the hindbrain (figure 2*d*) where they lie near the dorsal edge of the marginal zone.

(*c*) *Commissural interneurons*

Commissural interneurons can be filled with HRP applied to the contralateral hindbrain or cord, both rostral and caudal to the soma (figures 4 and 5). They are distributed throughout the cord with greatest density in the rostral half. In common with the other two classes of interneuron with commissural axons (see dorsolateral commissural interneurons and Giant dorsolateral commissural interneurons, below), they possess a ventral commissural axon which emerges from the cell body as a large tapered process, passing ventrally along the medial edge of the marginal zone, before crossing to the opposite side and ascending

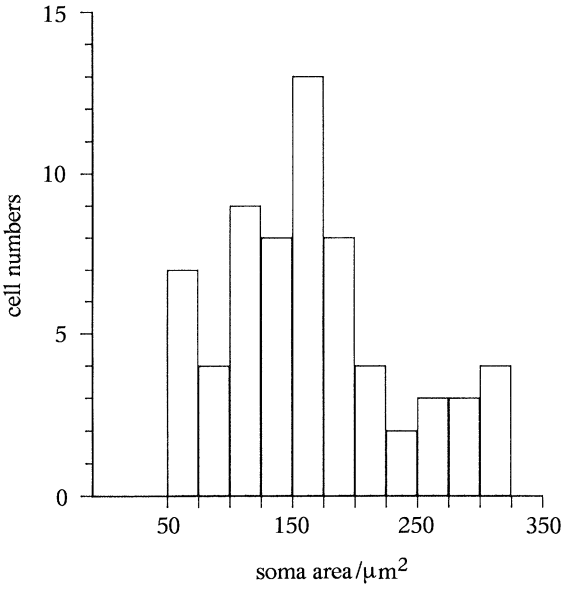

Figure 3. Histogram of motorneuron somata sizes at stage 34–35, represented by soma cross-sectional area in laterally and ventrally viewed fixed spinal cord preparations drawn in figure 2. Approximate measurements were made in micrometres by using a light microscope stage micrometer, and soma area was calculated as an ellipse where area $= \pi \times$ maximum length $\times$ maximum breadth $\times 0.25$.

towards the hindbrain in the contralateral marginal zone.

Anatomical characteristics which define this class and separate it from the other two classes with commissural axons, and in particular, from the dorsolateral commissural cells, are as follows.

1. The soma is usually unipolar (figure 4*b*), or can have a few dendrites which do not contact the dorsal tract axons (contrast figure 4*c* with dorsolateral commissural interneurons in figure 7).
2. Soma position is variable, but is typically in the mid third of the cord dorsoventrally (figures 4*a–c* and 5*b*), medial to the marginal zone and deeper in the cord than is normal for dorsolateral commissural interneurons (figure 7*e*).
3. The ventrally projecting part of the commissural axon characteristically bears radial dendrites extending a short distance into the marginal zone (figure 4*b–e*).
4. The main axon crosses to the opposite side of the cord where it may either ascend or T-branch (figure 4*d*, arrow) to ascend and descend the cord in the contralateral marginal zone.

Glycine-LI in commissural interneurons

ICC revealed a group of cells with glycine-like immunoreactivity (GLY-LI) in the spinal cord of *Triturus*. Cells were densely distributed (approximately seven per 100 μm cord length) in the rostral half of the cord (figure 6*a*) and occurred less frequently more caudally. GLY-LI was restricted to the soma and proximal axon where it emerged from the soma. In all cases the soma was unipolar, situated in the mid or ventral thirds of the cord, inside the marginal zone (figure 6*b–d*). The single, ventrally directed axon could not usually be traced more than a few tens of micrometres from the soma. In a few cases, this axon could be traced almost to the ventral surface of the cord, and in these instances the axon could be seen to bear short dendritic branches or spines protruding into the marginal zone (figure 6*c*). Rarely, stained ventral commissural axons were seen, but could not be traced to a specific soma because of dense staining of longitudinal axons in the marginal zone. As far as these cells can be visualised with glycine ICC, they show a marked similarity to the commissural interneurons described from HRP preparations (compare figure 6*b*,*c*,*d* with figures 4, 5 and 6*e*).

(*d*) *Dorsolateral commissural interneurons*

These neurons can be filled with HRP by application to the contralateral hindbrain or spinal cord rostral to the soma (figures 5*a* and 7). They occur along most of the cord, being distributed most densely in the rostral half. They are characterized by the following features.

1. The soma lies in a superficial dorsolateral position often separating the dorsal tract from the marginal zone (figure 7*e*).
2. Dendrites project dorsally and longitudinally from the soma into the dorsal marginal zone and the dorsal tract (figures 5*a*, arrow and 7*a–e*).
3. The ventrally projecting part of the commissural axon may bear dendrites emerging close to the soma and projecting into the dorsal marginal zone (figures 5*a* and 7*b*,*d*), but in most cases they are short and sparse.
4. The main axon crosses to the opposite side where it ascends in the contralateral marginal zone (figure 7*b–d*). T-branches have not been seen but some may occur as these neurons have occasionally been filled rostral to HRP applications (e.g. figure 5*a*).

(*e*) *Giant dorsolateral commissural interneurons*

These cells are filled by application to the hindbrain and rostral half of the cord. They merit special attention because no analogous cell type has been demonstrated in *Xenopus* embryos and Giant dorsolateral commissural cells have an unusual, possibly segmental distribution pattern (figures 8*a*,*b* and 9).

(i) *Anatomy of Giant dorsolateral commissural interneurons in Triturus*

The typical features of Giant dorsolateral commissural cells at stage 34 are illustrated in figures 8 and 9. The large ovoid soma (up to 40 μm × 12 μm) occupies a dorsolateral position just ventral to the Rohon-Beard cell bodies (figure 8*g*). The soma is oriented horizontally, extending from close to the dorsolateral surface of the cord towards the dorsal midline, and in the case of the largest ones, over the midline.

The portion of the soma nearest to the midline bears a long process which projects horizontally to the opposite side of the cord, under the Rohon-Beard cell

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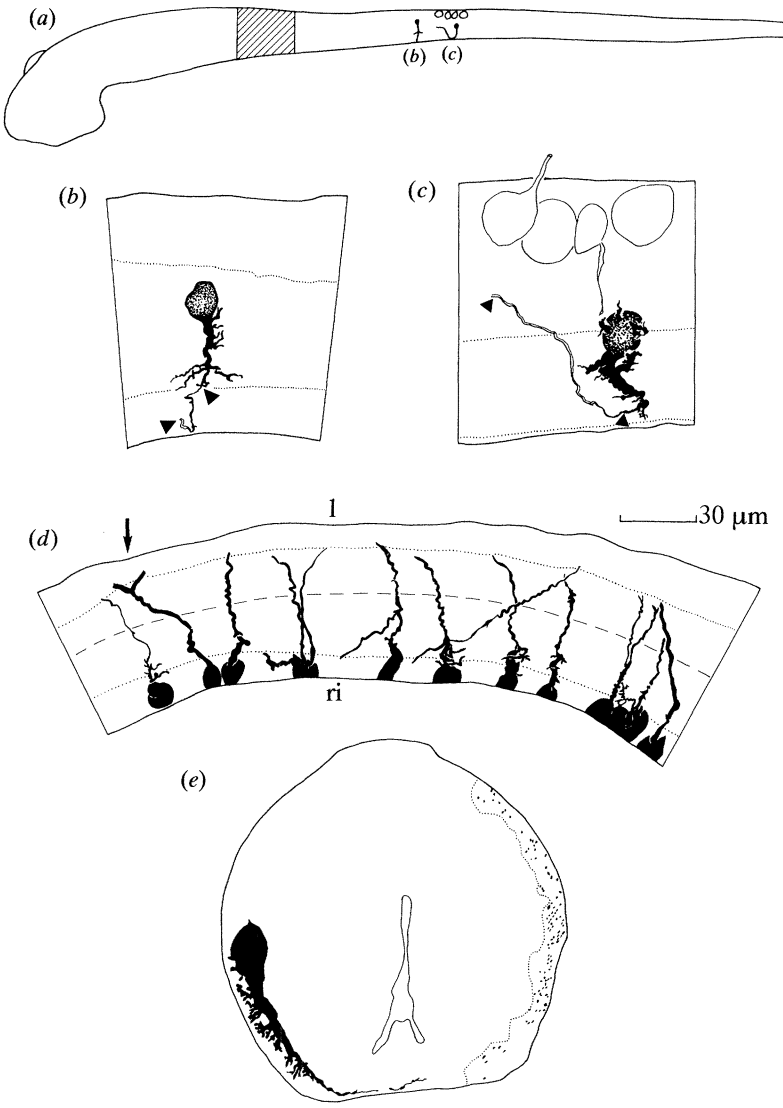

Figure 4. Commissural interneurons. (*a*) Low-power plan of spinal cord, lateral view, shows approximate positions of fill sites and cells drawn from two stage 34 spinal cords in (*b*) and (*c*). Fill site in (*b*) was right side, in (*c*) both sides. In (*b*) and (*c*) dotted lines show dorsoventral extent of marginal zone, and arrowheads show ventral commissural part of axon. (*c*) Commissural interneuron (shaded) and Rohon-Beard neurons (outlined). One Rohon-Beard neuron is unusual in extending a ventral process which could contact the commissural cell soma. (*d*) Drawing of stage 34–35 spinal cord, ventral view, fill site on left side showing ventral axons of filled commissural interneurons crossing from right (ri) to left (l). Arrow marks T-branch in commissural axon after crossing the cord. Dotted lines mark ventral borders of marginal zone on both sides. (*e*) Drawing made from three serial transverse sections of mid-cord. Fill site was rostral to sections on right side. Commissural interneuron on contralateral side and ipsilateral filled axons are shown.

bodies, and reaches the contralateral dorsal tract. Occasional examples have been found with two such dorsal commissural processes (figure 8*e*, stars). These processes typically have a few very short branches which could contact the ventral surfaces of Rohon-Beard cells. In older preparations the dorsal process branches on reaching the contralateral dorsal tract (see figure 11*b*).

