INTRODUCTION

Porifera is a taxon of morphologically diverse benthic animals that inhabit marine and freshwater environments from desert ponds to caves, from abyssal to littoral, and from arctic to tropical oceans. About 8500 species are recognized of some 11,000 described, but more than twice that number are estimated to exist based on current trends of discovery (Appeltans et al. 2012, Van Soest et al. 2012). Four classes are presently recognized, with Hexactinellida and Demospongiae (together loosely termed the Silicea) forming a sistergroup to Calcarea and Homoscleromorpha (Fig. 3.1A–D). Although some analyses have suggested sponges may be paraphyletic, the current consensus is that the phylum Porifera is monophyletic (Nosenko et al. 2013).

In contrast to the great range of gross morphologies of Porifera, the taxon is united in sharing a common internal form, consisting of flagellated cells whose beating action drives water from microscopic openings on the animal’s surface, through discrete epithelial-lined canals in the body, and out of larger collecting vents (Fig. 3.1A–E). Filtration of water is the primary mode of food uptake and excretion, and nutrient and gas exchange, and this strategy has only been lost in a few members of one deep-sea group of demosponges, which have turned to carnivory (these are not discussed in this chapter).

The ‘aquiferous’ or canal system forms the central polarizing structure of any sponge. Incurrent openings, termed ostia, litter the surface tissue and are invisible to the naked eye. The excurrent vent or osculum, in contrast, is a millimetre to half a metre in diameter and usually lies at the upper surface of the animal such that water filtered, as visualized by food colouring or fluorescent dye, is ejected in a jet above and directly away from the sponge body. The body of a sponge consists of (a) the aquiferous system (Fig. 3.1E–F), (b) soft tissues, including a compressible collagenous middle layer (mesohyl) and, where it is present, (c) an elastic and/or brittle skeleton of collagen and minerals.

The soft tissues of sponges vary in density from cobweb thin to massively thick (Fig. 3.1G). In all instances they consist of an outer epithelial layer formed by plate-like or in some cases T-shaped cells—both exopinacocytes—that enclose a collagenous region with wandering cells. Internally, bordering canals and feeding chambers, endopinacocytes form the inner epithelium. Sponges attach to substrates by a third type of epithelial cell—basopinacocytes— which also secrete a particular adhesive extracellular matrix. The collagenous middle region of sponge tissues is partly what gives the sponge body its elasticity. Elasticity (sponginess) is also provided by a type of collagenous skeleton called spicules. Three classes (Demospongiae, Hexactinellida, and Homoscleromorpha) have skeletal components, called spicules, made of silica—in contrast to the fourth class Calcarea, in which the spicules are made of calcium carbonate. There is one unusual group of coralline demosponges which, in addition to producing a siliceous skeleton, also constructs a massive skeleton of aragonite. Skeletogenesis differs so much in each of the groups that it is considered that the use of silica to form spicules arose independently in at least the Silicea and Homoscleromorpha, as did calcification in Calcarea and coralline demosponges.

Sponges are suspension feeders that filter water to extract bacteria and other picoplankton. Some species obtain a large portion of their nutrition from bacterial or algal symbionts, much as do stony corals, but generally a complement of food sources is required. The apparent simplicity of filtration as a process belies the complexity of having the precise dimensions of filtration system to maintain the correct pressure drop across the filter. The sponge’s principal sensory system is epithelial and where it is concentrated at particular locations in the aquiferous system as ‘organs’ (Ludeman et al. 2014), it allows modifications of the canal dimensions to either prevent uptake of bad water by closing ostia, or eject particles taken in inadvertently, by contracting. In some cases contractions are complicated behaviours involving coordinated inflations and contractions of the whole animal, which have been described as ‘sneezing’ (Elliott and Leys
with a speed indicative of electrical signalling (Bergquist 1978,
Reiswig and Mackie 1983). Modern molecular phylogenies have
confirmed that hexactinellids and demosponges form a clade
that is sister to the other two classes, but hexactinellids remain as
the only group with syncytial tissues, and so far they are the only
group known to use electrical signalling. Glass sponges, which
comprise 8% of described species, are also unusual in being
restricted to deep marine habitats where silica levels are high and
temperatures low and stable. Demosponges (83% of sponge spe-
cies) inhabit the greatest range of ecosystems, from freshwater
to marine, and have even adapted to the deep sea by adopting
carnivory in addition to filtration. Calcarea, which comprise 8%
of described sponges, and Homoscleromorpha (1% of sponge
species), the newest sponge class, are both marine groups with
wide distribution but often cryptic morphologies (Van Soest
et al. 2012).

