

# Origin and Evolution of the Nervous System Considered from the Diffuse Nervous System of Cnidarians

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## Abstract

Cnidarians have the most primitive nervous system in animal kingdom. Diffuse nerve net (called “diffuse nervous system”) covers the whole body. Cnidarians are considered the earliest metazoans to have evolved a nervous system and therefore offer possible insight into some of the fundamental properties of early nervous system. Recent studies show the cnidarian nervous system is much more complex than it was expected before. Genome projects and molecular developmental biological studies clarified indistinctness between Radiata and Bilateria. As to the nervous system, the situation is the same, namely there are many similarities between two animal groups. The cnidarian nervous system has all fundamental components with the molecular, morphological, and functional aspects. This might be also the case for the central nervous system, namely cnidarians are suggested to have primitive central nervous system.

I will make comprehensive description of the cnidarian nervous system based on morphological, functional, developmental studies in many biological levels from gene and molecule to individual, and compare with the concentrated nervous system of bilaterians. Subsequently, I will discuss the origin and evolution of the nervous system.

## Keywords

Nervous system • Evolution • Cnidarian • Nerve ring • Neuropeptide

## 6.1 Introduction

### 6.1.1 Diversity of Nervous Systems (Fig. 6.1)

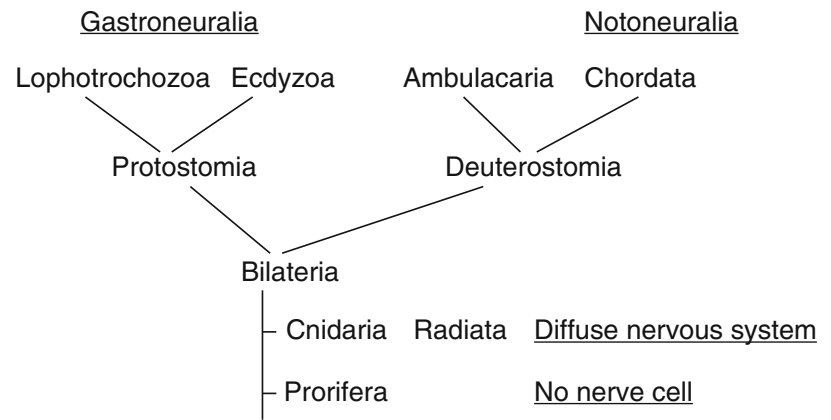
Nervous systems in animal kingdom are very diverse. Figure 6.1 shows the phylogenetic tree of animals’ nervous systems. Chordata including vertebrates have main neuronal components in the dorsal side, which is called notoneuralia. The evolution of this nervous system, tubular nervous system has started due to the emergence of a neural tube. Acadian in Urochordata and amphioxus in Cephalochordata has dorsal neural tube. Other Deuterostomia, Ambulacaria include Echinodermata and Hemichordata, have simpler nervous

systems. For example starfish in Echinodermata has a nervous system composed of a nerve ring and radial nerves, called radial nervous system.

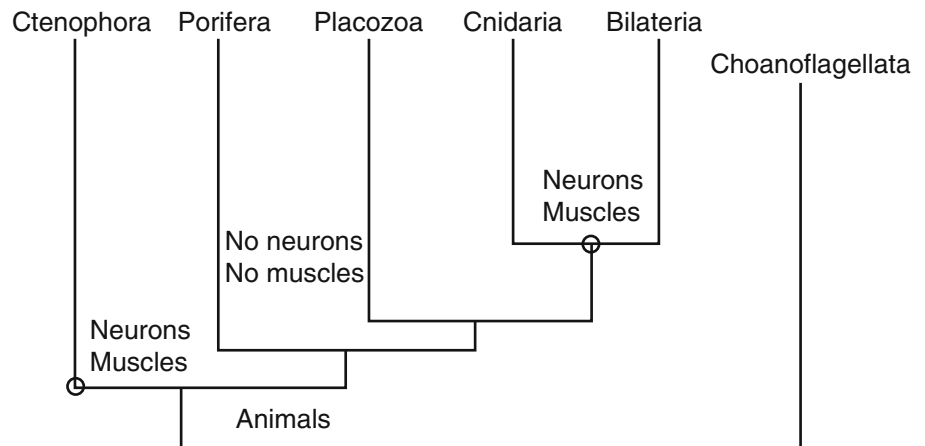
Protostomia among invertebrates have main neural components in the ventral side, which is called gastroneuralia. Insects in Ecdyzoa have micro-brain, which is a masterpiece of information processing equipment with small size, light weight, and low cost. This nervous system is called ladder-like nervous system, which is typical nervous system in gastroneuralia. Mollusca in Lophotrochozoa have modified type of ladder-like nervous system, called tetra neural nervous system. Cephalopoda including octopus and squid have large brain, which is called “vertebrate-like mega brain”. An earthworm in Annelida has typical ladder-like nervous system, and planarian *Dugesia japonica* has orthogonal nervous system, which is primitive type of ladder-like nervous system. Cnidaria have simple nervous system, diffuse nervous system, and Porifera have no neurons.

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**Fig. 6.1** Phylogenetic tree of the nervous systems of animals



**Fig. 6.2** Unique phylogenetic position of ctenophores and hypothesis of independent appearance of nervous systems in ctenophores and cnidarians. *Two circles* indicate emergences of the nervous system (Modified from Moroz et al. 2014)



### 6.1.2 Ctenophore's Hypothesis (Fig. 6.2)

Cnidarians and ctenophores (comb jellies) used to belong to one animal group, coelenterate, among basal metazoans traditionally, because they both have jelly-shaped forms and neurons. However, Cnidaria and Ctenophora are now divided into different phyla after many differences were recognized between the two animal groups. Moreover, very recently Moroz et al. (2014) showed that ctenophores are remarkably distinct from other animals in their content of neurogenic, immune and developmental genes. Their integrative analyses place Ctenophora as the earliest lineage within Metazoa. This hypothesis is supported by comparative analysis of multiple gene families, including the apparent absence of HOX genes, canonical microRNA machinery, and reduced immune complement in ctenophores.

Many bilaterian neuron-specific genes and genes of 'classical' neurotransmitter pathways either are absent or, if present, are not expressed in neurons in ctenophores. Hence it has been proposed that the nervous systems originated independently in Ctenophora and Cnidaria (Moroz et al. 2014). The nervous system of ctenophore is different from bilate-

rian nervous system, but those of cnidarian and bilaterian are similar (Watanabe et al. 2009).

### 6.1.3 Characters of Cnidarians

Recent molecular biological studies of cnidarian have given rise to doubt the clear distinguished between cnidarian and bilaterian. Cnidarian were considered to be a different animal group from the other higher animals, bilaterian. Cnidarian shows radial symmetry, and is diploblastic without the mesoderm, which are called radiate. On the other hand, all higher animals are bilaterian, and show bilateral symmetry with a dorsal-ventral axis, and are triploblastic with a mesoderm.

Genome projects of *Nematostella* and *Hydra* show genome sizes of these cnidarians are much larger than of nematode (*Caenorhabditis elegance*) or a fruit fly (*Drosophila melanogaster*) and comparable to vertebrates. Properties of genome structure of cnidarians were more similar to mammals than nematode and fruit fly (Putnam et al. 2007; Chapman et al. 2010).

Although cnidarians lack obvious mesoderm, many jellyfish have musculature with similarities to striated muscle in triploblastic animals at both ultrastructural and molecular levels. The presence and expression patterns of genes related to those involved in mesoderm and muscle specification in triploblastic animals were observed during jellyfish (hydrozoan medusa; *Podocoryne carnea*) muscle development. Similar results were observed in a sea anemone, *Nematostella vectensis* and coral, *Acropora millepora* (Ball et al. 2004; Martindale et al. 2004).

Expression of genes involved in patterning the dorsal-ventral (DV) axis were observed in cnidarians. The expression pattern of a gene clearly related to *dpp/Bmp4* – the key determinant of the DV axis in bilateral animals – has been asymmetrical during the embryonic development of *A. millepora* (Ball et al. 2004) and *N. vectensis* (Matus et al. 2006).

Many properties of bilateral symmetry could be observed in cnidarians. For example, formation and the structure of septa or mesenteries in the coelenteron of sea anemone, and rhopalial nervous system of box jellyfish *Tripedalia cyctophora*, show clear bilateral symmetry (Berking 2007; Skogh et al. 2006).

## 6.2 Cnidarian Nervous System

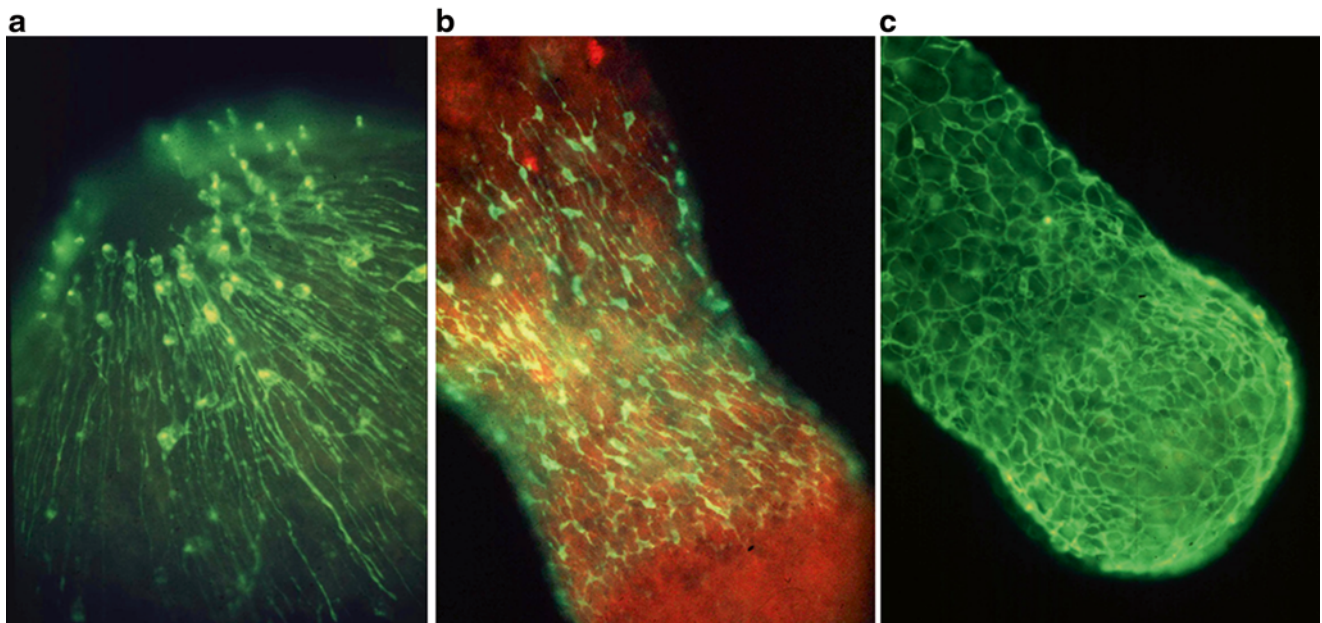
### 6.2.1 Cnidarian Nervous System (Fig. 6.3)

Immunohistochemistry using neuropeptide antisera and monoclonal antibodies specific to hydra neurons on whole

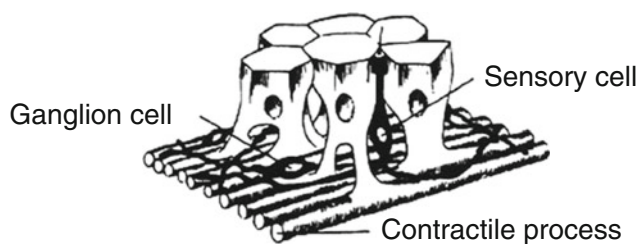
mounts has made it feasible to study the nerve net of hydra (Grimmelikhuijzen 1985; Dunne et al. 1985). These studies have shown that the hydra nerve net contains numerous subsets of neurons and that the spatial distributions are highly position-specific (Fig. 6.3). Numerous subsets of neurons containing different neuropeptides and several subsets of neurons defined by monoclonal antibodies were noted (Grimmelikhuijzen et al. 1982, 1995; Grimmelikhuijzen 1985; Dunne et al. 1985; Koizumi and Bode 1986, 1991; Koizumi et al. 1988; Yaross et al. 1986). The regional distribution of each subset tends to be constant (Koizumi and Bode 1986; Koizumi et al. 1988; Bode et al. 1988).

