Supplementary Material

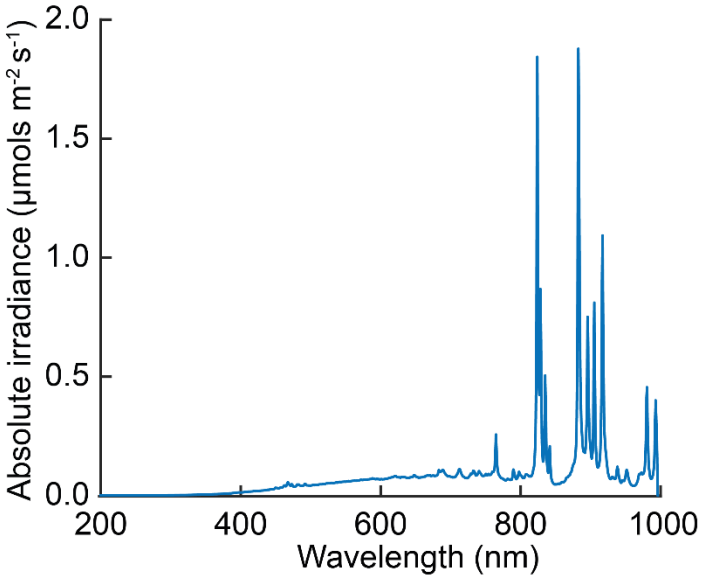

Figure S1. Spectrum of HPX-2000 that was used as the invariant white light in all experiments. The spectrum was measured using a spectrometer (QE *Pro*, Ocean Optics Inc., FL, USA) and the spectroscopy software OceanView (Ocean Optics Inc., FL, USA). The light from the HPX-2000 was delivered to the spectrometer via a fibre optic that passed light through a fibre optic variable attenuator (FVA-UV, Ocean Opitcs Inc., FL, USA) and finally to the HPX-2000 via a second fibre optic. The variable attenuator was set such that the light delivered to the spectrometer was of an intensity within the readable range of the spectrometer.

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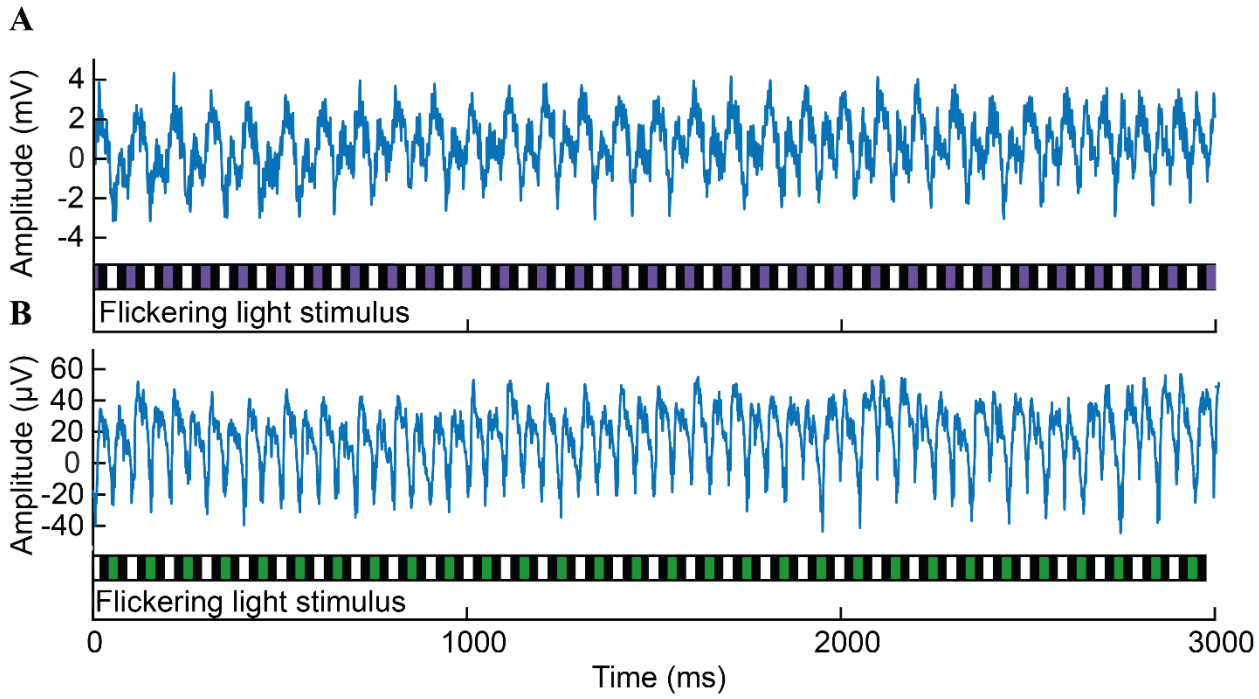

