Effect of Alzheimer’s Disease on Melanopsin Expression in the Lateral Geniculate Nucleus

Elizabeth Couser, Steven L. Bernstein, Michaela K. Mathews.

Purpose: While retinas from individuals with Alzheimer’s disease (AD) undergo degenerative changes, the effects of AD on the midbrain connecting eye and cortex are understudied. AD patients also experience a loss of melanopsin-containing retinal ganglion cells (mRGCs). We wanted to examine whether AD affects melanopsin expression in the lateral geniculate nucleus (LGN). We hypothesized that since AD affects RGCs, there should be a gradual loss of melanopsin expression as AD progresses. This information is essential in understanding the influence of AD on the central visual pathway.

Methods: Following IRB approval, we obtained human tissue samples from the Maryland Brain & Tissue Bank, which included age-matched normal, pre-clinical AD, and severe AD. We evaluated human LGN for the expression of melanopsin, as well as the number of normal neuron nuclei, using immunohistochemistry and confocal microscopy. Antibodies used were: anti-melanopsin (Thermo Scientific) and Brn3a (AbCam). Slides were analyzed using FluviewSoftware.

Results: Normal LGN tissue from age-matched controls showed abundant melanopsin expression in discrete deposits. In the parvocellular layer, Brn3a expression was abundant in small nuclei that were scattered throughout the section. Magnocellular layers showed many Brn3a(+) nuclei as well. In tissue from the preclinical and severely affected donors, there was a progressive decrease in the BrN3a marker in the LGN through the different stages of AD severity. Interestingly, while there was no reduction in melanopsin signal in the preclinical patients, this protein was drastically decreased in severe AD samples. Melanopsin was also seen to accumulate as smaller deposits in severe AD.

Conclusions: Similar to the retina and cortex, the LGN portion of the thalamic midbrain shows AD-related alterations, including melanopsin projections. AD also affects the number of LGN-Brn3a(+) neurons. The LGN-melanopsin signal appears to be preserved in pre-clinical AD. Since we previously showed that the LGN exhibits a progressive increase in inflammatory markers in AD, our current data supports a model whereby selective AD-related changes occur pre-clinically, but a threshold damage level is needed to affect melanopsin expression in the tissue samples. The impact of AD on the LGN including the progressive loss of magno and parvo neurons and melanopsin may further assist in explaining the visual deficits in early AD.

Commercial Relationships: Elizabeth Couser, None; Steven L. Bernstein, None; Michaela K. Mathews, None

Chemogenetic manipulation of ipRGCs reveals a primary role for this ganglion cell class in the visual systems ability to track slow changes in background light intensity

Nina Milosavljevic, Riccardo Storchi, Franck P. Martial, Robert J. Lucas. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Purpose: Melanopsin expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) are thought to provide the retina’s ability to measure background light intensity (irradiance). In order to determine the extent to which this is true for the sorts of gradual but high amplitude changes in irradiance we experience every day, we developed a new chemogenetic method of specifically and acutely...
Melatonin modulates M4-type intrinsically photosensitive retinal ganglion cells (ipRGCs)


Purpose: In the retina, the hormone melatonin is secreted at night by photoreceptors and serves as a dark-adaptive signal. Melatonin receptors have been found in many types of retinal neurons including ipRGCs (Sengupta et al. PLoS One 2011), suggesting it may modulate the physiology of ipRGCs. Here, we tested this possibility.

Methods: Eyecups were harvested from dark-adapted Sprague Dawley rats and superfused by Ames' medium. Whole-cell current clamp recording was made from M4-type ipRGCs which could be identified by their enormous somas. All stimuli were full-field 480nm light. In the experiments examining rod/cone-driven photoreponses, low-intensity stimuli too dim to activate melanopsin were used. In the experiments that studied melanopsin-based light responses, rod/cone input was blocked using the glutamate analogs L-AP4, DNQX and D-AP5, and stimulus intensities suprathreshold for melanopsin activation were used.