Porifera reproduce sexually and asexually. Sexual reproduc-
tion is by production of gametes—some species are hermaphro-
ditic and others have separate sexes—and while the majority of
species studied brood the fertilized egg and release a fully de-
veloped ciliated swimming larva (Fig. 3.1H), in other instances
both males and females release gametes. In the latter case oocyte
fertilization and development into a swimming larva occur in
the water column. Sperm can be taken up by feeding chambers
of conspecifics or, if released as sperm packets, can be ‘caught’
on the surface of conspecifics allowing sperm to penetrate the
body wall and fertilize oocytes. There is some indication that
like other metazoans sponges have and use pheromones, which
sperm use to locate conspecifics and recognize gametes (Riesgo
et al. 2014).

Sponge larvae are ciliated propagules 20 μm to 2 mm in
length (Fig. 3.1H). They are all non-feeding and can stay in the
water column for a month, but in laboratory settings larvae com-
monly metamorphose within 1–2 days of release from the par-
et. Sponge larvae can respond to gravity and light (Warburton
1966); where it has been identified, the sensory organ is a ring-
like arrangement of cilia and pigment at one pole (Leys and
et al. 2010).

Prior to molecular studies, Hexactinellida were proposed to
form a separate subphylum (or even phylum) because of their
unusual syncytial tissues and ability to arrest the feeding current
with a speed indicative of electrical signalling (Bergquist 1978,
Reiswig and Mackie 1983). Modern molecular phylogenies have
confirmed that hexactinellids and demosponges form a clade
that is sister to the other two classes, but hexactinellids remain as
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wide distribution but often cryptic morphologies (Van Soest
et al. 2012).

HISTORICAL INVESTIGATIONS
OF NERVOUS SYSTEMS

It is unclear exactly when people began to consider sponges
in the light of the evolution of nervous systems. From Parker
(1919), we know that Aristotle wondered at the mechanism of
the responsiveness of sponges, but Lendenfeld (1885) was one
of the first to publish a study of the histology of nerve-like cells
of sponges and to suggest that sponges should no longer be con-
sidered to be protists. The late 1800s was a time of reflection
on the origins of coordination and while most of those writing had studied cnidarians not sponges, they all contemplated the early origin of, and connection between, nerves and muscle, receptor, and effector (Moroz 2009, and references therein). Parker tied these thoughts together in a seminal review in 1919, but importantly he also carried out the first systematic experiments on sponge responsiveness to stimuli (Parker 1910). With that work there began a series of investigations on responsiveness to chemicals and histological evidence of nervous tissues in sponges, beginning with McNair (1923) and ending in Jones’ (1962) conclusion that there is no nervous system in sponges. This work included important contributions by Prosser (1967), Emson (1966), Lentz (1966), as well as Pavans de Ceccatty (1955, 1960, 1971, 1974b), and Pavans de Ceccatty et al. (1960). In recent years a very similar approach has been applied (histological studies, as well as effects of chemicals on behaviour) with little further advance in cellular sponges.

