PART I THE OUTGROWTH OF NERVE PROCESSES IN THE EMBRYO

CHAPTER 1 Introduction

The first part of this dissertation describes an investigation into the outgrowth of nerve processes in the region of the ventral nerve cord of C. elegans during embryonic development. The course of normal development was deduced from serial section reconstructions of a set of embryos fixed at different stages. Then laser ablation experiments were performed to remove specific neurons whose processes grew out during these early stages, in order to test whether the presence of these processes was necessary for correct subsequent development of the nervous system. Chapter 2 gives materials and methods. The observations from the wild type reconstructions are given in Chapter 3 and the results of the ablation experiments are described in Chapter 4. The two sets of results are discussed together in Chapter 5.

There are no previous direct results on the course of neural outgrowth in C. elegans, although disruption of the final arrangement of nerve processes has been observed in mutants (Hedgecock et al., 1985) and animals in which laser damage has prevented nerve cell migration (Chalfie et al., 1983). Below I first review previous work in other systems on neural guidance, and then give an introduction to C. elegans and its nervous system.

Review of neural guidance

1.1 A review of neural guidance

The building of a nervous system during development can be divided into three phases: the generation of the correct cells in the correct places, the outgrowth of nerve processes, and the formation of synapses. All of these phases show a high degree of specificity, which means that a large amount of information must be expressed by mechanisms that on the whole we do not yet understand, but would like to. In some ways the second phase, that of process outgrowth is the most clearly defined. This is because all neural branching structures are a consequence of a single phenomenon, the migration of growth cones during development, a truth which Cajal saw early and fought hard for (Hamburger, 1981), and which led Harrison to develop the first tissue culture techniques in order to follow outgrowing neurites directly (Harrison, 1910).

A growth cone is a specialised structure at the tip of any growing neurite that migrates through the animal, spinning out the nerve process behind it. This is not the only means by which nerve processes can be lengthened, since change in size and shape of the animal is matched by addition of new material to already existing processes. In many cases most of the length of nerve fibres is created in this way, but it is almost entirely passive, having at most a very small effect on the layout of the neurons axonal structure. For instance, most nerve processes grow along the ventral cord of C. elegans when it is only around 100 microns long, a tenth of its final length. However some changes in overall structure do occur by intercalary insertion; an example is the conversion of an initially bipolar cell to one that is pseudo-monopolar, by retraction of the cell body away from the branch (Kuwada, 1986 and with ventral nerve cord motor neurons in C. elegans). Such small alterations during subsequent development emphasise the importance of looking at outgrowth as it takes place, rather than making inferences from the finished pattern.

Growth cones in vitro

Growth cones are generally spread out, lamellar structures, which often extend fine microspikes, or filopodia (Letourneau, 1983). They mover over surfaces and as they move thevarious lamellar and filopodial extensions are retracted and new ones are extended out, so that the overall shape is continually changing (Bray and Chapman, 1985). It is easy to study growth cone migration in vitro using cultured neurons and a wide range of factors that affect migration have been observed. In order for motion to take place it appears that fairly tight adhesion to the substrate is necessary (Bray, 1979), and this leads immediately to the idea that differential adhesion may be important for growth cone guidance. Letourneau (1980) has shown that growth cones do indeed tend to grow along regions of higher adversity when faced with a choice in vitro. Although this may support the common suggestion that growth cones may in many cases be guided up an adhesive gradient in vivo (Nardi, 1983, Berlot and Goodman, 1984), it does not directly address that proposal, and there are several severe problems with the idea. Growth cones show different morphologies when migrating on artificial surfaces of different adhesivities, but even though the range of morphologies seen on different neurites in vivo is vast, any one growth cone does not change shape as it migrates over a uniform surface. In addition the strength of adhesivity would have to increase

exponentially, which would require an excessive magnitude range of adhesivity for a gradient of any substantial length. In fact growth cones in culture tend to grow in straight lines anyway, only changing directions when they branch. Based n an elegant combination of observations and experiments Bray has suggested that the neurite leaving the back of the growth cone exerts a tension, and that the growth cone always grows away from the source of tension (Bray, 1979). If the angle of the neurite is altered then the direction of growth coordinately changes, and if the tension is relaxed, by for example cutting the neurite, then the growth cones divides in two, the two halves growing off in opposite directions and exerting tension against each other. Together these results suggest that direction changes and branches may occur in vivo either where a path of higher adversity is crossed, or possibly at a point where the growth cone becomes tethered, so that growth in the new direction can pull against something.

Although there is a strong tendency to think of attractive forces on growth cones as being the principle tools of guidance control, it is equally possible for repulsive forces to be influential, and there are several examples that are known. There is a highly selective inhibitory effect when the neurotransmitter 5-HT is released from a micropipette near the advancing growth cone of an identified cell from the mollusc, Helisoma (Haydon et al., 1984). This has been proposed to have developmental significance in the detailed development of the Helisoma buccal ganglion (meinertxhagen, 1985). A retraction of the growth cone in vitro is also seen when rtinal and sympathetic axons meet each other in culture (Bray et al., 1980, Kampfhammer et al., 1986). Although retinal growth cones will cross retinal axons, and sympathetic growth cones will cross sympathetic axons, when one meets the other it shrinks back and withdraws its filopodial and lamellar extensions. Similar avoidance behaviour between different neurites of the same neuron could possibly explain the marvellous space filling, non-overlapping properties of many neurons' dendritic or axonal arborisations. Experimental evidence for such avoidance has been provided by studies of single sensory neurons in the leech, which fill a planar surface from several points in an apparently self-competitive fashion (Kramer and Stent, 1985). As yet there is no experimental evidence of such mechanisms acting between different neurons in vivo, but there are several cases in C. elegans where neurons abut against but do not overlap other members of their own classes; often there is a gap junction between the two abutting processes (see Chaper 7).

