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1 **Ca<sup>2+</sup>-activated K<sup>+</sup> channels reduce network excitability, improving adaptability and**  
2 **energetics for transmitting and perceiving sensory information**

3  
4 **Abbreviated title** (43 characters): How BK and SK channels benefit early vision

5  
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6

## 7 **Abstract**

8  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels (BK and SK) are ubiquitous in synaptic circuits, but their role in network  
9 adaptation and sensory perception remains largely unknown. Using electrophysiological and  
10 behavioral assays and biophysical modelling, we discover how visual information transfer in mutants  
11 lacking the BK channel (*dSlo*<sup>-</sup>), SK channel (*dSK*<sup>-</sup>) or both (*dSK*<sup>-</sup>;*dSlo*<sup>-</sup>) is shaped in the female fruit  
12 fly (*Drosophila melanogaster*) R1-R6 photoreceptor-LMC circuits (R-LMC-R system) through  
13 synaptic feedforward-feedback interactions and reduced R1-R6 *Shaker* and *Shab*  $\text{K}^+$  conductances.  
14 This homeostatic compensation is specific for each mutant, leading to distinctive adaptive dynamics.  
15 We show how these dynamics inescapably increase the energy cost of information and promote the  
16 mutants' distorted motion perception, determining the true price and limits of chronic homeostatic  
17 compensation in an *in vivo* genetic animal model. These results reveal why  $\text{Ca}^{2+}$ -activated  $\text{K}^+$   
18 channels reduce network excitability (energetics), improving neural adaptability for transmitting and  
19 perceiving sensory information.

20

## 21 **Significance statement:**

22 In this study, we directly link *in vivo* and *ex vivo* experiments with detailed stochastically operating  
23 biophysical models to extract new mechanistic knowledge of how *Drosophila* photoreceptor-  
24 interneuron-photoreceptor (R-LMC-R) circuitry homeostatically retains its information sampling and  
25 transmission capacity against chronic perturbations in its ion-channel composition, and what is the  
26 cost of this compensation and its impact on optomotor behavior. We anticipate that this novel  
27 approach will provide a useful template to other model organisms and computational neuroscience,  
28 in general, in dissecting fundamental mechanisms of homeostatic compensation and deepening our  
29 understanding of how biological neural networks work.

30

# 1 Introduction

2 Ca<sup>2+</sup>-activated K<sup>+</sup> channels are widely expressed in both the visual system and CNS and play  
3 important roles in cell physiology, such as modulating neuronal excitability and neurotransmitter  
4 release. Based upon their kinetics, pharmacological and biophysical properties, these channels can  
5 be divided into two main types: the “small”- (SK; 2-20 pS) and “big”-conductance (BK; 200-400 pS)  
6 channels. The SK channels are solely Ca<sup>2+</sup>-activated (Sah, 1996; Faber and Sah, 2003; Stocker,  
7 2004; Salkoff, 2006), while BK channels are both Ca<sup>2+</sup>- and voltage-dependent. At synapses, SK  
8 channels form negative feedback loops with Ca<sup>2+</sup> sources and are therefore essential regulators of  
9 synaptic transmission (Faber et al., 2005; Ngo-Anh et al., 2005). The functional role of BK channels  
10 in synaptic activities is less well understood, with various effects of blocking BK channels on  
11 neurotransmitter release having been reported (Fettiplace and Fuchs, 1999; Ramanathan et al.,  
12 1999; Xu and Slaughter, 2005).

13  
14 Although Ca<sup>2+</sup>-activated K<sup>+</sup> channels – through regulation of synaptic transmission between retinal  
15 neurons – seem to have conserved roles in early vertebrate (Shatz, 1990; Wang et al., 1999; Klocker  
16 et al., 2001; Pelucchi et al., 2008; Clark et al., 2009; Grimes et al., 2009) and invertebrate vision  
17 (Abou Tayoun et al., 2011), it has been difficult to work out how these channels advance *in vivo* circuit  
18 functions and what are their evolutionary benefits. This is because homeostatic processes that  
19 regulate electrical activity in neurons, in part, make communication in circuits surprisingly fault-  
20 tolerant against perturbations (Lemasson et al., 1993; Marder and Goaillard, 2006). Thus, the  
21 physical consequences of altering K<sup>+</sup> channel densities and those of homeostatic compensation are  
22 interconnected. Because *Drosophila* has single SK (*dSK*) and BK (*dSlo*) genes,  
23 electrophysiologically accessible photoreceptors and interneurons (large monopolar cells, LMCs)  
24 (Juusola and Hardie, 2001a; Zheng et al., 2006) with stereotypical connectivity (Meinertzhagen and  
25 O'Neil, 1991; Rivera-Alba et al., 2011), and readily quantifiable optomotor behavior (Blondeau and  
26 Heisenberg, 1982; Juusola et al., 2017), it provides an excellent model system to characterize how  
27 Ca<sup>2+</sup>-activated K<sup>+</sup> channels affect circuit functions and the capacity to see. Importantly, *Drosophila*  
28 photoreceptors and LMCs express both *dSK* and *dSlo* genes (Abou Tayoun et al., 2011; Davis et al.,  
29 2018). Here, we study to what extent intrinsic perturbations of missing one or both of these K<sup>+</sup>  
30 channels, through gene-deletion, can be neutralized by homeostatic processes trying to sustain

1 normal network functions, and what is the price of this compensation.  
2  
3 By using electrophysiological and behavioral assays and biophysical modelling, we uncover why  
4  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels improve communication between photoreceptors and Large Monopolar  
5 Cells (LMCs), which in the fly eye lamina network form stereotypical columns of feedforward and  
6 feedback synapses (R-LMC-R system) that process and route visual information to the fly brain. We  
7 show that although the loss of SK and BK channels does not diminish *Drosophila* photoreceptors'  
8 information sampling capacity *in vivo*, it homeostatically reduces other  $\text{K}^+$  currents and overloads  
9 synaptic-feedback from the lamina network. This makes communication between the mutant  
10 photoreceptors and LMCs inefficient, consuming more energy and distorting visual information flow  
11 to the brain. Thus, homeostatic compensation of missing SK and BK channels within the lamina  
12 network is suboptimal and comes with an unavoidable cost of reduced adaptability and altered  
13 (accelerated or decelerated) vision, which thereby must contribute to the mutant flies' uniquely tuned  
14 optomotor behaviors.  
15  
16 These results quantify the benefits of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels in improving robustness,  
17 economics and adaptability of neural communication and perception.  
18  
19

# 1 **Materials and Methods**

## 2 ***Drosophila melanogaster* strains and rearing**

3 *w+*; +; *dSlo4* (Gift from Nigel Atkinson laboratory, RRID: Addgene\_16173; other identifiers: CG10693,  
4 FBgn0003429).

5 *w+*; +; *dSlo4/dSlo18* (Gift from Allen Shearn laboratory, identifiers: Dmel\ash218, FBal0057820).

6 *w+*; *dSK-*; + (Patrick Dolph laboratory, RRID: AB\_2566830; other identifiers: CG10706,  
7 FBgn0029761).

8 *w+*; *dSK-*; +; *dSlo4* (in house).

9 *w+* *dSK-*; +; *dSlo4/dSlo18* (in house).

10

11 The flies were maintained in the stock as:

12 *w+*; +; *Slo4/TM6*

13 *w+* *dSK-*; +; +

14 *w+* *dSK-*; +; *dSlo4/TM6*

15 *w+*; +; *dSlo18/TM6*

16

17 The *dSK-* alleles were prepared as described earlier (Abou Tayoun et al., 2011). *Df7753* or  
18 *Df(1)Exel6290* line was obtained from Bloomington *Drosophila* stock center.

19

20 *dSlo4* null allele (Atkinson et al., 1991) was kindly provided by Dr. Nigel Atkinson. *dSlo4* mutants  
21 appear often unhealthy, with the *dSlo* channel being expressed both in muscles and the brain (due  
22 to its 2 independent control regions), making them hesitant fliers (Atkinson et al., 2000). Therefore,  
23 we generated transheterozygotes *dSlo4/dSlo18*, facilitating the flight simulator experiments.  
24 *dSlo4* and *dSlo18* (also called *ash2<sup>18</sup>*) are both mutations of slowpoke (Lajeunesse and Shearn, 1995;  
25 Atkinson et al., 2000). But slowpoke has multiple promoters: *dSlo4* is a loss of function, whereas  
26 *dSlo18* affects promoter C0 and C1 (neural-specific) yet leaves C2 promoter intact. *dSlo18* produces  
27 a functional channel in the muscle, thereby mostly rescuing the flight deficits. *dSlo18* only affects the  
28 brain control region and is homozygous lethal, and thus, both *dSlo4* and *dSlo18* were maintained over  
29 a *TM6b* balancer. For experimental flies, *dSlo4/TM6* or *dSK-;dSlo4/TM6* were crossed to *dSlo18* and  
30 we selected against the *TM6* balancer. When combined in a *dSlo4/dSlo18*, the mutations only affect

1 the expression of *dSlo* in the brain . All the flies were previously outcrossed to a common Canton-S  
2 background, which was the wild-type control. The overall yield of *dSlo*<sup>-</sup> mutants was lower than for  
3 the other flies, with the surviving adults flies being typically smaller, which suggested that  
4 homozygotic *dSlo*<sup>-</sup> mutants were less healthy.

5  
6 *Drosophila* were raised on molasses based food at 18 °C, under 12:12 h light:dark conditions. Prior  
7 to the experiments, the flies were moved to the laboratory (~21 °C) overnight or kept in a separate  
8 incubator at 25 °C. All electrophysiology (intracellular, electroretinogram and whole-cell recordings)  
9 was conducted at 20 ± 1 °C and optomotor behavior experiments at 21 ± 1 °C. During *in vivo*  
10 recordings, the fly temperature was feedback-controlled by a Peltier-system (Juusola and Hardie,  
11 2001a; Juusola et al., 2016). Moreover, the theoretical model simulations of the R-LMC-R system  
12 (see below) were also calculated for 20 °C, by adjusting the Q<sub>10</sub> of phototransduction reactions and  
13 membrane properties accordingly (Juusola and Hardie, 2001a; Song et al., 2012). Thus, by retaining  
14 effectively the same temperature for experiments and theory, we could compare directly the wild-  
15 type and mutant electrophysiology to their respective model predictions and optomotor behaviors.

16  
17 Because the intracellular response dynamics of *dSlo*<sup>4</sup> and *dSlo*<sup>4</sup>/*dSlo*<sup>18</sup> R1-R6 photoreceptors and  
18 LMCs, respectively, appeared consistently similar, differing in the same way from the wild-type  
19 responses, these responses were pooled in the main results (Figs 3-12). For the same reason, the  
20 corresponding responses of *dSK*<sup>;;dSlo</sup><sup>4</sup> and *dSK*<sup>;;dSlo</sup><sup>4</sup>/*dSlo*<sup>18</sup> R1-R6 and LMCs were also pooled.

21  
22 ***Why dSK and dSlo expression in photoreceptors or LMCs was not manipulated using Gal4-***  
23 ***drivers.*** When using mutant animals, it is standard practice to use cell-specific transgenic rescues  
24 to show that the described phenotype is causally linked to the used mutations (rather than genetic  
25 background effects linked to the mutation), and/or to complement classical mutants with cell-specific  
26 RNAi knockdown. This is normally done by using cell-specific Gal4 drivers to control expression of  
27 transgenes or RNAis. However, for the current study, we deemed these methods unviable. For LMCs,  
28 we could not use them because for technical reasons our recordings mix L1 and L2 cell types; see  
29 ***In vivo intracellular recordings*** below. Conversely, for common Gal4 photoreceptor lines (e.g.  
30 GMR, longGMR), our tests indicate that these compromise development (Bollepalli et al., 2017),  
31 causing reductions in sensitivity, dark noise, potassium currents, and cell size and capacitance, as

1 well as extreme variations in sensitivity between cells (Fig. 1). We have also found that another  
2 commonly used line (Rh1-Gal4), although not causing the same developmental abnormalities, leads  
3 to highly variable (~100-fold) UAS-transgene expression levels from cell to cell (Fig. 1F). In our hands,  
4 we have also found that effective RNAi in the photoreceptors is only reliably achieved with the strong  
5 GMR or longGMRGal4 drivers with their attendant developmental abnormalities (Fig. 1). Thus, the  
6 use of Gal4 drivers would be expected to induce experimental variability, altering photoreceptor  
7 output dynamics far more than the contribution of missing dSlo- or dSK-channels. Such controls  
8 would therefore increase uncertainty rather than reduce it, making their use here scientifically  
9 unsound.

10

## 11 **Electrophysiology and Analysis**

12 **Electroretinograms (ERGs).** ERGs were recorded from intact flies following the standard  
13 procedures (Dau et al., 2016). ≤1 week old adult female *Drosophila* were fixed into a conical holder  
14 (Juusola and Hardie, 2001b; Juusola et al., 2016), using low melting point beeswax, and stimulated  
15 by 1 s light pulses from a green (560 nm) LED with the brightest effective intensity, estimated to be  
16  $\sim 5 \times 10^6$  effective photons/photoreceptor/s. Both recording and reference electrodes were filled with  
17 *Drosophila* ringer (in mM): 120 NaCl, 5 KCl, 1.5 CaCl<sub>2</sub>, 4 MgCl<sub>2</sub>, 20 proline, and 5 alanine. The  
18 recording electrode was positioned to touch the cornea and the indifferent electrode the head  
19 capsule near the ocelli. Recorded signals were low-pass filtered at 200 Hz and amplified via a npi  
20 SEC-10LX amplifier (npi Electronics, Germany).

21

22 A wild-type ERG comprises two main components: a slow component and transients coinciding with  
23 changes in light stimuli (Heisenberg, 1971). The slow component (or maintained background  
24 potential) is attributed to photoreceptor output and has the inverse waveform of photoreceptors'  
25 intracellular voltage responses, while on- and off-transients originate from the postsynaptic cells in  
26 the lamina (Coombe, 1986). We further plotted the ERGs as dynamic spectra (Fig. 8H) to highlight  
27 how their oscillation frequencies changed in respect to light stimulation (Wolfram and Juusola, 2004).

28

29 **Whole-Cell Recordings.** Dissociated ommatidia were prepared from recently eclosed adult female  
30 flies and transferred to a recording chamber on an inverted Nikon Diaphot microscope (Hardie et al.,  
31 2002). The control bath solution contained 120 mM NaCl, 5 mM KCl, 10 mM N-Tris-(hydroxymethyl)-



1 methyl-2-amino-ethanesulphonic acid (TES), 4 mM MgCl<sub>2</sub>, 1.5 mM CaCl<sub>2</sub>, 25 mM proline, and 5 mM  
2 alanine. Osmolarity was adjusted to ~283 mOsm. The intracellular solution used in the recording  
3 pipette was composed of 140 mM K<sup>+</sup> gluconate, 10 mM TES, 4 mM Mg<sup>2+</sup> ATP, 2 mM MgCl<sub>2</sub>, 1 mM  
4 NAD, and 0.4 mM Na<sup>+</sup> GTP. Data were recorded with Axopatch 1-D or 200 amplifiers and analyzed  
5 with pClamp software (Axon Instruments). Cells were stimulated by a green-light-emitting diode with  
6 intensities calibrated in terms of effectively absorbed photons by counting quantum bumps at low  
7 intensities in wild-type flies.

8  
9 ***In vivo intracellular recordings.*** 3-7 days old (adult) female flies were used in the experiments; the  
10 female flies are larger than the males, making the recordings somewhat easier. A fly was fixed in a  
11 conical fly-holder with beeswax, and a small hole (6-10 ommatidia) for the recording microelectrode  
12 entrance was cut in its dorsal cornea and Vaseline-sealed to protect the eye (Juusola and Hardie,  
13 2001b; Zheng et al., 2006). Sharp quartz and borosilicate microelectrodes (Sutter Instruments),  
14 having 120–200 MΩ resistance, were used for intracellular recordings from R1-R6 photoreceptors  
15 and large monopolar cells (LMCs). These recordings were performed separately; with the electrodes  
16 filled either with 3 M KCl solution for photoreceptor or 3 M potassium acetate with 0.5 mM KCl for  
17 LMC recordings (Juusola et al., 1995b; Zheng et al., 2006), to maintain chloride battery. A reference  
18 electrode, filled with fly ringer, was gently pushed through ocelli ~100 μm into the head, in which  
19 temperature was kept at 19 ± 1°C by a Peltier device (Juusola and Hardie, 2001a).

20  
21 Only stable high-quality recordings were included. In darkness, R1-R6s' maximum responses to  
22 saturating bright pulses were characteristically >40 mV (wild-type, all mutants); the corresponding  
23 LMC recordings showed resting potentials <-30 mV and 10-40 mV maximum response amplitudes  
24 (wild-type and all mutants). Although the large maximum response variation is typical for *Drosophila*  
25 intracellular LMC recordings, their normalized waveforms characteristically display similar time-  
26 courses and dynamics (Nikolaev et al., 2009; Zheng et al., 2009). The smaller and more frequent  
27 responses are likely from LMC somata. These have larger diameters than the small and narrow LMC  
28 dendrites, in which responses should be the largest but the hardest to record from (Nikolaev et al.,  
29 2009; Zheng et al., 2009; Wardill et al., 2012). LMC subtypes were not identified, but most recordings  
30 were likely from L1 and L2 as these occupy the largest volume. Occasionally, we may have also  
31 recorded from other neurons or glia, which receive histaminergic inputs from photoreceptors (Shaw,

1 1984; Zheng et al., 2006; Zheng et al., 2009; Rivera-Alba et al., 2011). But because the selected  
2 recordings shared similar hyperpolarizing characteristics, LMC data for each genotype were  
3 analyzed together. Such pooling is further justified by the *Janelia Farm* gene expression data (Davis  
4 et al., 2018), which shows that both *dSK* and *dSlo* genes are rather highly expressed (in  
5 transcripts/million units) across all LMC types, and that all of these cells are expressing both genes.

6  
7 Light stimulation was delivered to the studied cells at center of its receptive field with a high-intensity  
8 green LED (Marl Optosource, with peak emission at 525 nm), through a fiber optic bundle, fixed on  
9 a rotatable Cardan arm, subtending 5° as seen by the fly. Its intensity was set by neutral density  
10 filters (Kodak Wratten) (Juusola and Hardie, 2001b); the results are shown for dim (estimated to be  
11 ~600), medium ( $\sim 6 \times 10^4$ ) and bright luminance ( $\sim 6 \times 10^5$  photons/s); or log -3, log -1 and log 0,  
12 respectively.

13  
14 Voltage responses were amplified in current-clamp mode using 15 kHz switching rate (SEC-10L  
15 single-electrode amplifier; NPI Electronic, Germany). The stimuli and responses were low-pass  
16 filtered at 500 Hz (KemoVBF8), and sampled at 1 or 10 kHz. The data were re-sampled/processed  
17 off-line at 1-2 kHz for the analysis. Stimulus generation and data acquisition were performed by  
18 custom-written Matlab (MathWorks, Natick, MA) programs: BIOSYST (Juusola and Hardie, 2001b;  
19 Juusola and de Polavieja, 2003).

20  
21 **Data Analysis.** The signal was the average of consecutive 1,000 ms long voltage responses to a  
22 repeated light intensity time series, selected from the naturalistic stimulus (NS) library (van Hateren,  
23 1997), and its power spectrum was calculated using Matlab's Fast Fourier Transform (FFT) algorithm.  
24 First 10-20 responses were omitted because of their adaptive trends, and only approximately steady-  
25 state adapted responses were analyzed. The noise was the difference between individual responses  
26 and the signal, and its power spectra were calculated from the corresponding traces (Juusola et al.,  
27 1994). Thus,  $n$  trials (with  $n = 20$ ), gave one signal trace and  $n$  noise traces. Both signal and noise  
28 data were chunked into 50% overlapping stretches and windowed with a Blackman-Harris-term  
29 window, each giving three 500-point-long samples. This gave 60 spectral samples for the noise and  
30 three spectral samples for the signal, which were averaged, respectively, to improve the estimates.  
31  $SNR(f)$ , of the recording or simulation was calculated from their signal and noise power spectra,

1  $\langle |S(f)|^2 \rangle$  and  $\langle |N(f)|^2 \rangle$ , respectively, as their ratio, where  $\|\cdot\|$  denotes the norm and  $\langle \cdot \rangle$  the average over  
 2 the different stretches (Juusola and Hardie, 2001b; Juusola and de Polavieja, 2003; Song and  
 3 Juusola, 2014).

