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REVIEW

Elements of a 'nervous system' in sponges

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ABSTRACT

Genomic and transcriptomic analyses show that sponges possess a large repertoire of genes associated with neuronal processes in other animals, but what is the evidence these are used in a coordination or sensory context in sponges? The very different phylogenetic hypotheses under discussion today suggest very different scenarios for the evolution of tissues and coordination systems in early animals. The sponge genomic 'toolkit' either reflects a simple, pre-neural system used to protect the sponge filter or represents the remnants of a more complex signalling system and sponges have lost cell types, tissues and regionalization to suit their current suspension-feeding habit. Comparative transcriptome data can be informative but need to be assessed in the context of knowledge of sponge tissue structure and physiology. Here, I examine the elements of the sponge neural toolkit including sensory cells, conduction pathways, signalling molecules and the ionic basis of signalling. The elements described do not fit the scheme of a loss of sophistication, but seem rather to reflect an early specialization for suspension feeding, which fits with the presumed ecological framework in which the first animals evolved.

KEY WORDS: Porifera, Neuroid conduction, Neural signalling, Nervous system evolution

Introduction

All animals and plants have tissues that conduct signals. Unicellular eukaryotes have a huge number of complex behaviours (Boenigk and Arndt, 2002) and even bacteria can coordinate to form multicellular arrays (Claessen et al., 2014). Therefore the ability to receive signals to coordinate behaviour and the mechanism of transmitting signals between cells has come about many times in very different lineages. But how related are the elements of these systems? Neuroid or non-nervous conduction in giant plant or algal cells such as *Mimosa* or *Nitella* (Fromm and Lautner, 2007) functions similarly to the neuroid conducting tissues of glass sponge syncytia, and to the gap junction-coupled epithelia of cnidarians, ctenophores and other animals (Mackie, 1965; Bassot et al., 1978; Hernandez-Nicaise et al., 1980; Leys and Mackie, 1997). Different ions form the basis of the action potentials (chloride and calcium potentials in the plant and alga, calcium in the sponge, and sodium or calcium in cnidarians and ctenophores) but the effect is similar – generating a rapid signal that effects a behavioural response. More specialized cellular conduction pathways – nerves – are suggested to have originated from these sorts of excitable conducting epithelia (Mackie, 1990; Mackie, 2004). Neuroid conduction is thought to have come about independently in different lineages (Mackie, 1970), but nerves appear to be a metazoan-specific feature, and are considered so specialized for their function that the idea that complex neural signalling may also have several independent or parallel origins (Moroz, 2009; Moroz et al., 2014) is not easily accepted.

For over a century our understanding of the evolution of complex systems such as nerves and nervous tissues has been reconstructed by studying elements found in extant representatives of the earliest evolving phyla – especially sponges and cnidarians (Parker, 1919). But recent phylogenetic analyses, which suggest that ctenophores may have evolved before sponges (Dunn et al., 2008; Ryan et al., 2013; Moroz et al., 2014) offer a new perspective because ctenophores have complex nervous systems and behaviour. This new scenario could mean there have been independent origins of complex neural signalling, or that sponges have lost nerves and the ability to send rapid directed signals. Other complex body systems have evolved in parallel in different lineages (e.g. Steinmetz et al., 2012), but whether the neuron could have evolved more than once is the main question. More sponge genomes, with more complete coverage and improved phylogenetic analysis will confirm in the coming years which group is more basal. But the current genomic data forces us to ask hard questions: what do sponges really have in terms of a 'neural toolkit' and could it reflect the remnants of a more-sophisticated coordination system? Alternatively, is the presence in genomes, and even expression in tissues, of 'neuronal' genes in sponges enough to even warrant the label 'pre-nervous system'? In an attempt to address these questions, I first briefly describe the nature of species from which data derives and then evaluate whether what we now know of the molecules, tissues and physiology of sponges best reflects elements of a potential (pre)nervous system, loss of one, or elements of a distinct system specialized for non-neural functions.

Model systems in Porifera

Marine sponges are typically difficult to maintain in tanks. Because of the large volumes of water they filter, unless water exchange is great, waste products quickly build up. Sponges rapidly detect poor water quality and reduce their filtration rates. But even for those that can be kept in tanks, few species release gametes (most of them brood embryos) and spawning events are usually unpredictable; only one, *Tetilla serica* (Watanabe, 1978) is known to have reproduced in captivity. *Tetilla* has a 2 year life cycle, maturing one year and spawning the next, and individuals can be separated into males and females – an almost ideal subject. Watanabe (Watanabe, 1978) reports that *Tetilla* was so abundant in the Aburatsubo Bay, Japan, that the eggs 'spawned by so many adults paint the sea surface red every two years'. Unfortunately the species is no longer known in those waters, and no other species has been found that is so tractable; so workers use the most readily obtained species locally (Fig. 1).

There are different reasons for selecting particular species for different kinds of work: *Amphimedon queenslandica* produces large numbers of embryos and larvae year round, larvae are large (up to 1 mm in length) and have differentiated morphology with anterior and posterior ends, cell layers and sensory cells that are involved in

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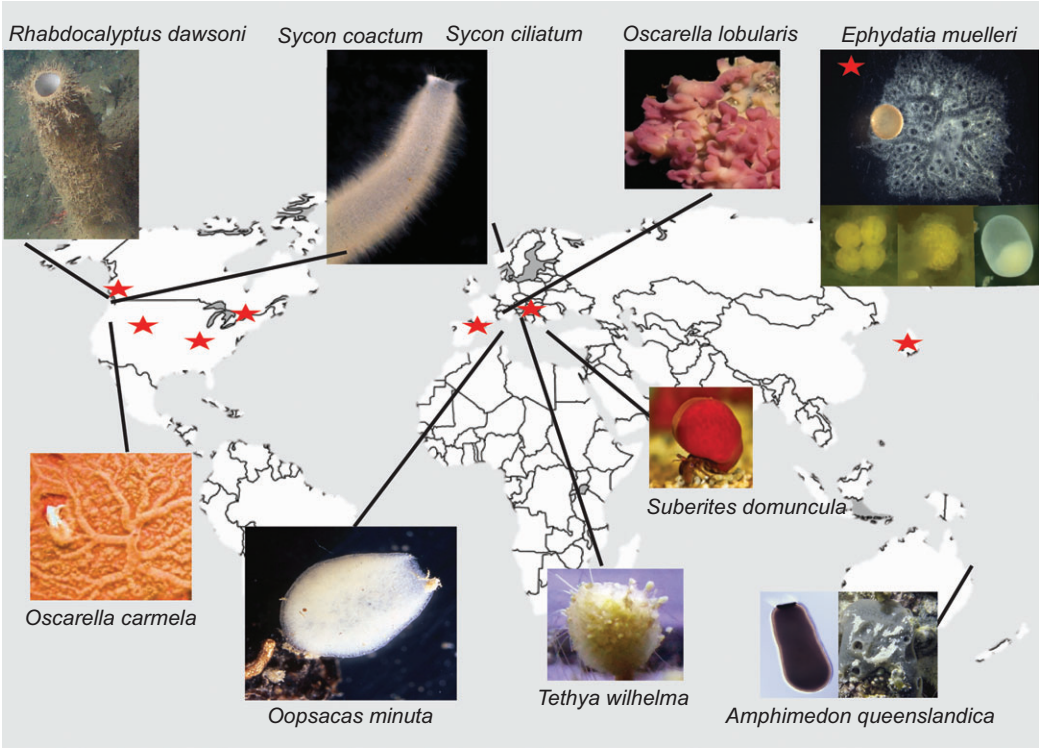