A major breakthrough was made in syncytial sponges (hexactinellids), however, with the discovery that they arrest their feeding current upon touch (Lawn et al. 1981) and that this is carried out by an electrically propagated action potential (Leys and Mackie 1997). This is not to say that there is no electrical signalling in demosponges. Loewenstein (1967) claims to have recorded coupling between cells in Microciona prolifera and Halichondria oculata, and although this work has not been reproduced, Reiswig also reported a rapid (<1 s) response of tissues covering the ostial (incurrent) pore fields in Phorbas amaranthus (formerly Hymedesmia) (Reiswig 1979). The recent finding of ionotropic glutamate receptors in expressed transcripts from several sponges (Riesgo et al. 2014), and functional work which shows that inward rectifying potassium channels have ionic properties that suggest they may function to rapidly reset membrane potential in demosponges (Tomkins-MacDonald et al. 2009), indicates there may be a more rapid mechanism of signalling in cellular sponges than is currently understood.

ARCHITECTURE

Sponges lack conventional nerves—but do sense and respond to stimuli. As Parker noted, the most obvious activity of a sponge is its filtration of vast volumes of water—up to 1000 times the body volume each day—which from some sponges pours out like ‘a vigorous spring’ (Parker 1919). The flagellated pump cells form one set of effectors. Another more gradual activity is contraction and relaxation of part of or the whole sponge body. This behaviour, which has been noted since the time of Aristotle, is controlled by a second set of effectors, cells forming the epithelia and/or cells of the middle layer (mesohyl) (e.g. Pavans de Ceccatty et al. 1970, Pavans de Ceccatty 1976, Nickel et al. 2011). It is easiest to address the architecture of the effectors in cellular and syncytial sponges separately, for although there is evidence that both effectors respond in both types of sponge, we know far more about the epithelial and mesohyl conduits in cellular sponges, and far more about flagella as effectors in hexactinellids.

CONDUCTION PATHWAYS AND EFFECTORS: EPITHELIA

Cellular sponges carry out contractions in response to a broad selection of stimuli; these are not just reflex responses, as described by Aristotle, in response to being torn off a rock (cited by Parker, 1919); they are also able to propagate contractions in a coordinated manner (Weissenfels 1990, Elliott and Leys 2007). Most past work has focused on instant responses to stimuli, but it is clear that there are much longer responses involving contraction of both epithelia and mesohyl cells, and that cells crawling in the mesohyl are also affected because they stop crawling during contractions (Elliott and Leys 2007). Studies have shown these contractions are propagated, and, most importantly, they are stereotypical and can be triggered by a threshold of stimuli (Elliott and Leys 2010, Ludeman et al. 2014).

The speed of contraction is typically slow—ranging from less than 1 to 130 µm/s (McNair 1923, Nickel 2004, Elliott and Leys 2007, Bond 2013)—well below the speed of electrically propagated events. This is what we fully expect for a signalling system based on membrane-bound receptors. Propagation requires divalent cations and in particular calcium (Elliott and Leys 2007). There are a couple of instances where more rapid propagation occurs: McNair (1923) reported that waves of contraction can move down the osculum of the freshwater sponge at 0.35 mm/s; the other is the rapid dropping of flaps over the ostial pore fields, previously discussed, as reported briefly by Reiswig (1979). Except for those two instances we have no expectation of electrical signalling in cellular sponges. Even in Calcarea the types of motility measured are extremely slow (Bond 2013).

CONDUCTION PATHWAYS AND EFFECTORS: SYNCYTIA AND FLAGELLA

Glass sponge syncytia provide uninterrupted conduits for electrical signalling. While it is quite certain some sponges lack nerves, the glass sponge syncytium functions analogously. About 75% of the glass sponge body consists of a single syncytial tissue that penetrates all parts of the sponge (Leys 1995, 1999). The few cellular regions are also joined to the syncytium by a continuous ‘cell’ membrane, but remain separated only by a protein plug in a narrow junction, which offers no impedance to electrical impulses (Mackie and Singla 1983). A stimulus to any part of the syncytium propagates via calcium channels at a rate of 0.17–0.3 cm/s throughout the syncytium and causes the flagellated pump units to stop beating (Leys et al. 1999). The 5 s-long action potential is slightly retarded by a 25–75% reduction in sodium concentration, but is completely blocked by the divalent ions Co²⁺ and Mn²⁺, and by the potassium channel blocker TEA (Leys et al. 1999). It is thought that calcium is the principal ion involved and that the slow rate of propagation is due to slow restoration of the membrane potential and or low channel abundance. The action potential is also sensitive to temperature with a Q10 of 3 (Leys and Meech 2006),
that sense changes in tension. This must be the case in Hexactinellida, which respond to sharp jabs and to clogging of the filtration system by arresting the flagella pumps (Lawn et al. 1981, Leys et al. 1999). In cellular sponges, a fish bite or clogging of the sponge filter by a sudden increase in particulate concentration may affect stretch sensitive channels, triggering the contraction responses noted above. McNair’s (1923) early work with freshwater sponges showed that the osculum contracted rapidly if pin pricks were applied to the top or to the bottom of the osculum chimney (Fig. 3.2A).

