THE POSTERIOR NERVOUS SYSTEM

OF THE NEMATODE CAENORHABDITIS ELEGANS

Thesis by

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ABSTRACT

Two young adult C. elegans have been serially sectioned and reconstructed from the tail tip forward through the anterior end of the pre-anal ganglion. Thirty-nine neurons can be identified in the tail, twelve cells in each lumbar ganglion, twelve cells in the pre-anal ganglion, and three cells in the dorso-rectal ganglion. Each cell in the tail can be reproducibly identified on the basis of a set of morphological features, including cell body position, fiber projections, fiber size, and cytoplasmic appearance. Eleven neurons in each lumbar ganglion are bilaterally homologous. Many lumbar cells have sensory dendrites in the tail. Two pairs of lumbar cells which lack sensory dendrites are prominent interneurons in the synaptic interactions of the tail. Virtually all synaptic contacts in the tail are found in the pre-anal ganglion. Most synapses involve lumbar fibers and fibers from cells whose cell bodies lie anterior to the reconstructed region. Pre-anal ganglion cells themselves are relatively minor participants in these synaptic interactions.

A complete connectivity matrix has been constructed for both animals, involving about 150 synapses in each case. Certain cells make repeated contacts with one another (up to thirteen contacts) in both animals. Other instances of non-reproducible synapses are found, usually involving one

contact in one animal and none in the other. No self-synapses are observed, but sensory cells frequently synapse onto their bilateral homologues. Homologously paired cells make similar sets of synaptic contacts. One class of reciprocal synapse formation is found.

Eighty per cent of the contacts are dyadic, with one pre-synaptic cell and two post-synaptic ones. Ten per cent of the contacts are triadic; the remaining ten per cent are apparently conventional synapses with a single post-synaptic element. Each dyadic synapse generally involves three different types of neurons - none homologous to another - such that $A \rightarrow \begin{matrix} B \\ C \end{matrix}$. Each type of pre-synaptic neuron (A) contacts only a few preferred pairs of fibers (B,C). Most dyadic contacts are involved in multiple routes of information flow, such that $A \rightarrow \begin{matrix} B \\ C \end{matrix}$ and, elsewhere, $B \rightarrow C$. The formation of dyadic synapses appears to follow strict rules which may reflect important factors in the development of the nervous system.

Most synaptic interactions can be included in a simple wiring diagram by which information flows from sensory cells through multiple routes to converge on a pair of interneurons which project forward into the ventral cord. Positional information is used to identify three pairs of interneurons which are important both in ventral cord synaptic patterns and in the synaptic interactions of the

pre-anal ganglion (White et al., 1976). The nematode's behavioral responses to sensory stimulation of the tail or the head are analysed combining known circuitry of the ventral cord, the tail, and the head.

Many neurons in C. elegans are derived after hatch from stereotyped sets of cell divisions (Sulston, 1976). Cell body positions and detailed morphologies are used to identify the cells of the lumbar ganglia which are derived from post-hatch cell divisions (in collaboration with Lois Edgar, John White, and John Sulston). Many of these cells are involved in sensory transduction in the adult tail; some as supporting cells of the phasmids, others as ciliated sensory neurons with synaptic output in the pre-anal ganglion.

Six cells in the pre-anal ganglion are derived from the post-hatch cell divisions of the most posterior pair of precursor cells of the ventral cord (Sulston, 1976). Daughter cells of homologous precursor cells which enter the anterior ventral cord are known to form particular classes of motoneurons (Sulston, 1976; White et al., 1976). Of the six pre-anal ganglion lineage cells (identified by their cell body positions à la Sulston, 1976), some do become motoneurons, possibly of the proper classes for their lineage. However, two of these cells definitely do not become motoneurons in the adult nematode, violating the

general pattern for their lineage.

Seven sensory neurons are identified in the tail. Several different modes of sensory transduction appear to be utilized.

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I. INTRODUCTION

In the complex behavioral array of a higher organism it seems inevitable that each element must be the consequence of a definite pattern of neuronal interactions. However, in most cases relatively little is known about the underlying pattern, and indeed the very complexity of higher nervous systems most often precludes a detailed analysis of this pattern, either in functional terms or in terms of the development of its component synaptic contacts. In consequence, many neurobiologists have turned instead to simpler invertebrate nervous systems, where individual neurons can often be reliably identified and where the behavioral roles and developmental origins of each can be reproducibly studied. The very variety of systems which have been chosen for these purposes testifies to the notion that no one of them is ideally suited to answer all of the types of behavioral and developmental questions which arise. Indeed, it appears at the moment as though the chosen systems divide themselves into two broad classes, 1) a group with relatively large, impalable cells, in which isolated ganglia contain cells which are few enough in number and sufficiently identifiable to permit an exhaustive electrophysiological study of the behavior role of each cell, and 2) a group which are small, rapidly reproducing, and genetically well suited for mutational analysis of neural development. The kinds of information currently

obtainable from these two classes of systems are complementary, although it is certainly to be hoped that greater overlap will develop in time.

Among systems of the first class, one of the earliest to be studied was the crayfish, in which Wiersma first recognized the occurrence of individual command interneurons (Wiersma, 1952; Wiersma and Ikeda, 1964). Eventually Wiersma and his colleagues were able to catalogue over one hundred types of crayfish interneurons, each with a characteristic behavioral role (Wiersma, Ripley and Christensen, 1955; Wiersma and Hughes, 1961; Wiersma and Bush, 1963). These early studies were particularly important for establishing the extent to which interneurons could be specifically involved in some behaviors but not in others.

Another system of the first class is the sea hare Aplysia, and more specifically its parietovisceral ganglion, where Hughes and Tauc (1962), Kandel et al. (1967) and Strumwasser (1967) have determined the constancy of cell number and position and have shown each morphologically identifiable cell to have a defined set of electrophysiological properties. Of special importance in this case has been the demonstration of cells with endogenous rhythmic properties appropriate for timing behavior (Strumwasser, 1963, 1968).

Still another system with large, impalable cells is

the segmental ganglion of the leech Hirudo, of which each leech contains twenty-one nearly identical copies with about 350 cells each. Each ganglion contains a complement of fourteen pairs of excitatory motoneurons and three pairs of inhibitory motoneurons. Each motoneuron innervates a territory of muscle fibers which is consistent in size and location from segment to segment (Stuart, 1970). Each ganglion also contains seven pairs of sensory cells, and each sensory cell has a receptive field along the body surface which is consistent from segment to segment. Three pairs of sensory cells are receptive to light touch, two pairs are receptive to maintained pressure and two pairs are receptive to noxious stimuli. These fourteen cells comprise all of the rapidly conducting sensory cells of the segmental ganglion (Nicholls and Baylor, 1968). Intracellular recordings of combinations of these cells indicate that the sensory and motor cells of the segmental ganglion are synaptically interconnected in a stereotyped pattern (Nicholls and Purves, 1970). Stereotyped synaptic interconnections are also found between cells in adjacent segmental ganglia. If the connectives between two segmental ganglia are severed and allowed to regrow, synaptic connections are reestablished in a consistent pattern but the function of these regenerated connectives is significantly altered from the function of normal connectives.

This altered function is then observed not only in the regenerated connectives, but in all of the connectives of the experimental animal (Jansen and Nicholls, 1972). The stereotyped patterns of synaptic interactions between sensory cells and motoneurons in the leech have been exploited to analyse some of the behavioral functions of the segmental ganglion, notably the shortening reflex (Nicholls and Purves, 1972) and the control of swimming motions (Kristan, Stent and Ort, 1974ab; Ort, Kristan and Stent, 1974). The leech is a promising system for exhaustive electrophysiological analysis of a given behavior, although it probably has too many cells to be studied to completion.

The stomatogastric ganglion of the lobster, which drives the lobster's gastric mill, is another interesting preparation for neurobiological study. This ganglion has about thirty cell bodies, most of which can be impaled with relative ease, and the synaptic interconnections of these cells are known to be very standardized, as measured by simultaneous intracellular recordings of pairs of cells (Morris and Maynard, 1970; Maynard, 1972; Mulloney and Selverston, 1974ab; Selverston and Mulloney, 1974; Hartline and Maynard, 1975; Maynard and Selverston, 1975). In view of the relative simplicity of the gastric mill's action, attempts are now in progress to make a detailed model of

the ganglion's output, based solely on the properties directly measured for each cell. If successful, this modelling should provide a particularly detailed view of the importance of each cell and each pairwise interaction in an overall behavioral pattern.

In the stomatogastric ganglion, attempts are also being made to examine the morphological basis of the observed functional contacts between cells. The ganglion is structured in typical invertebrate fashion, with a shell of cell bodies surrounding a core of synaptic neuropil. There are no synaptic contacts onto cell bodies; all cell bodies and proximal fibers are sheathed in glial cells. Only the distal fibers in the fine-textured synaptic neuropil have synaptic interactions (King, 1976a). Individual neurons identified by intracellular recording are known to have rather constant cell body sizes and positions, but the projections of these cells into the synaptic neuropil are highly complex and variable. This variability, plus the large number of synapses in the ganglion, (estimated to be one million (King,1976a)), has greatly increased the difficulty of demonstrating a morphological basis for the observed functional contacts.

Among systems of the second, genetically tractable class, the fruit fly, Drosophila melanogaster, is one of the most attractive (Benzer, 1971). Drosophila is routinely

maintained isogenically and has an abundance of interesting behaviors. Many behavioral mutations have been obtained, and the use of genetic tricks such as mosaicism has proved especially powerful in the analysis of these mutants. With mosaics, the site of gene function can be localized to specific tissues in the fly (Hotta and Benzer, 1972), and through the use of cell marker mutants, development of the nervous system can be revealed by fate mapping (Kankel and Hall, 1975). Particularly good progress has been made in the analysis of sensory reception (Harris, Stark, and Walker, 1975; Harris, 1976) and motor output (Jan and Jan, 1976), where the number of cells or cell types involved is sufficiently small. However, the study of central processing in the Drosophila brain remains relatively difficult, since individual central neurons cannot yet be easily identified.

Daphnia magna, a small crustacean, is an isogenic organism which has been studied by serial section reconstruction to examine the distribution and development of normal contacts in the visual system (Macagno, LoPresti and Levinthal, 1973; LoPresti, Macagno and Levinthal, 1973). The eye of Daphnia contains 176 receptor cells organized into twenty-two ommatidia, containing eight cells each. The receptors each send one fiber to the optic ganglion. The optic ganglion contains 110 neurons, five for each set of

ommatidial fibers. Primary synaptic contacts occur between sets of eight receptor cells and five lamina cells in a stereotyped fashion, which can be reproducibly identified morphologically. The fiber projections of the receptor cells show a limited degree of variation in branching pattern, but the synaptic connections between receptor cells and lamina cells show a very strong pattern which is stable in the four animals examined. There is some variation in the numbers of specific synapse types within the general pattern. Thus a limited degree of morphological variation does occur in isogenic animals raised under controlled conditions, but the pattern of synapse formation is highly conserved (Macagno, LoPresti and Levinthal, 1973).

In the development of the optic lamina in Daphnia, a single receptor neuron sends a fiber to the developing lamina cells which acts as a leader for the other seven receptor fibers. Only the lead fiber displays a growth cone during development; the other seven fibers follow the lead fiber by growing out in close apposition to it. The lead fiber becomes wrapped transiently in a glial fashion by the first two laminar neuroblasts which it contacts. The laminar neuroblasts undergo differentiation after contact with the lead fiber, in the same order in which they are contacted (LoPresti, Macagno and Leventhal, 1973). These studies seem particularly important because they

indicate rules for fiber growth and contact formation that may be general.

A final system of the second class is the nematode Caenorhabditis elegans, which is one of the simplest organisms to possess a neurally mediated repertoire of behavior. Its entire nervous system consists of fewer than 300 cells, and behavioral mutations are easily obtained and genetically analyzed (Brenner, 1974).

The nervous system of C. elegans is being studied in parts, using serial reconstruction techniques to detail each region. The bulk of the nervous system is in cephalic ganglia bordering the nerve ring; the sensory input and the motor output in this region have been described, (Ward et al., 1975; Ware et al. 1975) and the structure of the neuropil in the nerve ring has also been studied (Brenner, White and associates, unpublished). The structure of the pharyngeal nervous system and that of the ventral and dorsal nerve cords have also recently been described, including patterns of synaptic interaction (Albertson and Thomson, 1976; White et al., 1976).

These studies have revealed striking constancies in the organization of the nervous system in C. elegans. Sensory and motor structures are formed by well-defined sets of cells with very stable morphologies (Ward et al., 1975; Ware et al., 1975), and individual motoneurons and inter-

neurons can be reproducibly identified in the pharynx and in the ventral cord (Albertson and Thomson, 1976; White et al., 1976). Furthermore, the fibers in the ventral cord and in the sensory connectives of the snout are highly organized, and many can be identified solely by their positions relative to one another. In addition, the synaptic interactions are reproducible, and the synaptic patterns of the ventral cord permit some speculations concerning the roles of the identified motoneuron classes in behavior (White, 1975). On the developmental side, Sulston (1976) has recently developed a technique for following individual cell divisions in the living nematode by Nomarski optics, and has shown that many motoneurons in the ventral cord are not present at hatch, but are derived instead from the later cell divisions of a few precursor cells. Each of a set of thirteen precursor cells undergoes the same pattern of cell divisions to form thirteen similar sets of daughter cells, and within each set of daughter cells, the same subset of five cells become motoneurons of five different classes (Sulston, 1976; White et al., 1976). Thus the lineage of each cell in the ventral cord appears to govern its fate in the adult.

From the results obtained so far it appears that C. elegans will be particularly useful for obtaining a complete morphological description of an entire nervous

system and for examining the relationship between a cell's lineage history and its eventual function.

The work to be reported below is an extension of the anatomical analysis of the C. elegans nervous system into the tail, where a combination of limited cell number and favorable geometry has made a complete synaptic analysis possible. The tails of many other nematodes have been examined to a limited extent as a means of classification. (Many workers use the presence or absence of the sensory structures known as phasmids to divide the phylum Nematoda into two classes (Phasmidia and Aphasmidia). Many of the aphasmidial species have other specialized tail structures which secrete a substance from a posterior spinneret, so that the tail serves as a holdfast organ (Chitwood and Chitwood, 1950). Many of the phasmidial species have multiple sets of phasmids and anal papillae.) Much of the early nematode anatomy was done on two related phasmidial species, Ascaris lumbricoides and Ascaris megalocephala. These large parasitic nematodes can be studied by light microscopy and are now useful for neurophysiological studies as well. Hesse (1892) described the sensory endings and published a detailed drawing of the male tail of Ascaris m., but he did not dwell on the female tail. Voltzenlogel (1902) described the tails of both species more extensively, with many fine drawings of the male anatomy and a single

drawing of the female anatomy. Goldschmidt (1903, 1908, 1909, 1910), in connection with his detailed reconstructions of the anterior anatomies of both species, also reported briefly on the sensory anatomy of the tail of Ascaris. A complete picture of the nervous system of Ascaris has been put together by Chitwood and Chitwood (1950), based upon the work of Voltzenlogel and Goldschmidt. (As will be shown below, the overall organization of the Ascaris tail is quite similar to that reported here for C. elegans.)

Looss (1905) described the anatomy of Ancylostoma, including the nervous anatomy of the female tail, and was the first to describe innervation of the phasmids by lateral caudal nerves. Chitwood and Chitwood (1950) published a reconstruction of the nervous system of the female tail of Spironoura affina. Chitwood and Wehr (1934) reconstructed the nervous system of Rhabditis terricola, which is closely related to C. elegans. Drawings of the female tail of both species are shown by Chitwood and Chitwood (1950). (In both cases, the layout of the nervous system is very similar to that of C. elegans; the ganglia, commissures and nerve cords differ only in small details, as shown below.)

The work reported below on the C. elegans tail extends the previous studies to the electron microscopic level, where unambiguous cell identifications can be made and

where, in particular, synaptic interactions can be catalogued. The results confirm the general conclusions of the earlier studies and in addition suggest possibilities both for the function of the synaptic connections observed and for the rules governing their formation.

II. MATERIALS AND METHODS

NEMATODES: Caenorhabditis elegans (var. Bristol, strain N2) is a small free-living soil nematode. These animals are self-fertilizing hermaphrodites, facilitating the use of genetic crosses. Mutagenesis and handling techniques followed those of Brenner (1974) and Dusenbery et al. (1975).

Worms were grown monoxenically in petri plates containing nematode growth minimal medium (NGMM) agar pre-seeded with Escherichia coli strain OP50. Mutant stocks were stored frozen in small plastic tubes kept in liquid nitrogen.

FIXATION AND EMBEDDING: Worms were grown up to a desired stage in synchronous culture. Synchrony was obtained by collecting newly hatched animals over a period of approximately 45 minutes. Animals were fixed when they reach egg-laying stage, generally 50 hours post-hatch when grown at 20°C.

Methods of fixation and embedding followed those of Ware et al. (1975). Animals were rinsed off plates and anaesthetized for 15 minutes in 0.5% 1-phenoxy-2-propanol in 0.1M cacodylate, pH 7.4, for 1 to 4 hours. The tails were then cut off with an x-acto knife before staining for 45 minutes in 1% uranyl acetate, in 0.05M maleate, pH 6.1. The tails were then embedded in Epon-Araldite. After

sectioning, the thin sections were post-stained on carbon-coated formvar girds in lead citrate for 5 minutes (Reynolds, 1963).

RECONSTRUCTION: For microtomy, individual embedded tails were cut out of a flat mold and mounted onto support blocks with epoxy. Serial sections were cut with a Sorvall MT2B ultramicrotome and collected in strips on slot grids. Continuity of these series was very good, and the loss of sections was generally 3% or less. Sectioning was done perpendicular to the body axis. Most regions of the tail were reconstructed from 50-60 nm. sections, which best reveal synapses and commissures. Typical series were 1000-2000 sections long.

Electron microscopy was done with a Zeiss EM9, using a special roll film camera designed by Randle Ware (Ware et al., 1975). With this camera, it was possible to store 100 feet of 70mm. Rekordak microfilm and expose successive 200 frame series. The reconstruction of long fiber tracts (500-800 sections) was done by serial section cinematography, using methods and equipment described by Levinthal and Ware (1972) and Ware et al. (1975). Both the image combiner and the modified Vanguard M35 projector used for reconstruction were designed by Randle Ware. The ganglia were reconstructed from high-magnification prints of every section; this technique was required to catalogue every

synapse and to trace difficult commissures.

CATALOGUE OF RECONSTRUCTED ANIMALS: Eight adult hermaphrodites have been serially sectioned over major portions of the tail. The extent of these series is summarized in Figure 2c. Other animals were sectioned over shorter regions. The general features of each region of the tail have been confirmed in four or more animals. The most detailed reconstructions are those of B126 and B136, which feature complete series of all of the tail ganglia and their commissures.

I have been very fortunate to collaborate with Lois Edgar, who has extensively reconstructed the lumbar regions of another hermaphrodite tail. Many of my morphological findings have been confirmed by her work. Several morphological features were first recognized by Lois Edgar and later confirmed in my own reconstructions.

I have also sectioned the tails of wild-type males and some behavioral and morphological mutants. One extensive series of 90 nm. sections has been assembled to study the general anatomy of the adult male tail. Attempts to section juvenile tails have been unsuccessful, due to problems in membrane fixation.

MUTANT SELECTION: Ethylmethanesulfonate was used to mutagenize young nematodes, following the methods of

Brenner (1974) and Dusenbery et al. (1975). Mutagenized animals were then allowed to self-fertilize for two generations at 16°C, and recessive mutants were selected from the second generation progeny by a variety of methods.

Behavioral mutants insensitive to tail touch were sought among two populations; 1) those which fail to migrate to the bacteria on a half-seeded plate and 2) those which migrate to the bacteria well. Potential mutants of the first class were difficult to assess, since they generally did not have normal movement capabilities. Several mutants of the second class have been isolated.

Behavioral mutants unable to defecate normally have been selected by feeding prospective mutants on a bacterial lawn permeated with congo red particles. After 20 hours growth on this substrate, the animals were shifted to 25°C and continued to feed. After 4 hours at 25°C the animals were rinsed off the stain plate, moved to a fresh bacterial plate (without stain), and allowed to feed at 25°C. Normal animals defecate all of the congo red stain in a period of 5-10 minutes. Prospective ("constipated") mutants are selected from those animals holding stain after 30 minutes on the destaining plate. Temperature sensitive mutants and non-ts mutants should both be among these clones. Congo red does not permeate any of the nematode's tissues, nor does it appear to alter the animal's growth or development.

None of our defecation mutants have been ts-conditional, and none of them have shown a complete loss of defecation function. Total loss of defecation behavior is presumably a lethal condition.

Mutants with morphological defects in the tail, chemotaxis mutants and thermotaxis mutants have also been isolated by other workers in our laboratory. Their selection methods have previously been described by Dusenbery et al. (1975) and Hedgecock and Russell (1975).

FEULGEN STAINING: Nematode cultures were grown on plates to desired stages and than washed off into test tubes with MeOH/HAc fixative (3:1). After a 15 minute fixation the worms were treated with 1M HCl at 60°C for 10 minutes, stained with Schiff's reagent for 45 minutes, destained in bisulfite for 5 minutes and mounted in xylene on glass slides.

III. OBSERVATIONS: GENERAL ANATOMY OF THE ADULT HERMAPHRODITE

HYPODERMIS/CUTICLE: The hypodermis secretes an impermeable cuticle which protects C. elegans from its environment and which allows the animal to maintain a substantial turgor pressure. Cuticle covers the body and lines the openings of the pharynx and the anus. Small openings in the cuticle are found at some sensory structures; the amphids, some cephalic papillae and the phasmids (Ward et al., 1975; Ware et al., 1975). Hypodermal cells are restricted to dorsal, lateral and ventral ridges along most of the animal's length (Chitwood and Chitwood, 1950; White, 1975). An additional central hypodermal ridge is found above the anal region in the tail (Figure 1b). Posterior to the anus the anal, lateral and ventral ridges fill most of the available space. At the tip of the tail these ridges merge and become indistinguishable. Similar hypodermal patterns in the nematode tail have been previously reported by Looss (1905) and Chitwood and Chitwood (1950). Hypodermal nuclei are spaced along these ridges; the hypodermis appears to be syncytial in the tail. For clarity, I have not included the locations of hypodermal nuclei in the figures. However, three posterior hypodermal nuclei are very prominent in the tail tip and they can easily be viewed in feulgen-stained animals as the three most posterior nuclei.

The outer surface of the hypodermal cells in the tail

show frequent folded-membrane specializations, presumably for cuticle secretion. One of these specializations is evident in Figure 12, in the hypodermal tissue adjacent to the pre-anal ganglion. Thin hypodermal sheets connect the hypodermal ridges, lying between the body muscles and the cuticle - thus forming a thin cylinder of hypodermis around the whole body.

There are channels along the lateral lines of the animal, where the hypodermal ridges are pulled away from the cuticle. These channels are found along the length of the animal and are conspicuously enlarged in the tail (Figure 1b and Figure 5). They do not appear to be an artifact of fixation. There are gland-like cells (cells 7,8) which run laterally along these channels throughout the length of the tail. Similar cell types have been observed along these channels in anterior regions as well (White, 1975). White reports that there is a chain of these cells, and he suggests that they could be secreting a fluid into the channels. The phasmids are situated at the posterior ends of these channels, where the phasmids emerge laterally to open to the exterior. The channels could be important in transducing mechanical stimuli to the posterior body regions into pressure waves, which they could direct to the phasmids.

DIGESTIVE SYSTEM: C. elegans pumps bacteria into the

Figure 1.

(a) Side view of hermaphrodite tail (schematic). Eight transverse sections are shown in each of four panels (b), (c), (d), and (e). The locations of these sections are indicated in (a). Tail tip is foreshortened; the true tail tip (posterior to the phasmids) is considerably longer.

G33,G34,G35,G36 - Most posterior of the 36 intestinal cells

DC - dorsal cord

VC - ventral cord

LC - lateral cord

AC - anal cord

A - anus

V - rectal valve

VQ,DQ - ventral and dorsal quadrants of the longitudinal somatic muscles

DA - dilator ani

VM - valve muscle

PM - proctodeal muscles

P - phasmid

LG - lumbar ganglia

PAG - pre-anal ganglion

DRG - dorsorectal ganglion

IL - intestinal lumen

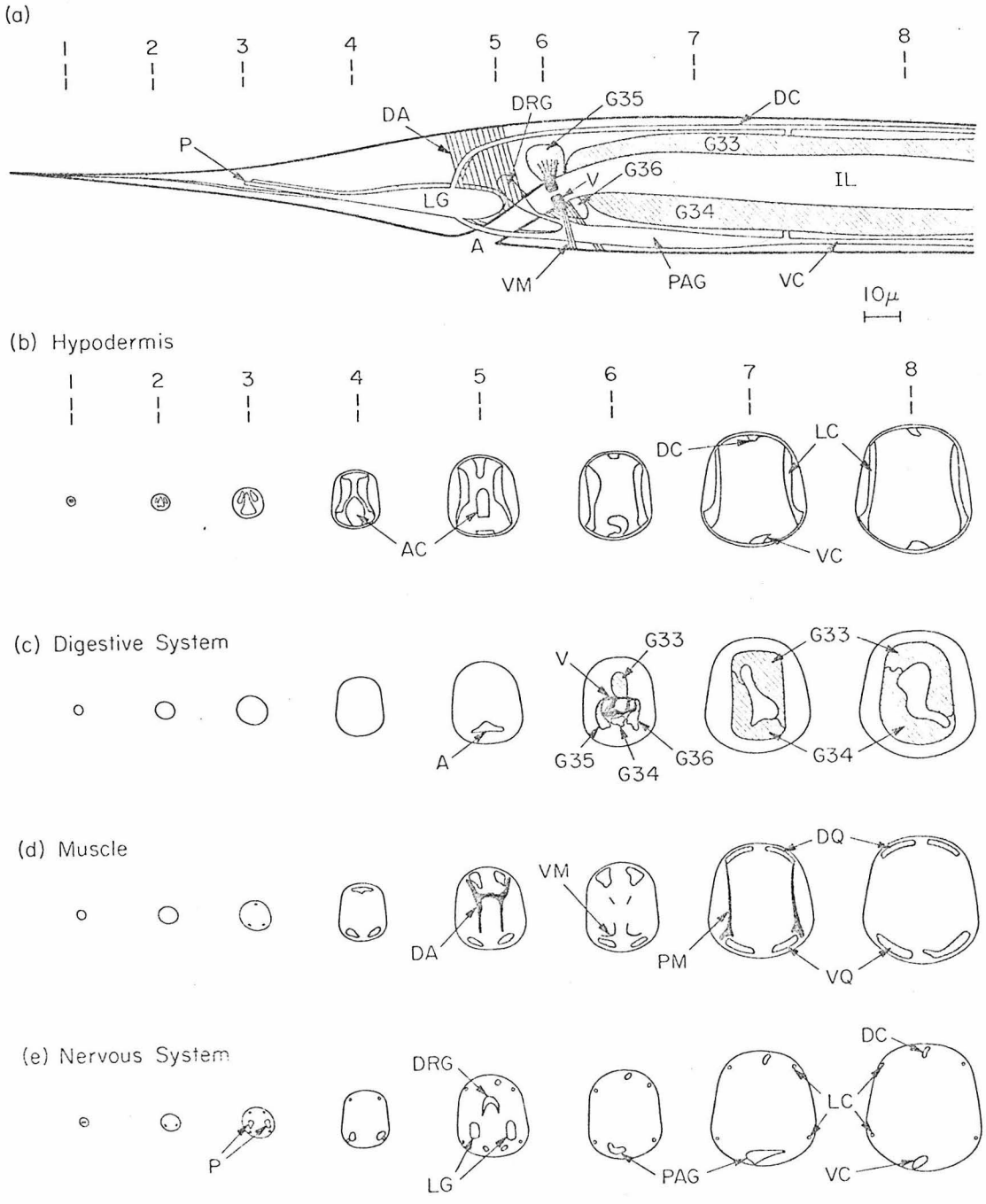

(a)
1
2
3
4
5
6
7
8
DA
DRG
G35
DC
P
G33
V
G36
IL
LG
G34
A
VM
PAG
VC
10μ
(b) Hypodermis
1
2
3
4
5
6
7
8
DC
LC
AC
VC
(c) Digestive System
V
G33
G33
A
G35
G34
G36
G34
(d) Muscle
DQ
VM
DA
PM
VQ
(e) Nervous System
DRG
DC
LC
P
LG
PAG
VC

Figure 1

intestine through a two-bulb pharynx. The structure of the pharynx has recently been described by Albertson and Thomson (1976). The intestine extends from the posterior pharynx to the tail, occupying roughly two-thirds of the animal's length. The intestine is separated from the anus by a rectal valve. The anus is lined with cuticle, as is the pharyngeal opening.

The intestine is formed by successive pairs of cells along its length. Seventeen pairs of intestinal cells have been counted by feulgen staining. A final pair of vestigial intestinal cells are observed in our reconstructed animals. This final pair of cells has a typical intestinal cell cytoplasm, but they are much smaller in size and these cells form intestinal villi for a very short length in the region of the rectal valve (Figures 1a,c).

The rectal valve consists of four dark staining cells. Chitwood and Chitwood (1950) have reported that these cells are modified intestinal cells in other species of nematode. The rectal valve seems to restrict the flow of material to several small channels (approximately five square microns in cross-section). The posterior intestine is enlarged to roughly 100 square microns in cross-section. This region of the intestine has previously been called the proctodeum (Chitwood and Chitwood, 1950). The anus has an internal cross-section of roughly 35 square microns.

Several specialized muscles are present in the tail which probably have a role in defecation (Figures 1a,d). A pair of muscle cells surround the proctodeum, presumably to flatten this region during defecation. A single small muscle cell has four groups of muscle fibers which attach to the four corners of the rectal valve. The two largest fiber bundles of this muscle cell attach the ventral half of the valve to the ventrolateral body cuticle. The smaller dorsal fiber bundles attach to the dorsal half of the valve, but their distal ends are not well anchored. This muscle cell probably serves to open the passage through the rectal valve during defecation. A similar muscle, found in other species of nematodes, is reported to act as a sphincter muscle (Chitwood and Chitwood, 1950). A larger H-shaped muscle cell has muscle fibers which connect the dorsal surface of the anus to the dorsolateral body cuticle. This cell probably serves to widen the anal opening during defecation. This cell has previously been called the depressor ani in other species of nematode (Voltzenlogel, 1903; Looss, 1905; Martini, 1916). Chitwood and Chitwood (1950) report that the depressor ani is universally present in all species of nematodes which have been examined.

