Steady-state VEP responses to uncomfortable stimuli

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Abstract

Periodic stimuli, such as op-art, can evoke a range of aversive sensations included in the term visual discomfort. Illusory motion effects **are** elicited by fixational eye movements, **but the cortex might also contribute to** effects of discomfort. **To investigate this possibility**, steady-state visually-evoked responses (SSVEPs) to contrast-matched op-art-based stimuli were measured at the same time as discomfort judgements. On average, discomfort reduced with increasing spatial frequency of the pattern. By contrast, the peak amplitude of the SSVEP response was around the midrange spatial frequencies. Like the discomfort judgements, **SSVEP responses** to the highest spatial frequencies were lowest amplitude, but the relationship breaks down between discomfort and SSVEP for the lower spatial frequency stimuli. This was not explicable by gross eye movements as measured using the facial electrodes. There was a weak relationship between the peak SSVEP responses and discomfort judgements **for some stimuli**, suggesting that discomfort can be explained in part by electrophysiological responses measured at the level of the cortex. However there is a breakdown of this relationship in the case of lower spatial frequency stimuli, which remains unexplained.

1. Introduction

Visual discomfort describes the adverse sensations including headache, eyestrain, and perception of illusory shapes and colours, that can be encountered on viewing certain visual stimuli, such as striped patterns (e.g. Wilkins et al, 1984). Visual discomfort is an umbrella term that includes a wide range of symptoms from various possible sources (Sheedy et al., 2003). Some instances of visual discomfort are well-documented, for example, the reports of eye-strain from accommodation-vergence conflict (Lambooij et al., 2009; MacKenzie et al., 2010; Shibata et al., 2011). As well as method of presentation, the spatiotemporal aspects of a stimulus can result in discomfort, for example contrast (Wilkins et al., 1984; Fernandez and Wilkins, 2008), and spatial frequency (O'Hare and Hibbard, 2011; O'Hare and Hibbard, 2013). In particular, spatially periodic stimuli have been shown to cause discomfort in both normal observers (Wilkins et al., 1984), and those with migraine (Marcus and Soso, 1989). Spatially periodic stimuli are encountered in everyday environments in the form of stripes, text (Wilkins et al., 2007; Jainta et al., 2010) and works of op-art (e.g. Wade, 2003; Kumar and Glaser, 2006; Troncoso, 2008; Zanker et al., 2010).

Spatially periodic stimuli can cause illusions of motion in the observer (McKay, 1957; Gori et al., 2006; Gori et al., 2008), typically reported as shimmering or scintillating, which are incorporated in the term visual discomfort (Wilkins et al., 1984). Shimmering effects and illusory motion are specifically measured as items on the Pattern Glare Test, a measure of instantaneous visual discomfort (Evans and Wilkins, 2008). There has been debate over the cause of these shimmering illusions. Accommodation fluctuations were suggested to have a role in the shimmering illusions arising from the 'Enigma' illusion (Gregory, 1993). There is also evidence that the source of shimmering illusions is likely to be due to fixational eye movements called microsaccades (Troncoso et al., 2008). When the effects of these eye-movements were reduced or negated, the strength of

the shimmering illusion was diminished. **This was found** using stimuli based on op-art, called riloids (Zanker et al, 2003). The illusion strength was diminished, not destroyed, suggesting there might be another contributor to the illusion strength in addition to eye movements. Additionally, the participants in the studies by Zanker et al., (Zanker et al., 2003; Zanker et al., 2004; Zanker et al., 2010) were asked specifically about shimmering, but there might be additional unpleasant effects experienced by the observers. As shimmering is not the only aspect of discomfort reported on viewing striped patterns, and it seems likely that microsaccades **are not the** sole contributor to discomfort from striped patterns.

Visual discomfort could also result from excessive neural responses (Juricevic et al., 2010). Sparse coding models suggest that the visual system is optimised to process stimuli with the characteristics of natural images (Field, 1987; Field 1994; Field 1999). Therefore stimuli that do not have these characteristics will not be efficiently processed, resulting in large neural responses and discomfort (Hibbard and O'Hare, 2015). **If this is true, uncomfortable stimuli should produce an increased response compared to comfortable stimuli**. There is evidence that discomfort from gratings is related to the haemodynamic brain response, for both black and white (Coutts et al., 2012) and chromatic gratings (Haigh et al., 2013b; Haigh et al., 2015). There is also some evidence for increased visual evoked potentials on viewing black-and-white riloid stimuli: stimuli that were most comfortable were also those **with a lower event-related potential (ERP) response amplitude, specifically the P100 response** (O'Hare et al., 2015). However the P100 **response** did not entirely follow the clear tuning for discomfort. **The responses to high spatial frequency stimuli were lowest for both the P100 ERP and discomfort. However, the ERP responses to the low spatial frequency stimuli did not match the discomfort judgements.**

