CHAPTER 5 Discussion

The last two chapters have described the results of a number of electron microscope reconstructions of the developing ventral nervous system in both normal and experimental C. elegans embryos. In a short space of around an hour the first nerve process grows back along the ventral cord from the front, the motor neurons in the ventral cord grow commissures around the body of the animal to form the dorsal cord, and a number of additional processes grow forward from the preanal ganglion at the back of the animal. The short time taken in laying down the skeleton of the ventral nerve cord and preanal ganglion reflects the rapid development of C. elegans embryos (13 hours total). The small number of cells present, and the simple morphologies of the nerve cells, allow precise suggestions to be made about the roles of individual cells during process outgrowth. Several possible intercellular interactions were investigated by killing the parents of specific cells with a focussed laser beam. Before discussing the pattern of process outgrowth in the ventral nervous system, and how it might be controlled, I will first consider the reliability of the observations on which the work is based.

5.1 Reliability

The approach of reconstruction from serial electron micrographs precludes the examination of a large number of individual animals, either in the wild type time series or in any particular experiment. It is reasonable to ask whether reliable conclusions can be drawn from the necessarily small number of reconstructed animals that have been presented: there are two possible sources of error or variation: experimental "noise" created by variability in the observational and experimental techniques, and natural variability of the phenomena themselves.

As far as the determination of process disposition is concerned the technique is very reliable; each individual reconstruction provides a large amount of information at a very fine level of detail, so that essentially all the nerve processes present can be positively identified and a complete picture of the relevant parts of each neuron determined (the exceptions are discussed in Chapter 2). The technique of laser ablation of individual identified cells is also very specific. It is unlikely that the killed cell has any residual influence, because, except for the DVC parent ablation, when no subsequent change was seen in any other cells anyway, the dead cell was observed to be excluded from the embryo when the hypodermis closed up (figure 2.2). It is in principle possible to damage neighbouring cells at the time of ablation, and in fact one of the embryos sectioned in the DD3/DD5 set showed signs of general morphological disorganisation, presumably due to such damage. However in all the cases discussed, except the AVG experiments, any changes that were observed were confined to a small number of neurons normally associated with the particular missing cell. Although control experiments in which random neighbouring cells were ablated were not performed, altogether six different cells all near together on the ventral surface of the 270 minute embryo (figure 2.1) were ablated without there being any overlap in the observed consequences.

As regards intrinsic variation, all the reconstructions are consistent with a fixed time sequence of normal axonal outgrowth. When a change in this pattern was seen in laser ablation experiments then, again excepting the AVG experiments, it was clean

and restricted in its extent, was generally observed in at least two cases, and was consistent, never being seen in one case but not another. The situation with respect to the removal of AVG appeared to show variability and is discussed more fully in the next section. However, taking all the results together and in conjunction with the known fixed adult anatomy, there is sufficient evidence to indicate that the developing C. elegans nervous system is simple and reproducible enough for the techniques used here to provide an accurate picture of events.

The high level of reproducibility and the generally restricted, fixed effect of removal of individual cells are typical of C. elegans development and anatomy. The cell lineage and the disposition of somatic cells at all stages of development are known to be nearly invariant (Sulston and Horvitz, 1979, Kimble and Hirsh, 1979, Sulston et al., 1983) the final antomy is equally stereotyped (White et al., 1986). Although a number of cases of adjustment in cell lineage after individual cell ablations are known (e.g. Sulston and White, 1980, Sulston et al., 1983) they are the exception rather than the rule, and in no case do they result in complete regulation back to the native form. All the results of ablation experiments performed here are consistent with only the daughters of the ablated cell being missing, and with there being no change of identity of any other cell. In addition they confirm the relevant cell assignments in the embryonic lineage, since in each case only the expected cell or cells was or were missing.

