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Experiment Title: Quantifying light perception via perceptual and physiologic measures in response to melanopsin and cone stimulation in migraineurs with interictal photophobia and in headache-free control subjects

Research Project: Photoreceptor directed light modulation: perception, pupillometry, EMG

Purpose and Approach

Migraine is a disabling neurologic disorder with a high prevalence. A core feature of migraine is photophobia. The purpose of this project is to determine the relative contribution of cone and melanopsin stimulation to brightness perception, as well as to photophobia in patients who suffer from migraine with aura and from migraine without aura. It is well established that patients with migraine, particularly migraine with aura, have increased sensitivity to light during the headache-free period. The proposed experiments will determine if these patients have increased sensitivity to cone stimulation, melanopsin stimulation, or both. Our central hypothesis is that melanopsin contributes to the sensation of brightness in humans, and that migraineurs (during their inter-ictal period) will demonstrate increased perceptual and physiologic sensitivity to melanopsin stimulation.

Melanopsin function in humans may be isolated using tailored modulations of the spectral content of light. While the cone photoreceptors and melanopsin have overlapping spectral sensitivities, it is possible to create sets of light spectra for which the absorption of photons is constant for some photoreceptor classes but not others. Using this "silent substitution" approach, we will create stimuli that target photoreceptor classes in combination or in isolation. The experiments proposed will use distinct modulations to selectively stimulate melanopsin, cones, or both.

Subjects

Participants will be recruited from the University of Pennsylvania campus, University of Pennsylvania Department of Neurology clinics, and greater Philadelphia community. Participants must be between the ages of 25 to 40 years old. To differentiate the status of participants as they move through the multiple stages of recruitment and screening we use the terms:

- *Recruited subject* to refer to someone who, prompted by advertising materials or personal contact, has provided responses to on-line screening materials that do not require an informed consent process to collect.
- *Candidate subject* to refer to someone who meets criteria based upon on-line screening materials and has completed an informed consent process, but has not yet satisfied all criteria for inclusion, including vision and pupillometry tests that are administered in person.
- *Enrolled subject* to refer to someone who has met all screening and inclusion criteria and has been scheduled for a primary data collection session.
- *Completed subject* to refer to those enrolled subjects who have completed the experimental protocol and have provided data that meet the retention criteria.

At the initial stage Recruited subjects are directed to a website (gkaguirre.com/poem) that presents them with a questionnaire on headache and associated symptoms called the "Penn Online Evaluation of Migraine" (POEM – v1.1s). The survey integrates the Choi 2009 questions

regarding visual sensitivity during and between headache. The survey responses are processed with an algorithm (https://github.com/gkaguirrelab/poemAnalysis.git) that assigns diagnostic labels: 1) migraine without aura; 2) migraine with visual aura; 3) migraine with non-visual aura; 4) headache, not-otherwise-specified; 5) mild non-migrainous headache; 6) headache free. It is possible for someone to receive more than one label (e.g., migraine with visual and non-visual aura).

Recruited subjects continue to be eligible for the study if they meet criteria for inclusion in one of three groups:

1) **Migraine with visual aura** -- meet all of these criteria: a) single label of migraine with visual aura; b) a Choi score of 6 or 7; c) a response of "yes" to the Choi query regarding the presence of light sensitivity during headache free periods.
2) **Migraine without aura** -- meet all of these criteria: a) single label of migraine without aura; b) a Choi score of 6 or 7; c) a response of "yes" to the Choi query regarding the presence of light sensitivity during headache free periods.
3) **Control** -- meet all of these criteria: a) single label of mild non-migrainous headache or headache free; b) a response of "no" or "I don't know" to the question regarding family history of migraine headaches; c) a response of "no" to the question regarding a history of childhood motion sickness.

These Recruited migraine and control subjects are then invited to complete the Visual Discomfort Score (VDS) survey. To continue to be eligible for the study, Recruited control subjects must score 7 or lower on the VDS; there is no required score for the recruited migraine subjects.