Fig. 1. Model sponge species studied world-wide. Hexactinellids: *Rhabdocalyptus dawsoni*, *Oopsacas minuta*; Calcarea: *Sycon coactum, Sycon ciliatum;* Homoscleromorphs: *Oscarella lobularis, Oscarella carmela;* Demosponges: *Tethya wilhelma, Suberites domuncula, Amphimedon queenslandica, Ephydatia muelleri*. Stars indicate locations where freshwater sponges are studied. Photos: *R. dawsoni*, *S. coactum*, *O. minuta*, *E. muelleri*, *T. wilhelma*, *A. queenslandica*, S. Leys; *O. carmella* courtesy of S. Nichols; *O. lobularis* reprinted with permission from Van Soest et al. (Van Soest et al., 2012); *S. domuncula*, reprinted with permission from Müller et al. (Müller et al., 2012).

larval behaviour (Leys and Degnan, 2001; Leys et al., 2002; Leys and Degnan, 2002). Investment in sequencing ESTs provided early hints of interesting genomic complexity (Degnan et al., 2008), which led the way to sequencing the first sponge genome (Srivastava et al., 2010). But *Amphimedon queenslandica* is only available in eastern Australia, cannot be cultured in the lab and broods its larvae, so embryos are inaccessible to manipulation. *Tethya wilhelma* lives easily in aquaria and has an interesting contraction behaviour (Nickel 2001; Nickel, 2004). Sponges in the genus *Tethya* are thought to be oviparous, but because reproduction has not been observed in lab specimens, so far work has been on buds. If these buds could be grown in thin 'sandwich' cultures under a coverslip, however, it would allow a greater range of experimental approaches. There is published work on the physiology (Lentz, 1966) and recently also the molecular biology (Leininger et al., 2014) of *Sycon*, a genus of calcareous sponge. The embryos and larvae are brooded and so are inaccessible to manipulation *in vitro*, but the sponge is small and therefore lives well in flow-through seawater tanks; it thus has the potential to be adapted more widely as a cold-water model species.

By far the easiest sponges to maintain and study in culture world-wide are spongillids. These are a group of haplosclerid demosponges which colonized freshwater between 183 and 141 million years ago (Meixner et al., 2007). A suite of papers describing the morphology and development of canals, choanocytes and spicules established this as an easy-to-use system (Weissenfels, 1976; Weissenfels and Landschoff, 1977; Weissenfels and Striegler, 1979; Weissenfels, 1980; Weissenfels, 1981; Weissenfels and Hündgen, 1981; Weissenfels, 1982; Weissenfels, 1983; Weissenfels, 1984; Wachtmann et al., 1990; Weissenfels et al., 1990; Weissenfels, 1992). The attractiveness of this model, which was highlighted by Yoko Watanabe through the film 'Life of the freshwater sponge' (Tokyo Film Corporation http://tokyocinema.net/EnglVieo.htm), has led to more recent studies on signalling and coordination of sponge behaviour (Elliott and Leys, 2007; Elliott and Leys, 2010), epithelia (Leys et al., 2009; Adams, 2010), patterning (Windsor and Leys, 2010) and most recently, sensory cells (Ludeman et al., 2014). And since freshwater sponges are easily obtained and cultured in Europe, Japan and North America, there is a body of knowledge on the genetics of development (Richelle-Maurer et al., 1998; Richelle-Maurer and Van de Vyver, 1999; Nikko et al., 2001; Funayama et al., 2005a; Funayama et al., 2005b; Mohri et al., 2008; Funayama et al., 2010; Holstien et al., 2010; Funayama, 2013) and even the possibility of using RNA interference methods (Rivera et al., 2011). Typically, gemmules are collected during winter months and kept refrigerated to hatch as needed in the lab, but it is also possible to keep a population over the long term by returning hatched batches to lakes. Individuals of freshwater sponges – and therefore all gemmules from one individual – are either male or female, and gametes can be obtained from cultures maintained in lakes (Mukai, 1989; Mukai, 1990).

Ecology of Ediacaran seas, sponge function and behaviour

What food would have been available to the first metazoans? Bacteria, flagellates and other early phytoplankton would probably have been the primary prey (Lenton et al., 2014). With no life yet on land, bacteria-rich seas fertilized by aggregates of faeces would not have existed and without that it is unlikely there would have been high levels of dissolved organic carbon (DOC). Paleontological evidence for high levels of dissolved organic matter in deep Ediacaran oceans is equivocal (Halverson et al., 2009), as is fossil evidence for larger animals at that time (e.g. Maloof et al., 2010). Capture of prey would be best achieved by filtration and concentration of food, which favours the idea of a filter/suspension feeder arising before the evolution of complex nervous systems. If filtration was the mechanism of feeding, it may have been energetically expensive (Leys et al., 2011), so it is unlikely to have originated in deep oxygen-poor oceans. Therefore, this animal would most likely have evolved in shallow waters in competition with other flagellates and have specialized to be efficient at filtering.

Sponges are primarily bacterivores – few suspension feeders other than flagellates specialize in capturing food less than 1 μm in size. The filter consists of microvilli that are linked laterally by a fine glycocalyx mesh 40–70 nm in diameter (e.g. Fjerdingstad, 1961; Leys et al., 2011; Mah et al., 2014). In sponges, and in some colonial choanoflagellates, neighbouring collars are also joined near the upper end by a second mucus mesh or by cells (Weissenfels, 1992). The tightness of the resulting filter means that filtration is efficient, and direct measurements of water filtered by sponges show up to 100% removal of bacteria (Maldonado et al., 2012). The sponge must therefore filter any particles that are in the water around it, including inorganic detritus such as fine sediments disturbed by fish or storms. Although the organic portion of resuspended material might be used by the sponge as food, sponges are irritated by concentrations greater than ~10 mg l^{-1} (Gerrodette and Flechsig, 1979; Tompkins-MacDonald and Leys, 2008) and contract ostia and/or canals during resuspension events, or in response to storms. The main behaviour of sponges, apart from filtering, is to prevent uptake of unwanted particles that might damage the filter: this occurs either by contractions of canals or, in the case of glass sponges, by arrest of the flagella pumps.

Sponges in all four classes – Calcarea, Demospongiae, Homoscleromorpha and Hexactinellida – contract (Nickel, 2010), and whereas contractions of the whole body take anywhere from 15 min to several hours, many sponges are constantly in motion, contracting portions of their body and relaxing others (Bond, 2013) (S.P.L., unpublished data). Contractions are usually triggered by storm events (turbulent water) and increased sediment in the water, but seasonal temperature changes (which are associated with changes in many water column properties) also cause reduced pumping and in some instances one species will stop pumping in response to a spawning event by another species (Reiswig, 1971).