Results: The rod/cone-driven light response of M4 cells consisted of a rapid, sustained depolarization accompanied by an increase in spike rate. Bath application of melatonin (10 nM) during subjective day increased both the duration and amplitude of this depolarization. Paradoxically, it attenuated the light-evoked increase in spiking. These effects persisted when melatonin was added in the presence of dopamine receptor antagonists (5 μM SCH23390 + 5 μM spiperone) but were abolished when it was applied in the presence of the melatonin receptor antagonist luzindole (2 μM). Luzindole added to normal Ames during subjective night had the opposite effects on rod/cone-cone-driven photoresponses: the duration of the depolarization was reduced while the spiking increase was enhanced. It also decreased the amplitude of the depolarization although this effect was not statistically significant (p=0.175). In the presence of rod/cone-signaling blockers, M4 cells generated sluggish, melanopsin-based depolarizing light responses, which were not affected by the addition of melatonin.

Conclusions: Endogenous melatonin modulates the rod/cone-mediated photoresponses of M4 cells. Melatonin is known to inhibit dopamine release from amacrine cells (Dubocovich Nature 1983) although such inhibition is not required for the effects observed here. Melatonin does not appear to act directly on M4 cells because its effects were abolished by rod/cone blockade. Instead, it probably acts on M4 cells’ presynaptic circuits.
**Melaonopin expressing intrinsically-photosensitive retinal ganglion cells (ipRGCs) project to many targets of the CNS, influencing both image forming and non-image forming portions of the visual system. Recent anatomical and electrophysiological studies have brought to light intra-retinal connectivity of ipRGCs with amacrine cells raising the possibility that ipRGCs could influence the activity of multiple ganglion cell types. Here, we characterise how widely ipRGCs drive light-dependent changes in the firing of retinal ganglion cells.**

**Methods:** Visual responses of RGCs were recorded *in vitro* in 5 isolated retinas of mice both lacking cone activity (Cgna3−/−) and displaying rod degeneration (rd1 mutation). Signals were acquired using a 256 channel multi-electrode array system (MEA) with electrodes spaced 200 μm apart, allowing a widespread and retinotopic sampling of the RGC population. Full field light stimuli were delivered to the ganglion cell layer (GCL) from below by a 470 nm LED. Stimulation epochs consisted of a 10 second light step followed by 120s of darkness.

**Results:** We found that full field light pulses induced changes in firing across a much wider fraction of RGCs than a series of spatially restricted stimuli covering the same area. Responses to the full field stimulus fell into two populations that differed in their reproducibility over multiple repeats. Decaying responses accounted for 48% of all recorded RGCs (n=615/1283) and responded robustly only for the first two stimulus presentations in any recording epoch. Robust responses accounted for 16% (n = 211/1283) of recorded RGCs and kept responding throughout all repeats of the stimulus. No light responses were observed in melanopsin knockout models (rd1;OPN4−/−; n = 6 retinas).

**Conclusions:** We recorded melanopsin driven responses in far more RGCs than the estimated <10% represented by ipRGCs. The extinction properties of the decaying response-type suggest that these may be indirect responses recorded in adjacent RGCs, which do not express any photopigment.

---

**Commercial Relationships:** Cyril G. Eleftheriou, None; Franck P. Martial, None; Robert J. Lucas, None

**Support:** ERC MeloVision grant

---

**Melanopsin expressing intrinsically-photosensitive retinal ganglion cells (ipRGCs) project to many targets of the CNS, influencing both image forming and non-image forming portions of the visual system. Recent anatomical and electrophysiological studies have brought to light intra-retinal connectivity of ipRGCs with amacrine cells raising the possibility that ipRGCs could influence the activity of multiple ganglion cell types. Here, we characterise how widely ipRGCs drive light-dependent changes in the firing of retinal ganglion cells.**

**Methods:** Visual responses of RGCs were recorded *in vitro* in 5 isolated retinas of mice both lacking cone activity (Cgna3−/−) and displaying rod degeneration (rd1 mutation). Signals were acquired using a 256 channel multi-electrode array system (MEA) with electrodes spaced 200 μm apart, allowing a widespread and retinotopic sampling of the RGC population. Full field light stimuli were delivered to the ganglion cell layer (GCL) from below by a 470 nm LED. Stimulation epochs consisted of a 10 second light step followed by 120s of darkness.

