Tailoring Organic LEDs for Bidirectional Optogenetic Control via Dual-Color Switching

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Revealing the intricate logic of neuronal circuits and its connection to the physiopathology of living systems constitutes a fundamental question in neuroscience. Optogenetics offers the possibility to use light of specific wavelengths to study the activity of neurons with unprecedented spatiotemporal resolution. To make use of this technique at its full potential, bidirectional proteins may be expressed across the neuronal membrane to provoke both enhancement and inhibition of neuronal activity depending on the excitation wavelength. This generates the demand for light sources with high spatial precision, high operation speed, and multi-color emission from the same location. To meet these requirements, the design, realization, and characterization of organic light-emitting diodes (OLEDs) are presented with switchable bicolor emission, exhibiting high irradiance and good efficiency. The OLEDs can switch between blue and red/green light upon changing the voltage polarity, triggering both optogenetic inhibition and excitation in ND7/23 cells and Drosophila melanogaster larvae expressing bidirectional optogenetic proteins. This work shows the potential of engineering OLEDs to enable multicolor optogenetics with a single, organic device, and provides a new avenue towards bicolor optical brain stimulation in vivo.

1. Introduction

Optogenetics is a widely used neuroscience technique that enables manipulation of cellular activity with high spatial and temporal precision by targeted photostimulation. For this, light-responsive ion channels are genetically expressed in the plasma membrane, where they regulate the flux of selected ions in response to light of specific color and intensity.[1-5] Optogenetics requires light sources such as lasers or LEDs, which are often combined with microscopes or optical fibers for light delivery to biological samples.[6] Although those devices are very powerful for in vitro studies, their bulkiness, rigidity, and low biocompatibility limit their use in vivo. Recently, organic light-emitting diodes (OLEDs) were proposed as alternative light sources for optogenetics.[7-12] OLEDs allow for a preferable biotic-abiotic interface owing to the possibility of fabrication on flexible substrates[13-18] and enable microsecond response times,[19,20] patterning to cellular scale,[21-23] operation at low voltage,[24,25] and tunable optical properties.[26,27]

Recently, light-sensitive proteins have been developed consisting of two different photosensitive cores that are fused into a single domain.[28,29] One example is BiPOLES[29]—a fusion of the red-light-sensitive cation-conducting channelrhodopsin Chrimson[30] for excitation and the blue-light-sensitive anion-conducting GtACR2[31,32] for inhibition. Another example is a fusion of the cation-conducting ChRmine[33] with GtACR2, called BiPOLES-ChRmine, for which green and blue light lead to excitation and inhibition, respectively.[29] Addressing such bidirectional channelrhodopsin pairs requires a light source that enables switching between two different emission colors. OLEDs are particularly attractive for this application since devices with different emission colors may be stacked on top of each other to enable multi-color emission from the very same area. Switching between OLED subunits is possible using the so-called AC/DC architecture, where two monochrome OLEDs share one electrode but require opposite polarity to be turned on individually.[34,35]

Here, we adapt this concept to stimulate the photosensitive proteins BiPOLES and BiPOLES-ChRmine expressed either in neuron-related ND7/23 cells or in Drosophila melanogaster larvae. Using optical simulations, we design bi-color AC/DC OLEDs[36] with optimized emission properties matching the action spectra of the selected proteins while avoiding optical crosstalk between emission domains. We then investigate the ion flux across the membranes of ND7/23 cells upon separate...
activation of the respective channelrhodopsins as a function of the OLED power density. Furthermore, we study the behavioral response of BiPOLES-expressing Drosophila larvae to excitation and inhibition of motoneurons and achieve switching between the two related behavioral modes within 1 s. Our work demonstrates that AC/DC OLEDs allow precise control of the new bidirectional light-gated ion channels, lending themselves as candidates to future implementation for co-located activation and inhibition of neuronal activity in vivo.

