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# A Key Innovation in Animal Evolution, the Emergence of Neurogenesis: Cellular and Molecular Cues from Cnidarian Nervous Systems

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## SUMMARY

**The emergence of neurogenesis led to the acquisition of an efficient neuromuscular transmission in eumetazoans, as shown by cnidarians that use evolutionarily-conserved neurophysiological principles to crumple, feed, swim. However, the cnidarian neuroanatomies are quite diverse and reconstructing the urcnidarian nervous system is not an easy task. Three types of characters shared by anthozoans and medusozoans appear plesiomorphic: 1) three cell types that all cnidarians differentiate, neurosensory cells, ganglionic neurons and nematocytes (cnidocytes) that combine mechano-chemosensation and venom secretion; 2) a chemical conduction through nerve nets and nerve rings, those being considered as annular central nervous systems; 3) a larval apical sensory organ that initiates metamorphosis. Other characters receive a disputed origin: 1) the neural stem cell(s), multipotent interstitial stem cell in hydrozoans, not identified in other classes; 2) the electrical conduction through neurons and epithelial cells present only in hydrozoans; 3) the embryonic origin of the nervous system; 4) the medusa sensory organs, ocelli or lens-eyes for light, statocysts for pressure, lacking in anthozoans. Nevertheless numerous gene families that regulate bilaterian neurogenesis are expressed during cnidarian neurogenesis, e.g. cnidarian eyes express *Pax, Six* and *opsin*, supporting a common origin for vision. However data establishing a clear picture of the cnidarian neurogenic circuitry are currently missing. Finally many "neurogenic" gene families likely arose and evolved in the absence of neurogenesis, as exemplified by Porifera that express them but lack synaptic transmission. Therefore some eumetazoan-specific families, missing in Porifera as *ParaHox/Hox*-like and *Otx*-like genes, might have contributed to the emergence of neurogenesis.**

## The key position of Cnidaria to trace back the first-evolved nervous systems

Despite numerous controversies, recent phylogenomic approaches have convincingly shown that cnidarians form with ctenophores (combjellies) a unique clade named coelenterates; as bilaterians coelenterates differentiate nerves and muscles and therefore group in eumetazoans (Philippe et al., 2009). In contrast, poriferans (sponges) and placozoans would occupy more basal positions in metazoans (Schierwater et al., 2009). Poriferans likely differentiate a proto-neuronal system: they are capable of chemical conduction (Leys et al., 1999), they differentiate sensory-like cells and express regulatory genes that are neurogenic in bilaterians (Gazave et al., 2008, Richards et al., 2008). However they do not display any cell types exhibiting synaptic conduction and usually feed by passive filtration. Therefore coelenterates provide appropriate model systems to trace back the first-evolved nervous systems that rely on synaptic conduction (Anderson & Spencer, 1989).

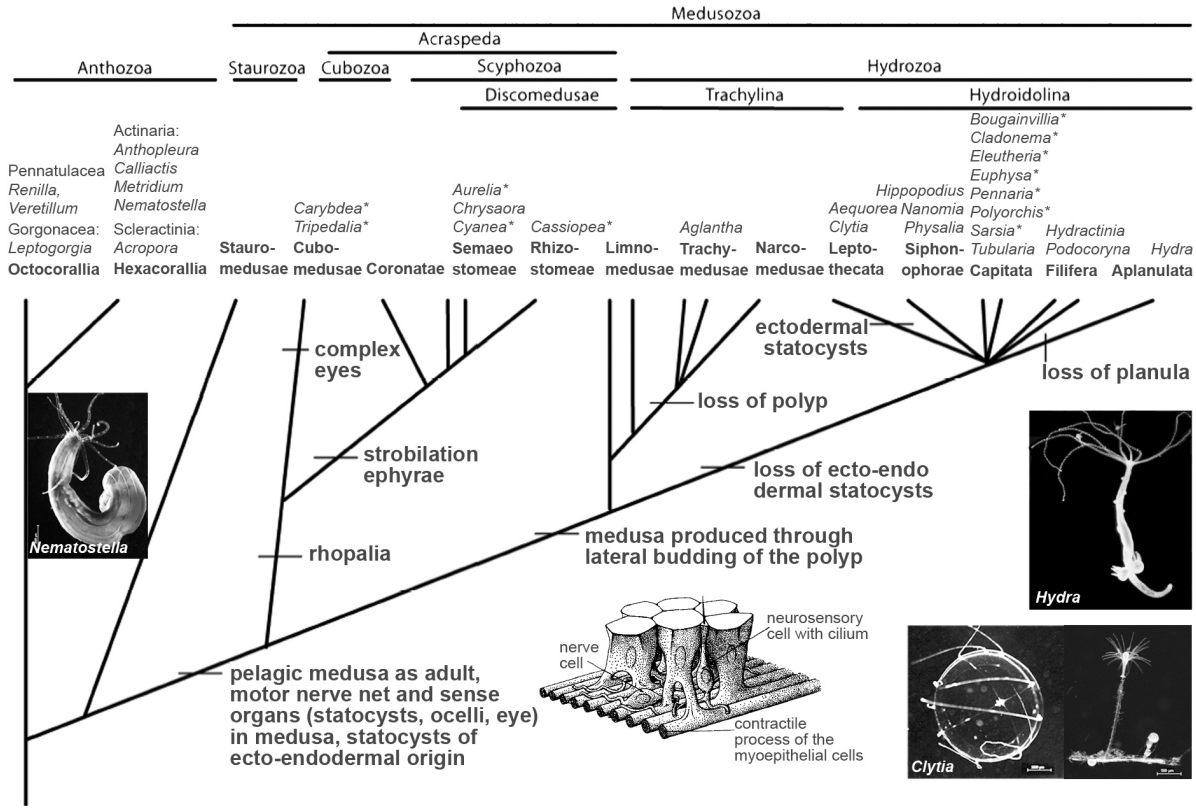


**Figure 1: Phylogeny of cnidarians and key steps in the evolution of their nervous systems.**
This tree reproduces the phylogeny established by Collins et al. (2006). The main changes affecting development and/or differentiation of the nervous system are indicated. Species used for investigating the anatomy, the physiology or the differentiation of the nervous system are listed on the top. In hydrozoans, *Hydractinia* and *Hydra* have lost the medusa stage. Asterisks indicate species that differentiate photo-sensory organs (eye-spots that detect light as ocelli or lens eyes). The scheme showing the basic cellular organization supporting neuromuscular transmission in cnidarians is taken from Mackie and Passano (1968).

The coelenterate phylum is supposed to have diverged about 650 million years ago, preceding the Cambrian explosion, the period when ancestors to most extant bilaterian phyla arose from a common hypothetical ancestor named Urbilateria (De Robertis, 2008). As a consequence Coelenterata form a sister clade to the bilaterians. Cnidarians are most often marine animals that are made up of two cell layers, the ectoderm and the endoderm, separated by an extracellular matrix named mesoglea (Bouillon, 1994b). However this “diploblastic” criterion is disputed as numerous cnidarian species actually differentiate “mesodermal” derivatives as striated muscle at one or the other stage of their life cycle (Seipel & Schmid, 2006). Cnidarian species cluster in two distinct groups (Bridge et al., 1995, Collins et al., 2006): anthozoans that live exclusively as polyps (corals, sea anemones) and medusozoans that display a complex life cycle with a pelagic parental medusa stage, a larval planula stage and a benthic polyp stage (Fig. 1). Among those, the cubozoans (box jellyfish) predominantly live as medusae, whereas scyphozoans and hydrozoan species usually follow a life cycle where they alternate between these two forms. The scyphozoan polyp produces through strobilation (transverse fission) a young flat ephyra that subsequently shape into medusa (Franc, 1994) whereas the hydrozoan polyp produces through budding a young medusa (Galliot & Schmid, 2002). The freshwater *Hydra* belongs to this latter group, but *Hydra* lost the medusa stage and the embryos produced by polyps develop directly, lacking the planula stage and the metamorphosis process. Cnidarian polyps are basically a tube with a single opening circled by a ring of tentacles, which has a mouth-anus function, whereas jellyfish display a more complex anatomy with the mouth-anus opening located at the extremity of the manubrium under the bell (Figs. 2Q, 2R, 3C).

In the mid XIX$^{e}$ century, with limited imaging tools, Louis Agassiz identified for the first time the cnidarian nervous system on two live hydrozoan jellyfish, *Sarsia* and *Bougainvillia* (Agassiz, 1850, Mackie, 2004a). Twenty five years later the pulsated swimming behavior of jellyfish was investigated experimentally by George Romanes, who proved that pacemakers were actually quite different in scyphozoans and hydrozoans, restricted to sense organs (the rhopalia) in the former, more diffuse and extending along the bell ring in the latter (Romanes, 1876 , Romanes, 1877). Surprised by what he named a dichotomy, his work undoubtedly highlighted the striking potential of studying “primitive”

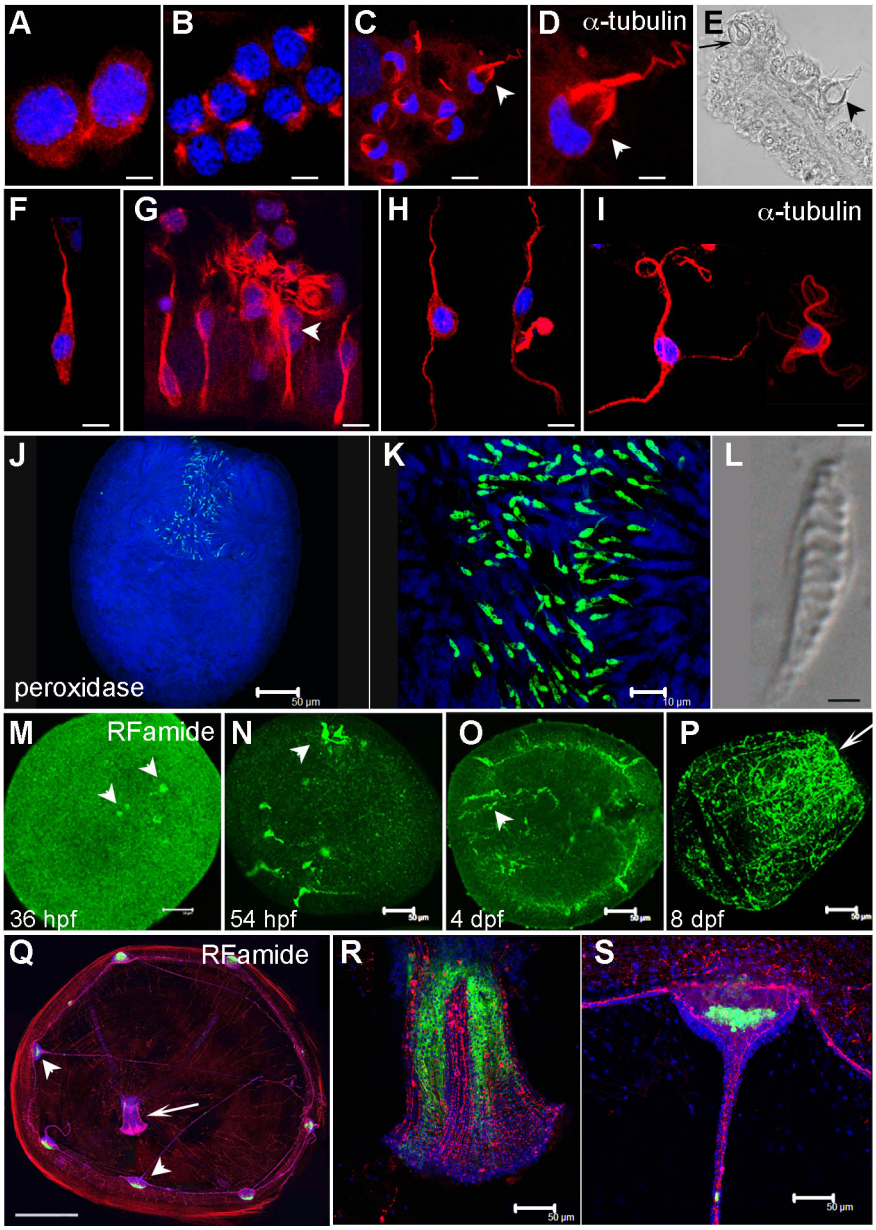


**Figure 2: The neuronal and nematocyte cell lineages.**
A-E) The *Hydra* nematocyte lineage : interstitial stem cells (A) synchronously divide, providing nematoblasts (B) that differentiate a typical capsule (nematocyst) as observed in mature nematocytes (C,D). Arrowheads : discharged nematocyst. E) Bright-field view of a tentacle with nematocytes either undischarged (arrow) or discharged (arrowhead) embedded in large epithelial battery cells. F-I) *Hydra* neurons detected after maceration with α-tubulin antibody (red) and DAPI (blue): sensory (F, G), bipolar (H) or multipolar (I) also named ganglionic cells. J-L) *Nematostella* spirocytes detected in larva thanks to their peroxidase activity (green). Spirocytes are anthozoan mechanosensory cells involved in adhesion to prey and non-prey (Kass-Simon & Scappaticci, 2002). M-P) Neurogenesis in the developing *Nematostella* : Early neurons expressing the neuropeptide RFamide (arrowheads) appear in the endodermal layer (M), then migrate to the ectoderm (N) where they form a net (O). In the newly metamorphosed polyp (P), the density of RF-amide neurons is higher in the oral region (arrow). Q-S) *Clytia* medusa showing a high density of RF-amide neurons (red) in the manubrium (Q arrow, R) and in the tentacle bulbs (Q arrowheads, S). Blue : DAPI staining ; green : endogenous GFP. Scale bars: 2 µm (A,B,D,L), 5 µm (E-I, C), 10 µm (K), 50 µm (J, M-P, R-S), 500 µm (Q).

nervous systems. Since then the history of the emergence of neurogenesis in the animal kingdom led to the elaboration of successive scenarios (Passano, 1963). The application of cellular and electrophysiological methods to coelenterates definitely proved that basically the same neurophysiological principles are valid from cnidarians to bilaterians

In this chapter, we will survey the following questions: What are the common anatomical and physiological characters shared by the cnidarian nervous systems? How, when and where neurogenesis and nematogenesis take place in cnidarians? What is currently known about the genetic circuitry that regulates these two processes at any stage of the cnidarian life cycle? What evolutionary

scenario can account for the key transition represented by the emergence of neurogenesis, presumably in the last common eumetazoan? Beside the evolutionary history of neurophysiology and neurogenesis, which biological questions can be investigated in cnidarian model systems?

