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REVIEW

Origin of the neuro-sensory system: new and expected insights from sponges

EMMANUELLE RENARD,[1] Jean VACELET,[1] Eve GAZAVE,[1] Pascal LAPÉBIE,[1] Carole BORCHIELLINI[1] and Alexander V. ERESKOVSKY[1,2]

Centre d'océanologie de Marseille, CNRS – Aix-Marseille Université, Marseille, France and [2]Department of Embryology, Saint-Petersburg State University, St. Petersburg, Russia

Abstract

The capacity of all cells to respond to stimuli implies the conduction of information at least over short distances. In multicellular organisms, more complex systems of integration and coordination of activities are necessary. In most animals, the processing of information is performed by a nervous system. Among the most basal taxa, sponges are nerveless so that it is traditionally assumed that the integrated neuro-sensory system originated only once in Eumetazoa, a hypothesis not in agreement with some recent phylogenomic studies. The aim of this review is to show that recent data on sponges might provide clues for understanding the origin of this complex system. First, sponges are able to react to external stimuli, and some of them display spontaneous movement activities. These coordinated behaviors involve nervous system-like mechanisms, such as action potentials and/or neurotransmitters. Second, genomic analyses show that sponges possess genes orthologous to those involved in the patterning or functioning of the neuro-sensory system in Eumetazoa. Finally, some of these genes are expressed in specific cells (flask cells, choanocytes). Together with ultrastructural data, this gives rise to challenging hypotheses concerning cell types that might play neuro-sensory-like roles in sponges.

Key words: animal evolution; choanocyte; flask cells; nervous system; Porifera; signal transduction.

INTRODUCTION

All living organisms are able to respond to some stimuli. This implies the existence of electrical or chemical mechanisms for conducting information at least over short distances at intracellular level.

Together with the acquisition of multicellularity, signal transduction over longer distances as well as intercellular communication mechanisms are required to ensure efficient coordination, movement or behavior of the whole organism. This has been well documented for both plants and animals where both chemical pathways and electrical signal transmissions are involved (Brenner *et al*. 2006).

In most metazoans (Cnidaria, Ctenophora and Bilateria forming Eumetazoa), integration and coordination is largely achieved by the nervous system, the fundamental unit of which is classically considered to be a specialized high velocity impulse conducting cell: the neuron. The term "neuro-sensory system" is also currently used to refer to the ensemble of tissues and organs involved in both perception (sense organs) and signal conduction to effectors. The remaining animal taxa, Porifera (sponges) and

Correspondence: Emmanuelle Renard, Centre d'Océanologie de Marseille, Station marine d'Endoume, Aix-Marseille Université – CNRS UMR 6540-DIMAR, rue de la Batterie des Lions, Marseille 13007, France.
Email: emmanuelle.renard@univmed.fr

Placozoa, are devoid of neurons (Pavans de Cecatty 1989; Schierwater 2005). Their relatively simple body plans (e.g. absence of organs, basement membrane and limited number of cell types) have suggested to zoologists that these two phyla may either be regarded as colonial protozoa or represent the first multicellular animals (Haeckel 1874, and reviewed in Schierwater 2005). According to the traditional gradualist view of evolution, it has generally been considered that the integrated neuro-sensory system was absent in the last common ancestor (LCA) of metazoans (later referred to as Urmetazoa, Müller *et al*. 2001) and would have appeared once in the LCA of so-called "true" Metazoa (referred to as Eumetazoa).

Nowadays, although both sponges and Placozoa are considered as indisputable metazoans (Srivastava 2008; Philippe *et al*. 2009), the relationships between early branching taxa is still uncertain.

Most molecular data, including very recent phylogenomic analyses, are consistent with a basal position of sponges (Srivastava *et al.* 2008; Philippe *et al*. 2009), while a few studies have proposed instead Placozoa (Schierwater 2005; Dellaporta *et al*. 2006; Schierwater *et al*. 2009) or, more surprisingly, Ctenophora (De Salle & Schierwater 2008; Dunn *et al*. 2008) (Fig. 1). We may note that the position of the two latter taxa has always been very doubtful because of suspected long branch attraction (LBA) biases. Traditionally, sponges are divided into three lineages (Hooper & Van Soest 2002): Demospongiae, Hexactinellida and Calcispongia. More recently, Homoscleromorpha have been proposed as a fourth sponge lineage phylogenetically distinct from the Demospongiae among which they were formerly classified (Borchiellini *et al*. 2004; Nichols 2005; Sperling *et al*. 2007; Dohrmann *et al*. 2008; Nielsen 2008; Ereskovsky *et al*. 2009; Philippe *et al*. 2009). The question of whether or not these four lineages form a monophyletic group is still under debate (Fig. 1) (Borchiellini *et al*. 2001, 2004; Medina *et al*. 2001; Dohrmann *et al*. 2008; Dunn *et al*. 2008; Nielsen 2008; Wang & Lavrov 2008; Philippe *et al*. 2009). These controversial relationships have led to several possible hypotheses concerning the origin and evolution of main body plan features, not always in agreement with the traditional scenario of gradual complexification. As far as the nervous system is concerned, three hypotheses are possible (Fig. 1):

1. If sponges form a paraphyletic group (whatever the position of the Placozoa), then the Urmetazoa might rather have been a nerveless animal and the nervous system would have appeared once in the LCA of Eumetazoa.

2. If sponges form a monophyletic group, contending with Placozoa to be a sister group of Eumetazoa, the most parsimonious scenario is the same.

3. In contrast, if Ctenophora are the earlier emerging animal group, then either the ancestral Metazoa was complex with a neuro-sensory system and a secondary simplification occurred in Porifera and Placozoa, as obviously occurred several times during animal evolution, or complexity (including neuro-sensory structures) appeared independently in Ctenophora and Cnidaria+Bilateria (Dunn *et al*. 2008; Jager *et al*. 2008; Pang & Martindale 2008).

