Constable, P. A., Gaigg, S. B., Bowler, D. M., Jägle, H. & Thompson, D. A. (2016). Full field electroretinogram in autism spectrum disorder. Documenta Ophthalmologica, 132(2), pp. 83-99. doi: 10.1007/s10633-016-9529-y

Full field electroretinogram in autism spectrum disorder

Paul A Constable [1,2], Sebastian B Gaigg [2], Dermot M Bowler [2], Herbert Jägle [3], Dorothy A Thompson [4]

1-Department of Optometry and Vision Science, Flinders University, Adelaide, Australia

2- Autism Research Group City University London, London UK

3- Department of Ophthalmology, University Clinic, Regensburg, Germany

4- Clinical and Academic Department of Ophthalmology, Great Ormond Street Hospital for Children, Great Ormond Street London UK

Corresponding author

Paul Constable

Optometry and Vision Science

Flinders University

GPO Box 2100

Adelaide SA 5001

Australia

Ph + 61 (0)8 7221 8398

Email: paul.constable@flinders.edu.au

Abstract

Purpose

To explore early findings that individuals with autism spectrum disorder (ASD) have reduced scotopic ERG b-wave amplitudes.

Methods

Dark adapted (DA) ERGs were acquired to a range of flash strengths, (-4.0 to 2.3 log phot cd.s.m^{-2}), including and extending the ISCEV standard, from two subject groups: (ASD) N=11 and (Control) N=15 for DA and N=14 for light adapted (LA) ERGs who were matched for mean age and range. Naka-Rushton curves were fitted to DA b-wave amplitude growth over the first limb (-4.0 to -1.0 log phot cd.s.m^{-2}). The derived parameters (V_{max}, K_m and n) were compared between groups. Scotopic 15 Hz flicker ERGs (14.93Hz) were recorded to 10 flash strengths presented in ascending order from -3.0 to 0.5 log Td.s to assess the slow and fast rod pathways respectively. LA ERGs were acquired to a range of flash strengths, (-0.5 to 1.0 log phot cd.s.m^{-2}). Photopic 30 Hz, flicker ERGs, oscillatory potentials (OPs) and the responses to prolonged 120 ms ON- OFF stimuli were also recorded.

Results

For some individuals the DA b-wave amplitudes fell below the control 5th centile of the controls with up to four ASD participants (36%) at the 1.5 log phot cd.s.m^{-2} flash strength and two (18%) ASD participants at the lower -2 log phot cd.s.m^{-2} flash strength. However, across the thirteen flash strengths there were no significant group differences for b-wave amplitude's growth (repeated measures ANOVA p=0.83). Nor were there any significant differences between the groups for the Naka-Rushton parameters (p>0.09). No group differences were observed in the 15Hz scotopic flicker phase or amplitude (p>0.1), DA ERG a- wave amplitude or time to peak (p>26). The DA b-wave time to peak at 0.5 log phot cd.s.m^{-2} were longer in the ASD group (corrected p=0.04). The single ISCEV LA 0.5 log phot cd.s.m^{-2} (p<0.001) was lower in the ASD group. Repeated measures ANOVA for the LA series was also significantly (p=0.01) different between groups. No group differences were observed for the LA a-wave, b-wave time to peak or the photopic negative responses (phNR) (p>0.08) to the single flash stimuli although there was a significant interaction between group and flash strength for the b-wave amplitude (corrected p=0.006). The prolonged 120 ms ON-responses were smaller in the ASD group (corrected p=0.003), but the OFF response amplitude (p>0.6) and ON and OFF times to peaks (p>0.4) were similar between groups. The LA OPs showed an earlier bifurcation of OP2 in the younger ASD participants, however no other differences were apparent in the OPs or 30Hz flicker waveforms.

Conclusion

Some ASD individuals show subnormal DA ERG b-wave amplitudes. Under LA conditions the b-wave is reduced across the ASD group along with the ON response of the ERG. These exploratory findings, suggest there is altered cone-ON bipolar signalling in ASD.

Keywords:

Autism Spectrum Disorder, electroretinogram, Naka-Rushton, ON-pathway 15Hz flicker

Introduction

Autism is a neurodevelopmental disorder of unknown aetiology with a prevalence of ~1:100 and a predilection for affecting males [1,2]. The term autism or autistic spectrum disorder (ASD) is characterised by impairments in reciprocal social interaction, imagination and language development. With early intervention and diagnosis [3] these difficulties can be helped, but ASD remains a lifelong condition that impacts upon an individual's and family's quality of life [4,5].

ASD individuals display morphological differences of the cerebral cortex [6,7] and Genome Wide Association Studies have linked ASD with variations in genes associated with neural development and synaptic transmission [8-11]. One important gene network links the metabolic glutamate receptor (mGluR) family and autism [12]. Of this family the mGluR6 receptors are recognised as being critical for the ERG b-wave generation [13]. Alterations in CNS development, final organisation and neurotransmitter signalling in ASD may manifest therefore as altered retinal signalling [14,15]. The retinal signalling pathways are broadly divided between vertical excitatory pathways, comprising photoreceptors, bipolar cells and ganglion cells and horizontal inhibitory pathways formed by amacrine and horizontal cells. Glutamate is the major excitatory neurotransmitter and γ-amino butyric- acid (GABA) and dopamine the main inhibitory neurotransmitters. Altered neurotransmitter receptor functions for these neurotransmitters have also been implicated in the ASD phenotype [16-18] and may additionally contribute to differences in retinal signalling and responses.

The ERG is a non-invasive clinical tool whose waveform can be related to specific neural pathways, neurotransmitters and their receptors [15,19]. Should the ERG detect differences in retinal signalling in ASD it could become a useful monitor of novel drugs targeting the CNS [20,21]; for example emerging therapeutic agents for ASD are targeting metabotropic glutamate gene networks and receptor groups [21]. Retinal studies of patients with schizophrenia and their children lend support to this hypothesis [22-25]. Schizophrenia and ASD have common genetic variants [26,27] and individuals with schizophrenia exhibit autism like traits [28]. Individuals with a high genetic risk of schizophrenia have reduced dark adapted (DA) b-wave amplitudes at the plateaux of the Naka-Rushton function [23]. This parallels a report of small amplitude scotopic ERG b-waves in ASD published 25 years ago, [29,30]. More recently, these findings were confirmed in schizophrenic individuals, who also showed reduced mixed rod-cone b-wave amplitude and reduced light adapted (LA) b-wave at the peak of the photopic hill [22]. Given the genotypic and phenotypic overlap between ASD and schizophrenia, it is of interest to see if these ERG profiles are found in ASD.

Individuals with ASD often show peculiarities in visual tasks, one of the strongest is their ability to find a specific shape or object within a hidden pattern [31] as well as displaying superior visual search strategies [32,33]. Their orientation discrimination thresholds are superior for detecting simple-first luminance defined grating patterns over more complex- second order texture defined grating patterns [34]. Cortical

electrophysiological findings [35] also support evidence for abnormal motion perception in ASD [36]. Whilst, the higher level visual tasks have been extensively studied: for reviews see Dakin and Frith (2005) [37] and Simmons et al (2009) [38], there have been fewer studies into the primary visual sense in ASD; and in particular the retinal response to luminance. This is despite evidence for increased pitch, tone [39,40] and decreased odour [41] discrimination in ASD supporting altered sensory processing in ASD. A recent study has found reduced pupillary constriction in ASD suggesting a difference in light sensitivity in this population [42] which is also supported by decreased peripheral visual field sensitivity in ASD [43]. Given the differences in sensory discrimination that are observed in ASD [44], the ERG may be able to discern if the retinal response to light contributes to these differences.

Our main aim was to follow up two early reports, predating ISCEV standards that showed reduced ERG scotopic b-waves in ~ 50-60% of autistic individuals [30]. The ERG may be able to identify a sub-set of individuals with ASD who have atypical neural signalling likely to be related to glutamate signalling. One limitation of the original studies of the ERG in ASD was that the authors did not conduct a full luminance response series. Therefore, we extended the DA ERG findings in a high functioning ASD population to test whether a reduced b-wave is a feature of these individuals and to see how the a- and b-wave amplitudes develop over an extended flash luminance range. In addition we explored the slow and fast rod pathways in ASD by determining the amplitudes of 15Hz flicker and the flash strength of phase reversal, where the temporal responses of the slow and fast rod pathways [45] cancel each other [46,47]. We also explored the LA ERG with particular emphasis on b-wave analysis given its dependence on glutamate signalling and two classes of receptors (*metabolic and ionotropic glutamate receptor families)* implicated in ASD [12,17].