2. Results

2.1. Design and Characterization of Bi-Color OLEDs

We selected a top-emitting device architecture since this may facilitate cell targeting in future patterned devices for high-resolution optogenetics.[11] The OLED structure is composed of two OLED units with three electrodes in which the outermost electrodes are set to a common potential (Figure 1a,b: details about materials and fabrication are given in Figure S1, Supporting Information, and the Experimental Section). 15 nm Ag central and top electrodes are evaporated on top of a gold seed layer for improving morphology, increasing transmittance, and lowering sheet resistance.[12] We used doped charge transport layers to facilitate efficient charge carrier injection and to enable optical tuning of the OLED microcavity without sacrificing electrical properties.[13] To produce the two emission colors required for stimulation of BiPOLES, our devices comprise a blue fluorescent stack on top of a red phosphorescent unit, while for the stimulation of BiPOLES-ChRmine, the red-emitting unit is replaced with a green-emitting system. In this way, the device emits red/green light stimulating the cation-channel of the tandem proteins when positive voltage is applied to the outer electrodes (i.e., the center electrode acts as cathode) and blue light targeting GaACR2 when the voltage is negative (i.e., the center electrode acts as anode).

Since green- and red-shifted photosensitive proteins also have considerable absorption in the blue wavelength regime, the emission spectra of the light sources need to be carefully adjusted in order to avoid substantial crosstalk between the two proteic cores in fused constructs. Else, stimulation of the blue-absorbing GaACR2 may lead to simultaneous activation of Chrimson and ChRmine. Organic emitters usually provide rather broad spectra with a significant tail towards longer wavelengths. Hence, in order to make OLEDs suitable for bicolor optogenetic experiments, careful tuning of the emission spectra is a particular challenge requiring strict design constraints.

Optical simulations were carried out using a transfer-matrix algorithm[41] to ensure narrow-band emission and high spectral separation between the two OLED units. We varied the hole transport layer (HTL, here Spiro-TTB:F6-TCNNQ) thickness of the upper units and the electron transport layer (ETL, here BPhen:Cs) thickness of the lower units of the device, while keeping the thickness of all other layers constant. Figure 1c shows the simulated spectra of the blue-red OLEDs compared to the action spectra of GaACR2 and Chrimson, which constitute the light-sensitive domains of BiPOLES. By increasing the ETL thickness, no significant change in the peak position of the blue unit is observed, while the spectrum of the red unit shifts to higher wavelengths with increasing thickness. Increasing the HTL thickness leads to spectral broadening of the blue unit and the rise of secondary peaks at around 500 nm (Figure 1c, bottom).

Figure 1d shows the spectra of the blue-green OLEDs compared to the action spectrum of the BiPOLES-ChRmine complex. Due to the more green-shifted action spectrum of ChRmine, its spectral overlap with GaACR2 is stronger compared to BiPOLES. As for the blue-red OLEDs, increasing the ETL thickness mainly affects the green unit, which shifts again to higher wavelengths. Increasing the HTL thickness leads to significant spectral changes in both the blue and the green units. While the spectrum of the blue unit becomes red-shifted and broadened, the spectrum of the green unit significantly blue-shifts. This shift leads to an overlap between the blue and green spectra at large HTL thickness (Figure 1d, bottom).

While spectral separation is key for targeting the individual components of fused optogenetic proteins, the overall light output of the OLED should be considered as well in order to ensure sufficient light output at low operating voltages, which is crucial to avoid damage to biological targets upon heating.[42] Figure 1e,f shows simulated EQE values for the blue-red and blue-green OLEDs, respectively. Again, the HTL largely influences the efficiency of the blue units, while changes in the ETL particularly influence outcoupling from the red/green cavity. For the blue-red OLEDs, we found that a device with HTL = 25 nm and ETL = 55 nm would provide minimum exposure of Chrimson to blue light (peak at 464 nm, FWHM = 27 nm) while exerting good EQEs for both the red (14.0 %) and blue (2.4 %) units. For the blue-green OLEDs, all configurations with HTL = 50 nm have poor optical matching with BiPOLES-ChRmine and strong overlap of the emission colors, making them undesirable for optogenetics. The configuration with HTL = 30 nm and ETL = 30 nm instead provides the smallest spectral overlap of the blue unit (peak at 462 nm, FWHM = 22 nm) with ChRmine, and may reach EQEs up to 2.4 % and 11.3 % for the blue and green OLEDs, respectively.

