Melanopsin-Driven Light Adaptation in Mouse Vision

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Summary

Background: In bright light, mammals use a distinct photopigment (melanopsin) to measure irradiance for centrally mediated responses such as circadian entrainment. We aimed to determine whether the information generated by melanopsin is also used by the visual system as a signal for light adaptation. To this end, we compared retinal and thalamic responses to a range of artificial and natural visual stimuli presented using spectral compositions that either approximate the mouse’s experience of natural daylight (“daylight”) or are selectively depleted of wavelengths to which melanopsin is most sensitive (“mel-low”).

Results: We found reproducible and reversible changes in the flash electroretinogram between daylight and mel-low. Simultaneous recording in the dorsal lateral geniculate nucleus (dLGN) revealed that these reflect changes in feature selectivity of visual circuits in both temporal and spatial dimensions. A substantial fraction of units preferred finer spatial patterns in the daylight condition, while the population of direction-sensitive units became tuned to faster motion. The dLGN contained a richer, more reliable encoding of natural scenes in the daylight condition. These effects were absent in mice lacking melanopsin.

Conclusions: The feature selectivity of many neurons in the mouse dLGN is adjusted according to a melanopsin-dependent measure of environmental brightness. These changes originate, at least in part, within the retina. Melanopsin performs a role analogous to a photographer’s light meter, providing an independent measure of irradiance that determines optimal setting for visual circuits.

Introduction

The visual system is charged with encoding visual information across the >10^5-fold change in background light intensity from starlight to cloudless midday. The switch between rod- and cone-based vision and adjustments in photoreceptor sensitivity are central to meeting this challenge. However, the behavior of circuits in the retina is also critical, with multiple examples of visual signals being shifted between parallel pathways with different computational characteristics, and of the behavior of individual elements within these pathways changing as a function of irradiance [1, 2]. Such network changes do not merely adjust sensitivity and avoid saturation, but optimize circuit behavior to ensure efficient extraction of visual information (see [3, 4]).

Effective adaptation relies upon an accurate measure of light intensity. One might expect that adaptation state would be defined by the most accurate available measure of irradiance; under many circumstances, this is provided by a particular class of retinal ganglion cell [5–8]. These intrinsically photosensitive retinal ganglion cells (ipRGCs) have their own melanopsin-dependent phototransduction mechanism [9–11], and employ this, along with extrinsic signals originating with rods and cones, to encode light levels over many decimal orders [6].

The information generated by ipRGCs is exported to the brain where it entrains circadian clocks and sets physiological and behavioral states [12, 13]. The hypothesis that ipRGCs also provide irradiance information to the retina contradicts a standard assumption of retinal function that information flows via ganglion cells to the brain, but not back into the retina. However, there is a growing body of evidence that ipRGCs do not obey this rule. In 2002, it was shown that a diurnal rhythm in an aspect of the human cone electroretinogram (ERG) may be regulated by a photoreceptor with melanopsin-like spectral sensitivity [14]. This was followed by data that revealed ipRGCs make gap-junction connections with neighboring amacrine cells [15, 16] and send axon collaterals to the retinal inner plexiform layer [17]. Meanwhile, there is also physiological evidence that ipRGCs excite dopaminergic amacrine cells, which themselves are influential modulators of retinal circuitry [18].

Establishing whether aspects of network light adaptation really are driven by ipRGCs and what impact (if any) this has on visual function is technically challenging. Historically, the starting point for assigning functions to ipRGCs has been eliminating rod and cone photoreception using genetic or pharmacological approaches [19, 20]. However, such preparations are ill suited to revealing ipRGC influences on conventional vision. Comparing visual responses of wild-type and melanopsin knockout mice could be more informative, but interpreting such data is complicated by evidence that retinal development and retinal circadian rhythms are disrupted in animals lacking melanopsin [21–24].

Here, we therefore set out to explore ipRGC influences on visual responses in animals with an intact retina. Our approach adopts the ideas of metamerism and receptor silent substitution from the field of human psychophysics. In brief, we employed a transgenic mouse (Opn1mwR [25]) in which the spectral sensitivity of cone photoreceptors is substantially shifted compared to that of melanopsin. Using a multispectral light source, we were able to produce background lighting conditions that were equivalent for cones but differed substantially in their effective photon flux for melanopsin. We found substantial differences in retinal and thalamic responses to visual stimuli presented under these two conditions. These differences could be explained, at least in part, by changes in visual feature selectivity of individual units and were associated with alterations in the dorsal lateral geniculate nucleus’ (dLGN’s) ability to encode natural scenes.

