Dopaminergic Neurons in the Nematode *Caenorhabditis elegans*

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ABSTRACT Dopamine is the putative transmitter of eight neurons in the hermaphrodite form of the nematode *Caenorhabditis elegans*. These include the cephalic and deirid neurons, which are believed to be mechanosensory. The male has an additional six dopaminergic neurons in the tail. Mutants have been selected which have defects in the formaldehyde induced fluorescence and lack dopamine to varying degrees, but they are not insensitive to touch. The dopaminergic neurons of *C. elegans* are compared with the homologous neurons in *Ascaris lumbricoides*.

The nervous system of the free living nematode *Caenorhabditis elegans* is being investigated by a combination of genetic and anatomical methods (Brenner, '74; Ward et al., '75). Reconstruction of the structure of the nervous system from serial section electron micrographs provides a wealth of accurate detail on the location and connections of neurons but does not allow easy identification of the chemical transmitters produced by nerve cells. The small size of *C. elegans* precludes electrophysiological investigation, but pharmacological agents can be applied to preparations of cut worms (J. Sulston, unpublished data). Using this method it has been shown that acetylcholine causes a contraction of the body wall musculature which is blocked by tubocurarine while γ-aminobutyric acid relaxes the body muscles. This corresponds well with the evidence obtained in *Ascaris lumbricoides* by electrophysiological methods, that acetylcholine is the excitatory neuromuscular transmitter and γ-aminobutyric acid or a related compound the corresponding inhibitory transmitter (Debell, '65).

In searching for other putative neurotransmitters in *C. elegans*, it is natural to consider the catecholamines. They can be detected by the Falck-Hillarp procedure (reviewed Fuxe and Jonsson, '73), and catecholamine containing neurons in several other invertebrates have been described (Cottrell and Laverack, '68; Sakharov, '70). This paper describes the catecholaminergic neurons in *C. elegans* and a number of mutants with defects in these nerve cells.

METHODS

Culture and genetics

The nematodes were handled as described previously (Brenner, '74; Sulston and Brenner, '74).

Formaldehyde induced fluorescence (FIF)

The general method has been reviewed recently (Fuxe and Jonsson, '73).

C. elegans

Two aluminum blocks (9 × 7 × 4 cm) were milled so as to fit together leaving a 1 mm gap between them. The blocks were cooled in liquid nitrogen. The nematodes were placed in the minimum volume of 0.1 M NaCl on a cover slip, quenched in liquid nitrogen, and transferred to the space between the blocks. The assembly was placed on a plastic petri dish over P_2O_5 in a desiccator, which was immediately evacuated to about 0.01 torr. After 30 minutes, the desiccator was sealed and left at room temperature. If the drying time was less than 24 hours, the blocks were warmed with a heat lamp before the desiccator was opened. The cover slip was transferred, with no special precautions, to a 1-lb preserving jar containing 1–2 g of paraformaldehyde equilibrated at 70% humidity. The jar was sealed, and placed in an oven at 67° for one hour. The nematodes, still supported on the cover slip,

were mounted in Zeiss immersion oil and allowed to clear for several hours before examination.

For multiple assays, each 22 × 64 mm cover slip carried ten samples separated from one another by Chinagraph lines and two cover slips were placed between a pair of blocks. Usually the nematodes were transferred from plate cultures and spread out by means of a paper strip, which also served to absorb excess saline. During transfers, the cover slip was kept on ice to prevent evaporation; if it became too wet, a plastic petri dish was interposed between it and the ice. When individual nematodes were examined, as in genetic mapping, they were transferred with a pointed stick to 10% ovalbumin.

A. lumbricoides

Adults. Heads and tails (3–5 mm in length) were cut from fresh *Ascaris*, rinsed in 0.1 M NaCl, blotted, and dropped into melting propane (Calor gas). The aluminium blocks were cooled in liquid nitrogen, and the frozen specimens were transferred to a depression in the lower block. The assembly was left over P_2O_5 in an evacuated desiccator for two days. The specimens were treated with formaldehyde as above, and vacuum embedded in Maraglas resin. After curing at 60° for 20 hours, the blocks were sectioned at 5 μm; the sections were mounted in immersion oil.

Larvae. Eggs were collected from adult uteri and incubated in 0.1 N sulphuric acid at 30° for 20 days (Rogers, '60). Some of the larvae were released by crushing the eggs with a glass rod and were incubated on Eagle's medium containing 5% foetal calf serum and 0.8% agar at 37° for one week; they were then treated in the same way as *C. elegans*.

Fluorescence microscopy

A Zeiss RA fluorescence microscope was equipped with a Philips CS200W4 lamp, a BG 12 excitation filter, and a 500 nm barrier filter. Photographs were taken on Ilford FP4 35 mm film, developed in Promicrol.

FIF — Feulgen correlation

C. elegans were freeze dried in ovalbumin and treated with formaldehyde as above. The cover slip was mounted on oil, with a spacer of lens tissue to minimize disturbance of the specimens. The fluorescent cell bodies and appropriate reference features were photographed in selected individuals by both phase and fluorescent illumination. The Zeiss Universal microscope used had a calibrated fine focus control which permitted the measurement of vertical distances to an accuracy of 1 μm. The cover slip was gently released from the slide with xylene, and passed through a graded series of alcohols to water. After Feulgen staining, dehydration, and clearing in methyl salicylate, the cover slip was mounted in methyl salicylate with a spacer as before; the same individuals were photographed by phase and bright field illumination.

