Chapter 27
Vision Made Easy: Cubozoans Can Advance Our Understanding of Systems-Level Visual Information Processing

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Abstract Animals relying on vision as their main sensory modality reserve a large part of their central nervous system to appropriately navigate their environment. In general, neural involvement correlates to the complexity of the visual system and behavioural repertoire. In humans, one third of the available neural capacity supports our single-chambered general-purpose eyes, whereas animals with less elaborate visual systems need less computational power, and generally have smaller brains, and thereby lack in visual behaviour. As a consequence, both traditional model animals (mice, zebrafish, and flies) and more experimentally tractable animals (*Hydra*, *Planaria*, and *C. elegans*) cannot contribute to our understanding of systems-level visual information processing—a Goldilocks case of too big and too small.

However, one animal, the box jellyfish *Tripedalia cystophora*, possesses a rather complex visual system, displays multiple visual behaviours, yet processes visual information by means of a relatively simple central nervous system. This—just right—model system could not only provide information on how visual stimuli are processed through distinct combinations of neural circuitry but also provide a processing algorithm for extracting specific information from a complex visual scene.

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27.1 Introduction

Choosing the right model system is a multifaceted Goldilocks conundrum: it has to be complex enough to answer the questions at hand, and putative future questions, yet simple enough for the resident researcher to understand. We propose that the cubozoan jellyfish *Tripedalia cystophora* is emerging as a close to ideal model to understand visual information processing in detail and with cellular resolution (Fig. 27.1). This animal possesses camera-type eyes structurally similar to vertebrate eyes and has multiple distinct visual behaviours and an experimentally tractable central nervous system (CNS).

So far, our understanding of visual information processing by interconnected neurons in the brain has been hampered by challenges inherent to the model systems currently available—too large and complex to understand in detail. Mice are the preferred model animals of medical and pharmaceutical research, and they admittedly possess a number of advantages, not least the evolutionary proximity to humans that allows for a direct comparison. Unfortunately, being a mammal, mice have huge brains comprising approximately 75 million neurons, interconnected by 1 billion synapses. In the cortex alone, mice have about 4 million neurons (Oh et al. 2014). But the greatest challenge is that the myelinated neurons render the brain opaque and that limits optical penetration to a few 100 μm. So even though the past two decades have given us wonderful advances in fluorescent tools and microscopy techniques, enabling simultaneous observation of large populations of neurons, our understanding of neuronal response to visual stimuli is largely limited to the outermost layer of the visual cortex.

Other conventional models have more tractable nervous systems, but even the brain of a 6-day-old zebrafish larvae comprises about 100,000 neurons (the adult has 10 million), and fruit flies have 250,000 brain neurons. While these systems seem more attractive, they are still too large to retrieve specific neuro-neuronal responses to a particular visual stimulus.

The much simpler model systems (*C. elegans*, *Planaria*, and *Hydra*) can be disregarded for the lack of image-forming eyes and distinct visual behaviour, and therefore they cannot help us understand biological image analysis.

In contrast, the cubozoan system offers a unique opportunity to provide answers to how complex image analysis could possibly be conducted by interconnected neurons, and with the small transparent CNS of *Tripedalia cystophora*, we can monitor an entire working CNS in response to specific visual stimuli. *T. cystophora* comes with its own set of challenges, though. The animals are highly sensitive to variations in osmolarity, temperature, oxygen tension, and water composition. But in our opinion, the possible gain in understanding greatly surpasses the disadvantages of working with this type of fragile marine organism, and hopefully the scientific community will soon realise the huge potential of these stunning mangrove-dwelling animals.

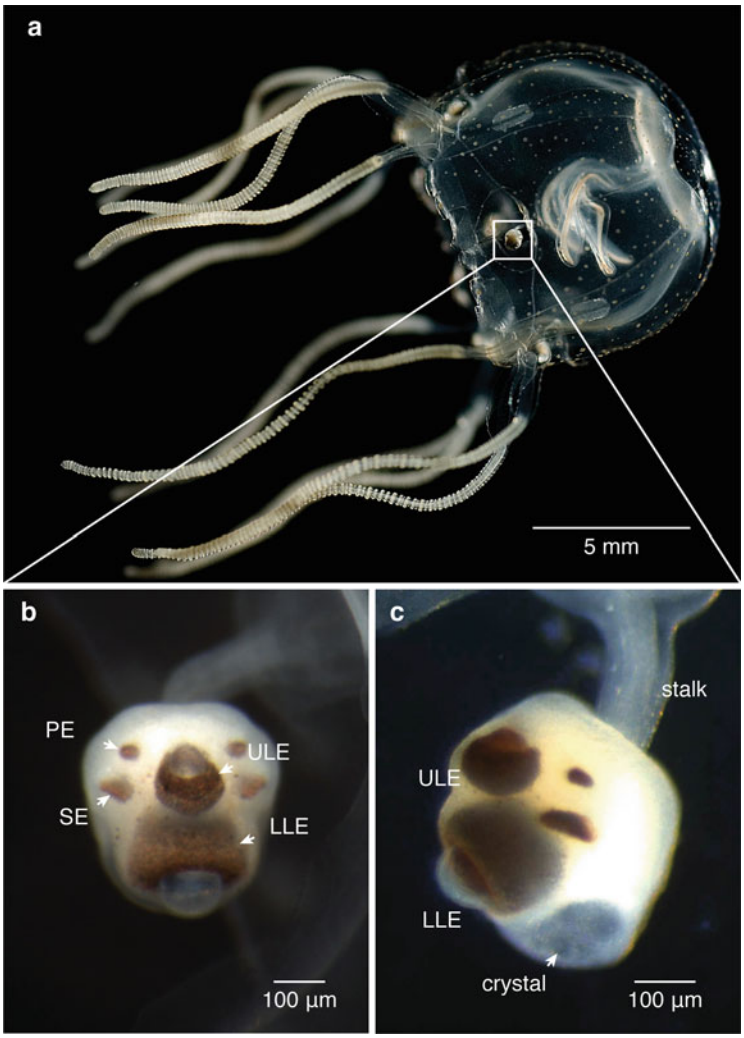

Fig. 27.1 Visual ecology of the box jellyfish *Tripedalia cystophora*. Box jellyfish are agile swimmers and predominantly guided by visual input. A sensory structure (rhopalium) is located on each side of the medusoid bell; (**a**) each of the rhopalia carries six eyes of four morphological types (*PE* pit eyes, *SE* slit eyes, *ULE* upper lens eye, and *LLE* lower lens eye), two of which are similar in structure to vertebrate eyes, (**b**) (front view) and (**c**) (side view). The transparent rhopalium also contains the processing neural network (CNS) of the animal. Merely 1000 processing neurons are available for analysing the visual information received by the eyes and relay motor signals to the bell musculature, yet *T. cystophora* display several robust visual behaviours (see Fig. 27.7). (**a**) and (**c**) modified after Bielecki et al. (2013b)

27.2 External Environment

The present account will attempt to elucidate the neural involvement in image analysis based on principles from neuroethology (Fig. 27.2) by guiding the reader through the neuronal processes from the environmental cues to the behavioural output. Neuroethology is the evolutionary and comparative approach to the study of animal behaviour and the underlying mechanistic control by the nervous system. This implies correlating sensory cues from the habitat to specific behavioural responses performed by the animal. *T. cystophora* is thought to possess chemo- and mechanoreceptors (Skogh et al. 2006), but vision is putatively the predominant behavioural modulator in box jellyfish. We must therefore specifically evaluate the visual environment of *T. cystophora*.

T. cystophora inhabits mangrove swamps in the Caribbean, where it can be found between the prop roots of *Rhizophora mangle* trees. The jellyfish must navigate the maze of roots to avoid collision thereby damaging their bell. The epidermis of the bell is merely one cell layer thick, and abrasion could prove fatal due to bacterial infection. Intuitively it would be wiser for *T. cystophora* to avoid the mangrove altogether, but sunlight penetrates the foliage and between the roots to create light shafts, where positive phototactic copepods congregate in high densities, and it is of course in the interest of *T. cystophora* to position itself here to prey on the abundant crustaceans. In congruity with the described habitat, *T. cystophora* performs at least four distinct, different, and visually modulated behaviours: obstacle avoidance

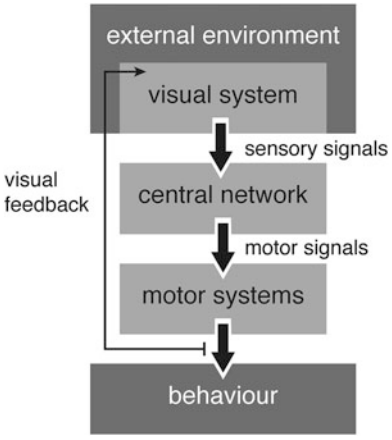

Fig. 27.2 Principles of visual neuroethology. The specific task of a **visual system** is to evaluate electromagnetic radiation with wavelengths in the detectable spectrum from the **external environment**. And subsequently transform the radiation into **sensory signals**, which can be processed by the neurons in the **central network** (CNS). Relevant sensory information will generate **motor signals** that are relayed to the effectors of the **motor system** and thereby regulate the **behaviour** of the animal. Own movements cause a significant disturbance of the recorded image in a visual system and must be considered. A **visual feedback** loop ensures that motor system-generated inconsistencies can be adjusted for or, in some cases, actively utilised by the visual system

(Garm et al. 2007c, 2013), prey location (Buskey 2003), long-distance navigation (Garm et al. 2011), and diurnal activity (Garm et al. 2012). The first three are the best studied and therefore the ones considered here.