Therefore, to be resolved, the question of the origin of the neuro-sensory system requires not only complementary data concerning non-bilaterian animals, but also a more solid phylogenetic background as a basis for interpretation.

The aim of this review is to contribute to this general debate by surveying major recent physiological, cytological, biochemical and molecular data concerning receptive-effective functions in sponges. As a result of the over-simplistic traditional view, these animals have long been neglected. Recent data suggest new and challenging hypotheses and clues that are discussed in the present paper. We hope that this survey will help and encourage further studies.

PERCEPTION/RESPONSE ABILITY AND BEHAVIOR: SPONGES ARE NOT SUCH PASSIVE ANIMALS!

Larvae

Sponge larvae are mobile and exhibit, like most eumetazoans, rapid responses to external stimuli (for review: Maldonado 2006): geotaxis (Warburton 1966), phototaxis (Warburton 1966; Bergquist & Sinclair 1968; Wapstra & Van Soest 1987; Woollacott 1993; Maldonado & Young 1999; Leys & Degnan 2001; Maldonado *et al*. 2003; Elliott *et al*. 2004; Uriz *et al*. 2008) and rheotaxis (Maldonado & Young 1999) have all been documented in sponge larvae. Phototaxis has been the most extensively studied so far, especially in demosponge parenchymella larvae, most complete studies being performed on *Amphimedon queenslandica* Hooper & van Soest, 2006 (formerly *Reniera* sp., Leys & Degnan 2001; Leys *et al*. 2002). This study evidenced the role of pigmented ciliated cells, forming a ring at the posterior pole of the larvae, in response to light: these cells are assumed to play both receptor and effector roles, which would explain the rapidity of behavior change. Similar processes might be involved in light

perception for other sponge larvae because posterior pigmented ciliated cells are found in various groups of demosponges (Wapstra & Van Soest 1987; Maldonado *et al*. 2003; Ereskovsky & Tokina 2004; Maldonado 2006). The organization of these ciliated pigmented cells is reminiscent of simple pigmentary cups of eumetazoans (Maldonado *et al*. 2003). However, other sponge larvae exhibit light perception capability, although they do not possess pigmented cells (Elliott *et al*. 2004; Gonobobleva & Ereskovsky 2004).

Therefore, in most cases, although larvae have been proved to be capable of perception of various stimuli, the

