CHAPTER 3 The Pattern of Outgrowth in Normal Embryos

The organisation of processes in the ventral nervous system is established during a short period of little more than an hour, at the same time as the animal is elongating in the eggshell from a stubby 'tadpole' to a worm. Electron microscope reconstructions of varying lengths were made from a series of four embryos at different developmental stages during this period (figure 3.1). Figure 3.4 shows a schematic picture of the state of the ventral nervous system in each of the reconstructions, which will be referred to by the letters A to D. During the period covered by these reconstructions the embryo increases by a factor of about two in length, being about one and a half fold in the egg (100 microns) at the time of the A reconstruction, and three and a half fold (220 microns) in the D reconstruction. At the beginning of the period under consideration here the nerve ring contains the majority of the final number of processes. Uncoordinated muscle activity has already started before the time of the A reconstruction (the onset of twitching is at about 430 mins). Movement becomes more organised around the stage of the final, D reconstruction, although since the embryo is restricted inside the egg shell it is not possible to assess fully the degree of coordination.

3.1 Morphology of growth cones

Growth cones are generally extended flattened lamellar structures that also have long thin filopodial extensions. In C. elegans the most extensive growth cones are seen on the growing tips of the motor neuron commissures. Typically they are a flattened sheet a few tenths of a micron thick and of variable shape and size in the plane of the sheet (figures 3.2, 3.5). The absence of normal looking filopodia may be due to the small scale (2-5 microns across); a vertebrate tissue culture growth cone could extend right round the
C. elegans embryo. However stubby finger-like extensions are seen in many cases, and these may perform an equivalent function. Figure 3.2 shows a three dimensional reconstruction of the complete cell DB4 from the B reconstruction, in which the thin sheet-like nature of the growth cone can be clearly seen.

Extended growth cones like those seen on commissures were not seen on processes growing along the ventral or dorsal cords, although some tips do have swollen or spread out endings (e.g. PVCL in figure 3.7). This corresponds to observations made in other organisms that process growing along pre-existing nerve bundles do not have such extended growth cones as those growing over virgin territory (Lopresti et al., 1973).

The quality of the cytoplasmic fixation in the embryos used for reconstruction was poor, since primary fixation is with OsO_4 follwed by tannic acid, which fixes membranes well but leaves little cytoplasmic structure. Therefore neither actin microfiliaments nor microtubules are preserved. However in some cases it is possible to see vesicles in growth cones, as for example in a commissural growth cone in the C reconstruction (figure 1.1). Studies by de Cino (1981) have indicated that transmitter is sometimes released by growth cones. An alternative explanation for the vesicles is simply that they may be a source of new membrane for insertion at the leading edge of the growth cone.

Figure 3.1

This shows the approximate ages of the embryos used in this study from which long series were reconstructed completely, and the parts of them that were reconstructed. Ages were determined as described in Chapter 2.5. The bracket below the embryo indicates the part of the ventral cord shown in figure 3.4.

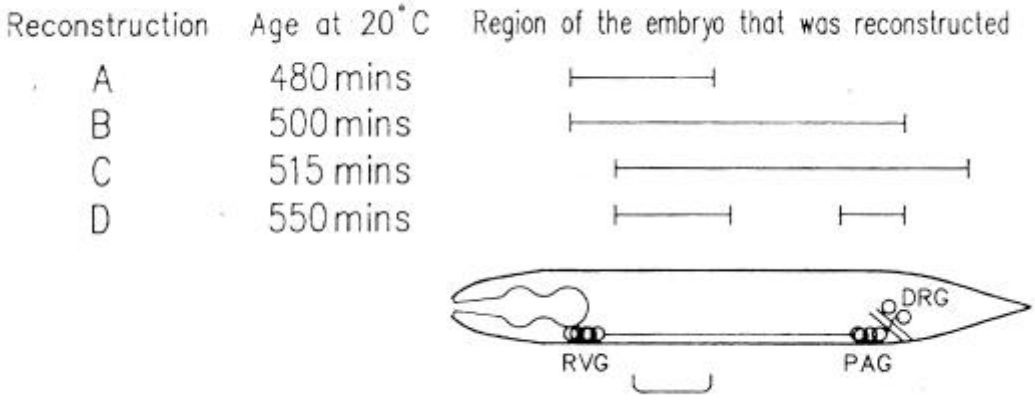

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Figure 3.2

A three-dimensional reconstruction of the motor neuron DB4 from the B series. The cell body is on the right. Out of this extends a growing commissure, terminating in the flattened extended structure at the left, which is the growth cone. This diagram was made with the aid of a 3-D reconstruction program written by J.G. White. The growth cone is approximately 5 microns across.

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A three-dimensional reconstruction of the motor neuron DB4 from the B series. The cell body is on the right. Out of this extends a growing commissure, terminating in the flattened extended structure at the left, which is the growth cone. This diagram was made with the aid of a 3-D reconstruction program written by J.G. White. The growth cone is approximately 5 microns across.