4  
 5 Information transfer rates,  $R$ , for each recording were estimated by using the Shannon formula  
 6 (Shannon, 1948), which has been shown to obtain robust estimates for these types of continuous  
 7 signals (Juusola and de Polavieja, 2003; Song and Juusola, 2014; Juusola et al., 2017). We analyzed  
 8 steady-state-adapted recordings and simulations, in which each response (or stimulus trace) is  
 9 expected to be equally representative of the underlying encoding (or statistical) process. From  $SNR(f)$ ,  
 10 the information transfer rate estimates were calculated as follows:

$$R = \int_2^{500 \text{ Hz}} \log_2(SNR(f) + 1) df \quad (1)$$

11  
 12 with the integral upper and lower bounds resulting from 1 kHz sampling rate and 500 points window  
 13 size, respectively. The underlying assumptions of this method and how the number and resolution  
 14 of spectral signal and noise estimates and the finite size of the used data can affect the resulting  
 15 information transfer rate estimates have been analyzed before (van Hateren, 1992a; Juusola and de  
 16 Polavieja, 2003; Song and Juusola, 2014) and are further discussed in (Juusola et al., 2017).

17  
 18 Using some longer recording series (to 50 stimulus repetitions), we further tested these  $R$  estimates  
 19 against those obtained by the triple extrapolation method (Juusola and de Polavieja, 2003). This  
 20 method, unlike SNR analysis, requires no assumptions about the signal and noise distributions or  
 21 their additivity. Voltage responses were digitized by sectioning them into time intervals,  $T$ , that were  
 22 subdivided into smaller intervals  $t = 1$  ms. In the final step, the estimates for the entropy rate,  $R_S$ , and  
 23 noise entropy rate,  $R_N$ , were then extrapolated from the values of the experimentally obtained  
 24 entropies to their successive limits, as in (Juusola and de Polavieja, 2003):

$$R = R_S - R_N = \lim_{T \rightarrow \infty} \frac{1}{T} \lim_{v \rightarrow \infty} \lim_{size \rightarrow \infty} (H_S^{T,v,size} - H_N^{T,v,size}) \quad (2)$$

25  
 26 where  $T$  is the length of the ‘words’,  $v$  the number of voltage levels (in digitized amplitude resolution)  
 27 and the *size* of the data file. The difference between the entropy and noise entropy rates is the rate  
 28 of information transfer,  $R$  (Shannon, 1948; Juusola and de Polavieja, 2003). Again, as shown before  
 29 for comparable data (Song and Juusola, 2014; Dau et al., 2016; Juusola et al., 2017), both methods

1 gave similar  $R$  estimates, implying that the Shannon method (Eq. 1) estimates were unbiased.

2

3 As expected, information transfer rates at 20 °C were lower than those at 25 °C (Song and Juusola,  
4 2014; Juusola et al., 2017), which is *Drosophila*'s preferred temperature (Sayeed and Benzer, 1996).  
5 Presumably, because of the tightly-compartmentalized enzymatic reactions inside each of its 30,000  
6 microvilli (phototransduction/photon sampling units), the  $Q_{10}$  of a *Drosophila* R1-R6's information  
7 transfer is high for many light stimuli;  $\geq 4$  for bright 200 Hz Gaussian white-noise stimulation (Juusola  
8 and Hardie, 2001a). Whereas, the  $Q_{10}$  of simple diffusion-limited reactions, such as ion channel  
9 currents, is lower,  $\sim 2$  (Lamb, 1984; Juusola and Hardie, 2001a). Critically here, stochastic R1-R6  
10 model simulations imply that warming accelerates microvilli recovery from their previous light-  
11 activation by shortening their refractory period (Song and Juusola, 2014). Therefore, for many bright  
12 fast-changing light patterns, a warm R1-R6 transduces characteristically more photons to quantum  
13 bumps than a cold one. And, with more bumps summing up bigger and faster macroscopic  
14 responses, extending their reliability to higher stimulus frequencies, information transfer increases  
15 (Juusola and Hardie, 2001a; Juusola et al., 2016; Juusola and Song, 2017).

16

## 17 **Behavioral Experiments and Analysis**

18 In the flight simulator experiments, we used 3-7 days old female flies, reared in 12:12 h dark:light  
19 cycle. A flying fly, tethered from the classic torque-meter (Tang and Guo, 2001), which fixed its head  
20 in a rigid position and orientation, was lowered by a manipulator in the center of a black-white cylinder  
21 (spectral full-width: 380-900 nm). It saw a continuous (360°) stripe-scene. After viewing the still scene  
22 for 1 s, it was spun to the counter-clockwise by a linear stepping motor for 2 s, stopped for 2 s, before  
23 rotating to clock-wise for 2 s, and stopped again for 1 s. This 8 s stimulus was repeated 10 times  
24 and each trial, together with the fly's yaw torque responses, was sampled at 1 kHz and stored for  
25 later analysis (Wardill et al., 2012). Flies followed the scene rotations, generating yaw torque  
26 responses (optomotor responses to right and left), the strength of which presumably reflects the  
27 strength of their motion perception (Götz, 1964). The moving stripe scenes had: azimuth  $\pm 360^\circ$ ;  
28 elevation  $\pm 45^\circ$ ; wavelength  $14.4^\circ$  (coarse) and  $3.9^\circ$  (fine-grained = hyperacute); contrast 1.0, as seen  
29 by the fly. The scene was rotated at  $45^\circ/\text{s}$  (slow) of  $300^\circ/\text{s}$  (fast).

30

## 31 **Biophysical Models for Estimating Wild-type and Mutant R1-R6s' Energy Consumption**

1 Our published (Song et al., 2012) and extensively tested (Song and Juusola, 2014; Juusola et al.,  
2 2015; Juusola et al., 2017; Song and Juusola, 2017) *Drosophila* R1-R6 photoreceptor model (Figs  
3 2A-D), was used to simulate both the wild-type and mutant voltage responses to naturalistic light  
4 intensity time series. It has four modules: (1) random photon absorption model, which regulates  
5 photon absorptions in each microvillus, following Poisson statistics (Fig. 2C, green); (2) stochastic  
6 quantum bump (QB) model, in which stochastic biochemical reactions inside a microvillus captures  
7 and transduces the energy of photons to variable QBs or failures (Figs 2A-B); (3) summation model,  
8 in which QBs from 30,000 microvilli integrate the macroscopic light-induced current (LIC) response  
9 (Fig. 2C, blue); and (4) Hodgkin–Huxley (HH) model of the photoreceptor plasma-membrane (Niven  
10 et al., 2003; Vähäsöyrinki et al., 2006; Song et al., 2012), which transduces LIC into voltage response  
11 (Fig. 2D). The model's open-source Matlab code can be downloaded from GitHub (the links below).

12  
13 Modules 1-3 simulate the stochastic phototransduction cascade in the rhabdomere. Because the  
14 mutants' phototransduction reactions were physiologically intact (as shown in the Results), all the  
15 parameters were fixed and kept the same in the simulations (50 parameters in 20 Equations); the  
16 mathematical details and parameters values are given in (Song et al., 2012; Juusola et al., 2015).  
17 Module 4 models the R1-R6 plasma membrane using deterministic continuous functions (HH model),  
18 in which parameters scale the model response to light stimulation, and now also to the estimated  
19 synaptic feedback (see below), approximating the recorded response (Figs 2D-F).

20

### 21 **Estimating excitatory synaptic feedback conductance**

22 Differences between the simulated and recorded responses (Fig. 2E) should reflect a real  
23 photoreceptor's synaptic feedback dynamics - input from LMCs (Zheng et al., 2006; Rivera-Alba et  
24 al., 2011; Dau et al., 2016), which the original R1-R6 model lacks (Juusola et al., 2017). Using these  
25 differences, one can work out the synaptic input current to R1-R6s.

26

27 The synaptic feedback current to each recorded R1-R6, whether wild-type or mutant, was  
28 extrapolated computationally by using the same fixed LIC (to the naturalistic light stimulus, Fig. 2C)  
29 with their specific *Shaker* ( $I_A$ ) and *Shab* ( $I_{Ks}$ ) current dynamics (Fig. 2D; see **Whole-Cell Recordings**,  
30 above, and Tables 1-2). In this procedure, a new flat (Fig. 2F, gray) conductance, representing the  
31 missing synaptic input, was injected to the full wild-type,  $dSK^-$ ,  $dSlor^-$  or  $dSK^-;dSlor^-$  R1-R6 model.

1 This conductance waveform was then shaped up in a closed-loop (Fig. 2F, wine), by our open-source  
2 Matlab software (GitHub access below), until the model's voltage response matched the  
3 corresponding recorded voltage signal (a single R1-R6's average response; see *In vivo*  
4 **intracellular recordings**, above) for the same light stimulus.

5  
6 Remarkably, here, the predicted synaptic input from LMCs to a specific R1-R6 was extracted purely  
7 from the difference between the photoreceptor recording and simulation. Yet, it much resembled  
8 typical intracellular LMC voltage responses to the same light stimulus (see Results). This implies  
9 that the computationally extracted synaptic feedback, which systematically and consistently differed  
10 between the wild-type and mutant R1-R6s (see Results), would closely resemble the real excitatory  
11 feedback these cells receive from the lamina network *in vivo*. Such a strong logical agreement  
12 between these two independently obtained results highlights the explorative power of this new hybrid  
13 simulation/recording approach, validating its use.

14  
15 Note, one cannot calculate the synaptic current from *ex vivo* dissociated cells, even if it was possible  
16 to retain their axon terminals, as these have different capacitance, input currents, extracellular milieu  
17 and voltage gradients than the *in vivo* photoreceptors. *In vivo*, the retina and lamina are partitioned  
18 by a glia-barrier, which keeps their respective extracellular fields at different potentials (Shaw, 1984).

#### 20 **Estimating ATP Consumption for Information Transmission in Wild-type and Mutant R1-R6s**

21 While the microvilli, which form the photosensitive R1-R6 rhabdomere (Fig. 2B), generate the LIC,  
22 the photo-insensitive plasma membrane uses many voltage-gated ion channels to adjust the LIC-  
23 driven voltage responses. In response to LIC, these open and close, regulating the ionic flow across  
24 the plasma membrane and further modulating tonic neurotransmitter (histamine) release at the  
25 photoreceptor-LMC synapse (Hardie, 1989; Uusitalo et al., 1995b). In return, tonic excitatory  
26 synaptic feedback from the LMCs participate in shaping the R1-R6 voltage response (Fig. 2F)  
27 (Zheng et al., 2006; Zheng et al., 2009; Dau et al., 2016). But to maintain the pertinent ionic  
28 concentrations in- and outside, R1-R6s rely upon other proteins, such as ion cotransporters,  
29 exchangers and pumps, to uptake or expel ions. The work of moving ions against their  
30 electrochemical gradients consumes energy (ATP), and a R1-R6's ATP consumption thus much  
31 depends on the ionic flow dynamics through its ion channels (Laughlin et al., 1998). To approximate

1 these dynamics during light responses, we used our HH R1-R6 body model (Niven et al., 2003; Song  
2 et al., 2012), which models the ion channels as conductances.

3

4 The HH model has these ion transporters:  $3\text{Na}^+/2\text{K}^+$ -pump,  $3\text{Na}^+/\text{Ca}^{2+}$ -exchanger and  $\text{Na}^+/\text{K}^+/2\text{Cl}^-$   
5 mechanisms to balance the intracellular ionic fluxes.  $\text{Na}^+/\text{K}^+/2\text{Cl}^-$  cotransporter balances with the  
6 voltage-dependent  $\text{Cl}^-$  and  $\text{Cl}^-$  leak conductances, maintaining intracellular  $\text{Cl}^-$  concentration.  $\text{Ca}^{2+}$   
7 influx in the LIC (~41%) is then expelled by  $3\text{Na}^+/\text{Ca}^{2+}$ -exchanger in 1:3 ratio in exchange for  $\text{Na}^+$   
8 ions. Although there is  $\text{K}^+$  influx in LIC (~24%), this is not enough to compensate  $\text{K}^+$  leakage through  
9 voltage-gated  $\text{K}^+$  conductances and  $\text{K}^+$  leaks. Apart from a small amount of  $\text{K}^+$  intake through  
10  $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ -cotransporter,  $3\text{Na}^+/2\text{K}^+$ -pump is the major  $\text{K}^+$  uptake mechanism. It consumes 1 ATP  
11 molecule to uptake 2  $\text{K}^+$  ions and extrudes 3  $\text{Na}^+$  ions. Because it is widely regarded as the major  
12 energy consumer in the cell, we use only the pump current ( $I_p$ ) to estimate the ATP consumption  
13 (Skou, 1965; Laughlin et al., 1998; Skou, 1998). For these estimates, we generated two separate  
14 photoreceptor membrane models: a conservative one (Table 1; containing the known voltage-  
15 sensitive and leak potassium conductances) and a speculative one (Table 2; by adding an  
16 unconfirmed chloride conductance and leak, now balanced with larger voltage-sensitive  $\text{K}^+$   
17 conductances). Their differences helped us to work out how the earlier proposed hypothetical  
18 homeostatic compensation through leak- or chloride channel expression (Niven et al., 2003;  
19 Vähäsöyrinki et al., 2006) would change a photoreceptor's ATP consumption.

20

21 From the equilibrium of  $\text{K}^+$  fluxes,  $I_p$  can be calculated as follows:

$$22 \quad I_p = \frac{1}{2}(I_A + I_{Ks} + I_{new} + I_{K\_leak} - I_{LIC\_K}) - \frac{1}{4}(I_{Cl} + I_{Cl\_leak}) \quad (3)$$

23 where  $I_A$ ,  $I_{Ks}$ ,  $I_{new}$ , and  $I_{K\_leak}$  are the currents through *Shaker*, *Shab*, new, and  $\text{K\_leak}$  channels,  
24 respectively,  $I_{LIC\_K}$  is the  $\text{K}^+$  influx in LIC and  $I_{Cl}$  and  $I_{Cl\_leak}$  are the currents through the voltage-gated  
25  $\text{Cl}^-$  and  $\text{Cl}^-$  leak channels, respectively. These currents can be calculated from the reverse potential  
26 of individual ions and their HH model produced conductances using Ohm's law:

$$27 \quad I_A = (E_m - E_K)g_A \quad (4)$$

$$28 \quad I_{Ks} = (E_m - E_K)g_{Ks}$$

$$29 \quad I_{new} = (E_m - E_K)g_{new}$$

$$30 \quad I_{K\_leak} = (E_m - E_K)g_{K\_leak}$$

$$I_{Cl} = (E_m - E_K)g_{Cl}$$

$$I_{Cl\_leak} = (E_m - E_K)g_{Cl\_leak}$$

Using  $I_p$ , the number of ATP molecules hydrolyzed per second can be calculated:

$$\frac{ATP_{molecules}}{s} = \frac{\int_0^T I_p dt}{T} \times \frac{N_A}{F} \quad (5)$$

where  $N_A$  is Avogadro's constant and  $F$  is Faraday's constant. The ATP usage per bit of information was calculated by dividing the estimated ATP molecules hydrolyzed in 1 s by the estimated information transfer rates (bits/s). We did not model the respective pump dynamics because, for the purpose of calculating ATP, only the time-integrated ionic fluxes count, not the time constants.

Previously, because of lack of a complete model for the photosensitive membrane, the LIC has only been estimated at the steady-state, or DC (Laughlin et al., 1998; Niven et al., 2007), when the sum of all currents across the model membrane equals zero:

$$I_A + I_{Ks} + I_{new} + I_{K\_leak} + I_{Cl} + I_{Cl\_leak} + I_p + I_{LIC_K} = 0 \quad (6)$$

Thus here, the conservative photoreceptor membrane model (Table 1) lacked  $I_{Cl\_leak}$  and  $I_{Cl}$  in Eqs. 3, 4 and 6, whereas the speculative model (Table 2) included them. But for both membrane models, because we estimated LIC directly from the stochastic phototransduction model (above), we could calculate a R1-R6's energy cost in response to any arbitrary light pattern, including naturalistic stimulation. Thus, our phototransduction cascade model provides the *functional* equivalence to the light-dependent conductance used in the previously published steady-state models (Laughlin et al., 1998; Niven et al., 2007).

Finally, we note that if the ion transporters, including  $3Na^+/2K^+$ -pump, were inherently noisy, their metabolic work would reduce a photoreceptor's information transfer rate. However, such effects are likely very small as the recorded and simulated photoreceptor information transfer rate estimates effectively match over a broad range of light stimuli (Juusola et al., 2017). Conversely, in concordance the data processing theorem (Shannon, 1948; Juusola and de Polavieja, 2003; Cover and Thomas, 2006), ion transporters cannot increase a photoreceptor's information transfer rate as they cannot increase sample (QB) rate changes that sum up the response, increasing its  $SNR(f)$ .

## Code and Software Accessibility



1 Biophysical *Drosophila* model (Matlab) is freely available from github:

2 [https://github.com/JuusolaLab/Microsaccadic\\_Sampling\\_Paper/tree/master/BiophysicalPhotorecep](https://github.com/JuusolaLab/Microsaccadic_Sampling_Paper/tree/master/BiophysicalPhotoreceptorModel)  
3 [torModel](https://github.com/JuusolaLab/Microsaccadic_Sampling_Paper/tree/master/BiophysicalPhotoreceptorModel)

4 LMC feedback to R1-R6 estimation code (Matlab) is freely available from github:

5 [https://github.com/JuusolaLab/SK\\_Slo\\_Paper](https://github.com/JuusolaLab/SK_Slo_Paper)

6 Information estimation code (Matlab) is freely available from github:

7 [https://github.com/JuusolaLab/Microsaccadic\\_Sampling\\_Paper/tree/master/SNRAnalysis](https://github.com/JuusolaLab/Microsaccadic_Sampling_Paper/tree/master/SNRAnalysis)

8

## 9 **Histology**

10 **Electron Microscopy.** 3-to-7-day-old dark/light-reared *Drosophila* were cold anesthetized on ice  
11 and transferred to a drop of pre-fixative [modified Karnovsky's fixative: 2.5% glutaraldehyde, 2.5%  
12 paraformaldehyde in 0.1 M sodium cacodylate buffered to pH 7.3 – as per (Shaw et al., 1989)] on a  
13 transparent agar dissection dish. Dissection was performed using a shard of a razor blade (Feather  
14 S). Flies were restrained on their backs with insect pins through their lower abdomen and distal  
15 proboscis. Their heads were severed, proboscis excised, and halved. The left half-heads were  
16 collected in fresh pre-fixative and kept for 2 h at room temperature ( $21 \pm 1$  °C) under room light.

17

18 After pre-fixation, the half-heads were washed ( $2 \times 15$  min) in 0.1 M Cacodylate buffer, and then  
19 transferred to a 1 h post-fixative step, comprising Veronal Acetate buffer and 2% Osmium Tetroxide  
20 in the fridge (4°C). They were moved back to room temperature for a 9 min wash (1:1 Veronal Acetate  
21 and double-distilled H<sub>2</sub>O mixture), and serially dehydrated in multi-well plates with subsequent 9 min  
22 washes in 50, 70, 80, 90, 95, and  $2 \times 100\%$  ethanol. Post-dehydration, the half-heads were  
23 transferred to small glass vials for infiltration. They were covered in Propylene Oxide (PPO) for  $2 \times$   
24 9 min, transferred into a 1:1 PPO:Epoxy resin mixture (Poly/Bed® 812) and left overnight. The  
25 following morning, the half-heads were placed in freshly made pure resin for 4 h, and placed in fresh  
26 resin for a further 72 h at 60 °C in the oven. Fixation protocol was provided by Professor Ian  
27 Meinertzhagen (Dalhousie University, Canada).

28

29 Embedded half-heads were first sectioned (at 0.5 µm thickness) using a glass knife, mounted in an  
30 ultramicrotome (Reichert-Jung Ultracut E, Germany). Samples were collected on glass slides,  
31 stained using Toluidine Blue and observed under a light microscope. This process was repeated and

1 the cutting angle was continuously optimized until the correct orientation and sample depth was  
2 achieved; stopping when approximately 40 ommatidia were discernible. The block was then trimmed  
3 and shaped for ultra-thin sectioning. The trimming is necessary to reduce cutting pressure on the  
4 sample-block and resulting sections, thus helping to prevent “chattering” and compression artifacts.

