CHAPTER 7 Synaptic Distributions and Reproducibility

7.1 Synaptic distributions

Altogether there are 3462 chemical synapses and 754 gap junctions between the 183 neurons in the H series database. The two different types of connection, gap junctions and chemical synapses, are distributed rather differently in the nervous system, as can be seen from table 7.1. Table 7.1 rows a) and b) show the proportion of pairs of adjacent processes that are connected by chemical synapses or by gap junctions.

Although there are 4.5 times as many chemical synapses as gap junctions, the proportion of pairs of adjacent cells connected by chemical synapses is similar to that connected by gap junctions (9% and 7% respectively). This is because a pair of cells is often connected by several chemical synapses, but rarely by more than one or two gap junctions. In other organisms there may be hundreds or thousands of synapses between a given pair of neurons (e.g. a cerebellar basket cell make many synapses on a Purkinje cell). The small number of synapses in C. elegans is probably due to the extremely small size of the entire nervous system.

7.2 Adjacency and synapse formation

It is immediately obvious from table 7.1 (rows a, b) that both the probability of being connected by a chemical synapse, and the number of synapses actually formed, are highly correlated with adjacency. There is a 14 fold increase in the proportion of connections formed when pairs of cells that have a high adjacency are compared with pairs that touch only briefly. This correlation is far less marked for gap junctions, for which the corresponding increase is only a factor of two. While the bast majority of chemical synapses are between pairs of cells with adjacency greater than 10, nearly half the gap junctions are between cells with adjacency less than or equal to 5.

It might be thought that the increase in the average number of synapses formed with higher adjacencies provides evidence that synapse formation is dependant on the area of contact. However, for any given pair of neurons it is possible to show that the number of synapses made between them does not substantially change with their adjacency. This proposal can be tested by considering pairs A, B which have contralateral homologues A', B'. In general A', B' will have a different adjacency from that of A, B, and they will often also form a different number of synapses. If we assume that the symaptic formation mechanisms for symmetrical pairs on the two sides are equivalent then we can test statistically whether the number of synapses formed tends to vary proportionally with the adjacency, or remains independent of adjacency. The details of the test are given in the appendix, but the results are as follows. There were 391 pairs of processes which formed synapses on both sides and showed different adjacencies on the two sides, and the value of the test statistic was 7103 with an expected standard error of around 1330. If the number of synapses varied proportionally with adjacency then the value should ideally be 0, while if it was independent the value should be around 7655. It is clearly many standard error values from 0, and only about half a standard error distance from 7655. Therefore there is strong evidence against a proportional system, and the data are consistent with the number of synapses between a given pair of neurons being determined independently of the adjacency. The slightly lower value of the calculated test

statistic than that expected by a wholly independent model can be explained by the fact that there must be some effect at very low adjacencies. If the adjacency is 0 then clearly no synapses can be made, and when it is only 1 or 2 there are spatial limitations preventing a large number of synapses being formed.

7.3 Reciprocal synapses and joint chemical/electrical connections

It is important to consider the correlation between synapse formation and adjacency when looking for statistical interactions between the different types of synapse. For instance, the frequency of reciprocal chemical synapses is consistent with synapses being independently specified in each direction. Although the overall probability of a connection from neuron B to neuron A given one from A to B is high (14% as opposed to 9%, see table 7.1 row c), this is because most of the pairs with a synapse from A to B have high adjacency, and so are more likely than normal to have a reverse synapse from B to A. When the probability of a reverse synapse is shown separately for each adjacency range, as in table 7.1, it can be seen to be essentially the same, or possibly slightly lower, as that for an unconditional synapse.

The same approach can be used to consider whether there is any statistical interaction between chemical synapses and gap junctions. In this case there is a slight negative correlation, since the probability of a gap junction between a pair of cells that are linked by chemical synapses is reduced by 1 or 2 percent from the unconditional probability in each adjacency range (table 7.1 row d). This difference can be partly explained by the fact that a significant proportion (66/284) of gap junctional connections are between members of the same neuronal class, while it is rare for there to be intraclass chemical synapses (discussed below).