Results

Mouse Cone Metamers

Our strategy for determining whether melanopsin modulates vision was to compare responses to the same visual stimuli...
presented against backgrounds appearing equivalent to conventional photoreceptors but differing substantially in effective irradiance for melanopsin. Given the similar spectral sensitivity of mouse medium-wavelength-sensitive (MWS) cone opsin and melanopsin (Figure 1B), to achieve this we employed transgenic mice (Opn1mwR) in which mouse MWS opsin is replaced by red-shifted human LWS cone opsin (red dotted line in B). A three primary LED light source (peak emission at 365, 460, and 600 nm) produced four spectrally distinct stimuli shown in (D) with log10 effective photon fluxes for each photopigment in inset. Spectra 1 and 2 approximated the mouse’s experience of natural light at solar angle +8°, while spectra 3 and 4 were selectively denuded of those wavelengths to which melanopsin is most sensitive. The individual elements of these daylight and mel-low stimulus pairs were designed to be rod and melanopsin isoluminant but to differ in effective irradiance for SWS opsin and LWS opsin. As a result, switching from either spectrum 1 to 2 or spectrum 3 to 4 produced a 58% Michelson contrast step to cones presented against backgrounds differing substantially in effective photon flux for rods and melanopsin. This was validated by measuring ERG responses to 200 repeats of 1 Hz, 50 ms transitions from either spectrum 1 to 2 and back again (daylight) or spectrum 3 to 4 and back (mel-low). Two control ERG measurements were made in response to daylight and mel-low stimuli: (1) in Opn1mwR mice at a moderate intensity (100-fold dimmer than maximum and hence with reduced melanopsin excitation; E and F) and (2) in Opn1mwt1/2;Opn1mwR mice at the maximum intensity (G and H). (E) and (G) show representative traces in daylight (black traces) and mel-low (blue traces); arrow indicates time of flash. Scale bars, 100 ms (x); 40 µV (y). Population response amplitudes are plotted in (F) and (H). Data were compared with paired t tests. In each control condition, responses to daylight and mel-low had equivalent amplitudes (p > 0.05).

From spectra matching this requirement, we chose two pairs (Figure 1D) that could be used to generate a 50 ms “flash” (stimuli 2 and 4) presented against backgrounds differing in melanopsin photon flux (stimuli 1 and 3). These combinations had the following characteristics. (1) The flash should be visible to cones but not rods or melanopsin. We were interested in modulatory rather than direct contributions of melanopsin to flash responses and thus aimed to make the elements of each background and flash combination melanopsin isoluminant. By working at high irradiances, we hoped to minimize rod influences on our recordings. Nevertheless, as a further precaution, we set background and flash elements to be rod isoluminant. (2) The cone experience of the flash stimulus should be equivalent for the two pairs. Setting both backgrounds and both flashes isoluminant for both SWS and LWS cone opsins.
LWS cone opsins across the pairs ensured that the two stimulus conditions were equivalent for each individual cone irrespective of its relative expression of the two pigments [26].

(3) One of the conditions approximates the mouse’s experience of natural daylight. We recorded spectral irradiance profiles in horizontal view over a dusk transition in an urban setting (solar angles from −9° to 30° under clear skies but outside of direct sunlight; Figures 1A and 1B) and modeled the mouse’s experience of these conditions by calculating the effective photon flux for each of the mouse photopigments (Figure 1C). At all positive solar elevations, the effective photon flux (melanopsin, rod opsin, and MWS opsin) was roughly equivalent and ~10 times greater than that for SWS opsin. Our first stimulus pair (spectra 1 and 3) maintained these activity ratios and recreated the mouse’s experience of a solar angle of +8° on our representative day. We therefore refer to this condition as “daylight.” In the other stimulus pair (spectra 2 and 4; termed “mel-low”), the effective photon flux for melanopsin was selectively reduced by 10 times.

