CHAPTER 9 Process Placement in the Nerve Ring

In order for nerve cells to make connections with each other they must be in physical contact. Therefore the physical arrangement of the neuropil is an important part of its design. The C. elegans nerve ring is essentially a large parallel bundle of fibres bent around the pharynx. A typical transverse section through the C. elegans nerve ring shows an apparently homogeneous group of process outlines on each side. Bilaterally symmetric processes occupy approximately symmetrical positions within the bundle, but there is local disorder on the scale of a few process diameters so that in general it is impossible to identify processes on the basis of their positions, even over fairly long stretches of reconstruction (although characteristic diagnostic properties of certain neurons do make them identifiable).

It is presumably unnecessary to specify the exact relative positions of all the processes, but important for there to be reasonably tight control over process position because processes do not branch, so the only way to make contact is to lie next to each other in the bundle. How is position controlled? There are essentially two different possible sources of order, either from contact with other processes or from an external source of information, such as a gradient (e.g. Bonhoeffer and Huf, 1982). The most likely form of contact mediated information would be a mutual adhesivity that kept two or more neurons together and therefore simplified the task of specifying their positions. Such selective fasciculation has been proposed as important in laying down other invertebrate nervous systems during development (see chapter 1 for review) and there are indications that it is important in process outgrowth in C. elegans (PVP/PVQ behaviour, discussed in chapter 5).

Figure 9.1

The distribution of adjacency in the database. The crosses connected by the heavy line indicate the number of cell pairs in the database with a particular adjacency. The fine lines are the corresponding numbers from the outcome of the random mixing stochastic model, using three different values of the parameter, p, and averaged over 10 runs to get smooth results. The best overall fit to the distribution is given by p – 0.08. This leaves two regions of misfit, X and Y, which are discussed in the text. Note that the vertical axis in this graph is nonlinear.

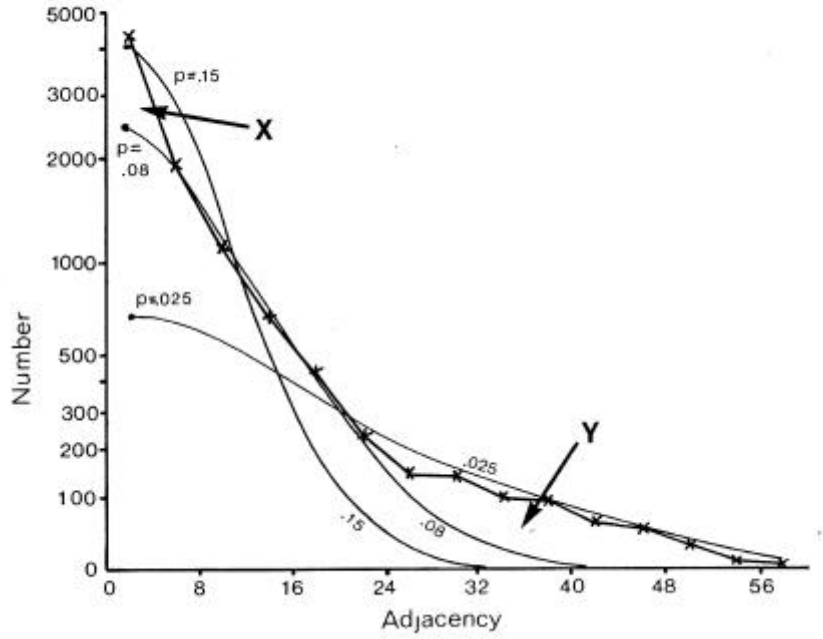
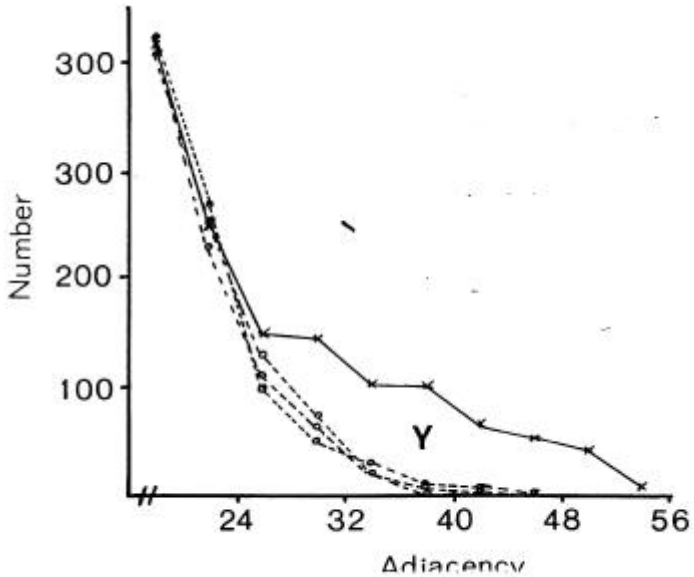


