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Human S-cone ERGs Obtained by Silent Substitution Stimulation

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We used triple silent substitution stimuli to characterize human S-cone ERGs in normal trichromats. S-cone ERGs were found to have different morphological features and temporal frequency response characteristics compared to ERGs derived from L-cone, M-cone and rod photoreceptors in normal participants. Furthermore, in two cases of retinal pathology, Blue Cone Monochromatism (BCM) and Enhanced S-cone Syndrome (ESCS), S-cone ERGs elicited by our stimuli were preserved and enhanced, respectively. The results from both normal and pathological retinæ demonstrate that triple silent substitution stimuli can be used to generate ERGs that provide an assay of human S-cone function.

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1. INTRODUCTION

The human electroretinogram (ERG) is a response which provides a measure of the global electrophysiological activity of the retina in response to light stimulation. Responses to diffuse flashes of light of the kind typically used in the clinical assessment of human ERGs [1] contain contributions from all the main classes of cone (long- (L), middle- (M) and short-wavelength (S) sensitive) and rod photoreceptors. Whilst such stimuli have undoubtedly proven to be useful in assessing retinal function in a global, non-selective manner, there has also been a great deal of interest in attempting to record ERGs that reflect the activity of individual photoreceptor populations.

The ERG that arises from isolated S-cone photoreceptors has been of particular interest. From a clinical perspective, this is driven primarily by the suggestion that S-cones are more vulnerable to damage in congenital and acquired retinal, as well as systemic, pathologies [2-8]. For example, several studies have demonstrated selective or more severe changes in S-cone mediated ERGs, compared to L- and M-cone responses, in certain forms of retinitis pigmentosa [9], type 1 and type 2 diabetes [6,7], glaucoma [10] and ocular hypertension [11,12]. Interest in isolating responses from S-cones has also been driven by the fact that the S-cone system forms part of a visual pathway that has several distinctive properties that set it apart from vision mediated by L- and M-cones [13]. For example, S-cones have different evolutionary origins to L- and M-cones [2,14]. The gene for the S-cone opsin is located on chromosome 7, rather than on the X chromosome, as it is for the L- and M-cone opsins [15]. S-cones also have distinctive anatomical [16-20] and functional [21] properties compared to L- and M-cones.

In the light of the special status of S-cone mediated vision, numerous attempts, adopting different methodologies, have been made at

isolating ERGs that reflect their operation. In some studies, S-cone isolation was achieved via chromatic adaptation [22-28]. This technique relies upon the use of a short wavelength incremental flash stimulus, superimposed on a high luminance broadband or longer wavelength background to which L- cones, M-cones and rods are adapted. In other studies, silent substitution techniques [29] were employed to isolate responses from S-cones [7,9,28,30-32]. The isolation of S-cone activity via silent substitution requires alternation between two stimuli that contain mixtures of wavelengths at different intensities which elicit no overall change in excitation in the L-cones, M-cones and rods, but do elicit changes in S-cone excitation. The isolation of 1 out of n classes of photoreceptor requires a minimum of n primaries tuned to different wavelengths. Therefore, to isolate human S-cones, a triple silent substitution stimulus is needed which necessitates generation by a four-primary system. Although two previous studies have used double silent substitution combined with sufficiently high background luminances to suppress the rods [31,32], none have, as yet, recorded an isolated S-cone ERG using triple silent substitution.

The aim of this study was to generate human S-cone ERGs using triple silent substitution stimuli generated on a four primary ganzfeld stimulator. Firstly, we wanted to characterize the morphology of the S-cone ERG in normal trichromats and compare it to the waveforms generated by L-cone, M-cone and rod isolating stimuli. Secondly, we wanted to examine how the morphology of the S-cone ERG is affected in two kinds of hereditary retinal pathology; blue cone monochromatism (BCM) and enhanced S-cone syndrome (ESCS). Both pathologies have relevance to S-cone mediated vision. BCM is an X-linked congenital cone dysfunction syndrome caused by L- and M-cone opsin gene array mutations which result in non-functional photopigments. This leads to an absence of L- and M-cone function in

affected individuals who are left with only preserved S-cone and rod function [33-38]. As a result, color discrimination is severely impaired in subjects with BCM, but there is some preservation of tritan discrimination [37,39]. ESCS is a rare genetic disease associated with an increase in the number and sensitivity of S-cones within the retina [40-44]. The ERG in individuals with ESCS is dominated by the S-cone response with reduced contribution from the L- and M-cones [40,42,45]. By comparing the responses elicited from patients with these pathologies with those from normal trichromats, we wanted to verify whether the S-cone ERGs generated by our triple silent substitution technique can provide responses that selectively reflect S-cone mediated visual function in the human retina.

