Pupil responses to hidden photoreceptor–specific modulations in movies

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Conflicts of interest

M.S. is listed as an inventor on a patent application filed by the Trustees of the University of Pennsylvania on September 11, 2015 (US Patent Application No. 14/852,001, "Robust Targeting of Photosensitive Molecules".

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Abstract

Under daylight light levels, the human pupillary light response (PLR) is driven by the activity of the L, M, and S cones, and melanopsin expressed in the so-called intrinsically photosensitive retinal ganglion cells (ipRGCs). However, the importance of each of these photoreceptive mechanisms in defining pupil size during natural viewing conditions remains to be established. To address this question, we embedded photoreceptor-specific modulations in a movie projected using a novel projector-based five-primary spatial stimulation system, which allowed for the precise control of photoreceptor activations in time and space. We measured the sensitivity of the PLR in eleven observers, who viewed short cartoon movies which contained hidden low-frequency (0.25 Hz) modulations of the L, M and S cones (no stimulation of melanopsin), melanopsin (no stimulation of L, M and S cones), both L, M, and S cones and melanopsin or no modulation at all. We find that all photoreceptors active at photopic light levels regulate pupil size under this condition. Our data imply that embedding modulations in photoreceptor contrast could provide a method to manipulate key aspects of the human visual system in everyday activities such as a movie watching.

Introduction

The human retina contains rods, cones and the photopigment melanopsin, which is expressed in a subset of so-called *intrinsically photosensitive retinal ganglion cells* (ipRGCs) [1-5]. All photoreceptors are thought to contribute to visual function, though their exact contributions in naturalistic behaviour depend on the spatial and temporal characteristics of the input into the retina, and the task at hand [6]. The aperture of the eye – the pupil – changes its size depending on the activity of the photoreceptors in the retina, and pupillometry represents a non-invasive and convenient method of assessing photoreceptor function. At photopic light levels, pupil size is controlled by an excitatory input of L+M (luminance), an inhibitory input of S cones (and possibly M cones, see [7, 8]), as well as a positive input from melanopsin (Fig. 1A).

These distinct retinal pathways have been rigorously described in analytical and parametric studies under extremely well-controlled but relatively reduced stimulus conditions typically employing spatially homogenous fields [7-16]. Nevertheless,the importance of each photoreceptor class in defining pupil size in more naturalistic viewing conditions remains to be established. Here, we asked whether it is possible to address this problem by embedding photoreceptor-specific modulations into movies seen under naturalistic viewing conditions.

The spectral tuning of the individual photoreceptors is relatively broadband, and therefore any given light will activate all photoreceptors (Fig. 1B). To assess the response of any given photoreceptor using narrowband, even monochromatic light, will therefore only yield non-specific responses. For example, while in the living human eye, melanopsin will respond maximally to a ~488 nm light (the shift from 480 nm of peak pigment absorbance to longer wavelengths being due to pre-receptoral filtering by the lens and ocular media), that light will also activate S cones at ~41%, M cones at ~43%, and L cones at ~26% of their maximal response, respectively. This experimental challenge is overcome by the method of silent substitution [17, 18]. In this method, pairs of spectra are presented to an observer such that

the exchange between the spectra only stimulates one class of photoreceptors (the *stimulated* photoreceptors), while not changing the activation of the set of *silent* photoreceptors. These pairs are also called metamers. Silent substitution is possible because of a property of photoreceptors called the *principle of univariance* [19]: a given photopigment integrates light depending on its spectral sensitivity, but it does not retain information about wavelength and intensity, producing only a scalar output.

Silent substitution can be achieved using any light source which has at least as many independent lights as spectral photoreceptors to be examined. In practice, for the human eye, which is five photoreceptor classes, one needs five independent lights; or under photopic conditions, in which orthodoxy assumes rods to be saturated [20] (but see [21] for evidence in mice and [22] for evidence in humans that this orthodoxy is challenged), four independent lights. Silent substitution can be realized in multiple ways, such as using a set of LEDs combined optically [7, 9-11, 13-16, 23-25], a combination of broadband light filtered through a diffraction grating and imaged on digital micromirror device [12, 26-28] (see [29] for technical details), or using modified projectors [30, 31].

