Connectivity changes in a class of motoneurone during the development of a nematode

THE ventral nerve cord of the nematode *Caenorhabditis elegans* contains a linear array of motoneurones (Fig. 1) which innervate the body muscles that mediate locomotion. The adult ventral cord has about three times as many cells as that of the first stage larva. The development events that generate the adult complement of cells occur in a period preceding the first larval moult. During this period we find that a class of pre-existing, juvenile motoneurones changes its pattern of connectivity. Neuromuscular junctions are removed from ventral muscles and are reformed onto dorsal muscles. Similarly the dendritic input to these neurones changes over from the dorsal to the ventral side.

The structure and connectivity of ventral cord motoneurones in adult hermaphrodites has been determined by serial section reconstructions of electron micrographs[1]. The salient features of the structure are summarised below:

Motoneurones in the ventral cord can be catagorised into five distinct morphological classes designated A, B, C, AS and D (Fig. 2). Classes A, B and D can be subdivided into those members that innervate ventral muscles (VA, VB and VD) and those that innervate dorsal muscles (DA, DB and DD). The members of a class are evenly distributed along the length of the cord such that all body muscles receive innervation from at least one member from each class. All motoneurones, except those in class D, receive their synaptic input from interneurones which have processes that run along the length of the ventral cord. Class D neurones, on the other hand, receive their innervation from the other classes of motoneurone. In the adult each DD motoneurone has a process which runs on the ventral side where it receives synaptic input from classes VA, VB and C motoneurones (Fig. 2). These connections are often made onto small dendritic spines which intercept neuromuscular junctions (Fig. 3*a*). The cell body is situated near the posterior end of the process and a branch comes off the anterior end. This branch leaves the ventral cord and runs round to the dorsal cord as a circumferential commissure. When it enters the dorsal cord it splits and runs both anteriorly and posteriorly, the posterior branch being longer. Many neuromuscular junctions (NMJs) are formed on this dorsal process (Fig. 3*b*) little, if any, synaptic input being received on it from the other neurone classes. The extent of both the dorsal and ventral cord processes is limited, each generally ending abruptly in a gap junction to the process of a neighbouring DD neurone. Thus the DD neurones receive their synaptic input from motoneurones in a well defined region on the ventral side and synapse onto muscles in the same region on the dorsal side. The VD neurones have a similar shape to the DD neurones although their processes are shorter (Fig. 2). These neurones are the converse of DD neurones, receiving their innervation from motoneurones on the dorsal side and forming NMJs on the ventral side.

When the first stage larva hatches there are a total of 22 motoneurones in the ventral cord and associated ganglia. This number increases to 76 in a period of post-embryonic development that commences about 9 h before the first larval moult[2,3]. It was found that all the VA, VB, C, AS and

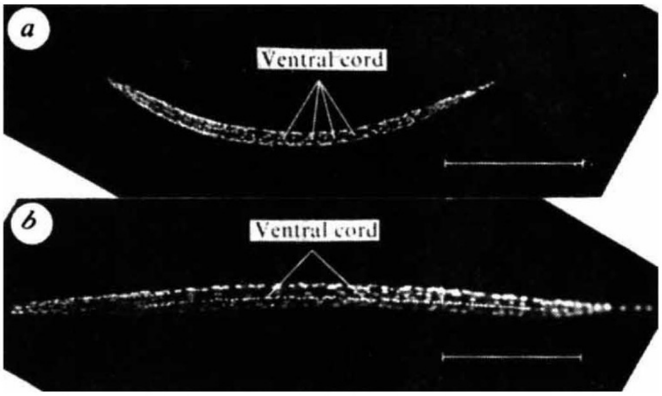

Fig. 1 Cell bodies in the ventral cords of first and second stage larvae. The number of motoneurones in the ventral cord increases in a period of post-embryonic development that commences about 9h before the first larval moult. *a*, A first stage larva before this period has started; *b*, a second stage larva that has completed all its ventral cord cell divisions. Animals were fixed in Carnoys and stained with the fluorescent nuclear stain Hoechst 33258. Scale bars, 100 μm.