5.2 The asymmetry of the ventral cord

One of the striking features of the C. elegans ventral nervous system is the almost, but not quite, complete asymmetry of the ventral nervous cord, which has around 55 neurons on the right side and only 4 or so on the left. If all the processes were together on the right hand side then it could be regarded as a single fused nerve that was displaced to one side for steric reasons, but since a small number of left/right pairs of processes are arranged symmetrically (PVQ, PVP, AVK and in the adult, HSN) the question arises of why not all the others? In fact the arrangement is essentially symmetrical anterior to the RVG; the cord splits into two to pass the excretory duct on both sides, with each bilateral pair of processes being split so there is one member on each side, an stays symmetrical throughout the ventral ganglion and into the bottom of the nerve ring.

Most animals with a symmetrical body plan have a symmetrical ventral nervous system, often consisting of a chain of ganglia linked by paired nerves, which are sometimes fused but clearly retain their symmetrical character. There are in fact some nematodes that have symmetrical paired ventral cords (Martini, 1916). Chitwood and Chitwood (1974), in discussing the differences amongst nematode species, state (p. 162):

Differences in the central nervous system lie chiefly in the degree of subdivision of the lateral ganglia, the form of the ventral ganglia, and the degree of fusion of the ventral nerves.

They go on to state that in many species both around the RVG and for some distance anterior to the PAG there are symmetrical paired nerves, though in most cases these are fused for the main part of the length of the body. They continue (p. 163):

The apparent doubleness in both anterior and posterior ends of the ventral nerve caused Meissner and many later authors to conclude that the entire nerve was at one time double. … (we) subscribe to the primitive double ventral nerve hypothesis.

Several observations that have been made in this study are relevant to the origin of cord asymmetry. Perhaps I should start with AVG. AVG is a unique neuron with its body in the RVG, it is the first neuron to send a process out along the ventral cord, and it sends it along the right hand side. When AVG was removed by ablating its parent the cord was seen to be disrupted in two ways.

First, as seen most clearly in the embryonic AVG reconstruction, the organisation of the embryonic ventral cord motor neurons was disturbed. In particular a DD process was seen to switch across to the left side of the cord and send its commissure round to the left rather than the right, and the DA and DB cells near this point also sent their commissures round the opposite side to normal. The switch of the DD process to the left cord confirms that AVG must normally grow out before the DD ventral cord processes. In the adult AVG reconstruction the DB3 and DD2 cells show abnormal process organisation. These effects would seem to be a direct consequence of the absence of AVG, because the outgrowth of DD processes and motor neuron commissures follow directly after the outgrowth of AVG. The postembryonic motor neurons do not seem so badly affected as the embryonic neurons, although the VD3 process in the adult reconstruction is switched from being on the right side to the left.

The second effect of removal of AVG, seen in the adult reconstruction, is a general disorganisation of the cord in which instead of a large ordered bundle on the right side and a very small one on the left there are several intermediate sized bundles at various positions on the left and right sides (figure 4.1). This indicates that AVG is ultimately necessary for correct organisation of the interneurons as well as motor neurons, whose disarray appears earlier. However AVG does not seem to be necessary for outgrowth of processes, since the total number of processes in a cross section of the experimental adult cord is within the expected range, and all the fully reconstructed cells send out processes in the correct direction, if not on the correct side.

It is also clear that AVG is not the sole determining influence for the left/right organisation of the ventral cord, because in the embryonic experimental reconstruction all the early interneurons from the back were growing forward correctly (PVQR, PVPL, DVA and DVC on the right, and PVQL and PVPR on the left). Also in the adult AVG reconstruction the majority of processes was at all times on the right, including the motor control interneurons (AVAL/R, AVBL/R, AVDL/R and AVEL/R). In the only positively identified case of interneurons growing on the wrong side, both AVF's were seen to grow on the left (they are normally both on the right, figure 4.1).