As these experiments rely upon the daylight and mel-low conditions appearing equivalent to mouse cones (at least within the resolution of our methods of assaying visual sensitivity), we first undertook control experiments to confirm that this was true (see also Figure S1 available online). Initially, we based these upon electroretinography (although see also Figure 3). As melanopsin is increasingly active at brighter backgrounds, our first control was to show that ERG responses to daylight and mel-low conditions (50 ms transition from “background” to “flash” spectra at 1 Hz) were indistinguishable at a moderate irradiance (Figures 1E and 1F). We next showed that responses to these stimuli were identical in mice lacking melanopsin (Opn4<sup>−/−</sup>; Opn1mw<sup>−/−</sup>) at both moderate (not shown) and high background irradiances (Figures 1G and 1H).

**Melanopsin-Driven Modulation of the Cone Flash ERG**

Having validated daylight and mel-low stimuli, we continued to present them to Opn1mw<sup>−/−</sup> mice at a high, melanopsin-active irradiance. ERG b-wave amplitude was reproducibly enhanced in the mel-low condition (Figure 2B). This change built up gradually over several minutes following transition from mel-low to daylight backgrounds (Figure 2C) and was reversible (Figures 2D and 2E). By changing our spectra to produce flash stimuli representing a range of increases in effective cone photon flux, we described contrast-response relationships under background and mel-low conditions for this flash ERG. Behavioral contrast sensitivity has recently been reported to be impaired in Opn4<sup>−/−</sup> mice [27]; however, we did not find an equivalent effect of dynamic modulations in melanopsin activity. Thus, b-wave amplitude was greater across most contrasts in the mel-low condition, indicating increased response gain, but no change in contrast sensitivity per se (Figures 2F and 2G).

**Melanopsin-Driven Changes in Visual Response Extend to the dLGN**

We next recorded responses of neurons in the dLGN, which allowed us to determine whether these changes were
propagated beyond the retina. Using multichannel electrodes, we recorded responses to daylight and mel-low flash paradigms across the contralateral dLGN (Figure 3A). To determine whether the change in ERG b-wave amplitude had a simple correlate in the dLGN response, we computed the mean change in firing of multiunit activity across the dLGN of each mouse. We found changes in this parameter across the experimental conditions very similar to those observed in the ERG. Thus, flash response amplitude was reduced for daylight versus mel-low in \textit{Opn1mwR} animals at high backgrounds, but not in any of the control conditions (Figures 3B and 3C; Figures S2A–S2C).

To explore the origins of this alteration in global response amplitude, we turned to examining responses at the single-unit level. Of 272 single units isolated from seven mice, 161 responded to the flash with, in all cases, increased firing. Many units showed qualitative differences in response to mel-low and daylight conditions (Figure 3D). In some cases, the temporal profile of stimulus evoked spikes changed (see Figure S3 for analysis of this feature). However, such changes in timing could not explain the change in global response amplitude (Figure 3B), as the predominant peak in firing was no less synchronous across the dLGN population in the daylight than mel-low condition (Figure 3E). Instead, we found that the increase in global response amplitude in mel-low could be attributed to a similar change at the single-unit level, with >75% of units showing greater light-evoked firing in this condition (Figure 3F).
that a predominant origin for this effect might be changes in trial-to-trial reproducibility. Indeed, there was an increase in response reproducibility mel-low (paired t test, p < 0.0001; Figure 3G) that correlated strongly with mean response amplitude (Pearson’s correlation coefficient R² = 0.16, p < 0.0001; Figure 3H). These effects were absent in melanopsin knockout mice (Figures S2D and S2E).

**Changes in Spatial Frequency Preference**

A predominant effect of switching from mel-low to daylight conditions was thus a reduction in trial-to-trial reproducibility. Indeed, there was an increase in response reproducibility mel-low (paired t test, p < 0.0001; Figure 3G) that correlated strongly with mean response amplitude (Pearson’s correlation coefficient R² = 0.16, p < 0.0001; Figure 3H). These effects were absent in melanopsin knockout mice (Figures S2D and S2E).

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orientations). In common with reports for mice dLGN [28, 29], however, we were unable to map robust surround components of the RF (Figure 4C). Because of this, we used an additional approach to determine spatial feature preference and presented contrast inverting gratings over a range of spatial frequencies.