For the optogenetic experiments, we fabricated blue-red OLEDs with HTL/ETL thicknesses of 25/55 nm and blue-green OLEDs with 30/30 nm with an active area of 17 mm × 17 mm. The device spectra are shown in Figure 2a,b. For both OLEDs, the measured curves are in good agreement with the simulations and thus should provide precise targeting of BiPOLES and BiPOLES-ChRmine. Current-voltage-irradiance characteristics of our OLEDs are shown in Figure 2c,d. The two units of the OLEDs are addressed separately by application of voltage with different polarity, which leads to dual-diode behavior and hence two different emission colors. At 5 V, the red unit exerts slightly higher irradiance than the green unit (91 and 84 µW mm⁻², respectively). For the blue unit, higher irradiance is achieved in the blue-green device (36 µW mm⁻² compared to 23 µW mm⁻² in the blue-red stack). While optogenetic studies often use significantly higher optical power density, it was shown that GaACR2, Chrimson, and ChRmine may also be stimulated with light intensities from 8/2 µW mm⁻² for GaACR2/Chrimson when expressed in sensory neurons and motoneurons of Drosophila larvae,[11,46] and 30 µW mm⁻² for ChRmine in hippocampal neurons.[13] Hence, the power density provided
by our OLEDs should be sufficient for stimulation in ND7/23 cells and Drosophila larvae. The maximum EQE of the blue-red devices is 2.9%/11.7% for the blue/red units, while the blue-green OLEDs reach 1.6%/7.5% (Figure 2e,f). Observed differences in current-voltage characteristics ($J$-$V$) and EQE between the blue-red and blue-green OLEDs may be due to differences in layer thicknesses and blocking layer materials.

Finally, the durability of the devices under AC operation was tested driving each subunit with a current density of 16.5 mA cm$^{-2}$ for 60 ms, followed by a black period of 140 ms while alternating between the two sub-units (i.e., repeated cycles of 60 ms blue, 140 ms black, 60 ms red/green, 140 ms black). With the chosen settings, the blue-red OLEDs exceed 691,000 cycles before failure, while the blue-green OLEDs fail shortly after 40,000 cycles (Figure 2g,h). We believe that the different device performance will have caused stronger heat generation in the blue-green OLEDs compared to the blue-red devices, thus accelerating device degradation. Note that heating of one sub-unit
Figure 2. Device characteristics of bi-color OLEDs. a,b) Comparison of simulated and experimental emission spectra of the blue-red OLEDs with 25/55 nm HTL/ETL thickness (a) and of the blue-green OLEDs with 30/30 nm HTL/ETL thickness (b). c,d) JV (solid) and voltage-irradiance curves (dotted) of the blue-red and blue-green devices. e,f) Measured EQEs of the blue-red and blue-green stacks. g,h) Stability of the blue-red and blue-green OLEDs under pulsed driving at 5 Hz. Voltages alternated between positive and negative windows with 60 ms on and 140 ms off, leading to a driving frequency of 2.5 Hz for each sub-unit. Irradiances were 10.9 and 20.1 µW mm⁻² for the blue and red sub-units of the blue-red OLED, respectively, and 8.7 and 17.5 µW mm⁻² for the blue and green sub-units of the blue-green OLED, respectively.
will also affect the other sub-unit. It is expected that this causes stronger degradation in stacked OLEDs when pulsed alternatingly compared to single-unit devices. With a view to future in vivo applications, we believe that devices with microsized pixels will outperform the big area devices in terms of stability because of the lower number of point defects and a reduced heat-up and current leakage over a smaller area.