One possible reason for this lies in the method of estimating ERP amplitude: Identifying ERP components can be difficult, depending on the nature of the waveform, and the waveforms themselves are subject to variations from noise, drift and change over the experiment (see Luck, 2005). An alternative form of EEG experimental design is to use steady-state responses as a tool. Steady-state visually evoked potentials (SSVEPs) **measure** the brain's response to periodic stimulation. There are methodological advantages of looking at the steady-state response, such as increased number of trials in a short time, and superior signal to noise ratio (see Vialatte et al, 2010; Norcia et al., 2015, for comprehensive reviews of the steady-state technique). **Reversing patterns will be used in the current study**, rather than onset/offset. **The response to reversing patterns is less variable than onset/offset** (Vialatte et al, 2010), and also avoids the asymmetry of the response between stimulus onset and offset (Norcia et al., 2015).

The choice of stimulation frequency is worth consideration. Norcia et al., (2015) argue that the choice of reversal rate is at some level arbitrary. Specifically, SSVEPs have been recorded with low frequencies (e.g. Norcia et al., 2002) and all the way up to 100Hz (Herrmann, 2001). Norcia et al., (2015) suggest that the critical aspect is that the stimulation and response are periodic, and therefore different researchers have used different frequencies, most commonly between 3 and 20Hz. Elegant work has matched the SSVEP response period to the ERP latency in the case of faces (Rossion and Jacques, 2011). Previous work has shown P100 **is** important component **in the context of visual discomfort** (O'Hare et al., 2015). **The reciprocal of P100 is 10Hz**, therefore 10Hz stimulation is one candidate frequency for investigation in the current study.

The visual system is differentially sensitive to spatial frequency content (e.g. Campbell and Robson, 1968). In other words, stimuli consisting of the spatial frequencies to which people are most sensitive will elicit the largest responses. This has been demonstrated in EEG responses (e.g. Plant, 1987; Parry et al, 1999; Sousza et al, 2007). Moreover, as well as spatial frequency, there are effects of the rate of flicker on the contrast sensitivity function (e.g. Kelly et al., 1964; Robson, 1966). Therefore, it is important to match stimuli for perceived contrast, so that any **effects** in either the discomfort ratings or EEG responses cannot be accounted for simply by contrast sensitivity.

In summary, the following experiment is to investigate the effects of op-art based uncomfortable stimuli on the steady-state EEG response amplitude. If efficient coding can explain some aspect of discomfort, then there will be some correspondence between discomfort and the relative magnitude of early cortical responses to more and less comfortable stimuli, when stimuli are controlled for differences in perceived contrast.

2. Method

2.1 Observers

20 young observers (4 male and 16 female), between 18 and 30 years of age, with corrected-to-normal vision took part in the study. None reported migraine or epilepsy. Informed consent was obtained from all individual participants included in the study. All experiments were in accordance with the ethical standards of the 1964 Helsinki Declaration and were scrutinised by the University of Lincoln School of Psychology ethics committee. Participants were reimbursed for their time.

2.2 Apparatus

EEG was recorded with 2048Hz sampling rate with a 64 channel Active Two system (BioSemi, Amsterdam), with 5 additional electrodes (two on the mastoids, two on the outer canthi of the eyes, and the final one infraorbital on the right eye). During recording channels were referenced to the common mode sense electrode (see http://www.biosemi.com/faq/cms&drl.htmfor more details). Incoming recordings were low pass filtered at 100Hz, and high pass filtered at 0.16Hz to remove artefacts. Experiments were conducted in a darkened, electrically insulated room, with the door slightly ajar to limit observer stress. Observers were seated approximately 50cm from the display, which was ensured using a table positioned in front of the observer. Head movements were not restricted, but participants were asked to remain as still as possible, and importantly, to focus on the fixation cross. The fixation cross was red, and subtended 0.38 x 0.38 degrees, with a line width of 0.15 degrees.