Catecholamine assay

Catecholamines and dopa were assayed by the methods of Anton and Sayre ('62, '64). Before adsorption onto alumina, dopamine and dopa were separated as follows. About 0.5 g of nematodes, which had been stored in liquid nitrogen, were thawed into 2 ml of 0.2 M perchloric acid — 0.02 M EDTA — 0.002 M sodium metabisulphite and sonicated in ice (Dawe sonifier, level 5, 20 seconds). The suspension was centrifuged at 10,000 rpm for five minutes, and the supernatant was passed through a 2.5 × 0.5 cm column of Amberlite CG50 Type II (Na^+). The column was washed with 2 ml of 0.01 M HCl, and the effluent and washings were pooled for the dopa assay. Catecholamines were eluted with 4 ml of 0.4 M perchloric acid. The alumina adsorption step was carried out on one fifth the scale recommended by Anton and Sayre. Overall recoveries were about 60% for catecholamines and 40% for dopa.

This procedure gave lower fluorescence blanks than the alternative method in which dopa and dopamine were separated after the alumina adsorption step. The separation greatly improved the sensitivity of the assay for dopamine in the presence of dopa.

The weight of the wet perchloric acid pellet was found to be approximately equal to the fresh wet weight of nematodes, and was used to quantify the input. Normalization by weight was more convenient, and gave more reproducible results, than normalization by number.

Chromatography

The alumina was eluted with 0.05 M HCl; the eluate was divided into two portions, to one of which was added 100 ng of dopamine or dopa, and dried in vacuo over P_2O_5 and NaOH. The residues were dissolved as far as possible in 5 μl of 95% ethanol, and spotted on separate prewashed 5 × 10 cm TLC cellulose plates (Merck). After electrophoresis in 2% acetic acid at 20 V/cm for 15 minutes in the short dimension, followed by chromatography in butanol-acetic acid-water (5:1.5:3) in the long dimension, the plates were heated at 105° over paraformaldehyde for one hour (Cowles et al., '68). Ten ng of catecholamine on the plate gave a visibly fluorescent spot.

Enzyme assays

Tyrosine hydroxylase was assayed by the method of Mandell et al. ('72). Extracts of *E. coli* strain B, on which the nematodes were grown, apparently caused tritium exchange without net synthesis of dopa in this assay. Therefore the reaction mixture was routinely supplemented with 0.1 mM NSD-1055 (generously donated by Smith and Nephews Ltd.) which was found to suppress this activity. In order to improve the sensitivity of the assay, centrifuged sonicates were precipitated with 60% saturated ammonium sulphate. The precipitate was dissolved in 300 μl 0.1 M sodium acetate — 0.5% mercaptoethanol and dialysed for 16 hours against 0.01 M sodium acetate — 0.1% mercaptoethanol.

Aromatic acid decarboxylase was assayed by a method similar to that of Waymire et al. ('71). The reaction mixture contained, in 100 μl:-sodium phosphate, 2 μM, pH 7.2; mercaptoethanol, 1 μl; dopa-1-^{14}C, 0.02 μM, 0.025 μC; pyridoxal phosphate, 0.02 μM. The incubation lasted one hour at room temperature.

RESULTS

Morphology

General

The distribution of FIF in a male *C. elegans* is shown in fig. 1. The cell bodies are arranged in bilaterally symmetrical pairs, and the processes also display bilateral symmetry. The FIF is distinctly green, indicating that it arises from a catecholamine rather than from 5-hydroxytryptamine (Corrodi and Jonsson, '67). The hermaphrodite differs from the male only in the tail, where it lacks the six cell bodies and the fluorescence in the ventral and lateral ganglia.

Cell bodies can be distinguished from processes by the use of simultaneous phase contrast and fluorescent illumination. In the same way, they can be distinguished from certain nuclei which frequently exhibit a yellow autofluorescence in older nematodes. Muscle, hypodermal and some-

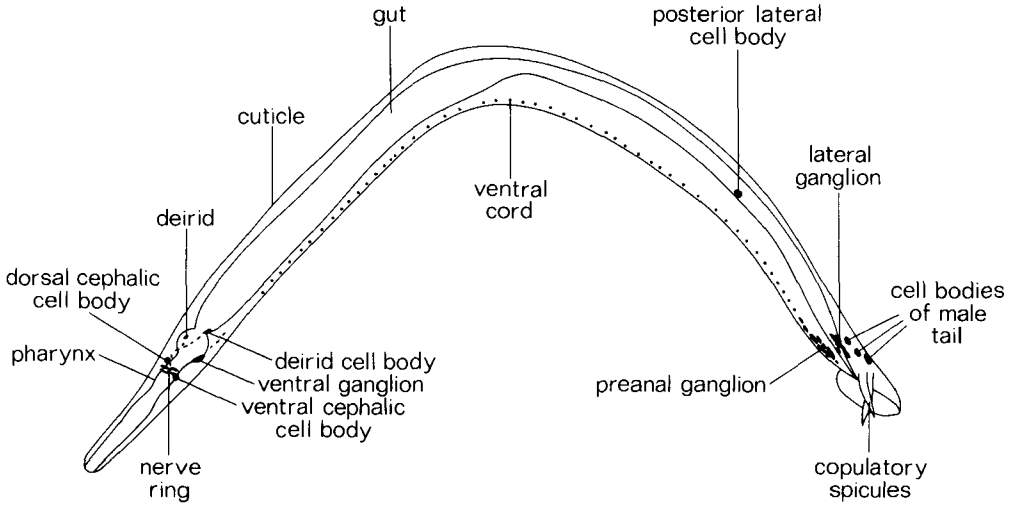

Fig. 1 FIF in male *C. elegans*, left lateral aspect, camera lucida × 170. The FIF of the left side only is drawn. The outline of the body and digestive tract is shown faintly.

times gut nuclei are affected, together with one pair of pharyngeal nuclei which lie in the posterior half of the anterior bulb.

