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Current advances in invertebrate vision: insights from patch-clamp studies of photoreceptors in apposition eyes

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Frolov RV. Current advances in invertebrate vision: insights from patch-clamp studies of photoreceptors in apposition eyes. J Neurophysiol 116: 709–723, 2016. First published June 1, 2016; doi:10.1152/jn.00288.2016.—Traditional electrophysiological research on invertebrate photoreceptors has been conducted in vivo, using intracellular recordings from intact compound eyes. The only exception used to be Drosophila melanogaster, which was exhaustively studied by both intracellular recording and patch-clamp methods. Recently, several patch-clamp studies have provided new information on the biophysical properties of photoreceptors of diverse insect species, having both apposition and neural superposition eyes, in the contexts of visual ecology, behavior, and ontogenesis. Here, I discuss these and other relevant results, emphasizing differences between fruit flies and other species, between photoreceptors of diurnal and nocturnal insects, properties of distinct functional types of photoreceptors, postembryonic developmental changes, and relationships between voltage-gated potassium channels and visual ecology.

insect photoreceptor; voltage-gated potassium channels; patch-clamp; visual ecology

Among invertebrates, the humble fruit fly Drosophila melanogaster stands out like a giant in the field of neurophysiological research, eclipsing contributions from all other model organisms. However, a few specialist areas have escaped the overwhelming dominance of the fruit fly, and visual ecology is a primary example of these (Cronin et al. 2014b). Here, D. melanogaster is only primus inter pares, for the intrinsically comparative character of visual ecology precludes an overly disproportionate influence by any single species, regardless of its experimental convenience.

Visual ecology is concerned with morphological and physiological adaptations of visual systems to the ecological needs of animals, treating visual systems from the molecular to behavioral levels, from specializations in the cornea to those in the optic ganglia, encompassing ontogenesis- and sex-related variables in the visual systems. However, this review focuses on only one narrow aspect of visual system function: the biophysical properties of photoreceptors in apposition-type compound eyes, as revealed in recent experiments using patch clamp as the main method. Overall, these biophysical properties include the following: general electrical characteristics, including input resistance, capacitance, resting potential; elementary current and voltage responses to discrete photons, known as current and voltage bumps, respectively; the molecular identities, conductances and kinetics of ion channels; absolute photoreceptor sensitivity; light-induced current (LIC) and voltage responses and their adaptation; and integral membrane properties, such as frequency bandwidth, gain, signal-to-noise ratio, and information capacity. Chronologically, the origins of this research area can be traced to the seminal patch-clamp works in Drosophila photoreceptors (Hardie 1991; Hardie and Minke 1992; Ranganathan et al. 1991) and comparative visual ecological intracellular recording studies of voltage-activated K⁺ (Kᵥ) conductances in fly photoreceptors (Laughlin and Weckström 1993; Weckstrom and Laughlin 1995). These studies were themselves preceded by extensive intracellular recordings from the horseshoe crab Limulus polyphemus (Dorlochter and Stieve 1997) and several insect species, including flies, locusts, and dragonflies (French and Jarvilehto 1978; Hardie et al. 1981; Laughlin 1976; Matić and Laughlin 1981; Pece et al. 1990).

The importance of comparative electrophysiological research into photoreceptors stems from the fact that the retina is the brain’s gateway for visual information, and that the biophysical properties of photoreceptors constrain the operational range and determine the temporal resolution of vision in accordance with complex natural selection pressures on the species. Ecologically, the physiological operational range is defined by light intensities normally encountered by the animal; these are manifestly different for diurnal and nocturnal animals (Cronin et al. 2014b). The lower boundary is arguably
the most important, as it sets the absolute sensitivity threshold of the visual system. While several morphological and dynamic physiological adaptations in the dioptric apparatus, including ommatidial acceptance angle, lens size, pigment screening and rhabdomere migration, are crucial for determining the threshold by influencing photon yield, there is evidence that photoreceptors of nocturnal species have higher quantum efficiency than photoreceptors of purely diurnal insects by virtue of having larger rhabdomeres (Frolov 2015). On the other hand, temporal contrast resolution (and photoreceptor information capacity) depends on the effective number of rhabdomeres, such as expression in photoreceptors play important roles in regulating signaling bandwidth (Niven et al. 2003; Vähäsöyrinki et al. 2006). Opening of ion channels during and after the light response causes transmembrane fluxes of Ca$^{2+}$, Na$^+$, K$^+$ and Cl$^-$ ions, according to their electrochemical potentials, which in turn drive ion pumps to recover the chemical gradients. The energy consumed by these pumps can constitute a very substantial fraction of the total metabolic cost of brain activity and has been proposed to be a natural selection factor that shapes photoreceptor channelomes (Laughlin et al. 1998; Niven et al. 2003).

Until recently, *Drosophila* was the only insect species whose photoreceptors had broadly characterized biophysical properties, but now detailed descriptions are available for half a dozen insects with apposition and neural superposition eyes. Most of the data discussed here were obtained from comparative intracellular and patch-clamp recordings in the laboratory of Professor Matti Weckström of the University of Oulu. Importantly, the shared and consistent methodology of these studies permits direct comparison of results between species in great detail. The insects studied have dissimilar phylogenies, visual habits, and behaviors. This review aims to explore several comparative physiological questions, which emerged from this body of work.

1) What are the differences in electrophysiological properties of photoreceptors from insects with different evolutionary histories, lifestyles and behaviors? Do such differences have any predictive value?

2) How do classes of specialized photoreceptors differ from each other and why?

3) What happens during photoreceptor ontogenesis, particularly in species whose incomplete metamorphosis causes prominent growth of the retina after hatching from the egg? Are photoreceptor properties at different stages consistent with constraints imposed by lifestyle/behavior?

4) Are there links between ancillary photoreceptor conductances, such as K$_v$ conductances, and visual ecology?

**Basic Phototransduction and Electrophysiology of Microvillar Photoreceptors**

While a number of excellent reviews provide in-depth discussions of phototransduction and electrophysiological properties of microvillar photoreceptors (see, e.g., Fain et al. 2010; Fain and Lisman 1981; Hardie and Juusola 2015; Laughlin 1989), several basic issues need to be addressed here.

Animal photoreceptors can be broadly separated into two distinct classes, microvillar and ciliary. Ciliary photoreceptors provide the basis of vertebrate vision, while microvillar photoreceptors are predominant among invertebrates. In insect photoreceptors, visual pigment rhodopsin is tightly packed into tens of thousands of microvilli, which form a highly convoluted brush-like structure called the **rhabdomere**. Phototransduction in *D. melanogaster* is initiated by absorption photons by the rhodopsin-associated chromophore. This triggers transformation of rhodopsin into metarhodopsin, which leads to activation of a G protein-coupled receptor. The G protein $\alpha$-subunit sheds GDP, binds GTP, and dissociates from the $\beta\gamma$-subunit complex. Then the $\alpha$-subunit activates membrane-associated phospholipase C (PLCb4), which, in turn, catalyzes the hydrolysis of phosphoinositol 4,5-bisphosphate into DAG (diacylglycerol) and IP$_3$ (inositol 3-phosphate). IP$_3$ is important for the long-term viability of the photoreceptors and modulation of PLC functioning. Eventually, certain forms of DAG or DAG metabolites open TRP-type (transient receptor potential) cationic channels via either a conventional ligand-binding mechanism (Fain et al. 2010) or the recently proposed photomechanical mechanism (Hardie and Franze 2012), triggering the LIC.

Electrical signaling by photoreceptors is enabled by light-activated conductances expressed in the rhabdomere (Hardie and Raghu 2001). The amplitude and kinetics of a voltage response to a flash of light depend on the corresponding parameters of the evoked LIC, momentary membrane resistance, and whole cell capacitance. The effects of the resistance are twofold: it provides ohmic gain and regulates the membrane time constant ($\tau_m = RC$). In the lower half of the physiological voltage range, at potentials less than $-30$ mV (and quite possibly over the entire physiological voltage range, as discussed below), membrane resistance during the light response is mainly determined by K$_v$ conductances, because a relatively small driving force for K$^+$ requires a large K$_v$ conductance to counteract the LIC driven by a comparatively large electrical force. As a consequence, insects that need to prevent excessive low-pass filtering at voltages near resting potential tend to express large early activating K$_v$ currents in their photoreceptors (see $K_v$ Conductances and Visual Ecology Revisited below).