Methods

Participants

A total of 11 ASD participants and 15 typically developing (Control) participants performed DA full field ERGs and 14 LA full field ERGs. The mean age and range of the control participants group were matched with the ASD group [ASD = 37.2 ± 13.2 (range 13.8-57.6 years, 10 male : 1 female, (p=0.90)) and Control (DA) = 36.9 ± 13.2 (range 12.0-58.0 years, 11 male : 4 female)] and Control (LA) = 35.3 ± 12.9 range 14.0-58.0, 11 male 3 female, p=0.96).

Participants with ASD were diagnosed according to conventional criteria and a review of available medical records and in 10/11 participants' assessment with the Autism Diagnostic Observational Schedule (ADOS) [48] confirmed that all met DSM-IV-TR [49] criteria for ASD (total score 11 ± 3 with range 5-15). One of the individual's diagnosis was based on a clinical assessment made by local health authorities and experienced clinicians. The ASD participants were high functioning adults with a mean ± SD full IQ of 116 ± 10 measured using the Wechsler Adult Intelligence Scale (WAIS-III^{UK}) [50]. Their autism quotient scores were 34 ± 9, which is within the ASD range; the cut-off between typical and ASD groups is 27 [51]. IQ data was not available for all of the control participants and we were not able to match participants on IQ. However, IQ is not known to affect the ERG recordings unlike other physical factors such as age [52]. Individuals gave their written and

informed consent before taking part and were paid standard University fees for their participation. Ethical approval was granted by University Ethics committee and complied with the tenets of the declaration of Helsinki. Participants were excluded if they had any history of ocular surgery, diabetes, epilepsy or were taking systemic or ocular medications that interact with the CNS. All included participants had normal monocular corrected Snellen acuities of at least 6/6, (logMAR equivalent 0).

ERG Recordings

ISCEV protocols for full field ERG recording were followed [53]. All eyes were fully dilated with 1.0% tropicamide. DTL Plus electrodes, (Diagnosys LLC, MA10854, USA) were placed across the limbus and referenced to Ag/AgCl gel sticker electrodes placed at each outer canthus and a ground electrode placed on the forehead. Each participant was dark adapted for 20 minutes before binocular stimulation to an ascending range of flash strengths with a colordome stimulator Diagnosys Espion^2 (Diagnosys, Oxford, UK). DA full field ERGs were elicited to flash stimuli of strengths -4.0 to 2.3 log phot cd.sec.m^{-2} in 0.5 log phot cd.sec.m^{-2} steps using 4 ms white flashes generated by LEDs (colour temperature 6,500K) or xenon tube as required (200 phot cd.s.m^{-2}) presented scotopically. ERGs were acquired in a time window of 250 ms, that included a 20 ms pre-stimulus interval and typically several trials were averaged for each flash strength. Additionally, DA scotopic 15 Hz flicker ERGs (14.93Hz) were elicited to 10 flash strengths presented in ascending order from -3.0 to 0.5 log Td.s testing slow and fast rod pathways respectively [54]. Flicker ERGs to each of these stimuli were acquired within a 402ms time window containing six periods and response magnitude, and phase were determined by Fourier analysis. Response waveform significance was estimated [55]. For the LA full field ERGs, participants were light adapted for 10 minutes (30 cd.m^{-2}) before binocular stimulation to an ascending range of flash strengths -0.5, 0.0, 0.5, 0.7 and 1.0 log phot cd.sec.m^{-2} using 4 ms white flashes. ERGs were acquired in a time window of 250 ms, including a 20 ms pre-stimulus interval with several trials averaged for the flash strengths. Additionally, photopic 30 Hz flicker ERGs at 0.5 log phot cd.s.m^{-2} and ON/OFF responses to an extended flash of 133 cd.m^{-2} presented for 120 ms at LA 43 cd.m^{-2} were acquired in a 345 ms time window, which included a 15 ms pre stimulus interval. LA OPs were extracted by filtering between 100 and 300Hz from the 0.5 log phot cd.s.m^{-2} single flash.

The position of the DTL electrode was checked following the recordings to ensure it was still positioned at the lower limbus with the subject's fixation monitored with an IR camera throughout the recordings.

ERG Analysis

The ERG traces were analysed in accordance with the ISCEV standard [53]. Traces were examined off-line for both eyes' recordings and individual traces removed manually if there were blink or movement artefacts. The ERG from the eye with the larger amplitude for each individual was used in the final statistical analysis. Amplitudes and time to peak for the a- and b- waves were measured from the averaged traces following artefact rejection. The 0.5 and 1.0 log phot cd.s.m^{-2} waveforms were used to calculate the b:a wave ratios and the 1.0 and

2.3 log phot cd.s.m^{-2} were used to compare a-wave parameters as the a-wave trough is more clearly defined to higher flash strengths. The phNR was measured from the b-wave peak to the subsequent trough.

Naka-Rushton Parameters

The Naka-Rushton function describes the change in retinal response with increasing flash luminance. It is a logistic growth function and is expressed by equation 1. The parameter V (µV) represents the b-wave amplitude to a flash strength I (cd.s.m^{-2}). The value V_{max} is the maximal response at the asymptote of the function. K_m is the semi-saturation constant at which the flash strength elicits a response equal to $0.5V_{max}$. The dimensionless constant *n* of defines the slope of the function. The parameters are believed to represent three aspects of retinal function with K_m associated with retinal sensitivity, *n* with retinal homogeneity and V_{max} with retinal responsiveness [56].

$$V= [V_{max}. I / (K_m + I)]^n \quad \ldots\ldots\ldots 1$$

The parameters for the Naka-Rushton function were derived from regression line analysis of the transformed fitted raw data [56]. To derive the three Naka-Rushton parameters b-wave amplitudes were plotted against flash strength and a natural log curve fitted and K_m calculated from equation 2 generating the first transformed function from which K_m could be derived. The value of V_{max} was set to 1% greater than the highest amplitude before the second limb in the single flash intensity series from -4.0 to -1.0 log phot cd.s.m^{-2} to ensure the logistic function only included values up until the first plateau derived from the rod driven pathway [56]. One participant, ASD69 (M, 54.3 yo; ADOS =10) showed an atypical drop at flash strength -1.0 log phot cd.s.m^{-2} which affected the fit of the Naka-Rushton function and so in this case the response to -0.5 log phot cd.s.m^{-2} was used to estimate the parameters.

$$V_{max}/2= a.\ln(K_m) + c \quad \ldots\ldots\ldots 2$$

With the values of V_{max} and K_m derived a second linear function was plotted using the calculated V_{max} value and the K_m from the first transformed function, with V/V_{max} vs $(1+K_m)^{-1}$. The slope of the fitted line represents the value *n* in the Naka Rushton equation: equation 3.

$$V/V_{max} = n[(1+K_m)^{-1}] + c \quad \ldots\ldots\ldots 3$$

In addition the b-wave amplitude was compared between groups at the plateau of the lower limb of the Naka-Rushton function which occurred at -1 log phot cd.s.m^{-2} which represents a mainly rod V_{max} response and at 1 log phot cd.s.m^{-2} representing a fixed point corresponding to a mixed rod-cone contribution to the DA b-wave. In addition a fixed point V_{max} for the LA responses as taken as the control peak of the photopic hill as previously described [22].

Statistics

For between group differences of amplitude, time to peak and flash strength the non-parametric Kruskal-Wallis test was used with follow-up pair wise Mann-Whitney test for significant group differences with post hoc Bonferroni correction for multiple comparisons, as well as a between group repeated measures for the DA and LA b-wave amplitudes (SPSS version 22.0). Figures show the group median and 5^{th} and 95^{th} centile range calculated by OriginPro2015 using resampling with replacement to determine the 5^{th} and 95^{th} confidence intervals (CI) for the ERG responses'.

Results

Dark Adapted ERG

The group results for a- and b-wave amplitudes and times to peak are shown in table 1. There were no significant group differences for the a- and b-wave parameters for the ISCEV standard flashes ($p>0.09$).