The greatest range of behaviour has been documented for freshwater sponges, from contractions of the osculum only (McNair, 1923) to a periodic contraction of the whole sponge called a ‘condensation rhythm’ (Weissenfels, 1990), as well as a behaviour that has been termed a ‘sneeze’ because of the biphasic inflation and then contraction of the aquiferous system to expel unwanted particles (Elliott and Leys, 2007). Larval behaviour is the other main activity known from sponges: larvae change swimming direction within seconds of a change in light intensity, some in response to gravity and other stimuli (reviewed in Maldonado and Bergquist, 2002). Sponge larval responses are not very different to responses seen by other invertebrate larvae that have nerves. How are they carried out? And how do adult sponges detect and respond to changes in water quality?

Neural toolkits

Sensory systems and conduction pathways

‘Systems’ and ‘pathways’ are terms that typically refer to a constant morphological structure: tissues. Understanding that sponges have ‘tissues’, which are groups of cells that are organized together to carry out a particular function - is essential to be able to consider and interpret evidence of the function of neural-like elements. There are at least 16 different cell types in sponges (Simpson, 1984) and whereas the function of some is well-known, many have a name but unknown function and yet others, such as archaeocytes, have subtypes whose function can only be identified by their behaviour or gene expression (e.g. Funayama et al., 2010). A number of types of sponge cells are organized and function together as tissues, as in other animals. For example choanocytes together with endopinacocytes form a highly effective, non-leaky, filtration unit. Epithelia are formed by many subtypes of pinacocytes, which form stable interactions. Those on the exterior of the sponge (exopinacocytes) have been shown to possess sealing junctions which allow the sponge to control the ionic milieu of its extracellular matrix, as in other animals (Prosser, 1967; Adams et al., 2010). Transport pathways in *Aplysina* are so distinct they can be lifted out of the sponge like a tendon (Leys and Reiswig, 1998), and in many sponges the cortex is such a distinct tissue of spicules, cells and ostia, it is termed a ‘rind’ (Boury-Esnault and Rützler, 1997). The most obvious tissue of a sponge is the epithelium, which has the sensory cells and is thought to be the conducting pathway.

Sensory cilia in the osculum

The osculum – excurrent chimney – is the most easily identified structure in all sponges. It also seems to be the main organ for sensing stimuli from the environment and triggering responses by the whole animal. Short (4–6 μm long), non-motile, ‘primary’ cilia have been found to line the inside of oscula in all sponges studied so far by scanning electron microscopy (Fig. 2A–D) (Nickel, 2010; Ludeman et al., 2014). Primary cilia are found on all cells in vertebrates and many cells in invertebrates, and are involved in sensing gradients of chemicals, light and flow (vibration) via ion channels of the transient receptor potential (TRP) family (Singla and Reiter, 2006).

Primary cilia in sponges are thought to function in a similar manner to the balancer cilia in ctenophores, or the sensilla of crustaceans, or the cilia on mammalian kidney epithelia by sending a signal, via a calcium wave, in response to a change in position of the cilium (Singla and Reiter, 2006). In sponges, primary cilia label with FM 1-43, a steryl dye, and also with a conjugate to the antibiotic neomycin sulphate, both non-specific calcium channel blockers (Ludeman et al., 2014; Fig. 2E,F), as do primary cilia in the lateral line of fish and inner-ear hair cells (Ou et al., 2009). The cilium is non motile and lacks a central pair of microtubules (Ludeman et al., 2014; Fig. 2G,H). Our understanding of the sensory role of primary cilia in animals and unicellular flagellates such as *Chlamydomonas* comes from behavioural assays (Fujiu et al., 2011). In the sponge, removing the whole osculum, or removing the cilia using chloral hydrate, eliminates the ability to respond to triggers of the ‘sneeze’ behaviour, the stereotypical inflation–contraction response that freshwater sponges use to rid themselves of wastes (Elliott and Leys, 2007). This links both the osculum and the cilia in the osculum with the sneeze behaviour. Furthermore, neomycin sulphate, FM 1-43 and gadolinium all reduce or block the ability of the sponge to carry out a ‘sneeze’ and the effect is reversible (Ludeman et al., 2014). The fact that cilia appear at the osculum of all sponges studied so far (even hexactinellids), suggests that this is a common sensory organ in Porifera.

Sensory cells in the larva

Sponge larvae come in a great range of forms, but are largely ciliated propagules, up to 3 mm in length; they often have differentiated anterior–posterior ends and may swim or crawl, usually rotating as a result of the metachronal beat of short cilia (Fig. 2I; Maldonado and Bergquist, 2002). In laboratory environments they are typically short-lived, settling within 12 h to 3 days, but *in situ* they may live much longer. Sponge larvae show phototaxis and geotaxis (Maldonado and Bergquist, 2002).Where phototaxis has been studied in depth, directional swimming has been shown to occur by a combination of rotation of the larva around its anterior–posterior (A–P) axis and the shading by pigment of a

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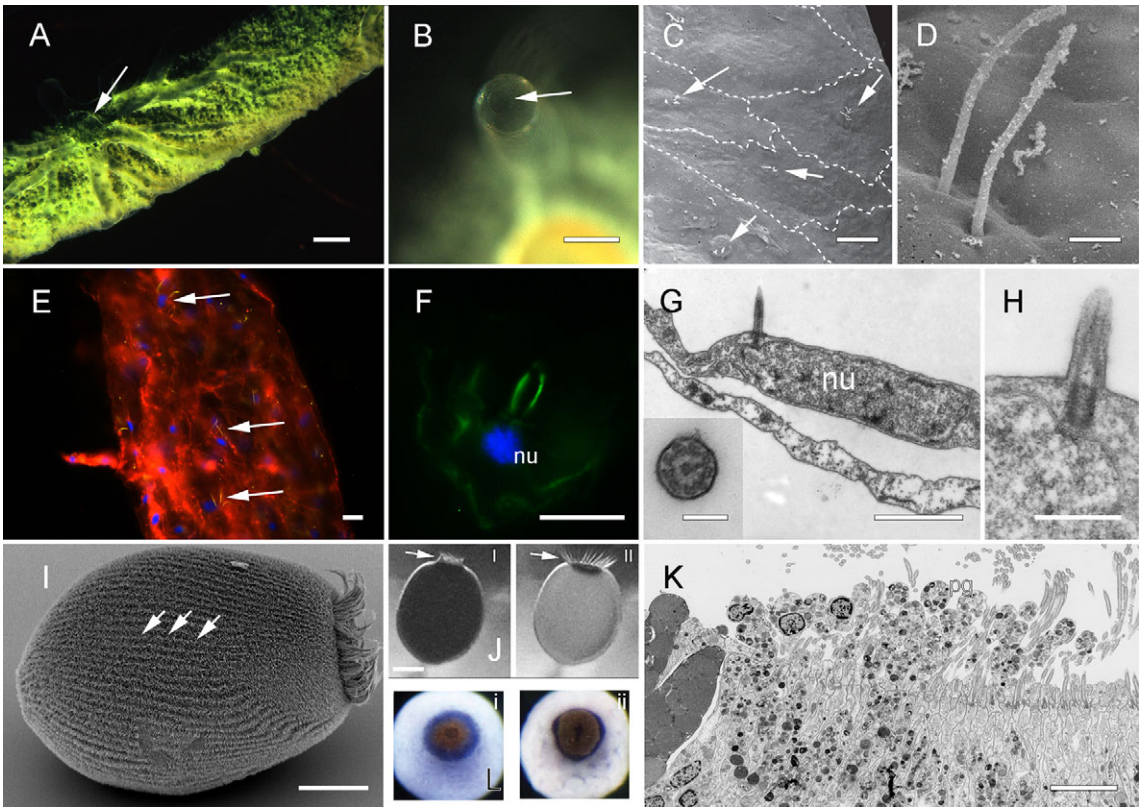