The properties of the second parameter controlling membrane filtering, capacitance, is complicated by the highly compartmentalized morphology of microvillar photoreceptors, comprising the rhabdomere, soma, axon, and presynaptic terminal. The latter two are usually absent in the dissociated ommatidial preparations used for patch-clamp recordings and are, therefore, not discussed further. The rhabdomere contains many thousands of elongated microvilli $\sim 60$ nm in diameter and 0.5–10 $\mu$m in length (Fain et al. 2010). Because of its highly convoluted topology, the light-sensitive rhabdomeric membrane is expected to be the main determinant of whole cell capacitance. This is supported by functional measurements of absolute sensitivity, which should be roughly proportional to the membrane area of the rhabdomere, since rhodopsin is a transmembrane protein. As shown previously in several species (Frolov 2015), membrane capacitance correlates moderately to strongly positively with absolute sensitivity, and such
correlations would not be possible if the main source of variation was the soma membrane and/or if the rhabdomere membrane area was relatively small. Interestingly, if cell-to-cell variations in capacitance were mainly due to the rhabdomere, then information capacity would be expected to correlate positively with capacitance, since the microvillus is the elementary sampling unit of the photoreceptor, and signal-to-noise ratio depends on the number of available sampling units. Indeed, such correlations have been observed repeatedly (Frolov 2015; Frolov et al. 2012a; Frolov and Weckstrom 2014; Immonen et al. 2014a).

Ion channel compositions of the soma and rhabdomere are different. In *D. melanogaster*, delayed-rectifier Shab K_+ channels are found in the basilar membrane of the rhabdomere (Hardie 1991; Hardie and Raghu 2001), while transient Shaker channels are expressed in the soma (Rogero et al. 1997). As shown in the following sections, correlations between capacitance and the magnitudes of the two potassium currents support the notion that the primary source of variability in capacitance between photoreceptors is indeed the rhabdomere.

However, there is an important and difficult experimental problem of space-clamp in the rhabdomere. The high length-to-diameter ratio of the microvillus creates a finite cable with high input impedance and dark space constant on the order of millimeters, much longer than the length of the microvillus (M. Kauranen, M. Vähäsöyrinki, unpublished observations). Upon activation of the microvillus by light, the space constant decreases dramatically but still remains relatively large. This implies that voltage clamp of photoreceptors is likely to be incomplete, resulting in underestimation of capacitance. Also, this evaluation does not account for the “neck” of the microvillus, a substantial narrowing at its base, which is bound to further increase microvillar input impedance. What could be the physiological purpose of such morphological organization of the rhabdomere? One hypothesis is that it reduces the effective capacitance as the microvillus serves more like a source of current into the photoreceptor, charging the membrane while partially bypassing the capacitor of the high-impedance rhabdomere. This also implies that LIC conductance may contribute much less to the total membrane conductance than its nominal magnitude implies. More studies are needed to investigate this matter.

The Case of *D. melanogaster*

*D. melanogaster* is a small fly with correspondingly small eyes, each containing ~800 facets. Although the fly is active during daylight, the peaks of its locomotor activity are observed at dusk and dawn, suggesting that the fly is crepuscular (Bahn et al. 2009). Recordings from *Drosophila* photoreceptors can be obtained by either patch-clamp or intracellular recording techniques, although both types of experiments are notoriously difficult to perform. Owing to the availability of unparalleled mutant libraries, covering virtually every gene with a rich arsenal of gene manipulation methods (Frolov et al. 2012b), electrophysiological studies in the fruit fly have yielded a finely detailed picture of phototransduction and the electrochemical transmission of light-induced signals (Fain et al. 2010).

In the *Drosophila* ommatidium, rhabdomeres are spatially separated from each other, forming a so-called open rhabdom, an arrangement occurring among diurnal insects, allowing each photoreceptor to sample its own field of view. This layout is associated with the neural superposition type of compound eye (Trujillo-Cenoz and Melamed 1966). Each ommatidium consists of eight photoreceptors, with two central photoreceptors exhibiting heterogeneity in wavelength discrimination and responsible for chromatic vision, and six peripheral green-sensitive photoreceptors responsible for motion detection (Yamaguchi et al. 2010), albeit this functional separation is not absolute (Schnaittmann et al. 2013; Wardill et al. 2012). *Drosophila* photoreceptors are relatively small, with average capacitance in the range between 50 and 65 pF (Jusola and Hardie 2001; Vähäsöyrinki et al. 2006), and contain about 30,000 microvilli, which serve as separate sampling units (Hochstrate and Hamdorf 1990; Song et al. 2012). Each microvillus contains a full suite of phototransduction machinery, from light-sensitive rhodopsin proteins to light-activated cationic channels, intricately organized into a supramolecular signaling complex dubbed the “signalplex”. Electrical activation of the microvillus by one or several simultaneously absorbed photons produces an all-or-nothing “quantum bump” after an ~30-ms latency period, which accommodates the biochemical reactions of the amplifying phototransduction cascade. This latency period shortens dramatically in light-adapted photoreceptors and with increased temperature. Each quantum bump is evoked by opening of about 15 light-activated channels (Henderson et al. 2000) and has a stereotypical shape and a mean amplitude of 8–10 pA at the holding potential of ~70 mV (henceforth, all quantum bump amplitudes are reported for this potential). Microvillus activation is followed by a refractory period, which sets the microvillus sampling rate and directly affects the size of the pool of operational sampling units, and, therefore, the information capacity (Hardie 2001; Song et al. 2012). Thus a photoreceptor containing fewer microvilli with shorter stochastic refractoriness may have information capacity on par with a photoreceptor containing more numerous but slowly recovering microvilli. This may be one of the reasons why relatively small-capacitance *Diptera* photoreceptors can vastly outperform the much larger photoreceptors of cockroaches and other species (Fain et al. 2010; Heimonen et al. 2012).

The fruit fly light-activated transduction channels belong to the ubiquitous TRP class of ligand-gated cationic channels (Frolov and Weckstrom 2016). Specifically, insect photoreceptor TRP channels are molecularly close to the “canonical” TRPC family of vertebrate TRP channels. Importantly, the entire history of this class of channels can be traced to the original discovery and description of these channels in *Drosophila* photoreceptors (Hardie and Minke 1992; Phillips et al. 1992). While at least three different TRP channels may be expressed in photoreceptors (Montell 2005), the bulk of the depolarizing inward current is carried by TRP and TRP-like (TRPL) channels, with the former clearly predominant (~90%) in the fruit fly (Hardie and Minke 1992; Niemeyer et al. 1996; Reuss et al. 1997). TRP channels are much more permeable to calcium over sodium than TRPL channels and can be blocked by low micromolar concentrations of lanthanum, which can serve as a convenient electrophysiological test during patch-clamp recordings. There is a differential operational expression of TRP and TRPL channels in the light-sensitive membrane, with TRPL present in the microvillar
membranes of dark-adapted flies but translocated from the rhabdomere to the soma and vice versa during light and dark adaptation, respectively, with time course of ~20 min (Bahner et al. 2002; Cronin et al. 2006). This indicates that TRPL channels may be more suitable for vision in the dark, and indeed the situation with TRP/TRPL channel expression seems to be reversed in the nocturnal cockroach (French et al. 2015).

Concerted opening of many light-activated channels produces a macroscopic LIC. Unfortunately, neither current nor voltage responses of Drosophila photoreceptors to prolonged contrast-modulated light stimulation can be recorded in the patch-clamp configuration due to rapid run-down of phototransduction. Thus properties of LIC in Drosophila, such as gain, dynamic intensity-, time-, and voltage-dependent adaptation, etc., are rather difficult to obtain, and the only source is modeling based on intracellular recordings. However, this setback seems to be limited to the fruit fly, with prolonged patch-clamp recordings of LIC and voltage responses obtainable in other fly species, such as the blowfly Calliphora vicina and the closely related Drosophila virilis (E.-V. Immonen, personal communications).