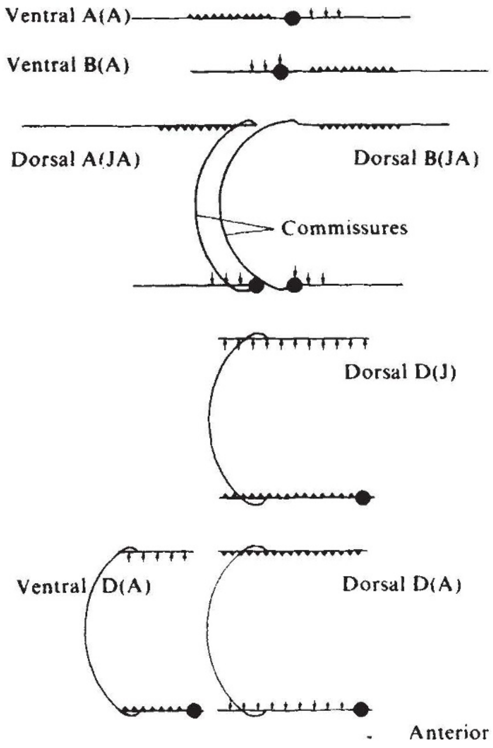

Fig. 2 Classes of motoneurone in the ventral cord. All the motoneurones of the ventral cord have a simple well defined shape. Neuromuscular junctions (▲▲▲) are made '*en passant*' along the length of a process. Similarly synaptic input occurs along other processes (↑ ↑ ↑). Muscle cells send out arms to the region of the cord where the motor neurone axons reside. All motoneurones which innervate the body muscles have cell bodies (-●-) which are situated in the ventral cord. Each motoneurone which innervates dorsal muscles sends out a commissure which leaves the ventral cord and runs circumferentially around the animal to the dorsal side. When these processes reach the dorsal midline they turn and together make up the dorsal nerve cord. There are five distinct classes of motoneurone in the ventral cord, A, B, C, AS and D, three of which are shown. Each class has a unique pattern of synaptic input[1]. Some classes are only present in adults (A), others are present in both juveniles and adults (JA), class DD neurones are present in different forms in juvenile and adult animals (J and A). Neurones in class A have axons that project anteriorly, whereas those in class B have axons that project posteriorly. Both these classes can be subdivided into neurones that innervate ventral muscles and those that innervate dorsal muscles. Class D also has both dorsal and ventral members. Neurones in this class do not receive any synaptic input from interneurones but rather from other classes of motoneurones. Ventral class D neurones receive synaptic inputs from class A, B and AS neurones on the dorsal side and innervate ventral muscles. Dorsal class D neurones in the adult (A) innervate dorsal muscles and receive their synaptic input from class A, B and C neurones on the ventral side. In the juvenile (J) they innervate ventral muscles and receive their synaptic input from DA and DB neurones on the dorsal side. Both the dorsal and ventral processes of the type D neurones end abruptly in gap junctions to the processes of neighbouring class D neurones.