Figure 9.2

An expansion of the region Y with three separate simulations of the random mixing model with p = 0.08. The gap between the true data and the model data is clearly significant. Since the vertical scale is linear in this case the area of region Y corresponds to the number of "extra" high adjacency contacts. This predicts around 400 extra persistent contacts, or 2.3 per neuron (178 neurons).



9.1 Specific persistent contacts

If selective fasciculation were important in organising the nerve ring, and the adhesive forces remained after early development, then one would expect to find pairs of processes with persistent contacts. These should be detectable in the database as pairs of neurons with exceptionally high adjacencies. If one looks at the distribution of all the adjacencies in the database it would be the sum of two components, a random mixing component, and a high adjacency component due to persistent contacts. The distribution of adjacencies is shown in figure 9.1. There is a clear change in slope at the curve at an adjacency of around 30.

In order to assess the significance of this shoulder, and to estimate its size, and hence the average number of persistent contacts made by a neuron, I produced a stochastic model of a collection of randomly mixing parallel fibres. This operates by recording the positions of the fibres in a hexagonal grid representing a slice through the process tract, and then moving to the next slice and allowing neighbouring processes to exchange positions with a certain probability. The adjacency of a pair of fibres is then taken to be the number of slices in which they are neighbours. The total number of slices was taken to be 75 to make the total adjacency (sum of all its adjacencies) of each fibre the same as the average total adjacency for the processes in the database. The second parameter, the probability of a process switching, p, was chosen so as to best match the model's distribution of adjacencies to that of the database. Thje best fit is given by $p = 0.08$.

There are two regions of misfit that cannot be eliminated, denoted by X and Y. Region X is due to a very large number of additional contacts of very short duration, which probably arise from processes crossing at an angle in the nerve ring. Such events are known to occur in the nerve ring but are not considered by the computer model. Region Y is the shoulder that includes longer contacts than predicted by the random model. Figure 9.2 shows an expansion of the shoulder region of the database distribution together with data from 3 simulations of the model. The shoulder is clearly significant beyond the variation in the simulations due to randomness in the model. However it is fit quite well by the random model with a low switching probability ($p= 0.025$, figure 9.1), which is not surprising, because low switching probabilities for a subset of process pairs are an approximation to specific adhesion between the processes, which is the sort of feature that we predicted might give rise to a shoulder beforehand.