The fact that removal of AVG leads to no major behavioural defect suggests that it has no critical function of its own. In the adult reconstruction, although it is a fairly

large cell, it has been seen to make very few connections to other neurons, the only consistent ones being large gap junctions to the two RIF interneurons and a small amount of synaptic input from the PHA phasmid neurons (probably chemosensory) (White et al., 1986). It has been postulated to be a sensory receptor itself on the basis of its adult extension beyond the dorsorectal ganglion into the tail, although no ultrastructural specialisation is seen there (ibid.). One might instead speculate that its main function is developmental. If one considers that it is just as important for a nervous system to be able to build itself as to function correctly in the end, it makes sense that there be selective pressure for neurons important in development even if they serve little or no purpose in the final circuitry. Another candidate for such a cell in the C. elegans nervous system is PVT. This is a large cell demarcating the front of the preanal ganglion and forming the most anterior link between the rectal epithelium and the ventral ectoderm, which has no observed synaptic output and only a couple of possible inputs. However no experiments have been performed to test the suggestion that it too may be primarily involved in developmental organisation. Of course one should beware of suggesting that every neuron must have a major function; it is quite likely that there are also redundant cells present that are not particularly important at any time.

The disarray seen in the ventral cord of the adult AVG reconstruction is very reminiscent of that seen in a reconstruction of a mutant in the gene unc-3 (e151) (figure 4.1, J G White, E Southgate and N Thomson, unpublished results). In that case too there were several subbundles, looking very similar to those of the AVG reconstruction; the majority of processes were on the right, including the identifidable cluster of major motor interneurons which again retained their internal organisation, but not their relative position in the bundle. The defect appears to be restricted to the ventral cord since the nerve ring was correctly organised according to several electron mircoscopic criteria, bu the phenotype of unc-3 mutants is much more severe than that after ablation of the AVG parent, and indeed in the reconstruction of the mutant it appeared that some postembryonic motor neurons might be missing or not properly made.

There are two other uncoordianted genes for which mutants show relevant defects. The DD and VD commissures can be visualised by immunocytochemical staining with antibodies against the neurotransmitter GABA (helping to confirm that the DD and VD classes are probably GABAergic and inhibitory); they normally all grow to the right. However in mutants for unc-71(e451) and unc-73(e936) a significant proportion of the commissures grow round the left side of the animal (25% and 35% respectively; S McIntire, pers. Comm.). The ventral cord is also seen to be disorganised, in that in some places in the cord the VD and DD processes, which normally run so close together that they are inseparable by light microscopy, are clearly separated. It would be interesting to see what happens in early ventral cord development, particularly to AVG, in all of these mutants.

The suggestion derived from the reconstructions of the adult AVG animal and the unc-3 mutant that left/right pairs of processes tend to stick together may be significant. When the lumbar neuronal processes meet in the PAG at the bottom of the lumbar commissures they “zip” together, each process in contact with its homologue, except for PVQL/R which remain apart (figure 3.9). Eventually PVQL/R end up on separate sides of the cord, while the others all stay together on the right

side. This affinity of a process for its opposite homologue provides a simple mechanism to ensure that processes stay together. Then perhaps only a slight bias is needed to send the pair to one side rather than the other. The experiments in which the parents of PVP cells were ablated reveal an underlying preference for the right side in at least one case. When PVPR was removed PVQL crossed to the right side rather than grew along the left side of the cord by itself, but when PVPL was removed PVQR still grew along the right side. It may be that the presence of preexisting fibres on the right rather than the left was the determining factor in this particular case, but after AVG, the DD axons, DVA and DVC have grown out on the right side, which might prove sufficient to continue to attract later arrivals.