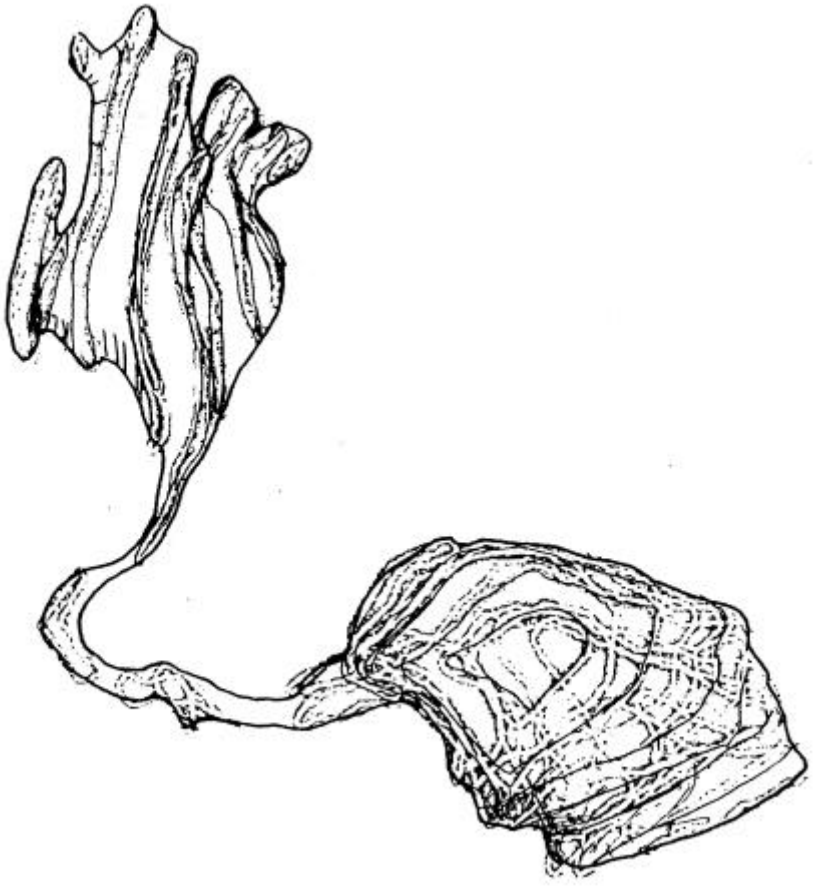
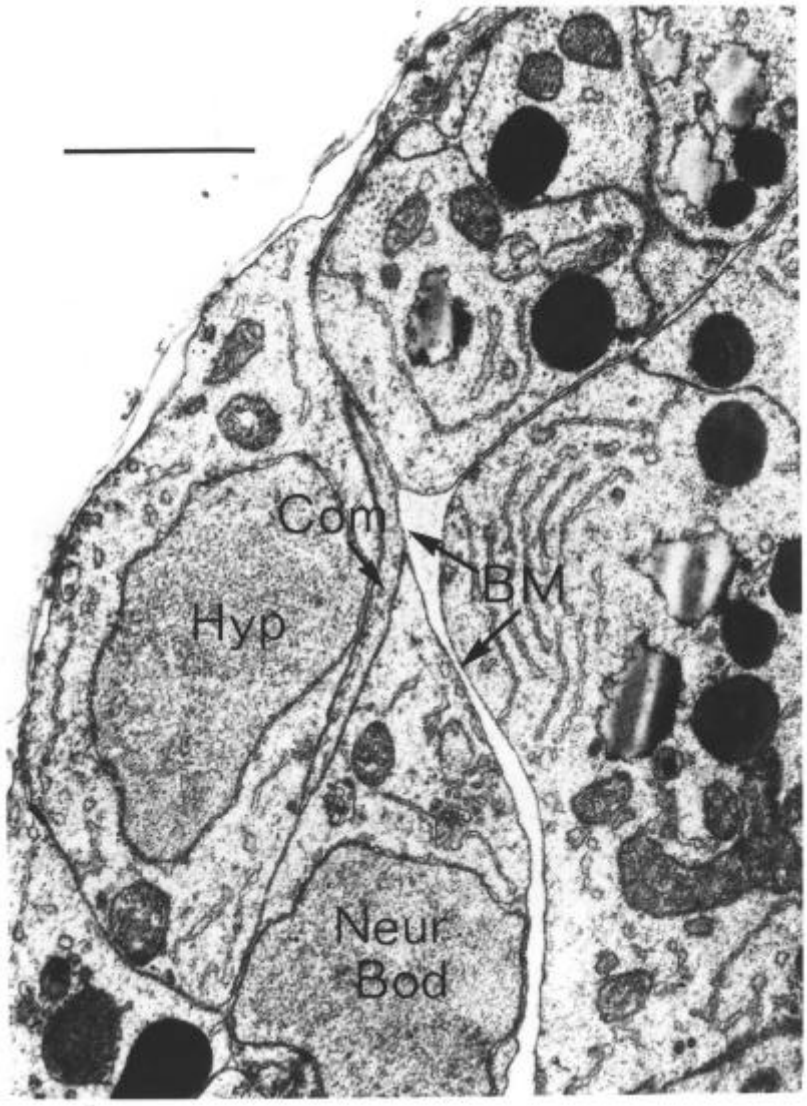


Figure 3.3

The growing DB5 commissure (Com) is forced to choose whether to pass the lateral neuron body of CANL (Neur Bod) on the side of the hypodermis (Hyp) or on the side of the basement membrane (BM). It passes on the hypodermal side, as do all motor neuron commissures in similar situations. This suggests that commissural growth cones attach to and move over cell surfaces rather than basement membrane. From the C reconstruction. Scale is 1 micron.



3.2 The attachment substrate for growth cones

Growth cones have been seen in vitro to extend very well over artifical substrates made of basement membrane components, such as fibronectin and laminin (Baron van Evercooren et al., 1982). This has led to the suggestion that basement membrane may provide a favoured substrate for growth cones to grow over. It is possible in at least one case in C. elegans to determine the substrate on which the growth cone moves. The motor neuron commissures grow out sandwiched between hypodermal cells and the basement membrane. There are several lateral neuronal cells that also lie between the hypodermis and the basement membrane, in the way of the growing commissures. Whenever a commissural growth cones reaches a lateral cell body it leaves the basement membrane and passes between the hypodermis and the lateral neuron (figure 3.3). There has never been observed an exception to this rule. Thus it seems that the growth substrate for these commissures is the surface of hypodermal cells, not the basement membrane.

A couple of similar results are provided by ablation experiments (Chapter 4) in which in one case DD5 moves from the right side of the cord to the left (after removing AVG), and in another case PVQL moves from the left to the right (after removing PVPR). In each case the process changing sides passes under a motor neuron cell body, rather than over it, again maintaining contact with the hypodermis rather than the basement membrane. There are many other examples where processes grow between cell bodies and other processes, well removed from the ectodermal basement membrane. During later development several posteembryonic processes grow the length of the ventral cord in the middle of the main bundle of embryonic processes. The embryonic reconstructions presented here show processes from the lumbar ganglia growing forward through the middle of the cluster of cell bodies in the preanal ganglion. In general wherever there is evidence on the subject of neuronal growth cone guidance in C. elegans it suggests that the substrate for growth is the surface of other cells, rather than a basement membrane. However this does not rule out the possibility that the basement membrane is important in certain cases.