27.3 Box Jellyfish Visual Ecology

Vision is based on the ability to perceive electromagnetic radiation or photons within a range of detectable wavelengths, which is transformed into neuronal relevant electrical signals in the photoreceptors. Two main classes of photoreceptors in image-forming eyes are found throughout the Metazoa: ciliated and rhabdomeric. The classification is based on the membrane folding strategy of the outer segment, the photoabsorption part of the photoreceptors. Membrane folding greatly expands the light-sensitive area making the photoreceptor more sensitive to light. In rhabdomeric photoreceptors, the cell membrane itself is extended by microvilli, whereas the ciliary receptors have a cilium extending from the cell, and the membrane of this cilium is then extended in microvilli (Land and Nilsson 2012). Both types of photoreceptors can be found throughout the Metazoa, and, contrary to the majority of invertebrates, vision in cubomedusae is mediated by ciliated photoreceptors.

27.3.1 Visual System Morphology

Within Cnidarians, cubozoans have the most elaborate visual system comprising 24 eyes located on 4 sensory structures, called rhopalia. In addition to four smaller eyes (two pit eyes and two slit eyes), each rhopalium carries two camera-type lens eyes, morphologically similar to vertebrate eyes (Fig. 27.1b and c). Among the four rhopalia, the visual field of cubozoans spans a complete sphere around the animal, but it is clearly divided between the upper lens eyes (ULE), directed upwards with a visual field of just less than 100°, and the lower lens eyes (LLE), which are directed downwards into the water with a visual field of 170° (Fig. 27.3). The smaller eyes share large parts of the visual field with the lens eyes; the pit eyes are directed upwards and the slit eyes into the water. Their function is not yet understood and therefore not considered here (cf. Garm and Bielecki 2008). The division of the visual field between the eyes is accomplished by the unique morphology of the rhopalium. It is suspended from the bell by a stalk and weighted by a heavy calcium sulphate crystal at its distal end, which ensures a constant vertical orientation of the rhopalium regardless of the orientation of the animal (Fig. 27.3; Garm et al. 2011).

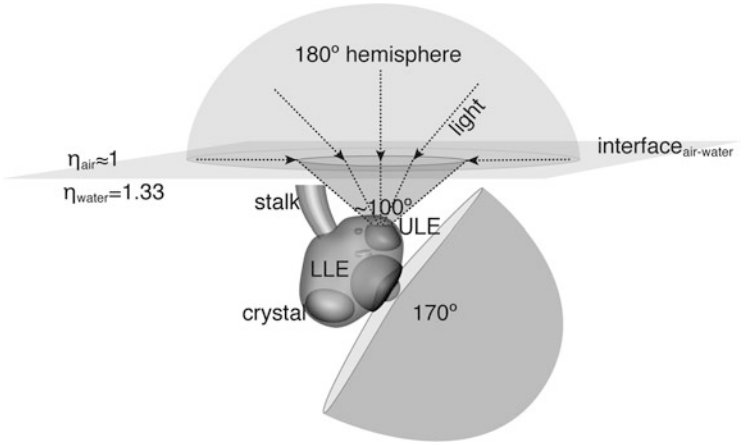

Fig. 27.3 Visual fields of the lens eyes. The rhopalium is suspended from the side of the bell by a flexible **stalk** and weighed down by a heavy **crystal** at the distal end. This morphological specialisation ensures a constant orientation of the rhopalium regardless of the orientation of the animal itself. This also ensures a strict division of the visual fields between the upper and lower lens eye. The upper lens eye (*ULE*) peers upwards, observing terrestrial cues through Snell's window; the lower lens eye (*LLE*) is directed into the water below the animal. Snell's window compresses the **180**° **hemisphere** above water into a 97° cone under water (*dashed lines*). The visual fields of the upper and lower lens eyes are **100**° and **170**°, respectively. The refractive indices (**η**) of air and water are indicated on the **air–water interface**

27.3.2 Optics

The quality of a visual system is determined by the sensitivity to light and the spatio-temporal resolution of the images produced. Usually the two are at an inverse relationship, but the evolution of lenses dramatically improved sensitivity by focusing the light onto the retinal photoreceptors. The acuity of the retinal image is determined by the number of photoreceptors and the angular width of their receptive field—smaller angle, higher acuity. The upper and lower lens eyes have nearly spherical lenses with graded refractive indices, which in the case of the upper lens eye can produce near aberration-free images (Nilsson et al. 2005). The lower lens eye has less than perfect optics, but the lenses of both eyes are capable of producing receptive fields of less than 1°. However, the retina in both the ULE and LLE is displaced in regard to the focal plane of the lenses causing a severe under-focus (Fig. 27.4). This is thought to eliminate high spatial frequencies (small details) from the retinal image, which diminish the needed processing capacity significantly. The displaced retina decreases the spatial resolution of the eyes to 10–20°, depending on the location of the photoreceptors in the retina, restricting the animals to see large objects in their field of view and not, e.g. their copepod prey (Nilsson et al. 2005). It has been suggested that box jellyfish uses matched filters to optimise the processing capacity and increase the signal-to-noise ratio in their sensory systems (Wehner 1987; Bielecki et al. 2013a). Matched filters are used to extract a known signal,

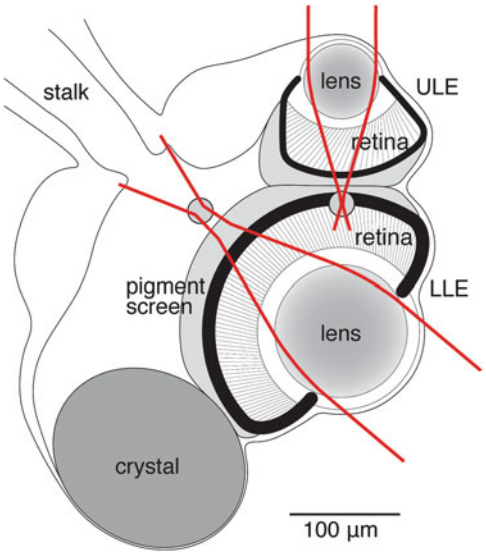

Fig. 27.4 Retinal displacement in the lens eyes of *Tripedalia cystophora*. Sagittal transection of the rhopalium reveals the components of the upper and lower lens eyes (*ULE* and *LLE*), respectively. The **retinas** are shielded from false light by a **pigment screen**. The spherical **lenses** of the upper and lower lens eyes have graded refractive indices and are capable of producing near aberration-free images. However, the retinas are displaced in relation to the focal point (in *light grey circles*) of the incident light (*red traces*). This displacement results in a much wider acceptance angle of the individual photoreceptors—10–20° dependent on the location on the retina. Modified after Nilsson et al. (2005)

whenever present, from an unknown signal. By disregarding redundant sensory information, the nervous system can process the presence and absence of the known signal rather than evaluate the entire unknown signal. This way the box jellyfish visual system can extract specific information from a complex visual scene with the limited neural capacity available to the animal (Wehner 1987; Bielecki et al. 2013a).

The iris of the LLE can contract in response to changing light intensities. This regulates the intensity of incident light in the LLE. In contrast, the iris of the ULE does not contract (Nilsson et al. 2005). The functional significance of regulating incident light intensities in the LLE but not in the ULE is not fully understood.

27.3.3 Opsins

Light has four characteristics: colour, intensity, direction, and polarity. The wavelength determines the perceived colour, and the amount of photons received within a given time defines the intensity. The visual neuroethology is of course based on what

the animal actually sees, and the signal transmission starts with the photoreceptive opsin located in the retinal photoreceptors. Opsins are transmembrane receptors binding an 11-*cis*-retinal molecule that changes configuration to all-*trans*-retinal in response to a photon, or quanta of photons, inducing an electrical response in the cell, and opsins are in this way light sensitive. Ciliary opsins (c-opsins) in general signal through a G protein and cyclic nucleotide (cNMP) pathway to hyperpolarise the photoreceptor membrane (Nilsson 2004; Kozmik et al. 2008; Koyanagi et al. 2008). Opsins have been categorised into eight groups by the amino acid sequence, which also corresponds to the type of G protein involved in the signal transduction pathway (Terakita 2005; Plachetzki et al. 2007; Suga et al. 2008), and one type of c-opsin, cnid-ops, was found exclusively in cnidarians (Plachetzki et al. 2007). The opsins expressed in box jellyfish lens eyes were demonstrated to utilise a G_s-cAMP phototransduction cascade (Koyanagi et al. 2008).

Opsin photon catch makes photoreceptors light sensitive, but additionally, opsins have peak responses to a given colour and are thereby tuned to a narrow range of wavelengths. In *T. cystophora* lens eyes, opsin responses peak in the blue-green spectrum (approximately 510 nm) and then progressively diminish to wavelengths diverging from this optimum (Garm et al. 2007a; Fig. 27.5). Single opsins expressed in the lens eyes, common among all the examined cubozoans, therefore suggest that the cubozoans are colour-blind and have peak sensitivity in the blue or blue-green spectrum of visible light (Garm et al. 2007a; Koyanagi et al. 2008). Curiously, Kozmik et al. (2008) found a blue 470 nm opsin in the *T. cystophora* rhopalial transcriptome and, by immunohistochemical staining, placed it in the retinas of the lens eyes. Since the electrophysiological evidence discourages dichromatic systems in the cubozoans (Garm et al. 2007a), these results are seemingly contradictory. However, we found the 470 nm opsin to be an extraocular opsin expressed in the neuronal mass of cells that comprise the neuropil of the rhopalium (Bielecki et al. 2014), where it presumably is involved in controlling the diurnal activity pattern (Garm et al. 2012).