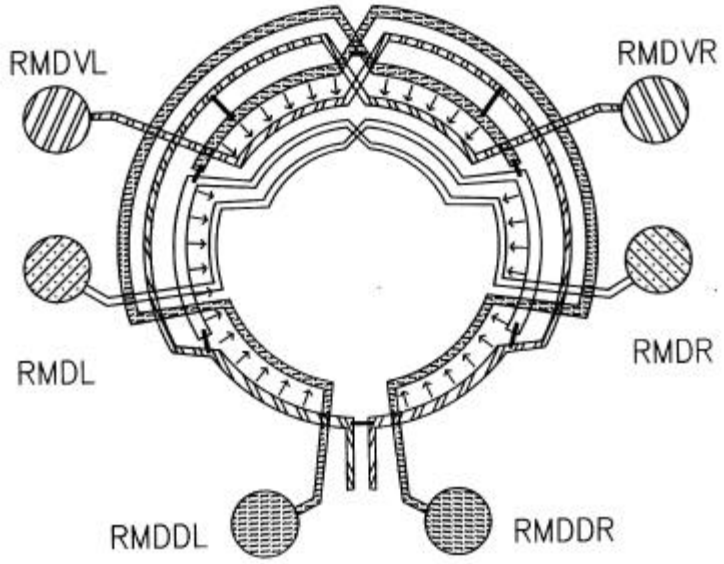
Therefore the presence of one type of connection between cells does not provide any indication of whether another type, either gap junctional or chemical in the opposite direction, will also be present. The only exception to this is when the cells are members of the same class, in which case if there are chemical synapses (only 5 examples) then they tend to be reciprocal (4/5), as would be expected. Together with the overall dependence of synapse formation on adjacency these results mean that there is a remarkably high probability of some form of connection between processes that have a high adjacency. If the adjacency is greater than 10 then the proportion of pairs forming either a gap junction or a chemical synapse one way or the other is 47% (478/1020).

7.4 Connections between members of the same neuronal class

Gap junctions are far more frequent than normal when both cells are members of the same neuronal class. The 302 neurons in C. elegans have been put into 118 different classes on the base of similar morphology and connectivity (White et al., 1986). Many of these classes, particularly those with processes in the nerve ring, consist of a pair of bilateral, symmetry related neurons. There are 61 such pairs in which the two cells make physical contact and 35 (57%) of these have gap junctions while only 5 (8%) have chemical synapses.

Figure 7.1

A schematic diagram showing the interconnections between the 6 members of the RMD class of neurons. The circle represents the nerve ring. At each radial position around the ring one of the RMD neurons has synaptic output (arrows), both onto muscle and onto other neurons, including the diametrically opposite RMD neuron, which "intercepts" the neuromuscular junctions (as with the DD process in figure 3.6). In general the RMD neurons are monopolar, with their proximal regions showing this intercepting behaviour, and their distal regions being synaptically active. Gap junctions (thin bars) link neighbouring neuromuscular regions but are not formed in general even where processes are close, sich as for instance where chemical synapses are made. Some variability was seen in this general pattern, since in the U series RMDL had output (both to muscle and other neurons) from the proximal as well as the distal part of its process).



It is common for there to be a gap junction where the ends of two processes from different members of the same neuronal class abut, such as when left and right symmetry related processes meet at the top of the nerve ring. This abuttal is interesting in itself, since it suggests that the processes might stop growing when they establish contact with the tips of their contralateral homologues. A similar phenomenon is seen in the ventral and dorsal nerve cords, where the processes from consecutive neurons of two classes of motor neuron (VD and DD) abut and make gap junctions but do not overlap; the other classes overlap (except AS), and sometimes make gap junctions. In the ring there are 24 pairs of cells whose processes end where they meet at the dorsal midline, and a gap junction is present in 22 of these cases. Of the 37 other classes of cells that have interclass cell contacts, but which do not abut, only 13 interclass gap junctions. None of the pairs that form internal chemical synapses abut, nor do any of interneurons that send adjacent processes back along the ventral cord; perhaps if they had inhibited each other's growth they could not have grown out together along the cord.