Studies in vivo

It is convenient to make a distinction between directional, tropic influences on neural guidance and spatially restricted, contact mediated influences. Both appear to play an important part. To oversimplify the situation, tropic influences are directionally constraining, while differential adhesivities are spatially constraining. There is also a division between specific and nonspecific factors. By nonspecific factors I mean those that would influence any of a large range of different neurons. Neither of these divisions is totally sharp, and in particular specificity is clearly a graded phenomenon.

The classical example of a non-specific factor would be a gradient of positional information (Wolpert, 1971), probably some chemical or surface marker, and the classical experimental system where there is evidence for such a gradient in neural development is in the establishment of a topographical mapping from the retina onto

the optic tectum of lower vertebrates. A series of experiments in which an ordered mapping reformed after parts of the retina and/or tectum were removed or grafted back in abnormal orientations suggested that the original chemo-affinity hypothesis of Sperry (1963), which proposed specific matching between corresponding sectors of the retina and tectum, was incorrect (see Gaze, 1970). More recently Bonhoeffer and Huf (1982) have shown using an in vitro axon growth choice assay that there is a gradient of affinity for temporal axons across the surface of the tectum, with highest affinity for the rostral part of the tectum, which is their normal target. Progressively more nasal axons show less specificity. The overall effect of these affinities would then be established by competition. There are many other proposed sources of information for the retino-tectal system, some also driven by competition (e.g. Willshaw and von der Malsburg, 1979).

However the situation during creation of the retino-tectal map on the surface of the tectum is different from the early outgrowth of processes that concerns the study of embryonic C. elegans outgrowth in this dissertation, since the axons have already reached their target tissue and are finding the correct place on it amongst a group of equivalent cells. For the rest of this review I will focus on the pathfinding properties of growth cones necessary to find their targets from the cell bodies, rather than the final stage as discussed here.

Directional and tropic effects

A very different function of a gradient is to specify a direction up which axons can travel. There are several examples where a general attraction that is not path specific has been indicated experimentally. Harris (1980) has shown this type of effect using the same retino-tectal projection in Xenopus menioned above as an experimental system, but at the earlier stage of development where the optic tract must be formed. Before axon outgrowth he implanted whole eye primordia into abnormal places in the brain, after which in most cases the retinal axons grew out and took a nearly direct route to the tectum, usually via a pathway totally different to the one they normally follow. If the implant was sufficiently caudal then the retinal processes ran instead down the spinal cord, in a particular dorsolateral tract, reproducing previous observations that this part of the spinal cord attracted displaced retinal axons (Constantine-Paton and Capranica, 1976). These results suggest that there is a general attraction of retinal axons to their target, and that this acts over a fairly wide zone, but that the mechanism may not be uniquely used for retino-tectal pathfinding; in the spinal cord, outside the normal range of retinal axons the same attraction system may be used for another set of processes.

A more specific attraction of neurons to their targets has been observed in the vertebrate peripheral nervous system (PNS). Lance-Jones and Landmesser (1981) showed that after a short piece of chick neural tube was reversed the motor neurons till largely found a way to the correct target muscles, crossing over each other on the way. However if the displacement is too great then they often grow to inappropriate muscles (ibid. and Summerbell and Stirling, 1981). Again this influence appears to be over a longer range than the reach of the filopodia, though still reasonably localised (Landmesser, 1984). There are also indications in the insect PNS that after the more specific cues are removed there is still a tendency for sensory neurons to grow

proximally towards the central nervous system (CNS), even along abnormal routes (Berlot and Goodman, 1984, Nardi, 1983).

One suggestion of a possible agent involved in the general attraction of a whole class of nerve fibres is nerve growth factor (NGF). Sympathetic fibres grow over abnormal territory towards a site of NGF injection in vivo (Gunderson and Barrett, 1980). However in both cases the amounts applied are much larger than the ovserved natural levels; NGF is much better known as a trophic agent necessary for neuron survival and a general promoter of neuron outgrowth, and the directional effect may be a subsidiary non-physiological consequence of an overdose of these other behaviours. In a careful set of experiments with explants from embryonic mouse trigeminal ganglia and their target tissue, maxiliary epithelium, Lumsden and Davies (1983, 1986) have shown a clear directional tropic attraction of trigeminal fibres to their target. This is diffusible through the colagen matrix in which the explants sit and the axons grow, and is separable from NGF, which appears to act later in development to preserve the connection. It also has no effect on axons from comparable neighbouring ganglia. Lumsden and Davies argue that NGF is active on too many cell types to be sensible as a tropic agent. However it might be countered that a general tendency for sympathetic axons to grow towards the periphery could be useful.