27.3.4 Spatio-Temporal Properties

About 400 and 600 photoreceptors make up the retinas of the *T. cystophora* upper and lower lens eyes, respectively. The spatio-temporal resolution of the lens eyes has been established (Nilsson et al. 2005; Coates et al. 2006; Garm et al. 2007a; O'Connor et al. 2010). The temporal resolution of the lens eye receptors has been investigated both indirectly (half width and time to peak of the response) and directly (flicker fusion frequency—fff) from ERG recordings (Garm and Ekström 2010). The receptors were found to have minimum half widths of approximately 30 ms from the lens eyes (Garm et al. 2007a) and flicker fusion frequencies of 10 and 8 Hz for the upper and lower lens eyes in *T. cystophora*, respectively (O'Connor et al. 2010).

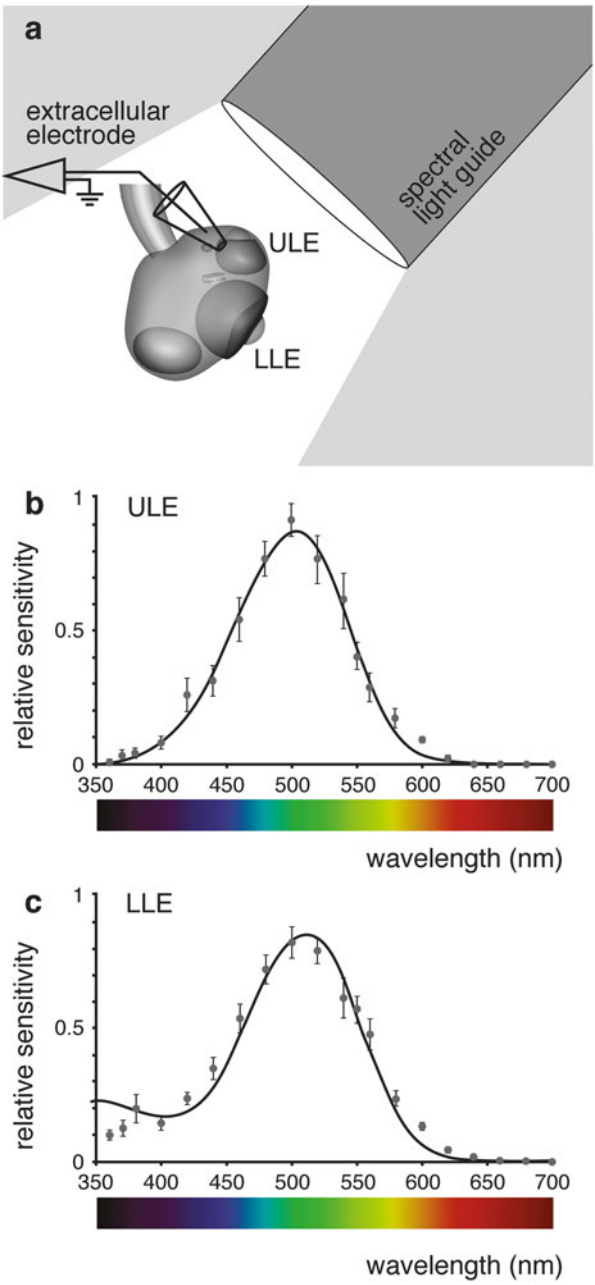

Fig. 27.5 Spectral sensitivity of the *Tripedalia cystophora* lens eyes. ERGs from the lens eyes (**extracellular electrode**) when exposed to narrow bandwidth light throughout the visible spectrum (350–700 nm) (**spectral light guide**) revealed that the box jellyfish have monochromatic vision with peak sensitivity in the blue-green spectrum, approximately 510 nm (506 nm for the upper lens eye (**b**, *ULE*) and 508 for the lower lens eye (**c**, *LLE*)). The average spectral sensitivity of each measured wavelength (grey circles ± SEM) in (**b**) and (**c**) was fitted to the absorption curve of single-opsin models (Govardovskii et al. 2000). (**b**) and (**c**) modified after Garm et al. (2007a)

27.3.5 Directional Vision

In most image-forming eyes, the photoreceptors are shielded by pigments to avoid light entering the eye from undesired directions. In mammalian eyes, these pigment screens are formed by specialised pigment cells encasing the photoreceptors in a dark orb. This adds architectural strain on the mammalian eye since all supportive cells (bipolar, amacrine, and ganglion cells) must also be located within the pigment screen, and the retina is therefore considered inverted. As a result light has to penetrate supportive cells and the cell bodies of the photoreceptors to strike the outer segment membranes. In cubozoan retinas, pigment granules are located within the photoreceptors themselves (Yamasu and Yoshida 1976; Bielecki et al. 2014), the retina of *T. cystophora* lens eyes is everted, and light strikes the outer segments directly. The photoreceptors articulate directly on putative second-order neurons, presumably the retinal-associated neurons described by Skogh et al. (2006).

27.4 Central Network

In model animals with large complex nervous systems, it is not only the sheer size and vast amount of synaptic connections that is an obstacle to understand neuronal image analysis in detail. Recent advances into the plasticity of complex nervous systems show that astrocytes modulate neuronal response and performance (Takano et al. 2007; Iliff et al. 2012; De Pittà et al. 2012; Volterra et al. 2014) and therefore play a significant role in nervous system plasticity. Needless to say, if the goal is to understand direct neuro-neuronal activity in response to a specific visual stimulus, highly plastic systems pose a much greater challenge. Astrocytes have never been observed in the cubozoan nervous system, indicating very limited plasticity. In fact, both behavioural and electrophysiological responses to specific visual stimuli are robust and consistent between individual preparations (Garm et al. 2007c, 2011; Garm and Bielecki 2008; Garm and Mori 2009; Petie et al. 2011, 2013).

27.4.1 Rhopalial Nervous System

Retinal images must be analysed by the nervous system for an animal to utilise the visual information for behavioural guidance (Fig 27.2). In many animals, the computational network resides within a centrally located brain. The central nervous system of cubozoans comprises a ring nerve and the rhopalial nervous system (RNS), where the latter is thought to be the centre for visual information processing and the ring nerve is involved in inter-rhopalial communication (Garm et al. 2006, 2007b). Each rhopalium contains about 1000 neurons available for the sensory information processing (retinal-associated, flank, and giant neurons), which must

accommodate all sensory systems (in addition to the eyes, the rhopalial system also includes a number of putative chemo- and mechanoreceptors (Skogh et al. 2006)). These three types were classified based on morphological characteristics and are located in different areas of the rhopalium. A large part of the volume of the rhopalium between the epidermis and gastrodermis is filled with a neuropil made up of neurites. Large numbers of bi- and unidirectional synapses have been found here using ultrastructural techniques, but due to the complex 3D structure, it is not possible to follow the neurites at any considerable length (Skogh et al. 2006). The neural architecture, or connectome, has not yet been described in detail, but ultrastructural and immunohistochemical studies have hinted at the morphology and physiology (Garm et al. 2006; Skogh et al. 2006; Gray et al. 2009; Parkefelt and Ekström 2009). Electrical synapses have never been associated with the cubozoan nervous system (Mackie et al. 1984), but chemical synapses are plentiful (Anderson and Grünert 1988; Skogh et al. 2006; Gray et al. 2009). This suggests that signal transduction and processing involve chemical synapses, and we made an effort to identify the involved neuroactive substances in visual processing of *T. cystophora*. We found that FMRFamide, serotonin, and dopamine all had inhibitory effect on the pacemaker signal frequency (Bielecki et al. 2013b), which is consistent with earlier behavioural and immunohistochemical studies (Plickert and Schneider 2004; Kass-Simon and Pierobon 2007; Parkefelt and Ekström 2009). Comparing our findings to the morphological data led us to suggest that putative second-order neurons and computational units use FMRFamide as transmitter, serotonin is involved at the retinal level, and this neuropil relies on dopamine to control the diurnal rhythm of the animal (Bielecki et al. 2013b).

27.4.2 Central Pattern Generators

The processing of sensory information, or "decision-making" (cf. Schall (2001)), is necessary to relay the correct motor signals to the musculature for the animal to appropriately navigate the immediate environment (Fig. 27.2; Briggman et al. 2005; Ijspeert 2008; Satterlie 2011). One of the major behavioural control systems in cubozoans is the swim pacemakers, which set the swim pace of the medusa (Satterlie 1979, 2002). Pacemakers are cells that produce a repetitive rhythmic output, and they control a wide variety of behavioural activities across the Metazoa, such as breathing, chewing, digestion, and locomotion (Delcomyn 1980; Ijspeert 2008). The pacemaker activity can be modified by both intrinsic and extrinsic neuromodulators (Morgan et al. 2000; Marder et al. 2005; Nusbaum and Blitz 2012). In the cubomedusae, pacemaker cells are located in the rhopalia, and the pacemaker signals control the discrete swim contractions in a one-to-one manner (Satterlie 1979). There is evidence for age-related pacemaker signal frequency in cubomedusae (Shorten et al. 2005), but the base frequency in the adult *T. cystophora* is approximately 1 Hz, corresponding to leisurely swimming activity by which the animal maintains the position in the water column and searches for food sources.