7.5 Reproducibility of connections

There are two sources of synaptic reproducibility that might be expected in the database. The first is from animal to animal, and the second is due to internal bilateral symmetry within one animal. Most neuronal classes that are associated with the nerve ring, and so represented in the database, consist of one or more bilateral pairs of homologous neurons. One problem with the comparison between animals is that the U series animal was an adult, while the H series animal was an L4 larva. Although almost all neural development in the nerve ring is embryonic the sex-specific circuitry concerned with egg laying is incomplete in the H series, and there may be other less obvious differences due to age.

The probability that a given pair of H series neurons A, B will be connected by chemical synapses if their contralateral homologues !' and B' are connected is 87%, while the probability that they will be connected if their U series homologues A^u and B^u are connected is only 75% (table 7.2 rows a, b). The corresponding figures for gap junctions are 89% and 79% (table 7.2 rows c, d). It therefore appears that gap junctional connections are slightly more reproducible than synaptic ones and that the two sides of the same animal are more similar than the two different animals. Part of the difference in chemical synaptic connectivity between the two animals is that there are more chemical synapse connections in the U series than the H series (916 as opposed to 819), and the extra connections are certain to be unmatched. However the difference between the animals is still probably significant because there are fewer U series gap junction connections than ones in the H series (260 against 284) but a lower proportion of U series gap junctions are matched in the H series than ones of the opposite side of the H series (above).

The difference between the "within animals" (left/right) comparison and the "between animals" (H/U) comparison can be further illustrated by considering the pairs that form connections in only two of the four possible places (each side of each animal). There are six ways that this can happen: both sides of the H series but not at all in the U series, both sides of the U series but not in the H series, and four different ways that there could be one synapse in the U series and one in the H series. If the similarity between animals were the same as that between sides of the same animal then the size

of all these classes should be the same: the number of pairs synapsing only in the H series, and the number synapsing only in the U series, should be a quarter of the number that synapse just once in the H series and once in the U series. In fact there are 23 pairs that synapse only in the H series, 51 only in the U series, and 64 that synapse once in each series. For gap junctions these numbers are 41, 22 and 14. In both cases there are more connections than expected that are U series specific or H series specific, indicating that there are significant differences between the two animals.

Similarly, a test can be performed to detect whether there is also a reproducible difference between the right and left sides of the nerve ring. If there was such a difference then one would expect that the connection between a pair of A^u,B^u in the U series would resemble more closely that between their exact equivalents in the H series, A,B, than that between the corresponding cells on the contralateral side, A' and B'. In fact the figures are 75% reproducibility to A,B, 74% to A'B' for chemical synapses, and 79% to A,B, 77% to A',B' for gap junctions. Therefore if there is consistent difference between the two sides it is very slight (1 or 2%). It would not be possible to identify the source of any consistent difference that may be reflected in this small change of reproducibility, because there is too much noise from the difference between the two animals.

7.6 Reproducibility depends on the number of synapses made, not on adjacency

Overall the observed levels of reproducibility suggest that there is an underlying regular pattern. In particular the two sides of the same animal appear to be sufficiently similar to allow us to assume that they were subject to the same synaptic specification procedure during development. This allows us to consider some aspects of the question of how neural connectivity is determined. By identifying the sources of inaccuracy of synaptic reproducibility it is possible to obtain information about, and thus to make suggestions about, the mechanisms for establishment of specific circuitry. This approach has already been used to indicate the independence of the number of synapses made between a pair of neurons from their adjacency. All the effects we will consider would not be altered significantly by one or two percent change due to possible slight genuine differences between the two sides.

Although it appeared at first sight that there was an unexpectedly high variation in the formation of chemical synapses, almost all the inconsistencies are due to the unreliability of weak connections, i.e. connections with only a small number of synapses. For pairs connected by three or more synapses (which is true for about two thirds of connected pairs) the probability that their homologues are connected is greater than 90%, whereas if there is only a single synapse then the probability that their homologues will be connected is only around a half. This difference exists both between and within animals (for exact numbers see table 7.2).

