Electrophysiological recordings of electrical activity and responses to stimulants from *Globodera rostochiensis* and *Syngamus trachea*

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**Summary**

Electrophysiological recordings of electrical activity have been taken from the anterior end of intact adult males of *Globodera rostochiensis* and from the cephalic papillae of intact adult females of *Syngamus trachea*. These are the first recordings from whole nematodes. In both species it was possible to detect a change in the electrical activity in response to the application of stimulants. The possible applications of electrophysiological techniques to nematode neurobiology are discussed.

**Résumé**

Enregistrements électrophysiologiques d'une activité électrique et réponses aux stimulants chez *Globodera rostochiensis* et *Syngamus trachea*

Des enregistrements électrophysiologiques correspondant à une activité électrique ont été obtenus à partir de l'extrémité antérieure de mâles intacts de *Globodera rostochiensis* et des papilles céphaliques de femelles adultes intactes de *Syngamus trachea*. Il s'agit là des premiers enregistrements obtenus à partir de nématodes entiers. Chez les deux espèces on a pu détecter une modification de l'activité électrique en réponse à l'application de stimulants. Les applications possibles des techniques électrophysiologiques à la neurobiologie des nématodes sont discutées.

Electron microscopy has provided a large amount of detail about the fine structure of the sense organs and nervous systems of nematodes (e.g. Ward *et al.*, 1975). Inferences about the functions of the sense organs have been made by examining changes in their ultrastructure during the life cycle (Endo, 1978, 1980). Use of the electron microscope in combination with other techniques has enabled the indirect study of the functional roles of parts of the nervous system. Lewis and Hodgkin (1977), for example, used behavioural mutants of *Caenorhabditis elegans* to examine the roles of various cells in the sense organs. Other workers have used laser ablation systems to destroy individual nerve cells and have been able to assign functional roles to these cells after examining the effect of the operation on the behaviour of the animal (Davis, 1986). However, little direct information on the functioning of the nervous system of nematodes is available.

Electrophysiology has been a valuable tool in the study of the nervous systems and sensory physiology of insects and other organisms since it has allowed the direct investigation of the roles of individual nerve cells in different behaviour patterns (e.g. Masson & Mustaparta, 1990). This technique has rarely been used with nematodes. Intracellular recordings were taken from nerves (Davis & Stretton, 1989a, b) and muscles (e.g. Byerly & Masuda, 1979) of dissected specimens of *Ascaris*. No recordings have been made from the sense organs of nematodes or from intact nematode preparations, partly because it was believed that the high internal pressure associated with the hydrostatic skeleton would cause individuals to burst if an electrode was inserted into their body. However, Tattar, Stack and Zuckermann (1977) found that 80% of *C. elegans* survived for 96 h after being pierced with glass microelectrodes and Kimble *et al.* (1982) and Fire (1986) have used glass microelectrodes to insert messenger RNA into *C. elegans*, some of which go on to reproduce successfully.

As part of a wider research programme on the sensory perception of nematodes including the development of electrophysiological bioassays, this paper reports our initial experiments to record electrical activity and changes in response to known stimuli in the plant parasitic nematode *Globodera rostochiensis*. It also reports our experiments to record directly from the sense...
Fig. 1. Diagram of equipment used to obtain electrophysiological recordings from *Globodera rostochiensis* and *Syngamus tracheae*.

organisms of the animal parasitic nematode *Syngamus tracheae*, chosen because of its unusually large and easily accessible sense organs.

**Materials and methods**

**GLOBODERA ROSTOCHIENSIS**

Adult male *G. rostochiensis* were obtained from plant cultures. Two weeks after planting chitted potato pieces, cv. Désirée, in sterile loam, the plants were inoculated with freshly hatched juveniles of *G. rostochiensis* Ro 1. After three weeks plants were removed, soil was washed from the roots and the plants were placed in supports with the roots in a plastic bowl containing continuously aerated water. Males were syphoned from the bottom of the bowl and used for electrophysiology within 72 h of collection.