The lateral end of the soma bears an axon which is of an unusually wide diameter, about 2 μm. A number of dendrites normally emerge from this end of the soma or from the axon close to the soma and project into the dorsal tract and marginal zone. The axon passes ventrally on the medial edge of the marginal zone and then crosses obliquely to the opposite side of the cord (figure 8*c–e*, commissural and contralateral portions of ventral axons are between arrowheads, and figure 9). The axon tapers slightly as it crosses the cord, but still remains unusually wide in diameter. The commissural portion is unbranched. On reaching the opposite side, the axon either ascends in the ventral part of the contralateral marginal zone, or more usually, T-branches a short distance before joining the contralateral tract, to ascend and descend

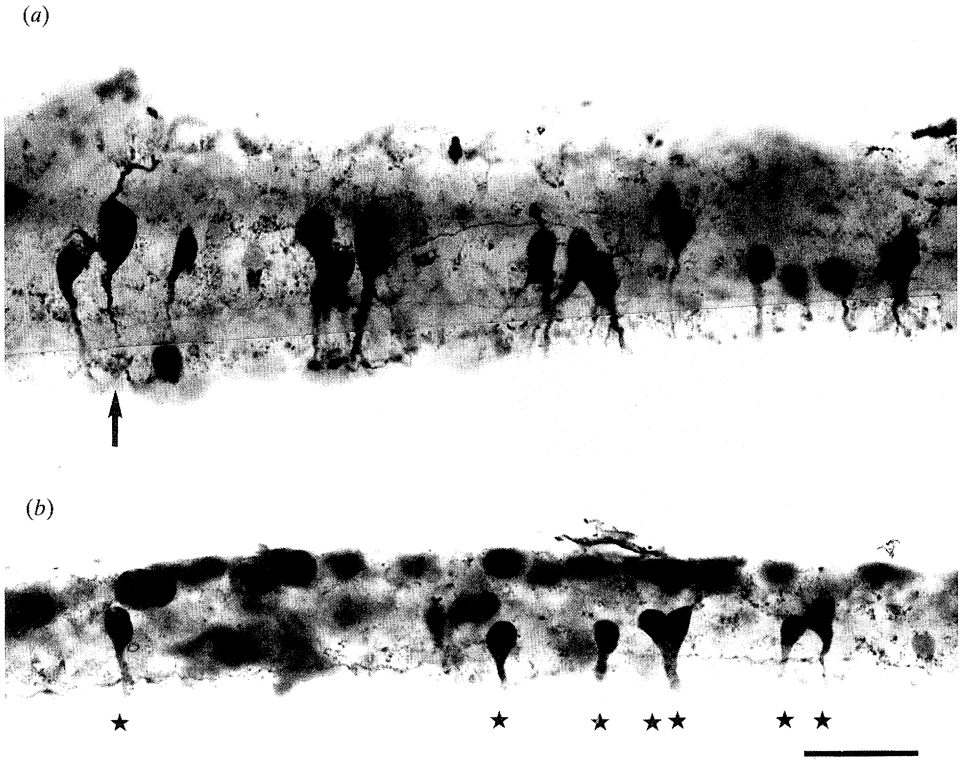

Figure 5. Commissural and dorsolateral commissural interneurons photographed in lateral view. (*a*) Commissural interneurons rostral and contralateral to HRP application at stage 35 with unipolar somata and ventral initial axon segment. At the arrow, a single dorsolateral commissural interneuron with more dorsal multipolar soma. (*b*) Commissural interneurons (at stars) caudal and contralateral to HRP application. Rohon-Beard somata can be seen dorsally out of focus on opposite side. Dorsal up, rostral to left. Scale bar, 50 μm.

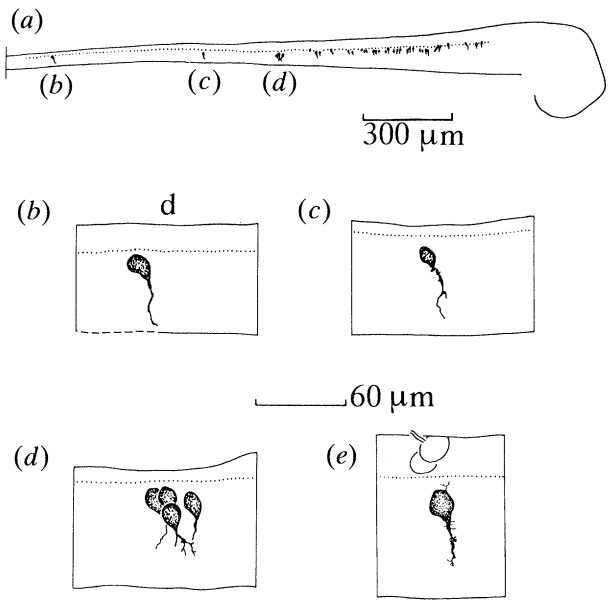

Figure 6. Glycine-immunoreactive cells. (*a*) Drawing of stage 35 spinal cord, oblique ventral view of right side showing distribution of GLY cells in rostral cord. Dotted lines mark dorsal limit of stained longitudinal axons. (*b–d*) Cells whose positions are indicated in (*a*). (*e*) HRP-filled commissural interneuron from stage 34–35 spinal cord, for comparison, mounted laterally. Cell is caudal to fill site, on opposite side.

in the ventral marginal zone. Where such T-branching occurs, the descending axon is small and unbranched and becomes obscured on merging with the marginal zone. The length of the ascending axons were up to 3 mm as cells were filled at a maximum distance of about 3 mm caudal to the fill site.

(ii) *Distribution of Giant dorsolateral commissural cells in Triturus*

Giant dorsolateral commissural cells are unusual because compared with other neurons their distribution is relatively regular and sparse (figures 8*a*,*b* and 9*a*). They did not occur in the caudal third of the cord, irrespective of the position of the fill site or the time allowed for HRP to be transported before fixation. At stage 34 the cells are relatively evenly spaced at approximately 130–230 μm intervals along the rostral cord (figure 8*a*), sometimes becoming closer together more caudally (figure 8*b*). This pattern could suggest a relation between the distribution of the giant neurons and the myotomes. However, measurements in fixed preparations with the myotomes left on one side do not support this idea.

The relatively evenly spaced pattern of cells is partly obscured in stages older than stage 34 where cell numbers increase and new cells infill between the existing ones (figures 10 and 11). The longitudinal

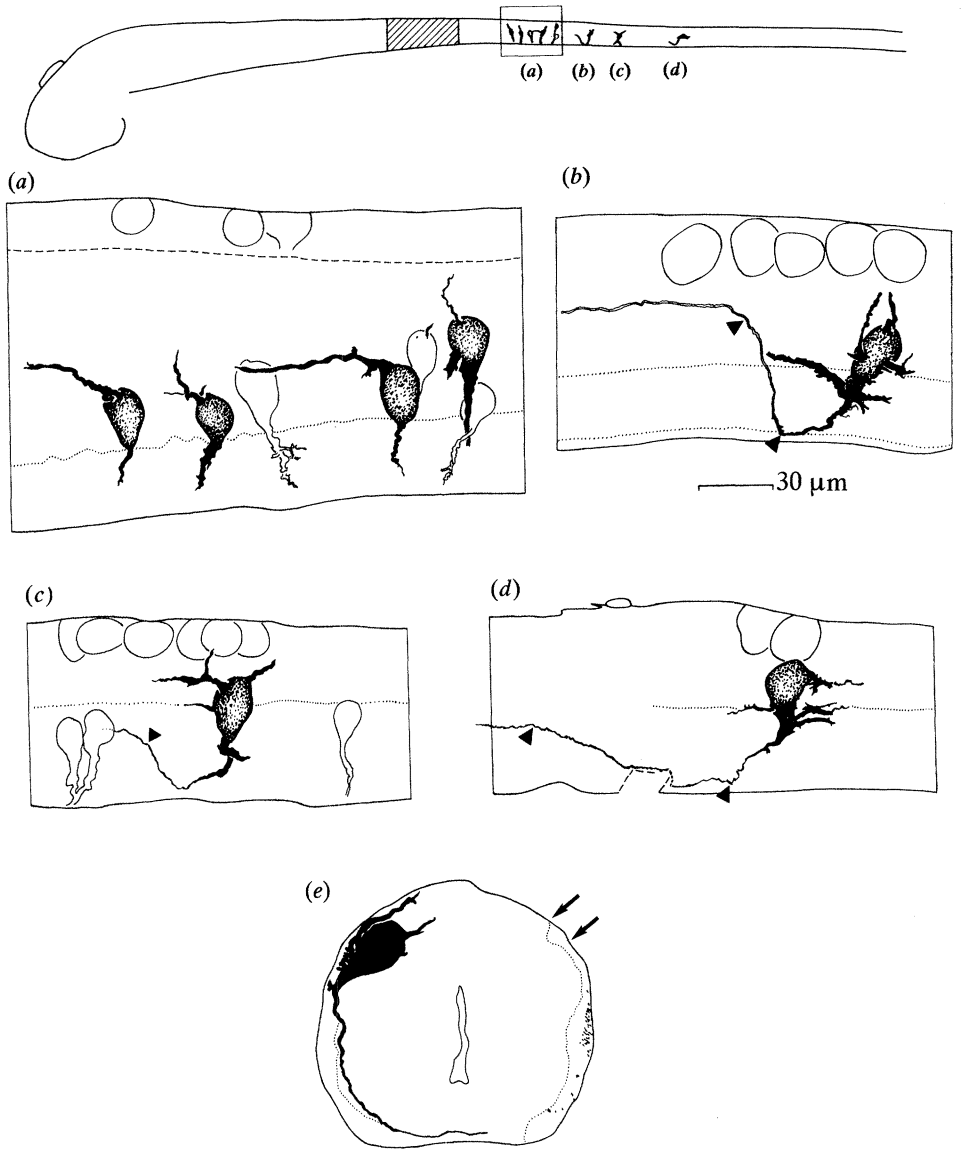

Figure 7. Dorsolateral commissural interneurons (shaded). Commissural interneurons and Rohon-Beard somata (outlined). Approximate positions of all cells drawn are shown on plan diagram. (*a*,*b*) Drawings from two different stage 33–34 spinal cords in dorsolateral view of left side. (*a*) Cells with dendrites dorsal to the dorsal edge of the marginal zone (dotted) to compare with commissural interneurons (outline) with dendrites in marginal zone. Commissural axons are obscured by heavily stained longitudinal axons (omitted). (*b*) Commissural axon (between triangles) crosses ventral cord and merges with ventral border of contralateral marginal zone (at upper triangle). (*c*,*d*) Drawings taken from two stage 33–34 spinal cords. Fill sites were right side, rostral cord. (*c*) is lateral, (*d*) is dorsolateral view. In (*d*), commissural axon (between triangles) could be traced crossing ventral cord and ascending in contralateral marginal zone. Rohon-Beard somata on opposite side are also shown. (*e*) Composite drawing of Dorsolateral commissural interneuron taken from two serial transverse sections of rostral spinal cord, stage 34, caudal to fill site on opposite side. Arrows mark dorsal tract.

distribution of these cells is generally not symmetrical but at later stages some Giant dorsolateral commissural cells are arranged in 'pairs' with one on either side (figure 10*d–f*). The dorsal processes of such pairs do not contact one another. By stage 47 groups of three cells can sometimes occur (figure 11*a*).