### 6.2.2 Neuron's Morphology: Sensory Cell and Ganglion Cell (Fig. 6.4)

The nerve net contains two types of nerve cells, ganglion cells and sensory cells (Mackie and Passano 1968; Davis et al. 1968; Koizumi and Bode 1991; Grimmelikhuijzen and Westfall 1995). Ganglion cells lie close to the muscle processes at the basal ends of the epithelial cells. Sensory cells have elongated cell bodies that extend from the level of the muscle processes in an apical direction and an elaborate ciliary cone at the apical end of the cell body (Fig. 6.4) (Westfall 1973; Westfall and Kinnamon 1978; Bode et al. 1988; Koizumi and Bode 1991; Grimmelikhuijzen and Westfall 1995).



**Fig. 6.3** Cnidarians nervous system visualized with different antibodies: a monoclonal antibody, JD1 (a), antiserum against neuropeptides, RFamide (b), antiserum against neuropeptides, GLWamide (c). a is hypostome of hydra, and (b, c) are foot part of hydra



**Fig. 6.4** Organization of a cnidarian epithelium and two types of nerve cells, a sensory cell and a ganglion cell (Modified from Mackie and Passano 1968)

### 6.2.3 Electrical Conduction

Most unique feature of the nerve cell is rapid conduction of electrical signals called electrical conduction along a nerve fiber. In cnidarians, differentiation of axon and dendrites is not observed. All nerve fibers are called neurites. However, action potentials and electrical impulses have been observed extracellularly and intracellularly from neurons and neurites (Horridge 1954; Bullock and Hrridge 1965; Josephson 1974; Mackie and Meech 1985; Anderson and Schwab 1983; Mackie and Meech 1995). Voltage dependent sodium channel was also reported, although some characters are different from other animals, such as TTX (tetrodotoxin, toxin of puffer fish)-insensitivity or very slow processes of repolarization of action potential (Anderson and Schwab 1983; Anderson 1987, 1989; Spencer et al. 1989; Anderson et al. 1993; Spafford et al. 1999; Barzilai et al. 2012). Voltage dependent potassium channel has also been demonstrated at electrophysiological and gene levels (Meech and Mackie 1993; Grigoriev et al. 1997; Klassen et al. 2008).

### 6.2.4 Chemical Transmission

Presence of the chemical directional synapse in cnidarian nerve nets are currently recognized (Anderson and Spencer 1989). Successive studies at ultrastructural level by Westfall's group contributed a lot to establish the presence of the chemical synapse in cnidarians (Westfall et al. 1971; Westfall 1973; Westfall and Kinnamon 1978; Kinnamon and Westfall 1982; Westfall 1987). Together with Westfall and Grimmelikhuijzen I also demonstrated that a neuropeptide is present only in neuronal dense-cored vesicles in hydra. Using immunogold electron microscopy, RFamide-peptide was present only in vesicles in nerve cells (Koizumi et al. 1989). Moreover, evidences for the presence of chemical synapse are accumulated at physiological and molecular levels today (see Grimmelikhuijzen and Westfall 1995 for review).

*En passant* synapses are general rather than synapse of the nerve terminal in cnidarian nerve net, and neurosecretory

nonsynaptic release of neurotransmitters was also observed in cnidarians. Bidirectional synapses and electrical synapses have also been demonstrated in addition to directional chemical synapse in cnidarians (Anderson 1985; Anderson and Spencer 1989; Spencer 1989).

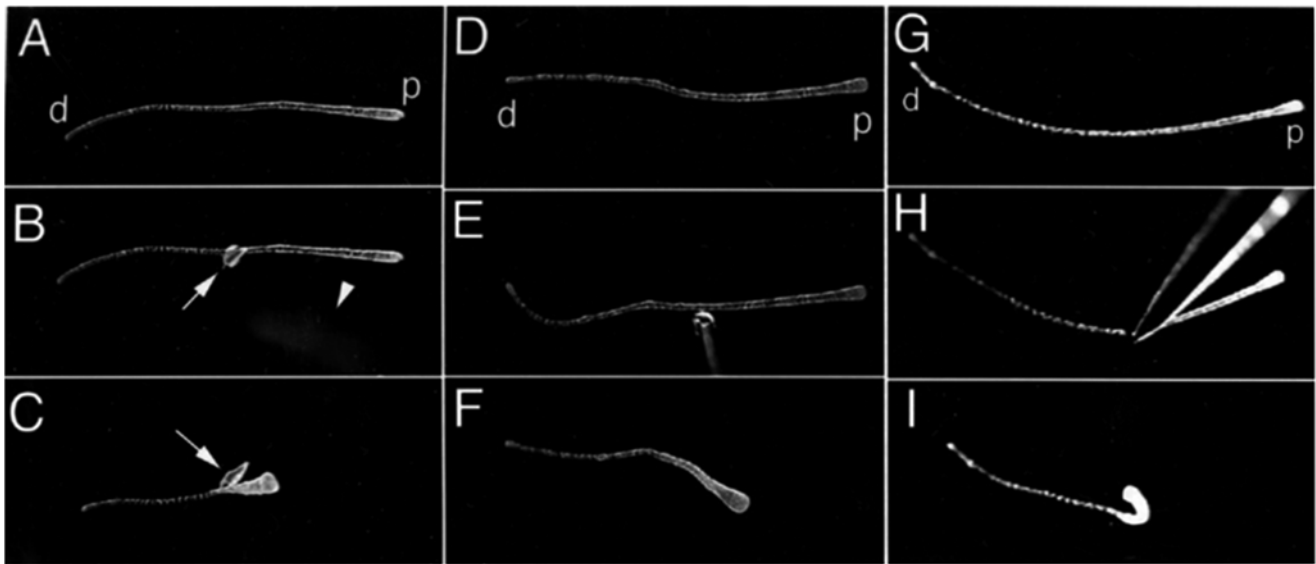
Various neuropeptides were reported as neurotransmitters in cnidarian nervous system. Grimmelikhuijzen and his collaborators contributed to establish the idea that peptides are major neurotransmitters in cnidarian nervous system (see Grimmelikhuijzen et al. 1989; McFarlane et al. 1989; Grimmelikhuijzen and Westfall 1995 for review). Peptide-gated ion channel was also identified in hydra (Golubovic et al. 2007), which suggests Hydra-RFamide neuropeptides work as first neurotransmitters in hydra.

Hydra peptide project also demonstrated that a huge number of peptide works as neurotransmitters in hydra. The project involves a systematic, large-scale screening of the peptide signal molecules in hydra, by adopting a novel comprehensive approach. Individual peptides were purified systematically to homogeneity without any biological assays using HPLC. The isolated peptides were subjected to structural analysis and then synthesized chemically. The synthetic peptides were then subjected to a series of biological tests to examine their functions in hydra. During the process, antibodies against each peptide were produced, and their locations were clarified. Half of peptides have been found to be neuropeptides, while half were epitheliopeptides. We estimated 200 neuropeptides in hydra (Takahashi et al. 1997, 2000, 2003; Yum et al. 1998; Morishita et al. 2003, see Koizumi 2002; Takahashi et al. 2008 for review).

Classical transmitters, such as acetylcholine and monoamines have been demonstrated in some species, but with many exceptions (see Ancia 1989; Grimmelikhuijzen et al. 1989; Scemes 1989 for reviews). However, analysis of neurotransmitter-related genes has shown cnidarians have all transmitters present in mammals (Watanabe et al. 2009).

### 6.2.5 Directional Conduction of Excitation (Fig. 6.5)

It is widely accepted that in a diffuse nervous system of hydra an external stimulus is conducted in all directions over the net. However, Shimizu (2002) reported 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. 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



**Fig. 6.5** One directional conduction of nerve response in a tentacle of hydra. Stimuli of *Artemia* (a–c), or glutathione (d–f), mechanical pinching (g–i) were applied on the middle of excised tentacle. a, d, and g: before stimulation, b, e, and h: on stimulation, and c, f, i: responses

after stimulation. Responses conduct one way only into proximal (p) direction, but not into distal direction (d). Tentacle excised from nerve free hydra showed no responses by application of these stimuli (Shimizu 2002)

for the unidirectional responses. These observations suggest that polarized property of the nerve net in hydra tentacles is responsible for the unidirectional tentacle contraction.

### 6.2.6 Non-directional Epithelial Conduction: Gap Junction Between Epithelia-Muscular Cells

One of the remarkable features of cnidarians nervous system is non-nervous epithelial conduction. Cnidarian epithelial cells are also muscular cells, called epitheliomuscular cells. Epithelial cells are connected with gap junction. Propagation in nonnervous epithelia is typically all-or-none, nondecremental, and unpolarized (Josephson 1974; Josephson and Schwab 1979). The spread of excitation in conducting epithelia is associated with effector responses. The nervous system interacts with the conduction system. Spontaneous activity appears to originate in the nervous system (Mackie 1965; Mackie and Passano 1968; Satterlie and Spencer 1987).

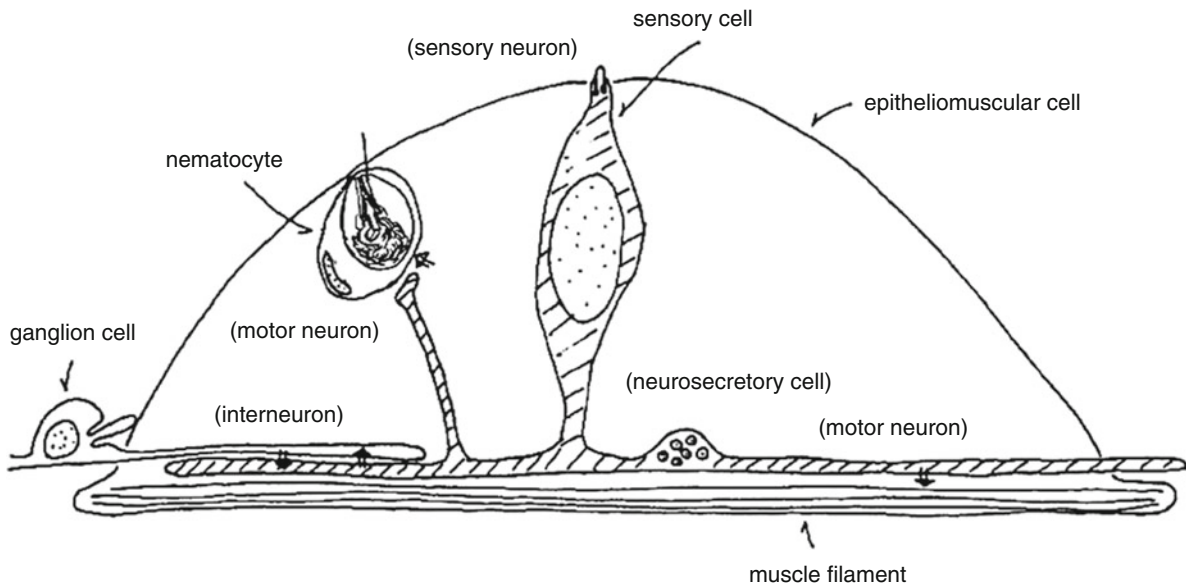
### 6.2.7 Multifunctionality of Neurons in Hydra (Fig. 6.6)

The nervous system of hydra has several unique features. The most remarkable of these is the multifunction of neurons. Each neuron in hydra possesses the entire repertory of nerve-cell functions (Westfall 1973; Westfall and Kinnamon 1978), i.e., the neurons are all sensory-motor-interneurons

with neurosecretory granules. For example, a sensory cell has sensory cilia as a sensory neuron, synaptic connections to the muscle layer as a motor neuron, synaptic connections to neurites or the cell body of a ganglion cell as an interneuron, and aggregations of granules in non-synaptic regions of proximal sites of the cell body as a neurosecretory cell (Fig. 6.6) (Westfall and Kinnamon 1978). Ganglion cells have the same features (Westfall 1973). Moreover, as is shown in Fig. 6.6, sometimes a single neuron innervates two different types of effectors: muscle fibers and a nematocyte (Westfall et al. 1971; Grimmelikhuijzen and Westfall 1995).

### 6.2.8 Distinction Between Endodermal and Ectodermal Nervous System

The presence of nerve cells of hydra has been demonstrated in the endoderm as well as in the ectodermal nerve net (Lentz 1968; Davis 1972; Westfall and Townsend 1977; Epp and Tardent 1978; Westfall and Rogers 1990; Westfall et al. 1991; Murate et al. 1996). However, the nerve nets visualized so far by neuropeptide antisera are all ectodermal nerve nets (Fig. 6.3). To examine the endodermal nerve net, we made inside-out hydras and visualized the endodermal nerve net using immunocytochemistry. Using a monoclonal antibody to  $\alpha$ -tubulin, we could visualize endodermal nerve net. However, we never detected endodermal nerve cells using neuropeptide antibodies. Neuropeptide antibodies show only an ectodermal nerve net. Hence, these experiments reveal a remarkable difference between the ectodermal and endoder-



**Fig. 6.6** Multifunctional nerve cells in hydra. A single sensory cell has synaptic connections to the muscle sheet of an epitheliomuscular cell, a nematocyte, and a ganglion cell. Moreover, it has sensory cilia and neu-

rosecretory granules. The *arrows* show synapses and their polarities (Koizumi 2002)

mal nervous system in regard to neuropeptide expression (Koizumi et al. 2004).