Dепolarization of a photoreceptor by LIC is counteracted and reversed by the delayed rectifier K+ channels (Hardie 1991; Laughlin and Weckström 1993; Weckstrom et al. 1991; Weckstrom and Laughlin 1995). At least two different channels with dissimilar kinetics and voltage dependencies of gating are expressed in Drosophila photoreceptors, a transient current with fast activation and inactivation (IA), and a sustained slowly activating and inactivating delayed rectifier (IDR). The molecular basis of IA is the Shaker gene, the homolog of the large KCNA family of mammalian genes, while IDR is based on the Shab gene, counterpart of the two mammalian KCNB channel genes (Niven et al. 2003; Vähäsöyrinki et al. 2006). Curiously, IDR in two central photoreceptors of each ommatidium seems to be based on another channel, possibly Shal (Anderson and Hardie 1996). In addition to the general repolarizing function, IA and IDR appear to play other roles. In the upper half of the physiological voltage range (greater than ~30 mV), IDR drastically increases total membrane conductance and shunts LIC-induced depolarization, improving temporal resolution of light responses (Vähäsöyrinki et al. 2006). On the other hand, IA is thought to be instrumental for the amplification of weak signals in the dark and reduction of metabolic costs of signaling (Niven et al. 2003).

**Periplaneta Americana: Superior Absolute Sensitivity**

The cockroach *P. americana* was the second insect species for which the biophysics of photoreceptor functioning was described using both intracellular recording and patch-clamp methods. Although molecular identification of ion channels and key proteins involved in phototransduction in the cockroach is greatly hampered by the lack of genetic variants, the recent development of RNA interference protocols allows targeted suppression of select retinal proteins, alleviating this problem (French et al. 2015). Even though intracellular recordings were initially the method of choice in the study of cockroach photoreceptors (Heimonen et al. 2006), it was quickly realized that patch-clamp can also be used to obtain high-quality data both in voltage- and current-clamp modes (Heimonen et al. 2012). In fact, longevity of some whole cell recordings was extraordinary, up to several hours of repeated light stimulation without deterioration of responses. This permitted recording of LIC and voltage responses to long contrast-modulated stimuli over the entire range of light intensities from the same photoreceptor.

*P. americana* is a nocturnal/crepuscular insect with large apposition compound eyes, containing around 2,000 facets in each (Ribi 1977). The animal demonstrates certain aspects of visually-guided behavior, such as avoidance of brightly illuminated spots when walking on an arena, and even moving to the target using visual cues in the surroundings (Kelly and Mote 1990; Mizunami et al. 1998). Remarkably, optomotor responses in the cockroach can be registered at light intensities that evoke bumps at the rate of less than one effective photon per photoreceptor per second (Honkanen et al. 2014).

The main qualitative difference between *Periplaneta* and *Drosophila* in organization of the ommatidium is that its rhabdomeres are not detached from each other and share the same field of view, with microvillar arrays tightly interlocked. This structure is called a closed or fused rhabdom and is found in most nocturnal and diurnal species, likely representing a primitive condition. The absolute majority of cockroach photoreceptors show broad spectral sensitivity to light, with the highest sensitivity to the green part of the spectrum (Heimonen 2008). Specialized UV- and violet-sensitive cells are encountered in less than 1 out of 10 recordings. *Periplaneta* photoreceptors are much larger than *Drosophila* photoreceptors: their average capacitance is ~350 pF (Heimonen et al. 2012; Immonen et al. 2014b), ranging from below 100 pF to 1,000 pF. The quantum bump is four to five times larger and has a slightly slower onset than in *Drosophila* (Immonen et al. 2014b). Although it is not known if the phototransduction cascade in the cockroach differs from that in the fruitfly, there are clear dissimilarities in bump generation. Quantum bumps in *Periplaneta* are much less sensitive to calcium than in *Drosophila*. For instance, a decrease in extracellular calcium concentration in *Drosophila* leads to a dramatic onset phase prolongation and decrease in bump amplitude (Henderson et al. 2000). In contrast, even a hundredfold decrease in extracellular calcium does not produce a comparable effect in *Periplaneta*, as quantum bumps become merely slightly slower and smaller. This difference may be due to the fact that the main light-activated channel in the cockroach is TRPL, as evidenced by insensitivity of LIC to lanthanum, permeability profile, and results of experiments involving double-stranded RNA against TRPL and TRP (Immonen et al. 2014b). Apart from the lower selectivity for calcium, TRPL channels have higher conductance than TRP channels and can be blocked by extracellular calcium in the voltage-dependent manner upon activation (Parnas et al. 2007). This channel block can explain the reduced bump amplitude when bumps are elicited from voltages more negative than resting potential, but not the relatively slow bump onset, which indicates that depletion of extracellular calcium interferes with the intrinsic phototransduction or channel-gating mechanisms unrelated to TRPL conductance per se. However, the effects of calcium removal manifest spectacularly at higher light intensities, as demonstrated by a drastic increase in LIC amplitude, absence of depolarizing transient, slow onset, and very slow IA (Immonen et al. 2014b). These findings suggest that external calcium is necessary for adaptive reduction of bump amplitude, since neither the absolute sensi-
tivity, nor individual bump amplitude at rest are altered strongly enough to account for such a large increase in LIC. In addition, these results reinforce the well-founded notion that calcium is involved in phototransduction and signal generation processes at multiple points and with different affinities.

Consistent with the nocturnal lifestyle and the need to reliably detect light in poorly lit environments, the gain of phototransduction in *Periplaneta* is about two times higher than in *Drosophila*, and information capacity seems to peak at \(~1,000\) effective photons/s per photoreceptor, without improvement in contrast resolution in brighter light (Heimonen et al. 2012). Likewise, the corner frequency of the response to randomly modulated stimulation (the frequency at which signal power falls by one-half) of cockroach photoreceptors is low, below 7 Hz, according to patch-clamp results (Heimonen et al. 2012). Thus the photoreceptor membrane is a strongly low-pass filter, a typical adaptation for vision in dim light that facilitates temporal summation.

Differences in the channelome between *Periplaneta* and *Drosophila* are not limited to light-activated channels. While photoreceptors in *Periplaneta* also express at least two types of K\(_c\) channels, the molecular identity of the delayed rectifier remains a mystery, and large variation in K\(_c\) current expression patterns is observed among photoreceptors. IA in *Periplaneta* is probably mediated by a Shaker-like channel, as its electrophysiological and pharmacological properties are very similar to IA in *Drosophila*. The situation is different with IDR, which has unusual pharmacological properties incompatible with Shab (Salmela et al. 2012). Two distinct channelome phenotypes have been observed. The first one, "normal", is found in \(~90\)% of photoreceptors and characterized by dominating IDR with relatively slow activation kinetics and a half-activation potential at around \(-20\) mV. IA in such cells is residual. These photoreceptors usually demonstrate sensitivity to green light and show regular responses to light with large depolarization and ordinary information capacity. The second, "hyperadapting" type is characterized by a prominent transient K\(_c\) current, with a very negative half-activation potential for IDR and relatively low capacitance. Such cells are characterized by very rapid adaptation of light responses, so that the maximal plateau depolarization does not exceed 10 mV (normally \(-20\)–\(-30\) mV in the first type of cells), and by low-information capacity. It is not known if the molecular origins of IA and IDR in these two cell types are the same.

Large physiological variability is the overriding theme in virtually all aspects of cockroach vision. It is observed at various levels of organization of the visual system periphery, from facets of variable sizes and irregular shapes to the rather disorderly terminations of photoreceptor axons in the lamina (Butler 1973; Ribi 1977). Prior to the intracellular recording study by Heimonen et al. (2006), it was thought that intrinsic variability within the visual systems of insects is quite small. For instance, in terms of coefficient of variation, photoreceptor acceptance angles, which determine the angular sensitivity and visual acuity, vary by 20% in flies and 25% in dragonflies (Laughlin and Hardie 1978), and even there the reported variation is not stochastic, but gradual, between different regions of the eye, i.e., from the acute zone toward the lateral retina. In contrast, the coefficient of variation for the horizontal acceptance angles in the cockroach was at least 40%, ranging from 2.2° to 12.7° (Heimonen et al. 2006). In this respect, the cockroach eye resembles the eye of the northeast-Asian wood white butterfly, *Leptidea amurensis* (Uchiyama et al. 2013). High variability was observed in two other traits: absolute sensitivity to light, and the dynamics of voltage response adaptation (Heimonen et al. 2006).