Stimuli were presented using a 22 inch Illyama HM204DTA Vision Master Pro 514 Diamondtron U3-CRT monitor, calibrated with a LS100 Mintola photometer. The refresh rate of the display was 60Hz. Screen resolution was 1024 x 786 pixels. Dell Optiplex780, running windows XP. All stimuli were generated and presented using MATLAB and the Psychtoolbox (Brainard, 1997, Pelli, 1997, Kleiner, 2007). Maximum luminance was 100.69 cd/m^2, and minimum luminance was 0.93 cd/m^2.

2.3 Stimuli

Stimuli consisted of nine riloid patterns, the same as used in O'Hare et al., (2015). These are based on the work of Zanker and colleagues (Zanker et al., 2003; Zanker et al., 2004; Zanker et al., 2010) see Equation 1:

$$I(x,y) = 0.5\big(1 + \sin\big[2\,\Pi\,\big(x - \Phi\,(y)\big)\big]\big)$$

Where:

$$\Phi(y) = A\sin\left(2\,\Pi\,\frac{y}{\mu}\right)$$

Where: $I_{(x,y)}$ defines luminance as a function of the horizontal *(x)* and vertical position *(y)*, resulting in a sine wave of frequency *f*, which varied between approximately 0.5, 3, and 9 cycles/degree, with phase modulated as $\Phi_{(y)}$, amplitude *A* was 0.94˚, and wavelength μ was 100, and 400 and 10000000000 pixels (2.93˚, 11.74˚ and 2.92.x10^8 degrees). μ relates to the waviness of the lines, with higher values indicating straighter lines, and λ relates to the thickness of the lines, with higher values indicating thinner lines. Stimuli were presented in a Gaussian-edged window subtending a visual angle central region 5.94˚ and σ of 0.74˚. This resulted in a circular aperture with a smoothed edge. Example stimuli can be seen in figure 1: left to right shows increasing λ values (thinner lines), top to bottom shows increasing μ values (straighter lines).

**********figure 1 here **********

2.4 Contrast Matching Procedure

Stimuli were matched for perceived contrast using a self-adjustment procedure. Test and standard stimuli were presented side-by-side at an eccentricity of 15.03˚ from the screen midline to the centre of the pattern. **The standard stimulus was always** presented on the right. The standard was always a 3cpd straight line grating **stimulus, presented** at 1Hz flicker rate, or a reversal every 500ms. The standard was set to a Michelson contrast level of 0.8. Stimuli were presented for 1 second duration, after which a mid-grey screen was presented. Observers increased or decreased the contrast of the test stimulus by 5% of the current level using the arrow keys, thus the step size was variable, as the percentage of the total contrast level determined the step size – higher contrasts had bigger step sizes The process was then repeated until the observer was satisfied that the stimuli were equal for perceived contrast, indicated using the space key. Each test stimulus was presented 3 times, and an average was taken of the three settings. Average results of contrast matching are presented in figure 2, demonstrating that higher frequencies need more contrast added to make them appear the same as the lower frequency stimuli. For the 5Hz stimuli, there is a main effect of λ ($F_{(1.3, 24.4)}$ = 26.978, **p < 0.001**). There was no statistically significant main effect of μ ($F_{(2,38)}$ = 1.146, **p = 0.329**). The Bayes factor is 2.510, meaning these data are 2.510 times more likely to be observed under the null hypothesis compared to the alternative hypothesis. **The Bayes factor was calculated using a custom-built MATLAB script.** There is a significant interaction between μ and λ ($F_{(4,76)}$ = 3.66, **p = 0.009**). Similarly, for the 10Hz stimuli, there is a significant effect of λ ($F_{(1.38, 26.25)}$ = 44.833, **p < 0.001**). There is no main effect of μ ($F_{(2,38)}$ = 0.165, **p = 0.849**). There is a Bayes factor of 4.158

meaning that the observed data are 4.158 times more likely to be observed under the null hypothesis compared to the alternative hypothesis. There is an interaction between μ and λ ($F_{(4,76)}$ = 4.345, **p = 0.003**). Although average settings are shown in figure 2, each observer was presented with their own individual settings during the main part of the experiment.