2. METHODS

A. Stimuli

Photoreceptor isolating stimuli were presented on a ColorDome (Diagnosys LLC, Lowell, MA, USA) four primary ganzfeld stimulator. The four LEDs had the following peak wavelengths: blue (460nm \pm 15 nm (half-bandwidth at half height)), green (514 nm \pm 20 nm), amber (590 nm \pm 8 nm) and red (635 nm \pm 10 nm)). The spectral characteristics, chromaticities and luminances of each class of LED were calibrated using a PR650 spectrophotometer (Photo Research Inc., Chatsworth, CA, USA). The stimuli used in these experiments comprised triple silent substitutions whereby responses from rods, L-, M- or S-cone photoreceptor populations were obtained in isolation using temporal modulations of color and luminance of the four LEDs [29,46]. Stimulus contrast (i.e. photoreceptor modulation) was defined as the Michelson contrast (equation 1) of rod or cone excitation (E) and was set at 0.25 for all stimuli:

$$\text{Contrast} = (\text{Emax} - \text{Emin}) / (\text{Emax} + \text{Emin}) \quad (1)$$

To create silent substitution stimuli, photoreceptor excitations were calculated by multiplying the emission spectra of the LEDs with cone fundamentals and the V_{λ}^{10} function [47,48] and integrating over a range of wavelengths [see: ref 49, for a fuller description of stimulus generation].

Two forms of temporal stimulation were used in this study; transient and steady-state. For the former, the luminance of the LEDs was modulated with a square-wave temporal profile (250 ms on, 250 ms off) to generate L-cone, M-cone, S-cone and rod isolating stimuli (see Figure 1). For the steady-state stimuli, the luminance of the four LEDs was modulated with sinusoidal profiles ranging from 5-70Hz. These stimuli allowed assessment of the temporal frequency response characteristics of the photoreceptor isolated ERGs. The modulation of photoreceptor excitation was kept constant at 0.25 for all stimuli. The retinal illuminance produced by each of the cone isolating stimuli was 8,000 photopic trolands (phot Td). The retinal illuminance of the rod isolating stimuli was 63 phot Td.

B. ERG Recording

ERGs were recorded from the right eye using a silver/nylon corneal fibre electrode (Dept. of Physics and Clinical Engineering, Royal Liverpool University Hospital, UK) referenced to a 9mm Ag/AgCl electrode (Biosense Medical, Chelmsford, UK) on the outer canthus; a similar electrode was affixed to the forehead to serve as ground. Impedance was maintained below 5 k Ω . Signals were recorded using the Espion E² system (Diagnosys LLC, Lowell, MA, USA) which amplified and filtered (bandwidth = 1 to 300 Hz) the ERGs and digitized them at a rate of 1000Hz. Retinal responses were acquired over 500 ms epochs with each response being composed of a

minimum of 256 repetitions. Participants viewed the stimuli monocularly with a dilated pupil (1% Tropicamide) and both a chin- and head-rest were used. Fixation was maintained on a central point which subtended approximately 0.5^o.