(2) Sensory cilia. Often cilia and flagella are considered identical organelles, but in sponges the different modes of function of flagella on choanocytes and of motile and non-motile cilia on epithelia reflect hidden ultrastructural differences (Linck 1973). In contrast to the flagella pumps, cells at the exit

Fig. 3.2. Sensory systems in adult and larval sponges. A: McNair’s 1923 drawings of the response of the osculum of *Ephydatia* to touch, showing presumed contraction of sphincter-like bands (a,b) equally along the osculum, (c,d) only at the tip of the osculum, and (e,f) in two places along the osculum and at its base (modified from McNair 1923, with kind permission of the Marine Biological Laboratory, Woods Hole). B, C: Primary cilia in the osculum of *Ephydatia muelleri*, shown in (B) thin section transmission electron microscopy (TEM), and (C) scanning electron microscopy (SEM). D: Relaxed and contracted *Tetysa wilhelma* (from Ellwanger et al. 2007, with kind permission by Springer). E: Primary cilium from the osculum of *Tetysa wilhelma* (from Nickel 2010, with kind permission by John Wiley and Sons). F: The ciliary response to a rapid increase (I) and decrease (II) in light intensity in a larva from *Amphimedon queenslandica* (from Leys et al. 2002, with kind permission by Springer). H: Cross-section through the posterior pole of the larva of *Amphimedon queenslandica* (TEM) (inset shows an SEM of the posterior pole of the larva). I,J: Cytoplasmic bridges connect pigment-filled projections at the posterior pole of the larva of *Sigmadocia caerulea* (TEM). The presence of microtubules stabilizing the bridges is visible in panel J (from Maldonado et al. 2003, with kind permission by Springer).
of choanocyte chambers in many sponges have cilia, which beat in a single direction with a single recovery stroke (SPL personal observation), as do most metazoan cilia. In addition, non-motile cilia occur in discrete locations in the aqueiferous system. Careful study of specimens, preserved and dissected so as to open their canal system to viewing by scanning electron microscopy, shows that there are non-motile cilia 4–6 µm long here and there along the aqueiferous system (Ludeman 2010) (Fig. 3.2B,C). The inner lining of the osculum in all demosponges studied so far possesses cilia (Nickel 2001, Ludeman et al. 2014) (Fig. 3.2D,E)—even glass sponge oscula have 6µm-long cilia (Ludeman et al. 2014). Experimental work now shows that the oscula cilia may function as sensory hair cells. These cilia label with dyes used to label ion channels in primary cilia, and chemicals used to block sensation by hair cells or lateral line cilia in fish, and also block propagated contractions that are usually easily triggered by mechanical stimuli or by L-glutamate (Ludeman et al. 2014). The defined location and specific function of the ciliary epithelium suggest that it is a region that receives signals from the environment and transmits them to the animal, and so in essence it functions as a sensory organ.

