

PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 37

JANUARY 1970

NUMBER 1

The Sensitivity and Responses of *Rhabditis* sp. to Peripheral Mechanical Stimulation*

N. A. CROLL AND J. M. SMITH

Department Zoology and Applied Entomology, Imperial College, University of London

ABSTRACT: The locomotory response of *Rhabditis* sp. to mechanical stimulation has been described. A technique for quantitating the intensity of the stimulus is outlined and the results of varying the stimulation are given. All stages of *Rhabditis* respond to mechanical stimulation, the direction of the response varying with the location of the stimulus. The whole sense shows adaptation, probably not at the sensory level, and the response is superimposed on the endogenous pacemaker system of the nematode. We interpret the reaction as an escape mechanism from predation, the mechanical threshold of the response approximating to that of contact with mites and predatory nematodes.

Tactile or mechanical stimuli must provide a significant part of the sensory information required by nematodes in their movement, hatching, feeding, mating and for many forms, their penetration into and migrations within hosts. The behavioral or physiological basis of the mechano-sensitivity in nematodes has, however, never been investigated (Lee, 1965). The setae and bristles of marine nematodes have been described as possible mechanoreceptors (Maggenti, 1964) and a peripheral nerve net has been demonstrated in *Deontostoma californicum* connecting the somatic setae (Croll and Maggenti, 1968). Inglis (1963), using the light microscope, observed 'campaniform-type receptors' in marine Cyatholaimidae, but this has been questioned by Wright and Hope (1968), the latter authors using electron micrographs.

Doubtless some of the tactile responses in the biological events listed above are the result of a complicated integration of senses, applying only to certain stages of nematodes. For these reasons we selected a simple, recognizable response: the short-lived locomotory response of *Rhabditis*, elicited by local mechanical stimulation.

Materials and Methods

Rhabditis sp. were originally isolated from leaf litter, in Ascot, Berkshire, U.K. They were cultured on 3–5% water agar; at lower concentrations excessive migration into the agar occurred, a hazard for the experiments. The nematodes carried sufficient bacteria when subcultured to support their growth and reproduction. All stages responded to mechanical stimulation, nevertheless adults gave the most reliable results, because of their greater size, and so were preferentially selected.

Mechanical stimulation of the nematodes by a consistent and measurable force was achieved by using the apparatus figured (Fig. 1). Stimulation was observed through a binocular microscope, and effected by dropping a pin, of known weight (5.0 mg), down a directed glass capillary at 85° to the horizontal. The capillary was marked in millimetres, and an electromagnetic coil was moved up and down the capillary, permitting the pin to be released from a series of measured heights. The coil was wired to a 6 volt dry cell, and included resistances (totalling 8.9 ohms) to prevent overheating of the coil. A switch was included to release the pin by breaking the current, and for drawing the pin back. This design allowed immobile individuals to be found on the agar

* This work was supported in part by a Royal Society grant providing a 16 mm cine camera.

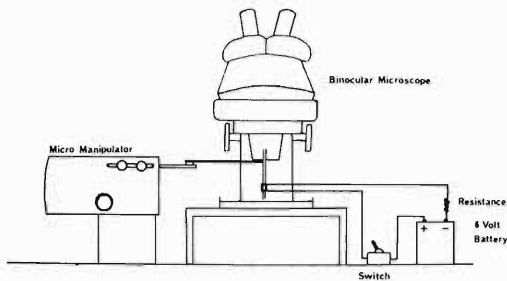


Figure 1. The design for stimulating nematodes, in a known region with a known kinetic force. A marked capillary was moved using the micromanipulator, and the electromagnetic coil was moved along the capillary, the switch released the pin (see text for further details).

cultures, the capillary to be aimed, and the pin released. Only stationary nematodes were used, and when the stimulated worms were activated to move continuously they were disregarded, this occurred in 3% of cases. The distance fallen was used to calculate the velocity in cm/sec., and expressed as kinetic energy in dynes/cm or ergs.

The nematode body was considered as being made up of 14 arbitrarily distinguished regions, each of equal length, from the anterior to the posterior tip. Sometimes it was difficult to be sure that the pin gave a 'square knock,' but we felt that any possible error was constant, and was compensated for in replication. Light elicited a slight photoresponse, when the nematodes were illuminated following a period of darkness, or after storage in dimmer light. A "cold light" of constant intensity was used, and the nematodes were always exposed to light for at least 10 min prior to experimentation.

