Developmental changes in biophysical properties of photoreceptors in the common water strider (Gerris lacustris): better performance at higher cost

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Frolov R, Weckström M. Developmental changes in biophysical properties of photoreceptors in the common water strider (Gerris lacustris): better performance at higher cost. J Neurophysiol 112: 913–922, 2014. First published May 28, 2014; doi:10.1152/jn.00239.2014.—Although the dependence of invertebrate photoreceptor biophysical properties on visual ecology has already been investigated in some cases, developmental aspects have largely been ignored due to the general research emphasis on holometabolous insects. Here, using the patch-clamp method, we examined changes in biophysical properties and performance of photoreceptors in the common water strider Gerris lacustris during postembryonic development. We identified two types of peripheral photoreceptors, green and blue sensitive. Whole cell capacitance (a measure of cell size) of blue photoreceptors was significantly higher than the capacitance of green photoreceptors (69 ± 20 vs. 43 ± 12 pF, respectively). Most of the measured morphological and biophysical parameters changed with development. Photoreceptor capacitance increased progressively and was positively correlated with sensitivity to light, magnitudes and densities of light-induced (LIC) and delayed rectifier K⁺ (I_{D,R}) currents, membrane corner frequency, and maximal information rate [Spearman rank correlation coefficients: 0.70 (sensitivity), 0.79 (LIC magnitude), 0.79 (I_{D,R} magnitude), 0.48 (corner frequency), and 0.57 (information rate)]. Transient K⁺ current increased to a smaller extent, while its density decreased. We found no significant changes in the properties of single photon responses or levels of light-induced depolarization, the latter indicating a balanced channelome expansion associated with I_{D,R} expression. However, the dramatic ~7.6-fold increase in I_{D,R} from first instars to adults indicated a development-related rise in the metabolic cost of information. In conclusion, this study provides novel insights into functional photoreceptor adaptations with development and illustrates remarkable variability in patterns of postembryonic retinal development in hemimetabolous insects with dissimilar visual ecologies and behaviors.

Gerris lacustris: water strider; photoreceptor; ion channels; photoreceptor function; accommodate behavioral challenges imposed by the growing animal’s visual ecology, which may be quite different from that of the adult.

We recently explored this issue in the stick insect Carausius morosus by comparing photoreceptor electrophysiology of first-instar nymphs and adults (Frolov et al. 2012). A number of intriguing differences between nymphs and adults were found, including larger voltage bumps, smaller delayed-rectifier K⁺ current (I_{D,R}), and lower information capacity of nymph photoreceptors compared with adults. Overall, developmental changes in stick insect photoreceptors appeared to enhance the shift from diurnal nymph to nocturnal adult lifestyle. However, stick insects have very specialized behavior and visual ecology, so these results might not be representative of hemimetabolous insects in general, with their wide morphological and behavioral diversity. Therefore, it is important to study visual development in other species with different lifestyles.

The common water strider or pond skater Gerris lacustris is a predatory insect, hunting on the water surface. Water striders can move extremely quickly on the water surface using hydrophobic legs (an average speed of 1.5 m/s corresponds to 150 body lengths/s), and observation suggests that they possess excellent vision. Water striders have round protruding apposition eyes each containing ~920 ommatidia. Each ommatidium includes eight photoreceptors, six peripheral and two central, forming an open rhabdom with perpendicularly oriented microvilli, hinting at polarization vision (Horvath 1995; Schneider and Langer 1969). A narrow acute zone exists in the equatorial plane of the eye (Dahmen 1991). The optics and arrangement of rhabdomeres in different facets, with photoreceptor contacts below the retina, all suggest the neural superposition mechanism of visual processing (Dahmen 1991; Wolburg-Buchholz 1979). During postembryonic development after hatching from the egg, water strider nymphs progress through five progressively longer lasting molts. Nymph water striders behave similarly to adults, occupying the same ecological niche and facing similar challenges, making them a useful model of typical growth-related changes in photoreceptor properties.