The inverting gratings revealed responses to a wide range of spatial frequencies in both conditions (Figures 4D and 4E). As a population, however, there was a tendency for larger-amplitude responses to finer gratings in the daylight condition. The origin of this effect was revealed by comparing the preferred spatial frequency of cells in mel-low and daylight (Figure 4F). Although many (42/112) cells showed maximal responses to the same spatial frequency in both conditions, there was an overall tendency for cells to prefer higher spatial frequencies in daylight compared to mel-low (Wilcoxon matched-pairs signed rank test, p < 0.05). Notably, those cells switching spatial frequency preference also had the largest reduction in full-field flash response (1.2 versus 7.5 spikes/s in daylight and mel-low, respectively; paired t test, p < 0.001) and accounted for all the change in spatial frequency tuning between conditions (Figure 4G). The most common effect (displayed by 51% of units changing) was a shift from preferring the lowest frequency (0.0175 cycles per degree [cpd]) to 0.035 cpd in the daylight condition. This optimal grating size, which equates to a visual angle of ~14', is smaller than the calculated RF center of these cells, but would be predicted to provide good contrast between the RF center and neighboring points of visual space. Such behavior could be readily explained by strengthening of an inhibitory surround to the RF. In any event, these data show that many dLGN neurons adjust spatial frequency tuning between mel-low and daylight conditions. For a substantial fraction this entails a fundamental realignment from responding most to full-field stimuli to preferring spatial patterns. Together, these changes provide a simple explanation for the surprising reduction in responses to full-field flashes in the daylight condition.

Changes in Temporal Frequency Tuning

We next asked whether the changes in temporal profile of responses to full-field flashes observed upon switching from mel-low to daylight conditions (see Figures 3D and S3) could reflect alterations in temporal frequency preference of dLGN units and therefore examined responses to drifting gratings at different temporal frequencies (spatial frequency fixed at 0.035 cpd over eight directions of motion). More than 80% of cells responding to drifting gratings responded to movements in all directions. Response amplitude increased across all temporal frequencies under the daylight condition (Figure 5A), consistent with the view that responses to stimuli with appropriate spatial structure are improved in this condition (Figure 4). However, there was no difference in temporal frequency tuning under mel-low and daylight spectra. Nor could we discern any relationship between responses to the full-field flash and drifting gratings to explain the changes in the temporal profile of flash responses between these conditions (Figures S3C–S3E).
These drifting gratings did, however, reveal another change in feature selectivity between mel-low and daylight conditions. As previously reported [29], a subset of units preferred movement in a particular direction (direction sensitive; see Experimental Procedures). Under both mel-low and daylight spectra, these direction-sensitive cells accounted for ~16% of the total. For all such cells, the degree of response suppression for motion in the null direction varied according to the temporal frequency of the stimulus (representative examples shown in Figures 5D and 5E). Under the mel-low condition the prevalence of direction sensitivity was highest for the slowest movement (0.2 Hz; corresponding to grating motion of 6°/s), while under daylight this occurred at 1 Hz (29°/s; Figures 5B–5D). This switch to preferring higher frequencies was also apparent in comparisons of direction selectivity (DS) index (Figure 5E). These data therefore indicate that, for at least a subset of direction-sensitive cells, the change in melanopsin effective photon flux drives an alteration in preferred speed of motion.

### Melanopsin-Dependent Changes in Response to Natural Movies

Having described differences in feature preference under daylight versus mel-low spectra using artificial stimuli, we finally set out to determine their implication for the dLGN’s ability to encode naturalistic stimuli. To this end, we projected movies of a natural scene (mice moving around an open arena; Figure 6A) in each condition. This short movie (30 s) was presented repeatedly over a 30 min period, enabling us to identify many units (~30% of all cells) with highly reproducible firing patterns across multiple presentations (see Supplemental Experimental Procedures, Natural Movie Correlation Analyses). We found many instances in which the firing pattern of units from Opn1mwR mice was reliable for multiple presentations of the movie under either mel-low or daylight spectra, but diverged substantially between the two conditions (Figure 6B). These were absent from recordings in Opn4^−/−;Opn1mwR animals (Figure 6E).
Based upon the improvement in trial-to-trial reproducibility of responses to artificial stimuli with appropriate spatial structure under the daylight spectrum (Figure 4C), we expected to see a similar increase in response reliability for the naturalistic movies in this condition. We therefore computed the trial-to-trial correlation for each unit under each condition (“autocorrelation”; see Experimental Procedures), and found that single-unit responses were indeed more reliable enhanced in the daylight condition (Figure 6C). Importantly, this effect was absent in Opn4−/−;Opn1mwR animals (Figure 6F).