These four defecation muscles send muscle arms into a common region above the ventral hypodermal ridge. (Some of these muscle arms are evident in Figure 12.) These muscle

arms appear to form gap junctions among themselves. (There may also be gap junctions connecting muscle arms of the valve muscle and the depressor ani with the muscle arms of the most posterior set of body muscles.) Neuromuscular junctions are formed by a large nerve fiber along the dorsal surface of the ventral hypodermal ridge, adjacent to the pre-anal ganglion (cell 33 in Figure 12). The association of the muscle arms with this motoneuron process is poorly preserved in my fixed animals, so the identification of these neuromuscular junctions must remain tentative.

Figure 1a indicates the distribution of contractile elements of the valve muscle and the depressor ani. Cross-sections of the depressor ani, the valve muscle and the proctodeal muscles are evident in Figure 1d.

BODY MUSCULATURE: C. elegans moves sinusoidally on an agar plate, lying on one side of its body. Cuticular treads along each lateral line help the animal to maintain traction. The sinusoidal motion is generated by longitudinal body muscles, which are organized into four quadrants, two dorsal and two ventral (Figure 1d). The cephalic body muscles are under dual control by ventral nerve cord motoneurons and nerve ring motoneurons. The cephalic muscles move the head through complex searching motions as well as initiating the sinusoidal body motion. The innervation of the cephalic muscles has previously been described by

Ware et al. (1975). The body muscles for the rest of the animal are controlled by motoneurons lying in the ventral nerve cord (White et al., 1976).

Each quadrant of body muscles is served by twenty-four cells, eight of which are cephalic. The muscle cells are arrayed sequentially along the animal's length so that most regions along the animal are served by the sarcomeres of two muscle cells per quadrant. The sarcomeres form a single layer against the cuticle, and are obliquely striated - a type which has previously been called meromyarian-platymyarian (Martini, 1903, 1906, 1909; Chitwood and Chitwood, 1950; Bird, 1971). Each muscle cell has about five sarcomeres running obliquely for most of the cell's length. The cytoplasmic appearance of the tail's body muscles is much the same as the description given by Ware et al. (1975).

The reconstructed region of the tail is served by the last two muscle cells of each quadrant. The most posterior pair of dorsal muscles is atypical, because the dorsal quadrants merge posterior to the anus (Figure 1d). A single muscle cell from the right dorsal quadrant continues posteriad as a single "quadrant" opposed to the two ventral quadrants. This situation lends the phasmidial region of the tail an appearance of three-fold symmetry.

Nematode muscles are unusual in that they send muscle

arms to the nerve cords to receive innervation (Schneider, 1866; Rosenbluth, 1965). In C. elegans the ventral muscle quadrants send arms to the ventral nerve cord for innervation, while the dorsal quadrants send arms to the dorsal nerve cord. All of the motoneurons which signal these muscles are located in the ventral nerve cord. The dorsal nerve cord contains no cell bodies, but receives all of its fibers by commissure from the ventral cord. The organization of the ventral nerve cord has recently been described by White et al. (1976).

In the tail, the body muscle cells send out muscle arms which travel to the nerve cords for innervation. Neuromuscular junctions occur at specialized muscle plates. The muscle arms of paired muscle cells divide into finger-like processes which interdigitate to form the muscle plate (pairs of dorsal muscle arms go to the dorsal cord; pairs of ventral muscle arms go to the ventral cord). Motoneurons form point junctions which signal two adjoining muscle fingers. Paired muscle arms also form gap junctions between themselves in these regions. The nature of these interactions suggests that dorsal pairs and ventral pairs of muscle cells generally receive synchronous input and maintain electrical synchrony by gap junctions. Similar muscle arm interactions have been noted in Ascaris lumbricoides (Rosenbluth, 1965; Stretton, 1976). Weisblat,

Byerly and Russell (1976) have recorded intracellularly from the muscle bellies in Ascaris and found that the many muscle cells in any given region along a muscle quadrant maintain close synchrony, and that the two ventral (or dorsal) quadrants also maintain synchrony at any given region along the animal's length.

In the posterior regions of C. elegans muscle plates and neuromuscular junctions are distributed sporadically along the lengths of the dorsal and ventral cords. The innervation of the defecation muscles is described in another section of this paper. These specialized muscles do not form their neuromuscular junctions in the nerve cords, nor with the same motoneurons.

NERVOUS SYSTEM OF THE TAIL: The nervous system of C. elegans has about 300 neurons, the majority being concentrated in several loosely structured ganglia in the head, between the first and second bulbs of the pharynx. The nerve ring contains some cell bodies, and it receives six major sensory fiber bundles from the sense organs of the snout. A pair of lateral ganglia, a ventral ganglion, and a retrovesicular ganglion lie just posterior to the nerve ring. The ventral nerve cord extends posteriad from the retrovesicular ganglion. The ventral nerve cord has 65 cell bodies distributed rather evenly along its length (Figure 2a). The ventral cord terminates in the tail in the

Figure 2.

(a) Feulgen-stained adult hermaphrodite, dark staining (compact) cell bodies only, animal is lying on ventral side; (b) schematic view of nervous system in the tail, cell body positions are indicated, dorsal cord is displaced to reveal ventral cord, three commissures connect dorsal cord to ventral cord and lumbar ganglia; (c) eight animals have been extensively sectioned, these series are mapped to show the region of the tail's nervous system which is included.

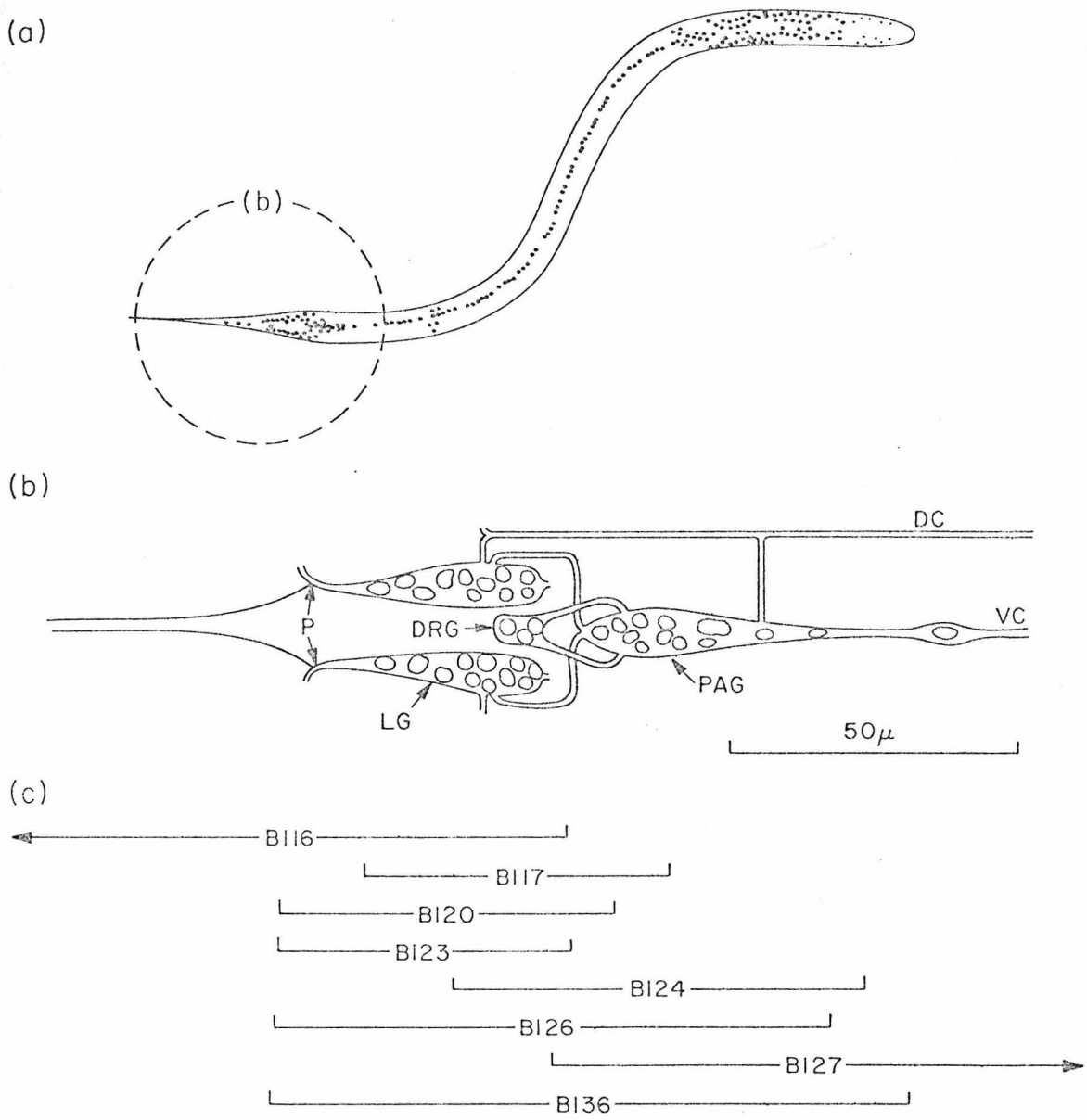

(a)
(b)
(b)
DC
VC
P
DRG
PAG
LG
50μ
(c)
B116
B117
B120
B123
B124
B126
B127
B136

Figure 2

pre-anal ganglion (PAG) which contains twelve loosely associated cell bodies (Figure 2b). Two commissures run in ventrolateral positions from the PAG posteriad to the paired lumbar ganglia, each of which contains twelve loosely associated cell bodies. The sensory cells of the phasmids have cell bodies in the lumbar ganglia. A small dorso-rectal ganglion (DRG) lies in a central position dorsal to the anal hypodermal ridge. The DRG has three cell bodies, and each sends a fiber to the PAG through a circum-rectal commissure. A dorsal cord (which contains no cell bodies) extends along the length of the body opposite to the ventral cord and receives all of its fibers by commissure from the ventral cord. The most anterior of these commissures comes from the retrovesicular ganglion (White, 1975). The dorsal cord terminates in the tail as a pair of circumferential commissures which merge with the lumbar-PAG commissures. A pair of caudal nerves run from the phasmids to the lumbar ganglia, generally in a ventrolateral position. Several nerve fibers run past the phasmids to the extreme tail tip. A pair of lateral nerves runs anteriad from the lumbar ganglion. These lateral cords are very small and their importance is unclear. The disposition of the tail's nervous system is shown in Figures 1e and 2b.

The ventral and dorsal cords are closely associated with the ventral and dorsal hypodermal ridges (White, 1975).

Each nerve cord runs as a tight bundle on one side of the hypodermal ridge. In the tail the ventral cord and the PAG always lie on the left side of the ventral hypodermal ridge, while the dorsal cord always lies on the right side of the dorsal hypodermal ridge (Figures 1b,e). Commissures also run in close association with hypodermal tissue, remaining between the body muscles and the thin hypodermal sheet in their paths from cord to cord. The lumbar ganglia and the lateral cords lie in the large lateral hypodermal ridges. The dorso-rectal ganglion lies against the anal hypodermal ridge, and its commissures run between the depressor ani and the anal hypodermal sheet.

Nerve fibers and neuronal cell bodies lie in close proximity to muscle cells along the length of the animal, but the nervous tissue seems to be isolated from the muscle tissue by a basement membrane (White, 1975). This basement membrane is especially evident along the ventral cord and the PAG. Motoneurons make direct contact with muscle at special muscle plates along the nerve cords. The motoneurons make point junctions which appear to contact two muscle fingers simultaneously. The cytoplasmic specializations at these neuromuscular junctions are very similar to the other synapses in the tail. The pre-synaptic motoneuron has an electron dense deposit along the inner membrane at the site of the junction, but there is no post-synaptic

specialization in the muscle finger. Clear round vesicles, approximately 25 nm. in diameter, are always evident. There are generally several motoneurons which make contacts at each muscle plate.

The pre-anal ganglion is the only well-defined region of neuropil in the tail. The PAG contains about 100 point synapses and a few gap junctions, occurring among forty unbranched fibers. Outside of the PAG there are very few neuron-neuron contacts found in the tail. The lumbar ganglia have no neuropil. The cells of the lumbar ganglia send fibers into the PAG to participate in synaptic interactions. Figure 3 shows the distribution of synaptic contacts along the length of the PAG in two animals. The anterior boundary of this region of neuropil also forms the anterior limit of many of the lumbar fibers which take part in these contacts (compare Figure 3 with the fiber projections shown in Figure 6). The posterior bound of the PAG neuropil also corresponds to the posterior limit of many interneurons which project into the PAG from the anterior ventral cord. The PAG neuropil is much simpler in design than most regions of invertebrate neuropil because there is no fiber branching and a minimum of specializations of any sort. There are very few gap junctions formed between neurons in the tail, a sharp distinction from earlier findings in the ventral cord (White, 1975; White et al.,

Figure 3.

(a) Schematic view of tail nervous system showing cell body positions of neurons. (b) and (c) Distribution of synapses in lumbar and pre-anal ganglia in two animals, B126 and B136. Numbers of synapses are summed over three micron intervals.

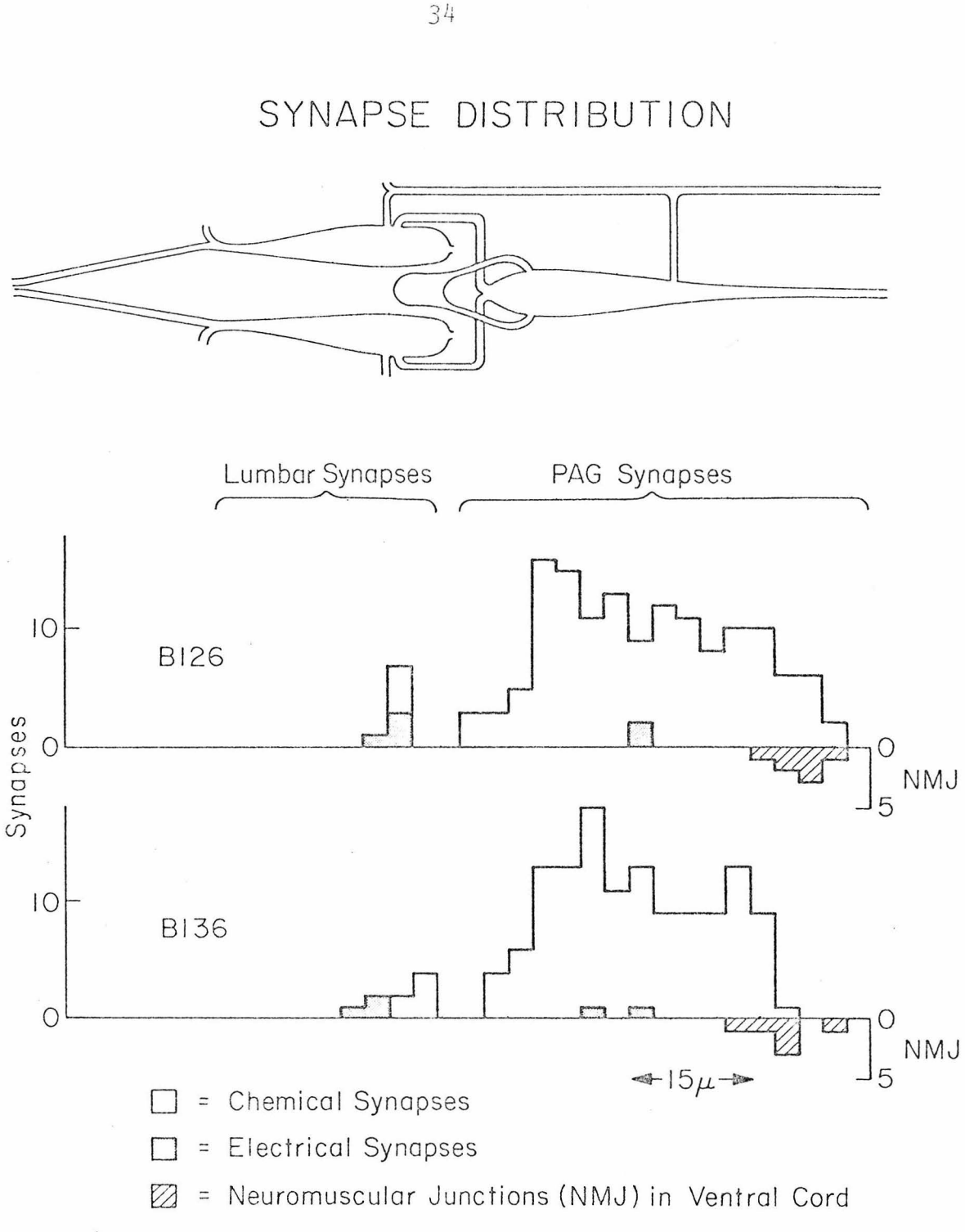

SYNAPSE DISTRIBUTION
Lumbar Synapses
PAG Synapses
B126
B136
Synapses
10
0
0
5
NMJ
15μ
= Chemical Synapses
= Electrical Synapses
= Neuromuscular Junctions (NMJ) in Ventral Cord

Figure 3

1976) and in the nerve ring (Ware et al., 1975). The gap junctions which do exist in the PAG are between the fibers of bilaterally symmetric lumbar sensory cells (cells 17, 18, 27-30). There are a few large chemical synapses, but the great majority of the chemical synapses are small point synapses. Many neurons in the PAG signal the same fibers repeatedly over short distances by multiple point synapses. Multiple synapses are also common in the ventral cord (White et al., 1976). Most of the synapses are dyadic, with a single pre-synaptic fiber and two post-synaptic fibers (Figure 4). Fewer synapses involve one or three post-synaptic fibers. There is always a pre-synaptic darkening along the inner membrane, but there is no post-synaptic specialization. The synapses are en passant, and the pre-synaptic element is usually enlarged locally and filled with clear round vesicles. Most synapses have 25 nm. vesicles, but the lumbar sensory fibers (17, 18, 27-30) have 50 nm. vesicles with more irregular outlines.

The fibers in the PAG neuropil also exhibit non-synaptic interactions in the form of small (approximately 0.2 micron) membrane invaginations between neighboring fibers and between fibers and cell bodies (Figure 4). These invaginations are frequent in the neuropil, uncommon elsewhere in the tail.

Similar structures have been reported previously in

Figure 4.

Typical synapse types in pre-anal ganglion. Open arrows indicate chemical synapses; most are dyads. Filled arrow indicates an electrical synapse. Thin arrows indicate intercellular "invaginations." Magnification ≈ 55,000X. Each panel is roughly 1.4μ x 1.0μ.

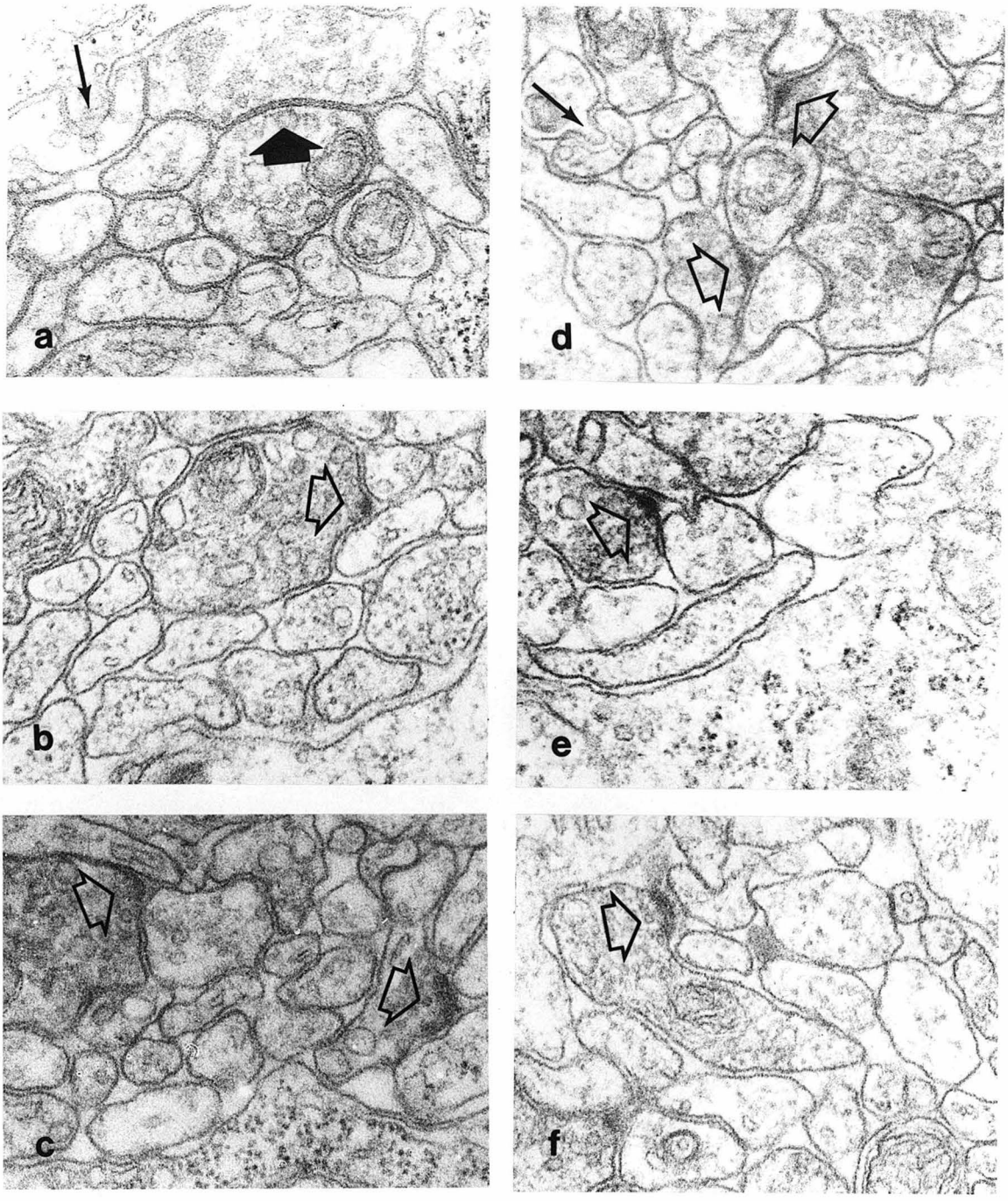


Figure 4

other neural systems, usually in association with coated vesicles. Landis and Reese (1974) observed similar protuberances associated with coated vesicles which are formed between neural elements in the glomerulus of the cerebellar cortex. Henkart (1975) observed coated vesicle invaginations between glial elements (donor) and neural elements (receiver) in Aplysia ganglia. Henkart reports that the frequency of these invaginations increases with increases in Ca^{++} concentration (or by substitution of Co^{++} or La^{+++} for Ca^{++}). She suggests that these invaginations may be the morphological basis for the transcellular transfer of macromolecules. Transfer of newly synthesized protein has been shown between Schwann cells and squid giant axon by Lasek et al. (1974). Membrane invaginations are numerous in the nematode PAG but are infrequently associated with vesicles. No obvious pattern was evident in the distribution of these structures among the neurons of the tail.

Outside of the PAG, there are very few synaptic contacts in the tail. A few electrical synapses and one class of chemical synapses are found in the lumbar ganglia. The dorsal cord contains some neuromuscular junctions, but no other synaptic interactions. No synapses have been found near the phasmids.

SENSORY ELEMENTS: Most of the hermaphrodite's sense organs are located in the head. The anterior tip of the animal is

innervated by two large amphids (each containing twelve ciliated dendritic endings) and by twenty-four other specialized dendritic endings which are assorted among sixteen papillae of six types. The detailed morphology of these endings and their supporting cells has previously been described by Ware et al. (1975) and Ward et al. (1975). A pair of cervical papillae, the deirids, are positioned just posterior to the nerve ring, with sensory endings embedded in the cuticle at the lateral lines. These organs are presumed to be pressure-sensitive, as they have no external opening (Ware et al., 1975). A pair of "post-deirids" may exist in the posterior half of the animal, also having sensory endings along the lateral lines. Sulston, Dew and Brenner (1975) have observed a pair of small dopamine-staining bodies in the posterior regions which they presume to be associated with the post-deirids. I have observed similarly positioned groups of cell bodies in my feulgen-stained animals (Figure 2a). My serial reconstructions of the tail have not extended to this region. The nature of these receptors (?) remains obscure.

The tail contains a pair of phasmids, each having two ciliated dendritic endings. No proprioceptors have been found in the posterior regions, nor have they been found elsewhere in C. elegans (White et al., 1976). There are no other "anal papillae" in the hermaphrodite tail, although

there are some non-papillary sensory endings near the phasmids.

The phasmids are very similar to the amphids and papillary organs of the head. Each phasmid consists of two ciliated dendritic endings within an extracellular pocket formed by a cap cell and a pocket cell (Figure 5) (Ware et al., 1975; also called the socket and sheath cells by Ward et al., 1975). A wing cell provides a wrapping process along the surface of each phasmid. The extracellular surround of the phasmidial cilia is in contact with the exterior, much like the amphidial pocket. The phasmidial cilia have filamentous endings which protrude very slightly from the phasmidial opening (Figure 5a). These cells do not have striated rootlets. Electron-dense material seals the cap and pocket cells together, much the same as the sealing of the amphidial cap and pocket cells (Ware et al., 1975) (Figure 5b). The cilia invade the pocket by means of a wrapping process of the pocket cell. The general cytoplasmic appearance of the phasmidial pocket is very similar to that of the amphidial pocket, but there is no "finger cell" associated with the phasmid (Ware et al., 1975)(Figure 5c). The cell bodies of the wing, cap, and pocket cells lie just anterior to the phasmid (cells 1-6) (Figure 9). The cell bodies of the phasmidial ciliated neurons are located in the anterior ends of the lumbar ganglia (cells 27-30) (Figure 9).

Figure 5.

Schematic drawing (center) shows a longitudinal reconstruction of the right phasmid. (a-f) Micrographs show transverse sections through different levels of the phasmid and tail tip; (a) Phasmidial opening to exterior, filamentous material from ciliated neurons extends into this opening (b) Phasmidial cross-section shows cilia enveloped by the cap cell, dark material inside cap cell cements it to the invading pocket cell, wing cell is wrapped around cap cell (c) Phasmidial cross-section shows cilia enveloped by pocket cell, wing cell is wrapped around pocket cell (d) shows basal body of cilium from cell 19, which is outside of the wing cell (e) shows fan-like ending of cilium from cell 19, which is embedded inside the wing cell (f) shows extreme tail tip of the nematode, dendrites of cells 17,18 are enveloped only in a little cuticle and hypodermis. Posterior tip is to the top of the drawing, lumbar ganglion is to the bottom of the drawing. Scale bar applies only to to drawing. Micrographs are enlarged approximately 13,000X.

C - cuticle

SC - subcuticular channel

H - hypodermis

W - wing cell

Pc - pocket cell

Cp - cap cell

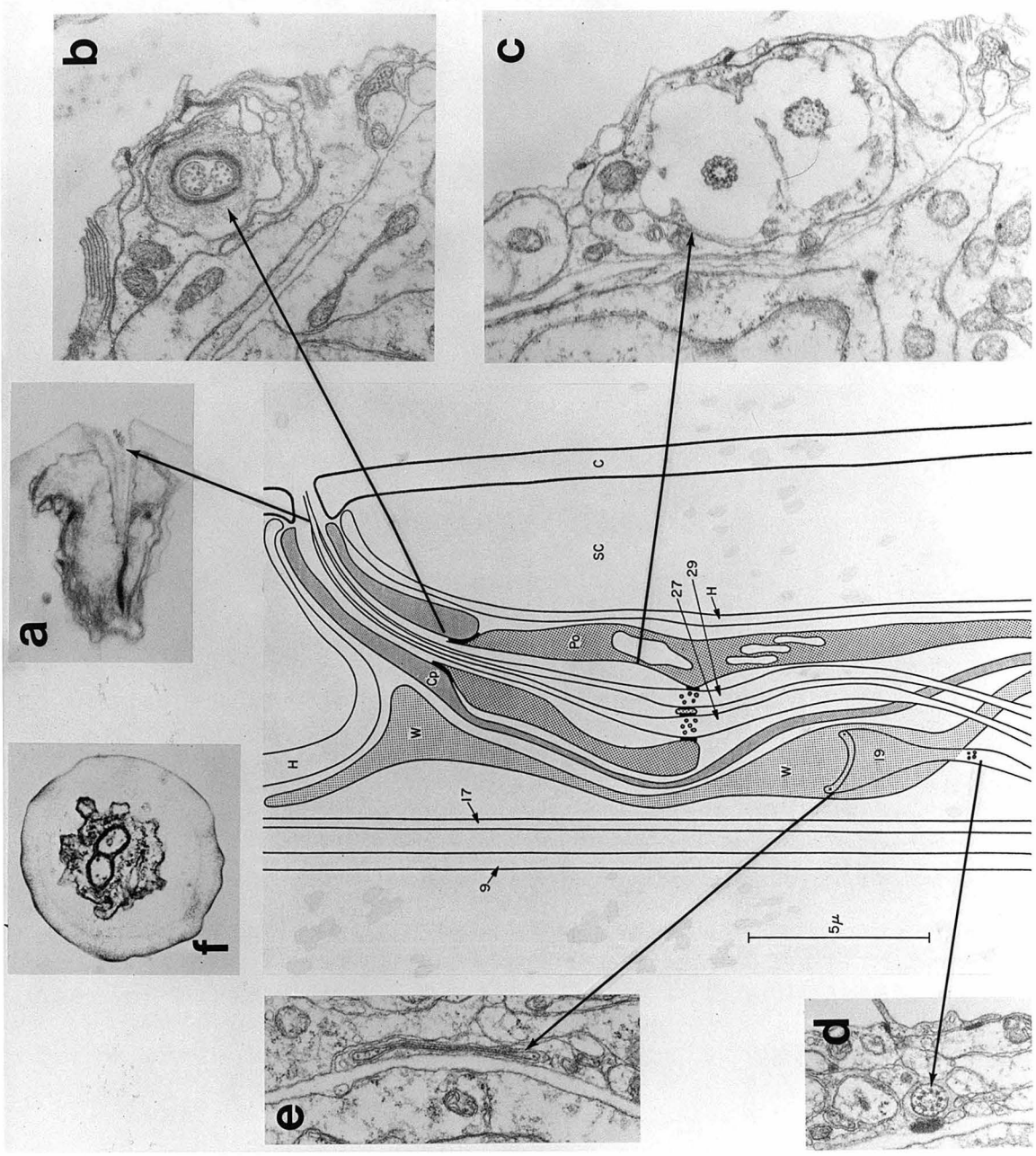


Figure 5

There is an asymmetric ciliated dendritic ending embedded in the wing cell of the right phasmid. The cilium ends as a broad fan-like electron-dense extension (Figures 5d,e). This ending is not exposed to the exterior, lying very close to the central axis of the tail. This dendrite has no striated rootlet, and has its cell body in the right lumbar ganglion (cell 19). The corresponding cell in the left lumbar ganglion has no dendrite (cell 20). This asymmetry has been confirmed in six animals, always with the same handedness.