2.2. Optogenetic Stimulation of ND7/23 Cells

Next, we investigated the capability of our OLEDs to elicit ionic currents in ND7/23 cells expressing either BiPOLES or BiPOLES-ChRmine. For this, we recorded the photocurrents with our OLEDs positioned at 2 mm distance from the cells delivering 500 ms light pulses for cell stimulation. Figure 3 shows data from patch-clamp recordings in whole-cell configuration.

The photocurrents of BiPOLES elicited by shining blue light at 15 μW mm⁻² and red light at 70 μW mm⁻² indicate that GtACR2 exhibits higher currents than Chrimson (Figure 3d), in agreement with previous observations. Current-voltage relationships were recorded to accurately estimate the voltage at which the net ion flux across the cellular membrane is zero (Reversal voltage, $E_{\text{rev}}$, Figure 3e,f). We find that with our OLEDs, the reversal voltages for BiPOLES are $-58.7 \pm 0.8$ mV using blue light (peak at 462 nm, FWHM = 25 nm) and $-6.1 \pm 0.6$ mV using red light (peak at 620 nm, FWHM = 38 nm). As depicted in Figure 3e, recordings at holding potentials over the corresponding $E_{\text{rev}}$ exhibit a reversal of currents from negative to positive, caused by inversion of the net direction of ions across the membrane. Since the [Cl⁻] between inside and outside the cell is asymmetric, the chemical inward gradient for anions across the cellular membrane (more Cl⁻ outside) dictates the net flux of chloride, and leads to a negative $E_{\text{rev}}$. On the other hand, the proton concentration across the cellular membrane is symmetric (intracellular and extracellular pH = 7.2); the direction of the proton flux is mainly determined by the voltage ($E_{\text{rev}}$ close to 0 mV).

Under the given buffer conditions, the recorded $E_{\text{rev}}$ thus evidences the activation of the chloride channel (GtACR2, $E_{\text{rev}} = -58.7$ mV) and the proton channel (Chrimson, $E_{\text{rev}} = -6.1$ mV) without much crosstalk in line with recordings previously performed with conventional illumination systems. Figure S2a–d, Supporting Information, shows the photocurrent response of BiPOLES-expressing cells as a function of OLED irradiance (dose). Here, the photocurrent density is increased with increasing irradiance for both GtACR2 and Chrimson.

Stimulation of BiPOLES-ChRmine with 16 μW mm⁻² blue light and 55 μW mm⁻² green light provides photocurrents with comparable amplitudes for GtACR2 and Chrimson (Figure 3g), again in accordance with previous studies. We measured the reversal voltages (Figure 3h,i) to be $-49.3 \pm 1.6$ mV using blue light (peak at 462 nm, FWHM = 24 nm) and $-8.3 \pm 2.3$ mV using green light (peak at 557 nm, FWHM = 65 nm). Since ChRmine is highly permeable for sodium ions, its $E_{\text{rev}}$ should be more positive than for Chrimson due to the slightly asymmetric internal and external Na⁺ concentration of 110 and 140 mM, respectively. Negatively shifted reversal voltages when compared to BiPOLES can be explained by a stronger overlap of GtACR2 and ChRmine absorption and do not result from spectral crosstalk of the employed OLEDs.