3) ‘Photosensory’ organelles. Almost all sponge larvae are covered with cilia, which beat unidirectionally and constantly to move the larva forward or prevent it from sinking. These ‘swimming cilia’ are approximately 20 µm long. In some sponge species, towards the posterior swimming pole of the larva, cilia vary in length from extremely short to those that are ten times the length of ‘swimming cilia’ (Leys and Degnan 2001, Collin et al. 2010). The ‘pole’ cilia are also responsive to changes in light. In some larvae, when light intensity is suddenly increased they straighten without beating, and when light intensity decreases they bend over the posterior pole (Leys and Degnan 2001). In other larvae the pole cilia do the reverse, bending under high light and straightening when light dims (Collin et al. 2010) (Fig. 3.2F,G). The cells from which long cilia arise have unusual bulbous extensions of the cell surface filled with pigment granules. It seems that these are well positioned to block light to the lower portion of the cell (Fig. 3.2H). Maldonado et al. (2003) showed that in some larvae cytoplasmic bridges connected neighbouring cytoplasmic extensions, raising the possibility that there might exist some need for rapid (electrical) signalling around the ciliary ring (Fig. 3.2I,J). Sponge larvae respond not only to light but also to gravity (Warburton 1966) and chemicals (Jackson et al. 2002), so other roles for cilia on the surface of sponges cannot be ruled out.

NEURO-MOLECULES AND NEUROTRANSMITTERS

Sponges may not have nerves but they do possess genes that suggest they either have the potential to carry out synaptic-like signalling or carry it out in an unconventional way. This would not be unexpected in light of the behavioural repertoire listed above. A near classic post-synaptic density (PSD) scaffold could be present in sponges based on what is present in their genomes and transcriptomes (Sakaraya et al. 2007, Alie and Manuel 2010)—even rapid acting channel receptors (ionotropic glutamate receptors or iGluRs) were found in three sponge transcriptomes (Riesgo et al. 2014). For synaptic signalling one would expect to see clusters of vesicles at the sites of contact of two cells. Several good examples of vesicle exchange between cells were shown by Pavans de Ceccatty et al. (1970) in a survey of cell–cell connections in demosponges, but so far a clear example of a PSD structure has yet to be found in a sponge (Sakaraya et al. 2007, Alie and Manuel 2010).

There is some evidence that sponges use molecules associated with synaptic transmission, though many of these studies are difficult to assess. Early histochemical work by Lentz (1966) on mesohyl cells in the osculum of Sycon showed serotonin (5HT), epinephrine (Epi), and norepinephrine (NE) staining in vesicles situated throughout the cytoplasm and near the cell membrane (Fig. 3.3A,B). Acetylcholinesterase activity was also demonstrated in cells near the osculum using a thiolacetic acid and lead nitrate assay, although these vesicles were located in the perinuclear area rather than at the cell membrane (Fig. 3, Lentz 1966). Recent analysis of several sponge transcriptomes has identified enzymes that synthesize these molecules including 5HT (e.g. tryptophan hydroxylase) (Riesgo et al. 2014), albeit none have been found so far in Sycon, the genus in which Lentz’s work was carried out.

Transmission electron micrographs (EM) showing exchange of vesicles between cells strongly suggest that sponges do have vesicle-based transmitter signalling (Pavans de Ceccatty et al. 1970) (Fig. 3.3C,D). More recent work has used immunohistochemical methods, which give staining patterns that are more diffuse, with labels seen generally throughout many cells (e.g. Ramoino et al. 2007). While antibodies are a promising technique for exploring ultrastructure, because sponge proteins are highly divergent from even their closest metazoan relatives, the use of antibodies raised against non-sponge antigens suffers from lack of cross-reactivity, so that in the absence of rigorous controls the results are difficult to interpret. Antibodies raised against sponge-specific epitopes and coupled to EM imaging (immunogold) in regions of cell–cell contact, such as those shown by de Ceccatty (Fig. 3C–E), promise to be informative, and controls, such as those carried out by Lentz (1966) by depleting 5HT, Epi, and NE, will be essential.

Reports of other neurotransmitter molecules in sponges have come from physiological experiments where behaviours such as contractions and alteration of pumping rate have been monitored (e.g. Emson 1966, Ellwanger and Nickel 2006). One question that emerges from these findings is why sponges would require signalling components typically associated with fast signalling at synapses, since few sponge responses are rapid. The simplest reason would be that molecules are involved in vesicle-based endo- and exocytosis, such as occurs during feeding, as explored by Ramoino et al. (2011). But it is also likely that contractions of the whole sponge body in response to irritants in the water would require coordination of all canals and therefore a signal that would travel across all epithelia within seconds to minutes.
Experiments to test for immunoreactivity of regions around the osculum to custom antibodies to these receptors and molecules are needed.