Two lumbar cells (cells 17, 18) each have a posterior process which travels in a bundle with the dendritic processes of the phasmidial and asymmetric cilia. These two processes are presumed to be sensory dendrites, although they do not show any specialized endings. These two processes run in ventrolateral positions past the phasmids and continue posteriad for another 65 microns to the extreme tip of the tail (Figures 2b and 5g). In these posterior regions the tail narrows to a fine hair-like process which undergoes frequent severe bends in the course of the nematode's activity, especially during reversal. This hair-like process bends in response to the slightest mechanical stimuli, and I presume that the dendrites of cells 17, 18 are sensitive to these mechanical stimuli.

IV. OBSERVATIONS:

DETAILED NERVOUS ANATOMY OF THE ADULT HERMAPHRODITE TAIL

CELL IDENTIFICATION: NEURONAL MORPHOLOGIES AND POSITIONS

Preliminary studies of feulgen-stained worms indicated that the neuronal cell bodies of the tail are constant in number and stable in general configuration. The lumbar ganglia and the PAG are visible in these preparations as separate collections of cell bodies. This level of analysis obviously cannot serve to demonstrate the reproducibility of individual neuron identifications. Reconstruction of serial thin sections of the tail has now confirmed that a complete list of individual cell types can be identified in each adult tail, and that each cell type has a relatively stable cell body position and a very stable set of fiber projections. Great care was required to match the proper set of sensory fibers from the lumbar ganglia with their synaptically active distal projections in the PAG. The success of this effort potentiates the manipulation of identified neurons in living animals, either by mutation or laser inactivation. The unbunched distribution of the cell bodies in the tail makes this region particularly favorable for these manipulations. Careful identifications by cell body position also allows the recognition of daughter cells from lineage cell divisions. This section is a catalogue of the morphological characteristics which distinguish each

neuronal cell type in the tail.

Most of the tail's cells can be identified solely on the basis of their cell body position, cytoplasmic features, and proximal fiber projections. All supporting cells, hypodermal cells, gland cells, muscle cells, gut cells, and most neurons can be quickly categorized on this basis. (Cell body positions of hypodermal cells, muscle cells, and gut cells here are not shown in the following figures for the sake of clarity.) There are some neurons, particularly lumbar neurons, which can be confused unless their distal fiber projections are traced through the commissures to the PAG. The lumbar fiber projections are very characteristic for each cell type. The fiber positions within the commissures and within the PAG are so stable that each lumbar neuron can now be identified within the PAG without tracing back to its cell body. Reconstruction of the lumbar-PAG commissures is particularly difficult in sections cut perpendicular to the body axis, but I have now accomplished this task in several animals. The commissures of the dorso-rectal ganglion are also very difficult to reconstruct in my sections, and I have been able to match these fibers to their cell bodies in the DRG in only one animal. However, the DRG fibers are easily distinguished and compared from animal to animal within the PAG. These DRG commissures have also been traced once by

Lois Edgar in another animal, and our results are in agreement.

All of the following cell types have been found to be morphologically identical in two or more animals; especially in B126 and B136. Cell body positions are summarized in Figure 9, and Figures 6, 7, and 8 contain schematic drawings of the processes of each identified cell.

LUMBAR GANGLIA: The phasmidial support cells have no anterior projections and are easily distinguished. There are three cell types: wing cells (1,2), cap cells (3,4) and pocket cells (5,6). Their cell bodies lie in the extreme tail tip, posterior to the lumbar ganglia (Figure 9).

Cells 7,8 have a gland-like appearance. They have lateral processes which extend along the lateral subcuticular channels. They may be analagous to similar cells noted in the anterior by White (1975). Their cell body positions are more variable than those of the lumbar neurons (Figure 9).

Two pairs of cells produce the ventro-lateral cords. Cells 9,10 have characteristic cell body positions at the posterior limits of the lumbar ganglia (Figure 9). Their ventro-

lateral fibers are filled with tubules, and these fibers extend from the extreme tail tip anteriad throughout our sections. (The tubule-filled projection of cell 9 can be seen in Figures 5b,c). Cells 11,12 have characteristic cell body positions in the central lumbar ganglia (Figure 9), and they have ventrolateral processes which travel in close association with the processes of cells 9,10. The processes of 11,12 are not tubule-filled.

Cells 13,14 lie dorsally in the tail, very near the lumbar region. These two cells have extensive dorsolateral processes, very much like the ventrolateral processes of cells 9-12. The processes of cells 13, 14 are not tubule-filled, and they extend from the tail tip anteriad throughout our sections.

The following information about the lumbar morphology of neurons 15-32 is probably sufficient to identify them in the reconstruction of other adult tails. None of this information is required to identify the processes of these cells in reconstructions of the PAG. Some details of fiber position for these cells are summarized in Figures 6, 9, 10 and 11.

Figure 6. Lumbar Ganglion Cells

Each panel depicts a schematic view of the nervous system of the tail, showing the cell body positions and fiber projections of a bilaterally homologous pair of lumbar neurons. (Exception: cells 19 and 20 are each unpaired neurons.) All of these cells send processes through lumbar PAG commissures into the pre-anal ganglion; only eight cells have anterior projections which enter the ventral cord. Seven cells have posterior sensory dendrites. Two cells (21,22) have short axons in the lumbar ganglia. All cell body positions and fiber positions are those of B136.

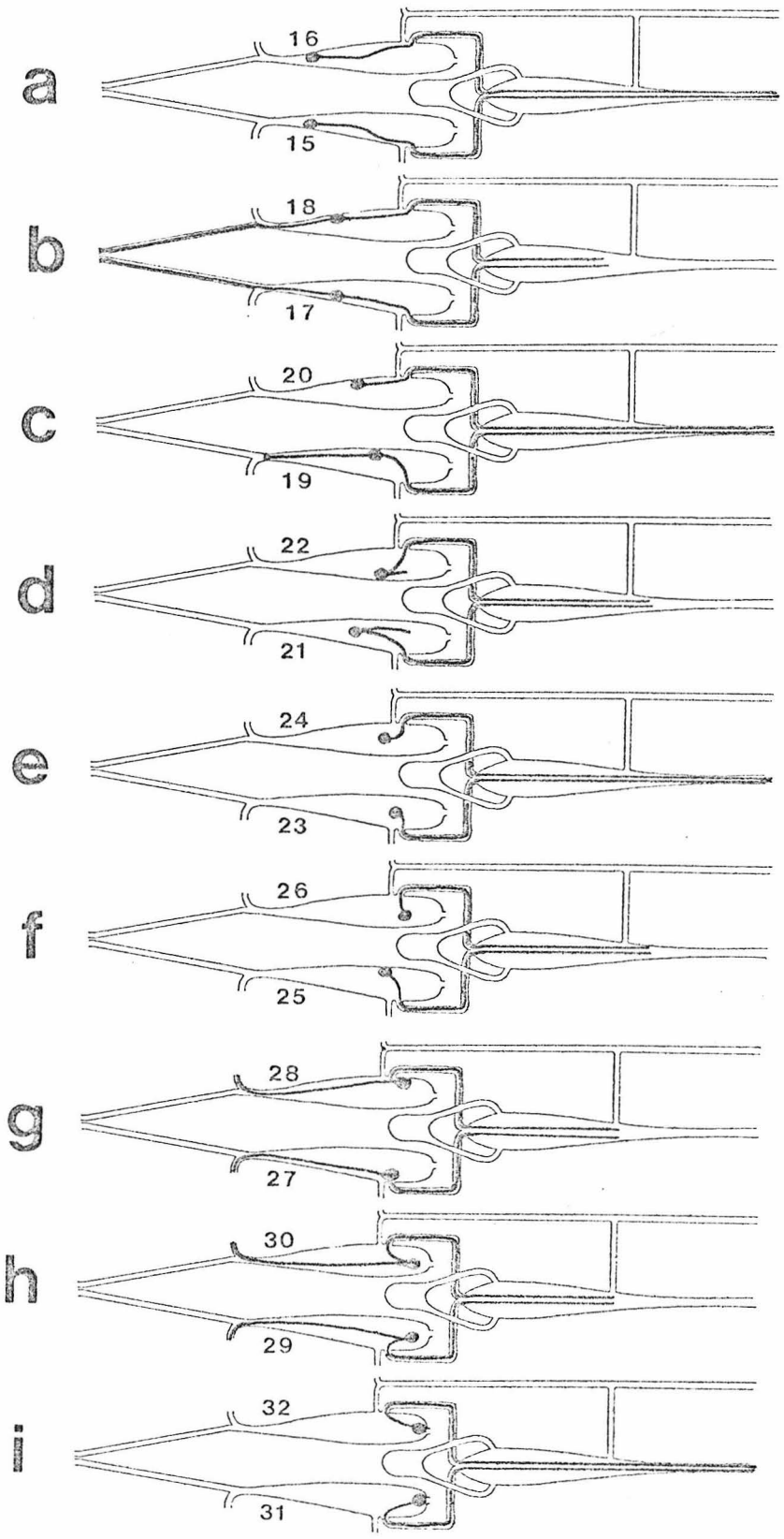


Figure 6

The cell bodies of cells 15,16 are located just posterior to the lumbar ganglia proper. The extent of their fiber projections into the PAG is shown in Figure 6a. These cells have no sensory specializations and their synaptic interactions are diffuse. The function of cells 15,16 is unclear.

Cells 17,18 are presumed to be sensory neurons, since they have "dendrites" which extend to the extreme tip of the tail. The fiber projections of these cells are shown in Figure 6b. These cells have extensive synaptic output in the PAG.

Cell 19 is the asymmetric cilium sensory neuron, located in the right lumbar ganglion. The corresponding cell in the left lumbar ganglion, cell 20, has no sensory dendrite. Both cells have projections into the PAG, shown in Figure 6c. Only cell 19 has many synaptic connections in the PAG, but both cells project anteriad into the ventral cord. Cell 19 is the only lumbar sensory neuron which does send a process into the ventral cord. The cell body of cell 20 is not easily distinguished from those of cells 22 and 24 - all

three cell bodies lie in similar dorsal positions within the left lumbar ganglion, but cell 20 is generally posterior to 22 and 24, and its process enters the lumbar-PAG commissure in a more posterior position.

Cells 21,22 have cell bodies which lie dorsally within the lumbar ganglia (Figure 6d). Their projections into the PAG do not participate in significant synaptic interactions. However, cells 21,22 each have a short axon which runs for a short distance anteriad along the dorsal margin of the lumbar ganglion. These axons become embedded in the cell bodies of cells 23,24, where they form a series of chemical synapses.

Cells 23,24 lie in dorsal positions within the lumbar ganglia. They have no dendrites within the lumbar ganglia, but send processes into the PAG which receive major synaptic input there (Figure 6e). The cell bodies of cells 23,24 receive direct synaptic contact from the proximal axons of cells 21,22. Cells 23,24 are thought to be major interneurons in the ventral cord.

The cell bodies of cells 25,26 also lie

in dorsal positions within the lumbar ganglia, very near those of cells 23,24. Cells 25,26 often appear somewhat smaller and rounder than neighboring lumbar cell bodies. These cells have no sensory dendrites; their projections into the PAG are shown in Figure 6f. Their proximal fibers are easily distinguished as they enter the lumbar-PAG commissures, since they form gap junctions there with the anterior processes of cells 9,10, the lateral tubule-filled fibers. The significance of these contacts is not clear. Cells 25,26 are important interneurons withing the PAG.

Cells 27,28 are phasmidial sensory neurons, whose dendrites form the more dorsal cilia within the phasmids (Figure 6g). Their cell bodies are somewhat larger than neighboring lumbar cell bodies, and they lie dorsolaterally in the lumbar ganglia. These cells have extensive synaptic output in the PAG. Their axons travel in extreme ventral positions within the lumbar-PAG commissures, under the neighboring fibers (Figure 10).

Cells 20,30 are phasmidial sensory neurons, whose dendrites form the ventral cilia within

the phasmids. Their cell bodies have anterior positions within the lumbar ganglia. The projections of these cells are shown in Figure 6h. They have extensive synaptic output in the PAG, quite distinct from that of cells 27,28.

Cells 31,32 have the most anterior cell bodies in the lumbar ganglia. They do not have sensory dendrites, but they do send processes into the PAG and anteriad into the ventral cord, shown in Figure 6i. Their fibers do not take part in PAG synapses. The morphology of these cells does not reveal any clear functional role.

Figure 6 presents schematic morphologies for all of the lumbar neurons which send projections into the PAG. (Cell body positions are those found in B136.) All of the fiber projections have been confirmed in two or more animals. The cell body positions of the lumbar ganglia of both B126 and B136 can be compared in Figure 9.

PRE-ANAL GANGLION: The pre-anal ganglion cells are relatively simple to identify in reconstructions of the PAG. Their cell bodies and their fiber configurations within the

PAG are all quite characteristic. Figure 7 displays the schematic morphologies of these twelve cells in two animals (B126 and B136). The cells are grouped into lineage and non-lineage cells. Some specific features are not easily depicted on the figure; these are listed below. Some of these cells have been identified as motoneurons by their junctional contacts with muscle arms from longitudinal body muscles. Most of the twelve cells have rather sparse synaptic contacts within the PAG; however, cell 43 is an active interneuron within the PAG.

Specific features of some PAG cells include:

Cell 36 has a large crenated nucleus and a unique cell body position at the posterior limit of the PAG. The dorsal process of cell 36 has motor output and runs in the DD position.

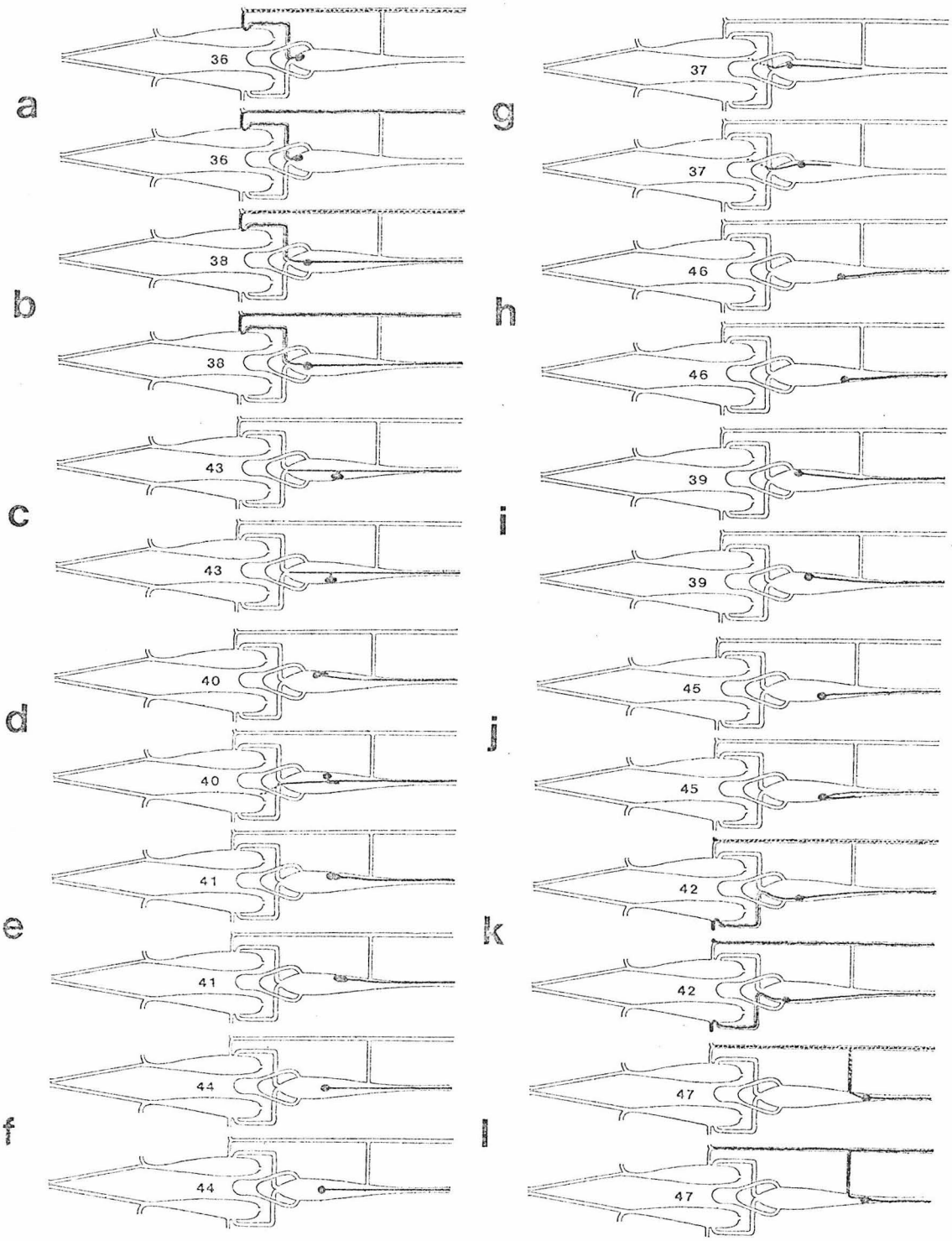
Cell 37 has a posterior process which continues in a ventral position along the inside surface of the anus after all other processes have commissured into the lumbar ganglion.

Cell 38 has a dorsal process which has motor output and runs in the DA position. The cell's ventral process run near other class A fibers.

Cell 39 has a process in the ventral cord

Figure 7. Pre-Anal Ganglion Cells

Each panel depicts two schematic views of the nervous system of the tail, showing the cell body positions and fiber projections of a pre-anal ganglion cell in two animals (B126 and B136). The cells shown in the left panels (a-f) are present at hatch in the pre-anal ganglion. The cells shown in the right panels (g-l) are the daughters of post-hatch lineage cell divisions. Four cells have commissures to the dorsal cord. Dashed lines indicate presumed projections (e.g. the dorsal cord of B126 has not been fully reconstructed). Two cells (37,40) have posterior processes which remain in lateral positions after other fibers have made circumferential commissures; these processes have not been fully reconstructed.


a
36
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b
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c
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d
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h
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i
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39
j
45
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k
42
42
l
47
47

Figure 7

which has motor output and runs in the VD position.

Cell 40 has a rather larger cell body which typically sends out several extra short processes into the PAG.

Cell 41 has the largest cell body in the PAG, an unmistakable feature.

Cell 42 has a distinctive posterior process which emerges from the cell body as a flattened sheet. This process travels through the right PAG-lumbar commissure to the dorsal cord. Gap junctions with cell 9 are sometimes observed in the lumbar ganglion. The dorsal cord process and the ventral cord process both travel near other class A fibers, but cell 42 has no apparent motor output in the tail.

Cell 43 is an interneuron. This cell body is positioned at the extreme right side of the PAG (see Figure 12).

Cell 44 has its cell body in a central position, lying just dorsal to the PAG neuropil.

Cell 45 has its cell body displaced far to the right side of the PAG. This cell body is somewhat flattened, especially at the anterior end.

Cell 46 has a very round cell body at the

anterior end of the PAG neuropil, and lies to the right.

Cell 47 has its cell body anterior to the PAG neuropil. This cell body is very round and lies to the right. The cell's processes in the dorsal and ventral cords run near other class A fibers, but no motor output has been found.

DORSORECTAL GANGLION: The dorsorectal ganglion has only three cell bodies, each sending a fiber into the PAG through one of the circumferential commissures. The morphologies of the DRG cells and their projections are shown in Figure 8. These data are taken from B126, where the circumferential commissures have been successfully reconstructed. These commissures cannot be reliably traced in B136. Lois Edgar first reconstructed these commissures in another animal (unpublished observations). My own findings in B126 confirm her earlier results. The fiber from cell 33 is greatly enlarged and filled with synaptic vesicles as it crosses the ventral hypodermal ridges to enter the PAG. This fiber's size makes it easily distinguished from the neighboring fiber of cell 35. Cell 33 probably acts as a defecation motoneuron, forming neuromuscular junctions with the muscle arms of the defecation muscles along the dorsal surface of the ventral hypodermal ridge.

Figure 8. Dorsorectal Ganglion Cells

Each panel depicts a schematic view of the nervous system of the tail, showing the cell body position and fiber projections of a dorsorectal ganglion cell in B136. The same configuration of cell bodies and fibers has been partially reconstructed in B126 and completely reconstructed in another animal by Lois Edgar. Each cell has a single projection which enters the PAG by way of a circumrectal commissure. All of these fibers travel anteriorly into the ventral cord.

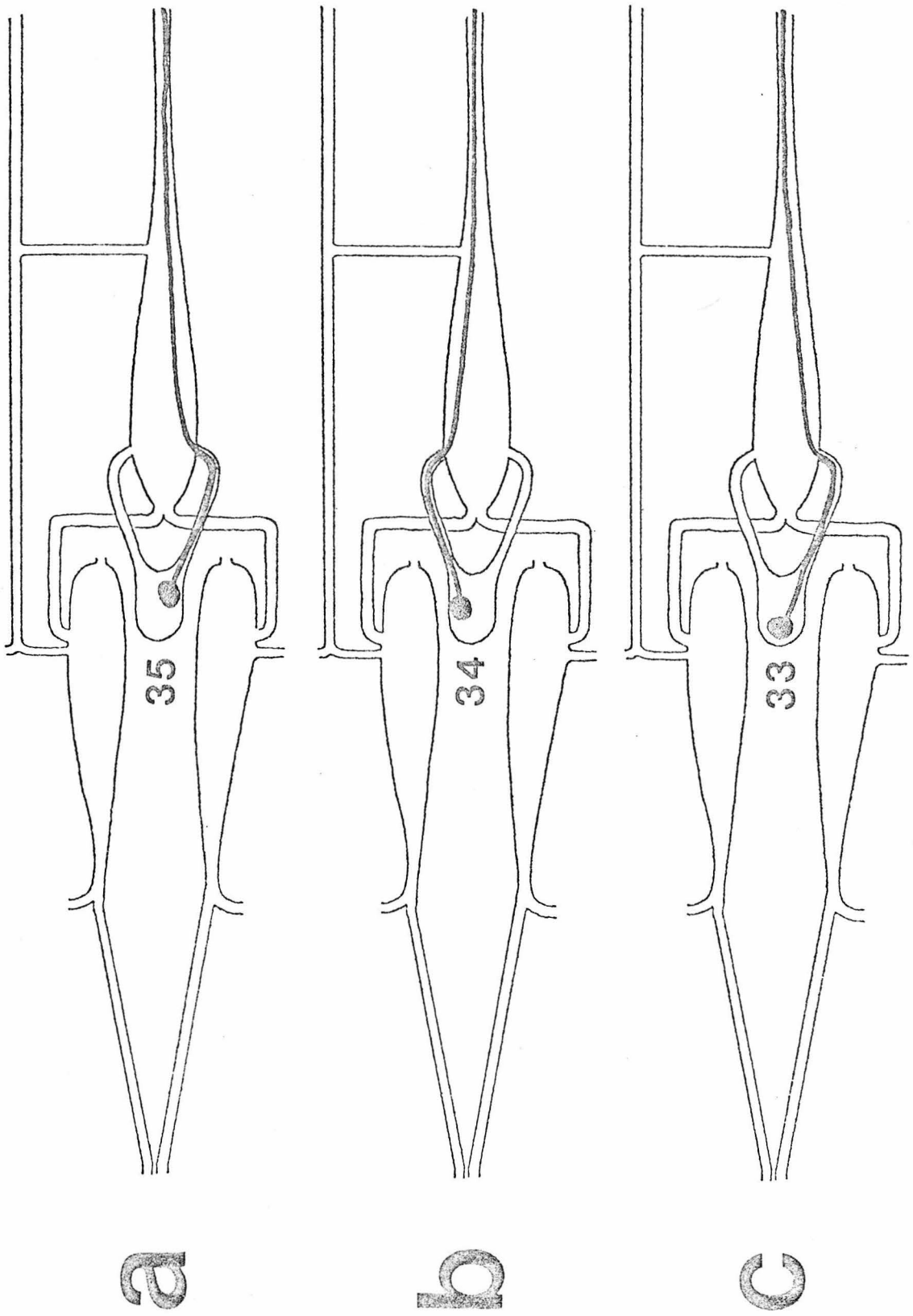


Figure 8

ANTERIOR CELLS: Thirteen or more fibers entering the PAG from the ventral cord are presumably distal projections from anterior neurons. Most of these fibers end blindly at the posterior limits of the PAG. One to three others enter circumrectal commissures and continue posteriad along the anal ridge. Thirteen fibers have been identified in the PAG in both B126 and B136. Each can be identified by its separate route through the PAG, its size, varicosities, cytoplasmic appearance, and synaptic connections (Figure 12). Four of these fibers represent two pairs of major interneurons. These four fibers travel together in a central position within the PAG; a pair of larger fibers running ventral to a pair of very small fibers (Figure 19). The larger fibers have a very light cytoplasm and many swellings filled with vesicles. The identity of these interneurons will be discussed further, but for the moment we will label these fibers as the α (larger) and δ (smaller) interneurons. One of the remaining fibers is clearly a motoneurons with junctions along the ventral cord to the most posterior body muscles - this fiber runs in the "ventral B" position of White et al. (1976), and is labelled B in subsequent figures.

The fibers which enter the circumferential commissures have been labelled t, u, and v. Fiber t passes under the DRG and continues posteriad along the anal hypodermal ridge.

This fiber has many varicosities along its length, especially along the anal ridge. (Lois Edgar first noted this feature.) Fibers u and v seem to end blindly at the DRG. V is present only in B136. Neither u nor v has been identified in the animal reconstructed by Lois Edgar. Fibers t and u receive significant synaptic input from sensory fibers in the PAG. None of these fibers appears to have any synaptic output in the tail.

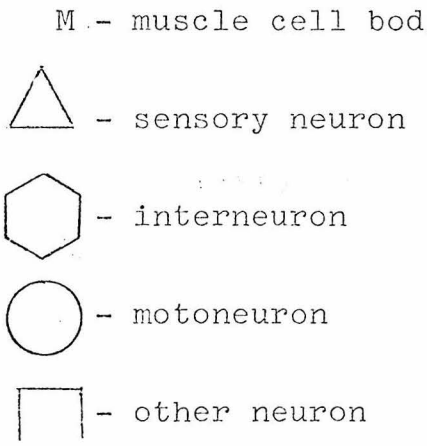
Fiber s is very striking in appearance, containing elaborate membranous inclusions. This fiber has no significant synaptic interactions and may not be a neuronal process.

Fiber n receives substantial synaptic contact from a PAG interneuron, and has no synaptic output in the tail. Three other fibers from the anterior, labelled p, q, and r, participate in very sparse synaptic interactions.

A complete list of the cells of the adult hermaphrodite tail is given in Appendix I. The cell body positions in the tail are illustrated in Figure 9, comparing the reconstructions of B126 and B136. The relative cell body positions show a certain degree of variability. This variability has not endangered our identifications of individual cells because of the concurrent stability of fiber positions. Fiber positions are very stable and often

Figure 9. Cell Body Positions in Two Animals (B126 and B136)

Each panel depicts a schematic view of the nervous system of the tail, showing the cell body positions of all neurons and supporting cells in the tails of B126 (above) and B136 (below). Cell bodies of supporting cells lie along the periphery of the lumbar ganglia. Cell bodies of one pair of bilaterally homologous neurons (cells 13,14) lie dorsally, outside of the lumbar ganglia.