All these results suggest that there may be general directional (often homing) guidance mechanisms that are not restricted to specific pathways, and apply to fairly broad classes of neurons. Interestingly the range of all the attractions is approximately the same, of the order of a few hundred microns. In cases that are more specific, such as the chick motor neuron guidance, the absolute size of the embryo is larger. Such distances correspond to a fairly small number of growth cone extensions, suggesting that a growth cone could detect a gradient on this scale. Since some specificity is involved and the directions of different sets of fibres can cross (as in the chick limb motor neuron experiments), it seems unlikely that a single gradient, such as a general adhesive gradient, provides the best explanation for them. In at least one case (Lumsden and Davies) the substrate if artificial and the factor is diffusible.

Before automatically explaining any experiment indicating a directional effect by a gradient, it should be born in mind, however, that there are at least two other ways in which a polarity or directionality could be specified. The first is intrinsic to the neuron, simply by the orientation in which it was created by its final cell division. This may often be important for initiating process outgrowth in the correct direction (Jan et al., 1985). The second is by a repeated sequence of more than two signals, in which case the direction can be determined by inspecting neighbouring sequence elements, or equivalently by a moving wave of some signal. This type of signal can operate over very long distances if it is actively maintained, and is the method is slime mould aggregation (Gerisch, 1982).

Fasciculation of nerve processes

A different sort of nonspecific influence that is important for neuronal outgrowth is the strong tendency of growth cones to grow along other neurons, which leads to the fasciculation of nerve processes. This is clearly one of the most important factors determining the structure of the peripheral nervous system, which is made of nerve bundles, and where closely studied it has also been seen to be important in the early

developing central nervous system at stages where processes are not dense (e.g. the insect CNS, Bate and Grunewald, 1981, Goodman et al., 1982). This has been seen by immunofluorescence to be expressed on many neuronal cell surfaces, and also on various epithelial and glial cells (Silver and Rutishauser, 1984). It has been claimed that the modulation of a single molecule such as NCAM could account for a very large proportion of the control of neural outgrowth (Edelman, 1983), but this appears unlikely because of the degree of specificity seen in many different but often adjacent and simultaneous interactions. However there is a large part to be played by fairly non-specific adhesion.

Almost a direct consequence of general neuronal fasciculation is the concept of the preservation of order within nerve bundles by a process tending to stay stuck to its neighbours. Many nerve projections show a general topographic order preservation, both in the central and peripheral nervous system (e.g. the retinal-tectal and spinal cord projections) and a simple method of correct guidance may be to place neurons in positions corresponding to a topological map of their targets and then to preserve the relative spatial arrangement in the outgoing bundle of fibres and rely on non-specific cues to spread the projection onto the target tissue(s). In fish retino-tectal projections Scholes (1979) has shown that order is in general maintained, but that there is a zone of active reorganisation near the tectum, and in other cases where ordering has been observed an active mechanism for correcting the final projection has also been detected (e.g. Landmesser, 1984).

Pioneers and specific fasciculation

The observation that fasciculation is a significant factor led to a realisation of the importance of the first nerve pioneers to grow out, called “pioneers” by Harrison (1910) and to the suggestion that they may be specialised in order to be able to lay down new paths. The pioneers in a various part of different insect peripheral nervous systems have been studied first by Bate (1976a), and subsequently by many others (e.g. Ho and Goodman, 1982, Bentley and Keshishian, 1982, Blari and Palka, 1985, Jan et al. 1985). Although in certain cases outgrowing central neurons grow out over new territory (Ho and Goodman, 1982), the majority of nerve bundles are pioneered by peripheral sensory neurons that essentially always follow a series of other neuronal cell bodies spaced out at intervals on the way to the CNS. This observation led to the “guidepost” hypothesis, that there are a class of specified cells in the periphery that are guideposts (maybe all neurons) and that pioneer growth cones search for and grow towards the nearest guidepost cell within reach at each stage (Bentley and Keshishian, 1982). In this case it appears that no single pioneer is essential, since various cell removal experiments resulted in satisfactory correction or adaptation (Keshishian and Bentley), 1983, Blair and Palka, 1985).

Ho and Goodman (1982) argue for a certain degree of specificity of fasciculation in the grasshopper PNS, particularly for outward growing CNS axons which must choose branches at points where afferent fibres have converged. There appears to be a much greater amount of specificity in the grasshopper CNS. Here again the earliest pioneer fibres have been identified (Bate and Grunewald, 1981), and the subsequent outgrowth of certain identified neurons has been followed (Goodman et al., 1982). A large number of closely adjacent fascicles are established and growth cones often cross a number of them before fasciculating with a particular one. This has lead to the

"labelled pathways" hypothesis (Ghysen and Jansen, 1979, Goodman et al., 1982), that the fascicles are differentially labelled by surface molecules and that growth cones are programmed to recognise a sequence of these labels and grow along them, thus defining a route through the developing nervous system. Ablations of neurons that generate the pathways for identified cells in this system have resulted in the stalling of growth cones (Raper et al., 1984, Bastiani et al., 1986). This contrasts with what has been seen in the PNS, and provides a genuine example of a specialised pioneer, whose presence is necessary for later axons to follow.

The chick PNS experiments described earlier provide another example of the requirement for a preexisting fascicle along which a subsequent neuron type will follow. In the experiments in which sections of neural tube, or limb buds, are displaced, sensory neurons that innervate muscle only follow the correct pathways to their muscles if the corresponding motor neurons do so (Honig et al., 1986). Furthermore, if instead of displacing motor neurons the whole motor neuron pool is removed before axon outgrowth, so that later there is no motor innervation of muscle, then there is effectively no sensory innervation of muscle either, and instead cutaneous sensory innervation is increased (Landmesser and Honig, 1986).