Clearly one cause of a mismatch in which a connection seen is present on one side but missing on the other might be that the neurons are not in contact in the case where the synapse is missing. However if they do touch each other then the dependence of reproducibility on the number of synapses made is practically independent of adjacency. If we consider A, B such that A' and B' are connected by only one

synapse then the probability of connection between A and B is around 60% in all adjacency ranges. At the other extreme if A' and B' are connected by three or more synapses then the probability that A and B are connected is 79% (11/14) if their adjacency is one or two, and more than 95% in any adjacency range greater than two (table 71 rows e, f). It is necessary to consider the data this way, because a direct comparison of reproducibility at different adjacencies would suggest that adjacency is an important factor (table 7.2 rows a, b, final columns). In fact this effect is mostly secondary, caused by the fact that connections with higher adjacency tend to have a larger number of synapses. If we combine this result with the earlier observation that the number of synapses made between a given pair of neurons is essentially independent of adjacency, then it appears that, except at very low adjacencies, the probability that two given processes will form a synapse is also practically independent of their adjacency. The overall correlation between synapse formation and adjacency (Table 1, row a) therefore implies that the physical organisation of processes is such that neurons that repoducibly synapse tend to have higher adjacencies. This is considered further in the discussion.

The picture that emerges is that pairs of neurons are programmed to make an approximately predetermined number of synapses, and that the connection will be more reliable if this number is greater than one or two. There is no significant similar set of results for gap junctions, because, as far as numbers of junctions are concerned, more than three quarters of gap junctional connections involve only one gap junction, and as regards adjacency, there is very little correlation between the presence of gap junctions and adjacency. It is however true that in the cases where there are two or more gap junctions on one side the chance of there being at least one on the other side is higher than normal (94%).

7.7 Mismatches of chemical synapses are due to extra connections more often than to missing connections

If we assume that there is an underlying "normal" pattern of connectivity then there are essentially two ways that a mismatch can occur. Either an additional abnormal connection can be made, or a normal connection can be missing. For any given connection involving a bilaterally represented neuronal class there are four equivalent possible locations that the connection could occur in the database, one on each side of each animal. If a mismatch exists in one animal then one can look in the other animal to get some idea of whether the connection is normally there or not. For chemical synapses there are over four times as many connections that occur in only one of the four cases as in three out of four cases (137 and 32 respectively). This implies that four times as many mismatches are due to a single extra connection being present as are due to a single connection being missing. The same is not true of gap junctional connections, for which there are 74 present in only one case and 58 in three cases.

These observations concerning unmatched chemical synaptic connections suggest that there are a set of extra synapses in addition to a fairly consistent set of basic connections. We can attempt to estimate the consistent set of synapses by counting only those synapses in addition to a fairly consistent set of basic connections. We can attempt to estimate the consistent set of synapses by counting only those synapses that are seen in three or four of the possible cases (both cases if both members of the pair of neurons are unique). There are 2890 of these synapses per animal, leaving 1184

extra synapses altogether in both animals, associated with 647 different cell pair types. Some of these synapses are between cells that are already connected by consistent synapses. However less than half (498) fall into this class; those that do so add fairly evenly to consistent connections with both more and fewer consistent synapses. The remainder (686) are formed between pairs that are not consistently connected. There is a tendency for these to be between processes that have higher adjacency; 437 are between processes with average adjacency 6 or greater.