Fig. 2. Sensory cilia in sponges. (A) Transparent raised excurrent canals leading to the osculum (arrow) in *Spongilla lacustris* encrusting on a branch in a lake. (B) The osculum (arrow) of a small lab-hatched individual of *Spongilla lacustris*. (C,D) Scanning electron micrographs of cilia (arrows) on the inner epithelium of an osculum cut open lengthwise. (E,F) Immunofluorescence of the whole osculum (E), and a single endopinacocyte (F) showing cilia labelled with the styrl dye FM 1-43 (green, arrows), nuclei (blue) and actin (red) (images, D. Ludeman). (G,H) Transmission electron micrograph of a section through the osculum showing the base of one cilium arising just above the nucleus (nu); inset shows a cross section of the cilium with no clear central pair of microtubules. (I) Scanning electron micrograph of the larva of *Amphimedon queenslandica* showing swimming cilia forming metachronal waves (arrows) and long posterior cilia (right). (J) Response of the long posterior cilia in *A. queenslandica* to changes in light intensity: (I) bent when suddenly dark and (II) straightened when suddenly light (from Leys et al., 2002). (K) Transmission electron micrograph through the pigment granules (pg) and long posterior cilia of the *A. queenslandica* larva. (L) Expression of the *AqCRY2* gene in two developmental stages (i,ii) at the posterior pole of the *A. queenslandica larva* (from Rivera et al., 2012). Scale bars: 5 mm (A); 50 µm (B); 10 µm (C,E,F); 1 µm (D); 2 µm (G); 100 nm (G, inset); 500 nm (H); 100 µm (I,J); 5 µm (K).

region of cilia (Fig. 2J) (Leys and Degnan, 2001; Maldonado et al., 2003). The pigment inclusions are intracellular, and appear to lie in a cell adjacent to the ciliated sensory cell (Fig. 2K). The simplest explanation for the 'steering' of the larva is that each cell responds independently to changes in light intensity as the larva rotates through the water (Leys and Degnan, 2001). But some larvae have cytoplasmic bridges between the protrusions containing the pigment (e.g. Maldonado et al., 2003), so some sort of more rapid communication between the pigment cells should not be ruled out because cytoplasmic bridges usually occur in tissues that need to maintain quicker communication (e.g. for coordinating developmental processes in sperm or in the embryo).

The photo pigment in the *Amphimedon queenslandica* larva has been studied more closely and is thought to be a cryptochrome with sensitivity at around 450 nm (Leys et al., 2002). Two cryptochromes AqCry1 and AqCry2 were purified from *A. queenslandica* and one, AqCry2, showed sensitivity to blue light and was expressed in a region around the pigment ring where the light sensitive cilia occur at the posterior pole of the larva (Fig. 2L) (Rivera et al., 2012). The interpretation is that the *Cry* genes encode proteins that are located in the ciliated cells in the larva, but further work using antibodies is needed to confirm this. It is possible that other proteins are involved in the light response of the larva, because a 600 nm peak was suggested to be due to an opsin-like molecule [see fig. 7 in Leys and Meech (Leys and Meech, 2006)]. So far, no true opsin has been found in either the *Amphimedon queenslandica* or *Oscarella carmela* genomes nor in any transcriptome from sponges (Feuda et al., 2012).

Other sponge larvae also have phototactic behaviour (Maldonado et al., 2003; Collin et al., 2010). Amphiblastula larvae of calcareous sponges show negative phototaxis (Elliott et al., 2004) and have curious 'cross cells' which express *Smad1/5* (Leininger et al., 2014) as well as *SoxB* (Fortunato et al., 2012), genes that are also expressed in vertebrate sensory systems. In early work, Tuzet suggested that the cross cells were involved in photosensation (Tuzet, 1973), but no experiments have tested this. The absence of any opsins in sponges is curious because opsins are known from plants and fungi (microbial, type I opsins) and are thought to be convergent with animal type II opsins (Heintzen, 2012). At least two rhabdomeric (type II) opsins have been found in ctenophores (Schnitzler et al., 2012). Were opsins, like nerves, also lost in sponges?

Conducting pathways and effectors

If sensory cilia receive signals, how is the signal transmitted through the sponge and what is the effector? In glass sponges the syncytial

tissues transmit electrical signals, and the effectors are the flagella of choanocytes, which stop beating. Cellular sponges have no electrical signals, and are not known to arrest their flagella beating, so the effectors are contractile cells that reduce the size of the canals and chambers, effectively reducing flow into and through the sponge. Earlier workers identified the effectors of contractions in sponges as a type of smooth muscle cell called a myocyte (Bagby, 1966; Prosser, 1967); it was thought that these could be both in the mesohyl and epithelium. Recent work has referred to them as actinocytes and there is some evidence that actinocytes are largely epithelial, i.e. are pinacocytes, and that mesohyl cells play a passive role in contractions (Nickel et al., 2011). Where canals are wide, 'sphincters' made from one or more specialized pinacocytes arise from the canal epithelium, allowing the sponge to constrict a portion of the canal. In other places, sieve cells function in the same way to reduce the dimensions of the incurrent space. In *Tethya wilhelma*, for example, a sieve-like cell (sometimes two) forms the apopyle or excurrent passage of chambers and this cell expresses genes for myosin (Steinmetz et al., 2012).

Whereas pinacocytes are stationary and maintain contact with neighbours via adherens and septate junctions, many cells in the sponge mesohyl are in constant motion and do not seem to stay in contact with epithelia or with other cells for long. Both Prosser (Prosser, 1967) and Adams et al. (Adams et al., 2010) have shown that sponges control the ionic milieu of the extracellular space, so signalling is expected to be juxtacrine – being released from one cell to trigger a response in a neighbouring cell without direct passage of material from cell to cell. In fact, few examples exist of direct exchange of materials between sponge cells and this seems to be one of the main puzzles given the description of a near complete set of scaffolding proteins involved in post-synaptic densities (PSDs) in the *Amphimedon queenslandica* genome (Sakaraya et al., 2007; Alié and Manuel, 2010) as well as in other sponge transcriptomes (Riesgo et al., 2014).