In this work, we used a modified projector system, the design of which was described previously [31], to examine the pupil responses to photoreceptor-selective sinusoidal modulations embedded in short greyscale cartoon clips. Under photopic conditions, we examined responses to stimuli targeted at the *LMS* cones (with no modulation of melanopsin), *melanopsin* (with no modulation of LMS cones), and a modulation of all photoreceptors, which we term *Light Flux*. We find evidence that all photoreceptors regulate pupil size to some degree even during high spatiotemporal frequency modulations.

Methods and materials.

Visual stimuli. Stimuli were presented in free-viewing, and participants were free to move their eyes throughout the study. Participants were seated 128 cm from a canvas onto which two spatially aligned projectors projected a movie which had in it embedded a 0.25 Hz modulation of photoreceptor excitation. The stimulus was a rectangle of dimensions 81 x 109 cm, yielding a total visual angle of approximately 35 x 46 deg^2. The targeted photoreceptors were the LMS cones at equal contrast (*LMS*), melanopsin (*Mel*), or all photoreceptors in unison (*Light Flux*). A fourth condition (*Reference*) contained no modulations. In our low-frequency modulations, we did not control for the effect of cones in the shadow of the retinal blood vessels, so-called penumbral cones, which are known to be stimulated in high-frequency (8-16 Hz) melanopsin-directed modulations [27].

Projector system. The design of the projector system was first described in [31], but this concrete realization was slightly different. Two Hitachi CP-X5022WN 3LCD were stacked on top of each other and the boundaries of their respective projected outputs were aligned by the experimenters. The projectors were modified using optical filters. In Projector 1, the blue primary was modified using a 470 nm cut-off yellow longpass filter (PIXELTEQ, Largo, FL; part # LP470-r40x25x1), making a cyan primary; the green primary was modified using a 463-571 nm notch filter (MidOpt, Palatine, IL; part number #102340892), making yellow. In

Projector 2, the blue primary was modified using a 463-571 nm magenta notch filter (MidOpt, Palatine, IL; part #102340892), making violet; and the green primary was modified using a 550 nm bandpass filter (PIXELTEQ, Largo, FL part # Bi550-r40x25x1), producing a narrower green primary. This yielded a dual projector system with five independent primaries (Fig. 1C, D). At maximum output, the primaries had CIE 1931 *xy* chromaticities: (0.65, 0.34), (0.50, 0.49), (0.08, 0.22), (0.28, 0.70), and (0.16, 0.02), and photopic luminances 54.36, 131.64, 20.84, 185.66, and 8.18 cd/m^2, respectively.

Naturalistic movies. The movie selected was the Tom & Jerry cartoon "Pent-House Mouse" (1963) [32] (Fig. 1E, length 6m11s). The film was first turned into a greyscale movie and then processed in MATLAB to embed photoreceptor-directed modulations into the luminance variations (Fig. 1F, G, H). The photoreceptor contrast of the modulation was 28% for all directions.

Photoreceptor spectral sensitivities. For the LMS cones, we assumed that physiologically-relevant CIE 2006 photoreceptor sensitivities [33]. Melanopsin spectral sensitivity was derived using the Govardovskii nomogram with λ_{max} = 480 nm, assuming low photopigment density, and applying the same pre-receptoral filtering as for the cones. The receptor spectral sensitivities are available in the Silent Substitution Toolbox (http://github.com/spitschan/SilentSubstitutionToolbox).

Age adjustment. To account for age-dependent differences in prereceptoral filtering (specifically age-dependent lens-density), stimuli were adjusted in age group for each observer, with discrete categories 20-24 years of age, (22 years reference age) 25-29 years of age (27 years reference age), and 30-34 years of age (32 years of age). The age adjustment was performed following the calculations set by the CIE [33]. Within those age groups, lens density does not abruptly change and all subjects had 6/5 or better visual acuity and normal red-green colour vision as assessed by the Ishihara test [34].

Protocol and procedure. After completing giving informed consent, observers were tested for visual acuity and normal colour vision. Sitting down, they then viewed the four video conditions consecutively, with a two-minute break in between each video. The conditions were randomized between participants. All testing took place during regular daytime working hours (9 to 5 pm).

Pupillometry. The participants' pupil responses were measured using a light-weight binocular eye tracking headset (Pupil Labs GmbH, Berlin, Germany), recording pupil responses at 30 Hz, and also a front-facing view of what the subject saw. Using software provided by the manufacturer, 2D ellipses were fit to the resulting videos off-line, and time-locked to the onset of the stimuli using open-source software provided by the manufacturer (https://github.com/pupil-labs/pupil).