**********figure 2 here **********

2.5 Main Experiment Procedure

Each individual was presented with their own perceptually matched stimuli based on their settings from the contrast matching experiment. Each stimulus was presented centrally for 10s. There were 18 stimuli to measure the steady-state responses, 3 levels of μ x 3 levels of λ, x 2 rates of flicker (stimuli flickered at 5Hz and 10Hz). Each stimulus was presented once per block in randomised order, and there were seven blocks, resulting in a total of 70 seconds of presentation time for each of the 18 stimuli. The starting phase of the stimulus was the same each time it appeared. A red fixation cross was presented throughout, and observers were asked to keep as still as possible, fixating the cross. There were no specific instructions regarding blinks, as viewing was to be as natural as possible. The observer initiated each trial with a keyboard press. There was varying onset time, a randomly chosen value between 0.5 and 1.5 seconds for each trial to prevent EEG locking to the start of the presentation (Parker, 1982). After stimulus offset, the observer was asked to enter their responses using the computer keyboard. This involved rating the stimuli using an open-ended magnitude estimation scale – participants could use any value between 0 and 99 to enter their response. In order to limit the effects of observers trying to 'please' the experimenter, observers were deliberately not given any further instruction, but asked to interpret the scale as they saw fit, and it was stressed that there might also be no discomfort to report from these stimuli.

2.6 Analysis

Results were analysed using BrainVision Analyser and MATLAB. EEG responses were decimated to 256Hz, for ease of analysis, and band-pass filtered using a Butterworth filter with 12dB/octave and a time constant of 1.5915 between 0.1 and 70Hz to remove gross artefacts (following Sousza et al, 2007), and notch filtered at 60Hz to remove possible line noise. Channels were re-referenced to the average of all EEG channels (with the exception of the facial electrodes). For the steady-state responses, there were 35 x 2 second segments. A Gratton-Coles (1983) procedure was implemented to correct for eye movement artefacts, namely blinks and saccades. The procedure takes the EOG and EEG signals for each trial, and removes variability related to the stimulus, i.e. subtracts the ERP from all channels (including EOG). Then a “propagation factor” is calculated to describe the relationship between EOG and EEG, and so be able to correct the EEG signal by removing the variability that is explained by the EOG. This is done for both saccades and blink artefacts separately. This technique is claimed to enable researchers to minimise trial rejection, including trials containing eye movement artefacts (Gratton et al., 1983). A threshold of +/-100μV was used as criterion for automatic rejection of artefacts: **Any segment exceeding this threshold was removed**. Data were exported to MATLAB for Fourier analysis. Data from all remaining trials were averaged to obtain a single vector. The amplitude of the response was defined as the amplitude of the 2 x fundamental frequency (second harmonic), 10 and 20Hz respectively. SPSS and also the LME4 function (Bates et

al., 2015), in the package R **(R Core Team, 2013)** were used for the statistical analysis. **In addition, the visreg function (Breheny and Burchett, 2012) and ggplot function (Wickham, 2009) were used to make some of the figures.** Both steady-state VEP, and behavioural responses, were subjected to a 2-way repeated measures ANOVA, with λ and μ as factors, each with three levels. Greenhouse Geisser corrections were used where assumptions of sphericity had been violated.

3. Results

3.1 Discomfort Judgements

Mean discomfort judgements are plotted in Figure 3. For the 5Hz stimuli, there was a statistically significant main effect of λ ($F_{(2,38)}$ = 32.589, **p < 0.001**), but not of μ ($F_{(2,38)}$ = 0.683**, p = 0.511**). The Bayes factor for μ **was 3.141,** meaning the data were **3.141** times more likely to be observed under the null hypothesis compared to the alternative hypothesis. There was no significant interaction effect ($F_{(4,76)}$ = 1.344**, p = 0.261, Bayes factor 2.258 in favour of the null**). **All post-hoc tests averaged over μ were significant (see figure 3 caption).** For the 10Hz stimuli, there is a statistically significant main effect of λ ($F_{(2,38)}$ = 45.616, **p < 0.001**), and a main effect of μ ($F_{(2,38)}$ = 3.524, **p = 0.039**), but no interaction effect ($F_{(4,76)}$ = 1.096, **p = 0.365, Bayes factor 2.553 in favour of the null**). **For significant post-hoc tests see figure 3 caption. Pairwise comparison scatterplots can be seen in supplementary information.**

**********figure 3 here **********

3.2 Steady-State Responses

The average amplitude spectrum for all observers can be seen in figure 4. The 5Hz stimuli are plotted on the top row. This shows clear peaks at 10Hz, 20Hz, 30Hz and 40Hz. The 10Hz flicker is shown on the bottom row. This shows peaks at 20Hz and 40Hz. The spectra of four representative observers can be seen in the supplementary information. There was no difference shown when pooling across electrodes O1, O2, and Oz. This is also included in the supplementary information.