Therefore, in addition tot he nonspecific general tropism and fasciculation that were discussed earlier, there is substantial evidence for specific interactions between neurons and bundles of other neurons with which they will fasciculate. In the case of the insect CNS the specificity appears to be almost certainly mediated by contact; not only are the differing choices too tightly packed for a longer range influence to be sufficiently selective, but there have also been seen in the electron microscope direct interactions of growth cone filopodia inserting themselves deep into the surfaces of cells they wille ventually fasciculate with (Bastiani and Goodman, 1984). Monoclonal antibodies have recently been made that appear to recognise specific fascicles in the grasshopper CNS, and the growth cones that will join them (harrelson et al., 1986). Interestingly in each case several different bundles stain with the same antibody. If the antigens are involved in determining fasciculation then this would be reminiscent of the observation with ectopic retinal implants that there seems to be an affinity of retinal axons for an abnormal target in the spinal cord, as well as the tectum.

Interactions with non-neuronal surfaces

Up until now the interactions between growth cones and their targets, or other neurons, have been stressed, but clearly their relationship to non-neuronal substrates may also be important, particularly for pioneer neurons. In various different situations growth cones have been proposed to migrate over basement membrane, glial cells, epithelial cells, and mesenchyme. One of the strong reasons for proposing basement membrane as a possible neuronal substrate is that both raw basement membrane and several purified basement membrane components, such as fibronectin and laminin, have been shown to provide good surfaces for outgrowth in vitro (Varon-van Evercooren et al., 1982). Also in vitro processes are often found growing in spaces adjacent ot a limiting basement membrane (e.g. the CNS pioneers in the grasshopped, Bate and Grunewald, 1981, or the first fibres in the fish spinal cord, Kuwada et al., 1986). However this region almost always also contains a large number of glial processes, and at least in the case of retinal axons, the nerve fibres

seem to be particularly strongly attached to these glial endfeet (Krayanek and Goldberg, 1981), which have been shown to stain early on for NCAM (Silver and Rutishauser, 1984). The ordered outgrowth of retinal axons can be disrupted by injection of anti-NCAM antibodies (ibid.). In addition Silver and Ogawa (1981) have shown that a preformed glial bridge is ncessary and sufficient for growth of neocortial fibres across the corpus callosum.

On the basis of this type of observation, Singer et al. (1979) proposed the blueprint hypothesis, suggesting that there was a preformed meshwork of favoured pathways established on the glial and neuroepithelial external surface, which would channel growth cones in the same sort of way as Letourneau's adhesive grid in vitro (Letourneau, 1980). As with fasciculation, to which this type of concept is clearly related, non-neuronal blueprints could come in a complete range of specificities, from generally available for all axons to completely specific for a particular growth cone. In the case of the grasshopper CNS it has been possible to implicate a particular glial cell, the segment border cell, as determining the exit site for one of the main connectives to the periphery (Bastiani and Goodman, 1986). It effectively acts as a specific labelled pathway itself.

Summary

There is no case where the underlying mechanisms that control a nontrivial outgrowth pattern for a particular neuron or type of neuron have been determined in detail. One of the reasons for this is that we still know too little about the molecular and cellular basis of growth cone movement and guidance (Letourneau, 1983). On a larger scale, there are a number of experiments suggesting various sources of influence for process outgrowth. These experiments normally involve perturbation of particular factors in vivo and the results can sometimes be open to variable interpretation, depending on the hypotheses being addressed by the interpreter. One certain conclusion, however, is that a large range of different mechanisms can be used to influence neural guidance, usually in various combinations, and often in a redundant fashion. The information necessay for determining the outgrowth of any particular neuron will be expressed via a subset of these factors, the relevant subset probably differing in different stages of outgrowth.

Therefore the best that can be done at the general level is to identify the basic forms of the different types of relevant influence and interaction, and provide a list of tools that are available to whatever program controls development. In generating such a list I again restrict myself to outgrowth from the cell to the target, rather than interactions on the target tissue in which competition and neural activity may well play a part. With this restriction there currently seems to be evidence for the following list:

1. Much of the necessary organisation can be achieved by the initial positioning and orienting of the neurons.

2. There is a general tendency for axons to extend in straight lines unless otherwise influenced.

3. There can be local inhibitory influences on growth cone extension, either humoral or contact mediated.

4. Adhesion is clearly important for growth cone migration, and it seems likely that preformed generally adhesive pathways provide a set of preferred highways for processes to grow along.

5. Also in the realm of general adhesivity, there is a strong tendency for extending neurites to fasciculate together.

6. Both these last two influences can also act in a specific, as well as a non-specific, fashion, for example when a growth cone joins one particular fascicle out of several.

7. There can be a directional attraction of axons, normally from some fairly broad class of neurons, to some target or region, and this can function when a normal route is unavailable. At least in some cases this attraction is mediated by diffusible factors.

For those elements of the list where there is specificity, as in the last two cases, it seems that the same specificity mechanism may be used in more than one place.