Flash Strength (log phot cd.s.m^{-2}) ERG measurement	**ASD**		**Control**		**p**
	median	**5^{th} to 95^{th} percentile**	**median**	**5^{th} to 95^{th} percentile**	
-2.0 b-wave amplitude (μV)	204	126-326	241	157-457	0.18
-2.0 b-wave time to peak (ms)	90	65-120	91	78-105	0.88
0.5 b-wave amplitude (μV)	270	195-504	352	227-596	0.24
0.5 b-wave time to peak (ms)	53	45-58	47	35-53	0.04*
0.5 a-wave amplitude (μV)	155	125-272	207	113-340	0.26
0.5 a-wave time to peak (ms)	15	14-23	16	14-25	0.31
1.0 b-wave amplitude (μV)	278	222-538	370	260-528	0.13
1.0 b-wave time to peak (ms)	52	37-59	46	33-57	0.06*
1.0 a-wave amplitude (μV)	227	144-350	230	153-420	0.74
1.0 a-wave time to peak (ms)	13	11-16	13	11-22	0.56
2.3 a-wave amplitude (μV)	296	157-451	316	220-482	0.47
2.3 a-wave time to peak (ms)	9	8-13	8	7-10	0.59

Table 1. Amplitudes and time to peak of the a- and b-waves of the DA full field ERGs for the ASD and control group at ISCEV flash luminances are tabulated. Amplitudes in microvolts and time to peaks are in milliseconds. * indicates Bonferroni corrected p-value for multiple tests.

a-wave

The a-waves of the control and ASD group were not significantly different in their amplitudes or time to trough. The 1.0 and 2.3 log phot cd.s.m^{-2} ERGs were used for these inter-group comparisons as the a-wave is well defined and timing unambiguous at higher flash strengths. The median ASD amplitude and time to trough were 296µV (157-451µV) and 9ms (8-13ms) and for the control group 316µV (220-482µV) and 8ms (7-10ms) at 2.3 log phot cd.s.m^{-2}. There were no significant group differences for time to trough ($p>0.56$) nor amplitude ($p>0.47$) indicating no differences in phototransduction in the DA state.

b-wave

The b-wave amplitudes at low flash strengths were not significantly different between groups ($p>0.13$), although the b-wave amplitudes of two of the ASD group at the -2 log phot cd.s.m^{-2} were below the 5th centile of the control group (157 µV) with b-wave amplitudes of 126 (ASD105) and 152 µV (ASD 121). The range of these control data for the ISCEV standard flash ERGs were comparable to published data recorded from 53 healthy 25-50 year olds [52] (See Fig 1). Following the rod dominated lower limb the b-waves of the ASD group had a higher proportion (4/11 or 36%) that fell below the control 5th centile at flash strengths of 1.0 and 1.5 log phot cd.s.m^{-2}.

There was a slight delay in the time to peak of the b-waves at higher flash strengths (corrected $p=0.04$ and $p=0.06$) for the 0.5 and 1.0 log phot cd.s.m^{-2} flash strengths respectively. Overall there was a trend for lower b-wave amplitudes in the ASD group compared to the control group. Figure 1 shows the full luminance response range from -4.0 to 2.3 log phot cd.s.m^{-2}.

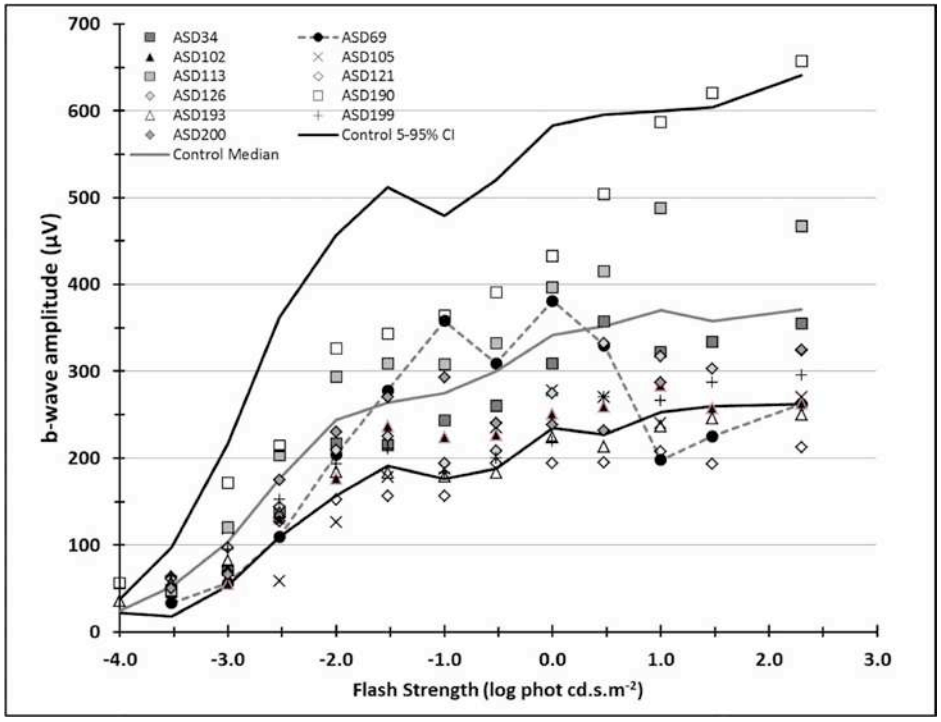

Fig 1 The full DA luminance response series for the ASD and control group demonstrating that some of the ASD participants responses were outside the 5% confidence interval of the control group. ASD69 (filled circles)

showed a dip in the luminance response function at -0.5 log phot cd.s.m^{-2} (dotted line). The control 5-95% CI (black solid line) and the control median (solid grey line) shown.

Atypical Electroretinograms

Figure 2 shows the DA luminance response series for ASD121 (M aged 26.3 years, ADOS: 5) and ASD69 (M aged 51.7 years, ADOS: 10). For ASD69 there is a normal growth of the lower limb that is rod dominated up until -1.0 log phot cd.m.s^{-2} where there is a decrease in the second limb as flash strength increases and the cone responses contributes. Throughout the recording the eye position with respect to the electrode was monitored and this alteration is not attributable to electrode position. In comparison ASD121 exhibits a reduced b-wave amplitude profile across the series of flash strengths from -4.0 log phot cd.s.m^{-2} to 2.3 log phot cd.s.m^{-2}. The LA responses for ASD69 were at the lower limit of the 5% ASD centile but we assume that there were additional unknown factors affecting the cone response under DA conditions in this case given electrode placement was not a factor.