White (1983) suggested that mismatches might arise because synaptic connections were only specified between processes that are normally adjacent, so that if a nerve process was misplaced and acquired new unexpected neighbours it might make additional, incorrect connections to some of them. There is quite a large variation in the set of cells contacted by any neuron, since if A is adjacent to B then the probability of A' being adjacent to B' is only about 75% (7028/9214). However this is not the predominant source of mismatches. If A' is not adjacent to B' the probability of a connection from A to B is far smaller than normal (table 7.1 row g compared to row a). Only about a quarter of all mismatches can be explained in this fashion (24/108 for the bilateral comparison and 65/224 for the H/U comparison – 5th column of table 7.2). The proportions are similar for gap junctions (8/32 and 10/55 respectively). These results suggest first that specificity is probably determined for at least an extension of the normal set of neighbours, covering processes with which a neuron is likely to stray into contact, and second that physical contact is not a major limiting factor in determining synapse formation, since most of the marginal synapses are made with processes with which the neuron is normally in contact. Instead the earlier observations suggest that additional synapses are mostly made between processes that normally have reasonable adjacency.

7.8 Connections are not determined purely by neuronal classes

So far we have compared pairs of neurons that are directly symmetry related, such as A, B to A' B'. There are also many situations where a neuron B contacts both A and its contralateral homologue A'. These contacts are not symmetry related, but if connections are determined purely by the classes of the two neurons, then one would expect a connection between A and B whenever there was one between A' and B. In fact the overall measures of this type of reproducibility are 67% for chemical synapses and 57% for gap junctions (table 7.2 rows e and f respectively), significantly less than the levels of reproducibility for are 67% for chemical synapses and 57% for gap junctions (table 7.2 rows e and f respectively), significantly less than the levels of reproducibility for symmetry related connections (rows a, c). The given frequencies of a matching connection from A to B are conditional on A and B being adjacent, since there is no automatic reason why A and B should be adjacent whenever A' and B are, ecause there is no geometrical symmetry relating to the two pairs. This difference is due neither to the number of connections from A' to B tending to be low nor to the adjacency of A and B being low, since there is still a difference when the frequency is tabulated with respect to either of these categories (columns 2 and 6 of table 2). Indeed the reproducibility is down to 70% even in the cases where there are 3 or more synapses, which are 95%

Figure 7.1

Figure 7.1

A schematic diagram showing the interconnections between the 6 members of the RMD class of neurons. The circle represents the nerve ring. At each radial position around the ring one of the RMD neurons has synaptic output (arrows), both onto muscle and onto other neurons, including the diametrically opposite RMD neuron, which "intercepts" the neuromuscular junctions (as with the DD process in figure 3.6). In general the RMD neurons are monopolar, with their proximal regions showing this intercepting behaviour, and their distal regions being synaptically active. Gap junctions (thin bars) link neighbouring neuromuscular regions but are not formed in general even where processes are close, sich as for instance where chemical synapses are made. Some variability was seen in this general pattern, since in the U series RMDL had output (both to muscle and other neurons) from the proximal as well as the distal part of its process).

reproducible in the symmetric left/right comparison. The only set of conditions under which there is a respectable degree of reproducibility is when the adjacency of A to B is high (column 7).

What might cause the difference between A and A'' in synaptic specificity with respect to B? If the difference is cell intrinsic, as opposed to being activity related, then either A and A' must be inherently distinguishable or else either they or B must be regionally specialised. Regional specialisation would account for the observations because in general different parts of A and A' contact B, and they contact it in different places (see figure 7.1 for an example concerning cells all of the same class). There are several indications that suggest that this might be a correct explanation. Although few neurons in the C. elegans nerve ring have a classical bipolar morphology, with a presynaptic dendritic structure and a predominantly postsynaptic axonal structure, there are many neurons whose processes show regional differences (White et al., 1983, White et al., 1986), and most of the cases of differential specificity that we are considering, such as that between the RMD neurons shown in figure 7.1, involve these cells. In addition, if the differences were due to regional specialisation, then there would be the highest chance of a mismatch if the cells only touched in one place, and much less chance if they were in contact over a large proportion of their axonal structure. This suggests that the mismatch frequency would be lower when the adjacency of A and B was high, which is indeed the case (table 7.2 column 7). Such an effect would not be expected if the difference between A'B and AB synapses was due to distinct identificatory labelling of A and A'.

