Feeding and wounding responses in *Hydra* suggest functional and structural polarization of the tentacle nervous system

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Abstract

The nervous system of *Hydra*, a freshwater cnidaria, occurs as dispersed, or diffuse, nerve net throughout the animal. It is widely accepted that in a diffuse nervous system an external stimulus is conducted in all directions over the net. Here I report observations that *hydra* tentacles respond to feeding and wounding stimuli in a unidirectional manner. Upon contact of a tentacle with a brine shrimp larva during feeding, tissue on the proximal side of the point of contact contracted strongly, whereas tissue on the distal side contracted only very weakly. Feeding a tentacle to which a second tentacle was grafted to the proximal end in the reversed orientation showed that unidirectional conduction, once initiated, was blocked by the reversal of polarity, demonstrating that the distal to proximal polarity of tissue is crucial for unidirectional conduction. Unidirectional conduction was obtained also by mechanically pinching the tissue. The response of tentacles devoid of neurons examined was bidirectional, demonstrating that the nervous system is responsible for the unidirectional responses. These observations suggest that polarized property of the nerve net in *hydra* tentacles is responsible for the unidirectional tentacle contraction. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

A common view in neurobiology is that diffuse nerve net of *hydra* represents the most primitive nervous system (Matthews, 1997). The nerve net is a diffuse, two-dimensional network of nerve cells which combine sensory and motor functions. The nerve cells which comprise the net are diverse in morphology, represented by multipolar, bipolar and unipolar processes extending from the cell body (Epp and Tardent, 1978). The processes are not differentiated into axons and dendrites although they have ending in synaptic junctions (Mackie, 1990; Robinson, 1998). *Hydra*'s nervous system is under construction and disruption continuously, because epithelial tissue of *hydra* is continuously dislocated towards and sloughed off at both apical and basal ends (Mackie, 1990; Campbell, 1967). To compensate the loss of nerve cells, new differentiation of nerve cells occurs constantly (Mackie, 1990).

External stimuli applied to this system are assumed to spread in all directions over the net (Matthews, 1997). It has also been generally accepted that the diffuse system is found typically in animals having radial symmetry while a centralized system is found in animals having bilateral symmetry (Matthews, 1997). There are, however, observations which contradict the viewpoint of a diffuse nerve net in *hydra*. First, TEM observations showed that ganglion cells in the tentacles are connected to one another by synaptic junctions,
which often have synaptic vesicles only on one side of the junction, thereby allowing unidirectional conduction of the stimulus between two nerve cells (Westfall et al., 1971; Westfall, 1996). Second, the morphological observation of nerve cells showed that there are bipolar but asymmetrically shaped neurons distributed in the animal (Epp and Tardent, 1978). Third, when prey is caught by a tentacle during the feeding response, contraction of the tentacle was observed only on the proximal side of the tentacle, that is the side closer to the head (Josephson, personal communication; Rushforth and Hofman, 1972; Rushforth, 1973). These observations suggest that hydra tentacles have the property of unidirectional conduction. In an attempt to investigate the basic physiological properties of hydra's nervous system, I examined the contractile behavior of hydra tentacle. Also, the involvement of nervous system in response to feeding and wounding was examined. Results obtained support the view that unidirectional conduction occurs in the hydra diffuse nerve net. This unidirectional response is inconsistent with the widely accepted view that unidirectional conduction first arose during metazoan evolution in animals with bilateral symmetry.

2. Materials and methods

2.1. Strains and culture

A wild type strain of H. magnipapillata (strain 105, Sugiyama and Fujisawa, 1977) was used. Animals were cultured in modified ‘M’ solution (Takano and Sugiyama, 1983) in 500 ml beakers at 18.0±0.5 °C. They were fed daily with brine shrimp larva (Artemia nauplii) (Shin-Toa Koeki Co Ltd.) and were transferred to fresh culture medium 2–3 h after feeding. Polyps used for the experiments were collected 24–30 h after the last feeding.

Animals devoid of nerve cells, termed epithelial hydra, were generated by treatment of animals of 105 strain with colchicine (Campbell, 1976). Once produced, they were hand fed and maintained as described by Campbell (1976).