To return to Meissner's suggestion the the primitive ventral nerve was double, it may be worth discussing the advantages and disadvantages of a fused cord over paired nerves. The obvious disadvantage of a single cord like that of C. elegans is the loss of possible left/right control over body movement. Although there are four bands of muscle in
C. elegans both ventral quadrants receive the same input from the right hand ventral cord, as do both dorsal quadrants from the single dorsal cord. Therefore the body of the animal moves only in the dorsal/ventral plane, although the head can and does move freely in all directions. However there are extra cross connecting motor neuron and interneuronal classes in the head, and it is likely that in order to obtain reasonable left/right coordination, something similar would be needed in the body. There is no sign of this, even in vestigial form. On the other hand, if, as seems likely, the putative primitive twin-nerved ancestor did not have the capability for left/right body control (I have found no mention of any nematode that does), then there is a strong case for bringing the motor circuitry elements together in one nerve. First it allows an effective halving of the number of motor neurons; with the system as it is in C. elegans there is only one active motor neuron of each class at each cross section of the body. Second it removes at source any loss of synchrony between wave generation on the left and right sides of the body. Third it provides back up in an extremely important part of the animal's nervous system by having twofold redundancy of each motor circuitry driving interneuron. However there is no obvious reason why the interneurons not involved in the motor circuitry should join together or not, since they serve no function in the cord but merely use it as a route from one end of the cord to the other. Indeed this view is supported by the fact that a minority of three apparently unrelated classes (AVK, PVP and PVQ) are still bilateral in C. elegans.

In conclusion I would like to speculate that the primitive nematode ventral cord was double and symmetric, and that the selection pressure for the currently more common asymmetric cord came from the motor circuitry. It appears that AVG plays a critical role in organising the left/right asymmetry of the motor neurons. An important factor for the interneurons appears to be the mutual affinity of left/right pairs (and of the motor circuitry interneuron classes for each other, since they preserve their approximate relative structure under perturbation by AVG parent ablation and unc-3 mutation). The interneuron pairs of groups may then tend to go to the right side either directly or under the influence of AVG, the motor neurons, or other previously determined processes, such as that of DVA. If this picture is correct then the fact that so many left/right pairs of non-motor circuitry interneurons also join up and grow together on the right would suggest that, even in situations like this where all the cells

are individually distringuishable, neural guidance may be often controlled by non-specific factors that affect a large number of neurons.

5.3 Motor neuron outgrowth and formation of the dorsal cord

The preceding section described how the presence of AVG appears to help determine the side of the cord that the DD processes grow along. A second question concerns how the DD processes growing along the ventral cord know where to stop and send out their commissures. Although they have short posterior processes, the main DD ventral cord processes extend forward from the cell bodies, eventually making contact with the next DD cell along. However there is a certain amount of evidence to suggest that the determining factor for DD ventral cord growth may not be the next DD cell, but the position of the next DB cell body. First the DD commissures always exit from next to DB cell bodies, even when these are not immediately behind the next DD cell (e.g. DD3/DB4 in figure 3.4). Second there often seems to be some sort of recognition event involving DD process tips inserting themselves into DB cells at the time of and soon after process outgrowth, particularly at the back of the cord (figure 3.12). Third, in Acaris, where distances are much greater, all the DD commissures exit opposite DB cell bodies together with DB commissures, which are all on the right hand side behind the RBG (Johnson and Stretton, 1987). VD and AS commissures also grow out together in Ascaris (ibid.). Neighbouring VD and AS cells are sisters, but there is no lineal relationship whatsoever between DB and DD cells (in C. elegans, and presumably also in Ascaris, whose early lineage is identical to that of C. elegans, Sulston et al., 1983). Fourth, after DD3 and DD5 were removed by ablating their parent, DD4 did not extend to fill the whole space left by DD3, but instead stopped and began sending out a commissure at an only very slightly anterior position to normal (figure 4.3). This experiment does not prove DB involvement, however, because it remains possible that the normal growth length is intrinsically determined, as appears to be the case with the postembryonic touch cells AVM and PVM (Chalfie et al., 1983). A more conclusive, but unperformed, experiment would be to remove a DB cell.