Participants. Eleven observers (mean age±SD 24.5±6.5 years, 7 female) were recruited from the wider University of Manchester community. Observers were entered into a raffle to win a hamper. Two participants were authors of this study (A.A. and M.G.).

Data analysis. Raw pupil data were processed using custom MATLAB software as follows. Missing data reported from the 2D fitting algorithm were interpreted as eye blinks; to account

for transient mis-estimations of the pupil size before and after eye lid closure, we removed additional samples around these events (padding window ±5 samples at 30 Hz). These missing samples were then linearly interpolated. Data were them smoothed with a Savitzky-Golay filter (11th order, frame length 21 samples) using MATLAB's built-in sgolayfilt() function. Raw pupil data (expressed in pixels of the 2D ellipse) were mean-normalised across the entire time series and expressed as percent change around that mean. We then extracted the per-cycle average pupil responses, collapsing across all cycles during the entire cartoon. This per-cycle average was then fit, in a least-squares sense, with a sum of sine and cosine at the stimulation frequency (0.25 Hz); the weights of the sine and cosine were then transformed into amplitude and phase of the evoked pupil response.

Ethics. Ethical approval was obtained from the University of Manchester Ethics commission (approval number #2017-2958-43594359).

Code and data availability. All data and code to perform the data analyses reported here can be found at https://github.com/spitschan/Spitschan_2018_JOV.

Results

Photoreceptor-selective modulations embedded in movies produce distinct pupil responses. The 0.25 Hz photoreceptor-specific modulations stimuli evoked distinct responses in all observers (Fig. 2A-D). The strongest response (in amplitude expressed as percent change in pupil diameter relative to the mean) was elicited by the *Light flux* modulations (mean±1SD = 5.0616±1.2554%), followed by *L+M+S* (mean±1SD = 4.1340±0.9263%). This difference is significantly different ($t(10) = -2.6852$, $p = 0.0229$) (Fig. 3A). In many observers we find a small melanopsin-evoked pupil response and in aggregate, there is a significant difference between the *Melanopsin* (mean±1SD = 2.1182±1.3096%) and the *Reference* condition (mean±1SD = 1.0792±0.7652) ($t(10) = 2.6885$, $p = 0.0228$). We note that the melanopsin-evoked pupil response is somewhat inconsistent across observers, pointing to individual differences in the observers' melanopsin sensitivity (Fig. 2B; 3A, B).

Amplitude and phase of pupil responses. Expressed as amplitude and phase, we find that LMS and light flux pupil responses are well above the noise floor defined by the aggregate fit of the pupil response to the stimulus containing no stimulus at the modulation frequency (0.25 Hz). For all observers, the phase of the LMS and light flux response is delayed relative to the stimulus onset (Fig. 2A, C; 3B), which is consistent with the "dead time" of the human pupil response (~200-300 msec). For the melanopsin-evoked pupil response, we find variability in both amplitude and phase (Fig. 2B; 3A, B), which warrants further investigation.

Summation of pupil responses. We examined to what extent the sum of the responses to the *LMS* and *Mel* stimuli resembles the response to the *Light Flux* stimulus, which is, when expressed in contrast, the sum of the *LMS* and *Mel* stimuli in the stimulus domain: The *Light Flux* stimulus modulates the L, M, and S cones in the same way that the LMS modulation does; and it modulates melanopsin in the same way that the *Mel* modulation does. We applied a simple linear model by simply adding the amplitude and phase, expressed as

complex sum, of the *LMS* and *Mel* responses, and reading out the amplitude again (Fig. 4). Conceptually, this is a vector sum model, where the pupil response to each modulation corresponds to a vector (defined by a given phase and amplitude), and we simply add vectors (Fig. 4, inset), yielding a new vector. We find that the amplitude of the complex sum of the *LMS* and *melanopsin* pupil response can account for the *Light Flux* pupil response while the *LMS* response only does so in a limited way (Fig. 4). We note that a better fit of the amplitude of the complex sum already requires that the phase of the two summands is aligned in such a way as to yield a better-fitting amplitude, we did not consider the goodness-of-fit of phase.