(iii) *Giant dorsolateral commissural cells in Ambystoma*

Cells with similar anatomical features (figure 12*b*,*c*) and regular distribution have been filled in the rostral cord of *Ambystoma mexicanum* embryo CNS of an equivalent developmental stage. Figure 12*a* shows the rostral cord from an early stage 39 (Schreckenberg & Jacobson 1975) *Ambystoma* CNS, in which HRP was applied to the left side of the cord, rostral to the filled cells. The distance between filled Giant dorsolateral commissural cells decreases from 350 μm rostrally to 150 μm in the mid-cord region as the myotome width decreased from approximately 350 μm, to 250–300 μm over the same region.

In a slightly older stage 39/40 embryo the interval

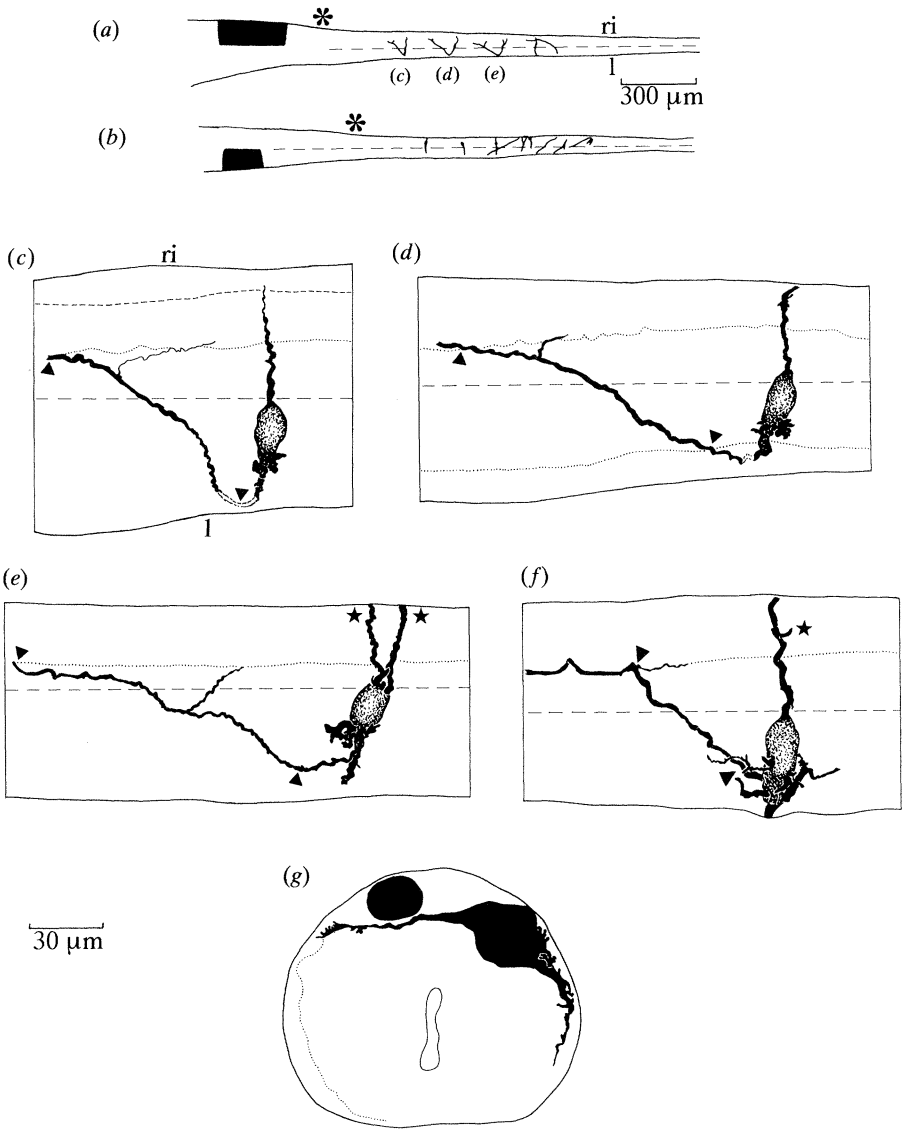

Figure 8. Giant dorsolateral commissural interneurons, stage 34. (*a*,*b*) Drawings of two spinal cords mounted dorsoventrally, showing regular distribution of Giant dorsolateral commissural cells in the rostral cord (fill sites black). Position of cells in (*c*), (*d*) and (*e*) is indicated. (*c*–*e*) Cells are shown in dorsal view. Ventral axons crossing cord from left (l) to right (ri) and joining lateral tract on right side are also shown (ventral portion is between triangles). Dotted lines mark ventral borders of marginal zone. (*e*) has two dorsal processes crossing the cord (stars). (*f*) Giant dorsolateral commissural cell from another stage 34 spinal cord. Star marks dorsal process which could contact Rohon-Beard cells. (*g*) Composite picture from two adjacent transverse sections of mid-spinal cord, caudal to fill site (left hindbrain), showing Giant dorsolateral commissural interneuron and Rohon-Beard cell.

between filled Giant dorsolateral commissural cells in the rostral cord is only about 100 μm (figure 12*b*).

(*f*) *'Ascending' and 'dorsolateral ascending' interneurons*

Both cell types are filled in the same way via their ascending axons, from the ipsilateral hindbrain or rostral cord (figure 13). Because the applications are ipsilateral, and the somata are usually deep to the marginal zone, soma and processes are frequently obscured by longitudinal axons from other filled cells. In the *Xenopus* embryo dorsolateral ascending interneurons lie in a dorsolateral superficial position and have multipolar somata while ascending interneurons have more ventral unipolar somata. In *Triturus*, neurons with ipsilateral ascending axons do not fall into two distinct classes; some more ventral somata have dorsal dendrites (figure 13*b*ii,*ci* and *d*), while some dorsolateral somata are unipolar (figure 13*c*ii, iii).

Neurons with unipolar somata are numerous, particularly in the rostral cord, but only a small number of multipolar dorsolateral ascending interneurons were filled in the rostral cord. These interneurons are distiguished by having a dorsolateral superficial soma giving rise to dendrites in the dorsal tract (figure 13*c*i,*e*).

Both of these categories of cell possess an ascending ipsilateral axon, which may bear short processes

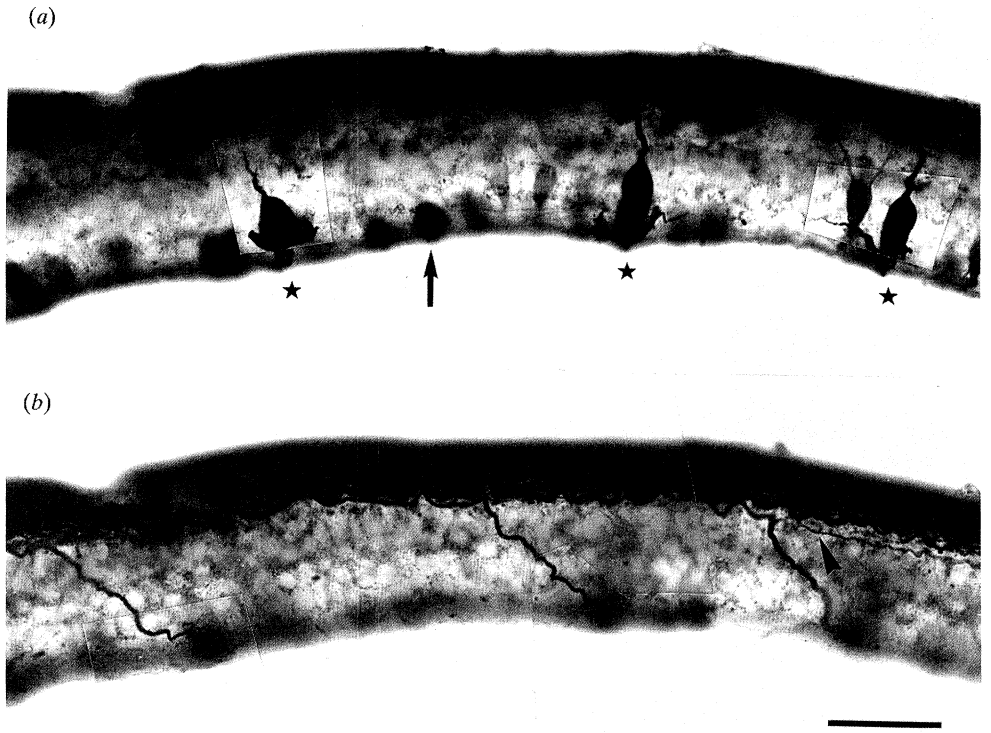

Figure 9. Giant dorsolateral commissural interneurons at stage 34 photographed in dorsal view at two levels of focus to show: (*a*) three dorsal somata (at stars) on left side with commissural processes crossing to filled right side (upper); somata of commissural interneurons can also be seen out of focus on left side (e.g. at arrow), and (*b*) ventral commissural axons of these three cells turning rostrally to join ventral edge of right marginal zone. Note caudal axon branch at arrowhead. Most caudal cell drawn in figure 8*f*. Rostral to left. Scale bar 50 μm.

extending to the inner border of the marginal zone. The axon emerges ventrally, then turns to ascend ipsilaterally (figure 13*b*i and *c*i), or T-branches to ascend and descend (figure 13*b*).

GABA-LI in 'ascending' interneurons

Interneurons with an ascending ipsilateral axon were immunostained for GABA throughout the rostral half of the cord, and in the most caudal part of the hindbrain. The soma characteristically lies in the mid-third of the cord dorsoventrally, medial to the marginal zone. Details of soma anatomy are often obscured by overlying GABA-stained axons which also made it difficult to follow stained axons emerging from the soma individually. Where details can be seen, the soma is most often unipolar (figure 14*b*,*d*). However, there are a few examples of cells with additional processes projecting laterally or dorsally from the soma (figure 14*c*). The axon emerges ventrally, then turns to ascend the cord, or T-branches (figure 14*b*). There are sometimes short spines emerging radially from the most proximal part of the axon.

(*g*) *Descending interneurons*

Descending interneurons are filled by HRP application to the spinal cord ipsilateral and caudal to their somata. They extend from the hindbrain through the trunk spinal cord (figures 15 and 16).

They have a bipolar or multipolar soma positioned inside the dorsal two thirds of the marginal zone. Dorsal and ventral dendrites spread throughout the marginal zone and always reach into the dorsal tract, where contacts with Rohon-Beard axons would be possible (arrowed in figures 15*b*,*c*,*e*,*f*,*g*).