### 6.2.9 Sensory System (Fig. 6.12)

Cubozoan have elaborate sensory systems, rhopallia, at the margin of the bell. The system has camera eyes like mammalian eyes and a statocyst shown in Fig. 6.12. This jellyfish species, *Tripedalia cystophora* have four rhopallia connected with the nerve ring (Garm et al. 2007). The neuronal organization in rhopallium is like ganglion and is used for integration of plural sensory information containing light and gravity stimulus (Garm et al. 2006, 2007; Nilsson et al. 2006; Skogh et al. 2006).

All cnidarian medusae have eye-spots, and even cnidarian polyps have sensory cells. There is evidence that all cnidarian including polyps have sensitivity to light and gravity (see Rushforth 1973b; Josephson 1974; Satterlie 2011 for reviews).

### 6.2.10 Motor System

Many physiological studies show the evidence of neuromuscular system in cnidarians (Bullock 1943; Hoyle 1960; Singla 1978; Spencer 1979; Weber et al. 1982; Spencer 1982; Kerfoot et al. 1985).

In hydra, ectodermal epitheliomuscular cells have longitudinal muscles and endodermal digestive cells have circular muscles in the proximal part of the cell. Contraction of lon-

gitudinal muscle causes contraction of the polyp, and contraction of circular muscle causes elongation of the polyp. Both are antagonistic to each other. When we want to separate two layers of hydra, procaine, an activator of muscle contraction, is applied to the body column. Simultaneous contraction of both layers give us separated layer: a contracted ectodermal ring and an elongated endodermal bar.

Micro-connections between ectodermal muscle sheet and endodermal muscle sheet crossing through mesoglea were observed, which suggest the structure to carry out the antagonistic interactions between both muscles (Takahashi-Iwanaga et al. 1994).

## 6.3 Functional Ability of the Diffuse Nervous System

### 6.3.1 Hydra's Behavior

Hydra has many abilities to perform neuronal functions that might be comparable to those performed by the central nervous system of bilaterians (Rushforth 1973a, b). For example, inhibitions and modifications of feeding behavior in satiated hydra were observed (Koizumi and Maeda 1981; Blanquet and Lenhoff 1968). Figure 6.4 shows the rise of feeding threshold in satiated hydra. Habituation was also observed in hydra by Rushforth (1973a, b). Habituation (inhibition of response by repeated neutral stimuli) is not due to adaptation of sensory receptor or fatigue of motor muscle, but due to function of central nervous system.

### 6.3.1.1 Locomotion

Hydra shows a various modes of locomotion although it usually has sessile life as other polyps. Surprisingly, as many as seven different forms of locomotion have been reported. They are inchworm or caterpillar movements, walking on substratum using tentacles, somersaulting, positive gliding downward from water surface, floating by means of gas bubble, active gliding along substratum on base with the aid of tentacles and body movements, active gliding along substratum on base without aid of tentacles and body movements, walking along water surface film (for review, Rushforth 1973a). During this locomotion hydra selectively uses a specific one type of four nematocysts.

### 6.3.1.2 Feeding Behavior (Figs. 6.7 and 6.8)

Feeding activities in hydra, like several modes of locomotion, consist of a series of complex behavioral sequences. These sequences of the capture and engulfment of *Artemia salina* nauplia by hydra are enumerated as (1) nematocyst discharge; (2) tentacular movement; (3) mouth opening, creeping over the prey and closure; (4) inhibition of endogenous tentacle and body contractions (Fig. 6.7a). Hydra use two types of nematocyst in the first step of feeding behavior;

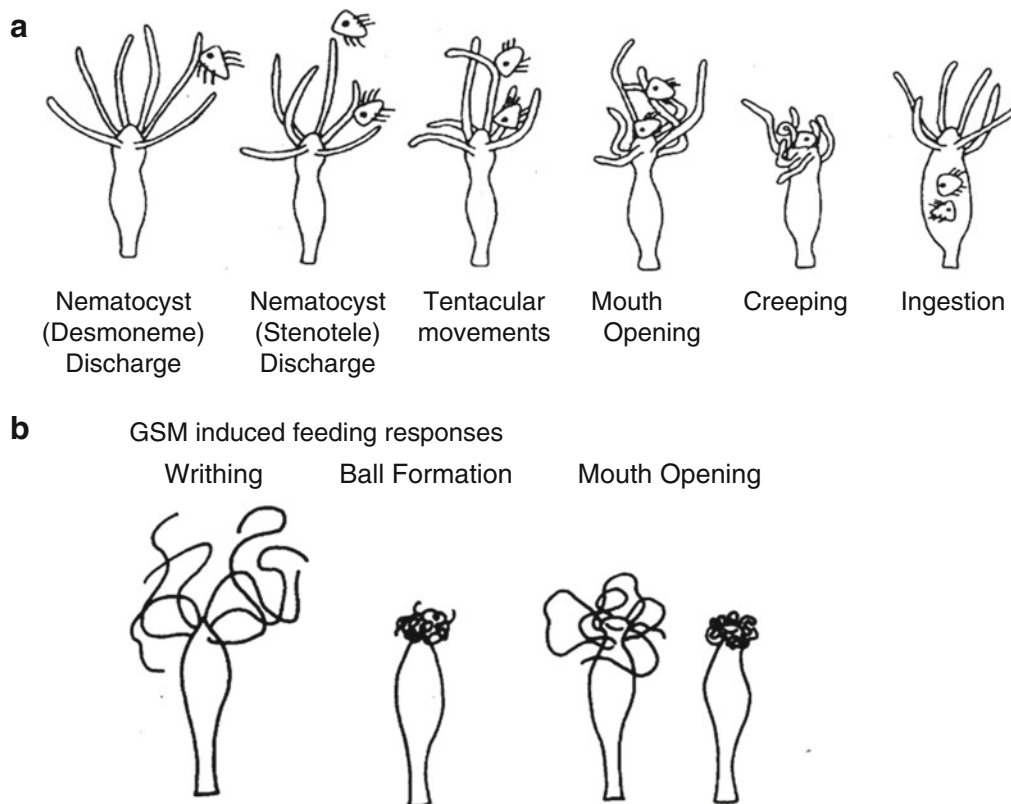
desmoneme to capture preys and stenotele to kill preys by injection of toxin from the tip of discharged thread.

Discharge of nematocysts is evoked by mechanical stimulus to a cnidocyte, but chemical stimulus modifies the discharge. A nematocyte is mechano- and chemo-sensory cell in addition to effector cell. The nematocyte is elaborate masterpiece, and is found only in cnidarians, and is not found in other animal groups.

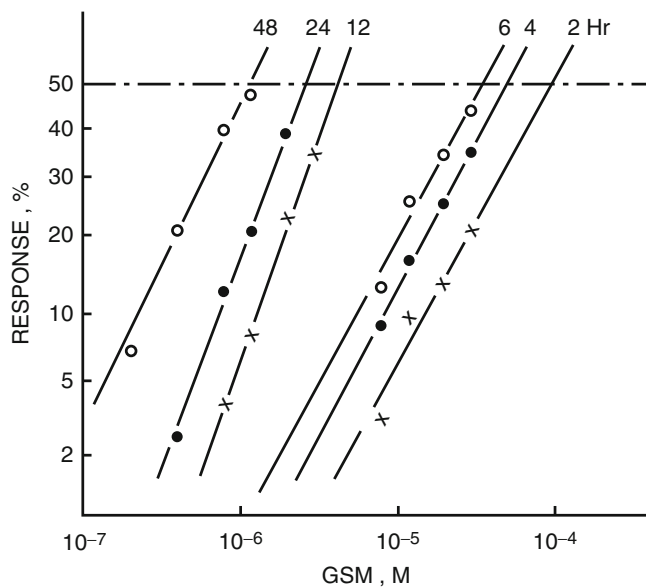
Second and third steps of feeding behavior, tentacular movements and mouth opening, are evoked by feeding stimulus, glutathione released from the injured hole of prey (Fig. 6.7b).

Central nervous system is involved in the control of feeding responses in bilaterians, e.g., fly or mouse. Various modifications of feeding responses are observed in hydra in a similar manner to fly or mouse. Various types of inhibitions of feeding responses were observed in hydra. Rise of threshold of feeding stimulus in satiated hydra (Fig. 6.8), elongation of time to ingest a brine shrimp in satiated hydra, inhibition of nematocysts discharge, and so on (Koizumi and Maeda 1981; Smith et al. 1974).

Neck formation is also modification of feeding behavior appeared in satiated hydra. Hydra that still contains partially digested food in their gastrovascular cavity undergoes a



**Fig. 6.7** Feeding behavior of hydra. (a) Feeding behavior of ingestion of *Artemia* brine shrimps. (b) Feeding responses induced by chemical feeding stimulus, S-methyl-glutathione (GSM)



**Fig. 6.8** Inhibition of feeding response in satiated hydra. Shift of regression lines of feeding response along the abscissa by shortening the starvation periods. Vertical axis indicates fractions of feeding response (feeding stimulus-induced mouth opening response), and abscissa indicates log concentrations of feeding stimulus, S-methylglutathione (GSM). Number attached with each line indicates the starvation periods (hours). Concentration where 50% response is obtained is the mean value of the threshold (Koizumi and Maeda 1981)

modified feeding response when it ingests additional prey. A localized constriction is formed just below the sites of tentacle attachment, preventing the loss of gastrovascular contents. This response involves the interaction of an ectodermal receptor system for glutathione and an endodermal receptor system for tyrosine (Blanquet and Lenhoff 1968).

### 6.3.1.3 Habituation (Fig. 6.9)

Rushforth (1965, 1967, 1973a, b) reported hydra, *Hydra pirardi* and *H. viridis* have the ability of habituation, a primitive type of learning as shown in Fig. 6.9. The contraction response of the animals to intermittent mechanical stimulation was represented by a response curve: a plot of the proportions of a group of hydra contracting at various rotation speeds (Fig. 6.9a, response curve). If hydras are exposed to repeated stimulation at fixed rotation speed, they become less responsive to continued exposure (Fig. 6.9b, habituation curve). After habituation, a lowering of the response curve is observed (Fig. 6.9c). Recovery from the habituation needs 4 h (Fig. 6.9d).

Increasing the strength of the stimulus produces a slower rate of habituation. Contractions are readily evoked in hydras habituated to mechanical stimulation by a different stimulus, that of light, which is similar to dishabituation. These observations show this process is not considered to be one of muscular fatigue. Rushforth (1973a, b) argues the lowering the sensitivity to mechanical agitation fit to criteria of habituation. Habituation is recognized as a function of brain in higher animals, bilaterians, and as a primitive learning.

### 6.3.1.4 No Classical Conditioning

We have no evidences of associative learning of cnidarian's nervous system. Classical conditioning was also not demonstrated. This nervous system might not reach the level of complex learning although it has abilities of integration of plural sensory information and of memory (see Rushforth 1973a, b; Mackie 2004 for review).