3.3 AVG pioneers the ventral cord

The first nerve process to grow along the ventral cord belongs to the interneuron AVG. AVG has its cell body in the retro-vesicular ganglion at the front of the ventral cord; it is an unpaired cell, and is the most posterior interneuron in the front of the animal to send a process back along the cord. The cell body and process were identified in both the A and and B reconstructions. By the A reconstruction the process has already grown back along the cord. At this stage the DD ventral cord processeshave also grown out on the right side of the cord (figure 3.4). However inspection of a younger embryo revealed that there was a single continual process in the ventral cord at a stage at which DD processes had not grown out (not shown). In the B reconstruction the AVG process grows the full length of the cord and up out of the pre-anal ganglion into the dorso-rectal ganglion, where it stops by the DVC cell body. IT reaches no further than the DRG in all the latter embryonic reconstructions (B to D), although in the adult it is seen to extend right back into the tail spike (White et al., 1986). Therefore there must be a second period of extension postembryonically or during late embryogenesis.

3.4 Motor neurons

The next event after the appearance of AVG is the growth of processes from the DD motor neurons forward alongside the AVG process on the right side of the ventral cord. In the A reconstruction these extend until they nearly touch the next DD cell body (figure 3.4). In the adult, adjacent DD neurons overlap for a short stretch and are linked by gap junctions, but in all the embryonic reconstructions there are small spaces between them of the order of a micron in length (figures 3.4, 3.5). It is of course possible that contact has been made and the processes subsequently withdrawn. Another possible correlate of DD extension in the ventral cord, discussed further later, is that all the DD ends of DD processes are by DB cell bodies. Ventral cord processes from the DA and DB motor neurons do not grow out until later, after the commissures and dorsal cord processes are made.

The first signs of commissure outgrowth can also be seen in the A reconstruction. All the motor neurons have lamellar extensions poking laterally under the ventral musculature at the site where their commissural growth cones will leave the ventral cord (figure 3.4). These nascent growth cones leave from the DA and DB cell bodies, and from near the anterior end of DD axons. Outgrowth of commissures from the motor neurons of all classes is synchronous; in the B series, only about 20 minutes older than the A series, they have all reached the lateral hypodermis, and in the C series they are just about to reach the dorsal hypodermal ridge (figure 3.4). RID, the only process to grow along the full length of the dorsal cord, is not present at this time, and the commissures apparently turn of their own accord, DA ones anteriorly, DB posteriorly, and DD in both directions, and link up to form the dorsal cord. Although they are reproducible, there is no regular anterior/posterior order to the positions of the commissures from the different classes of cells (figures 1.3, 3.5), so the direction in which they turn cannot be simply determined on the basis of the classes of neighbouring processes (e.g. DA's and DB's towards each other). Since the dorsal hypodermis is syncytial and does not contain apparent landmarks it seems that the direction must be intrinsically determined. In addition, if we assume that the direction is determined in the same fashion for all the members of a class, then it must be specified in terms of the anterior and posterior of the animal, rather than whether to turn left or right, since some of the DB axons go round the left hand side of the body and turn right when they reach the dorsal midline, while others go round the right side and turn left.

Figure 3.4

Schematic diagrams of the part of the body indicated in figure 3.1 from the A, B, C and D reconstructions (1), b), c) and d) respectively). The diagrams are cylindrical projections in the same basic form as figure 1.3, with the positions of the muscle bands being shown as hatched areas. The DA3, DB4 and DD2 cell bodies and the DA3, DB4 and DD3 commissures are shown in each case. Initially only the AVG and DD processes in the ventral cord are present (a). Then the commissures grow out simultaneously from all three motor neuron classes (b, c), followed eventually by the ventral cord processes of DA and DB motor neurons and other ventral cord interneurons (d). ALM and CAN are lateral neurons, which migrate back from the front and then send processes forward.