Regional Sensitivity and Response to a Single Stimulus

Using a fresh individual for each record, a pin was dropped 3.0 cm (with a force of 14.5 ergs) onto each of the 14 regions of the body. At least 25 replications were made for each region. After stimulation, the number and direction of waves of locomotory movement was counted, to the nearest 0.5 of a wave. The waves were sufficiently slow to permit accurate counting through the binocular microscope, and cine films were analysed by frame analysis.

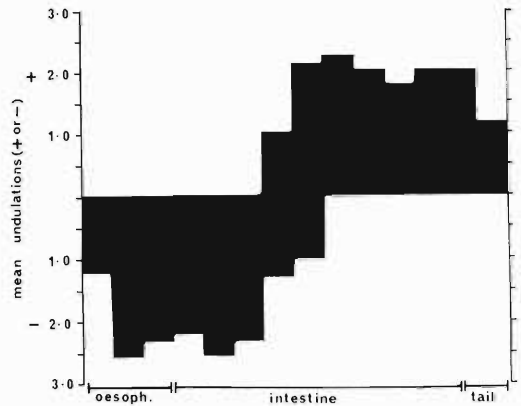


Figure 2. The relative response, forwards (+) or backwards (-), when different parts of *Rhabditis* were given a uniform stimulus. Each worm was immobile, having not been previously stimulated.

Results

All the nematodes responded when stimulated, irrespective of the regions of stimulation, but the extent and direction of the response varied (Fig. 2). If stimulated on the anterior half, the nematodes moved backwards, and they moved forwards if stimulated posteriorly. The direction of the wave was assumed simultaneously by all parts of the nematode, and it would be incorrect to assume that the wave was initiated posteriorly or anteriorly. Around the mid point, movement was forwards or backwards, but almost always unidirectional. Very rarely (3 individuals only) a hit half way down the body caused the waves in each half to move simultaneously in opposite directions. The extent of the response for the anterior regions ranged from 1.0 to 2.5 undulations (mean 2.19), and for all the posterior regions it was 1.0 to 2.2 undulations (mean 2.19). It was therefore treated as being of the same order in both halves. The first and fourteenth regions of the body, as well as the median region showed somewhat lower responses than did the remainder of the body.

Response to Stimulation of Variable Intensity

By dropping the pin from varying heights onto motionless individuals the response in undulations was measured. It was established

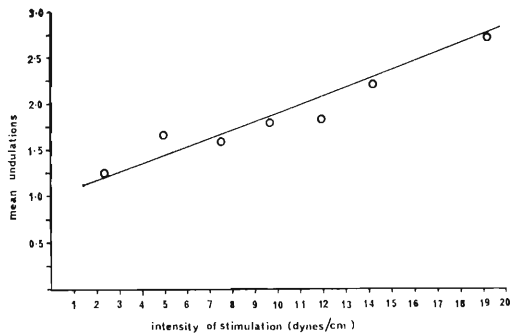


Figure 3. The variation in response, using an increasing intensity of stimulation. All individuals were stimulated at the oesophago-intestinal junction.

above that the response varied with the region of stimulation, all nematodes were therefore stimulated at the oesophago-intestinal junction. Each kinetic force was applied to at least 25 individuals.

Results

While individual responses varied quite widely, there was a consistently higher (and faster) response with an increasing intensity of stimulation. The relationship between ergs and undulations approximated to linearity up to 4 cm or 19.2 ergs (Fig. 3), this being the upper limit of our system.

Adaptation to Successive Stimulation

Individuals were repeatedly stimulated (using a drop of 0.5 cm, or 2.30 ergs) at the oesophago-intestinal junction, each successive stimulus being applied immediately following the response to the previous stimulation. The direction and extent of successive responses to stimulation were observed. Other individuals were stimulated at the tail end, following inactivity through repeated stimulation at the oesophago-intestinal junction. When no longer responsive to successive stimulation at the same point, some individuals were immediately immersed in a drop of hot (approximately 35°C) water, to expose them to a second source of stimulation.