### 6.3.1.5 Autonomic Nervous Functions

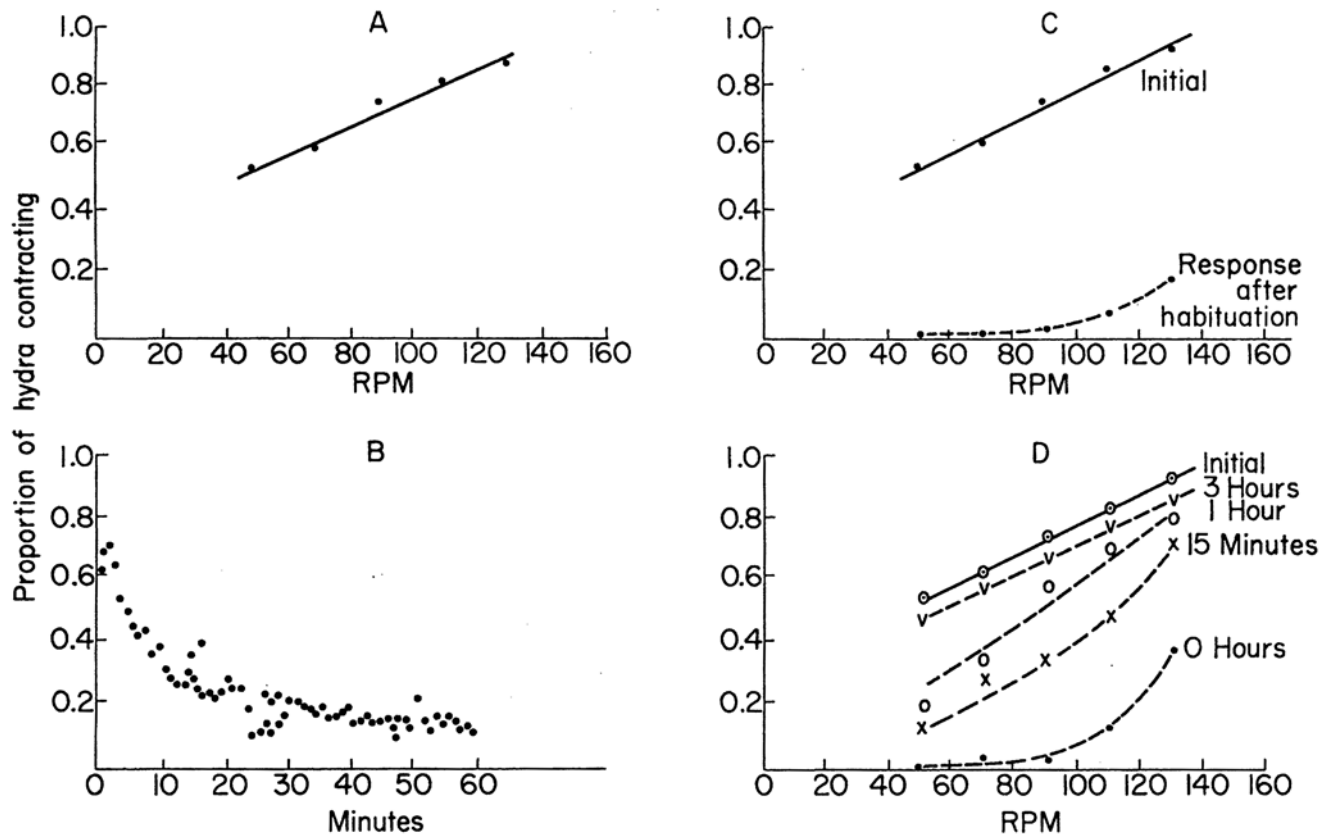
The mammalian digestive tract undergoes various digestive movements such as peristalsis and segmentation movement. A widely accepted view has been that, early in evolution, the digestive process was static based upon diffusion, and later it became dynamic involving digestive movements. Here, we report digestive movements which occur in hydra. We find that the body column of hydra undergoes a series of movements when fed with *Artemia*. Comparison of the movements to those in mammals showed similarities in appearance to esophageal reflex, segmentation movement, and defecation reflex. When nerve cells were eliminated, polyps did not show these movements, demonstrating that the diffuse nerve net in the body column of hydra primarily regulates the movements just as the netlike enteric nervous system does in mammals (Shimizu et al. 2004).

Moreover, antagonistic interactions between sympathetic nervous system and parasympathetic nervous system were demonstrated in hydra (Shimizu and Okabe 2007). Autonomic regulation is most developed in mammals, in which a part of peripheral nervous system, termed the autonomic nervous system plays the dominant role. Circulatory activity and digestive activity in vertebrates change in opposite phases to each other. The stage where circulatory activity is high and digestive activity is low is termed the “fight or flight stage” while the stage where circulatory activity is low and digestive activity is high is termed the “rest and digest stage”. It has been thought that the autonomic nervous system originated in early vertebrate phyla and developed to its greatest extent in mammals. They compared the pattern of change of circulatory and digestive activities in several invertebrates and found that the two stages seen in mammals are also present in a wide variety of animals, including evolutionarily early-diverging invertebrate cnidarian taxa. They proposed a novel possibility that the basic properties of the autonomic nervous system were established very early in metazoan evolution (Shimizu and Okabe 2007).

## 6.3.2 Cnidarian's Behavior (Fig. 6.10)

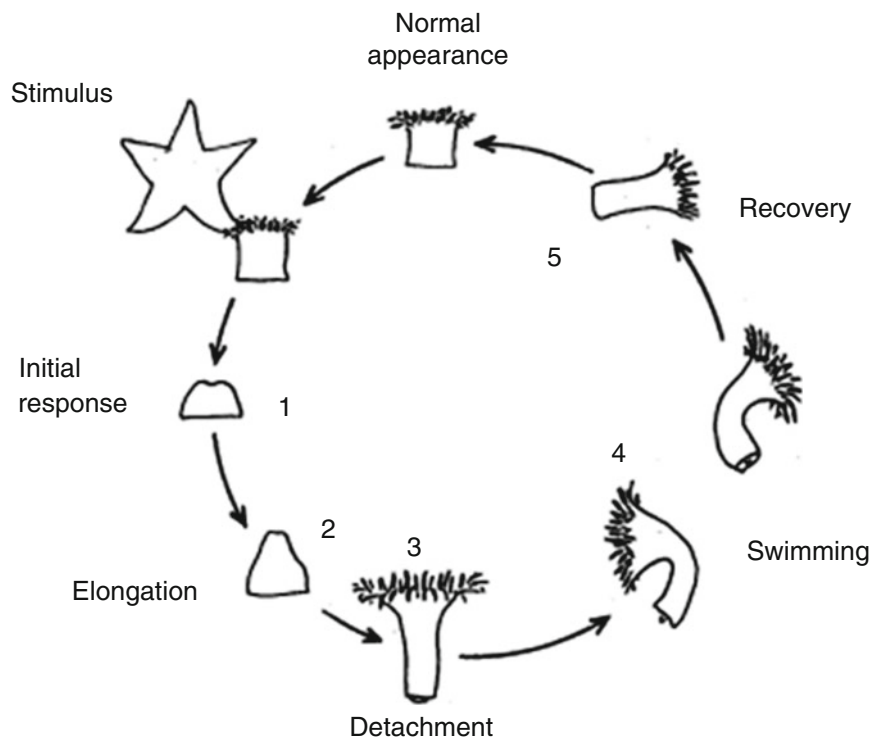
Other groups of cnidarians show wide variety of more complex behaviors. For example, a sea anemone, *Stomphia coccinea*, shows complex swimming behavior when it contact with a starfish as shown in Fig. 6.10 (Robson 1961). The behavior after stimulus, the contact with a starfish, is com-





**Fig. 6.9** Habituation of contraction response by intermitted mechanical stimulations. (a) Response curve, (b) Habituation curve, (c) Response curve after habituation, (d) Recovery curve after habituation. See text in detail (Rushforth 1973a)

**Fig. 6.10** Sea anemone behavior after contact with a starfish (Robson 1961)



posed of the following responses: initial response, elongation, detachment, swimming, and recovery. The behavior is initiated by chemoreception from starfish.

Another example is various actions of hydrozoan medusa, *Stomatoca*. They are swimming, crumpling (protective involution), tentacle posture, pointing (unilateral reciprocal flexing of the manubrium and margin), and visceral movements (Mackie and Singla 1975). There are now many examples of series of complex behaviors of cnidarians, as bilaterians show (see Rushforth 1973a, b; Spencer and Schwab 1982; Passano 1982; Satterlie 1985; Arkett and Spencer 1986; Shelton 1982 for review).

## 6.4 Developmental Characters of Cnidarian's Nervous System (Fig. 6.11)

### 6.4.1 Hydra's Developmental Dynamics of Diffuse Nervous System

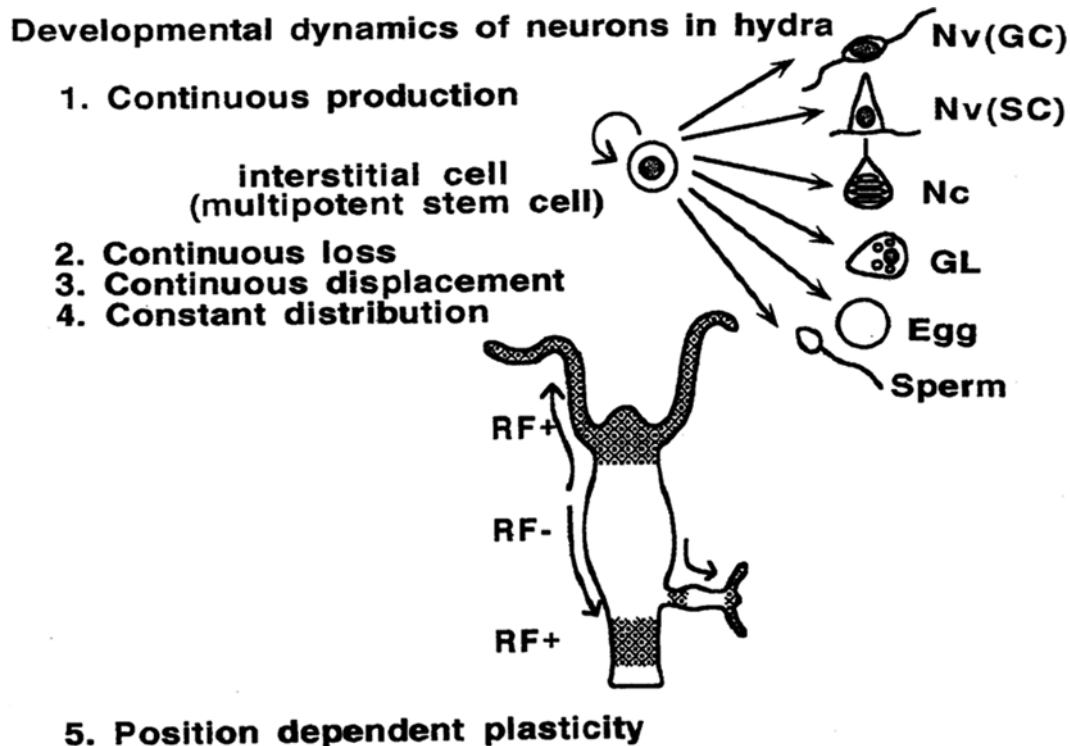
Hydra has three types of cell lineages: an ectodermal epithelial cell lineage, an endodermal epithelial cell lineage, and an interstitial cell lineage. The interstitial cell lineage is composed of interstitial cells, nerve cells, nematocytes, gland cells, and gametes. Interstitial cells are multipotent stem cells, committed precursors, and differentiating

intermediates (David and Gierer 1974; Bode and David 1978; Bode 1996).

In an adult hydra, nerve cells are produced continuously by constant differentiation from interstitial cells (Bode et al. 1988; David and Hager 1994). Nerve-cell production in the nerve net is balanced by a loss of neurons at the extremities and by the supply of neurons to young buds. Therefore, neurons are continuously changing their axial location by moving with epithelial cells either towards the apical end (the apex of the hypostome or the tip of a tentacle) or towards the basal end (the basal disk) (Campbell 1976 1973; Bode et al. 1988; Bode 1992). However, the distribution of each subset of neurons expressing a certain neural phenotype is maintained (Bode et al. 1988; Bode 1992).

How is the constant nerve net maintained in spite of the active growth dynamics in hydra described above? In experiments related to this question, it was demonstrated that neurons can change the expression of FMRF amide-like peptide and vasopressin-like peptide depending upon their position in hydra (Koizumi and Bode 1986, 1991). Moreover, it was demonstrated that ganglion cells were converted to sensory cells when the neurons were moved from the body column to the hypostome (Koizumi et al. 1988). These dynamic features of neurons in the adult hydra correspond to properties of developing nerve cells in embryos of higher animals.

Hydra has high capacity to regenerate: if it is decapitated, new head appears within a few days, and head-specific nervous



**Fig. 6.11** Developmental dynamics of neurons in hydra. See text in detail

system reappears at the same time, which can perform the normal feeding behavior (Koizumi 2002). These active features of reorganization and plasticity of neurons are remarkable in cnidarians as described as ‘Hydra is steady state embryo’.