5  
6 Ultra-thin sections (85 nm thickness) were cut using a diamond cutting knife (DiATOME Ultra 45°,  
7 USA), mounted and controlled using the ultramicrotome. The knife edge was first cleaned using a  
8 polystyrol rod to ensure integrity of the sample-blocks. The cutting angles were aligned and the  
9 automatic approach- and return-speeds set on the microtome. Sectioning was automatic and  
10 samples were collected in the knife water boat. Sections were transferred to Formvar-coated mesh-  
11 grids and stained for imaging: 25 min in Uranyl Acetate; a double-distilled H<sub>2</sub>O wash; 5 min in  
12 Reynolds’ Lead Citrate (Reynolds, 1963); and a final double-distilled H<sub>2</sub>O wash.

13  
14 **Conventional microscopy.** Heads of 8-day-old dark/light-reared female and male flies were  
15 bisected, fixed, and embedded as explained previously (Chinchore et al., 2009). 1 μm eye cross  
16 sections were cut using a Sorvall ultra microtome MT-1 (Sorvall, CT), stained with toluidine blue, and  
17 inspected using a Zeiss Axioplan2 microscope. Digital images were taken using Optronics DEI-750  
18 camera (Optronics) and MetaVue (Universal Imaging) software.

## 19 20 **Experimental Design and Statistical Analysis**

21 Figures show mean ± SD (or SEM) for each group (wild-type, *dSK*<sup>-</sup>, *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>), and  
22 typically also the individual values for each recordings (marked as ○). Significance between two  
23 groups was calculated using 2-tailed paired Student’s t-test (both with and without the equal variance  
24 assumption). In Figs 1 and 4, we used also one-way ANOVA for multiple comparisons between each  
25 group. For the analyses, we used OriginLab 2018b, Graphpad Prism v5 and Matlab statistical toolbox.  
26 Specific p-values and sample sizes are indicated in the relevant figures and/or legends.

## 27 28 **Results**

### 29 **Absence of dSK and dSlo Shapes Photoreceptor Responses**

30 To examine how Ca<sup>2+</sup>-activated K<sup>+</sup> channels shape *Drosophila* photoreceptor voltage output, we

1 performed *in vivo* intracellular recordings (Fig. 3A) from R1-R6 somata (Fig. 3B) in the retinae of  
2 *dSlo*<sup>-</sup>, *dSK*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> null mutants and wild-type flies, using conventional sharp  
3 microelectrodes. Briefly dark-adapted (~20 s) mutant R1-R6s responded to logarithmically  
4 brightening light flashes with increasing graded depolarizations (Fig. 3C), having wild-type-like or  
5 slightly smaller amplitudes (Fig. 3D). However, both *dSK*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6 outputs peaked  
6 faster (Fig. 3E; mean time-to-peak) and decayed earlier (Fig. 3F; mean half-width) to their respective  
7 resting potentials than the wild-type. While those of *dSlo*<sup>-</sup> R1-R6s, in contrast, showed decelerated  
8 dynamics, lasting longer than the wild-type except at the highest intensities (Figs 3C and 3F).

9  
10 Notably, however, in all the corresponding recordings, the early light-induced depolarizations (Fig.  
11 3C; light grey area) were similar, implying that the mutant R1-R6s sampled light information normally.  
12 Thus, phototransduction reactions inside a R1-R6's ~30,000 microvilli (photon sampling units; Fig.  
13 3B), which form its light-sensor, the rhabdomere (Hardie and Juusola, 2015), seemed unaffected by  
14 the absence of Ca<sup>2+</sup>-activated K<sup>+</sup> channels. But, instead, these mutant genotypes influenced more  
15 the subsequent neural information modulation phase (Fig. 3C; light brown area).

16

### 17 **Response Differences not from Homeostatic Ion Channel Expression**

18 If a R1-R6 photoreceptor was an isolated system, missing Ca<sup>2+</sup>-activated K<sup>+</sup>-conductances would  
19 directly increase its membrane resistance,  $R_m$ , and consequently its time constant ( $\tau_m = R_m \cdot C_m$ ;  $C_m$   
20 is membrane capacitance). This would slow down voltage responses to light changes. However, *in*  
21 *vivo*, as each R1-R6 features complex bioelectric interactions within its membrane and with its neural  
22 neighbors, the mutant responses showed far more sophisticated dynamics (Fig. 3), presumably  
23 reflecting homeostatic changes in these interactions (Marder and Goaillard, 2006; Vähäsöyrinki et  
24 al., 2006). Therefore, to work out what made the mutant R1-R6 outputs differ, we analyzed changes  
25 both in their intrinsic (membrane) properties and extrinsic (synaptic) feedback from the surrounding  
26 network.

27

28 We first asked whether the differences in *dSlo*<sup>-</sup>, *dSK*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6 voltage responses  
29 resulted from homeostatic somatic conductance changes. These would affect their membrane  
30 resistances, accelerating or decelerating signal conduction. For example, missing dSK channels in  
31 *dSK*<sup>-</sup> photoreceptors could be compensated by up-regulating dSlo channel expression, for which

1 these cells carry a normal gene; and *vice versa* in  $dSlo^-$  photoreceptors. Alternatively, the cells could  
2 increase  $K^+$ - or  $Cl^-$ -leak-conductances (Niven et al., 2003; Vähäsöyrinki et al., 2006). While such  
3 intrinsic homeostatic mechanisms could accelerate  $dSK^-$  R1-R6 output, these would also lower their  
4 resting potentials; by reducing depolarizing  $Ca^{2+}$ -load and/or increasing hyperpolarizing  $K^+/Cl^-$  loads.  
5 Equally, a lack of such homeostatic ion channel expression changes could have contributed to  $dSlo^-$   
6 photoreceptors' slower signaling.

7  
8 To test these hypotheses, we measured *in vivo* somatic electrical membrane properties in dark-  
9 adapted mutant and wild-type R1-R6s (Fig. 4A) using single-electrode current-clamp (e.g. Juusola  
10 and Weckström, 1993). We found that all the mutant R1-R6s charged smaller, but broadly wild-type-  
11 like voltage responses to injected current pulses (Fig. 4B). Depolarization to positive currents  
12 showed characteristic outward rectification (arrows), caused by activation of voltage-dependent  $K^+$   
13 channels (Hardie, 1991a; Hardie et al., 1991; Juusola and Hardie, 2001b; Vähäsöyrinki et al., 2006),  
14 while hyperpolarization to negative currents, in effect, charged their membranes passively.

15  
16 The membrane input resistances of the mutant R1-R6s (Fig. 4C), as determined by small  
17 hyperpolarizing responses to -0.02 nA current steps, were characteristically lower than in the wild-  
18 type (Juusola and Hardie, 2001b; Niven et al., 2003), with the mean resistance of  $dSK^-$  R1-R6s being  
19 the lowest (*cf.* Abou Tayoun et al., 2011). Most crucially, however, the mutant ( $dSK^-$ ,  $dSlo^-$  and  
20  $dSK^-;;dSlo^-$ ) photoreceptors' resting potentials (Fig. 4D), instead of being more hyperpolarized,  
21 were >10 mV more depolarized than the wild-type. Here, if  $dSK^-$  or  $dSlo^-$  R1-R6s' intrinsic signaling  
22 properties were regulated homeostatically, by ion channel expression (as hypothesized), then their  
23 resting potential in darkness should have been below the wild-type range, rather than above it. Also,  
24 the higher resting potentials (Fig. 4D) and lower membrane resistances (Fig. 4C) should have  
25 accelerated signal conduction. Yet, the mean  $dSlo^-$  R1-R6 voltage response time-to-peak values to  
26 intermediate light flash intensities were, in fact, slower than in the wild-type (Figs 3E and 3F).

27  
28 Hence, collectively, these results suggested that the accelerated ( $dSK^-$  and  $dSK^-;;dSlo^-$ ) and  
29 decelerated ( $dSlo^-$ ) light-induced voltage response dynamics of the mutant photoreceptors (Figs 3B  
30 and 3C) unlikely resulted from compensatory expression of leak- or  $Ca^{2+}$ -activated  $K^+$  channels at  
31 the somata, but required other/further mechanisms. Notice, however, these results were recorded

1 from dark-adapted R1-R6s at relative rest; without light-induced conductance (LIC) interactions.  
2 During brightening light stimulation, LIC and other conductances increase progressively with  
3 membrane depolarization (e.g. Song et al., 2012; Juusola et al., 2017), reducing resistance further  
4 by >>10-fold (e.g. Juusola and Weckström, 1993), as our recordings and simulations clarify later on.

5

## 6 **Response Differences not by Transduction or K<sup>+</sup> Conductance Differences**

7 To eliminate the possibility that developmental morphological defects in the mutant R1-R6s would  
8 have caused their altered responses, we assessed the mutant and wild-type eyes/retinae using both  
9 electron- (Fig. 5A, above) and light-microscopy (below). We found no obvious morphological  
10 differences between the eyes; with each method displaying highly ordered ommatidia with normal  
11 looking intact R1-R7 photoreceptor rhabdomeres.

12

13 Nevertheless, deletion of *dSlo*, *dSK* or both could still affect intracellular [Ca<sup>2+</sup>] regulation, and thus  
14 potentially alter microvillar phototransduction functions indirectly (Song et al., 2012; Hardie and  
15 Juusola, 2015), modifying sampling, amplification or integration of light-induced currents (LIC). We,  
16 therefore, used whole-cell recordings in dissociated ommatidia (Hardie, 1991b) (Fig. 5B) to compare  
17 the mutant and wild-type R1-R6s' elementary responses (quantum bumps, QBs) to single photons  
18 (Fig. 5C) and macroscopic LICs to light pulses (Figs 5D and 5E). In this preparation, photoreceptor  
19 axon terminals were severed, cutting off any synaptic feedback from the lamina network to R1-R6s  
20 (Zheng et al., 2006).

21

22 We found the mutant R1-R6s' bump amplitudes and waveforms (Fig. 5C) and macroscopic LICs  
23 (Figs 5D and 5E) to increasing light intensities wild-type-like, showing normal dynamics within the  
24 normal experimental variation. Here, the smaller *dSlo*<sup>-</sup> LIC maxima likely resulted from the smaller  
25 size of these homozygotic mutant flies due to their lower yield/reduced health. Thus, deletion of *dSlo*,  
26 *dSK* or both channels neither disrupted the microvillar R1-R6 morphology nor its phototransduction  
27 functions, again suggesting that the mutant R1-R6s would sample light information like their wild-  
28 type counterparts (see: Song et al., 2012; Hardie and Juusola, 2015; Juusola and Song, 2017).

29

30 Intriguingly, however, K<sup>+</sup> conductances in dissociated *dSK*<sup>-</sup> and *dSlo*<sup>-</sup> R1-R6s showed slightly  
31 reduced (19-36%) fast A- (*I<sub>A</sub>* or *Shaker*) and delayed rectifier currents (*I<sub>KS</sub>* or *Shab*) (Figs 5F-H), while

1 these currents were broadly wild-type-like in *dSK<sup>-/-</sup>;dSlo<sup>-/-</sup>* R1-R6s. The decrease in the  $I_A$  and  $I_{Ks}$   
2 currents together with dSK or dSlo current removal should, with other things being equal, increase  
3 membrane resistance and its time constant, leading to slower voltage responses. Instead *in vivo*, we  
4 found resistance in all the mutant R1-R6s below the wild-type (Fig. 4C), with both *dSK<sup>-/-</sup>* and  
5 *dSK<sup>-/-</sup>;dSlo<sup>-/-</sup>* R1-R6s responding faster and only *dSlo<sup>-/-</sup>* R1-R6s slower (Fig. 3E), implying that  
6 homeostatic changes in K<sup>+</sup> channel expression alone cannot explain their response differences.

7  
8 Together, the observed normal rhabdomere morphology, wild-type-like LIC dynamics and only partly  
9 reduced photo-insensitive membrane conductances implied that the mutant R1-R6s' accelerated or  
10 decelerated voltage responses, higher resting potentials and lower membrane resistance *in vivo*  
11 could not be induced by homeostatic ion channel expression changes in photoreceptor somata alone.  
12 But this would more require network adaptation (Nikolaev et al., 2009; Zheng et al., 2009), parallel  
13 changes in the synaptic network activity. In such scenarios, missing one or both Ca<sup>2+</sup>-activated K<sup>+</sup>  
14 channels would cause a homeostatic (automatic) rebalancing of the bidirectional signal transfer  
15 between photoreceptor axon terminals and the lamina interneurons (Shaw, 1984; Zheng et al., 2006;  
16 Zheng et al., 2009; Abou Tayoun et al., 2011; Dau et al., 2016).

17

### 18 **dSK or dSlo Absence Changes Network Adaptation**

19 In the adult *Drosophila* brain, dSlo and dSK share similar expression patterns with higher expression  
20 in the lamina and medulla neuropils and weaker in the retina (Becker et al., 1995; Abou Tayoun et  
21 al., 2011). Thus, theoretically, dSlo and dSK could co-participate in shaping the bidirectional signal  
22 transfer between R1-R6 photoreceptor axons and LMCs, which form columnar R-LMC-R network  
23 processing units in the lamina (Nikolaev et al., 2009; Zheng et al., 2009). Here, the deletion of one  
24 or the other ion channel could disrupt this balance.

25

26 We, therefore, next asked how Ca<sup>2+</sup>-activated K<sup>+</sup> channels might contribute to network adaptation in  
27 the R-LMC-R system. We recorded *dSK<sup>-/-</sup>*, *dSlo<sup>-/-</sup>*, *dSK<sup>-/-</sup>;dSlo<sup>-/-</sup>* and wild-type R1-R6 responses to a  
28 repeated 1 s naturalistic light intensity time series stimulus (NS) (van Hateren, 1997) *in vivo*, and  
29 found each of them adapting differently (Fig. 6A).

30

31 The mean of the wild-type response (Fig. 6B, black trace; measured at each second) decreased

1 approximately exponentially as the cells adapted to NS (Fig. 6C), reaching a relative steady-state in  
2 15-20 s (Figs 6B and 6C). In contrast, the corresponding means of the mutant responses declined  
3 faster but then displayed unique genotype-specific undershooting. The means of  $dSK^-$  (red trace)  
4 and  $dSK^-;;dSlo^-$  (orange) responses first decreased to their minima in  $<10$  s, and then increased, as  
5 the cells gradually further depolarized, reaching a relative steady-state in 35-40 s;  $\sim 20$  s later than  
6 the wild-type. While the mean of  $dSlo^-$  photoreceptor output (blue) decayed slower than in the other  
7 mutant R1-R6s and undershot less.

8  
9 Concurrently, the wild-type and mutant R1-R6 output ranges - measured as the standard deviation  
10 (Fig. 6D) of their response waveforms (Fig. 6C) at each second of NS - adapted with distinctive  
11 dynamics and speeds.  $dSlo^-$  R1-R6 outputs desensitized the slowest, slower than the wild-type, with  
12 their ranges compressing with different average time courses ( $\tau_{dSlo^-} = 3.41 \pm 3.28$  s,  $n = 19$  cells [22  
13 recordings];  $\tau_{Wild-type} = 1.47 \pm 0.67$ ,  $n = 7$  cells [10 recordings]; mean  $\pm$  SD) (Fig. 6D). Conversely,  
14  $dSK^-$  and  $dSK^-;;dSlo^-$  R1-R6 output ranges first compressed as rapidly as the wild-type ( $\tau_{dSK^-} = 1.45$   
15  $\pm 0.66$ ,  $n = 7$  cells [7 recordings];  $\tau_{dSK^-;;Slo^-} = 1.44 \pm 0.32$ ,  $n = 8$  cells [9 recordings]), but then slowly  
16 begun to expand, reflecting their rather similar mean voltage dynamics (Fig. 6B). The adaptive range  
17 reduction occurred most severely in  $dSK^-;;dSlo^-$  and  $dSlo^-$  R1-R6s, leaving their steady-state  
18 responses  $\sim 10\%$  smaller than those of the wild-type.

19  
20 These results highlight the complex role of  $Ca^{2+}$ -activated  $K^+$  channels in regulating R1-R6 output in  
21 network adaptation. While the absence of  $dSlo$  channel slowed adaptation in  $dSlo^-$  R1-R6s, the  $dSK^-$   
22 and the double-mutant  $dSK^-;;dSlo^-$  R1-R6s adapted faster but showed overshooting dynamics.  
23 Consequently, as an overall sign of compromised gain control, the mutant R1-R6s reached their  
24 steady-state responsiveness 20-30 s later than the wild-type. Thus, each mutant R-LMC-R system  
25 adapted suboptimally, constrained to its own unique dynamics.

26

### 27 **$dSK$ or $dSlo$ Absence Leaves Information Sampling Intact**

28 A R1-R6's information transfer rate depends mostly on its photon-absorption rate changes, set by  
29 the number of individual sampling units (rhabdomeric microvilli) and the speed and refractoriness of  
30 their phototransduction reactions (Song et al., 2012; Juusola et al., 2017; Juusola and Song, 2017).  
31 In contrast, obeying the data processing theorem, any changes in membrane filtering affect signal

1 and noise equally, and therefore cannot increase information (Shannon, 1948; Juusola and de  
2 Polavieja, 2003; Cover and Thomas, 2006). Accordingly, information transfer rates of mutant  
3 photoreceptors with normal phototransduction but without specific  $K^+$  channels, such as the slow  
4 delayed rectifier *Shab* ( $I_{KS}$ ) (Vähäsöyrinki et al., 2006), are broadly wild-type-like. But mutations that  
5 damage ion channels can destroy information. For example, *Sh* mutant R1-R6s' "nonfunctional"  
6 *Shaker* ( $I_A$ )  $K^+$  channels appears to truncate signal amplification while generating noise, reducing  
7 information flow (Niven et al., 2003). Critically, however, the R-LMC-R system has intrinsic potential  
8 to combat detrimental changes within its parts. A R1-R6's impaired function can be compensated in  
9 part by extra light information (through gap-junctions and feedback synapses) from its neighbors, in  
10 which receptive fields face the same visual area (Shaw, 1984; Zheng et al., 2006; Wardill et al., 2012;  
11 Juusola et al., 2017).

12  
13 Because *dSlo*<sup>-</sup>, *dSK*<sup>-</sup> and *dSK*<sup>-</sup>;;*dSlo*<sup>-</sup> mutant R1-R6s lack completely their functional channels (which  
14 thus should not generate extra noise) and have normal rhabdomere morphology and LIC dynamics  
15 (Fig. 5), theoretically, their somatic information transfer rates should be wild-type-like, or slightly  
16 lower; in case, their LMC feedback was compromised.

17  
18 To test this hypothesis, we compared *dSlo*<sup>-</sup>, *dSK*<sup>-</sup> and *dSK*<sup>-</sup>;;*dSlo*<sup>-</sup> R1-R6s' encoding performance to  
19 the wild-type control using the same recordings as above. In each case, the first 20-30 responses  
20 with the adapting trends were removed. The signal was taken as the average of the next 20  
21 responses, which thus had settled to a relative steady-state, with its power spectrum calculated by  
22 Fourier transform. The corresponding noise power spectrum was estimated from the difference  
23 between each response and the signal (see Materials and Methods ).

24  
25 We found that the mutant R1-R6s' signal-to-noise ratios (Fig. 6E) and information rates (Fig. 6F)  
26 were broadly wild-type-like; increasing in parallel with brightening light, as tested for dim, middle and  
27 bright NS. Thus, as hypothesized, after the initial ~20-30 s adaptation phase, the loss of *dSK*, *dSlo*  
28 or both channels affected only marginally a R1-R6's encoding performance. These results highlight  
29 the R-LMC-R system's robustness and compensatory ability to withstand internal damage.

30

31 ***dSK* or *dSlo* Absence Increases Synaptic Feedback**



1 To work out in theory how synaptic feedback from the lamina interneurons should shape the wild-  
2 type R1-R6 output and how homeostatic feedback changes should shape mutant R1-R6 outputs,  
3 we next combined biophysical R1-R6 modelling with intracellular recordings.