## Cellular organization of the cnidarian nervous systems

***The nematocytes (or cnidocytes) are phylum-specific mechanoreceptor cells***

Cnidarian nervous systems are made of mechanoreceptor cells named nematocytes or cnidocytes (giving their name to the phylum), and neurons. The nematocytes are highly specialized mechano-receptor cells, which play a key role in the capture of preys and defense (Fig. 2A-2E) – reviewed in (Bouillon, 1994a, Tardent, 1995). These stinging cells are abundant, representing 35% of the cells in *Hydra* (David, 1973). Mature nematocytes function as receptor-effector cells, receptor thanks to their cnidocil that can be stimulated chemically or mecanically (by preys), effector thanks to their nematocyst (or cnidocyst), a thick-wall capsule that respond in nanoseconds by discharging its toxic content (Fig. 2C-2E) (Ozbek et al., 2009). This venom that is released as large droplets into the prey by an everting tubule, immobilises the prey, which, by releasing the peptide glutathione, induces the feeding response, i.e. tentacle bending and mouth opening (Loomis, 1955, Lenhoff et al., 1982, Shimizu, 2002). How the information sensed by the cnidocil apparatus is transduced to target the discharge function is not clear. The nematocyst discharge seems to operate in the absence of neuronal control indicating that nematocytes can behave autonomously (Aerne et al., 1991). However nematocytes are under neuronal control as evidenced by the presence of two-cell as well as three-cell synaptic pathways in the tentacle epidermis (Holtmann & Thurm, 2001, Westfall et al., 2002). This neuronal control is actually rather playing an inhibitory function to reduce the spontaneous firing activity of nematocytes. Mechanosensory cells display variable morphologies and functions; in anthozoans, spirocytes are mechanosensory cells (Fig. 2J-L) involved in adhesion to prey and non-prey (Kass-Simon & Scappaticci, 2002). Finally mechanoreception in cnidarians is likely not restricted to nematocytes, some medusa differentiate clusters of hair cells at the base of tentacles and on the velum, quite similar to the vertebrate hair cells of the inner ear (Arkett et al., 1988). These hair cells are possibly involved in the feeding response and the locomotion behavior.

***Several neuronal cell types in cnidarians: neurosensory, sensory-motor and ganglionic***

In *Hydra* neurons that can be bipolar, tripolar or multipolar, are of three distinct types, neurosensory, ganglionic and neurosecretory (Lentz & Barrnett, 1965, Davis, 1974). Sensory cells characterized by their cilium that reach the surface, are located within the ectodermal layer (Fig. 2F,G). By contrast, ganglion neurons (bi, tri or multipolar), which are the most common type of neuronal cells in this animal, are spread in both cell layers, along the mesoglea and function as interneurons (Fig. 2H,I). However the sensory cells might also be considered as multifunctional cells, which indeed receive external inputs but also produce secretory granules and possibly function as motorneuron and interneuron (Westfall & Kinnamon, 1978). Similarly in sea anemones, sensory neurons also associate with smooth muscle fibers, suggesting that they behave as sensory and motoneurons (Grimmelikhuijzen et al., 1989). Medusozoans share a common structure, the motor nerve net, initially identified by Passano (who named it the giant fiber nerve net), which conducts the excitation through the subumbrellar muscles of the medusae (Satterlie, 2002). In the jellyfish *Cyanea*, the motor nerve net establish bidirectional non-polarized synapses with their target cells, namely myoepithelial cells and nematocytes, thus functioning as sensory-motoneurons (Anderson, 1985).

***A common progenitor cell for nematocytes and neurons?***

In *Hydra*, neurons and nematocytes derive from the same multipotent stem cell, the interstitial stem cell, which also provides progenitors for two other cell lineages, the gland cells and the gametes (Bode, 1996). However contrary to Lentz's statement, interstitial stem cells in *Hydra* cannot differentiate into epithelial cells. This restricted multipotent property of intersitial stem cells was nicely demonstrated in "nerve-free" or "epithelial" polyps obtained after chemical (Campbell, 1976, Yaross & Bode, 1978) or genetic (Sugiyama & Fujisawa, 1978, Terada et al., 1988) elimination of the interstitial stem cells. As a result the interstitial derivatives, namely nematocytes and neurons, progressively disappear, the animals become completely epithelial as evidenced by the loss of autonomous feeding behavior and the need for force-feeding to maintain them alive. Upon transplantation of interstitial stem cells in such animals a complete nervous system differentiate *de novo* (Minobe et al., 1995). Neuronal progenitors and nematoblasts, which express a common set of regulatory genes (Fig. 4), might actually share a common bipotent progenitor that expresses *Gsx/cnox-2* (Miljkovic-Licina et al., 2007). All together these data suggest that nematocytes and neurons can be considered as "sister cell types that evolved from a common precursor by cell type diversification" (Arendt, 2003).

But here again the same rules do not seem to apply to all cnidarians: First in some hydrozoan species (*Pennaria, Physalia*), sensory cells but neither ganglionic neurons nor nematocytes can differentiate in the absence of interstitial stem cells, suggesting that these sensory cells arise from epithelial cells (Martin & Thomas, 1981, Thomas, 1987). Whether the experimental conditions that eliminate the intersitial cells (colchicine treatment, surgical resection of the entoderm) enhance the plasticity of the epithelial cells is not known. Therefore a separate origin for sensory cells and ganglionic neurons should definitively be tested in wild-type conditions. Second, interstitial stem cells were only characterized in hydrozoans so far and it was proposed that epithelial cells would provide the progenitors for the nematocyte and neuronal cell lineages in anthozoans (Marlow et al., 2009), as well as in cubozoans and scyphozoans (Nakanishi et al., 2008). However cell lineage analyses were mostly performed in hydrozoan species, namely *Hydra*, therefore similar studies are definitively needed to clarify what is shared between cnidarian species during nematogenesis and neurogenesis.

***The complex and highly variable anatomic organization of cnidarian nervous systems***

Classically the cnidarian nervous system is described as a "diffuse nerve net" (Pantin, 1952), which can indeed be visualised by the neuron-specific RFamide immunostaining as in developing *Nematostella* (Fig. 2M-P). However in adults and larvae, the distribution of neurons is neither random nor uniform. First significant variations of the general neuron density can easily be noted along the anatomy: in medusae neurons are denser in tentacle bulbs and tentacles, in the nerve rings along the bell margin and in the manubrium as in *Clytia* medusa where the RF-amide neurons are denser in the manubrium and the tentacle bulbs (Fig. 2Q-S), in polyps neurons are denser at the extremities than in the body column (at least six fold higher in the head region in *Hydra)*. Second immunostaining help identify distinct subsets of neurons with specific spatial distribution (Grimmelikhuijzen et al., 1989, Koizumi et al., 1990), suggesting a much higher complexity than anticipated. Third, nerve rings that correspond to compression of the nerve-net architecture were identified at the base of tentacles in some *Hydra* species (Koizumi et al., 1992), around the mouth opening in *Nematostella* (Marlow et al., 2009). Nerve rings were also characterized along the bell margin of some but not all medusae (Table 1): In hydromedusae, there are two nerve rings (inner and outer), in cubozoans a single one and none in scyphozoans (Satterlie, 2002). These nerve rings that are connected to the sense organs allow a fast conduction and coordinate the swimming behaviors. More generally in all classes nerve rings are considered as an annular form of central nervous system (Passano & McCullough, 1965, Koizumi, 2007, Garm et al., 2007). These observations clearly indicate that cnidarian nervous systems are already quite sophisticated, organized in a more complex fashion than random nerve nets. However given the numerous variations in their anatomies, capturing the portrait of the ancestral cnidarian nervous system remains quite elusive. We will discuss that point once we will know more about behaviors, cell differentiation and gene regulatory pathways.

| | ***Anthozoans*** | ***Scyphozoans*** | ***Cubozoans*** | ***Hydrozoans*** |
|---|---|---|---|---|
| **Medusa anatomy** | (no medusa stage) | No velum | velum | velum |
| **Nerve nets (NN)** | Diffuse NN | Motor NN<br>Diffuse NN | Motor NN<br>Diffuse NN | Motor NN<br>Diffuse NN |
| **Nerve rings (NR) at the bell margin** | (no medusa stage) | none | a single NR | two NRs:<br>inner (subumbrellar),<br>outer (exumbrellar) |
| **Swim pacemakers** | (no swim) | rhopalia<br>(8, 16 or >4x4) | rhopalia (4x, each with 6 eyes) | inner nerve ring |
| **Neuronal electrical conduction** | (?) | (?) | (?) | Electrical and dye coupling in neurons |
| **Epithelial / neuroid conduction** | inherent muscle contraction | (?) | (?) | (+)<br>myoid conduction |
| **Gap junctions (GJ)** | no conventional GJ | no conventional GJ | no conventional GJ | neuro-epithelial GJ,<br>neuro-neuronal GJ<br>epithelial GJ |

**Table 1:** Anatomical and physiological differences between the nervous systems of the four cnidarian classes (Satterlie, 2002). Concerning electrical conduction and gap junctions, please see references in the text.

## Complex behaviors rely on chemical and electrical conduction in cnidarians

***Contraction bursts and elongation in polyps, crumpling behavior in hydromedusae***

Abraham Trembley, who was the first to definitively establish the animality of *Hydra*, reported about its spontaneous contractile activity, having observed that polyps regularly contract to form a ball before extending again (Trembley, 1744). Passano applied electrophysiological methods to *Hydra* to show that indeed these contraction bursts and extension movements that represent the main activity of the polyps, are initiated by two interacting pacemakers, one named CB (Contraction Bursts) located in the nerve ring at the base of the hypostome and inducing contractions of the longitudinal myofibers, and a second one, named RP (Rhythmic Potential), endodermal, close to the basal disc that leads to extension by inducing contraction of the circular myofibers (Passano & McCullough, 1964, Passano & McCullough, 1965, McCullough, 1965). The contraction bursts are enhanced by touch or water movement, repressed under starvation, transiently inhibited by blue light, whereas a third pacemaker, the tentacle pulse (TB) induces tentacle contraction. More recently pharmacological approaches showed that GABA, glutamate and glycine receptors modulate the activity of these pacemakers (Kass-Simon et al., 2003, Ruggieri et al., 2004). Sea anemones (anthozoans) also display spontaneous contraction bursts for which pacemakers and conduction systems with similarities to those characterized in *Hydra* were identified (McFarlane, 1974a). Hydromedusae also display a spontaneous contractile activity named “crumpling” when they contract their tentacles, manubrium and umbrella margin. However this behavior, which is supposed to be protective, involves epithelial conduction through the radial muscles but no pacemaker activity (Mackie & Passano, 1968, Mackie, 1975, Spencer, 1979).

***The swimming behavior of medusae***

Over the past 50 years, neurobiologists characterized in several cnidarian species the complex wiring that support the swimming behavior, they characterized the pacemaker activity that initiates this behavior but also the motor nerve net (MNN) that transmits the excitation, the various conduction pathways that enhance or inhibit this transmission, and finally the neuro-muscular transmission (Satterlie, 2002). As anticipated from Romanes’ work, these studies confirmed the anatomical and physiological variations that can be observed between hydromedusae and cubomedusae /scyphomedusae (see Table 1). Moreover some species evolved unique behaviors, such as the hydrozoan jellyfish *Aglantha* that can swim either fast or slow, fast to escape and slow similarly to the other medusae (Mackie, 2004b). This behavioral plasticity relies on a complex neural circuitry with giant neurons involved in fast swimming and 14 distinct but interacting conduction systems.

***The feeding response***

Beside swimming, cnidarians also actively catch their food, a behavior that requires venom discharge from the nematocytes to immobilize the preys (Tardent, 1995, Ozbek et al., 2009) and coordinated movements of the tentacles to bring the food at the mouth opening to be ingested (Westfall & Kinnamon, 1984, Mackie et al., 2003). Similarly in medusae, one or several tentacles bend towards the manubrium to transfer the prey to the digestive track and this feeding behavior usually slow downs or inhibits swimming. In the sea anemone *Calliactis* three conduction systems interact to regulate the feeding behavior (McFarlane, 1975). Once the preys are ingested, the animals exhibit satiety (Grosvenor et al., 1996) and the digestion of nutrients requires neurally controlled movements of the digestive tract (Shimizu et al., 2004).

***The light response***

Finally the light response is another important aspect of cnidarian behaviors: light can regulate the rhythmic contractions of *Hydra* polyps (Passano & McCullough, 1962) and induce phototaxic behavior as demonstrated by *Hydractinia* planulae or *Tripedalia* medusae that are attracted by light (Plickert & Schneider, 2004). Indeed electrophysiological analyses confirmed that visual inputs regulate swimming of hydromedusae and cubomedusae (Anderson & Mackie, 1977, Garm & Bielecki, 2008). In fact many medusozoan species differentiate light-sensing organs at the adult medusa stage, organs that are connected to nerve rings. These organs are either simple ocelli (multicellular organ composed of several photosensitive cells) as in *Aurelia*, or camera lens-eyes as in *Cladonema* and *Tripedalia* (Martin, 2002) (Fig. 1). Moreover cubozoans and scyphozoans differentiate multifunctional sense organs named rhopalia where structures sensing gravity and light are grouped together the swimming pacemaker (Nakanishi et al., 2009). In cubozoans, the rhopalia are the most complex, containing each a statolith, sensory epithelia, two lensed eyes and up to four pigment-cup ocelli; they regulate obstacle avoidance and mating, and were proposed to be part of the central nervous system (Garm et al., 2007). However light regulation can also take place in the absence of light-sensing organs as in *Hydra*

and *Hydractinia* where neurosecretory cells possibly regulate myoepithelial activity through RFamide neuropeptides (Plickert & Schneider, 2004). Alternatively light can also directly act on photosensitive neurons (Mackie, 1975, Anderson & Mackie, 1977).