Here, we studied photoreceptors from dissociated ommatidia of water striders at different developmental stages using the patch-clamp method. We found two classes of peripheral photoreceptors, with spectral sensitivity maxima to blue and green light. Photoreceptor growth was associated with gradual changes in most parameters measured, such as capacitance, light sensitivity, ionic conductance, and information processing. However, we also found significant differences compared with the developmental pattern of stick insect eyes.
MATERIALS AND METHODS

Preparation and Morphological Measurements

Nymph and adult water striders (Gerris lacustris) were captured from two small artificial ponds in the Botanical garden at the University of Oulu, Oulun Yliopisto, Finland, over the course of two consecutive seasons, from May to September. Both nymphs and adults were studied during each season. No differences in experimental results were observed between the 2 yr. However, since the experiments were conducted over two seasons and interseasonal weather dissimilarities can not only affect insect growth, but also even alter instar numbers (Esperk et al. 2007), we did not group water strider nymphs into instar stages but instead used body size and photoreceptor capacitance (as a proxy of size/membrane area) as developmental indicators.

Ommatidia were dissociated as described previously (Frolov et al. 2012, 2014; Hardie et al. 1991). In brief, after decapitation the eyes were cut off and retinas scooped out and cut into several pieces. The retinal fragments were then gently triturated until ommatidia started to fall off. Separate ommatidia were allowed to settle in the recording chamber on the stage of an inverted microscope (Axioskop 35M; Zeiss, Oberkochen, Germany). Eye and body sizes were measured under the preparation microscope along the anteroposterior axis using an electronic caliper. Ommatidial measurements were taken using a microelectrode mounted on a Sensapex micromanipulator (Sensapex, Oulu, Finland) as described in the Fig. 1 legend.

Electrophysiology

Whole cell recordings from dissociated ommatidia were performed at room temperature exactly as described previously (Frolov et al. 2014). Because of the geometry of the recording situation, all data are probably from peripheral photoreceptors. In brief, Sensapex micromanipulators, an Axopatch 1-D patch-clamp amplifier, and pClamp 9.2 software (both Axon Instruments/Molecular Devices) were used for data acquisition and analysis. Patch electrodes with resistance of 5.0–9.0 MΩ were fabricated from thin-walled borosilicate glass (World Precision Instruments, Sarasota, FL). Bath solution contained the following (in mM): 120 NaCl, 5 KCl, 4 MgCl2, 1.5 CaCl2, 10 N-Tris(hydroxymethyl)-methyl-2-amino-ethanesulfonic acid (TES), 25 proline, and 5 alanine, pH 7.15 (with NaOH). Patch pipette solution contained the following (in mM): 140 KCl, 10 TES, 2 MgCl2, 4 Mg-ATP, 0.4 Na-GTP, and 1 NAD, pH 7.15 (with KOH). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The liquid junction potential between bath and intracellular solution was ~4 mV. All voltage values cited in text were corrected for liquid junction potential. The series resistance was compensated by 80% and after compensation was typically <10 MΩ. No additional correction of presented voltage values was performed. Membrane capacitance was calculated from the total charge flowing during capacitive transients for voltage steps between −104 and −8474 mV. A K+ reversal potential of ~84 mV was used for calculating K+ conductances.

For light stimulation, 10 monochromatic light-emitting diode (LEDs; Roithner Laser Technik), covering a range from 355 to 639 nm, were used in combination with a series of neutral density filters (ranging from 0 to 8 log units of attenuation; Kodak). To estimate photoreceptor spectral sensitivity, a stimulation protocol was used to deliver 50-ms flashes of light from all 10 LEDs. With the use of a spectrometer USB4000 (Ocean Optics) the driving voltage of each LED was adjusted to give approximately the same number of photons as the ultraviolet LED driven with 10 V using the spectrometer software. To measure photoreceptor performance, two contrast-modulated stimuli were used: a computer generated a 90-s stimulus consisting of 30 repetitions of 2 s of Gaussian randomly modulated band-limited white noise (f3dB = 250 Hz) preceded by 30 s of steady light with the intensity equal to the mean intensity of the white noise contrast (WN1), and a 116-s light contrast series (WN2) made of 58 repetitions of a 2-s sequence obtained from the WN1 by filtering it using a Chebyshev 8-pole filtering algorithm at 15 Hz and multiplying the resulting contrast by factor of 4 to maximize the stimulus power within the available bandwidth.