**Results:** We found that full field light pulses induced changes in firing across a much wider fraction of RGCs than a series of spatially restricted stimuli covering the same area. Responses to the full field stimulus fell into two populations that differed in their reproducibility over multiple repeats. Decaying responses accounted for 48% of all recorded RGCs (n=615/1283) and responded robustly only for the first two stimulus presentations in any recording epoch. Robust responses accounted for 16% (n = 211/1283) of recorded RGCs and kept responding throughout all repeats of the stimulus. No light responses were observed in melanopsin knockout models (rd1;OPN4−/−; n = 6 retinas).

**Conclusions:** We recorded melanopsin driven responses in far more RGCs than the estimated <10% represented by ipRGCs. The extinction properties of the decaying response-type suggest that these may be indirect responses recorded in adjacent RGCs, which do not express any photopigment.
the Hoxd10-GFP line labeling ON and ON-OFF RGCs that innervate the accessory optic system (Dhande et al. J. Neurosci. 2013). Dark-adapted retinas were superfused with Ames’ medium. GFP+ cells were targeted using a multiphoton laser for whole-cell recording. All stimuli were receptive field center-selective white spots 200 μm in diameter and 10 sec in duration, with intensities too low to activate melanopsin.

**Results:** In darkness, prolonged depolarizing current injection induced equally sustained depolarization and spiking in all cell types, whereas bath application of the group III metabotropic glutamate receptor (mGluR) antagonist CPPG depolarized ipRGCs substantially more than the other RGCs. In response to the light spots, ipRGCs depolarized throughout the 10 sec whereas TRHR and Hoxd10 cells seldom depolarized for more than several seconds. The mGluR7 antagonist MMPIP made the photoresponses of ipRGCs and ON Hoxd10 cells more transient but did not affect TRHR or ON-OFF Hoxd10 cells. GABA and glycine receptor antagonists enabled TRHR and Hoxd10 cells to depolarize for the duration of the 10-sec light but only slightly enhanced ipRGC photoresponses. When RGCs were voltage-clamped at E \(_{\text{F}}\) to isolate bipolar-driven light responses, the GABA/glycine antagonists dramatically prolonged ON-OFF Hoxd10 cells’ light-evoked currents but affected the other cells minimally. Adding AMPA/kainate desensitization inhibitors on top of the GABA/glycine antagonists had no effect on any of the cells tested.

**Conclusions:** The CPPG result suggests that ipRGCs are postsynaptic from sustained bipolar cells whereas TRHR and Hoxd10 cells get input from transient bipolars (Awatramani & Slaughter J. Neurosci. 2000). mGluR7 signaling and the relative lack of amacrine-driven inhibition enhance the sustained nature of ipRGC photoresponses. By contrast, AMPA/kainate receptor desensitization and intrinsic membrane properties do not shape photoresponse kinetics.

**Commercial Relationships:** Xiwu Zhao, None; Kwoon Y. Wong, None

**Support:** Research to Prevent Blindness Scientific Career Development Award, NIH Grants EY023660 and EY007003

**Program Number:** 5569 Poster Board Number: B0140

**Presentation Time:** 8:30 AM–10:15 AM

**Assessment of rod, cone, and intrinsically photosensitive retinal ganglion cell (ipRGC) contribution to the canine chromatic pupillary response**

**Connie Yeh**1, 2, **Kristin L. Koehl**1, **Christine Harman**1, **Simone Iwabe**1, **Jose Guzman**1, **Simon M. Petersen-Jones**1, **Andras M. Komaromy**1, 2

1 Small Animal Clinical Sciences, Michigan State University, East Lansing, MI; 2 Clinical Studies, University of Pennsylvania, Philadelphia, PA

**Purpose:** The objective of this study was to develop a chromatic pupillometry protocol for specific functional assessment of canine rods, cones, and ipRGCs by evaluating both normal dogs and dogs with well-defined retinal and optic nerve (ON) disease phenotypes.

**Methods:** Thirty five dogs with different stages of primary loss of rod- (mutations in CNGB1, PDE6A, PDE6B, RD3), cone- (CNGB3 – achromatopsia), combined rod/cone- (RPE65, STK38L, IQCB1, RPGR), and optic nerve function (ON head coloboma) were tested and compared to normal animals (n=5). Chromatic pupillometry was performed bilaterally under isoflurane general anesthesia using a Q450 Ganzfeld stimulator (Roland Consult). This system also contained a computerized pupillometer to record the movement of the pupil. After 20 min of dark adaptation, the eyes were stimulated with a 1-sec dim (1 cd/m\(^2\)) and bright (400 cd/m\(^2\)) blue light stimuli (470 nm). Following 5 min of light adaptation to a blue background (480 nm, 25 cd/m\(^2\)) the eyes were stimulated with a 1-sec bright red (640 nm, 400 cd/m\(^2\)) light.