***Chemical conduction relies on classical neurotransmitters and neuropeptides***

The electrophysiological work that clearly demonstrated the role of chemical synapses in conduction systems as the motor nerve net in hydromedusae (Anderson, 1985), was completed by pharmacological and cellular studies that searched for the chemical support of this conduction (see the detailed review by (Kass-Simon & Pierobon, 2007). In short, these studies showed that ion channels (calcium, potassium and sodium) function similarly in jellyfish and vertebrates (Grigoriev et al., 1999, Jeziorski et al., 1999, Spafford et al., 1999), that neurotransmitters like glycine (Pierobon et al., 2001), nitric oxide (Colasanti et al., 1997), endocannabinoid (De Petrocellis et al., 1999), glutamate (Bellis et al., 1991) and acetylcholine (Kass-Simon & Pierobon, 2007, Denker et al., 2008a) likely play a physiological role in hydrozoan behavior. Indeed NO affects the swimming behavior of *Aglantha digitale* (Moroz et al., 2004) whereas pharmacologically-induced modulations of the glutamate, glycine and GABA receptors affect the pacemaker activity in *Hydra* (Bellis et al., 1991, Pierobon et al., 1995, Kass-Simon et al., 2003, Pierobon et al., 2004, Ruggieri et al., 2004). Similarly in anthozoans aminergic-like receptors were pharmacologically characterized and a G-protein coupled receptor was identified (Bouchard et al., 2003). One striking aspect of cnidarian nervous systems is that they are strongly peptidergic, i.e. neurosecretory cells release peptides as signaling molecules (Grimmelikhuijzen et al., 2002, Koizumi, 2002, Fujisawa, 2008), which is providing an additional level of complexity. At the neurophysiological level, neuropeptides indeed affect the neuromuscular transmission (McFarlane et al., 1991, McFarlane et al., 1993, Takahashi et al., 1997, Yum et al., 1998, Takahashi et al., 2003, Hayakawa et al., 2007), the visual and photic response (Plickert & Schneider, 2004, Parkefelt & Ekstrom, 2009) and possibly the feeding response (Pernet et al., 2004).

***Conventional gap junctions and electrical conduction are restricted to hydrozoans***

Beside chemical conduction, electrical conduction takes place in cnidarian nervous systems as demonstrated by the electrical and dye coupling observed in photosensitive neurons of *Polyorchis* that allows the photoregulation of the swimming behavior (Anderson & Mackie, 1977, Spencer & Satterlie, 1980). This electrical conduction presumably relies on gap junctions and indeed neuro-epitheliomuscular and neuro-neuronal gap junctions were observed in *Hydra*; the same neurons using actually both chemical and electrical conduction (Westfall et al., 1980).

However gap junctions are also involved in electrical conduction between non-neuronal cells, allowing epithelial or neuroid conduction. This type of conduction was proposed to be at work in hydrozoans as *Hydra* and siphonophores (Passano & McCullough, 1965, Mackie, 1965). In hydromedusae (*Sarsia, Euphysa*) and *Hydra,* gap junctions between epithelial cells were identified (Mackie & Passano, 1968, Hand & Gobel, 1972) and myoid conduction could be recorded in *Nanomia* as well as in *Euphysa* (Spencer, 1971, Josephson & Schwab, 1979). In *Hydra*, dissociated myoepithelial cells exhibit electrical activity (Kass-Simon & Diesl, 1977) and nerve-free animals are excitable (Campbell et al., 1976), suggesting that this neuroid conduction indeed relies on epithelial gap junctions although their presence is disputed (de Laat et al., 1980). Nevertheless molecular data seem to support this scenario, gap junctions are built on connexins in vertebrates, innexins in invertebrates; innexins are indeed expressed in *Hydra* and the fusion protein Inx1-GFP was found along membranes of epithelial cells (Alexopoulos et al., 2004). Therefore it is conceivable that epithelial conduction makes use of similar cellular and molecular basis since the eumetazoan ancestor.

However again hydrozoans seem to play solo: Similarly to *Hydra* “inherent muscle contractions” occur in the absence of any nerve-net activity in the sea anemone *Calliactis* (McFarlane, 1974b), but in contrast to hydrozoans, evidences for conventional gap junctions and innexin genes are missing in anthozoans (Mackie et al., 1984, Magie & Martindale, 2008). Conventional gap junctions are also missing in scyphozoans and cubozoans. However genes encoding non-conventional gap junction proteins wre cloned from two distinct anthozoan species (*Renilla* and *Nematostella*) (Germain & Anctil, 1996, Magie & Martindale, 2008); hence non-conventional gap junctions might account for neuroid conduction in anthozoans. At the evolutionary level, neuroid conduction, which is able to rapidly integrate external informations, is present in plants, protists, sponges, coelenterates and a variety of bilaterian phyla (molluscs, tunicates, amphibians); therefore neuroid conduction was proposed to have evolved earlier and independently of synaptic conduction (Horridge, 1968, Mackie, 1970, Leys et al., 1999). If true this would mean that neuroid conduction was maintained in hydrozoans but lost or significantly diverged in other cnidarian classes. Alternatively electrical conduction would have arisen multiple times independently.

## Where and when neurogenesis and nematogenesis take place in cnidarians ?

***Neurogenesis and nematogenesis during embryonic and larval development***

Data about neurogenesis and nematogenesis during development are available in a number of hydrozoan species as *Clytia (Phialidium)* (Thomas, 1987)*, Pennaria (Halocordyle)* (Martin & Thomas, 1981, Kolberg & Martin, 1988), *Hydractinia* (Plickert, 1989), *Hydra* (Brumwell & Martin, 1992), *Podocoryne* (Groger & Schmid, 2001), but also anthozoan species as *Anthopleura* (Chia & Koss, 1979)*, Nematostella* (Marlow et al., 2009), and scyphozoan species as *Aurelia* (Yuan et al., 2008, Nakanishi et al., 2008). These analyses clearly established that anthozoan, hydrozoan and scyphozoan larvae differentiate a nervous system. In these species neuronal markers as RFamide start to be expressed quite early, at the late gastrula stage (Figs. 2M-2O). In hydrozoans, some endodermal cells, named interstitial stem cells give rise to nematoblasts and neuroblasts, which rapidly migrate towards the ectodermal layer (Martin et al., 1997, Groger & Schmid, 2001) (Fig. 3A). In *Hydractinia*, this differentiation pathway is regulated by the canonical Wnt pathway (Teo et al., 2006).

In *Podocoryne* planula, tyrosin-tubulin neurons develop progressively, forming repetitive units from anterior to posterior, reminiscent of the formation of the central nervous system in bilaterians. A similar asymmetric development was noted in *Aurelia* (scyphozoan) (Nakanishi et al., 2008) and *Acropora* (anthozoan) where the multipolar and sensory nerve cells expressing the *Gsx* ortholog *cnox-2Am* are restricted to the mid-body region (Hayward et al., 2001), while the sensory nerve cells expressing RF-amide, *Pax-C* or *Emx* are denser at the aboral pole but rare or absent from the oral pole (Miller et al., 2000, de Jong et al., 2006). However in non hydrozoan classes, evidences for interstitial stem cells are missing and the neurons are supposed to differentiate from epithelial cells; moreover in *Aurelia* (scyphozoan) and *Nematostella* (anthozoan) the sensory nervous system is believed to differentiate from the ectodermal layer, a situation that is again reminiscent to that observed in vertebrates (Nakanishi et al., 2008, Marlow et al., 2009). Therefore an asymmetry in the distribution of neuronal populations along the anterior to posterior axis appears to be the rule in the cnidarian larvae and further analyses in representative cnidarian and ctenophore species should tell us whether the ectodermal origin of sensory cells can be considered as plesiomorphic.

***The apical tuft, a larval chemosensory organ shared by anthozoans and medusozoans***

Most cnidarian species undergo metamorphosis during their development, i.e. the swimming larvae transform into sessile polyps, a complex process that invoves cell death, cell proliferation and morphogenesis. The nervous system plays an essential role in this transition: anthozoan and scyphozoan larvae differentiate at the anterior/aboral larval pole a transient neuronal structure, named the apical tuft or the apical sensory organ (Yuan et al., 2008, Marlow et al., 2009). In hydrozoans, sensory neurons also densely pack at the aboral pole. This sensory anterior structure, which differentiates under the control of the FGF pathway (Matus et al., 2007, Rentzsch et al., 2008), can sense environmental cues to promotes the settlement of the swimming larva before it undergoes metamorphosis into polyp (Pang et al., 2004). Several molecular components involved in this response were identified in the hydrozoan *Hydractinia* (Walther et al., 1996, Frank et al., 2001). External clues such as lipids of bacterial source actually trigger a signaling cascade that leads to the release of LWamide neuropeptides (Leitz et al., 1994, Leitz & Lay, 1995, Schmich et al., 1998, Plickert et al., 2003). These neuropeptides synchronize the cellular response that leads to metamorphosis. Serotonin was also proposed to be part of this response in *Phialidium* (McCauley, 1997). In addition RFamide neuropeptides can inhibit the process (Katsukura et al., 2003). During metamorphosis of hydrozoan and scyphozoan larvae, large parts of the larval nervous system degenerate and a new wave of neuronal differentiation is observed with complex migration patterns (Kroiher et al., 1990, Martin, 2000, Nakanishi et al., 2008). A similar process also probably occurs in metamorphosing anthozoans (de Jong et al., 2006), suggesting that all cnidarian larvae follow similar developmental processes.

***Adult neurogenesis and nematogenesis in polyps***

The cnidarian polyps continuously differentiate a nerve net that exhibits an oral-aboral polarity, with nerve rings on the oral/apical side, pharyngeal and oral in *Nematostella*, apical in *Hydra* but no sensory organs as recognized in medusae (Galliot et al., 2009, Marlow et al., 2009). In some *Hydra* species the apical nerve ring was not characterized anatomically but Passano considered that even in such species a “functional” apical nerve ring is actually active, corresponding to a less-compressed network of ganglionic cells (personal communication). Over the past decades adult neurogenesis and nematogenesis were mostly investigated in *Hydra*; the interstitial cells, which are multipotent stem cells restricted to the ectoderm of the body column, continuously providing neurosensory cells, neurosecretory cells, ganglionic cells, mechanoreceptor cells (nematocytes), gland cells and gametes

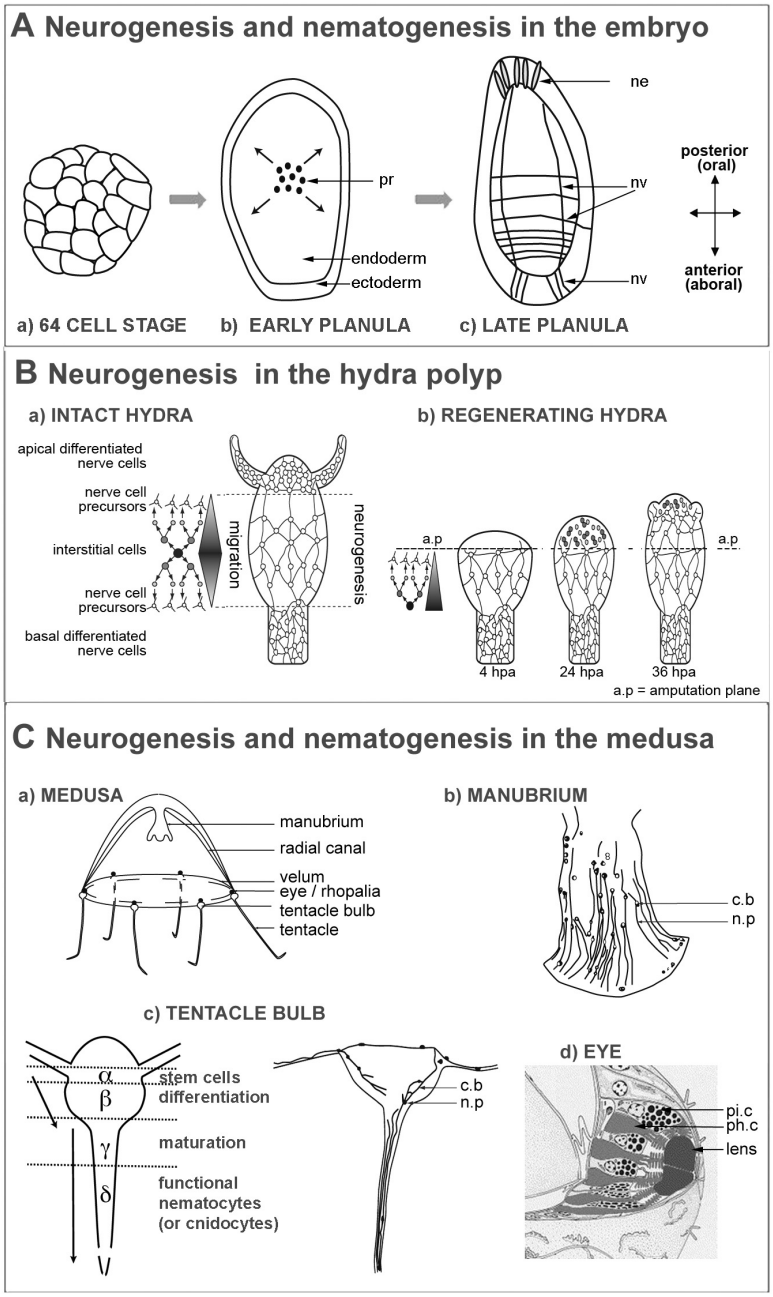


**Figure 3: Neurogenesis and nematogenesis during the cnidarian life cycle**.
A) Neurogenesis in developing *Podocoryne*. In mid-gastrula (b) the precursors of nerve cells and nematocytes (pr) arise in the endoderm, rapidly differentiate and migrate to the ectoderm, forming a diffuse network throughout the planula larva (swimming larva, c). At the planula stage (c), the nerve cells (nv), detected here with an anti-tyrosine tubulin antibody, show laterally oriented neurites that form a ladder (Groger & Schmid, 2001). The ectodermal anterior pole (up) contains nerve cells and the posterior pole (bottom) contains large mature nematocytes (ne). Upon metamorphosis, the anterior pole of the larva will become the aboral region of the polyp (also named foot) and the posterior pole of the larva will provide the oral region (also named head). B) Diagram showing the hydra nerve net, much denser in apical and basal regions. In intact hydra (a), neurogenesis takes place in the body column where interstitial stem cells provide progenitors for neurons and nematocytes. Neuronal progenitors migrate and differentiate in the upper and lower regions of the body column. In head-regenerating hydra (b), de novo neurogenesis takes place at the tip to reform in two days the nerve net that disappeared from the tip immediately after amputation. Progenitors are detected at 24 hpa and apical neurons after 32 hpa. C) Neurogenesis in the hydrozoan medusa: Shematic view of an adult hydromedusa (a) where neurogenesis takes place in three regions: the manubrium (b), the tentacle bulb (c) and the eye (d) located in the vicinity of the tentacle bulb. b) Closer view of a *Clytia* manubrium with the mouth opening directed to the bottom and the nerve net detected with the anti-RFamide antibody; cell bodies (c.b) and neuronal projections (n.p). c) Closer views of tentacle bulbs where, on the left, nematocytes differentiate from stem cells ($\alpha$) in the proximal area ($\beta$), migrate distally in the maturation area ($\gamma$) and finally reach the tentacle when mature ($\delta$) as shown by (Denker et al., 2008b). Tentacle bulbs are also the site of intense neurogenesis, as depicted on the right with RF-amide nerve cells that project from the bulb to the tentacle. d) Drawing of a *Cladonema* eye after (Weber, 1981) with the tripartite lens, the ciliated photoreceptor cells (ph.c) and the pigment cells (pi.c). Figure reproduced from Galliot et al., Dev. Biol. 2009.

when the animals follow the sexual cycle (Bode, 1996). These interstitial stem cells that can now be traced in transgenic *Hydra* (Khalturin et al., 2007), divide faster than the epithelial stem cells, once a

day. In non-hydrozoan species, interstitial stem cells were not characterized so far and neurons are supposed to differentiate directly from the epithelial cells.