Even if this list were complete, it would only provide a framework for two further lines of inquiry. The first is to search for the molecular and cellular mechanisms involved in each type of interaction, and the nature of their possible diversity and specificity. The second is to investigate how the consequent repertoire of available influences intricate outgrowth patterns for the huge variety of different neurons. One way to attack these problems is to choose an organism where the types of interaction involved and the different levels of specificity can be made as clear as possible, and then use the experimental power of molecular genetics as a technique to probe both the nature of the molecules concerned and the internal control structure of the genome. A good candidate for that organism is C. elegans.

So far in this introduction I have mixed examples from invertebrate and vertebrate model systems fairly freely, since many of the results can be directly compared, and it seems likely that factors which control growth cone guidance at the cellular level may well be analogous, if not identical, between even very widely diverged species. The significant difference between invertebrate and invertebrate nervous systems for the purposes of experimentation on axon guidance is that, in addition to in general containing orders of magnitude fewer cells than vertebrate ganglia, many and in some cases all, neurons in an invertebrate ganglion are reproducibly identifiable from one animal to the next. Often there will be only one or a small reproducible number of cells with any particular set of characteristics. Therefore repeatable experiments can be undertaken concerning a known individual neuron and the specific factors involved in controlling the outgrowth of its processes. C. elegans contains only 302 neurons altogether, all of which are identifiable, and for all of which the complete audit anatomy is known at the electron microscope level (White et al., 1986).

Finally, but not least importantly, we turn to the use of genetic techniques to study neural outgrowth. The primary reason for choosing C. elegans as a model organism

for the study of neural development was not the simplicity of its nervous system, but that it is well suited to genetic analysis (Brenner, 1974). The reason that genetics has not been mentioned before this point is that, although it can provide an extremely powerful tool for studying biological function and control and has been extensively used to study neuronal cell determination (e.g. Lehmann et al., 1983, Hedgecock, 1985), it has as yet provided very little insight into neural guidance. In vertebrates a few known mutations affect neuronal branching patterns and guidance, such as mouse mutants weaver, staggerer and reeler, which affect the structure of various cell types in the cerebellum (Caviness and Rakic, 1978). In Drosophila there are several mutations that have been used as experimental tools to remove neurons, or produce them in abnormal places (e.g. the homeotic mutants, Palka, 1982) but the only published mutation that seems to directly affect neuronal guidance is bendless, in which one of the neuron types involved in the escape jump response fails to reach its target (Thomas and Wyman, 1982). However it is not known whether other processes are affected, nor is the wild type development of the particular neuron known. In fact the organism in which the greatest number of neural guidance specific mutants are known is C. elegans (Hedgecock et al., 1985, S. McIntire, J. White, E. Hedgecock, personal communications, discussed further in the next section). In addition to any intrinsic interest and possible significance, it was in order to provide the developmental framework for further characterisation of the molecular mechanisms involved in guidance via this genetic approach that the study described in this thesis was undertaken.

1.2 The C. elegans nervous system

C. elegans is a small nematode, or roundworm, approximately 1mm long in the adult form. It has a simple body structure and a small number of cells: 959 somatic cells including 302 neuons. Development from egg to fertile adult takes only three and a half days at room temperature. Wild type animals used in this study are isogenic, since the egglaying sex is a self-fertilising hermaphrodite, rather than a female, with the consequence that strains are normally propogated asexually, forming clones. Males occur naturally at low frequencies. Ther hermaphroditism also facilitates genetic analysis, and many mutants have been studied. Together these facts make C. elegans a favourable model organism for the detailed study of development at the level of single cells, using both anatomical and genetic techniques, and it was chosen as such by Sydney Brenner (1974).

The life cycle consists of an embryonic stage, inside the egg, which takes about 16 hours, followed by four larval stages, named L1 to L4. The course of development is extremely reproducible. The pattern of cell divisions from the fertilised egg to the adult has been determined completely (Sulston and Horvitz, 1979, Kimbe and Hirsh, 1979, Sulston et al., 1983) and is essentially invariant.

Not only are the pattern of cell division and the general body plan of C. elegans simple and reproducible at a cellular level, but so is its nervous system. The complete nervous system of the adult hermaphrodite has been reconstructed by White et al. (1986) from electron micrographs of serial thin sections. The neurons have simple branching structures, and both the dispositions of cell processes, and the connections they make, appear to be largely invariant between animals. They can be assigned to 118 different neuronal classes on the basis of morphology and synaptic connectivity

(the system of nomenclature is described in Chapter 2). An overview of the nervous system of an L1 larva is shown in Figure 1.2. Its central processing region is a loop of neuropil around the pharynx, called the nerve ring, containing around 175 nerve processes. Running from this is a set of longitudinal process bundles that connect the ring to sensory receptors, the body motor nervous system, and several small ganglia in the tail. There are also circumferential commissures carrying processes from one lonitudinal bundle to another. The most important of the longitudinal bundles is the ventral nerve cord, which runs from the retrovesicular ganglion (RVG) just behind the nerve ring to the preanal ganglion (PAG) at the beginning of the tail, and containing the motor neuron cell bodies for the body motor circuitry.

Nerve cells in C. elegans are small (less than 5 microns in diameter) and it is not currently practical to impale them with microelectrodes. However intracellular recording from selected neurons has been possible in the larger nematode, Ascaris lumbricoides. Attention has been focussed on the ventral cord motor circuitry (reviewed in Stretton et al., 1985), and the distribution of cell types seen there corresponds anatomically very closely to that in C. elegans.