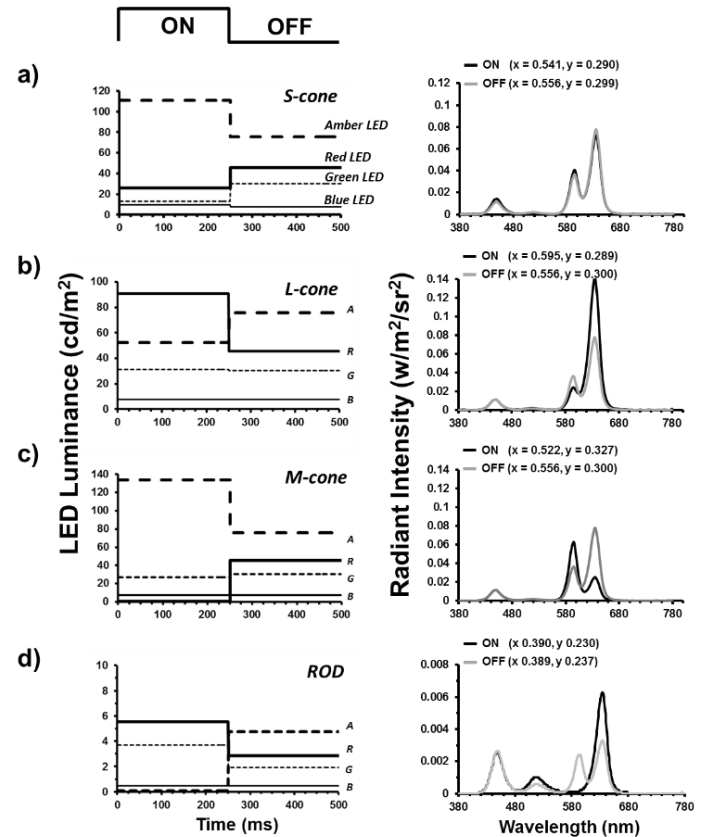


Fig. 1. Left-hand column; LED luminance profiles used to generate the (a) S-cone, (b) L-cone, (c) M-cone and (d) rod isolating transient ERGs. The right-hand column shows the spectral characteristics of the onset and offset phases of the same stimuli along with the CIE (1931) x y chromaticity co-ordinates of the onset and offset phases.

C. Data Analysis

The averaged steady-state ERGs were subjected to a two-stage offline analysis involving, firstly, resampling of the traces and secondly, Fourier analysis. ERG responses were recorded using a sampling rate of 1000Hz with an epoch of 4000ms, but because the FFT uses a sampling rate of 1024Hz, a simple interpolation was required to produce 4096 samples. The resampled traces were imported into Signal software (version 2.16; Cambridge Electronic Design, Cambridge, UK) and subjected to a FFT. This analysis provided a measure of the amplitude at the fundamental (stimulating) frequency. Noise (N) was defined as the mean amplitude (A) of the response \pm 1 Hz from the stimulation frequency (F):

$$N = (A(F-1) + A(F+1))/2 \quad (2)$$

A response was considered significant if the measured ERG amplitude was at least 2.82 times greater than the computed noise amplitude for that frequency [50].

D. Participants

A total of 16 color normal trichromats (5 males, 11 females; mean age: 33 yrs, age range: 20-60 yrs) participated in this study. In addition, 2 participants diagnosed with blue cone monochromacy (BCM) and 1 participant diagnosed with enhanced S cone syndrome (ESCS) were tested. The participants with BCM have an L opsin gene, with a novel point mutation p.Pro196Ala, predicted to account for the phenotype. The participant with ESCS has bi-allelic loss of function mutations in *NRL*. This is a transcription factor which positively regulates *NR2E3* and the loss of function is likely to cause the phenotype. Color vision in all subjects (except the participant with ESCS) was assessed using CAD color test (City University, UK). The 16 trichromats had normal red/green and yellow/blue colour thresholds. The BCM subjects had highly elevated red/green thresholds (BCM1 35.78 x normal; BCM2 38.80 x normal). Their yellow/blue thresholds were also slightly elevated compared to normals (BCM1 3.96 x normal; BCM2 3.51 x normal).

All subjects gave informed consent prior to the commencement of the experiments which were conducted (both in terms of stimulation and use of the recording electrodes) in accordance with the Declaration of Helsinki and were approved by the University of Bradford Ethics Committee.

