

Supplemental Data

Title: Enhanced, ipRGC-mediated reflexive eye closure is specific to migraine with aura

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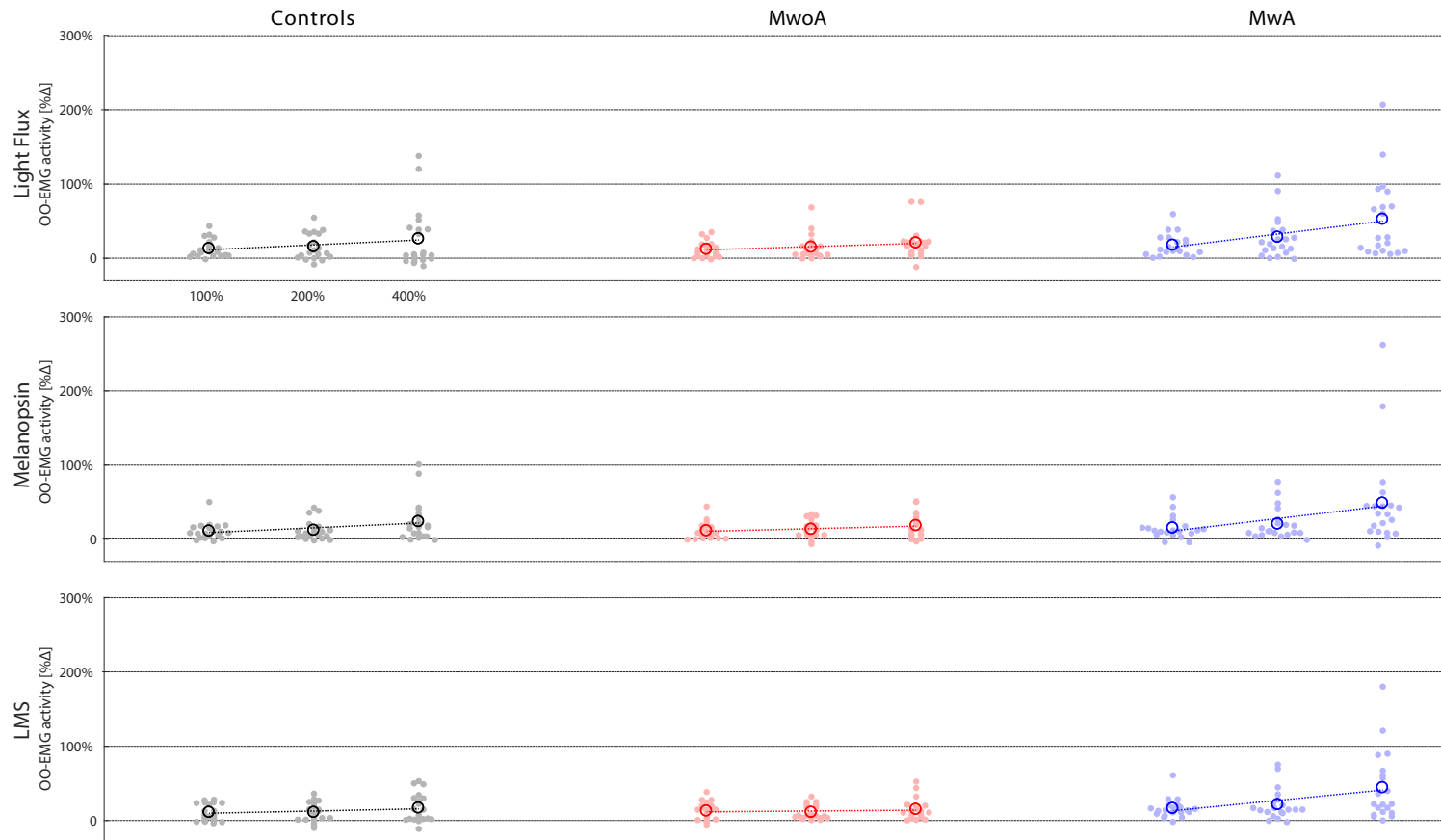
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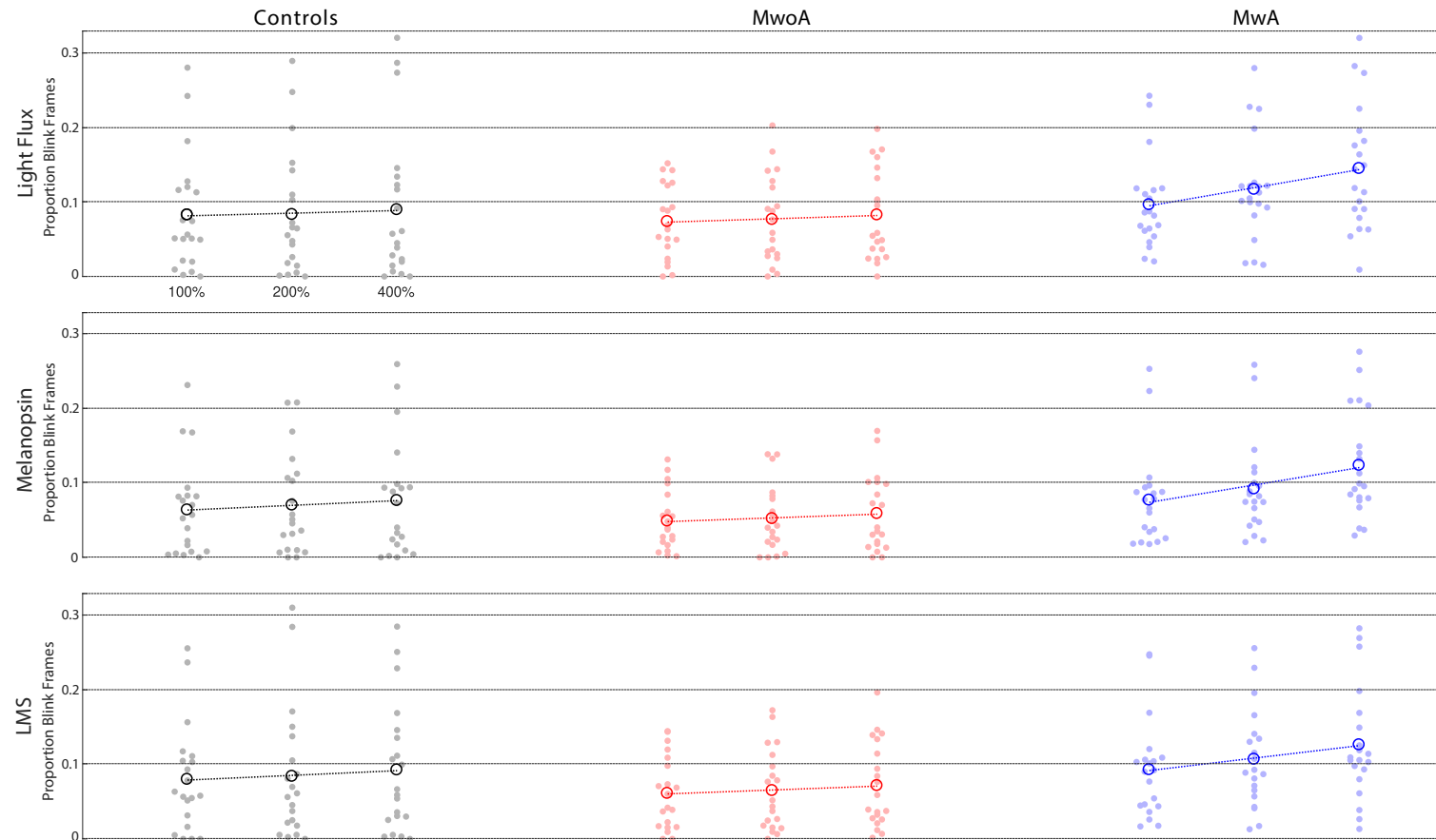
Analysis results using the pre-registered parameters:

Our pre-registration had proposed a 4000-msec window, shifted 1000 msec after stimulus onset. We quantified the effect of our stimuli upon the OO-EMG and blink measure by calculating the average response over this 4000-msec window starting 1000 msec after pulse onset and ending 1000 msec prior to pulse offset. This in contrast to our primary analysis in which we analyzed the data within a 3400-msec window starting 300 msec after pulse onset and ending 300 msec prior to pulse offset. That window was selected for our primary analysis to avoid the influence of blinking at stimulus onset or offset in the measured response.

When the OO-EMG data are analyzed using this 4000-msec temporal window, we find similar results to 3400 msec window, albeit somewhat attenuated by the incorporation of the non-specific blinking response at stimulus offset (Supplementary Figure 1, Supplementary Tables 1-2). We also observe similar results with the blinking activity data, but again find they are attenuated due to inclusion of the non-specific blinking response at stimulus offset (Supplementary Figure 2, Supplementary Tables 3-5).



Supplemental Figure 1. Orbicularis oculi electromyography (OO-EMG) activity by stimulus and group. Each row presents the OO-EMG activity evoked by stimuli that targeted a particular combination of photoreceptors, and each column contains the data from each individual group ($n = 20$ participants per group). The stimuli were presented at three different contrast levels (100, 200, and 400%), and these (log-spaced) values define the x-axis of each subplot. The mean (across trial) OO-EMG activity for a given stimulus and contrast is shown for each participant (filled circle), as is the mean OO-EMG activity across participants (open circle). The best fit line to the mean OO-EMG activity across participants as a function of log contrast is shown in each subplot. OO-EMG activity over time was quantified by calculating the standard deviation of voltage activity within a 500 msec window, sliding across time, which is expressed as percentage change [%Δ] relative to activity occurring prior to pulse onset. From these normalized time series, we calculated the average evoked response over a 4000 msec window starting 1 second after pulse onset.



Supplemental Figure 2. Blink activity by stimulus and group. Each row presents the blink activity evoked by stimuli that targeted a particular combination of photoreceptors, and each column contains the data from each individual group ($n = 20$ participants per group). The stimuli were presented at three different contrast levels (100, 200, and 400%), and these (log-spaced) values define the x-axis of each subplot. The mean (across trial) blink activity for a given stimulus and contrast is shown for each participant (filled circle), as is the mean blink activity across participants (open circle). The best fit line to the mean blink activity across participants as a function of log contrast is shown in each subplot. Blink activity is expressed as the proportion of frames of the video recording in which the eyelid covered the eye over a 4000 msec window starting 1 second after pulse onset.

Group comparison	Mean difference	t-statistic	p-value
MwA - Controls	26.0	2.385	0.053
MwA - MwoA	30.9	2.830	0.017*
MwoA - Controls	-4.9	0.445	0.897

Supplemental Table 1. Post-hoc analyses of group effects in orbicularis oculi EMG activity at 400% contrast. We examined the effect of group upon the mean OO-EMG activity using a 3-by-3 mixed effects ANOVA. This analysis was conducted over a 4000-msec response window starting 1 second after pulse onset as per our preregistration document. Measured EMG activity across stimulus types (melanopsin, cones, and light flux) were averaged within subjects. The independent variables were by group (MwA, MwoA, and control) as a between subjects factor. Post-hoc testing was conducted using the Tukey procedure. *p-value < 0.05.