**********figure 4 here **********

Responses were normalised by dividing each signal by the signal for the lowest spatial frequency, waviest line stimulus (λ = 0.5 and μ = 100). Figure 5 shows the normalised responses to the stimuli. For 5Hz stimuli, there is a significant main effect of spatial frequency ($F_{(1.48,28.13)}$ = 4.579, **p < 0.028**). There was no significant effect of μ ($F_{(1.45,27.58)}$ = 0.277, **p = 0.759**, **Bayes factor 3.867 in favour of the null**). There was also no significant interaction effect ($F_{(4,76)}$ = 0.907, **p = 0.465, Bayes factor 2.813).** 10Hz stimuli were also normalised by dividing the peak amplitude by the stimulus of lowest spatial frequency, and highest waviness (λ = 0.5 and μ = 100). For the 10Hz stimuli, there was a significant main effect of spatial frequency ($F_{(2,38)}$ = 8.014, **p = 0.001**). The effect of line waviness was not statistically significant ($F_{(1.37,26.07)}$ = 3.642, **p = 0.055**), however the Bayes factor for the interaction term is **0.774,** suggesting these data are **1.293** times more likely to be observed under the

alternative hypothesis compared to the null hypothesis. There was a significant interaction effect ($F_{(2.00,37.91)}$ = 3.789, **p = 0.032**). **Pairwise comparisons can be seen in the figure caption for figure 5, see also supplementary information for visualisation of pairwise comparisons.**

**********figure 5 here **********

3.3 Eye movements

In order to investigate if there is a systematic effect of the stimulus on eye movements, a bipolar HEOG was calculated. The raw data for the facial electrodes on the left and right outer canthi was taken. Initially this was filtered between 0.1 and 70Hz using a Butterworth filter with 12dB/octave and a time constant of 1.5915, with a notch filter at 60Hz, as in the main EEG analysis. To calculate the bipolar HEOG signal, the signal from the left canthus was subtracted from the right canthus signal. This resulting signal was then divided into one-second epochs, time-locked to the onset of the stimulus, in a similar fashion to event-related potential (ERP) analysis. Segments were averaged to obtain an average time series for each stimulus for each observer, and then averaged again to obtain a time series for each stimulus averaged over all observers. This can be seen in figure 6.

**********figure 6 here **********

From the time series of the average bipolar HEOG signal, it can be seen that the average time course of the eye **movements does not show a systematic response time-locked to the signal**. In order to quantify the response from the bipolar HEOG, the standard deviation of the response over the whole **time series for each observer was taken. The average of the standard deviation over all observers was then taken.** This should show the magnitude of the eye movements. The average of the standard deviation for each stimulus type is plotted in figure 7.

**********figure 7 here**********

There was no statistically significant effect of μ ($F_{(2,38)}$ = 1.809, **p = 0.178**, **Bayes factor 1.8012**). There was also no statistically significant effect of λ ($F_{(2,38)}$ = 0.646**, p = 0.530, Bayes factor 3.2018).** Although there was a statistically significant interaction effect ($F_{(4,76)}$ = 3.053, **p = 0.022**, **Bayes factor 1.008**) on the magnitude of eye movements from the 5Hz stimuli. For the 10Hz stimuli, there was no significant effect of μ ($F_{(1.45,27.59)}$ = 1.029, p = 0.348**, Bayes factor 2.639**). There was no significant effect of λ ($F_{(1.42,27.04)}$ = 0.084, p = 0.857**, Bayes factor 4.278**), and there was no significant interaction effect ($F_{(2.49,47.26)}$ = 1.401, p = 0.256**, Bayes factor 2.196**) on the magnitude of eye movements for the 10Hz stimuli.

3.4 Linear Mixed Effects Model

A linear mixed effects model was used to explore the relationship between discomfort, O1 amplitude, and eye movements. Fixed effects were defined as amplitude of the SSVEP response, spatial frequency, and line waviness of the stimuli. The observers were included as a random variable in the model. A Likelihood Ratio Test was used to compare the model against a null model, with spatial frequency, line waviness as fixed factors, and observer as a random factor. SSVEP amplitude for the 5Hz stimuli did not relate significantly to discomfort judgements ($\chi^2_{(1)} = 1.025$, $p = 0.311$). The difference in Bayesian Information Criterion was 4.2 in favour of the null hypothesis. **Figure 8 shows discomfort judgements against SSVEP amplitude for three levels of spatial frequency for the 5Hz stimuli.**

**********figure 8 here **********

For the 10Hz stimuli, O1 amplitude was found to have a relationship with discomfort ($\chi^2_{(1)} = 5.507$, $p = 0.0189$), lowering it by 13.596 ± 5.534 (standard error). **Figure 9 shows discomfort judgements against SSVEP amplitude for three levels of spatial frequency, low, midrange and high for the 10Hz stimuli.** The difference in Bayesian Information Criterion was 0.3 in favour of the alternative hypothesis, although this is not a strong effect.