The causes of variability in photoreceptor responses were determined in later patch-clamp studies. In the first study, most of variation in voltage response waveforms had been explained by variation in the corresponding LIC kinetics (Fig. 1) (Heimonen et al. 2012). In the follow-up study, variability in photoreceptor size as measured by whole cell capacitance could explain the variability in both amplitude of macroscopic LICs and absolute sensitivity, with larger photoreceptors demonstrating larger LICs and higher sensitivity (Fig. 2) (Immonen et al. 2014b). It was also found that quantum bump size did not correlate with capacitance, implying that differences in capacitance and hence absolute sensitivity originate from differences in the number of microvilli between photoreceptors. The relationship between capacitance and sensitivity was also quantified in dark-adapted photoreceptors, using prolonged low-intensity stimuli evoking discontinuous bump responses (Fig. 3). The Spearman rank order correlation coefficient (SRCC) of 0.82 suggests a very strong positive correlation. These results suggest that increasing the number of microvilli may be a viable evolutionary strategy to improve vision in the dark.

Having a relatively large number of microvilli entails at least two performance-related implications for the photoreceptor. First, increasing the number of sampling units improves detection of fine aspects of the stimulus, i.e., increases contrast resolution and information capacity. Indeed, it was shown in several species that capacitance can strongly positively correlate with information capacity (Frolov 2015; Immonen et al. 2014a). Second, increasing the number of microvilli increases...
capacitance, which decelerates and attenuates membrane responses due to augmented low-pass filtering. However, the actual effect of increased capacitance on filtering depends on the expression levels and voltage-dependence of sustained K_\text{v} channels, which can moderate or even completely negate the detrimental effects of high capacitance. On the other hand, photoreceptors with fewer sampling units do not just have lower absolute sensitivity, but also can saturate faster than their counterparts with more microvilli.

The observed differences between Drosophila and Periplaneta are striking, and, while it is highly likely that they represent specialized evolutionary adaptations, they are quite difficult to explain. For instance, what could possibly justify the extraordinarily large current quantum bumps in the cockroach, linked to the proportionally large ionic fluxes and, respectively, cellular energy spent to restore the ionic equilibriums? Is it an adaptation to the nocturnal lifestyle, or is it caused by an unrelated factor and does not metabolically burden the photoreceptor, especially considering that bump size decreases drastically with increased illumination and depolarization from resting potential, and that input resistance at rest is so large (>300 MΩ according to patch-clamp studies) that metabolic costs of large bumps are rather inconsequential, unlike in C. vicina, where dark consumption is already very costly (Pangrsic et al. 2005)? Such questions require more studies of different species with dissimilar lifestyles and behaviors.

**Diurnal vs. Nocturnal**

As soon as the unusual properties of photoreceptors in the dark-active cockroach P. americana became apparent, we undertook further comparative studies of insects with dissimilar lifestyles and behaviors. Diurnal/nocturnal is a major line dividing visual systems not only in regard to lifestyle, but also structurally and functionally, since specific adaptations enabling optimal visual operation in each illumination environment are generally mutually incompatible (Cronin et al. 2014b; Frolov et al. 2016). Although such adaptations at the level of photoreceptor biophysics have been studied extensively using intracellular recordings (Cronin et al. 2014a), the patch-clamp experiments discussed in this section provided new information.

First, two rather stereotypical nocturnal and diurnal species, the stick insect Carausius morosus and the water strider Gerris lacustris (Frolov et al. 2012a; Frolov and Weckstrom 2014), were compared. The stick insect lives in foliage, is active during daytime, and undertook further comparative studies of insects with dissimilar lifestyles and behaviors. Diurnal/nocturnal is a major line dividing visual systems not only in regard to lifestyle, but also structurally and functionally, since specific adaptations enabling optimal visual operation in each illumination environment are generally mutually incompatible (Cronin et al. 2014b; Frolov et al. 2016). Although such adaptations at the level of photoreceptor biophysics have been studied extensively using intracellular recordings (Cronin et al. 2014a), the patch-clamp experiments discussed in this section provided new information.

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facets per eye in *G. lacustris*. The photoreceptors form a closed rhabdom in the stick insect and an open rhabdom in the water strider.

Consistent with findings in the diurnal/crepuscular *Drosophila* and nocturnal cockroach, photoreceptors of *G. lacustris* are much smaller than photoreceptors of *C. morosus*: 60 vs. 175 pF average, with capacitance in both species strongly positively correlated with absolute sensitivity (Fig. 3). Importantly, median absolute sensitivity was 10 times lower in the diurnal *G. lacustris* than in the nocturnal *C. morosus*, raising the question whether photoreceptor size alone can provide clues about the animal’s lifestyle or behavior. Table 1 gives average capacitances of photoreceptors from *D. melanogaster* and 16 other species obtained in patch-clamp experiments. If purely nocturnal and diurnal species are segregated (with *C. punctata* and *N. glauca* set aside), and their average capacitances compared using a t-test assuming unequal variances, a statistically significant difference emerges (*P* = 0.012). However, if *C. punctata* and *N. glauca* are considered “diurnal”, the relationship becomes statistically insignificant (*P* = 0.11). The case of *N. glauca* is treated in the next section, concluding that organization of its ommatidia is not consistent with a strictly diurnal lifestyle, but the situation of *C. punctata* is more ambiguous (Frolov 2015). Still, the trend for diurnal insects to have smaller photoreceptors and nocturnal larger ones seems clear, although its formal confirmation will depend on further testing of species from diverse families. It should be noted that, although capacitance values of a number of other insects have been obtained in various intracellular recording studies using injections of current in current-clamp mode, such data are poorly compatible with patch-clamp results (see comparison of capacitance values in Immonen et al. 2014a).

Figure 3 shows correlations between absolute sensitivity and capacitance values obtained from patch-clamp experiments in five species with dissimilar visual ecologies. Importantly, since all of these measurements were performed in the same setup under nearly identical illumination conditions, the absolute sensitivities can be directly compared. It follows from the plot that the highest median absolute sensitivity is found in the nocturnal stick insect, while the lowest is found in *C. punctata* and *G. lacustris*. The crepuscular/nocturnal cockroach and *N. glauca* demonstrate intermediate medians. If data from all five species are combined, a moderate positive correlation emerges (SRCC = 0.38, *P* < 10⁻²). Overall, these results imply that 1) cell size is an important general factor determining absolute sensitivity; 2) the absolute sensitivities of these species are consistent with their visual ecologies; and 3) variation in capacitance may have functional significance.

However, the scatterplots (Fig. 3) suggest that other factors add variability between and within species. Considering that measurements were performed from dissociated ommatidia under unnatural side-on illumination, and assuming that in such a setting effects on absolute sensitivity of interspecies dissimilarities in the corneal morphology, structure of the ommatidium, photoreceptor arrangement, and mechanisms of adaptation might be reduced or even completely negated, the variability could plausibly originate from experimental error, differences in screening pigment concentration, visual pigment concentration, or size of light-insensitive membrane. While pigment density can be studied by conventional photometry and microspectrophotometry methods, rhabdom-to-nonrhabdom membrane ratios cannot easily be determined. This latter factor might be responsible for size differences between photoreceptors of the day-active *G. lacustris* and *C. punctata*: the average capacitance of *C. punctata* photoreceptors is 7 times higher than that of *G. lacustris* photoreceptors (Table 1), but the average maximal sustained LIC is only 3.5 times higher (700 pA vs. 200 pA) (Frolov 2015; Immonen et al. 2014a). Moreover, photoreceptor size in *G. lacustris* is probably constrained by the size of the head, as smaller animals are unlikely to invest heavily into the size of photoreceptors.

However, these patch-clamp results must be interpreted with caution, since side-on stimulation is inevitably attenuated by screening pigment granules surrounding the rhabdom. By contrast, intracellular recordings of dark-adapted rhabdoms are not shielded by screening pigment, implying that they might have even higher absolute sensitivity. Further intracellular experiments are needed for replication of the in vitro findings.