Pacemaker responses diverge dependent on whether a visual stimulus is presented to the entire rhopalium or the lens eyes individually. In the context of the visual environment of *T. cystophora*, a sharp change in light intensity presented to the entire rhopalium resembles a sudden change in ambient light intensity. Contrary, stimulating the eyes individually implies a specific and directional change in the visual environment of the animal. A light-on stimulus presented to an entire rhopalium produces a distinct arrest of swim pacemaker signals for about 10–20 s, after which the signals return to the base frequency of approximately 1 Hz. Conversely, a light-off stimulus results in a steep and instantaneous increase in pacemaker frequency (Garm and Bielecki 2008). Similarly, a light-on stimulus decreases pacemaker signal frequency when presented to the lower lens eye, and light-off increases pacemaker signal frequency (Garm and Mori 2009). Contrary, stimulating the upper lens eye causes the opposite response: instantaneous increased signal frequency to light-on and decreased to light-off stimulus (Garm and Mori 2009). Additionally, the pit eyes and the neuropil are light sensitive and capable of modulating the pacemaker frequency. The pit eyes are located laterally on either side of the upper lens eye, also directed upwards, and with overlapping visual fields (Garm and Bielecki 2008). The neuropil is not directionally screened from incident light but expresses a 470 nm opsin, indicating extraocular light detection with peak sensitivity in the blue spectrum (Skogh et al. 2006; Bielecki et al. 2014). When the pit eye and neuropil are stimulated individually, they respond similar to the upper lens eye: increased pacemaker signal frequency in response to light-on stimulus and decreased frequency to light-off (Garm and Mori 2009). Curiously, both the pit eye and neuropil respond slower to the light-on stimulus than to the upper lens eye. They do not reach peak response until 10–20 s after the light-on stimulus and show a strong pacemaker signal inhibition in response to the light-off stimulus (Garm and Mori 2009). The extraocular opsin found in the neuropil has peak absorbance in the blue spectrum of visible light (470 nm). This is consistent with opsins controlling diurnal rhythm (Levy et al. 2007), and the neuropil could be the modulator of diurnal activity in *T. cystophora* (Garm et al. 2012). It would also explain the slower response time when stimulating the neuropil individually. The function of the pit eye is not yet understood (Garm and Bielecki 2008; Garm and Mori 2009).

The identical response patterns when stimulating the lower lens eye individually and the entire rhopalium indicate an override mechanism that favours the response in the lower lens eye over the other eyes of the rhopalium (Garm and Bielecki 2008; Garm and Mori 2009).

Even though their neural output is well described (Satterlie 1979; Garm and Bielecki 2008), the pacemaker cells themselves have not yet been positively identified. Excision experiments place them at the proximal part of the rhopalium near the insertion of the rhopalial stalk (Yatsu 1917), where Skogh et al. (2006) found approximately 30 giant neurons on each side of the rhopalium. These putative pacemakers have several afferent synapses indicating that a number of input channels articulate to one or a limited number of pacemaker cells producing a number of pacemaker subsystems (Skogh et al. 2006). These subsystems probably have neuromodulatory effect on each other to produce the complex neural output

necessary for each of the specialised behavioural tasks. The pacemaker cells subsequently transmit their complex signal to the ring nerve through the bidirectional epidermal stalk nerve (Fig. 27.6; Garm et al. 2006; Garm and Bielecki 2008). Only pacemaker signals elicit swim contractions, but a wide variety of signals can be recorded from the stalk. The pacemaker signal has a distinct different electrical signature compared to other more action potential-like afferent signals (Fig. 27.6; Bielecki et al. 2013b). Little is known about the action potential-like signals beyond

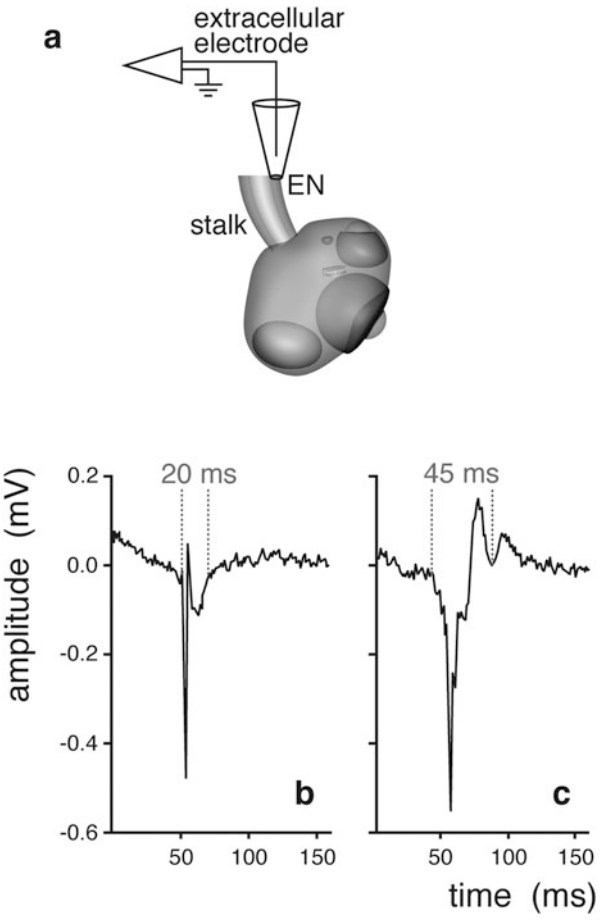

Fig. 27.6 Neuronal signals from the rhopalial nervous system. Various neuronal signals can be recorded from the epidermal stalk nerve (**a**, *EN*) by an **extracellular** suction **electrode**. Some signals resemble action potentials in duration and electrical signature (**b**), but the specific function remains uncertain. However, the more complex pacemaker signal (**c**) controls the bell contractions in a 1:1 manner and thereby the behaviour of the animal. The duration and electrical signature diverge significantly from action potentials, and where an action potential has a duration of approximately 20 ms and originates from one cell (**b**), the pacemaker signal has a duration of 45 ms and a profile indicating multiple cell origin (**c**). It is thereby possible to predict the behaviour of the animal in response to visual stimuli just by assessing the occurrence of pacemaker signals. Modified after Bielecki et al. (2013b)

the epidermal stalk nerve, and only the pacemaker signals will be discussed in this account (Petie et al. 2011).

27.4.3 Motor Systems

Once the visual information has been filtered, processed, and modulated to a neural output, it is finally transmitted to the effectors, which are normally the muscular system (Fig. 27.2). The ring nerve is located orally on the bell and is responsible for transmitting neural signals between rhopalia (Satterlie 1979; Garm et al. 2007b). The ring nerve directly innovates the velarium, which controls directional swimming, and connects to a nerve net that extends to cover the entire bell (Garm et al. 2007b). It is suggested that the motor signal is first transmitted to the ring nerve and then to the muscular tissue since numerous neurons extend from the ring nerve to the muscular tissue. Innervation of the velarial opening is especially prominent, but nerve net neurons from the bell also articulate on the ring nerve (Satterlie 2002). Excision experiments showed that even with a severed ring nerve, the animal could still perform swim contractions (Satterlie 1979); however, it must be assumed that the ring nerve aids in propagating the nervous signal and contributing to contraction synchrony since the nerve net neurons articulate on the ring nerve (Satterlie 2002). Such a system is known from the hydromedusa *Aglantha digitale*, where special action potentials propagate in the ring nerve which influence the synaptic transmission time and thereby allow for synchronous bell contractions (Mackie and Meech 1995a, 1995b).

In *T. cystophora*, rhopalial pacemaker signals produce a swim contraction in a 1:1 manner, but the animal possesses four rhopalia, and a higher level of motor signal control is necessary to prevent multiple inputs to the motor effectors simultaneously (Stöckl et al. 2011). Since the rhopalia are interconnected through the diffuse bipolar nerve net of the bell and the ring nerve (Satterlie 1979, 2011; Garm et al. 2007b), it has been suggested that the driving rhopalium inhibits the others by hyperpolarizing the pacemaker cells of subordinate rhopalia (Satterlie 1979; Stöckl et al. 2011). The role as driving rhopalium alternates and is determined by which rhopalial pacemaker is currently modulated by visual input (Satterlie 1979; Petie et al. 2011; Stöckl et al. 2011).

In accordance with earlier studies on the cubozoan nervous system (Plickert and Schneider 2004; Kass-Simon and Pierobon 2007), we could merely induce inhibition of the swim pacemaker system of *T. cystophora* by bath-applying neuroactive substances (Bielecki et al. 2013b). Thus, it is attractive to propose a rhopalial pacemaker system that comprises an intrinsic signal frequency of about 3.5 to 4 Hz, which is downregulated according to the sensory input to the given rhopalium and the neighbouring rhopalia. The suggested intrinsic frequency is based on recorded maximum frequencies and the fact that one bell contraction has duration of 200–250 ms (Petie et al. 2011; Bielecki et al. 2013a) making a higher frequency unlikely. Directional swimming is accomplished by asymmetrical swim contractions and directional constriction of the velarial opening to produce a jet of water that propels the animal in the right direction (Petie et al. 2011).