Figure 1.1

Transverse section of a 515 minute embryo (the C reconstruction of Chapter 3). The gut, muscle quadrants (M) and outer hypodermis (h) are all labelled. There are two nerve processes in the ventral nerve cord (AVG and DD3), and one motor neuron cell body (DB4). A left handed commissure is growing out from the DB4 cell body towards the dorsal hypodermis. In its growth cone can be seen a number of small vesicles. Scale bar is 2 microns

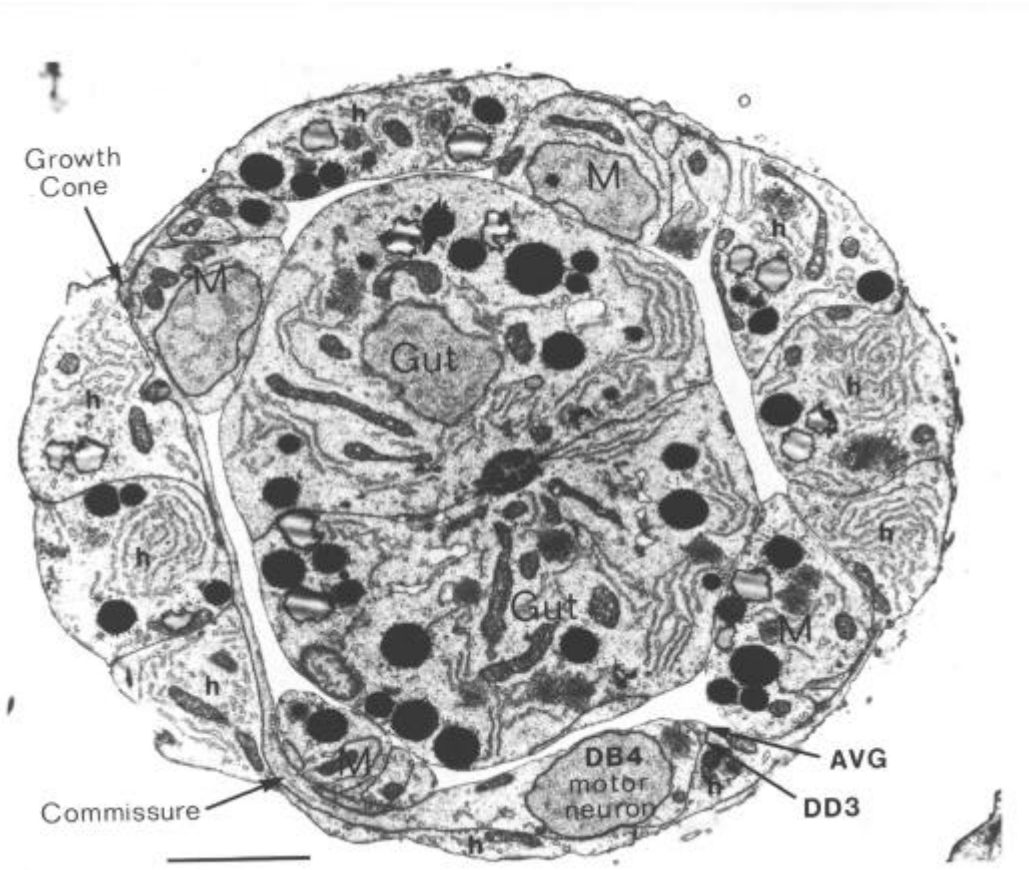
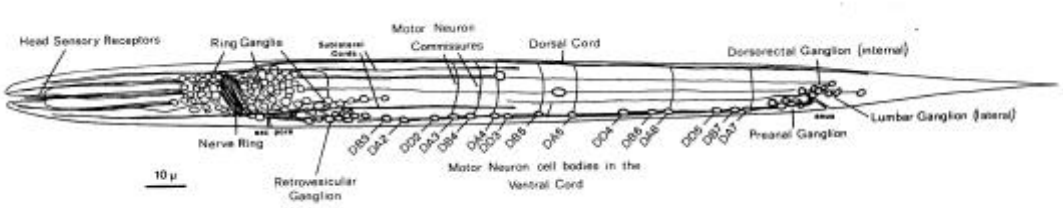


Figure 1.2

A general view of the L1 larva and its nervous system. All the neuronal cell bodies and process tracts behind the retrovesicular ganglion on the midline or the left side are shown. The main region of neuropil is the nerve ring, which is a loop around the pharynx. The ventral cord runs back from this and contains motor neuron cell bodies in addition to processes. Those ventral cord motor neurons that do not send a commissure around the left side of the body to the dorsal cord send one to the right side. There are four small tail ganglia: the preanal ganglion, the dorsorectal ganglion, and two lumbar ganglia, one on each side.