### Table 1. Average photoreceptor capacitance values and notable behavioral aspects of 17 insect species

<table>
<thead>
<tr>
<th>Species</th>
<th>Capacitance, pF</th>
<th>n</th>
<th>Lifestyle</th>
<th>Notable Behavior(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apis mellifera</em> (honeybee)</td>
<td>74 ± 18</td>
<td>6</td>
<td>Diurnal</td>
<td>Fast-flying, complex aerobatics</td>
</tr>
<tr>
<td><em>Calliphora vicina</em> (blowfly)</td>
<td>105 ± 54</td>
<td>9</td>
<td>Diurnal</td>
<td>Fast-flying, complex aerobatics</td>
</tr>
<tr>
<td><em>Carausius morosus</em> (stick insect)</td>
<td>201 ± 142</td>
<td>16</td>
<td>Nocturnal</td>
<td>Light-induced thanatosis</td>
</tr>
<tr>
<td><em>Cerica punctata</em> (lesser water boatman)</td>
<td>455 ± 210</td>
<td>23</td>
<td>Low light/diurnal</td>
<td>Crepuscular/diurnal Complex aerobatics</td>
</tr>
<tr>
<td><em>Drosophila melanogaster</em> (fruitfly)</td>
<td>50–65*</td>
<td>17</td>
<td>Diurnal</td>
<td>Crepuscular/diurnal Complex aerobatics</td>
</tr>
<tr>
<td><em>Drosophila virilis</em> (fruitfly)</td>
<td>85 ± 13</td>
<td>17</td>
<td>Diurnal</td>
<td>Complex aerobics</td>
</tr>
<tr>
<td><em>Ectobius lapponicus</em>, male (cockroach)</td>
<td>60 ± 4</td>
<td>3</td>
<td>Diurnal</td>
<td>Flying</td>
</tr>
<tr>
<td><em>Gerris lacustris</em> (water strider)</td>
<td>61 ± 23</td>
<td>25</td>
<td>Diurnal</td>
<td>Very rapid water sliding</td>
</tr>
<tr>
<td><em>Gomphadorhina portentosa</em> (cockroach)</td>
<td>377 ± 32</td>
<td>3</td>
<td>Nocturnal/crepuscular</td>
<td></td>
</tr>
<tr>
<td><em>Gryllus bimaculatus</em> (cricket)</td>
<td>124 ± 54</td>
<td>13</td>
<td>Nocturnal</td>
<td>Complex aerobatics</td>
</tr>
<tr>
<td><em>Gryllus integer</em> (cricket)</td>
<td>88 ± 63</td>
<td>7</td>
<td>Nocturnal</td>
<td>Rapid escape, predator</td>
</tr>
<tr>
<td><em>Mecoptera roeselli</em> (cricket)</td>
<td>109 ± 20</td>
<td>5</td>
<td>Diurnal</td>
<td></td>
</tr>
<tr>
<td><em>Notonecta glauca</em> (backswimmer)</td>
<td>246 ± 145</td>
<td>29</td>
<td>Diurnal/noctidial</td>
<td>Rapid escape, predator</td>
</tr>
<tr>
<td><em>Panchlora nivea</em> (cockroach)</td>
<td>265 ± 79</td>
<td>52</td>
<td>Nocturnal/crepuscular</td>
<td>Flying</td>
</tr>
<tr>
<td><em>Papilio xuthus</em> (butterfly)</td>
<td>129 ± 36</td>
<td>5</td>
<td>Diurnal</td>
<td>Fast-flying, complex aerobatics</td>
</tr>
<tr>
<td><em>Periplaneta americana</em> (cockroach)</td>
<td>407 ± 199</td>
<td>44</td>
<td>Nocturnal</td>
<td>Extremely rapid running</td>
</tr>
<tr>
<td><em>Periphasma schultei</em> (stick insect)</td>
<td>224 ± 53</td>
<td>6</td>
<td>Nocturnal</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD; *n*, no. of insects. All data with the exception of *D. melanogaster* were obtained in the author’s laboratory. *A range of capacitance values of *D. melanogaster* photoreceptors is provided (see Juusola and Hardie 2001; Vähäöyriinki et al. 2006).
**Notonecta glauca: Ordered Variability for Versatile Vision**

Highly visual dark-active insects with apposition/neutral superposition eyes are evolutionarily pressured to maximize absolute sensitivity of the retina, while retaining sufficiently fast light responses. This constitutes a dilemma: for better sensitivity, a bigger rhabdomere is needed, but large rhabdomeres are associated with high capacitance that attenuates fast voltage signals by RC-filtering. This problem can be avoided, for instance, by reducing input resistance, although it is a metabolically expensive strategy. In this section, I examine the case of the water boatman *Notonecta glauca*, also known as the backswimmer, which has alleviated the problem of around-the-clock vision by further specialization of photoreceptors within the ommatidium.

Backswimmers are freshwater predators, feeding upon various aquatic and terrestrial organisms, with vision crucial for prey detection and capture as well as for recognition of suitable bodies of water during flight. They have large eyes with two high acuity and mainly binocular zones, ventral and dorsal. Morphology of the cornea facilitates forming sharp images in both air and water (Fischer et al. 2000; Horvath 1989; Schwind 1980). Backswimmers are active around the clock, although in the dark their hunting ability is diminished (Gergs et al. 2010). To adapt to diurnal changes in illumination, backswimmers extensively modulate their eye structure. Photoreceptors in each ommatidium are surrounded by pigment cells that form the entrance aperture. During the day, pigment cells move and close the aperture, shielding the peripheral photoreceptors, which are exposed in the dark (Immonen et al. 2014a). Circadian adaptation is amplified by periodic rhabdomere movements: distally during the night to increase the photoreceptor acceptance angle (the angle subtended by the photoreceptor entrance aperture) and thus improve absolute sensitivity, and proximally during the day to improve the visual acuity at the expense of sensitivity. These two mechanisms modulate light influx by two to three orders of magnitude (Laughlin 1989).

In contrast to the intrinsically random variability of the visual system of *P. americana*, the retina of *N. glauca* is a fine example of an alternative organization. The ommatidium consists of two fused central UV- or blue-sensitive rhabdomeres surrounded by six detached green-sensitive peripheral rhabdomeres, which systematically vary in cross-sectional size: two retinula cells are small, two are of intermediate size, and two are large (Fischer et al. 2000; Horridge 1968; Schwind et al. 1984). This arrangement is regular and ubiquitous and can be described as ordered variation. If it could be shown that the largest photoreceptors have the widest acceptance angles and are intrinsically more sensitive to light than their smallest counterparts, such organization could be considered an adaptive mechanism that increases the operational range of a backswimmer’s vision by amplifying the effects of the above-mentioned physiological adaptations. For this, it was necessary to demonstrate the existence of positive mutual correlations between photoreceptor capacitance, absolute sensitivity and acceptance angle. The question was studied using both patch-clamp and intracellular recording methods in a complementary fashion. Recordings from dissociated ommatidia, with light impinging from the side and not through the optics, provide quite reliable measures of capacitance and absolute sensitivity, but not of acceptance angles. On the other hand, intracellular recordings, while in theory allowing measurement of all three parameters of interest, turned out to be very challenging in the backswimmer, with capacitance and absolute sensitivity determined less robustly than in patch-clamp experiments (Immonen et al. 2014a). The results showed moderate to strong positive correlations between capacitance, sensitivity and acceptance angles, thus confirming the hypothesis and suggesting that the outer rhabdomeres can function as a scotopic system, while the central and possibly the smaller peripheral rhabdomeres can provide a basis for the higher acuity photopic system. Among other interesting findings, the average quantum bump was very large, as in the cockroach; in addition, capacitance and sensitivity to light positively correlated with information capacity, consistent with findings in other species.