**********figure 9 here **********

The relationship between eye movements and discomfort judgements was also explored, using eye movements, spatial frequency, and line waviness as fixed effects, and observer as a random variable. A Likelihood Ratio Test was used to compare the model with a null model consisting of fixed factors of spatial frequency and line waviness, and observer as a random factor. Eye movements showed no relationship with discomfort judgements ($\chi^2_{(1)} = 0.005$, $p = 0.945$) for the 5Hz stimuli. The difference in Bayesian Information Criterion was 5.2 in favour of the null hypothesis. **Figure 10 shows discomfort judgements against eye movements for three spatial frequencies, low, midrange and high.**

**********figure 10 here **********

There was also no relationship between discomfort judgements and eye movements for the 10Hz stimuli ($\chi^2_{(1)} = 3.0625$, $p = 0.080$), although this was approaching statistical significance. The difference in Bayesian Information Criterion was 2.2 in favour of the null hypothesis. **Figure 11 shows discomfort judgements against eye movements for the 10Hz stimuli for three levels of spatial frequency, low midrange and high.**

**********figure 11 here **********

4. Discussion

The aim of this study was to investigate the contribution of cortical activity to visual discomfort using the relative amplitude of steady-state visual evoked potentials as a measure of overall response to op-art based stimuli. There are advantages of using the SSVEP technique over traditional transient methods, in terms of higher signal to noise ratio (Vialette et al., 2010; Norcia et al., 2015). In addition, using dynamic stimuli may limit the effect of fixational eye movements normally present in the static versions of these kind of op-art based stimuli (Troncoso et al., 2008; Zanker et al., 2003; Zanker et al., 2004; Zanker et al., 2010). In the current study, both spatial frequency (λ) and waviness (μ) of the lines were manipulated. If the theory of excessive neural responses being a cause of visual discomfort is correct, the spatial frequency of the underlying grating would have an effect on the electrophysiological response.

4.1 The effect of spatial frequency (λ)

On average, there was a statistically significant effect of spatial frequency (λ) on discomfort judgements, with thinner stripes being judged more comfortable. This is similar to the findings of previous investigations (O'Hare et al., 2015). The peak SSVEP response from the O1 channel was greater for the midrange spatial frequencies compared to the high and low spatial **frequencies. This peak at midrange spatial frequencies resembles the contrast sensitivity function. However, stim**uli in the present study were matched for perceived contrast and therefore any spatial frequency tuning cannot be accounted for by simple differences in contrast sensitivity. If only the midrange and high spatial frequency stimuli were to be considered, then the pattern of O1 channel peak power would match the pattern of discomfort judgements. This result would support the argument of Juricevic et al., (2010), who argued that discomfort could be the result of excessive neural activity.

However, the low spatial frequency stimuli results in this experiment do not support the assertion that discomfort results from excessive cortical activity. Whilst the low spatial frequency stimuli were judged as more uncomfortable than the other spatial frequency stimuli, this was not matched by the O1 channel response magnitude. In the case of the 5Hz stimuli, there was a clear peak in O1 channel amplitude for midrange spatial frequency stimuli compared to both lower and higher spatial frequencies. For the 10Hz stimuli there was again a clear peak for midrange spatial frequency stimuli compared to higher and lower spatial frequencies.

The discrepancy could potentially be explained in terms of eye movements. Flickering stimuli can induce the optokinetic effect, a type of **nystagmus. This is a phenomenon** where the eye follows a moving stimulus and then quickly saccades back repeatedly (commonly seen in observers looking out the windows on a moving train, for example). These eye movements are best elicited using moving stimuli around 10Hz (Bergmann et al., 1963). However, it can also be induced using stationary stimuli, that flicker, for example from stationary stimuli that elicit 'phi motion' (Spillman et al., 1997), from stroboscopic lighting of gratings (Behrens and Grüsser, 1979) and also from reversing gratings (Tong et al., 2003). **The optokinetic effect** is seen with low spatial frequency, high contrast striped stimuli (e.g. Abadi et al., 2005). The optokinetic effect depends on both spatial frequency and