**Results:** The median constriction amplitude (%) induced by the dim blue, bright blue, and bright red light stimuli in normal dogs were 18% (range: 6.7-41.2%), 58.9% (23.8-67.6%), and 18.6% (9.3-32.2%) respectively. In dogs with loss of rod function, the pupillary response to dim blue light stimulus was absent, followed by a decrease in bright red light response with retinal disease progression. In dogs with achromatopsia, an absent pupil response to bright red light stimulus but well-preserved response to blue light stimulus was observed. Except for the animal with ON disease, in which all pupillary responses were absent, the melanopsin response was maintained in all dogs, even when rod- and cone-mediated retinal function was not detectable. Characteristic for ipRGC function, the melanopsin response was sustained for several minutes (median: 7 min 36 sec, range: 50 sec-17 min 46 sec) after the offset of the bright blue light stimulus.

**Conclusions:** Pupil responses elicited by light stimuli of different color and intensity allow functional assessment of canine rods, cones and ipRGCs. Chromatic pupillometry offers an effective diagnostic tool for retinal and ON diseases in both clinical and research settings.

**Commercial Relationships:** Connie Yeh, None; Kristin L. Koehl, None; Christine Harman, None; Simone Iwabe, None; Jose Guzman, None; Simon M. Petersen-Jones, None; Andras M. Komaromy, None

**Support:** NIH Grants EY06855, EY017549, EY019304, FFB, MSU-CVM Endowed Research Funds, Myers-Dunlap Endowment for Canine Health

**Program Number:** 5570 Poster Board Number: B0141

**Presentation Time:** 8:30 AM–10:15 AM

**Effect of blue light cut-off filter on the electroretinogram from intrinsically photosensitive retinal ganglion cells**

Manami Kuzu1, 2, Hisashi Matsubara2, Masahiko Ayaki3, Mineo Kondo4, Kazuo Tsutota1, Takeshi Morita4

1 Ophthalmology, NHO Mie Chuo Medical Center, Tsu, Japan; 2 Dept of Ophthalmology, Mie Univ Grad School of Medicine, Tsu, Japan; 3 Dept of Ophthalmology, Keio Univ, Tokyo, Japan; 4 Dept of Living and Environmental Science, Fukuoka Women’s Univ, Fukuoka, Japan

**Purpose:** We have previously succeeded in recording melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) response to light stimuli from human eyes using four-primary illumination system, which modulates stimulus levels to the ipRGC and cones independently (Fukuda et al. 2010; 2012). The aim of this study was to attest effect of the blue light cut-off filter, which can selectively remove the wavelengths including the peak spectrum sensitivity of melanopsin on the electroretinogram (ERG) obtained from ipRGCs using our system.

**Methods:** We used the four-primary illumination system to stimulate ipRGCs independently of other photoreceptors using four kinds of light-emitting diodes (LEDs) and the filter (380-495nm, cut-off 50%) to cut-off blue light in this study. The peak wavelengths of the four LEDs 633 nm, 593 nm, 507 nm and 468 nm and the light stimulus with sinusoidal wave modulation was used. Six subjects (age, 30.2±2.6 years) were recruited. Power and phase spectra of ipRGC responses were calculated by using Fast Fourier Transformation (FFT). The power indicates degree and the phase expresses latency of the response.

**Results:** The power (mV rms) without and with filter was 1.4±0.7 and 0.77±0.37, respectively. The phase (degree) without and with filter was 217.5±92 and 262.2±62.2, respectively. The reduction of power with filter was 41.8±21.6% and the phase did not show differences.
homoscedasticity, there were significant differences between with and without filter (<0.05).

**Conclusions:** The effect of blue light cut-off filter on electrical response from ipRGC showed not only the reduction of the power and also the alteration in the phase as well. This results validate the blue light cut-off filter influence on the electrical response of ipRGC in human.