Subjects who reach this stage are invited to visit the laboratory, complete an informed consent process, and become Candidate subjects. Candidates will be excluded for a history of glaucoma, generalized epilepsy, negative reaction to dilated eye drops, a concussion in the last 6 months, or on-going symptoms from head trauma/concussion. Candidate subjects will undergo a test of best-corrected distance acuity using a Snellen eye chart and a test of color vision using the Ishihara color plates. Candidate subjects will be excluded if best corrected acuity is below 20/40 or if they do not have normal color vision as judged by the Ishihara plates. Finally, candidate subjects will be invited to participate in a pupillometry screening session. During this session they will be presented with 12 trials of the 200% light flux stimulus. We will judge the ability of the subject to participate in the experiment and provide good quality data; generally, we will retain subjects if they are able to provide acceptable pupillometry data on at least 9 of the 12 trials. Subjects who complete this last stage of screening become Enrolled subjects.

Enrolled subjects are invited to participate in the primary experiment (described below). Those Enrolled subjects who complete the experiment and provide data that meet retention criteria will be considered Completed subjects. We will continue to recruit and test subjects until we have 40 completed subjects in each of three groups (migraine with visual aura, migraine without aura, control).

The number of subjects to be studied is based upon a power analysis. A prior report (https://tinyurl.com/y94bhq8s) showed a reduction in melanopsin-mediated pupil function in a Parkinson's disease population. We took this effect as an estimate of a group difference effect size that we would try to detect in our study. We used the variation in pupil response observed in a previous study from our lab (https://osf.io/9umq4/) to simulate how power varies as a function of the number of subjects studied within each group. With an alpha value of 1%, this simulation suggested we need ~40 subjects to achieve 80% statistical power.

We will recruit migraine with visual aura subjects without regard to gender or age. Recruitment and enrollment of subjects for the other groups (migraine without aura and control) will be guided in an attempt to match the gender ratio and median age that is obtained in the migraine with aura group.

Subject preparation

At the start of a data collection session, subjects will be acclimated to the experimental room. The ambient light level in the room is adjusted so that the measured luminance from a standard location on the wall close to where the subject sits will be similar to the background luminance of the light flux stimuli. Subjects will enter the testing room at least 20 minutes prior to data collection. Pharmacologic dilation of the right eye is achieved with 1% tropicamide ophthalmic solution following administration of 0.5% proparacaine as a local anesthetic. The operator will then review the experimental procedure with the subject and adjust the apparatus for subject comfort. The IR video camera will adjusted to view the left eye. Surface EMG electrodes will be applied to the subject's skin to record orbicularis oculi muscle activity under both the left and right eye. To ensure correct EMG placement, a test EMG recording will be made for 5 s during which subjects will be ask to blink and squint.

Stimulus creation

We will deliver spectral modulations designed through silent substitution that will selectively target either melanopsin (Mel), cones (LMS), or both (light flux). These stimuli will be a subset of those described in prior reports by our group (Spitschan 2017). For each targeted photoreceptor mechanism, we will generate stimuli of 3 different contrast levels (400%, 200%, and 100% Weber contrast). The stimuli have similar chromaticity.

The overall intensity of the stimulus background can be adjusted by placing neutral density filters in the light path. We will monitor the measured luminance of the light flux background and adjust our apparatus as needed over the course of the entire experiment with the goal of maintaining the luminance of the light flux background within a narrow range.

Stimulus delivery

All of the stimuli will be generated with a digital light synthesis engine (OneLight Spectra) under computer control. The stimuli will be presented through a custom-made eyepiece with a circular, uniform field of 27.5° diameter. The central 5° diameter of the field will be obscured to block the effects of the foveal macular pigment which can cause variation in photoreceptor spectral sensitivity. The subject will view the stimulus through a 6mm diameter artificial pupil. Visual stimuli are tailored to an individual observer’s age, taking their predicted lens density into account in the pre-receptoral filtering (see Spitschan 2014, 2015, 2016 for details).

Trial design

Each trial will consist of the following sequence. Subjects will view the relevant background spectrum for an average of 2 s. The precise duration of this background spectrum will vary between 1.5 and 2.5 s to prevent subjects from being able to anticipate the start of the modulation. Next, subjects will be presented with a 4-second pulse of spectral change. The transition from the background to the stimulation spectrum (Mel, LMS, or light flux) and the subsequent return to the background are subjected to a 500 ms half-cosine window. This step minimizes perception of a Purkinje tree percept in the melanopsin-directed stimulus. EMG and pupillometry recordings will begin 1.5s prior to the stimulus pulse and continue until 12 s after the pulse has ended. At this point, subjects will be prompted via auditory cue to speak aloud their discomfort rating. This audio will be recorded for the duration of the 4-s response window,

which will be marked by another auditory cue to notify the subject of the end of the response window.