Similar results are obtained when comparing synaptic specificity of dorsal and ventral members of the same neuronal class. Once again their interactions with other neurons are not symmetry related, and so the lower levels of reproducibility that are observed (69% for chemical synapses and 52% for gap junctions) can be explained by regional specialisation of some of the neurons.

DISCUSSION

The main conclusions of this set of investigations are:

1. The overall pattern of connectivity between neurons is fairly reproducible. However at a detailed level there are differences. There is a greater difference between the two animals than between the two sides of one animal.

2. Although chemical synapse formation is correlated with adjacency when all pairs of neurons are considered, between any particular pair both the probability of forming a connection, and the number of synapses made, are essentially independent of adjacency.

3. Synaptic reproducibility is very high (>95%) if several synapses are normally made. However there is a substantial number of unmatched single synapses, present in only one of two symmetrical cases. A significant majority of unmatched chemical synapses appear to be due to the formation of abnormal or infrequently made connections, rather than the loss of a normal connection.

4. The formation of a connection between two neurons is not purely dependent on the classes of the neurons involved and whether they are adjacent. A possible additional factor is that in many cases synaptic specificity is regionally localised on neurons.

I shall discuss these points in turn.

7.9 Differences between repeats of equivalent circuitry

There are two significant questions concerning the overall reproducibility of the synaptic connections in the nerve ring. The first is whether the differences seen are systematic and functional, or merely random variation due to looking at an essentially fixed pattern at too fine a level of detail. The second is whether the left and right sides of the nerve ring are developmentally equivalent in terms of synaptic circuitry. Some parts of the nervous system show significant reproducible left/right asymmetry (e.g. the ventral nerve cord, see Chapter 5), but at a gross level the two sides of the ring are symmetrical. These questions could be addressed by considering the four examples of each cell pair interaction present in the database, one for each side of each animal.

To consider the second question first, a comparison was made to search for consistent synaptic differences between the left and right sides across the two animals. This indicated that they were very similar, although there may be a slightly greater similarity overall between the same sides of the two animals than between their opposite sides (approximately 1%, the effect for instance of a single possible asymmetric neuron class). This possible minor difference was ignored for the rest of the analysis in order to consider the two sides as developmentally equivalent when investigating their differences. There was a certain amount of unsystematic variation between the two sides. Clearly it is not easy to assess the functional significance of anatomical changes in synaptic connectivity in a circuit whose function is largely unknown. However there was no obvious observable pattern to the differences that might have suggested a rewiring of any piece of the circuitry, such as loss of one connection but a gain of a compensating connection to a parallel interneuron, and the vast majority of differences involved connections with just one or two synapses, or a single gap junction. It seems reasonable to suggest that the variation seen here is mostly due to random fluctuation in process positioning and synapse formation.

The equivalent comparison between the two difference animals, however, revealed that the two sides of each animal were more similar than the same sides of different animals, suggesting possible real differences between the two animals. The U series is from an older animal but again, except for the introduction of the major egg-laying motor neurons (HSN's) and a generally raised number of connections from the labial receptors (IL1, IL2 classe), the differences appear to be scattered randomly throughout the circuitry, and to consist mostly of only one or two synapses for any

particular pair of cells. Since the animals are isogenic, the systematic differences must be either age related, or a consequence of environmental differences during development. Laboratory specimens of C. elegans are cultured on bacterial lawns grown on agar plates; their environment varies only in the level of food supply and the degree of dessication! There have been no studies to determine whether any of the C. elegans neural circuitry shows variation under different conditions. A possible approach would be to compare the ventral nerve cord circuitry of animals that have been raised swimming freely in liquid culture with the standard nerve cord of animals raised on agar plates, which has been reconstructed many times.

7.10 Connection formation and adjacency

A simple model for formation of synapses between two neurons would be that there is a cell-cell recognition event where the cells contact, and that synapse formation is a local event, so that the probability of forming a synapse, and the number formed, would increase with increasing length of mutual contact. However there is clear evidence from the variation in adjacency and synapse formation in equivalent pairs of cells in the database that this is not the case. Both the probability of forming a synapse, and the number of synapses formed, are essentially independent of the degree of adjacency, providing that some contact is made. This implies that there is internal regulation of synapse formation at a cell-wide level. Such regulation is biologically reasonable, because it would be asking a lot of a process placement mechanism to finely control the exact amount of contact between different neurons, and the circuitry is reasonably fixed. Indeed the adjacency of a particular pair of neurons is much more variable than their connectivity. The next chapter pursues further the question of process placement in the nerve ring.