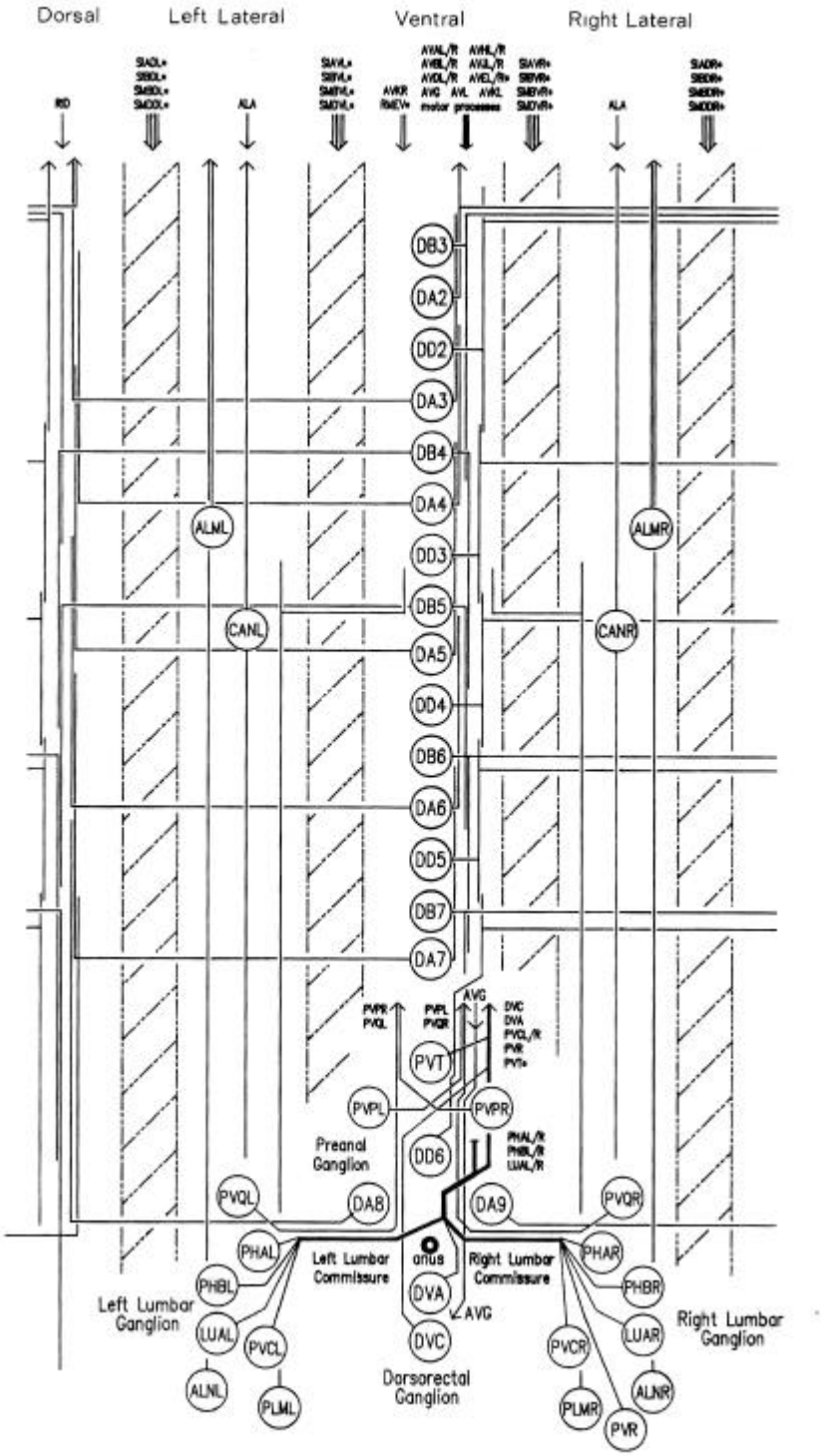
Previous studies on neural process guidance in C. elegans have been restricted to examining the structure of the adult nervous system in both wild type animals and mutants in which processes go astray. White (1983) discusses some possible factors that may be important in neural guidance on the basis of the adult electron microscope reconstructions. Chapter 9 of this thesis also considers process placement in the nerve ring using data from the adult reconstructions. Several techniques (mostly unpublished) have been developed to visualise processes by light microscopy, and these have been used to screen mutants that have possible neural defects, such as uncoordinated mutants that do not move well. Hedgecock et al. (1985) filled certain classes of sensory neurons with fluorescein by simple immersion of animals in the dye. Mutants in five unc genes showed guidance defects in these neurons, with processes either growing erratically in abnormal locations, or stopping prematurely. Several mutants are also known in which the outgrowth of the touch neurons is defective (Chalfie and Suslton, 1983). Further studies have been undertaken using monoclonal antibodies (S. McIntire, S. Siddiqui and J. Culotti, unpublished) and by electron microscopereconstruction of mutants (J. White, unpublished).

The study of neural outgrowth undertaken here has concentrated on the ventral cord, and to a lesser extent the ganglia at either end (RVG and PAG). Figure 1.3 shows in schematic form all the neurons and nerve processes behind the RVG in a newly hatched L1 larva. The ventral cord contains the motor neurons that innervate body muscles as well as interneuron processes that run to and from the nerve ring. There are two groups of processes in the ventral cord, one on each side of the hypodermal ridge. They are very asymetrical. The right hand cord contains 25 to 30 processes, including the motor neuron processes and many pairs of interneurons which are bilaterally symmetric in the nerve ring, while the left hand cord contains only 3 or 4 processes. The other main longitudinal bundle is the dorsal cord, which contains motor neurons processes and just one interneuron, RID.