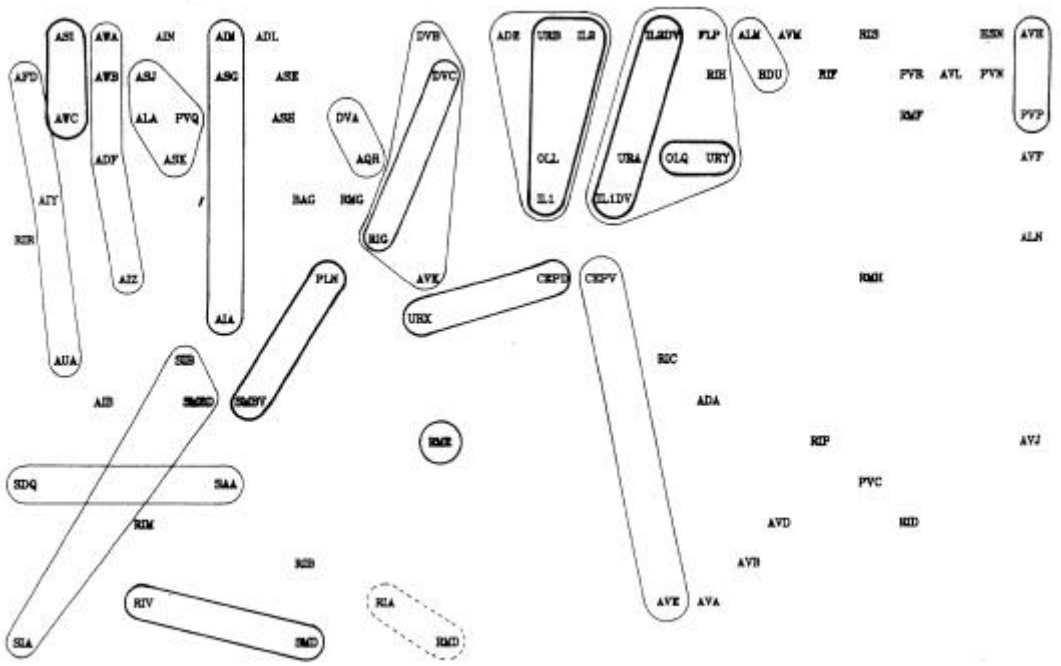
It is possible to estimate the number of significantly persistent contacts from the graph in figure 9.2 as about 400, and thus to arrive at a figure of on average 2.3 persistent specific contacts per neuron. This is very crude – there may be many specific contacts of shorter length – but it gives an indication that there may be fascicles or bundles of mutually adhesive processes in the C. elegans nerve ring. However if such bundles are common then they cannot contain very many processes, because the average number for long bundles must be only 3 or 4. A second test suggests the same result. The average number of contacts made by a neuron is 52.1, most of which are short. If we compare the adjacencies of all the contacts with that of the longest contact then we see that on average 12.6 are longer then 25% of the maximum, only 4.8 are longer than 50% of the maximum, but 2.4 are longer than 75$

of the maximum. Thus it seems that a very small number of contacts are comparatively consistent.

Figure 9.3

Figure 9.3

Clusters of neuronal classes obtained by hierarchical clustering of the adjacency data. There are three thicknesses of line, corresponding to an association measure of 25 or more for the thickest, 15 or more for the intermediate one, and 8 or more for the thinnest one. All these clusters were seen on both sides of the nervous system. In some cases the dorsal and ventral members of the same class ended up reproducibly in different clusters (e.g. CEP, IL1, SMB). The positions of classes were moved as little as possible from those in figure 8.1, in order to show the relationship between possible bundle assignments and circuitry. The RME class is ringed because the four RME neurons form a tight bundle with each other. The RIA and RMD classes are linked in a dashed cluster because there are a number of RIA/RMD pairs that have associations just under 8.



9.2 Identified bundles

It is hard to tell with individual process pairs whether their high adjacency in accidental or not, but if several processes combined in a bundle it should be objectively deducible from the adjacency information in the database. A bundle will consist of a group of processes with the property that all pairs in the group are highly adjacent, but no other process is very adjacent to the group as a whole.

The number of possible groups goes up exponentially with the size of the group, so it is not possible to try every one even with small groups. However there is a branch of multivariate statistics called cluster analysis that is specifically designed to handle this type of problem, and a variant of a standard algorithm from this theory was used to extract clusters of highly mutually adjacent processes that are likely candidates for bundles. The details of this algorithm are given in the appendix, but the final result is a hierarchial set of nested clusters with a measure of the degree of association at each level, which corresponds to an average internal adjacency. Any real clusters, such as the proposed bundles, should stand out as having a high association measure at the level of the group, but not combine well with an external process or group at the next level down. Figure 9.3 shows the bundles detected by the algorithm in the C. elegans database at associations measure cutoffs of 25, 15 and 8. In the case of contralateral homologues, either bundles were seen on both sides, or the same bundle included both homologues.

In order to provide an objective significance criterion for the association measure of a cluster, I used the same algorithm on data from a simulation of the random mixing model described in the last section (with $p = 0.08$). The maximum association measure obtained was 12.75 and less than 10% of the values were greater than 7.5. Thus according to this criterion all the bundles shown in figure 9.3 at an associational level of 15 are likely to be significant, as are most of those at a level of 8, especially when they occur on both sides of the animal.