The next event after DD process outgrowth is the growth of the motor neuron commissures. All the commissures grow out synchonously and reach the dorsal midline at the same time, well before any other longitudinal process has grown along the dorsal cord (RID will do so eventually). There is therefore a problem of recognising the correct point at which to turn, and a subsequent problem of deciding the direction in which to turn. Although adjacent to the basement membrance, the commissural growth cones appear to grow on the surface of the hypodermis, rather than the basement membrane (section 3.2). Similar behaviour was inferred from experiments on early optic nerve outgrowth (Krayanek and Goldberg, 1981). When the growth cones reach the dorsal ridge they have been seen to insert finger-like extensions into the hypodermis, indicating that some cell recognition event may have taken place (figure 3.11). Therefore it seems that the best candidate for the source of the required information is the dorsal hypodermal ridge itself, and that the growth cone “tastes” the hypodermis as it advances, eventually recognising the dorsal ridge.

The suggestion that there is a specific property of the dorsal hypodermal ridge that is recognised, while simplifying the explanation of how the dorsal cord is formed, creates problems of its own. The dorsal hypodermis is a syncytium containing many

nuclei and covering the dorsal side of the animal from head to tail and from one lateral ridge to ther other (the lateral boundaries can be seen in the section in figure 1.1). The commissure therefore grows on the surface of this syncytium for some time before it recognises a specific part of it. In so doing it crosses the path of some later longitudinal nerves, such as the ALM process, and the sublateral bundle (SAAD, SABD, SIBD, SMDD, see figure 1.2). Hence it appears that some property of the membrane must be localised to only that part of the cell surface covering the dorsal ridge. The syncytium is formed in the embryo in a curious fashion by two rows of cells passing between each other and then fusing. Mutations in two genes, unc-83 and unc-84, are known to affect this process (Sulston and Horvitz, 1982). Although mutant L1 larvae move well, they have been seen in electron microscope reconstructions to contain defects in the structure of the dorsal cord (J. G. White, unpublished observation), which might be due to the failure in the correct localisation of recognition components in the dorsal hypodermal ridge.

Once the motor neurons have turned onto the dorsal cord, they seem to grow out rapidly along it and, if they are DA or DB neurons, start making neuromuscular junctions (D reconstruction, figure 3.6). It is only at around this time or later that their dendrites grow out in the ventral cord, so they start neuromuscular activity receiving organised synaptic input. A system in which neurons generate synaptic activity before they receive their controlling input would be expected to generate a lot of random signals, but would allow the whole nervous system to be built simultaneously instead of sequentially, starting with sensory neurons and progressing along the processing pathway.

5.4 Discussion

Decussation of nerve processes, in which an entire group of cell processes cross the midline, is a standard phenomenon in most animal nervous systems, and a scaled down version of the same type of behaviour can be seen in C. elegans in the crossing over of processes from paired interneurons in the preanal and retrovesicular ganglia. The PVP processes cross in the PAG (figure 3.8) and the RIF, RIG and SABV processes cross in the RVG (figure 3.10). Since the general property of decussation appears to be functionally unnecessary, it may give some insight into general constraints on developmental organisation.

It is very clear in C. elegans that there is no ultimate functional advantage to be gained from the decussation. The crossovers are not used to facilitate transfer of information from one side of the nervous system to the other by receiving input on one side and having output on the other, since in almost every case all the synapses and gap junctions observed in the adult wild type reconstructions are on the parts of the processes beyond the cross over point. The exception is that the RIF cells both make gap junctions to AVG on their cell bodies, but this also would not be logically different if the cell body positions were reversed. It is not even the case that the symmetrical body positions of the neurons involved are preserved into later development; in fact the cell bodies in both the PAG and the RVG get squashed into a single row as the muscles mature.