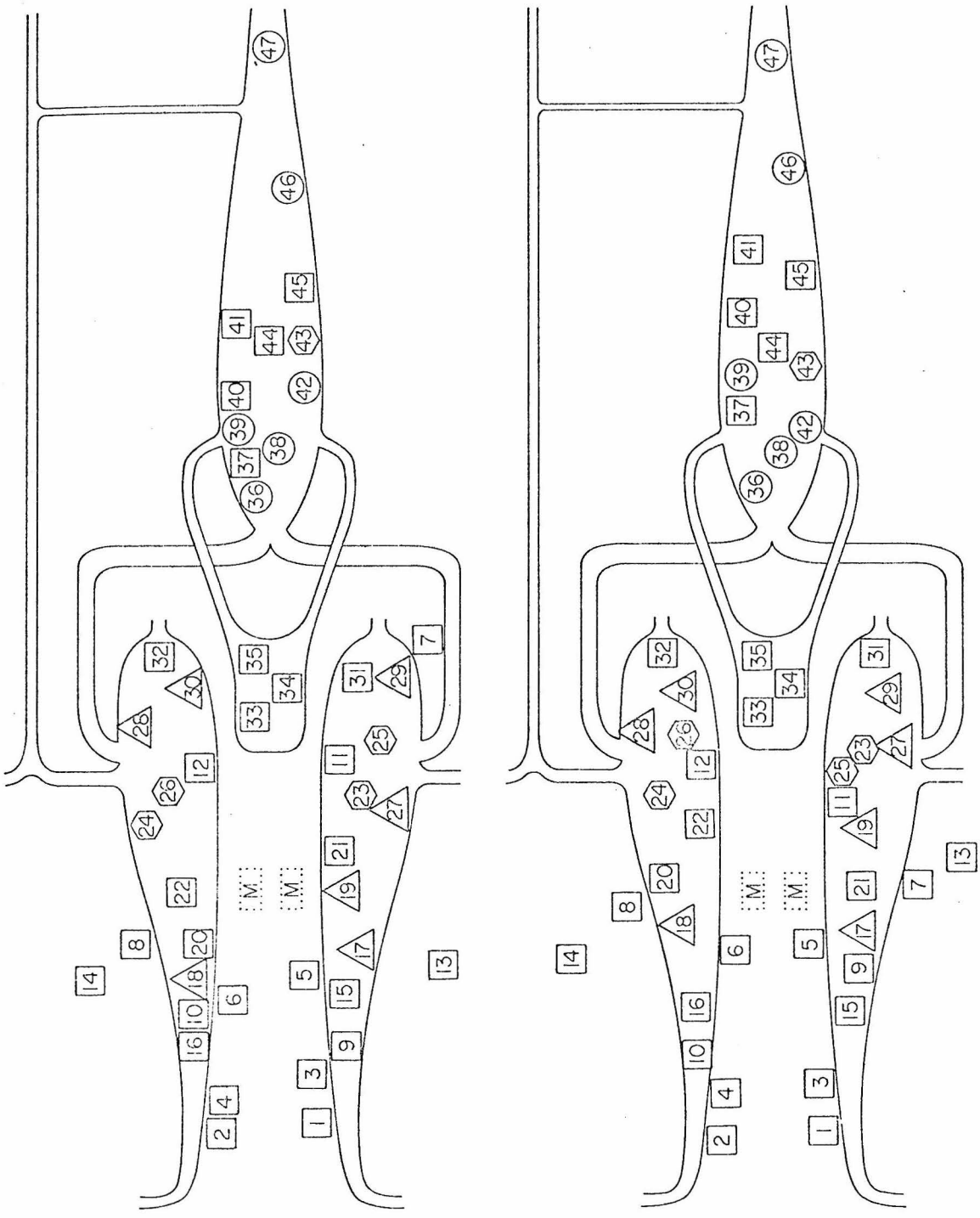

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Figure 9

more useful than cell body position in making identifications. White et al. (1976) found in the anterior ventral cord of C. elegans that fiber positions are stable enough to identify individual types of motoneurons and interneurons over great distances from their cell bodies. The relative sizes of these fibers are also characteristic of each cell type.

Fiber positions within the PAG are stable enough to identify all cells without retracing the commissures. It is not possible to reproduce all of these data in these few pages. In my reconstructions I relied upon cinematographic techniques to quickly explore the routes by which each fiber makes its way through the PAG. B136 was compared to B126 in this manner, and all fibers were identified unmistakably. Corresponding pairs of lumbar cell projections generally have symmetrical placements within the PAG, and some pairs have gap junction interactions with their homologues.

Commissures to the dorsal cord are identical in the two animals examined. The dorsal cord of B136 has been reconstructed extensively, covering 80 microns anterior to the commissures from the lumbar regions. Relative fiber positions in the dorsal cord are extremely stable over the entire reconstructed region. Four members of the dorsal cord come from identified PAG neurons by way of commissures in the tail. The rest of the dorsal cord fibers (approxi-

mately five others) must come from commissures anterior to the reconstructed region; additional PAG neurons which have ventral projections could be participating in some of these commissures.

Figures 10 and 11 display the ordering of fibers in the lumbar-PAG commissures at two levels, comparing the organization in B126 and B136. Figure 12 displays the disposition of fibers and cell bodies at a position midway along the PAG in the same two animals. Figures 19a,b show the positions of the important motoneurons and interneurons at the anterior end of the PAG in both animals. These positions should be compared to the organization of the anterior ventral cord, shown in Figure 19c (taken from White et al., 1976).

SYNAPTIC ORGANIZATION OF THE PRE-ANAL GANGLION NEUROPIL:

Complete surveys of the PAG synapses were made in B126 and B136 by examining prints of high magnification EM photographs of every PAG section in each animal. All neuronal cell bodies and processes had been previously identified by cinematography, so that all participating elements in each synapse could be noted. Occasional sections were missing, and occasional sections were relatively poor in quality, but there were no serious gaps in either series. A list of synapses was made for each animal, noting the position of each synapse by section number. Listed synapses show

Figure 10. Fiber Positions in Lumbar-PAG Commissures (1)

Left and right lumbar-PAG commissures are shown for B126 (a) and B136 (b) at corresponding levels in each animal. These cross-sections depict only the extreme ventral edge of the animal (see Figure 1, panel (e), cross-section 5). Magnification is about 8000X. A tracing of the fibers of each commissure is shown above each micrograph. Fiber positions of homologous cells are similar from side to side, and from animal to animal.

Each commissure carries fibers from the lumbar ganglion ventrally to pass the ventro-lateral cord (cells 9,11 or 10,12), the fibers then pass under the ventral body muscle quadrant and then anteriorly to join the PAG. The level shown here is the most anterior lumbar entrance to the commissures, where the most anterior lumbar cells (27-32) are just entering the commissure (from the extreme left and extreme right). Because the fibers are cut obliquely in this region, they are commonly elongated and sometimes found discontinuously at several points in the same section.

Some PAG cells (36,38,42) use these commissures to travel to the dorsal cord. Other PAG cells (37,40) sometimes continue posteriorly near the positions shown, without passing under the muscle cells.

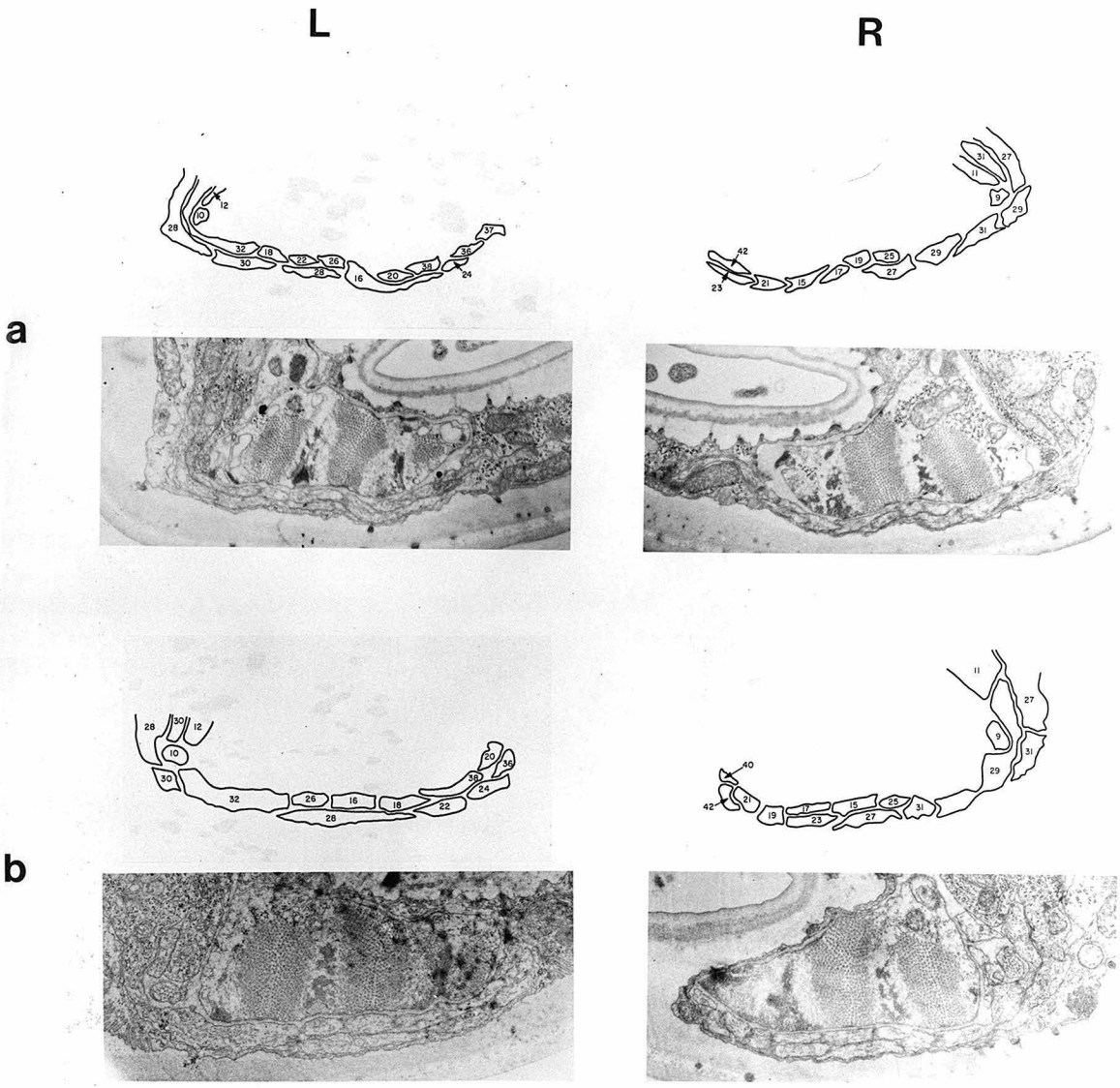

Figure 10

Figure 11. Fiber Positions in Lumbar-PAG Commissures (.

Left and right lumbar-PAG commissures are shown in B126 (a) and B136 (b) at corresponding levels in each animal. Magnification is about 25,000X. These cross-sections are just anterior to those in Figure 10, near the extreme ventral edge of the animal. Here the fibers are in an orderly bundle between the passage under the muscle quadrant and the entrance to the posterior limits of the PAG. Lumbar fibers (15-32) are in relatively constant positions with respect to each other. Bilaterally ho gous pairs of fibers take similar positions in these commissures. Fibers from PAG cells (36,37,38,40,42) are not rigidly ordered.

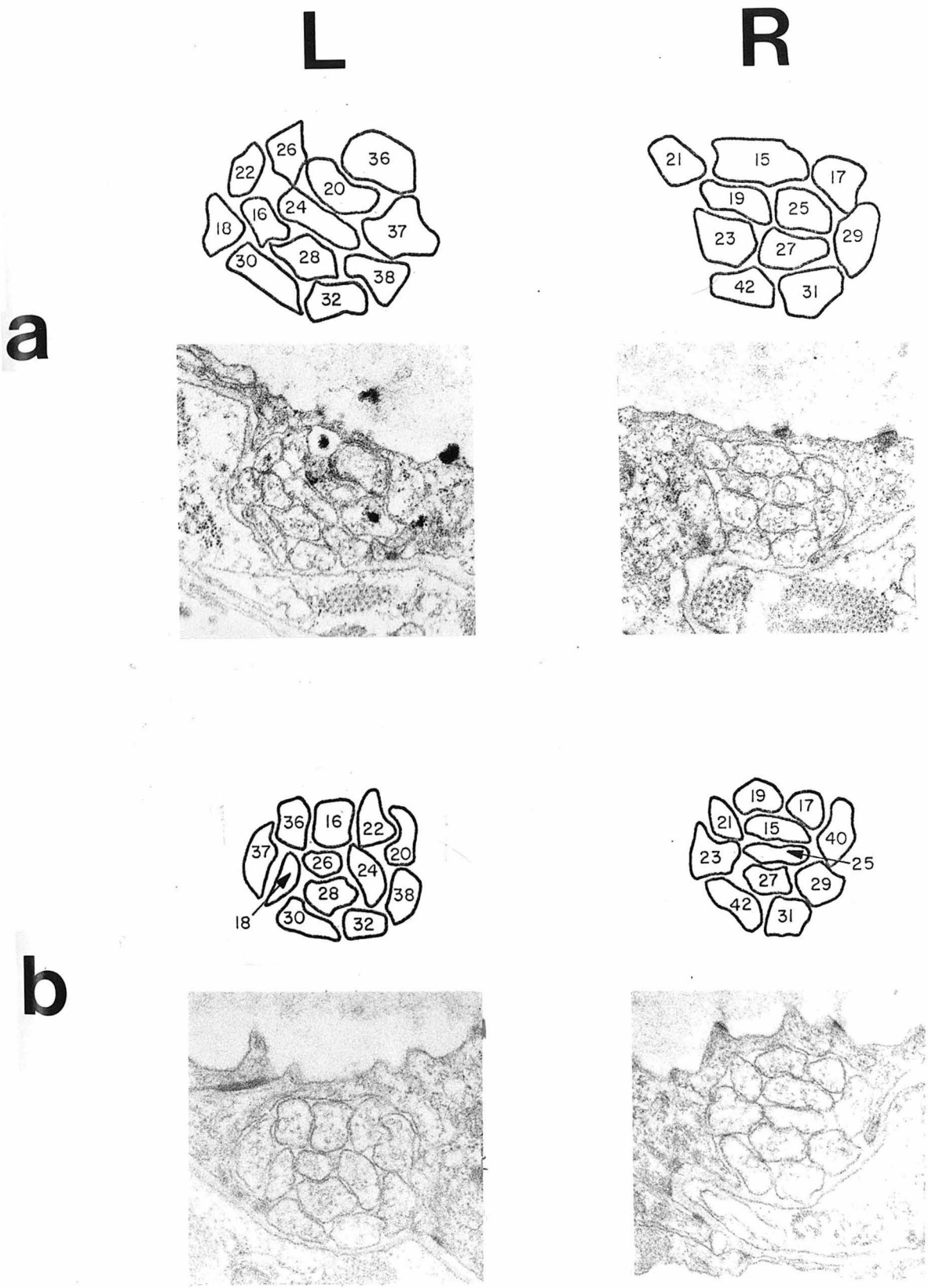
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Figure 11

Figure 12. Fiber Positions in the Pre-Anal Ganglion

Fiber positions are compared at corresponding levels in B136, (a) and (b), and B126, (c). Magnification is about 16,000X. Each cross-section is from the extreme ventral edge of the animal (see Figure 1, panel (e), cross-section 7). Larger profiles are those of cell bodies. Slight shifts in cell body positions appear very striking in these comparisons (40 vs. 41).

H - hypodermis

M - muscle cell (ventral quadrant)

G - gut cell

DMF - muscle arms from defecation muscles

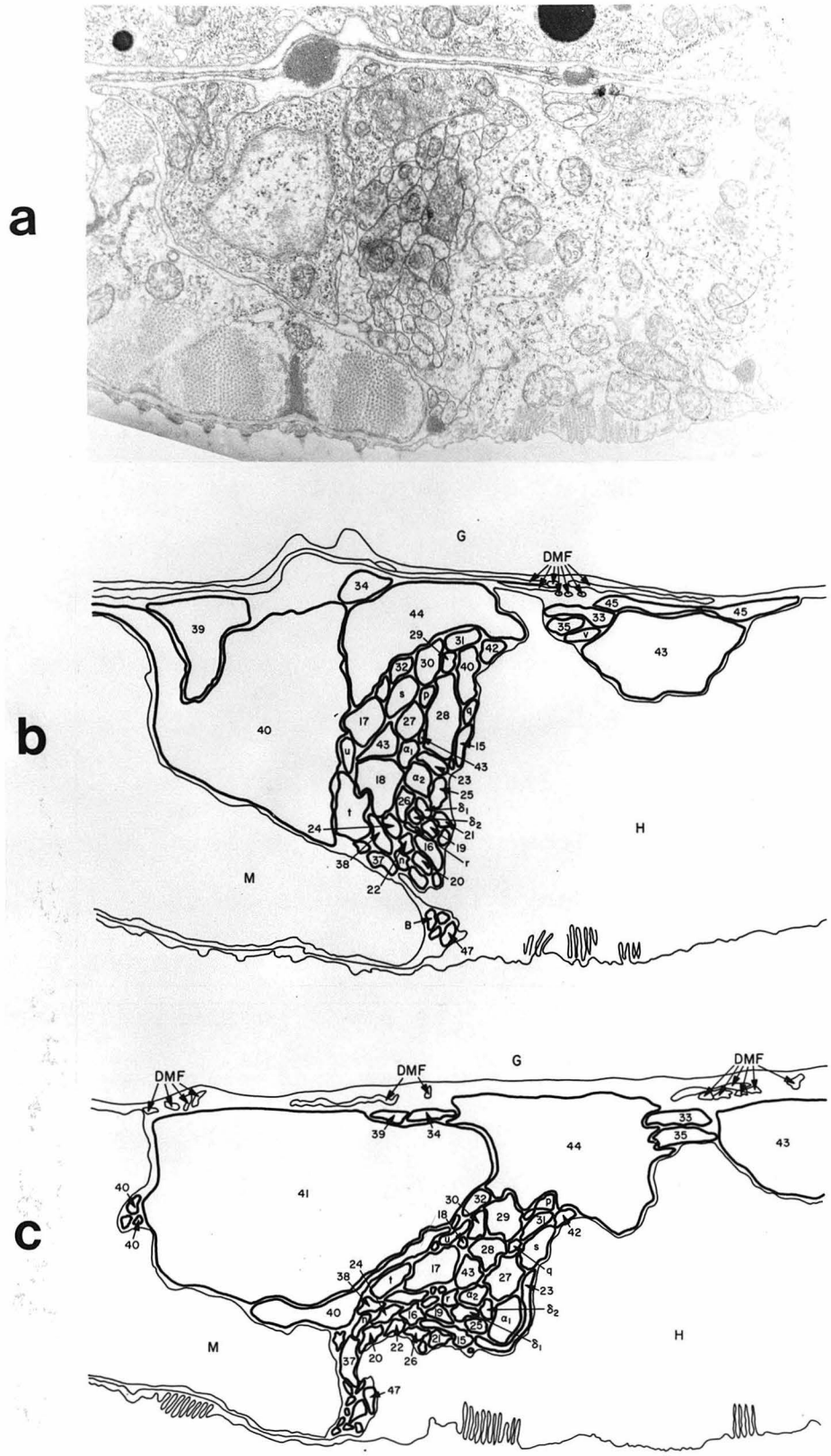


Figure 12

pre-synaptic specializations for three to ten successive sections (electron-dense deposits along the inside of the pre-synaptic membrane, usually accompanied by a cluster of vesicles). A second list of possible synapses was made, where two or three sections adjoining missing or damaged sections displayed indications of a pre-synaptic specialization. The pattern of these interactions is not noticeably different from the pattern of the primary synapse list. Together these two lists probably include every synapse in the PAG for each animal.

Figure 13 shows a chart of the synaptic patterns in B126 and B136, where the numbers of synapses are compared for each possible combination of pre- and post-synaptic elements. Most synapses are dyadic; these are listed twice in separate boxes, once for each post-synaptic element. Figure 13 includes all chemical synapses from both the primary and secondary lists for each animal. Multiple point synapses are common; these are listed in Figure 13 as multiple numbers - that is,a serial synapse made up of three point synapses is added into the listing as three separate synapses. Despite these corrections, Figure 13 should not be interpreted as a representation of true electrophysiological synapse strengths. The reconstructed series are quite complete, so the observed variations in synapse multiplicity are presumed to represent real variations.

Figure 13. Chemical Synapses 47 x 47

This chart displays every chemical synaptic contact in the tail for two animals (see Figure 3). Each pre-synaptic fiber is listed at the left, each post-synaptic fiber is listed above. Synapses for B126 are listed above the diagonal, for B136 below the diagonal, in each box - note the illustration in upper right corner. Each line indicates a single synaptic contact in one animal (pre → post). A dyadic synapse, with two post-synaptic fibers, is listed in each of two separate boxes, once for each post-synaptic fiber. Unidentified participants in synapses are lumped into the category "Others." The numbers of contacts in the most heavily filled boxes are as high as 12 vs. 13, 10 vs. 11, 11 vs. 12. All chemical synapses are located in the pre-anal ganglion except for the following contacts: 21 → 23 and 22 → 24, which are in the lumbar ganglia.

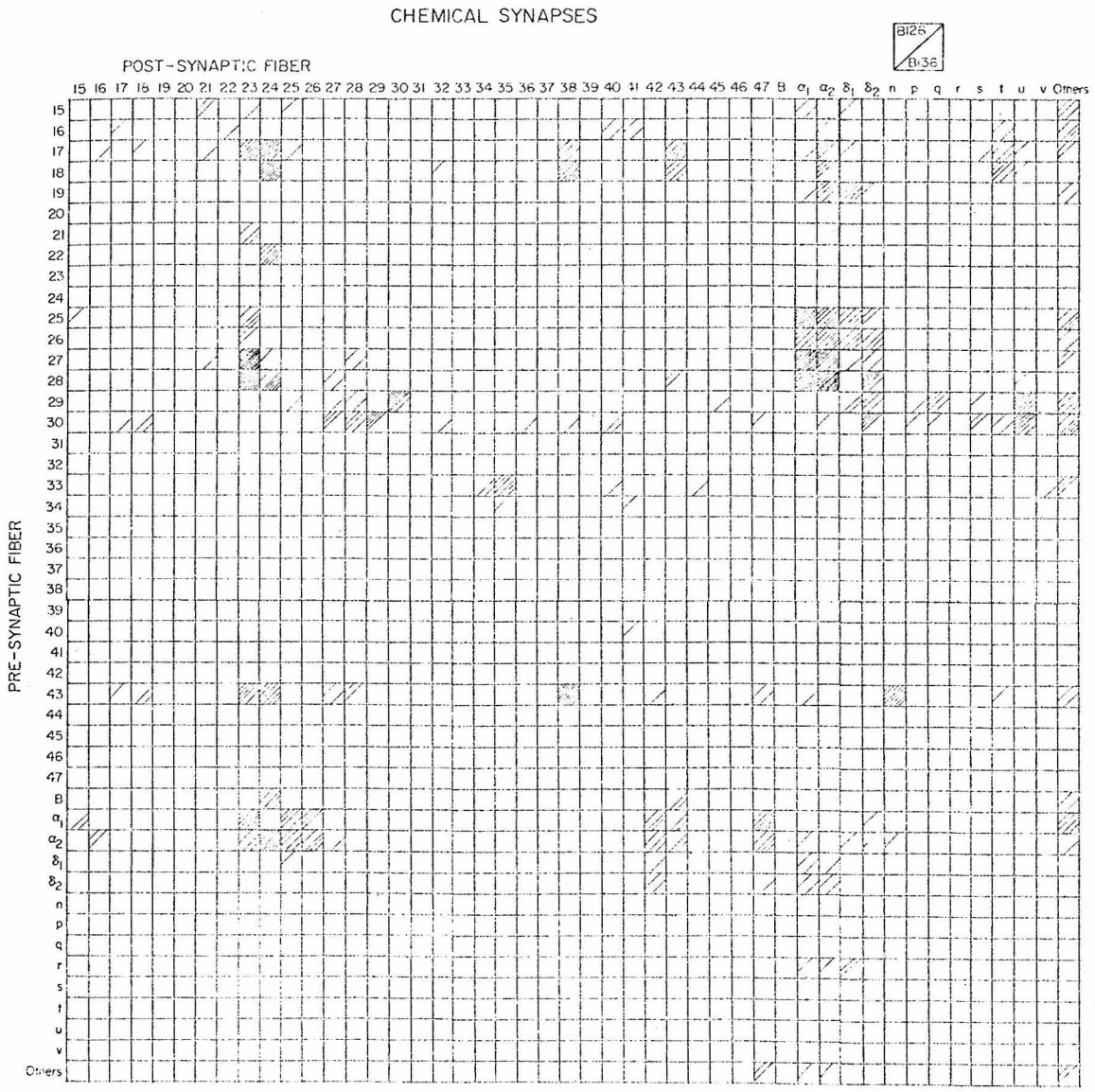

CHEMICAL SYNAPSES
8126
8136
POST-SYNAPTIC FIBER
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 B α1 α2 δ1 δ2 n p q r s t u v Others
PRE-SYNAPTIC FIBER
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 B α1 α2 δ1 δ2 n p q r s t u v Others

Figure 13

This chart is a simplification which neglects the spatial configuration of the synapses - these features will be dealt with separately.

Comparison of the two animals reveals a very strong pattern of synaptic interactions. The pattern is clearly non-random, with 74% of the synapses falling into the same few classes (3.4%) in both animals. Several synapse types are present in very high multiples in each animal (seven or more times). Roughly 26% of the synapses are scattered singly among the uncommon classes (96%) of interaction. These synapses may be important, or they may represent the background noise of mistaken synapse formation (It is not known whether a given class of interaction must be multiply-represented to have functional significance in the nematode's behavior.)

Some neurons are involved in many types of synaptic interactions in the PAG, while others are involved in few or no interactions. Individual patterns of interaction typify each neuron. Most bilaterally symmetric pairs of lumbar cells have rather similar patterns, and these patterns are recognized to be even more similar upon further analysis. Several PAG neurons receive similar patterns of input. A few neurons are active as both pre- and post-synaptic elements; these are the interneurons, α, δ, ϕ (cells 25,26), and χ_0 (cell 43). Cells 23,24 are labelled

the γ interneurons, despite their total lack of synaptic output in the PAG. (Nonetheless, have patience and note the diverse and extensive sensory input which the γ interneurons receive in the PAG.)

Because the nerve processes in the PAG are generally quite straight and unbranched, there is little topological possibility for self-synapses. Indeed, no self-synapses have been found. However, there are frequent synapses between some bilaterally homologous neurons, particularly among the lumbar sensory neurons. These sensory fibers also form a few gap junctions among symmetric pairs (Figure 14). These sensory fibers travel through the PAG in close opposition to their symmetric partners, and all of them terminate without entering the ventral cord. Thus the PAG represents the prime site of sensory processing for the sense organs of the tail. (Cell 19, the neuron with the asymmetric cilium, does appear to send a process into the ventral cord, a possible exception to this rule.) The synaptic output of the two types of phasmidial ciliated neurons are strikingly different and may be serving very different behavioral functions. The synaptic output of sensory cells 17,18 somewhat resembles the output of phasmidial cells 27,28. Very little feedback control impinges upon these sensory neurons from non-sensory cells.

Figure 15 displays a somewhat simplified synapse chart

Figure 14. Gap Junctions

This chart displays every electrical gap junction in the tail for two animals (see Figure 3). Dark shading indicates a synapse in the pre-anal ganglion. Light shading indicates a synapse in the lumbar ganglion. No more than one gap junction of a given type is found per animal. As in Figure 13, synapses for B126 are shown above the diagonal, for B136 below the diagonal, in each box. All gap junctions are shown twice on this chart, since both participating fibers are presumably both pre- and post-synaptic elements here.

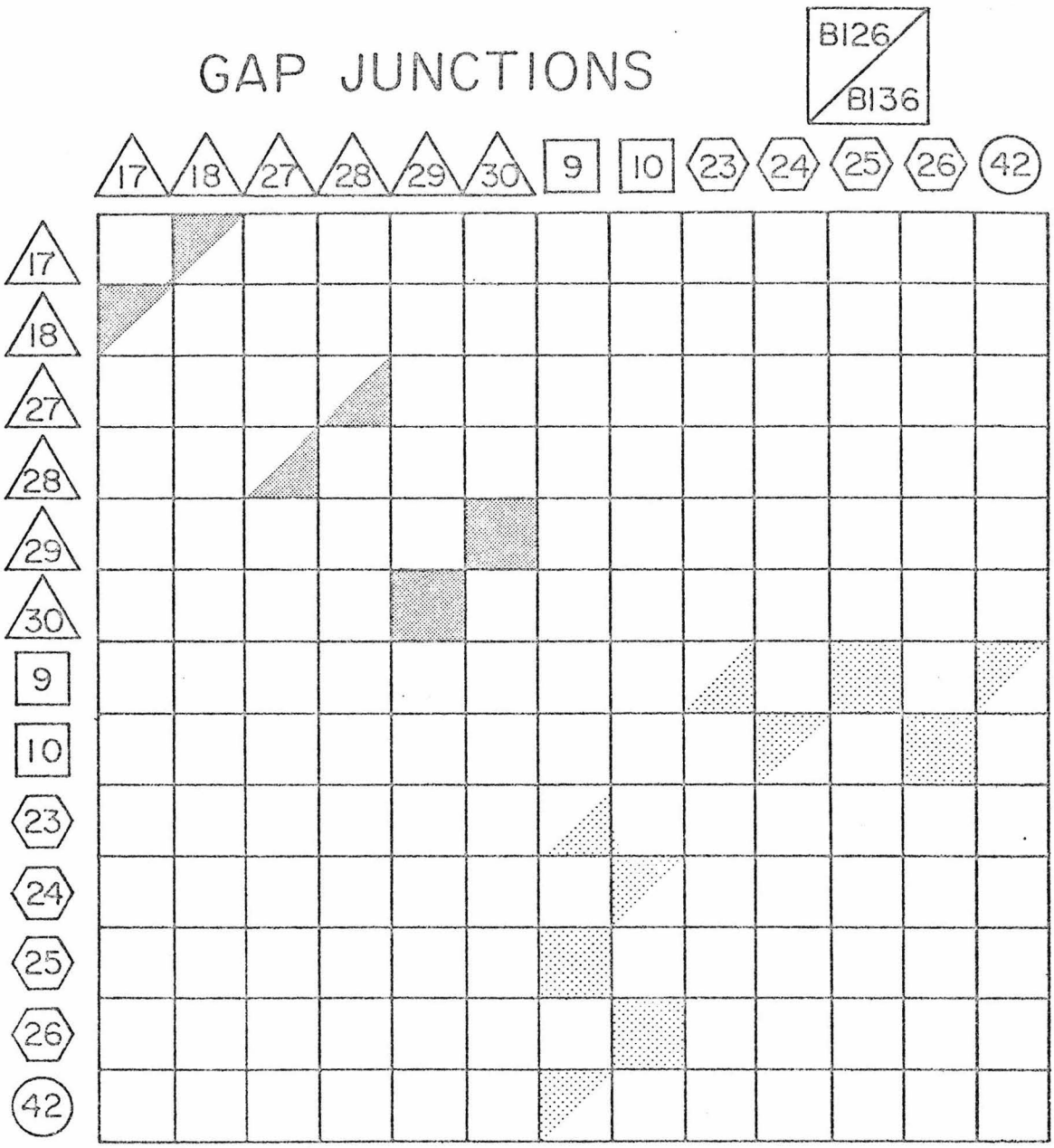


Figure 14

Figure 15. Simplified Synapse Chart 23 x 23

This chart displays the sum of the chemical synapses found in B126 and B136 among the active fibers. As in Figure 13, pre-synaptic fibers are listed at the left, post-synaptic fibers at the top. Dyadic synapses are again listed twice, once for each post-synaptic fiber. All synapses are in the PAG.