4  
5 Our biophysical R1-R6 model (Fig. 7A) incorporates 30,000 computational microvilli (Song et al.,  
6 2012), each of which implements full stochastic phototransduction reactions to transduce absorbed  
7 photons into QBs. Essentially, this model samples light information much like a real R1-R6 (Song et  
8 al., 2012; Song and Juusola, 2014; Juusola et al., 2017; Juusola and Song, 2017). Its QBs sum up  
9 realistic macroscopic LIC, with the best performance for naturalistic stimuli at  $1-8 \times 10^5$  photon  
10 absorptions/s (Song and Juusola, 2014; Juusola et al., 2017). LIC then charges a Hodgkin-Huxley-  
11 type photoreceptor membrane circuit (Figs 7B and 8) (Niven et al., 2003; Vähäsöyrinki et al., 2006;  
12 Song et al., 2012; Song and Juusola, 2014), generating output that approximates intracellular  
13 recordings to comparable light stimulation (Song et al., 2012; Song and Juusola, 2014; Juusola et  
14 al., 2017; Song and Juusola, 2017). Most differences in the simulated and recorded response  
15 waveforms would then be caused by the real R1-R6s' synaptic feedback currents - input from LMCs  
16 (Zheng et al., 2006; Rivera-Alba et al., 2011; Dau et al., 2016), which the model lacks (Juusola et al.,  
17 2017). Moreover, given that the mutant R1-R6s' phototransduction is wild-type-like and voltage-  
18 sensitive conductances either wild-type-like or only moderately reduced (Fig. 5), their voltage  
19 response differences should also mostly reflect synaptic feedback differences (Fig. 6).

20  
21 Therefore, we could extrapolate the synaptic feedback current to each recorded R1-R6, whether  
22 wild-type or mutant, computationally (Fig. 7C) by using the same fixed LIC with their specific  $I_A$  and  
23  $I_{SK}$  current dynamics (Figs 5 and 8). In these simulations, we first injected a new flat (zero)  
24 conductance, representing the missing synaptic input, to the full R1-R6 model. The software then  
25 shaped up this conductance waveform in a closed-loop until the model's voltage response matched  
26 the recorded response for the same light stimulus. Thus, theoretically, the resulting (predicted)  
27 current should closely mimic the real synaptic feedback, which the tested R1-R6 would have  
28 received from the lamina network *in vivo*.

29  
30 Fig. 7D shows the corresponding mean LIC and synaptic feedback estimates to repeated light  
31 stimulation for the tested wild-type and mutant photoreceptors, and the concurrent voltage-sensitive

1 K<sup>+</sup> currents and K<sup>+</sup> leak estimates. In these simulations, whilst the LIC was the same (fixed; dark red  
2 traces) for every genotype, their synaptic feedback and K<sup>+</sup> (dark green) currents balanced out  
3 differently to reproduce their respective *in vivo* voltage signals (Fig. 7E).

4  
5 We found that in every simulation the predicted synaptic feedback to R1-R6s was excitatory, graded  
6 and phasic (Figs 7D and 7F). It rapidly increased (“switched-on”) during light decrements and  
7 decreased (“switched-off”) during light increments. This accentuated transient (phasic) light changes  
8 in photoreceptor output (Fig. 7E; cf. Fig. 7A). Moreover, the predicted synaptic excitatory load to R1-  
9 R6s (Fig. 7F) was unique for each mutant and the wild-type flies with the highest mean to *dSK*<sup>-</sup> (red)  
10 and *dSlo*<sup>-</sup> (blue) photoreceptors. Thus, the enhanced excitatory feedback conductance from the  
11 lamina interneurons is the most probable mechanistic explanation of why and how the mutant  
12 photoreceptors were more depolarized than their wild-type counterparts, both in darkness (cf. Fig.  
13 4D) and during light stimulation (Fig. 7E).

14  
15 Remarkably, these feedback dynamics (Fig. 7F), which were extrapolated using only photoreceptor  
16 data (Figs 7A-C), closely resembled postsynaptic intracellular LMC responses to the same light  
17 stimulus (Fig. 7G). This implied that L2, L4 and lamina intrinsic amacrine neurons (Lai), all of which  
18 receive inhibitory inputs from R1-R6 but form excitatory feedback synapses to R1-R6 (Kolodziejczyk  
19 et al., 2008; Raghu and Borst, 2011; Hu et al., 2015; Davis et al., 2018), could alone or together be  
20 the major source of this feedback. Thus, these new findings are consistent with our theory of how  
21 the R-LMC-R system, by dynamically balancing its inhibitory and excitatory synaptic loads, shapes  
22 the early neural representation of visual information (Zheng et al., 2006; Nikolaev et al., 2009; Zheng  
23 et al., 2009; Dau et al., 2016).

### 24 25 **dSK and dSlo Lower Neural Information Energy Cost**

26 In response to LIC and synaptic feedback, ion channels open and close, regulating the ionic flow  
27 across the photoreceptor membrane. Meanwhile its ion cotransporters, exchangers and pumps  
28 uptake or expel ions to maintain ionic concentrations in- and outside. The work of the pumps in  
29 moving ions against their electrochemical gradients consumes ATP (Laughlin et al., 1998). For a R1-  
30 R6, a reasonable estimate of this consumption can be calculated from the ionic flow dynamics  
31 through its ion channels; details in Materials and Methods (see also: Song and Juusola, 2014).

1

2 Using our biophysical R1-R6 model, which now included the synaptic feedback, we calculated how  
3 much each recorded wild-type and mutant R1-R6 consumed metabolic energy (ATP molecules/s) to  
4 encode bright naturalistic light changes (Fig. 7H, left). We discovered that because their enhanced  
5 synaptic feedback held  $dSK^-$ ,  $dSlo^-$  and  $dSK^-;dSlo^-$  R1-R6s at higher operating voltages, where  
6 signaling is more expensive, they consumed on average 13.3%, 18.3% and 10.2% more ATP than  
7 the wild-type, respectively.

8

9 We also estimated each tested R1-R6's ATP consumption by using the method of balancing out the  
10 ionic currents for its light-induced mean (flat) depolarization level, or DC (Laughlin et al., 1998). This  
11 produced a metric, which followed quite a similar trend (Fig. 7H, right). But because it discarded how  
12 much the dynamic ion fluctuations increase the work to maintain transmembrane ionic concentration,  
13 it underestimated the total ATP consumption by  $\sim 1/3$ .

14

15 Next, using the full biophysical models (Fig. 9), we calculated how the mutant R1-R6s'  
16 homeostatically reduced *Shaker* and *Shab*  $K^+$  conductances (Figs 5F-H) affect their neural  
17 information costs. We fixed the *Shaker* and *Shab* conductance dynamics of the  $dSK^-$ ,  $dSlo^-$  and  
18  $dSK^-;dSlo^-$  R1-R6 models to match typical wild-type R1-R6 VC-recordings (Fig. 8A). This increased  
19 the mutant photoreceptors' energy consumption, but only slightly (*cf.* Figs 9H to 7H). Hence, the  
20 observed homeostatic 19-36% *Shaker* and *Shab* current reduction in  $dSK^-$  and  $dSlo^-$  R1-R6s (Figs  
21 5F and 5H) made evolutionary sense, as it cut both their hyperpolarizing drive, which therefore would  
22 require less excitatory synaptic feedback to depolarize the cells, and neural information costs. But  
23 this saving was small, only 4.5-6.2%. And somewhat unexpectedly, its homeostatic effect, in fact,  
24 increased the  $dSK^-$  and  $dSlo^-$  R1-R6s' synaptic feedback overload slightly in respect to  $dSK^-;dSlo^-$   
25 R1-R6s, which had wild-type-like *Shaker* and *Shab* conductance dynamics (Fig. 7F).

26

27 Furthermore, simulations about other possible homeostatic changes (Fig. 10) indicated that by  
28 increasing leak and voltage-sensitive  $K^+$  conductances, or adding an extra  $Cl^-$ -leak, in the R1-R6  
29 membrane would strengthen and accentuate synaptic feedback (Fig. 10F), and by that increase both  
30 the wild-type R1-R6s' ATP consumption (Fig. 10H; now by 23.2%) and the mutant photoreceptors'  
31 neural information costs in respect to the wild-type (Figs 10I and 10J), now by 22.3% ( $dSK^-$ ), 37.0%

1 (*dSlo*) or 57.6% (*dSK*;;*dSlo*). Therefore, as energy wasting reduces fitness, the earlier proposed  
2 leak-conductance overexpression alone (Niven et al., 2003; Vähäsöyrinki et al., 2006) seems an  
3 unlikely homeostatic strategy here.

4  
5 These results establish the extra energy, which a mutant R1-R6 must spend to function without Ca<sup>2+</sup>-  
6 activated K<sup>+</sup> channels, as a major cost for homeostatic compensation of neural information (Fig. 7I).  
7 To maintain similar information rates (Fig. 6F), an average mutant R1-R6 consumed at least 13.1%  
8 (*dSK*; p = 0.114), 28.0% (*dSlo*; p = 0.016) or 42.7% (*dSK*;;*dSlo*; p = 9.56 x 10<sup>-4</sup>) more ATP for each  
9 transmitted bit than its wild-type counterpart (Fig. 7J). Notably, these costs would only increase  
10 further if homeostatic compensation of the missing dSK and dSlo channels further entailed over-  
11 expression of additional K<sup>+</sup> or Cl<sup>-</sup> conductances or leaks (Figs 9 and 10). Thus, in *Drosophila*  
12 photoreceptors, Ca<sup>2+</sup>-activated K<sup>+</sup> channels reduce the energy cost of neural information.

#### 14 **dSlo and dSK Co-Regulate Feedforward Transmission to LMCs**

15 Thus far, we have provided experimental and theoretical evidence that both BK (*dSlo*) or dSK  
16 channel deletions enhance synaptic feedback from the lamina interneurons to R1-R6s (Figs 3-10).  
17 But these results still leave open the corresponding changes in the post-synaptic LMC output, which  
18 initiates the motion vision pathways to the fly brain (Joesch et al., 2010; Wardill et al., 2012). To test  
19 how dSK and dSlo deletions affect such feedforward transmission directly, we recorded intracellular  
20 voltage responses of dark-adapted LMCs in the mutant and wild-type laminae to brightening light  
21 flashes, which covered a 4-log intensity range (Fig. 11A).

22  
23 Expectedly, light rapidly hyperpolarized LMCs and darkness depolarized them (Fig. 11B) (Zettler and  
24 Järvillehto, 1973; Juusola et al., 1995a; Zheng et al., 2006), driven by the photoreceptors' inhibitory  
25 transmitter, histamine (Hardie, 1989; Dau et al., 2016). Yet, these dynamics varied somewhat  
26 systematically between the genotypes, with the mutant LMCs often showing oscillating responses  
27 (ringing) around specific frequencies. L1 (on-pathway) and L2 (off-pathway) responses are thought  
28 to be largely similar at the dendritic (lamina) level (Hardie and Weckström, 1990; Uusitalo et al.,  
29 1995a; Nikolaev et al., 2009) (*cf.* Fig. 7G), with their medulla terminals' light-on and -off preference  
30 (Joesch et al., 2010; Freifeld et al., 2013) most likely arise through specific medulla circuit processes.  
31 Therefore, with most penetrations likely from L1 and L2, which are the largest LMCs, our recordings

1 should mostly depict mutation-induced variations and less LMC-type-dependent differences.

2

3 *dSK*<sup>-</sup> LMC output was consistently the most transient, even to dim flashes (Figs 11B-E), showing  
4 accelerated (most “light-adapted”) dynamics with the fastest time-to-peak values (Fig. 11D). By and  
5 large, the size (Fig. 11C) and half-width (Fig. 11E) of these responses were wild-type-like, but, unlike  
6 the wild-type, they often showed rapid oscillation bursts to dim flashes (see also Abou Tayoun et al.,  
7 2011).

8

9 In contrast, both *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> LMC responses to dimmer flash intensities were on average  
10 smaller than those of the wild-type and *dSK*<sup>-</sup> LMCs (Figs 11B and 11C). But as their amplitudes  
11 increased with light intensity, the brightest flashes evoked about the same size responses from all  
12 the genotypes (Figs 11B and 11C). Therefore, during dim (but not bright) stimulation, the excitatory  
13 feedback from L2 and L4 cells to R1-R6s (Zheng et al., 2006), if directly following the recorded *dSlo*<sup>-</sup>  
14 and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> LMC responses, could be driven by smaller *dynamic* modulation (Fig. 11C) on a  
15 larger *static* load (as their mean LMC responses would thus also be more depolarized). This would  
16 reduce R1-R6 membrane impedance and, presumably, synaptic gain in R1-R6 output; consistent  
17 with the smaller *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6 responses to dim naturalistic light stimulation (Fig. 6C).  
18 Furthermore, *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> LMC response dynamics were also slower and less tightly time-locked (Fig.  
19 11D); often ringing sluggishly (Fig. 11B), prolonging the response half-width (Fig. 11E) and peaking  
20 later than the other corresponding LMC responses (Fig. 11D). Such desynchrony would add noise  
21 in the synaptic feedback, and may have contributed to the slightly lower signal-to-noise ratios and  
22 information transfer rates of *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6s (Fig. 6E).

23

24 Thus, deletion of *dSK*, *dSlo* or both led to suboptimal network adaptation in the R-LMC system, seen  
25 as accelerated or decelerated LMC responses and mutation-specific oscillations. Crucially, these  
26 oscillations, with their characteristic frequencies, were also regularly observed in the mutant eyes’  
27 global electrical activity (electroretinograms, ERGs) (Figs 11F-H), supporting the intracellular results.  
28 Nevertheless, the observed differences cannot be directly attributed for missing *dSK*, *dSlo* or both in  
29 the mutant LMCs (cf. Abou Tayoun et al., 2011). The respective functional channels (*dSK* in *dSlo*-  
30 mutants, *dSlo* in *dSK*- mutants, and both channels in wild-type) could act remotely in the circuit, or  
31 their LMC response dynamics could result from combinatorial effects on both R1-R6s and LMCs.

1

## 2 **Mutants' Optomotor Responses Reflect Early Vision Defects**

3 To test whether the mutation-specific network adaptations influence visual perception, we measured  
4 the flies' optomotor behavior in a classic flight simulator system (Fig. 12A). Notably here, *dSK*<sup>-</sup>, *dSlo*<sup>-</sup>  
5 and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> mutants lack their respective channel activity throughout their brains, and thus are  
6 likely to have perceptual deficits beyond their distorted LMC inputs - and in case of *dSlo*<sup>-</sup>, reduced  
7 health/motility (see Materials and Methods). But it is the LMC input, which sets their absolute motion  
8 vision limit (Rister et al., 2007; Joesch et al., 2010; Wardill et al., 2012). So, whilst any observed  
9 phenotype is convolving the channel contributions in some complex, unknown way across cell types,  
10 LMC input to the mutant brain is still its motion vision bottleneck, driving optomotor behaviors in a  
11 closed loop. Therefore, as a mutant's optomotor behavior cannot be better than, and must ultimately  
12 reflect, its LMC input, it is informative to compare their respective optomotor response to their LMC  
13 input at different stimulus conditions to determine the generic behavioral differences across the  
14 different phenotypes. Moreover, these comparisons tell us further each mutant phenotype's capacity  
15 to compensate its specific mutation effects.

16

17 The tethered wild-type and mutant flies generated yaw torque by attempting to follow left and right  
18 rotating panoramic scenes, which showed either coarse (14.4°) or fine-grained (3.9°) vertical black-  
19 and-white stripe patterns, facing the flies. The resulting optomotor response waveforms and sizes  
20 were used to quantify how well individual flies and their respective populations (genotypes) saw  
21 these scenes rotating either slowly (45 °/s) or fast (300 °/s). Note that although the average inter-  
22 ommatidial angle (the eyes' optical limit) is 4.5° (Gonzalez-Bellido et al., 2011), photomechanical  
23 photoreceptor microsaccades enable *Drosophila* to see much finer (hyperacute) details (Juusola et  
24 al., 2017).

25

26 We found that flies of each genotype could follow these stimuli (Fig. 12A), indicating that their visual  
27 systems represented and motor systems reacted to the opposing (left and right) image motion  
28 appropriately. However, the relative optomotor response sizes (Fig. 12B) and waveforms (Fig. 12C)  
29 showed genotype-specific sensitivities, or stimulus preferences, which were both repeatable and  
30 independent of the stimulus presentation order. Thus, these response differences could not be  
31 caused by stimulus salience, neural habituation or flight muscle fatigue.

1  
2 Wild-type flies preferred, on average, the fast coarse stripe field rotations (Fig. 12B, black;  $96.6 \pm$   
3  $8.5\%$  maximum response, mean  $\pm$  SD,  $n = 15$  flies) over the slow coarse ( $87.8 \pm 12.6\%$ ) and slow  
4 hyperacute ( $66.1 \pm 15.2\%$ ) stimuli, but only just. Even their responses to fast hyperacute rotations  
5 were substantial ( $28.9 \pm 9.0\%$ ), consistent with *Drosophila*'s high visual acuity even at saccadic  
6 speeds ( $>200$  °/s) (Juusola et al., 2017). Such an all-round optomotor performance over a broad  
7 motion stimulus range is consistent with the idea that the optomotor behavior scales with the sensory  
8 input strength and dynamics from the eyes (Wardill et al., 2012). Thus, the adaptive signal scaling in  
9 the early visual system, seen as amplitude and time-normalized LMC contrast responses to different  
10 stimulus intensity and speed conditions (Zheng et al., 2006; Zheng et al., 2009), would be a  
11 prerequisite for consistent perception (optomotor behavior) over a broad image motion range.

12  
13 In contrast, *dSK*<sup>-</sup> mutants responded far more strongly to the fast coarse rotating field (Fig. 12B, red;  
14  $99.8 \pm 7.6\%$  maximum response,  $n = 13$  flies) than the other stimuli (19.9-68.0%), with their slow  
15 and fast hyperacute field rotation responses being significantly weaker than those of the other  
16 genotypes (Fig. 12E). Interestingly and distinctively, the *dSK*<sup>-</sup> responses were further dominated by  
17 large and fast body saccades (\* in Fig. 12A), which appeared at seemingly regular intervals from the  
18 stimulus onset onwards and could make  $>50\%$  of their total amplitude (Fig. 12D). Thus, the  
19 accelerated *dSK*<sup>-</sup> photoreceptor and LMC dynamics (cf. Figs 3C and 11C), and tendency to oscillate,  
20 seem preserved in the *dSK*<sup>-</sup> visual system, with these motion perception distortions possibly  
21 compelling their “spiky” optomotor responses.

22  
23 The optomotor behavior of *dSlo*<sup>-</sup> mutants showed similarly suggestive correlations to their R-LMC-R  
24 network adaptation dynamics. These flies, which boast slightly decelerated photoreceptor (Fig. 3E)  
25 and LMC (Fig. 11D) dynamics, preferred slow field rotations, and, surprisingly, were most sensitive  
26 to the slow hyperacute stimulus (Fig. 12B, blue;  $94.8 \pm 9.0\%$ ,  $n = 3$  flies). Although *dSlo*<sup>-</sup> mutants, in  
27 absolute terms, generated the weakest flight simulator torque responses of the tested genotypes,  
28 the mutants that flew did so over the whole experiments, making these stimulus preferences genuine.

29  
30 Finally, the sensitivity of *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> mutant responses (Fig. 12B, orange) followed the average of  
31 *dSK*<sup>-</sup> and *dSlo*<sup>-</sup> mutants' optomotor responses (Fig. 12B, purple dotted line) more closely than the

1 mean wild-type responses (black). In particular, their responses were relatively more sensitive to  
2 hyperacute stimuli than the corresponding wild-type responses (Fig. 12E) but rose and decayed  
3 slower (Fig. 12F, arrows), consistent with *dSK*; *dSlo* having slower LMC dynamics (Figs 11D-E).  
4 Thus, suggestively, their optomotor dynamics differences reflected more differences in early visual  
5 network adaptations rather than in other systems, such as the sensorimotor.

6

## 7 **Discussion**

8 Our results indicate that *dSlo* (BK) and *dSK* (SK) reduce excitability and energy (ATP) consumption  
9 while increasing adaptability and dynamic range for transmitting neural information at the lamina  
10 network, ultimately stabilizing visual perception in changing light conditions. Here, single- and  
11 double-mutant photoreceptors showed either accelerated or decelerated responses and more  
12 depolarized resting potentials during steady-state adaptation. Such changes likely emerged from  
13 suboptimal homeostatic rebalancing of synaptic feed-forward and feedback signaling between  
14 photoreceptor axon terminals and the rest of the lamina network. Notably, this network compensation  
15 was unique for each mutation, resulting in distinctive adaptive regimes; with their respective LMCs  
16 showing oscillating accelerated or decelerated responses with reduced output ranges. These altered  
17 LMC response dynamics, and thus the flow of visual information, most probably distorted the mutants'  
18 rotating scene perception, and their optomotor responses, in relation to the wild-type.