Acquisition design

An acquisition refers to a sequence of trials that targets a specific photoreceptor class, either LMS, Mel, or light flux stimulation. A given acquisition will consist of 10 trials. The last 9 trials will consist of 3 trials at each of the three previously described contrast levels and their ordering will be counterbalanced using DeBruijn sequences. The first trial will be identical to the last trial of the acquisition. This trial is included to ensure the rest of the trials are preceded by similar stimulus history; the data from this first trial will be discarded. The total length of an acquisition will be 3 minutes 40 seconds.

Session design

A single session will consist of 6 acquisitions (2 of each stimulus type, Mel, LMS, and light flux), ordered such that consecutive acquisitions are not of the same stimulus class. The entire experiment will consist of 4 sessions. We anticipate that subjects will complete two sessions in a given testing day, and thus the entire data collection over two testing days, although this may be varied by subject. Subjects will be invited to take a break between sessions.

Pupillometry

We will measure the consensual pupillary light reflex from the subject's left eye while their right eye is stimulated through the eyepiece as described above. The pupil response of the unstimulated left eye will be measured using an infrared camera (Pupil Labs EyeTracker). This video output will be processed by *transparentTrack* (https://github.com/gkaguirrelab/transparentTrack) to extract pupil size as a function of time on each trial.

Measures of Squint

There will be two independent physiologic measures of squint: 1) EMG recording of orbicularis oculi activity and 2) distance between the upper and lower right eye lid (i.e., the height of the palpebral fissure). The orbicularis oculi muscle is the sole muscle controlling both blinking and forced eye closure, and its electrical activity has been used as an objective measure of visual discomfort. For the EMG recording, we will record EMG responses of both the left and right orbicularis oculi muscles (BioNomadix 2-Channel EMG, Biopac Systems, Inc). The two reference leads will be placed just inferior to the lower lid with the ground lead placed on the neck. The width of the palpebral fissure will be extracted from the IR video data.

Discomfort ratings

We will ask subjects to rate any discomfort that they experience from the stimuli at the conclusion of each trial. They will be asked to report this percept on a 0 – 10 scale. The following instructions will be provided regarding this rating:

> *Following each trial, please rate the degree of discomfort that you experienced from the light pulse on a scale of zero to ten. A score of zero means that the light was not at all uncomfortable. A score of five means that the light pulse was moderately uncomfortable. A score of ten means that the light pulse was extremely uncomfortable.*

Responses will be recorded automatically by the experimental apparatus, and will be transcribed into numeric values by either speech-to-text software and / or by a member of the

laboratory staff. In the latter instance, the transcriber will kept unaware of the particular stimulus that was presented on each trial to which the subject responded.

Validation

Before and after each session, we will take spectroradiometric measurements of the stimuli and their backgrounds. For each stimulus type, we will determine contrast on the following post-receptoral mechanisms: LMS, L-M, S, and melanopsin. For each stimulus type we will perform 5 validation measurements, both before and after the experiment. This validation procedure will be performed for each stimulus type at 400% contrast only.

These validation measurements will also provide measurements of the background luminance for each stimulus. We will use these measurements to determine when our apparatus should be modified to maintain the targeted background luminance. We will adjust the apparatus in an attempt to maintain the background luminance of the light flux stimulus within a ±0.1 log unit range of this value at the start of data collection.

Exclusion criteria

Following a single session for a given subject, the following criteria will be applied to determine if data from that session will be excluded from subsequent analysis. One source of exclusion is if a subject's post-session validation shows too much inadvertent contrast on nominally silenced post-receptoral mechanisms or too little contrast on the targeted photoreceptor class.