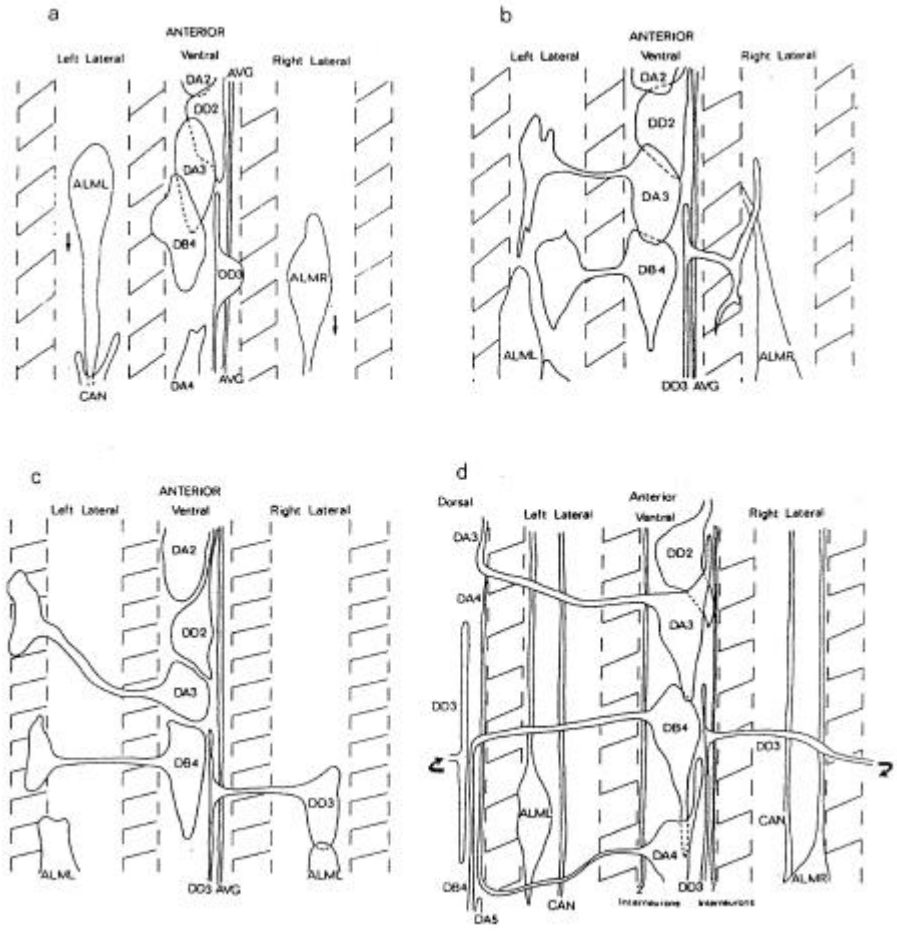
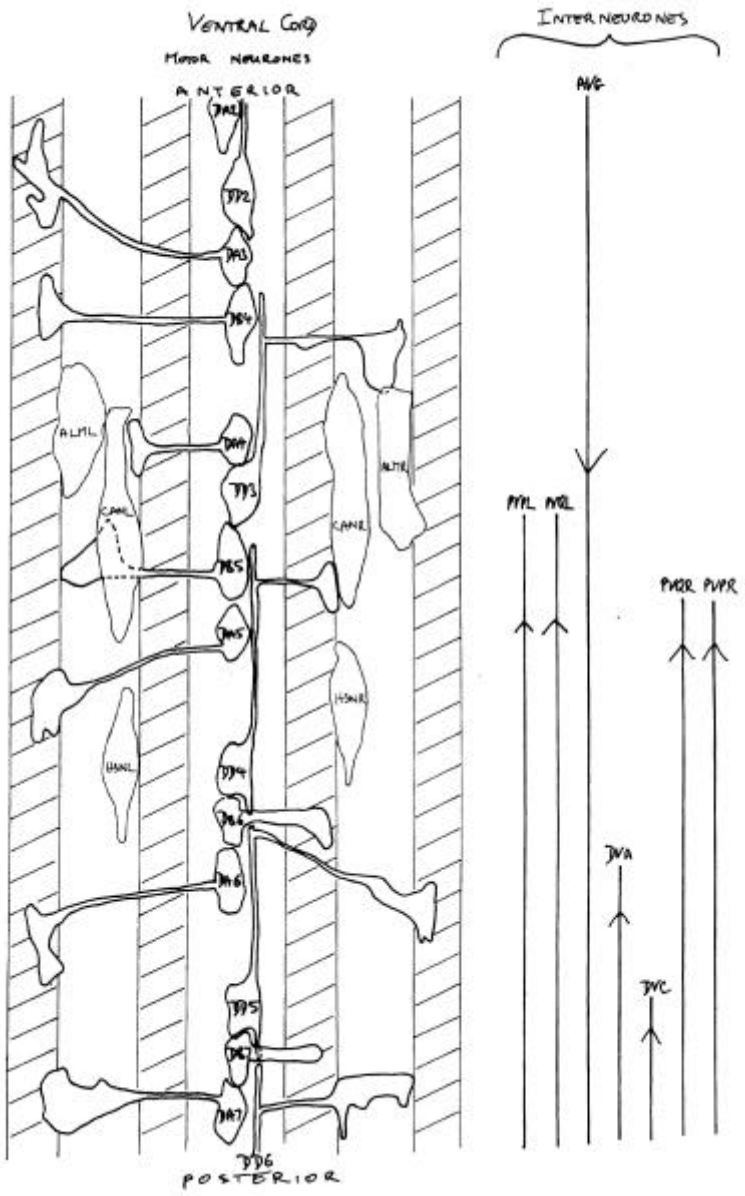


Figure 3.5

A schematic diagram of the entire ventral cord region from the C reconstruction. The motor neurons and lateral neurons are shown on the left in the same form as in figure 3.4. (HSNL/R are postembryonic neurons that grow out processes in the L4 larva to innervate vulval muscles). The positions reached by all the interneurons that have grown substantially into the ventral cord are shown on the right. In addition to the PVT, PVCL and PVCR processes have also grown just past the front of the preanal ganglion in this reconstruction.



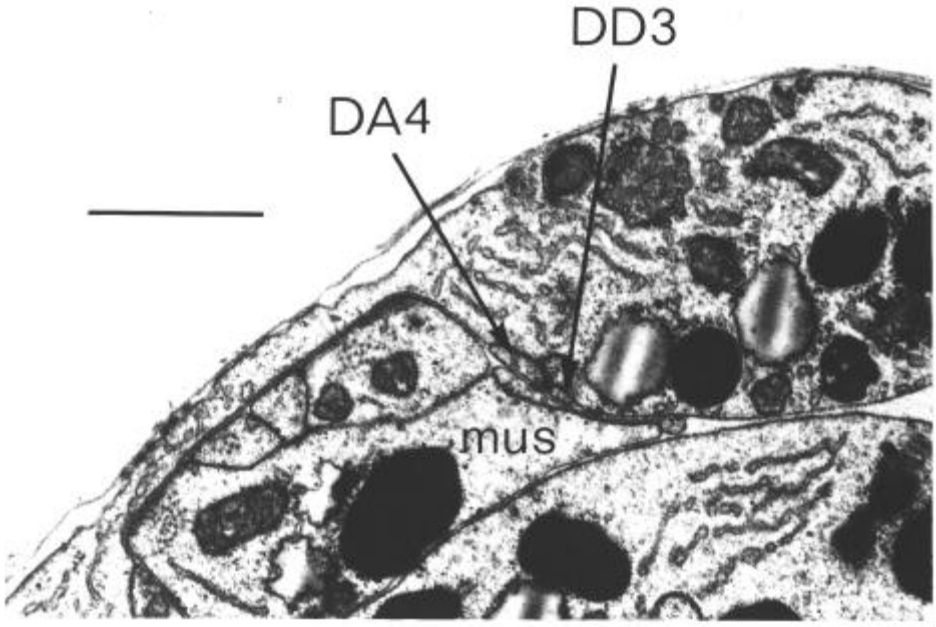
There appears to be a possible correlation between the position of DA commissures and the location of hypodermal cell boundaries. The embryonic ventral hypodermis consists of 6 left/right pairs of cells, known as P cells, which are joined at front and back to the main body hypodermal syncytium, called hyp7. DA3 to DA7 lie on the boundaries between adjacent P cells, and they send solitary commissures to the left directly from their cell bodies out along the P/P cell boundary. In contrast DA2, which is on the boundary between the most anterior P cells and hyp7, sends its commissure forward and out to the right together with those of DB3 and DD2. Similarly DA1, DA8 and DA9, none of which are near a P/P cell boundary, send their commissures together with processes from other cells (DB2 and DD1 in the case of DA1, the lumbar commissures for DA8 and DA9).
There are no corresponding visible cues for DB commissure guidance, and only weak ones for DD commissures. One possibility is that DB cells are involved; DD commissures all leave the cord from approximately opposite DB cells (figure 3.5, note especially DD3, whose commissure exit point is quite a long way behind the DD2 cell body, but opposite DB4). It is hard to tell whether a DD commissure is created by diversion of the growth cone that generated the ventral cord process, or by a genuine branching. The commissure always comes from near the anterior tip of the ventral cord process, the extension of which is essentially complete when the commissure starts growing, suggesting that only a single growth cone is used. However there is a definite T junction in the final structure, and the DD process can make branches, since one is certainly made when the commissure reaches the dorsal cord (figure 1.3). The side of the animal that the DD commissures go round is easier to explain. They all go round the right side of the animal, which is consistent with the position on the right side of the cord of their ventral cord processes, from which the commissures diverge or branch.