3. RESULTS

A. Morphology of the Transient S-cone ERG in Normal Trichromats

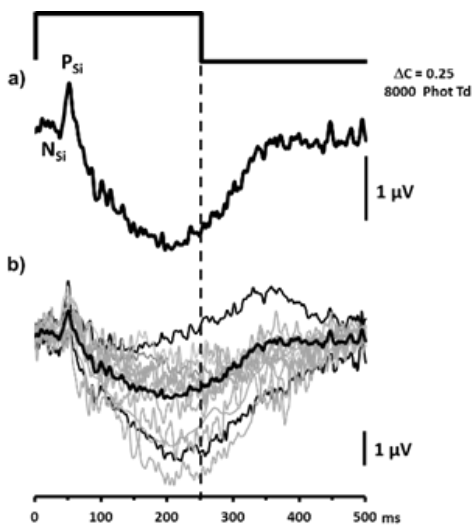


Fig. 2. (a) The group averaged ($n=16$) S-cone ERG. The onset response consists of an initial negative peak (N_{Si}) followed by a positive component (P_{Si}) after which there is a large negativity. At stimulus offset there is no discernible d-wave and the response returns to baseline levels. (b) To illustrate the response variability across the subject cohort, individual (grey lines) and group averaged (thick black line) ERGs elicited from 16 normal participants by a silent substitution S-cone isolating stimulus are plotted. The thin black lines represent ± 1 S.D. from the mean. S-cone contrast = 0.25 and the stimulus had a retinal illuminance of 8000 photopic trolands.

Figure 2 shows ERGs obtained from 16 trichromats in response to a silent substitution S-cone isolating stimulus with a square-wave temporal profile comprising an onset (i.e. S-cone excitation increment) duration of 250ms and a 250ms offset (S-cone excitation decrement)

period. The S-cone ERG elicited by this stimulus has a waveform with an initial negative a-wave (which we have termed N_{Si}) which has a peak implicit time of 31.36 ms (s.d. = 5.95 ms). This negativity is followed by a b-wave component (termed here P_{Si}) with a peak implicit time of 53.8 ms (s.d. = 5.36 ms). Following these main onset components, the S-cone ERG then appears to be dominated by a large negativity. Following stimulus offset, the S-cone ERG exhibits a slow recovery of the negativity back to baseline approximately 350 ms after the stimulus onset.

In order to compare the S-cone ERGs with responses derived from the other photoreceptor populations, Figure 3 shows the group-averaged ($n = 16$) S-cone responses with those elicited by the L-cone, M-cone and rod silent substitution stimuli. As can be observed, the ERGs from the four photoreceptor populations have different morphological features. The ERGs elicited using the S-cone isolating stimuli have the smallest amplitudes compared to the other photoreceptors, with b-waves (P_{Si}) typically of the order of approximately $1\mu V$. There are also differences in terms of the peak implicit times of the main onset response components. For example, the S-cone a- and b-waves (N_{Si} and P_{Si} in our nomenclature) are longer (31.36 ms and 53.8 ms, respectively) than those for the equivalent components (and in the L-cone isolated ERG ($N_{Li} = 20.1$ ms (s.d. = 1.449 ms); $P_{Li} = 39.4$ ms (s.d. = 3.34 ms)). By comparison the rod b-wave (P_{Ri}) has the longest implicit time at 85.95 ms (s.d. = 6.88 ms) and has no discernible a-wave under these recording conditions. Another distinctive feature of the S-cone ERG is the lack of any prominent positive d-wave following stimulus offset. This contrasts with the L- and M-cone ERGs, which exhibit a clear offset response component (P_{Ld} and P_{Md}). The rod mediated ERG appears to exhibit a prominent negative response (N_{Rd}) to stimulus offset.

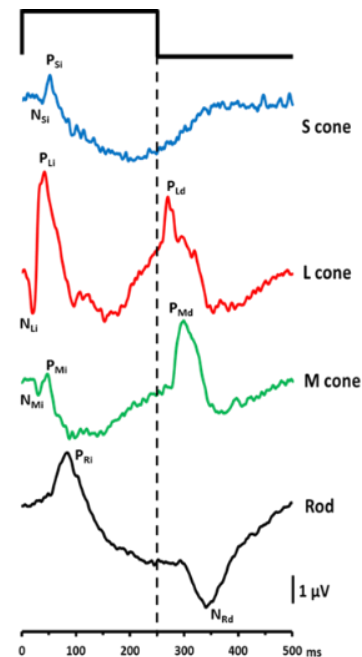


Fig. 3. Comparison of the ERGs elicited from the four classes of human photoreceptors using silent substitution stimuli. Each of the traces is group averaged ($n = 16$) from trichromatic observers. For all stimuli, photoreceptor modulation was 0.25. The L-, M- and S-cone isolating stimuli resulted in a mean retinal illuminance of 8000 photopic Trolands. The rod-isolating stimulus resulted in a retinal illuminance of 63 photopic Trolands.