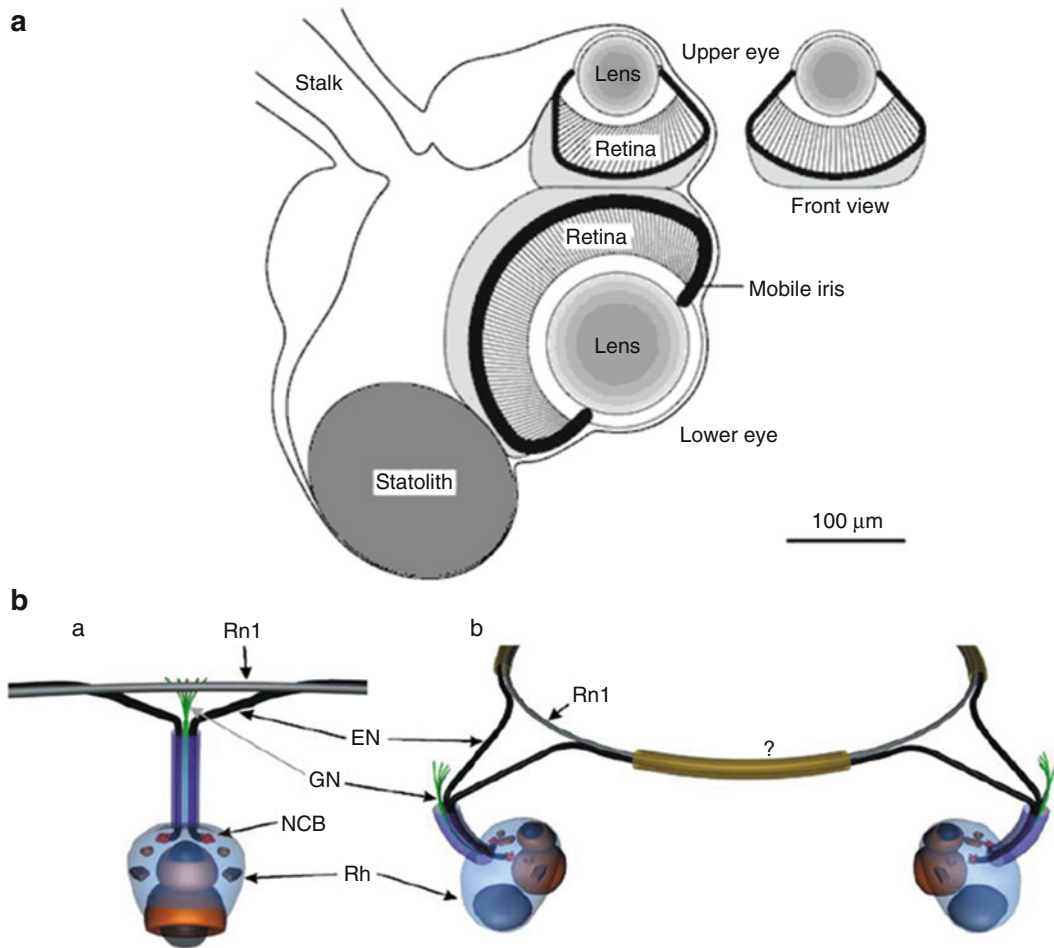
#### 6.4.2 *Nematostella*: Model Animal of Developmental Neurobiology

A sea anemone, *Nematostella* is a new model animal in cnidarians, because it has an advantages to carry out developmental biology of embryo very easily. Molecular developmental biology of this animal now is successful and productive. As to developmental neurobiology, importance of this animal has begun to be recognized (Technau and Steele 2011; Nakanishi et al. 2011; Layden et al. 2012; Watanabe et al. 2014).

### 6.5 Central Nervous System of Cnidarians

#### 6.5.1 Cubozoa Rhopalium-Ring Nerve Complex (Fig. 6.12)

A nerve ring connecting the rhopalia has been reported in cubozoan medusae (Garm et al. 2006; Nilsson et al. 2006; Skogh et al. 2006; Satterlie 2011). Garm et al. (2007) wrote: “What are the distinctions, if any, between the peripheral and the central nervous system in cnidarians? This is by no means a trivial question, especially since cnidarians possess multifunctional neurons. Here, we define the central nervous system as the part of the nervous system in which several information channels are integrated and processed, with an output signal being produced to control other parts of the body, e.g., the motor neurons. The receptor cells and motor neurons therefore make up the bulk of the peripheral nervous system. The rhopalial nervous system situated within the



**Fig. 6.12** Rhopalia and nerve ring in a cubozoan medusa, *Tripedalia cystophora*. (a) Complex eyes in a rhopalium. (b) Nerve ring connecting rhopalia (Nilsson et al. 2005; Garm et al. 2006)

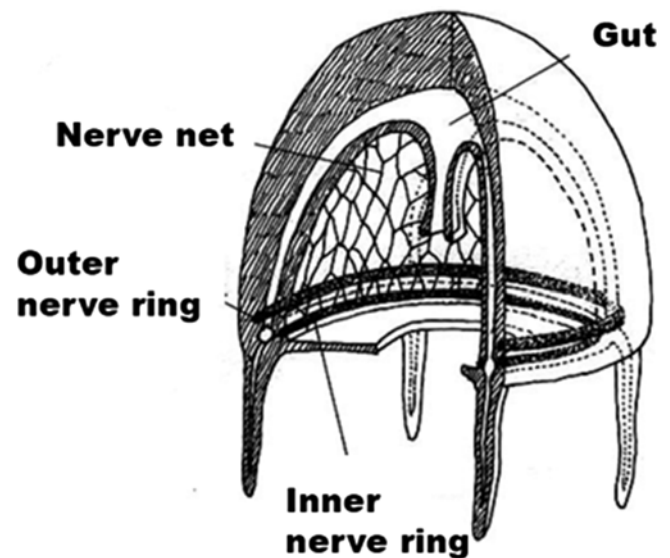
eye-bearing rhopalia probably fulfils the requirements for being a central nervous system, since it receives input from several eyes and part of its output is a pacemaker signal that directly controls swim pulses. The cubozoan nerve ring is structurally similar to the hydrozoan ring nerve and, on the assumption that this indicates similar functions, the ring nerve also constitutes a part of the central nervous system.”

### 6.5.2 Hydrozoan Nerve Ring (Figs. 6.13 and 6.14)

As to the hydrozoan medusae, two types of nerve ring are observed: the inner nerve ring and the outer nerve ring running through the marginal zone of the bell (Figs. 6.13 and 6.14) (Mackie et al. 2003; Koizumi et al. 2014). Hydrozoan jellyfish, such as *Polyorchis penicillatus* and *Aequorea victoria*, have inner and outer nerve rings. The inner nerve ring is considered to be a fused single giant axon, in which neurons show dye and electrical coupling with gap junctions (Spencer and Satterlie 1980). This morphological feature of the inner nerve ring enables rapid conduction of electrical signals and simultaneous movements of tentacles called crumpling (Roberts and Mackie 1980; Satterlie and Spencer 1983; Mackie 2004). The outer nerve ring of hydrozoan jellyfish is known to integrate pleural sensory information (Mackie and Meech 2000; Mackie 2004).

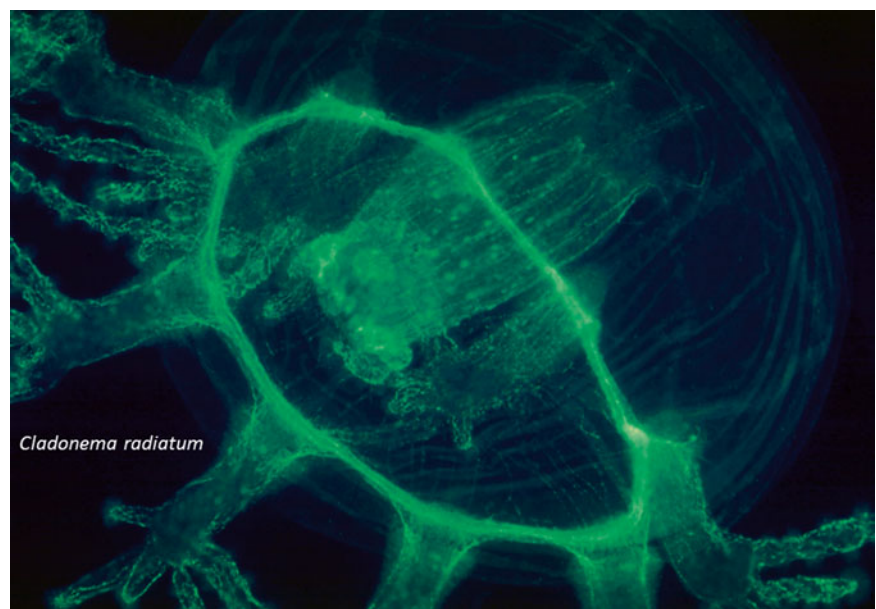
Mackie (2004) stated: “The term ‘central nervous system’ can legitimately be applied to hydromedusan nervous systems as these animals have concentrations of hundreds of axons running in parallel forming ‘nerve rings’ in the margin. There are two such rings, an inner and an outer, but axonal processes cross between them at many points and the two

rings essentially function as single unit. In cross sections of the nerve rings of *Aequorea victoria*, a total of about 800 axon profiles are seen. The nerve rings include several functionally distinct nerve pathways, and they often interact in complex ways. The fact that the central nervous system takes the form of an annulus rather than a single, compact ganglion does not make it any less ‘central’ in terms of the functions carried on within it. The annular configuration is simply an adaptation to radial symmetry. It does mean, however, that pacemakers and synaptic interactions are replicated at numerous points around the ring, rather than being localized to specific zones as in the neuropil of a conventional ganglion.”



**Fig. 6.13** Illustration of hydrozoan jellyfish containing two nerve rings: inner nerve ring and outer nerve ring around the margin of the bell

**Fig. 6.14** Picture of the nerve ring observed in the margin of the bell of a hydrozoan medusa, *Cladonema radiatum*. Nervous system is visualized immunohistochemically using RFamide antibody



### 6.5.3 Hydra's Nerve Ring (Fig. 6.15)

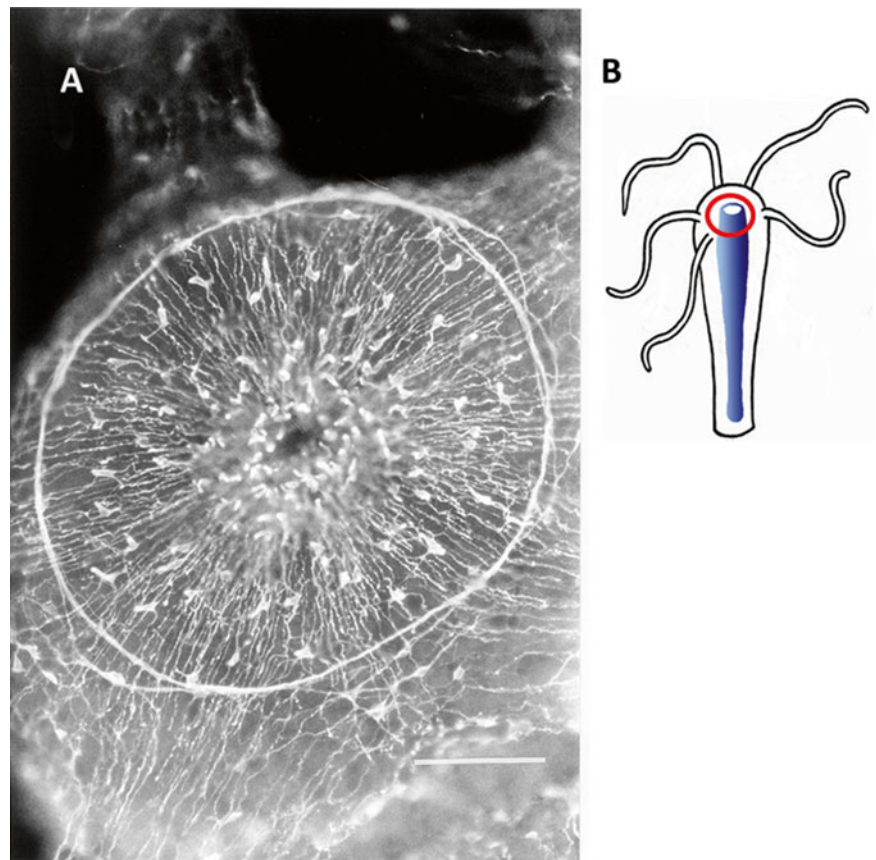
In addition to diffuse nerve nets, a nerve ring is observed in the lower hypostome of hydra. It is a ring composed of ganglion cells, whose neurites make a bundle and run circumferentially around a mouth (Koizumi et al. 1992). Ultrastructural studies have shown around 30 neurites make a bundle (Yuura 2008). Hence the nerve ring is a sole neural structure showing a tight association of neurons in contrast to the diffuse nerve net seen in other regions.

This nervous structure has unique features in the hydra nervous system (Koizumi et al. 1992; Koizumi 2007). It shows static developmental characters in contrast to the dynamic features of hydra nerve net present in other regions. This character is similar to higher animal's nervous system. Moreover, its structure and location are similar to the well-known central nervous system of other animals without a complex central nervous system such as nematodes and starfishes. As to functions of the hydra nerve ring, the identified function is a crumpling of the tentacles, corresponding to the function of the inner nerve ring of hydrozoan jellyfish. The jellyfish nerve ring is considered to be a primitive central nervous system of radiates.