It is only after the dorsal cord processes have extended for some distance that we begin to see growth of the ventral cord dendrites from DA and DB neurons, (D reconsruction, figure 3.4). This coincides with the growth back along the cord of some of the ring interneurons, possibley including the motor circuitry interneurons that innervate the DA and DB ventral cord processes. However there are no visible synaptic connections between the interneurons and the growing DA and DB dendrites.

The dorsal cord, in the other hand, does show signs of synaptic activity in the D reconstruction. The DA and DB neurons are already making small, but clear, neuromuscular junctions from their dorsal cord axons (figure 3.6). As in the final adult version these involve a joint synapse onto muscle and a DD process. No corresponding DD neuromuscular junctions in the ventral cord have been seen in this reconstruction. The dorsal cord processes have not reached their final length in the D reconstruction, and in fact are at a rather interesting stage: each DA axon stops where the next one arrives at the dorsal cord and turns. Later the axons overlap considerably, but the regions of neuromuscular output do not; instead they correspond closely to the regions where the axons are present at this stage. It is possible to speculate that there is a pause in axon extension while the zones of neuromuscular activity are being set up, but more data would be required to provide respectable evidence!

Figure 3.6

A neuromuscular junction from the DD reconstruction. Although small this shows all the characteristics of normal neuromuscular junctions in the developed nervous system. The dorsal cord process of the motor neuron DA4 is synapsing jointly onto muscle (mus) and the DD3 process. There is also a DB4 process present in this section. Scale bar is 1 micron.



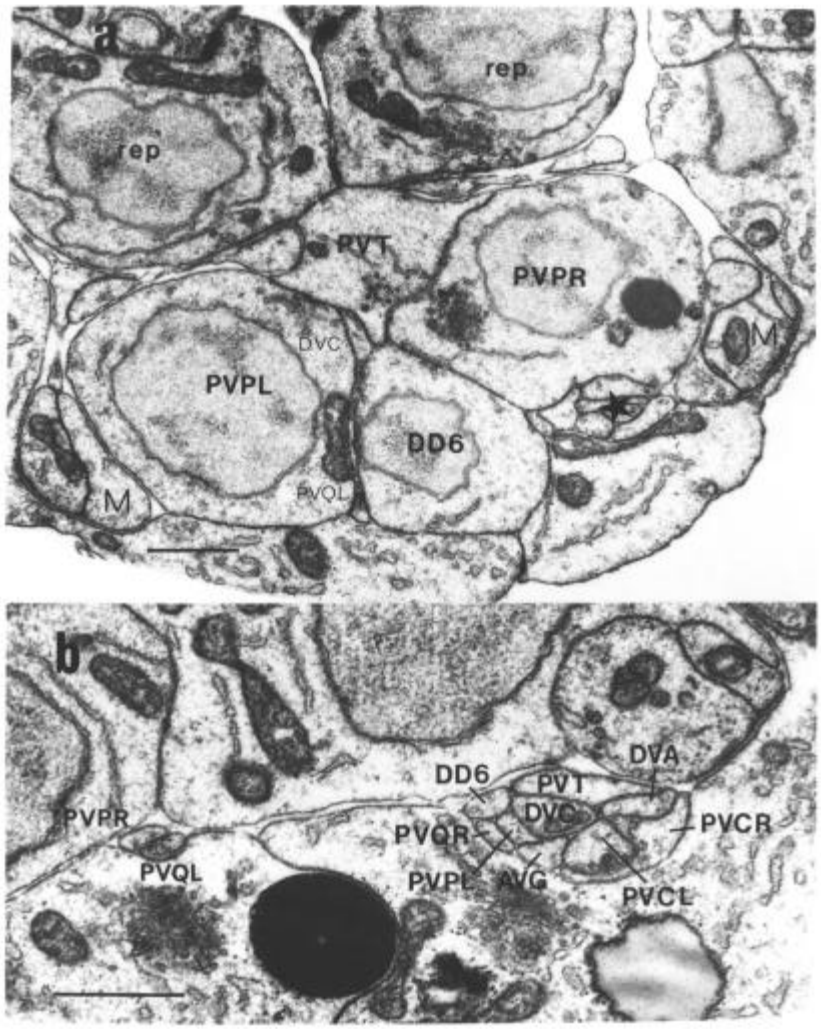
3.5 Later ventral cord interneurons

While the motor neuron commissures are growing out, a set of interneurons are growing forward along the ventral cord from the pre-anal ganglion (PAG) at the back (figure 3.5). The most advanced of these are two pairs of processes, PVPR and PVQL on the left side of the ventral cord and PVPL and PVQR on the right side. These are followed by DVA and DVC, which are both unpaired neurons that run on the right. Figure 3.7 shows a cross section of the posterior cord from the C reconstruction, after these posterior neurons have reached the front of the cord, that we see other anterior interneurons growing back along the cord (figure 3.4).
PVP and PVQ: The PVQ neurons are the most anterior cells in the lumbar ganglia (PVQL in the left lumbar ganglion, PVQR in the right, figure 1.3); their processes descend the lumbar commissures and then run forward through the PAG and along the cord. PVPR and PVPL have cell bodies in the PAG, where they are the only bilateral pair of interneurons. Their processes cross over when they leave the bodies, joining up with the PVQ process on the opposite side, and then run forward along the cord. The structure of PVP decussation is discussd below together with that of three pairs of neurons in the retrovesicular ganglion.