B. Temporal Response Properties of the S-cone ERG

Figure 4 shows the ERG temporal response functions obtained using steady-state (sinusoidally modulated) L-, M- and S-cone isolating stimuli. The S-cone ERG function (figure 4a) is low-pass in appearance and response amplitude falls below our threshold criterion ($< 2.82 \times$ noise) beyond 28 Hz. Figure 4b shows the S-cone ERG temporal response function along with those obtained for the L- and M-cone isolating stimuli under the same conditions. The L-cone ERG has the largest magnitude and the function has a band-pass appearance with peak responses obtained between 20 – 25 Hz. Even at the highest stimulation rates tested (70 Hz) the L-cone ERG remains above the threshold criterion. The M-cone ERG exhibits response amplitudes at the lowest stimulation frequencies that are comparable to the L-cone responses. However, beyond 10 Hz the M-cone ERG falls to a minimum value then the temporal response function exhibits a secondary peak at 30 Hz. The response falls below threshold above 46 Hz.

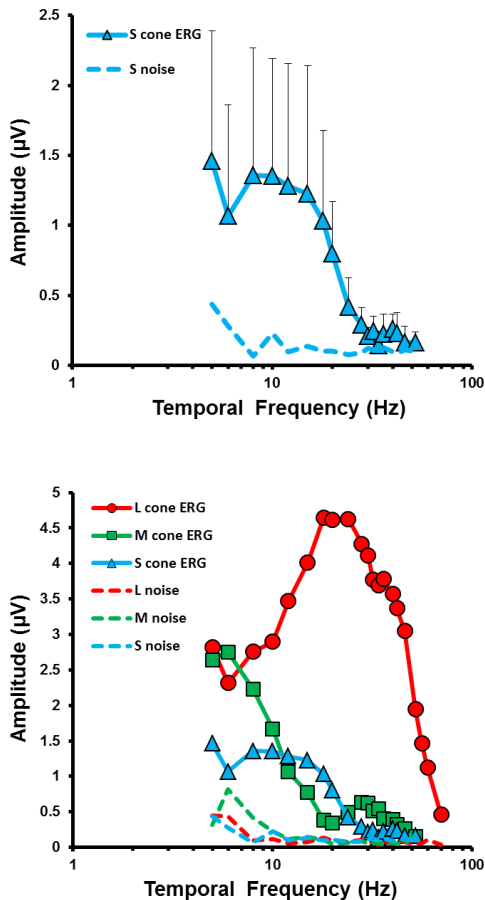


Fig. 4. (a) Temporal frequency response function for the fundamental of the S-cone ERG. The dashed line represents noise levels (see methods for definition). The data represent the group averaged responses ($n=4$). (b) For comparison the temporal frequency response functions for all cone-isolating stimuli (L-, M- and S-cones) are plotted together. For all stimuli photoreceptor modulation = 0.25. The retinal illuminance of the L-, M- and S-cone isolating stimuli = 1000 ph Td.

C. The S-cone ERG in Blue Cone Monochromacy and Enhanced S-Cone Syndrome

ERG recordings from BCM patients in response to our silent substitution cone isolating stimuli have the potential of providing us with a means of checking the suitability of these stimuli in eliciting

selective responses from the different human photoreceptor classes. Individuals with BCM only have one operational population of cones contributing to photopic vision, the S-cones. Hence, the prediction is that S-cone ERGs should be preserved in these individuals but there would be negligible responses to L- and M-cone stimulation. Figure 5 shows the ERGs obtained from the two participants with BCM in response to L-, M- and S-cone isolating stimuli, alongside the group averaged responses from normal trichromats to the same stimuli. In line with predictions, there is little or no discernible response to L- and M-cone stimulation. However, there does appear to be a response in both BCM patients to S-cone stimulation – consistent with the preserved S-cone photoreceptors in this condition. In terms of the ERG response components to S-cone onset there are similarities between the waveforms obtained from normal trichromats and those from the BCM patients. Both groups have ERGs with clear b-waves (P_{Si}) occurring between 50-60 ms post stimulus onset. However, there are some differences between the responses from the normals and BCM patients. For example, the descending portion of the P_{Si} in the BCM ERGs exhibits a steep decline followed by a broad, low amplitude positivity. This is contrary to the typical response from the normal group which consists of a gradual decline into a broad negativity. The offset response is also different, consisting of a more prominent d-wave compared to the slower recovery phase of the response in the trichromats.

