

## CHAPTER 8 The Logical Organisation of the Circuitry

As well as looking at typical local interactions between neurons in the nerve ring the completeness of the information in the database allows us to investigate the large scale structure of the neural circuitry. We can ask such questions as: what is the processing depth from sensory input to motor output, i.e. how many intermediary neurons are there?, and to what extent is the circuitry unidirectional, progressing linearly from input to output? It is unlikely to be completely directional; one expects to find a certain amount of feedback, both in control-type circuits, in analogy to electrical engineering, where feedback can be used to adjust gain and bias to optimise the response to stimuli, and in rhythmic pattern generators if they exist. In fact the questions of processing depth and directionality are related, since it is necessary to have ordered the circuit diagram before being able to count the number of intermediary neurons between sensory input at the top and motor output at the bottom. I shall therefore consider first the extent to which the circuit diagram can be directionally ordered.

### 8.1 Directionality

Let us consider the network of chemical synapses and ignore gap junctions for the time being since, although they can be rectifying and directional, one can not detect any directionality from electron micrographs. The aim is to vertically order the neurons so that as many as possible of the synapses point downwards, with their presynaptic neuron above the postsynaptic neuron in the ordering. This was done with a sorting algorithm that is described in the appendix.

In fact the operation was carried out with combined data for neuronal classes, rather than with the individual cells, since all the members of the same class should occupy the same functional position in the circuitry. Since the earlier analysis suggested that there was a core set of connections that were almost always present, to which were added a number of sporadic connections consisting of only one or two synapses, orderings were obtained for two sets of data, one made by adding all the synapses that were seen in at least three out of the four possible places (left and right sides in both H and U series). In the latter case the few synapses between two unpaired cells were only counted if they appeared in both the H and the U series. This more stringent synapse set is the same as the set of consistent synapses discussed in Chapter 7. For ease of comparison the summed data were divided by two and the stringent data multiplied by two so that both sets gave numbers in terms of synapses per animal.

The results suggest that on a large scale the circuitry is very directional, and that almost all the chemical synaptic feedback that does take place is in the form of reciprocal synapses. The more stringent set of data could be arranged so that more than 95% of the connections pointed downward, leaving only 140 out of 2890 (4.8%) pointing upward (figure 8.1). Of these, 116 were involved in reciprocal connections, which have to contribute upward synapses because they have synapses in both directions. This leaves only 24 persistently seen synapses that are involved in upward connections, 12 per side. These 12 synapses are distributed over 11 connections, so there is no case in the whole nerve ring of strong, consistently seen, indirect synaptic feedback. However it appears that direct feedback using reciprocal connections is an important feature of the circuitry,