In *Hydra*, the nematocyte and neuronal differentiation pathways follow distinct regulations (Fig. 4): interstitial cells committed to the nematocyte lineage undergo up to five synchronous cell cycle divisions, forming clusters of syncitial nematoblasts. Once they stop proliferating, the nematoblasts start differentiating their nematocyst vacuole, which can be of four distinct types (Holstein & Emschermann, 1995). Differentiated nematocytes then migrate to their definitive location, namely the tentacles, according to a process that relies on contact guidance from surrounding tentacles (Campbell & Marcum, 1980). In the tentacles, nematocytes are embedded within large epithelial cells named battery cells, each battery cell containing several nematocytes, themselves connected to sensory neurons by synapses. After discharge of their capsule, nematocytes are eliminated and replaced by new ones.

In contrast, the differentiation of neuronal cell appears simpler: neuronal progenitors are located along the body column, more numerous in the upper and lower (peduncle) regions but absent from the tentacles or the basal disc. Indeed transplantation of interstitial stem cells in nerve free *Hydra* have shown that neurogenesis but not nematogenesis is strongly influenced by the position of the graft along the body column, i.e. enhanced at the lower and upper positions of the body column where nerve cell density is higher (Yaross & Bode, 1978). Interestingly this position-dependent regulation of neurogenesis seems to be largely under the control of epithelial cells (Koizumi et al., 1990). Neuronal progenitors get arrested in G2 until a signal let them divide and terminally differentiate as a sensory or ganglionic cell (Schaller et al., 1989, Bode, 1996). Mature neurons receive signals from the head and foot regions to migrate, explaining the higher neuronal densities recorded at the extremities (Fig. 3Ba). After mid-gastric bisection, nematocytes and neurons disappear from the head regenerating tip and a wave of *de novo* neurogenesis occurs in the presumptive head region on the second day (Figs. 3Bb, 5D-G), preceding the emergence of the regenerated head (Miljkovic-Licina et al., 2007). A similar wave of *de novo* neurogenesis is also observed in the presumptive head region during budding, the asexual form of reproduction in *Hydra*. Therefore *Hydra* provides a model system where adult neurogenesis can be investigated in homeostatic and two distinct developmental contexts.

***Differentiation and regeneration of light-sensing organs in medusae***

In cnidarians, sensory organs that detect light and pressure differentiate at the time the medusae develop as ephyrae in scyphozoans or as buds in hydrozoans. The sensory organs that can detect light exhibit a variable complexity in their anatomy (Martin, 2002): they can be clustered photoreceptor cells named ocelli or more complex lens-eyes (Fig. 3C). A distinct sensory organ named statocyst (or lithocyst) can also measure pressure. In scyphozoans and cubozoans these two types of sensory organs are grouped together within a structure named rhopalia that also contains the swim pacemaker. In *Aurelia* (syphozoan) the gravity-sensing organ, the swim pacemaker and the ocelli differentiate following a strict temporal order (Nakanishi et al., 2009). Whether the differentiation of cubozoan rhopalia follows a similar temporal order is currently unknown. In all medusae analyzed so far the photoreceptor cells are ciliated as in vertebrate visual photoreceptor cells and not rhabdomeric as predominantly observed in invertebrate ones. Moreover cnidarians express c-opsins, suggesting that these two types of photoreceptors were already present in the Cnidaria Bilateria ancestor (Suga et al., 2008). Also the cubozoan and hydrozoan eyes express *Pax* and *Six* genes (Kozmik et al., 2003, Stierwald et al., 2004), suggesting a common regulation of vision in eumetazoans. Nevertheless there is one strange case, that of *Tripedalia* (cubozoan), whose larva exhibits pigmented rhabdomeric photoreceptor cells, while the adult medusa eye uses ciliated ones (Nordstrom et al., 2003, Kozmik et al., 2008). As cnidarian larvae do not differentiate eyes, the *Tripedalia* larva might thus represent an ancestral rhabdomeric light-sensing organ that was lost in most cnidarian species. Finally lens-eyes can regenerate as in *Cladonema* (Stierwald et al., 2004), which thus offers an experimental model system to investigate the specification of eyes in developmental and regenerative context in cnidarians (Fig. 3C).

***Adult neurogenesis and nematogenesis in medusae***

In the mature medusa, the manubrium and the tentacle bulbs are the sites of intense production of neurons and nematocytes as observed in the hydrozoan jellyfish (Figs. 2Q-S, 3C). In contrast to *Hydra* polyps where all stages of nematogenesis overlap along the body column, the differentiation stages in *Clytia* follow a proximo-distal gradient along the tentacle bulbs (Denker et al., 2008b). Moreover the tentacle bulb isolated from the medusa has the capacity to survive for several days in culture, opening the possibility for manipulations and functional studies.

## A tentative view of neurogenesis in early eumetazoan evolution

***Can we trace back in cnidarians an ancestral neurogenic circuitry?***

In the absence of genetically tractable model systems in cnidarians, the characterization of a neurogenic circuitry shared by cnidarians first, by coelenterates second and by coelenterates and bilaterians third, will rely on five criteria:

1) the orthologous character of the cnidarian and bilaterian gene families;
2) for each gene family, the stage and cell type specific regulation in the nervous systems of anthozoan and medusozoan species;
3) the biochemical characterization of the functional domains of a given gene product;
4) the characterization of the regulatory elements and trans-acting factors involved in cell-specific expression;
5) the functional proof of the neurogenic function through loss-of-function (possibly completed by gain-of-function assays) in anthozoan and medusozoan species.

So far studies that would fulfill all these criteria are missing but tools are now available in several cnidarian species to investigate each of these criteria.

Gene cloning in cnidarians was initially targeted to evolutionarily-conserved developmental genes, then gene sequences were obtained through EST projects as in *Hydra* (Hwang et al., 2007)*, Clytia* (Jager et al., 2006)*, Hydractinia* (Soza-Ried et al., 2009), *Acropora* and *Nematostella* (Technau et al.,

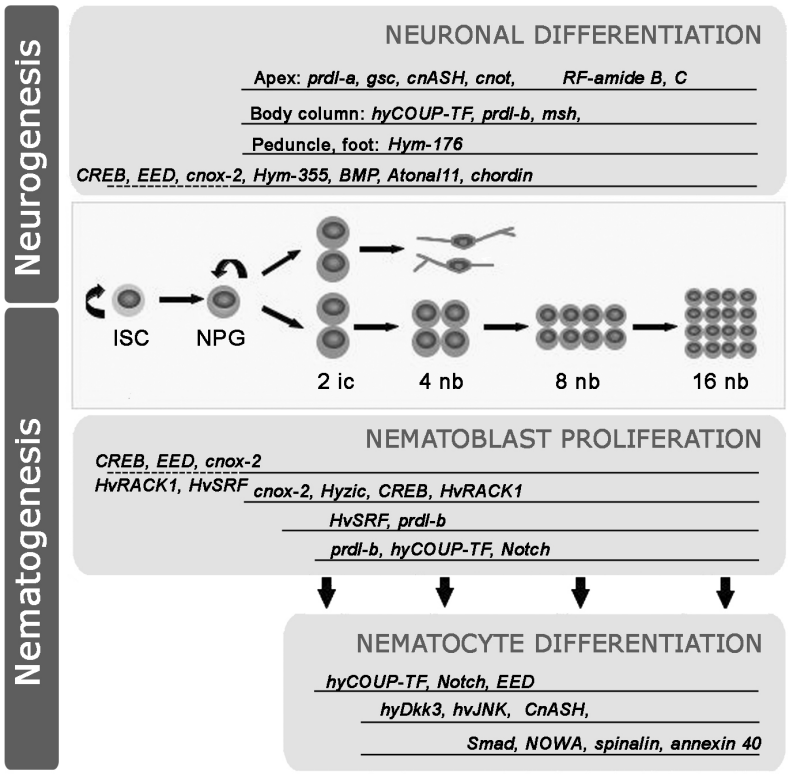


**Figure 4**: **Neurogenesis and nematogenesis in *Hydra*.**
In hydrozoans interstitial stem cells (ISC) provide progenitors for neurons and nematocytes, but also for gland cells and gametes (not shown). ISC express orthologs to *RACK1* (Hornberger & Hassel, 1997)*, SRF* (Hoffmann & Kroiher, 2001), EED (Genikhovich et al., 2006), CREB (Chera et al., 2007). Neuronal progenitors (NPG) are fast-cycling cells that differentiate into neurons and nematocytes; they express the ParaHox gene *Gsx/cnox-2* (Miljkovic-Licina et al., 2007). Hym-176 is a myoactive neuropeptide but the Hym-355 and RF-amide neuropeptides regulate neuronal differentiation positively and negatively respectively. The homeobox genes *msh, prdl-b* (Miljkovic-Licina et al., 2004) and the nuclear receptor *COUP-TF* (Gauchat et al., 2004) are restricted to neurogenesis in the body column whereas *prdl-a* (Gauchat et al., 1998), *Gsc* (Broun et al., 1999), *cnASH* (Hayakawa et al., 2004), *cnot* (Galliot et al., 2009) and *Gsx/cnox-2* (Miljkovic-Licina et al., 2007) are expressed in distinct neuronal subsets in the apical region. Regulation of neurogenesis by epithelial or gland cells (Guder et al., 2006, Fujisawa, 2008) are not indicated here.
During nematogenesis, *Gsx/cnox-2* (Miljkovic-Licina et al., 2007), *Hyzic* (Lindgens et al., 2004) and *prdl-b* (Gauchat et al., 2004) are successively required for keeping the clustered nematoblasts synchronously dividing (horizontal arrows). In contrast, *hyCOUP-TF* that acts as a transcriptional repressor (Gauchat et al., 2004), *HvJNK* (Philipp et al., 2005) and the Notch pathway (Kasbauer et al., 2007, Khalturin et al., 2007) likely promote arrest of proliferation and entry into differentiation, a switch that can take place after 2, 3, 4 or 5 runs of cell division (vertical arrows). The nematocytes that differentiate the typical venom-filled capsule (nematocyst) express Annexin40 (Schlaepfer et al., 1992), the Achaete scute homolog *CnASH* (Grens et al., 1995, Lindgens et al., 2004), *Smad* (Hobmayer et al., 2001) and *dickkopf-3* (Fedders et al., 2004). A number of phylum/species-specific genes are expressed in nematocytes (Hwang et al., 2007), among them spinalin (Koch et al., 1998) and NOWA (Engel et al., 2002) are structural proteins of the nematocyst. Genes are written italic and proteins plain.

2005). Given the importance of peptides as neurotransmitters in cnidarians, a peptide sequencing project was launched, which identified significant regulators of neurophysiology, neurogenesis and morphogenesis (Takahashi et al., 1997, Fujisawa, 2008). In parallel genome sequencing projects were launched in *Nematostella* (Putnam et al., 2007) and *Hydra* (http://hydrazome.metazome.net). Analyses from high throughput sequencing have provided two major conclusions, first that the complexity of a large number of gene families was already established in the Cnidaria Bilateria ancestor (Technau et al., 2005, Putnam et al., 2007); second that this ancestral diversity was secondarily reduced in some bilaterian phyla as Ecdysozoa (Miller et al., 2005). Concerning the gene families that regulate neurogenesis in bilaterians, the origin of most of them predates Porifera divergence (Fig. 6) (Sakarya et al., 2007, Simionato et al., 2007, Larroux et al., 2008, Richards et al., 2008, Sullivan et al., 2008) and their diversity was indeed already established in cnidarians, although it was still increased after coelenterate divergence (Galliot et al., 2009).