Data Analysis

Photoreceptor performance was examined in the frequency domain using MatLab (MathWorks) exactly as described previously (Frolov et al. 2014). In brief, responses to a 2-s white noise modulated light signal, S(f), were estimated by averaging voltage responses to repetitions of the 2-s white-noise sequence. The noise was then obtained by subtracting the signal estimate from the original (noise-containing) sequences (Kouvalainen et al. 1994). The signal-to-noise ratio [SNR(f) = S(f)/N(f)] was obtained in the frequency domain as SNR(f) = |S(f)|^2/N(f)^2. The contrast gain of voltage responses T(f) was calculated by dividing the cross-spectrum of photoreceptor input [white-noise contrast, C(f)] and output (photoreceptor signal) S(f)·C*(f) (f denotes the complex conjugate), by the autospectrum of the input C(f)·C*(f) and taking the absolute value of the resulting frequency response function T(f): T(f) = |S(f)·C*(f)/C(f)·C*(f)|. The Shannon information rate (IR) for white-noise modulated responses was calculated according to the equation IR = −∫log2[S(f)/N(f)] df within a frequency range from 0.98 to 15.14 Hz [window length, 3,000 ms; nonuniform fast Fourier transform (NFFT), 4,096 points]. The 3-dB frequency was obtained by fitting the gain function with a first-power Lorentzian function.

All values are given as means ± SD unless stated otherwise; n indicates the number of experiments. Statistical analysis was performed by unpaired t-test (for samples that passed the Shapiro normality test at P < 0.05 level) or Mann-Whitney U-test for other data.
RESULTS

General Properties

We studied water striders at different developmental stages, from a body length of slightly above 1 mm, to adults of 8- to 10-mm long. More than 20 adults and 20 nymphs of all stages were used in electrophysiological experiments. Figure 1 shows that the eye size, length and width of ommatidia, and whole cell capacitance of photoreceptors (a measure of cell size) increased progressively with body length (ρ = 0.93, P < 10^{-5}, n = 38; ρ = 0.96, P < 10^{-6}, n = 8; ρ = 0.87, P < 0.05, n = 8; and ρ = 0.56, P < 10^{-5}, n = 51, respectively). In particular, capacitance was 29 ± 7 pF (n = 14) in first instar nymphs and 60 ± 21 nF (n = 33) in adults. Because average photoreceptor capacitance correlated linearly with body length (Fig. 1D), it provides a useful immediate indicator of photoreceptor development.

Responses were recorded from 23 green-sensitive photoreceptors and 48 blue-sensitive photoreceptors originating from water striders of different developmental stages (Fig. 2). Spectral sensitivities [approximated by the amplitude of light-induced current (LIC) responses to different wavelength LEDs] of nymph and adult photoreceptors of each spectral class were identical. Interestingly, in adults blue photoreceptors were noticeably larger and had a significantly higher whole cell capacitance (a measure of cell size) than green ones (69 ± 20 vs. 43 ± 12 pF, respectively, P < 0.001; n = 23 and 10, respectively). Moreover, it appears that water strider individual ommatidia contain peripheral photoreceptors of a single spectral type as, without exception, all ommatidia where blue photoreceptors were recorded were substantially longer and more slender than ommatidia from which green photoreceptor recordings were obtained. This difference was so salient that it was possible to discern the spectral classes of photoreceptors in the dissociated ommatidia very reliably by microscope examination alone. In addition, blue photoreceptors (and blue-like ommatidia by visual observation) were more numerous than green ones, in agreement with the numbers cited above.

Figure 3 shows typical light responses of adult and nymph photoreceptors to steady light of increasing intensity (Fig. 3, A and B) and quantifies transient and sustained depolarization of five photoreceptors from adult water striders (Fig. 3C). The resting potential was ∼60 ± 4 mV (n = 17). No significant differences in the levels of “shot noise” or voltage bump amplitudes were observed between nymphs and adults.

Sensitivity to Light

Despite the relatively small size and capacitance, variation in capacitance among water strider photoreceptors correlated with variation in absolute sensitivity to light. Photoreceptor sensitivity was measured by counting voltage responses to single photons (“voltage bumps”) evoked by continuous stimulation at low light intensities (eliciting responses at a rate that was low enough to allow reliable bump counting; on average, the rate of evoked bumps was 3.3 ± 1.8 s^{-1}). For each photoreceptor, sensitivity to light was defined as the reciprocal of light intensity that would induce one effective bump per second. The relative light sensitivity was then calculated as a fraction of the sensitivity of the most sensitive cell in the experiments. Figure 4 shows the correlation between sensitivity to light and capacitance. For the combined sample of green and blue photoreceptors from water striders of all developmental stages, the Spearman rank order correlation coefficient was...
quantum bumps, the increased magnitude of whole cell currents in either amplitude or kinetics of
increase with growth (Fig. 5). Photoreceptor capacitance correlated strongly with the magnitude of maximal steady-state LIC shown in Fig. 5. Photoreceptor capacitance correlated with the idea that the growth of photoreceptors during development mainly occurs in the rhabdom, possibly in its length, as well as in the number and size of microvilli, increasing the area of the light-absorbing membrane.