Does the backswimmer’s predatory behavior, with its heavy reliance on vision, affect the properties of its photoreceptors, and, if so, how? To properly explore this question, a comparative study of photoreceptors in a closely related but not predating species with otherwise similar lifestyle was needed. However, *Notonectidae* are true bugs, the *Hemiptera*, and no common nonpredator species is known in this family. Fortunately, another *Nepomorpha* (aquatic bug) family, *Corixidae*, mainly consists of herbivore/scavenger species. *Corixidae*, also known as lesser water boatmen, share many morphological and behavioral features with backswimmers, including flight during night, rapid swimming and escape reactions. *Corixa punctata* photoreceptors were examined in a patch-clamp study (Frolov 2015). While several differences were found between *C. punctata* and *N. glauca*, the most relevant ones here were the differences in sustained K* Current density within the physiological voltage range, corner frequency and information capacity, with all three parameters approximately two times smaller in the lesser water boatman than in the backswimmer (Frolov 2015). Although LIC magnitudes were not available in the *N. glauca* study, a much higher IDR density, together with a similar sustained depolarization level, indicates that LIC in the backswimmer is also approximately two times higher than in the lesser water boatman. Considering that visual recognition of prey plays an essential role in predation by the backswimmer (Gergs et al. 2010), whereas visually-triggered escape reactions in both species clearly do not require any particular visual prowess, it is plausible that the reported differences in photoreceptor performance between the two species are specifically associated with the predatory lifestyle of *N. glauca*.

**Distinct Biophysical Properties of Specialized Photoreceptors**

How do classes of specialized photoreceptors differ from each other? In the mammalian retina, photoreceptors are separated into two major functional types, rods and cones, with rods underlying low-resolution scotopic achromatic vision, and cones responsible for high-acuity chromatic vision in well-lit environments. Likewise, specialized ommatidia and photoreceptors exist in abundance in the compound eyes of insects, serving a variety of functions from polarized light detection to precise color discrimination (Arikawa 2003; Brunner and Labhart 1987; Labhart et al. 2009; Mappes and Homberg 2004; Rossel 1989). Specialized ommatidia can either be seemingly randomly distributed in the retina, or segregated into distinct
regions, such as the dorsal rim area (DRA) in the compound eye of the cricket *Gryllus bimaculatus* used for sensing polarized light for navigation, or the high-acuity zones in the backswimmer (Labhart et al. 1984; Schwind 1980). However, how these and other specializations are reflected in biophysical properties of photoreceptors is largely unknown. One of the best studied specializations is the differences between photoreceptors responsible for chromatic vs. achromatic vision: as in mammals where temporal resolution of chromatic vision is relatively poor (Boynton 1979), predominantly blue- and UV-sensitive photoreceptors underlying color vision in insects have lower frequency resolution than green-sensitive photoreceptors that usually perform achromatic vision and motion detection (Anderson and Laughlin 2000; Srinivasan and Lehrer 1985). Differences between specialized ommatidia can go beyond such well-studied dissimilarities as spectral sensitivity. For instance, ommatidia in the cricket’s DRA are completely free of internal screening pigment and contain blue- and UV-sensitive photoreceptors, with large angular sensitivities and overlapping visual fields. Such broad spatial summation of incident light suggests high absolute sensitivity and poor contrast resolution. Indeed, two intracellular recording studies showed that blue-sensitive photoreceptors from the DRA are characterized by much slower voltage responses to light, and hence greater temporal summation, than green- and UV-sensitive photoreceptors (Labhart et al. 1984). Nevertheless, the full extent of differences between DRA and green photoreceptors remained obscure in the absence of voltage-clamp recordings. Recently, however, a comprehensive comparative patch-clamp analysis in *G. bimaculatus* revealed striking differences between these types of photoreceptors (Frolov et al. 2014).

First, inconsistent with the relatively large size of facets, absolute sensitivity of DRA photoreceptors was 35 times smaller (~1.5 log units) than that of green photoreceptors. One explanation could be that relatively low sensitivity is needed to avoid saturation of screening pigment-free DRA photoreceptors under normal illumination conditions. Moreover, the properties of elementary responses differed substantially: quantum bumps recorded from DRA photoreceptors had slightly smaller amplitudes, longer time to peak and decay constants, and longer latency than green photoreceptors (Fig. 4, A and B). As a result, responses of DRA photoreceptors to brief flashes of light of varying intensity (impulse responses) were relatively slow and prolonged, indicating inferior temporal and contrast resolution. All this implies a comparatively poor synchronization of light response in DRA photoreceptors, which are responsible for polarization sense and obviously do not mediate situational optomotor responses to the same extent as green photoreceptors. The differences in the properties of current bumps also imply that evolutionary pressures, which presumably shape photoreceptor performance by preventing unnecessary metabolic expense, can operate even at the level of consolidation of elementary responses.

The reason for low absolute sensitivity of DRA photoreceptors became clear when macroscopic LICs were examined. Depending on light intensity, LIC in DRA photoreceptors was 5–10 times smaller than in green photoreceptors (Fig. 4, C and D). Considering that the amplitudes of quantum bumps in the two types of cells were not significantly different (Fig. 4A), these results strongly suggest that the number of microvilli in a DRA photoreceptor is comparatively small. However, this conclusion is difficult to reconcile with the nearly identical average whole cell capacitance values of polarization-sensitive and green photoreceptors.

On the other hand, the relatively low absolute sensitivity of DRA photoreceptors is probably compensated by the loss of screening pigment, which provides optical isolation of the adjacent ommatidia. As a result, photons that fail to be absorbed by the visual pigment in the facet they impinge upon can be absorbed by neighboring rhabdoms. Although this would reduce acuity, the concomitant increase in absolute sensitivity of the entire region could render such a trade-off evolutionarily beneficial, especially considering that polarization vision is used not for motion detection but rather for general navigation and detection of bodies of water, as well as objects of interest (Egri et al. 2012; Krapp 2007).

Consistently with this notion that a smaller repolarizing current is required to offset the depolarizing effect of a smaller LIC, DRA photoreceptors were characterized by two to three times smaller sustained $K_v$ conductance and even smaller IA conductance than green photoreceptors. However, the less than proportional decrease in IDR conductance restricted the upper boundary of the physiological voltage range of DRA photoreceptors, which did not depolarize beyond 10 mV from resting potential, even in the brightest light. In contrast, maximal sustained depolarization in green photoreceptors averaged 25 mV. The combination of small LIC and small IDR restricted both the signal-to-noise ratio and the speed of membrane responses in DRA photoreceptors, resulting in smaller information capacity than in green photoreceptors.

While findings in the cricket illustrated profound dissimilarities between highly specialized DRA and green photoreceptors, it was also interesting to compare photoreceptors that differ less dramatically. The water strider *G. lacustris* provided
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such an opportunity (Immonen et al. 2014a). The eye of G. lacustris does not have distinct regions, although there is a narrow high acuity zone around the eye equator with high vertical resolution used for examination of nearby objects on the water surface (Dahmen 1991). Water strider ommatidia contain blue- and green-sensitive photoreceptors, with the former clearly more numerous than the latter. Dissociated ommatidia linked to blue photoreceptors are consistently more elongated and slender than ommatidia containing green photoreceptors, indicating that distinct spectral classes of peripheral photoreceptors are not combined in the same ommatidium. Blue and green photoreceptors demonstrated substantial differences: blue photoreceptors were larger than green ones (on average 69 vs. 43 pF), had a 4.6 times higher absolute sensitivity, and higher maximal LIC and IDR amplitude values. These differences appear to be quantitative in nature, arising from dissimilar average cell sizes as approximated by capacitance, and not qualitative, as in the cricket, as evidenced by the properties of quantum bumps, which were identical in amplitude and kinetics for both types of water strider photoreceptors. Interestingly, maximum membrane frequency response was significantly higher in green photoreceptors, 8.9 Hz vs. 7.3 Hz in blue photoreceptors.

While the patch-clamp studies in G. bimaculatus and G. lacustris provided detailed comparative information on specialized photoreceptors in two species, three earlier intracellular recording studies examined differences between distinct photoreceptor classes in blowfly and bumblebees. In the blowfly, Anderson and Laughlin compared properties of the central photoreceptors (long visual fibers, R7–R8) responsible for chromatic and polarization vision with those of peripheral photoreceptors (short visual fibers, R1–R6) responsible for achromatic contrast coding and motion detection (Anderson and Laughlin 2000). R1–R6 photoreceptors were found to have higher absolute sensitivity, faster responses and lower gain than R7–R8 photoreceptors. Likewise, in two species of bumblebees, green-sensitive photoreceptors that putatively provide achromatic input outperformed blue- and UV-sensitive photoreceptors involved in color vision in terms of response speed (Skorupski and Chittka 2010, 2011). All of these results are consistent with differences between the achromatic motion detection and chromatic visual processing subsystems (Anderson and Laughlin 2000).