Regarding the hydrozoan adult nervous system, four types of cellular expression patterns were recorded (Fig. 4): *1) restricted to the neuronal cell lineage, 2) restricted to the nematocyte lineage*, *3) co-expressed in these two cell lineages, 4) expressed in the nervous system but not restricted to it* (Seipel et al., 2004, Miljkovic-Licina et al., 2004, Gauchat et al., 2004, Galliot et al., 2006, Chera et al., 2007, Denker et al., 2008b, Galliot et al., 2009). It should be noted that expression in the nematocyte lineage can mask neuronal expression, often more difficult to detect. Therefore genes from the second class might actually belong to the third one. In summary the candidate regulators of neurogenesis in cnidarians are bHLH (*Achaete-Scute*, *atonal-like*), ANTP-class (*Gsx, emx, msx*, *not),* PRD-class (*aristaless*-like, *gsc, rx, repo, PaxA/C, PaxB)* and SIN-class (*Six1/2, Six3/6, Six4/5*) transcription factors whereas nematogenesis would specifically require *Zic*, *Dickkopf3, JNK, Smad*, *CnASH/Ash1, Notch* (see Fig. 4).

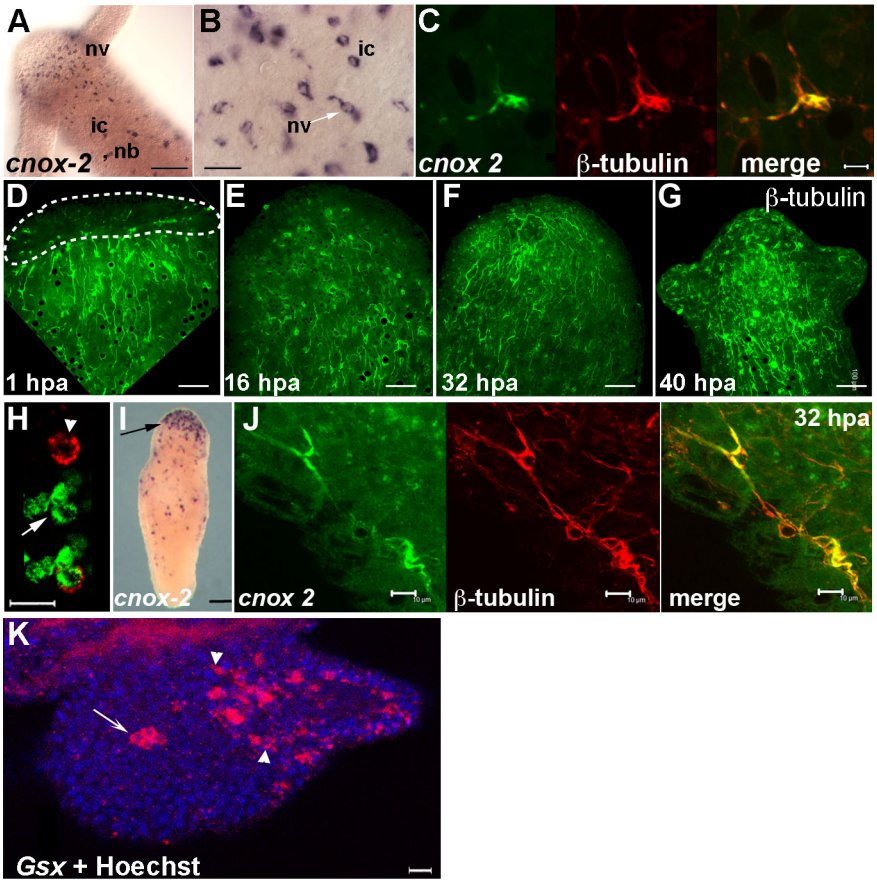


**Figure 5: Adult neurogenesis in hydrozoans**
A-C) Neurons (arrow) located at the base of the head region between the tentacle roots express the ParaHox *Gsx* orthologue, *cnox-2*. Along the body column, *cnox-2*+ cells are interstitial cells (ic) and proliferating nematoblasts (nb). D-G) *De novo* neurogenesis during *Hydra* head regeneration. Following bisection the tip of the head-regenerating half is depleted of neurons (D, outline), progressively repopulated with neuronal progenitors (U) and mature neurons (V) detected with anti β-tubulin (green). The apical nervous system at 40 hpa is still less dense than in adult polyps (W). hpa : hours post-amputation. H-J) During head regeneration, *cnox-2* is expressed in progenitors and differentiating neurons in the presumptive head region (arrow) shown here at 32 hpa. I) Apical BrdU labeled cells (red) expressing *cnox-2* (green). J) Apical neurons co-expressing *cnox-2* transcripts (red) and β-tubulin (green). K) In the *Clytia* medusa, the proliferative (arrow) and differentiating (arrowheads) zones of the tentacle bulbs express the ParaHox gene *Gsx*, detected here with FastRed (red). Scale bars: 200 µm (A), 10 µm (B, I, J), 20 µm (K), 50 µm (D-G).

To better characterize the cellular status of the cells expressing a given gene, protein colocalization and cellular analyses can be coupled to in situ hybridization. Except one candidate in *Polyorchis* (Lin et al., 2001), no pan-neuronal markers that would cross-hybridize between cnidarian species was reported yet but several antibodies provide useful tools to detect large subsets of neurons (Fig. 2; Fig. 5). Moreover cell proliferation markers as *in vivo* BrdU-labeling or anti-phoshoH3, which detect S-phase cells and mitotic cells respectively, can also be combined to expression analyses (Fig. 5H). Such approaches, well established in *Hydra* (Koizumi, 2002, Lindgens et al., 2004, Gauchat et al., 2004, Miljkovic-Licina et al., 2007) and *Clytia* (Denker et al., 2008b), tell us whether a given gene is rather expressed in proliferating progenitors, differentiating cells and/or terminally differentiated cells. Moreover studying cellular behaviors live is now possible in *Hydra* thanks to the transient expression of reporter constructs (Bottger et al., 2002, Miljkovic et al., 2002, Muller-Taubenberger et al., 2006) or the obtention of stable transgenic lines (Wittlieb et al., 2006, Khalturin et al., 2007).

Biochemical analyses as gel retardation are useful *in vitro* approaches as they can identify among tissue extracts the protein complexes that are activated at specific stages. Such methods also confirmed the expected function of predicted protein domains as the specific DNA-binding activity of the CREB, bHLH, paired-like, Pax, RXR, COUP-TF transcription factors that are expressed in cnidarian nervous systems (Galliot et al., 1995, Grens et al., 1995, Gauchat et al., 1998, Kostrouch et al., 1998, Miller et al., 2000, Sun et al., 2001, Plaza et al., 2003, Kozmik et al., 2003, Gauchat et al., 2004, Kaloulis et al., 2004). The transactivation potential of some of these could also be tested in transfected mammalian cells (Kozmik et al., 2003, Gauchat et al., 2004) or in bilaterian model systems (see Table 2).

| **Gene name** | **Cnidarian species** | **Function in cnidarians** | **References** |
|---|---|---|---|
| *LWamide (Hym-54)* | *Hydra vulgaris, Hydractinia* | neuropeptide required for metamorphosis | (Takahashi et al., 1997, Plickert et al., 2003) |
| *RFamide* | *Hydractinia* | neuropeptide inhibiting metamorphosis | (Katsukura et al., 2003) |
| *RGamide (Hym-355)* | *Hydra vulgaris* | neuropeptide enhancing neurogenesis | (Takahashi et al., 2000) |
| *PWamide (Hym-33H)* | *Hydra vulgaris* | epitheliopeptide inhibiting neurogenesis | (Takahashi et al., 1997, Takahashi et al., 2009) |
| *Notch pathway* | *Hydra vulgaris* | Required for the post-mitotic differentiation of nematocytes | (Kasbauer et al., 2007, Khalturin et al., 2007) |
| *Frizzled, Wnt3* | *Hydractinia* | Wnt3 overactivation by paullones induces nerve cell and nematocyte differentiation | (Teo et al., 2006) |
| *Wnt3, Dickkopf1/2/4* | *Hydra vulgaris* | Dickkopf1/2/4 antagonize Wnt3 in the body column, inducing a neurogenic zone | (Guder et al., 2006) |
| *FGF (NvFGFa1, NvFGFa2), FGFR (NvFGFRa)* | *Nematostella vectensis* | NvFGFa1, NvFGFRa support apical sensory organ formation in *Nv* planula; NvFGFa2 inhibits its ectopic formation | (Rentzsch et al., 2008) |
| *Zic* | *Hydra vulgaris* | Promotes proliferation of nematoblasts and prevents their differentiation | (Lindgens et al., 2004) |
| *ParaHox Gsx (cnox-2)* | *Hydra vulgaris* | Promotes proliferation of progenitors for apical neurons and nematoblasts in intact and regenerating *Hydra*; upstream to *Zic* | (Miljkovic-Licina et al., 2007) |
| | | **Overexpression in bilaterians** | |
| *Achaete-scute (CnASH)* | *Hydra vulgaris* | Proneural activity in *Drosophila*: induction of ectopic sensory organs, partial rescue of the achaete and scute double mutant | (Grens et al., 1995) |
| *Brachyury (HyBra2)* | *Hydra vulgaris* | Neural inducing activity in *Xenopus* | (Bielen et al., 2007) |
| *Pax B (Pax2/5/8 - Pax6 like)* | *Tripedalia cystophora* | Proneural activity in *Drosophila*: induction of small ectopic eyes, partial rescue of spa(pol), a Pax2 eye mutant. | (Kozmik et al., 2003) |

**Table 2:** Cnidarian genes regulating neurogenesis in cnidarians and/or in bilaterians as deduced from functional analyses.

A limited number of autologous functional studies have been performed so far, they provided results (Table 2) regarding the following aspects: 1) the RGamide and PWamide peptides play opposite roles on neuronal differentiation in *Hydra*; 2) the LWamide and RFamide neuropeptides play opposite roles on metamorphosis in *Hydractinia*; 3) the canonical Wnt3 pathway regulates the stock of interstitial stem cells in *Hydractinia*; 4) the inhibition of the Wnt3 pathway might specify a neurogenic region in *Hydra* (Guder et al., 2006); 5) the FGF pathway controls aboral specification in the *Nematostella* planula; 6) the *Gsx/cnox-2* homeobox gene controls the proliferation of bipotent neuronal progenitors in *Hydra*; 7) the Zic gene promotes proliferation of nematoblasts and the Notch pathway supports nematocyte differentiation in *Hydra*.

Which of the candidate genes mentioned above belong to evolutionarily-conserved genetic circuitries? It is certainly too early to discuss any picture but some candidates are promising as the *Pax / Six / opsin* cascade in eye specification. Similarly in developing mammals the *Gsx* orthologs specify brain neuronal progenitors (Toresson & Campbell, 2001, Yun et al., 2003), whereas in *Drosophila* and mammals *Gsx/Ind* promotes dorsal ventral patterning along the neural tube through negative epistatic relationships with the *NK2/Vnd* and *Msx/msh* homeobox genes (Mieko Mizutani & Bier, 2008). As all these genes are expressed in cnidarian species, including the *Hydra msx* in neurons, further studies testing the regulatory interactions between *Gsx/cnox-2, msx* and *NK-2* genes in cnidarian nervous systems should tell us whether this block of genes was already functional in the Cnidaria Bilateria ancestor.

***How to draw the Urcnidarian nervous system?***

Given the variations observed in the neuroanatomies and neurophysiologies of cnidarians, drawing the ancestral cnidarian nervous system is not an easy task. The common features that are shared by anthozoans and medusozoans include four main characters, 1) *the differentiation of three main cell types*, the ectodermal sensory cells that are often multifunctional, the basal ganglionic cells and the nematocytes; 2) the functional organization of these cell types in *conduction systems that use nerve nets and nerve rings*; 3) the conduction through *chemical synapses* that display strikingly evolutionarily-conserved properties with bilaterian synapses; 4) the development of *an anterior sensory organ* in the swimming larva required for settlement and metamorphosis.

Now a number of characters that are not shared between anthozoans and medusozoans receive a controversial origin. Two scenarios can be discussed for each of them, either they arose by convergent evolution, i.e. were acquired independently in various phyla, or they are considered as homologous and were submitted to divergent evolution, meaning in Cnidaria, some loss in one or the other cnidarian group. In this review, we have identified at least four of these convergent/divergent characters:

1) *the stem cell(s) of the neuronal and interstitial cell lineages*, identified as interstitial in hydrozoans, and possibly epithelial in other cnidarian classes. Also the question of a unique stem cell for all cells of the various hydrozoan nervous systems remains open as a possible epithelial origin of sensory cells was documented. For both questions further comparative cellular and molecular studies will help clarify these issues;

2) *the embryonic origin of the nervous system* is also disputed: endodermal in hydrozoans and possibly ectodermal in anthozoans and scyphozoans, at least for some cell lineages. If confirmed, this would indicate that the hydrozoan situation is derived and that an ancestral ectodermal origin might actually correspond to the prevalent bilaterian situation;

3) *the electrical conduction through neurons and epithelial cells* appears to be restricted to hydrozoans. Given that gap junctions support this electrical conduction and that innexins that form conventional gap junctions are widely conserved between invertebrates including hydrozoans, convergent evolution seems unlikely. Consequently, conventional gap junctions would have been lost in anthozoans and hydrozoans would better represent the ancestral situation for this feature;

4) *the presence of light-sensing organs and vision in medusozoans,* but not in anthozoans. Light sensing is widely spread in non-metazoan species but the clustering of photoreceptor cells to form sensory organs was a major innovation in animal evolution. The question of a unique origin for all animal eyes or a repeatedly convergent evolution is a long-standing one (Nilsson & Arendt, 2008). However several key components of the genetic circuitry driving eye specification and phototransduction in bilaterians (*Pax, Six, c-opsins*) are already available and properly regulated in cnidarians, strongly supporting the hypothesis of a unique origin in the Cnidaria Bilateria ancestor. Nevertheless the repetitive recruitment of orthologous genes to perform the same task was proposed (Kozmik et al., 2008), suggesting then some higher hierarchical order in the accessibility to developmental processes.