Other prominent autofluorescent structures are the gut (green cytoplasm with yellow droplets) and parts of the cuticle (notably the bright yellow bursa, spicules and rays of the male tail).

In nematodes exposed to 30 μg/ml of reserpine for several hours, FIF is absent from the processes and is found only in the cell bodies. Reserpine has no effect on the behaviour of the nematodes.

The head. Figures 2, 3 and 4 show the distribution of FIF in the head of *C. elegans*. In a well preserved young adult, many of the cell bodies which lie in front of and behind the nerve ring can be resolved in phase contrast illumination. Thus the four fluorescent cell bodies which lie close to the ring can be located in the general pattern. Since this pattern is strictly conserved, the corresponding cell bodies can then be identified in electron micrographs of another individual; they prove to belong to the cephalic neurons (Ward et al., '75).

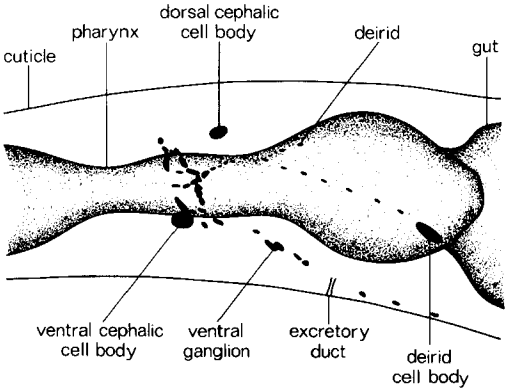

Fig. 2 FIF in head of hermaphrodite *C. elegans*, camera lucida × 750. The outline of the body and pharynx is shown faintly. Left lateral aspect, FIF of left side only.

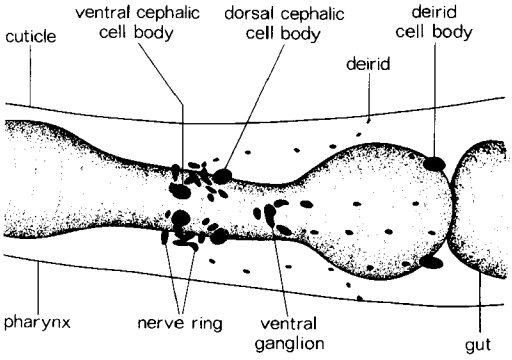

Fig. 3 FIF in head of hermaphrodite *C. elegans*, camera lucida × 750. Ventral aspect.

An alternative method for assigning the fluorescence to particular cell bodies is to follow the formaldehyde treatment with Feulgen staining. Usually, three out of four nematodes survive the staining procedure without displacement. They shrink by some 20%, but there are many phase contrast landmarks in the region of the nerve ring (e.g. the outline of the pharynx; muscle and hypodermal nuclei), and the fluorescence can easily be correlated with the nuclei of the four cephalic neurons.

These methods are less effective for the third pair of cell bodies in the head, and only permit their assignment to the middle region of the sub-lateral ganglia. However, in older animals a series of varicosities allows a process from each cell body to be traced forwards to the nerve ring (fig. 4C). A branch from this process ends in a bright dot on the dorsal ridge of the "tread" (the lateral cuticular ridges, visible in phase contrast illumination), which is exactly the position of the deirid receptor; we conclude that the sub-lateral cell bodies belong to the deirid neurons.

The processes from these six cell bodies can then be traced in electron micrographs of serial sections. This has been accomplished for two individual nematodes (J. White, N. Thomson, and E. Southgate, unpublished data), and the result is shown schematically in figure 5. All the FIF can be accounted for by the cell bodies and synaptic vesicles of these neurons. Two significant differences between the two individuals were found. The first is in the connectivity of the ventral cephalic neurons. In one nematode, both neurons are connected as shown in the main diagram: each axon forks to form the anterior and posterior synaptic endings. In the other, both neurons are connected as shown in the inset: each axon passes directly to the posterior synaptic ending which is in turn joined to the anterior ending by a vestigial fork. The second difference is in the form