The axon emerges ventrally from the soma before turning to descend in the marginal zone. The ventrally directed portion may have dendrites emerging from it into the marginal zone. It then tapers and descends in the ventral marginal zone. Figure 15*a* shows a descending interneuron with a clear axon extending about 750 μm to the fill site. In other cases descending interneurons were filled up to 1.1 mm rostral to the HRP fill site. In some cases an ascending axon was clear but more often it was not resolved (figure 15*c*). The longitudinal column of descending interneurons extends into the caudal hindbrain (figure 15*d*).

(*h*) *Kolmer-Agduhr cells*

These cells are filled by application to the ipsilateral spinal cord rostral to the soma. They are ependymal cells abutting the ventrolateral border of the neurocoel, distributed along the whole length of the cord (figure 17)

The soma is typically pear-shaped, and the ependymal surface bears short tufts of fine processes which project into the neurocoel. These processes are of two

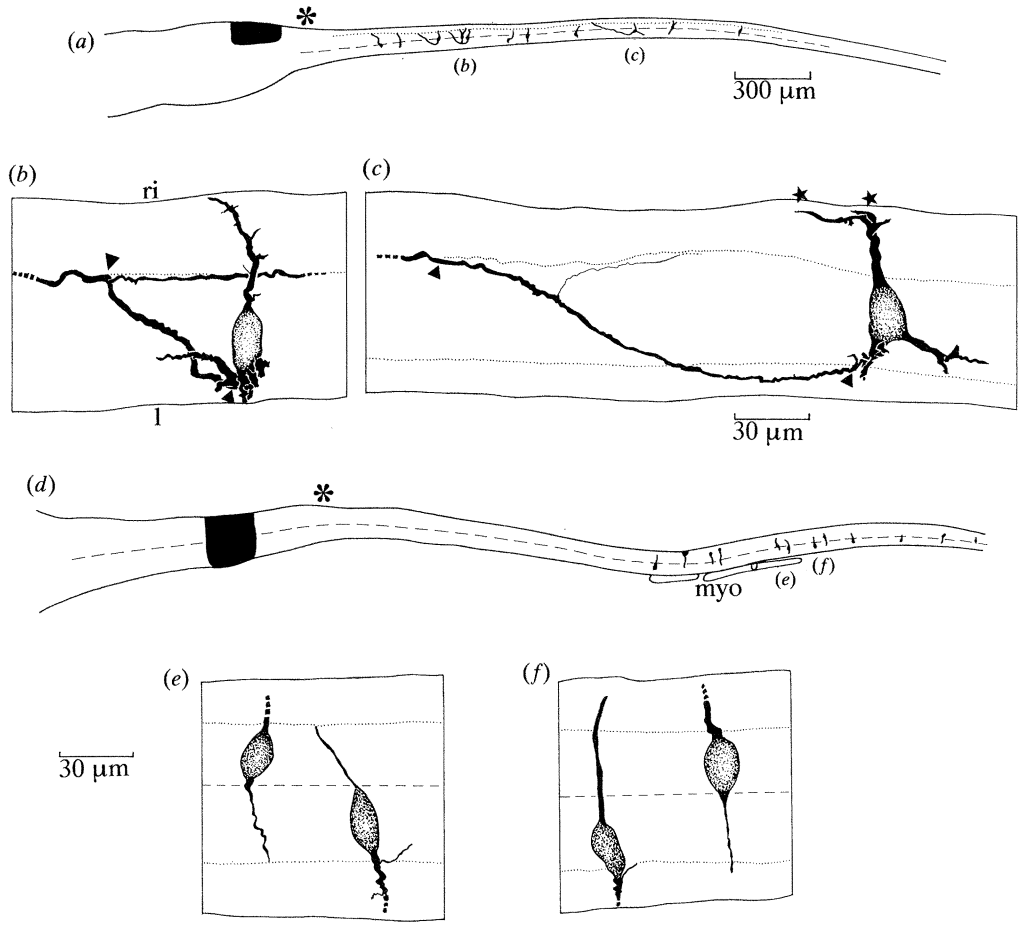

Figure 10. Giant dorsolateral commissural interneurons, stage 37. (*a*) Drawing of spinal cord mounted dorsoventrally. Regular distribution of rostral Giant dorsolateral commissural cells is now less apparent. Positions of cells in (*b*) and (*c*) are indicated. (*b*,*c*) Dorsal view, Giant dorsolateral commissural cell somata with ventral crossing axons (ventral portion lies between triangles). In (*c*), dorsal commissural process branches to ascend after crossing the cord (marked by stars). (*d*) Double-sided fill shows arrangement of Giant dorsolateral commissural cells on both sides of cord. A few myotomal muscle fibres (myo) have been left in position to show relation between Giant dorsolateral commissural cells and myotomes at this developmental stage. (*e*) and (*f*) mark positions of cell 'pairs' drawn in (*e*) and (*f*).

distinct lengths (figure 17*a*) and probably correspond to the microvilli and cilia found in similar cells in *Xenopus* (Dale *et al.* 1987*a*).

The axon runs into the ventral portion of the marginal zone and ascends ipsilaterally.

GABA-LI in Kolmer-Agduhr cells

Gaba-ICC reveals a nearly continuous double row of these cells, running the full length of the cord one on either side of the ventral neurocoel (figure 17*d*,*e*). The pear-shaped soma, the ependymal surface with tufts of fine processes which project into the canal, and the ascending axon can all be seen clearly in GABA-stained preparations. These features identify this type of GABA cell unequivocally as the Kolmer-Agduhr cells of HRP preparations.

4. DISCUSSION

(*a*) *Triturus and Xenopus embryo spinal cords compared*

The aim of this work was to compare the classes of neuron in the embryonic spinal cords of a urodele and anuran. The neurons of the *Xenopus laevis* embryo spinal cord had been described on the basis of broad anatomical features (Roberts & Clarke 1982). Similar methods have now been used to characterize nine neuron classes in *Triturus vulgaris* (figure 18) Eight neuron classes appear to be common to both so the names introduced for *Xenopus* have been adopted for *Triturus*. Examination of neurons close to fill sites reveals no differences in soma size and shape, or dendritic field when compared with those further away. There is therefore no evidence of distinct short-

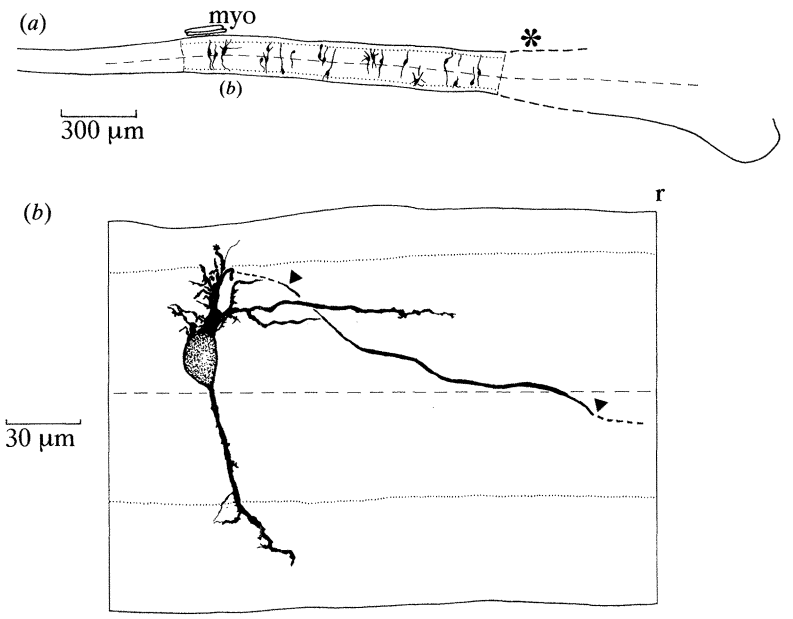

Figure 11. Giant dorsolateral commissural interneurons, stage 47–48. (*a*) Drawing of rostral spinal cord mounted dorsoventrally, double-sided fill shows paired arrangement of cells. There appears to be considerable infilling by new cells at this stage, and dendritic branching is more complex, particularly where the dorsal process reaches the contralateral axon tract. Position of cell drawn in (*b*) is indicated. (*b*) Dotted lines mark dorsal border of dorsal tract. Ventral portion of axon lies between triangles.

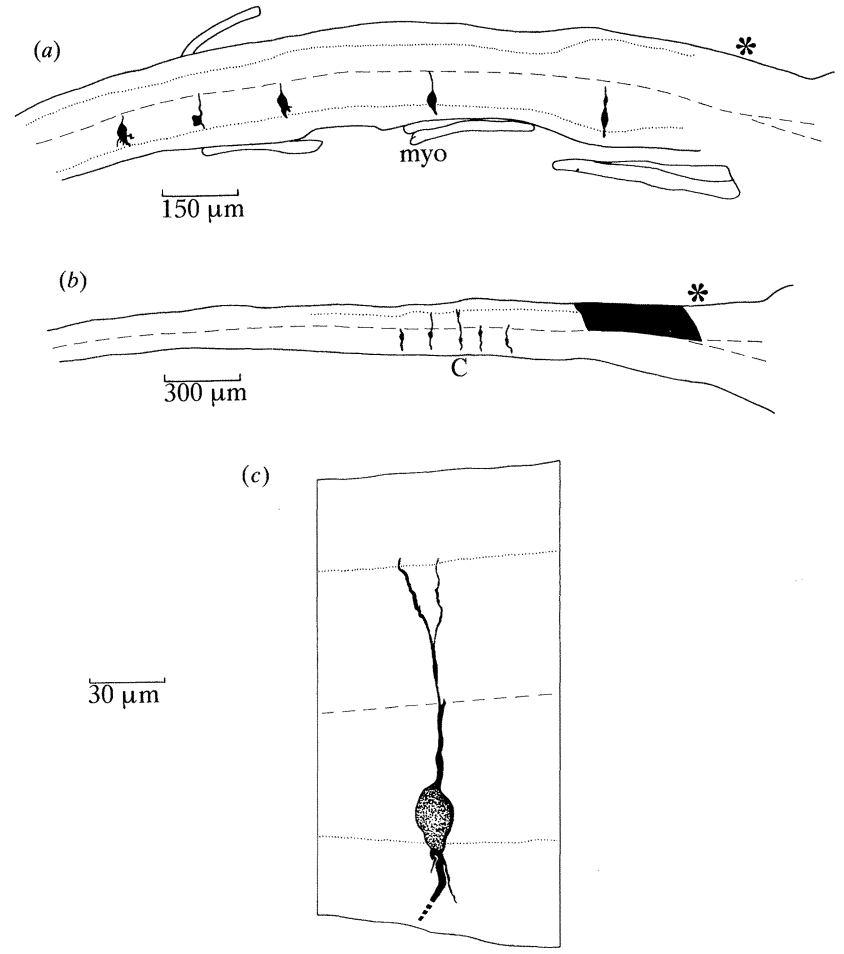

Figure 12. Giant dorsolateral commissural interneurons in *Ambystoma mexicanum*. (*a*) Drawing of early stage 39 rostral spinal cord, dorsal view. Dotted lines mark dorsal borders of dorsal tracts. Some myotomes (myo) have been left in place to show relation of filled cell distribution to muscle blocks. At this stage, dorsal processes just cross midline. (*b*) Late stage 39 rostral spinal cord, dorsal view. Dorsal processes now reach contralateral dorsal tract in some cases. (*c*) Cell (marked in *b*) which shows typical anatomy.