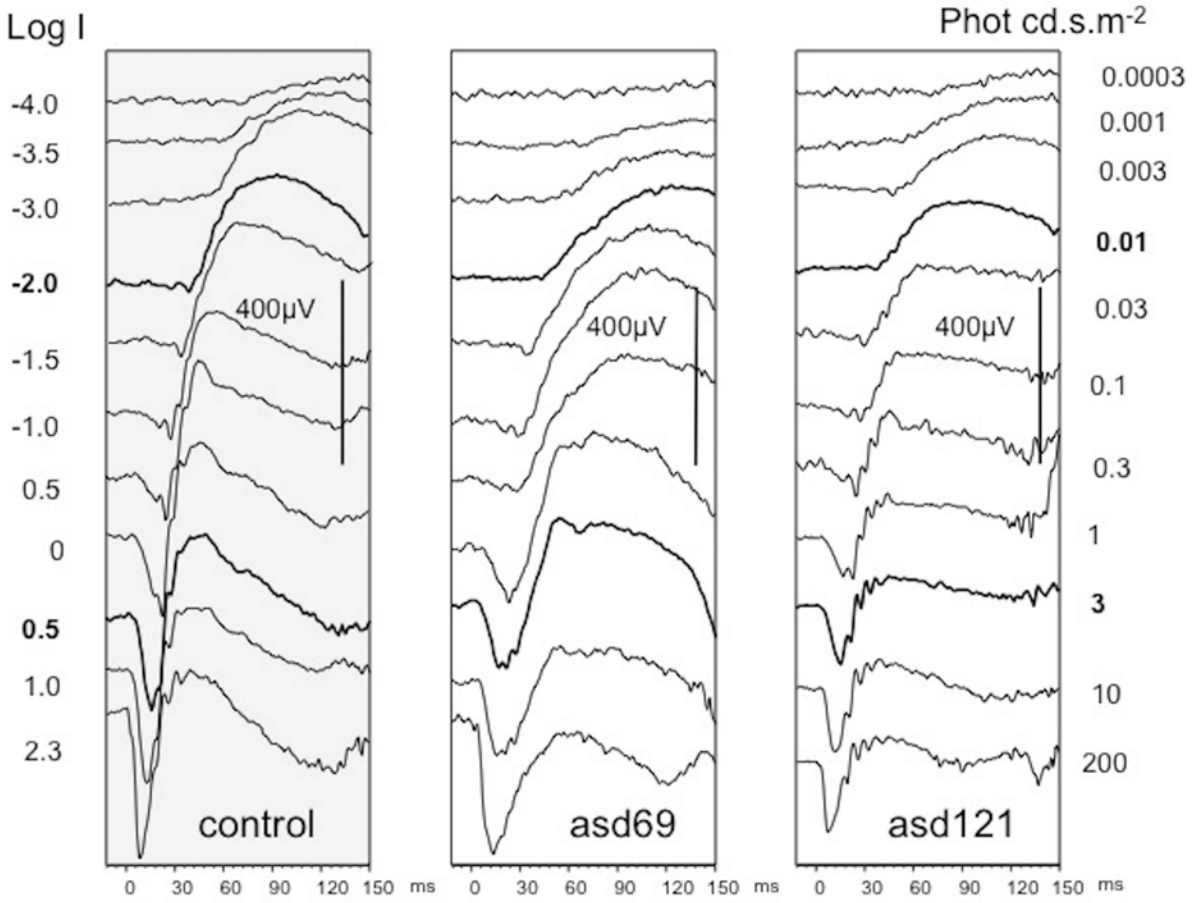

Fig 2 Luminance response series of a typical control and two atypical participants. One individual (ASD69) the b-wave amplitude is reduced after -1.0 log phot cd.s.m^{-2} and restricted to the second limb of the luminance response series. In comparison ASD121's b-wave amplitudes are reduced across the series from -4.0 to 2.3 log phot cd.s.m^{-2}.

Figure 3 shows the 0.5 log phot cd.s.m^{-2} waveform for ASD69. For comparable a-wave amplitude and time to peak, the ASD b-wave is relatively smaller. This suggests a post-receptoral difference in function in the individuals with reduced DA b-wave amplitudes as all a-wave parameters were non-significant between groups ($p>0.47$).

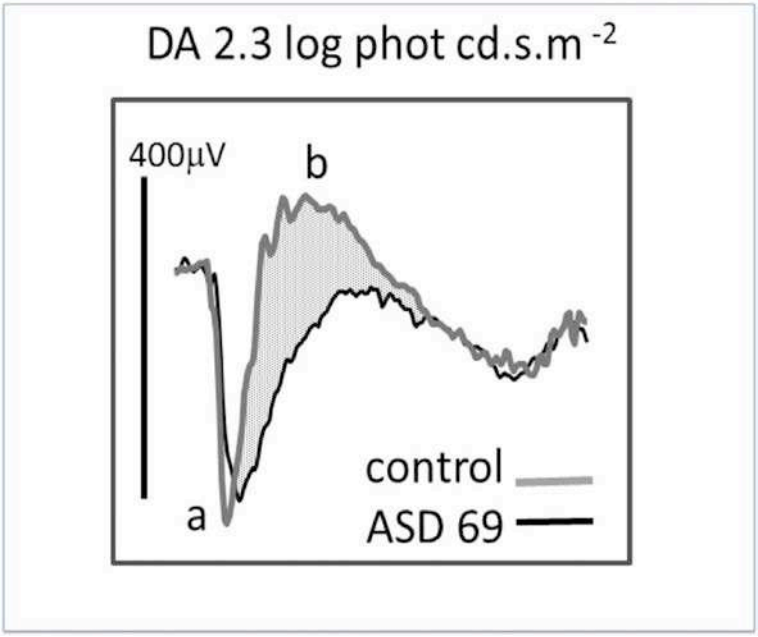

Fig 3 Waveform for ASD69 at 2.3 log phot cd.s.m^{-2} (DA 200) and a control trace from Figure 2. Are overlaid to illustrate the selective reduction in the b-wave compared to the a-wave. (a: a-wave; b: b-wave).

b:a ratios

Group b:a ratios for ASD participants and controls to ISCEV dark adapted 0.5 log phot cd.s.m^{-2} were not significantly different: median b:a ratio ASD 1.79, range 1.38-2.48 compared with controls 1.75, range 1.44-2.29; $p=0.88$. For the 1.0 log phot cd.s.m^{-2} the ASD b:a ratios were lower than the control median 1.39, range 1.10-1.76 compared with controls 1.53, range 1.26-1.80 ($p=0.05$).

Naka Rushton

The results of the Naka-Rushton parameters are shown in table 2 with the V_{max}, n and K_m parameters calculated by the method of Evans et al (1993) [56]. There were no significant group differences in the Naka-Rushton parameters. The median V_{max} was lower in the ASD group although the half maximal flash strength to elicit V_{max} was the same between groups ($p=0.31$). The power of the function n which was also not significantly different between groups ($p=0.09$).

In order to further compare the two Naka-Rushton functions a fixed point at the first plateau occurred, (-1 log phot cd.s.m^{-2}), was taken as representative of the rod dominant V_{max}. A second fixed point on the second limb representing the mixed rod-cone response was then selected at 1 log phot cd.s.m^{-2} for further comparison. A between groups one-way ANOVA at these two intensities for the group median (CI) b-wave amplitudes was not

significant at the -1 log phot cd.s.m^{-2} flash strength [ASD 234 (156-364 µV); Control 275 (176-479 µV p=0.42] nor the 1log phot cd.s.m^{-2} [ASD 286 (207-587 µV); Control 370 (253-600 µV p=0.13].

To look at the effect of increasing flash strengths across the 13 step luminance range (-4.0 to 2.0 log phot cd.s.m^{-2}) the b-wave amplitude was analysed using a 13 responses x 2 (Group [ASD, Control]) repeated measures ANOVA. This showed no significant main effect of Group, $F(1,12) = 0.06$, $p = 0.82$, $\eta_p^2 = 0.03$, with b-wave amplitude for the flash strengths.

The Naka Rushton parameters are consistent with previous findings from 30 normal subjects [56] that reported the following values from the regression analysis (V_{max} 512.9; log K_m -2.43 and n 1.03). However, the V_{max} values reported here are lower using DTL electrodes compared to Evans et al (1993) [56] who used Burian-Allen corneal electrodes.

	V_{max} (µV)	**$Log_{10}(K_m)$**	***n***
ASD (11)	239 (157 to 367)	-2.78 (-2.15 to -3.24)	0.97 (0.89 to 1.05)
Control (15)	287 (195 to 517)	-2.78 (-2.31 to -3.19)	1.00 (0.91 to 1.26)
p	0.31	1.00	0.09

Table 2. The Naka-Rushton parameters as determined by regression analysis (median with range).. V_{max} (maximal b-wave amplitude), K_m (Flash strength required to reach $0.5V_{max}$), n (power of the Naka-Rushton function).

15 Hz slow and fast rod pathways

The scotopic 15Hz flicker responses showed no group differences in amplitude or phase for the 10 flash strengths presented in ascending order from -3.0 to 0.5 log Td.s (fig 4). There was also no difference in the flash strength at the point of phase reversal indicating that neither the slow (rod-ON-bipolar/amacrine AII) nor the fast (rod-cone gap junction) pathway are affected (p>0.1) [54].

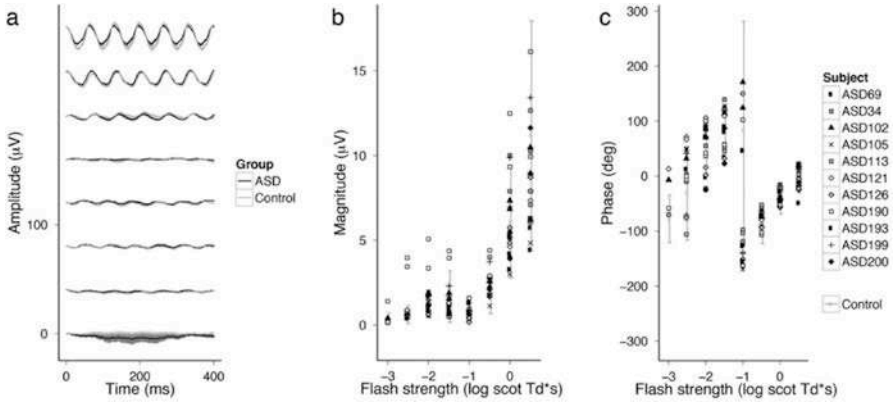

Fig 4a Group averages for amplitude of the 15Hz flicker and phase of the response showing no significant group differences. **Fig 4b** plots the amplitude of the flicker response for the ASD individuals that exhibit a typical response profile compared to the controls. **Fig 4c** shows a similar phase reversal as the slow and fast rod

pathways cancel each other and the 15-Hz amplitude minimum for both groups. (ASD: Autism, Spectrum Disorder). (Error bars are mean ± SD).