Discussion

Summary of results. Pupillary responses to melanopsin-directed stimuli using the method of silent substitution have been documented in the literature [7-16, 26], including by the authors [12, 26]. These studies typically have employed very carefully controlled artificial stimuli. However, past work has not addressed the question of how these photoreceptors each define pupil size during natural viewing. In this study, we find that by embedding photoreceptor-selective modulations in naturalistic movie stimuli, we can elicit pupil responses specific to the receptor type. Consistent with what we would predict from studies employing spatially homogenous stimuli, we find a subtle but significant response to the melanopsin-mediated stimulus at the stimulation frequency (0.25 Hz). The response to the *Light flux* modulation is in amplitude larger than the response to the *LMS* modulation, providing additional evidence that there are melanopsin contributions to early visual processing that can be detected in our paradigm. Further, we find that we can model the pupil response to the *Light flux* modulation as the complex sum of the responses to the *Melanopsin* and the *LMS* modulation, which is corroborated by an earlier finding [12] (see their Fig. S8).

Defining pupil size during free viewing of naturalistic stimuli. By embedding the modulations in a naturalistic "entertainment" movie, we have placed the task in an ecologically-relevant context for using a video display unit (VDU). Allowing the participants to freely view the movie and not adhere to specific fixation procedures gives the paradigm an additional naturalistic aspect to it. While much emphasis has been placed on examining the photoreceptor inputs into pupillary control under well-controlled but relatively reduced stimulus conditions, our approach provides an understanding of these inputs which is *complementary* to more parametric and analytical approaches. Using this pragmatic approach to see if we could modulate the activity of photoreceptors in a task that human participants might engage in, in a normal day-to-day sense, we indeed confirm that all photoreceptors known to be active at photopic light levels (as established in previous studies, e.g. [12, 13]), can be manipulated using modulations embedded in naturalistic movies.

Individual differences. We note that there appear to be large individual differences which warrant further investigation, both in amplitude and phase. For one observer, S07 and also author of this study, pupil modulation induced by the melanopsin stimulus was of opposite phase to most other observers (Fig. 2B). This observer has had extensive experience in viewing melanopsin-directed stimuli or modulations on the projector system. Because of the

distribution of macular pigment in the central region of the retina, Maxwell's spot [35, 36] may be visible for some observers and we hypothesize that this might be the reason for the odd out-of-phase response in this observer. In aggregate, however, we find that these stimuli can drive photoreceptor-specific mechanisms of human pupillary control.

Challenges to silent substitution. The silent substitution nominally allows selective stimulation of a given photoreceptor class. Silent substitution requires, however, a good estimate of the human participants' spectral sensitivities. In practice, these are assumed based on standard cone fundamentals such as the physiologically-relevant CIE cone fundamentals [33]; some investigators also allow for an individual-observer calibration routine (e.g. [9-11, 13, 25]). In this work, for practical and pragmatic reasons, we forwent calibration at the level of the individual observer, and simply accounted for age-dependent differences in prereceptoral filtering, which largely affects the amount of short-wavelength filtering, within a given age bracket spanning 5 years. In our modulations, we did not account for the potential intrusion of rods at daylight levels [21, 22]. We note that if rods were considered to contribute to these types of modulations, they would do so in a similar way for any practical application involving VDUs.

Opportunities for translation. Given the broad impact of light on aspects of human physiology and behavior (such as melatonin suppression, circadian phase-shifting and modulation of alertness), the system and paradigm described here to modulate visual responses in a photoreceptor-selective fashion represents an opportunity to modulate these while people are engaging in everyday tasks such as movie-watching [31]. One powerful further application in the future could be as a method for low-impact assessment of photoreceptor health in clinical contexts, specifically in pediatric and geriatric populations.

Towards a revision of colorimetry? In this study, we have employed a 5-primary projector-based system for silent substitution with spatial control at the pixel level, and controlling the activation of the photoreceptors active at daylight: the LMS cones and melanopsin-containing ipRGCs. Commercially available VDUs have three primaries, which reflects the fact that human colour vision in photopic light-levels is trichromatic. However, this study and a recent one [31] employing a similar system finds that non-visual responses can be manipulated with a modified VDU with more than three primaries. Additionally, there is more and more evidence that melanopsin contributes to "classical" visual functions such as the perception of brightness [24, 37], colour [9, 38], space (humans: [9]; mice: [39]), and other visual attributes (humans: [26], mice: [40-42]). It remains an interesting question to what extent the classical colour matching functions, which are the basis of our current cone fundamentals, reflect the activity of melanopsin (though the amount of rod contributions have been discussed extensively [43]). Given the numerous roles of melanopsin in visual perception and in regulating physiology and behavior, and the emergence of tetrachromatic or higher-order primary displays, we expect that colorimetry may need some revisions to account for these effects, visual or non-visual.