**Figure 8.1**

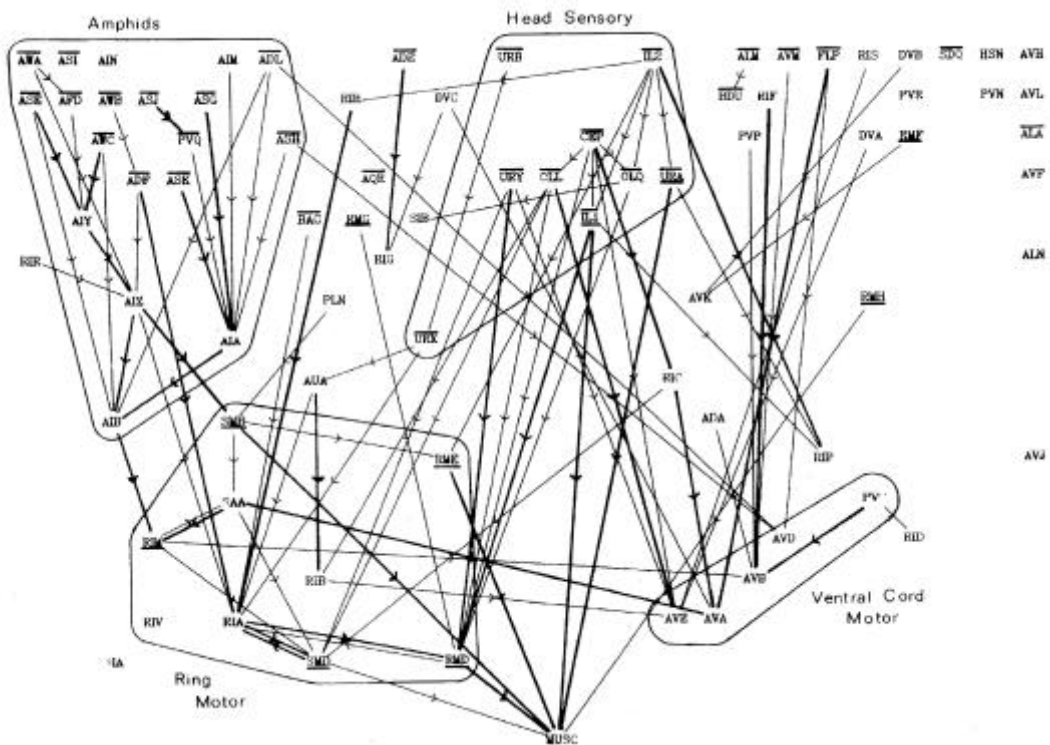
All the neuronal classes in the nerve ring arranged in the optimal vertical ordering to minimise the number of upward chemical synapses. The next three pages contain 8.1 (a), (b) and (c). Each has the classes arranged in the same positions, but shows different sets of connections.

(a) shows all the chemical synaptic connections in the stringent set with 5 or more synapses with fine lines, and those with 10 or more synapses with heavy lines. Sensory neurons are indicated by a bar above the cell name, and motor neurons by a bar underneath the class name. Four subjective groupings of related neurons are encircled. These are discussed further in the text.

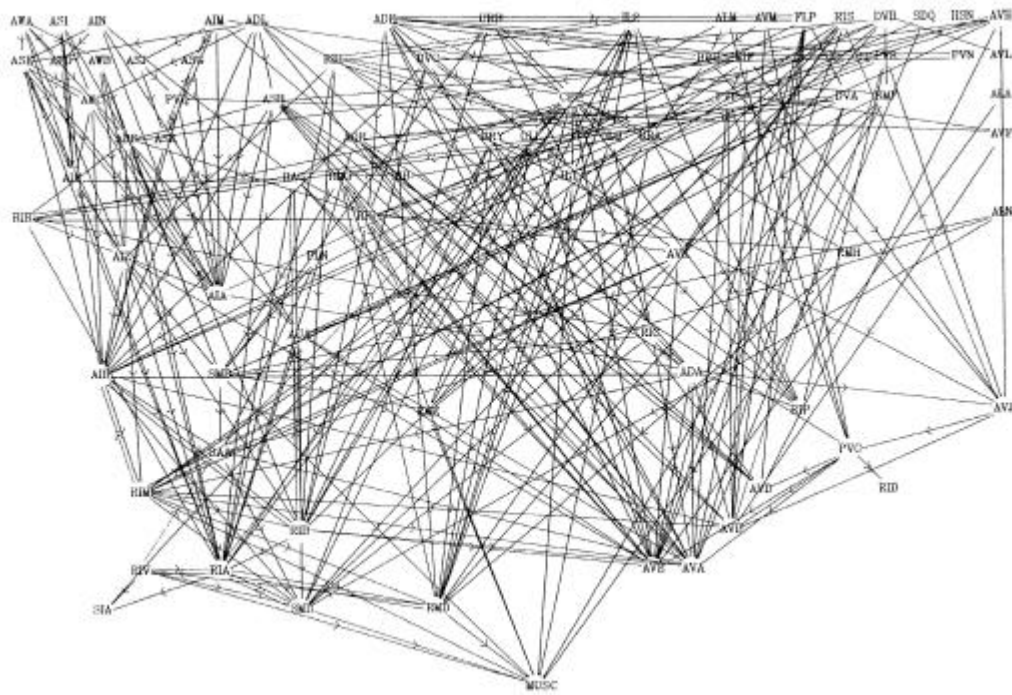
(b) shows all the chemical synaptic connections in the stringent set.