- sensory cell


- interneuron


- motoneuron


- other neuron

CHEMICAL SYNAPSES

(BI26+BI36)

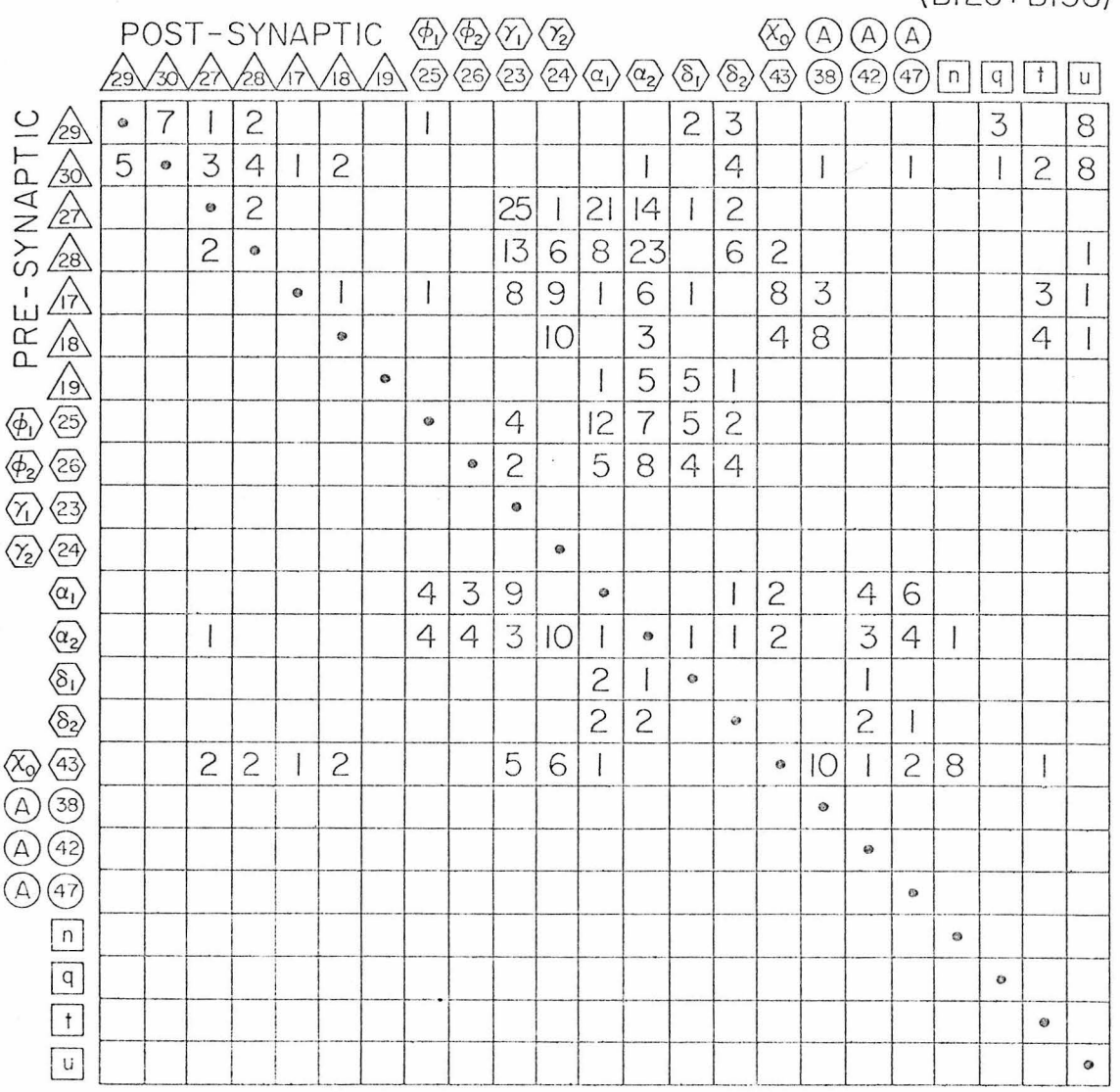


Figure 15

for the most synaptically active neurons of the PAG. The data from B126 and B136 have been summed for this chart, using the numbers given in Figure 13. These few neurons form 85% of all the PAG synapses among themselves. Dyadic synapses are again represented twice, once for each post-synaptic element. The similarities between bilateral pairs of neurons are again evident. Ipsilateral connections are roughly twice as frequent as contralateral connections. Most of these left-right differences are probably a reflection of the lack of left/right mixing of lumbar fibers in the posterior half of the PAG, where the fibers retain some of their commissural organization. Even if the left/right differences do arise from passive causes, they could have some functional significance. This topic will be pursued in another section.

Figure 16a is a simplified synaptic chart of the most active neuron classes, where the synapses of bilaterally homologous pairs of neurons have been combined. This treatment ignores left/right distinctions. The averaged data from Figure 15 have been summed to obtain the numbers in Figure 16a. In this chart, the convergence of all synapses into a very small number of classes is evident, with the α and γ interneurons receiving the bulk of all sensory input. Dyadic synapses are again represented twice, once for each post-synaptic element. The connectivity of

Figure 16. Chemical Synapses: Homologues Combined

(a) This chart displays the sum of the chemical synapses found in B126 and B136 among the most active fibers, where all contacts of bilaterally homologous fibers are combined. Homologous motoneurons are grouped as (A). As before, pre-synaptic fibers are listed at the left, post-synaptic fibers at the top. As before, dyadic synapses are listed twice, once for each post-synaptic fiber. (b) shows a wiring diagram based upon the data in (a). Heavy lines indicate the most common synaptic connections (15 or more occurrences in the two animals combined).

CHEMICAL SYNAPSES: HOMOLOGUES COMBINED

(a)

PRE-SYNAPTIC \ POST-SYNAPTIC	27,28	17,18	19	φ	γ	α	δ	χ_0	A
27,28	4				45	66	9		
17,18		•			27	10		12	11
19			•			6	6		
φ				•	6	32	15		
γ					•				
α				15	22	•			17
δ						7	•		4
χ_0	4	3			11			•	13
A									•

(b)

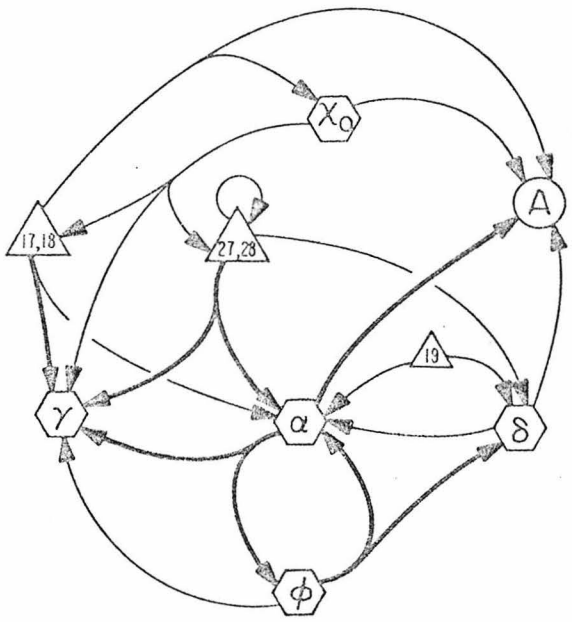


Figure 16

this simplified chart can be summarized by the wiring diagram shown in Figure 16b. (The layout and symbols used in Figure 6b are those of White et al. (1976). Justification for borrowing their nomenclature will be forthcoming.)

A cursory glance at the wiring diagram reveals that there are several types of simple feedback loops involved in the initial processing of sensory signals by five interneuron types. Of these interneurons, the α, δ, γ, and χ_0 types have processes which proceed anteriad from the PAG into the ventral cord. The feedback loops could be used as a negative feedback circuit to filter sensory signals, or as a positive feedback circuit to prolong and enhance sensory signals. Alternately, the spatial configuration of synaptic contacts could be a controlling feature in using feedback loops to select certain timing features of sensory signals. The multiple routes by which sensory signals can be routed to the α and γ interneurons suggest the potential significance of timing features. All of these subjects are discussed below. For the moment, we shall examine the spatial configuration of synapses, which is important in many of these possibilities.

There are two major aspects to the spatial configuration problem: the spatial separation of individual contacts and the predominance of dyadic contacts. There are relatively few non-dyadic contacts; about eight monads and

twelve triads per animal. The non-dyadic contacts do not follow any consistent pattern, but appear to be just aberrations from the dyadic norm. Other aberrant contacts are observed where hypodermal tissue substitutes for one of the two post-synaptic elements. Triads are generally difficult to assign, since there is generally one clear post-synaptic element and two other possible post-synaptic elements. In 75% of the triads, two of the post-synaptic fibers are from homologous cells.

The complete synaptic chart of dyadic contacts requires three dimensions to properly display the possible combinations of contacts. In Figure 17 these data are presented as six different planes from an 8x8x8 chart of the dyadic contacts. (Two planes are devoid of data points and have been left out.) This data has been assembled from an aggregate of all the dyadic contacts in B126 and B136. (Now each synapse is listed once, ignoring the two possible permutations of the post-synaptic elements. I have not analysed the left/right ordering of these elements.) This level of analysis produces a proliferation of possible synapse types (288), yet there is again a clear restriction of contacts into a small number of classes. This restriction appears to have important implications in both the functional circuitry and the developmental specification of synaptic contacts.

Figure 17. Preferred Dyadic Synapse Combinations

Six planes are shown from a three-dimensional chart of dyadic synapses. Each plane represents the summed synaptic output from a given cell type in B126 and B136; this pre-synaptic element is shown above its array of dyadic outputs. Bilaterally homologous nerves and homologous motoneurons are grouped as single cell types. Each array of output is bordered by two lists of possible post-synaptic elements. (Since these 8 x 8 arrays are redundant, half of each array is not shown; i.e.,A → B,C is considered equivalent to A → C,B). Each dyadic contact is shown only once on this chart; monads and triads are not included. Two planes are not shown because two cell types have no synaptic output in the PAG (cells γ and (A)).

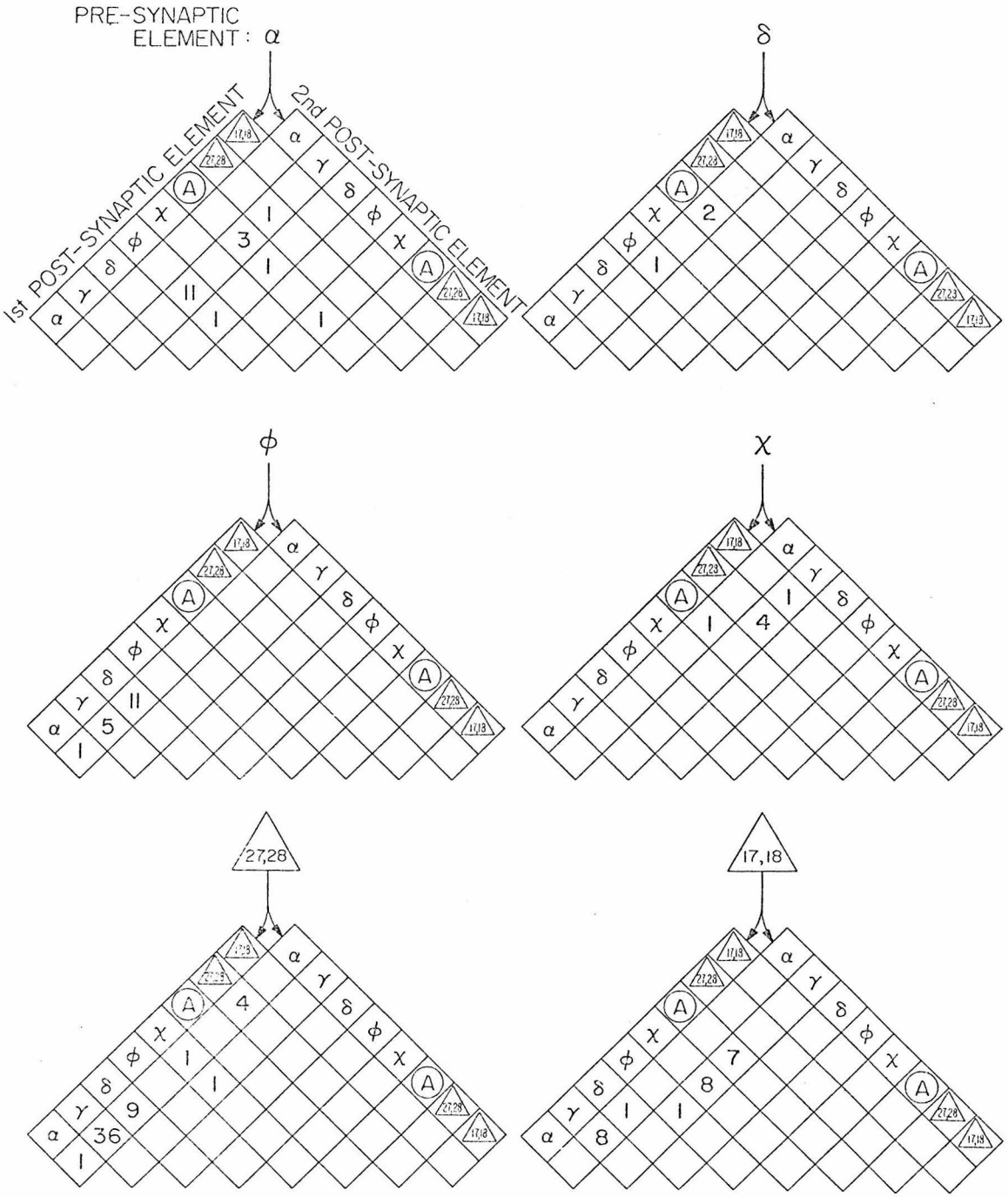
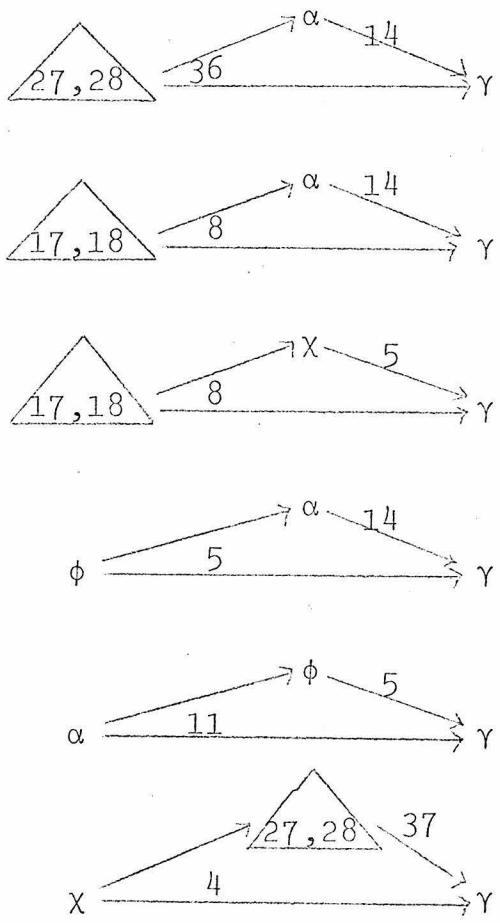

PREFERRED DYADIC SYNAPSE COMBINATIONS
PRE-SYNAPTIC ELEMENT: α
1st POST-SYNAPTIC ELEMENT
2nd POST-SYNAPTIC ELEMENT
δ
φ
χ
27,28
17,18

Figure 17

Very few dyads involve two homologous post-synaptic elements. Post-synaptic elements are rarely homologues of the pre-synaptic fiber. Thus synapses can generally be represented as $A \rightarrow \begin{matrix} B \\ C \end{matrix}$ where A, B, and C are each from different neuronal types. Beyond this general restriction, each type of pre-synaptic fiber generally contacts only a few possible combinations of neuron types, and each type of neuron has a different spectrum of preferred combinations. Three sets of pre-synaptic elements contact an α interneuron at almost every dyadic (and triadic) synapse. The other three sets of pre-synaptic elements contact a γ interneuron at almost every dyadic (and triadic) synapse. Most pre-synaptic elements show a very strong preference for one particular pair of post-synaptic targets. (In more than 75% of all triads, the same pair of targets are contacted: $A \rightarrow \begin{matrix} C_1 \\ B \\ C_2 \end{matrix}$).

In a typical PAG dyad, $A \rightarrow \begin{matrix} B \\ C \end{matrix}$, the two post-synaptic elements (B,C) are generally involved in another PAG synapse. $B \rightarrow C$. In this manner, the dyadic synapse mediates the simultaneous stimulation of two routes of information flow which converge at C: $A \overset{\nearrow B \searrow}{\longrightarrow} C$. These multiple routes from A to C may represent the morphological basis for processing information through timing comparisons and timing delays. The following multiple routes are common:



The γ interneurons are the targets of each multiple route of information flow. The α interneurons are most often the intermediate elements in those multiple routes. The timing features of sensory stimulation of the tail may be scanned by the γ interneurons, comparing direct sensory input with delayed input from the α interneurons. The functional possibilities of dyadic circuitry are discussed in a subsequent section.

The spatial separation of the dyads may also be important. The complex possibilities offered by this variable

are difficult to comprehend. Without the ability to do intracellular recording in this system, it is not possible to evaluate the physiological constraints which rule the circuits. Nonetheless, the α interneurons are favorably placed to display the nature of the problem.

Figure 18 shows the placement of synaptic contacts along the two α fibers in B126 and B136. The synaptic output of each α fiber is shown above the fiber, and the synaptic input to each α fiber is shown below the fiber. The varicosities of the fibers are indicated; bending of these fibers is insignificant. Several spatial features are immediately evident. The posterior connections show a strong ipsilateral bias, while the anterior connections seem evenly mixed. Alpha fibers are pre-synaptic at varicosities spread intermittently along their length, while they receive synaptic input more continuosly along their lengths. There are many closely spaced reciprocal synapses with ϕ interneurons which set up feedback loops with minimum time delays. Contacts with γ interneurons remain strongly ipsilateral throughout the PAG, but these contacts do not demonstrate any obvious spatial pattern. Sensory input from cells 17,18,19 seems to be restricted to small regions, but the larger number of contacts from phasmidial neurons 27,28 are spread along the length of the α fibers without an obvious pattern. Alpha fiber output to motoneurons seems

Figure 18. Synaptic Input and Output of α Interneurons

The spatial distribution of synaptic contacts involving α interneurons is shown schematically for B126 (a) and B136 (b). The varicosities of each α fiber are shown schematically; bending and intertwining of these fibers are not significant and are not shown. Sensory inputs to the α interneurons are shown by short arrows below each fiber. Other inputs to the α interneurons are shown by long arrows below each fiber. Alpha interneuron synaptic outputs are shown by arrows above each fiber, showing all post-synaptic members of each output. Most inputs to α interneurons involve concurrent inputs to other fibers - these other contacts are not shown. These fibers extend for the length of the PAG, and in one case α_2 extends somewhat further into a PAG-lumbar fiber bundle.

SYNAPTIC INPUT AND OUTPUT OF α INTERNEURONS

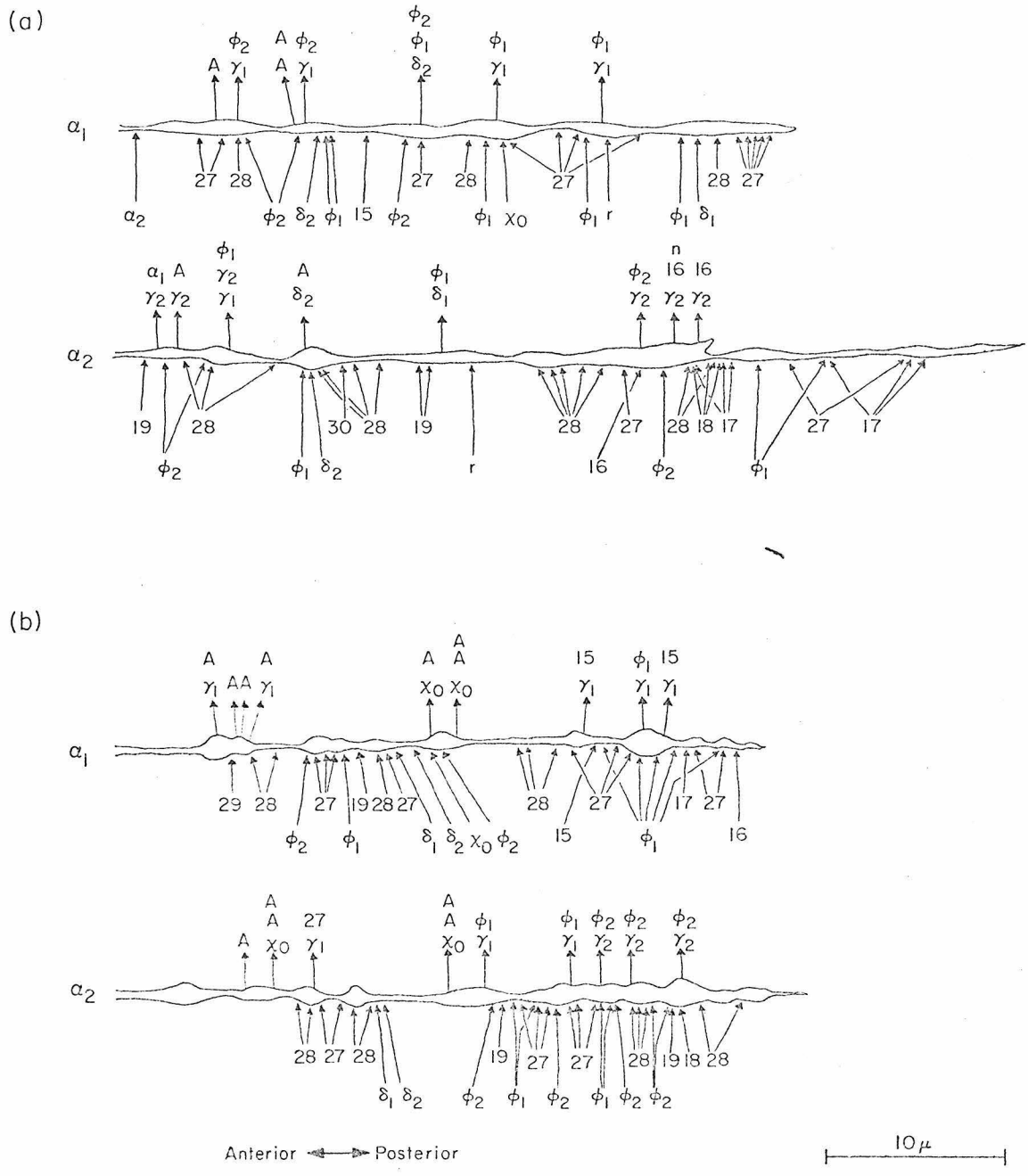


Figure 18

restricted to the anterior half of the PAG, but the number of these contacts is too variable for the spatial configuration to be the factor of interest. In fact, spatial configuration has not emerged as a useful tool in studying the role of the α interneurons within the PAG.

FURTHER IDENTIFICATION OF TAIL NEURONS BY FIBER POSITION AND LINEAGE: The neuronal circuits which we have reconstructed in the nematode tail are only a part of the larger circuitry which governs the behavior of the whole animal. It is inconvenient to discuss the functional properties of the PAG circuits without knowing how these circuits are connected to the ventral cord and the nerve ring. The comparable circuits in the ventral cord between motoneurons and interneurons have recently been reconstructed by White et al. (1976). The lineage of many of the PAG neurons has been identified recently by Sulston (1976). Sulston, White and Edgar have recently followed the lineage of many of the lumbar cells (unpublished observations). The sensory inputs from the anterior sense organs into the ventral cord circuitry are currently being investigated, and the general route of these inputs is known (White, personal communication). Reconstructions of the ventral cord have revealed a remarkably consistent pattern of fiber distribution and synaptic interactions along the length of the ventral and dorsal cord. This pattern has permitted White and his

colleagues to recognize all of the major interneurons and motoneuron classes in most regions along the length of the animal by reconstructing relatively short series. Only in the regions nearest to the PAG were they unable to recognize the same pattern.

In the ventral cord there are 65 motoneuron cell bodies which fall into seven classes. Each class has an individual distribution of axonal and dendritic processes. Each class of motoneuron neurite travels in a unique position in the ventral and/or dorsal cord. In a given region along the animal, there is only one motoneuron of each class which is synaptically active. Neighboring members of each class contract their homologues by gap junctions at the distal ends of their neurites.

There are ten interneurons of four classes which are functional along the ventral cord (none in the dorsal cord). Each interneuron class travels along the ventral cord in a unique position. The synaptic pattern by which the interneurons contact each other and the motoneurons is essentially unchanged along the length of the cord, although various types of connections do vary in relative frequency. The interneurons reportedly receive a great number of synaptic contacts in the nerve ring. White has designated the four classes of interneurons as α, β, γ, and δ (Figure 19c). The α interneurons are a bilaterally symmetric pair of monopolar

cells, with cell bodies in the anterior regions of the lateral ganglia in the head. Their processes are very large and run down the center of the ventral cord (White, 1975). The β interneurons are a bilaterally symmetric pair of monopolar cells, with cell bodies in the posterior regions of the lateral ganglia. Their processes are also large and run prominently above the α processes (White, 1975). The γ interneurons do not have cell bodies in the head, but appear to come from cell bodies somewhere in the tail of the animal. The γ processes run under the α processes in the ventral cord. There are four δ interneurons, two bilaterally symmetric pairs, with cell bodies in the anterior regions of the lateral ganglia. The δ processes are smaller in diameter and they run between the α and β processes in the ventral cord. Two of the δ processes terminate in the anterior ventral cord, while the other two δ processes continue through the posterior ventral cord (White, 1975; White et al., 1976).

INTERNEURONS: In my own reconstructions of the PAG, I have noted the presence of four synaptically active interneurons which enter the PAG from the ventral cord; two large clear processes and two small processes which run just above them as they enter the PAG (Figures 19a,b). The two large processes travel in a central position in the cord and I have identified them as the α interneurons of White et al.

Figure 19. Fiber Positions in the Ventral Cord

The fiber positions of prominent interneurons and motoneurons are shown in our most anterior reconstructions of B126 (a) and B136 (b) and in a typical cross-section of the anterior ventral cord (c) (White et al., 1975). Magnification in these tracings is about 25,000X. In the ventral cord, major motoneuron classes (A,B,C,DD,VD) adopt characteristic positions with respect to each other and to the muscle plate (M); major interneurons adopt characteristic positions with respect to each other and have characteristic sizes; α and β interneurons being larger than the δ and γ interneurons. In B136 (b), one of the proposed δ interneurons appears unnaturally large, due to a mitochondrion in the cytoplasm; this fiber is much smaller along the rest of its length, in accordance with a typical δ interneuron.

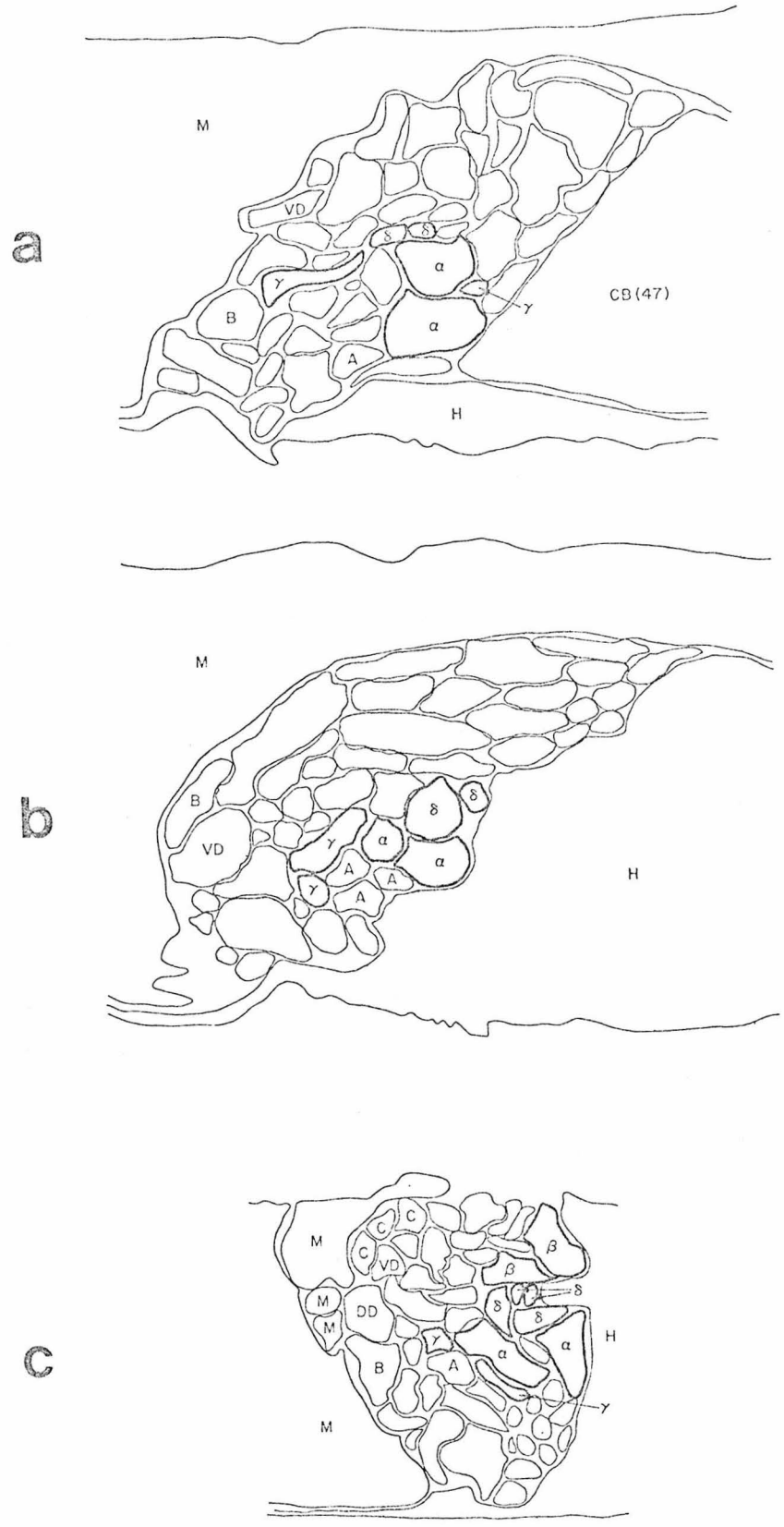

a
M
VD
δ
δ
α
γ
CB(47)
B
α
γ
A
H
b
M
B
VD
γ
α
δ
δ
A
α
A
γ
A
H
c
M
C
C
C
VD
β
β
δ
M
DD
δ
δ
M
γ
α
α
H
B
A
γ
M

Figure 19

(1976). The two smaller fibers are identified as the δ interneurons of White et al. (1976). No correlates to the β interneurons have been observed in the PAG; I assume that the β interneurons terminate in the posterior ventral cord. Two bilaterally symmetric monopolar neurons from the lumbar ganglia (cells 23,24) send processes anteriad into the ventral cord just beneath the α fibers; I have identified these cells as the γ interneurons of White et al. (1976). Figure 19 displays drawings of the relative fiber positions in the anterior PAG of B126 and B136 and drawings of the relative fiber positions in the ventral cord (taken from White et al., 1976).