2.2. Feeding and wounding stimuli

Tentacles were excised from adult animals and placed in the culture medium for approximately 1 h before being used in the experiments. Feeding stimulus was elicited by placing a brine shrimp larva on a tentacle with a glass micropipette. The feeding response was also stimulated by exposing a tentacle to 5×10⁻⁶ M glutathione (Lenhoff, 1961). The response of tentacle and body column tissue to a wounding stimulus was examined by pinching the tissue with a pair of forceps. Care was taken so that the wound did not crush the tissue and separate it into two pieces.

2.3. Grafting tentacles

Grafting of tentacles to one another was carried out using human hair of approximately 60 μm in diameter. The two tentacle tissue pieces were threaded onto the hair and pressed from both ends using small squares of parafilm. The basic process was similar to the procedure of grafting of body column tissue (Shimizu and Sugiyama, 1993).

2.4. Recording and analyzing pattern of feeding and wounding responses by VCR

The feeding and wounding responses of tentacle and wounding response of body column were recorded under dissecting microscope with a VCR. The whole process was analyzed later as digitally captured still images (Digital Still Recorder DKR700, Sony Corp.). After determining that a tentacle was lying flat on the bottom of plastic dish, the axial length of tentacle tissue was measured on the captured still images.

3. Results

When a brine shrimp larva contacts the tentacle of a hydra, nematocytes in the battery cells of the tentacle ectoderm are discharged into the brine shrimp thereby capturing it, and attaching the brine shrimp to the tentacle. Subsequently, the tentacles bend towards the mouth and the captured shrimp is ingested. The initial steps of this feeding response were initiated by attaching an Artemia to the surface of a tentacle using a thin plastic tube. An immediate response to the attachment of a brine shrimp larva to the tentacle was the extensive contraction of the side of the tentacle proximal to the prey attachment site (Fig. 1A–C). The extent of contraction on the proximal side was significantly greater than on the side distal to the point of attachment (Fig. 1C and Fig. 2a). A similar pattern of contraction was obtained at all positions.
of prey attachment in the tentacle (data not shown). The tentacle remained contracted for 10–30 min followed by release of the brine shrimp larva and subsequent elongation of it to the length before contraction.

Treatment of animals with glutathione is known to elicit the feeding response, including the movement of the tentacles towards the mouth as well as the opening of the mouth in the hypostome (Lenhoff, 1961). The proximal contraction of the tentacle was also induced by injecting a glutathione solution of $5 \times 10^{-6}$ M onto the surface of tentacles (Fig. 1D–F), suggesting that the response was provoked, at least in part, by chemical stimulus by glutathione and not solely by physical contact of Artemia with tissue surface.

The contraction of the tentacle proximal to the site of attachment of the brine shrimp larva suggests the existence of a unidirectional conduction of stimulus. To further examine this possibility, two isolated tentacles were grafted to one another with their distal/proximal polarities either in alignment or in opposite directions (Fig. 3A,D). Twenty-four hours after grafting, an Artemia was attached to the distal end of the grafts (Fig. 3B,E). In grafts with opposite polarities contraction occurred on the distal side, but not on the proximal side of the graft junction (Fig. 3C). In contrast, in grafts in which the distal/proximal polarities were aligned, contraction spread across the graft junction along the entire grafted tentacle (Fig. 3F). These results suggest that distal-to-proximal conduction of feeding response is a stable and intrinsic property of tentacle tissue.

Response of a tentacle to mechanical stimulus was examined by pinching a region of the tentacle approximately 100–150 $\mu$m in axial length of tissue using a pair of forceps, and was termed pinching response. This stimulus also provoked a
polarized response as in the feeding response (Fig. 1G–I), whereby the part of tentacle proximal to the pinched site contracted extensively. The part distal to the pinched site also contracted, but to a significantly lower extent (Fig. 1I and Fig. 2B). Overall, a tentacle reacted more quickly to the pinching response than to the feeding response (data not shown). Further, in response to pinching, the whole tentacle slowly diminished in size, most likely due to the tissue damage provoking the leakage of tissue medium (data not shown). To determine whether a mechanical stimulus in other parts of the animal would also provoke a unidirectional response as in the tentacles, the body column was pinched at a position (Fig. 4A,B). The apical side and basal side contracted to a similar extent (Fig. 4C), demonstrating that unidirectional contraction and conduction is restricted to tentacle tissue. The same tendency was seen at all positions along the body column (data not shown).