Results

Each anterior stimulus consistently evoked a backwardly directed locomotion. After re-

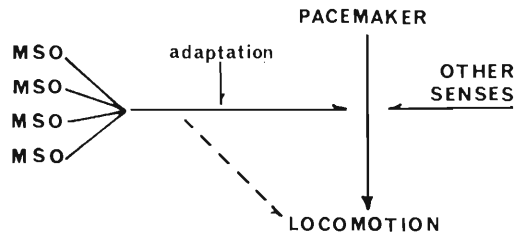


Figure 4. A hypothetical model to explain the relationship between mechanical sensitivity and responses. Mechanical stimulation, from a number of different mechanical sensory receptors, elicits a co-ordinated response, superimposed on the pacemaker. Adaptation occurs in the whole sense, not at sensory level, and does not interfere with other senses. The dotted line represents an alternate system which connects the MSOs directly to motor nerves. (MSO = mechanical sensory receptor)

peated stimulation the nematodes became unresponsive. The mean number of stimuli required before there was no further response was 12, but varied from 4 to 21. The unresponsiveness lasted for about 15 sec, after which a locomotory response again resulted from further mechanical stimulation. Although the number of waves occasionally increased over the number resulting from the initial stimulus, the response always becomes weaker prior to unresponsiveness.

Nematodes that were unresponsive through successive stimulation at the oesophago-intestinal junction, were not responsive when stimulated posteriorly, the whole tactile sense becoming equally unresponsive. Individuals that were continuously moving forwards, could be stimulated to reverse when hit anteriorly. When adapted to successive stimuli, no further response could be observed and the active worms still moved forwards normally.

When unresponsive to mechanical stimulation, the worms responded vigorously to sudden immersion in water, 15°C above the ambient temperature.

Biological Interactions and direct observations

Mites and predatory nematodes in the Mononchidae were added to agar cultures of *Rhabditis sp.* and the interactions between *Rhabditis* and these predators observed.

Results

We found no evidence to support predatory mechanisms in prey locations, all the contacts appeared to be the result of random encounters. Some *Rhabditis* sp. were seen to draw away, both anteriorly and posteriorly, when suddenly contacting mites or predatory Dorylaimoidea, the reaction being more marked with the mites.

Responses of Isolated Pieces

In attempting to investigate the general neural pathways of the mechano-sensitivity, some individuals were cut using a fine pair of scissors and the resultant pieces were mechanically stimulated.

Results

Most pieces of the worm did not respond to mechanical stimulation, the pieces often collapsed, everting all the contents, although there were individuals which did respond after being cut. The anterior pieces largely remained normal in their sensitivity and responses, but most posterior halves collapsed. There were a few definite examples where posterior pieces, severed behind the nerve ring, showed distinct responses to mechanical stimulation.

Discussion

Rhabditid nematodes occur freely in the soil, feeding microbivorously, and are found in large numbers in areas of organic decay. Here they undoubtedly fall prey to other nematodes, mites, dipterous larvae and other small carnivores (Esser, 1963). It is tempting to think that the mechanical response described in this paper enables *Rhabditis* sp. to avoid these predators. Bilaterally symmetrical vermiform organisms tend toward cephalization, but in the sense described here, both extremities evoke a lower response to the same stimulation than the general body, and the sense does not show cephalization (Fig. 2). It is also possible that the fewer undulations resulting from stimulation of the head and tail, which are the narrowest fourteenths, may be due to more near misses or glancing blows, than at the wider parts of the nematode. Such blows would yield a stimulus of somewhat lower intensity, and would result in fewer undulations, in accordance with the data in Figure 3. If the biological basis of mechanoreception is in predator avoidance these observations may relate to

escape from lateral attack, and the probing head would have a higher threshold for its response, as it is continuously exposed to mechanical sensory stimulation. The threshold for the response is very low, being elicited by contact with predatory nematodes and arthropods. The mechanical stimulation required is very small, and we have observed the response being elicited when mites and nematodes touch *Rhabditis* sp.

From our observations we postulate a peripheral nerve system, of connected mechano-receptors, although we were unable to demonstrate a nerve net using the silver technique of Croll and Maggenti (1968). The peripheral sense shows adaptation but not at the receptor level, as an individual adapted in one area shows total adaptation to further stimulation in other regions. Adapted worms do however respond to other stimuli (e.g. heat), or revert to their original state of activity or inactivity. We therefore believe that the tactile sensitivity evokes a response which is superimposed on the innate endogenous rhythm, a conclusion which could be applied to the data for *Ditylenchus dipsaci*, reported by Doncaster and Webster (1968).