27.5 Integrative Approach to Behaviour

For an integrative approach, all activity of an animal must be viewed in this context and correlated to the restraints of the sensory habitat. In the case of *Tripedalia cystophora*, three behaviours justify these considerations and will be discussed here: long-distance navigation, light shaft detection, and obstacle avoidance. All these behaviours are integrated parts of the diurnal activity of the animal, and all are tractable for systems-level understanding of visual information processing (Fig. 27.7): robust, repeatable, and instantaneous in response to visual stimulation. In addition, *T. cystophora* displays diurnal activity. In response to diminishing ambient light, the activity of *T. cystophora* decreases, and the animal seeks the bottom of the creek where it presumably attaches to the sea grass (Fig. 27.7 position c) (Garm et al. 2012). When the light returns to the habitat, the activity of *T. cystophora* increases, and the animal returns to the daytime behavioural repertoire. However, the behavioural response to diminishing light is slow, possibly controlled by the extraocular opsin found in the neuropil (Garm et al. 2012; Bielecki et al. 2014), and cannot be used for visual information processing purposes.

27.5.1 Long-Distance Navigation

At sunrise, when the light conditions enable visual orientation, *T. cystophora* rise to the surface water layer of the mangrove/creek habitat (Fig. 27.7 position a). Since the murky water does not offer any directional cues, the animals are reliant on the ability to detect the mangrove canopy for long-distance navigation (Garm et al. 2011; Fig. 27.8). This behaviour is supported by the upper lens eye, which scans Snell's window for the contrast line between the bright open sky and the darker canopy (Garm et al. 2011; Bielecki et al. 2013a). Snell's window is a phenomenon produced by the difference in refractive indices of air and water causing the visual input from the hemisphere above water to compress into a cone of about 97° under water (Fig. 27.3). The mangrove creeks are affected by tidal currents that could carry animals into the middle of the creek and onwards to the open sea. If this happens, the animal will be unable to find food and, since all the other animals of the species are located under the mangrove canopy, will be far in between reproductive opportunities. This way long-distance navigation behaviour serves the animal to remain within the habitat and to avoid starvation.

An additional challenge to the long-distance behaviour comes from the fact that all known photoreceptor cells adapt to constant light stimulus (Matthews et al. 1988; Fain et al. 2001), and without countermeasures an animal would be unable to detect stationary objects in its visual field (Land 1999). To avoid this photoadaptation, a strategy must be integrated into any visual system that refreshes the retinal image congruent to the spatio-temporal resolution. In humans and primates, a series of muscular-controlled fixational eye movements (microsaccades, ocular drift, and

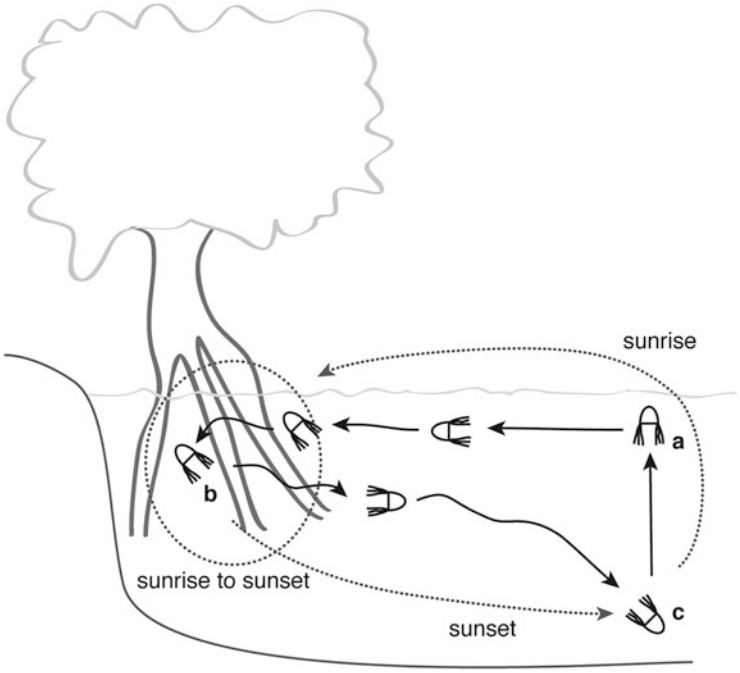

Fig. 27.7 Behaviour of *Tripedalia cystophora*. The mangrove-dwelling box jellyfish display several robust visual behaviours: long-distance navigation, light shaft detection, obstacle avoidance, and diurnal activity. At sunrise the animal rises to the surface (position **a**), scans Snell's window (see Fig. 27.3) for the contrast line between the canopy and the open sky, and swims under the canopy for the duration of the day (**sunrise** dashed line, see Fig 27.8). During the day *T. cystophora* passively hunts copepods in light shafts created by sunlight penetrating the foliage of the mangrove trees (position **b**, see Fig. 27.9), all the while avoiding contact with the prop roots penetrating their marine habitat (**sunrise to sunset** dashed circle, see Fig. 27.10). At sunset when light conditions no longer support visual behaviour, the activity of *T. cystophora* significantly diminishes, and the animal seeks to the bottom of the creek (position **c**) where it attaches itself to sea grass (**sunset** dashed line). The described behaviours, long-distance navigation, light shaft detection, and obstacle avoidance, can be used for exploring neuro-neuronal response to visual stimuli since they are robust, instantaneous, and repeatable. Diurnal activity has a much slower response to changing light conditions, and is presumably controlled by extraocular opsins, making it less suitable for vision experiments

ocular microtremors) prevent adaptation of the photoreceptors (Land 1999). Box jellyfish do not have muscles associated with their visual system, and the animals are too slow to refresh the retinal image by swim speed alone. Instead cubomedusae refresh the retinal image of the upper lens eye by utilising their swim contractions. The contractions cause the rhopalium to swing within the rhopalial niche where it is situated, and the amplitude of the swing matches the acceptance angle of the individual photoreceptors in the upper lens eye. The consequent displacement of the retinal image causes a contrast line between light and dark to shift to an adjacent

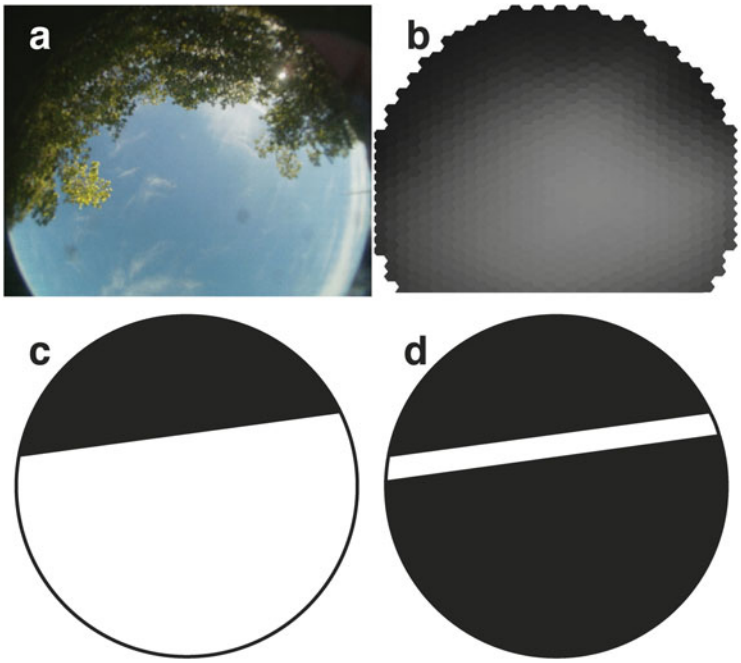

Fig. 27.8 Long-distance navigation. At the water surface (Fig. 27.7 pos. **a**), *Tripedalia cystophora* peers through Snell's window to locate the mangrove canopy (**a**) (see Fig. 27.3). The upper lens eye is severely under focused due to the displaced retina, but the canopy can still be discerned with box jellyfish acuity (**b**). *T. cystophora* scans the visual field for the contrast line between the canopy and the open sky (**c**) to assess the direction and the distance to their habitat under the trees. An additional challenge must be met, since all known photoreceptors adapt to constant light conditions and the swim speed of the animal alone is insufficient to refresh the retinal image. However, the bell contractions cause the rhopalium to swing within its niche, and the amplitude was shown to match the acceptance angle of the individual photoreceptors. The canopy-sky contrast line would then shift to an adjacent row of photoreceptors, creating a local light-on stimulus. The contrast line will then light up on the retina as a bright line on a dark (adapted) background (**d**). **a** and **b** modified after Garm et al. (2011)

line of photoreceptors (Fig. 27.8c and d; Bielecki et al. 2013a). This creates a local light-on stimulus on the affected receptors of the upper lens eye and results in an increased in pacemaker signal frequency (Fig 27.8d; Garm and Mori 2009). The animal will thereby increase swim contractions and swim in the direction of the canopy cover until the contrast line is located on the peripheral photoreceptors of the retina in the upper lens eye (Garm et al. 2011).