To determine if the unidirectional conduction as part of the feeding response is due solely to the nervous system, or due to both the nervous and epithelial systems, the following experiment was carried out. A graft was constructed consisting of a normal distal half, and a proximal half that was nerve-free (Fig. 3G). If unidirectional conduction is due solely to the epithelial cells, then capturing an Artemia by the normal distal part of the tentacle (Fig. 3H) should result in contraction of both the normal distal half and the nerve-free proximal half of the tentacle. As a result, contraction occurred in the normal distal half, but not in the nerve-free proximal half (Fig. 3I). In contrast, grafts consisting of normal tissue in both the distal and proximal halves of a tentacle contracted to a similar extent in both halves as already mentioned (Fig. 3D–F), demonstrating that the nervous system is responsible for the unidirectional contraction.
Involvement of the nervous system in the pinching response was also examined using nerve-free tentacles (Fig. 1J–L). In the majority of cases, the site of pinching thickened but no overall contraction of the tentacle was observed (Fig. 1L). The limited extent of contraction on one side appeared to be a little greater than on the other side, but never as great as in normal tentacles (data not shown). These observations support the view that nervous system is crucial for the unidirectional pinching response. In some cases, the whole tentacle contracted, presumably because of the damage of leaking intracellular fluid out into the surroundings.

4. Discussion

To summarize, we examined the pattern of response of tentacles to feeding and pinching, as well as the response of body column tissue to pinching stimulus. The response in tentacle tissue was unidirectional in a distal-to-proximal direction (Fig. 1). This unidirectional response was not observed in the body column tissue (Fig. 4). Analysis of these treatments using nerve-free animals indicated that the nervous system was involved in these responses in the tentacles (Fig. 3G–I). These observations indicate that the nerve net in tentacles is polarized functionally in a distal-to-proximal direction. This possibility is consistent with previous observation made by Westfall with TEM (Westfall et al., 1971; Westfall, 1996). Nerve cells of *hydra* extend processes in two or more directions to make contact with other cells via synaptic junctions (Epp and Tardent, 1978). They found that the junctions in *hydra* tentacles are, in many cases, asymmetrical where dense cored vesicles are found only on one side of the junction, thus, providing a structural basis for unidirectional conduction. These TEM results coupled with the functional tests presented here raise the possibility that *hydra* nervous system in the tentacles is both structurally and functionally polarized in distal-to-proximal orientation enabling unidirectional conduction.

A question remains as to what this unidirectional response of the fed tentacle means physiologically. Obviously, the contraction and bending of tentacle toward the hypostome after feeding shortens the distance between the prey and hypostome, helping the contact of the prey with the hypostome. In addition, unidirectional contraction keeps the distal part of the fed tentacle intact, thus, enabling the capture of more Artemia on this part of the tentacle. Overall contraction of the tentacle, if it occurs, could make the search of more Artemia relatively difficult by the shortening of the tentacle. Thus, the unidirectional conduction and contraction could be evolutionally of advantage. Neurologically, unidirectional conduction of stimulus into the head could be related to the fact that nerve cells are most densely populated in *hydra* head as the most elementary brain system as also proposed in *Podocoryne carnea* (Groeger and Schmid, 2000), although there has not yet been any direct proof. The tentacle nervous system, which transmits external stimulus into the head region might, thus, represent afferent nerves to the central nervous system.

Although freshwater *hydra* is a highly diverged member of phylum Cnidaria, the animal has been considered a typical example of good correlation between the radially symmetrical body plan and diffuse nervous system opposite to the correlation between bilaterally symmetrical body plan and central nervous system (Matthews, 1997). This comes from the view that the control of behavior of bilateral animals is easier, with the concentrated nervous system in the middle showing bilateral
symmetry in nervous system itself (Matthews, 1997). The present results suggest that even in radially symmetrical animals the centralized nervous system is of greater advantage than completely diffuse system as to, for instance, capturing prey.

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