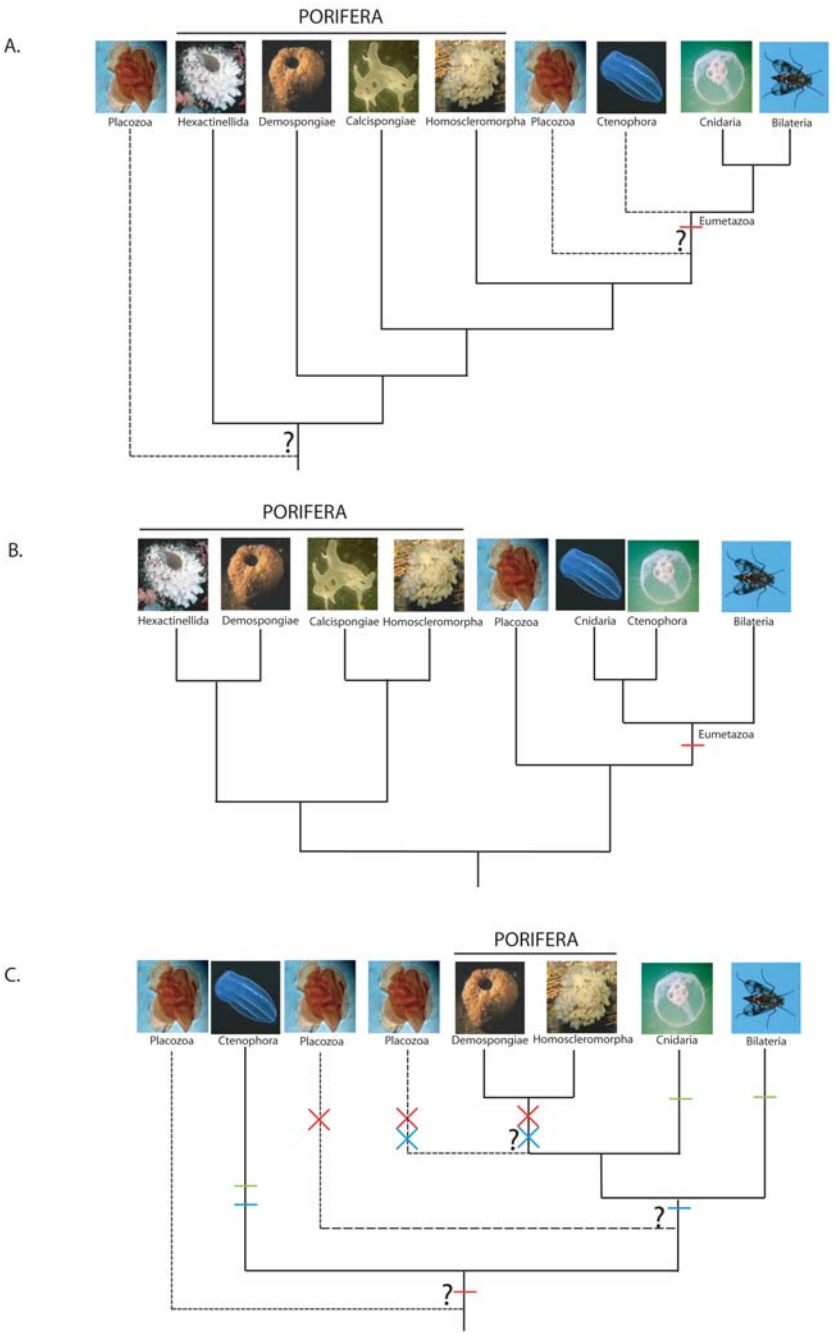

Figure 1 Hypothesized phylogenetic relationships between the first emerging lineages of Metazoa and their implications for scenarios concerning the origin of the neuro-sensory system (according to the parsimony principle): (A) Sponges forming a paraphyletic group at the base of the metazoan tree (Borchiellini *et al*. 2004; Sperling *et al*. 2007; Nielsen *et al*. 2008), the Urmetazoa is assumed to be devoid of nervous system (whatever the position of Placozoa). (B) Porifera forming the first emerging monophyletic group of Metazoa, Placozoa being sister-group of Eumetazoa (Philippe *et al*. 2009): the Urmetazoa is also thought to be devoid of a neuro-sensory system; (C) Ctenophora being the first emerging metazoan lineage (Dunn *et al*. 2008): either the Urmetazoa may have been complex with simplification in sponges and Placozoa (position not tested in this study) or the complexity of Ctenophora may represent a convergence with Cnidarians and Bilaterians. Apparition events are represented by colored lines, loss events by colored crosses (red, blue or green on C represent alternative scenarios).

receptor cells or the structures involved remain generally unknown.

Adults

Despite their sessility, sponge adults also display different behavior patterns and types of reaction involving cell–cell communication and coordination. Since Aristotle (384–322 BC), it has been observed that sponge adults are capable of reacting. Responses to various stimuli were observed: mechanical (e.g. injury), electrical and chemical stimuli, changes of light, temperature, oxygen, salt concentration, presence of sediment (for review: Jones 1962; Leys & Meech 2006; Elliott & Leys 2007). Responses might affect the aquiferous system (opening/closure of oscula (exhalant pores) and ostia (inhalant pores), current velocity, flagellar activity of choanocytes), as well as more or less localized tissue contractions (Simpson 1984; Leys & Meech 2006; Pfannkuchen *et al*. 2008). Whereas specific sensory cells have not yet been clearly identified, the effector cells involved thus seem to be various: pinacocytes (Elliott & Leys 2007), contractile cells called actinocytes or myocytes (Bagby 1966; Elliott & Leys 2007), spherulous cells (Bonasoro *et al*. 2001), as well as choanocytes, even though not directly demonstrated (De Vos & Van de Vyver 1981; Leys & Meech 2006).

In addition to larvae and adult response capability to environmental stimuli, in various species, adults display spontaneous movement (Merejkowsky 1878; review in Jones 1962). Intrinsic rhythmic contractions have been well documented in *Tethya* (Demospongiae), resulting in contraction of the body volume up to 70% within 20 min in *Tethya wilhelma* Sarà *et al*., 2001 (Lieberkühn 1859; Schmidt 1866; Reiswig 1971; Sarà & Manara 1991; Nickel 2001, 2004, 2006; Nickel & Brummer 2003). *Ephydatia muelleri* Lieberkühn, 1855 (Demospongiae), even if it exhibits more discrete activity, has also been studied because its partial transparency makes observation at the cellular level easier (De Vos & Van de Vyver 1981; Weissenfels 1983, 1990; Simpson 1984; Elliott & Leys 2007). These rhythmic contractions of the body are assumed to enable more efficient renewal of water in the aquiferous system, which might be advantageous for species living in low current waters (Sarà 1990) or might limit obstruction of canals in areas under strong sedimentation (Elliott & Leys 2007; Leys & Tompkins 2005; Leys & Meech 2006; Simpson 1984). Pinacocytes and/or actinocytes might be involved by means of an actin–myosin mechanism (Nickel 2001; Elliott & Leys 2007). The rhythm of contractions can be modified by external stimuli, such as mechanical attacks (Nickel 2004; Elliott & Leys 2007) or chemicals (Ellwanger & Nickel 2006). These experiments provide evidence that, nonetheless, some sponges are capable of coordinated movement, but also that this coordination constitutes an integrative response to environmental factors.

Even more unexpectedly for sessile animals, a few species are capable of crawling along a substratum (Bond & Harris 1988; Pansini & Pronzato 1990; Nickel 2006), albeit rather slowly: 1–4 mm per day for *Chondrilla nucula* Schmidt, 1862 (Bond & Harris 1988); and 4 mm per day for *T. wilhelma* (Nickel 2006). Experiments show that locomotion is modulated by environmental factors such as the nature of the substrata or the light intensity (Pronzato 2004; Nickel 2006) and that *T. wilhelma* is capable of changing direction almost instantaneously (Nickel 2006). Once again, this coordinated behavior, even if exceptional in sponges, implies efficient integrated perception–conduction mechanisms.

CONDUCTION MECHANISMS IN THESE ANEURAL ANIMALS

The question of how sponges perform conduction and coordination was a major subject of debate among the spongiologist community for about 50 years (Parker 1910; Pantin 1952; Jones 1962; Lentz 1966; Pavans de Ceccatty 1974, 1979; Mackie 1979, 1990). The conclusion was that sponges do not possess a nervous system, because they lack neuronal cells (Pavans de Ceccatty 1989). In the absence of neurons, various hypotheses were proposed to try to explain the experimental observations (signal propagation from a few mm to 0.3 cm s^{-1} observed in sponges versus several hundred cm s^{-1} often observed in neuronal conduction ; Leys & Mackie 1997; Leys *et al*. 1999; Elliott & Leys 2007): (i) cellular conduction: even if the velocity of contraction propagation observed is generally much slower than typical neuronal conduction, other cell types or particular cell–cell communication structures might be involved; (ii) chemical diffusion was envisaged, but the velocity and the unlocalized character of responses observed in some species seemed not to be consistent with diffusion mechanisms only, thus leading to the hypothesis of (iii) electrical conduction, where the velocity of conduction in hexactinellids was thought to be compatible with electrical mechanisms (Lawn *et al*. 1981; Simpson 1984) even if action potentials were not monitored in sponges until 1997 (Leys & Mackie 1997; Leys *et al*. 1999).