Light-Induced Currents

Figure 5A compares average quantum bumps from adults and first instar nymphs triggered by 1-ms flashes of light. Typical maximal macroscopic LIC in adult and first instar nymph photoreceptors, obtained with 4-s long light pulses, are shown in Fig. 5B. Photoreceptor capacitance correlated strongly with the magnitude of maximal steady-state LIC (LICss) determined as the average current during the last 3 s of the 4-s long current response to the light intensity that elicited the largest LIC (ρ = 0.79, P < 10⁻⁶) for all cells combined, n = 30; ρ = 0.78, P < 10⁻⁴ for blue photoreceptors only, n = 20; Fig. 5C). From first instar to adult water striders, average maximal LICss increased by 2.8-fold from 73 ± 24 pA (n = 5) to 204 ± 139 pA (n = 10). Although no statistically significant correlation was found between capacitance and maximal LICss density obtained by dividing maximal LICss by cellular capacitance (ρ = 0.32, P = 0.09 for all cells combined; ρ = 0.29, P = 0.2 for blue photoreceptors only), LIC density tended to increase with growth (Fig. 5D and Table 1).

As no substantial difference was observed between nymph and adult water striders in either amplitude or kinetics of quantum bumps, the increased magnitude of whole cell currents suggests that growth-related changes in the rhabdom are limited to increasing the number and size of microvilli.

Voltage-Activated K⁺ Currents

Voltage-activated K⁺ channels in the photoreceptor membrane are responsible for resting potential, prevention of excessive membrane depolarization, and regulation of voltage responses in a voltage- and time-dependent manner (Niven et al. 2003b; Vähäsöyrinki et al. 2006; Weckström et al. 1991; Weckström and Laughlin 1995). As in many other insect species, we found two types of voltage-activated outward currents, with hallmarks of a rapidly activating and inactivating A-type K⁺ current (IA) and a slowly-inactivating (at positive potentials) delayed rectifier type K⁺ current (IDR) in water strider photoreceptors (Fig. 6). IA could be completely removed with an inactivating prepulse to −34 or −44 mV between 0.5 and 2 s in duration (prepulse duration and voltage can be selected during recordings depending on the magnitude of the remaining IA so as to abolish it without altering IDR substantially). Figure 6, A and B, shows the total K⁺ current, IDR, and IA recorded from typical adult and first instar nymph photoreceptors. In adults, the magnitudes of maximal IDR and IA were similar, although with large variability from cell to cell (Fig. 6C). The half-activation potential was −19 ± 6 mV for IA and −18 ± 10 mV for IDR (n = 23).

Developmental changes we found in K⁺ conductances were a prominent increase in IDR and a smaller increase in IA: photoreceptor capacitance correlated strongly with the maximal IDR conductance (ρ = 0.81, P < 10⁻⁶ for all cells combined, n = 62; ρ = 0.79, P < 10⁻⁶ for blue photoreceptors only, n = 36), whereas only a weak correlation was observed between capacitance and IA (ρ = 0.30, P = 0.019 for all cells combined, n = 59; ρ = 0.28, P = 0.1 for blue photoreceptors only, n = 35; Fig. 6, D and E). In particular, maximal IDR conductance increased by 7.6-fold from first instar nymphs to adults [from 2.3 ± 1.4 nS (n = 12) to 17.5 ± 7.5 nS (n = 23), respectively] while capacitance only increased twofold. At the same time, maximal IA increased from 9.2 ± 3.9 nS in first instar nymphs to 17.8 ± 10.8 nS in adults or less than twofold (P < 0.01). When changes in current density (maximal current divided by capacitance) were examined, positive correlation was found between IDR density and capacitance (ρ = 0.53, P < 10⁻⁵ for all cells combined; ρ = 0.45, P = 0.006 for blue photoreceptors only) and a weak negative correlation was
Physiological Development of Gerris lacustris Photoreceptors