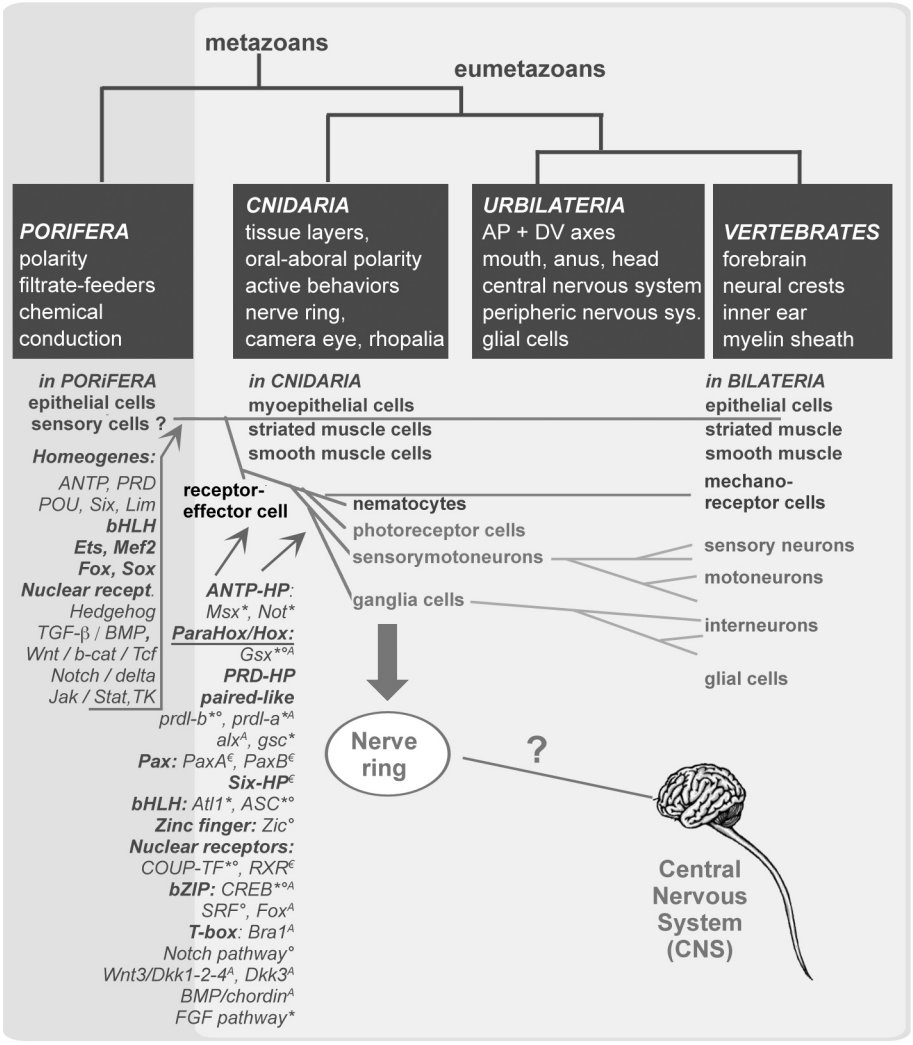


**Figure 6: The early evolutionary steps of neurogenesis from Porifera to Bilateria.**
Sponges (porifera) that differentiate sensory-like cells but no nervous system feed through passive filtration. Emergence of cells with synaptic conduction likely preceded the divergence of Cnidaria. The various active behaviors of cnidarians rely on complex conduction systems using nerve nets, nerve rings and sensory organs as well as on mechanoreceptor cells (nematocytes) that are densely packed in the tentacles. Cnidarian neurons are already diversified in sensorymotoneurons, ganglionic cells and photoreceptor cells that in medusae can cluster to form ocelli or more complex camera-type eyes. Ganglionic cells that function as interneurons can compress together to form nerve rings, recognized as an annular form of cephalisation. However this scheme does not reflect the wide anatomical and physiological diversity among cnidarian nervous systems (see text). The cnidarian genes expressed during neurogenesis (*), nematogenesis (°), eye differentiation (€) and/or apical patterning (A) are listed (brown). The presence in sponges (red) and placozoans (Schierwater et al., 2009) of gene families that regulate neurogenesis in bilaterians, indicates that their conservation across evolution was constrained even in the absence of neurogenesis.

### *Perspectives: the paradigmatic value of the cnidarian nervous system*

Cnidarians nervous systems provide unique experimental paradigms not only to trace back the evolutionary history of their differentiation and their physiology, but also to decipher some striking properties that might have major biomedical impact. In fact cellular studies in *Hydra* polyps have demonstrated an unusual plasticity of the nervous system as neurons constantly change their phenotype while migrating towards the extremities (Bode et al., 1986, Bode, 1992). In animals totally depleted of their neuronal progenitors after exposure to antineoplastic drugs, differentiated neurons of the body column change their phenotype in de novo regenerated heads. More strikingly, ganglionic neurons of the body column can transdifferentiate into apical neurosensory cells after regeneration, as evidenced by the de novo differentiation of a cilium (Koizumi et al., 1988). Similarly the striated muscle cells of the jellyfish *Podocoryne* can be induced to transdifferentiate to neurons and smooth muscle cells (Schmid & Reber-Muller, 1995). Whether this cellular plasticity is restricted to hydrozoans remains to be investigated.

Beside transdifferentiation, cnidarians permanently renew their nervous system in adulthood and can even regenerate it after injury, including complete eyes. The biology of stem cells at the various stages

of the life cycle is one aspect of this potential that requires careful comparative investigations. Whether there are some common molecular basis in cnidarians for this unusual regenerative potential is at the moment not clear, but the fact that cnidarian genomes contain most of the signaling pathways and regulatory genes active in bilaterians, together with the recent development of potent functional tools are promising conditions to help uncover some ancient principles about developmental and adult neurogenesis.

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## References

Aerne B, L., Stidwill RP, Tardent P. 1991. Nematocyte discharge in Hydra does not require the presence of nerve cells. J Exp Zool 258: 137-41

Agassiz L. 1850. Contributions to the natural history of the acalephae of North America, Part 1: on the naked-eyed medusae of the shores of Massachusetts, in their perfect state of development. Memoirs of the American Academy of Arts and Sciences 4: 221–316

Alexopoulos H, Bottger A, Fischer S, Levin A, Wolf A, et al. 2004. Evolution of gap junctions: the missing link? Curr Biol 14: R879-80

Anderson PA. 1985. Physiology of a bidirectional, excitatory, chemical synapse. J Neurophysiol 53: 821-35

Anderson PA, Mackie GO. 1977. Electrically coupled, photosensitive neurons control swimming in a jellyfish. Science 197: 186-8

Anderson PA, Spencer AN. 1989. The importance of cnidarian synapses for neurobiology. J Neurobiol 20: 435-57

Arendt D. 2003. Evolution of eyes and photoreceptor cell types. Int J Dev Biol 47: 563-71

Arkett SA, Mackie GO, Meech RW. 1988. Hair cell mechanoreception in the jellyfish Aglantha digitale. J Exp Biol 135: 329-42

Bellis SL, Grosvenor W, Kass-Simon G, Rhoads DE. 1991. Chemoreception in Hydra vulgaris (attenuata): initial characterization of two distinct binding sites for L-glutamic acid. Biochim Biophys Acta 1061: 89-94

Bielen H, Oberleitner S, Marcellini S, Gee L, Lemaire P, et al. 2007. Divergent functions of two ancient Hydra Brachyury paralogues suggest specific roles for their C-terminal domains in tissue fate induction. Development 134: 4187-97

Bode H, Dunne J, Heimfeld S, Huang L, Javois L, et al. 1986. Transdifferentiation occurs continuously in adult hydra. Curr Top Dev Biol 20: 257-80

Bode HR. 1992. Continuous conversion of neuron phenotype in hydra. Trends Genet 8: 279-84

Bode HR. 1996. The interstitial cell lineage of hydra: a stem cell system that arose early in evolution. J Cell Sci 109: 1155-64

Bottger A, Alexandrova O, Cikala M, Schade M, Herold M, David CN. 2002. GFP expression in Hydra: lessons from the particle gun. Dev Genes Evol 212: 302-5

Bouchard C, Ribeiro P, Dube F, Anctil M. 2003. A new G protein-coupled receptor from a primitive metazoan shows homology with vertebrate aminergic receptors and displays constitutive activity in mammalian cells. J Neurochem 86: 1149-61

Bouillon J. 1994a. Cellules urticantes. In Traité de Zoologie. Anatomie, Systématique, Biologie. CNIDAIRES, Hydrozoaires, Scyphozoaires, Cubozoaires, CTENAIRES., ed. PP Grassé, pp. 65-107. Paris: Masson

Bouillon J. 1994b. Embranchement des cnidaires (Cnidaria). In Traité de Zoologie. Cnidaires, Cténaires, ed. PP Grassé, pp. 1-28. Paris: Masson

Bridge D, Cunningham CW, DeSalle R, Buss LW. 1995. Class-level relationships in the phylum Cnidaria: molecular and morphological evidence. Mol Biol Evol 12: 679-89

Broun M, Sokol S, Bode HR. 1999. Cngsc, a homologue of goosecoid, participates in the patterning of the head, and is expressed in the organizer region of Hydra. Development 126: 5245-54

Brumwell GB, Martin VJ. 1992. Immunocytochemically defined populations of neurons progressively increase in size through embryogenesis of Hydra vulgaris. Biol Bull 203: 70-9

Campbell RD. 1976. Elimination by Hydra interstitial and nerve cells by means of colchicine. J Cell Sci 21: 1-13

Campbell RD, Josephson RK, Schwab WE, Rushforth NB. 1976. Excitability of nerve-free hydra. Nature 262: 388-90

Campbell RD, Marcum BA. 1980. Nematocyte migration in hydra: evidence for contact guidance in vivo. J Cell Sci 41: 33-51

Chera S, Kaloulis K, Galliot B. 2007. The cAMP response element binding protein (CREB) as an integrative HUB selector in metazoans: clues from the hydra model system. Biosystems 87: 191-203

Chia FS, Koss R. 1979. Fine-structural studies of the nervous-system and the apical organ in the planula larva of the sea-anemone Anthopleura elegantissima. J Morph 160: 275-98

Colasanti M, Venturini G, Merante A, Musci G, Lauro GM. 1997. Nitric oxide involvement in Hydra vulgaris very primitive olfactory- like system. J Neurosci 17: 493-9

Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. Syst Biol 55: 97-115

David CN. 1973. A quantitative method for maceration of hydra tissue. Wilhelm Roux´ Archives of Developmental Biology 171: 259-68

Davis LE. 1974. Ultrastructural studies of development of nerves in hydra. Am Zool 14: 551-73

de Jong DM, Hislop NR, Hayward DC, Reece-Hoyes JS, Pontynen PC, et al. 2006. Components of both major axial patterning systems of the Bilateria are differentially expressed along the primary axis of a 'radiate' animal, the anthozoan cnidarian Acropora millepora. Dev Biol 298: 632-43

de Laat SW, Tertoolen LG, Grimmelikhuijzen CJ. 1980. No junctional communication between epithelial cells in hydra. Nature 288: 711-3

De Petrocellis L, Melck D, Bisogno T, Milone A, Di Marzo V. 1999. Finding of the endocannabinoid signalling system in Hydra, a very primitive organism: possible role in the feeding response. Neuroscience 92: 377-87

De Robertis EM. 2008. Evo-devo: variations on ancestral themes. Cell 132: 185-95

Denker E, Chatonnet A, Rabet N. 2008a. Acetylcholinesterase activity in Clytia hemisphaerica (Cnidaria). Chem Biol Interact 175: 125-8

Denker E, Manuel M, Leclere L, Le Guyader H, Rabet N. 2008b. Ordered progression of nematogenesis from stem cells through differentiation stages in the tentacle bulb of Clytia hemisphaerica (Hydrozoa, Cnidaria). Dev Biol 315: 99-113

Engel U, Oezbek S, Engel R, Petri B, Lottspeich F, Holstein TW. 2002. Nowa, a novel protein with minicollagen Cys-rich domains, is involved in nematocyst formation in Hydra. J Cell Sci 115: 3923-34

Fedders H, Augustin R, Bosch TC. 2004. A Dickkopf- 3-related gene is expressed in differentiating nematocytes in the basal metazoan Hydra. Dev Genes Evol 214: 72-80

Franc A. 1994. La strobilation. In Traité de Zoologie. Cnidaires, Cténaires, ed. PP Grassé, pp. 635-8. Paris: Masson

Frank U, Leitz T, Muller WA. 2001. The hydroid Hydractinia: a versatile, informative cnidarian representative. BioEssays 23: 963-71.