We identified “crumpling” as a function of nerve ring of *Hydra oligactis* so far. Destruction experiment also supports the results (Koizumi 2007). Hydrozoan jellyfish, *Polyorchis penicillatus* and *Aglantha digitale*, have inner and outer nerve rings (Fig. 6.3). The inner nerve ring is considered to be a fused single giant axon, in which neurons show dye- and electrical-couplings with gap junctions (Spencer and Satterlie 1980). This morphological feature of the inner nerve ring enables rapid conduction of electrical signals and simultaneous movements of tentacles, which is called as “crumpling” (Roberts and Mackie 1980; Satterlie and Spencer 1983; Mackie 2004). The outer nerve ring of hydrozoan jellyfish is known to integrate pleural sensory information. Crumpling of hydra corresponds to the function of the inner nerve ring of hydrozoan jellyfish (Roberts and Mackie 1980; Spencer and Satterlie 1980; Satterlie and Spencer 1983; Koizumi 2007).

Hydra has many abilities to perform neuronal functions that might be comparable to those performed by the central nervous system of bilaterians as described in Sect. 6.3.1. Considering all the information available, we proposed a hypothesis, ‘The nerve ring in the head of hydra is a central nervous system-like neural structure’ (Koizumi 2007).

**Fig. 6.15** Nerve net observed in the hypostome of *Hydra oligactis* by means of immunohistochemistry using antiserum against Rfamide neuropeptides. (a) Picture of the nerve ring, (b) Schematic illustration showing the position of the nerve ring in hydra. Scale indicates 100  $\mu$ m in (a)



### 6.5.4 Anthozoan Nerve Ring

Recent molecular phylogenetic studies show that anthozoans are the most primitive group among cnidarians (Bridge et al. 1992). Especially as anthozoans have well-developed nerve ring, which was the fundamental neural structure since the beginning of cnidarians.

The nerve ring can be visualized in the hypostome of a sea anemone, *Aiptasia sp.* visualized with RFamide antiserum. A clear circle of nerve ring in the hypostome, running circumferentially around the mouth was observed. This is the first observation of nerve ring in anthozoans including cell bodies and neurites. About 70 neurons are present in the nerve ring. Similar nerve ring were observed in the hypostome of other sea anemone species, *Aiptasiomorpha minuta*, and *Actinia equina* (Koizumi et al. 2011).

A new model cnidarian, sea anemone, *Nematostella vectensis* was reported to have two nerve rings around a pharynx although the circular bundle was not visualized because it was examined by in situ hybridization not by immunohistochemistry (Marlow et al. 2009).

As to coral, fluorescent immunolabeling is not easy because of the intensive endogenous fluorescence of both green and red wavelength and hard skeleton. But these difficulties can be managed using various techniques such as enzyme-immunostaining and decalcification. Unexpectedly, a coral polyp of *Pocillopora damicornis* shows nerve ring in the hypostome around the mouth. Using antibodies against

RFamide, GLWamide, and vasopressin, nerve ring was visualized in addition to dense nerve net in tentacles. GLWamide and vasopressin antibodies visualized two nerve rings, inner nerve ring and outer nerve ring. The other coral, *Galaxea fascicularis* also showed the nerve ring (Koizumi et al. 2011).

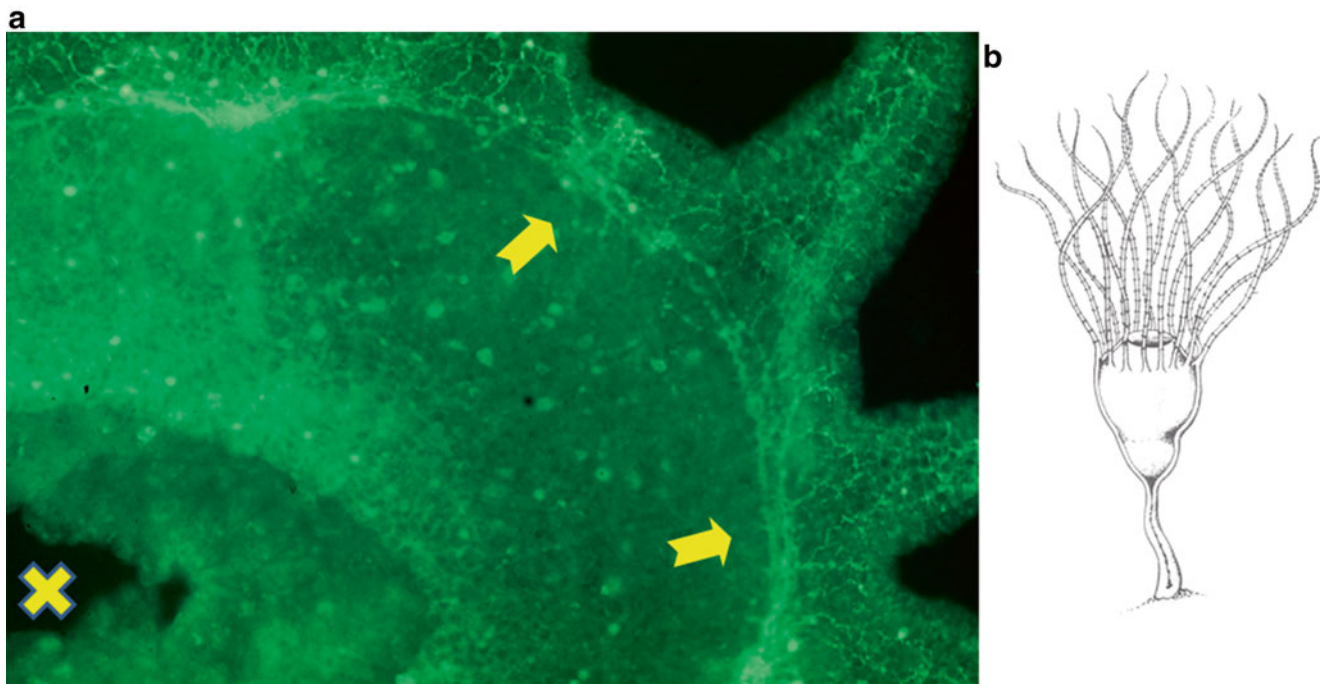
### 6.5.5 Scyphozoan Nerve Ring (Fig. 6.16)

The nerve ring around the mouth in the head has first time observed in a polyp of scyphozoan, *Aurelia auria* (Fig. 6.16). The nerve ring runs circumferentially around the mouth and shows thicker bundle like other polyp of cnidarians. More than 70 neurons were counted in the nerve ring (Koizumi, unpublished data).

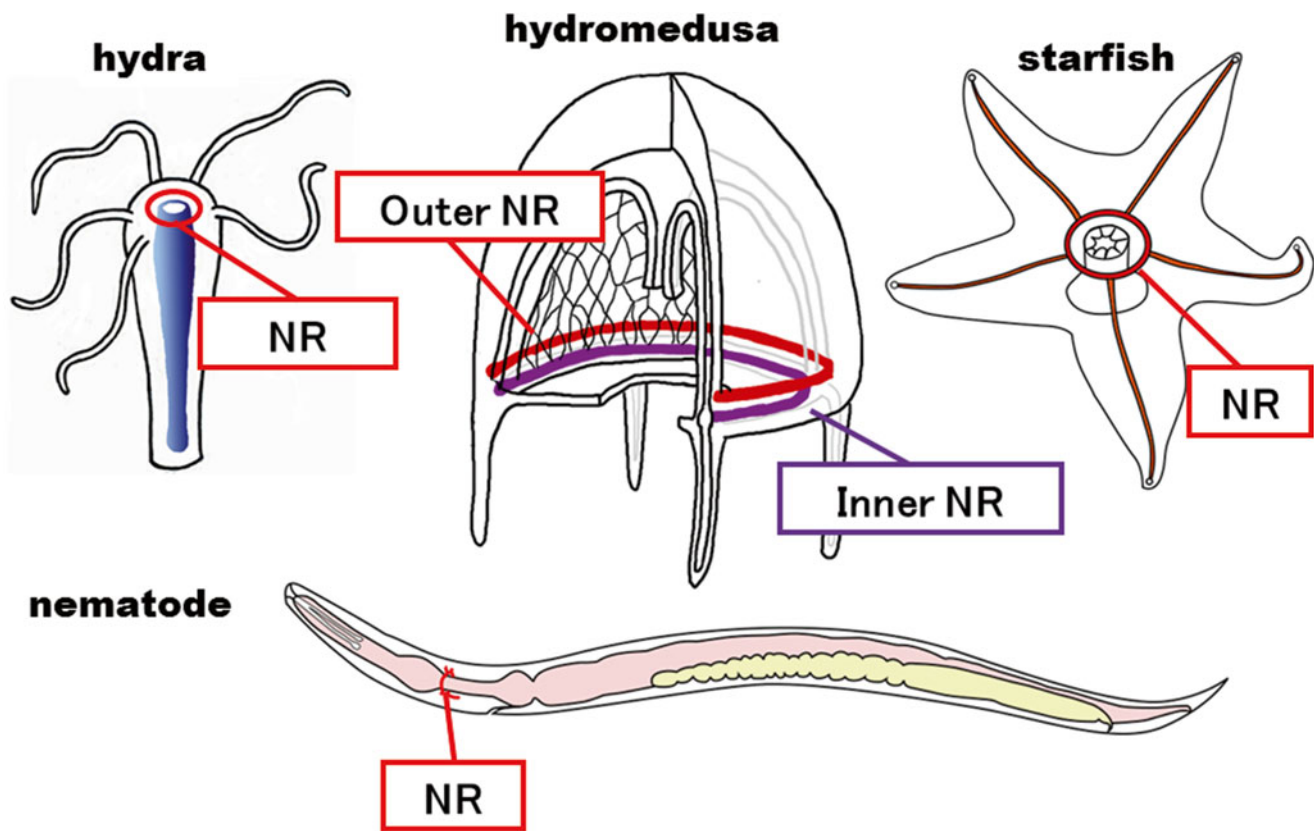
### 6.5.6 A Hypothesis That Cnidarian Nerve Ring Is a Central Nervous System-Like Neuronal Structure

Satterlie (2011) has proposed the presence of a central nervous system in cnidarian medusae. The rhopalium-nerve ring complex of cubomedusae, the rhopalia in the scyphomedusae, and the two nerve rings in hydromedusae have all been proposed to be primitive central nervous systems.

In our preliminary experiments, polyps of the scyphozoan *Aurelia auria* showed well-developed nerve rings in the



**Fig. 6.16** Nerve ring in a polyp of scyphozoan, *Aurelia aurita*. (a) Nerve ring visualized with RFamide antiserum. (b) Sketch of a polyp. Arrows show nerve ring, and cross shows mouth opening



**Fig. 6.17** Illustration of nerve rings observed in two cnidarians, hydra and hydromedusae, starfish, and nematode. *NR* nerve ring

hypostome (Figs. 6.16 and 6.17), and anthozoan polyps, the sea anemone *Aiptasia sp.*, and the coral, *Pocillopora damicornis* also showed nerve rings around the mouth (Koizumi et al. 2011). A new model cnidarian, the sea anemone, *Nematostella vectensis* has been reported to have two nerve rings around pharynx (Marlow et al. 2009).

Recent molecular phylogeny shows that Anthozoa is an animal group first evolved in the cnidarians, and Medusozoa including Hydrozoa and Scyphozoa is a latecomer (Kayal et al. 2013). Hence, it is suggested that the nerve ring is an ancestral feature of Cnidaria.

We have already demonstrated the presence of a nerve ring in the polyp of hydra, which runs circumferentially around the mouth in the hypostome (Koizumi et al. 1992). All these results strongly suggest that there are two kinds of the neural structure of the nerve ring, one running around the mouth in the polyp head (type 1) and the other running around the margin of the medusa bell (type 2). In my opinion they are general neuronal structures in cnidarians (Koizumi et al. 2014).

As to their morphological properties, both nerve rings are nerve associations with bundles of neurites and cell bodies. As to their physiological properties, the nerve rings in the

medusa (type 1) have been proposed as a central nervous system by Mackie (2004) and Satterlie (2011). The nerve ring in the hydrozoan polyp head (type 1) has also been proposed as a central nervous system-like system by Koizumi (2007). Here, I propose a working hypothesis that the neural association called the nerve ring is a primitive central nervous system-like neural structure in cnidarians.

## 6.6 Origin and Evolution of the Nervous System Considered from the Diffuse Nervous System of Cnidarians

Three epoch-making incidents can be expected when we think about the history of the nervous system, a long time when huge various nervous systems established after the first nervous system appeared in the earth. First event is the emergence of nerve cells and the nervous system. Second event is the emergence of brain, which causes prosperity of gastro-neuralia. Third event is the emergence of a neural tube, which causes prosperity of notoneuralia. In this section, origin and evolution of the nervous system will be discussed, based on the studies on cnidarian nervous system.

### 6.6.1 Centralization and Functional Specialization

By viewing nervous systems from the bottom of phylogenetic tree to top of gastroneuralia or notoneuralia, we can easily imagine their evolutionary process. Centralization and specialization are considered the direction of the nerve evolution. In fact, cnidarians have not remarkable large brain although they have its primitive form.