(c) shows all the gap junctional connections in the stringent set.

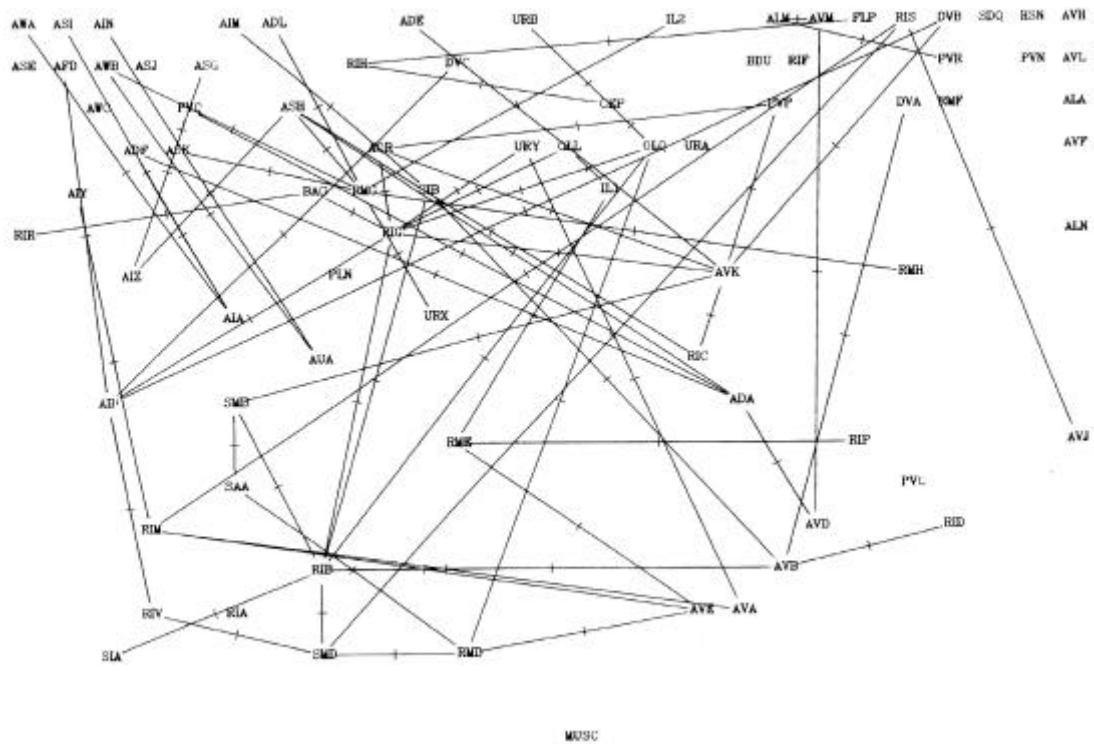
(a)



(b)



(c)



since reciprocal connections contained 378 downward synapses as well as the 132 reverse synapses already mentioned, therefore accounting for 20% (494/2890) of the persistently observed synapses.

It was not possible to order the averaged data so clearly. In that case there were 3898 synapses per animal, of which 386 (9.9%) pointed upwards in the arrangement that had been found to be best for the stringent data. When the ordering was specifically optimised for the averaged data the number of upward synapses was only reduced to 328 (8.4%), nearly twice the percentage that was seen with the stringent data. These observations support the suggestion made in Chapter 7 that at least a proportion of the additional synapses are different in nature from the consistently observed synapses, having perhaps a more random distribution.