**Commercial Relationships:** Manami Kuzu, None; Hisashi Matsubara, None; Masahiko Ayaki, None; Mineo Kondo, None; Kazuo Tsubota, None; Takeshi Morita, None

---

**Program Number:** 5571 **Poster Board Number:** B0142  **Presentation Time:** 8:30 AM–10:15 AM  **Seasonal variation in the response of intrinsically photosensitive retinal ganglion cells**  
Adam Elias Brondsted1, 2, Birgit A. Sander1, Henrik Lund-Andersen1, 2, 1Dept. of Ophthalmology, Glostrup Hospital, Glostrup, Denmark; 2Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

**Purpose:** Intrinsically photosensitive retinal ganglion cells (ipRGCs) are responsible for photoentrainment of the circadian rhythm and form the afferent limb in the pupil light response. The ipRGCs are sensitive to blue light and exhibit circadian variation in the response to blue light. Seasonal rhythms in mammals are controlled by the circadian rhythm and hence, seasonal variation in the light response of the ipRGCs may exist. The purpose of this study was to investigate the effect of seasonal change on the ipRGCs measured by blue light pupillometry. As the ipRGC’s are known to respond with latency and a sustained activity, we quantified the post illumination pupil response (PIPR) as a surrogate marker of ipRGC stimulation.

**Methods:** Twenty five healthy participants with a mean age of 44 years (range, 23 – 69) were recruited for the study and examined at baseline (summer) and at 3 control visits until next summer. Consensual normalized pupil responses to bright blue light (470 nm, 300cd/m²) were recorded and the mean pupil constriction was calculated from 10-30 s after stimulus offset. Maximal and sustained pupil contraction was also calculated. Control recordings of red light were also obtained. The post-illumination pupil response (PIPR) was recorded for 6 s. The 6 s PIPR and maximum pupil constriction were expressed as percentage baseline (Mean ± SD) and the mean difference (± SD) in phase amplitude percentage (PAP) between blue and red stimuli was calculated.

**Results:** The blue PIPR was significantly less sustained (p<0.01) in the early AMD group (75.49 ± 7.88%) than the control group (58.28 ± 9.05%). The red PIPR was not significantly different (p>0.05) between the early AMD (84.79 ± 4.03%) and control groups (82.01 ± 5.86%). Maximum constriction amplitude in the early AMD group for blue (43.67 ± 6.35%) and red (48.64 ± 6.49%) stimuli were not significantly different to the control group for blue (39.94 ± 3.66%) and red (44.98 ± 3.15%) stimuli (p>0.05). The PAP difference in the early AMD group (4.49 ± 1.85%) was lower than the control group (6.53 ± 3.49%).

**Conclusions:** These results are suggestive of inner retinal mRGC deficits in early AMD. This non-invasive, objective measure of pupil responses may provide a new method for quantifying mRGC function and monitoring AMD progression.

**Commercial Relationships:** Michelle L. Maynard, None; Andrew J. Zele, None; Beatrix K. Feigl, None

**Support:** ARC-DP140100333

---

**Program Number:** 5572 **Poster Board Number:** B0143  **Presentation Time:** 8:30 AM–10:15 AM  **Melanopsin ganglion cell function in early age-related macular degeneration**  
Michelle L. Maynard1, Andrew J. Zele1, Beatrix K. Feigl1, 1Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia; 2Queensland Eye Institute, Brisbane, QLD, Australia.

**Purpose:** Melanopsin-expressing retinal ganglion cells (mRGCs) have non-image forming functions including mediation of the pupil light reflex (PLR). There is limited knowledge about mRGC function in retinal disease. Initial retinal changes in age-related macular degeneration (AMD) occur in the paracentral region where mRGCs have their highest distribution, making them vulnerable during disease onset. In this cross-sectional clinical study, we measured the PLR to determine if mRGC function is altered in early stages of macular degeneration.