Fujisawa T. 2008. Hydra peptide project 1993-2007. Dev Growth Differ 50 Suppl 1: S257-68

Galliot B, Miljkovic-Licina M, de Rosa R, Chera S. 2006. Hydra, a niche for cell and developmental plasticity. Semin Cell Dev Biol 17: 492-502

Galliot B, Quiquand M, Ghila L, de Rosa R, Miljkovic-Licina M, Chera S. 2009. Origins of neurogenesis, a cnidarian view. Dev Biol 332: 2-24

Galliot B, Schmid V. 2002. Cnidarians as a model system for understanding evolution and regeneration. Int J Dev Biol 46: 39-48

Galliot B, Welschof M, Schuckert O, Hoffmeister S, Schaller HC. 1995. The cAMP response element binding protein is involved in hydra regeneration. Development 121: 1205-16

Garm A, Bielecki J. 2008. Swim pacemakers in box jellyfish are modulated by the visual input. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 194: 641-51

Garm A, Poussart Y, Parkefelt L, Ekstrom P, Nilsson DE. 2007. The ring nerve of the box jellyfish Tripedalia cystophora. Cell Tissue Res 329: 147-57

Gauchat D, Escriva H, Miljkovic-Licina M, Chera S, Langlois MC, et al. 2004. The orphan COUP-TF nuclear receptors are markers for neurogenesis from cnidarians to vertebrates. Dev Biol 275: 104-23

Gauchat D, Kreger S, Holstein T, Galliot B. 1998. prdl-a, a gene marker for hydra apical differentiation related to triploblastic paired-like head-specific genes. Development 125: 1637-45

Gazave E, Lapebie P, Renard E, Bezac C, Boury-Esnault N, et al. 2008. NK homeobox genes with choanocyte-specific expression in homoscleromorph sponges. Dev Genes Evol 218: 479-89

Genikhovich G, Kurn U, Hemmrich G, Bosch TC. 2006. Discovery of genes expressed in Hydra embryogenesis. Dev Biol 289: 466-81

Germain G, Anctil M. 1996. Evidence for intercellular coupling and connexin-protein in the luminescent endoderm of Koellikeri (Cnidaria, Anthozoa). Biol Bull 191: 353-66

Grens A, Mason E, Marsh JL, Bode HR. 1995. Evolutionary conservation of a cell fate specification gene: the Hydra achaete-scute homolog has proneural activity in Drosophila. Development 121: 4027-35

Grigoriev NG, Spafford JD, Spencer AN. 1999. Modulation of jellyfish potassium channels by external potassium ions. J Neurophysiol 82: 1728-39

Grimmelikhuijzen CJ, Graff D, Koizumi O, Westfall JA, McFarlane ID. 1989. Neurons and their peptide transmitters in Coelenterates. In Serie A: Life Sciences Vol. 188, ed. NA Series, pp. 95-109. New York: Plenum Press

Grimmelikhuijzen CJ, Williamson M, Hansen GN. 2002. Neuropeptides in cnidarians. Can J Zool 80: 1690-702

Groger H, Schmid V. 2001. Larval development in Cnidaria: a connection to Bilateria? Genesis 29: 110-4

Grosvenor W, Rhoads DE, Kass-Simon G. 1996. Chemoreceptive control of feeding processes in hydra. Chem Senses 21: 313-21

Guder C, Pinho S, Nacak TG, Schmidt HA, Hobmayer B, et al. 2006. An ancient Wnt-Dickkopf antagonism in Hydra. Development 133: 901-11

Hand AR, Gobel S. 1972. The structural organization of the septate and gap junctions of Hydra. J Cell Biol 52: 397-408

Hayakawa E, Fujisawa C, Fujisawa T. 2004. Involvement of Hydra achaete-scute gene CnASH in the differentiation pathway of sensory neurons in the tentacles. Dev Genes Evol 214: 486-92

Hayakawa E, Takahashi T, Nishimiya-Fujisawa C, Fujisawa T. 2007. A novel neuropeptide (FRamide) family identified by a peptidomic approach in Hydra magnipapillata. Febs J 274: 5438-48

Hayward DC, Catmull J, Reece-Hoyes JS, Berghammer H, Dodd H, et al. 2001. Gene structure and larval expression of cnox-2Am from the coral Acropora millepora. Dev Genes Evol 211: 10-9

Hobmayer B, Rentzsch F, Holstein TW. 2001. Identification and expression of HySmad1, a member of the R-Smad family of TGFbeta signal transducers, in the diploblastic metazoan Hydra. Dev Genes Evol 211: 597-602

Hoffmann U, Kroiher M. 2001. A possible role for the cnidarian homologue of serum response factor in decision making by undifferentiated cells. Dev Biol 236: 304-15

Holstein T, Emschermann P. 1995. Zytologie. In Cnidaria: Hydrozoa, Kamptozoa, ed. J Schwoerbel, P Zwick, pp. 5-15. Stuttgart: Gustav Fisher Verlag

Holtmann M, Thurm U. 2001. Mono- and oligo-vesicular synapses and their connectivity in a Cnidarian sensory epithelium (Coryne tubulosa). J Comp Neurol 432: 537-49

Hornberger MR, Hassel M. 1997. Expression of HvRACK1, a member of the RACK1 subfamily of regulatory WD40 proteins in Hydra vulgaris, is coordinated between epithelial and interstitial cells in a position-dependent manner. Dev Genes Evol 206: 435-46

Horridge GA. 1954. The nerves and muscles of medusae. I. Conduction in the nervous system of Aurellia aurita Lamarck. J Exp Biol 31 594-600

Horridge GA. 1968. The origins of the nervous system. In The Structure and Function of Nervous Tissue, ed. GH Bourne, pp. 1-31. New York: Academic Press Inc.

Hwang JS, Ohyanagi H, Hayakawa S, Osato N, Nishimiya-Fujisawa C, et al. 2007. The evolutionary emergence of cell type-specific genes inferred from the gene expression analysis of Hydra. Proc Natl Acad Sci U S A 104: 14735-40

Jager M, Queinnec E, Houliston E, Manuel M. 2006. Expansion of the SOX gene family predated the emergence of the Bilateria. Mol Phylogenet Evol 39: 468-77

Jeziorski MC, Greenberg RM, Anderson PA. 1999. Cloning and expression of a jellyfish calcium channel beta subunit reveal functional conservation of the alpha1-beta interaction. Recept Chann 6: 375-86

Josephson RK, Schwab WE. 1979. Electrical properties of an excitable epithelium. J Gen Physiol 74: 213-36

Kaloulis K, Chera S, Hassel M, Gauchat D, Galliot B. 2004. Reactivation of developmental programs: The cAMP-response element-binding protein pathway is involved in hydra head regeneration. Proc Natl Acad Sci U S A 101: 2363-8

Kasbauer T, Towb P, Alexandrova O, David CN, Dall'armi E, et al. 2007. The Notch signaling pathway in the cnidarian Hydra. Dev Biol 303: 376-90

Kass-Simon G, Diesl VK. 1977. Spontaneous and evoked potentials from dissociated epithelial cells of Hydra. Nature 265: 75-7

Kass-Simon G, Pannaccione A, Pierobon P. 2003. GABA and glutamate receptors are involved in modulating pacemaker activity in hydra. Comp Biochem Physiol A Mol Integr Physiol 136: 329-42

Kass-Simon G, Pierobon P. 2007. Cnidarian chemical neurotransmission, an updated overview. Comp Biochem Physiol A Mol Integr Physiol 146: 9-25

Kass-Simon G, Scappaticci AA. 2002. The behavioral and developmental physiology of nematocysts. Can J Zool 80: 1772-94

Katsukura Y, David CN, Grimmelikhuijzen CJ, Sugiyama T. 2003. Inhibition of metamorphosis by RFamide neuropeptides in planula larvae of Hydractinia echinata. Dev Genes Evol 213: 579-86

Khalturin K, Anton-Erxleben F, Milde S, Plotz C, Wittlieb J, et al. 2007. Transgenic stem cells in Hydra reveal an early evolutionary origin for key elements controlling self-renewal and differentiation. Dev Biol 309: 32-44

Koch AW, Holstein TW, Mala C, Kurz E, Engel J, David CN. 1998. Spinalin, a new glycine- and histidine-rich protein in spines of Hydra nematocysts. J Cell Sci 111: 1545-54

Koizumi O. 2002. Developmental neurobiology of hydra, a model animal of cnidarians. Can J Zool 80: 1678-89

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: 151-9

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: 358-71

Koizumi O, Itazawa M, Mizumoto H, Minobe S, Javois LC, et al. 1992. Nerve ring of the hypostome in hydra. I. Its structure, development, and maintenance. J Comp Neurol 326: 7-21

Koizumi O, Mizumoto H, Sugiyama T, Bode HR. 1990. Nerve net formation in the primitive nervous system of Hydra--an overview. Neurosci Res Suppl 13: S165-70

Kolberg KJ, Martin VJ. 1988. Morphological, cytochemical and neuropharmacological evidence for the presence of catecholamines in hydrozoan planulae. Development 103: 249-58

Kostrouch Z, Kostrouchova M, Love W, Jannini E, Piatigorsky J, Rall JE. 1998. Retinoic acid X receptor in the diploblast, tripedalia cystophora. Proc Natl Acad Sci U S A 95: 13442-7

Kozmik Z, Daube M, Frei E, Norman B, Kos L, et al. 2003. Role of pax genes in eye evolution: a Cnidarian PaxB gene uniting Pax2 and Pax6 functions. Dev Cell 5: 773-85

Kozmik Z, Ruzickova J, Jonasova K, Matsumoto Y, Vopalensky P, et al. 2008. Assembly of the cnidarian camera-type eye from vertebrate-like components. Proc Natl Acad Sci U S A 105: 8989-93

Kroiher M, Plickert G, Müller WA. 1990. Pattern of cell proliferation in embryogenesis and planula development of Hydractinia echinata predicts the postmetamorphic body pattern. Roux´s Arch. Dev. Biol. 199: 156-63

Larroux C, Luke GN, Koopman P, Rokhsar DS, Shimeld SM, Degnan BM. 2008. Genesis and expansion of metazoan transcription factor gene classes. Mol Biol Evol 25: 980-96

Leitz T, Lay M. 1995. Metamorphosin A is a neuropeptide. Roux' Arch Dev Biol 204: 276-9

Leitz T, Morand K, Mann M. 1994. Metamorphosin A: a novel peptide controlling development of the lower metazoan Hydractinia echinata (Coelenterata, Hydrozoa). Dev Biol 163: 440-6

Lenhoff HM, Heagy W, Danner J. 1982. Bioassay for, and characterization of, activators and inhibitors of the feeding response. In Hydra: Research Methods, ed. HM Lenhoff. New York: Plenum Press

Lentz TL, Barrnett RJ. 1965. Fine Structure of the nervous system of Hydra. Am Zool 5: 341-56

Leys SP, Mackie GO, Meech RW. 1999. Impulse conduction in a sponge. J Exp Biol 202 (Pt 9): 1139-50

Lin YC, Gallin WJ, Spencer AN. 2001. The anatomy of the nervous system of the hydrozoan jellyfish, Polyorchis penicillatus, as revealed by a monoclonal antibody. Invert Neurosci 4: 65-75

Lindgens D, Holstein TW, Technau U. 2004. Hyzic, the Hydra homolog of the zic/odd-paired gene, is involved in the early specification of the sensory nematocytes. Development 131: 191-201

Loomis WF. 1955. Glutathione control of the specific feeding reactions of hydra. Ann N Y Acad Sci 62: 209-28

Mackie G. 2004a. The first description of nerves in a cnidarian: Louis Agassiz's account of 1850. Hydrobiologia 530/531: 27-32

Mackie G, Anderson PA, Singla CL. 1984. Apparent absence of gap junctions in two classes of Cnidaria. Biol Bull 167: 120-3

Mackie GO. 1965. Conduction in the Nerve-Free Epithelia of Siphonophores. Am Zool 5: 439-53

Mackie GO. 1970. Neuroid conduction and the evolution of conducting tissues. Q Rev Biol 45: 319-32

Mackie GO. 1975. Neurobiology of Stomotoca. II. Pacemakers and conduction pathways. J Neurobiol 6: 357-78

Mackie GO. 2004b. Central neural circuitry in the jellyfish Aglantha: a model 'simple nervous system'. Neurosignals 13: 5-19

Mackie GO, Marx RM, Meech RW. 2003. Central circuitry in the jellyfish Aglantha digitale IV. Pathways coordinating feeding behaviour. J Exp Biol 206: 2487-505

Mackie GO, Passano LM. 1968. Epithelial conduction in hydromedusae. J Gen Physiol 52: 600-21

Magie CR, Martindale MQ. 2008. Cell-cell adhesion in the cnidaria: insights into the evolution of tissue morphogenesis. Biol Bull 214: 218-32

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: 235-54

Martin VJ. 2000. Reorganization of the nervous system during metamorphosis of a hydrozoan planula. Inv Biol 119: 243-53

Martin VJ. 2002. Photoreceptors of cnidarians. Can J Zool 80: 1703-22

Martin VJ, Littlefield CL, Archer WE, Bode HR. 1997. Embryogenesis in hydra. Biol Bull 192: 345-63

Martin VJ, Thomas MB. 1981. The origin of the nervous-system in Pennaria tiarella, as revealed by treatment with colchicine. Biol Bull 160: 303-10

Matus DQ, Thomsen GH, Martindale MQ. 2007. FGF signaling in gastrulation and neural development in Nematostella vectensis, an anthozoan cnidarian. Dev Genes Evol 217: 137-48

McCauley DW. 1997. Serotonin plays an early role in the metamorphosis of the hydrozoan Phialidium gregarium. Dev Biol 190: 229-40

McCullough CB. 1965. Pacemaker Interaction in Hydra. Am Zool 5: 499-504

McFarlane ID. 1974a. Control of the pacemaker system of the nerve-net in the sea anemone Calliactis parasitica. J Exp Biol 61: 129-43

McFarlane ID. 1974b. Excitatory and inhibitory control of inherent contractions in the sea anemone Calliactis parasitica. J Exp Biol 60: 397-422

McFarlane ID. 1975. Control of mouth opening and pharynx protrusion during feeding in the sea anemone Calliactis parasitica. J Exp Biol 63: 615-26

McFarlane ID, Anderson PA, Grimmelikhuijzen CJ. 1991. Effects of three anthozoan neuropeptides, Antho-RWamide I, Antho-RWamide II and Antho-RFamide, on slow muscles from sea anemones. J Exp Biol 156: 419-31

McFarlane ID, Hudman D, Nothacker HP, Grimmelikhuijzen CJ. 1993. The expansion behaviour of sea anemones may be coordinated by two inhibitory neuropeptides, Antho-KAamide and Antho-RIamide. Proc Biol Sci 253: 183-8

Mieko Mizutani C, Bier E. 2008. EvoD/Vo: the origins of BMP signalling in the neuroectoderm. Nat Rev Genet 9: 663-77

Miljkovic M, Mazet F, Galliot B. 2002. Cnidarian and bilaterian promoters can direct GFP expression in transfected hydra. Dev Biol 246: 377-90

Miljkovic-Licina M, Chera S, Ghila L, Galliot B. 2007. Head regeneration in wild-type hydra requires de novo neurogenesis. Development 134: 1191-201

Miljkovic-Licina M, Gauchat D, Galliot B. 2004. Neuronal evolution: analysis of regulatory genes in a first-evolved nervous system, the hydra nervous system. Biosystems 76: 75-87

Miller DJ, Ball EE, Technau U. 2005. Cnidarians and ancestral genetic complexity in the animal kingdom. Trends Genet 21: 536-9

Miller DJ, Hayward DC, Reece-Hoyes JS, Scholten I, Catmull J, et al. 2000. Pax gene diversity in the basal cnidarian Acropora millepora (Cnidaria, Anthozoa): implications for the evolution of the Pax gene family. Proc Natl Acad Sci U S A 97: 4475-80