The ventral and dorsal cords contain the motor circuitry controlling body movement. There are three classes of motor neuron at the L1 stage, DA, DB and DD (five more classes are added during postembryonic development). In addition to having their cell body and a process in the ventral cord, all these motor neurons send a commissure round the body of the animal to the dorsal cord, where they have another process. Muscle arms from ventral muscles extend to the ventral cord, while those from dorsal muscles extend to the dorsal cord. Movement of the body is limited to the dorsal-ventral plane. The head has more freedom of movment, owing ot more complex innervation of the muscles in the head directly from the nerve ring, but motion of the whole animal is caused by propogating dorsal-ventral waves along the body. DA and DB neurons both have their neuromuscular output in the dorsal cord, and receive input from (different) interneurons in the ventral cord. However they have different polarities: both ventral and dorsal DA processes grow forward, while DB processes grow backward. DD motor neurons receive input in the dorsal cord from the DA and DB neurons, by "intercepting" their neuromuscular junctions, and have output in the ventral cord, which is thought to be inhibitory, ensuring relaxation of the ventral musculature while the dorsal musculature is contracted.

Figure 1.3

All the nerve processes and cell bodies behind the RVG. This diagram is a schematic cylindrical projection of the inner surface of the hypodermis and nervous system, obtained by conceptually cutting along the dorsal midline and unfolding flat. The dorsal cord is shown at the left hand edge, anterior is at the top, and posterior at the bottom. The positions of the four longitudinal muscle quadrants are shown by hatched regions. Nerve processes in C. elegans branch only rarely and reproducibly and all the branches in this region are shown. Processes entering the ventral, lateral or dorsal cords from the front are indicated at the top. Those with asterisk after the neuron's name only run part way back along the body. Posterior interneuron processes running forward along the ventral cord are indicated at the top. Those with an asterisk after the neuron's name only run part way back along the body. Posterior interneuron processes running forward along the ventral cord are indicated similarly at the front of the preanal ganglion. All the anterior axons in the ventral cord without an asterisk terminate in the preanal ganglion, except for that of AVG, which is shown ascending into the dorsorectal ganglion. The PHA and PHB neurons from the lumbar ganglia also have posteriorly directed processes that terminate in the phasmid sensilla. Note the different directionalities of outgrowth of the different ventral cord motor neuron classes.


Dorsal
Left Lateral
Ventral
Right Lateral
RID
ALA
AVG
motor processes
DB3
DA2
DD2
DA3
DB4
DA4
DD3
DB5
DA5
DD4
DB6
DA6
DD5
DB7
DA7
ALML
ALMR
CANL
CANR
PVT
PVPL
PVPR
DD6
Preanal Ganglion
DA8
DA9
PVQL
PVQR
PHAL
PHAR
PHBL
PHBR
LUAL
LUAR
PVCL
PVCR
ALNL
ALNR
PLML
PLMR
PVR
anus
DVA
DVC
Left Lumbar Commissure
Right Lumbar Commissure
Left Lumbar Ganglion
Right Lumbar Ganglion
Dorsorectal Ganglion

In addition to those in the ventral and dorsal cords there are a few neuronal cells and processes on the lateral hypodermal ridge and four small ganglia at the back of the animal (figure 1.3). The lateral neurons ALM and PLM are touch receptor classes (Chalfie and Sulston, 1980), while CAN and ALA are associated with the excretory canals, which run through the lateral ridges. In the front half of the animal there are four processes running back under each muscle quadrant from the nerve ring. These sublateral processes are possibly proprioceptive, involved in controlling head movement, since the neurons they belong to are closely associated with the head motor circuitry, SMBD and SMDD being motor neuron classes themselves. The preanal ganglion contains three interneuron cell bodies, DD6, DA8 and DA9. The lumbar ganglia on the sides at the back contain the cell bodies of the ALN and PLM neurons, which have lateral processes, and of the phasmid chemoreceptors PHA and PHB and the ventral cord interneurons PVQ, PVC, LUA and PVR, all of which send anterior processes down to the preanal ganglion and the ventral cord via the lumbar commissures. Finally there are two neurons in the dorsorectal ganglion on the top surface of the rectal epithelium behind the anus, DVA and DVC.

There are both practical and strategic reasons for choosing the ventral cord as the target for study. First, although the final anatomy of the nerve ring has been reconstructed, it is too complex a structure to be able to easily study its development. Its final structure is, however, discussed with respect to developmental considerations in the second part of this thesis. Second, the method of observation used has been reconstructed from electron micrographs, and it is relatively easy to reconstruct the ventral cord region from transverse sections, since processes are mostly lonitudinal, any commissures containing only a few processes. Third, and perhaps most importantly, it is possible to at least some extent to examine functionality defects in ventral cord structure, which allows the combining of work on structure and function. A reasonable functional model of the ventral cord motor circuitry has been proposed, both by analogy to the results in Ascaris and as a result of ablation experiments in which components of the circuitry were removed (Chalfie et al., 1985). Movement is very easily observed, and a large number of uncoordinated mutants have been ovtained that have various defects in movement (Brenner, 1974).

As mentioned previously, some of these mutants have been seen to have defects in nerve process morphology (Hedgecock et al., 1985 S. McIntire, J. White unpublished observations). Particular examples are that some or all circumferential commissures go astray in unc-5, unc-6 and unc-33 mutants, and the PHA and PHB processes get stuck at the bottom of the lumbar commissures in unc-33, unc-44, unc-51 and unc-76 mutants. These defects suggest that the affected genes may be involved in the processes of neural outgrowth that have been studied here. Genes defined in this way provide a possible link between the anatomical experiments and observations described here and the molecular mechanisms involved. The defects they induce are compared with the wild type development and the effects of cell ablations in Chapter 5.