The ChRmine photocurrents show slower dynamics compared to GtACR2 and Chrimson (Figure S2, Supporting Information). Stimulation of BiPOLES-ChRmine with blue light leads to small negative currents after light turn-off (Figure S2e, Supporting Information). We hypothesize that partial co-activation of ChRmine is taking place under blue illumination. Also, partial co-activation could justify the lower Δ$E_{\text{rev}}$ of BiPOLES-ChRmine with blue light as compared to BiPOLES (Figure S3a, Supporting Information). After light turn-off, the slow kinetics of ChRmine cause measurable tail currents persisting after illumination in BiPOLES-ChRmine (Figures S2g and S3b, Supporting Information). However, the residual currents observed are minimal in comparison to the photocurrents during illumination, regardless of irradiance. Therefore, the small co-activation of GtACR2 and ChRmine does not prevent our OLEDs from independent control of the single proteins when linked together. Furthermore, the OLED drive voltages are low enough to avoid significant heat-up of the device. At the range of irradiances and driving conditions used in our cell experiments, the temperature increase during illumination does not exceed 1 °C in all cases (Figure S4, Supporting Information), which ensures that our devices are also good candidates for cell stimulation in vivo.

2.3. Optogenetic Control of Drosophila melanogaster Larvae

After successful application of our bi-color OLEDs to drive bi-directional photocurrents in cell cultures, we next prove their functionality for activation and inhibition of neuronal activity. We expressed UAS-BiPOLES in motoneurons (OK371-GAL4) of Drosophila melanogaster and employed our blue-red OLEDs for optogenetic stimulation. Reports on the use of OK371-GAL4 showed that activation leads to muscle contraction and immobilization, while inhibition causes muscle relaxation with paralysis.

For the experiments, we placed third instar larvae onto a thin layer of agarose (0.5 mm thick), which was positioned on top of the devices. Before stimulation, we waited 5 min to accustom the larvae to the new environment. Then, we applied 5 s-long illumination cycles of increasing light intensity with 1 min of resting time between the stimulations. Figure 4a shows a larva on top of the OLED before illumination and during illumination with red and blue light. As expected, BiPOLES-expressing larvae contracted during activation of motoneurons with red light and relaxed during inhibition with blue light. This effect leads to larval paralysis (Figure 5, Supporting Information) and an increase in larval length upon blue light due to GtACR2 stimulation (Figure 4b). Upon illumination with red light, BiPOLES-expressing larvae showed strong contractions already at very low irradiance (Figure 4c) and stopped crawling (Figure 5, Supporting Information). The minimal irradiance to evoke contraction and relaxation in BiPOLES-expressing larvae with our OLEDs was 1.3 and 5.9 μW mm⁻² for red and blue illumination, respectively. Experiments were repeated with control
w118 larvae, which did not show any significant length or speed variation upon stimulation with blue or red light, even at the highest irradiance (Figure S6, Supporting Information).

Next, we investigated larval response upon switching between activation and inhibition at different stimulation frequencies (Videos S1 and S2, Supporting Information) driving the blue and red OLEDs at constant optical power density (7 µW mm⁻²). Figure 4d and Figure S7, Supporting Information, show the normalized length of larvae stimulated with red and blue light at a switching frequency of 0.2 and 1 Hz, respectively. In all stimulation cycles, we observed clear and rapid contraction of the larvae during red illumination, followed by relaxation under blue light. Larval response occurred within approximately 0.5 s and reached the same extent of contraction and relaxation during each stimulation cycle. These results demonstrate that our OLEDs are able to induce consistent bi-directional neuronal stimulation with switching frequencies up to 1 Hz.

Since prolonged driving of the OLED may lead to heat-up of the device, we measured the temperature variation of the OLED under 25 color-switching cycles keeping the optical power density for both device units constant at 7 µW mm⁻². As a result, the temperature on top of the glass surface increased by 2.3 °C after 25 cycles (Figure 4e). This is well within the acceptable temperature range of Drosophila rearing and will not lead to heat-induced behavioral changes.48