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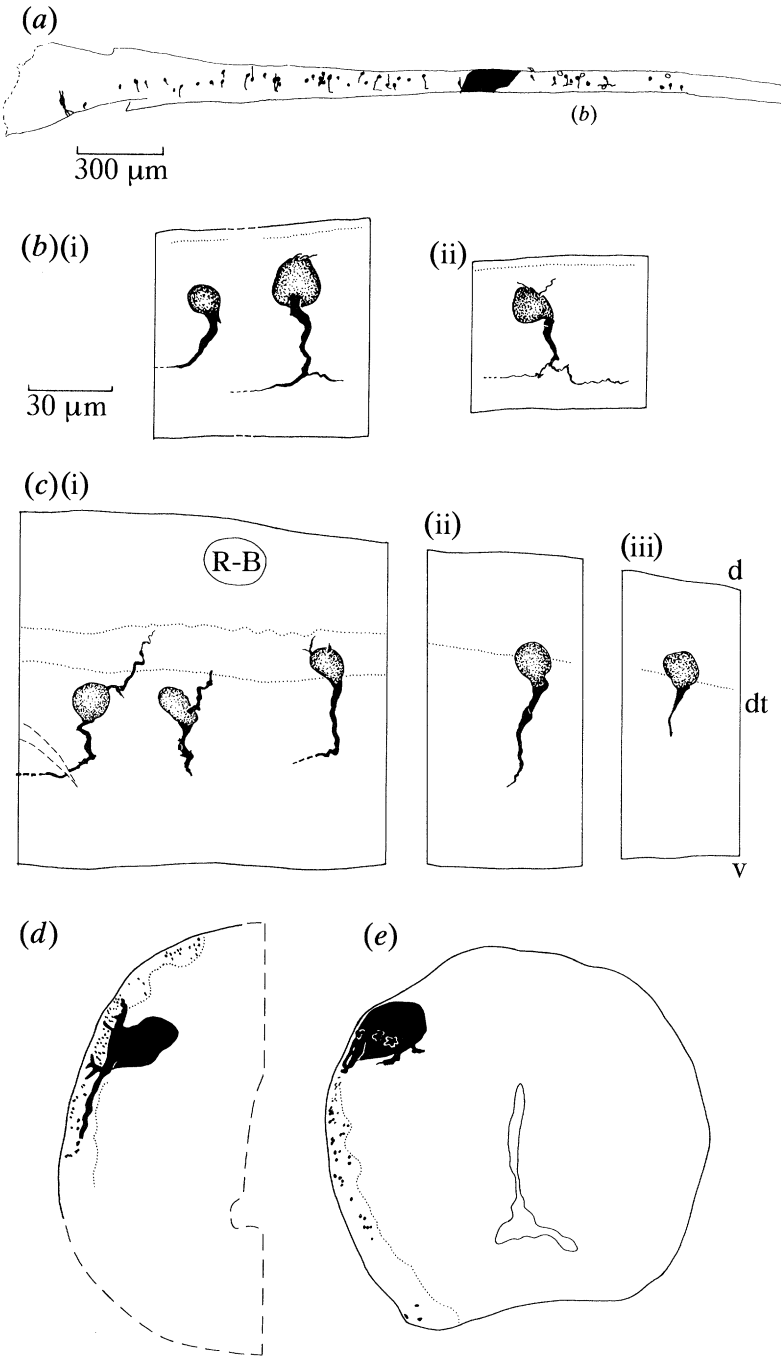

Figure 13. Ascending and dorsolateral ascending interneurons. (*a*) Drawing of whole mount at stage 34, mounted oblique ventrally. HRP-filled cells rostral to fill site and extending into hindbrain are descending interneurons, cells caudal to fill are ascending interneurons. Position of cells in (*b*) is indicated. (*b*) Possible ascending interneurons with somata ventral to dorsal tract and lying deep to marginal zone. Distal parts of ascending axons are obscured by overlying longitudinal axons (omitted). (*c*) Interneurons in rostral cord from another spinal cord, stage 34, viewed laterally. Fill site is rostral hindbrain. Extent of stained dorsal tract axons (dt) and the position of a Rohon-Beard soma are also shown. The caudal three interneurons have a dorsolateral soma position. (*d*,*e*) Transverse sections, stage 34. Both are mid-cord, caudal to fill site. (*d*) A typical ascending interneuron; (*e*) is dorsolateral ascending.

axoned neuron types in addition to the nine classes described here.

(i) *Rohon-Beard neurons*

The primary mechanosensory Rohon-Beard neurons in *Xenopus* have dorsal somata, ascending and descending central axons and peripheral neurites with free nerve endings in the skin which respond to rapid, local deformation (Hughes 1957; Roberts & Hayes 1977; Clarke *et al.* 1984). The morphology and distribution of Rohon-Beard neurons in the spinal cord of *Xenopus* are indistinguishable from those found in the *Triturus* embryo. The *Triturus* Rohon-Beard neurons have similar free nerve endings in the skin and responses to skin deformation (Roberts & Clarke 1983).

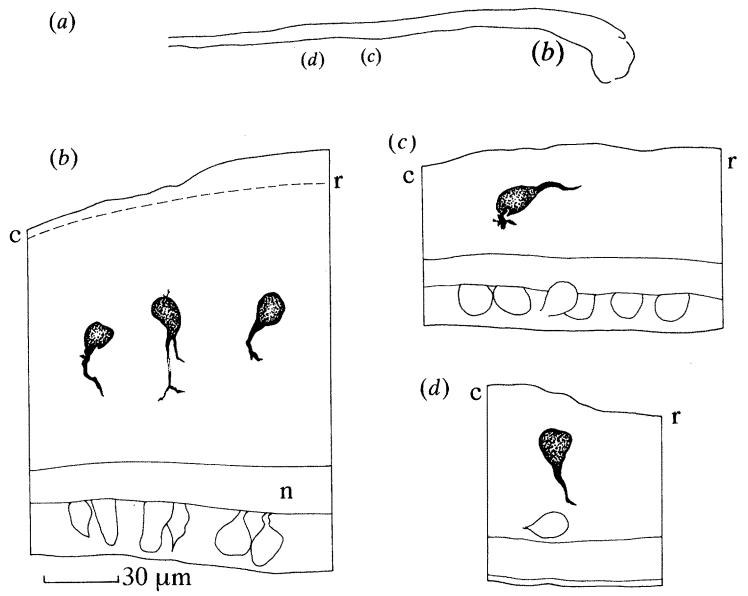

Figure 14. 'Ascending' interneurons with GABA-LI. (*a*) Drawing of rostral whole mount, stage 34–35, immunostained for GABA and mounted laterally. Letters (*b*,*c*,*d*) show approximate rostrocaudal positions of cells drawn in (*b–d*). (*b–d*) GABA-immunoreactive 'ascending' interneurons. Kolmer-Agduhr cells with GABA-LI are drawn in outline bordering the neurocoel (n). Axons of individual cells are obscured in the marginal zone by dense staining of strongly immunoreactive longitudinal axons, not drawn here. Middle cell in (*b*) shows axon T-branching to ascend and descend. Neuron in (*c*) is unusual in having a dorsal dendrite, but this process does not project far enough to contact the dorsal tract.

(ii) *Motorneurons*

Motorneurons in *Triturus* are defined by the possession of an axon which projects ipsilaterally to the myotomes. The general features of their anatomy and distribution are similar for both *Triturus* and *Xenopus*, although in both cases there is considerable variation in size, shape and position of somata and in the nature and extent of dendritic fields (see figure 2). Blight (1978) describes 'primary' motorneurons with large, multipolar somata and smaller 'secondary' motorneurons with unipolar somata in *Triturus*. The concept of primary motorneurons, which appear relatively early in development, and of morphologically distinct secondary motorneurons, which develop later, is well known (see Youngstrom 1940 (*Ambystoma*); Van Mier *et al.* 1985 (*Xenopus*); Forehand & Farel 1982; Stehouwer & Farel 1983 (Rana); Myers et al 1986 (zebrafish)). The two extremes described by Blight are certainly present in our preparations but many intermediate forms are also present (see figures 2 and 3). This suggests that a straightforward separation of primary and secondary motorneurons based on our anatomical evidence would be difficult for the developmental stages considered here.

(iii) *Commissural interneurons*

Commissural interneurons with unipolar somata lying deep to the marginal zone, an axon which projects ventrally, bearing short radial dendrites and then crosses ventrally to ascend or T-branch are present in both animals (Roberts & Clarke 1982). Although the glycine-LI in *Triturus* was not strong, the anatomical features of the soma and proximal portion of the axon where visible in *Triturus* are identical to those of the commissural interneurons revealed with HRP and correspond well to the glycine-immunoreactive commissural interneurons described for *Xenopus* (Dale *et al.* 1986; Roberts *et al.* 1988). In the absence of ventral commissural axons immunostained for glycine in *Triturus*, it is impossible to identify this group conclusively using ICC, but it would seem reasonable to propose that they are commissural interneurons where glycine distribution is concentrated in the somata. The role of commissural interneurons in producing reciprocal inhibition during swimming has been defined physiologically for *Xenopus* (Dale 1985) and suggests a similar inhibitory role for commissural interneurons in *Triturus*.

(iv) *Dorsolateral commissural interneurons*

Glycine ICC in *Triturus* does not reveal any glycine-immunoreactive multipolar somata like those of dorsolateral commissural interneurons. This suggests that the choice of anatomical criteria used to distinguish between commissural and dorsolateral commissural interneurons was appropriate and points to a physiological distinction between the two groups. Such conclusions are supported by similar findings in *Xenopus* embryos (Clarke & Roberts 1984; Roberts *et al.* 1988) where physiological evidence shows that dorsolateral commissural interneurons are excited by sensory Rohon-Beard neurons and excite rhythmic neurons on the opposite side (Roberts & Sillar 1990).