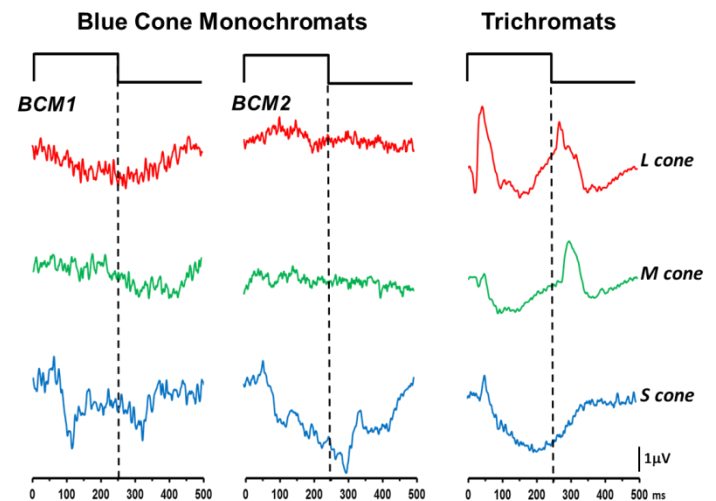


Fig. 5. L-, M- and S-cone ERGs recorded from two Blue Cone Monochromats (BCM1 & BCM2). Also shown are the group averaged ($n=16$) responses from normal trichromatic observers to the same stimuli. For all stimuli photoreceptor modulation = 0.25. The L-, M- and S-cone isolating stimuli had a retinal illuminance of 8000 photopic Trolands.

Figure 6 shows the S-cone ERG elicited from a single participant with ESCS using the triple silent substitution stimulus. Also shown is the group averaged ERG response obtained from the normal trichromats to the same stimulus. This response is very different compared to the normal S cone response from the trichromatic group. The first unique feature of the S cone response in ESCS is the large a-wave and b-wave, with peak amplitudes of $1.41\mu\text{V}$ and $1.28\mu\text{V}$ respectively. The ESCS a-wave is four times the amplitude of a-wave obtained from the trichromatic group. The second feature is the d-wave (peak amplitude $2.56\mu\text{V}$) at stimulus offset, a feature that is not usually present in the normal S cone ERG.

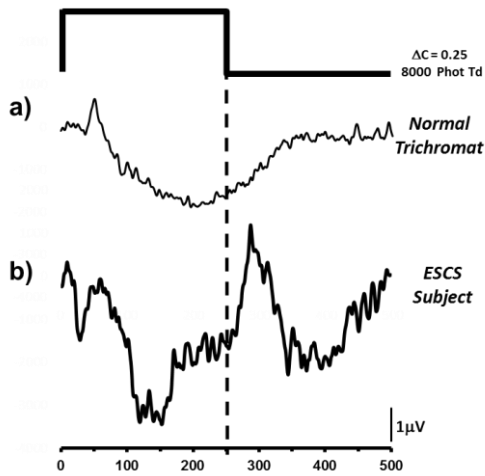


Figure 6. (a) The group averaged ($n=16$) S-cone ERG obtained from normal trichromats. (b) The S-cone ERG obtained from a participant with enhanced S-cone syndrome (ESCS). All of the responses were elicited using a stimulus with S-cone contrast = 0.25 and a retinal illuminance of 8000 photopic trolands.

3. DISCUSSION

In this study we have used triple silent substitution stimuli to elicit ERGs that selectively reflect S-cone function in the normal trichromatic human retina. We have described the basic morphology of the S-cone response and have shown that the use of silent substitution stimuli enables the generation of responses from each of the rod, L- and M-cone photoreceptor classes that have different morphological features and temporal frequency response characteristics. We have been able to validate the selective nature of our S-cone stimulation paradigm by the examination of responses from patients with genetically verified Blue Cone Monochromatism (BCM). The preservation of the S-cone response in these subjects, coupled with the abolition of the L- and M-cone ERGs, is consistent with the fact that the S-cones are the only functional group of cone photoreceptors in BCM. In addition, the S-cone ERG in ESCS exhibits changes in response amplitude and waveform morphology that are consistent with previous reports [42,51,52]. Overall, these findings demonstrate that our triple silent substitution stimuli provide a clinically useful means via which we can assay human S-cone mediated visual function.