PVQL and PVPR pioneer the left hand ventral cord; this appears to be a joint action, since their anterior tips are never more than a few tenths of a micron apart in any of the reconstructions (e.g. figure 3.5). The tips of the PVQR and PVPL are similarly close to each other, but there is no such tight relationship between the left hand pair and the right hand pair (figure 3.5). The fact that the left hand pair are often more advanced than the right hand pair suggests that the prior presence of AVG and DD processes on the right side of the cord has little effect on PVPL and PVQR outgrowth. The two processes in each pair are tightly associated all the way along the cord back to the point where the PVP processes cross over in the PAG. This association is also seen in the adult reconstructions of the ventral cord; the processes diverge only when they reach the nerve ring (White et al., 1986, unpublished data). Together these observations suggest that PVP and PVQ growth in the ventral cord might be cooperative, and a number of ablation experiments were performed to test this hypothesis (Chapter 4). In general the PVP process is on top of the PVQ process, i.e. there is a ventral to dorsal order of: hypodermis, PVQ, PVP, basement membrane (figure 3.7).
DVA and DVC: DVA and DVC are the two embryonic neurons with cell bodies in the dorso-rectal ganglion, above the rectum (1.3). They both grow forward along the right hand side of the cord behind PVPL and PVQR, but their tips are not close together like those of a PVP/PVQ pair. DVA grows down around the right side of the rectum back along the track of AVG, and in all cases keeps to the outside of the main right hand bundle of processes in the ventral part of the PAG (figure 3.7). DVC, on the other hand, grows down the left side of the rectum and crosses from dorsal left to ventral right, in the same place that two PVP processes cross over (3.8). Neither DVA nor DVC appear to be particularly tightly associated with any other process in the ventral cord. In the adult cord DVA is always at the ventral right hand exremity of the main right hand bundle, whilst DVC runs in the middle of the bundle, in association with DVB, which is the postembryonic dorso-rectal ganglion neuron (White et al., 1986). Other lumbar

Figure 3.7

Typical transverse sections through (a) the preanal ganglion and (b) the back of the ventral cord (from the C reconstruction). In (a) the star indicates the main group of processes that will become the right hand ventral cord. The other cells and processes are individually labelled. This section comes from just posterior to the point where the PVP processes and DVC will cross over (shown in figure 3.8). In (b) the independent growth of PVPR and PVQL along the left hand cord is clear. This section comes from very close the the preanal ganglion, and the PVCL/R and PVT processes are near their front tips, and are a little swollen, particularly PVCR, which is showing signs of wrapping around other processes. Scale bar is 1 micron in each case.



commissure processes: In addition to the DA8, DA9 and PVQ processes the lumbar commissures contain processes descending into the pre-anal ganglion from the following lumbar ganglion cells: PHAL/R, PHBL/R, LUAL/R, PVCL/R and PVR. Of these the PVC cells and PVR eventually grow the full length of the cord; the others stop at the front of the pre-anal ganglion. There is a very characteristic pattern at the back of the pre-anal ganglion where the lumbar processes from the two sides meet, which has been seen whenever the region has been reconstructed (figure 3.9). The processes from each side meet slightly to the right of the midline and "zip up", each contacting its contralateral analogue. The exceptions to this rule are the PVQ processes, which are at the top of the row one each side but stay at opposite corners of the structure and do not make contact. The dorsal to bentral order of this structure is PVQ, PHA, PHB, PVC, with the unpaired process of PVR wrapping around the ventral side of the whole group. This suggests that the contralateral pairs other than the PVQ's have a strong affinity for each other, and it is probably significant that the PVQ's are the only lumbar processes to run up the cord with one process on each side of the cord, as opposed to the more normal pattern of both processes being on the right. Anterior interneurons: Anterior interneurons other than the AVG are only seen growing back along the cord in the D reconstruction. As described in the section on cell identification in Chapter 2 it is not possible to identify these processes. However it is clear that there are at least PVPR and PVQL on the left side. There is one is one process growing part way back along the cord on the left, which may be RMEV or AVKR, and there are 11 interneurons on the right, some of which probably come from the back (e.g. PVPL and PVQR), but others of which are from the front; in particular 4 from the front stop within the anterior D reconstruction. The anterior interneurons seen her may include (some of) the major interneurons that innervate the ventral cord motor neurons.

3.6 Descussation in the preanal and retrovesicular ganglia

The PVP processes cross over in the pre-anal ganglion where they leave their cell bodies and then grow forward on the opposite side of the cord. This crossing over is at the back of PVT and above DD6, at the same place that the DVC process crosses from left to right. PVQL, DVC and, to a lesser extent, PVQR flatten out on the surface of DD6; DVC is always between the PVP crossover and DD6 (figure 3.8). The PVP cross over was observed in the adult reconstruction (White et al, 1986), but it is much less clear there since there are extra cells in the adult preanal ganglion (6 postembryonic motor neurons) and the arrangement of cells and processes is much less well organised. This loss of symmetry and order is already visible in the difference between the B and D reconsructions. In the B reconstruction the cells in the left/right pairs (PVPL/R and DA8/9) are nearly opposite one another, while in the D recosntruction there is a definite tilt to each pair and the whole preanal ganglion is becoming more linear. This is probably caused by a lateral constriction due to elongation of the embryo and an increase in the amount of space taken up by the muscle cells as they mature. In athe adult the symmetry and order present in the early embryo when processes first grow out is almost entirely lost.

Figure 3.8

The crossover of PVP processes in the preanal ganglion (from the C reconstruction). (a) to (d) are a posterior to anterior series, each being separated from the next by about 0.5 microns. (e) to (h) are tracings of (a) to (d) showing the positions of significant processes. The sequence of events is as follows: at the front of its cell body (a) PVPR sends a process across to the left side (b), in front of which DVC and PVPL cross from left to right (c, d). The PVPL process invariably crosses in front of the PVPR process. Scale bar is 1 micron.