Numerous ultrastructural studies on different sponges show regions of density between neighbouring cells – cells apparently exchanging large vesicles, some with distinct clathrin-coated pits (Pavans de Ceccatty et al., 1970; Lethias et al., 1983) – but no obvious synaptic structure with a post-synaptic density has been found. Many PSD proteins are also found in unicellular eukaryotes where there is clearly no pre-neuronal role (Burkhardt et al., 2014). So a neuronal context is not necessarily implied by gene content. But knowing whether PSD genes occur and function together in sponges would help determine when components of a proper PSD arose. In this vein, correlation analysis by Conaco et al. (Conaco et al., 2012) suggested that although there is a lack of global co-regulation of the entire set of PSD genes, small modules are co-expressed. But there is some circularity in this reasoning, because the same analysis suggests there is no co-regulation of epithelial genes in sponges based on the fact that the authors did not consider sponges to possess proper epithelia. A number of PSD genes (*Homer*, *CRIPT*, *DLG* etc.) are expressed in globular cells of the epithelium of the larva of *Amphimedon queenslandica*, which are interpreted to be potential sensory cells receiving signal cues that guide settlement behaviour (Sakaraya et al., 2007; Richards et al., 2008). Normally PSDs are in the cell receiving the signal, not the sensory cell, so their location in the globular cell of *Amphimedon* is confusing. Globular cells in *Amphimedon* also express many other genes [(e.g. NF-κB (Gauthier and Degnan, 2008); bHLH and Delta (Richards et al., 2008); Frizzled (Adamska et al., 2010); TIRs (Gauthier et al., 2010)] so experimental work is needed to determine whether the gene expression is linked to sensory function.

Ionic physiology and signalling molecules

Only glass sponges (Hexactinellida) use electrical impulses to rapidly send signals arresting the feeding current (Leys and Mackie, 1997). All attempts to determine the mechanism of contractions and signal propagation in other sponges, including bath application of chemicals, substitution of ions in the medium and triggering with mechanical and electrical stimuli, so far show that electrical signalling does not occur in cellular sponges. Loewenstein (Loewenstein, 1967) reported that aggregating cells of *Haliclona* spp. could pass current to one another in the presence of calcium and magnesium, suggesting that something like a gap junction exists in these cells, but the work has never been repeated. Innexins of gap junctions have so far not been found in sponge genomes or transcriptomes and dye coupling, usually an indication of gap-junction-coupled cells, was not seen in dissociated cells of *Haliclona* cf. *permollis* (Leys, 1995).

If electrical signalling occurred in cellular sponges, some faster behavioural response to changes in the ionic medium would be suspected, but this does not seem to be the case. Prosser (Prosser, 1967) showed that for sponges to contract, the water must have a univalent ion (sodium could be replaced by potassium or lithium) and a divalent cation (magnesium and calcium were usually both required, although reduced contractions only occurred in the absence of magnesium and strontium could replace calcium) (Fig. 3A). Importantly, Prosser showed that contractions can occur at 10-fold higher external potassium concentrations (100 mmol l^{-1}), which would normally depolarize cells, so he concluded it was unlikely that action potentials were involved in contractions (Prosser, 1967). Therefore, slower signalling pathways are expected, and these could involve either small molecule transmitters (SMTs, including amino acids, biogenic amines and gaseous molecules) or neuropeptides (usually 3–40 amino acids long).

Although many SMTs are well known from plants and fungi, the evolutionary origins of metazoan representatives of these molecules are not entirely clear. Some of these molecules are found in sponge transcriptomes and have been shown to function in the contraction behaviour of sponges, but others do not seem to be produced by sponges and may come from the sponges' bacterial symbionts. For example, there is evidence for the presence of metabotropic glutamate and GABA receptors in the genomes of both *Amphimedon queenslandica* and *Oscarella carmela*, and physiological experiments show that glutamate triggers contractions and GABA inhibits contractions in the freshwater sponge (see below). Despite an initial report that serotonin and dopamine receptors were present in *Amphimedon* (Srivastava et al., 2010), none have been found in transcriptomes of eight sponges or the *Amphimedon* genome (Riesgo et al., 2014). Anti-serotonin immunoreactivity was suggested for a sponge larva, but distribution of the label was difficult to associate with any particular cell or cells, and specificity of the antibody was not confirmed by western blotting (Weyrer et al., 1999). Oddly, many papers report serotonin or serotonin-like molecules (brominated cyclodipetides) in chemical extracts from sponges (e.g. Hedner et al., 2006). As sponges are rich sources of novel metabolites (Taylor et al., 2007), the majority of which are produced by bacterial symbionts, we should consider whether the major source of serotonin in sponges may actually be bacterial symbionts.

Of the other SMTs (e.g. histamine, aspartate, ATP, cAMP GABA, glutamate and the gaseous molecule NO) the function of glutamate and GABA has been studied in most detail in the freshwater sponge *E. muelleri* (Elliott and Leys, 2010). The sponge can be triggered to 'sneeze' by vigorous shaking (2–4 Hz) or by adding dilute Sumi

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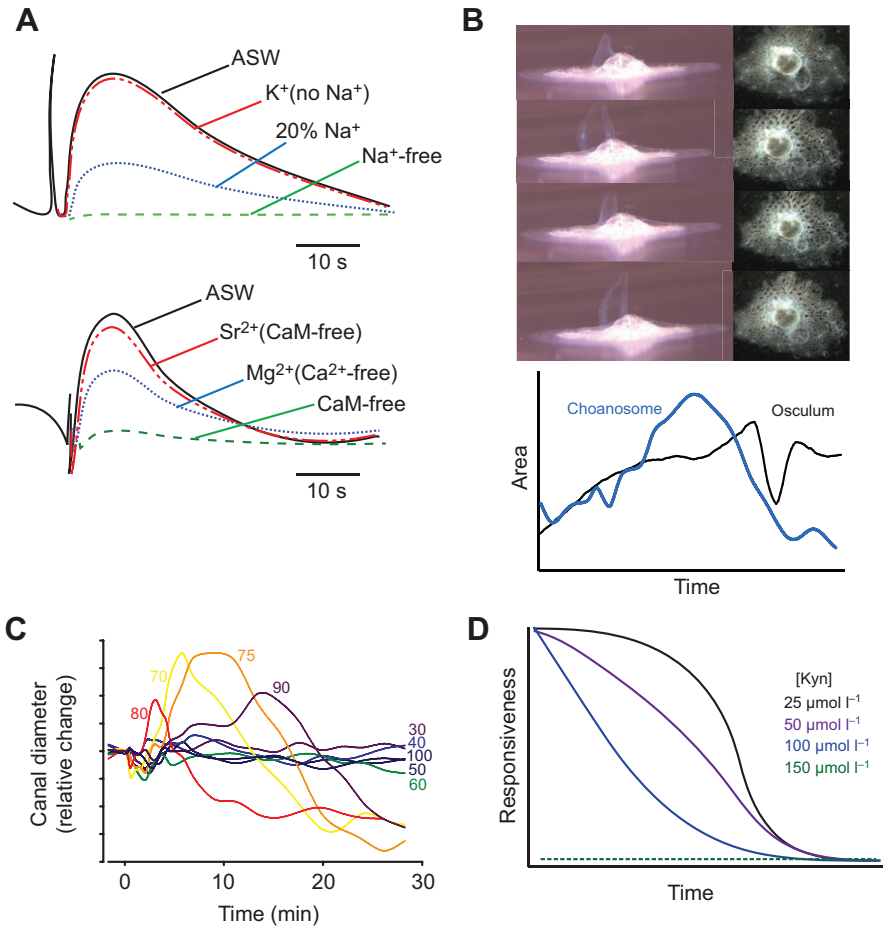