A cnidarian neuron is multifunctional, each of which has a set of all nerve functions. Muscle cells are epithelial cells or digestive cells at the same time. In contrast, neurons in higher animal work as either a sensory neuron, or an interneuron, or a motor neuron. Moreover, each motor neuron controls a few specific muscular cells.

### 6.6.2 Specialization of Nerve Cells at Developmental and Morphological Level

Specialization of nerve cells in higher animals appears clearly by comparison of cnidarians and mammals. Neurons are produced from interstitial cells, multipotent stem cells in an adult cnidarians continuously appear and die. Hence, neurons show active turn-over in cnidarians like various cells except neurons of higher animals. Developmental strategies of the nervous system in higher animals are long-term survival of differentiated nerve cells and consequential loss of ability of division and differentiation.

Hydra's neurons show active dynamics such as neurotransmitter phenotypic plasticity in an adult (Bode 1992). These characters are observed only in developing processes in bilaterians.

Specialization of nerve cells is also related to the polarity of neurons. Neurons have precise polarity: side of input of neural information, dendrite, and side of output of neural information, axon. Cnidarian neuron does not have such differences, but all have only neurites. Hence, during evolution of the nervous system, neurons might become specialized in many aspects from unspecialized nerve cells like as observed in cnidarian.

### 6.6.3 From Peptides to Classical Transmitter

Neuropeptides are more remarkable than classical transmitter in cnidarian nervous system. We thought the origin of the neurotransmitter might be peptides, but not choline or monoamine (Grimmelikhuijzen et al. 1989; Scemes 1989). Peptides are produced from precursor protein via protein synthesis from DNA. As to monoamines, specific enzymes for synthesis from amino acids (tyrosine or phenylalanine) are essential.

I expect this system emerged later than peptide biosynthesis. However this hypothesis needs to be verified in more detail.

Genomic analysis of ctenophore's nervous system suggests that an amino acid, glutamate is used as neurotransmitter and probably neuropeptide but not classical transmitters, such as acetylcholine and any monoamines (Moroz et al. 2014).

### 6.6.4 All Fundamental Components are Present in Cnidarian Nervous System

If cnidarian nervous systems were compared with those of mammals, insects or octopus, there are huge differences in complexities and functional levels. However, we can see all fundamental components of this nervous system. Rapid electrical conduction along neurites, chemical transmission in synapses, information processing in nerve nets, sensory systems, and motor systems. It is also in the case of central nervous system at morphological, physiological, and molecular levels as described in the present review. Functional abilities of integration of plural sensory information, and primitive learning are remarkable examples observed in cnidarian.

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### References

- Anctil M (1989) The antiquity of monoaminergic neurotransmitters: evidence from Cnidaria. In: Anderson PAV (ed) Evolution of the first nervous system. Plenum Press, New York, pp 141–156
- Anderson PA (1985) Physiology of a bidirectional, excitatory, chemical synapse. *J Neurophysiol* 53:821–835
- Anderson P (1987) Properties and pharmacology of a TTX insensitive Na<sup>+</sup> current in neurones of the jellyfish *Cyanea capillata*. *J Exp Biol* 133:231–248
- Anderson PAV (1989) Ionic currents of the Scyphozoa. In: Anderson PAV (ed) Evolution of the first nervous system. Plenum Press, New York, pp p267–p280
- Anderson PAV, Schwab WE (1983) Action potential in neurons of the motor nerve net of *Cyanea* (Coelenterata). *J Neurophysiol* 50:671–683
- Anderson PAV, Spencer AN (1989) The importance of cnidarian synapses for neurobiology. *J Neurobiol* 20(5):435–457
- Anderson PA, Holman MA, Greenberg RM (1993) Deduced amino acid sequence of a putative sodium channel from the scyphozoan jellyfish *Cyanea capillata*. *Proc Natl Acad Sci U S A* 90:7419–7423
- Arkett SA, Spencer AN (1986) Neuronal mechanisms of a hydromedusan shadow reflex. II. Graded response of reflex components, possible mechanisms of photic integration, and functional significance. *J Comp Physiol A* 159:215–225
- Ball EE, Hayward DC, Saint R, Miller DJ (2004) A simple plan- cnidarians and the origins of developmental mechanisms. *Nat Rev Genet* 5:567–577



- Barzilai MG, Reitzel AM, Kraus JEM, Gordon D, Technau U, Gurevitz M (2012) Convergent evolution of sodium ion selectivity in metazoan neuronal signaling. *Cell Rep* 2:242–248
- Berking S (2007) Generation of bilateral symmetry in Anthozoa: a model. *J Theor Biol* 246:477–490
- Blanquet RS, Lenhoff HM (1968) Tyrosine enteroreceptor of Hydra: its function in eliciting a behavior modification. *Science* 159(3815):633–634
- Bode HR (1992) Continuous conversion of neuron phenotype in hydra. *Trends Genet* 8:279–284
- Bode HR (1996) The interstitial cell lineage of hydra: a stem cell system that arose early in evolution. *J Cell Sci* 109:1155–1164
- Bode HR, David CN (1978) Regulation of a multipotent stem cell, the interstitial cell of hydra. *Prog Biophys Mol Biol* 33:189–206
- Bode HR, Heimfeld S, Koizumi O, Littlefield CL, Yaross MS (1988) Maintenance and regeneration of the nerve net in hydra. *Am Zool* 28:1053–1063
- Bridge D, Cunningham CW, Schierwater B, DeSalle R, Buss LW (1992) Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. *Proc Natl Acad Sci U S A* 89:8750–8753
- Bullock TH (1943) Neuromuscular facilitation in scyphomedusae. *J Cell Comp Physiol* 22:251–272
- Bullock TH, Horridge GA (1965) Structure and function of the nervous system of invertebrates. Freeman, San Francisco
- Campbell RD (1973) Vital marking of single cells in developing tissues: India ink injection to trace tissue movements in Hydra. *J Cell Sci* 23:651–661
- Campbell RD (1976) Elimination of hydra interstitial and nerve cells by means of colchicine. *J Cell Sci* 21:1–13
- Chapman JA et al (2010) The dynamic genome of Hydra. *Nature* 464:592–596
- David CN, Gierer A (1974) Cell cycle kinetics and development of *Hydra attenuata*. III. Nerve and nematocyte differentiation. *J Cell Sci* 16:359–375
- David ND, Hager G (1994) Formation of a primitive nervous system: nerve cell differentiation in the polyp hydra. *Perspect Dev Neurobiol* 2:135–140
- Davis LE (1972) Ultrastructural evidence for the presence of nerve cells in the gastrodermis of Hydra. *Z Zellforsch Mikrosk Anat* 123(1):1–17
- Davis LE, Burnett AL, Haynes JF (1968) Histological and ultrastructural study of the muscular and nervous system in Hydra. II. Nervous system. *J Exp Zool* 162:295–332
- Dunne J, Javois LC, Huang LW, Bode HR (1985) A subset of cells in the nerve net of *Hydra oligactis* defined by a monoclonal antibody: its arrangement and development. *Dev Biol* 109:41–53
- Epp L, Tardent P (1978) The distribution of nerve cells in *Hydra attenuata* Pall. *Wilhelm Roux' Arch* 185:185–193
- Garm A, Ekstroem P, Boudes M, Nilsson DE (2006) The rhopalia are integrated parts of the central nervous system in box jellyfish. *Cell Tissue Res* 325:333–343
- Garm A, Poussart Y, Panafelt L, Ekstroem P, Nilsson DE (2007) The ring nerve of the box jellyfish, *Tripedalia cystophora*. *Cell Tissue Res* 329:147–157
- Golubovic A, Kuhn A, Williamson M, Kalbacher H, Holstein TW, Grimmelikhuijzen CJP, Guender S (2007) A peptide-gated ion channel from the freshwater poly Hydra. *J Boil Chem* 282(48):35098–35103
- Grigoriev NG, Spafford JD, Gallin WJ, Spencer AN (1997) Voltage sensing in jellyfish Shaker K<sup>+</sup> channels. *J Exp Biol* 200(Pt 22):2919–2926
- Grimmelikhuijzen CJP (1985) Antisera to the sequence Arg-Pheamide visualize neuronal centralization in hydroid polyps. *Cell Tissue Res* 241:171–182
- Grimmelikhuijzen CJP, Westfall JA (1995) The nervous systems of Cnidarians. In: Bredbach O, Kutsch W (eds) *The nervous systems of invertebrates: an evolutionary and comparative approach*. Birkhauser Verlag, Basel, pp 724–24
- Grimmelikhuijzen CJP, Dierickx K, Boer GJ (1982) Oxytocin/vasopressin-like immunoreactivity in the nervous system of Hydra. *Neuroscience* 7:3191–3199
- Grimmelikhuijzen CJP, Graff D, Koizumi O, Westfall JA, McFarlane ID (1989) Neurons and their peptide transmitters in coelenterates. In: Anderson PAV (ed) *Evolution of the first nervous system*. Plenum Press, New York, pp 95–109
- Horridge GA (1954) The nerves and muscles of medusae I. Conduction in the nervous system of *Aurellia aurita*. *J Exp Biol* 31:594–600
- Hoyle G (1960) Neuromuscular activity in the swimming sea anemone, *Stomphia coccinea* (Muller). *J Exp Biol* 37:671–688
- Josephson RK (1974) Cnidarian neurobiology. In: Muscatine L, Lenhoff HM (eds) *Coelenterate biology, reviews and new perspectives*. Academic, New York, pp 245–280
- Josephson RK, Schwab WE (1979) Electrical properties of an excitable epithelium. *J Gen Physiol* 74:213–236
- Kayal E, Roure B, Philippe H, Collins AG, Lavrov DV (2013) Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evol Biol* 13(5):1–18
- Kerfoot PAH, Mackie GO, Meech RW, Roberts A, Singla CL (1985) Neuromuscular transmission in the jellyfish *Aequorea victoria*. *J Exp Biol* 116:1–25
- Kinnamon JC, Westfall JA (1982) Types of neurons and synaptic connections at hypostome-tentacle junctions in Hydra. *J Morphol* 173:119–128
- Klassen TL, O'Mara ML, Redstone M, Spencer AN, Gallin WJ (2008) Non-linear intramolecular interactions and voltage sensitivity of a KV1 family potassium channel from *Polyorchis penicillatus* (Eschscholtz 1829). *J Exp Biol* 211:3442–3453
- Koizumi O (2002) Developmental neurobiology of hydra, a model animal of cnidarians. *Can J Zool* 80:1678–1689
- Koizumi O (2007) Nerve ring of the hypostome in hydra: is it an origin of the central nervous system of bilaterian animals? *Brain Behav Evol* 69:151–159
- Koizumi O, Bode HR (1986) Plasticity in the nervous system of adult hydra. I. The position-dependent expression of FMRFamide-like immunoreactivity. *Dev Biol* 116:407–421
- Koizumi O, Bode HR (1991) Plasticity in the nervous system of adult hydra. III. Conversion of neurons to expression of a vasopressin-like immunoreactivity depends on axial location. *J Neurosci* 11:2011–2020
- Koizumi O, Maeda N (1981) Rise of feeding threshold in satiated Hydra. *J Comp Physiol* 142:75–80
- Koizumi O, Heimfeld S, Bode HR (1988) Plasticity in the nervous system of adult hydra. II. Conversion of ganglion cells of the body column into epidermal sensory cells of the hypostome. *Dev Biol* 129:358–371
- Koizumi O, Wilson JD, Grimmelikhuijzen CJP, Westfall JA (1989) Ultrastructural localization of RFamide-like peptides in neuronal dense-cored vesicles in the peduncle of Hydra. *J Exp Zool* 249(1):17–22
- Koizumi O, Itazawa M, Mizumoto H, Minobe S, Javois JC, Grimmelikhuijzen CJP, Bode HR (1992) The nerve ring of the hypostome in hydra. I. Its structure, development and maintenance. *J Comp Neurol* 326:7–21
- Koizumi O, Sato N, Goto C (2004) Chemical anatomy of hydra nervous system using antibodies against hydra neuropeptides: a review. *Hydrobiologia* 530–531:41–47
- Koizumi O, Yatabe M, Minobe S, Kurumata M, Hamada S, Nakamura M, Namikawa H (2011) Origin and evolution of the nervous system viewed from the diffuse nervous system: Nerve ring of cnidarians