Light Adapted ERG

The group results for amplitudes and time to peaks for the phNR, the a- and b-waves are shown in table 3 for the ISCEV standard 0.5 log phot cd.s.m^{-2} single flash. Independent samples for the 5 flash strengths using found no significant interaction between the a-wave parameters of amplitude or time to trough ($p>0.08$) and group. Similarly the phNR amplitude and time to trough were non-significant ($p>0.07$). However, there was a significant interaction between groups and flash strengths for the b-wave amplitude (corrected $p=0.006$) across the flash strength series. Follow up Mann-Whitney test for the b-wave amplitude of the 0.5 log phot cd.s.m^{-2} revealed a significantly (corrected $p<0.001$) lower b-wave amplitude: median (95% CI) [ASD 94 (87-186 µV); Control 137 (80-234 µV)]. No group differences were observed in the time to peak of this response ($p=0.72$). Figure 5 shows the group data (median with 95% CI) for the LA ERGs across the flash strengths of -0.5 to 1.0 log phot cd.s.m^{-2}.

0.5 log phot cd.s.m^{-2} single flash	**ASD**		**Control**		**p-value**
	median	**5th to 95th percentile**	**median**	**5th to 95th percentile**	
a-wave amplitude (µV)	29	18-48	35	14-53	0.08
a-wave time to peak (ms)	14	13-16	13	13-15	0.50
b-wave amplitude (µV)	94	87-186	137	80-234	<0.001*
b-wave time to peak (ms)	29	27-31	28	27-29	0.72
phNR amplitude (µV)	94	85-173	134	74-223	0.07
phNR time to peak (ms)	41	37-47	40	36-46	0.30

Table 3. Results of time to peak of the a- and b-waves and the photopic negative response (phNR) of the full field ISCEV standard 0.5 log phot cd.s.m^{-2} single flash for ASD (N=11) and control (N=14) groups. The b-wave amplitude was significantly lower in the ASD group (corrected $p<0.001$). * indicates Bonferroni corrected p-value for multiple tests.

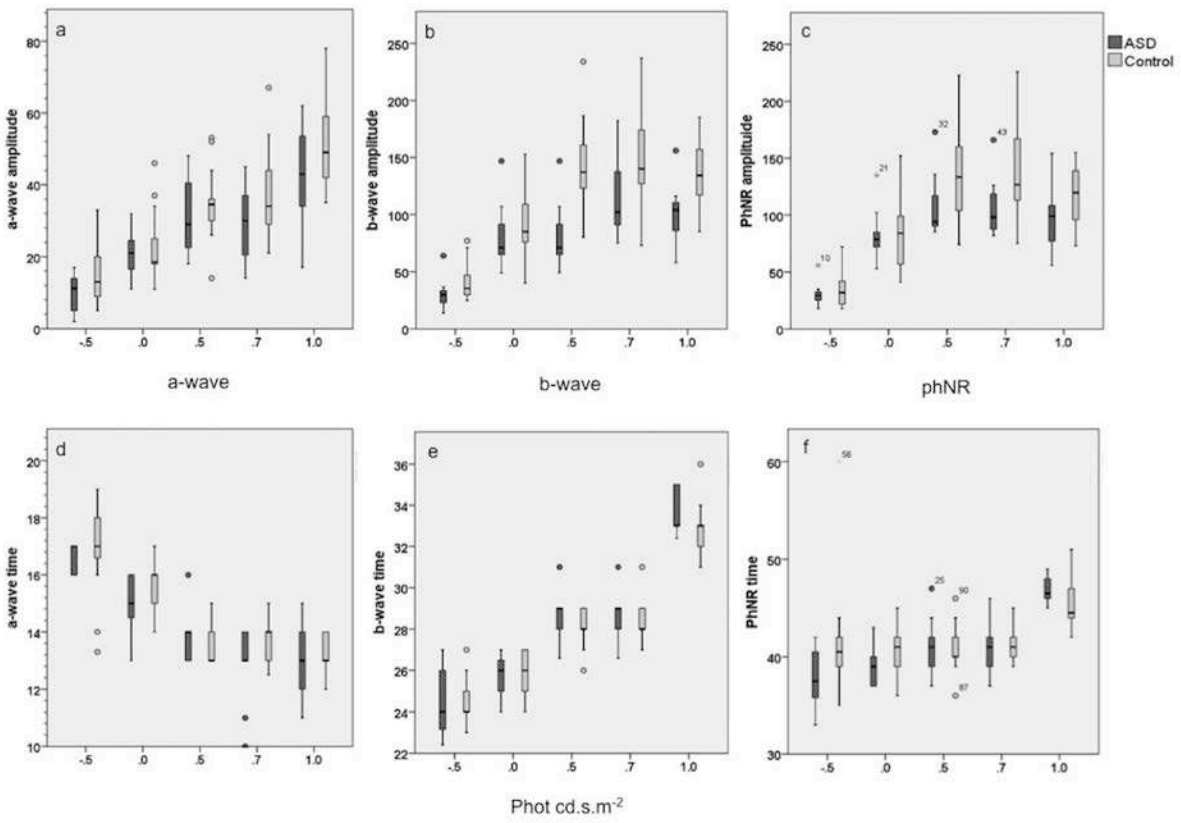

Fig 5 Group data for each photopic flash strength at -0.5, 0, 0.5, 0.7 and 1.0 log phot cd.s.m^{-2} with the 5th - 95th centile range. A significant difference (corrected p=0.006) between groups was observed for the b-wave amplitude (fig 1b) across the luminance series. At 0.5log phot cd.s.m^{-2} the ASD group's b-wave amplitude was significantly lower than the control group's (p<0.001).

Figure 6 shows the LA ERG luminance series for ASD121 (male, aged 28.8; ADOS=7) illustrating the reduced b-wave amplitudes compared to an age equivalent control from this study across the flash strength series.

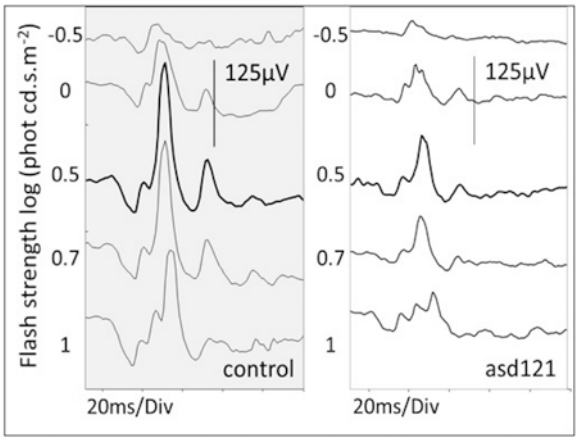

Fig 6 A typical series of photopic ERGs from flash intensities (-0.5 to 1.0 log phot cd.s.m^{-2}) for ASD121 and age matched control participant showing the reduction in the b-wave across the flash series. The bold trace highlights the ISCEV standard 0.5 log phot cd.s.m^{-2} single flash.

ON and OFF response

The b-wave of the LA ERG is formed by the depolarisation of post-receptoral ON (at light onset) and OFF (at light offset) bipolar cells. Given the significant group differences in the b-wave amplitude this could be due to a reduction in depolarisation either in the ON- or OFF- bipolar cell pathway or both. In order to separate these two pathways prolonged 120 ms flash was used to define the ON- and OFF- responses to light onset and offset respectively. Figure 7 shows a recording from ASD121 (M aged 26.3 years, ADOS: 5) with a reduced b-wave at the onset of the flash, but not at offset.