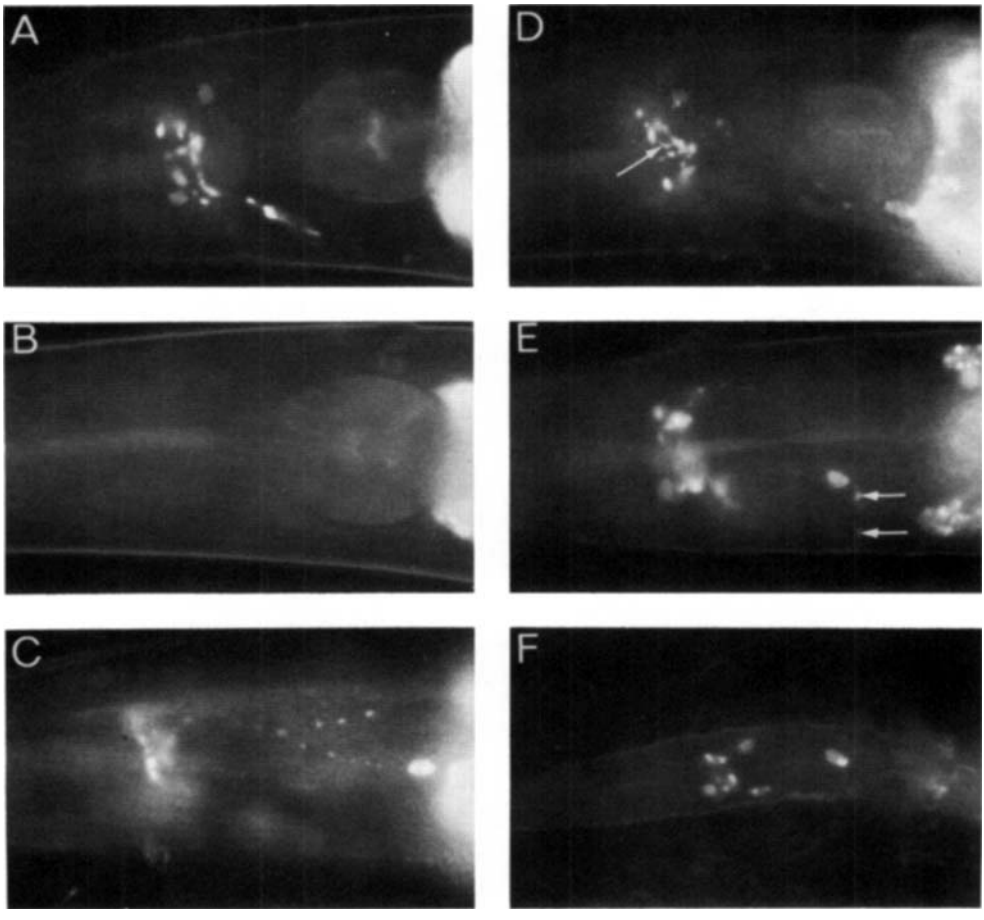

Fig. 4 FIF in head of hermaphrodite *C. elegans*, left lateral aspect, × 750. (A) Nerve ring, cephalic cell bodies, and ventral ganglion. (B) Same plane of focus as (A), not treated with formaldehyde. (C) Deirid cell body, process, and receptor. (D) Varicosity (arrowed) in process leading to anterior synapses of cephalic neuron. (E) Varicosities (arrowed) in deirid commissure. (F) L2 larva, showing absence of deirid process in nerve ring.

of the anterior deirid process, which always synapses extensively at its first entry into the nerve ring, but does not always form the longitudinal lateral spike. The deirid endings can also be seen by FIF, and are quite variable in size; they are often asymmetric in a single individual.

Occasionally, a fine process which normally lacks FIF contains a varicosity and can be visualized in the light microscope: thus the deirid commissure is seen in figure 4E (arrowed) and the link between the anterior and posterior ring endings of the dorsal cephalic neuron is visible in figure 4D (arrowed). Varicosities are always seen in the anterior deirid process of the adult, and small groups of vesicles in this process were found on electron micrographs.

The body. The posterior lateral cell bodies lie in small ganglia immediately under the tread. When visualised by Feulgen staining, the left ganglion is seen to contain six cell bodies and the right ganglion four cell bodies. Frequently a short chain of varicosities extends ventrally from each cell body, and less commonly a chain extends dorsally. It is likely that the chain of varicosities in the ventral cord is an extension of the posterior lateral processes, since no backward projecting deirid or cephalic processes have been found in

serial electron micrographs. However, there is no indication of a T-junction in the ventral cord at the posterior lateral level. This region has not yet been studied in detail by electron microscopy, but preliminary observations suggest an association between the posterior lateral ganglia and sub-cuticular sensory endings similar to those of the deirids. These may be the post-deirids (Chitwood and Chitwood, '37).

The tail. Only the male has the elaborate pattern of FIF shown in figures 6 and 7. There are 6 cell bodies; the fluorescence of the preanal and lateral ganglia is probably in synaptic processes. The detailed anatomy is not yet known.

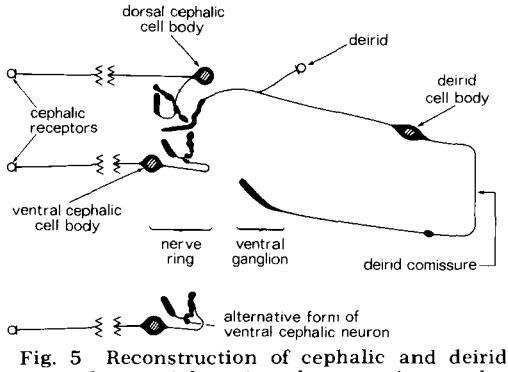

Fig. 5 Reconstruction of cephalic and deirid neurons from serial section electron micrographs, schematic, left lateral aspect. The swellings of the processes shown in the nerve ring and ventral ganglion represent synaptic endings with abundant vesicles.

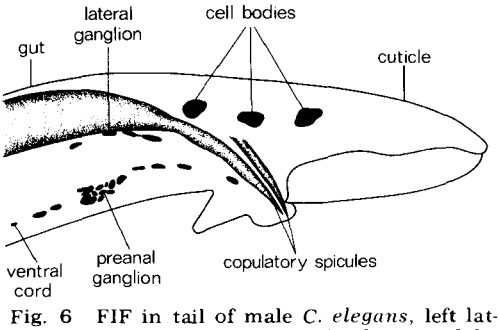

Fig. 6 FIF in tail of male C. *elegans*, left lateral aspect, camera lucida × 750. The FIF of the left side only is drawn. The outline of the body, copulatory spicules and gut is shown faintly.

Development

The preceding account refers to the adult animal. In L1 and L2 larvae the anterior deirid processes, the posterior lateral cells and the varicosities of the ventral cord are invisible. They are first seen in the L3 and reach full intensity only in the young adult. The posterior lateral ganglia are absent from Feulgen stained young L1 larvae; they are generated by cell division in the L1 and L2. The FIF in the cephalic processes, on the other hand, is present from the earliest times, and is sometimes visible before hatching. The FIF in the male tail appears during L4, when the other male structures are being formed.