Cellular conduction hypothesis: presence of specific cells or junctions?

Pavans de Ceccatty (1966, 1974) suggested that neu-

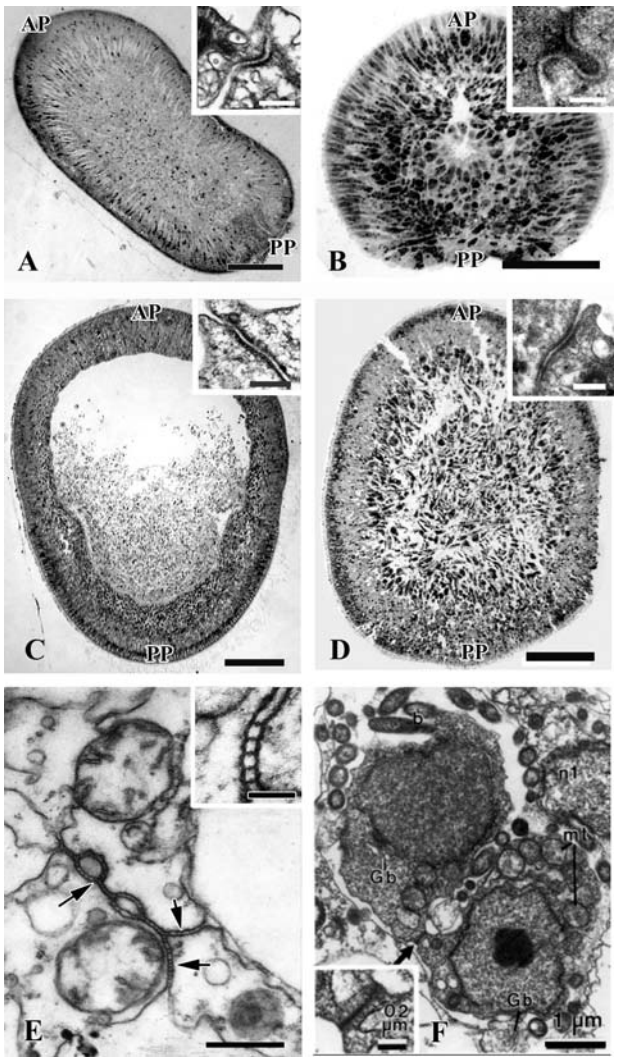

Figure 2 Specialized cell junctions in sponge larvae (A–D) and adults (E, F). (A) Parenhymella of *Ircinia oros* (Demospongiae, Dictyoceratida); (B) Dispherula of *Halisarca dujardini* (Demospongiae, Halisarcida); (C) Cinctoblastula of *Corticium candelabrum* (Homoscleromorpha); (D) Parenchymella of *Pleraplysilla spinifera* (Demospongiae, Dictyoceratida) with the desmosom-like cell junctions (insets). (E) Septate junctions (arrows) between sclerocytes of *Sycon ciliatum* (Calcispongia, Calcaronea); inset, septate junction (from: Ledger 1975). (F) Two mesohyl choanoblasts of *Farrea occa* (Hexactinellida) are plug-connected (arrow) (TEM). *Mt*, mitochondria; *Gb*, Golgi bodies; inset, plug junction (from: Reiswig & Mehl 1991).

Abbreviations: AP, anterior pole, PP, posterior pole. Scale bar, A, 100 µm; Inset, 0.2 µm; B, 50 µm; Inset, 25 nm; C, 50 µm; Inset, 0.2 µm; D, 50 µm; Inset, 0.2 µm; E, 0.5 µm; Inset, 0.1 µm; F, 1 µm; Inset, 0.2 µm.

roid (bipolar) cells in the mesohyl of *Tethya* possessing vesicles, microfilaments and microtubules were neuroid cells. This hypothesis was controversial partly due to both lack of functional evidence and similarities with another cell type, the myocytes (Simpson 1984).

The most rapid responses were observed in hexactinellid species (0.2–0.3 cm s^{-1}, Lawn *et al*. 1981). The syncytial nature of the tissues was, at first, thought to explain this velocity. In the other groups of sponges that have a cellular organization pattern, this explanation cannot be accepted. Moreover, no gap junctions facilitating cell–cell communications as in eumetazoans could so far be identified in cellular sponges (Green & Bergquist 1979; Garrone *et al*. 1980; Lethias *et al*. 1983). However, specialized cellular junctions, such as zonula adhaerens (Boury-Esnault *et al*. 2003; Ereskovsky & Tokina 2004, 2007; Gonobobleva & Ereskovsky 2004; Ereskovsky & Willenz 2008), septate junctions (Ledger 1975; Green & Bergquist 1979) and plug-junctions (Mackie & Singla 1983) do occur in sponges (Fig. 2), even if they are generally underestimated. The cell cohesion is strengthened in homoscleromorph sponges by the presence of a zonula adhaerens and a basement membrane containing type IV collagen and laminin (Boute *et al*. 1996; Boury-Esnault *et al*. 2003; Aouacheria *et al*. 2006), so that some authors propose considering this taxon with true epithelia, as "epitheliosponges" (Ereskovsky & Tokina 2007). No homoscleromorph species model has been studied so far in stimuli response experiments, but this tight cohesion of cells might favor the sponge's coordinated responsiveness.