The presence of peripheral sensory cells has been established many times, in nematodes (reviewed by Bullock and Horridge, 1965), and the evidence here is that local excitation can propagate for some distance. The response may be a reflex, but the interpretation of direct responses from isolated pieces of nematode, must at best be tenuous, as there must be a gross sensory stimulation. The area of stimulation in each worm was constant, using a pin of constant size, although the extent of the response varied depending on the region of stimulation.

Rhabditids, often largely inactive in agar, are highly mobile in water. It is possible that a thigmo-orthokinesis occurs, when there is a low substrate feedback, leading to a maximum activity when a small area of the body is in contact with a solid substrate.

Neurophysiological work on many nematodes other than *Ascaris* (Jarman, 1959) is lacking, and is often complicated by small size and the nematode hydrostatic skeleton. The morphological and physiological basis of this response will await confirmation by electrophysiologists and electron microscopists.

A hypothetical system for the neural connections of the sense is presented (Fig. 4).

Literature Cited

- Bullock, T. H., and G. A. Horridge.** 1965. Structure and Function in the Nervous System of Invertebrates. Vol. I and II, pp. 1719. W. H. Freeman and Co., San Francisco.
- Croll, N. A., and A. R. Maggenti.** 1968. A peripheral nervous system in Nematoda with a discussion of its functional and phylogenetic significance. Proc. Helm. Soc. Wash. 31(1): 108-115.
- Doncaster, C. C., and J. M. Webster.** 1968. Clumping of the plant parasitic nematode *Ditylenchus dipsaci* in water. Nematologica, 14: 131-136.
- Esser, R. P.** 1963. Nematode interactions in plates of non-sterile water agar. Proc. Soil. Sci. Soc. Fla. 23: 121-138.
- Inglis, W. G.** 1963. 'Campaniform-type' organs in nematodes. Nature, 197: 618.
- Jarman, M.** 1959. Electrical activity in the muscle cells of *Ascaris lumbricoides*. Nature, Lond. 184: 1244.
- Lee, D. L.** 1965. The Physiology of Nematodes. Oliver & Boyd, London.
- Maggenti, A. R.** 1964. Morphology of somatic setae: *Thoracostoma californicum* (Nemata: Enoplidae). Proc. Helm. Soc. Wash. 31(2): 159-166.
- Wright, K. A., and W. D. Hope.** 1968. Elaborations of the cuticle of *Acanthonchus duplicatus* Wieser, 1959 (Nematoda: Cyatholaimidae) as revealed by light and electron microscopy. Can. J. Zool. 46(5): 1005-1011.

Two New Species of Strigeid Metacercariae from an Indian Fresh Water Fish, *Xenentodon cancilla* (Ham.)

K. K. CHAKRABARTI

Department of Zoology, University of Lucknow, Lucknow (India)

ABSTRACT: Two new species of strigeid metacercariae from the Indian fresh water fish, *Xenentodon cancilla*, are described. They were found as concurrent infections in four of 81 fish from the reservoir, Nishatganj Bundha, and the local fish market. Encysted *Neascus hepatica* sp. n. were found in the liver, and encysted *Tetracotyle xenentodoni* sp. n. attached to the mesentery.

Sixty-one specimens of *Xenentodon cancilla* (Ham.) were examined by the writer from September 1966 to January 1968. Most of the fish were obtained from the local fish market, but eight were collected from "Nishatganj Bundha," a reservoir of the Gomti river at Lucknow; of the latter, only two specimens (12 August 1967) were found to have double infection with cysts of strigeid metacercariae. The metacercariae belonged to two separate larval strigeid groups, viz., *Neascus* Hughes, 1927 and *Tetracotyle* Fillipi, 1859. Twenty more specimens of *X. cancilla* were examined from the local fish market and "Nishatganj Bundha"; of these, two from fish market and one from "Nishatganj Bundha" were infected with both species of strigeid metacercariae. This apparently shows the occurrence of double infection in *Xenentodon cancilla* not

to be accidental, but a usual phenomenon in nature. The metacercariae are described here as new species. All measurements are in millimeters.

Neascus hepatica sp. n. (Figs. 1-6)

Cyst

Oval and transparent cysts (Fig. 1) containing the metacercariae were found embedded only in the liver of the fish. They numbered 15 to 25 in individual hosts. Cysts single layered, devoid of pigment, taking light stain with Mallory triple stain, measuring 0.72-0.87 by 0.44-0.52. Metacercarial body much smaller than cyst, consequently metacercaria performs free movements in the spacious accommodation available. Cyst also contains transparent fluid laden with excretory products. When