## 8.2 The organisation of the feedback

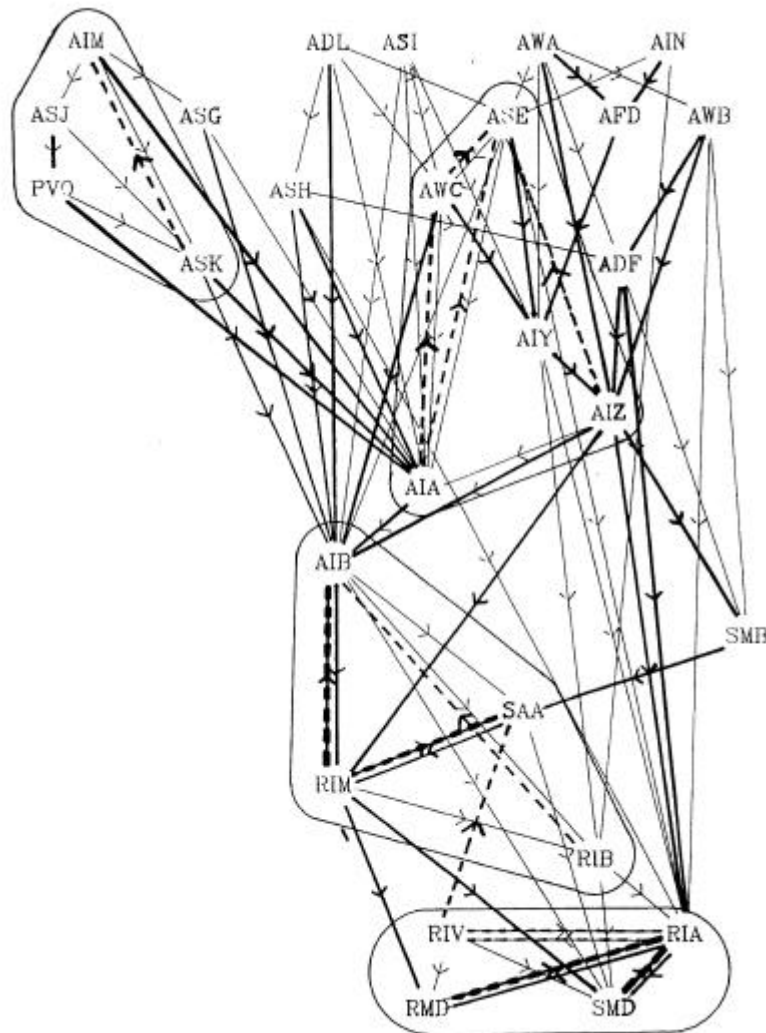
Several groups of neurons have been indicated in figure 8.1 on subjective grounds because they seem to be involved in a particular part of the circuitry. Two sensory and two motor groupings have been defined. One contains neurons associated with the amphid sensilla, which are multiply innervated sensilla on either side at the front of the head, probably concerned mainly with chemo- and osmo-detection (Ward et al., 1975). As well as sensory receptor neurons there are a number of interneuron classes that appear to be predominantly concerned with processing information from these receptors. The other sensory grouping contains a second set of neurons with sensory receptors at the front of the head, distinct from the amphids. These all have their cell bodies in front of the nerve ring, and tend to form direct connections to motor circuitry (or directly to muscle in the cases of IL1, URA). There are additional sensory neurons, such as the touch neurons (ALM, AVM, Chalfie et al., 1985) that are not assigned to either of these classes. The two motor circuitry groupings contain the interneurons that innervate ventral cord motor neurons on the one hand, and some of the circuitry that controls neuromuscular activity onto head muscles from the nerve ring on the other hand.

Figure 8.2 shows the amphid circuitry and part of the ring motor circuitry in greater detail, including all the synaptic connections. Although there are several examples of reciprocal feedback they do not interlink the whole circuitry. The groups of neuronal classes that are connected so that each neuron could potentially influence all the others in group are outlined in figure 8.2 (the connection between RIV and SAA breaks this rule, but only contains one consistent synapse). Let us call such groups “modules”. Neurons higher in the directional ordering are isolated from modules that lie below them. Thus all the amphid circuitry apart from AIB is isolated from motor activity. In this sense even the reciprocal feedback that is seen only has a limited effect on the overall directionality of the circuitry.