**Methods:** Pupil responses were measured in 8 early AMD patients (AREDS 2001 classification; mean age 72.6 ± 7.2 years, 5M and 3F) and 12 healthy control participants (mean age 66.6 ± 6.1 years, 8M and 4F) using a custom-built Maxwellian-view pupillometer. Stimuli were 0.5 Hz sinewaves (10 s duration, 35.6° diameter) of short wavelength light (464nm, blue; red; blue; retinal irradiance = 14.5 log quanta.cm⁻².s⁻¹) to produce high melanopsin excitation and of long wavelength light (638nm, red; retinal irradiance = 14.9 log quanta.cm⁻².s⁻¹), to bias activation to outer retina and provide a control. Baseline pupil diameter was determined during a 10 s pre-stimulus period. The post illumination pupil response (PIPR) was recorded for 40 s. The 6 s PIPR and maximum pupil constriction were expressed as percentage baseline (Mean ± SD) and the mean difference (± SD) in phase amplitude percentage (PAP) between blue and red stimuli was calculated.

**Results:** The blue PIPR was significantly less sustained (p<0.01) in the early AMD group (75.49 ± 7.88%) than the control group (58.28 ± 9.05%). The red PIPR was not significantly different (p>0.05) between the early AMD (84.79 ± 4.03%) and control groups (82.01 ± 5.86%). Maximum constriction amplitude in the early AMD group for blue (43.67 ± 6.35%) and red (48.64 ± 6.49%) stimuli were not significantly different to the control group for blue (39.94 ± 3.66%) and red (44.98 ± 3.15%) stimuli (p>0.05). The PAP difference in the early AMD group (4.49 ± 1.85%) was lower than the control group (6.53 ± 3.49%).

**Conclusions:** These results are suggestive of inner retinal mRGC deficits in early AMD. This non-invasive, objective measure of pupil responses may provide a new method for quantifying mRGC function and monitoring AMD progression.

**Commercial Relationships:** Michelle L. Maynard, None; Andrew J. Zele, None; Beatrix K. Feigl, None

**Support:** ARVO 2015 Annual Meeting Abstracts

---

**Program Number:** 5573 **Poster Board Number:** B0144  **Presentation Time:** 8:30 AM–10:15 AM  **The post-illumination pupil response of melanopsin expressing retinal ganglion cells**  
Prakash Adhikari1, Andrew J. Zele1, Beatrix K. Feigl1, 1Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia; 2Queensland Eye Institute, Brisbane, QLD, Australia.

**Purpose:** The post-illumination pupil response (PIPR) has been quantified in the literature by four metrics. The spectral sensitivity of only one metric is known and this study quantifies the other three. To optimize the measurement of the PIPR in humans, we also determine the stimulus protocol producing the largest PIPR, the duration of the PIPR, and the metric(s) with the lowest coefficient of variation.

**Methods:** The consensual pupil light reflex (PLR) was measured with a Maxwellian view pupillometer (35.6° diameter stimulus). Experiment 1: Spectral sensitivity of four PIPR metrics [plateau, 6 s, area under curve (AUC) early and late recovery] was determined from a criterion PIPR (n = 2 participants) to a 1 s pulse at five wavelengths (409-592 nm) and fitted with Vitamin A nomogram (max

©2015, Copyright by the Association for Research in Vision and Ophthalmology, Inc., all rights reserved. Go to iovs.org to access the version of record. For permission to reproduce any abstract, contact the ARVO Office at pubs@arvo.org.
in the plasma membrane and produces an intracellular calcium increase upon light exposure, similar to WT Opn4. Pupil constriction in Opn4S310A (Mean IC50=13.55, SD=0.25, N=5) is significantly attenuated at 550nm compared to Opn4WT controls (Mean IC50=13.05, SD=0.12, N=4) compared using a two-tailed Student’s t-test, t(7) = -3.59, p = 0.009. This IC50 difference is consistent with a 14nm hypsochromic shift in the spectral sensitivity of S310A Opn4.

Conclusions: We have identified a putative spectral tuning mutant, S310A Opn4. This forms a functional photopigment, capable of coupling to a Gq/11 signalling pathway. Pupillometry data suggests S310A Opn4 may be spectrally shifted to shorter wavelengths, consistent with evidence that mutation of the equivalent position in other opsins causes hypsochromic shifts in spectral tuning.

Commercial Relationships: Jessica Rodgers, None; Steven Hughes, None; Carina A. Potheecary, None; Laurence A. Brown, None; Michael Parsons, None; Patrick Nolan, None; Stuart N. Peirson, None; Mark W. Hankins, None

Support: EPSRC, Wellcome Trust, MRC