This situation is different from that in most vertebrate decussations, in which the cells remain on the opposite side from their axonal termini, and have some functionality on

both sides. However, even there it is clear that, considering the whole organism, there is more crossing over than is necessary. An engineer would have the right side of the brain receive information from, and control, the right side of the body. Some communication between the two dies is certainly necessary, and this is seen for example in the corpus callosum between the two hemispheres of the cerebral cortex (and in the C. elegans nerve ring). However such connections are inherently different from the general sensory and motor decussations, for which the argument can still be made that they are functionally necessary, and are more likely to reflect developmental than functional constraints.

One common factor between the four miniature examples of decussation in the PAG and RVC of C. elegans is that they are all between pairs of neurons touching across the ventral midline. It might be suggested that their mutual affinity causes their processes to grow towards the opposite cell, and therefore cross over. However after either PVPL or PVPR was removed by ablating its parent the other stayed in position and still sent its process across the midline and along the opposite side of the cord as normal. In addition there are three pairs of cells in the ventral ganglion in front of the excretory duct which are also adjacent across the midline (AIA, SMBV and SAAD) and none of them cross over. Instead the simplest unifying property of the decussating pairs is regional: they comprise all the left/right pairs of interneurons associated with the ventral hypodermal ridge between the excretory duct and the anus. This, however, suggests neither a mechanism nor a reason for the crossing over.

One possibility is that the crossing is ballistic: both processes are attracted to some point or region on the midline and once they get there they keep on growing in the same direction and thus cross over. Nerve processes in vitro tend to grow in straight lines (Bray, 1979). The attraction of the ballistic hypothesis is that it permits there to be no intrinsic distinction between the two cells. The fact that all the decussating pairs in the RVG cross in the same place supports the hypothesis. Also the PVP crossing point in the PAG seems to be special, since the DVC process crosses from top left to bottom right in the same place, on its way forward through the preanal ganglion. The change in position of DVC does not define the site, because removal of DVC by ablating its parent had no effect on the PVP processes and their crossover. Neither did removal of PVQL, which normally contacts PVPR as soon as it crosses to the left and grows forward with it.

If we accept the ballistic hypothesis then it seems likely that PVT defines the site in the preanal ganglion, since the PVP processes cross between PVT and the processes of DVC and PVQ neurons, which are flattend out over the surface of DD6, partially separating the PVP cells from DD6 (figure 3.8). Alternatively it may be that the site is defined by the DVC and PVQ processes in a redundant manner, so that removal of any one of them makes no difference. The affinity of these three processes for the DD6 cell body is striking; they spread over its surface whereever it is available, and when DVC was removed the PVQ processes spread further to mostly fill the gap (figure 4.7). Further experimentation removing either PVT or DD6 might prove illuminating.

A variant of the ballistic hypothesis is that the initial directions of outgrowth of the processes are both intrinsically towards the midline, and so the processes simply cross over before turning forward. All the cells involved migrate ventrally from lateral positions as the hypodermis closes over the ventral surface of the embryo. It might be that the growth cones start out continuing the direction of migration of the cell and thus cross the ventral midline. This argument would apply equally well to the ventral ganglion cell pairs that do not cross, and it is certainly not necessary for an axon to leave a cell body in the same direction that the cell has been migrating. For example the ALM cell bodies are seen migrating backward along the lateral hypodermis in the C and D reconstructions, and in the E reconstruction they are sending axons forward along the same path they have just followed but in the opposite direction (figure 3.4). However, even if this does not provide a complete explanation, it does suggest how intrinsic opposite polarities of the two cells in each pair may be established.

5.5 Selective fasciculation

I have already suggested that AVG helps organise the ventral cord by providing a preferential parth for growth of, at the least, the DD axons. The wildtype outgrowth of PVP and PVQ processes from the back of the cord, in which their tips always were found very close together along the cord (section 3.5), suggested that there might be some interaction involved. Therefore a series of ablation experiments were performed to investigate PVP and PVQ outgrowth (section 4.3).