The synaptic interactions of the identified α, δ, and γ interneurons in the PAG are not identical to their ventral cord interactions, but they do have some strong features in common. In White et al.'s (1976) reconstructions of the ventral cord, class A motoneurons typically receive synaptic input only from α interneurons, δ interneurons, and other class A motoneurons. In my reconstructions of the PAG, the α and δ fibers contact cells 38 and 42 (class A motoneurons), and cell 47 (a suspected class A motoneuron). (I will explain my identifications of cells 38,42,47 shortly.) Delta interneurons in the ventral cord have only one other synaptic target, α interneurons. My PAG δ fibers have only one other synaptic target, α fibers. The most striking

difference in the PAG is the multiplicity of $\alpha \rightarrow \gamma$ contacts. In the ventral cord, there are many $\gamma \rightarrow \alpha$ contacts but no $\alpha \rightarrow \gamma$ contacts.

PRE-ANAL GANGLION LINEAGE: Many of the ventral cord motoneurons develop and become wired into the ventral cord circuitry after the nematode has hatched from the egg. The post-embryonic development of these motoneurons from their precursor cells has recently been reported by Sulston (1976). Each precursor cell undergoes a fixed set of cell divisions in every animal examined, and the progeny of these cell divisions can be recognized individually by Nomarski optics in the living animal. Reconstructions of identified cells in the anterior ventral cord have demonstrated that each developing cell is destined to become a particular predictable type of motoneuron. Thirteen precursor cells (P0 to P12) go through these programmed cell divisions, usually to give five motoneurons (VA,VB,VC,DAS,VD) and a hypodermal cell; the motoneurons migrate into prescribed positions along the ventral cord and become wired in (Figures 21,22). Some lineage cells undergo programmed cell deaths and do not survive in the adult animal. Some of the most anterior lineage cells which lie in the retrovesicular ganglion do not assume the typical morphologies of other lineage cells - some are not motorneurons at all. However, even these atypical cells do have reproducible fates. The

other motoneurons of the ventral cord, which are present at hatch are called "J" cells and become the DA, DB, and DD motoneurons in the adult ventral cord.

The adult pre-anal ganglion contains six lineage cells from the most posterior pair of precursor cells, P11 and P12 (Sulston, 1976). The other six PAG cells are present at hatch, along with fifteen ventral cord motoneurons and twelve other retrovesicular cells. Sulston has followed the cell divisions of P11 and P12. He reports that their cell divisions are atypical with a total of six programmed cell deaths occurring, plus the production of two extra hypodermal cells. Figure 20 shows Sulston's drawing of the adult PAG beside drawings of the PAG's of B126 and B136. We can readily identify the lineage cells in the PAG as follows:

Cell 37 comes from P12d-predicted to be a DAS motoneuron
Cell 39 comes from P12e-predicted to be a VD motoneuron
Cell 42 comes from P12a-predicted to be a VA motoneuron
Cell 45 comes from P11e-predicted to be a VD motoneuron
Cell 46 comes from P11d-predicted to be a DAS motoneuron
Cell 47 comes from P11a-predicted to be a VA motoneuron

Each of these motoneuron types can be immediately identified by its morphology and pattern of synaptic input and neuromuscular output. The details of these morphologies

Figure 20. PAG Cell Body Positions - Lineage

The cell body positions of the pre-anal ganglion cell bodies are shown in longitudinal reconstructions of B126 (a) and B136 (b), and in a "typical" animal (c) followed by Nomarski optics for lineage cell divisions (from Sulston, 1976). Six lineage cells can be identified, products of cell divisions by precursor cells P11 and P12.

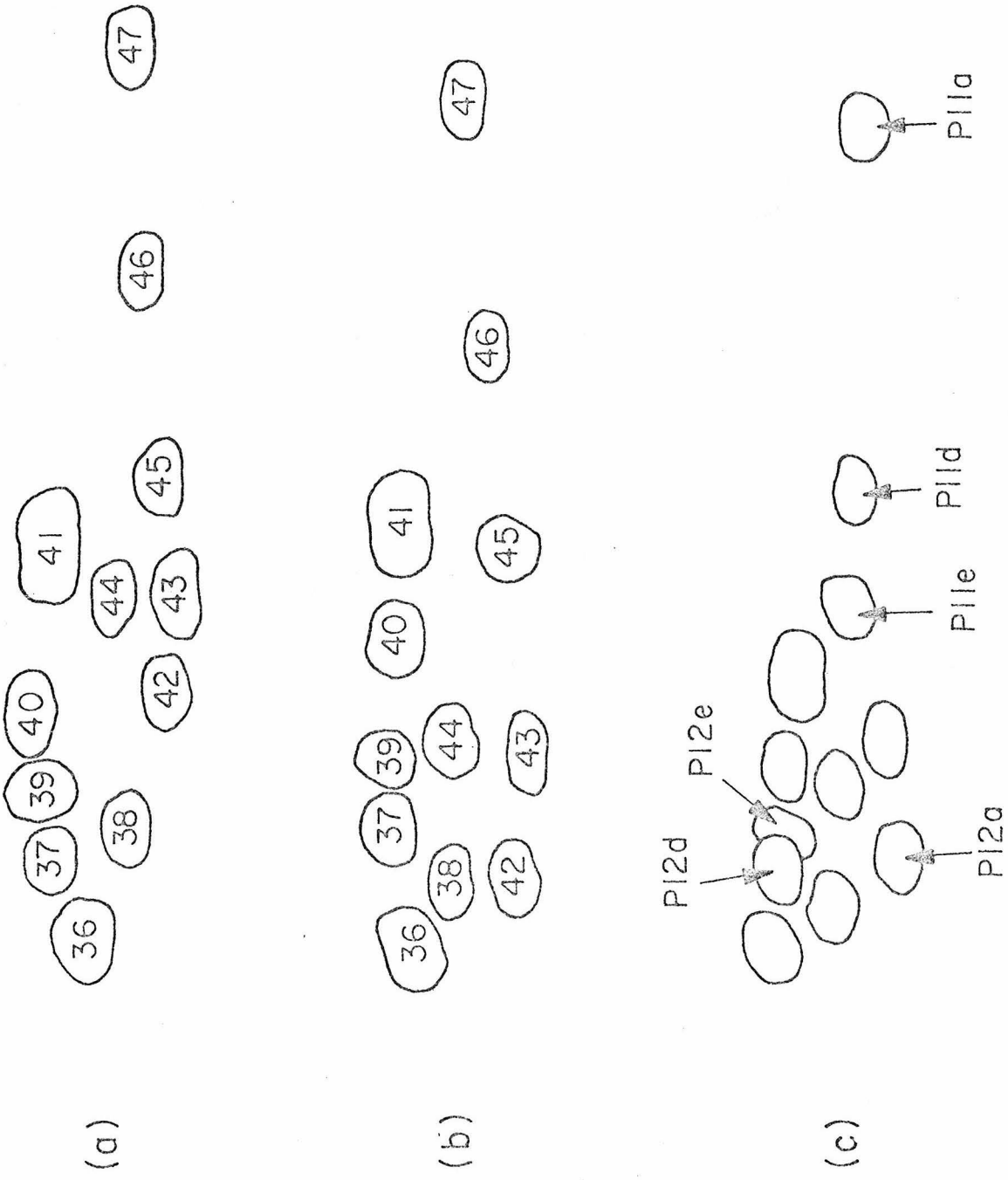

(a)
36
37
39
40
38
41
44
43
42
45
46
47
(b)
36
37
39
40
38
41
44
43
42
45
46
47
(c)
P12d
P12e
P12a
P11e
P11d
P11a

Figure 20

Figure 21. Motoneuron Types - Morphologies

Typical motoneuron morphologies are shown for the eight types of ventral cord motoneurons, displayed in schematic views of the head and the ventral cord (taken from White et al., 1976). All motoneurons have their cell bodies in the ventral cord. (Cell bodies are shown as dark dots.) Some typically have anterior projections, others have posterior projections, some typically have projections into the dorsal cord. Neuromuscular outputs are indicated by ⏃⏃⏃.

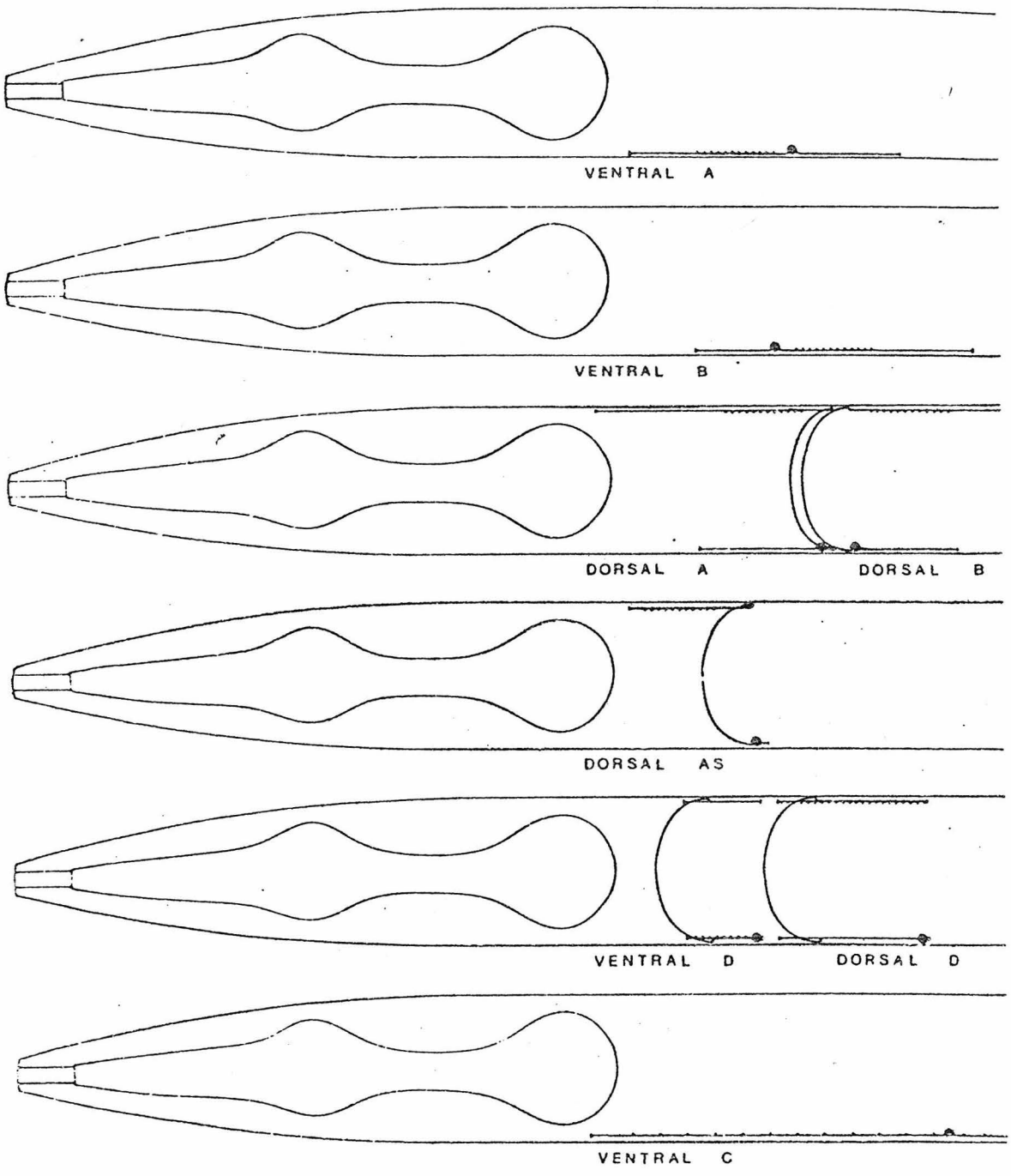

VENTRAL A
VENTRAL B
DORSAL A
DORSAL B
DORSAL AS
VENTRAL D
DORSAL D
VENTRAL C

Figure 21

Figure 22. Lineage Cell Divisions

(a) Lineage cell divisions donate a set of daughter cells into each lumbar ganglion; here the set of daughter cells in the right lumbar ganglion is shown, with their lineage. The left lumbar ganglion receives an exactly homologous set of daughter cells which become bilaterally homologous cells. Cell 19 is the daughter of a separate set of cell divisions in the anterior regions of the nematode, and migrates into the right lumbar ganglion. No corresponding cell migrates into the left lumbar ganglion (Edgar, White and Sulston, unpublished data).

H - hypodermal cell

X - programmed cell death

P - precursor cell

(b) Lineage cell divisions donate two homologous sets of daughter cells into the pre-anal ganglion. Three programmed cell deaths occur in each lineage; three daughters become potential motoneurons, types VA,DAS,VD; one cell becomes a hypodermal cell.

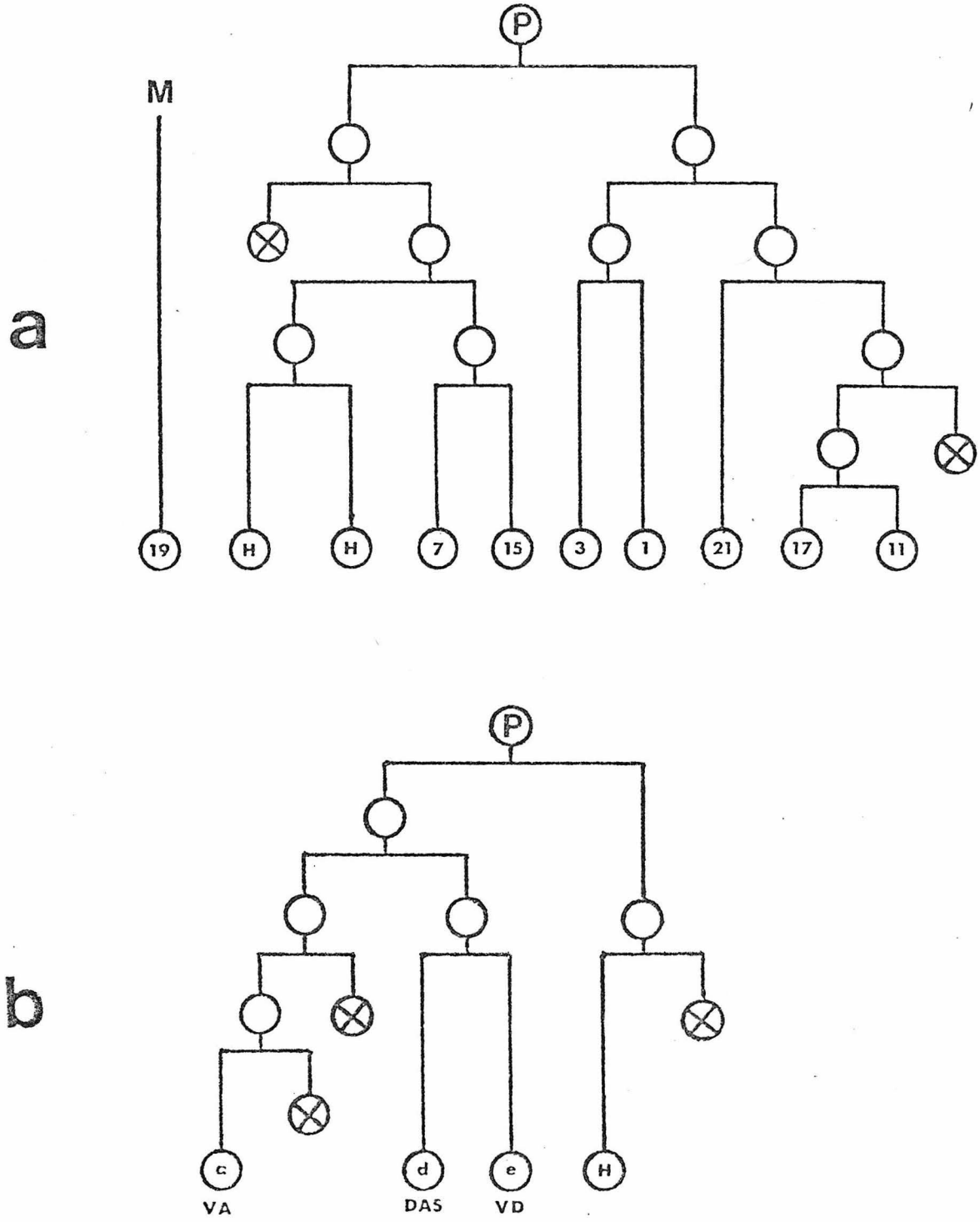


Figure 22

and contacts are summarized in Figure 21 (taken from White et al., 1976). Each class of motoneuron (A,B,D,AS) travels in a standard position within the nerve cords (Figure 19c). The prefix V designates that the motoneuron forms neuromuscular junctions (NMJ) in the ventral cord; the prefix D designates NMJ output in the dorsal cord. Thus a DB motoneuron travels in the B position to form NMJ in the dorsal cord, and has the typical morphology of a class B motoneuron. Class D motoneurons have neurites in both cords, maintaining a dendrite in the opposite cord to its own axon. The D dendrites associate closely with the muscle plate and receive synaptic contact from class A and class B motoneurons - these contacts are simultaneous to the class A and B contacts to the muscle plates.

To determine the true fates of the PAG lineage cells, I re-examined the positions in the cords which their neurites assume, and compared their cell morphologies to those of the standard motoneuron classes (see Figures 7,21).

Cell 37 is predicted by lineage to become a DAS motoneuron. Cell 37 should have a short proximal dendrite in the ventral cord travelling anteriad to a commissure to the dorsal cord, where it should maintain an axonal process with NMJ output. In fact, cell 37 has only a short proximal process which appears to end blindly without synaptic contacts or commissural connections. Cell 37 does not

appear to be a DAS motoneuron, nor any type of motoneuron in either animal. No other nearby PAG neuron maintains a DAS type process either, so I conclude that lineage cell P12d does not follow the prescribed lineage fate.

Cell 46 is also predicted to become a DAS motoneuron. Cell 46 does send a process anteriad into the ventral cord. No commissure to the dorsal cord is observed in either animal in our reconstructions, but such a commissure could possibly occur further anterior. A DAS type axon is observed in the dorsal cord of B136 which has NMJ output and which extends from some commissure anterior to the reconstructed region. The process might really be a part of cell 46, or it might be an extension of the DAS motoneuron arising from lineage cell P10d. No other nearby PAG cell appears to be a DAS motoneuron. The fate of lineage cell P11d remains indeterminate, but is possibly in accordance with the expected program.

Cell 39 is predicted by lineage to become a VD motoneuron, with an anterior axon and an anterior commissure to a dorsal dendrite. Cell 39 does send an axon anteriad and NMJ output has been found in both animals. No commissures have been found in my reconstructions, but such a commissure may well occur further anterior (the figures in White et al. (1976) strongly predict this). A dorsal VD dendrite is evident in the dorsal cord of B136, coming from a commissure

anterior to the reconstructed region. No other nearby PAG cell is a good candidate for a VD motoneuron. The fate of P12e remains indeterminate, but is very possibly in agreement with the expected program.

Cell 45 is also expected to be a VD motoneuron. Cell 45 sends a process anteriad which is not in position as a class D fiber, but more like the position of a class C fiber. No NMJ output is observed. One nearby PAG cell (cell 41) might make a better candidate for a possible VD motoneuron - although there is no strong evidence that cell 41 is a motoneuron at all. Cell 41 cannot be mistaken for cell 45 in my reconstructions, nor in the lineage picture of Sulston (1976) (Figure 16), since cell 41 has a much larger cell body than any other PAG cell. I conclude that lineage cell P11e, cell 45, does not follow the normal lineage program.

Cell 42 is predicted to become a VA motoneuron, with a ventral axon anterior to the cell body and a ventral dendrite posterior to the cell body. Cell 42 sends an anterior process into the ventral cord which is roughly in the proper position to be a class A axon, but no NMJ output is observed in either animal. Cell 42 also has a dendrite which extends posteriad from the cell body and which continues through the PAG, through the right lumbar-PAG commissure and then travels dorsally to the dorsal cord. This dendritic

process continues anteriad in the dorsal cord for at least eighty microns in a class A position without synaptic activity. This dendritic process does receive proper class A synaptic input from α and δ fibers in the PAG. Cell 42 may really be following the fate of becoming a VA motoneuron. The aberrant dorsal commissure may be due to the boundary problems associated with the cell body's extreme posterior location - which does not allow the normal room for a posterior dendrite. Similar boundary problems appear to occur for the most anterior VB motoneurons in the retrovesicular ganglion, whose anterior dendrites travel aberrantly to the dorsal cord and/or the lateral ganglia (see the figures in White et al. (1976)). No other PAG cell nearby to cell 42 appears to be as good a candidate for a VA motoneuron. The fate of P12a remains indeterminate, but is probably in accordance with the expected program.

Cell 47 is also predicted to be a VA motoneuron. Cell 47, like cell 42, has a dorsal commissure from a posterior dendrite. The commissure travels around the left side of the body and the dorsal process travels as a class A fiber in the dorsal cord without any synaptic interactions. The posterior dendrite does receive class A input from fibers in the PAG. Cell 47 sends a process anteriad in the ventral cord in the class A position, but no NMJ output is observed in either animal. Cell 47 has a unique cell body position

which precludes mistaking its lineage. I conclude that the fate of lineage cells is indeterminate, but possibly in agreement with the expected fate.

Of the six lineage cells in the PAG, two are clearly not following their predicted fates, and may not even be motoneurons. Two other cells have aberrant dorsal fibers, but these cells could still be following their predicted fates within the confines of certain boundary problems. The remaining two cells may be correctly following their predicted fates, but I cannot follow their processes far enough anterior to be certain.

Six other PAG cells are present at hatch. Some of these cells might be expected to become DA, DB, or DD motoneurons in the adult, if they are analogous to the "J" cells of the ventral cord. Cell 36 does resemble a DD motoneuron, with dorsal D type NMJ output. However, cell 36 does not have a class D dendrite in the ventral cord. Cell 38 appears to be a DA motoneuron. Cell 38 has class A NMJ output in the dorsal cord and maintains a process in the ventral cord in the class A position. Cell 38 receives synaptic input which differs somewhat from the VA fibers (42,47). None of the other four cells (40,41,43,44) have dorsal commissures of NMJ output in my reconstructions. None of these four cells appears to be a motoneuron. Cell 43 is clearly an interneuron, and has been labelled χ. This

interneuron has not been mentioned as an important element in the anterior ventral cord (White et al., 1976), although this cell does send a process anteriad into the ventral cord.

The PAG has no VB lineage cells, since P11b and P12b undergo programmed cell deaths (Figure 22). DB motoneurons have not been found either. The PAG represents a boundary problem where class B motoneurons would have no room to send a functional posterior axon anyway (Sulston, 1976). There does exist a VB type fiber at the most posterior NMJ regions in the ventral cord which presumably is a process from P10b in the posterior ventral cord. This fiber enters the PAG in a class B position and ends blindly at the posterior end of the PAG. This fiber receives no synaptic input in the PAG. The most posterior β → B synapses are expected to occur anterior to the P10b cell body (White et al., 1976; Sulston , 1976). This interaction may somehow help to signal the β fibers to end without reaching the PAG.

A DB type axon with NMJ output is found in the dorsal cord of B136. This presumably is a process from a "J" cell anterior to the reconstructed region. No DB type motoneurons are found in the PAG, but this is not unexpected because of the boundary problems involved.

To summarize, the general organization of motoneuron types at the most posterior neuromuscular junction regions

in the ventral and dorsal cords does not appear to be any different from the organization reported previously for the anterior regions of the nematode cords (White et al., 1976). All of the same motoneuron classes are observed to be in their proper positions at the muscle plate, and many of these motoneurons receive typical synaptic inputs from identified interneurons.

LUMBAR LINEAGE: Many of the cells of the lumbar regions of the tail are not present at hatch, but develop from precursor cells during the first larval stages. A series of ordered cell divisions of two bilaterally homologous precursor cells produces two bilaterally homologous sets of cells which migrate into position in the lumbar regions to become hypodermal cells, supporting cells and neurons in the adult tail. The lineage of these cells has recently been observed by John Sulston and John White by Nomarski optics in developing animals (unpublished data). Lois Edgar has sectioned two animals in which the cell lineages had been followed. She has partially reconstructed the lumbar regions and identified the cell bodies of the lineage cells (unpublished data). I have been fortunate to compare her reconstructions to my own work on the lumbar regions. Together, Lois Edgar and I have been able to identify all of the lineage cells in the lumbar regions and determine the fate of each daughter cell. The lineage cells of each

lumbar region are derived from identical sets of cell divisions from single precursor cells, and bilaterally homologous daughter cells can be unambiguously assigned as bilaterally homologous pairs of a known cell type. The lineage cell division pattern is shown in Figure 22, and the fate of each daughter cell is shown.

An additional unpaired lumbar cell is the daughter cell from a separate set of lineage cell divisions. This daughter cell migrates into the tail from a small set of lineage cells which originate near the center of the nematode. Two other daughters of this lineage migrate into the posterior lateral ganglion (Lois Edgar, personal communication from John White). The daughter cell which migrates into the tail has been identified by cell body position as cell 19, in the right lumbar ganglion. Cell 19, of course, is the most striking asymmetric cell in the tail, having a sensory ciliated dendrite embedded in the wing cell of the right phasmid. The independent lineage of cell 19 demonstrates that cells 19,20 should not be considered to be bilaterally homologous in any sense. Instead, cell 19 and cell 20 are now recognized as two asymmetric unpaired lumbar neurons. Cell 20 is present at hatch, while cell 19 develops after migrating into the tail post-hatch. Cell 20 has no clear functional role in the adult tail.

In examining the set of cells which are present in the lumbar ganglia at hatching, one immediately notes that the two pairs of major interneurons of the adult tail (γ and ϕ) are present in the first larval stage. The phasmidial sensory neurons, 27-30, are also present, although some of the phasmidial support cells are absent. Thus the primary elements of the sensory circuits in the PAG are present in both the juvenile and the adult.

V. DISCUSSION

COMPARATIVE ANATOMY: The tail of C. elegans is strikingly similar in general organization to the tails of several larger nematodes (Chitwood and Chitwood, 1950). Particularly interesting are the close similarities with the tail of Ascaris, a large parasitic nematode which has already been studied extensively and has favorable prospects for intracellular recording of its neuronal activity. At the level of gross anatomy, Goldschmidt (1908) and Chitwood and Chitwood (1950) found that the female tail of Ascaris has the same set of ganglia (two lumbar, one pre-anal, and one dorsorectal) as reported above for the hermaphrodite tail of C. elegans.

More detailed comparison is complicated by the fact that accurate cell counts have not been reported for Ascaris, and by the fact that the light-microscope methods used in Ascaris do not permit the following of fine fibers. However, the approximate Ascaris cell counts reported (Bullock and Horridge, 1966) are close to those found in C. elegans (e.g. pre-anal ganglion: Ascaris 11, C. elegans 12; lumbar ganglia: Ascaris 6 each, C. elegans 12 each) and the real degree of correspondance may indeed be very high. More detailed study of Ascaris might establish whether homology is sufficiently great to permit electrophysiological testing in Ascaris of functional hypotheses

generated by the synaptic anatomy of C. elegans.

CHEMOSENSORY TRANSDUCTION IN THE TAIL: C. elegans is known to have a variety of chemosensory responses (Ward, 1973; Dusenbery, 1974; Dusenbery et al., 1975; Dusenbery, 1976).

The great concentration of sensory receptors in the snout (Ward et al., 1975; Ware et al., 1975), particularly the concentration of amphids and internal labial papillae in which ciliated sensory dendrites are directly exposed to the exterior, strongly suggests that anterior receptors play a role in chemosensory responses. However, the phasmids of the tail also contain ciliated sensory dendrites directly exposed to the exterior, raising the possibility of tail receptor involvement as well. On the snout the close placement of the bilaterally or hexaradially symmetrical homologues argues against their differential use to detect gradients; instead it seems most likely that these homologues act in concert. On the tail, receptors are also very closely spaced, but between head and tail there is a sufficiently large spatial separation (approximately 1.5 mm. in adult C. elegans) to suggest the possibility that head-tail comparison might be used in chemosensory responses.

Just how the comparison might be carried out is uncertain, but it is noteworthy that two of the major interneuron classes which receive sensory input in the tail (α and δ) also receive input from some snout receptors with

exposed sensory dendrites (inner labial papillae; White, personal communication) (Figure 23c). Head-tail comparisons carried out through these interneurons could represent a component of chemosensory responses quite distinct from the mechanism by which an animal orients accurately up a gradient. For instance, White (1975) has suggested that accurate orientation may be brought about by strengthening body contractions coincidentally with head movements up a gradient. White has argued that such strengthening might be carried out using β interneurons, which receive strong input from the ciliated neurons of the amphids, putative chemoreceptors of the snout (Figure 23c). But independent of this refined orientation mechanism there could also be a cruder one based on head-tail comparisons of chemical stimuli, serving to get the animal going in roughly the right direction. (The relatively small number of possible chemosensory cells in the tail would be in keeping with a crude orientation mechanism.) Whether the tail is indeed sensitive to chemical stimuli, whether head-tail comparisons are indeed made, and if so, whether the α and δ interneurons mediate the comparisons are all matters which remain to be resolved.