- (Abstract). *Comp Physiol Biochem (suppl)* 28, 131 Abstract of ICCPB2011
- Koizumi O, Hamada S, Minobe S, Hamaguchi-Hamada K, Kurumata-Shigeto M, Nakamura M, Namikawa H (2014) The nerve ring in cnidarians: its presence and structure in hydrozoan medusae. *Zoology (Jena)* 118:115–124. doi:10.1016/j.zool.2014.10.001
- Layden ML, Boekhout M, Martindale MQ (2012) Nematostella vectensis achaete-scute homolog NvashA regulates embryonic ectodermal neurogenesis and represents an ancient component of the metazoan neural specification pathway. *Development* 139:1013–1022
- Lentz T (1968) Primitive nervous systems. Yale University Press, New Haven
- Mackie GO (1965) Conduction in the nerve-free epithelia of siphonophores. *Am Zool* 5:439–453
- Mackie GO (2004) Central neural circuitry in the jellyfish *Aequorea victoria*. A model simple nervous system. *Neurosignals* 13:5–19
- Mackie GO, Meech RW (1985) Separate sodium and calcium spikes in the same axon. *Nature* 313:791–793
- Mackie GO, Meech RW (1995) Central circuitry in the jellyfish *Aequorea victoria*. II. The ring giant axon and carrier systems. *J Exp Biol* 198:2271–2278
- Mackie GO, Meech RW (2000) Central circuitry in the jellyfish *Aequorea victoria*. III. The rootlet and pacemaker systems. *J Exp Biol* 203:1797–1807
- Mackie GO, Passano LM (1968) Epithelial conduction in hydromedusae. *J Gen Physiol* 52:600–621
- Mackie GO, Singla C (1975) Neurobiology of *Stomatocysta*. I. Action systems. *J Neurobiol* 6(4):339–356
- Mackie GO, Marx RM, Meech RW (2003) Central circuitry in the jellyfish *Aequorea victoria*. IV. Pathways coordinating feeding behaviour. *J Exp Biol* 206:2487–2505
- Marlow HQ, Srivastava M, Matus DQ, Rokhsar D, Martindale MQ (2009) Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev Neurobiol* 69(4):235–254
- Martindale MR, Pang K, Finnerty JR (2004) Investigating the origins of triploblasty: ‘mesodermal’ gene expression in a diploblastic animal, the sea anemone *Nematostella vectensis* (phylum, Cnidaria; class, Anthozoa). *Development* 131(10):2463–2474
- Matus D, Pang K, Marlow H, Dunn CW, Thomas GH, Martindale MQ (2006) Molecular evidence for deep evolutionary roots of bilaterality in animal development. *Proc Natl Acad Sci U S A* 103(30):11195–11200
- McFarlane ID, Graff D, Grimmelikhuijzen CJP (1989) Peptidergic neurotransmitters in the Anthozoa. In: Anderson PAV (ed) Evolution of the first nervous system. Plenum Press, New York, pp 111–128
- Meech RW, Mackie GO (1993) Potassium channel family in giant motor axons of *Aequorea victoria*. *J Neurophysiol* 69:894–901
- Morishita F, Nitagai Y, Furukawa Y, Matsushima O, Takahashi T, Hata M, Fujisawa T, Tunamoto T, Koizumi O (2003) Identification of a vasopressin-like immunoreactive substance in hydra. *Peptides* 24:17–26
- Moroz L et al (2014) The ctenophore genome and the evolutionary origins of neuronal systems. *Nature*. doi:10.1038/nature13400
- Murate M, Takahashi-Iwanaga H, Kurosaki R, Takeda M, Koizumi O (1996) Scanning electron microscopy of endodermal sensory cells of *Hydra magnipapillata*. *Cell Tissue Res* 283:455–459
- Nakanishi N, Renfer E, Technau U, Rentzsch F (2011) Nervous systems of the sea anemone *Nematostella vectensis* are generated by ectoderm and endoderm and shaped by distinct mechanisms. *Development* 139:347–357
- Nilsson DE, Gislén L, Coates MM, Skogh C, Garm A (2005) Advanced optics in a jellyfish eye. *Nature* 435:201–205
- Nilsson DE, Gislén L, Coates MM, Skogh C, Gram A (2006) Advanced optics in a jellyfish eye. *Nature* 435:201–205
- Passano LM (1982) Scyphozoa and cubozoa. In: Shelton GAB (ed) Electrical conduction and behavior in ‘simple’ invertebrates. Clarendon Press, Oxford, pp 149–202
- Putnam NH et al (2007) Sea anemone genomes reveals ancestral eumatazoan gene repertoire and genomic organization. *Science* 317:86–94
- Roberts A, Mackie GO (1980) The giant axon escape system of a hydrozoan medusa, *Aequorea victoria*. *J Exp Biol* 84:303–318
- Robson EA (1961) Some observations on the swimming behavior of the sea anemone *Stomphia coccinea*. *J Exp Biol* 38:343–363
- Rushforth NB (1965) Behavioral studies of the coelenterate *Hydra pirardi* Brien. *Anim Behav Suppl* 1:30–42
- Rushforth NB (1967) Chemical and physical factors affecting behavior in *Hydra*: interactions among factors affecting behavior in *Hydra*. In: Corning WC, Ratner SC (eds) Chemistry of learning. Plenum Press, New York, pp 369–390
- Rushforth NB (1973a) Behavior. In: Burnett AL (ed) Biology of *Hydra*. Academic Press, New York, pp 3–41
- Rushforth NB (1973b) Behavioral modifications in coelenterates. In: Corning WC, Dale JA, Willows AOD (eds) Invertebrate learning. Plenum Press, New York, pp 123–170
- Satterlie RA (1985) Central generation of swimming activity in the hydrozoan jellyfish *Aequorea victoria*. *J Neurobiol* 16:41–55
- Satterlie RA (2011) Do jellyfish have central nervous systems? *J Exp Biol* 214:1215–1223
- Satterlie RA, Spencer AN (1983) Neuronal control of locomotion in Hydrozoan medusae. A comparative study. *J Comp Physiol* 150:195–206
- Satterlie RA, Spencer AN (1987) Organization of conducting systems in simple invertebrates: porifera, cnidaria and ctenophora. In: Ali MA (ed) Nervous system in invertebrates. Plenum Press, New York, pp 213–264
- Scemes E (1989) Rethinking the role of cholinergic neurotransmitters in the Cnidaria. In: Anderson PAV (ed) Evolution of the first nervous system. Plenum Press, New York, pp 157–166
- Shelton GAB (1982) Anthozoa. In: Shelton GAB (ed) Electrical conduction and behavior in ‘simple’ invertebrates. Clarendon Press, Oxford, pp 73–148
- Shimizu H (2002) Feeding and wounding response in *Hydra* suggest functional and structural polarization of the tentacle nervous system. *Comp Biochem Physiol A Mol Integr Physiol* 131:669–674
- Shimizu H, Okabe M (2007) Evolutionary origin of autonomic regulation of physiological activities in vertebrate phyla. *J Comp Physiol* 193:1013–1019
- Shimizu H, Koizumi O, Fujisawa T (2004) Three digestive movements in *Hydra* regulated by the diffuse nerve net in the body column. *J Comp Physiol A* 190:623–630
- Singla CL (1978) Locomotion and neuromuscular system of *Aequorea victoria*. *Cell Tissue Res* 188:317–327
- Skogh C, Garm A, Nilsson DE, Ekstroem P et al (2006) Bilaterally symmetrical rhopalial nervous system of the box jellyfish *Tripedalia cystophora*. *J Morphol* 267:1391–1405
- Smith S, Oshida J, Bode H (1974) Inhibition of nematocyst discharge in *Hydra* fed to repletion. *Biol Bull* 147:186–202
- Spafford JD, Spencer AN, Gallin WJ (1999) Genomic organization of a voltage-gated Na<sup>+</sup> channel in a hydrozoan jellyfish: insights into the evolution of voltage-gated Na<sup>+</sup> channel genes. *Receptors Channels* 6(6):493–506
- Spencer AN (1979) Neurobiology of Polyorchis. 11. Structure of effector systems. *J Neurobiol* 10(2):95–117
- Spencer AN (1982) The physiology of a coelenterate neuromuscular synapse. *J Comp Physiol* 148:353–363
- Spencer AN (1989) Chemical and electrical synaptic transmission in the cnidaria. In: Anderson PAV (ed) Evolution of the first nervous system. Plenum Press, New York, pp 33–54

- Spencer AN, Satterlie RA (1980) Electrical and dye coupling in an identified group of neurons in a coelenterate. *J Neurobiol* 11:13–19
- Spencer AN, Schwab WE (1982) Hydrozoa. In: Shelton GAB (ed) *Electrical conduction and behavior in 'simple' invertebrates*. Clarendon Press, Oxford, pp 73–148
- Spencer AN, Przysiecki J, Acosta-Urquidi J, Basarsky TA (1989) Presynaptic spike broadening reduces junctional potential amplitude. *Nature* 340:636–663
- Takahashi T, Muneoka Y, Lohmann J, deHaro LM, Solleder G, Bosch TCG, David CN, Bode HR, Koizumi O, Shimizu H, Hatta M, Fujisawa T, Sugiyama T (1997) Systematic isolation of peptide signal molecules regulating development in hydra: LWamide and PW families. *Proc Natl Acad Sci U S A* 94:1241–1246
- Takahashi T, Koizumi O, Ariura Y, Romanovitch A, Bosch TCG, Kobayakawa Y, Mohri S, Bode H, Yum S, Hatta M, Fujisawa T (2000) A novel neuropeptide, Hym355, positively regulates neuron differentiation in hydra. *Development* 127:997–1005
- Takahashi T, Kobayakawa Y, Muneoka Y, Fujisawa Y, Mohri S, Hatta M, Shimizu H, Fujisawa T, Sugiyama T, Takahara M, Yanagi K, Koizumi O (2003) Identification of a new member of the GLWamide peptide family: physiological activity and cellular localization in cnidarian polyps. *Comp Biochem Physiol Part B* 135:309–324
- Takahashi T, Hayakawa E, Koizumi O, Fujisawa T (2008) Neuropeptides and their functions in Hydra. *Acta Biol Hung* 59:227–235
- Takahashi-Iwanaga H, Koizumi O, Fujita T (1994) Scanning electron microscopy of the muscle system of *Hydra magnipapillata*. *Cell Tissue Res* 277:79–86
- Technau U, Steele RE (2011) Evolutionary crossroads in developmental biology: Cnidaria. *Development* 138:1447–1458
- Watanabe H, Fujisawa T, Holstein TW et al (2009) Cnidarians and the evolutionary origin of the nervous system. *Develop Growth Differ* 51:167–183
- Watanabe H, Kuhn A, Fushiki M, Agata K, Oezbek S, Fujisawa T, Holstein TW (2014) Sequential actions of b-catenin and Bmp pattern the oral nerve net in *Nematostella vectensis*. *Nature Commun*. doi:10.1038/ncomms6536
- Weber C, Singla CL, Kerpoort PAH (1982) Microanatomy of subumbellar motor innervation in *Aglantha digitale* (Hydromedusae: Trachylina). *Cell Tissue Res* 223:305–312
- Westfall J (1973) Ultrastructural evidence for a granule-containing sensory-motor-interneuron in *Hydra littoralis*. *J Ultrastruct Res* 42:268–282
- Westfall JA (1987) Ultrastructure of invertebrate synapses. In: Ali MA (ed) *Nervous systems in invertebrates*. Plenum Press, New York
- Westfall J, Kinnamon JC (1978) A second sensory-motor-interneuron with neurosecretory granules in *Hydra*. *J Neurocytol* 7:365–379
- Westfall JA, Rogers RA (1990) A combined high-voltage and scanning electron microscopic study of two types of sensory cells dissociated from the gastrodermis of *Hydra*. *J Submicrosc Cytol Pathol* 22:185–190
- Westfall JA, Townsend JW (1977) Scanning electron stereomicroscopy of the gastrodermis of *Hydra*. *Scan Electron Microsc* 2:623–629
- Westfall JA, Yamataka S, Enos PD (1971) Ultrastructural evidence of polarized synapses in the nerve net of *Hydra*. *J Cell Biol* 51:318–323
- Westfall JA, Wilson JD, Rogers RA, Kinnamon JC (1991) Multifunctional features of a gastrodermal sensory cell in *Hydra*: three-dimensional study. *J Neurocytol* 20:251–261
- Yaross MS, Westerfield J, Javois LC, Bode HR (1986) Nerve cells in hydra: Monoclonal antibodies identify two lineages with distinct mechanisms for their incorporation into head tissue. *Dev Biol* 114:225–237
- Yum S, Takahashi T, Koizumi O, Ariura Y, Kobayakawa Y, Mohri S, Fujisawa T (1998) A novel neuropeptide, Hym-176, induces contraction of the ectodermal muscle in *Hydra*. *Biochem Biophys Res Commun* 248:584–590
- Yuura H (2008) Ultrastructural study on the nerve ring in hydra. Master thesis, Fukuoka Women's University