Figures

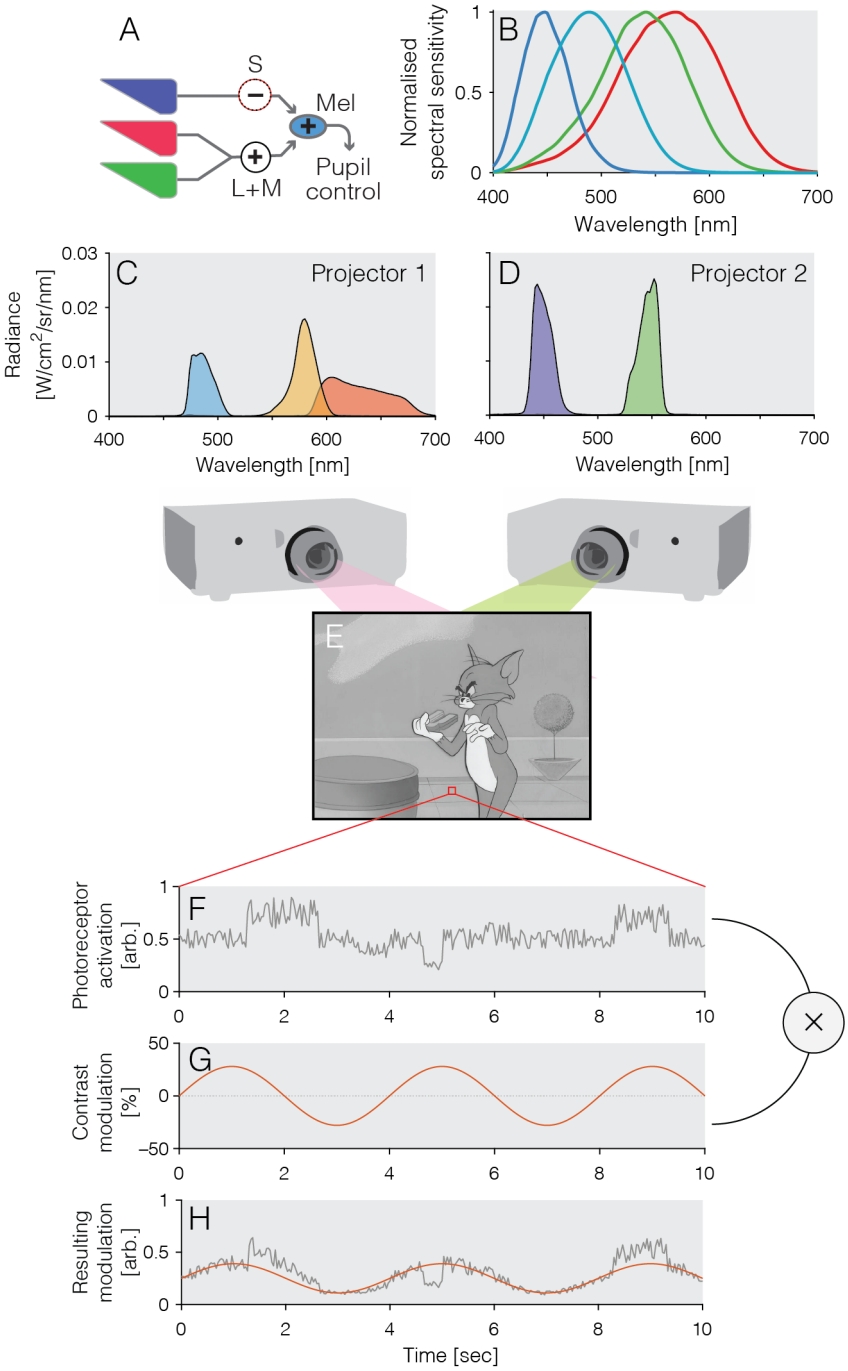

Figure 1. ***Fundamentals and methods***. *A*) Spectral sensitivities of the human photoreceptors; *B*) Schematic circuit diagram of the human pupillary light response, with L+M cones, and melanopsin, providing an excitatory drive to pupil constriction, and S cones yielding an inhibitory input; *C and D*) Spectral radiance of the five primaries for the two projectors; *E*) Sample frame from the Tom & Jerry cartoon; *F*) Sample time course over 10 sec of one pixel of the cartoon, displaying local variations in luminance; *G*) Time course of a pure sinusoidal stimulus (0.25 Hz) stimulating a given photoreceptor class; *H*) The sinusoidal stimulus get

superimposed with the variations in pixel luminance so as to yield an embedded sinusoidal modulation while retaining image contrast.