Another possibility, a priori somewhat less likely, is that chemosensory cells in the tail might be used to guide backward movement, in simplified analogy to the role played

by snout receptors in forward movement. However, there are no known chemical orientation responses during backward movement. Preliminary experiments with a reversing mutant have shown that an animal moving backwards does not orient to a salt gradient (Hodgkin and Lewis, unpublished observations). Further studies of backward motion in these mutants might be worthwhile.

Crude guidance of movement by head-tail comparisons may involve some broad-range chemotactic stimulus. However, the large number of separable chemotactic responses (nine or more) and the many genetically distinct chemosensory mutants (six or more) (Dusenbery, 1976) argue that the two pairs of phasmidial ciliated neurons are probably too few to be useful in most of the nematode's chemotactic behaviors. The twelve pairs of closely-spaced amphidial neurons, which are well-exposed to the exterior, probably detect chemical gradients by temporal comparisons, independent of any input from the tail. The nematode exhibits a pronounced "searching" motion of the head which is thought to increase the amphidial capabilities in detecting chemical gradients (Ward, 1973). Each bilaterally homologous pair of amphidial neurons might be specialized to utilize a different receptor, giving the nematode a broad range of sensory capabilities.

MECHANOSENSORY TRANSDUCTION IN THE TAIL: According to their placement in the animal, the various apparent mechanoreceptors of C. elegans can be divided into two classes. The first are the groups at the extremes of the animal (head and tail), which have been thought to mediate responses to externally applied touch, and the second are the deirids and post-deirids, more centrally placed and confined to the lateral lines. The placement of the deirids and post-deirids suggests that they may serve to indicate how tightly the animal is held down by surface tension; the lateral lines are in direct contact with the surface on which the animal moves and are therefore most subject to mechanical deformation when the animal is held down. (Indeed, C. elegans changes its behavior very dramatically when suspended in liquid, and preliminary observations suggest also that C. elegans can distinguish between areas of an agar surface with different degrees of hydration.)In this study, the post-deirids were not studied because they lie just anterior to the sectioned region (probably associated with a small group of cell bodies shown in Figure 2a). However, two cells of the lateral cord, cells 9,10, might be carrying sensory signals from the post-deirids to the ϕ interneurons, with which they make extensive gap junctions; a more anteriorly extended set of sections would be required to explore this possibility. (Hesse (1892) previously noted

a posterior projection from the post-deirids into the lateral cord in Ascaris megalocephala).

The more posterior receptor group of the tail includes the phasmidial endings (cells 27-30), the buried ending (cell 19), and a pair of fine posterior processes (cells 17, 18). The mechanical responses thought to be mediated by one or more of these receptor types pose an interesting problem; forward moving responses can be generated by touch to any part of the posterior part of the body, even though the apparent mechanoreceptors are confined to the tail tip. The necessary transfer of mechanical energy to the receptors might be accomplished by the cuticle itself, but an alternative possibility presents itself. The phasmids lie at the posterior ends of large fluid-filled lateral channels, whose function is uncertain. (A chain of gland-like cells lying along these channels have been suggested to secrete their contained fluid (White, 1975)). In the region of the phasmids, the tail is narrowed considerably and the channels are relatively large, suggesting that the channels may serve to focus pressure waves from distant mechanical stimuli onto the phasmids. Interestingly, the buried ending of cell 19 may also be in a position to be stimulated by such waves.

The dendrites of cells 17,18 do not appear to be located in a sensitive position for stimuli transduced by

the lateral channels. These dendrites extend posteriorly for another 65 microns past the phasmids. At their posterior limits these fibers are sheathed in a very fine cuticular process, less than four microns thick (Figure 5f). This process is dragged behind the animal in a thread-like fashion. Each time the animal reverses, this process is bent severely, usually forming a sharp 180° bend, and other mechanical stimuli batter it about very easily. Although these dendrites show no cytoplasmic specializations, it seems likely that they are responsive to mechanical stimulation of the tail tip.

Mechanical stimulation of the tail causes striking behavioral responses. An animal which is stationary or moving backwards will stop and move rapidly forward in response to tail tap and an animal moving forward will increase its forward speed. Habituation to tail tap may occur after twenty or more repeated stimuli (1/second). Mechanical stimulation of the posterior half of the body will cause similar responses. Mechanical stimulation of the snout of the anterior half of the body will cause the animal to move backwards. Backwards motion is not as coordinated nor as fast as forward motion in response to tail tap. Habituation to head tap also occurs after repeated stimulation. Mutants insensitive to light touch of the head have been reported by Hodgkin and Lewis

(unpublished observations). I have isolated several similar mutants which are insensitive tc light touch of the tail. Study of these mutants is continuing.

All of the sensory neurons of the tail may be mechanosensory, utilizing a variety of methods for transducing mechanical stimuli. The cell bodies of these neurons are favorably positioned for laser inactivation studies.

DEVELOPMENT OF THE TAIL: CHANGES AFTER HATCH: The hermaphrodite tail does undergo some developmental changes after hatching, but these changes are relatively minor in comparison to the extensive changes post-hatch in the male tail of C. elegans. The hermaphrodite's PAG has six lineage cells from the P11 and P12 precursor cells. Four of these lineage cells may become motoneurons in the adult tail in accordance with their lineage fates. The other two lineage cells do not appear to be functional neurons. The PAG has six other cells which are present at hatching. Two of these cells are motoneurons and may undergo some rewiring post-hatch, much like the 'J' cells of the ventral cord (Sulston, 1976). Three of the other four original PAG cells seem to lack neuronal function in the adult tail. Perhaps these cells have more important roles in the male tail - the male tail has a pre-anal ganglion which contains a similar number of cell bodies while mediating a much more complex set of behaviors. Alternately, they may have some function

in the juvenile tail which is no longer required in the adult.

The lumbar ganglia in the hermaphrodite gain many new lineage cells post-hatch. Many of these cells appear to be involved in sensory transduction. Cells 1-4 are supporting cells for the phasmids. Cells 7,8 are postulated to be important in sustaining sensory transduction in the subcuticular channels. Cells 17,18,19 are sensory neurons in the adult. Many cells analogous to these lineage cells are known to be present in the cephalic regions, in association with other sensory ganglia (Ware et al., 1975). One wonders which of the cephalic cells are also derived from post-hatch cell divisions. Each of these lineage cells could be adding to the sensory capabilities of the adult nematode. No behavioral changes are known to occur post-hatch in the hermaphrodite which would correspond to the addition of these cells, but a more careful search might be fruitful.

Three other pairs of lumbar lineage cells become neurons which have few apparent functions in the adult. Cells 11,12 are associated with the ventro-lateral cords. These cells have no apparent synaptic interactions in the tail, and have no sensory specializations. Cells 15,16 have no sensory dendrites, and have a small number of synaptic interactions in the PAG. Cells 21,22 have no sensory

dendrites and no synaptic inputs, but they have a single major synaptic output to the cell bodies of the α interneurons - the only chemical synapses of the lumbar ganglia. Presumably this synaptic input to α could have some pacemaker function in the adult. Overall, these three pairs of lineage neurons seem rather useless in the adult. Perhaps they are just a vestige of some of the important lineage changes in the male tail.

The male nematode is most easily identified and distinguished from the hermaphrodite by the specializations of its tail. The male tail undergoes elaborate changes during the third and fourth larval stages to form the copulatory bursa. Before these changes take place, the male nematode is not visibly distinguishable from the hermaphrodite, and the juvenile male's behavior is apparently no different from that of the juvenile hermaphrodite. The adult male performs complex searching behaviors and mating behaviors while moving backwards, guided by sensory structures of the bursa. The layout of the nervous system in the male tail is rather similar to the layout of the hermaphrodite tail, but the number of sensory structures is greatly increased. There is also a proliferation of specialized muscle cells. The neuropil of the male PAG is enlarged, but the PAG contains only a few extra cell bodies (Hall, unpublished observations). The number of inter-

neurons in the male tail cannot be assessed from my preliminary reconstructions. The nature of these lineage changes are currently being examined morphologically by Albertson, White and Sulston (personal communication).

DEVELOPMENT OF THE TAIL: RULES OF FIBER GROWTH AND SYNAPSE FORMATION: Synaptic organization in the tail of C. elegans reveals a striking constancy. Synapse formation is restricted to a 1x1x40 μ^3 region of neuropil. In the pre-anal ganglion there are about forty unbranched neurites which form dyadic synapses among themselves, and a very small number of synapse types are heavily favored. Motoneuron processes within the PAG are strictly dendritic, having no synaptic outputs. The interneurons have single processes which often function both pre- and post-synaptically, generally without any spatial distinction within the PAG (probably common in invertebrate ganglia, as found in the lobster by King (1976ab)). Since all of these processes are unbranched, they can achieve their constant synaptic organization only by maintaining close proximity to their synaptic partners as they travel through the PAG. The set of genetic specifications which govern fiber growth in the PAG may intersect with the set of specifications which govern synapse formation.

Previous studies of the nematode's ventral cord have already shown that the important interneurons travel in

close proximity to the active motoneuron axons and dendrites, while other inactive fibers are grouped at the periphery of the ventral cord. The active fibers all maintain rigid spatial relationships among themselves and with the muscle endplates throughout the length of the ventral cord (White et al., 1976). These spatial relationships are not unrelated to the synaptic organization of the ventral cord. However, no genetic factor has yet been shown to govern the organization of the ventral cord or the formation of synapses.

Ordered patterns of fiber growth also appear to underlie the organization of the PAG. Each lumbar ganglion contains nine cells which send single fibers in an ordered bundle into the PAG. The more active lumbar fibers associate with one another before reaching the PAG. Within the PAG the lumbar fibers intermingle in a stereotyped fashion with the ventral cord interneurons. Active sensory fibers and interneurons are closely grouped in the center of the PAG, while the inactive fibers travel along the periphery. Bilaterally symmetric pairs of sensory neurons generally run in close opposition through the PAG, with the interneurons clustered around them. As in the ventral cord, pre-synaptic fibers display local swellings which bring them into close proximity with the proper pairs of post-synaptic fibers.

The anterior bound of the PAG corresponds to the limits of fiber growth for many lumbar cells. The posterior bound of the PAG corresponds to the limits of fiber growth for many of the ventral cord interneurons (whose cell bodies are in the lateral ganglia in the head). Within these bounds synapses are distributed rather evenly; outside these bounds synapses are sparse. Almost every PAG synapse involves both lumbar ganglion cells and lateral ganglion interneurons. These synapses can only occur in this short region where the distal processes overlap. Dyadic chemical synapses are the major form of synapse in the PAG, but they are not reported to be as common in the ventral cord. The ventral cord has a much higher frequency of electrical synapses (White et al., 1976). The classes of chemical synapses which are typical in the ventral cord are not very common in the PAG and some classes are entirely absent in the PAG. The interactions of the α and γ interneurons with each other are particularly striking in this regard. In the PAG, dyadic $\alpha \rightarrow \gamma$ synapses are common, while in the ventral cord there are many $\gamma \rightarrow \alpha$ synapses instead. Some factor specific to the PAG may exert a general influence which regulates fiber growth and the probability of synapse formation (perhaps including the suppression of some specific synapse types). The higher synapse density within the PAG may be a function of the unique collection of fibers which are brought together here.

Our studies of synapses in the tail of C. elegans reveal that chemical synapses are far more common than gap junctions (electrical synapses). Among the chemical synapses, a majority are dyadic - each pre-synaptic fiber contacting two post-synaptic fibers at the same point. A continuous synaptic cleft is bordered by all three fibers. No morphological specializations identify either post-synaptic fiber. An intracellular electron-dense tuft is associated with the pre-synaptic membrane, and a cluster of synaptic vesicles is generally apparent in the pre-synaptic terminal. The pre-synaptic tuft generally "points" between the two post-synaptic elements. These dyadic synapses appear to play a central role in the functional organization of the tail's circuitry, and probably reflect important developmental processes in synapse specification.

Several types of dyadic synapses have previously been noted in other organisms, both in invertebrate and vertebrate nervous systems. Ribbon synapses in the vertebrate retina are dyadic, and comprise 10% of all the contacts in the inner plexiform layer of the retina (Dowling and Boycott, 1966; Dowling and Werblin, 1969). Ribbon synapses are also found in invertebrates, as in the ocellus of the fleshfly, B. peregrina (Toh and Kuwabara, 1975), but these synapses are not all dyadic. The ribbon synapse is characterized by an electron-dense "ribbon" which lies

adjacent to the synaptic cleft in the pre-synaptic terminal. All of the cell membranes at a ribbon synapse are rather electron-dense, and the spacing between cells is somewhat wider at the cleft. Synaptic vesicles cluster along the ribbon. In the fleshfly, the pre-synaptic ribbon often appears T-shaped in cross-section (Toh and Kuwabara, 1975). Similar ribbon dyads have also been observed in the second optic ganglion of the blowfly (Smith, 1966), in the fly lamina (Trujillo-Cenoz, 1965b; Boschek, 1971), and in the optic lamina of the lobster (Hamori and Horridge, 1966).

The button synapse is another common form of dyadic synapse, best characterized in the ocellus of the dragonfly (Dowling and Chappell, 1972). The button synapse is similar in appearance to the dyadic contacts in C. elegans. The button synapse has an electron-dense tuft attached to the pre-synaptic membrane, adjacent to the synaptic cleft. The cell membranes at a button synapse are relatively electron-dense, and the synaptic cleft is often somewhat wider and more uniform than the unspecialized intracellular gap. Synaptic vesicles cluster in the pre-synaptic terminal near the tuft. Similar tufted dyadic synapses have recently been described in the lobster stomatogastric ganglion, where they comprise the predominant class of chemical synaptic contacts (King, 1976a). Tufted dyadic contacts have also been reported in the first synaptic relay of the spider eye

(Trujillo-Cenoz, 1965a) and in the neural plexus of the lateral eye of Limulus (Whitehead and Purple, 1970).

Most of these examples of dyadic synapses involve sensory ganglia, generally at a primary level of sensory processing. Many of these dyadic synapses are believed to be involved in reciprocal synapses, similar to the reciprocal connection which I have found in the nematode tail. Reciprocal dyads have previously been found in the vertebrate retina (Dowling and Boycott, 1966; Dowling, 1970), in the spider visual system (Trujillo-Cenoz, 1965e), in the Limulus plexus (Whitehead and Purple, 1970), in the dragonfly ocellus (Dowling and Chappell, 1972), and in the fleshfly ocellus (Toh and Kuwabara, 1975). Chappell and Dowling (1972) suggest that the reciprocal synapse in visual systems could mediate lateral inhibition among sensory cells to enhance image discrimination or mediate "on" or "off" responses - behavioral functions which are well known from electrophysiology of the same visual systems. Reciprocal dyadic synapses in the nematode tail may prove to be the morphological basis for mediating "on" or "off" responses to sensory stimulation of the tail. This subject is discussed further in another section.

The functional status of individual synapses cannot be assessed on morphological grounds. In synapse counts I have included some synapses which can be found on only a few

sections near breaks in the series. Most of the counted synapses have groupings of synaptic vesicles nearby. Previous studies by Mark (1970) and Cass, Sutton, and Mark (1973) have suggested that some morphologically intact synapses can be functionally inactive in the frog. It is possible that some of the PAG synapses could also be non-functional. Most PAG synapses are grouped into a small number of highly repeated classes. These multiple connections may well be required for a given class of synapses to be functionally significant. There is also a dearth of functional data concerning dyadic synapses, and I cannot be certain whether both post-synaptic members of a dyadic synapse actually receive functional input. All of the eight cell types which are involved in most of the PAG dyads are known to have significant reproducible input and output, which lessens the chance that any of the eight types might lack an active functional role in these synapses.

Dyadic synapses in the nematode tail generally involve three non-homologous fibers, producing muliple routes of information flow. Most of these dyads involve a few preferred combinations of fibers. Strict rules for dyad formation may also govern the synapses of other organisms, but these rules can only become apparent when total reconstruction is successful (all fibers must be identified). Few regions of neuropil have yet been studied so

of neurons appear to participate in the same sets of synaptic interactions, providing the same cellular signal when acting post-synaptically and requiring the same pairs of cellular signals to act pre-synaptically.

There is no evidence that individual synapses are uniquely specified. Instead, the formation of dyadic synapses in the PAG appears to be a probabilistic phenomenon. Synapses occur intermittently in regions where the proper set of fibers are grouped together. Certain dyads are much more common than others despite apparently similar morphological chances for interaction. Each dyadic type appears to have a separate probability of formation. Dyads of a given type are not generally spaced apart in an organized pattern, and dyads of different types are distributed independently of one another. The dynamics of synapse formation in the PAG are unknown. These dyads may be formed only at certain times in development, or they may be continuously formed and destroyed.

The dynamics of synapse formation may be a very important feature in understanding the synapse pattern found in the adult. The orderly growth of the nerve fibers into a stereotyped array may precede synapse formation, limiting the number of possible synapse combinations - perhaps involving a transient recognition process similar to that observed in the Daphnia visual system (LoPresti,

Macagno and Levinthal, 1973). Proper groups of pre- and post-synaptic fibers might grow together as they encounter one another, increasing their chances for interaction and excluding many other possible combinations. Alternately, fiber positions could be altered after synapse formation is relatively complete, with the completed synapses causing certain groups of fibers to hang together during a non-specific adjustment of relative fiber positions. The formation of a dyadic synapse may require the pre-synaptic fiber to identify two proper synaptic fibers within a broad array of choices, or the post-synaptic fibers may use a prior recognition system to pair off before searching together for a proper pre-synaptic fiber.

How many cellular recognition factors must be involved in specifying the dyadic synapses of the eight major PAG cell types? If eight different factors are secreted, one per cell type, then each pre-synaptic fiber would have to independently assign probabilities of synapse formation to thirty-six possible pairs of dyadic partners - a cumbersome task. However Figure 17 clearly indicates that most pre-synaptic fibers choose a very restricted set of preferred dyadic contacts. There appear to be two different levels of specific control: first, there is a very strong requirement for one particular type of post-synaptic fiber (B) to be involved in every dyad - perhaps donating a

primary factor (b); second, there is a restricted set of post-synaptic fibers which are acceptable as the other member of the dyad (C) - perhaps donating a supplemental factor (c). We have previously noted that 90-95% of all dyads do include the proper donor of primary factor (b). The proper supplementary factor (c) appears to be donated in only about 61% of the dyads, as can be seen in the following listing:

TABLE 1

(Data from Figure 17)

PRE- (A)	POST- (B)	% of DYADS	POST- (C)	% of DYADS
α	γ	83	φ	67
φ	α	100	δ	65
27,28	α	98	γ	70
δ	α	100	A	67
χ	γ	83	27,28	67
17,18	γ	96	α,χ or A?	32?
	Average	95%		61%

Cells 17,18 clearly do not follow these rules so well, as their second post-synaptic fibers (C) are not so strictly specified (see Figure 17). Seven different types of fibers are listed above as donating recognition factors; the eighth cell type, cells 17,18, receives very few synaptic inputs

and may not supply a functional recognition factor. (Since these are lineage cells, their lack of synaptic inputs might also reflect a cessation of synapse formation by the other, non-lineage cell types when cells 17,18 wire into the PAG.) Fewer than seven separate recognition factors may be required, since some cell types appear to receive similar classes of input: note the similarities in input to γ and δ, and to ϕ and A, in Figure 17. The δ fibers may be donating the same recognition factor as the γ fibers, but in lesser concentrations, or supplying a very similar factor. Likewise, the ϕ fibers and A fibers may be donating the same factor in slightly different form. These similarities are not entirely convincing on the basis of so little data, and no compelling, quantitative model of dyadic synapse formation has yet emerged. Efforts are continuing along these lines.

A small number of cellular recognition factors may be utilized to govern the formation of most of the synapses in the PAG. These same factors could also be important in the orderly fiber growth which must form the PAG. A mutation of one of these factors could cause multiple morphological alternations and associated behavioral changes (such as paralysis or severe uncoordination), possibly with lethal consequences. It may prove to be very difficult to isolate viable mutants which will be useful for exploring the roles of these factors.

The study of recessive lethal mutations is currently quite difficult in the nematode. Future work with lethal mutants may become more feasible with the advent of balancer mutants which could maintain these genes as heterozygotes (Herman et al., 1976).

The simplicity of the defecation circuit in the PAG suggests that this system may be amenable to genetic dissection. The defecation circuit is monosynaptic: cell 33 → defecation muscles. Mutations of the recognition factor(s) responsible for these synapses might be expressed as relatively simple morphological variants of cell 33 and the defecation muscle arms, with associated deficits in defecation behavior. Better characterization of defecation mutants could be useful in this regard. Balancer mutants again could be helpful in maintaining "constipated" mutants.

FUNCTIONAL ANAYLYSIS OF THE PRE-ANAL GANGLION CIRCUITRY: Most of the PAG circuitry serves primarily to direct sensory information to ventral cord interneurons, with resulting behavioral responses by the whole animal. To analyse the function of the PAG circuitry, one must first consider the function of the ventral cord interneurons and motoneurons, which control the animal's body motion. The organization of the ventral cord circuitry has recently been reported by White (1975) and White et al. (1976). Their descriptions of the motoneuron and interneuron types have been given in

previous sections. The basic wiring diagram of the ventral cord circuitry is shown in Figure 23b (White et al., 1976). The various synapse types are distributed sparsely along the ventral cord. Some synapse types are more common in anterior regions of the cord, others in posterior regions. Some of these synapse types are conspicuously missing in the PAG.

Analysis of any part of the circuitry of C. elegans is hampered by the inability to obtain electrophysiological recordings of neuronal activity or muscle activity. The large parasitic nematode, Ascaris, is more amenable to electrophysiology and numerous studies have been made upon Ascaris muscle. The structure of Ascaris muscle is surprisingly similar to that of C. elegans and the layouts of the nervous systems of the two species are very similar. It is expected that the physiology of Ascaris muscles is much the same as in C. elegans.

The physiology of Ascaris muscle has been studied by del Castillo and coworkers (DeBell, Castillo and Sanchez, 1963; del Castillo and Morales, 1967; del Castillo, de Mello and Morales, 1967), and more recently by Stretton (Stretton, 1976; personal communications) and by Weisblat, Byerly and Russell (1976). Acaris muscle displays mycgenic activity, even when all nerve activity has been blocked. Modulation of this spontaneous muscular activity can be achieved by

application of neurotransmitters to the muscle plate: acetylcholine is excitatory and GABA is inhibitory. Gap junctions between adjoining muscle arms permit adjoining muscles to maintain close synchrony. Waves of muscular contraction can possibly be propagated along the length of the animal even in the absence of nervous activity. Crofton (1966) was the first to suggest that the nematode's normal body motion is produced by myogenically propogated muscular contraction. The nervous system may act only to modulate the spontaneous muscle activity. Proprioception is probably stretch-activated, and is probably mediated by the muscles themselves or by the motoneurons of the ventral and dorsal cords. Current experiments confirm that muscular activity is highly sensitive to stretching and bending (Weisblat, Byerly and Russell, unpublished observations).

White et al. (1976) suggest that the B and A motoneurons are best situated to be excitatory and inhibitory motoneurons respectively, one using acetylcholine and the other using GABA as neurotransmitters. Class D motoneurons are driven exclusively by the A and B motoneurons (Figure 23b) and have NMJ output to the set of body muscles opposing their dendritic region, suggesting that they set up reciprocal inhibition of opposing sets of muscles - allowing the dorsal side of the animal to relax as the ventral side contacts and vice versa (White, 1975). White suggests that

this reciprocal inhibition is required to generate the initial set when body motion is begun, but that it is unimportant during the self-propagated motion thereafter.

Forward and backward motion are both achieved by sinusoidal waves of muscular contractions. The nematode lies on one side so that the dorsal body muscles work in opposition to the ventral body muscles. The leading end of the body generally appears to be undergoing stronger contractions than the trailing end. White et al. (1976) suggest that this gradient of muscle activity is induced by a gradient of motoneuron activity, with muscle oscillations at the more active end of the body entraining oscillations of the remaining body muscles to become the leading end. In this model, sensory stimuli could produce behavioral reversal by acting upon the interneurons of the ventral cord to reverse the gradient of motoneuron activity.

Gradients of motoneuron activity must be established by gradients of interneuron activation, which in turn might be achieved either by gradients of specific synapse types between interneurons and motoneurons or by other mechanisms. White et al. (1976) mention only a few synapse gradients along the ventral cord; notably $\beta \rightarrow \alpha$ and $\gamma \rightarrow \alpha$ synapses are more common in the posterior ventral cord. No other synapse gradients are reported to exist between interneurons and motoneurons. One might expect that δ interneuron output

would be more concentrated in the anterior ventral cord because there are four δ interneurons there, versus two in the posterior ventral cord, but no gradient involving the δ interneurons has been reported.

Among other possible mechanisms for activation gradients, one must consider the possibility of passive decremental spread of activity (without action potentials) in ventral cord interneurons. This mechanism would provide the ventral cord with graded sensory activation (only). Nematode nerves have not yet been impaled reliably with microelectrodes, so there is no applicable data concerning the generation of action potentials. Furthermore, the nematode nervous system is so small that graded potentials could conceivably be utilized to perform much of the system's information processing. The space constant (λ) of C. elegans nerve fibers cannot be determined directly, but estimates can be made using data from other systems and the general equation:

$$\lambda = \sqrt{\left(\frac{RR_m}{2R_i}\right)}$$

where R = radius of fiber (cm)

R_m = membrane resistance (Ω cm^2)

R_i = cytoplasm resistivity (Ω cm)

Table 2 shows the expected range of the space constant for the 0.2-0.4μ (diameter) nematode nerves in the PAG, assuming that the cable properties are similar to those of four selected invertebrate preparations.

TABLE 2

If Nematode Cable Properties =	Cable Properties	Space Constant (Expected Range in C. elegans)	Data Source
	Squid Nerve	100-140μ	Katz (1966)
	Lobster Nerve	125-180μ	Katz (1966)
	Crab Nerve	200-290μ	Katz (1966)
	Cockroach Giant Axon	50-70μ	Pichon (1969)

These estimates vary rather widely and they may not be valid since the reference preparations are all quite large compared to the very fine fibers of the nematode. However, they do give some indication that synaptic interactions within the PAG may be quite capable of integrating sensory data with graded potentials (since most PAG synapses are within 0.5-30μ of each other, probably less than the space constants of the fibers involved). King (1976b) has previously suggested that similar neurites in the lobster stomatogastric ganglion, which also lack clearly separate dendritic and axonal differentiation, also perform normal synaptic integration of passively conducted potentials.

Preliminary evidence (White, 1975) suggests that chemosensory signals transduced by amphidial neurons are conducted by a multisynaptic route to the β interneurons (Figure 23c). White suggests that the B motoneurons are excitatory, stimulating stronger contractions of the body muscles to speed the animal's forward motion during chemotaxis. The circuit:

amphidial neurons $\overset{\pm}{\rightarrow}$ β $\overset{\pm}{\rightarrow}\overset{\pm}{\rightarrow}$ B motoneurons

could produce stronger contractions each time the head moves up the gradient of attractant, thus keeping the animal oriented to the gradient.

White also found evidence that mechanosensory neurons in the head direct signals by a multisynaptic route to the α and δ interneurons (Figure 23c). White presumes that the class A motoneurons are inhibitory. The circuit:

cephalic mechanosensors $\overset{+}{\rightarrow}$ α,δ $\overset{+}{\rightarrow}\overset{+}{\rightarrow}$ A motoneurons

could mediate reversal of the animal in response to head tap by inhibiting the motion of the anterior body muscles through the A motoneurons.

My own data show that mechanosensory stimulus of the tail is routed particularly to the α, γ, and δ interneurons, with no β interneurons involvement (Figure 23a). The γ interneurons do happen to have synaptic targets similar to those of the β interneurons in the ventral cord (Figure 23b) (White *et al.*, 1976).

When all of these studies on the nervous circuitry of *C. elegans* are combined, one can begin to speculate on the

Figure 23. Wiring Diagrams

(a) Wiring diagram for the pre-anal ganglion. Most common connections shown as heavy arrows. (b) Wiring diagram for the ventral cord (White et al., 1976). (c) Wiring diagram (partial) for sensory inputs to ventral cord interneurons in the head (White, personal communication).

Ph - phasmidial neurons (27,28)

TT - tail tip mechanosensors (17,18)

As - asymmetric mechanosensor (19)

A,B,D - motoneurons

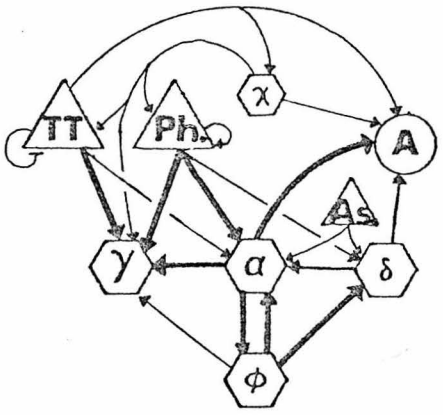
Am - amphidial neurons (dotted line indicates output from finger and wing cells)

Ce - mechanosensory (and chemosensory?) neurons from papillae in the head

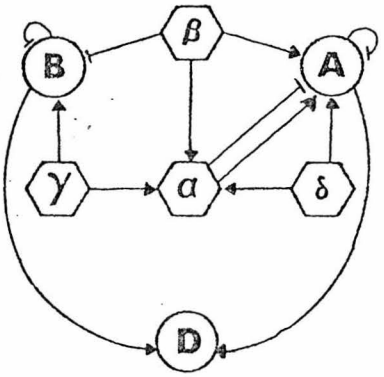
RI - nerve ring interneurons

OI - "other" interneurons

a



b



c

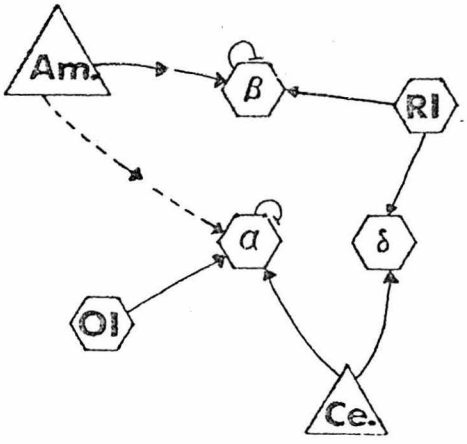


Figure 23

possible functions of the ventral cord interneurons in mediating behavioral responses to sensory stimulation. In making these speculations, the following assumptions are used:

1. Muscle cells propagate contactile waves along the length of the animal by their muscle arm interactions with neighboring muscle cells.