In addition to these general considerations on adult sponge histology (syncytia of Hexactinellida, epithelia of Homoscleromorpha and peculiar junctions in Demospongiae and Calcispongia), some authors have reported other peculiar cohesive structures assumed to play a role in cell–cell communication and coordination: in demosponge parenchymellae, Maldonado *et al*. (2003) report cytoplasmic bridges between posterior ciliated cells thought to enable intercellular communication and thus coordinate the cell activity of these putative photoreceptor–effector cells.

Studies at the molecular level also provide evidence that sponges share with other animals the main ingredients for intercellular communication: (i) major extracellular matrix molecules such as collagens (Aouacheria *et al*. 2006; Exposito & Garrone 1990), laminin (Nichols *et al*. 2006), fibronectin domain (Labat-Robert *et al*. 1981); and (ii) and other molecules implicated in cell adhesion (Nichols *et al*. 2006).

Chemical signaling hypothesis: implication of calcium and neurotransmitters

Quite early, studies provided evidence of sponge reactivity to chemicals known to influence the nervous system activity of eumetazoans. For example, Emson (1966) showed in *Cliona celata* Grant, 1826 the effect of various chemicals on the water circulation. Acetylcholine, histamine and gamma-aminobutyric acid (GABA) modify the filtering activity. The presence of acetylcholine in sponges was first demonstrated by Mitzopolitanskaya (1941). Not only were other neurotransmitters subsequently discovered (epinephrine, norenephrine, epineurin, norepineurin, 5-oxytriptamin and serotonin), but also enzymes necessary for their synthesis, such as monoaminoxydase and cholinesterase (Mitzopolitanskaya 1941; Lentz 1966; Thiney 1972; Guerriero *et al*. 1993; Schäcke *et al*. 1994; Weyrer *et al*. 1999; Müller *et al*. 2004); as well as receptors: in *Geodia cydonium* Jameson, 1811 a metabotropic glutamate (mGlu) receptor-like protein is present and able to react to glutamate exposure by increasing the intracellular calcium concentration (Perović *et al*. 1999). Various neuroactive compounds also alter the rhythm of contraction in *T. wilhelma* (Ellwanger & Nickel 2006; Ellwanger *et al*. 2007), confirming the probable presence of numerous receptor types in sponges. Ramoino *et al*. (2007) performed western blotting staining of GABA, glutamate decarboxylase (GAD), vesicular GABA transporter (vGAT) and metabotropic $GABA_B$ receptors in *Chondrilla nucula.* Pinacocytes, choanocytes and scattered archeocytes show clear GABA immunoreactivity. Therefore, it is obvious that, like other animals, sponges use a complex neuromediator signaling system for cell communication and coordination.

Another classical mechanism implicated in cell reactivity to stimuli is the regulation of intracellular calcium concentration ($[Ca^{2+}]_i$). The activation of the mGlu receptor of *G. cydonium* (Perović *et al*. 1999) and activation of the integrin receptor in *Suberites domuncula* Olivi, 1792 (Wimmer *et al*. 1999) were shown to result in an increase of $[Ca^{2+}]_i$. In the second case, the $[Ca^{2+}]_i$ was shown to be regulated by a calmodulin. Temperature stress also induces an increase in $[Ca^{2+}]_i$, thought to be mediated by a conserved abscisic acid/cyclic ADP-ribose (ABA/cADPR) signaling pathway (Zocchie *et al*. 2001). While studying photoresponses in the larva of *Amphimedon queenslandica*, Leys and Degnan (2001) observed that an increased external potassium concentration caused reversible arrest of the beating of the long cilia. They hypothesized the intervention of depolarization of the membrane potential resulting in possible influx of calcium in the cilium, as reported in other Eukaryotes.

Taken together, these results show that sponges possess a complex chemical signaling system involving the intervention of several neuromediators acting in the eumetazoan nervous system, as well as a pivotal role for Ca^{2+}, implicating conserved pathways such as cADPR and cAMP (Zocchie *et al*. 2001; Ellwanger & Nickel 2006).

Electrical signaling: action potential in Hexactinellida

In a Hexactinellida, *Rhabdocalyptus dawsoni* Lambe, 1892, as in other studied sponges (Pavans de Ceccatty *et al*. 1960; Simpson 1984), water flow has been shown to be stopped rapidly (within 20 s) by mechanical or electrical stimuli (Lawn *et al*. 1981; Mackie & Singla 1983; Leys *et al*. 1999) and the response spreads through the whole sponge. However, the main difference between syncytial hexactinellids, compared to the other sponges (cellular tissues), is the velocity of the signal propagation, which is generally much higher (e.g. from 4 to 350 mm s^{-1} in *Ephydatia muelleri* (Demospongiae), compared to approximately 0.26–0.28 cm s^{-1} in *R. dawsoni* (Hexactinellida). This might be explained by the fact that all attempts to record electrical signals in sponges have so far failed except in this hexactinellid. In *R. dawsoni*, action potentials have been recorded (Lawn *et al*. 1981; Leys & Mackie 1997; Leys *et al*. 1999) through the trabecular syncytium. The conduction velocity of 0.27 cm s^{-1} is slow compared with conduction in nerves, whereas the absolute and relative refractory periods (29 and 150 seconds, respectively) are very long. This electrical conduction is temperature sensitive. Together with drug treatment experiments, this observation led the authors to hypothesize the involvement of calcium channels (instead of sodium channels) (Leys *et al*. 1999).

To date, action potentials have not been recorded in other sponges, but Tompkins-MacDonald *et al*. (2009) report for the first time the physiological study of inward-rectifier K^+ (Kir) channels in *Amphimedon queenslandica* (Demospongiae). The authors emphasize their conserved fundamental properties of ion selectivity, block and rectification. They hypothesize that cells possessing such channels (not identified so far) should be able to maintain a stable resting potential and to sustain prolonged depolarization of their membrane without massive loss of internal K^+.