Table 1. Comparison of photoreceptor properties in water strider and stick insect adults and first instar nymphs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water Strider, Adult</th>
<th>Water Strider, First Instar</th>
<th>Stick Insect, Adult</th>
<th>Stick Insect, First Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitance, pF</td>
<td>60 ± 21</td>
<td>29 ± 7</td>
<td>212 ± 104</td>
<td>37 ± 21</td>
</tr>
<tr>
<td>I_{DR}, nA</td>
<td>17.5 ± 7.5</td>
<td>2.3 ± 1.4</td>
<td>11.9 ± 0.9</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>I_{A}, nA</td>
<td>17.8 ± 10.8</td>
<td>9.2 ± 3.9</td>
<td>7.5 ± 1.0</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>I_{DR} density, nS/pF</td>
<td>0.29 ± 0.12</td>
<td>0.08 ± 0.04</td>
<td>0.10 ± 0.08</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>I_{A} density, nS/pF</td>
<td>0.31 ± 0.19</td>
<td>0.33 ± 0.13</td>
<td>0.07 ± 0.08</td>
<td>0.19 ± 0.10</td>
</tr>
<tr>
<td>LIC_{SS}, pA</td>
<td>204 ± 139</td>
<td>–73 ± 24</td>
<td>–691 ± 440</td>
<td>–82 ± 45</td>
</tr>
<tr>
<td>LIC_{SS} density, pA/pF</td>
<td>–2.8 ± 1.1</td>
<td>–2.3 ± 0.7</td>
<td>–3.2 ± 1.9</td>
<td>–2.6 ± 1.0</td>
</tr>
<tr>
<td>Current bump, pA</td>
<td>–12.2 ± 3.6</td>
<td>–11.1 ± 2.5</td>
<td>–19.1 ± 1.7</td>
<td>–26.9 ± 3.4</td>
</tr>
<tr>
<td>Voltage bump, mV</td>
<td>1 – 2*</td>
<td>1 – 2*</td>
<td>1.9 ± 0.2</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>LIC_{SS}-to-current bump ratio</td>
<td>16.7 (2.53)</td>
<td>6.6</td>
<td>36.2 (12.1f)</td>
<td>3.0</td>
</tr>
<tr>
<td>IR(_T), bits/s</td>
<td>60 ± 6 (2.3f)</td>
<td>26 ± 12</td>
<td>34.1 ± 5.0 (11.8f)</td>
<td>2.9 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. I_{DR}, delayed rectifier type K\(^+\) current; I_{A}, A-type K\(^+\) current; LIC_{SS}, steady-state light-induced current. *In the water strider, as voltage bumps in nymphs and adults at resting potential level were very similar, no attempt was made to analyze them beyond rough size estimation. †Information rate (IR) as measured by different stimuli: WN1 in the stick insect and WN2 in the water strider. ‡Adult-to-nymph ratios are shown for each species.

Fourier allows transmission of faster signals with less attenuation. Membrane noise can be assumed not to be similarly amplified, and the unavoidable photon shot noise only increases as a function of square root of light intensity (see, e.g., Juusola et al. 1994). Accordingly, we found that both f_{34dB} and IR increased with photoreceptor size (Fig. 7, D and E). There were moderate positive correlations between capacitance and f_{34dB} (ρ = 0.48, P = 0.004 for all cells combined, n = 34; ρ = 0.64, P = 0.0004 for blue photoreceptors only, n = 26) and between capacitance and IR_{max} (ρ = 0.57, P = 0.002 for all cells combined, n = 27; ρ = 0.51, P = 0.018 for blue photoreceptors only, n = 21). The greater number of points for adults in Fig. 7D than in Fig. 7E is due to using values of f_{34dB} from responses to both WN1 and WN2 stimuli.

Developmental Changes in the Cost of Signaling

Metabolic expenses associated with processing visual stimuli in the form of membrane potential modulation can be estimated by calculating the magnitude of the total voltage-activated K\(^+\) current at the light-induced steady-state depolarization when other types of voltage-activated conductances are absent (Niven et al. 2003a). Using this method we examined the general developmental trends in metabolic signaling cost in response to contrast-modulated light near IR_{max}. Only I_{DR} was used for calculations because at potentials corresponding to IR_{max} the contribution of I_{A} was negligible due to inactivation. No correlation was found between photoreceptor capacitance and IR_{max} depolarization level (Fig. 8A). However, the increase in I_{DR} with development caused strong correlation between capacitance and I_{DR} at the IR_{max} depolarization level (ρ = 0.75, P < 10^{-6} for all cells combined, n = 32; ρ = 0.84, P < 10^{-6} for blue photoreceptors only, n = 24; Fig. 8B). This increase in I_{DR} was moderately correlated with improvement in membrane bandwidth (ρ = 0.63, P = 10^{-3} for all cells combined, n = 32; ρ = 0.65, P = 10^{-3} for blue photoreceptors only, n = 24) and IR_{max} (ρ = 0.49, P = 0.01 for all cells combined, n = 27; ρ = 0.54, P = 0.012 for blue photoreceptors only, n = 21; Fig. 8, C and D). Finally, when capacitance was plotted against estimated relative metabolic cost (Fig. 8E), defined as I_{DR} divided by the corresponding IR_{max} as a proxy for bits/ATP-consumed, a moderate positive correlation was detected (ρ = 0.39, P = 0.045 for all cells combined, n = 27; ρ = 0.47, P = 0.032 for blue photoreceptors only, n = 21),
suggesting that developmental increase in $I_{DR}$ was faster than improvement in $IR_{max}$.