(v) *Giant dorsolateral commissural interneurons*

Giant dorsolateral commissural interneurons were found in embryos of *Triturus* and *Ambystoma mexicanum* and similar interneurons have been illustrated for

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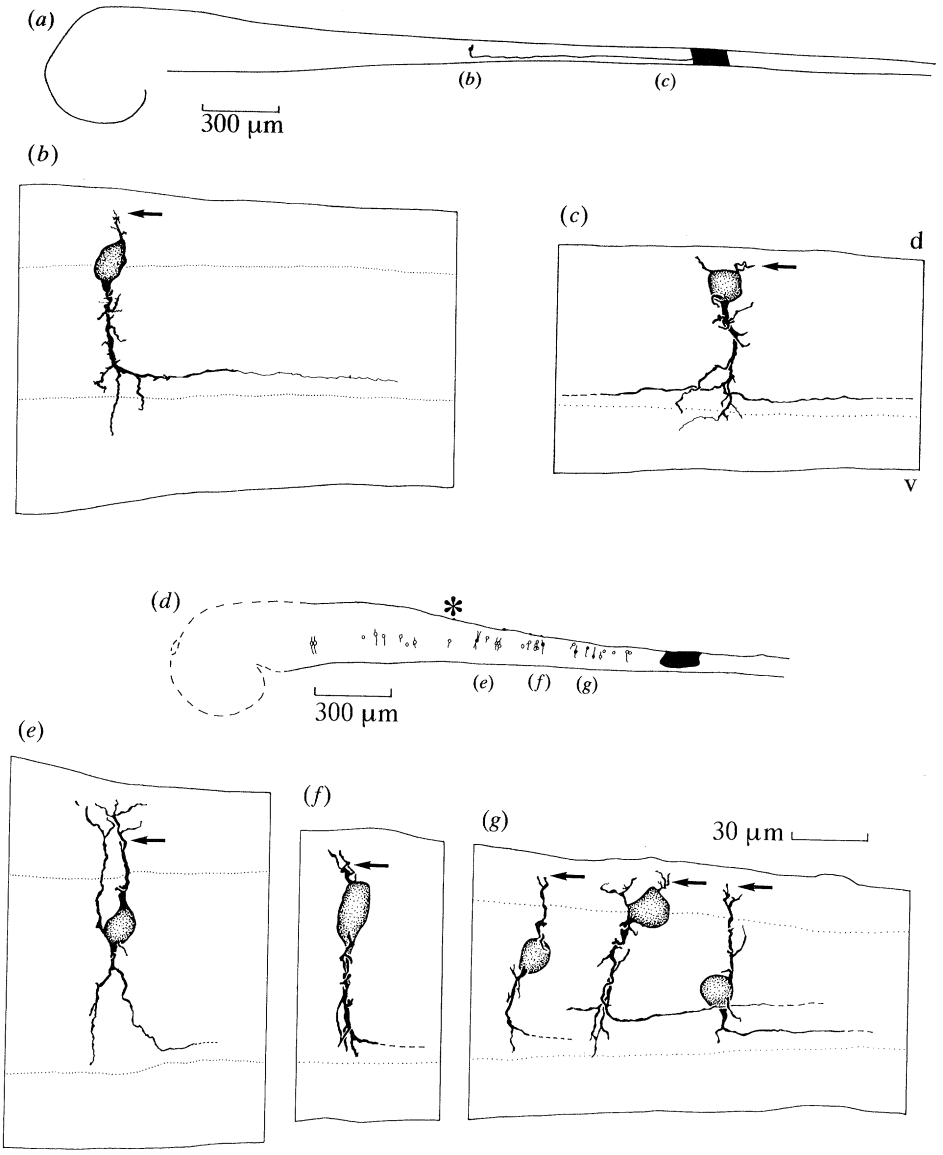

Figure 15. Descending interneurons in lateral view at stage 35. (*a*) Plan diagram to show extent of axon of cell drawn in (*b*). Positions of cell (*c*) in relation to fill site is also indicated. (*c*) Example where an ascending axon is present. (*d*) Low-power plan of descending interneurons filled from site indicated (black). (*e–g*) Examples to show range of dorsoventral soma positions and morphologies. Horizontal arrows indicate level on dendritic arbor of ventral edge of dorsal tract of Rohon-Beard axons.

embryos of the tiger salamander, *Ambystoma punctatum* (Youngstrom 1940). However, there is no evidence for these cells in the embryonic spinal cord of either *Xenopus* or *Rana temporaria* (Roberts & Clarke 1982; C. E. Harper, unpublished results).

As the anatomy of the two spinal cords and the behaviour of *Triturus* and *Xenopus* embryos are strikingly similar, why does *Triturus* require this extra class of spinal interneurons? The dorsolateral position of the ipsilateral dendrites, and the termination of the dorsal commissural process(es) in the contralateral dorsal tract, suggest that these cells may receive input from Rohon-Beard axons, possibly on both sides of the body (see figure 8*g*). An excitatory function is likely as ICC reveals that they do not show immunoreactivity to GABA or glycine, the two most likely inhibitory transmitters in the cord. The large diameter of the ventral commissural and ascending axon suggests rapid conduction, perhaps in response to strong, or bilateral stimuli. One possibility is that they could function like Mauthner neurons, which occur in the hindbrain of many vertebrates including teleosts (Kimmel 1982; Nissanov *et al.* 1990), amphibians, and reptiles (Ten Donkelaar 1982), and trigger fast-startle escape swimming. However, if the sensory input from Rohon-Beard neurons is on both sides, it is unlikely that such movements would be particularly directional.

(vi) *Ascending and dorsolateral ascending interneurons*

In the *Triturus* embryo spinal cord, at least two anatomical cell groups show GABA-LI; one group is

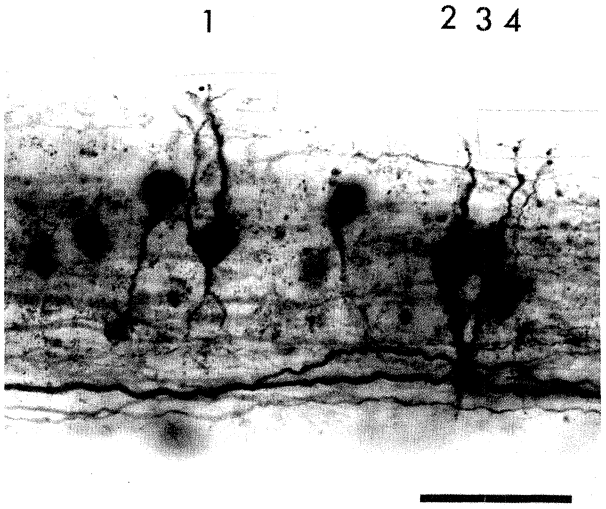

Figure 16. Descending interneurons photographed in lateral view at stage 35 rostral to HRP fill site on same side. Four descending interneurons (numbered) can be recognized by the extent of their dendrites. These lie throughout and dorsal to the marginal zone (clear from HRP filled axons). Other unidentified neurons can be seen. Same preparation drawn in figure 15*d*,*e* where dorsal and ventral edges of cord are indicated. Dorsal up, rostral to left. Scale bar 50 μm.

clearly identifiable as Kolmer-Agduhr cells (figure 17). The second group consists primarily of cells which correspond to the ascending interneurons of HRP preparations. However, there are a few examples of GABA-immunoreactive interneurons which have laterally or dorsally directed processes emerging from the soma (figure 13*c*), and correspond more closely to our definition of dorsolateral ascending interneurons. It is therefore not clear whether ascending and dorsolateral ascending interneurons should be classed as one quite variable morphological group, or as two separate groups. In this case, as in *Xenopus* (Roberts *et al.* 1987), GABA ICC does little to make the picture any clearer. The problem is compounded by the position of the soma in many cases, because details are frequently obscured by overlying axons. In the absence of physiological evidence on function, it seems safest to assume that dorsolateral ascending and ascending interneurons in *Triturus* may eventually prove to have separate physiological roles in the CNS, despite the fact that they are not clearly distinguishable at present on morphological grounds.

(vii) *Descending interneurons*

Descending interneurons with bi- or multi-polar somata, extensive dorsal and ventral dendrites and a descending axon on the same side are present in the

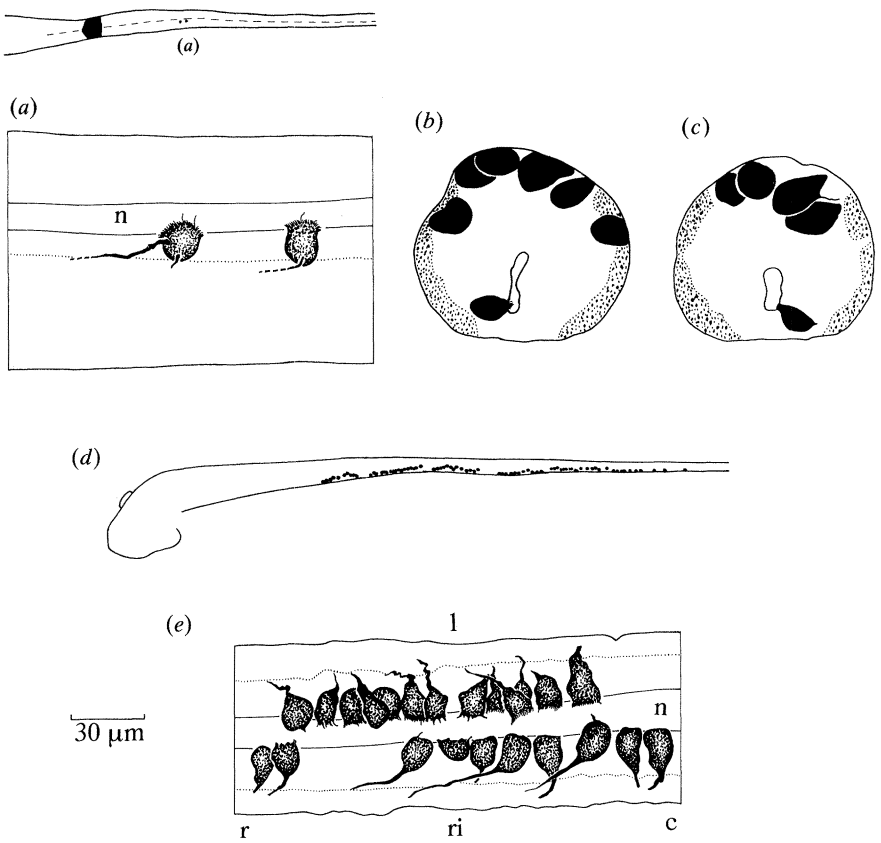

Figure 17. Kolmer-Agduhr cells. (*a*) HRP-filled cells from stage 33 spinal cord, ventral view. Approximate positions of cells and fill site are shown on plan diagram. (*b*,*c*) Drawings of transverse sections of mid-trunk spinal cord, showing filled Kolmer-Agduhr cells caudal to fill site and contacting ventral edge of neural canal. (*d*,*e*) Kolmer-Agduhr cells immunostained for GABA. (*d*) Drawing of whole mount stage 34–35, immunostained for GABA and mounted laterally, showing distribution of GABA-LI in Kolmer-Agduhr cells on right side. Other GABA cells are omitted. (*e*) Drawing from stage 33–34 mid-spinal cord, immunostained and mounted ventrally to show Kolmer-Agduhr cells with GABA-LI on both sides.