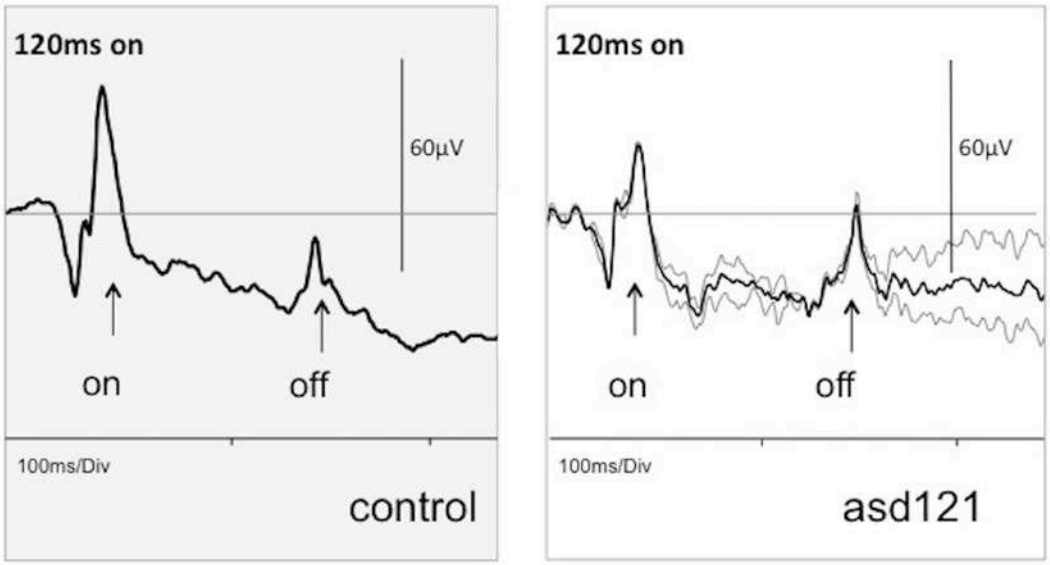

Fig 7 The ON and OFF responses to an extended 120 ms flash for an age matched control in comparison to ASD121 with representative repeat traces in light grey demonstrating the reduced ON component with preserved OFF component. Note smaller amplitude scale for ASD121.

Table 4 reports the group parameters for the extended 120ms flash for the groups. The ON-b-wave amplitude was significantly lower (corrected p=0.003) in the ASD [median (CI): 34 µV (26-53) and control 49 µV (35-77)] (fig 8). All other parameters were non-significant between groups (p>0.3). The number of control participants was reduced in this sample (N=10) as some individuals found the extended flash protocol aversive or were too tired to continue.

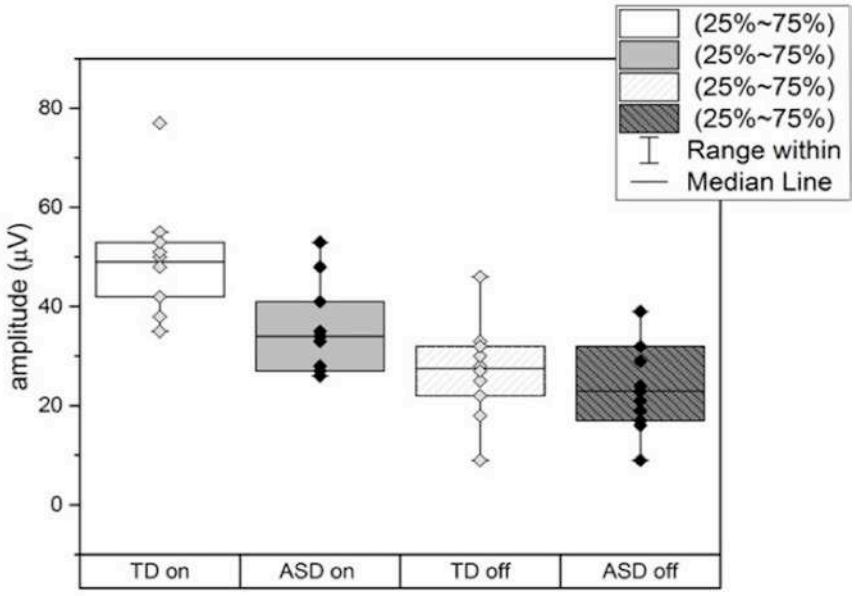

Fig 8. The ON and OFF amplitudes between groups. The ASD group had a significantly reduced ON response (p=0.003) with no differences between groups for the OFF response. Box plots show the 25-75 % centiles the horizontal black line the median.

	a-wave (µV)	**a-wave (ms)**	**ON b-wave (µV)**	**ON b-wave (ms)**	**OFF b-wave (µV)**	**OFF b-wave (ms)**
ASD (11)	17 (10-27)	19 (17-22)	34 (26-53)	35 (29-36)	23 (9-39)	140 (139-143)
Control (10)	22 (12-32)	18 (17-21)	49 (35-77)	34 (31-36)	28 (9-46)	140 (140-143)
p	0.37	0.94	0.003*	0.82	0.60	0.82

Table 4. Parameters for the 120ms extended flash which enables the ON and OFF bipolar cell contribution to the photopic ERG to be separated. A signifcant group difference was present for the b-wave at light onset (corrected p=0.003). * indicates Bonferroni corrected p-value for multiple tests.

Photopic Hill

The effect of increasing flash strengths across the 5 step luminance range (-0.5 to 1.0 log phot cd.s.m^{-2}) the b-wave amplitude was analysed using a 5 responses x 2 (Group [ASD, Control]) repeated measures ANOVA. This showed a significant main effect of Group, $F(1,4) = 7.3$, $p = 0.01$, $\eta_p^2 = 0.24$, with b-wave amplitude for the flash strengths. The V_{max} peak for the control group occurred at 0.7 log phot cd.m.s^{-2} and figure 9 illustrates the peak with the control group, but the ASD group did not establish a clear peak until approximately ~1 log phot cd.s.m^{-2}. The V_{max} b-wave amplitudes for the ASD group were; median (CI) [102 (75-182) and for control 140 (73-277)] which was non-significant (p=0.06). The a-wave amplitude at the fixed point V_{max} peak of the photopic hill was also not different between groups (p=0.15).

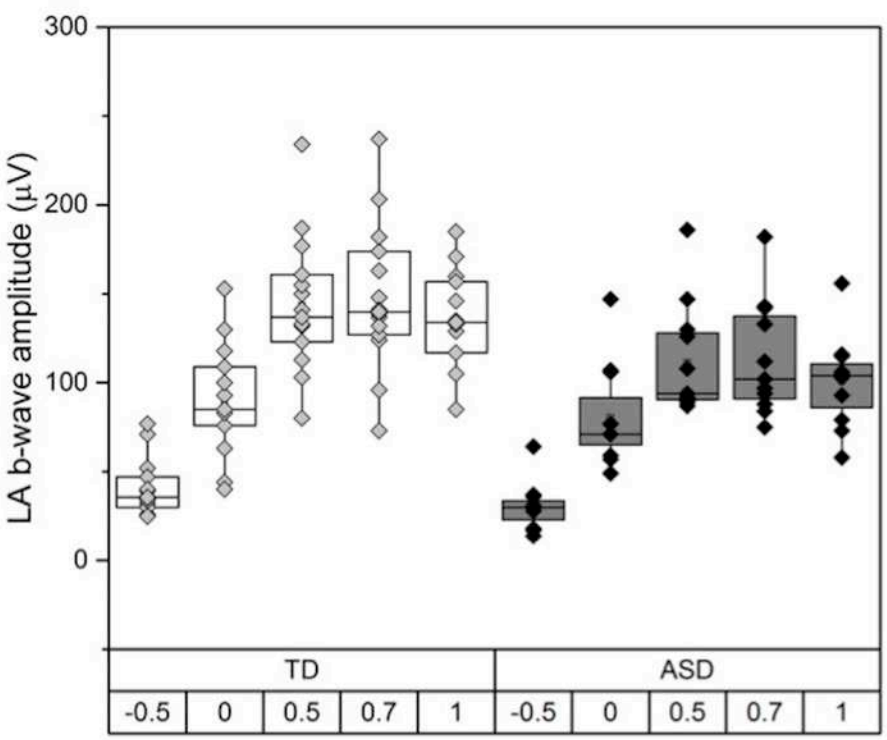

Fig 9. The photopic hill represented by the median with 5-95% CI. for each group. The median represented by the dark line in the box reaches a maximum at 0.7 phot log cd.s.m^{-2} for the control group. In contrast the mediam peak for the ASD group occurs at ~ 1.0 log phot cd.s.m^{-2} with a noticable flattenning of the photopic hill. Repeated measures ANOVA revealed a signifivant (p=0.01) difference between groups although no significant difference was observed at the peak of the hill (fixed V_{max} p=0.06).