A couple of features of the response to artificial stimuli suggest that the visual representation of visual scenes across dLGN neurons could be more diverse (richer) in the daylight condition: changes in spatial frequency tuning (Figure 4) indicate that a subset of dLGN neurons provide finer spatial resolution of visual features; and alterations in the behavior of direction-sensitive units (Figure 5) imply that this aspect of motion is tracked over a wider range of velocities. To determine whether responses across the population were indeed more varied in the daylight condition, we computed the correlation between mean firing response patterns of pairs of units from each animal (“signal correlation”). To limit the risk of sampling bias, we did not restrict our analysis to units with equivalent spatial RFs or related feature selectivity; our only constraint was that the units included had an RF centered within the projection screen and that they responded consistently to at least some aspect of the movie (see Experimental Procedures). As might be predicted, therefore, we found substantial variability in the signal correlation between pairs of units within a single animal. Nevertheless, even in this unfiltered data set there was a significant increase in signal correlation in the mel-low compared to daylight condition in Opn1mwR animals (Figure 6D). This effect was enhanced if one restricted analysis to those pairs with relatively high pairwise correlations in either condition (Pearson’s linear correlation coefficient > 0.25). Once again, Opn4−/−;Opn1mwR animals lacked this change (Figure 6G). We conclude that melanopsin-driven adjustments in the visual response allow the visual code to provide a richer, less redundant representation of natural scenes.

Discussion

Here, we have applied the concepts of metamerism and receptor silent substitution to compare responses to visual stimuli presented under conditions that differ only in their effective photon flux for melanopsin. The first condition (daylight) approximates the mouse’s experience of natural daylight, while in the second condition (mel-low), those wavelengths to which melanopsin is most responsive, were selectively depleted. Switching between daylight and mel-low conditions resulted in substantial alterations in visual responses in the retina and dLGN. These were caused by fine changes in stimulus selectivity in spatial and temporal dimensions at the single-neuron level. Such changes in feature preference were associated with quantitative improvements in the dLGN’s ability to encode natural scenes when presented in the daylight condition.

To reveal the impact of melanopsin on conventional vision, it was necessary to devise a method of selectively modulating the activity of melanopsin in an animal with a fully functional visual system. Our approach adopts the concept of metamerism: that stimuli differing markedly in spectral composition can appear indistinguishable for one or more classes of photoreceptor. In this case, our mel-low and daylight conditions are designed to appear equivalent for cones. One feature of the approach is that it cannot reveal any contribution of ipRGCs to adaptation at lower irradiances when their activity depends upon rods and cones. Our data thus likely underestimate the contribution of ipRGCs to retinal physiology.

While our conclusions do not require that the rate of photon capture by all cones is absolutely identical under mel-low and daylight conditions (a practical impossibility), it is important that they are sufficiently similar as to make any difference in cone response fall below the detection limits of our methods. We are confident that this is the case. We have designed these stimuli based upon extensive measurements of the in vivo spectral sensitivity of mouse cones [25, 30, 31]. Moreover, both ERG and dLGN responses are indistinguishable between mel-low and daylight conditions when working at light levels below those favored by melanopsin and under all conditions in mice lacking melanopsin. Finally, the changes in response properties we observe build up over several minutes of exposure to the new background (Figure 2), consistent with a gradual, melanopsin-dependent adjustment in visual response, but not with a fundamental difference in the cone experience of the two conditions, which should be apparent from the very first presentation of the new stimulus.

What functional advantage could be gained by adjusting vision according to a melanopsin-dependent assessment of irradiance? Light adaptation in visual circuits involves changes in the behavior of individual synapses and the nature and extent of connections between pairs of neurons. One function of this adaptation is to conserve the visual code against changes in irradiance; another is to adjust circuitry to take advantage of irradiance-dependent increases in signal reliability and/or changes in photoreceptor temporal resolution [1, 2, 32–34]. However, these two features are in some ways contradictory. Viewed from the perspective of a circuit element charged with achieving the second of these goals, the fact that light adaptation upstream in the circuit minimizes the impact of changes in background light levels on incoming signals makes it more difficult to accurately assess irradiance. Basing adaptation instead on an independent measure of irradiance not subject to the same light adaptation processes, as shown here for melanopsin, is one solution to this problem. If such a general mechanism were to explain the influence of melanopsin in the mouse retina, one might expect to find independent irradiance codes also in other visual systems. Indeed, this seems to be the case. Thus, in fish and other lower vertebrates, a range of non-rod, non-cone photopigments are expressed widely in inner retinal neurons [35–38]. Meanwhile, in Drosophila, a recent publication reports regulation of vision by cryptochrome, the fly’s version of an irradiance measurement system [39].