27.5.2 Foraging: Light Shaft Detection

Another robust visual behaviour in *T. cystophora* is related to foraging in their habitat between the prop roots of the *Rhizophora mangle* mangrove trees. During the day *T. cystophora* prey on the copepod *Dioithona oculata* by swimming through a swarm and catch them on outstretched tentacles (Buskey 2003). The copepods are positive phototactic and congregate in huge numbers within light shafts created by sunlight penetrating the foliage of the mangrove trees (Figs. 27.7 and 27.9). The foraging behaviour is mediated by the lower lens eye in *T. cystophora* and, since the spatial resolution is insufficient for direct prey location (Buskey 2003; Nilsson et al. 2005), entails detection of the light shafts (Fig. 27.9; Garm and Bielecki 2008; Garm and Mori 2009).

Light shaft detection does not necessarily involve image formation but rather a rapid increase in ambient light intensity corresponding to an animal swimming from a shadow area to an area with full sunlight. The flush of light causes the swim contractions to cease and the animal to slowly glide through the water. Cubomedusae are negatively buoyant and will sink through the water column, so within approximately 10–15 s, the swim contractions will resume (Buskey 2003). This behaviour was supported by electrophysiological data (Garm and Bielecki 2008), where a sudden increase in light intensity (light-on) presented to the entire rhopalium resulted in a decreased pacemaker frequency (Fig. 27.9b). This response resembles the modus operandi of the lower lens eye when stimulated individually, indicating that light shaft detection is controlled by the lower lens eye. In contrast the upper lens eye modulates an increased pacemaker frequency when presented to a light-on stimulus (Garm and Mori 2009). Should the animal inadvertently exit the light shaft, it will perform a number of fast bell contractions, turn 180°, and try to relocate the light shaft (Fig. 27.9a; Buskey 2003). This is also supported by the electrophysiological data, where a light-off stimulus results in an instantaneous increase in pacemaker signal frequency (Fig. 27.9b; Garm and Bielecki 2008; Garm and Mori 2009). This behavioural modulation ensures most possible time spent in light shafts and thereby access to prey items.

27.5.3 Obstacle Avoidance

In the medusa stage, *T. cystophora* does not have natural enemies but face threats nonetheless. While searching for light shafts and prey, the animal must avoid colliding with the prop roots of their underwater habitat. The epidermis of their bell is merely one cell layer thick, and abrasion can cause infection and subsequently death. So when *T. cystophora* encounter an obstacle, the animal will perform 4–5 fast bell contractions, turn 120–180°, and swim away (Garm et al. 2007c, 2013; Fig. 27.10). Their obstacle avoidance behaviour is modulated by the lower lens eye and based on true spatial vision (Garm et al. 2013). In behavioural assays,

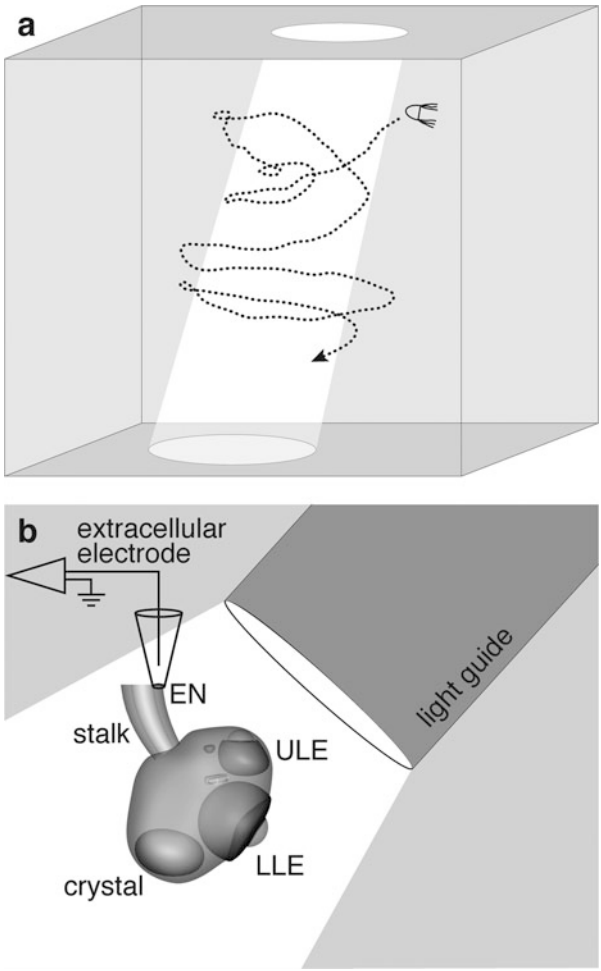

Fig. 27.9 Light shaft detection. *Tripedalia cystophora* prey on positive phototactic copepods that congregate in light shafts created by sunlight penetrating the foliage of the mangrove trees (**a**). When the animal enters a light shaft, it experiences a sudden increase in the general light intensity, and the swim contractions cease for 10–20 s, and the animal drifts through the light shaft passively hunting copepods with outstretched tentacles. Once outside, the animal is faced with a sharp decrease in ambient light intensity and responds by increasing swim contraction frequency, circling back, and attempting to re-enter the light shaft (**a**). The visual stimuli that the animal is subjected to when interacting with light shafts were recreated in the lab (**b**). When the entire rhopalium was exposed to a light-on stimulus, a sudden decrease in pacemaker signal frequency could be recorded from the epidermal stalk nerve (**EN**) using extracellular techniques (**extracellular electrode**). Upper lens eye (**ULE**) and lower lens eye (**LLE**). (**a**) Inspired by Buskey (2003)

T. cystophora was exposed to vertical, horizontal, and oblique bars on the perimeter wall of a behavioural arena (Fig. 27.10a; Garm et al. 2013). Here, the animals displayed graded obstacle avoidance behaviour, depending on the direction of the

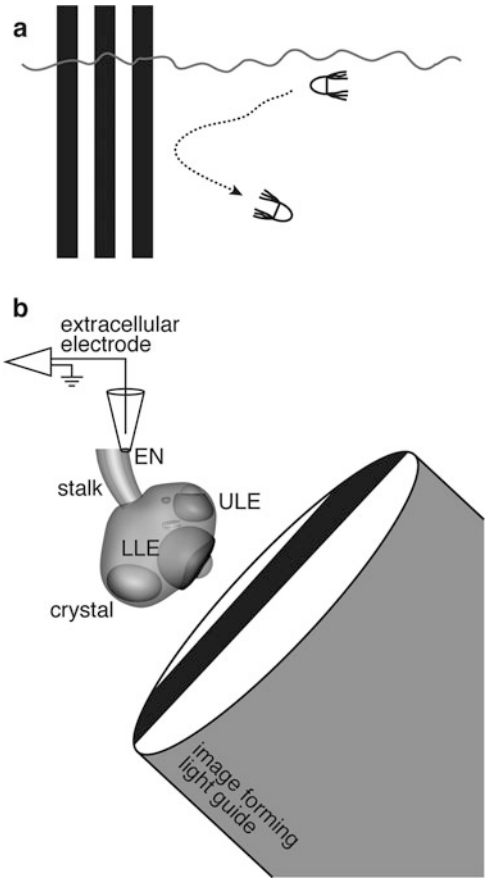

Fig. 27.10 Obstacle avoidance. When *Tripedalia cystophora* is confronted with an obstacle, it will perform four to five fast bell contractions, turn 120–180°, and swim away (**a**). Electrophysiological experiments confirm the behavioural data (**b**). When simultaneously showing the lower lens eye (*LLE*) a moving bar and recording the pacemaker signals from the epidermal stalk nerve (*EN*), a sharp increase in pacemaker signal frequency was observed when the bar travelled across the middle of the visual field. This is consistent with the obstacle avoidance response observed in behavioural experiments

depicted bars. The avoidance response in *T. cystophora* was most prominent when exposed to vertical bars, then oblique, and the least response to horizontal bars (Garm et al. 2013). This response pattern corresponds to the directionality of the roots in the habitat, and it seems that the visual system of the animal is tuned to detect this specific pattern on the retina (cf. matched filters in Wehner (1987)). In addition to the directionality, *T. cystophora* shows a graded avoidance response to varying contrasts of the bars, with higher contrast yielding greater response. It has been suggested that this response is an inherent measure of distance under water. Contrast dissipates quickly through the water column, so objects with less contrast are further away than objects of more contrast (Garm et al. 2013).

Obstacle avoidance behaviour has been confirmed by electrophysiological experiments. Moving bars projected into the lower lens eye result in a sharp increase in swim pacemaker signal frequency when the bar travels across the middle of the visual field (Fig. 27.10b). This instantaneous increase matches the behavioural response to an obstacle. A uniform decrease in light intensity of the entire visual field, of the same magnitude as the decreased intensity when the bar was moving

across the visual field, did not elicit the same type of response from the behavioural modulators proving that the animals see the root as an object in an image (manuscript in prep).

27.6 Future Research Perspectives

We want to obtain a system-level understanding of the cubozoan visual system and follow the information pathway from the retinal photoreceptors through the rhopalial nervous system (RNS) to the motor neurons. Neural physiology and signal transmission strategies are highly conserved throughout metazoan evolution, so we can possibly gain a general understanding about nervous systems by studying cubozoan signal transduction mechanisms and network organisation. If we can understand the algorithm used in neural image analysis in a simple system such as *Tripedalia cystophora*, we have a high chance of discovering universal filtering and processing units used by all animal visual systems including humans. However, at present we are merely able to measure the retinal response to different light stimuli by performing ERGs on the retinas (Garm et al. 2007a; O'Connor et al. 2010; Bielecki et al. 2013a) and to determine the neural output by monitoring the activity in the epidermal stalk nerve (Garm and Bielecki 2008; Garm and Mori 2009; Bielecki et al. 2013b). Unfortunately, what happens between the visual input and the neural output (Fig. 27.2) is contained within the proverbial black box.