Mutants

Nematodes were treated with ethyl methanesulphonate as described by Brenner ('74). F2 individuals were cloned and a sample from each clone was examined for FIF. When defects were seen, several individuals were selected for re-cloning. From a total of about 1,000 F2 clones, six mutants were ultimately selected and were assigned to five different genes (fig. 8):

cat-1. (two isolates) FIF is seen only in the cell bodies, and not in the processes; similar to the effect of reserpine.

cat-2. FIF is abolished.

cat-3. FIF is reduced in the processes,

Fig. 7 FIF in tail of male C. *elegans*, left lateral aspect, × 750. Note strong autofluorescence in gut, copulatory spicules, and bursa.

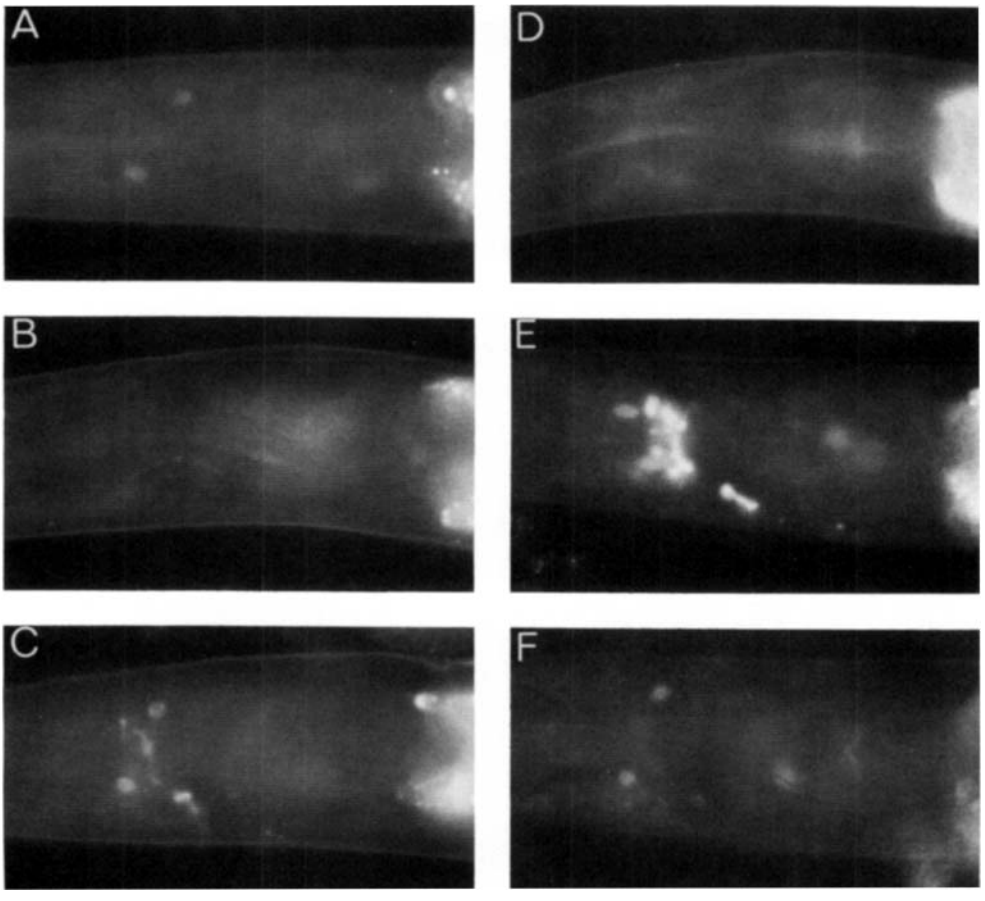

Fig. 8 FIF in head of hermaphrodite *C. elegans*, left lateral aspect, × 750. (A) *cat-1*. (B) *cat-2*. (C) *cat-3*. (D) *cat-4*. (E) *cat-5*. (F) Wild type, exposed to 30 μg/ml reserpine.

but not in the cell bodies; similar to the effect of low concentrations of reserpine. The defect is more pronounced in larvae and at low temperature. This mutant is slightly uncoordinated.

cat-4. FIF is greatly reduced, but still faintly visible in some individuals.

cat-5. The normal number of cell bodies is present, but, in 60% of individuals, one, and, in 20% of individuals, both of the dorsal cephalic cell bodies lie in front of the nerve ring. The intensity of the FIF and the pattern in the nerve ring are unaltered. Some individuals are uncoordinated. The lesions are not confined to the cephalic cell bodies, since Feulgen stained specimens display other variable defects (e.g. loss of other nerve cell bodies from their accustomed sites, malformation of one oviduct).

With one exception (see below) the mutants were epistatic to all the markers tested, and mapping was straightforward. Since the only available assay is destructive, direct selection of a double with a closely linked marker is difficult and was not attempted. However, the *trans* three factor cross described by Brenner ('74) can be applied in the usual way both to order the genes and to yield such closely linked doubles. The genetic map is shown in figure 9. *Cat-2* is not closely linked to *bli-4*, in conflict with the original assignment of *bli-4* to the left hand end of chromosome II (Brenner, '74). The error proved to be due to a mis-labelled strain, and *bli-4* has now been unambiguously re-assigned to the position shown (S. Brenner and J. Hodgkin, unpublished data). *Cat-5* appears to be expressed poorly in both *dpy-11*/

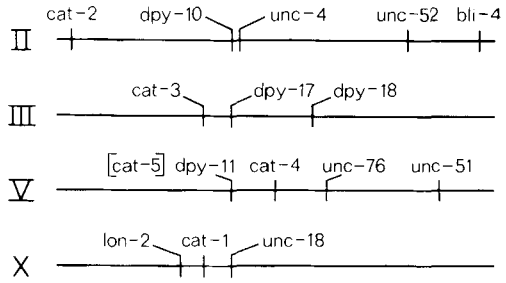

Fig. 9 Partial genetic map of *C. elegans*, showing location of *cat* mutants and known markers used in mapping them. 1 mm = 1 map unit.

dpy-11 and dpy-11/+; for this reason the exact distance between *cat-5* and *dpy-11* is uncertain.