The simplest effect we observe is smaller responses to a full-field flash in the daylight condition. This is apparent in the ERG and in the LGN at both population and single-unit levels. Using spatially structured stimuli reveals that this effect represents a widespread shift in spatial frequency tuning. Many dLGN units that respond well to full-field flashes and gratings at the lowest spatial frequencies in mel-low switch to preferring higher spatial frequencies under daylight (~0.035 cpd). Future work will be required to define the changes in visual circuits underlying these effects. There are multiple reports of luminance- and/or irradiance-dependent changes in spatial frequency preference (although generally at lower light levels than...
studied here [1, 2, 40–42]). Where elucidated, mechanisms for such changes involve alterations in the inhibitory surround provided by retinal horizontal and amacrine cells. These cell types therefore seem likely targets of melanopsin control. Inhibitory amacrine cells are also responsible for establishing direction selectivity [43, 44], implicating changes in their activity also as a likely origin of the melanopsin-dependent change in temporal frequency tuning of direction sensitive units that we observe (Figure 5).

While our ERG data reveal that at least some melanopsin-driven changes in visual response originate with the retina, our study does not preclude a contribution of central processing within the brain. These retinal changes could themselves involve feedback via centrifugal histaminergic [45, 46] and/or serotonergic [47] projections. Moreover, thalamic and thalamocortical circuits allow plenty of opportunity for fine-tuning visual responses. It may therefore be that some of the changes in visual feature selectivity we see in the dLG N reflect an impact of melanopsin-based assessments of irradiance within the brain itself. These could extend to melanopsin-driven increases in arousal or attention, although any such effect would likely be specific to vision as responses to another sensory stimulus were indistinguishable under the two lighting conditions (Figure S4).

Our findings add to the growing evidence that the sensory requirements of pRGCs should influence lighting design [48]. Most incandescent and fluorescent lighting is long wavelength biased compared to natural light and thus deficient in those wavelengths to which melanopsin is most sensitive, effectively recapitulating our mel-low condition. While the degree to which melanopsin excitation is reduced in our experimental mel-low condition is probably several times greater than in most artificial lighting, these data do imply that choosing light sources that more closely approximate melanopsin’s experience of daylight may bring qualitative improvements in visual performance.

Experimental Procedures

In Vivo Electrophysiology

Animal care was in accordance with the UK Animals, Scientific Procedures, Act (1986). ERGs and dLG N responses were recorded concurrently from 13 Opn1mw<sup>16</sup> and eight Opn4<sup>14−</sup>;Opn1mw<sup>16</sup> male mice (aged 3–6 months) anesthetized with urethane (1.6 g/kg, 30% w/v; Sigma-Aldrich). dLG N recordings employed a 32-channel probe (A4x8-5mm-50-200-413; Neuronexus). Recording methods as previously reported [49]. In addition, a separate set of mice (six Opn1mw<sup>17</sup> and three Opn4<sup>14−</sup>;Opn1mw<sup>16</sup> males) was used to record responses in the dLG N to spatially structured stimuli.

Visual Stimuli

Full-field visual stimuli were generated using three independently controlled LEDs (β<sub>max</sub> 365, 460, and 600 nm; Cairn Research). LEDs were combined to generate two background and stimulus combinations that are summarized in Figure 1D. The approach is equivalent to that described in [48], using spectral efficiency functions available at http://lucasgroup.lab.ls.manchester.ac.uk/research/measuringmelanopicilluminance. Structured images were presented using a custom-made light source containing four independently controlled LEDs (β<sub>max</sub> 405, 455, 525, and 630 nm; Phlatlight PT-120 Series, Luminus Devices), directed into a digital mirror device projector (DLP LightCommander).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.09.015.

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