Oscillatory Potentials and 30Hz

The LA OP2 showed an early bifurcation in the ASD group compared to the control group in which the bifurcation occurred at a later age (Fig 10). The 30Hz flicker responses were within normal limits of the control group with the ASD group's amplitudes ranging from 46-109 µV (Control 49-144 µV) and the time to peak from 24-30 ms (Control 24-30ms).

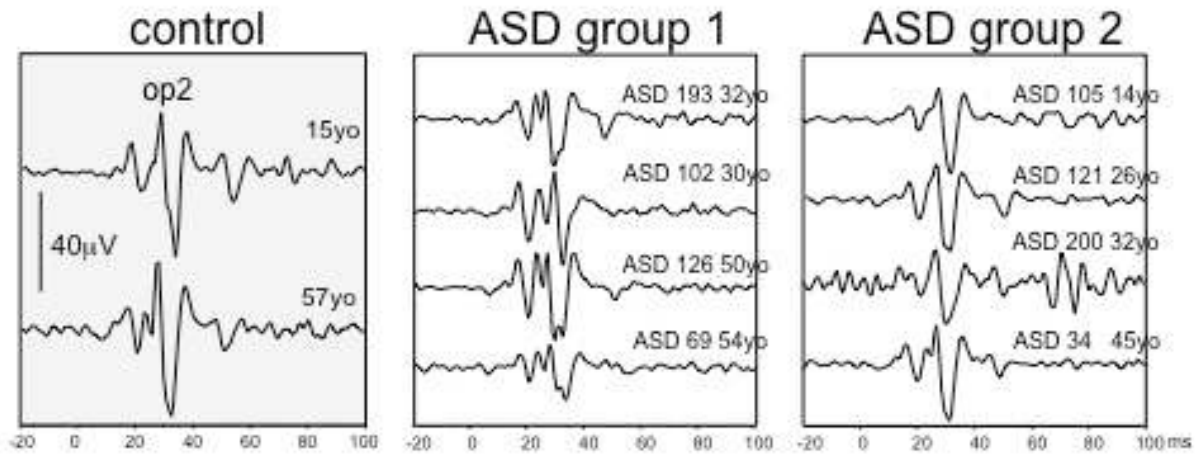

Fig 10. The morphological differences in the photopic OPs. The ASD group falls into two groups, group 1 shows a bifurcation of the OP2. Control examples of the limits of the particpant age range are shown.

Discussion

The findings of our exploratory study of the scotopic luminance response function in ASD individuals support the earlier work of Ritvo et al [30]. They found scotopically balanced red and blue full field flash ERG b-waves to be smaller from individuals with ASD compared to controls and attributed this to the glutamate rod driven ON- bipolar pathway[30]. We also found ASD DA b-waves to be low amplitude, but a smaller proportion 2/11 (18%) fell below normal range compared with 13/28 (48%) in Ritvo's study [30]. The different rates may be due in part to the lower power of this exploratory report but also the severity of ASD. The individuals with ASD in our study represent the higher functioning end of the ASD spectrum whilst Ritvo et al assessed a range of low functioning ASD individuals. If neurological behaviour and retinal function are linked then we may not expect to see as a higher proportion showing marked b-wave differences. A larger sample would provide the opportunity to see if specific autistic traits measured by the ADOS [48] or AQ [51] were associated with changes in the ERG. However, the new findings reported here are the first to investigate the ERG responses across a wide range of stimulus strengths and the low b-wave amplitudes under LA conditions points to a difference in the cone-bipolar synapse.

The linked gene networks associated with ASD provide evidence for a role of altered neurotransmitter signalling affecting the behaviour of ASD individuals [11,57], and the metabotropic glutamate pathway has been specifically implicated in the pathogenesis of ASD [11,12]. In high functioning adults with ASD we found reduced DA b-wave amplitude to the -2.0 log phot cd.s.m^{-2} flash in two ASD individuals. This represented 18% of the group, falling below the control 5th centile lower amplitude of 172µV. Although mGluR6 receptors are strongly expressed in the ON-bipolar cells, the expression of mGluR6 is *not* widespread in the cortex [58]. Cortical changes in ASD have been well documented [59] in ASD with particular differences evident in the frontal cortex and superior temporal sulcus [60] which are cortical regions that are not rich in mGluR6 [61]. However, Hanna & Calkin's (2007) [62] study of the expression profile of glutamate receptors and transporters in primate rod bipolar cells found mGluR3 receptors in 74% of cells. Given that mGluR3 is strongly expressed

in the frontal and temporal cortex where it affects cognitive [63] and language development [64] in ASD, this mGluR receptor may offer a potential link between reduced b-waves and cortical development in ASD.

The b-wave amplitude or PII component of the ERG could be reduced by an increase in the a-wave amplitude/ fast PIII. However, we did not find any differences between the groups for the a-wave parameters which suggests these ERG differences are the result of post-receptoral signalling that contributes to the b-wave [65]. A greater proportion (4 /11 or 36%) of ASD individuals had reduced b-wave amplitudes at higher flash strengths when the cones contribute to the b-wave [65,66]. This implied a reduced contribution of the cone ON-bipolar cells at the higher flash strengths under DA conditions. ASD69 in particular exhibited a fall in the second limb of the luminance response function at flash strengths greater than -0.5 log phot cd.s.m^{-2}.

The Naka-Rushton function parameters, V_{max} representing retinal responsiveness and K_m representing retinal sensitivity were not different between groups for the scotopic limb between -4.0 and -1.0 log phot cd.s.m^{-2}. This finding along with no group differences in the repeated measures of the b-wave amplitude provides evidence that the response to increasing luminance by the retina is not affected in ASD under DA conditions. Overall there were no significant differences in the b-wave amplitudes for the DA single flash series (p=0.83) and as such the DA responses may only differ in a sub-set of individuals on the ASD spectrum. At the plateau of the first limb at -1 log phot cd.s.m^{-2} and at the mixed rod-cone fixed flash strength of 1 log cd.s.m^{-2} there were no group differences in contrast to the findings in schizophrenia [22]. In addition we did not find any group differences in the slow or fast rod pathway amplitudes or phases including the flash strength at which the phase shift occurs. In future studies a counter-phase paradigm to control rod and cone receptor stimulation in the 15Hz flicker ERG may be used to look for subtle differences in these signalling pathways [67].

The importance of the LA-cone pathway is that it is possible to explore the ON- and OFF- bipolar cells that utilise different glutamate receptors. The ON bipolar cells express the metabotropic G-protein which enable the ON-bipolar cell to depolarise when post synaptic glutamate levels decrease when there is an increment in illumination [68]. In contrast, the OFF-bipolar cell expresses the ionotropic iGluR receptors that are non-selective cation channels that depolarise the membrane when there is an increase in post synaptic glutamate following a decrease in illumination [13,65]. Both metabotropic [12] and ionotropic [17] receptors are implicated in ASD. Therefore, any imbalances in these glutamate signalling pathways may help explain the pattern of responses observed at the higher DA flash strengths.

The principle findings under LA conditions were a significant group difference in the b-wave amplitudes across the 5 flash strengths in this high functioning ASD adult group. This is in contrast to the DA series in which no group differences were observed. There was also a significant difference between groups for the LA ISCEV standard 0.5 log phot cd.s.m^{-2} flash and development of the photopic hill in response to increasing flash strengths which was not evident in the DA series. These findings are significant because they point to a difference in the LA responses in the group rather than in individuals. This preliminary finding supports the potential use of the LA ERG to discriminate between groups and implies a difference in the cone-ON-bipolar cell-signalling pathway. Further evidence for the cone-ON bipolar cell pathway being selectively impaired in

ASD was evident in the extended 120 ms flash that revealed a selective significant group difference for the initial ON-bipolar b-wave but not the later OFF-bipolar cell driven 'd'-wave amplitude. Furthermore, the phNR that originates from amacrine, glial and retinal ganglion cell activity [69,70] and defines the fall from the peak of the b-wave to the subsequent trough was equivalent between groups. The normal phNR suggests normal inner retinal activity in ASD as opposed to the outer plexiform layer where cones synapse with ON-bipolar cells. The a-waves amplitudes and times to peak were not different between groups which implied normal phototransduction in cones which was also observed for rods in the DA luminance series.