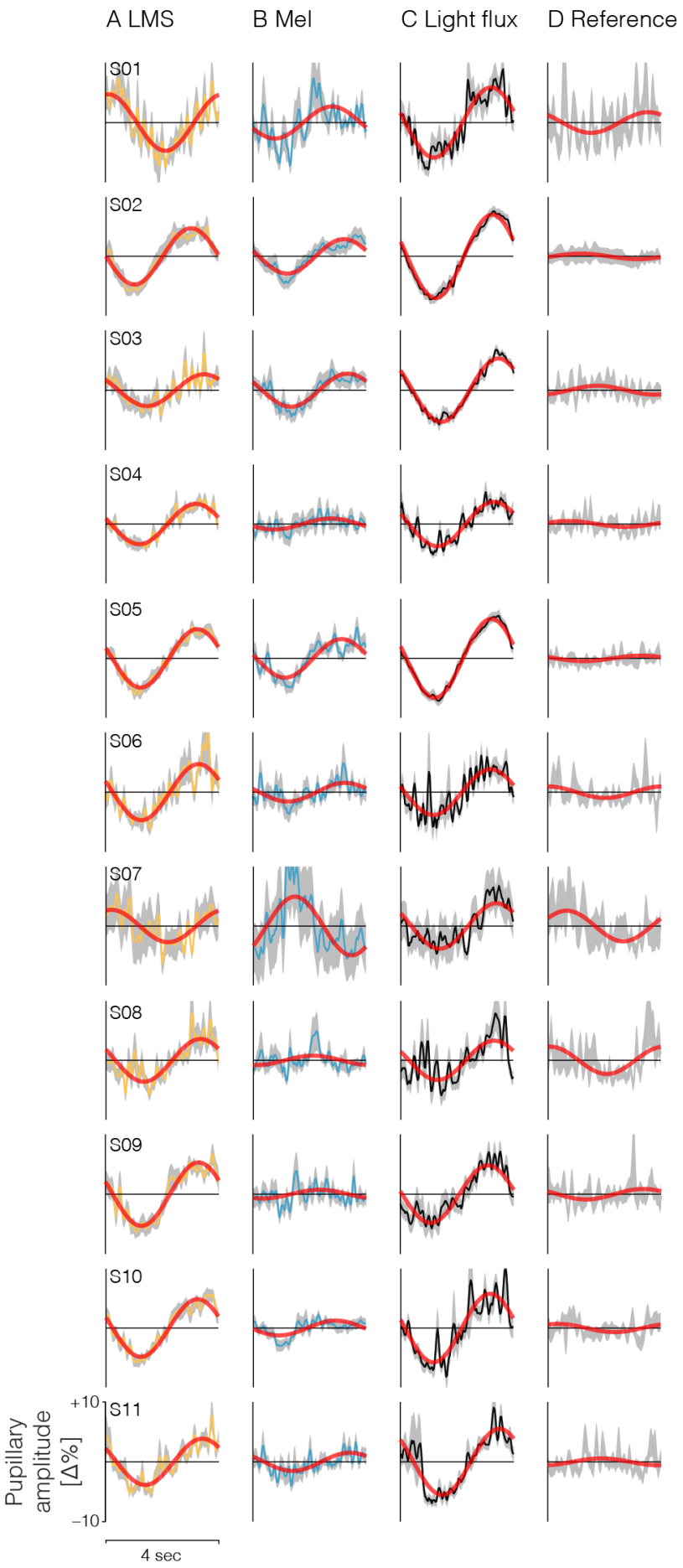
A LMS
B Mel
C Light flux
D Reference
S01
S02
S03
S04
S05
S06
S07
S08
S09
S10
S11
+10
−10
Pupillary amplitude [Δ%]
4 sec

Figure 2. ***Individual subject pupil data***. Data are collapsed (column-wise) across stimulus types. A) L+M+S, B) Melanopsin (*Mel*), C) Light flux (all photoreceptors are modulated), D) no modulation. Red lines indicate the least-squares fit of a sum and cosine at the modulation frequency to cycle-average response.