One important consideration that might invalidate the suggestion of a highly directed flow is that we have ignored gap junctions. Figure 8.1 © shows the distribution of all the gap junctional connections in the nervous system. Some of these are within groupings identified previously. There are also some classes that make a lot of gap junctions, but very few chemical synapses (e.g. RIG, RMG, AVK). It is of course not known whether or not any of these gap junctions are rectifying and thus possibly directional themselves.

**Figure 8.2**

A more detailed view of a particular part of the synaptic circuitry. This shows the amphid neurons and their main paths of connectivity to the ring motor circuitry. All connections present in the stringent data set are shown; connections with 5 or more synapses have heavier lines. All upward synaptic connections are dashed, again with the dashes being heavier if there are 5 or more upward synapses to the connection. Groups of classes that are potentially connected by feedback are encircled. The only exception is that the upward connection between RIV and SAA was not used to link the AIB, SAA, RIM, RIB module to the RIV, RIA, RMD, SMD module, since it only contains one synapse.



However in at least one case a gap junctional connection has been shown to be functionally important in one direction, by removing the cell involved with a focussed laser beam (Chalfie et al., 1985).

### **8.3 The processing depth**

The ordering of the circuitry allows us to count the number of synapses between sensory input and motor output. The method used calculates a hypothetical flow of information through the synaptic connections down through the nervous system (see Appendix for details). It necessarily treats all the observed synapses as having equal functional effect and so the resulting estimates are probably physiologically very inaccurate. However they provide a reasonable basis for a broad comparison of the flow of information from different sensory modalities.

There are on average 3.5 chemical synapses between sensory neurons and the head muscles, basing the calculation on the more stringent data and the ordering derived for in section 8.1. As might be expected, there is a lot of variation in the number of intermediary connections. The actual number can vary from a minimum of one in the case of neuromuscular output from the sensory motor neurons IL1 and URA to a maximum of 16 for a particular sequence of synaptic connections starting from the amphid receptor ASI. However this upper band is somewhat misleading; the average distance from muscle of any given neuronal class is never greater than 5.9 synapses (for ASJ). Nevertheless there is clear systematic variation dependent on the type of sensory receptor being considered. Input from the amphid receptors takes the longest time on average to reach muscle (4.4 synapses), reflecting the extra stage of amphid specific interneuron processing shown in figure 8.2. The other head sensory input is rapid, taking only 2.2 synapses on average, and the average value for the remaining sensory neuronal classes is 3.2 synapses.

The same method that calculates depth of processing also generates an estimate of the proportion of “sensory influence” that reaches different final types of output (see the appendix for details). There are three major discernible targets for output from the nerve ring: the head musculature via direct neuromuscular synapses, the ventral cord motor circuitry interneurons, and the RIP class of neurons, which provide the sole connection to the pharyngeal nervous system, which is thought to pump constitutively unless repressed by RIP. In general the number of synapses connecting sensory neurons to the ventral cord circuitry or RIP is about one less than the number needed to reach the head musculature, possibly because there is an extra layer of motor pattern generating circuitry (considered further in the discussion). The “fast” head sensory neurons have proportionally more output onto the head muscles, and provide the majority of output onto RIP. It seems reasonable to suggest that they carry out much of the short range sensing involved in moving the head to feed and searching out a path round obstacles for the body to follow when moving. The amphids generate a balanced number of connections to both the head muscles and ventral cord interneurons, and have no link with RIP. The other sensory neurons provide comparatively more input onto the ventral cord interneurons. This is perhaps reasonable because many of their sensory endings are in various other parts of the body, rather than being at the tip of the head.