PVP and PVQ processes grow on both sides of the ventral cord. The left hand cord contains only three processes at hatching, PVPR, PVQL, and AVKR (plus RMEV at the front, see fig. 1.3). The normal sequence of events is that PVPR and PVQL grow forward together, and AVKR was only seen to be growing back after they had reached the front. It appears that PVPR is needed for the other two to grow on the left side, because when it is removed no processes are seen n either the embryonic or adult left hand cords (figure 4.4). If PVQL is removed then PVPR still grows forward along the cord by itself. Therefore, although PVQR is not a unique pioneer in normal development because the PVQL growing tip is parallel with its own, it does appear to have a primary role in establishing the left hand cord. When VPR is removed the PVQL process still grows forward along the cord, but on the right side rather than the left, and apparently somewhat delayed compared to PVQR and PVPL which normally grow on the right. In this case therefore the ability to grow and the basic directionality of growth are preserved, although the actual path taken was altered, as when AVG was removed. This corresponds to what is seen when guideposts are removed in the insect PNS (Berlot and Goodman, 1984), or motor neurons in the chinck embryo (Landmesser and Honig, 1986). It is not known whether the AVKR process also extended along the right hand cord in the absence of PVPR and PVQL on the left side.

These results are not symmetrically reproducible on the other side of the ventral cord, since PVQR still grows forward along the right side in the absence of PVPL. However, as discussed above, the cord is not symmetrical. While PVQR and PVQL are the first processes to grow along the left side of the cord, there are other preexisting processes on the right at the time when PVQR grows forward (AVG and DD axons) which might provide some degree of non-specific affinity that assisted

PVQR in growing along the right side. This could in principle be tested by removing PVPL, AVG and DD6.

Although the removal of PVQL had no effect on the outgrowth of PVPR along the left hand ventral cord, it did appear to affect the growth of other processes down the lumbar commissure from the left lumbar ganglion to the preanal ganglion (see figure 1.3 for a schematic plan of the normal situation). In neither of the reconstructed embryos in which PVQL had been removed did any of the left lumbar processes grow down lumbar commissure, although they had done so on the right side. As well as containing processes descending from the lumbar ganglion, the lumbar commissures contian a DA motor neuron process ascending from the preanal ganglion. This was present in both the PVQL^{-} reconstructions.

These results suggest that there is a specific need for PVQL in order for the other lumbar ganglion cells to grow correctly in the right direction. Similar behaviour is seen in the developing grasshopper CNS, where in several cases it has been shown that an identified neuronal growth cone normally fasciculates with a specific preexisting fascicle, in the absence of which it fails to grow in any organised fashion (Raper et al., 1984, Bastiani et al., 1986, duLac et al., 1986). In one case it was shown that a specific subset of the processes in the preexisting fascicle is required (Raper et al., 1984). This corresponds to the observation that the DA process in the lumbar commissure is not sufficient to promote growth of other processes down the commissure.

If PVQL provides guidance for the left lumbar processes by some process of selective fasciculation, then this fasciculation does not last for long. When processes from the two lumbar commissures meet in the preanal ganglion all the cell types other than PVQ immediately form contact with their bilateral homologues, “zipping up” with each other (figure 3.8). The other left lumbar processes then leave PVQL to join their right hand homologues and PVQR on the right hand side. Therefore it seems that there is a hierarchy of affinities that applies the left lumbar processes other than PVQL; first they follow, and in fact require, PVQL, then they leave PVQL in order to join their right hand homologues.

These observations all fit the “labelled pathways” hypothesis (Ghysen and Jansen, 1979, Goodman et al., 1982), that growth cones are programmed to recognise a sequence of surface labels on fascicles, possibly in some adhesive hierarchy, and that this determines their path through the developing nervous system. The situation when the left and right lumbar processes meet is somewhat novel, in that then two equivalent sets of processes fasciculate together, and must decide which of the two PVQ neurons to follow. There is no good clue as to what determines this (discussed earlier in the section on cord asymmetry.