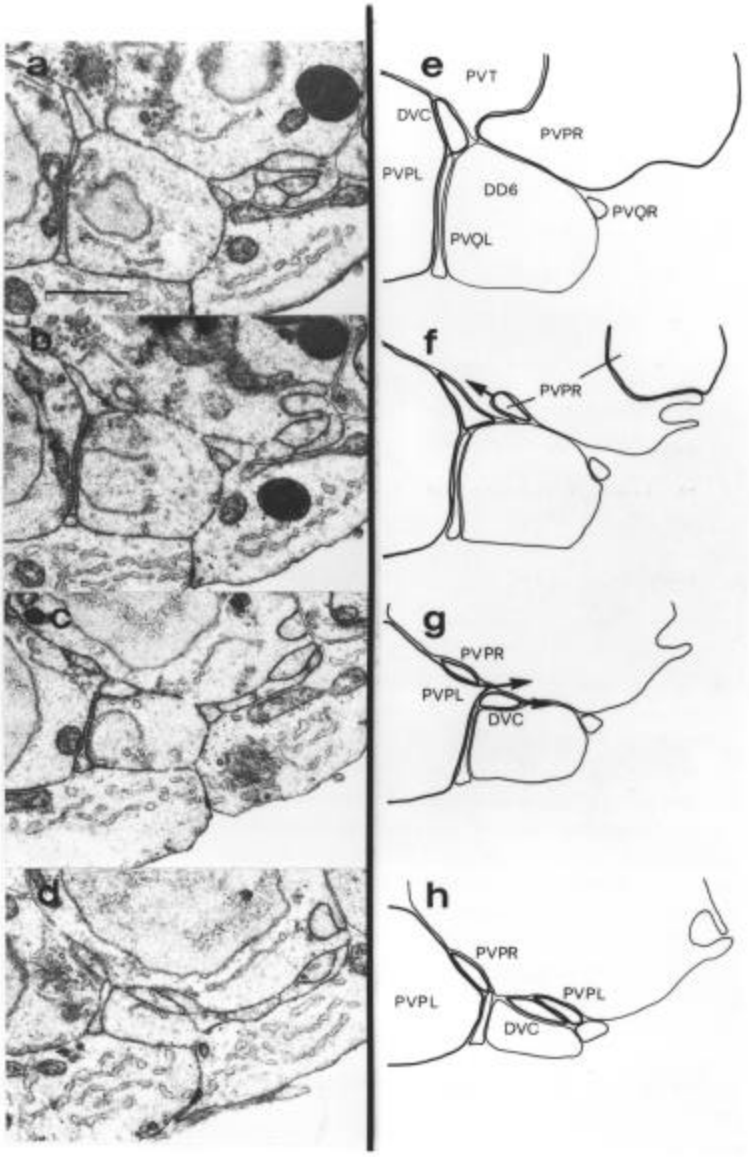
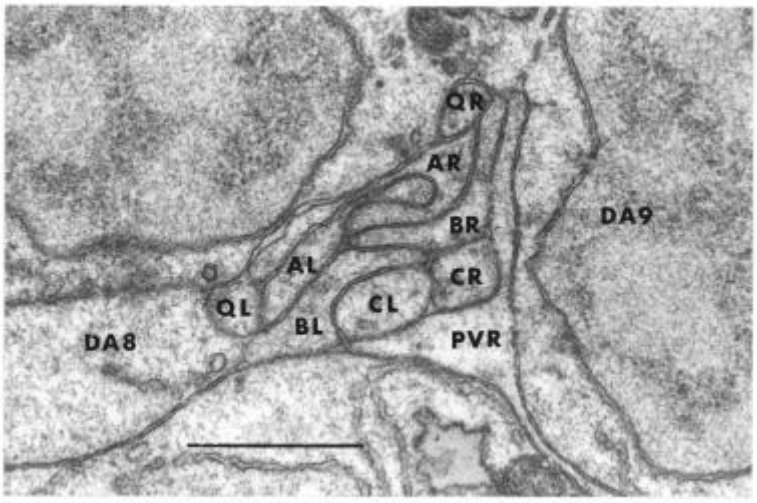


Figure 3.9

The place at the back of the preanal ganglion where the two lumbar commissures meet (from the C reconstruction). All the processes are labelled: QL/R are PVQL/R, AL/R are PHAL/R, BL/R are PHBL/R, CL/R are PVCL/R, and PVR, DA8 and DA9 are all the correct full names. The PHA, PHB and PVC processes all line up against each other, to some extent wrapping around their partners and thus increasing the area of contact (particularly the PHA and thus increasing the area of contact (particularly the PHA processes). In contrast the PVQ processes appear to have no mutual affinity. More anterior to this, the PHA, PHB and PVC processes all grow along the right side together, while the PVQ processes split, one growing on he left and one on the right. Scale bar is 1 micron.



A phenomenon equivalent to the PVP crossover is seen in the retrovesicular ganglion at the front of the ventral cord, which is once again more symmetrical in the embryo than in the adult. There are three bilateral pairs of neurons in the embryonic retrovesicular ganglion: RIGL/R, RIFL/R and SABVL/R, all of whose processes run forward. The RIF processes have grown out in the A and B reconstructions and once again they are seen to cross over by their cell bodies (figure 3.10). Following this observation, careful comparison of the positions of cell bodies in embryonic reconstructions and the embryonic lineage study (Sulston et al., 1983) with those in the adult reconstructions (White et al., 1986) confirmed that the RIF processes did indeed cross over in the adult reconstructions, and that the RIG and SABV processes do the same. The Processes from all three classes cross in nearly the same place in the adult reconstruction, by the SABV cell bodies. The SABV processes are just beginning to grow out and cross in the B reconstruction (figure 3.10), but the RIG processes have not yet grown out.

Therefore crossing over is seen in all the embryonic left/right pairs of interneurons in what might be termed the extended ventral cord, i.e. everything on the ventral hypodermal ridge between the excretory duct and the anus. During postembryonic development another interneuron pair, AVFL/R, is added in the retrovesicular ganglion, but their processes are not bilaterally symmetrical; they are bipolar, running back together down the right side of the ventral cord and forward also together to the left of the excretory duct and round the left side of the nerve ring.

The crossing over, or decussation, of processes to the opposite side of the body from the soma is a property of many nerve types in higher animals, and the cases observed here may provide extremely simple examples of the same event that are susceptible to experimental manipulation of both the cells involved and of their environment. Several cell ablation experiments were performed to investigate factors involved in the PVP cross over in the preanal ganglion (Chapter 4).