**DISCUSSION**

**General Aspects**

Although much is known about the development of patternning, wiring, and molecular mechanisms in compound eyes, especially in the fruit fly (see reviews by Meinertzhagen 2000 and Pichaud 2014), the functional aspects of photoreceptor ontogenesis are poorly understood. Here, we have for the first time described the biophysical properties of water strider photoreceptors and provided electrophysiological analysis of their postembryonic development. Several features of *Gerris lacustris* photoreceptors differ compared with photoreceptors of other species. First, ommatidia containing peripheral photoreceptors of different spectral classes could be easily distinguished morphologically, suggesting an unusual specialization with consistent segregation of green- and blue-sensitive photoreceptors into separate ommatidia. We tried to test this hypothesis by recording from two photoreceptors in the same ommatidium, but these attempts were unsuccessful. There were also morphological differences between green and blue photoreceptors, as whole cell capacitance of the latter was significantly higher than that of green cells. As photoreceptor size appears to be linked to photoreceptor performance (see Fig. 8 in Frolov et al. 2012), this suggests that contrast resolution is primarily performed by blue photoreceptors. The prevalence of ommatidia with blue-like appearance in the recording chamber after dissociation, and among recorded cells, is consistent with this hypothesis.
Poor Information Processing

A surprising result was the relatively low corner frequency and average IR produced by photoreceptors of animals that are commonly thought to possess excellent vision. These results also seem to contradict observations made in the field, which show extremely fast escape reactions by water striders approached by an observer or in response to any movement. Data from other species, using similar white-noise modulated light contrasts, indicate that responses of water strider photoreceptors to WN1 (IR of 12 bits/s) and even to WN2 (60 bits/s) are inferior to those of the common backswimmer Notonecta glauca (WN1, 90 bits/s; unpublished observations), the lesser water boatman Corixa punctata (WN1, 36 bits/s; unpublished observations), and the stick insect (WN1, 34 bits/s; Frolov et al. 2012) but similar to those of the black cricket Gryllus bimaculatus (WN1, 13 bits/s; WN2, 36 bits/s; Frolov et al. 2014). However, the information capacity of Gerris lacustris photoreceptors was superior to Periplaneta americana photoreceptors (Heimonen et al. 2012). The corner frequency in all these species was ~7 Hz, except for the backswimmer where it was close to 20 Hz. Of course, all these IR values are lower than IR estimates obtained using even broader-band stimuli from fast-flying insects, like flies (Niven et al. 2007).

One explanation for the poor performance of water strider photoreceptors could be that despite their high “running” speed and sensitivity to large moving objects, water striders do not need truly superior vision due to their particular visual ecology. Gerridae occupy the two-dimensional world of the water surface and, correspondingly, their eyes possess a narrow acute vision zone consisting of facets ±5° around the eye equator with high vertical resolution used for detailed examination of objects of interest at close distance (Dahmen 1991). However, their primary sense appears to be vibratory perception and discrimination of signals conveyed by water ripples through which they discern mates from potential prey and predators and which serves as their main means of communication (Wilcox and Spence 1986). Therefore, apart from close-distance resolution, vision is likely to be instrumental for detection of larger distant or smaller nearby predators, which move without producing significant vibrations, such as birds above water or fish and other aquatic species underwater. Since triggering of avoidance reactions may only require visual motion detection, high IR photoreceptors might be unnecessary for mediating escape reactions in the water strider. They may instead depend on temporal and spatial processing of edges of shadows by on- and off-photoreceptor responses in higher order visual centers.