Fig. 3. Ionic basis of contractions in freshwater sponges. (A) Substitution of sodium (top panel) and calcium and magnesium (bottom panel) in marine sponges (after Prosser, 1967). Solid line (both panels): ASW control. Top panel: dotted blue line, 80% reduction in sodium (sodium replaced with sucrose); dashed green line, no sodium, 100% sucrose; dash-dotted red line, potassium instead of sodium. Bottom panel: dotted blue line, magnesium but no calcium; dashed green line, neither calcium nor magnesium; dash-dotted red line, strontium instead of calcium and magnesium. (B) Contraction of the osculum (left) and choanosomal region with feeding chambers (right) of *Ephydatia muelleri* with tracings showing the time of both events below. (C) Concentration-dependent effect of glutamate on the inflation–contraction behaviour of *E. muelleri*. A full 'sneeze' is triggered by 75 µmol l^{-1} L-Glu; lower concentrations generate localized contractions and higher concentrations cause the surface of the sponge to tear, whereas the canals continue a full inflation–contraction event (from Elliott and Leys, 2010). (D) Concentration-dependent effect of glutamate blocker kynurenic acid on contractions in *E. muelleri*. Longer incubation in Kyn reduces responsiveness to L-Glu, even at lower concentrations of the inhibitor. High concentrations of the inhibitor block all contractions.

calligraphy ink. Ink clogs the canals and it takes the sponge some hours to remove it, but the effect of ink is informative because the repeated inflation–contraction events eventually push the undigested and mucus-coated clumps of particles out of the osculum to litter the bottom of the dish. Whereas shaking causes the osculum to contract, 70–80 µmol l^{-1} L-Glu causes the osculum to contract vigorously and triggers the full stereotypical inflation–contraction ('sneeze') behaviour in *Ephydatia muelleri* (Fig. 3B,C) (Elliott and Leys, 2007). Higher concentrations caused such a vigorous contraction that the top of the sponge tore, although the canals continued through their full inflation and contraction.

Two glutamate receptor inhibitors, AP3 (a competitive inhibitor) and kynurenic acid (Kyn, a non-competitive or allosteric inhibitor) both blocked the sneeze behaviour in a concentration-dependent manner (Elliott and Leys, 2010) (Fig. 3D). These experiments suggested that clogging of chambers with dye must trigger stretch receptors or reduce flow enough to make the sensory cells in the osculum (Ludeman et al., 2014) respond and cause the osculum to contract; the hypothesis is that glutamate receptors lie at the base of the osculum and along the entire epithelium of the sponge incurrent canal system. Transmission is presumed to be by localized release from cells into the mesohyl, then binding mGluR receptors, which triggers calcium to enter neighboring cells, which in turn release glutamate, much as envisioned by Nickel (Nickel, 2010). There are at least three mGluR candidates for this in the sponge (Sakaraya et al., 2007). GABA applied directly causes the sponge to flinch, but incubation in GABA (1 mmol l^{-1}) for 10 min prevents any sneeze when stimulated either by shaking or by L-Glu (70–80 µmol l^{-1}) (Elliott and Leys, 2010).

Many molecules are known to trigger contractions of the osculum, ostia or whole body of sponges (Emson, 1966; Prosser, 1967; Ellwanger et al., 2004; Ellwanger and Nickel, 2006). *Tethya wilhelma* has pacemaker-like activity with repeated innate contractions every hour to several hours depending on the individual. Contractions can also be triggered by a suite of chemicals including caffeine, AchE, nicotine, nitric oxide, cAMP and serotonin (Ellwanger and Nickel, 2006). Although no molecules prevent contractions in *Tethya* and most trigger an immediate contraction, some molecules have an interesting modulating effect – for example, NOC-12 a nitric oxide donor and caffeine both reduce the amplitude and period of the contractions (Ellwanger and Nickel, 2006). Bath application of chemicals can also have very different effects on different sponge species: in *Tethya*, for example, both glutamate and GABA clearly trigger abrupt contractions of the sponge (Ellwanger et al., 2007), whereas in *Ephydatia*, GABA distinctly inhibits contractions (Elliott and Leys, 2010). We do not yet know the role of aspartate, histamine or ATP in sponges and this is where continued research should focus.

The role of biogenic amines (e.g. catecholamines dopamine, epinephrine and norepinephrine) in signalling in the sponge is unclear. Bath application of both dopamine and epinephrine causes contractions (Prosser, 1967; Ellwanger and Nickel, 2006) and portions of the catecholamine synthesis pathway were found in most, but not all, of eight sponge transcriptomes, yet the complete pathway was not found in any sponge transcriptome or genome (Riesgo et al., 2014). It is possible that some of these molecules are so divergent that they remain undetected with BLAST searches.

Neuropeptides have not yet been found in sponges, although as with catecholamines, some enzymes of the synthesis pathways are

present. Peptidergic signalling plays a large role in ctenophore and cnidarian nervous systems (Anctil, 1987; Spencer, 1989), but sponges could use peptides as signalling molecules even without nerves. Sponge larvae settle and metamorphose more rapidly in the presence of GLW-amide peptides (Whalan et al., 2012), so peptides may be used by sponge larva for locating the right settlement substrate. Generally biofilms and coralline algae trigger metamorphosis in invertebrate larvae and the same was found for *Amphimedon queenslandica* larvae (Jackson et al., 2002), but exactly how this works is unknown. Nevertheless, if sponges use peptidergic signalling, larval cells would be the place to look for receptors.

In sum, there is currently only physiological and genomic evidence for amino acid transmitters in coordination of behaviour in sponges. But without nerves to study and as sponge tissues show low cross-reactivity to commercial antibodies, there are still few tools available to study this, as has been found in ctenophores.

Ion channels

Ion channels are responsible for all rapid ionic changes across membranes. The simplest for cloning and therefore easiest to study in sponges have been potassium channels. Potassium channels are responsible for stabilizing membrane potential, and so are indicators of electrical behaviour. Intriguingly, no voltage-sensitive channel has yet been identified in sponges, although it hardly seems likely that they are entirely absent from the group. A K_v channel was said to be present in *Amphimedon queenslandica* (Alié and Manuel, 2010) but the voltage sensor domain is absent in that sequence. No channels with a voltage sensor have been found in the transcriptomes of eight other sponges, so at present we do not know of a K_v channel from a sponge. However other K channels have been studied, including inward rectifying and two pore K channels (K_{ir} and K_{2P}). The K_{ir} channel isolated from *Amphimedon* shows rapid inactivation, which indicates that the channel resets the membrane potential quickly – as though it might respond to depolarization, a hint that electrical signalling may occur in *Amphimedon* (Tompkins-MacDonald et al., 2009). The K_{2P} channel shows sensitivity to amino acids and to pH, but not to temperature, rather like other animal K_{2P} channels (Wells et al., 2012). A temperature- and mechano-sensitive cation channel has been found in *Axinella polypoides*, but it is not known to have a role in directional signalling or coordination of behaviour (Zocchi et al., 2001).