**EVOLUTIONARY CONSIDERATIONS OF NEUROGENESIS**

The expression of genes involved in neural development in sponges, which do not have neurons, is intriguing. Probably the most interesting of these is bHLH, which has been shown to convert cells of a non-neuronal fate to a neuronal phenotype; but bHLH also has roles in muscle development (and mesodermal cell differentiation), hematopoiesis, and skeleton development (Simionato et al. 2007). A sponge bHLH, AmqbHLH1, was shown to have neurogenin-like activity in *Xenopus* and proneural activity in *Drosophila*, where it was able to induce the formation of more sensory bristles than in wild types (Richards et al. 2008). In the *Amphimedon* demosponge embryo, bHLH
genes are expressed in cells populating an unusual ‘middle layer’ of cells which differentiate into ciliated swimming cells, flask-shaped cells, and cells that contain mucous inclusions, all of which populate the outer epithelium of the swimming larva. Notch Delta expression was also studied in the homoscleromorph sponge Oscarella lobularis and found to largely occur in choanocytes, the pump cells of the feeding chambers (Gazave et al. 2008). It is not possible to draw inferences about neurogenic cell lineages from these data without additional characterization of the signalling properties of the cells that these genes go on to define. This sort of work has not yet been done, but is likely to yield exciting data, especially if coupled with studies following the histogenesis of cells that form oscula and sensory structures in the sponge.

In the absence of a link between neurogenic genes and functional sensory systems in sponges, we can imagine that, as with the PSD scaffold, neurogenic proteins may be involved in developing some aspect of the sponge that is not specifically involved with coordination (beyond the usual need for each cell to signal to its neighbours), which could have been co-opted to construct more rigid designs for signalling in later evolving animals. Immunogold-EM studies with custom antibodies to sponge proteins will help clarify some of the PSD proteins’ roles in sponges.

There are other genes such as the axon guidance molecules (AGMs), however, that must play alternative roles in sponges because clearly neural patterning and synapse formation are not required. Homologues of plexinA1, semaphorin3b, and EphB1 are AGMs expressed in A. queenslandica larvae (Conaco et al. 2012). AGMs create permissive and repulsive zones for migrating cells and processes during development, and are known to contribute to the formation of the vertebrate vascular system, itself an elaborate set of interconnected tubes (Carmeliet and Tessier-Lavigne 2005). It may be that AGMs participate in the patterning and formation of the aquiferous canals in sponges by restricting the ways in which the canal system can develop.

Why do sponges have a large set of genes typically associated with nervous systems? If ctenophores are basal even to sponges, as recently suggested (Ryan et al. 2013), then sponges may be secondarily simplified having lost a nervous system. The residual components may have been used in a simpler, non-neural signalling system that is well suited to the sponge’s physiological needs, and is less energetically costly. Alternatively, the signalling genes possessed by sponges may be used in a more conventional way, and these ‘signalling units’ have been co-opted into the later evolving nervous systems. A third framework, not entirely separate from the former, is that sponges use ‘neural’ components in an unconventional manner. It may be that some sponge cells are coupled through gap junctions which allow electrical signalling through epithelia, and this system is linked to mesohyl cells via slower vesicle-based signalling, as is suggested by some cell ultrastructure work. This would serve to effectively couple electrical and chemical signalling, but at speeds far slower than seen in typical electrically coupled synapses, and more consistent with the speed of behaviours observed in sponges. Unconventional use of neural molecules may also point to convergent mechanisms, though without a more complete characterization of the pathways this remains an open question. Sponges have no nervous system, yet appear to respond to some neurotransmitters and encode genes used to build and maintain nervous systems. How these genes shape the morphology and physiology of the sponge remains an active area of research.

REFERENCES