Strictly speaking, the use of triple silent substitution has not been previously employed to isolate the S-cone ERG. More commonly, double silent substitution stimuli have been employed with sufficiently high luminances to saturate the rod response [10,31,32]. The use of triple silent substitution stimuli, generated by a four-primary stimulator, offers the advantage of maintaining net excitation in all four photoreceptor types at a constant level. This negates the use of a high luminance adapting light used to suppress the rod system, which can introduce post-receptoral non-linearities. This may be particularly relevant in the case of S-cone isolation as the S-cones and rods share a common post-receptoral pathway [53]. The S-cone ERG elicited by our paradigm is broadly consistent with previous results [7,10,28,32] and comprises a small a-wave with a larger b-wave. Overall, the S-cone ERG is of smaller amplitude than those generated by rod, L- and M-cone isolating stimuli of the same photoreceptoral contrast. The average peak implicit timings of the S-cone ERG a- and b-wave components (31 ± 2 and 52 ± 2 ms, respectively) are longer than for other cone types and consistent with some earlier studies [7,10,27,28,45] but not others [26,30,32,54,55]. Where longer b-wave implicit times have been noted,

it has been postulated that this is most likely to be related to rod intrusions [27]. However, some studies have shown that the S-cone ERG b-wave implicit time is up to 10ms earlier than that reported in this study [32]. Differences in stimulus intensities used across studies may partially explain these discrepancies, but intrusions from L- and M-cones have also been suggested as a reason for some of the earlier S-cone b-wave implicit times reported [32]. These intrusions are minimized in our measurements.

In contrast to L- and M-cone ERGs, the S-cone ERG elicited by our triple silent substitution method exhibits no obvious d-wave offset component in normal trichromats. This is consistent with previous S-cone ERG studies [10,28,30,32,56,57]. The absence of an offset d-wave response in the S-cone ERG has been traditionally interpreted as providing evidence for a lack of direct S-cone input to an OFF pathway [56,57]. This tended to corroborate the notion of a poorly established S-cone OFF pathway in the primate retina [e.g. 58]. In fact, the presence/absence of an S-cone offset response in the ERG has been previously employed as an indicator of S-cone isolation and a measure of L- or M-cone intrusion into the S-cone ERG [10,32,56]. However, subsequent anatomical studies have established the presence of an S-cone OFF pathway in the macaque, based on S-cone inputs to an OFF midget bipolar, which in turn are connected to OFF midget ganglion cells [59]. Although the S-OFF pathway has not been described in the human retina, it is postulated that a similar physiological substrate exists to convey S-off signals [60–63], presumably forming the basis of a mechanism for the detection of S-cone decrements which has been psychophysically established [64]. Whilst this OFF midget bipolar- OFF midget ganglion cell circuitry might exist in the central fovea, in the retinal periphery OFF midget ganglion cells receive most of their input from L- and M-cones. Thus, S-cone OFF midget ganglion cells are something of a rarity in the peripheral retina [65]. This lack of access to an S-cone OFF pathway in the periphery may be the reason for the reduced d-wave offset response in our S-cone ERGs which are generated by spatially extensive stimuli which extend to a retinal eccentricity of approximately 60–70°.

S-cone ERGs from patients diagnosed with BCM have previously been used to characterize S-cone responses which are free of intrusions from L- and M-cones [26,54,55]. The results from the participants with BCM presented in this study show a clear preservation of the S-cone ERG with abolished L- and M-cone mediated responses. This is an important finding as it provides validation of the selective nature of our cone isolating stimuli. Nevertheless, whilst the morphology and timing of the onset b-waves (P_{Si}) are similar to the S-cone ERG elicited from normal trichromats, there are features of the S-cone responses in BCM that are clearly very different. Firstly, the descending portion of the b-wave (P_{Si}) as it develops into the PhNR is steeper and larger compared to the gradual decline seen in the S-cone ERG elicited from the normal trichromats. Secondly, the response following the steep decline is positive compared to a gradual negative trough in the trichromats. A similar response was recorded in two patients with BCM using a chromatic adaptation paradigm [55]. Several studies in primates have shown that the second order neurons, particularly hyperpolarizing bipolar cells and horizontal cells contribute to the negative trough following the b-wave [66–68]. The presence of what clearly resembles an offset response in both BCM patients is unusual. The fact that this component is not present in the normal trichromats demonstrates that this is not an artefact of our methodology. Furthermore, the fact that the offset response was reproducible in both subjects, on two separate recording sessions, suggests that it is a genuine physiological response. Currently, we are unsure of its origins but speculate whether it may be related to an S-OFF pathway, where in the absence of functioning L-