19

### 20 **Homeostatic Compensation Shapes both Electrical Responses and Synaptic Release**

21 Because of the continuous bidirectional adapting interactions between photoreceptors and different  
22 lamina interneurons, the altered LMC responses cannot be explained simply by the absence of *dSK*  
23 and *dSlo* channels in the LMCs. In particular, both *dSlo* and *dSK* genes are well expressed in all  
24 LMCs (L1-L5) and photoreceptors (R1-R8) (Davis et al., 2018), underlying the interdependence of  
25 R1-R6 and LMC response dynamics and the need for systems level analyses to untangle them.  
26 Moreover, different neurons' expression levels in the lamina terminals could vary dynamically, be  
27 tuned by circadian clock (Agrawal et al., 2017) or influenced (up- or down-regulated) by the Gal4-  
28 lines used to identify the cells (e.g. see  $I_A$  and  $I_{Ks}$ -currents in Fig. 1). Nevertheless, irrespective  
29 whether these processes happen or not, homeostatic changes in the mutant R-LMC-R systems must  
30 involve both R1-R6s' and LMCs' electrical response waveforms and their synaptic release



1 machineries. For example, in the *dSK* R-LMC-R system, the homeostatically rebalanced synaptic  
2 feedforward-feedback interactions (Fig. 7) and reduced R1-R6 *Shaker* and *Shab* K<sup>+</sup> conductances  
3 (Fig. 5) alone would make their electrical response waveforms (Fig. 11B) different from the wild-type;  
4 as seen in *dSK* LMC waveforms peaking faster (Fig. 11D) and often oscillating to dim light.

5

## 6 **Ca<sup>2+</sup>-activated K<sup>+</sup> Channels Reduce Costs of Adaptation and Increase Its Range**

7 Adaptability is critical for animal fitness. In sampling and transmission of sensory signals, it reduces  
8 communication errors, such as noise and saturation, by continuously adjusting new responses by  
9 the memories of the past stimuli (Song et al., 2012; Juusola and Song, 2017). To ensure reliable  
10 perception of visual objects in changing conditions, retinal adaptation exploits visual world similarities  
11 and differences (van Hateren, 1992b; Song and Juusola, 2014) through characteristic visual  
12 behaviors (Schilstra and Hateren, 1999; Blaj and van Hateren, 2004; Juusola et al., 2017) and  
13 employs costly codes (de Polavieja, 2002) through multiple layers of feedbacks. This gives  
14 emergence for *homeostatic* network gain regulation, in which photoreceptor adaptation is mediated  
15 both by intrinsic (Juusola and Hardie, 2001b; Vähäsöyrinki et al., 2006; Song et al., 2012; Hardie  
16 and Juusola, 2015) and synaptic feedbacks (Zheng et al., 2006; Zheng et al., 2009). Here, the  
17 absence of *dSK*, *dSlo* or both channels left the phototransduction cascade essentially intact but  
18 reduced the intrinsic photoreceptor *Shaker* and *Shab* conductances, which should have made  
19 voltage responses larger and slower. Yet, *in vivo* recordings refuted these predictions, showing  
20 instead distinctive mutation-specific dynamics. Therefore, the observed defects in photoreceptor  
21 adaptability - including response fluctuations and altered dynamic ranges - seem mostly attributable  
22 to the R-LMC-R system's suboptimally balanced synaptic feedforward inhibition and feedback  
23 excitation; reflecting homeostatic compensation at the network level. The resulting excitatory  
24 feedback overload also provided a plausible explanation why the mutant photoreceptors' resting  
25 potentials and response speeds differed from the wild-type (Zheng et al., 2006; Abou Tayoun et al.,  
26 2011).

27

28 The primary effects of mutations can be difficult to separate from the secondary effects of  
29 homeostatic compensation (Marder and Goaillard, 2006). Nonetheless, the overall consistency of  
30 our findings suggest that many differences in *in vivo* response properties of the mutants' R1-R6s and  
31 LMCs result from homeostatic gain regulation, whereupon differently balanced synaptic excitatory

1 and inhibitory loads in the lamina network generate unique adaptive dynamics (encoding regimes);  
2 see also (Abbott and Lemasson, 1993; Lemasson et al., 1993). In the double-mutant, the most  
3 depolarized photoreceptors (Fig. 4D) and the slowest LMC output (Figs 11D-E) imply that the  
4 network gain was particularly challenging to regulate, providing the most compromised adaptability  
5 and response range (Fig. 12). In the single-mutants, adaptability of early vision was better  
6 compensated by enhanced network excitation, as seen by more wild-type-like LMC response  
7 dynamics (Figs 11C-E). But this still came with the cost of increased ATP consumption (Figs 7H-I).  
8 Moreover, in each case, the *dSK* and/or *dSlo* channel deletions affected optomotor behavior (Fig.  
9 12), suggesting that the mutants' distinct LMC output dynamics distorted their motion perception;  
10 alike what we have previously shown to occur with different color channel mutants (Wardill et al.,  
11 2012). Here, *dSK*<sup>-</sup> mutants' accelerated LMC responses (Figs 11B-C) presumably drove their fast  
12 hyper-saccadic optomotor responses (Figs 12A-D), while *dSlo*<sup>-</sup> mutants' decelerated LMC responses  
13 (Figs 11B-C) most probably sensitized their vision to slow scene rotations (Figs 12A-D).

14  
15 We have shown how Ca<sup>2+</sup>-activated K<sup>+</sup> channels serve local and global neural communication,  
16 improving economics and adaptability. Locally, they help to reduce calcium load and repolarize  
17 membrane potentials in synaptic terminals. Globally, they reduce the overall network excitability and  
18 the cost of transmitting information, while increasing the range of neural adaptation and reliable  
19 perception.

20

## 21 **Genetic control limitations**

22 Finally, our results showed that the standard genetic rescue controls themselves - by using Gal4-  
23 lines and RNAi - can affect cellular form and function, causing larger neural response variability than  
24 what is observed in the tested phenotypes (Fig. 1). Thus, we could not use such controls to make  
25 *reductionist* conclusions about information processing at the network level; when analyzing neural  
26 response and homeostatic compensation dynamics in fine detail. And because of this, there still  
27 remains the formal possibility that the reported photoreceptor phenotypes are not actually associated  
28 with *dSK* and *dSlo*, but rather reflect other mutations on the same chromosome (or in the genetic  
29 background). Nevertheless, our results highlight the importance of carefully testing the viability and  
30 usefulness of the planned control methods, both at the cellular and systems (network) level, so that  
31 the scientific rationale and reliability of the study becomes defined *constructionistically* (Marr and

1 Poggio, 1977), within the experimental/methodological limits.

2

### 3 **Author Contributions**

4 This research was initiated by M.J., P.D. and R.C.H. Genetics: A.A.T., P.D. and F.B. Electrophysiology:  
5 X.L., A.D., D.R., A.N., L.Z., M.B., B.C., R.C.H. and M.J. Electron microscopy: S.D. Histology: P.D.  
6 Optomotor behavior: D.J. Modeling and data analyses: Z.S., A.D. and M.J. M.J., P.D. and R.C.H.  
7 designed the experiments. M.J. wrote the manuscript with all authors contributing in editing. M.J.,  
8 P.D. and R.C.H. procured funding.

9

### 10 **References**

- 11 Abbott LF, Lemasson G (1993) Analysis of neuron models with dynamically regulated conductances. *Neural Comput*  
12 5:823-842.
- 13 Abou Tayoun AN, Li X, Chu B, Hardie RC, Juusola M, Dolph PJ (2011) The *Drosophila* SK channel (dSK) contributes  
14 to photoreceptor performance by mediating sensitivity control at the first visual network. *J Neurosci* 31:13897-  
15 13910.
- 16 Agrawal P, Houl JH, Gunawardhana KL, Liu TX, Zhou J, Zoran MJ, Hardin PE (2017) *Drosophila* CRY Entrain Clocks  
17 in Body Tissues to Light and Maintains Passive Membrane Properties in a Non-clock Body Tissue Independent  
18 of Light. *Current Biology* 27:2431-+.
- 19 Atkinson NS, Robertson GA, Ganetzky B (1991) A component of calcium-activated potassium channels encoded by the  
20 *Drosophila* slo locus. *Science* 253:551-555.
- 21 Atkinson NS, Brenner R, Chang WM, Wilbur J, Larimer JL, Yu J (2000) Molecular separation of two behavioral  
22 phenotypes by a mutation affecting the promoters of a Ca-activated K channel. *J Neurosci* 20:2988-2993.
- 23 Becker MN, Brenner R, Atkinson NS (1995) Tissue-specific expression of a *Drosophila* calcium-activated potassium  
24 channel. *J Neurosci* 15:6250-6259.
- 25 Blaj G, van Hateren JH (2004) Saccadic head and thorax movements in freely walking blowflies. *J Comp Physiol A*  
26 190:861-868.
- 27 Blondeau J, Heisenberg M (1982) The 3-dimensional optomotor torque system of *Drosophila Melanogaster* - studies on  
28 wildtype and the mutant optomotor-blind H31. *J Comp Physiol* 145:321-329.
- 29 Bollepalli MK, Kuipers ME, Liu CH, Asteriti S, Hardie RC (2017) Phototransduction in *Drosophila* Is Compromised by  
30 Gal4 Expression but not by InsP(3) Receptor Knockdown or Mutation. *Eneuro* 4.
- 31 Chinchore Y, Mitra A, Dolph PJ (2009) Accumulation of rhodopsin in late endosomes triggers photoreceptor cell  
32 degeneration. *PLoS Genet* 5:e1000377.
- 33 Clark BD, Kurth-Nelson ZL, Newman EA (2009) Adenosine-evoked hyperpolarization of retinal ganglion cells is  
34 mediated by G-protein-coupled inwardly rectifying K<sup>+</sup> and small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel  
35 activation. *J Neurosci* 29:11237-11245.
- 36 Coombe PE (1986) The large monopolar cells L1 and L2 are responsible for ERG transients in *Drosophila*. *J Comp*  
37 *Physiol A* 159:655-665.
- 38 Cover TM, Thomas JA (2006) *Elements of information theory*, 2nd Edition. Hoboken, N.J.: Wiley-Interscience.
- 39 Dau A, Friederich U, Dongre S, Li XF, Bollepalli MK, Hardie RC, Juusola M (2016) Evidence for dynamic network  
40 regulation of *Drosophila* photoreceptor function from mutants lacking the neurotransmitter histamine. *Front*  
41 *Neural Circuit* 10:1-22.

1 Davis FP, Nern A, Picard S, Reiser MB, Rubin GM, Eddy SR, Henry GL (2018) A genetic, genomic, and computational  
2 resource for exploring neural circuit function. bioRxiv:385476.

3 de Polavieja GG (2002) Errors drive the evolution of biological signalling to costly codes. J Theoret Biol 214:657-664.

4 Faber ES, Sah P (2003) Calcium-activated potassium channels: multiple contributions to neuronal function.  
5 Neuroscientist 9:181-194.

6 Faber ES, Delaney AJ, Sah P (2005) SK channels regulate excitatory synaptic transmission and plasticity in the lateral  
7 amygdala. Nat Neurosci 8:635-641.

8 Fettiplace R, Fuchs PA (1999) Mechanisms of hair cell tuning. Annual review of physiology 61:809-834.

9 Freifeld L, Clark DA, Schnitzer MJ, Horowitz MA, Clandinin TR (2013) GABAergic lateral interactions tune the early  
10 stages of visual processing in *Drosophila*. Neuron 78:1075-1089.

11 Gonzalez-Bellido PT, Wardill TJ, Juusola M (2011) Compound eyes and retinal information processing in miniature  
12 dipteran species match their specific ecological demands. Proc Natl Acad Sci U S A 108:4224-4229.

13 Götz KG (1964) Optomotor studies of the visual system of several eye mutants of the fruit fly *Drosophila*. Kybernetik  
14 2:77-92.

15 Grimes WN, Li W, Chavez AE, Diamond JS (2009) BK channels modulate pre- and postsynaptic signaling at reciprocal  
16 synapses in retina. Nat Neurosci 12:585-592.

17 Hardie RC (1989) A histamine-activated chloride channel involved in neurotransmission at a photoreceptor synapse.  
18 Nature 339:704-706.

19 Hardie RC (1991a) Voltage-sensitive potassium channels in *Drosophila* photoreceptors. J Neurosci 11:3079-3095.

20 Hardie RC (1991b) Whole-cell recordings of the light-induced current in dissociated *Drosophila* photoreceptors -  
21 evidence for feedback by calcium permeating the light-sensitive channels. Proc R Soc B-Biol Sci 245:203-210.

22 Hardie RC, Weckström M (1990) 3 classes of potassium channels in large monopolar cells of the blowfly *Calliphora*  
23 *Vicina*. J Comp Physiol A 167:723-736.

24 Hardie RC, Juusola M (2015) Phototransduction in *Drosophila*. Curr Opin Neurobiol 34:37-45.

25 Hardie RC, Voss D, Pongs O, Laughlin SB (1991) Novel potassium channels encoded by the Shaker locus in *Drosophila*  
26 photoreceptors. Neuron 6:477-486.

27 Hardie RC, Martin F, Cochrane GW, Juusola M, Georgiev P, Raghu P (2002) Molecular basis of amplification in  
28 *Drosophila* phototransduction: Roles for G protein, phospholipase C, and diacylglycerol kinase. Neuron 36:689-  
29 701.

30 Heisenberg M (1971) Separation of receptor and lamina potentials in electroretinogram of normal and mutant *Drosophila*.  
31 J Exp Biol 55:85-100.

32 Hu W, Wang T, Wang X, Han J (2015) Ih channels control feedback regulation from amacrine cells to photoreceptors.  
33 PLoS Biol 13:e1002115.

34 Joesch M, Schnell B, Raghu SV, Reiff DF, Borst A (2010) ON and OFF pathways in *Drosophila* motion vision. Nature  
35 468:300-304.

36 Juusola M, Weckström M (1993) Band-pass filtering by voltage-dependent membrane in an insect photoreceptor. Neurosci  
37 Lett 154:84-88.

38 Juusola M, Hardie RC (2001a) Light adaptation in *Drosophila* photoreceptors: II. Rising temperature increases the  
39 bandwidth of reliable signaling. J Gen Physiol 117:27-42.

40 Juusola M, Hardie RC (2001b) Light adaptation in *Drosophila* photoreceptors: I. Response dynamics and signaling  
41 efficiency at 25 degrees C. J Gen Physiol 117:3-25.

42 Juusola M, de Polavieja GG (2003) The rate of information transfer of naturalistic stimulation by graded potentials. J Gen  
43 Physiol 122:191-206.

44 Juusola M, Song Z (2017) How a fly photoreceptor samples light information in time. J Physiol-London 595:5427-5437.

45 Juusola M, Uusitalo RO, Weckstrom M (1995a) Transfer of graded potentials at the photoreceptor interneuron synapse. J  
46 Gen Physiol 105:117-148.

1 Juusola M, Song Z, Hardie RC (2015) Phototransduction Biophysics. In: Encyclopedia of Computational Neuroscience  
2 (Jaeger D, Jung R, eds), pp 2359-2376. New York, NY: Springer New York.

3 Juusola M, Kouvalainen E, Järvillehto M, Weckström M (1994) Contrast gain, signal-to-noise ratio, and linearity in light-  
4 adapted blowfly photoreceptors. *J Gen Physiol* 104:593-621.

5 Juusola M, Dau A, Zheng L, Rien DN (2016) Electrophysiological method for recording intracellular voltage responses  
6 of *Drosophila* photoreceptors and interneurons to light stimuli *in vivo*. *Jove-J Vis Exp* 112:e54142.

7 Juusola M, Weckstrom M, Uusitalo RO, Korenberg MJ, French AS (1995b) Nonlinear models of the first synapse in the  
8 light-adapted fly retina. *Journal of Neurophysiology* 74:2538-2547.

9 Juusola M, Dau A, Song ZY, Solanki N, Rien D, Jaciuch D, Dongre S, Blanchard F, de Polavieja GG, Hardie RC, Takalo  
10 J (2017) Microsaccadic sampling of moving image information provides *Drosophila* hyperacute vision. *Elife*  
11 6:e26117.

12 Klocker N, Oliver D, Ruppertsberg JP, Knaus HG, Fakler B (2001) Developmental expression of the small-conductance  
13 Ca(2+)-activated potassium channel SK2 in the rat retina. *Mol Cell Neurosci* 17:514-520.

14 Kolodziejczyk A, Sun X, Meinertzhagen IA, Nassel DR (2008) Glutamate, GABA and acetylcholine signaling  
15 components in the lamina of the *Drosophila* visual system. *PLoS One* 3:e2110.

16 Lajeunesse D, Shearn A (1995) Transregulation of thoracic homeotic selector genes of the antennapedia and bithorax  
17 complexes by the trithorax group genes - absent, small, and homeotic Disc-1 and Disc-2. *Mech Develop* 53:123-  
18 139.

19 Lamb TD (1984) Effects of temperature-changes on toad rod photocurrents. *J Physiol-London* 346:557-578.

20 Laughlin SB, de Ruyter van Steveninck RR, Anderson JC (1998) The metabolic cost of neural information. *Nat Neurosci*  
21 1:36-41.

22 Lemasson G, Marder E, Abbott LF (1993) Activity-dependent regulation of conductances in model neurons. *Science*  
23 259:1915-1917.

24 Marder E, Goaillard JM (2006) Variability, compensation and homeostasis in neuron and network function. *Nat Rev*  
25 *Neurosci* 7:563-574.

26 Marr DC, Poggio T (1977) From Understanding Computation to Understanding Neural Circuitry. *Neurosci Res Prog B*  
27 15:470-491.

28 Meinertzhagen IA, O'Neil SD (1991) Synaptic organization of columnar elements in the lamina of the wild type in  
29 *Drosophila melanogaster*. *J Comp Neurol* 305:232-263.

30 Ngo-Anh TJ, Bloodgood BL, Lin M, Sabatini BL, Maylie J, Adelman JP (2005) SK channels and NMDA receptors form  
31 a Ca<sup>2+</sup>-mediated feedback loop in dendritic spines. *Nat Neurosci* 8:642-649.

32 Nikolaev A, Zheng L, Wardill TJ, O'Kane CJ, de Polavieja GG, Juusola M (2009) Network adaptation improves temporal  
33 representation of naturalistic stimuli in *Drosophila* eye: II mechanisms. *PLoS One* 4:e4306.

34 Niven JE, Vähäsöyrinki M, Kauranen M, Hardie RC, Juusola M, Weckström M (2003) The contribution of Shaker K<sup>+</sup>  
35 channels to the information capacity of *Drosophila* photoreceptors. *Nature* 421:630-634.

36 Pelucchi B, Grimaldi A, Moriondo A (2008) Vertebrate rod photoreceptors express both BK and IK calcium-activated  
37 potassium channels, but only BK channels are involved in receptor potential regulation. *J Neurosci Res* 86:194-  
38 201.

39 Raghu SV, Borst A (2011) Candidate glutamatergic neurons in the visual system of *Drosophila*. *PLoS One* 6:e19472.

40 Ramanathan K, Michael TH, Jiang GJ, Hiel H, Fuchs PA (1999) A molecular mechanism for electrical tuning of cochlear  
41 hair cells. *Science* 283:215-217.

42 Rister J, Pauls D, Schnell B, Ting CY, Lee CH, Sinakevitch I, Morante J, Strausfeld NJ, Ito K, Heisenberg M (2007)  
43 Dissection of the peripheral motion channel in the visual system of *Drosophila melanogaster*. *Neuron* 56:155-  
44 170.

45 Rivera-Alba M, Vitaladevuni SN, Mishchenko Y, Lu Z, Takemura SY, Scheffer L, Meinertzhagen IA, Chklovskii DB, de  
46 Polavieja GG (2011) Wiring economy and volume exclusion determine neuronal placement in the *Drosophila*

1 brain. *Curr Biol* 21:2000-2005.

2 Sah P (1996) Ca(2+)-activated K<sup>+</sup> currents in neurons: types, physiological roles and modulation. *Trends Neurosci*  
3 19:150-154.

4 Salkoff L (2006) A tail of multiple calcium-sensing domains. *J Gen Physiol* 128:387-388.

5 Sayeed O, Benzer S (1996) Behavioral genetics of thermosensation and hygrosensation in *Drosophila*. *P Natl Acad Sci*  
6 USA 93:6079-6084.