Other explanations may be found in the structure of the eyes in the water strider. In flies, the males have a high acuity area in the frontal part of the retina, where the photoreceptors are also faster than elsewhere (Burton et al. 2001). A similar situation, improving the performance of photoreceptors in the acute zone near the equator of the eyes, may exist also in Gerris but could not be distinguished by our in vitro recordings because the anatomical origin of the ommatidia could not be determined. Another intriguing possibility is suggested by the evidence for neural superposition-like optics and photoreceptor connections in the first visual ganglion, the lamina (Dahmen 1991; Wolburg-Buchholz 1979). This may improve the SNR by the square root of the number of superpositioned signals. These possibilities could significantly increase IR from the
The functional correlations between capacitance and other biophysical properties of photoreceptors are another interesting finding. Both the mean capacitance and its range were relatively small (60 ± 21 nF) compared with 255 ± 132 pF in the backswimmer (unpublished observations), 421 ± 203 pF in the lesser water boatman (unpublished observations), 212 ± 104 pF in the stick insect (Frolov et al. 2012), 165 ± 102 pF in the black cricket (green photoreceptors only; Frolov et al. 2014), and 323 ± 88 pF in the American cockroach *Periplaneta americana* (Salmela et al. 2012). Among insects, only *Drosophila melanogaster* was shown to have a comparable photoreceptor capacitance, 50 to 65 pF (Juusola and Hardie 2001; Väähäöyriö et al. 2006).

Despite the small variability in capacitance between cells, we found a strong dependency of many photoreceptor properties, e.g., *I*_{DR} and LIC magnitudes, on capacitance. This appears remarkable, especially considering anticipated experimental errors in measuring capacitance. The growth of the eye is likely to increase the number of the microvilli in all photoreceptors, thereby increasing their surface area and the measured capacitance. The fact that many biophysical properties, and especially IR, correlated with capacitance (Fig. 7) fits well with the theory of Song et al. (2012) and emphasizes the importance of photoreceptor membrane geometry for photoreceptor functioning.

**Photoreceptor Development in the Water Strider vs. the Stick Insect**

One of the most prominent features of developmental retina growth in hemimetabolous insects is the growth of photoreceptors per se. This growth is accompanied by changes in absolute and relative sizes of light-sensitive and light-insensitive parts of photoreceptor membrane and in the distribution of ion channel expression, entailing profound changes in biophysical properties of photoreceptors. In those hemimetabolous insects that rely on vision from the earliest larval stages, growth-related changes in retina must occur in a balanced way to maintain photoreceptor function and to optimize it with respect to developmental changes in visual ecology and behavior. In fact, retinal development in hemimetabolous insects could be unique in that there might not be other autonomous parts or organs of the nervous system in which functionally important cells must grow and change their properties to a similar extent. Interestingly, growth of the retina can be independent of the molting cycle (Stark and Mote 1981).

Developmental changes in photoreceptor biophysical properties have only previously been explored in one other species, *Carausius morosus* (Frolov et al. 2012). As the water strider has very different behavior and visual ecology from the stick insect, it is instructive to compare photoreceptor characteristics between first instar nymphs and adults of these two species as well as the developmental trends discovered in electrophysiological experiments.

In general, *K*⁺ conductances and LIC changed in a similar manner in the stick insect and the water strider: *I*_{DR} and LIC magnitudes were strongly and positively correlated with capacitance, whereas *I*₈ was correlated to a much lesser extent (no correlation was found in the stick insect). These results support the notion that 1) *I*₈ is predominantly expressed in the light-insensitive part of photoreceptor membrane, while *I*_{DR} channels are closely associated with the rhabdom, consistent with previously published observations (Hardie 1991; Krause et al. 2008; Rogero et al. 1997); and 2) photoreceptor growth mainly occurs in the rhabdom. However, none of these trends explain the three most salient developmental differences between the two species: the anomalously large voltage bumps, very low levels of steady-state depolarization, and poor responses to white-noise modulated light stimulation in stick insect first instar nymphs compared with adults. In contrast, first instar nymphs of the water strider demonstrated only one of these developmental differences, the relatively low IR.