Although there is no apparent link at the level of individual process pairs, there is a strong general correlation between synapse formation and adjacency. Neuron pairs that are synaptically connected tend to have high adjacencies, and the more synapses they form the higher the adjacency tends to be. The amount of contact is in general much larger than that needed to make the synapses in. In a sense adjacency seems to depend on the likelihood of forming synapses, rather than synapse formation on adjacency. This also makes biological sense, since the goal of positioning nerve processes is to place in contact with each other those neurons that will form connections. However the same correlation is not seen for gap junctions, for which this post hoc biological rationale is just as relevant. This seems to suggest an effect on neuronal placement, and thus possibly process guidance, of either chemical synapses or gap junctions (or something involved in forming them). One might postulate either process attraction linked with synapse formation, or a repulsion, or stopping of growth, associated with gap junctions. When considering connections within the same class it was observed that bilaterally symmetric processes that gap junction with each other often terminate when they meet at the midline, while those few classes that form intraclass synapses all substantially overlap. Elsewhere in C. elegans the muscle arms in the body are clearly attracted to the presynaptic processes, since when motor neuron axons are displaced the muscle arms still go to them and receive neuromuscular input (Chapter 4, and J White, S Brenner, unpublished observations). However, although muscle arms resemble postsynaptic processes, they are phylogenetically anomalous and possibly a special example.

7.11 An underlying pattern of connectivity with additions?

The average number of chemical synapses between a pair of synaptically connected neurons 4.2, and there are many cases with more than 10 synapses, whereas there are rarely more than one or two gap junctions between a pair of neurons. We have seen that in individual cases the level of adjacency between two processes does not appear to be important to synaptic reproducibility. Instead the most significant general factor as an indicator of reproducibility is the number of synapses made. Connections with only one or two synapses are unreliable, while those normally containing many synapses are nearly all present in all cases. This result is statistical; there may be individual pairs of neurons that always connect but only form one synapse (e.g. OLQV and RIC only contact briefly but in each case seen make a single very large synapse full of vesicles). However overall there is evidence that the probability of forming a connection and the number of synapses are linked.

If we consider mismatches in which a connection is present on one side but not the other, there are four times as many cases where the connection is seen on neither side of the other animal as ones in which it is seen on both sides. An explanation of this result would be provided by the hypothesis that there is an underlying pattern of circuitry that is consistently present, but there are also always a number of additional synapses that are selected from a wide range of possibilities, and which therefore do not generate reproducible connections. The distribution of this set of additional synapses was estimated by subtracting away consistently seen synapses. Since this operation was performed with synapses rather than connections it leaves some additional synapses between processes which have a consistent connection, for example where in one of the four cases 10 synapses were made, and in others only 6. However the majority of additional synapses are between processes that are not reproducibly connected, which implies that the variation observed in the circuitry is not simply due to modulation in level of a small restricted set of possible connections. The new connections normally contain only one or two synapses. This would provide an explanation for the low reproducibility of synaptic connections containing only one or two synapses. Both the adjacency distribution of the additional synapses, and a direct count of the proportion of mismatches for which the unconnected pair of neurons are not adjacent, indicate that most of the additional connections are between pairs of neurons that normally have a reasonable adjacency, rather than being due to a process wandering away from its normal circle of connections.

One possible alternative explanation for the extra “random” connections is that they are due to errors in scoring synapses during the reconstructions. It is sometimes hard to unambiguously identify whether or not a process is postsynaptic to a particular synapse. However the fact that the comparison between the U and H series contains many differences with similar characteristics to those described above, but many of which are reproducible from side to side of each animal, suggests that they are real.

