PART II The Organisation of the Adult Nerve Ring

CHAPTER 6 Introduction and Methods

6.1 Introduction

Around the beginning of this century several attempts were made to map all the nerve processes in a nematode nervous system, using light microscopy of methylene blue stained animals, most notably by Goldschmidt (1908), who argued erroneously that a nervous system was a syncytial network of anastomatosed cells. That view was soon disproved, but it was not until recently that the goal was realised of determining the anatomical structure of a complete nervous system at the level of individual processes and synaptic connections (White et al., 19860. As part of that achievement the entire central nervous system of two C. elegans specimens was reconstructed from electron micrographs of serial sections. The purpose of the investigation reported here was to extract information about the organisational structure of the C. elegans nervous system from the resulting anatomical data, concentrating in particular on the syaptic circuitry.

The approach taken was to construct a computer database containing information about all the synapses and gap junctions between the neurons, and also form one animal an indication of the amount of contact between each pair of neurons. This information was used for three separate lines of investigation. The first was to study the distribution of symapses within the nervous system in order to investigate the variability of the circuitry in different circumstances, and the type of variation seen, and to use that variability to make inferences about possible factors involved in determining whether connections are made. This work is described in Chapter 7. The second line of study, described in Chapter 8, considered the general organisational structure of the synaptic circuitry, and how it might relate to function. The third, described in Chapter 9, used the data on the contact between neurons to investigate the physical organisation of nerve processes in the neuropil.

The date for all these investigations are purely anatomical; there are no physiological studies on the nerve ring neurons in either C. elegans or Ascaris. Some of the functional circuitry involved in the motion response to a touch stimulus in C. elegans has been deduced by a combination of laser ablation experiments and the detailed anatomy (Chalfie et al., 1986). However practically all of the discussion concering possible function of parts of the ring circuitry has to be based on the electron microscope anatomical data showing sensory endings, synapses and gap junctions, and motor output onto muscle. As will be shown in Chapter 8, there are some fairly broad statements that can be made about the organisation of connections at the level of the whole nervous sytem, or groups of neuronal classes, but caution must be exercised in interpreting plausible connectivity patterns in any detail. In particular no attempt is made to predict the inhibitory or excitatory nature of particular synaptic connections, or to stimulate, even conceptually, any piece of circuitry.

The type of study undertaken here is novel because the data available are unique in their completeness at such a fine level of detail. There have been many studies of circuitry at the physiological level in other animals (research on a number of well defined invertebrate systems is reviewed in Selverston, 1985) and it is often possible to dye fill the neurons from which recordings have been made to determine their anatomy at the light microscope level. However the overall distribution of connectivity between all the different identified neurons in even a part of a central nervous system has not previously been analysed at an electron microscopic level, owing largely to the much greater complexity of other animals' nervous tissue. Perhaps the system about which most is known is the vertebrate retina, which has been studied in detail at both a physiological and electron microscope level (Dowling and Boycott, 1966, McGuire et al., 1986, reviewed in Sterling, 1983). Around 50 types of cell falling into a few basic classes have been identified, and much is known about typical connections between these cell types. However particular cells and situations are not reproducible and electron microscope studies have necessarily concentrated on the properties of single cells (McGuire et al., 1986).

6.1 Methods

Data from two electron microscope reconstructions, the H series and the U series of White et al. (1986), were used to construct a computer database. The part of the animal that is represented in the database is the whole of the central processing region, or nerve ring, which consists of a ring of neuropil around the pharynx in the head of the animal that contains about 175 nerve fibres and the bast majority of the synapses in the entire nervous system. A general description of the C. elegans nervous system can be found in Chapter 1. Chapter 2 introduces the nomenclature of C. elegans neurons. The database contains the following information about the nerve processes in the ring: for each pair of neurons it stores the number of gap junctions between them and the number of chemical synapses in each direction. In addition, for the H series, there is a measure of the adjacency or degree of mutual contact between each pair of processes. This adjacency was obtained by looking at every 5^{th} micrograph in the reconstruction series and counting the number of these pictures on which the given pair of neurons were in contact.