temporal frequency, and it might be that higher spatial frequencies might not strongly elicit the optokinetic effect at the speeds used in the current study (Waddington and Harris, 2015). There was evidence in the time series of the HEOG of a periodic response. However, **this is not conclusive evidence for the** optokinetic effect, as there are many possible contributors to changes in amplitude in the HEOG response. **The bipolar HEOG was greatest for the midrange spatial frequencies, but these are not the optimal stimuli for eliciting such eye movements. As outlined above**, the lower spatial frequency stripes would be more likely to elicit the optokinetic effect. An eye-tracker would be needed to establish exactly what eye movements are in **response to these stimuli. Evidence of a typical saw-tooth movement would indicate the optokinetic effect.**

4.2 The effect of line waviness (μ)

There was a statistically significant effect of line waviness (μ) on average discomfort judgements for the 10Hz stimuli, but this was not statistically significant in the case of the 5Hz stimuli. The Bayes factor for the 5Hz stimuli was 3.1414 meaning that there **may be an effect** of line waviness on discomfort, but it is 3.1414 times more likely that there is not. There is also a significant effect of μ on O1 SSVEP responses in the case of the 10Hz stimuli, but not for 5Hz stimuli. The Bayes factor for the 5Hz stimuli was 3.8669 meaning that it is more likely that there is no effect of line waviness on O1 amplitude than the possibility that there is an effect. In the case of the 10Hz stimuli, the straighter the lines, the greater the discomfort, and the greater the O1 response. This is interesting as previous research has shown μ to have more of an effect on the perception of illusory motion than λ (Hermens and Zanker, 2012). It might therefore be the case that illusory motion and shimmering effects are separate from discomfort originating in the visual cortex, and the two stimulus parameters affect different sources of discomfort, however, at this stage this is speculative.

4.3 The relationship between discomfort and SSVEP

In the current study, there was a statistically significant relationship between the discomfort judgements and O1 amplitude for the 10Hz stimuli only. This effect is very small, and according to the Bayesian Information Criterion difference (approximately 0.3 in favour of the alternative) although statistically significant, only just worth mentioning**. No effect was found** for the 5Hz **stimuli. This is possibly because** there is a lower signal to noise ratio for the 5Hz stimuli compared to the 10Hz stimuli, as there are more reversals overall for the 10Hz stimulation.

4.4 The relationship of discomfort and eye movements

Previous research has shown that eye movements have a role in causing discomfort from periodic stimuli. It has been suggested that illusory motion effects, such as 'shimmering' would arise from fixational eye movements, specifically microsaccades, on viewing these stimuli (Patzwahl and Zanker, 2000; Zanker et al., 2003; Zanker et al., 2004; Troncoso et al., 2008; Zanker et al., 2010). In the current study, gross eye movement artefacts, like blinks and saccades were measured by the facial electrodes, and corrected using the Gratton-Coles (1983) procedure. A separate analysis of bipolar HEOG was conducted, to see if there was any systematic effect of the stimuli on the eye movements themselves. **There were no statistically significant main effects found, and also there was no evidence of a relationship between discomfort judgments and gross eye movements as measured with HEOG. However, HEOG is not sensitive tool for the detection and analysis of eye movements.**

Microsaccades are *fixational* eye movements (Martinez-Conde et al., 2004; Martinez-Conde et al., 2006**), meaning that these will increase when the eyes are static. The current study uses methods to detect gross eye movements, meaning these small movements will likely be missed.** It is possible to detect microsaccades and other fixational eye movements using the facial electrodes of the EEG **(Dimigen et al., 2009). However**, this technique is not widely used. A more convincing method would be to use a high-fidelity eye-tracker. Hermens and Zanker (2012) study measuring microsaccadic response to riloid patterns directly using such an eye tracker no relationship between the stimulus parameters λ and μ and the rate of microsaccades. Additionally, they found either no relationship, or an inverse relationship, between the rate of microsaccades and the reported strength of the illusory motion. The presence of the fixation cross was the one factor reliably influencing the illusion strength, and so the role of microsaccades is less clear. There are many movements of the eyes including blinks, drift, tremor and microsaccades (Martinez-Conde et al., 2004), and the bipolar HEOG is not the optimum tool to characterise them. The main focus of this study is not to dispute that there are eye movements that will contribute to the discomfort judgements, as this has been shown in previous research, but to suggest that there may be a role for neural responses as well.