Perhaps the most intriguing molecular find in terms of ion channels is that ionotropic glutamate receptor (iGluR)-like molecules were found in transcriptomes of three out of eight sponge species: *Sycon coactum, Oscarella carmela* and *Ircinia fasciculata* from Calcarea, Homoscleromorpha and Demospongiae classes, respectively. These have the Q/R site and the pore motif SYTANLAAF (Riesgo et al., 2014). Ionotropic receptors imply there is a need for fast signalling, yet where this happens is not clear because contractions and indeed responsiveness in demosponges is not fast. In demosponges, contractions travel at 2–20 µm s^{-1} along epithelia [12.5 µm s^{-1} in *Tethya wilhelma* (Nickel, 2004) and 0.3–5 µm s^{-1} in *Ephydatia muelleri* (Elliott and Leys, 2007)] except in the osculum, where a wave of contraction was reported to travel at 6–122 µm s^{-1} in *E. muelleri* (Elliott and Leys, 2007) and at 170–350 µm s^{-1} in *E. fluviatilis* (McNair, 1923). Propagation across a whole animal can take 30 min to 1 h, so a signal cascade via metabotropic glutamate receptors (mGluRs), which binds glutamate via a GPCR is expected to be sufficiently rapid for transmitting signals between cells. The fastest rate of contraction in sponges is still ten times slower than action potential propagation in plants (Fig. 4A), so it is unlikely that an electrical signal is involved. If iGluRs enable a rapid response then one might speculate that it could be in response to injury – like the pin prick that McNair (McNair, 1923) used in his studies – and if so, perhaps the primary response is to release chemical defences, something that has not been studied. In that case, the 'slow' contractions could be a secondary response, causing the sponge to be smaller and appear less palatable to a predator.

Glass sponges – electrical signalling

Glass sponges, Hexactinellida, use electrical signalling. Unlike all other sponges the whole body of a glass sponge forms a single continuous syncytium (Leys, 1999). Syncytial tissues allow electrical signals to travel unimpeded by membrane barriers throughout the whole animal and these cause the feeding current to stop within seconds of a mechanical or electrical stimulus; the effect is 'all or none' (Leys and Mackie, 1997). Glass sponges are thought to have diverged from a common ancestor shared with demosponges during the late Neoproterozoic early Cambrian period (Mehl, 1996; Antcliffe et al., 2014). We know the development and morphogenesis of tissues from two species: *Farrea occa* (Ijima, 1904) and *Oopsacas minuta* (Boury-Esnault and Vacelet, 1994; Leys et al., 2006). Glass sponges form cellular embryos, which become syncytial after the 64-cell stage (6th cleavage) by fusion of macromeres (Leys et al., 2006). This tissue, the trabecular reticulum, forms an extremely thin giant multinucleated cell that forms the outer skin (called the dermal membrane), and all the incurrent canals, flagellated chambers, excurrent canals and oscula tissues (Ijima, 1904; Mackie and Singla, 1983; Leys, 1999). All tissues are cytoplasmically connected and cytoplasm streams throughout the tissues along giant tracts of microtubules (Fig. 4B) (Leys, 1995).

Arrests of the glass sponge pumping system were first noted by G. Silver who in the 1970s put thermistor flow meters into the osculum of *Rhabdocalyptus dawsoni in situ* at 25 m depth. As divers approached the sponges and stirred up sediment, the sponges stopped pumping. Experiments in tanks confirmed this behaviour and the speed of conduction and ability to travel circuitous paths, but not to jump between distinct pieces of sponge suggested there must be an electrical signal (Mackie, 1979; Lawn et al., 1981), but the thinness (2–10 µm) and elasticity of the trabecular tissue made it difficult to record from. It was only by developing a novel preparation of sponge tissue aggregates fused to the body wall that it was possible to attach suction electrodes and record electrical signals (Fig. 4C) (Leys and Mackie, 1997).

The characteristics of glass sponge conduction are as follows. (1) The action potential (AP) is 5 s long and travels at 0.27 cm s^{-1}. The absolute refractory period, the period during which a second AP cannot be generated, is 29 s. The second of a pair of APs with delays between 30 s and 150 s have a lower amplitude and slower conduction velocity, indicating that 150 s is the relative refractory period (Fig. 4D). The slowness of the AP may be attributed to the immensely circuitous path that it has to take through the syncytial strands of the tissues, but it is also considered to reflect a low density of ion channels in the syncytial tissues. This is substantially slower than the conduction systems of plants (Fig. 4A).

(2) The action potential is dependent on calcium and potassium (Leys et al., 1999). Reduction in Na^+ to 25% of normal seawater has very little effect on the AP – the amplitude is slightly reduced and delayed (Fig. 4E). In contrast, perfusion with 10 mmol l^{-1} Co^{2+}, 1 mmol l^{-1} Mn^{2+} or 24 µmol l^{-1} nimodipine – all calcium-channel blockers – eliminate the AP reversibly. Similarly, perfusion with the potassium channel blocker TEA (1–5 mmol l^{-1}) also blocks the AP

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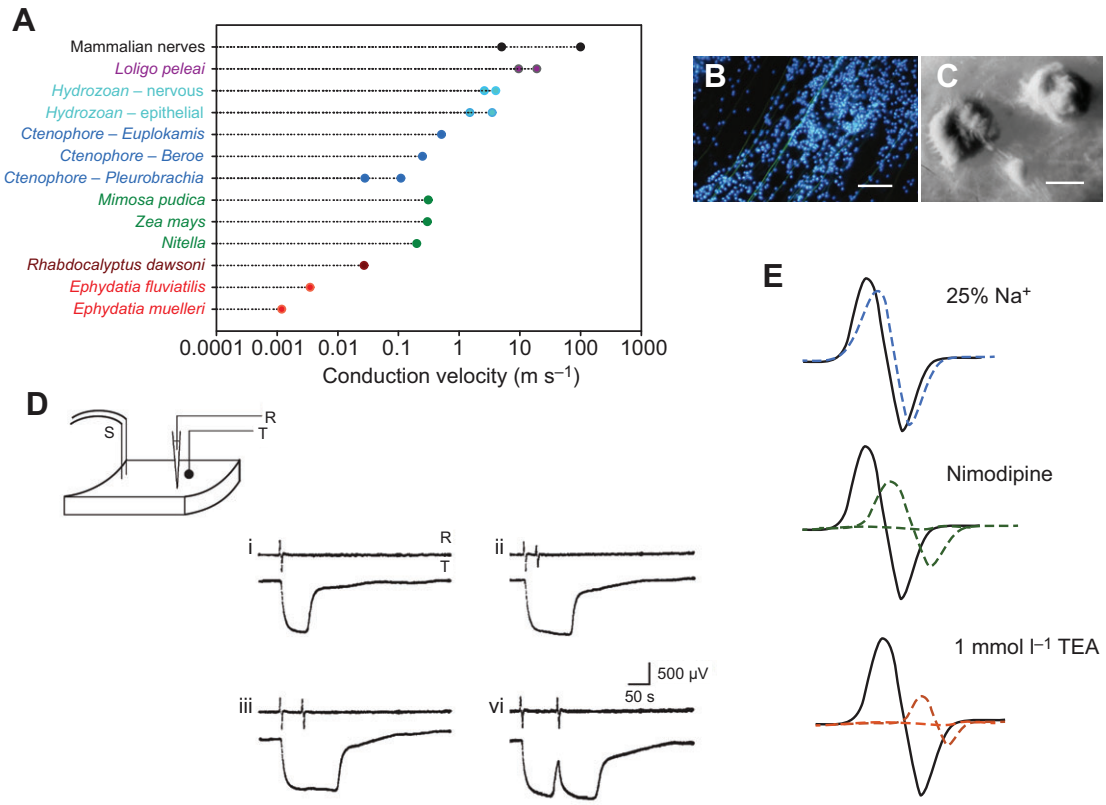