7 Schilstra C, Hateren JH (1999) Blowfly flight and optic flow. I. Thorax kinematics and flight dynamics. *J Exp Biol* 202  
8 (Pt 11):1481-1490.

9 Shannon CE (1948) A mathematical theory of communication. *Bell Syst Tech J* 27:379-423.

10 Shatz CJ (1990) Competitive interactions between retinal ganglion cells during prenatal development. *J Neurobiol*  
11 21:197-211.

12 Shaw SR (1984) Early visual processing in insects. *J Exp Biol* 112:225-&.

13 Shaw SR, Frohlich A, Meinertzhagen IA (1989) Direct connections between the R7/8 and R1-6 photoreceptor subsystems  
14 in the *dipteran* visual-system. *Cell Tissue Res* 257:295-302.

15 Skou JC (1965) Enzymatic Basis for Active Transport of Na<sup>+</sup> and K<sup>+</sup> across Cell Membrane. *Physiol Rev* 45:596-617.

16 Skou JC (1998) Nobel Lecture. The identification of the sodium pump. *Biosci Rep* 18:155-169.

17 Song Z, Juusola M (2014) Refractory sampling links efficiency and costs of sensory encoding to stimulus statistics. *J*  
18 *Neurosci* 34:7216-7237.

19 Song Z, Juusola M (2017) A biomimetic fly photoreceptor model elucidates how stochastic adaptive quantal sampling  
20 provides a large dynamic range. *J Physiol-London* 595:5439-5456.

21 Song Z, Postma M, Billings SA, Coca D, Hardie RC, Juusola M (2012) Stochastic, adaptive sampling of information by  
22 microvilli in fly photoreceptors. *Curr Biol* 22:1371-1380.

23 Stocker M (2004) Ca(2+)-activated K<sup>+</sup> channels: molecular determinants and function of the SK family. *Nat Rev Neurosci*  
24 5:758-770.

25 Tang S, Guo A (2001) Choice behavior of *Drosophila* facing contradictory visual cues. *Science* 294:1543-1547.

26 Uusitalo RO, Juusola M, Weckstrom M (1995a) Graded responses and spiking properties of identified first-order visual  
27 interneurons of the fly compound eye. *J Neurophysiol* 73:1782-1792.

28 Uusitalo RO, Juusola M, Kouvalainen E, Weckstrom M (1995b) Tonic Transmitter Release in a Graded Potential Synapse.  
29 *Journal of Neurophysiology* 74:470-473.

30 Vähäsöyrinki M, Niven JE, Hardie RC, Weckström M, Juusola M (2006) Robustness of neural coding in *Drosophila*  
31 photoreceptors in the absence of slow delayed rectifier K<sup>+</sup> channels. *J Neurosci* 26:2652-2660.

32 van Hateren JH (1992a) Theoretical predictions of spatiotemporal receptive-fields of fly LMCs, and experimental  
33 validation. *J Comp Physiol A* 171:157-170.

34 van Hateren JH (1992b) A theory of maximizing sensory information. *Biol Cybern* 68:23-29.

35 van Hateren JH (1997) Processing of natural time series of intensities by the visual system of the blowfly. *Vision Res*  
36 37:3407-3416.

37 Wang GY, Olshausen BA, Chalupa LM (1999) Differential effects of apamin- and charybdotoxin-sensitive K<sup>+</sup>  
38 conductances on spontaneous discharge patterns of developing retinal ganglion cells. *J Neurosci* 19:2609-2618.

39 Wardill TJ, List O, Li X, Dongre S, McCulloch M, Ting CY, O'Kane CJ, Tang S, Lee CH, Hardie RC, Juusola M (2012)  
40 Multiple spectral inputs improve motion discrimination in the *Drosophila* visual system. *Science* 336:925-931.

41 Wolfram V, Juusola M (2004) Impact of rearing conditions and short-term light exposure on signaling performance in  
42 *Drosophila* photoreceptors. *J Neurophysiol* 92:1918-1927.

43 Xu JW, Slaughter MM (2005) Large-conductance calcium-activated potassium channels facilitate transmitter release in  
44 salamander rod synapse. *J Neurosci* 25:7660-7668.

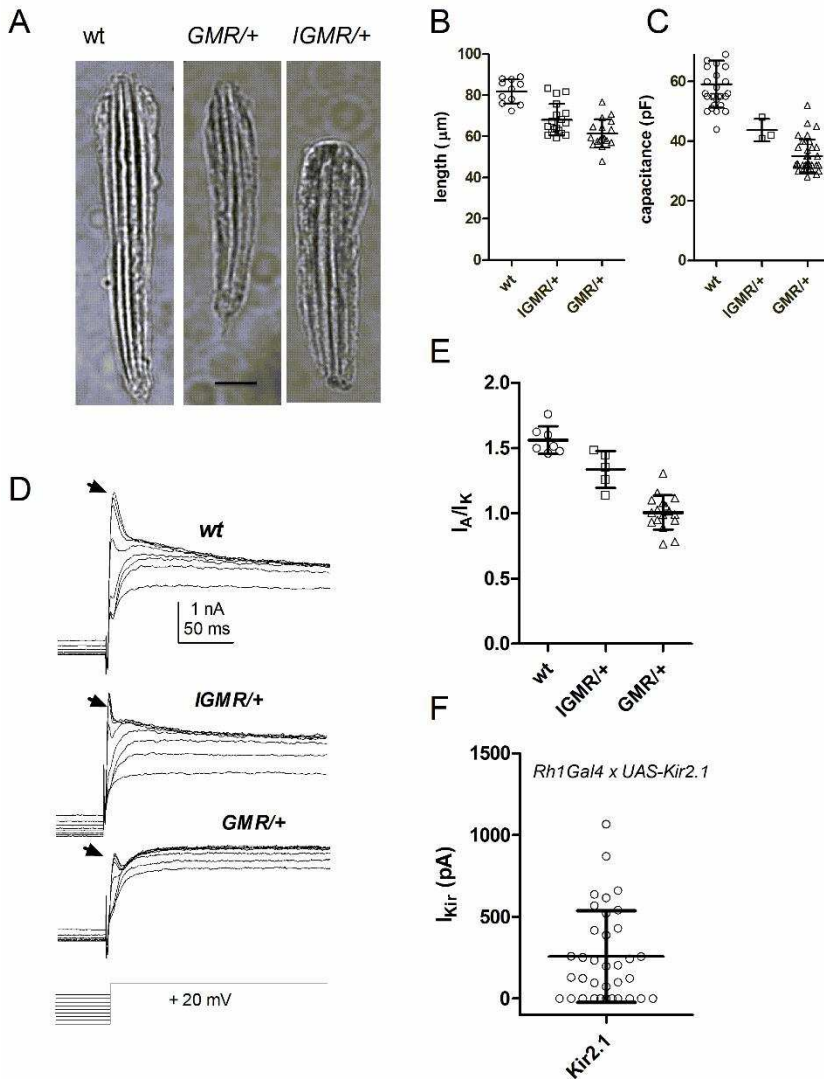
45 Zettler F, Järvillehto M (1973) Active and passive axonal propagation of non-spike signals in retina of *Calliphora*. *J Comp*  
46 *Physiol* 85:89-104.

1 Zheng L, de Polavieja GG, Wolfram V, Asyali MH, Hardie RC, Juusola M (2006) Feedback network controls  
2 photoreceptor output at the layer of first visual synapses in *Drosophila*. *J Gen Physiol* 127:495-510.  
3 Zheng L, Nikolaev A, Wardill TJ, O'Kane CJ, de Polavieja GG, Juusola M (2009) Network adaptation improves temporal  
4 representation of naturalistic stimuli in *Drosophila* eye: I dynamics. *PLoS One* 4:e4307.

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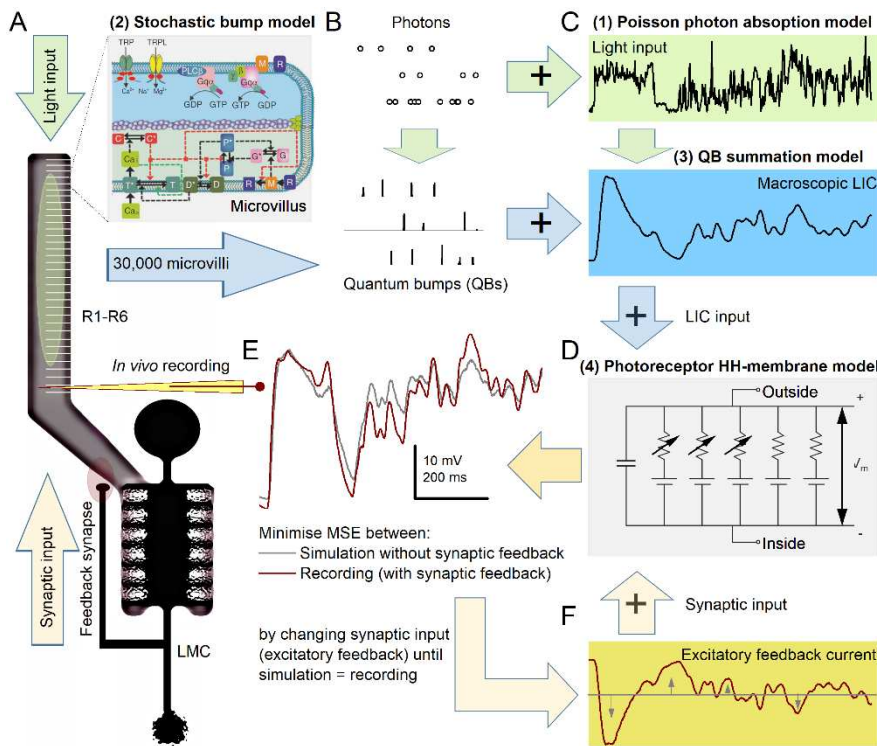
1 **Figure Legends**



2

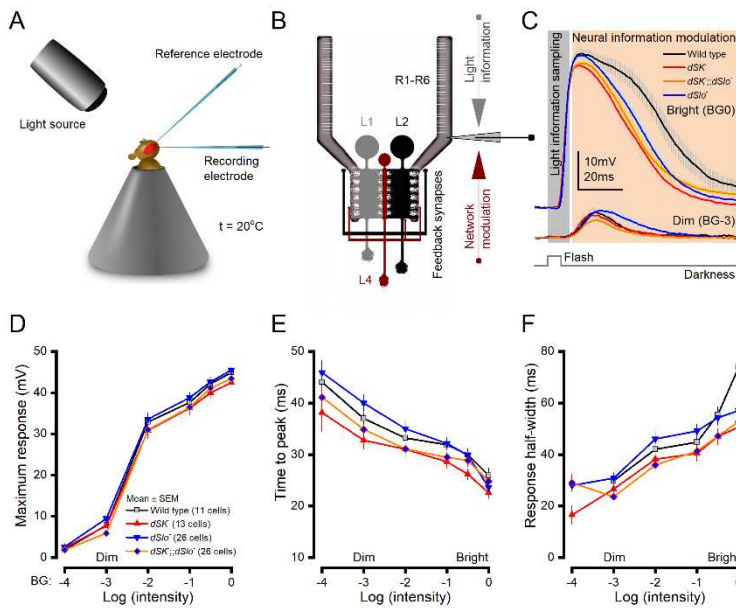
3 **Figure 1.** Gal4-controls alter photoreceptor structure and function. **A**, Images of dissociated  
 4 ommatidia from wild-type (wt) and flies expressing one copy of GMRGal4 (GMR/+)  
 5 longGMRGal4 (IGMR/+). Scale bar 10  $\mu\text{m}$ . **B**, Ommatidial length (wt, n=11; longGMR, n=18; GMR/+,  
 6 n=17;  $p < 0.0001$ ) and **C**, whole-cell capacitance (wt, n=30; longGMR, n=3 [ $p = 0.002$ ]; GMR, n=35  
 7 [ $p < 0.0001$ ]), **C**, are substantially reduced by expression of one copy of both GMRGal4 and  
 8 longGMRGal4. **D**, Native *Shaker* ( $I_A$  arrows) and delayed rectifier, *Shab* ( $I_{KS}$ ) current profiles are  
 9 altered in both GMRGal4 and longGMRGal4 flies. **E**, Summary data: ratio of *Shaker* ( $I_A$ ) to *Shab* ( $I_{KS}$ )  
 10 currents (wt, n=7; longGMR/+, n = 5 [ $p = 0.01$ ]; GMR/, n=17 [ $p = 0.002$ ]). **F**, Expression levels of a K  
 11 channel transgene (UAS-Kir2.1) driven by Rh1Gal4 was extremely variable (inward rectifier currents  
 12 measured from n = 36 cells), with ~30% of photoreceptors cells showing no detectable expression  
 13 at all. **B-C** and **E-F**: Mean  $\pm$  SD, two-tailed t-test. All significant at  $p < 0.05$  also on ANOVA plus Tukey's  
 14 Multiple comparison.





1

2 **Figure 2.** Schematic R1-R6 model structure with excitatory synaptic feedback from lamina  
 3 interneurons (LMCs: L2 and L4). R1-R6s signal to LMCs synaptically, using inhibitory transmitter,  
 4 histamine (Hardie, 1989), while the feedback synapses from LMCs to R1-R6s use excitatory  
 5 transmitters (Davis et al., 2018). **A**, R1-R6 rhabdomere has 30,000 microvilli. Each microvillus (inset)  
 6 is a semi-independent photon sampling unit with full transduction reactions to stochastically absorb  
 7 incoming photons and adaptively transduce them to quantum bumps (QBs). These reactions are  
 8 modelled by 20 differential equations with 50 fixed parameters (no free parameters) using the  
 9 Gillespie algorithm (Song et al., 2012). **B**, Because each microvillus recovers from its previous QB  
 10 within ~50-200 ms (refractoriness), its probability to convert its next absorbed photon (dot rows) to  
 11 a QB increases in time from 0 to 1, with not every absorbed photon causing a QB (Song et al., 2012).  
 12 **C**, The photons follow Poisson statistics to sum up the light stimulus (green) and the QBs sum up  
 13 the macroscopic light-induced current (LIC, blue). **D**, LIC drives a HH R1-R6 membrane model  
 14 (parameters in Tables 1-2), simulating a voltage response. **E**, This simulation (gray) is compared to  
 15 a real recording (purple; intracellular voltage signal to same light stimulus. See *In vivo intracellular*  
 16 *recordings*). **F**, Flat (tonic) feedback conductance (gray; mimicking synaptic input from the LMCs)  
 17 is injected into the R1-R6 model (with LIC) and its waveform is shaped dynamically (wine) in a  
 18 closed-loop until the resulting simulated photoreceptor voltage response matches the real recording  
 19 (**E**).



1

2 **Figure 3. R1-R6 Photoreceptors of Different  $Ca^{2+}$ -Activated  $K^+$  Channel Null-Mutants Show Distinctive**  
 3 **Response Dynamics to Light Flashes.**

4 **A,** Recordings were performed *in vivo* from R1-R6 somata using conventional sharp microelectrodes.

5 **B,** 30,000 *microvilli*, which form a R1-R6's light-sensor, the rhabdomere (comb-like structure), sample light  
 6 information (incoming photon rate changes). R1-R6 axon terminals then transmit these signals to the lamina  
 7 network through sign-inverting (histaminergic) output synapses (to L1-L3 monopolar cells and amacrine cells)  
 8 and receive synaptic feedback (network modulation) in return (Meinertzhagen and O'Neil, 1991; Rivera-Alba  
 9 et al., 2011); the schematic highlights excitatory feedbacks from L2, lamina intrinsic amacrine neurons (Lai)  
 10 and L4 to photoreceptor terminals. Because R1-R6s are short and have large length constants, synaptic  
 11 feedback also influences their somatic response waveforms (Zheng et al., 2006; Dau et al., 2016).

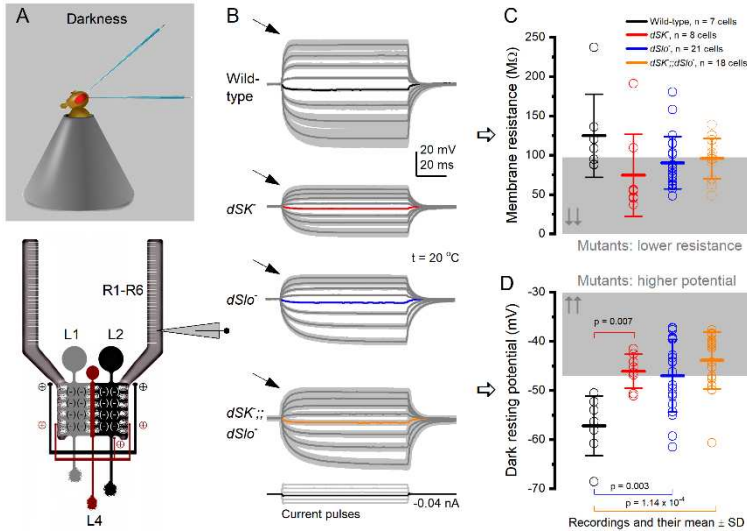
12 **C,** The average *dSlo*, *dSK*, *dSK;;dSlo* and wild-type responses to 10 ms bright and dim flashes. Their  
 13 corresponding delay and rise times ( $\leq 20$  ms from the flash onset; light gray area) were similar, suggesting  
 14 intact light information sampling. But the mutant R1-R6 responses decayed (in light brown area) either faster  
 15 or slower than the wild-type, suggesting differences in neural tuning.

16 **D,** Mutant and wild-type R1-R6 responses had comparable maximum amplitudes over the tested flash intensity  
 17 range, resulting in similar  $V/\log(I)$  saturation curves.

18 **E,** *dSK;;dSlo* and *dSK* responses peaked, on average, sooner than the wild-type to all test intensities, with  
 19 significantly shorter *dSK* values for BG-0.5 ( $p = 0.028$ ) and BG-1 ( $p = 0.047$ ). Conversely, *dSlo* responses  
 20 peaked later than the wild-type to all but the two brightest flashes. Moreover, these responses peaked  
 21 significantly later than those of *dSK* at BG-0.5 ( $p = 0.035$ ) and BG-3 ( $p = 0.013$ ), and *dSK;;dSlo* at BG-2 ( $p =$   
 22  $0.038$ ) and BG-3 ( $p = 0.013$ ).

1 **F**, Wild-type response half-widths to the brightest flash (BG0) were significantly longer than those of *dSK*<sup>-</sup> ( $p =$   
 2  $0.003$ ) and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> ( $p = 8.56 \times 10^{-4}$ ) R1-R6s. Conversely, *dSlo*<sup>-</sup> responses, on average, lasted the longest  
 3 over a broad flash intensity range; vs. *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>: at BG-1 ( $p = 0.042$ ) BG-2 ( $p = 0.011$ ) and BG-3 ( $p = 0.007$ ).

4 **D-F**: Mean  $\pm$  SEM, two-tailed t-test.



5  
 6 **Figure 4.** In Darkness, R1-R6 Photoreceptors of  $\text{Ca}^{2+}$ -Activated  $\text{K}^+$  Channel Null-Mutants Have  
 7 Lower Membrane Resistances and Higher Resting Potentials.

8 **A**, *In vivo* R1-R6 recordings. R1-R6 terminals provide histaminergic feedforward inhibition to LMCs.  
 9 In return, R1-R6s receive excitatory feedback from L2 and L4 monopolar cells.