Minobe S, Koizumi O, Sugiyama T. 1995. Nerve cell differentiation in nerve-free tissue of epithelial hydra from precursor cells introduced by grafting. I. Tentacles and hypostome. Dev Biol 172: 170-81

Moroz LL, Meech RW, Sweedler JV, Mackie GO. 2004. Nitric oxide regulates swimming in the jellyfish Aglantha digitale. J Comp Neurol 471: 26-36

Muller-Taubenberger A, Vos MJ, Bottger A, Lasi M, Lai FP, et al. 2006. Monomeric red fluorescent protein variants used for imaging studies in different species. Eur J Cell Biol 85: 1119-29

Nakanishi N, Hartenstein V, Jacobs DK. 2009. Development of the rhopalial nervous system in Aurelia sp.1 (Cnidaria, Scyphozoa). Dev Genes Evol 219: 301-17

Nakanishi N, Yuan D, Jacobs DK, Hartenstein V. 2008. Early development, pattern, and reorganization of the planula nervous system in Aurelia (Cnidaria, Scyphozoa). Dev Genes Evol 218: 511-24

Nilsson DE, Arendt D. 2008. Eye evolution: the blurry beginning. Curr Biol 18: R1096-8

Nordstrom K, Wallen R, Seymour J, Nilsson D. 2003. A simple visual system without neurons in jellyfish larvae. Proc Biol Sci 270: 2349-54

Ozbek S, Balasubramanian PG, Holstein TW. 2009. Cnidocyst structure and the biomechanics of discharge. Toxicon 54: 1038-45

Pang K, Matus DQ, Martindale MQ. 2004. The ancestral role of COE genes may have been in chemoreception: evidence from the development of the sea anemone, Nematostella vectensis (Phylum Cnidaria; Class Anthozoa). Dev Genes Evol 214: 134-8

Pantin CF. 1952. The elementary nervous system. Proc R Soc Lond B Biol Sci 140: 147-68

Parkefelt L, Ekstrom P. 2009. Prominent system of RFamide immunoreactive neurons in the rhopalia of box jellyfish (Cnidaria: Cubozoa). J Comp Neurol 516: 157-65

Passano LM. 1963. Primitive Nervous Systems. Proc Natl Acad Sci U S A 50: 306-13

Passano LM, McCullough CB. 1962. The Light Response and the Rhythmic Potentials of Hydra. Proc Natl Acad Sci U S A 48: 1376-82

Passano LM, McCullough CB. 1964. Co-ordinating systems and behaviour in Hydra. I. Pacemaker system of the periodic contractions. J Exp Biol 41: 643-64

Passano LM, McCullough CB. 1965. Co-ordinating Systems and Behaviour in Hydra. II. The Rhythmic Potential System. J Exp Biol 42: 205-31

Pernet V, Anctil M, Grimmelikhuijzen CJ. 2004. Antho-RFamide-containing neurons in the primitive nervous system of the anthozoan Renilla koellikeri. J Comp Neurol 472: 208-20

Philipp I, Holstein TW, Hobmayer B. 2005. HvJNK, a Hydra member of the c-Jun NH(2)-terminal kinase gene family, is expressed during nematocyte differentiation. Gene Expr Patterns 5: 397-402

Philippe H, Derelle R, Lopez P, Pick K, Borchiellini C, et al. 2009. Phylogenomics revives traditional views on deep animal relationships. Curr Biol 19: 706-12

Pierobon P, Concas A, Santoro G, Marino G, Minei R, et al. 1995. Biochemical and functional identification of GABA receptors in Hydra vulgaris. Life Sci 56: 1485-97

Pierobon P, Minei R, Porcu P, Sogliano C, Tino A, et al. 2001. Putative glycine receptors in Hydra: a biochemical and behavioural study. Eur J Neurosci 14: 1659-66

Pierobon P, Sogliano C, Minei R, Tino A, Porcu P, et al. 2004. Putative NMDA receptors in Hydra: a biochemical and functional study. Eur J Neurosci 20: 2598-604

Plaza S, De Jong DM, Gehring WJ, Miller DJ. 2003. DNA-binding characteristics of cnidarian Pax-C and Pax-B proteins in vivo and in vitro: no simple relationship with the Pax-6 and Pax-2/5/8 classes. J Exp Zool B Mol Dev Evol 299: 26-35

Plickert G. 1989. Proportion-altering factor (PAF) stimulates nerve cell formation in Hydractinia echinata. Cell Differ Dev 26: 19-27

Plickert G, Schetter E, Verhey-Van-Wijk N, Schlossherr J, Steinbuchel M, Gajewski M. 2003. The role of alpha-amidated neuropeptides in hydroid development--LWamides and metamorphosis in Hydractinia echinata. Int J Dev Biol 47: 439-50

Plickert G, Schneider B. 2004. Neuropeptides and photic behavior in Cnidaria. Hydrobiologia 530/531: 49-57

Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, et al. 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 317: 86-94

Rentzsch F, Fritzenwanker JH, Scholz CB, Technau U. 2008. FGF signalling controls formation of the apical sensory organ in the cnidarian Nematostella vectensis. Development 135: 1761-9

Richards GS, Simionato E, Perron M, Adamska M, Vervoort M, Degnan BM. 2008. Sponge genes provide new insight into the evolutionary origin of the neurogenic circuit. Curr Biol 18: 1156-61

Romanes GJ. 1876 Preliminary observations on the locomotor system of medusae. Phil Trans Roy Soc Lond [B] 166: 269–313

Romanes GJ. 1877. Further observations on the locomotor system of medusae. Phil Trans Roy Soc Lond [B] 167: 659-752

Ruggieri RD, Pierobon P, Kass-Simon G. 2004. Pacemaker activity in hydra is modulated by glycine receptor ligands. Comp Biochem Physiol A Mol Integr Physiol 138: 193-202

Sakarya O, Armstrong KA, Adamska M, Adamski M, Wang IF, et al. 2007. A post-synaptic scaffold at the origin of the animal kingdom. PLoS ONE 2: e506

Satterlie RA. 2002. Neuronal control of swimming in jellyfish: a comparative story. Can J Zool 80: 1654-69

Schaller HC, Hoffmeister SA, Dubel S. 1989. Role of the neuropeptide head activator for growth and development in hydra and mammals. Development 107: 99-107

Schierwater B, Eitel M, Jakob W, Osigus HJ, Hadrys H, et al. 2009. Concatenated analysis sheds light on early metazoan evolution and fuels a modern "urmetazoon" hypothesis. PLoS Biol 7: e20

Schlaepfer DD, Bode HR, Haigler HT. 1992. Distinct cellular expression pattern of annexins in Hydra vulgaris. J Cell Biol 118: 911-28

Schmich J, Trepel S, Leitz T. 1998. The role of GLWamides in metamorphosis of Hydractinia echinata. Dev Genes Evol 208: 267-73

Schmid V, Reber-Muller S. 1995. Transdifferentiation of isolated striated muscle of jellyfish in vitro: the initiation process. Semin Cell Biol 6: 109-16

Seipel K, Schmid V. 2006. Mesodermal anatomies in cnidarian polyps and medusae. Int J Dev Biol 50: 589-99

Seipel K, Yanze N, Schmid V. 2004. The germ line and somatic stem cell gene Cniwi in the jellyfish Podocoryne carnea. Int J Dev Biol 48: 1-7

Shimizu H. 2002. Feeding and wounding responses in Hydra suggest functional and structural polarization of the tentacle nervous system. Comp Biochem Physiol A Mol Integr Physiol 131: 669-74

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 Neuroethol Sens Neural Behav Physiol 190: 623-30

Simionato E, Ledent V, Richards G, Thomas-Chollier M, Kerner P, et al. 2007. Origin and diversification of the basic helix-loop-helix gene family in metazoans: insights from comparative genomics. BMC Evol Biol 7: 33

Soza-Ried J, Hotz-Wagenblatt A, Glatting KH, Del Val C, Fellenberg K, et al. 2009. The transcriptome of the colonial marine hydroid Hydractinia echinata. FEBS J in press

Spafford JD, Spencer AN, Gallin WJ. 1999. Genomic organization of a voltage-gated Na+ channel in a hydrozoan jellyfish: insights into the evolution of voltage-gated Na+ channel genes. Recept Chann 6: 493-506

Spencer AN. 1971. Myoid conduction in the siphonophore Nanomia bijuga. Nature 233: 490-1

Spencer AN. 1979. Neurobiology of Polyorchis. II. Structure of effector systems. J Neurobiol 10: 95-117

Spencer AN. 1989. Chemical and electrical synaptic transmision in the Cnidaria. In Evolution of the First Nervous Systems, ed. PAV Anderson, pp. 33-53. New York: Plenum Press

Spencer AN, Satterlie RA. 1980. Electrical and dye coupling in an identified group of neurons in a coelenterate. J Neurobiol 11: 13-9

Stierwald M, Yanze N, Bamert RP, Kammermeier L, Schmid V. 2004. The Sine oculis/Six class family of homeobox genes in jellyfish with and without eyes: development and eye regeneration. Dev Biol 274: 70-81

Suga H, Schmid V, Gehring WJ. 2008. Evolution and functional diversity of jellyfish opsins. Curr Biol 18: 51-5

Sugiyama T, Fujisawa T. 1978. Genetic analysis of developmental mechanisms in Hydra. II. Isolation and characterization of an interstitial cell-deficient strain. J Cell Sci 29: 35-52

Sullivan JC, Sher D, Eisenstein M, Shigesada K, Reitzel AM, et al. 2008. The evolutionary origin of the Runx/CBFbeta transcription factors--studies of the most basal metazoans. BMC Evol Biol 8: 228

Sun H, Dickinson DP, Costello J, Li WH. 2001. Isolation of cladonema pax-b genes and studies of the dna-binding properties of cnidarian pax paired domains. Mol Biol Evol 18: 1905-18

Takahashi T, Kobayakawa Y, Muneoka Y, Fujisawa Y, Mohri S, et al. 2003. Identification of a new member of the GLWamide peptide family: physiological activity and cellular localization in cnidarian polyps. Comp Biochem Physiol B Biochem Mol Biol 135: 309-24

Takahashi T, Koizumi O, Ariura Y, Romanovitch A, Bosch TC, et al. 2000. A novel neuropeptide, Hym-355, positively regulates neuron differentiation in Hydra. Development 127: 997-1005

Takahashi T, Koizumi O, Hayakawa E, Minobe S, Suetsugu R, et al. 2009. Further characterization of the PW peptide family that inhibits neuron differentiation in Hydra. Dev Genes Evol 219: 119-29

Takahashi T, Muneoka Y, Lohmann J, Lopez de Haro MS, Solleder G, et al. 1997. Systematic isolation of peptide signal molecules regulating development in hydra: LWamide and PW families. Proc Natl Acad Sci U S A 94: 1241-6

Tardent P. 1995. The cnidarian cnidocyte, a high-tech cellular weaponry. BioEssays 17: 351-62

Technau U, Rudd S, Maxwell P, Gordon PM, Saina M, et al. 2005. Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. Trends Genet 21: 633-9

Teo R, Mohrlen F, Plickert G, Muller WA, Frank U. 2006. An evolutionary conserved role of Wnt signaling in stem cell fate decision. Dev Biol 289: 91-9

Terada H, Sugiyama T, Shigenaka Y. 1988. Genetic analysis of developmental mechanisms in hydra. XVIII. Mechanism for elimination of the interstitial cell lineage in the mutant strain Sf-1. Dev Biol 126: 263-9

Thomas JT. 1987. The embryonic origin of neurosensory cells and the role of nerve-cells in metamorphosis in Phialidium gregarium (Cnidaria, Hydrozoa). Int J Invertebr Reprod Dev 11: 265-85

Toresson H, Campbell K. 2001. A role for Gsh1 in the developing striatum and olfactory bulb of Gsh2 mutant mice. Development 128: 4769-80

Trembley A. 1744. Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes. Leiden

Walther M, Ulrich R, Kroiher M, Berking S. 1996. Metamorphosis and pattern formation in Hydractinia echinata, a colonial hydroid. Int J Dev Biol 40: 313-22

Weber C. 1981. Structure, histochemistry, ontogenetic development and regeneration of the Ocellus of *Cladonema radiatum* Duj. (Cnidaria, Hydrozoa, Anthomedusae). J Morphol 167: 313-31

Westfall IA. 1996. Ultrastructure of synapses in the first-evolved nervous systems. J Neurocytol 25: 735-46

Westfall JA, Elliott CF, Carlin RW. 2002. Ultrastructural evidence for two-cell and three-cell neural pathways in the tentacle epidermis of the sea anemone Aiptasia pallida. J Morphol 251: 83-92

Westfall JA, Kinnamon JC. 1978. A second sensory--motor--interneuron with neurosecretory granules in Hydra. J Neurocytol 7: 365-79

Westfall JA, Kinnamon JC. 1984. Perioral synaptic connections and their possible role in the feeding behavior of Hydra. Tissue Cell 16: 355-65

Westfall JA, Kinnamon JC, Sims DE. 1980. Neuro-epitheliomuscular cell and neuro-neuronal gap junctions in Hydra. J Neurocytol 9: 725-32

Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TC. 2006. Transgenic Hydra allow in vivo tracking of individual stem cells during morphogenesis. Proc Natl Acad Sci U S A 103: 6208-11

Yaross MS, Bode HR. 1978. Regulation of interstitial cell differentiation in Hydra attenuata. III. Effects of I-cell and nerve cell densities. J Cell Sci 34: 1-26

Yuan D, Nakanishi N, Jacobs DK, Hartenstein V. 2008. Embryonic development and metamorphosis of the scyphozoan Aurelia. Dev Genes Evol 218: 525-39

Yum S, Takahashi T, Koizumi O, Ariura Y, Kobayakawa Y, et al. 1998. A novel neuropeptide, Hym-176, induces contraction of the ectodermal muscle in Hydra. Biochem Biophys Res Commun 248: 584-90

Yun K, Garel S, Fischman S, Rubenstein JL. 2003. Patterning of the lateral ganglionic eminence by the Gsh1 and Gsh2 homeobox genes regulates striatal and olfactory bulb histogenesis and the growth of axons through the basal ganglia. J Comp Neurol 461: 151-65