Figure S2. ERG and intracellular signal over three seconds of recording in response to a flickering light stimulus. (a) Filtered intracellular signal is displayed in blue and the amplitude of the response is before amplification. The raw intracellular signal was filtered with a 50 Hz notch filter and a low pass filter with a 70 Hz cutoff frequency. (b) A raw ERG signal is displayed in blue and the amplitude of the response is before amplification. In both figures the flickering light stimulus is displayed below the signal showing the invariant white light, followed by no light, followed by a coloured light, followed by no light. In (a) the coloured light was a 380 nm light that produced a weaker response than the invariant white light and in (b) the coloured light was a 530 nm light that produced approximately the same response as the invariant white light. In both intracellular and ERG recordings, responses to the light were delayed by approximately 60 ms.

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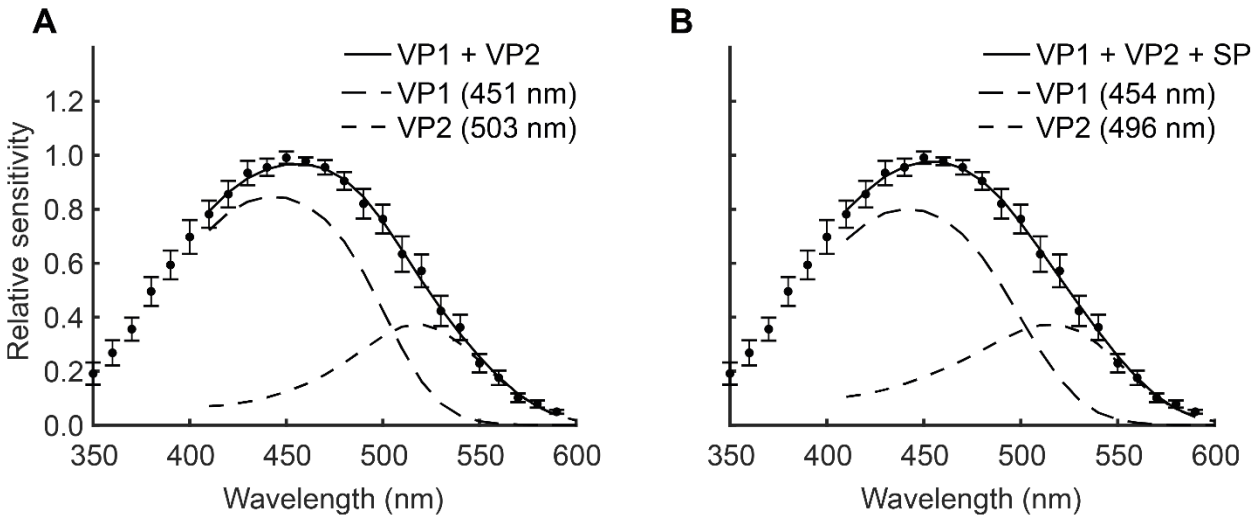

Figure S3. Mean ERG recordings from all times of day for *G. dampieri* fitted with a model excluding a screening pigment and including a screening pigment. (a) Mean ERG recordings fitted with a model excluding a screening pigment. (b) Mean ERG recordings fitted with a model including a screening pigment with an absorbance coefficient of 0.005 (mean ± SEM, N = 8). Model fit is displayed as a black curve and dashed curves show the two visual pigments in their relative contributions.