The advantages of using the small transparent connectome of *T. cystophora* seem obvious, but the RNS comprises small neurons and is protected by a defiant impenetrable epidermis, which discourages intracellular electrophysiological recordings and makes it impossible to identify cells until backfilling them post-recording. Other approaches are therefore warranted. Recently developed optogenetic reporters, genetically encoded calcium indicators (GECIs) and voltage indicators (GEVIs), have provided tools to monitor neuronal activity in real time. In contrast to conventional calcium- or voltage-sensitive dyes, optogenetic reporters can be expressed in specific cell types and will reliably report on the state of the cell (Akemann et al. 2010; Ahrens et al. 2012, 2013; Alford et al. 2013; Akerboom et al. 2013; St-Pierre et al. 2014; Lin and Schnitzer 2016; Yang et al. 2016).

The central nervous system of *T. cystophora* is probably the poster child of live cell imaging: transparent, small, and separated from the motor effectors. Due to the proximity of the CNS and the eyes, we can monitor the neural response to a visual stimulus from the photoreceptors, across the processing circuits and integration in the pacemaker cells, to the motor neuron output signal, all in the same imaging frame and with single cell resolution.

For the imaging approach, it is imperative that the model animal is completely immobile. To resolve this issue, mice are head fixed, and zebrafish are embedded in agarose under the microscope. At best, this causes discomfort for the animal, but in worst-case scenario, it produces an unnatural response pattern. In *T. cystophora* the motor effectors can be separated from the motor signal, and the behaviour related to

a specific visual stimulus is accurately predicted by electrophysiological monitoring of the pacemakers inherent to the RNS. Of course, feedback information is lost from the other rhopalia and putative sensory systems on the bell, but since one driving rhopalium tends to override the output of the others (Petie et al. 2011; Stöckl et al. 2011), valuable information can still be extracted from one transected rhopalium.

Unfortunately, we have not yet succeeded in culturing mature females and therefore do not have access to recently fertilised embryos for microinjection. Granted, the obstacle to microinjection resides with the inabilities of the researchers, not the animals, but the issue has to be resolved nonetheless. A solution to the lack of embryos could be utilising a viral vector to express the optogenetic reporters in the RNS neurons, and one such option is the vesicular stomatitis virus (VSV). The virus' genome is antisense RNA coding for the necessary molecular machinery for viral gene transcription and RNA metabolism and thereby independent of interaction with the host cell nucleus and potentially applicable for use in a wide variety of organisms (Mundell et al. 2015). In most applications using VSV, a GFP is situated behind the ubiquitous T7 promoter and will infect and express in all cell types but spreads anterograde transsynaptically (with the native glycoprotein). This means that it is possible to follow interconnected neurons and the putative signal from point of injection to the processing units. Currently, the VSV is used as a neuro-neuronal tracing tool and has not yet been designed to carry optogenetic reporters. However, we succeeded in transducing GFP in the photoreceptors of the lower lens eye using the VSV vector (Fig. 27.11), but, while it is quite remarkable that a vector produced for mammalian transduction purposes works in the cnidarian system, unfortunately transsynaptic transfection still eludes us (Mundell et al. 2015).

Other viral vectors carry optogenetic reporters: adeno-associated virus (AAV), the preferred transduction workhorse for the mouse scientific community (Gao et al.

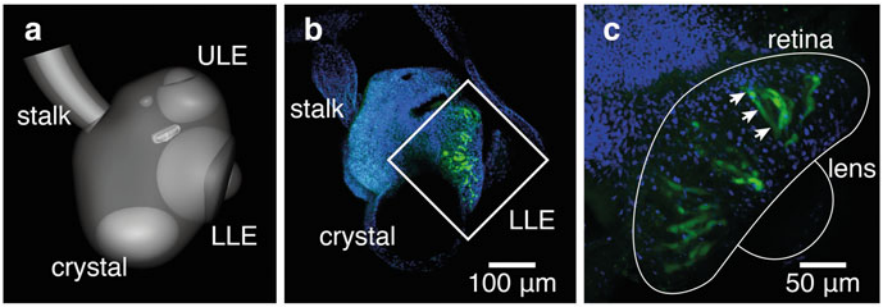

Fig. 27.11 GFP expression in the lower lens eye in vivo. A challenge to using *Tripedalia cystophora* as a model for visual information processing is the limited access to genetic tools. Specifically, the lack of stable transgenic adult jellyfish. However, we have successfully expressed green fluorescent protein (*GFP*) in the photoreceptors of the lower lens eye (*LLE*) in vivo using a vesicular stomatitis virus (VSV) vector (**b**). Higher magnification (**c**) of the square area in (**b**) shows individual photoreceptors expressing GFP (*arrowheads*). The orientation of the rhopalium in (**b**) is depicted in the schematic (**a**). VSV(VSV-G)GFP (green), post-fixed (PFA), and DAPI stained (blue). (**b**) and (**c**) modified after Mundell et al. (2015)

2005), translocates into the host cell nucleus where it forms a replicative double-stranded DNA of the AAV genome. AAV infects all cell types, but the inserted gene will only be expressed if it is situated behind a cell type-specific promoter. We do not yet know if AAV will infect cells of the cnidarian system, but even if it did, the genome of *T. cystophora* has never been sequenced, and we therefore lack promoter sequences specific to the RNS neurons. It is possible that out of pure serendipitous luck that the commercially available AAV-Synapsin-GCaMP construct (Akerboom et al. 2013) will readily transduce *T. cystophora* rhopalial neurons, but this remains to be determined.

Only time will tell, whether it is possible to produce stable lines of transgenic box jellyfish expressing dynamic optogenetic reporters, but it could greatly enhance our understanding of visual information processing on the cellular level, as current model systems do not yet offer the imaging resolution, depth of field, and field of view necessary to contain the entire processing network within the same recording. This is the true advantage of the box jellyfish model system, and, even though the present account is far from exhaustive, we hope to have inspired box jellyfish awareness for future research.

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References

Ahrens MB, Li JM, Orger MB, Robson DN, Schier AF, Engert F, Portugues R (2012) Brain-wide neuronal dynamics during motor adaptation in zebrafish. Nature 485:471–477

Ahrens MB, Orger MB, Robson DN, Li JM, Keller PJ (2013) Whole-brain functional imaging at cellular resolution using light-sheet microscopy. Nat Methods 10:413–420

Akemann W, Mutoh H, Perron A, Rossier J, Knöpfel T (2010) Imaging brain electric signals with genetically targeted voltage-sensitive fluorescent proteins. Nat Methods 7:643–649

Akerboom J, Carreras Calderón N, Tian L, Wabnig S, Prigge M, Tolö J, Gordus A, Orger MB, Severi KE, Macklin JJ, Patel R, Pulver SR, Wardill TJ, Fischer E, Schüler C, Chen TW, Sarkisyan KS, Marvin JS, Bargmann CI, Kim DS, Kügler S, Lagnado L, Hegemann P, Gottschalk A, Schreiter ER, Looger LL (2013) Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. Front Mol Neurosci 6:2

Alford SC, Wu J, Zhao Y, Campbell RE, Knöpfel T (2013) Optogenetic reporters. Biol Cell 105:14–29

Anderson PA, Grünert U (1988) Three-dimensional structure of bidirectional, excitatory chemical synapses in the jellyfish *Cyanea capillata*. Synapse 2:606–613

Bielecki J, Høeg JT, Garm A (2013a) Fixational eye movements in the earliest stage of metazoan evolution. PLoS One 8:e66442

Bielecki J, Nachman G, Garm A (2013b) Swim pacemaker response to bath applied neurotransmitters in the cubozoan *Tripedalia cystophora*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 199:785–797