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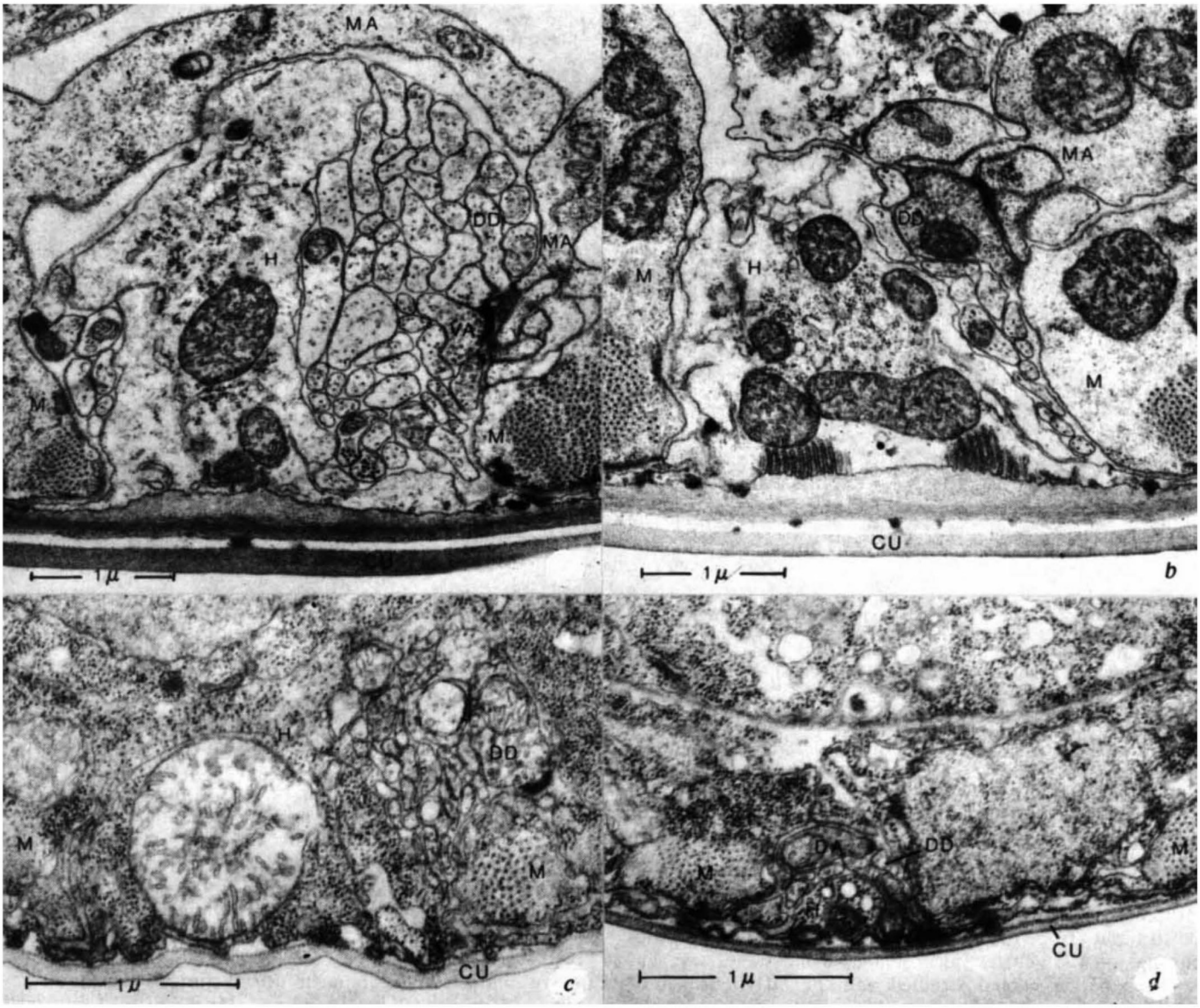

Fig. 3 Interneurones which come from the nerve ring send processes which run alongside a hypodermal ridge (H) which underlies the cuticle (CU). These interneurones synapse onto class A, B and AS motoneurones. The body muscles (M) send out arms (MA) which go to the endplate region of the motoneurone to receive their synaptic input. *a*, A VA motoneurone in the ventral cord of an adult hermaphrodite synapsing onto some muscle arms, the synapse being intercepted by the dendritic spine of a dorsal type D neurone (DD). *b*, The same DD neurone synapsing onto muscle arms in the dorsal cord (the view has been rotated 180°). There are far fewer processes in the dorsal cord as all the interneurones run in the ventral cord. *c*, A DD neurone synapsing onto muscle arms in the ventral cord of a first stage (L1) larva. This provides the sole synaptic input for the ventral side in the L1 stage yet these synapses disappear completely by the second (L2) stage. *d*, The same DD cell receiving its synaptic input on the dorsal side from a DA motoneurone. There are two classes of motoneurone which synapse onto dorsal muscles and DD cells at this stage, dorsal type A (DA) and dorsal type B (DB). Scale bars, 1 μm.