Figure 3. OLED-driven photocurrents of ND7/23 cells expressing BiPOLES and BiPOLES-ChRmine. a) Buffer conditions for the experiments on ND7/23 cells. Internal buffer contained 110 mM [NaGlu] and the bath solution contained 140 mM [NaCl], both were adjusted to pH 7.2. b) Schematic representation of BiPOLES acting under blue and red light. c) Schematic representation of BiPOLES-ChRmine acting under blue and green light. d) Exemplary photocurrents of GaCr2 (N = 10) and Chrimson (N = 10) at increasing membrane holding potentials (from −80 to +40 mV in 20 mV steps). e,f) Normalized photocurrents as a function of the holding potential (e) and reversal voltage (f) for both components of BiPOLES activated with the blue-red OLED. g) Exemplary photocurrents of GaCr2 (N = 9) and Chrimson (N = 10) at increasing holding potentials activated by the blue-green OLED. h,i) Normalized photocurrents as a function of the holding potential (h) and reversal voltages (i) for both components of BiPOLES-ChRmine. Line: mean; shaded area: s.e.m. (e and h); central black line: mean; whiskers: s.e.m. (f and i). N = number of cells. Photocurrent traces and current-voltage relationship of BiPOLES were obtained using blue light at 15 µW mm⁻² and red light at 70 µW mm⁻²; for BiPOLES-ChRmine, blue light at 16 µW mm⁻² and green light at 35 µW mm⁻² were used.
3. Conclusions

We developed bi-color organic LEDs to stimulate the subdomains of tandem optogenetic proteins, through irradiation by two different colors from a single OLED pixel. The OLEDs were designed by tailoring the emission spectra via optical simulations to match the activation spectra of BiPOLES and BiPOLES-ChRmine, while also ensuring adequate EQEs and sufficient stability. We proved that bi-color OLEDs are versatile tools for optogenetic control of both non-electrogenic cells and neurons. Selective stimulation of GtACR2 and Chrimson in BiPOLES-expressing ND7/23 cells was achieved with our blue-red OLEDs, while our blue-green devices provided reliable stimulation of the fused GtACR2 and ChRmine channelrhodopsins. The latter demonstrates that by careful tuning of the spectral width and position, bi-color OLEDs may also reliably target fused optogenetic proteins that provide only narrow spectral separation. Moreover, our OLEDs provided reliable stimulation of neurons in Drosophila, enabling direct switching between activation and inhibition of the locomotor system in the same animal.

With the capability of BiPoles to enable almost exclusive red-light activation with only minimal residual activation in the blue, our devices are also ideal for selective targeting of cultures hosting multiple cell types with spectrally distinct channelrhodopsins. In the future, OLED patterning to microscopic size may be combined with a soma-targeted version of BiPOLES to enable bidirectional optogenetic control of only selected neurons. Moreover, targeting single neurons with micropatterned bicolor OLEDs would allow indirect modulation of the electrical activity in postsynaptic neurons. Finally, the combination of our bi-color OLEDs with flexible substrates would unlock the full potential of these devices as functional implants for in vivo applications.

4. Experimental Section

**OLED Simulation, Fabrication, and Characterization:** Optical simulations were carried out using Simoji, a software developed in-house. The software uses the transfer matrix method to calculate the reflection and transmission of the multi-layered stacks, assuming a finite number of dipoles sitting at different positions within the emission layers. To compute the EQE, electrical and radiative efficiencies were set to 1 and spin factor to 1 and 0.25 for phosphorescent and fluorescent emitters, respectively.