Since the clustering method is hierarchial and continues to make larger and larger clusters it does generate further amalgamations of the bundles seen in figure 9.3. Although the association measure for such bundles falls below our significance test level there is evidence that some of them are real, primarily because the same groupings are seen for homologous bundles on the two different sides of the same animal. The fact that they have a low association measure implies that they are not true completely mixing bundles, but they may be either super-bundles – bundles of bundles – or cases where processes are shared by several bundles. The suggestion that particular processes might belong to more than one bundle is taken further in the discussion section.

9.3 Discussion

There is some evidence in the database for the presence of reproducible persistent contacts between nerve fibres, both between pairs of neurons and between groups of three or more processes that run together round the ring as sub-bundles within the complete process tract. The largest grouping of neurons which all had fairly high adjacency to each other contained seven cell types (figure 9.3) but most of the likely

bundles generated by cluster analysis of the neighbourhood information contained only two or three cell types. The average number of high adjacency contacts per neuron was also small (2.3).

The analysis presented here suffers from its reliance on identifying specific contacts by unusually high adjacencies. It would therefore miss any important short term contacts, and would also be confused by processes that for half their length are in one part of the neuropil, and for the other half in another part. There is a clear example of such behaviour in the case of the interneuron AIB, which runs near AIA in the proximal part of its trajectory, and near RIB in the distal part (White, 1983). This is consistent with AIB's role as the major linking interneuron between the amphid receptor circuitry and the motor control circuitry (figure 8.2). Such switching of bundles could be used by other processes that carry information between sufficiently different groups of processes.

Another example of the possible presence of sub-bundles in the nervous system is provided by the motor neuron processes in the ventral nerve cord. The VA and VB classes of motor neuron are both bipolar, with an axonal process that produces neuromuscular output for part of its length (the other part neither makes nor receives connections) and also receives some input, and a dendritic process that is purely postsynaptic. All the dendrites run together in one place, under the main motor neurons, while all the axons run in a group against the basement membrane. Although these two groups of processes are adjacent they rarely mix. In addition there are a number of places where a motor neuron commissure cuts across the entire nerve cord; when this happened the commissure usually runs between the dendritic and axonal groups, separating one group from the other, but splitting neither (7/13 cases; in 5/13 a VB dendrite is on the wrong side – in only one case is the axonal bundle split). In this case a general adhesion between like processes may be useful in keeping all the dendrites near their source of inervation, and keeping the axons near the basement membrane, where neuromuscular junctions are made.

There is a strong relationship between the proposed groupings of the neurons into bundles and the circuitry. Figure 9.3 has been organised so as to show the extent to which the bundles are formed from neurons that are near in the processing diagram in figure 8.1, which was obtained purely from connectivity data. However it is by no means true that all persistent pairwise contacts are between neurons that are connected, either by chemical synapses, or by gap junctions (e.g. CEP and URX, or the ventral cord motor neuron bundles). In some cases bundles correspond to parts of the processing modules defined previously on the basis of internal feedback, but they also often contain vertical groupings of neuronal classes from the directional ordering, sometimes with elements from two modules, one of which feeds into the other. Such organisation is to be expected if the main criterion for process placement is to maximise the adjacency of symaptic partners, since the main flow of information is down through the network, across the modules.

The observation that there are a small number of persistent contacts suggests that specific fasciculation mechanisms are significant in the C. elegans nerve ring, and appears to rule out the specification of process position by a general mechanism that acts equivalently on all cells. This is perhaps not surprising in an organism with such a small number of cells, almost all of which are distinct, forming different sets of

specific connections. The data certainly do not allow the prediction of a set of hierarchical forces that could determine position in the nerve ring. There is also the problem present in all the analysis of the database of trying to investigate the underlying mechanisms involved in building a structure (the nerve ring) by looking at the finished product. However, taken together with the evidence for the role of specific fasciculation in embryonic neural outgrowth presented in the first part of this thesis, there are strong grounds for believing that the organisation of the nerve ring may make use of small specific bundles to correctly position processes so that synaptic connections can be made.