VD neurones present in the adult are generated in this period[2]. We deducted by subtracting these neurones from the total adult complement that juveniles must have only DA, DB and DD motoneurones in their ventral cords. This seemed paradoxical as the lack of motoneurones on the ventral side would imply that neither the ventral muscles nor the DD neurones receive any innervation.

To resolve this paradox two first stage larvae each about 10 h old were fixed, sectioned and reconstructed as described in ref. 4. Motoneurones in the juvenile cord could be unambiguously related to their counterparts in adults because of the low level of variability in the sequences of cell types along the cord[1,2]. Cells which are DA and DB neurones in the adult were found to be the same in the larvae. This was not the case with the DD neurones, however; the morphology of the processes was the same but the

Table 1 Disposition of neuromuscular junctions in *C. elegans* larvae

		Dorsal NMJs	Ventral NMJs
First stage larva *a*	DD 1	0	20
First stage larva *b*	DD 1	0	14
	DD 2	0	15
	DD 3	0	9
Adult	DD 1	31	0
	DD 2	28	0
	DD 3	20	0

Two first stage larvae were reconstructed, *a* and *b*. The first reconstruction (*a*) covered the first DD motoneurone, the second (*b*) was longer and covered the first three DD neurones in the anterior ventral and dorsal nerve cords. The number and location of NMJs formed by these neurones is shown and compared with their counterparts in a reconstructed adult.