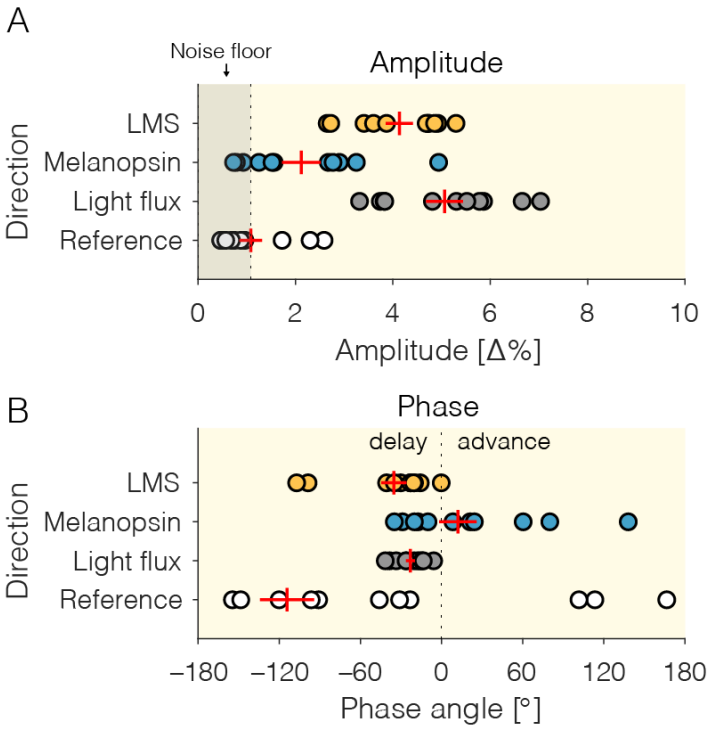

Figure 3. ***Extracted response properties of the pupil responses to photoreceptor-directed modulations embedded in naturalistic movies.*** (*A* – amplitude, *B* – phase). Data shown as red crosses are represent mean±SEM across participants. *A*) Amplitude results. Noise floor is given by the mean amplitude of the fitted pupil response to the *Reference* modulations, which contained no additional power at the stimulation frequency. *B*) Phase data, with negative angles corresponding to a phase delay (pupil response lags behind the stimulus), and positive angles corresponding to a phase advance (pupil response precedes the stimulus). The amplitude and phase data shown here correspond directly to the red fit lines in Fig. 2.

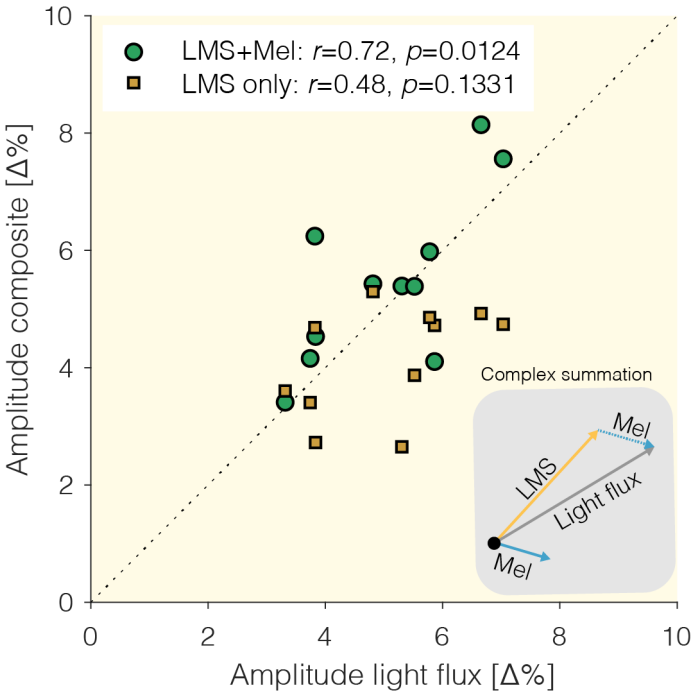

Figure 4. Linear summation model. We compare the amplitude of the pupil response to the *Light flux* modulation to the amplitude of two candidate models: The response to the *LMS* modulation, and the complex sum of the response to the *LMS* modulation and the response to the *Mel* modulation. *Inset*: Visualization of the vector summation model.

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