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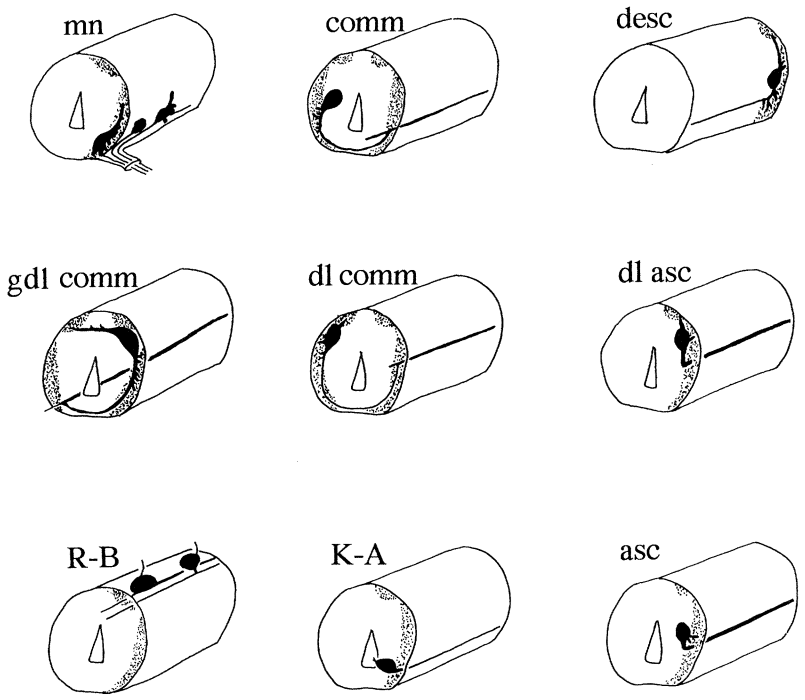

Figure 18. Schematic drawings of spinal cord (rostral to right) to show characteristic anatomical features and positions of the nine neuron types described in *Triturus*. Commissural interneuron (comm), descending interneuron (desc), giant dorsolateral commissural interneuron (gdl comm), dorsolateral commissural interneuron (dl comm), dorsolateral ascending interneuron (dl asc) and ascending interneuron (asc); Rohon-Beard (R-B), Kolmer-Agduhr (K-A).

rostral spinal cord of both species (Roberts & Clarke 1982). Present evidence in *Xenopus* suggests that they are active during fictive swimming and provide the excitatory component of the spinal central pattern generator (Dale & Roberts 1984; Roberts & Alford 1986). Intracellular fills in *Xenopus* suggest that it is likely that many descending interneurons also have ascending axons but the evidence from extracellular fills is at present unclear.

(viii) *'Kolmer-Agduhr' cells*

'Kolmer-Agduhr' cells with somata on the lateral edge of the floor plate, microvilli and cilia projecting into the neurocoel, and an ascending axon, are present in both *Triturus*, and *Xenopus* (Dale *et al.* 1987*a*,*b*). Their specific GABA-LI suggests an inhibitory function but their role in either species remains obscure. The fact that they project microvilli and cilia into the neurocoel and have no clear dendrites suggests a sensory role, monitoring some mechanical or chemical condition of the neurocoel but there is no evidence for this at present. Ependymal cells of the floor plate have been implicated in a development role, regulating patterns of cell differentiation and the orientation of axons (Yamada *et al.* 1991), but it remains to be seen whether Kolmer-Agduhr cells are involved.

(*b*) *Spinal segmentation in Triturus?*

A segmental organization of motorneurons and interneurons has been described in the spinal cord of zebrafish embryos (Myers *et al.* 1986; Bernhardt *et al.* 1991), but has not been found in adult goldfish (Fetcho 1990) or in the higher vertebrate spinal cord. Segmental organization of neurons is characteristic of the embryonic hindbrain of higher vertebrates, and studies have revealed recently that this organization may be paralleled by segmental gene expression (for review see Lumsden & Keynes 1989). It was therefore of interest to consider whether Giant dorsolateral commissural neurons in *Triturus* provide an example of segmentally distributed neurons in the spinal cord of amphibians, which could be utilized in future studies of segmental gene organization.

The distribution pattern of Giant dorsolateral commissural cells in *Triturus* is unusual. The other spinal neuron classes at this stage of development form more or less continuous, rather irregular, longitudinal columns, with neuron density greatest rostrally, where newly differentiating cells fill in gaps between existing ones (as in *Xenopus* (Dale *et al.* 1986,1987*a*,*b*; Roberts *et al.* 1987 1988; Roberts 1988)). In contrast, Giant dorsolateral commissural interneurons at stage 35 have a sparse but regular distribution in the trunk cord. In at least some cases they appear to become closer together more caudally (figure 7*b*), a distribution pattern reminiscent of the arrangement of myotomes which become shorter caudally. This is perhaps all the more remarkable because Giant dorsolateral commissural interneurons are usually filled by application of HRP to the rostral cord or hindbrain and it might be expected that more cells would stain nearer to the fill site.

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When the distribution of filled Giant dorsolateral commissural interneurons is related to that of myotomes, they do not appear to correspond well to one another at stage 35 (see figure 8). This could be because the myotomes elongate more than the spinal cord during development as has been shown in *Xenopus* embryos (Westerfield & Eisen 1985). If this was the case, it is possible that Giant dorsolateral commissural interneuron distribution in *Triturus* corresponds to the segmentation of myotomes at an earlier stage in development. It is also possible that muscle and nervous tissue undergo fixation-induced shrinkage at different rates which could contribute to any discrepency. At later developmental stages (figures 11 and 12) the infilling by newly differentiated Giant dorsolateral commissural interneurons obscures even further any relation between myotome and Giant dorsolateral commissural interneuron distribution. All we can safely say is that our results are compatible with the possibility that Giant dorsolateral commissural interneurons or their precursors are segmentally distributed at early stages in their differentiation.

(*c*) *Spinal neurons in other species*

(i) *Giant dorsolateral commissural interneurons*

In the hagfish, *Myxine*, Bone (1963) described large dorsal interneurons with dorsal dendrites, some of which cross the roof of the spinal cord to reach the contralateral white matter, and a ventral commissural axon which could not be followed on the opposite side. Interneurons with similar somata and dendrites have been seen in young larval brook lampreys, *Lampetra planeri*, but their axons did not appear to cross the cord in the ventral commissure (Whiting 1948). Embryos of the Australian lungfish *Neoceratodus forsteri* appear to be the only other non-urodele vertebrate with similar interneurons though again the projection of the commissural axon is uncertain (Whiting *et al.* 1992). The animals with interneurons closest in morphology to the Giant dorsolateral commissural interneurons of *Triturus* are the lancelet and dogfish larvae. In *Branchiostoma*, Bone (1959,1960) has shown that the nerve cords of both the adult and larva contain Rohde cells (Rohde, 1888) which, in caudal regions, have all the features of Giant dorsolateral commissural interneurons including a large ventral commissural axon which ascends contralaterally. In *Squalus acanthias* larvae, Bone (1977) has reported similar giant neurons in the spinal cord.

Large cells with ventral commissural axons and dorsal processes have been reported in *Rana catesbiana* embryos (Campbell *et al.* 1987), but the dorsal processes extend only as far as the midline, and do not reach the contralateral tract as Giant dorsolateral commissural interneuron processes do. Neurons with dorsal commissural processes are reported in the spinal cords of adult frogs and teleosts (Johnston 1908), but they do not appear to resemble the Giant dorsolateral commissural interneurons of urodele embryos in other respects.

(ii) *Other spinal neurons*

All fish and amphibian embryos so far described appear to have Rohon-Beard neurons and motorneurons. Parallels in the interneurons of the embryonic spinal cord are difficult to establish on the basis of earlier studies using silver staining because the axonal projection patterns of the neurons could not be established. However, two recent studies on teleost embryos suggest that they may have very similar spinal neuron classes to those we have described in *Xenopus* and *Triturus*. In the Japanese medaka, *Oryzias latipes*, three classes of neuron were described with axonal projections which probably correspond to those of commissural, descending and ascending (or dorsolateral ascending) interneurons (Kuwada 1986). In a more complete study in zebrafish embryos seven classes of interneurons are described (Bernhardt *et al.* 1991). Three of these (CoPA, DoLA and CiA) could correspond directly to our dorsolateral commissural, dorsolateral ascending and ascending classes. The commissural interneurons are subdivided into two classes in the zebrafish embryo depending on whether the axon ascends or bifurcates (CoSA and CoB). Similarly, descending interneurons are also subdivided into two classes (VeLD and CiD). These comparisons suggest that with further study the two groups may be shown to have homologous neuron classes at early stages of development.