The ventral ganglion of *cat-2*, and both the nerve ring and the ventral ganglion of *cat-1*, have been studied by electron microscopy. No differences from the wild type were found: the normal processes were present, and they contained normal vesicles.

All the mutants are indistinguishable from the wild type in their response to touch. The same is true for the double mutant *cat-1 cat-2*. In order to test for the possibility of synthetic touch insensitivity, involving more than one class of mechanosensory receptor, *cat-1 cat-2* was re-mutagenised. Approximately 20,000 F2's were examined, yielding eight mutants which responded poorly or not at all to touch with a fine hair but which responded normally to more vigorous stimulation with a pointed stick. Upon outcrossing, touch insensitivity was found in every case to be independent of the presence of *cat-1* and *cat-2*. These mechanosensory mutants fall into seven complementation groups and will be described elsewhere.

Catecholamine assays

Several strains of *C. elegans* were grown up on batches of 20 9 cm plates. Their contents of dopa and dopamine are shown in table 1. The mutant *E152*, which has a defective dorsal cord (S. Brenner, unpublished data) but has the normal FIF pattern, is included for comparison. There are no significant differences in the level of dopa, but a clear correlation of the level of dopamine with the intensity of FIF.

Since only limited quantities of nematodes can be grown on plates, more extensive measurements were made on nematodes grown in liquid culture. However, in clearing cultures (containing both adults and larvae) only a slight correlation of dopamine level with FIF was found, because the mutant levels were elevated nearly to those of the wild type. This effect was associated with very high levels of dopa (up to 1,000 ng/g), of which no more than 1% could be accounted for by bacterial contamination. Histochemically, the nematodes were identical with those grown on plates, so that the excess dopa and dopamine were not situated in the neurons; probably the large amount of dopa was hidden in the autofluorescent gut.

TABLE 1

The dopamine and dopa contents of nematodes grown on plates

C. elegans strain	Dopamine (ng/g)	Dopa (ng/g)
wild type	145	16
E152	95	18
cat-1	30	54
cat-4	<10	30
cat-2	<10	26

When liquid cultures are aerated for several days after clearing, the adult nematodes are eaten by internally hatched larvae; the product is a mixture of L2 and dauer larvae which are active and viable but have much reduced gut fluorescence. Dopamine assays on such nematodes gave the results shown in figure 10; dopa, on the other hand, varied randomly from undetectable levels to 200 ng/g. Once again the level of dopamine is correlated with the intensity of FIF.

The identification of the catecholamine was made more certain by recording the excitation and emission spectra of the oxidation product formed in the assay and subtracting the spectra of the non-oxidized blank. The difference spectra so obtained are identical with those of oxidised dopamine. Furthermore, after the desalting procedure described, the catecholamine from 1 g of wild type nematodes was just detectable by chromatography; it co-chromatographed with authentic dopamine, and was absent from extracts of *cat-2*. The presence of dopa was confirmed similarly.

Epinephrine and norepinephrine were

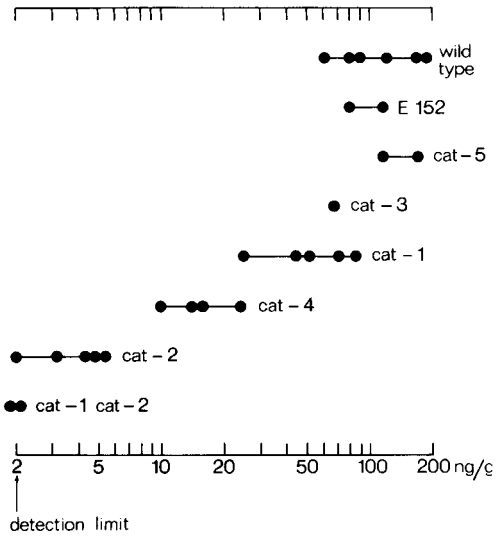

Fig. 10 Dopamine content, in ng/g wet weight, of *C. elegans* larvae grown in liquid culture. Each point represents a separate culture, and coincident points have been separated horizontally for clarity. The abscissa is logarithmic.

not detected in either *cat-2* or wild type larvae at an assay sensitivity of 2 ng/g.

In a larval culture, the average weight of a nematode is 8×10^{-8} g. Therefore, each wild type larva contains about 9×10^{-6} ng of dopamine and each *cat-1* about 4×10^{-6} ng. From measurements on photomicrographs, the total volume of the fluorescent cell bodies of a larva is estimated to be 40 μm^3 on average and the volume of the fluorescent processes about 4 μm^3. Since the intensity of the fluorescence in the cell bodies is approximately the same in *cat-1* and the wild type, it follows that the concentration of dopamine is about 100 $\mu g/g$ in the cell bodies, and about 1,000 $\mu g/g$ in the endings. These figures are intended only to be very approximate estimates, and are in any event subject to variations of perhaps two-fold according to the condition of the nematodes.