In conclusion, the molecular, physiological and biochemical data accumulated since the end of the 1990s do not provide an adequate basis to fully understand the

mechanisms involved, but it would appear that they are more complex than previously imagined. Cellular, chemical and electrical mechanisms do seem to be involved, as in eumetazoans.

CANDIDATE SPONGE CELLS FOR NEUROSENSORY-LIKE ROLES

In larvae

We referred earlier to the pigmented ciliated cells of the posterior pole of some demosponge parenchymellae envisaged as playing a photoreceptor and effector role at the same time (Leys *et al*. 2002; Maldonado *et al*. 2003). Similar types of multifunctional cells are also found in cnidarians and ctenophorans where they enable reactivity and coordination without the intervention of neurons (Aerne *et al*. 1991; Hernandez-Nicaise 1991; Nordström *et al*. 2003). Some authors thus propose that in the Urmetazoa, assumed to have a limited number of cell types, multifunctionality of cells would have been frequent, and that segregation of cell functions evolved together with gene duplications and functional divergence (Arendt 2008).

Nevertheless, the ring organization of the posterior pigmented ciliated cells is characteristic only for parenchymella larvae of some demosponge orders, such as Haplosclerida and Dictyoceratida (Ereskovsky 2005; Maldonado 2006), whereas in other orders the pigmented cells are not organized as a ring, and in other larval types a uniform color is observed. However, all these larvae show responses to various stimuli, including light (Boury-Esnault *et al*. 2003; Elliott *et al*. 2004; Gonobobleva & Ereskovsky 2004; Uriz *et al*. 2008). When pigmented cells are present they are assumed to be responsible for light sensitivity, but in view of the variety of stimuli to which larvae are able to react, other cell types might be involved. New candidate cells were recently proposed on the basis of biochemical and molecular data: (i) serotonergic archeocytes, which were discovered for the first time in the inner part of the parenchymella of *Tedania ignis* Duchassaing & Michelotti, 1864 (Weyrer *et al*. 1999); and (ii) "flask cells" of the larva of *A. queenslandica* (Fig. 3A). These cells

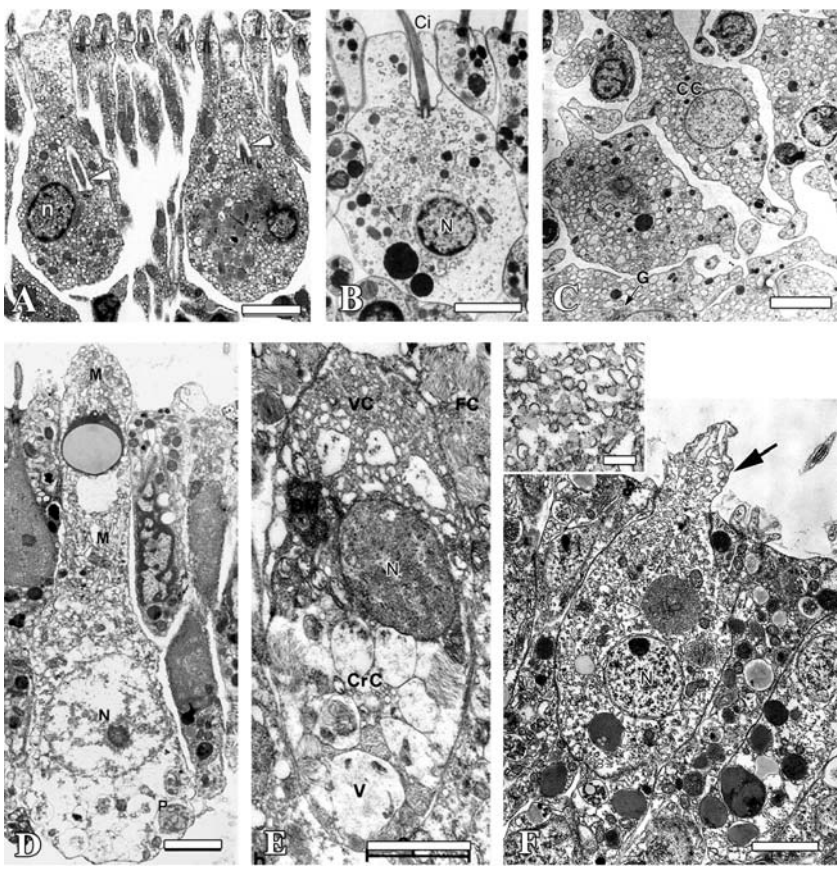

Figure 3 Transmission electron micrographs (TEM) of sponge larval cell types - putative candidates for sensory roles. (A) the flask cells of *Amphimedon queenslandica* (Demospongiae, Haplosclerida), *arrowhead* - cilium (from: Leys & Degnan 2001); (B) the globular flagellated cell of *Haliclona tubifera* (Demospongiae, Haplosclerida) (from: Woollacott 1993); (C) the vesicular cells of *Haliclona* sp. (Demospongiae, Haplosclerida) (from: Amano & Hori 1994); (D) the "bottle cell" of calciblastula of *Soleneiscus* sp. (Calcinea, Calcispongia) (from: Amano & Hori 2001); (E) the cruciform cells (CrC) of amphiblastula of *Scypha ciliata* (Calcaronea, Calcispongiae) (from: Franzen 1988); (F) the non-ciliated ovoid vacuolar cells in cinctoblastula of *Oscarella tuberculata*: arrow – (Homoscleromorpha); inset – vesicles within the cytoplasm of vacuolar cell.