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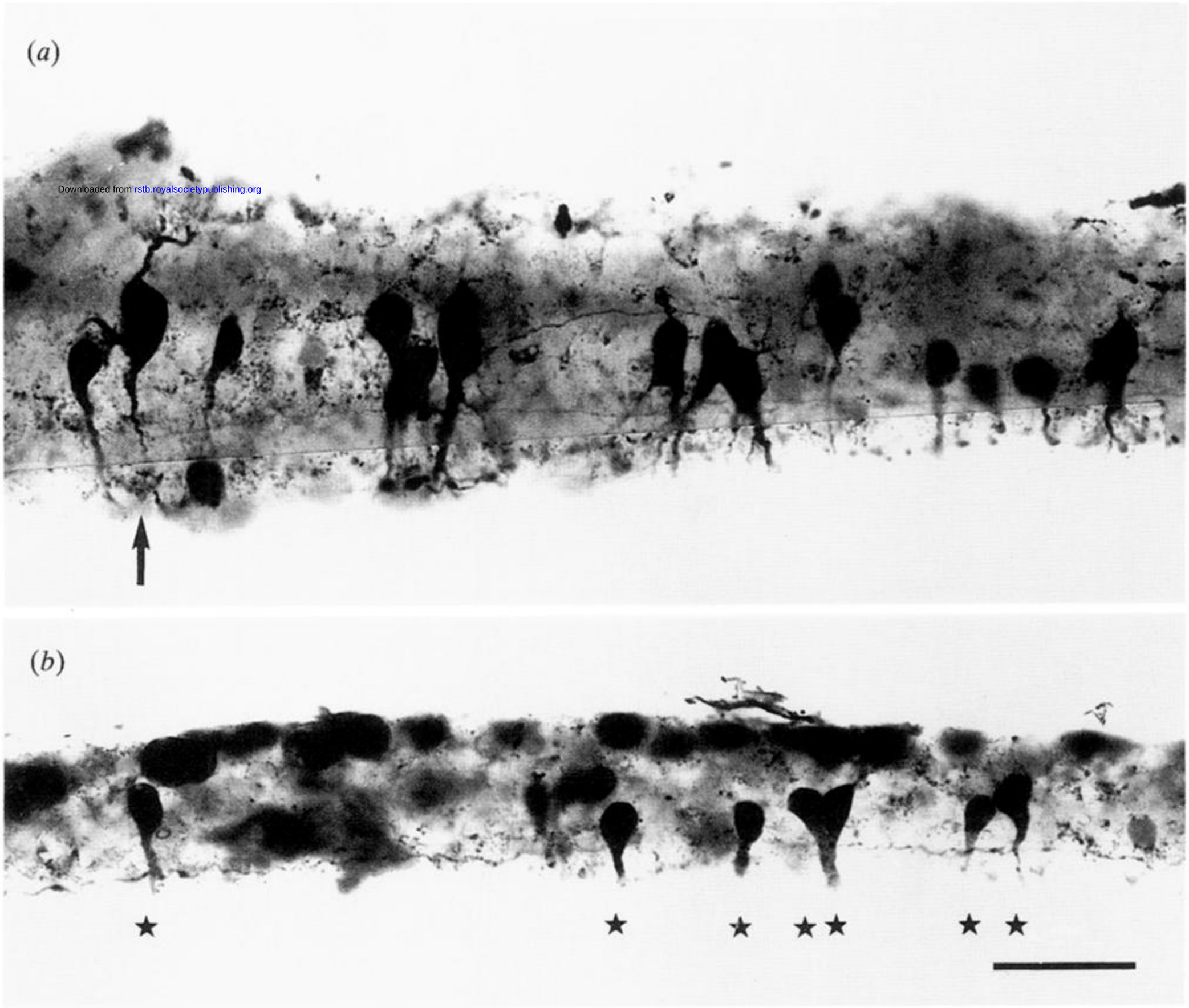
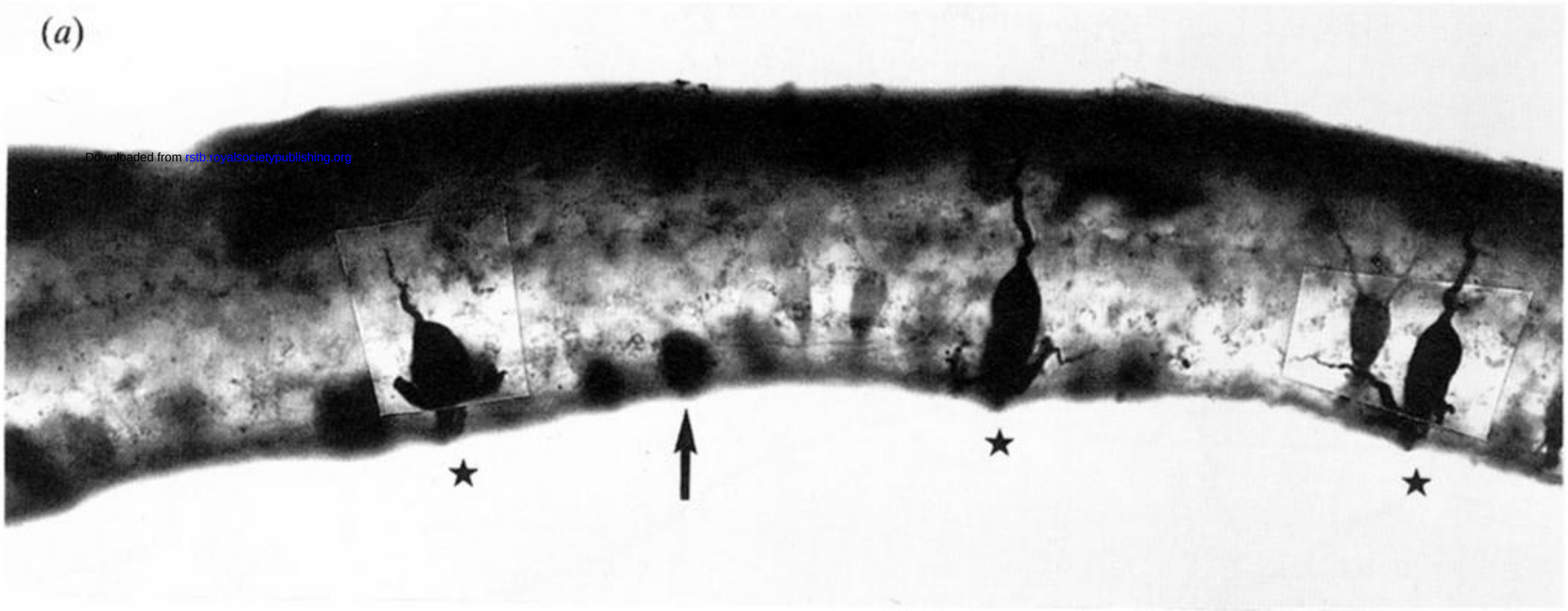
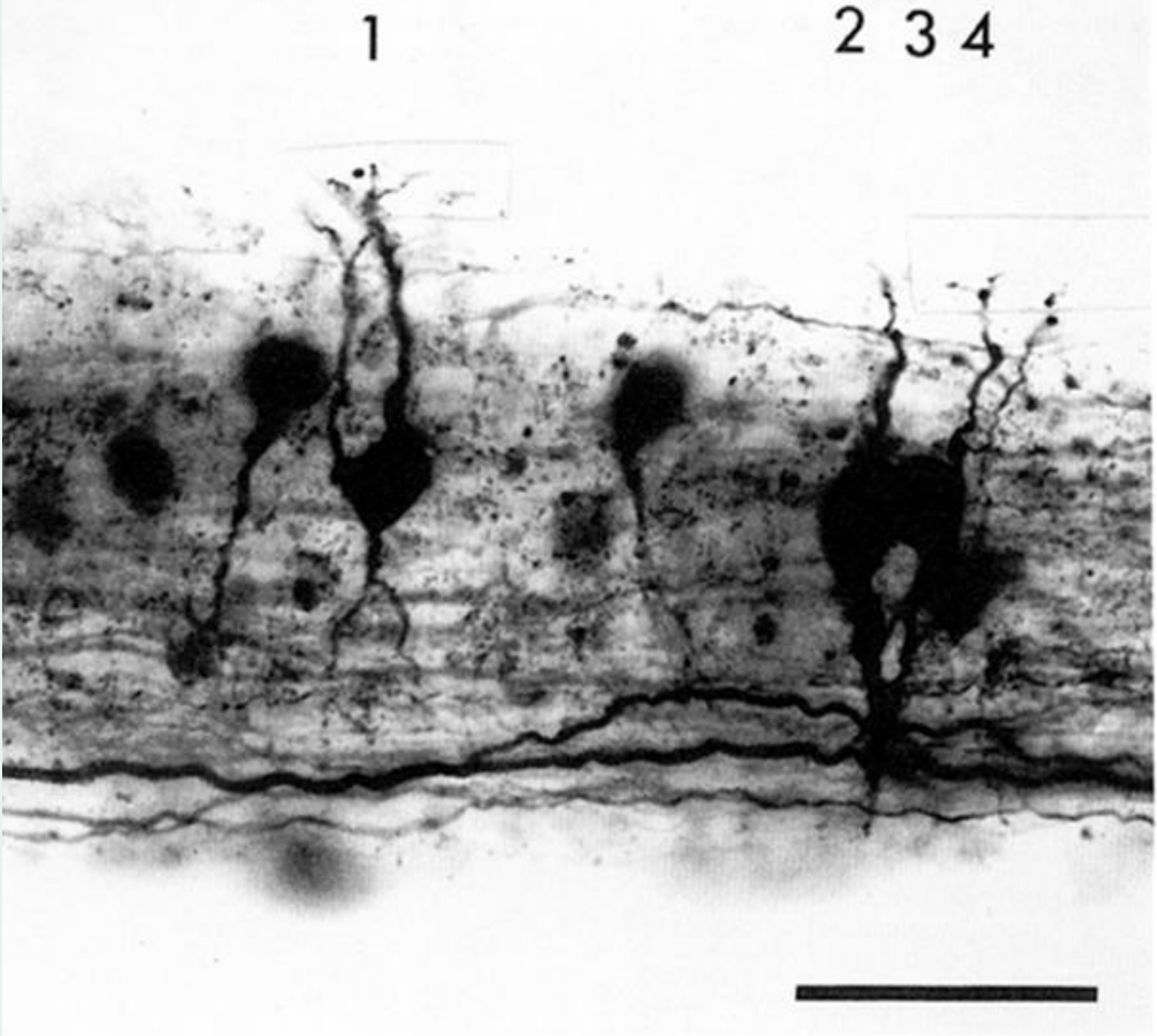


gure 5. Commissural and dorsolateral commissural interneurons photographed in lateral view. (*a*) Commissural terneurons rostral and contralateral to HRP application at stage 35 with unipolar somata and ventral initial axon gment. At the arrow, a single dorsolateral commissural interneuron with more dorsal multipolar soma. (*b*) ommissural interneurons (at stars) caudal and contralateral to HRP application. Rohon-Beard somata can be seen orsally out of focus on opposite side. Dorsal up, rostral to left. Scale bar, 50 μm.



igure 9. Giant dorsolateral commissural interneurons at stage 34 photographed in dorsal view at two levels of focus o show: (*a*) three dorsal somata (at stars) on left side with commissural processes crossing to filled right side (upper); omata of commissural interneurons can also be seen out of focus on left side (e.g. at arrow), and (*b*) ventral ommissural axons of these three cells turning rostrally to join ventral edge of right marginal zone. Note caudal axon ranch at arrowhead. Most caudal cell drawn in figure 8*f*. Rostral to left. Scale bar 50 μm.



gure 16. Descending interneurons photographed in lateral ew at stage 35 rostral to HRP fill site on same side. Four scending interneurons (numbered) can be recognized by e extent of their dendrites. These lie throughout and rsal to the marginal zone (clear from HRP filled axons). ther unidentified neurons can be seen. Same preparation awn in figure 15*d*,*e* where dorsal and ventral edges of cord e indicated. Dorsal up, rostral to left. Scale bar 50 μm.

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