Stimulus	Group comparison	Mean difference	t-statistic	p-value
<i>Light Flux</i>	MwA - Controls	26.2	2.036	0.113
	MwA - MwoA	32.3	2.508	0.039*
	MwoA - Controls	-6.1	-0.472	0.885
<i>Melanopsin</i>	MwA - Controls	24.7	1.893	0.150
	MwA - MwoA	30.7	2.352	0.057
	MwoA - Controls	-6.0	-0.458	0.891
<i>Cones</i>	MwA - Controls	27.1	2.854	0.016*
	MwA - MwoA	29.6	3.116	0.008*
	MwoA - Controls	-2.5	-0.262	0.963

Supplemental Table 2. Post-hoc analyses of group effects by stimulus type in orbicularis oculi EMG activity at 400% contrast. We examined the effect of group and stimulus upon the mean OO-EMG activity using a 3-by-3 mixed effects ANOVA. This analysis was conducted over a 4000-msec response window starting 1 second after pulse onset as per our preregistration document. Independent variables included group (migraine with aura, without aura, and control) as a between subjects factor and photoreceptor target (melanopsin, cones, and light flux) as a within subjects factor. Post-hoc testing was conducted using the Tukey procedure. *p-value < 0.05.

Group comparison	Mean difference	t-statistic	p-value
MwA - Controls	0.0459	1.958	0.132
MwA - MwoA	0.0614	2.614	0.030*
MwoA - Controls	-0.0154	0.656	0.790

Supplemental Table 3. Post-hoc analyses of group effects in blink activity at 400% contrast. We examined the effect of group upon the mean proportion of blink frames using a 3-by-3 mixed effects ANOVA. This analysis was conducted over a 4000-msec response window starting 1 second after pulse onset as per our preregistration document. Responses across stimulus types (melanopsin, cones, and light flux) were averaged within subjects. The independent variables were by group (MwA, MwoA, and control) as a between subjects factor. Post-hoc testing was conducted using the Tukey procedure. *p-value < 0.05.

Stimulus	Group comparison	Mean difference	t-statistic	p-value
<i>Light Flux</i>	MwA - Controls	0.0559	2.109	0.097
	MwA - MwoA	0.0630	2.378	0.053
	MwoA - Controls	-0.0072	-0.270	0.961
<i>Melanopsin</i>	MwA - Controls	0.0479	2.193	0.081
	MwA - MwoA	0.0657	3.007	0.011*
	MwoA - Controls	-0.0178	-0.814	0.696
<i>Cones</i>	MwA - Controls	0.0341	1.434	0.330
	MwA - MwoA	0.0554	2.329	0.060
	MwoA - Controls	-0.0213	-0.894	0.646

Supplemental Table 4. Post-hoc analyses of group effects by stimulus type in blink activity at 400% contrast. We examined the effect of group and stimulus upon the mean proportion of blink frames using a 3-by-3 mixed effects ANOVA. This analysis was conducted over a 4000-msec response window starting 1 second after pulse onset as per our preregistration document. Independent variables included group (migraine with aura, without aura, and control) as a between subjects factor and photoreceptor target (melanopsin, cones, and light flux) as a within subjects factor. Post-hoc testing was conducted using the Tukey procedure. *p-value < 0.05.

Group	Stimulus comparison	Mean difference	t-statistic	p-value
Controls	LightFlux - Melanopsin	0.0133	1.770	0.189
	LightFlux - LMS	-0.0030	-0.514	0.865
	LMS - Melanopsin	0.0163	2.403	0.050
MwoA	LightFlux - Melanopsin	0.0239	3.187	0.007*
	LightFlux - LMS	0.0111	1.889	0.151
	LightFlux - Melanopsin	0.0128	1.889	0.151
MwA	LightFlux - Melanopsin	0.0213	2.835	0.017*
	LightFlux - LMS	0.0187	3.189	0.006*
	LMS - Melanopsin	0.0025	0.373	0.926

Supplemental Table 5. Post-hoc analyses of stimulus type effects by group in blink activity at 400% contrast. We examined the effect of group and stimulus upon the mean proportion of blink frames using a 3-by-3 mixed effects ANOVA. This analysis was conducted over a 4000-msec response window starting 1 second after pulse onset as per our preregistration document. Independent variables included photoreceptor target (melanopsin, cones, and light flux) as a between subjects factor and group (migraine with aura, without aura, and control) as a within subjects factor. Post-hoc testing was conducted using the Tukey procedure. *p-value < 0.05.