3.7 Growth cone insertions into other cells

An observation previously made in other animals is the insertion of thin processes from growth cones into other neuronal processes or target tissues that might be important for guidance (Bastiani and Goodman, 1983). The same phenomeno9n has been observed in the developing C. elegans nervous system, and there are two cases in particular where it is especially noticeable and correlates with possible guidance decision taking.

The first case is when the motor neuron commissures reach the dorsal midline. In one reconstruction (wild type but not on of A to D), a single commissure has just reached the dorsal hypodermal ridge, on emerging from underneath the left dorsal muscle quadrant. The growing tip of this commissure inserts two stubby finger-like processes about 0.1 – 0.2 microns in diameter and 0.4 – 0.8 microns long into the dorsal hypodermal ridge (figure 3.11). It is at this stage that the growth cone must turn through a right angle and grow along the ridge.

Figure 3.10

The decussation in the retrovesicular ganglion (from the B series). The RIF processes have both just crossed over posterior to this section, and we can see the back of the SABVR cell body and its process also thrusting across the midline. In contrast to the preanal ganglion decussation here processes cross on the surface of the neuropil. Scale bar is 1 micron.

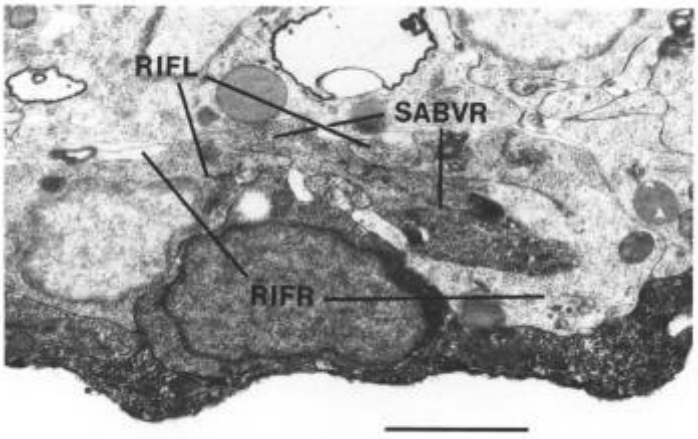


Figure 3.11

A finger from the DA6 commissural growth cone inserting into the dorsal hypodermal ridge as it comes out from under the left dorsal muscle quadrant. The particles to the left of the finger are not vesicles (determined by viewing at higher resolution).

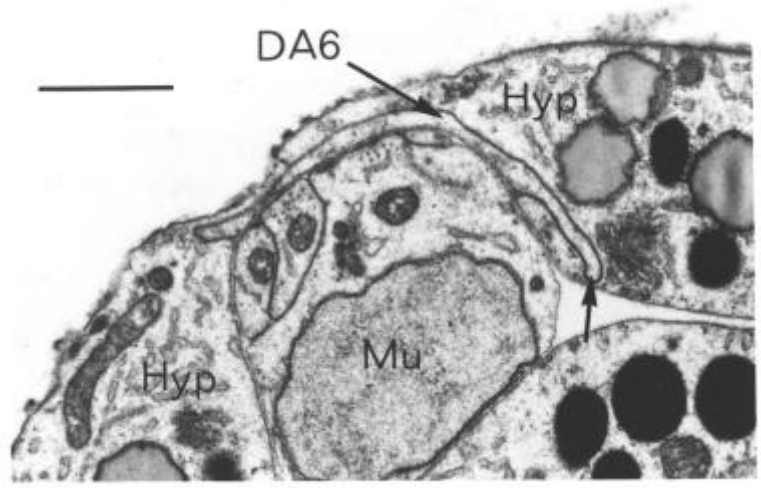
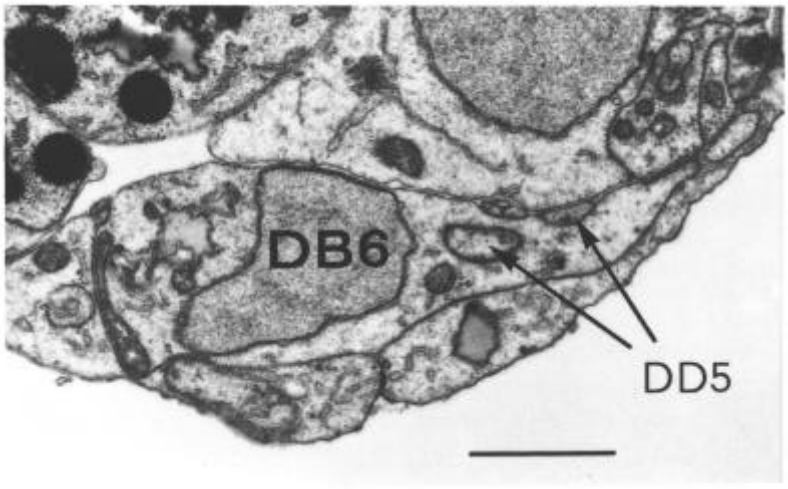


Figure 3.12

An insertion of an extension at the front of the DD5 ventral cord process into the cell body of DB6. DD5 stops growing forward along the cord at about the point that it reaches DB6. Both DD5 and DB6 send commissures out to the right around the same place. A part of the DB6 commissure can be seen. From the C construction. Scale B 1 micron.



The second case concerns what happens when DD processes meet DB cell bodies. In all cases that have been reconstructed the anterior tips of DD6 and DD5 insert into the bodies of DB7 and DB6 respectively. Insertion of DD4, DD3 and DD2 into DB5, DB4 and DB3 also takes place but less frequently and in a less pronounced fashion. An example of DD5 insertion into DB6 is shown in (figure 3.12). These DD insertions into DB cells reinforce the suggestion made above that DB cells might be involved in DD morphology. Where the insertions are most pronounced (DB6 and DB7), the DB commissures grow to the right next to the DD commissures.

There are occasional other insertions into the ventral hypodermis from growth cones of processes growing along the ventral cord (data not shown), but no particular pattern is discernible. Also I have seen no cytological correlates of the insertions, such as vesicles clustering around the insertion in the cell into which the insertion is made, which have been seen in the corresponding phenomenon in insects (Bastiani and Goodman, 1983).