Enzymes of dopamine synthesis

Tyrosine hydroxylase was just detectable in clearing liquid cultures; the results were very variable and no consistent differences between the strains were found.

The activity of aromatic acid decarboxylase was consistently 7 ± 2 nM/mg protein/hour at 25°, regardless of the strain and the method of culture.

Ascaris lumbricoides

Little or no FIF can be demonstrated in the larva of *A. lumbricoides* immediately after release from the egg, but during one week's growth on Eagle's medium a characteristic pattern (fig. 11A) gradually develops. By analogy with *C. elegans*, the nerve ring, ventral ganglion, four cephalic cell bodies, two deirid cell bodies and two posterior lateral cell bodies can be recognized; the arrangement of these structures is found to be very similar in the two organisms. In the tail a faint pair of cell bodies is visible, close to the anus.

In the adult, the four cephalic neurons are very easily traced after formaldehyde

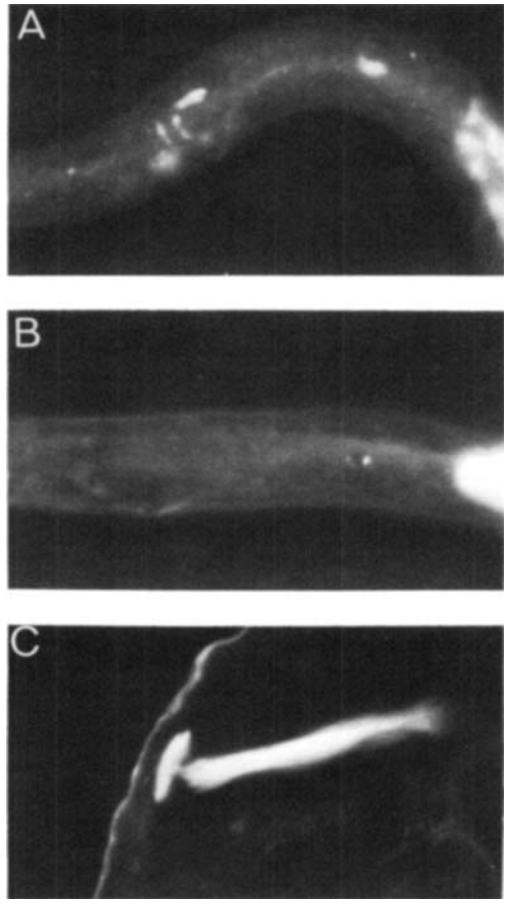

Fig. 11 FIF in *A. lumbricoides*, × 750. (A) Head of 7-day larva, left lateral aspect. (B) Same as (A), but not treated with formaldehyde. (C) Cephalic receptor in adult, with intense FIF, lying beneath autofluorescent cuticle.

treatment, since their entire length is intensely fluorescent. The cell bodies of the dorsal pair lie just behind the nerve ring, and those of the ventral pair just in front; the presumptive sensory endings are beneath the cuticle in the submedian papillae (fig. 11C) and the presumptive synaptic processes form a branched network in the nerve ring. The correlation of these neurons with either of the two sets of submedian papillary neurons described by Goldschmidt ('03, '08) is uncertain. The cephalic sensory receptor corresponds in morphology to his medial submedian receptor, yet it appears to lie laterally in the papilla.

The FIF is green, like that in *C. elegans;* the fluorescence is maximal at an excitation wavelength of ca. 420 nm and an emission wavelength of ca. 480 nm, as expected for the derivative of a catecholamine (Corrodi and Jonsson, '67).

The only other FIF in the head of the adult is in a pair of processes which run in the ventral cord and ramify in the ventral ganglion, apparently without connection to the cephalic neurons. Possibly these are the ventral deirid processes, but the deirid cell bodies, lateral processes, receptors and commissures lack FIF entirely.

The injection of 5 μg reserpine (100 μg/ml in 0.02% acetic acid) into the pseudocoelom halfway between the head and the vulva five hours before sacrifice causes the loss of FIF from the processes in the nerve ring but not from the cell bodies and axons. This indicates that in the ring the catecholamine is largely bound in vesicles, but that elsewhere it is free.

The organization of the tail is rather different from that in *C. elegans*. The male has a pair of prominent neurons which ramify in the preanal ganglion and supply papillae immediately behind the anus. In the female a possibly homologous pair of neurons follow a similar course around the anus, but do not ramify so extensively in the preanal ganglion and end without any specialized structures in the wall of the pseudocoelom.

DISCUSSION

Intensity of FIF is closely correlated with dopamine content in the wild type and several mutants of *C. elegans* (fig. 10); other catecholamines are much less abundant. The dopamine concentration in the nerve endings (ca. 1,000 μg/g) is in the range of catecholamine concentrations in mammalian varicosities (Fuxe and Jonsson, '73); the entensity of FIF in the endings appears comparable with that in the varicosities of mouse iris. Taken together, these observations show that dopamine is responsible for the FIF in the nematode; the distribution of FIF within the neurons implies that dopamine is a neurotransmitter.

Reserpine apparently unloads the catecholamine containing vesicles in the nematodes, just as it does in mammals; presumably, therefore, the two types of vesicle share at least some common features. They differ in appearance, however; the vesicles in *C. elegans* are spherical, about 300 Å in diameter, and lack the dark cores characteristic of mammalian catecholamine containing vesicles; furthermore, they are identical with the vesicles in many other neurons of the nematode (fig. 12).