2. Muscle cells produce spike activity independently, in response to stretch activation.

3. Three classes of motoneurons modulate muscle activity: Class A motoneurons are inhibitory.

Class B motoneurons are excitatory.

Class D motoneurons set up reciprocal inhibition of parallel dorsal and ventral muscle cells. Class D motoneurons must be excited by Class A and inhibited by Class B motoneurons, or vice versa.

4. In the ventral cord:

$\alpha, \delta \overset{+}{\rightarrow} A$

$\beta, \gamma \overset{+}{\rightarrow} B$

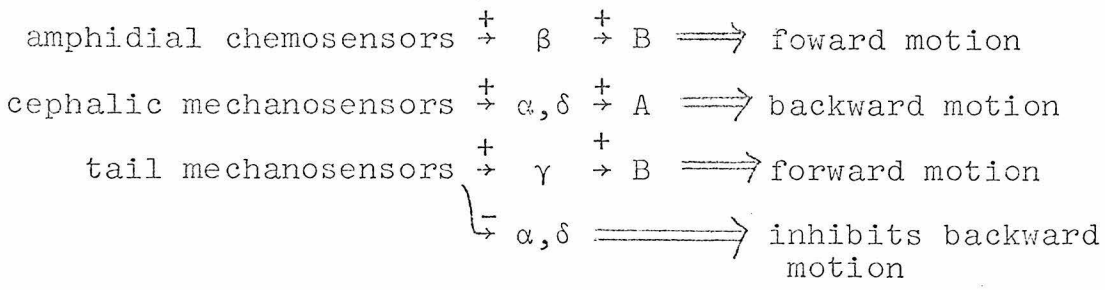
$\beta, \gamma \overset{+}{\rightarrow} \alpha$ (Synapse gradients make these more common in posterior regions.)

$\beta \overset{-}{\rightarrow} A$

5. Gap junctions between neighboring motoneurons of a given class are sufficient to maintain synchrony among them, but not sufficient to propagate waves of excitation or inhibition without decrement.

When these assumptions are taken into account, there are two general alternatives which could explain the operation of the ventral cord interneurons.

Alternative 1. Sensory stimuli which activate the β or γ interneurons automatically produce forward motion. Sensory stimuli which activate the α or δ interneurons automatically produce backward motion. The following excitatory and inhibitory connections would be likely:



By what means could β and γ interneurons promote forward motion? They might have more $\beta,\gamma \overset{+}{\rightarrow} B$ synapses in the anterior end to selectively excite the anterior body muscles. However, no synapse gradient of this type has been observed (White et al., 1976). Instead, the observed synapse gradients favor more $\beta,\gamma \rightarrow \alpha$ synapses in the posterior ventral cord. These synapse gradients might mediate selective inhibition of the posterior body muscles if the α interneurons are excited to subthreshold levels which are still sufficient to cause $\alpha \overset{+}{\rightarrow} A$ stimulation in the posterior regions. If the stimulation of α interneurons did reach

threshold, their action potential would cause general inhibition of the whole animal and failure to move forward.

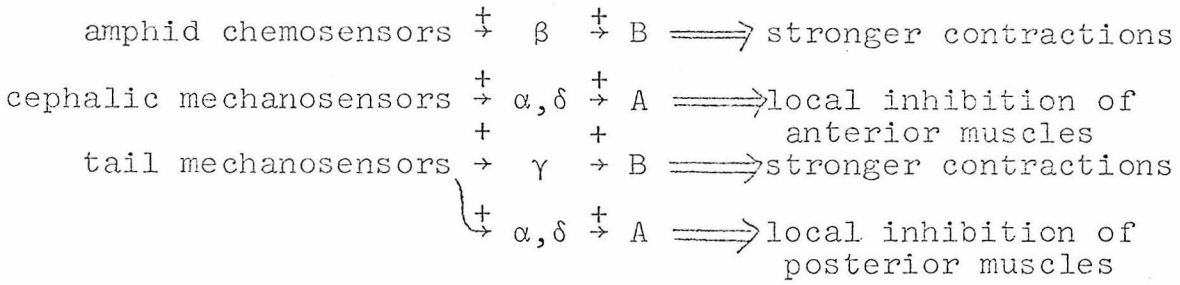
By what means could α and δ interneurons promote backward motion? They might have more α,δ $\overset{+}{\rightarrow}$ A synapses in the anterior ventral cord to selectively inhibit the anterior body muscles. However, no synapse gradient of this type has been noted (White et al., 1976).

Alternative 1 will only be a useful hypothesis if new synapse gradients can be found in the ventral cord, otherwise it seems unsatisfactory.

Alternative 2. Mechanosensory stimuli to the head and tail are compared by the α and δ interneurons. The end of the animal receiving stronger mechanical stimuli becomes relatively quiescent through local α,δ $\overset{+}{\rightarrow}$ A inhibitory effects. These comparisons could be accomplished best if the α and δ interneurons conduct signals only by passive spread without action potentials. The α and δ interneurons are postulated to be quite inexcitable, with thresholds too high to allow action potentials despite massive multiple inputs.

β and γ interneurons could set the general level of excitation of the nematode's behavior. These interneurons respond to sensory stimulation by carrying signals with little or no decrement (by action potentials) to the entire ventral cord.

The following connections would be likely:



In this model, the observed gradients for $\beta,\gamma \overset{+}{\rightarrow} \alpha$ synapses could serve to reinforce the dominance of the anterior body muscles. (This dominance is known to cause the animal to strongly favor forward motion.) The predominance of $\beta,\gamma \overset{+}{\rightarrow} \alpha$ contacts in the posterior ventral cord should lead to selective inhibition of posterior body muscles by $\alpha \overset{+}{\rightarrow} A$ contacts.

Alternative 2 is dependent upon the differing thresholds and conduction properties of the interneurons, although it does not violate any general principles of neurophysiology. The detailed behavior of the hermaphrodite is not well known, but this alternative is sufficient to explain the known responses in a general manner. In this model, the feedback circuits of the PAG can mediate filtering or enhancement of sensory stimulation of the tail. Also, the γ interneurons could be useful in guiding backwards motion towards an attractive chemosensory stimulus transduced by the phasmids, in the same manner that the β interneurons are thought to guide forward chemotaxis. This same circuit

could be elaborated in the male tail to provide a range of attractive responses during backward motion.

Accepting Alternative 2 as the more probable hypothesis, one makes the following additional assumptions:

6. In the PAG: $(17,18,19,27,28) \overset{+}{\rightarrow} \gamma,\alpha,\delta$

$\alpha,\delta \overset{+}{\rightarrow} A$

$\alpha \overset{+}{\rightarrow} \phi,\gamma$

$\phi \overset{+}{\rightarrow} \gamma,\alpha,\delta$

7. α and δ interneurons have no action potentials, but passively conduct graded sensory signals along their length. β and γ interneurons generate action potentials in response to sensory stimuli.

The additional assumptions are redundant in some respects. For instance, the properties of the δ interneurons may be somewhat different from the α interneurons without altering the general mode of operation of the whole system. The interaction between α and ϕ interneurons is postulated to be positive feedback, but could instead be performing more complex sensory filtering. Inhibitory feedback and the proper timing delays in the $\alpha \circlearrowright \phi$ circuit might mediate an "on" or "off" response.

The operation of the ϕ interneurons is quite striking. Their synaptic output goes to very similar targets when compared to the output of the sensory cells (17,18,19,27,28). The interaction of the ϕ interneurons with the lateral

cords (cells 9,10) suggests that the ϕ interneurons may convey sensory signals from the post-deirids to the same set of interneurons which receive other sensory input in the tail. If these ϕ outputs are excitatory, they could be responsible for the animal's responses to mechanical stimulation of the post-deirids. These responses are similar to the animal's responses to tail tap.

There are multiple feedback loops, multiple point synapses and multiple routes leading to the excitement of the ventral cord interneurons α, γ, and δ. When one assumes that most or all of the major PAG synapses are excitatory, as in Alternative 2, the response to any tail stimulus will be maximized. The multiple synapses and multiple routes to γ excitation insure that these fibers reach their threshold for producing action potentials (subthreshold γ excitation will be counterproductive to the generation of forward motion). The formidable multiplicity of inputs to α interneurons may reflect their relative inexcitability. Highly repeated synapses may also reflect the need for a "safety factor" to insure proper function in a system with many mistaken connections.

Multiple routes of α and γ interneuron excitation could also mediate more complex sensory filtering, using timing features of the stimuli. Inhibitory circuits might act to restrict the sensitivity of the interneurons to stimuli with

the proper intensity. The spatial configuration of the feedback loops is so variable that timing features would seem to be muddled in these circuits. There is no data concerning the range of stimulus sensitivity possessed by the tail. Without better physiological data, these speculations are not worth carrying further.

Our assumptions about the PAG circuitry presume a predominance of excitatory connections. In some other invertebrate systems, notably in the lobster stomatogastric ganglion (Mulloney and Selverston, 1974a,b; Selverston and Mulloney, 1974), inhibitory connections are known to be very commonplace. The true circumstances in nematode neuropil are unknown, but I could interpret the PAG circuitry in similar terms without tearing apart all of the functional interactions. (For instance, the B motoneurons could be spontaneously active inhibitory motoneurons. β and γ interneurons could respond to sensory activiation by sending inhibitory signals to the B motoneurons, with the net effect of increasing muscle excitation.) Modelling all of the PAG connections as inhibitory synapses is rather cumbersome, so I have neglected this analysis until I have stronger reasons to do so.

This hypothetical model of the nematode's nervous system does not seem to make full use of the possibilities

provided by the dyadic synapse. Multiple routes of information flow and simultaneous synaptic signals offer a wealth of potential timing features. Computer modelling of the complete nematode wiring would probably be required to fully assess these capabilities, but these efforts are premature until more animals have been reconstructed to better judge the variability of the spatial separation of dyads. Even in these two animals, B126 and B136, the degree of variability seems rather large to allow for sophisticated timing mechanisms, especially since there are so few synapses involved. The random addition or loss of just a few synapses of a given type might be sufficient to greatly upset the normal timing delays. Complex timing features are well-known in the thirty-cell stomatogastric ganglion of the lobster (Morris and Maynard, 1970; Mulloney and Selverston, 1974b), but these few neurons utilize one million synapses in the operation of the ganglion (estimated by King, 1976a).

SUMMARY

1. All of the cells in the C. elegans tail can be individually identified and are basically identical in several animals from an isogenic strain.

2. Sensory neurons, motor neurons, and interneurons can be identified and classified by their characteristic morphologies and synaptic connections.

3. Complete synaptic interactions among identified cells in a sensory-motor ganglion can be catalogued and compared from animal to animal.

4. Synaptic patterns are stable with minor variations - perhaps reflecting the probabilistic nature of synapse formation. Twenty-five per cent of all synapses are apparently single random "mistakes." "Important" synapse types are highly repeated.

5. The dyadic synapse is the predominant class of chemical synapse in the tail. This synapse is the morphological basis for the simultaneous stimulation of multiple routes of information flow in sensory processing, probably as a means of sensory filtering or sensory enhancement.

6. Dyadic synapses display very strict rules of synapse

formation. A very few types of dyads are favored over all others. Two types of interneurons (α and γ) are always the primary synaptic targets.

7. Projections from anterior interneurons can be identified by their characteristic fiber positions and synaptic interactions. The γ interneurons, with cell bodies in the lumbar ganglion, can also be identified by their fiber positions in the anterior end of the reconstructed region.

8. Rather complete wiring diagrams are now available for the tail and the ventral cord; the wiring diagram for the cephalic region is not known in detail. These wiring diagrams allow us to explore the behavior of the nematode in terms of defined motoneurons, interneurons, and sensory inputs.

9. Lineage cells in the lumbar ganglia have been identified. Many of these cells are involved in sensory transducion in the adult nematode.

10. Lineage cells in the pre-anal ganglion have been identified. Two of these cells do not follow the general pattern for their lineage; four other lineage cells become motoneurons, possibly of the proper classes for their lineage.

11. Phasmidial cilia (cells 27-30), the asymmetric cilium (cell 19), and the unciliated dendrites of sensory cells 17 and 18 may all be sensitive to mechanical stimuli. Specific chemosensory responses have not yet been identified. Several different means of sensory transduction may be utilized in the reception of mechanical stimuli by these neurons.

REFERENCES

Albertson, D.G. and Thomson, J.N. (1976) The pharynx of Caenorhabditis elegans, Proc. Roy. Soc. London, Series B, in press.

Benzer, S. (1971) From gene to behavior, JAMA, 218, 1018.

Bird, A.F. (1971) The Structure of Nematodes, Academic Press, New York.

Boschek, C.B. (1971) On the fine structure of the peripheral retina and lamina ganglionaris of the fly, Musca domestica, Z. Sellforsch. Mikrosk. Anat., 118, 369.

Brenner, S. (1973) The genetics of behavior, Br. Med. Bulletin, 29, 269.

Brenner, S. (1974) The genetics of Caenorhabditis elegans, Genetics, 77, 71.

Bullock, T.H. and Horridge, G.A. (1966) Structure and Function in the Nervous System of Invertebrates, W.H. Freeman and Co., San Francisco, 2 vol.

Cass, D.T., Sutton, T.J. and Mark, R.F. (1973) Competition between nerves for functional connexions with Axolotl muscles, Nature, 243, 201.

Chappell, R.L. and Dowling, J.E. (1972) Neural organization of the median ocellus of the dragonfly, I. Intracellular electrical activity, J. Gen. Physiol., 60, 121.

Chitwood, B.G. and Chitwood, M.D. (1950) An Introduction to Nematology, Monumental Printing, Baltimore.

Chitwood, B.G. and Wehr, E.E. (1934) The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea, Z. Parasitol., 7, 273.

Crofton, H.D. (1966) Nematodes, Hutchinson University Library, London.

DeBell, J.T., Castillo, J. del, and Sanchez, V.L. (1963) Electrophysiology of the somatic muscle cells of Ascaris lumbricoides, J. Cell. Comp. Physiol., 62, 159.

del Castillo, J., de Mello, W.C., and Morales, T. (1967) The initiation of action potentials in the somatic musculature of Ascaris lumbricoides, J. Exp. Biol., 46, 263.

del Castillo, J. and Morales, T. (1967) The electrical and mechanical activity of the esophageal cell of Ascaris lumbicoides, J. Gen. Physiol., 50, 603.

Dowling, J.E. (1970) Organization of vertebrate retinas, Invest. Ophthalmol., 9, 655.

Dowling, J.E. and Boycott, B.B. (1966) Organization of the primate retina: electron microscopy, Proc. Roy. Soc., London, Series B, 166, 80.

Dowling, J.E. and Chappell, R.L. (1972) Neural organization of the median ocellus of the dragonfly, II. Synaptic structure, J. Gen. Physiol., 60, 148.

Dowling, J.E. and Werblin, F.S. (1969) Organization of the retina of the mudpuppy, Necturus maculosa, I. Synaptic structure, J. Neurophysiol., 32, 315.

Dusenbery, D.B. (1973) Countercurrent separation: A new method for studying behavior in small aquatic organisms, PNAS, 70, 1349.

Dusenbery, D.B. (1974) Analysis of chemotaxis in the nematode Caenorhabditis elegans by countercurrent separation, J. Exp. Zool., 188, 41.

Dusenbery, D.B. (1976) Genetic dissection of chemotaxis of the nematode Caenorhabditis elegans, I. Chemotactic behavior of mutants altered in their response to NaCl, in press.

Dusenbery, D.B., Sheridan, R.E., and Russell, R.L. (1975) Chemotaxis-defective mutants of the nematode, Caenorhabditis elegans, Genetics, 80, 297.

Goldschmidt, R. (1903) Histologische untersuchungen an nematoden, Zool. Jahrb. Anat., 18, 1.

Goldschmidt, R. (1908) Das nervensystem von Ascaris lumbricoides und megalocephala, I., Ztschr. Wiss. Zool., 90, 73.

Goldschmidt, R. (1909) Idem. II., Ztschr. Wiss. Zool., 92, 306.

Goldschmidt, R. (1910) Idem. III., Festschrift Hertwig, 2, 256.

Hamori, J. and Horridge, G.A. (1966) The lobster optic lamina, J. Cell Sci., 1, 257.

Harris, W. (1976) Color vision in Drosophila, Ph.D. thesis, Caltech.

Harris, W., Stark, W., and Walker, J. (1975) Genetic dissection of the photoreceptor system in the compound eye of Drosophila melanogaster, J. Physiol., in press.

Hartline, D.K. and Maynard, D.M. (1975) Motor patterns in the stomatogastric ganglion of the lobster Panulirus argus, J. Exp. Biol., 62, 405.

Hedgecock, E.M. and Russell, R.L. (1975) Normal and mutant thermotaxis in the nematode, Caenorhabditis elegans, PNAS, 72, 4061.

Henkart, M. (1975) A morphological basis for transcellular transfer of macromolecules, Neuroscience Abstracts 1, no. 1172, 762.

Herman, R.K., Albertson, D.G., and Brenner, S. (1976) Chromosome rearrangements in Caenorhabditis elegans, Genetics, 83, 91.

Hesse, R. (1892) Ueber das nervensystem von Ascaris megalocephala, Z. Wiss. Zool., 54, 548.

Hodgkin, J.A. (1974) Genetic and anatomical aspects of the Caenorhabditis elegans male, Ph.D. thesis, Cambridge.

Hotta, Y. and Benzer, S. (1972) Mapping of behavior in Drosophila mosaics, PNAS, 67, 1156.

Hughes, G.M. and Tauc, L. (1962) Aspects of the organization of central nervous pathways in Aplysia depilans, J. Exp. Biol., 39, 45.

Jan, V.N. and Jan, L.Y. (1976) A synaptic transmission mutant of Drosophila melanogaster, in press.

Jansen, J.K.S. and Nicholls, J.G. (1972) Regeneration and changes in synaptic connections between individual nerve cells in the central nervous system of the leech, PNAS, 69, 636.

Kankel, D.R. and Hall, J.C. (1975) Fate mapping of nervous system and other tissues in genetic mosaics of Drosophila melanogaster, Develop. Biol., in press.

Kandel, E.R., Frazier, W.T., Waziri, R., and Coggeshall, R.E. (1967) Direct and common connections among identified neurons in Aplysia, J. Neurophysiol., 30, 1352.

Katz, B. (1966) Nerve, Muscle and Synapse, McGraw-Hill, New York.

Kennedy, D., Selverston, A.I., and Remler, M.P. (1969) Analysis of restricted neuronal networks, Science, 164, 1488.

King, D.G. (1976a) Organization of crustacean neuropil, I. Patterns of synaptic connections in lobster stomatogastric ganglion, J. Neurocytol., 5, 207.

King, D.G. (1976b) Organization of crustacean neuropil, II. Distribution of synaptic contacts on identified motor neurons in lobster stomatogastric ganglion, J. Neurocytol., 5, 239.

Kristan, W.B., Jr., Stent, G.S., and Ort, C.A. (1974a) Neuronal control of swimming in the medicinal leech, I. Dynamics of the swimming rhythm, J. Comp. Physiol., 94, 97.

Kristan, W.B., Jr., Stent, G.S., and Ort, C.A. (1974b) Neuronal control of swimming in the medicinal leech, III. Impulse patterns of the motor neurons, J. Comp. Physiol., 94, 155.

Landis, D.M.D. and Reese, T.S. (1974) Differences in membrane structure between excitatory and inhibitory synapses in the cerebellar cortex, J. Comp. Neurol., 155, 93.

Lasek, R.J., Gainer, H., and Przybylski, R.J. (1974) Transfer of newly synthesized proteins from Schwann cells to the squid giant axon, PNAS, 71, 1188.

Levinthal, C. and Ware, R.W. (1972) Three-dimensional reconstruction from serial sections, Nature, 236, 207.

Looss, A. (1905) The anatomy and life history of Agchylostoma duodenale, Rec. Egypt. Gov't. School Med., 3, 1.

LoPresti, V., Macagno, E.R., and Levinthal, C. (1973) Structure and development of neuronal connections in isogenic organisms: Cellular interactions in the development of the optic lamina of Daphnia, PNAS, 70, 433.

Macagno, E.R., LoPresti, V., and Levinthal, C. (1973) Structure and development of neuronal connections in isogenic organisms: Variations and similarities in the optic system of Daphnia magna, PNAS, 70, 57.

Mark, R. (1970) Chemospecific synaptic repression as a possible memory store, Nature, 225, 178.

Martini, E. (1903) Ueber furchung und gastrulation bei Cucullanus elegans, Ztschr. Wiss. Zool., 74, 501.

Martini, E. (1906) Ueber subcuticula and seitenfelder einiger nematoden. I., Ztschr. Wiss. Zool., 81, 699.

Martini, E. (1909) Idem. IV., Ztschr. Wiss. Zool., 93, 535.

Martini, E. (1916) Die anatomie der Oxyuris curvula, Ztschr. Wiss. Zool., 116, 137.

Maynard, D.M. (1972) Simpler networks, Ann. N.Y. Acad. Sci., 193, 59.

Maynard, D.M. and Selverston, A.I. (1975) Organization of the stomatogastric ganglion of the spiny lobster, IV. The pyloric system, J. Comp. Physiol, 100, 161.

Morris, J. and Maynard, D.M. (1970) Recordings from the stomatogastric nervous system in intact lobsters, Comp. Biochem. Physiol., 33, 969.

Mulloney, B. and Selverston, A.I. (1974a) Organization of the stomatogastric ganglion of the spiny lobster, I. Neurons driving the lateral teeth, J. Comp. Physiol., 91, 1.

Mulloney, B. and Selverston, A.I. (1974b) Organization of the stomatogastric ganglion of the spiny lobster, III. Coordination of the two subsets of the gastric system, J. Comp. Physiol., 91, 53.

Nicholls, J.G. and Baylor, D.A. (1968) Specific modalities and receptive fields of sensory neurons in the central nervous system of the leech, J. Neurophysiol., 31, 740.

Nicholls, J.G. and Purves, D. (1970) Monosynaptic chemical and electrical connexions between sensory and motor cells in the central nervous system of the leech, J. Physiol., 209, 647.

Pichon, Y. (1969) Ph.D. thesis, U. of Rennes; in Parnas, I. and Dagan, D. (1971) Functional organization of giant axons, Adv. Insect Physiol., 8, 95.

Ort, C.A., Kristan, W.B., Jr., and Stent, G.S. (1974) Neuronal control of swimming in the medicinal leech, II. Identification and connections of motor neurons, J. Comp. Physiol., 94, 121.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, J. Cell Biol., 17, 208.

Rosenbluth, J. (1965) Ultrastructure of somatic muscle cells in Ascaris lumbricoides, J. Cell Biol., 26, 579.

Schneider, A. (1866) Monographie der nematoden, Berlin.

Selverston, A.I. and Kennedy, D. (1969) Structure and function of identified nerve cells in the crayfish, Endeavour, 28, 107.

Selverston, A.I. and Mulloney, B. (1974) Organization of the stomatogastric ganglion of the spiny lobster, II. Neurons driving the medial tooth, J. Comp. Physiol., 91, 33.

Smith, D.S. (1966) The organization of the insect neuropile; in Wiersma, C.A.G. (Ed.), Invertebrate Nervous Systems, U. Chicago Press, Chicago, 79.

Stretton, A.O.W. (1976) Anatomy and development of the somatic musculature of the nematode Ascaris, in press.

Strumwasser, F. (1963) A circadian rhythm of activity and its endogenous origin in a neuron, Fed. Proc. (FASEB), 22, 220.

Strumwasser, F. (1967) Types of information stored in single neurons; in Wiersma, C.A.G. (Ed.), Invertebrate Nervous Systems, U. Chicago Press, Chicago, 291.

Strumwasser, F. (1968) Membrane and intracellular mechanisms governing endogenous activity in neurons; in Carlson, F.D. (Ed.), Physiological and Biochemical Aspects of Nervous Integration, Prentice-Hall, New Jersey, 329.

Stuart, A.E. (1970) Physiological and morphological properties of motoneurons in the central nervous system of the leech, J. Physiol., 209, 627.

Sulston, J.E. (1976) Post-embryonic development in the ventral cord of Caenorhabditis elegans, Proc. Roy. Soc. London, Series B, in press.

Sulston, J.E. and Brenner, S. (1974) The DNA of Caenorhabditis elegans, Genetics, 77, 95.

Sulston, J.E., Dew, M., and Brenner, S. (1975) Dopaminergic neurons in the nematode, Caenorhabditis elegans, J. Comp. Neurol., 163, 215.

Toh, V. and Kuwabara, M. (1975) Synaptic organization of the fleshfly ocellus, J. Neurocytol., 4, 271.

Trujillo-Cenoz, O. (1965a) Some aspects of the structural organization of the arthropoid eye, CSH Symp. Quant. Biol., 30, 371.

Trujillo-Cenoz, O. (1965b) Some aspects of the structual organization of the intermediate retina of Dipterans, J. Ultrastruct. Res., 13, 1.

Voltzenlogel, E. (1902) Untersuchungen ueber den anatomischen und histologischen bau des hinterendes von Ascaris megalocephala und Ascaris lumbricoides, Zool. Jahrb., Abt. Anat., 16, 481.

Ward, S. (1973) Chemotaxis by the nematode Caenorhabditis elegans: Identifications of attractants and analysis of the response by use of mutants, PNAS, 70, 817.

Ward, S., Thomson, J.N., White, J.G., and Brenner, S. (1975) Electron microscopical reconstruction of the anterior sensory anatomy of the nematode, Caenorhabditis elegans, J. Comp. Neurol., 160, 313.

Ware, R.W., Clark, D., Crossland, K., and Russell, R.L. (1975) The nerve ring of the nematode Caenorhabditis elegans: Sensory input and motor output, J. Comp. Neurol., 162, 71.

Ware, R.W. and LoPresti, V. (1975) Three-dimensional reconstruction from serial sections, Int. Rev. Cytol., 40, 325.

Weisblat, D.A., Byerly, L., and Russell, R.L. (1976) Ionic mechanisms of electrical activity in somatic muscle of the nematode Ascaris lumbricoides, J. Comp. Physiol., in press.

Weisblat, D.A. and Russell, R.L. (1976) Propagation of electrical activity in the nerve cord and muscle syncytium of the nematode Ascaris lumbricoides, J. Comp. Physiol., in press.

White, J.G. (1975) The structure of the ventral cord of Caenorhabditis elegans, Ph.D. thesis, Cambridge.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1976) The structure of the ventral nerve cord of Caenorhabditis elegans, Proc. Roy. Soc. London, Series B, in press.

Whitehead, R. and Purple, R.L. (1970) Synaptic organization in the neuropil of the lateral eye of Limulus, Vision Res., 10, 129.

Wiersma, C.A.G. (1952) Neurons of arthropods, CSH Symp. Quant. Biol., 17, 155.

Wiersma, C.A.G. and Bush, B.M.H. (1963) Functional neuronal connections between the thoracic and abdominal cords of the crayfish, Procambarus clarkii, J. Comp. Neurol., 121, 207.

Wiersma, C.A.G. and Hughes, G.M. (1961) On the functional anatomy of neuronal units in the abdominal cord of the crayfish, Procambarus clarkii (Girard), J. Comp. Neurol., 116, 209.

Wiersma, C.A.G. and Ikeda, K. (1964) Interneurons commanding swimmeret movements in the crayfish, Procambarus clarkii (Girard), Comp. Biochem. Physiol., 12, 509.

Wiersma, C.A.G., Ripley, S.H., and Christensen, H.B. (1955) The central representation of sensory stimulation in the crayfish, J. Cell. Comp. Physiol., 46, 307.

APPENDIX I: Cells in the Hermaphrodite Tail

(Compare with Figures 6,7,8,9)

Accessory Cells

Cell No.	Left Side	Right Side	Lineage
1		Wing Cell	L212
2	Wing Cell		L212
3		Cap Cell	L211
4	Cap Cell		L211
5		Pocket Cell	
6	Pocket Cell		
7		Lateral Gland	L1221
8	Lateral Gland		L1221

Lumbar Neurons

9		Tubule Filled, Lateral Cord	
10	Tubule Filled, Lateral Cord		
11		Lateral Cord	L22212
12	Lateral Cord		L22212
13		Dorso-lateral Cord	
14	Dorso-lateral Cord		
15		Neuron	L1222
16	Neuron		L1222

Cell No.	Left Side	Right Side	Lineage
17		Tail Tip Dendrite (Sensory)	L22211
18	Tail Tip Dendrite (Sensory)		L22211
19		Asymmetric Cilium (Sensory)	Migrant Cell
20	Unpaired Neuron		
21		Neuron (Possible Pacemaker for 23)	L221
22	Neuron (Possible Pacemaker for 24)		L221
23		γ_1 Interneuron	
24	γ_2 Interneuron		
25		ϕ_1 Interneuron	
26	ϕ_2 Interneuron		
27		Phasmidial Cilium A	
28	Phasmidial Cilium A		
29		Phasmidial Cilium B	
30	Phasmidial Cilium B		
31		Neuron	
32	Neuron		

Dorsorectal Neurons

33	Defecation Motoneuron (?)	
34	Neuron	
35	Neuron	

PAG Neurons

Cell No.		Lineage
36	DD Motoneuron (?)	J cell (?)
37	Neuron	P12d
38	DA Motoneuron	J cell (?)
39	VD Motoneuron (?)	P12e
40	Neuron	
41	Neuron	
42	VA Motoneuron (?) Inactive	P12a
43	χ Interneuron	
44	Neuron	
45	Neuron	P11e
46	DAS Motoneuron (?)	P11d
47	VA Motoneuron (?) Inactive	P11a