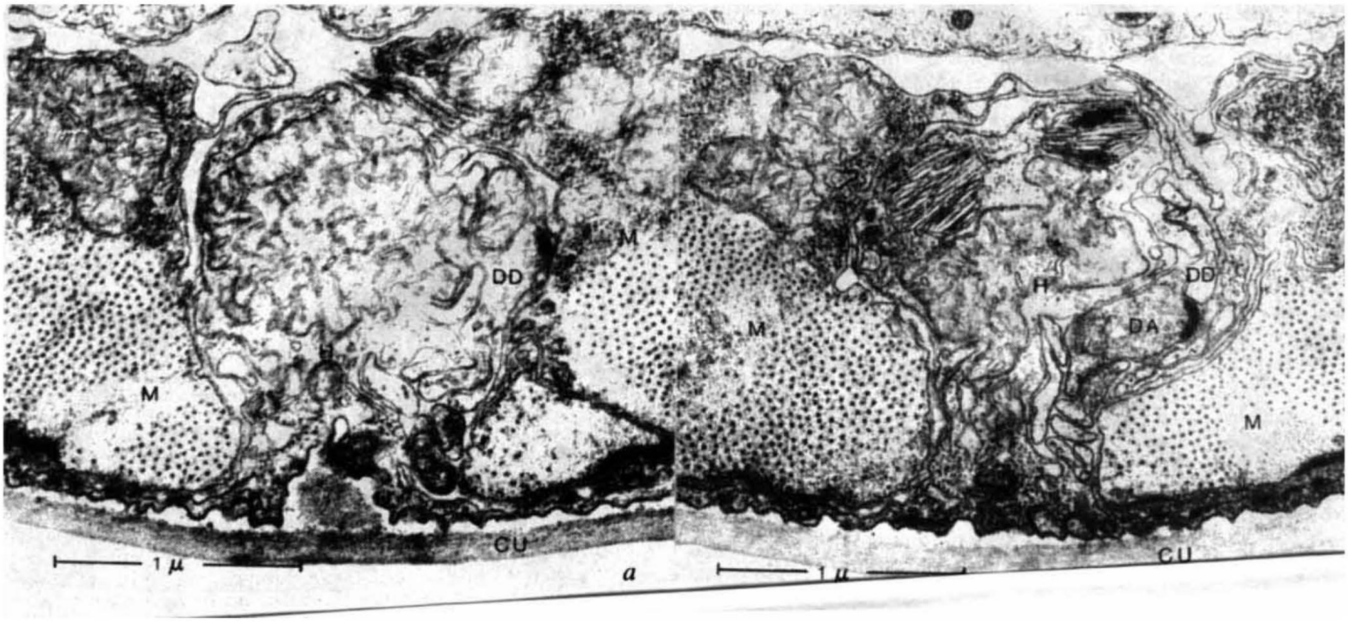

Fig. 4 *a*, The dorsal cord of a fourth stage larva of the mutant *e1466*. This mutant is defective in post-embryonic development and none of the late developing motoneurones are formed. The DD neurone is synapsing onto muscles on the dorsal side with no synapses on the ventral side as in wild-type adult animals. Some of the original synaptic input to the DD neurones (that is, from the other motoneurone classes on the dorsal side) seems to persist (*b*), a feature which is seen rarely in wild-type adults. Scale bars 1 µm.

disposition of the synapses was different (Table 1). In juveniles DD neurones had neuromuscular junctions on their ventral processes (Figs 2 and 3*c*) whilst their dorsal processes received synaptic input from DA and DB motoneurones (Fig. 3*d*) resembling the VD neurones of adults. Thus during the course of post-embryonic development all the ventral NMJ's from the DD cells disappear and new NMJ's are formed on the dorsal side. Similarly the synaptic input changes from the DA and DD neurones on the dorsal side to inputs from the newly formed VA, VB and C motoneurones on the ventral side. A late second stage larva has also been partially reconstructed and the DD neurones of this animal were found to be the same as those of the adult. It seems likely that the change in the connectivity of the DD motoneurones takes place between the first and second larval stages at the same time as the late developing motoneurones are formed[2,3].

Mutants have been isolated which are defective in various aspects of post-embryonic development (H. R. Horvitz, and J. E. Sulston, unpublished). In one of these mutants, *lin-6 I (el466)* none of the late-developing motoneurones are produced. The ventral cord was reconstructed in a fourth stage larva of this mutant and we found that the DD motoneurones formed NMJs on the dorsal side (Fig. 4*a*) as they do in wild-life adult animals. The DD neurones of the mutant received no synaptic input on the ventral side because the late developing motoneurones which provide all the synaptic input in wild-type adults were absent. It therefore seems likely that the DD motoneurones displace their NMJs from the ventral to the dorsal side in this mutant as they do in wild-type animals leaving the ventral muscles with no synaptic input. This is consistent with the observation that the first stage larvae of this mutant have apparently normal locomotory behaviour, whereas all the later stages are uncoordinated in their body movements (J. E. Sulston, personal communication). There are some indications the DD neurones of the mutant continue to receive some synaptic input from DA and DB neurones on the dorsal side (Fig. 4*b*) unlike wild-type adults that rarely have such connections[1]. The observations that the DD neurones in *el466* change those sites where NMJs are formed in the same way as those in wild-type animals suggest that this process is independent of the formation of NMJs from the late developing neurones. This may not be the case for the sites of synaptic input for the DD neurones since some of the juvenile sites seem to persist in this mutant.

Connectivity changes in normal[5] and abnormal development[6] have been described in other organisms. The behaviour of the DD neurones in *C. elegans* provides a rather extreme example of neuronal plasticity during development where not only are connections altered but also the direction of information flow in processes is reversed.

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Dopamine receptors localised on cerebral cortical afferents to rat corpus striatum

STRIATAL dopamine receptors, monitored by dopamine-sensitive adenylate cyclase activity[1,2] or binding of ^{3}H-haloperidol[3-5] apparently represent distinct entities because of differences in drug sensitivity and the pattern of their ontogenetic development[6,7]. Dopamine also elicits both excitatory and inhibitory effects on striatal neurones[8,9]. We have examined the effects of selective degeneration of striatal intrinsic neurones with the neurotoxin, kainic acid[10-12], and elimination of cortico-striate afferents by cortical ablation[13] on the dopamine receptors in rat striatum.

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