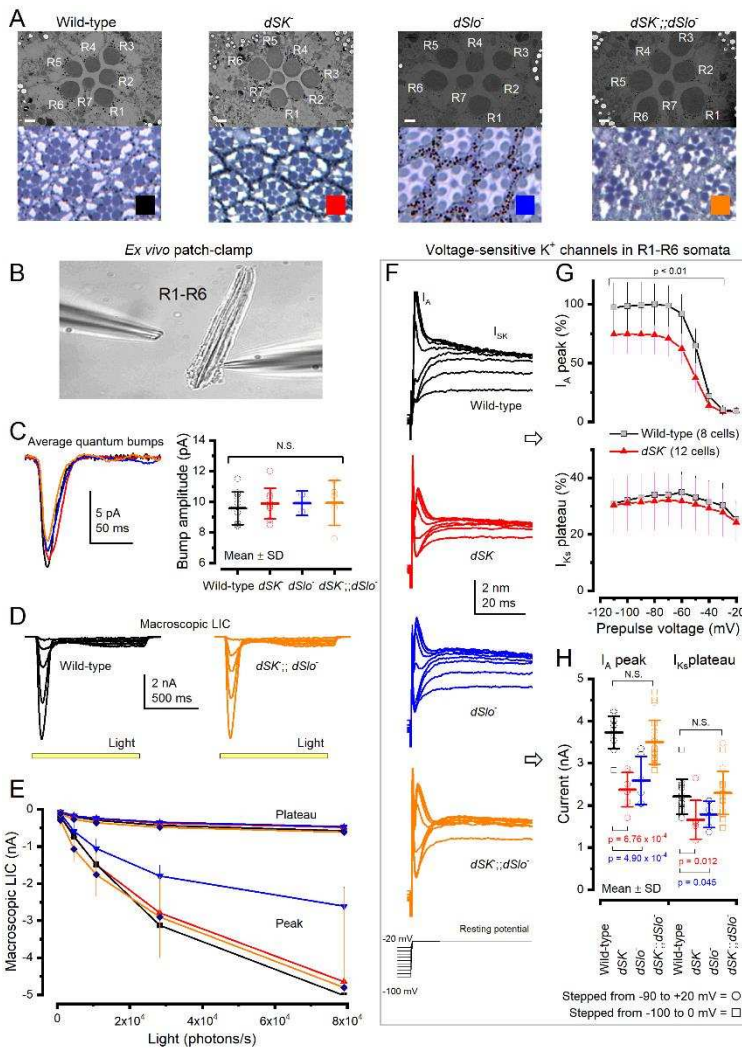
10 **B**, Voltage responses of dark-adapted wild-type and mutant R1-R6s to intracellular current pulse  
 11 injections. The arrows indicate outward rectification; caused by voltage-sensitive *Shaker* and *Shab*  
 12  $\text{K}^+$ -conductance activation to fast membrane depolarizations.

13 **C**, Mean wild-type R1-R6 input resistance ( $124.8 \pm 52.7 \text{ M}\Omega$ ,  $n = 7$  cells) is significantly higher than  
 14 that for all the mutant recordings ( $89.8 \pm 34.5 \text{ M}\Omega$ ,  $n = 47$ ,  $p = 0.024$ ), but not for each mutant-type  
 15 separately (*dSK*<sup>-</sup>,  $74.6 \pm 52.0 \text{ M}\Omega$ ,  $p = 0.071$ ,  $n = 8$ ; *dSlo*<sup>-</sup>,  $90.4 \pm 33.4 \text{ M}\Omega$ ,  $p = 0.232$ ,  $n = 21$ ;  
 16 *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>,  $95.9 \pm 25.6 \text{ M}\Omega$ ,  $p = 0.519$ ,  $n = 18$ ).

17 **D**, Mutant R1-R6s are more depolarized than the wild-type photoreceptors in darkness (wild-type, -  
 18  $57.2 \pm 6.1 \text{ mV}$ ; *dSK*<sup>-</sup>,  $-46.1 \pm 3.5 \text{ mV}$ ; *dSlo*<sup>-</sup>,  $-47.0 \pm 7.4 \text{ mV}$ ; *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>,  $-43.9 \pm 5.8 \text{ mV}$ ).

19 **C-D**: Mean  $\pm$  SD, p-values from ANOVA for multiple comparisons with Bonferroni-test.

20



1

2 **Figure 5.** *dSK*<sup>-</sup>, *dSlo*<sup>-</sup> and *dSK;;dSlo*<sup>-</sup> Photoreceptors Have Normal Morphology, Light-Induced  
 3 Currents (LIC) but 8-40% Reduced Light-Insensitive *I*<sub>A</sub> Currents.

4 **A**, The mutant retinae appear structurally intact, with R1-R7s having wild-type-like rhabdomeres and  
 5 pigmentation; 1 μm EM scale bars.

6 **B**, Whole-cell recordings were performed from dissociated ommatidia.

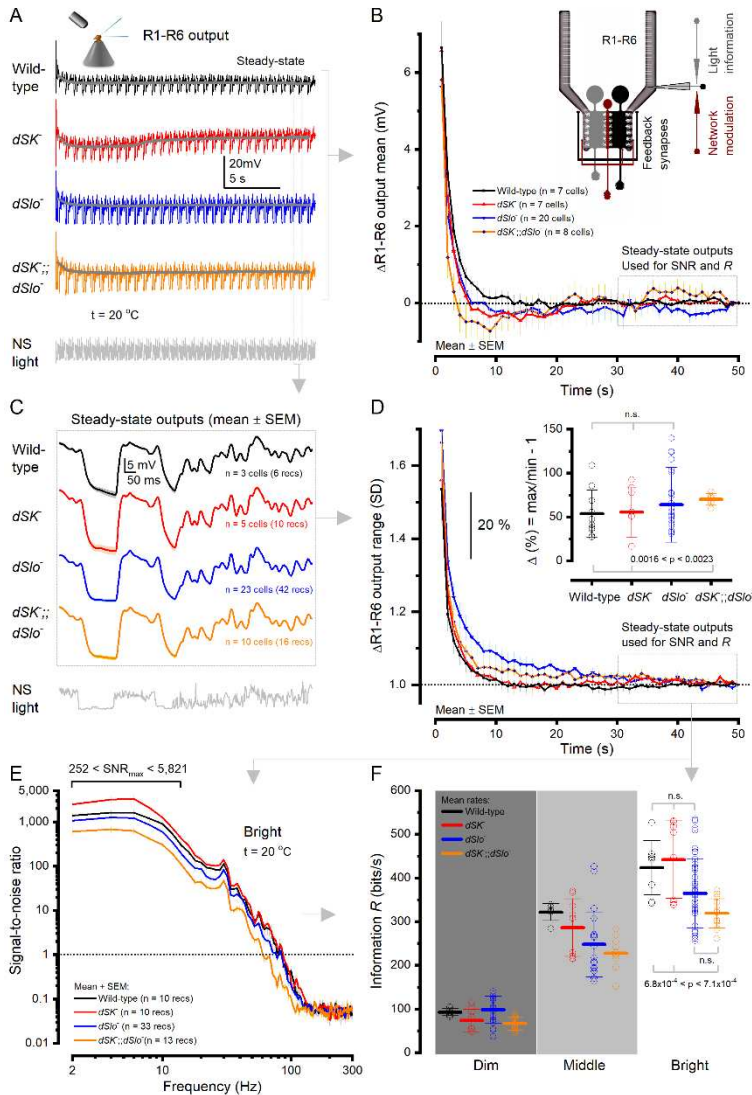
7 **C**, Mutant and wild-type R1-R6 quantum bump (QB) waveforms and their amplitude distributions to  
 8 dim flashes are similar.

9 **D**, Wild-type and *dSK;;dSlo*<sup>-</sup> R1-R6 LIC responses to 1 s light pulses of different brightness.

10 **E**, Macroscopic LIC peak and plateau responses were similar, with the normal experimental variation,  
 11 indicating that *dSK* and *dSlo* deletions do not affect phototransduction. The smaller *dSlo*<sup>-</sup> max LIC  
 12 probably resulted from these ommatidia being smaller, reflecting *dSlo*<sup>-</sup> mutants reduced yield/health.

13 **F**, Wild-type and mutant R1-R6s' voltage-sensitive outward K<sup>+</sup> currents to increased voltage steps  
 14 contain both the transient *Shaker* (*I*<sub>A</sub>) and sustained delayed rectifier, *Shab*, (*I*<sub>Ks</sub>) components.

- 1 **G**, *dSK* R1-R6  $K^+$ -currents have a reduced  $I_A$  but near normal-sized  $I_{KS}$ .
- 2 **H**, On average, the maximum  $I_A$  and  $I_{KS}$  currents in *dSK* and *dSlo* R1-R6s are a bit smaller than the
- 3 wild-type (*dSK*  $I_A$ : 36.4% < wild-type,  $I_{KS}$ : 24.9% < wild-type; *dSlo*  $I_A$ : 30.6% < wild-type,  $I_{KS}$ : 19.0%
- 4 < wild-type) but wild-type-like in *dSK*;;*dSlo* R1-R6s.



5

6 **Figure 6.** Adaptation Dynamics and Information Rates of Wild-Type and  $Ca^{2+}$ -Activated  $K^+$  Channel

7 Null-Mutant R1-R6 Photoreceptors.

8 **A**, Intracellular voltage responses to a repeated 1-s-long bright naturalistic light intensity time series

9 stimulus (NS).

10 **B**, Change in the response mean ( $\pm$  SD) over the repeated stimulation. Mean wild-type and *dSlo*

11 R1-R6 outputs declined near exponentially to steady-state, whereas adaptation in mean *dSK* and

12 *dSK*;;*dSlo* R1-R6 outputs depicted unique undershoots.

13 **C**, Mean waveforms  $\pm$  SD of steady-state adapted 1 s responses.

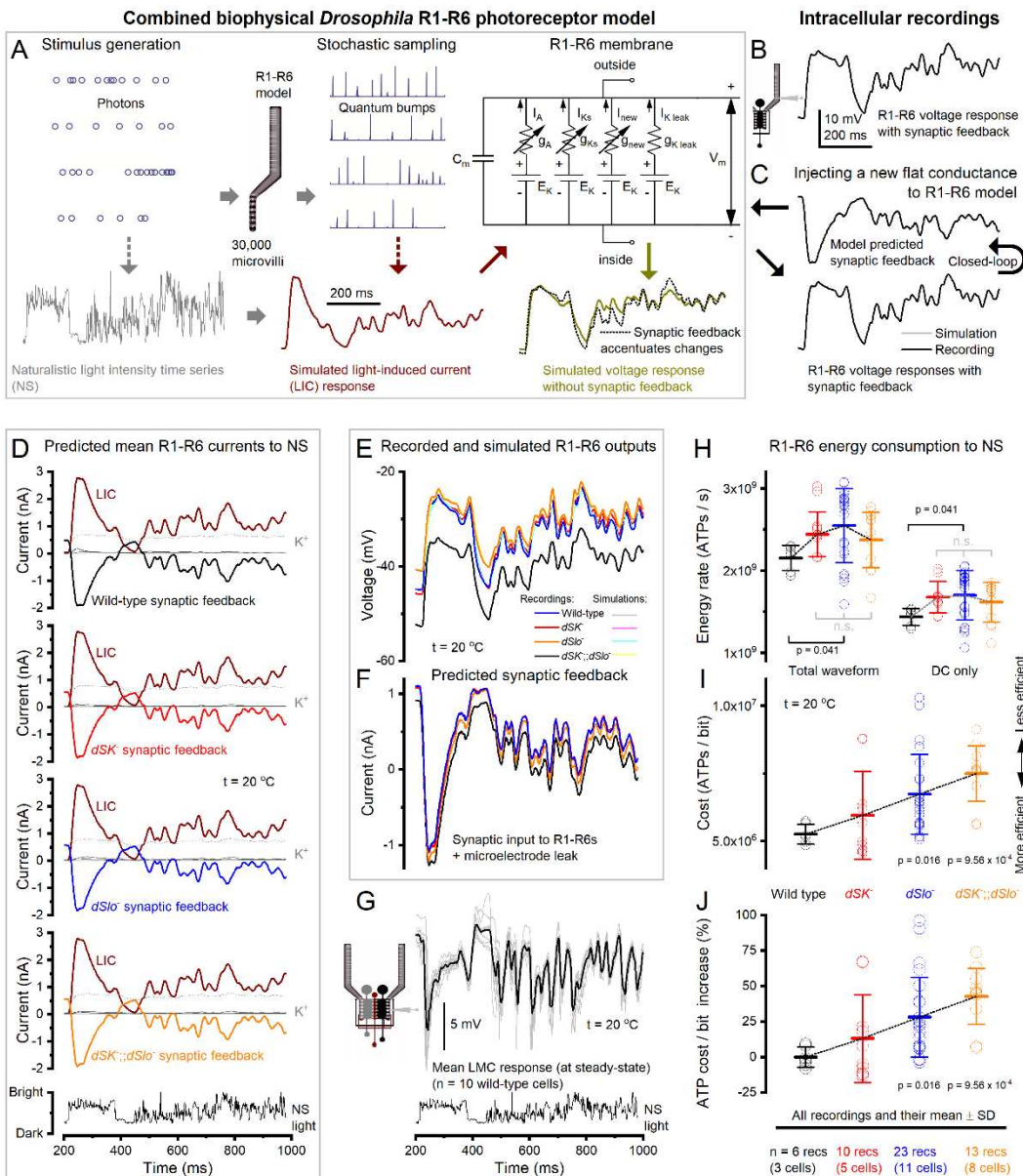
14 **D**, Relative change in R1-R6 output range, measured as the standard deviation (SD) of the



1 responses at each s during 50 s of stimulation (mean  $\pm$  SD). Wild-type and *dSlo*<sup>-</sup> R1-R6s  
2 desensitized during repeated stimulation, following exponential time constants. Wild-type R1-R6  
3 output range contracted from 114% to 100% in about 26 s ( $\tau_{\text{Wild-type}} = 1.96 \pm 0.39$  s); *dSlo*<sup>-</sup> from 134%  
4 in about 19 s ( $\tau_{\text{dSlo4}} = 8.3 \pm 1$  s); *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> from 133% in 8 s ( $\tau_{\text{dSK};\text{dSlo4}} = 3.4 \pm 0.4$  s). *dSK*<sup>-</sup> and  
5 *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6s' output ranges showed further sensitizing trends, reaching a steady-state after  
6 ~40 s.

7 **E**, Mutant and wild-type R1-R6s' average signal-to-noise ratios, measured from their steady-state  
8 outputs to bright NS, are high and broadly similar.

9 **F**, Mutant and wild-type R1-R6s sampled information from dim, moderately intense (middle) and  
10 bright naturalistic stimulation in a comparable manner (mean  $\pm$  SD; n = 10-33 recordings).  
11 *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6s had a marginally lower mean information transfer rate than the other genotype  
12 photoreceptors. In each genotype, R1-R6 information rates to the given stimulation vary naturally  
13 (up to ~200 bits/s) as each cell receives different amount of information from the network (Juusola  
14 et al., 2017).



1

2 **Figure 7.** Predicted Synaptic Feedback and ATP Consumption of Wild-Type and Mutant R1-R6 Photoreceptors.

3 **A,** Biophysically realistic R1-R6 model has four modules: stimulus generation, stochastic photon  
 4 sampling/quantum bump generation, bump integration and voltage-sensitive membrane. But it lacks the  
 5 synaptic feedback from the lamina network (Juusola et al., 2017; Song and Juusola, 2017), which affect the  
 6 real R1-R6 output. R1-R6 simulations (dark yellow trace) and recordings (dotted trace) to a repeated  
 7 naturalistic light intensity time series (NS) were analyzed at relative steady-state adaptation (*cf.* Fig. 6C).

8 **B,** Characteristic recording waveform to bright NS (BG0).

9 **C,** Synaptic feedback to each recording was estimated computationally by linking it to the photoreceptor model,  
 10 which had no free parameters. A new flat (zero) conductance, representing the synaptic input, was then  
 11 injected to the model. This conductance waveform was shaped in a closed-loop until the model output (gray)  
 12 matched the recorded output (black).

1 **D**, The fixed light-induced (dark red), K<sup>+</sup> currents and the average predicted synaptic feedback and of wild-  
2 type and mutant R1-R6 recordings.

3 **E**, Together, these currents charged up their respective simulated R1-R6 voltage responses. The simulations  
4 (light colors) match the recordings (bright colors) near perfectly.

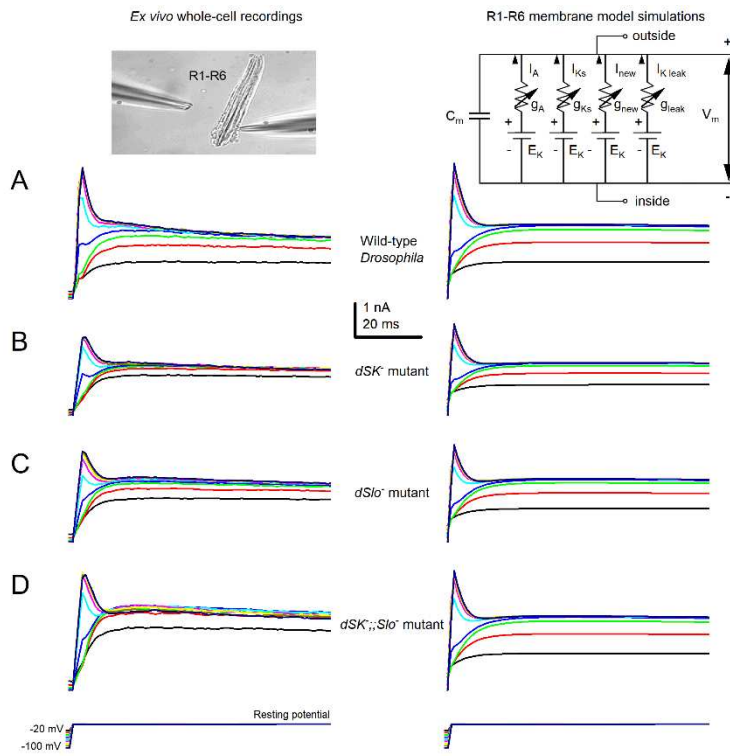
5 **F**, The average predicted synaptic feedback was unique to the mutant R1-R6s and showed stronger  
6 modulation on a higher mean (tonic excitatory background) than the wild-type (see also Fig. 10F). Testing the  
7 feedback means across all recordings: wild-type vs *dSK*<sup>-</sup>,  $p = 0.041$ ; *dSK*<sup>-</sup> vs *dSlo*<sup>-</sup>,  $p = 0.033$ ; *dSlo*<sup>-</sup> vs  
8 *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>,  $p = 0.009$ ; testing the mean feedback waveforms against each other,  $p < 2.274 \times 10^{-62}$ .

9 **G**, Separately recorded large monopolar cell (LMC) response waveforms to the same NS much resemble the  
10 predicted feedback waveforms (in F), suggesting that L2 and L4 cells, which form feedback synapses with R1-  
11 R6s (Meinertzhagen and O'Neil, 1991; Rivera-Alba et al., 2011), would contribute to R1-R6 output modulation  
12 (Zheng et al., 2006).

13 **H**, With these conductances included in each separate wild-type, *dSK*<sup>-</sup>, *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6 models,  
14 the metabolic energy (ATP) consumption of each recording was calculated for its full waveform (left) (Song  
15 and Juusola, 2014) and DC voltage (right) (Laughlin et al., 1998), respectively. Notably, the original DC voltage  
16 method, which does not consider how the dynamic ion fluctuations add to the electrochemical pumping work,  
17 underestimates ATP consumption by 1/3 (33.2%; see Materials and Methods ).

18 **I**, The cost of neural information, was calculated for each recording by dividing its information rate estimate  
19 with its full ATP consumption rate estimate. On average, the absence of *dSK* or *dSlo* or both increased the  
20 cost of neural information in a mutant R1-R6 by 13.1% (*dSK*<sup>-</sup>), 28.0% (*dSlo*<sup>-</sup>) or 42.7% (*dSK*<sup>-</sup>;*dSlo*<sup>-</sup>).





1

2 **Figure 8.** R1-R6 photoreceptors' characteristic voltage-sensitive *Shaker* and *Shab* K<sup>+</sup> current  
 3 responses *ex vivo* and their HH-models to voltage commands, under whole-cell voltage-clamp  
 4 conditions.