Bielecki J, Zaharoff AK, Leung NY, Garm A, Oakley TH (2014) Ocular and extraocular expression of opsins in the rhopalium of *Tripedalia cystophora* (Cnidaria: Cubozoa). PLoS One 9:e98870
Briggman KL, Abarbanel HD, Kristan WB (2005) Optical imaging of neuronal populations during decision-making. Science 307:896–901
Buskey E (2003) Behavioral adaptations of the cubozoan medusa *Tripedalia cystophora* for feeding on copepod (*Dioithona oculata*) swarms. Mar Biol 142:225–232
Coates MM, Garm A, Theobald JC, Thompson SH, Nilsson DE (2006) The spectral sensitivity of the lens eyes of a box jellyfish, *Tripedalia cystophora* (Conant). J Exp Biol 209:3758–3765
De Pittà M, Volman V, Berry H, Parpura V, Volterra A, Ben-Jacob E (2012) Computational quest for understanding the role of astrocyte signaling in synaptic transmission and plasticity. Front Comput Neurosci 6:98
Delcomyn F (1980) Neural basis of rhythmic behavior in animals. Science 210:492–498
Fain GL, Matthews HR, Cornwall MC, Koutalos Y (2001) Adaptation in vertebrate photoreceptors. Physiol Rev 81:117–151
Gao G, Vandenberghe LH, Wilson JM (2005) New recombinant serotypes of AAV vectors. Curr Gene Ther 5:285–297
Garm A, Bielecki J (2008) Swim pacemakers in box jellyfish are modulated by the visual input. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 194:641–651
Garm A, Ekström P (2010) Evidence for multiple photosystems in jellyfish. Int Rev Cell Mol Biol 280:41–78
Garm A, Mori S (2009) Multiple photoreceptor systems control the swim pacemaker activity in box jellyfish. J Exp Biol 212:3951–3960
Garm A, Ekström P, Boudes M, Nilsson DE (2006) Rhopalia are integrated parts of the central nervous system in box jellyfish. Cell Tissue Res 325:333–343
Garm A, Coates MM, Gad R, Seymour J, Nilsson DE (2007a) The lens eyes of the box jellyfish *Tripedalia cystophora* and *Chiropsalmus* sp. are slow and color-blind. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 193:547–557
Garm A, Poussart Y, Parkefelt L, Ekström P, Nilsson DE (2007b) The ring nerve of the box jellyfish *Tripedalia cystophora*. Cell Tissue Res 329:147–157
Garm A, O'Connor M, Parkefelt L, Nilsson DE (2007c) Visually guided obstacle avoidance in the box jellyfish *Tripedalia cystophora* and *Chiropsella bronzie*. J Exp Biol 210:3616–3623
Garm A, Andersson F, Nilsson Dan-E (2008) Unique structure and optics of the lesser eyes of the box jellyfish Tripedalia cystophora. Vision Research 48 (8):1061–1073
Garm A, Oskarsson M, Nilsson DE (2011) Box jellyfish use terrestrial visual cues for navigation. Curr Biol 21:798–803
Garm A, Bielecki J, Petie R, Nilsson DE (2012) Opposite patterns of diurnal activity in the box jellyfish *Tripedalia cystophora* and *Copula sivickisi*. Biol Bull 222:35–45
Garm A, Hedal I, Islin M, Gurska D (2013) Pattern- and contrast-dependent visual response in the box jellyfish *Tripedalia cystophora*. J Exp Biol 216:4520–4529
Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K (2000) In search of the visual pigment template. Vis Neurosci 17:509–528
Gray GC, Martin VJ, Satterlie RA (2009) Ultrastructure of the retinal synapses in cubozoans. Biol Bull 217:35–49
Ijspeert AJ (2008) 2008 Special issue: Central pattern generators for locomotion control in animals and robots: a review. Neural Netw 21:642–653
Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 4:147ra111
Kass-Simon G, Pierobon P (2007) Cnidarian chemical neurotransmission, an updated overview. Comp Biochem Physiol A Mol Integr Physiol 146:9–25
Koyanagi M, Takano K, Tsukamoto H, Ohtsu K, Tokunaga F, Terakita A (2008) Jellyfish vision starts with cAMP signaling mediated by opsin-G(s) cascade. Proc Natl Acad Sci U S A 105:15576–15580

Kozmik Z, Ruzickova J, Jonasova K, Matsumoto Y, Vopalensky P, Kozmikova I, Strnad H, Kawamura S, Piatigorsky J, Paces V, Vlcek C (2008) Assembly of the cnidarian camera-type eye from vertebrate-like components. Proc Natl Acad Sci U S A 105:8989–8993

Land MF (1999) Motion and vision: why animals move their eyes. J Comp Physiol A 185:341–352

Land MF, Nilsson D-E (2012) Animal eyes. Oxford University Press, New York

Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, Miller DJ, Hoegh-Guldberg O (2007) Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. Science 318:467–470

Lin MZ, Schnitzer MJ (2016) Genetically encoded indicators of neuronal activity. Nat Neurosci 19:1142–1153

Mackie G, Meech R (1995a) Central circuitry in the jellyfish *Aglantha*. I: The relay system. J Exp Biol 198:2261–2270

Mackie G, Meech R (1995b) Central circuitry in the jellyfish *Aglantha*. II: The ring giant and carrier systems. J Exp Biol 198:2271–2278

Mackie GO, Anderson PAV, Ssingla CL (1984) Apparent absence of gap junctions in two classes of cnidaria. Biol Bull 167:120–123

Marder E, Bucher D, Schulz DJ, Taylor AL (2005) Invertebrate central pattern generation moves along. Curr Biol 15:R685–R699

Matthews HR, Murphy RLW, Fain GL, Lamb TD (1988) Photoreceptor light adaptation is mediated by cytoplasmic calcium concentration. Nature 334:67–69

Morgan PT, Perrins R, Lloyd PE, Weiss KR (2000) Intrinsic and extrinsic modulation of a single central pattern generating circuit. J Neurophysiol 84:1186–1193

Mundell NA, Beier KT, Pan YA, Lapan SW, Göz Aytürk D, Berezovskii VK, Wark AR, Drokhlyansky E, Bielecki J, Born RT, Schier AF, Cepko CL (2015) Vesicular stomatitis virus enables gene transfer and transsynaptic tracing in a wide range of organisms. J Comp Neurol 523:1639–1663

Nilsson DE (2004) Eye evolution: a question of genetic promiscuity. Curr Opin Neurobiol 14:407–414

Nilsson DE, Gislén L, Coates MM, Skogh C, Garm A (2005) Advanced optics in a jellyfish eye. Nature 435:201–205

Nusbaum MP, Blitz DM (2012) Neuropeptide modulation of microcircuits. Curr Opin Neurobiol 22:592–601

O'Connor M, Nilsson DE, Garm A (2010) Temporal properties of the lens eyes of the box jellyfish *Tripedalia cystophora*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 196:213–220

Oh SW, Harris JA, Ng L, Winslow B, Cain N, Mihalas S, Wang Q, Lau C, Kuan L, Henry AM, Mortrud MT, Ouellette B, Nguyen TN, Sorensen SA, Slaughterbeck CR, Wakeman W, Li Y, Feng D, Ho A, Nicholas E, Hirokawa KE, Bohn P, Joines KM, Peng H, Hawrylycz MJ, Phillips JW, Hohmann JG, Wohnoutka P, Gerfen CR, Koch C, Bernard A, Dang C, Jones AR, Zeng H (2014) A mesoscale connectome of the mouse brain. Nature 508:207–214

Parkefelt L, Ekström P (2009) Prominent system of RFamide immunoreactive neurons in the rhopalia of box jellyfish (Cnidaria: Cubozoa). J Comp Neurol 516:157–165

Petie R, Garm A, Nilsson DE (2011) Visual control of steering in the box jellyfish *Tripedalia cystophora*. J Exp Biol 214:2809–2815

Petie R, Garm A, Nilsson D-E (2013) Contrast and rate of light intensity decrease control directional swimming in the box jellyfish *Tripedalia cystophora* (Cnidaria, Cubomedusae). Hydrobiologia 703:69–77

Plachetzki DC, Degnan BM, Oakley TH (2007) The origins of novel protein interactions during animal opsin evolution. PLoS One 2:e1054

Plickert G, Schneider B (2004) Neuropeptides and photic behavior in Cnidaria. Hydrobiologia 530–531:49–57

Satterlie RA (1979) Central control of swimming in the cubomedusan jellyfish *Carybdea rastonii*. J Comp Physiol 133:357–367

Satterlie RA (2002) Neuronal control of swimming in jellyfish: a comparative story. Can J Zool 80:1654–1669
Satterlie RA (2011) Do jellyfish have central nervous systems? J Exp Biol 214:1215–1223
Schall JD (2001) Neural basis of deciding, choosing and acting. Nat Rev Neurosci 2:33–42
Shorten M, Seymour JE, Cross MC, Carette TJ, Woodward G, Cross TF (2005) J Zool 267:371–381
Skogh C, Garm A, Nilsson DE, Ekström P (2006) Bilaterally symmetrical rhopalial nervous system of the box jellyfish *Tripedalia cystophora*. J Morphol 267:1391–1405
Stöckl AL, Petie R, Nilsson DE (2011) Setting the pace: new insights into central pattern generator interactions in box jellyfish swimming. PLoS One 6:e27201
St-Pierre F, Marshall JD, Yang Y, Gong Y, Schnitzer MJ, Lin MZ (2014) High-fidelity optical reporting of neuronal electrical activity with an ultrafast fluorescent voltage sensor. Nat Neurosci 17:884–889
Suga H, Schmid V, Gehring WJ (2008) Evolution and functional diversity of jellyfish opsins. Curr Biol 18:51–55
Takano T, Han X, Deane R, Zlokovic B, Nedergaard M (2007) Two-photon imaging of astrocytic Ca2+ signaling and the microvasculature in experimental mice models of Alzheimer's disease. Ann N Y Acad Sci 1097:40–50
Terakita A (2005) The opsins. Genome Biol 6:213
Volterra A, Liaudet N, Savtchouk I (2014) Astrocyte Ca^{2+} signalling: an unexpected complexity. Nat Rev Neurosci 15:327–335
Wehner R (1987) 'Matched filters' – neural models of the external world. J Comp Physiol A: Neuroethol Sens Neural Behav Physiol 161:511–531
Yamasu T, Yoshida M (1976) Fine structure of complex ocelli of a cubomedusan, Tamoya bursaria Haeckel. Cell Tissue Res 170:325–339
Yang HH, St-Pierre F, Sun X, Ding X, Lin MZ, Clandinin TR (2016) Subcellular imaging of voltage and calcium signals reveals neural processing in vivo. Cell 166:245–257
Yatsu N (1917) Notes on the physiology of *Carybdea rastonii*. J Coll Sci Tokyo Imp Univ 40:1–12