Changing Photoreceptors

The life cycle of insects of the superorder Endopterygota, also known as Holometabola, which include butterflies, flies, bees, etc., is characterized by complete metamorphism with distinctive larval, pupal, and imago stages. Consequently, the eyes of the newly closed adults do not appear to undergo such dramatic changes with age as the eyes of hemimetabolous species that grow continuously during postembryonic development, with accompanying profound changes in the retina (Keskinen et al. 2002; Keskinen and Meyer-Rochow 2004; Meyer-Rochow and Keskinen 2003; Stark and Mote 1981). However, until recently, virtually nothing was known about developmental changes in the electrical properties of photoreceptors.

Compound eyes grow at their periphery, gradually adding new facets (Stark and Mote 1981). At the same time, the overall retinal thickness and facet size in the middle of the eye also increase, indicating that the ontogenetically older photoreceptors are unlikely to grow completely until the late larval stages. However, due to the tiered structure of the ommatidium, it cannot be excluded that at least some photoreceptors reach maturity relatively early without a noticeable effect on the overall retina thickness. What differences between larval and adult photoreceptors can be predicted a priori? First, the absolute sensitivity of larval photoreceptors is expected to be lower than that of adult photoreceptors for the trivial reason of differing rhabdom sizes. Second, growth entails an increase in whole cell membrane capacitance and a decrease in resistance. However, since the timelines for the expansion of photoreceptor membrane and expression of different channels are not necessarily identical, the changes in specific membrane resistance might not match the increasing capacitance, so that membrane time constant can be different at different instar stages. Third, the chanelome expansion might be unbalanced, with consequences for membrane depolarization during light response. Finally, if larval photoreceptors have a smaller number of microvilli than adult photoreceptors, which is naturally anticipated, then both the temporal resolution and light-voltage gain in larvae should be relatively lower.

Postembryonic developmental changes in photoreceptors were examined in C. morosus and G. lacustris and discussed at length in Frolov and Weckström (2014). Figure 5, A–E, shows pictures of typical first instar and adult stick insects, with micrographs of dissociated ommatidia. Developmental patterns discovered in C. morosus and G. lacustris contain conspicuous differences. Common trends include the foreseen increases in absolute sensitivity, capacitance, amplitudes of LIC and IDR, and maximal information rate. The density of IA obtained by dividing the maximal conductance by capacitance decreases with growth in both species, probably reflecting the decrease in the relative area of light-insensitive membrane where IA channels are thought to be expressed (Rogero et al. 1997). There were two main developmental dissimilarities. First, in C. morosus, the level of steady-state depolarization in response to light increased dramatically from the first-instar larva to the imago stage, while in G. lacustris no such change was observed. Second, the average membrane corner frequency increased in the water strider, from 3.9 Hz in the first instar larvae to 7.7 Hz in adults, but decreased in the stick insect from 7.4 Hz in the first instars to 5.1 Hz in adults.

It appears that both of these differences can be explained by developmental changes in IDR. In the stick insect, IDR density was higher in the first instar larvae than in the adults. In contrast, IDR density in G. lacustris first instar larvae was more than three times lower than in adults. At the same time, the density of LIC increased by 1.2-fold from first instar to adult in both animals. As a result, depolarization is dampened stronger in stick insect larvae than in adults (Fig. 5F), while the situation in the water strider is opposite. Furthermore, in stick insect larvae, the IDR and capacitance are correspondingly sufficiently large and small enough to yield a corner frequency higher than that in adults. Again, the situation is opposite in the water strider. Interestingly, in C. morosus and G. lacustris first instar larvae, the ratio of average corner frequencies determined by fitting light-voltage gain functions (1.77) is close to the ratio of frequencies obtained by dividing the maximal
the population, a feat hardly feasible for large, up to 10 cm in length, slow-moving adults (Meyer-Rochow and Keskinen 2003). Interestingly, developmental changes in stick insect photoreceptors are consistent with those in the lifestyle: the relatively high corner frequency in the first instar larvae facilitates processing of visual information in bright-lit environments, while the low corner frequency in adults is more appropriate for vision in the dark as it augments temporal summation.

Finally, it is instructive to examine the available information about the post-eclosion changes in basic electrophysiological properties of photoreceptors in holometabolous insects. Using intracellular recordings, Rudolf and coauthors recorded from dark- and light-reared blowflies and found that membrane time constant decreased by a third during the first week as a result of decreasing membrane resistance (Rudolf et al. 2014). Since dark consumption of energy in the blowfly photoreceptors is already very high, the metabolic savings due to the relatively high resistance in immature flies may be quite substantial. Surprisingly, membrane resistance was slightly lower in dark-reared flies than in light-reared flies consistently over the entire lifespan. It would be interesting to examine input resistance at the immediate preeclosion pupal stage, but such data are not yet available.

**Kv Conductances and Visual Ecology Revisited**

It was noticed in the early 1990s that different flies express distinct suites of Kv conductances, and that this could be explained in terms of lifestyle and behavior. Weckström and Laughlin probed photoreceptors from 20 species of Diptera using intracellular recording and concluded that rapid, maneuverable diurnal fliers tend to have fast photoreceptors characterized by relatively high corner frequencies, low gain, low input resistance, low sensitivity and fast light adaptation (Laughlin and Weckström 1993; Weckstrom and Laughlin 1995). These properties were in contrast with the characteristics of photoreceptors in the slow-flying, mostly nocturnal Tipulid flies. Crucially, the differences between Kv channelomes were consistent with the perceived differences in functional requirements of fast and slow photoreceptors. Diurnal fast-fliers predominantly expressed a non-inactivating Kv current, which facilitates rapid membrane response, low input resistance and low gain. In contrast, nocturnal and slow-flying flies featured a largely inactivating Kv current, which prevents excessive K\textsuperscript{+} influx and thus saves metabolic energy. This theory of differential Kv channel expression was later reinforced by finding circadian changes in the properties of Kv conductance in the locust, which was markedly more inactivating during night than day (Cuttle et al. 1995). Likewise, the diurnal/crepuscular, slow-flying fruit fly D. melanogaster was characterized by a prominent transient Kv current that also fits the proposed model. However, it was not clear if the trends discovered in Diptera were true for insects in other orders. Indeed, patch-clamp recordings from P. americana (Salmenla et al. 2012) and D. virilis (E.-V. Immonen, personal communications) presented a challenge: the nocturnal cockroach, for whom vision is clearly a secondary sense, displayed a large non-inactivating delayed rectifier, while the diurnal D. virilis showed a Kv current with strong IA indistinguishable from that in its smaller counterpart.

Fig. 5. Changing photoreceptors of the stick insect. A: photographs of adult and first instar stick insects. B and C: photographs of eyes from first instar and adult stick insect, respectively. D and E: micrographs of typical dissociated ommatidia from first instar and adult stick insect, respectively. F: representative voltage responses to white-noise-modulated light contrast in nymphs and adults at a light level yielding the maximal information rate. [Adapted with changes from Frolov et al. 2012a with permission.]

average IDR values by average capacitances (1.97) (see Table 1 in Frolov and Weckström 2014).

However, one developmental aspect in C. morosus cannot be explained satisfactorily. Current quantum bumps in the first instars were 30% larger than in adults. This, together with more permissive membrane filtering due to a 5.4 times lower average capacitance but only 2 times higher input resistance, yielded voltage quantum bumps of 5 mV amplitude on average, an extremely high value for insect photoreceptors. While the physiological purpose, if any, of this feature is not understood, it serves as a reminder that growth-related changes may be nontrivial, and that more comparative studies are needed to reveal additional developmental patterns.