Abbreviations: Ci, cilium, G, Golgi complex, M, membranous structures, N, nucleus, V, vacuole, VC, vesicular cytoplasm. Scale bar: A, 2 μm, B, 2 μm, C, 3 μm, D, 2 μm, E, 2 μm, F, 2 μm, inset, 0.5 μm.

express simultaneously five messengers corresponding to post-synaptic genes, leading the authors to suggest they might play neuro-sensory-like roles (Sakarya *et al*. 2007). Richards *et al*. (2008) show that flask cells (reported by authors as "globular cells") express three genes that are important in the nervous system patterning of Eumetazoa: *AmqbHLH1*, a gene with conserved proneural activity and its supposed (according to the eumetazoan Notch pathway) upstream regulators *AmqNotch* and *AmqDelta1*. Flask cells show remarkable ultrastructural features: they have a clear apico–basal polarity, a general bottle shape and a cilium (Fig. 3A) (Leys & Degnan 2001). Nevertheless, unlike typical ciliated cells, neither a longitudinal or horizontal rootlet nor an accessory centriole is associated with their basal body, which rules out a locomotor role and, therefore, might reflect a sensory role (Woollacott 1993; Leys & Degnan 2001). In parenchymellae of other demosponge species, cells with similar ultrastructure are present, although not always ciliated. Whether ciliated or not, one of the noteworthy peculiarities of these flask cells is the abundance of small vesicles and membranous tubules in the cytoplasm that are reminiscent of synthesis–exocytosis of molecules. It should be stressed that cell types sharing a characteristic bottle or oval shape and a large quantity of small electron transparent vesicles and membranous structures (with or without cilia) are in fact found in nearly all sponge larvae, but the variety of names renders comparison difficult in the literature: globular flagellated cells (Fig. 3B) (Woollacott 1993), vesicular cells (Fig. 3C) (Amano & Hori 1994), flask-like cells (Maldonado 2006), vacuolar cells (Lévi 1964) and urn-shaped cells (Boury-Esnault 1976) in Demospongiae; the bottle cells in calcinean Calcispongia (Fig. 3D) (Amano & Hori 2001; Ereskovsky & Willenz 2008); cruciform cells in calcarean Calcispongia (Fig. 3E) (Duboscq & Tuzet 1938; Franzen 1988; Gallissian & Vacelet 1992; Amano & Hori 1992); and non-ciliated ovoid vacuolar cells in Homoscleromorpha (Fig. 3F) (Boury-Esnault *et al*. 2003; De Caralt *et al*. 2007). We do not suggest that all these cell types are homologous to flask cells, but the sharing of abundant vesicles is reminiscent of a capacity for synthesis and exocytosis of chemicals (Woollacott 1993; Amano & Hori 1994; Leys & Degnan 2001). Together with the interesting results of Sakarya *et al*. (2007) and Richards *et al*. (2008), this suggests that it might be worth paying particular attention to these microvesicle-rich cells when exploring neuro-sensory-like functions in sponge larvae.

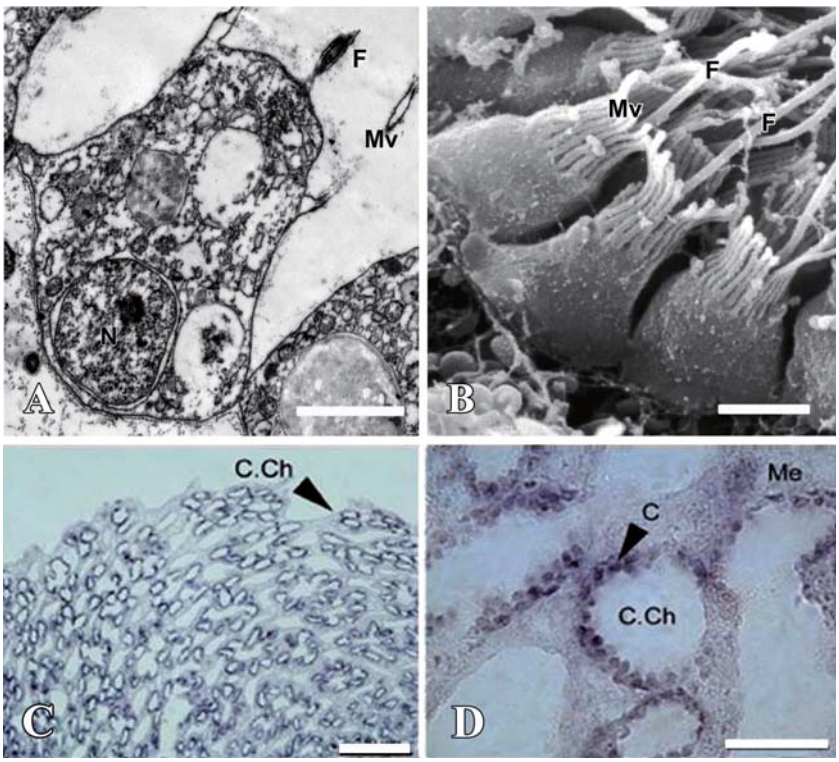

Figure 4 Choanocytes and choanocyte chambers of *Oscarella lobularis* (Homoscleromorpha). Ultrastructure: TEM (A) and SEM (B) of the choanocytes. C, D, *In situ* hybridization pattern in *O. lobularis*. Expression pattern of the gene *OlobNK* observed on sections at low magnification, only choanocyte chambers (CCh) are stained (from: Gazave *et al*. 2008).
Abbreviations: C, choanocyte, F, flagellum, Me, mesohyl, Mv, microvilli, N, nucleus. Scale bar: A, 2 μm, B, 5 μm, C, 250 μm, D, 40 μm.