4.5 The effect of flicker rate

The steady-state VEP responses were higher overall for 5Hz stimuli compared to 10Hz stimuli. This is expected for a typical steady-state VEP response; it has been shown previously that higher frequency stimulation with SSVEP techniques results in smaller response amplitudes (Herrmann, 2001). There are other factors that might contribute to the increased power in the 5Hz compared to the 10Hz stimulation, for example **the** placing of the electrodes. **It is possible that the generators of the response to the 5Hz stimulation are not the same as the generators of the faster 10Hz response, and therefore different electrodes might also show greater response amplitude to one frequency compared to another.**

4.6 Measuring subjective discomfort

Maximum discomfort reported by observers in the current study was the lowest spatial frequency tested (λ = 0.5cpd). Although 3cpd is typically thought of as being the spatial frequency of maximum discomfort (e.g. Evans and Wilkins, 2008), there is some variation in the spatial frequency of maximum discomfort across the previously published literature. The Pattern Glare test is a measure of subjective reports of distortions and discomfort from three striped stimuli, 0.5cpd, 3cpd and12cpd gratings (Evans and Wilkins, 2008). The 3cpd grating is chosen as the standard for maximum discomfort in this test, with the 0.5cpd pattern being used as a control. Using striped patterns in the range of approximately 0.4 to 22 cpd, the observers in the Wilkins et al., (1984) study reported approximately 3cpd as maximally uncomfortable. A separate group demonstrated 4cpd striped patterns to be most uncomfortable (Conlon et al., 2001), whereas Adjamian et al., (2004) quote a range 2-4cpd for the maximally uncomfortable stimuli according to their observers.

Observers in the study by Huang et al., (2003) found 1.2cpd most uncomfortable, compared to higher and lower frequencies. This study compared migraine to control groups, and demonstrated a difference in haemodynamic response between migraine and control for 0.3cpd and 1.2cpd stimuli. This is important because individuals with migraine tend to report more extreme discomfort compared to controls (Marcus and Soso, 1989). In the current study, no chinrest was used for fear of

artefacts, and so the spatial frequencies quoted are approximate. Given the possibility of slight variation of the spatial frequencies in the current study, the quoted values might not be far from the range reported in previous literature. For example, leaning the head backwards by as much as 10cm would result in the stimuli being 0.62, 3.73 and 11.19cpd instead of 0.5, 3, and 9cpd. This is a difference, but not a substantial one.

As there are different contributing factors to discomfort, the task of the observers was to respond only to discomfort. The expected source of discomfort was not specified, in order to limit bias from experimenter expectations. This has the disadvantage that the observer responses are not specific. For example, one individual might report more eyestrain, possibly due to excessive saccadic eye movements, whereas another might be more susceptible to headache-type symptoms. A recent study by Monger et al., (2015) showed high spatial frequency grating (19cpd) to have the most number of people reporting certain illusions such as shimmering, and fading, even though maximum overall discomfort is reported from the 2.3cpd gratings used in their study. This could be explained as the discomfort arising from the higher spatial frequencies are using a different mechanism compared to the midrange spatial **frequencies. For example**, there might be increased microsaccades in the higher spatial frequency stimuli that contribute to the discomfort response, whereas other sources of discomfort e.g. excessive neural responses, might be contributing in the case of the midrange spatial frequency stimuli. This study highlights the need for future work to investigate the qualitative aspects of discomfort as well, to try and assess which mechanism is predominantly causing discomfort in any particular stimulus.

5. Conclusion

Discomfort judgements showed clear spatial frequency tuning with least discomfort from the high spatial frequencies compared to lower spatial frequencies. Ignoring the low spatial frequency stimuli, discomfort judgements follow the same pattern as the steady-state VEP responses – lower responses for the high compared to the midrange spatial frequencies. Although a small effect, discomfort shows some relationship to steady-state VEP responses.

For the low spatial frequencies there is a different pattern of results. These are the most uncomfortable but they do not show the greatest steady-state VEP response from the early visual areas. Although low spatial frequency flickering stimuli **can drive** the optokinetic response, **there was no increase in either gross eye movements.** In summary, there is a contribution of early visual area brain responses to discomfort from op-art based stimuli, but also it is likely that eye movements affect the discomfort arising from these stimuli.

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Abbreviations

EEG: electroencephalogram; EOG: electrooculogram; SSVEP: steady state visual evoked potential; VEP: visual evoked potential.

Data Accessibility

Supporting information and raw data are available through Figshare.

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