All OLEDs were fabricated by thermal evaporation in a vacuum tool (Kurt J. Lesker Co.; base pressure of $4 \times 10^{-8}$ mbar) on pre-cleaned glass substrates. Thickness and deposition rates of the organic materials were measured via quartz crystal monitors and doping was assured by co-evaporation. A 2-methyl-9,10-bis(naphthalene-2-yl)anthracene (MADN) matrix doped with 1.5 wt.% 2,5,8,11-tetra-tert-butylperylene (TBPee) was used as blue emitter system. N,N'-di(naphthalene-1-yl)-N,N'-diphenylbenzidine ($\alpha$-NPD) was doped with 10 wt.% iridium(III) bis(2-methyldibenzo-[f,h]chinoxaline)(acetylacetonate) (Ir(MDQ)$_2$(acac)) to obtain the red emission layer. A combination of a 4,4′,4″-tris(carbazol-9-yl)-triphenylamine (TCTA) matrix and 2,2′,2″(1,3,5-benzenetriyl)tris(1-phenyl-1H-benzimidazole) (TPBi) matrix each doped with 8 wt.% of the green emitter bis(2-phenylpyridine)(acetylacetonate)iridium(III) (Ir(ppy)$_2$(acac)) was used as green-emitting system.
2,2′,7,7′-tetrakis-[N,N-di-methylphenylamino]-9,9′-spirobifluorenone (Spiro-TTB) doped with 4 wt. % 2,2′-(perfluoronaphthalene-2,6-diyldiene)-dimalononitrile (F2-TCNNQ) was used as HTL, whilst 4,7-diphenyl-1,10-phenanthroline (Bphen) doped with Cesium (Cs) was chosen as ETL. For the blue-red OLEDs, Bphen and α-NPD were used as hole blocking and electron blocking layers (HBL/EBL), respectively. For the blue-green OLEDs, aluminum(III) bis(2-methyl-8-quinolinato)-4-phenylphenolate (BALq) and TPBi were used as HBL, while α-NPD and 2,2′,7,7′-tetrakis[diphenylamino]-9,9′-spirobifluorenone (Spiro-TAD) were used as EBL. The transparent electrodes consisted of 15 nm of silver (Ag) evaporated on 2 nm of gold (Au). The reflective back electrode consisted of 35 nm of Ag evaporated on top of 35 nm of aluminum (Al). All samples were encapsulated under nitrogen atmosphere with glass lids and epoxy resin directly after fabrication before use in ambient conditions. All the devices had an active area of 303 mm².

JV was measured using a source meter unit (SMU2450, Keithley Instruments) whereas the irradiance was recorded with a calibrated Si photodiode (Thorlabs). EQE and spectra of the OLEDs were obtained in an integrating sphere (LMS-100, Labsphere Inc.) equipped with a calibrated spectrometer (CDS-600, Labsphere Inc.). Device temperature was tracked with an infrared thermometer (Optris CT laser LT). Temperature data was smoothed over 5 adjacent points to reduce noise. Lifetime measurements were conducted using a source meter unit (SMU2450, Keithley Instruments) equipped with a calibrated Si photodiode (Thorlabs) and a digital multimeter (DMM6500, Keithley Instruments).

Mammalian Cell Culture and Transfection: PCNA3.1 vectors contained Chrimson/ChRmine and GtACR2 coding sequences together with mCerulean3/eYFP for the detection of expression.[29] The expression of the coding sequences together with the opsins sequences (Chrimson/ChRmine and GtACR2) were fused through a sequence coding for ß-HK (rat gastric H+/K+ ATPase beta subunit). ND7/23 cells (Sigma-Aldrich, St. Louis, MO, USA) were seeded on poly-D-lysine coated glass coverslips in 35 mm Petri dishes containing Dulbecco’s Modified Eagle Medium (DMEM; Biochrom GmbH) with 5% (v/v) fetal bovine serum (FBS superior; Biochrom, Berlin Germany), glutamine (Biochrom, Berlin Germany), and 100 μg ml⁻¹ penicillin/streptomycin (Biochrom, Berlin Germany). Growth media were further supplemented with 1 mM all-trans Retinal. For transfection, 6 μL FuGENE HD transfection reagent (Promega, Madison, WI) was mixed with 2 μg of vector DNA and added to the cells ~48 h prior to measurements.