There is a visual ecological question related to lifestyle dissimilarities between the first instar larvae and imagoes of the stick insect. First, instar larvae are diurnal, ground-roaming animals, while adults are nocturnal. It is believed that diurnal lifestyle of small inconspicuous larva facilitates expansion of the population, a feat hardly feasible for large, up to 10 cm in length, slow-moving adults (Meyer-Rochow and Keskinen 2003). Interestingly, developmental changes in stick insect photoreceptors are consistent with those in the lifestyle: the relatively high corner frequency in the first instar larvae facilitates processing of visual information in bright-lit environments, while the low corner frequency in adults is more appropriate for vision in the dark as it augments temporal summation.

Finally, it is instructive to examine the available information about the post-eclosion changes in basic electrophysiological properties of photoreceptors in holometabolous insects. Using intracellular recordings, Rudolf and coauthors recorded from dark- and light-reared blowflies and found that membrane time constant decreased by a third during the first week as a result of decreasing membrane resistance (Rudolf et al. 2014). Since dark consumption of energy in the blowfly photoreceptors is already very high, the metabolic savings due to the relatively high resistance in immature flies may be quite substantial. Surprisingly, membrane resistance was slightly lower in dark-reared flies than in light-reared flies consistently over the entire lifespan. It would be interesting to examine input resistance at the immediate preeclosion pupal stage, but such data are not yet available.

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In the companion paper (Frolov et al. 2016), new results from studying Kv conductances in 15 phylogenetically diverse species are presented. In brief, it was confirmed that rapid diurnal flyers, such as the blowfly C. vicina, the honeybee A. mellifera, and the butterfly P. Xuthus, indeed express relatively large non-inactivating Kv conductances. However, nocturnal and less visually demanding insects did not generally demonstrate strong IA of Kv current in the physiological voltage range, and no substantial trend in regard to visual performance could be identified for the transient fraction of Kv conductance. Instead, photoreceptors in species that are known to rely more on vision behaviorally were found to have high densities (conductance per capacitance ratio) of sustained Kv conductance than photoreceptors of less visual species.

Interestingly, strong negative correlations were found between photoreceptor capacitance and specific membrane conductance for both sustained and inactivating components of Kv conductance (Fig. 6), which, considering that increased capacitance enhances low-pass filtering, raises questions regarding the character of changes in the photoreceptor membrane. In D. melanogaster, IA and IDR channels are expressed in different membrane regions: the former in light-insensitive membrane, and the latter thought to be primarily around microvilli. If such differential expression also occurs in other species, then the only plausible explanation for the observed negative correlations is the predominant increase in the total membrane area of the rhabdom. It should be noted that an ordinary increase in the number of microvilli is unlikely to result in a prominent decrease in IDR density with capacitance, unless the length of the average microvillus is also increased. Indeed, it follows from Fig. 6A that IDR conductance does not correlate negatively with capacitance, and that IDR density decreases more slowly with capacitance than IA density, which is consistent with the above hypothesis. Comparative morphological studies of rhabdoms are necessary for more definite test of this hypothesis. Nevertheless, since the increased capacitance is opposed by a similarly increased Kv conductance in the physiological voltage range (Fig. 6A), it appears that there might be an evolutionary trade-off between increased absolute sensitivity, which is associated with high capacitance, and the negative low-pass filtering effects of that high capacitance on the speed of photoreceptor responses.

Conclusions and Future Directions

Patch-clamp studies of photoreceptors in dissociated ommatidia can provide a wealth of new information, some of which is difficult or impossible to obtain in vivo, such as recording of voltage- and light-activated currents from the same cells, manipulation of extracellular and intracellular solutions, and pharmacological interventions, with analysis of ensuing changes in LIC and voltage responses. In this author’s view, one of the least explored and most promising aspects of such experiments is the possibility to study photoreceptor properties comprehensively on an individual basis, by recording all kinds of responses, from current and voltage bumps to macroscopic responses to contrast-modulated stimuli, with consecutive search for trends and patterns. As shown here, such analyses have already yielded interesting results.

However, while comparative physiologists can usually explain their findings at the cellular level in terms of general physiological differences between studied species, the true test of scientific understanding would be a reliable prediction of relevant lifestyle/behavior on the basis of findings at the cellular level in the sensory system of interest. What do we know about photoreceptor biophysics from the patch-clamp studies that would allow us to predict the insect’s visual ecology? Currently, there are only a handful of parameters unambiguously linked to certain lifestyles/behavioral traits. First is absolute sensitivity: high sensitivity is a feature of dark-active insects (Fig. 3). Another parameter is low input resistance at voltages near resting potentials due to high sustained Kv conductance; this is strongly associated with highly-maneuverable fliers (Fig. 5) and not found in other species, even such visually guided predators as N. glauca. High information capacity with high membrane corner frequency is a feature of flying predators (Niven et al. 2007). Finally, a relatively high photoreceptor capacitance is indicative of noc-

![Fig. 6.](http://jn.physiology.org/)

**Fig. 6.** Kv conductances of insect photoreceptors. A and B: correlations between photoreceptor capacitance and sustained conductance (A) and conductance density (B), determined as averages over the physiological voltage range, from −60 to −20 mV. C and D: correlations between photoreceptor capacitance and inactivating conductance (C) and conductance density (D), determined as averages in the range from −50 to −20 mV. The same data are shown in Frolov et al. (2016), with addition of data for the Cuban cockroach *Panchlora nivea.*
urnal or crepuscular lifestyle, as discussed above. Thus a species with high absolute sensitivity and low input resistance of photoreceptors is likely to be a nocturnal flier, whereas a combination of comparatively low capacitance, low absolute sensitivity and high input resistance would betray a diurnal insect with limited flying and visual abilities. However, a genuinely blind electrophysiological testing of insect photoreceptors in dissociated ommatidia (or any other primary cellular preparation) is difficult to establish in the conventional laboratory setting.

A number of substantive questions need to be addressed in future comparative electrophysiological studies of photoreceptors. These include, but are not limited to, the following: evolution-driven differences in the molecular workings of the phototransduction cascade; the identities and roles of specific light-activated and Kv conductances; their differential expression within and between photoreceptors in the context of photoreceptor specialization and between species in the context of evolution and visual ecology; the functional electrical interaction between photoreceptors; and characterization of photoreceptors in superposition eyes.

For example, it is now clear that channelomes of photoreceptors can vary widely between insect species, but how they evolved and how specific conductances optimize photoreceptor function remain largely unknown. Even the immediate functions of some channels remain poorly understood: for instance, although it was claimed that IA in D. melanogaster strongly influences the information capacity of photoreceptors (Niven et al. 2003), this question requires more scrutiny because, in other species that express strong IA, it rapidly and completely inactivates at low depolarization voltages and can plausibly affect neither the sustained depolarization level, nor resolution of contrast. As another example, it is not yet clear why in some species the main light-activated channel is TRP, like in D. melanogaster (Hardie and Minke 1992), while in others, a TRPL-like one, like in the cockroach (French et al. 2015: Immonen et al. 2014b). Although the most plausible hypothesis is that the high-conductance TRPL channels support large quantum bumps putatively instrumental for vision in the dark, this question still requires a thorough comparative electrophysiological and molecular investigation.

Little is known about electrical properties of photoreceptors in the superposition compound eyes, with all information available coming from intracellular recordings (see e.g., Belusic et al. 2013: Warrant and McIntyre 1990). We have attempted dissociation and patching of such retinas on several occasions but without much success, although it is possible that proper investment of time and efforts will yield tangible results.

There is one methodological issue that needs attention: correspondence between patch-clamp and intracellular recording results. Specifically, studies in the cockroach revealed two major discrepancies, in input resistance values and corner frequencies of light responses to contrast-modulated light. In intracellular recordings, the input resistance was usually on the order of 100 MΩ, whereas in whole-cell patch-clamp recordings it was rarely below 300 MΩ (Heimonen et al. 2012). The likely reason for this difference is the leaky membrane-glass interface in the intracellular setting, which adds a localized leak conductance. The second discrepancy is that between the corner frequencies: in intracellular recordings, the corner frequency was typically close to 20 Hz, while in patch-clamp experiments the average maximal corner frequency was usually below 7 Hz. Although the relatively large instrumental leak conductance associated with intracellular experiments might facilitate the greater bandwidth, more research is required in this direction.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.V.F. prepared figures; R.V.F. drafted manuscript; R.V.F. edited and revised manuscript; R.V.F. approved final version of manuscript.

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