- If the absolute value of the median of the contrast values over the 5 post-session validation measurements on any of the nominally silenced post-receptoral mechanisms (for melanopsin-directed stimuli, this includes LMS, L-M, and S post-receptoral channels; for LMS-directed stimuli, this includes melanopsin, L-M, and S) exceeds 20%, data from this session will be discarded
- If the median contrast value over the 5 post-session validation measurements of any of the targeted photoreceptor classes (i.e., melanopsin, the combined LMS postreceptoral channel, or both for melanopsin-directed, LMS-directed, and light flux stimuli, respectively) is less than 87.5% of the called-for contrast (i.e., if our 400% modulations achieve contrast of less than 350%), data from this session will be discarded.
- For light flux stimuli, if the median contrast value for LMS and melanopsin post-receptoral channels is less than 350% or the absolute value of L-M or S cone contrast is greater than 20%, data from this session will be discarded.

We also take 5 validation measurements prior to the experimental session. If these measurements fail to meet the criteria described above, the session will be re-scheduled and we will attempt to re-generate stimuli that meet criteria.

We will also exclude subjects on the basis of poor quality pupil data. Data from a single trial will be discarded if 25% of data frames are deemed "bad" by the tracking algorithm. If for a given session, 75% of all trials within a given stimulus type are discarded (Mel, LMS, or light flux, regardless of contrast level), or 50% of total trials across all stimulus types are discarded, all data from this session will be excluded.

If a session is excluded either due to poor stimulus validation or poor quality pupil data, the subject may be re-scheduled for additional sessions; this decision will be made on a subject-by-subject basis after consideration of the factors that contributed to poor data quality.

We will aim to collect acceptable data from a total of four sessions in each subject. To be considered a Completed subject, acceptable data from two or more sessions must be obtained.

Primary outcomes to be tested

Primary response metric

There are several indices that could be derived from each measurement type. We define here the primary response metric that will be obtained and used in our primary planned comparison:

- Pupillometry*:* We will fit the pupil response using a three-component temporal model implemented previously by the lab (Spitschan 2017). We will fit this model to the mean response for each subject for each contrast level within each stimulus type. To derive error bounds on the parameter estimates of these fits, we will use bootstrap resampling across trials to obtain the +/- interquartile range. While several parameters of pupil response amplitude and timing may be derived from the model fit, our primary test will examine the "percent persistent" of the response for a given stimulus type. This is the proportion of the area of the pupil response attributable to the slow return to baseline after peak constriction.
- Electromyography (EMG): Similar to prior studies of light-induced squint (JM Stringham et al, 2013) we will define a response window that begins 1 s after stimulus onset and ends 4 s later (1 s after stimulus offset). Within this window, we will calculate the root-mean-squared (RMS) of the EMG voltage. We will obtain the median and +/- interquartile range of the RMS across trials for each subject in response to each stimulus condition.
- Discomfort Ratings: We will report the median and +/- interquartile range for each subject in response to each stimulus condition.

Primary planned comparison

Our study includes three groups (migraine with visual aura, migraine without aura, and controls), three stimulus directions (melanopsin, LMS, light flux), and three contrast levels (400%, 200%, 100%). We define here a single, planned primary test. Additional, control and elaboration tests are also envisioned.

Our primary test is if subjects with migraine with visual aura differ in their response to 400% contrast melanopsin stimulation as compared to headache free controls.

For each of the measurement types (pupil, EMG squint, discomfort ratings) we will ask if the median value of the primary response metric (defined above) across subjects evoked by the 400% melanopsin stimulus differs between the migraine with aura and control populations. Our primary interest is in measuring the effect size and establishing bounds on the measurement. We will also obtain a p-value for this comparison by label-permutation. To do so, we will take the set of observed values for the migraine with aura and control subjects and randomly re-assign the group labels. For each permutation of group labels we will compute the difference in the median response metrics between the simulated groups. Across 1000 permutations, we will identify the percentage of simulations in which the simulated difference in median values is more extreme than the observed, veridical difference in medians. We will report this as a p-value.

The additional data collected in this study will be used to examine the specificity of any group differences we observe. To examine if the effect is specific to migraine with aura, we will compare the observed effect to that between the migraine without aura and control groups. To

examine if the effect is specific to melanopsin, we will examine the response to LMS directed stimulation. To examine if the effect interacts with cone stimulation, we will examine the response to light flux stimulation. Finally, we will examine if the effect varies as a function of stimulus contrast.