Whole-Cell Voltage Clamp Recordings of ND7/23 Cells: Fire-polished, borosilicate glass capillaries (Science Products GmbH, Hofheim, Germany) were used as an EBL. Glass micropipettes were filled with a solution of 200 mM KCl and 10 mM TES. The resistance of the pulled pipettes was 1.5-2.8 MΩ. Light from a PE-4000 CoolLED (CoolLED, Andover, UK) was used for fluorescence excitation. The CoolLED light path was coupled into an Axiovert 100 TV inverted microscope (Carl Zeiss, Oberkochen, Germany), which was used to search for fluorescent cells through a 40x/1.0 water objective (Carl Zeiss, Oberkochen, Germany). Before a patch was established, the objective was replaced by the OLED, which illuminated cells from below the glass coverslip. The OLED was driven by a T-Cube LED Driver (LEDDB; Thorlabs, New Jersey, United States) delivering a constant current. Whole-cell patch-clamp recordings were performed at room temperature with an access resistance < 10 MΩ and a membrane resistance generally > 500 MΩ. A CLC-03X5 amplifier (npi Electronic, Tamm, Germany) was used for signal amplification and digitization was done using a Digidata 1440 A (Molecular Devices, Sunnyvale, CA). A reference AgCl electrode embedded in agar containing 140 mM NaCl was submerged in the bath solution, which contained 140 mM NaCl, 1 mM KCl, 1 mM CsCl, 2 mM CaCl2, 2 mM MgCl2, and 10 mM HEPES and was adjusted to pH 7.2 and 310 mOsm (using glucose). The internal buffer solution contained 110 mM NaGluconate, 1 mM KCl, 1 mM CsCl, 2 mM CaCl2, 2 mM MgCl2, 10 mM EGTA, and 10 mM HEPES adjusted to pH 7.2 and 290 mOsm (with glucose). All measurements were synchronized and controlled using the pCLAMP software (Molecular Devices, Sunnyvale, CA), which was also used for calculating and pre-correcting lipid junction potentials. Membrane currents as a function of the holding potential were normalized with respect to the cation channel current obtained at ~80 mV. Photocurrents as a function of the OLED irradiance were divided by the cell membrane capacitance. Data were analyzed and plotted using Clampfit 10.4 and Origin Pro.

Drosophila Rearing and Optogenetic Stimulation: The following fly strains were used: OK371-GAL4, UAS-BIPOLES (gift from Peter Soba), and w¹¹¹8. All fly crosses were raised in the dark at 25 °C on conventional cornmeal-agar medium supplemented with 0.5 mM all-trans-retinal (ATR). Control w¹¹¹8 flies were also grown on 0.5 mM ATR-supplemented food. All optogenetic measurements with Drosophila larvae were recorded underneath a stereomicroscope (Nikon SMZ25, P2-SHR Plan Apo 0.5x/0.075 NA objective) with an sCMOS camera (Andor Zyla 4.2 PLUS). A 695 nm long-pass filter was mounted in front of the camera to avoid overexposure due to OLED illumination. Using this filter, only a small portion of light from the red OLED was detected by the camera. Larvae were imaged under infrared light using an LED bar light source (LUMIMAX LSB-Series; 850 nm peak), causing a light intensity of approximately 0.02 mW mm⁻² at the sample. Videos were acquired from the sCMOS camera using Nikon NIS-Elements software at a frame rate of 10 Hz except for the high-frequency switching experiment, which used 20 Hz. For optogenetics and control experiments, third instar larvae were taken out of the vials in dim green light, gently washed in DI water, and placed on a thin sheet of agarose (1.5% w/v in DI water) located on top of the OLED. Before starting optogenetic experiments, animals got accustomed to their new environment for approximately 5–10 min in the dark. Larval length was measured by manual tracking of head, centroid, and tail positions every second (every 0.05 s for the high-frequency experiment) in ImageJ using the plugin MTrack, and calculating the geometric distance of the three points. Subsequently, the length and speed were normalized to the mean value measured before the start of the first stimulation for each larva for all the cases except the color-switching experiments, where data were normalized with respect to the first datapoint. Speed and length were calculated as mean values for the duration of each stimulation/resting period. Statistics and significance were calculated via one-sample t-test using Origin Pro. All p-values are given in Tables S1 and S2, Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.