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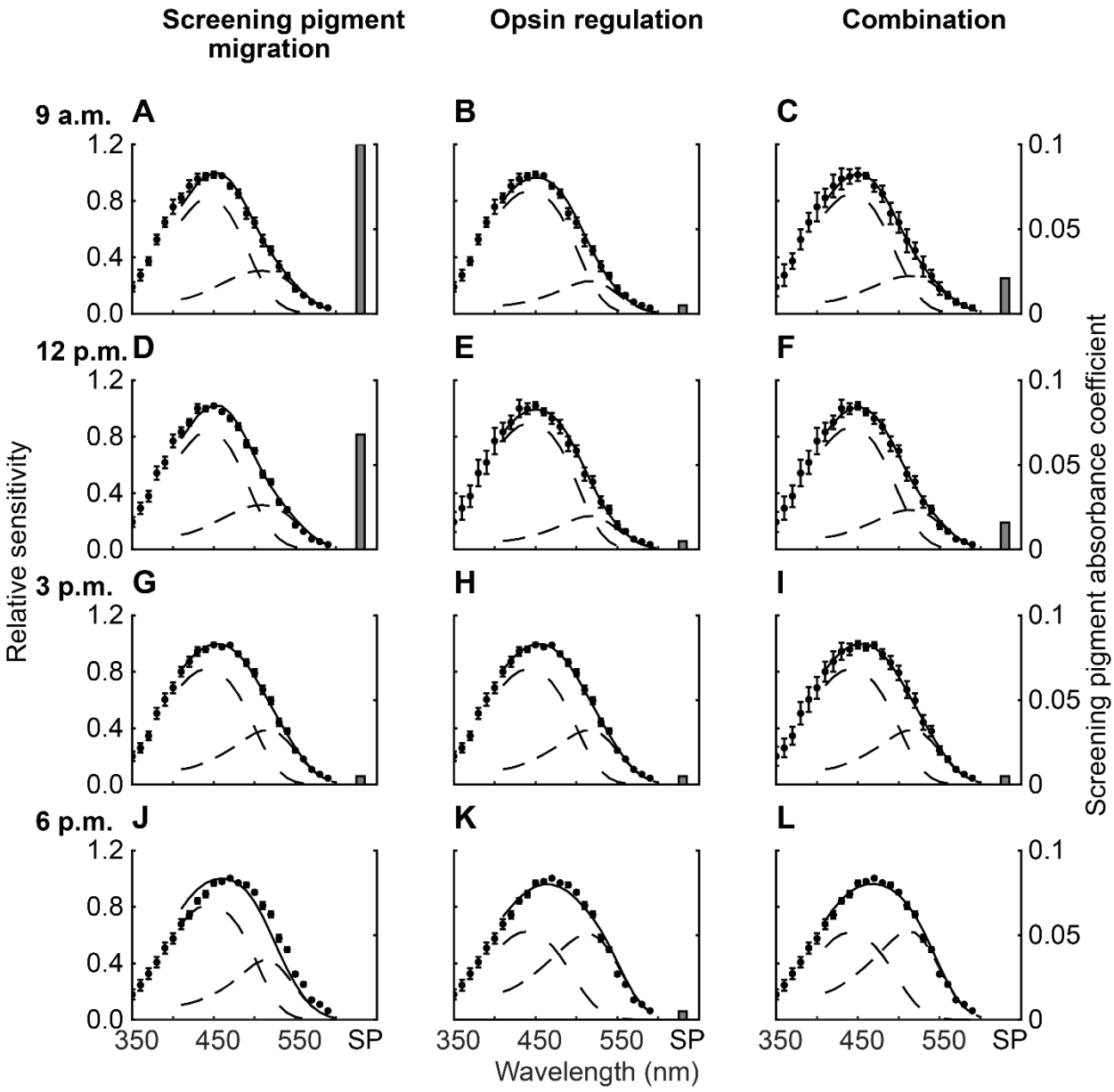

Figure S4. Mean ERG recordings from eight *G. dampieri* at 9 a.m., 12 p.m., 3 p.m., and 6 p.m., fitted with three different models. Mean ERG recordings (left y axis) are displayed as points (mean ± SEM, N = 8) and each model is displayed as the black curve. Dashed curves show the visual pigment templates after screening pigment effects for VP1 (454 nm) and VP2 (496 nm) in their relative contributions as shown in Table S1. Grey bars represent the screening pigment absorbance coefficient for each model (right y-axis).

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Table S1. Best fitting model results for each of the three models fitted to the dataset from each time of day.

Model	Time	p_i ratio VP1:VP2	$k_{s,max}$
Screening pigment migration	9 a.m.	0.73:0.27	**0.1**
	12 p.m.	0.73:0.27	**0.068**
	3 p.m.	0.73:0.27	**0.005**
	6 p.m.	0.73:0.27	**0**
Opsin regulation	9 a.m.	**0.84:0.16**	0.005
	12 p.m.	**0.84:0.16**	0.005
	3 p.m.	**0.73:0.27**	0.005
	6 p.m.	**0.53:0.47**	0.005
Combination	9 a.m.	**0.78:0.22**	**0.021**
	12 p.m.	**0.78:0.22**	**0.016**
	3 p.m.	**0.73:0.27**	**0.005**
	6 p.m.	**0.53:0.47**	**0**

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