The observations about lumbar commissure formation contrast with those made in the ventral cord that, even if normal cues are missing, processes tend to keep on growing in the correct direction. A plausible explanation of this difference is tat there is a non specific property of the ventral cord which permits or promotes neuron growth along it. Apart from the presence of other processes, at the relevant time there is a continuous line of motor neuron cell bodies along the ventral midline, which may act

as general guideposts in the same way as neuronal cell bodies that have been proposed to facilitate neuron outgrowth in the insect PNS (Bentley and Keshishian, 1982).

There are a number of uncoordinated mutants that are known to be defective in outgrowth of processes from the lumbar ganglion cells, on the basis of fluorescent staining of the PHA and PHB phasmid sensory neurons by direct uptake of fluorescein isothiocyanate (Hedgecock et al., 1985). Mutants in unc-33, unc-44 and unc-76 all show the same phenotype. Rather than growing forward into the preanal ganglion the phasmid axons stop abruptly where they meet at the bottom of the lumbar commissures, often with swollen endings. This is at the point where the resorting of the fibres takes place, with the majority of the left lumbar processes leaving PVQL to grow forward with their contralateral homologues. The fact that there are several genes with both this phenotype and also defects in movement is interesting in relation to a suggestion made earlier (in the discussion of ventral cord asymmetry). This proposed that the mutual affinity of ventral cord bilateral homologues may be a basic general mechanism whose biological purpose is to bring together the motor circuitry interneurons, and which affects other neurons incidentally. A prediction of this hypothesis would be that the anterior motor circuitry interneurons would also be afected by the mutations. In mutants for unc-6 (referred to as unc-106 in Hedgecock et al.), the PHA and PHB axons normally fail to grow down the lumbar commissures, but instead wander forward along the lateral hypodermis. This is reminiscent of the defect seen in the left lumbar commissure when PVQL was removed. However the defect in unc-6 mutants is more general than that following PVQL removal, since axons from the postembryonic PVD neurons on the lateral hypodermis also fail to reach the ventral cord, and motor neuron commissures are also disrupted (S McIntire, personal communication).

5.6 Conclusion

In the introduction to this part of the dissertation it was proposed that a number of different mechanisms could be used to influence neuronal guidance, often concurrently, and a list of possible types and sources of influence was provided. The behaviour of outgrowing neurites in both normal and experimental C. elegans embryos that has been described here has suggested new examples of several different types of influence.

The formation of the dorsal cord could be explained by the presence of a preexisting preferred pathway along the dorsal hypodermal ridge. This would essentially be an epidermal blueprint, as proposed by Singer et al. (1979). DD growth along the ventral cord may be limited by some inhibitory effect of DB cells, although from the observations that are available the inhibition seems more likely to be caused by selective recognition accompanied by membrane insertion than by the retraction of growth cones as seen by Kampfhammer et al. (1986) in vitro. The decussation of processes in the preanal and retrovesicular ganglia may be due to the tendency of growth cones to grow in straight lines, as discussed by Bray (1979). In the lumbar commissures and the determination of which processes grow along the left and right nerve cords there appear to be several examples of selective fasciculation, similar to that proposed in the labelled pathways hypothesis (Ghysen and Jansen, 1979). There also appeared to be a general directionally or premissive property of the ventral cord

region that meant that, even when specific cues were removed, processes still grew out along the cord.

All these proposed interactions fall broadly into some class of interaction that has been suggested previously. Further experiments of the same type as described here, some of which I have mentioned in the discussion, could be carried out to define more precisely the characteristics of particular interactions. The other possible approach to furthe rwork is to use the existing picture as a basis for an investigation of the genetic factors controlling neural outgrowth, eventually uncovering the critical molecular mechanisms involved using molecular genetic techniques (Greenwald, 1985). In this discussion I have mentioned a number of mutants that affect neural guidance in the ventral nervous system, in some cases in ways that are partially interpretable in terms of the mechanisms proposed here. The genetic approach is discussed further in the final conclusion after part II.