The word synapse is reserved for chemical synapses in this discussion; electrical connections are referred to as gap junctions because they are identified as such from the electron micrographs. White et al. (1986) presents the criteria used in identifying synapses and gap junctions in C. elegans electron microscope reconstructions. Synapses are made en passant between adjacent processes. Although synaptic boutons are not seen, synapses can be recognised in electron micrographs by the presence of presynaptic density and the accumulation of vesicles. The chemical synapse count in the database combines data from monadic and dyadic synapses. In dyadic synapses, which are seen frequently in C. elegans, there are two postsynaptic partners. It excludes cases where the only connection seen between two cells is half a dyadic synapse, since such observations have been seen to be unreliable.

In general throughout the presentation and discussion of results a distinction is made between a connection between two neurons, and a synapse between them. There is a connection if there are one or more synapses. All the results that do not concern comparison between the two different animals represented in the H and U series were

obtained with data from the H series alone, because the adjacency information, which is only available for that animal, is often an important factor in the analysis. For a general H series neuron, A, I will refer to its contralateral homologue as A', and to the corresponding in the U series as A^u.

The database program is written in C and implemented on a VAX-8600 minicomputer. The main data is stored in a large array in virtual memory, together with a set of referencing arrays that allow easy access and cross comparison. All the analysis softwre is contained in one program that is modular in design and uses a free format command input system developed previously (Durbin et al., 1986). An analysis requiring a new algorithm is implemented by writing a new subprogram and entering it as an option in the command tree.

This investigation relies on much previous hard work by Nichol Thomson, Eileen Southgate, and John White in performing the original reconstructions, and crosschecking all the data. I would also like to thank Barbara Cross and Mabel Eggo for assisting in typing some of the data into the computer.

Table 7.1 Distributions of connections

	All	Ranges of Adjacency of A to B 1–2	3–5	6–10	>10
a) Chem A->B given A adjacent to B	.09 (8778) [4.2]	.02 (3374) [1.8]	.06 (1832) [2.6]	.11 (1497) [3.6]	.23 (2075) [5.1]
b) Gap junction A–B given A adjacent to B	.07 (4350) [1.3]	.04 (1677) [1.0]	.08 (915) [1.2]	.07 (738) [1.4]	.09 (1020) [1.7]
c) Chem B->A given chem A->B	.14 (819) [4.7]	.00 (56)	.07 (112) [3.0]	.11 (169) [3.2]	.19 (482) [5.2]
d) Gap junction A–B given chem A->B	.06 (819) [1.5]	.02 (56) [1.0]	.06 (112) [1.3]	.05 (169) [1.6]	.07 (482) [1.5]
e) Chem A->B given 1 synapse A'->B'	.55 (84) [1.7]	.65 (20) [1.2]	.56 (18) [2.0]	.64 (14) [1.9]	.61 (23) [1.7]
f) Chem A->B given >2 synapses A'->B'	.95 (523) [5.3]	.79 (14) [2.9]	.96 (53) [3.2]	.98 (100) [4.3]	.97 (351) [6.0]
g) Chem A -> B given A' not adj to B' but A adjacent to B	.01 (1886) [2.2]	.01 (1274) [1.4]	.02 (366) [1.9]	.02 (176) [3.3]	.04 (70) [4.3]

On the left is indicated the type of connection being considered and the range of cell pairs A,B over which to calculate the frequency of the connection being made. Each entry in the table has three figures: the first is a frequency, the second in parentheses is the number of cell pairs over which that frequency was calculated, and the third in brackets is the average number of synapses (gap junctions) made in those cases where a connection is made. The first column gives the overall figures, while columns 2 to 5 break down these numbers according to the adjacency of A to B. A' is the symmetrical homologue of A. Since gap junctions are asymmetrical, unordered rather than ordered pairs A, B are considered for row (b). Also adjacencies to muscle are not considered for row (b) because gap junctions are not made through the basement membrane.