The chain of varicosities extending from the deirid cell body to the ring (fig. 4C), and the occasional varicosities seen in otherwise invisible processes (figs. 4D,E), are presumably due to loaded vesicles travelling from the cell bodies to the synaptic processes. No evidence for chemical synapses in these areas was found in electron micrographs, but occasional clusters of vesicles were found. The vesicles in the branch leading to the deirid receptor, however, appear to have entered a blind alley — unless the receptor is neurosecretory, which seems unlikely on morphological grounds. It may be that vesicles moving

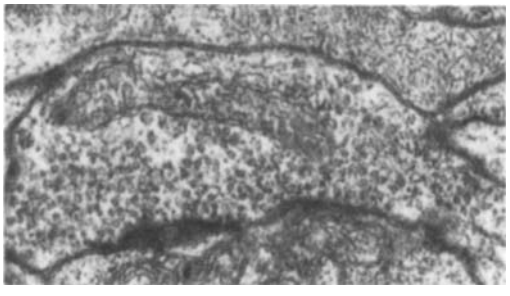

Fig. 12 Dorsal cephalic neuron in *C. elegans*, anterior synaptic ending, × 30,000. The nematode was fixed in 1% osmium tetroxide and embedded in Araldite; thin sections were stained in uranyl acetate and lead citrate.

away from a cell body can find their way into any part of the neuron, regardless of whether or not they are used there. The absence of varicosities from the anterior cephalic processes implies that there is directional control of transport close to the cell body.

The two mutants which lack FIF in the endings are likely to be defective in the production, loading or transport of vesicles. The more extreme mutant, *cat-1*, proved to have vesicles normal in appearance and distribution, and so is likely to be defective in loading.

The cephalic and deirid receptors are club shaped structures lying beneath the cuticle, and so are very likely to be mechanosensory. It is surprising, therefore, that *cat-1* and *cat-2* are no less sensitive to touch than the wild type. Even when the cuticle is explored with the tip of a fine hair, no difference between the responses of the mutants and the wild type can be detected. It may be thought that some synaptic activity can persist, even when the transmitter concentration has been reduced to 3% of the normal level. However, the double mutant *cat-1 cat-2* is likely to have a transmitter concentration less than 0.1% of normal in the nerve endings, and yet it too is just as sensitive as the wild type. A more probable explanation is that the sensitive areas of the dopaminergic receptors overlap with those of others. The outer labial receptors, which lie close to the cephalic receptors and are also club shaped and subcuticular, are already known. In addition, there must be some mechanosensory elements along the entire length of the body to account for its sensitivity, and they may overlap with the deirids. However, since mutagenesis of *cat-1 cat-2* failed to yield synthetic mechanosensory mutants, this interpretation remains speculative.

There are also grounds for expecting a behavioural change in dopamine deficient males, for the six extra neurons are a prominent feature of the male tail. In fact, there is some loss of mating efficiency: in order to produce progeny, *cat-1* and *cat-2* males must be placed on a smaller bacterial lawn and be presented with older (more receptive) hermaphrodites than is necessary for the wild type (J. Hodgkin, unpublished data). However, since there is no obvious change in activity and the mutant males are able to mate eventually, there are probably once again alternative mechanosensory elements.

One trivial explanation for the absence of a clear visible phenotype in these mutants is that dopamine may not be a functional neurotransmitter. Thus, Hanley et al. ('74) have recently presented evidence for the synthesis of both acetylcholine and serotonin by a giant neuron in Helix. In view of the observed concentration of dopamine and its apparent storage in vesicles close to synapses, such an explanation does not seem very likely in this case. We would prefer to conclude that we have just not found the correct environmental conditions for making the phenotypic distinction between wild type and mutants.

In the mutants *cat-1*, *cat-2*, *cat-3*, and *cat-4*, all the dopaminergic neurons are affected in the same way and, by visual or electron microscopic examination, the physical form of the neurons remains unaltered. The lesions are likely, therefore, to affect biosynthesis or storage of the transmitter, at steps which are common to all the neurons. Assays of the two known enzymes of dopamine biosynthesis, tyrosine hydroxylase and aromatic acid decarboxylase, gave no indication of strain differences. However, differences are scarcely to be expected, because the continued synthesis of dopa in *cat-2* and *cat-4* and the high level of aromatic acid decarboxylase in the wild type indicate that both enzymes are involved in other pathways.

The specificity of the FIF reaction and its application to whole mounts of many individuals enable one to appreciate directly the extreme conservatism of the nervous system of *C. elegans*. This contrasts with the catecholaminergic nerve nets in higher animals, where invariance of form of individual neurons would not be apparent even if it existed. Underlying this conservatism, however, is the possibility of limited variation. The ventral cephalic neurons of the two wild type individuals which have been examined by electron microscopy are depicted in figure 5. The ring endings are similar in size and position in the two animals; more significantly the T-junction linking them is unique in location and orientation, and can be picked out from other processes in

the area before its connection to the cell body has been traced. Thus we have an example of a neuron which can form very similar structures by two alternative routes. At the light microscope level, a similar phenomenon is observed in the mutant *cat*-5, in which displacement of the dorsal cell bodies does not affect the pattern of endings in the ring.

The cephalic sensilla in many nematodes depart from the six-fold symmetry of the other papillary sensilla, there being only four. Ward et al. ('75) suggested that on morphological criteria the two deirids could belong to the same set, thus completing the hexaradiate pattern. This is confirmed by the present work, which shows that only this set of six anterior sensory neurons contains dopamine.

In general plan, the nervous system of *A. lumbricoides* appears to be very similar to that of *C. elegans*. The homology of the catecholaminergic neurons lends support to the possibility that, notwithstanding their great disparity in size, there is a strong conservation of the properties of the nervous system between these two nematodes and that the electrophysiological results which could be obtained with *A. lumbricoides* can be usefully extrapolated to *C. elegans*.

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