7.12 Localisation of synaptic specificity within neurons

There are a significant number of cases where synapses or gap junctions are formed between members of two classes of neurons when they make contact in one place on their processes, but not when equivalent cells touch in other places. It seems more likely that this is due to regional localistion of the potential to form the relevant type of synapse in the same way for all neurons in a class, than to all the different members of each class having different recognition properties. A more direct example of clear localisation is seen in the adult ventral cord, where the VA and VB motor neurons are bipolar, sending out processes in one direction that only receive input, and only have neuromuscular output from their other process, a classical axon/dendrite morphology. The interneurons are closely available to both processes. However many of the ring neurons are not bipolar, and it would appear that many of the specialisations, presumably involving differential localisation of surface components, would have to be between distal and proximal regions of the same nerve process, as in figure 1. The following neuronal classes can be tentatively implicated as candidates for regional specialisation on the grounds that they are involved in more than one mismatch and have a suggestive axonal structure: RMD, SMD, SAA, SIB, SMB, RIC. There are many others with apparently discontinuous axonal structure (White et al., 1986). An example of a different type of change between proximal and distal regions of the same nerve process is given by the single neurite of the major interneuron AIB, which changes relative position in the neuropil, running for the first half of its length with chemical receptor axons, and th distal half with motor neurons and motor circuitry interneurons (White et al., 1983).

7.13 Conclusion

Two types of information can be inferred from the foregoing analysis. First a picture can be drawn of the degree of overall synaptic rigidity in the C. elegans nerve ring, and how the connectivity might vary or be modified. Second there are a number of observations suggesting factors that might be important for the formation of synapses between individual pairs of neurons.

It is apparent that synaptic circuitry in C. elegans is not so rigidly reproducible as the positioning and lineal origin of cells, which are practically identical from animal to animal. However the data support the suggestion that there is a broad core of connections that are constant, including most of the strong synaptic connections containing many synapses. This core is subject to a reasonably low level of variation itself (at the level of one or two synapses per connection), but there are also a number of additional chemical synapses (about 10% of the number in the core pattern) connecting other pairs of processes, which are not reproducible. Gap junctions show less variation, and no indication of a strong additional component.

There is some evidence that the changes and additions might not be random, since there are many consistent ones, each small in itself, between the H series animal and the U series animal. Since these two animals are isogenic they must be due to either environmental or age-related differences. If the additions are functional then there are two possible ways in which they could be used. The first is to “tinker” with a standard pattern, slightly altering the influence of various parts of the nervous system

on each other but not changing the functional roles of cells. The second is to introduce a new behaviour, or to flexibly wire a particular task so that it is performed in a different way in different animals. This is different from the question of functional flexibility in a single neuron taking part in more than one task, for which there is plenty of evidence: the IL1 and URA classes are for instance both sensory and motor neurons. Although no data are available on the variability of function of nerve ring neurons in different animals, I believe that two observations point towards the tinkering rather than the respecification theory. First, in no case is a systematic pattern of connectivity change discernible (other than the introduction of the egg-laying circuitry in the sexually mature U series animal). Second, the great majority of changes consist of only one or two synapses per connection.

There are several observations concerning the formation of connections between pairs of neurons. It seems likely that the potential to form synapses and gap junctions may be localisable to particular parts of a single process. The probability of a chemical synaptic connection being formed, which may depend on environmental as well as genetic factors, and the number of synapses formed, appear to be regulated so as not to depend on the amount of contact between a pair of cells. However the factors involved in synapse formation may influence adjacency, because synapse formation, but not gap junction formation, is correlated with adjacency, suggesting that one or the other of the two processes may have an effect on nerve process placement. The evidence for these properties generated by this study is statistical and indirect, and therefore inappropriate as a basis for a series of direct intrusive testing experiments. However it is not susceptible to the bias that might be generated by studying intensively a single connection. Also, since a very large number of cell pairs were considered, it is statistically significant; the conclusions do almost certainly reflect pervasive properties of one simple nervous system, and they may also be relevant to other organisms.