and M-cones in BCM, the S-OFF midgenet pathway is the only viable OFF mechanism present in these patients.

The S-cone ERG recorded from the participant with ESCS using the triple silent substitution stimulus also appears to exhibit morphological features consistent with those that have been previously described for this condition [e.g. 51,52,69]. ESCS is a rare inherited degenerative retinal condition that, in addition to other retinal changes, is associated with increased S-cone sensitivity [45] resulting from an increased number of S-cones in the retina compared to normals [42-44]. Although there are functional L- and M-cones, their contributions to the ERG are very much reduced [42,52]. A consequence of this S-cone domination of the retina is that individuals with ESCS exhibit supra-normal ERGs mediated largely by the S-cone system [42]. Consistent with this increase in S-cones, the response elicited from the ESCS subject using the S-cone isolating triple silent substitution stimulus is of greater amplitude than that found in normal. In line with previous studies, there is a prominent, large amplitude a-wave component [42].

Another feature of the S-cone ERG recorded from the participant with ESCS, but not in normal trichromats, is a large positive d-wave component generated following stimulus offset. This has been previously reported in ESCS [51,52] and the presence of a prominent positive offset response forms a possible electrophysiological correlate of the re-organization of post-receptor circuitry that is purported to take place in this condition [42,43]. As noted above, in the normal retina S-cones in the central retina have connections with both ON- and OFF - bipolar neurons, whilst peripheral S-cones are largely restricted to ON-bipolars [65]. The presence of an OFF response in ESCS clearly suggests that the outputs of the more numerous S-cones have access to both ON and OFF response pathways just like L- and M-cone do in the normal trichromatic retina [70]. Given the highly disorganized retinal structure associated with this pathology such re-organization of S-cone outputs remains a possibility [52]. Interestingly, the S-cone ERG offset response in the BCM subjects consists of an initial negative component, almost 180° out of phase with the OFF response observed in the ESCS participant, suggesting differences in post-receptor re-organization across the two pathologies.

In addition to transient ERGs we also recorded cone isolating steady-state responses from L-, M- and S-cones in normal trichromats. This was in an attempt to ascertain whether S-cone ERGs could be differentiated from L- and M-cone mediated responses on the basis of their temporal frequency response characteristics. Each of the cone photoreceptor populations generated ERG temporal response functions with different features. The S-cone ERG exhibits a low-pass temporal frequency response function with a resolution limit lower than that obtained for either the L- or M-cone responses. This would appear to be in keeping with traditional views of the S-cone system which characterize it as a temporally sluggish system [71,72] compared to vision mediated by the L- and M-cones. This reduced temporal resolution may reflect limitations on the S-cone signal imposed by an anatomically segregated processing pathway which has its origins in the retina [13,18,60] and is maintained in retino-cortical projections via the koniocellular processing pathway [73]. One theory that has been advanced is that the slow temporal responsiveness of the S-cone system is the result of response delays between ON and OFF responses at the ganglion cell level [74]. The fact that this temporal limitation is manifest in the steady-state ERG appears consistent with its imposition on the S-cone system at a relatively early retinal level.

In summary, we have demonstrated in this study that triple silent substitution stimuli provide an effective means via which we can selectively investigate S-cone function. The responses we have elicited from the S-cone population exhibit different morphological features and have different temporal frequency response characteristics compared to ERGs derived from L-cone, M-cone and rod photoreceptors. Furthermore, in cases of retinal pathology, which either isolate or enhance S-cone function, the responses elicited by our stimuli provide an appropriate assay of the functional integrity of the S-cone system.

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