Individual adult males were placed in a drop of artificial tap water (Greenaway, 1970) on a cavity slide. The anterior end of a nematode was than sucked into a suction Ag/AgCl microelectrode (made by breaking off the tip of a sharp electrode until the aperture is approximately 40 μm in diameter) connected to a vacuum pump operating at low suction. A sharp Ag/AgCl microelectrode filled with 0.05 M KCl was then inserted into the body of the nematode, as close as possible to the anterior end (Fig. 2 A) and the suction was turned off, thus breaking the seal around the head and allowing test substances to reach the sense organs. Either electrode could be used as the recording electrode. Substances known to elicit responses were presented to the nematode by placing a few drops of the appropriate solution into the liquid surrounding the nematode. D-Tryptophan (1 mM), which *C. elegans* is known to avoid (Dusenbury, 1975), was used as a repellant. Sex pheromone extracted by the method of Greet, Green and Poulton (1968) was used as an attractant.

Electrical signals detected were amplified up to 1 000 times using a Grass P 16 microelectrode amplifier, displayed on a Medelec FOR-1004 cathode ray oscilloscope (CRO) and stored on magnetic tape (Fig. 1). Permanent copies of traces were made using a Siemens Oscillomink L chart recorder. A successful preparation was recognised when a complex signal consisting of spikes of various magnitudes was detected. Suitable settings of the recording instruments were made initially and never altered during a recording period. Before treatment with a stimulant a trace was recorded to establish amplitude and frequency of spikes and recording was continued to determine changes in electrical activity after stimulation. The duration of recordings was between 5 and 10 minutes. Recordings were made from over 25 individuals. Differences in traces were recognised qualitatively but were also quantified by counting frequency and amplitude, the latter in arbitrary units depending on the gain settings initially chosen for the preparation.
Fig. 2. A: Adult male *Globodera rostochiensis* with a suction microelectrode around the anterior end and a sharp microelectrode inserted into the body; Scales bar = 200 micrometres. B: Diagram of the head of *Syngamus trachea* with a recording microelectrode on a cephalic papilla. Diameter of head region = 0.75-1 mm depending on age of adult.

Fig. 3. Electrophysiological recordings from the anterior end of the body of intact adult males of *Globodera rostochiensis* showing normal spike activity from unstimulated adults (A) and activity before (B) and after (C) exposure to D-Tryptophan.
Fig. 4. Electrophysiological recordings from the anterior end of the body of adult males of *Globodera rostochiensis* showing spike activity before (A) and after (B) exposure to female sex pheromone.

**SYNGAMUS TRACHEA**

Adult worms (between 14 and 28 days post-infection) were extracted from the tracheae of young rooks, *Corvus frugilegus*, and were stored for up to 5 days at a temperature of 27 °C in Hanks' balanced salt solution (BSS) containing 1% glucose and 0.8% bicarbonate. Adults of *S. trachea* are unusual, the male being attached to the much larger female (Fig. 1); all recordings were from the female. Paired adults were immobilised in a small amount of Hanks' BSS on a wax bed using fine gauge entomological pins. A recording microelectrode was then placed on or as near as possible to the cephalic papillae (Fig. 2 B) which in this species contain nerve endings from the inner labial, outer labial and cephalic sensilla (Jones, 1975). The indifferent (or non-recording) electrode was placed into the solution surrounding the nematode (Fig. 1). Mammalian blood was used as a stimulant and was presented by placing a few drops into the solution surrounding the nematode. The resulting electrical signals were amplified, displayed and recorded in the same way as for *G. rostochiensis*. Recordings were made from over ten females.

Changes in electrical activity were detected in response to both D-Tryptophan (Fig. 3 B, C) and sex pheromone (Fig. 4 A, B); in both cases more spike activity was recorded after stimulation than before.

Individual nematodes vary in their electrical activity. For example the recordings of two nematodes before stimulation may be different (e.g. Fig. 3 A, B). Therefore interpretation of the results concentrates on changes observed before and after stimulation of each individual.