**Differences in voltage responses.** Why are voltage bumps so large in the stick insect nymphs, both in absolute terms and compared with the regular-sized bumps in adults, whereas water strider nymphs are no different from adults in this respect? Although we have argued that large voltage bumps are caused by permissive membrane filtering (Frolov et al. 2012), the second part of this question requires a comparison of relevant biophysical properties of water strider and stick insect photoreceptors (Table 1). Obviously, relatively large current
quantum bumps found in stick insect nymphs can explain most if not all of the difference in voltage bump size between the two species. In addition, water strider nymph photoreceptors were characterized by the relatively high $I_A$, which is expected to attenuate isolated voltage bumps evoked from the dark resting potential in dim illumination. In fact, the difference in $I_A$ was even greater at negative voltages than at the nominal voltage (not corrected for the access resistance voltage error) of $+36 \text{ mV}$ (Table 1). For instance, at $-24 \text{ mV}$, the chord conductance of $I_A$ was $1.6 \pm 0.7 \text{ nS}$ in stick insect nymphs and $3.0 \pm 1.4 \text{ nS}$ in water strider nymphs.

In contrast to water strider nymphs, prolonged voltage responses of stick insect nymphs occur at relatively low potentials. Two membrane characteristics mostly determine the level of steady-state depolarization during light response if we ignore ion exchanger currents: the magnitudes (and densities) of LIC and $I_{DR}$. Although LICs were of similar magnitude and density in the first instar nymphs of both species, $I_{DR}$ was more than twofold higher in the stick insect (Table 1). Again, as in the case with $I_A$, the difference was even more prominent at negative voltages: at $-24 \text{ mV}$, $I_{DR}$ was $2.4 \pm 1.2 \text{ nS}$ in stick insect nymphs and $0.7 \pm 0.6 \text{ nS}$ in water strider nymphs. As $I_{DR}$ determines membrane gain and regulates the level of depolarization (Vähäsöyrinki et al. 2006), the relatively small $I_{DR}$ in water strider nymphs could be useful for maintaining higher steady-state depolarization with the same LIC. Low $I_{DR}$ should also result in a smaller membrane corner frequency (Fig. 8C), which is instrumental for filtering out high-frequency voltage noise (van Hateren 1992). On the other hand, a relatively low $f_{3dB}$ in dim light would not affect the IR strongly since the contrast resolution, under intensities where photoreceptors act more like photon counting units, is limited by the number of absorbed photons rather than membrane speed (see, e.g., Frederiksen et al. 2008).

**IRs and metabolic costs.** The main cause of the anomalously low IR in the stick insect nymphs appears to be the relatively small number of phototransduction units available for resolving light contrasts. Dividing the average maximal LIC$_{SS}$ by the size of the current quantum bump yields a figure proportional to the number of such units, individual bump light adaptation notwithstanding. Table 1 shows that for the stick insect the LIC$_{SS}$-to-current bump ratio is $12.1$-fold higher for adults than for nymphs. For the water strider this ratio is much smaller, only $2.5$-fold. Moreover, there are more transduction units available in water strider nymphs than in stick insect nymphs but this is reversed for adults. There is a surprisingly good match between these differences in “transduction unit” ratios and the differences between the actual IRs within each species (Table 1), indicating a direct relationship between peak IR of photoreceptors and the number of contrast-resolving units, as suggested previously (Song et al. 2012). Note that the IR values shown were obtained by different stimuli (we did not use WN1 in experiments with water strider nymphs) so a direct comparison of these IR values between the two species is not possible. Nevertheless, the LIC$_{SS}$-to-current bump ratio for the adult stick insect was $2.2$-fold higher than that for the adult water strider, whereas the corresponding IR ratio is $2.8$ (using the adult water strider’s IR of $12 \pm 5 \text{ bits/s}$ and the stick insect’s IR of $34.1 \pm 5.0$ for WN1). Altogether, these data demonstrate that photoreceptor performance of water strider first instar nymphs is superior to that of their stick insect counterparts due to differences in the photoreceptor channelome (the set of ion channels expressed in the photoreceptor), which, in turn, is shaped by evolution under differing behavioral and visual ecological pressures.

In contrast to the stick-insect Carausius, the growth of water strider photoreceptors was associated with increasing metabolic cost of signaling when measured with $I_{DR}$, both in absolute terms and per bit of information. Analysis of developmental changes in $I_{DR}$ reveals the cause of this increase: with growth, the density of $I_{DR}$ is actually increased (Fig. 6F) rather than decreased as in the stick insect. This increase in the cost of information can be contrasted with the previous observations in flies, which show that although flies with better vision expend more energy to maintain the high performance of their photoreceptors, the cost of information per bit steadily decreases with improvement in the overall information processing across species (Niven et al. 2007). However, that result is not necessarily applicable to the developmental context, as opposite trends are found in two different species. This situation calls for more comparative and developmental research on the question of photoreceptor performance and energy consumption.

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