In adults

Apart from the controversial bipolar cells described by Pavans de Ceccatty (1966) in the mesohyl of *Tethya*, no cells with obvious ultrastructural features reminiscent of eumetazoan neuro-sensory cells have been reported. Therefore, adult sponges are considered to be devoid of specialized conduction cells. Different types of pigmented cells are present in adult sponges, but without evidence of sensory functions, whereas flask-like cells have not been reported. Nevertheless, an emerging hypothesis proposes choanocytes as potential sensor-effector cells (Fig. 4A, B). Their ultrastructure is quite similar to that of eumetazoan mechanoreceptors so that Jacobs *et al*. (2007) suggest that collar cells might represent the cell type that gave rise to eumetazoan sensory cells. Even if this hypothesis is consistent with the view of an ancestral multifunctionality (i.e. crucial role of choanocytes in nutrition and reproduction) giving rise secondarily to separated specific cell functions (Arendt 2008), common ancestrality of cell lineage would be difficult to demonstrate. Even if possible co-option or secondary loss of functions cannot be ruled out, the conservation of several gene expression patterns (together with other data) might provide clues to potential common ancestrality. Only two genes with choanocyte-associated expression have been reported so far:

1. *Annexin* in *Ephydatia fluviatilis* L., 1759 (Demospongiae) is expressed in archeocytes differentiating in choanocytes during dissociation/reaggregation experiments (Funayama *et al*. 2005). Interestingly, *Annexin* genes encode a family of proteins with numerous roles all involving interactions with cell membranes and activity regulation by cytosolic [Ca^{2+}] (Futter & White 2007).

2. A $NK_{6,7}$-related gene has been shown to be expressed strictly in choanocytes of the Homoscleromorpha *Oscarella lobularis* Schmidt, 1862 (Gazave *et al*. 2008). The authors draw our attention to the fact that NK_6 and NK_7 families have a predominantly neural expression pattern in bilaterians (Fig. 4C,D).

This is far from sufficient to test Jacobs' hypothesis, but these first results must be kept in mind for further essay investigations. Nevertheless, considering the internal position of choanocytes, the proposition of Jacobs *et al.* (2007) to consider these cells as possible sensors can only be valid for water change perception; they can hardly be involved in responses to external stimuli. Therefore, other cell types might be proposed: for example, pinacocytes that are directly exposed to the environment. Of note is that pinacocytes and choanocytes are the two most immunolabeled cell types in the experiments of Ramoino *et al*. (2007). The expression of GABA receptors in cells in direct contact with the medium, together with the increase in GABA release after K^+-induced membrane depolarization, has led the authors to suggest that these cells are able to respond to chemical stimuli.

Of course, proof is lacking for all the cells formerly hypothesized as candidates for neuro-sensory functions (posterior pigmented cells and flask-cells in larvae; and choanocytes and pinacocytes in adults). It will be necessary, in the coming years, to obtain a larger set of physiological and molecular data to test these new and challenging hypotheses.

CONCLUSIONS AND PERSPECTIVES

Over the past 20 years, our knowledge concerning sponge features at molecular, biochemical, histological and physiological levels has greatly increased. For conduction mechanisms as well as other aspects of sponges, it has become more and more obvious that these animals are not as simple as generally described in zoological textbooks. The absence of neurons and obviously identified sensory cells does not indicate the absence of an efficient perception-conduction system enabling adaptive responses to environmental changes. On the basis of the data surveyed in this review, it should be obvious that sponges are not devoid of sensory cells, and use cellular, chemical and/or electrical signals to coordinate their activities, even if we have still got a long way from identifying all the cells and understanding the whole processes involved. This is partly due to the fact that sponges are not always convenient animals for all classical experimentation methods, such as physiological experiments, calling for the time consuming adaptation of protocols. It is to be expected that more and more molecular data might favor comparison with the Eumetazoa, providing clues to putative conserved mechanisms that might be involved in the patterning or functioning of sponge conducting systems. From the functional point of view, post-synaptic orthologous gene expression patterns are still being studied (Sakarya *et al*. 2007), providing us with interesting new hypotheses regarding the cells that might be involved in larvae. It would also be worth studying their expression in adults. We detected in our expressed sequence tag (EST) dataset of *O. lobularis* various genes that are known to be implicated in eumetazoans in the regulation of vesicle formation and exocytosis, in particular during neurotransmitter emission. We hope that the expression patterns of these genes under various conditions, in both larvae and adults, will give us insights into the cells concerned. Concerning the body plan patterning genes,

several genes known to play a role in nervous system differentiation have been reported in sponges: *Frizzled* (Adell *et al*. 2003); *Sox* (Jager *et al*. 2006); *Pax* (for review see Kozmik 2008); $NK_{6,7}$ (Gazave *et al*. 2008; Larroux *et al*. 2006); *bHLH* (Richard *et al*. 2008; Simionato *et al*. 2007); and *Tlx* apparented genes (Coutinho *et al*. 2003; Larroux *et al*. 2006; Richelle-Maurer *et al*. 2006). Nevertheless, expression data remain scarce and are not always easy to compare to data from eumetazoans. Even if we are all aware that conserved coexpression of genes is not sufficient to permit doubt-free homology assignation to known eumetazoan cell types, these data will help us (together with other data) to propose new candidate cells and hypotheses to be tested.

As well as other non-bilaterian models, sponges have been neglected for many years and do merit their recent "rehabilitation". In the light of recent results, in the context of a more integrated view of eukaryote evolution, where one may dare to speak of "neurobiology" in plants (Brenner *et al*. 2006), we may expect that in the future a better understanding of perception and signal conduction mechanisms in sponges will lead the zoological community to question the appropriate criteria for referring to a nervous system: is the historical "presence of neurons" necessary and sufficient? We hope that this review may serve to convince the reader that despite their lack of identified neuroid cells, sponges are promising models for understanding the origin of the neuro-sensory system in the animal lineage.

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