Fig. 4. Electrical conduction in glass sponges. (A) Conduction velocities in plants and animals. (B) Microtubules (green) and nuclei (blue) in giant syncytia of the glass sponge *Rhabdocalyptus dawsoni*. (C) Adherent aggregates fusing with the syncytial tissue of *R. dawsoni*, a preparation that allows extracellular recording from the sponge. (D) Diagram of the recording setup and records of action potentials in *R. dawsoni* (from Leys et al., 1999). S, stimulating electrode; R, recording electrode; T, thermistor flow probe. Top traces, electrical records; bottom traces, thermistor flow records: (i) a single stimulus causes and AP and arrest of flow; (ii,iii) repeated stimuli cause further APs even though the flow is still arrested; (iv) after pumping resumes a second stimulus causes a second AP and arrests the flow again. (E) Effect of sodium, calcium and potassium on the action potential in *R. dawsoni* (after Leys et al., 1999). Top, 75% reduction of sodium (replacement with choline chloride); middle, the calcium blocker nimodipine (24 µmol l^{-1}) delays and blocks the AP, reversibly; bottom, the potassium channel blocker TEA reduces, delays and then blocks the AP, also reversibly. Scale bars: 20 µm (B); 1 mm (C).

reversibly. These results suggest that the AP relies on influx of calcium and repolarization of the membrane by potassium.

(3) The action potential is temperature sensitive. *Rhabdocalyptus dawsoni* studied in tanks at the Bamfield Marine Sciences Centre, B.C., had a Q_{10} of ~3; the sponges did not pump at temperatures below 7°C, and would not arrest pumping at temperatures above 12.5°C. Presumably, other glass sponges have a slightly wider temperature tolerance because they inhabit colder waters in Hecate Strait, B.C. (5–7°C) and in Antarctica, but a limited range of function is still expected based on the constraints of calcium channel operation (Leys and Meech, 2006).

These characteristics do not seem to reflect a prior history of nerves that have been lost and replaced by syncytia. Although syncytia are common in animals, their method of formation by fusion during embryogenesis is not seen in other sponges or other animals. Epithelial conduction in the comb plates of ctenophores has similar velocities and is also calcium based (Moss and Tamm, 1987), but travels through cells connected by gap junctions. The temperature dependence of the action potential in glass sponges is thought to reflect an adaptation to deep, cold water. Recently, we have wondered whether syncytia and electrical conduction may have arisen as a low-cost system to prevent damage to tissues by clogging. Glass sponges can contract but very slowly (Nickel, 2010), and contraction may not be effective to prevent damage by a sudden resuspension event. Our recent work (Leys et al., 2011) suggests that the high cost of pumping may have led, over time, to reducing the resistance through the sponge by evolving very large canals. Could the cost of filtering in the deep sea have triggered the evolution of syncytia concurrent with electrical signalling as a way to prevent intake of materials that might damage the filter? Ongoing work by A. Kahn (Kahn and Leys, 2013) on the energetics of filtration promises new data on this question.

Common elements in different coordination systems

The sum of knowledge of sponge coordination systems shows that sponges are largely epithelial animals, with sensory cells that are epithelial, effectors that are contractile epithelial cells as well as flagellated collar bodies lining the feeding chambers of glass sponges; signalling pathways also seem to use the epithelia. There is evidence for slow signalling in cellular sponges, probably using metabotropic receptors and calcium waves, which are slow, but effective at closing the intake system to prevent damage to feeding chambers and sufficiently fast to eject inedible material that may have entered and clogged chambers. In glass sponges, electrical signalling is by action potentials which travel via syncytia and also prevent damage to feeding chambers.

The sort of signalling seen in sponges is simple in comparison to a nervous system, but the main need for signalling seems to be protection of choanocytes and tissues from clogging and damage. The sponge sensory system also provides a highly tuned control of

canal diameter to vary the amount of water processed, and this suggests that there may be an energetic benefit to reduce filtration if food is limited, for example during winter months. Larvae have other sensory needs, which are attuned to helping them find the best settlement sites, but even these are morphologically simple compared with those of Cnidaria or Ctenophora. If one compares just the sensory systems of sponges and ctenophores, it hardly seems likely that sponges have lost nerves. Sensory organs in ctenophores are sophisticated – both the balancer organ of the cydippid larva and of the adult in *Pleurobrachia* (Tamm and Tamm, 2002) and the photosensory molecules, including opsins of *Mnemiopsis* (Schnitzler et al., 2012) reflect a complexity not seen in any sponge. Ctenophore nerves use glutamate in signalling, while GABA appears in muscle (Ryan et al., 2013; Moroz et al., 2014). Serotonin is apparently absent, but ctenophores have a broad range of neuropeptides and clearly identifiable nerves with synapses; they also have gap junctions with a large number of innexin molecules used in epithelial conduction (Moroz et al., 2014). These innovations both enhance the agility of ctenophores and their ability to respond to and capture prey. In short, the two systems are not easily compared.

The fossil record does not give any insight into early ctenophore body plans – except for the idea that frond-like animals of the Ediacaran may have had ctenophoran affinities (Dzik, 2002) – but if ctenophores were predatory as extant species are, then what would they have eaten? The environment in which the first multicellular animals evolved was presumably oxygenated at the surface, as a result of photosynthesis and turbulence, but the only food would have been picoplankton – flagellates, bacteria and viruses (Lenton et al., 2014). It is difficult to think of an animal that could have existed prior to sponges and which would also have fed on bacteria and or unicellular flagellates, but which did not have a sponge-like body plan. If efficient filtering without damaging the filter was important to early animals, then mechanisms to protect the filter would have arisen and these would probably have been the first type of signalling system to use elements that are now recognized from nervous systems. The next step would have involved innovation of more agile movement, including muscle and signalling systems (possibly epithelial); these body plans may have co-opted the elements found in sponges but would have required more sophisticated gene regulatory networks (Peter and Davidson, 2011) to build. A study of these networks in both sponges and ctenophores might shed some light on this transition.

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Competing interests

The authors declare no competing or financial interests.

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