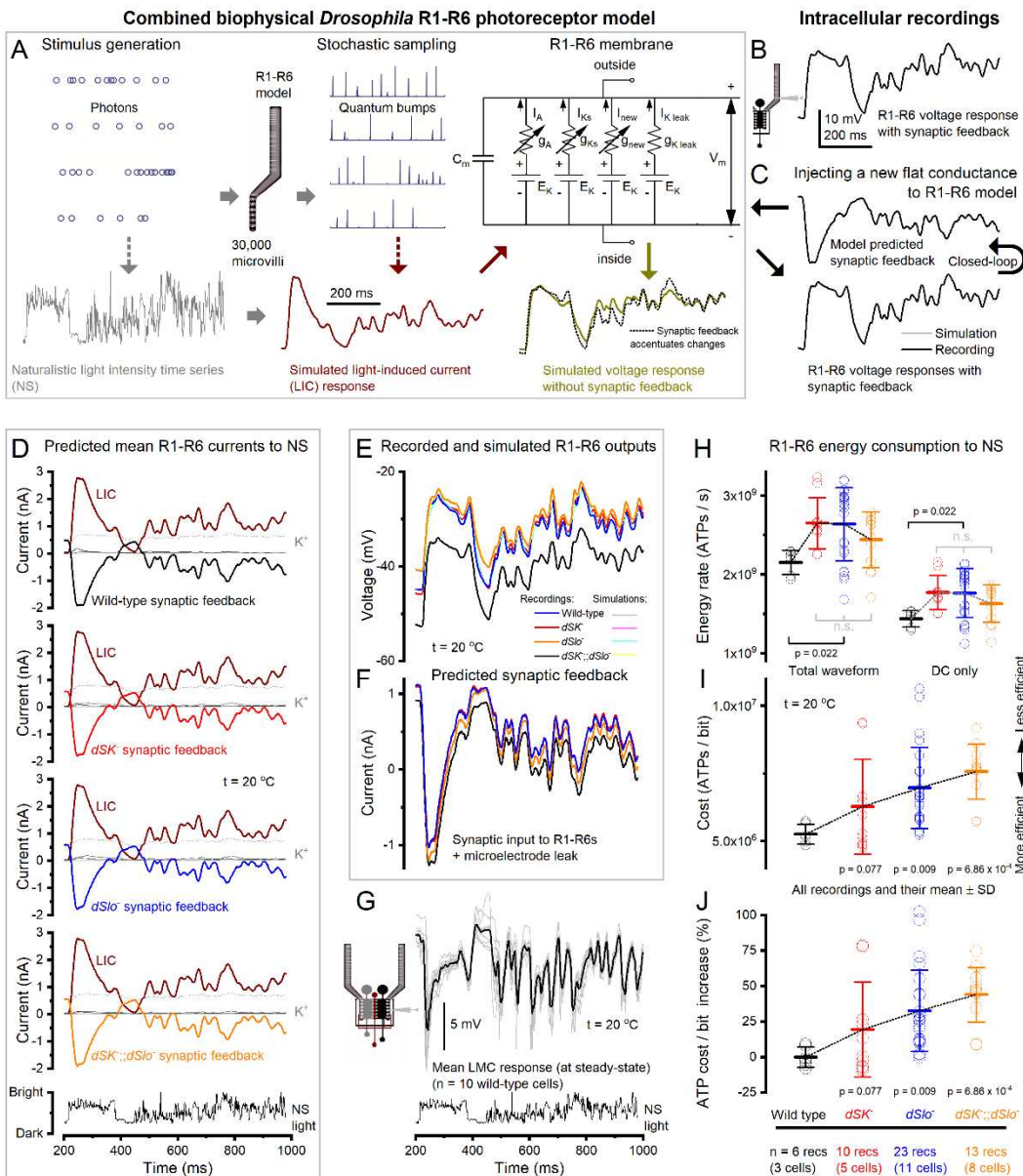
5 **A**, wild-type

6 **B**, *dSK*<sup>-</sup>

7 **C**, *dSlo*<sup>-</sup>

8 **D**, *dSK*<sup>-</sup>; *dSlo*<sup>-</sup>

9



1

2 **Figure 9.** Examples of the wild-type (black), *dSK*<sup>-</sup> (red), *dSlo*<sup>-</sup> (blue) and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> (orange data)

3 photoreceptor models' *Shaker* ( $I_A$ ), *Shab* ( $I_{Ks}$ ),  $K^+$ -leak, LIC and synaptic feedback currents to  
 4 naturalistic light stimulation, when using the same wild-type *Shaker* and *Shab* conductance  
 5 dynamics in all the models; cf. Figs 7 and 10.

6 **A**, In these simulations, the while-type, *dSK*<sup>-</sup>, *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> R1-R6 models had identical  
 7 light induced current (LIC) and voltage-sensitive membrane conductances (the models used the  
 8 wild-type *Shaker* and *Shab* dynamics as in Fig. 8A; cf. Fig. 7A).

9 **B**, Characteristic recording waveform to bright NS (BG0).

10 **C**, Again, synaptic feedback was computed through the R1-R6 model, which had no free  
 11 parameters, in a closed-loop until the model output (gray) matched the recorded output (black).

1 **D**, The fixed light-induced (dark red), K<sup>+</sup> currents and the average predicted synaptic feedback of  
2 wild-type and mutant R1-R6 recordings.

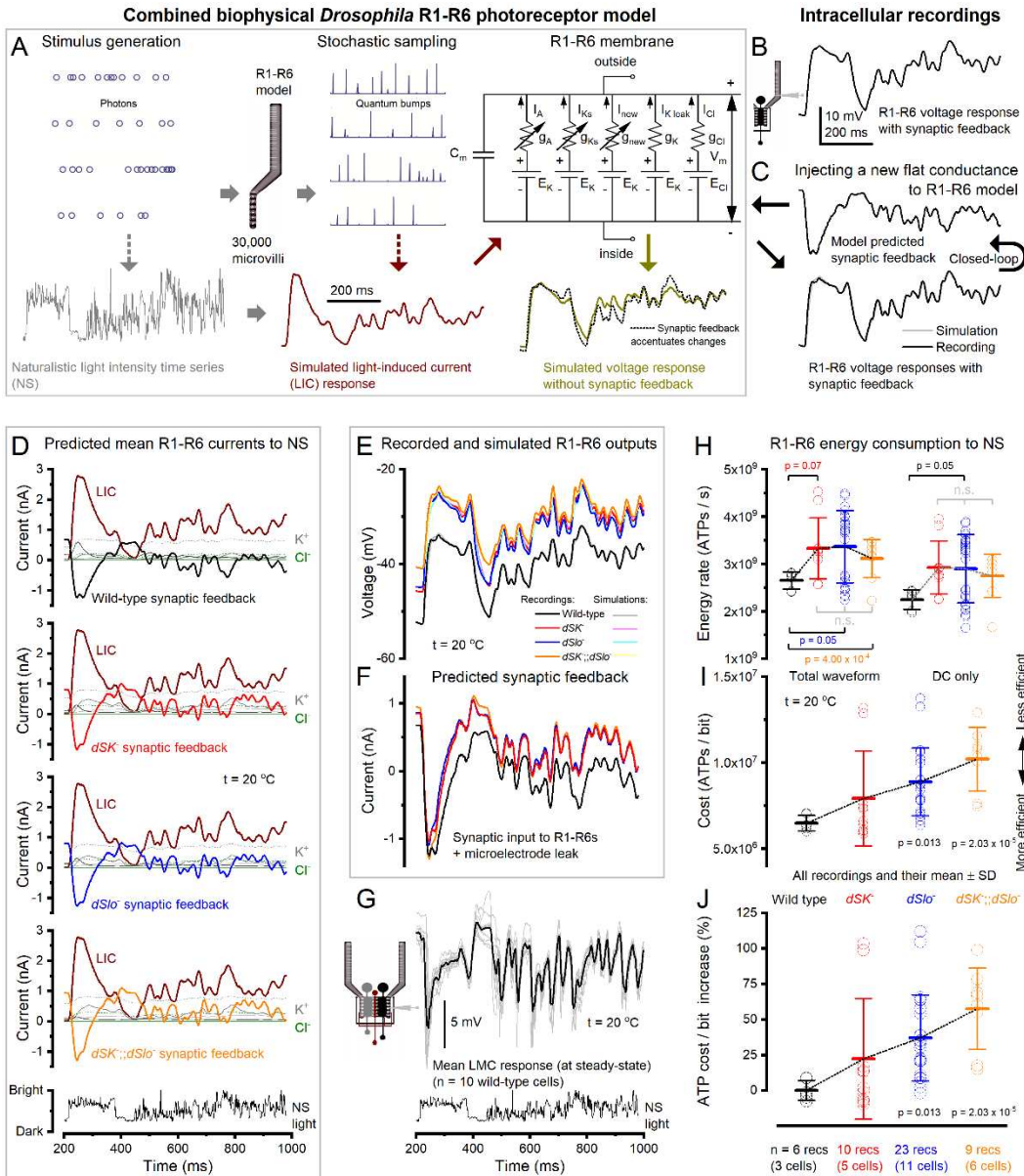
3 **E**, Together, these currents charged up their respective simulated R1-R6 voltage responses. The  
4 simulations (light colors) matched the recordings (bright colors).

5 **F**, Again, the average predicted synaptic feedbacks to the mutant R1-R6s were stronger, having  
6 higher means (tonic excitatory background) than the wild-type.

7 **G**, The recorded large monopolar cell (LMC) response waveforms to the same NS resembled the  
8 predicted feedback waveforms (in F).

9 **H**, The ATP consumption of these mutant R1-R6 models was 3.5-8.6% higher than in those  
10 models, which had their recorded (smaller) *Shaker* and *Shab* conductances (*cf.* Figs 5F-H and 7H).

11 **I**, The cost of neural information, was calculated for each recording by dividing its information rate  
12 estimate with its full ATP consumption rate estimate. On average, the absence of dSK or dSlo or  
13 both increased the cost of information in a mutant R1-R6 by  $19.3 \pm 33.4\%$  (*dSK*<sup>-</sup>),  $32.6 \pm 28.6\%$   
14 (*dSlo*<sup>-</sup>) or  $43.9 \pm 19.3\%$  (*dSK*<sup>-</sup>;;*dSlo*<sup>-</sup>). Thus, 19-36% homeostatic reductions in *Shaker* and *Shab*  
15 currents in *dSK*<sup>-</sup> and *dSlo*<sup>-</sup> R1-R6 photoreceptors caused only 4.5% and 6.2% savings in their ATP  
16 consumption per each bit of transmitted information (*cf.* Figs 7I and 7J).



1

2 **Figure 10.** Examples of the wild-type (black), *dSK*<sup>-</sup> (red), *dSlo*<sup>-</sup> (blue) and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> (orange data)  
 3 photoreceptor models' *Shaker*, *Shab*, K<sup>+</sup>-leak, Cl<sup>-</sup>-leak LIC and synaptic feedback currents to  
 4 naturalistic light stimulation, when using larger wild-type *Shaker* and *Shab* conductance dynamics  
 5 (as in Table 2) in all the models; cf. Figs 7 and 9.

6 **A**, In these simulations, we further added a Cl<sup>-</sup>-leak and Cl<sup>-</sup> conductance (here combined to  $g_{Cl^-}$   
 7 simply the HH-diagram) in the R1-R6 photoreceptor membrane model and balanced these by  
 8 increasing voltage-sensitive K<sup>+</sup> conductances (Materials and Methods: speculative photoreceptor  
 9 membrane model), and again the synaptic feedback (Juusola et al., 2017; Song and Juusola, 2017)  
 10 was computed in a closed-loop until the simulations matched the recordings (cf. Fig. 6C).

11 **B**, Characteristic recording waveform to bright NS (BG0).

12 **C**, Synaptic feedback to each recording was estimated computationally by linking it to the

1 photoreceptor model, which had no free parameters.

2 **D**, The fixed light-induced (dark red),  $K^+$  and  $Cl^-$  currents and the average predicted synaptic  
3 feedback and of wild-type and mutant R1-R6 recordings.

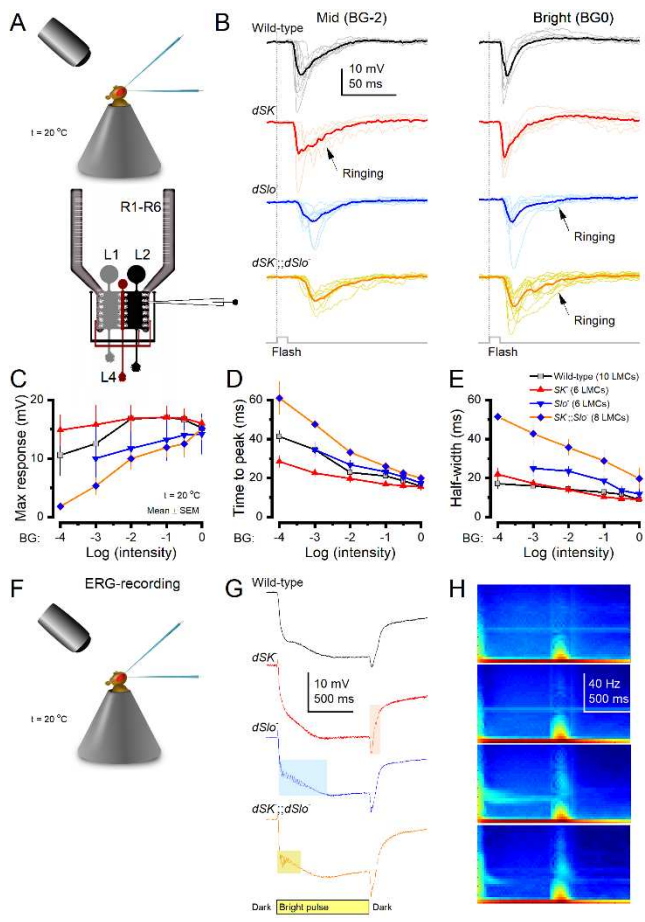
4 **E**, These currents charged up their respective simulated R1-R6 voltage responses (light colors),  
5 which matched the actual recordings (bright colors).

6 **F**, Similar to the other simulations (*cf.* Figs 7E and 9E), the predicted synaptic feedback to the mutant  
7 R1-R6s was larger (carrying bigger modulation) with a higher mean (tonic excitatory background)  
8 than the wild-type. However, the modulation in these simulations was even more transient.

9 **G**, Separately recorded large monopolar cell (LMC) response waveforms to the same NS much  
10 resemble the predicted feedback waveforms (in F).

11 **H**, Energy (ATP) consumption of each recording was calculated for its full waveform (left) (Song and  
12 Juusola, 2014) and DC voltage (right) (Laughlin et al., 1998), respectively. Notably, the added extra  
13  $Cl^-$ -leak and  $Cl^-$  conductance (with rebalanced  $K^+$  conductances) increased the photoreceptors'  
14 energy usage by ~26.3% (*cf.* Fig. 7H, left: from  $2.10 \times 10^9$  ATP/s to  $2.65 \times 10^9$  ATP/s.). Whilst the  
15 original DC voltage method (right), which does not consider how the dynamic ion fluctuations add to  
16 the electrochemical pumping work, now underestimated ATP consumption by about 15%.

17 **I**, The cost of neural information, was calculated for each recording by dividing its information rate  
18 estimate with its full ATP consumption rate estimate. Here, homeostatic increase in  $K^+$  and  $Cl^-$  leak  
19 conductances (to compensate the loss of  $Ca^{2+}$ -activated  $K^+$  channels) increased the cost of  
20 information in a mutant R1-R6 by  $22.2 \pm 42.3\%$  (*dSK*),  $37.0 \pm 30.3\%$  (*dSlo*) or  $57.6 \pm 28.8\%$   
21 (*dSK;;dSlo*), in respect to the comparable wild-type model.

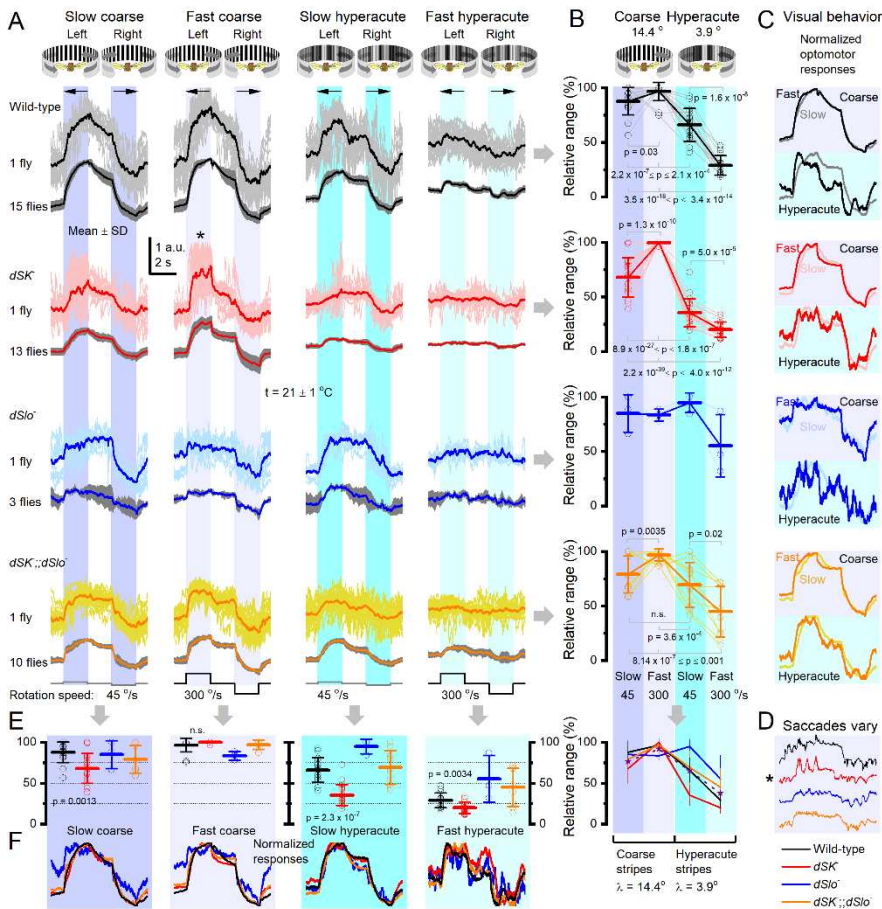


1  
2 **Figure 11.** Large Monopolar Cell (LMC) Output in the Wild-Type and Mutant Flies Differ Consistently.  
3 **A**, Intracellular LMC recordings were carried out from *in vivo* (above). The schematic (below)  
4 highlights synaptic feedbacks from L2/AC and L4 to photoreceptors terminals.  
5 **B**, Voltage responses of the wild-type and mutant LMCs to Bright and Middle intensity flashes.  
6 **C**, *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> LMCs generated smaller responses to the dim and middle intensity flashes  
7 than the wild-type (*dSlo*<sup>-</sup>:  $p < 0.01$ , BG0-4,  $n = 8$ ; *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>:  $p < 0.03$ , BG0-4,  $n = 5$ ).  
8 **D**, *dSK*<sup>-</sup> LMC responses peaked the fastest, whereas *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> LMC responses were  
9 slower than the wild-type ( $p < 0.03$ ) over the tested intensity range.  
10 **E**, *dSK*<sup>-</sup> LMC responses lasted as long as the wild-type responses, while *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> LMCs  
11 took much longer to repolarize than wild-type (*dSlo*<sup>-</sup>,  $p < 0.04$ , BG2-3; *dSK*<sup>-</sup>;*dSlo*<sup>-</sup>,  $p < 0.04$ , BG0-3).  
12 **F**, *In vivo* electroretinograms (ERG), depicting global light-induced eye activity, were recorded from  
13 the corneal surfaces of intact *Drosophila*.  
14 **G**, *dSK*<sup>-</sup>, *dSlo*<sup>-</sup> and *dSK*<sup>-</sup>;*dSlo*<sup>-</sup> ERGs often showed characteristic oscillations after the light on- and  
15 off-transients, consistent with the corresponding intracellularly recorded light-induced LMC  
16 oscillations (B).  
17 **H**, Dynamic spectra of the ERGs (G) reveal the frequency-dependency and duration of the



1 oscillations.

2 **C-E:** Mean  $\pm$  SEM, two-tailed t-test.



3  
4 **Figure 12.** Wild-Type Flies and  $Ca^{2+}$ -Activated  $K^+$  Channel Mutants Differ in Their Sensitivity to Track  
5 Different Field Rotations.

6 **A,** Optomotor responses to slow (45 %/s) and fast (300 %/s) left/right rotating fields of either coarse  
7 (14.4°) or fine-grained (hyperacute: 3.9°) black-and-white vertical stripes (100% contrast), as seen  
8 by the flies. Above: mean (thick line) and 19-32 responses (thin lines) of the same fly to the four  
9 stimuli. Below: population means of many flies of the same genotype. Each fly was tested with these  
10 four stimuli to work out its relative optomotor sensitivity. In the experiments, the stimulus presentation  
11 order was actively varied to reduce novelty, flight fatigue or adaptation induced bias.

12 **B,** The relative output (%) range (max-min) of each tested fly, as scaled by its maximum optomotor  
13 response to the four stimuli. Wild-type, *dSK* and *dSK;;dSlo* flies typically responded the strongest  
14 to the fast coarse field rotation, whilst *dSlo* flies were most sensitive to slow hyperacute field rotations.

15 **C,** Normalized responses of each genotype to each test stimuli.

16 **D,** The relative output range (max-min) of the different genotype of each test stimuli.

17 **E,** Normalized responses of the different genotypes, compared to each other, for each test stimuli.