## 8.4 Discussion

The strongest general feature of the *C. elegans* nerve ring circuitry is its extremely high directionality. The neuronal classes can be ordered in such a way that less than 5% of the synapses point backward. This organisation is clearly very different from that of many higher organisms. For example in the mammalian cortex every projection from one area of cortex to another seems to be matched by a reverse projection (van Essen, 1979). However there are also structures that are only a few synapses deep that seem to be fairly directional, such as the vertebrate retina (Sterling, 1983).

Almost all the synapses that do point backward are members of reciprocal connections, which were shown in Chapter 7 to be almost as frequent as would be expected on the basis of the distribution of directed synapses. There are almost no persistently seen synapses involved in indirect feedback. In addition the feedback that is seen appears to be largely restricted to affecting small groups of neuronal classes, or modules. In the case of the circuitry shown in figure 8.2 it is possible to suggest functions for the observed modules in terms of the different stages of processing needed. However in discussing the possible function of elements of the circuitry one should bear in mind that all the data is anatomical; there is no functional or physiological data.

The outputs of the amphid receptors shown in figure 8.2 appear to be processed fairly independently from the rest of the sensory input. Their output is eventually concentrated onto the interneurons AIZ, AIA and AIB. AIZ and AIB synapse onto RIB, RIM and RIA, the major interneurons that appear to be involved in controlling the head musculature. These RIX neurons then synapse onto the RMD and SMD which are two of the main head motor neuron classes, each containing 6 neurons interconnected in a complex fashion (see figure 7.1 for the RMD connections).

Feedback in the AIM, ASJ, PVQ, ASK module could best be used to modulate their own receptor output. The ASE, AWC, AIY, AIZ, AIA group combines the output of a set of, mainly chemosensory, amphid receptors. The AIB, SAA, RIB, RIM group receives input from other modalities as well as the processes chemosensory data from the neurons seen in figure 8.2, and has output to both the head and body motor circuitry (RIM is itself a head motor neuron). It may make the basic decision on body movement, which the RIA, RMD, SMD, RIV module then executes. The feedback in this final motor output module, both between the interneurons RIA and RIV and the motor neurons, and within the motor neuron classes themselves, may be involved in the generation of oscillatory head movements that then propagates backward as waves. In several respects the connections seen here resemble those seen in central pattern generators (oscillation generators) in other invertebrate systems (for several examples see Model neural networks and behaviour, ed Selverston, 1985). In these other systems reciprocal connections between neurons also appear to play an important part, and it is often seen that neurons are multifunctional, for instance with both motor neurons (Miller and Selverston, 1985) or command interneurons (Getting and Degin, 1985) taking part in the pattern generating circuitry.

One of the proposed modules, the one containing AIB, SAA, RIB and RIM, contains a mixture of neurons that subjectively appear to be part of the amphid circuitry (AIB), and the motor control circuitry (RIB and RIM). Much of the amphid receptor circuitry shown at the top of figure 8.2 is concentrated onto AIA and AIB. It appears that one of these two neurons may be involved in feedback within the amphid circuitry, possibly to “tune” its output, while the other is involved in feedback that may determine the relative importance of the amphid output to the movement of the animal. Another suggestion to explain the organisation of AIA and AIB, made initially by J G White, is that they receive joint input and that AIA inhibits AIB, thus causing the combination to act as a differentiating circuit which could be used to detect gradients during side to side head movement.

The principle of a highly directed network containing small processing modules provides for only a very limited use of circuit feedback. This use is to improve the versatility of small scale processing units made out of a very few neurons. One reason that C. elegans does not have longer loop neural feedback may be that, having only a shallow overall processing depth, it uses sensory feedback to perform this role. The advantage of this is that it measures the actual, rather than the intended, outcome of motor activity. Indeed it is thought that proprioceptive feedback may be important in the propagation of locomotory waves down the body. However, even if more complicated nervous systems do have more sophisticated circuit structures, it may still be useful to effectively isolate functional units as much as possible from the internal working of other parts of the nervous system, even if those working are somewhat relevant. If this is done then additional operations can be added easily at any stage without perturbing the rest of the system.