One trace was also obtained showing spikes of regular amplitude occurring at regular intervals (4 spikes s⁻¹) which is characteristic of a pacemaker neurone (Fig. 5).

**SYNGAMUS TRACHEA**

Electrical activity was recorded directly from the sense organs of this species. Spike activity was recorded from the cephalic papillae (Fig. 6 A), the first recordings of electrical activity from the sense organs of a nematode. As a control, the recording electrode was also placed into various points on the cephalic inflation away from the sense organs where it is known that there is no nervous tissue present (Jones, 1975); no electrical activity was recorded at any of these points.

A change in electrical activity in the cephalic papillae was detected in response to blood, the main food source of the adult females, when it was introduced into the liquid surrounding the nematode (Fig. 6 B, C). The response was recorded as an increase in spike frequency (from approximately 23 spikes s⁻¹ to 66 spikes s⁻¹ in the

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**Results**

**GLOBODERA ROSTOCHIENSIS**

Electrical activity was recorded from the anterior end of the body of intact *G. rostochiensis*. Spikes were recorded from unstimulated individuals (Fig. 3 A).
Electrical activity in *Globodera rostochiensis* and *Syngamus trachea*

**Fig. 5.** Recording from *Globodera rostochiensis* showing spikes of regular amplitude occurring at regular intervals, characteristic of a pacemaker neurone. The trace was drifting and was corrected where indicated (arrow).

example shown in Fig. 6) with no apparent change in spike amplitude.

**Discussion**

The small size of adult male *G. rostochiensis* (approximately 1 mm long) made direct recording from the sense organs impossible. Intracellular recordings similar to those made by Davis and Stretton (1989a, b) on *Ascaris* were also not possible. However, extracellular recordings of electrical activity inside the body have been obtained and changes in electrical activity have been recorded in response to the presence of stimulants. It is therefore possible that the technique could be developed for use as a bioassay for the presence of naturally occurring stimulants, such as sex pheromones, and their analogues. Characterisation of these chemicals would then be more straightforward since there would be no need for behavioural assays.

The recording of spikes of regular amplitude occurring at regular intervals is characteristic of a pacemaker cell, i.e. a cell capable of independently generating rhythmic electrical activity. Such cells may control the activity of muscles or of other neural circuits. Recordings from muscle cells of *Ascaris* have been obtained with a similar activity pattern and a similar spike frequency (Jarman, 1959). Whether the recording obtained from *G. rostochiensis* is from the same type of cell as the recordings from *Ascaris* is uncertain.

*S. trachea* is much larger (up to 15 mm long), and hence much easier to manipulate than *G. rostochiensis* and its anterior sense organs are more easily accessible. These attributes have enabled us to record directly from the cephalic papillae of this nematode in an approach similar to that used to record from insect gustatory receptors (e.g. Wadhams, Angst & Blight, 1982; Frazier, 1986). The traces obtained in this way from *S. trachea* are likely to be recordings of electrical activity in the whole cephalic papilla rather than recordings from the individual dendritic processes. However the results, which are similar to those that can be obtained from insects, indicate that a more extensive direct study of the functioning of the sense organs of this nematode should be feasible. Such a study may include analysis of the spikes recorded to ascertain whether more than one type is present, and if so whether different populations of spikes respond to different stimulants as is the case in some insects (Blight et al., 1989). It may also be possible in future to record from the amphids of *S. trachea*; these are deep inside the body of the nematode making them less accessible than the cephalic papillae but they are unusually large (Jones, 1979), which may facilitate accurate insertion of an electrode. If the system could be developed to allow recordings of amphidial activity as well as activity in the cephalic papillae the large size and unusual life cycle of this nematode, during which it encounters a wide range of environmental conditions and has to respond to a wide range of stimulants, may make it an excellent model for the study of nematode sensory physiology.

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Fig. 6. Electrophysiological recordings from the cephalic papillae of intact adult females of *Syngamus trachea* showing normal spike activity from unstimulated individuals (A) and spike activity before (B) and after (C) exposure to blood.

**REFERENCES**


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