The normal LA a-wave parameters in the ASD group contrast with schizophrenia where reduced a-waves [22,24,25] have been reported. The LA a-wave has a contribution from the OFF-bipolar cells [71] which implies the OFF-bipolar cell pathway is not affected in ASD. The ASD group did not establish a clear photopic hill peak until ~+0.3 log phot cd.s.m^{-2} higher than control. The shape of the photopic hill appeared to be more sigmoidal which may be in response to a loss of the logistic growth component that is derived from the ON-bipolar pathway [72] and consistent with the findings of reduced cone-ON bipolar contribution in ASD. One weakness of our study is that we did not extend the LA flash strength series to > 1 log phot cd.s.m $^{-2}$ and cannot fully analyse the characteristics of the photopic hill into its component parts [72,73]. Overall the ERG findings in ASD were not as striking as those observed in schizophrenia with the main similarity being a reduced b-wave under LA conditions [22,23].

One observation was an earlier bifurcation of the main LA OP2 in the ASD group that is normally seen in a more aged population [74]. Autistic individuals show early changes in memory than TDs and this may be a sign of earlier ageing in the CNS in ASD [75,76]. This may also be due to differences retinal ageing in ASD given cortical volumes show different growth and thinning profiles to neurotypical individuals [59]. To date no study has looked at the retinal layer thickness in ASD across a wide range of ages and this is an area of future work.

The significant ASD group findings are a selective decrease in the single flash and ON component of the extended flash b-wave in the presence of normal photopic a-waves, phNR, OFF-response, 30Hz and the later OPs. Taken together, these findings indicate impairment in either ON-bipolar cell metabotropic receptors or downstream signalling cascades that reduce the amplitude of the depolarisation of the ON-bipolar cell following an increment in light under LA conditions. However, reconciling the observed electrophysiological findings with CNS proteins that are common to both the ON-bipolar cell and have been implicated in ASD is speculative.

A possible mechanism could be downstream components of the metabotropic signalling cascade such as G_{α} and $G_{\beta\gamma}$ that regulate the non-selective cation channel transient receptor potential melastatin (TRPM1) channel in the ON-bipolar cell that when gated open results in depolarisation [77]. SNPs associated with mGluR5 and $G_0\alpha$ and phospholipase-β have recently been postulated as possible markers for identifying ASD [78]. Deletion of the $G_0\alpha$ subunit abolishes the DA and LA b-wave in mouse [79,80] and therefore potentially a partial loss of function in $G_0\alpha$ may contribute to the diminished b-waves observed in the ASD group. Knock out mice for Gβ(3) exhibit a more pronounced loss of the LA b-wave amplitude compared to the DA b-wave which is similar to the pattern of results observed in this study population [81]. Therefore, whilst Gβ(3) knock-out mice

result in a similar clinical ERG profile to the ASD group there is currently no direct evidence linking the G-protein β subunit gene *GNB3* with ASD. Further genetic studies would be required to determine which signalling or channel proteins were responsible for the reduced LA b-waves.

Conclusion

This exploratory study of the ERG in ASD support those previously reported by Ritvo [29,30] that the DA ERG b-wave is reduced in some individuals on the ASD spectrum. This study has shown reduced b-wave amplitudes related specifically to the cone-ON-bipolar cell synapse with no group differences in the a-wave parameters that imply normal phototransduction in ASD. The reduced 'ON' response to the extended flash further supports this pathway as being atypical in ASD and this could be further explored using higher flash strengths to define the photopic hill in ASD. To fully assess the clinical utility of the ERG in ASD a larger study exploring the LA responses in children would be needed to demonstrate that the ERG could be used as a measure of CNS function in this neurodevelopmental disorder.

Acknowledgements

This study was funded by the College of Optometrists and parts of this work were presented at the 52nd ISCEV conference in Boston. The study was nominated for the Marmor prize for clinical innovation in electrophysiology at the meeting. The authors thank the anonymous reviewers for their helpful comments on this paper.

Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

1. Baird G, Simonoff E, Pickles A, Chandler S, Loucas T, Meldrum D et al. (2006) Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). The Lancet 368 (9531):210-215. doi:10.1016/s0140-6736(06)69041-7

2. Nicholas JS, Charles JM, Carpenter LA, King LB, Jenner W, Spratt EG (2008) Prevalence and characteristics of children with autism-spectrum disorders. Ann Epidemiol 18 (2):130-136

3. Rattazzi A (2014) The importance of early detection and early intervention for children with autism spectrum conditions. Vertex (Buenos Aires, Argentina) 25 (116):290-294

572 4. Lin LY (2014) Quality of life of Taiwanese adults with autism spectrum disorder. PloS one 9 (10):e109567.
573 doi:10.1371/journal.pone.0109567

574 5. Dillenburger K, Jordan JA, McKerr L, Keenan M (2015) The Millennium child with autism: Early childhood
575 trajectories for health, education and economic wellbeing. Developmental neurorehabilitation 18 (1):37-46.
576 doi:10.3109/17518423.2014.964378

577 6. Ecker C, Marquand A, Mourao-Miranda J, Johnston P, Daly EM, Brammer MJ et al. (2010) Describing the
578 brain in autism in five dimensions--Magnetic Resonance Imaging-assisted diagnosis of Autism Spectrum
579 Disorder using a multiparameter classification approach. J Neurosci 30 (32):10612-10623.
580 doi:10.1523/jneurosci.5413-09.2010

581 7. Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N et al. (2010) Longitudinal
582 Magnetic Resonance Imaging study of cortical development through early childhood in autism. J Neurosci 30
583 (12):4419-4427. doi:10.1523/jneurosci.5714-09.2010

584 8. Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS et al. (2009) Common genetic variants on
585 5p14.1 associate with autism spectrum disorders. Nature 459 (7246):528-533. doi:10.1038/nature07999

586 9. St. Pourcain B, Wang K, Glessner JT, Golding J, Steer C, Ring SM et al. (2010) Association between a high-
587 risk autism locus on 5p14 and social communication spectrum phenotypes in the general population. Am J
588 Psychiatry 167 (11):1364-1372. doi:10.1176/appi.ajp.2010.09121789

589 10. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S et al. (2009) Autism genome-wide copy
590 number variation reveals ubiquitin and neuronal genes. Nature 459 (7246):569-573

591 11. Autism Genome Project Consortium. (2007) Mapping autism risk loci using genetic linkage and
592 chromosomal rearrangements. Nat Genet 39 (3):319-328. doi:10.1038/ng1985

593 12. Hadley D, Wu ZL, Kao C, Kini A, Mohamed-Hadley A, Thomas K et al. (2014) The impact of the
594 metabotropic glutamate receptor and other gene family interaction networks on autism. Nature communications
595 5:4074. doi:10.1038/ncomms5074

596 13. Gerber U (2003) Metabotropic glutamate receptors in vertebrate retina. Doc Ophthalmol 106 (1):83-87

597 14. Ulrike G (2000) Distribution of GABA and glycine receptors on bipolar and ganglion cells in the
598 mammalian retina. Microscopy Research and Technique 50 (2):130-140

15. Lavoie J, Maziade M, Hébert M (2014) The brain through the retina: the flash electroretinogram as a tool to investigate psychiatric disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry 48:129-134. doi:10.1016/j.pnpbp.2013.09.020

16. Collins A, Ma D, Whitehead P, Martin E, Wright H, Abramson R et al. (2006) Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. Neurogenetics 7 (3):167-174

17. Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B et al. (2002) Linkage and association of the glutamate receptor 6 gene with autism. Mol Psychiatry 7 (3):302-310

18. Mariken de K, Wouter GS, Roel AO, Judith H, Jan B, Barbara F et al. (2009) A common variant in DRD3 receptor is associated with Autism Spectrum Disorder. Biol Psychiatry 65 (7):625-630

19. Nguyen CT, Vingrys AJ, Wong VH, Bui BV (2013) Identifying cell class specific losses from serially generated electroretinogram components. BioMed research international 2013:796362. doi:10.1155/2013/796362

20. Dhingra A, Vardi N (2012) "mGlu Receptors in the Retina" - WIREs Membrane Transport and Signaling. Wiley interdisciplinary reviews: Membrane transport and signaling 1 (5):641-653. doi:10.1002/wmts.43

21. Gregory KJ, Dong EN, Meiler J, Conn PJ (2011) Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential. Neuropharmacology 60 (1):66-81. doi:10.1016/j.neuropharm.2010.07.007

22. Hébert M, Merette C, Paccalet T, Emond C, Gagne AM, Sasseville A et al. (2015) Light evoked potentials measured by electroretinogram may tap into the neurodevelopmental roots of schizophrenia. Schizophrenia Research 162 (1-3):294-295. doi:10.1016/j.schres.2014.12.030

23. Hébert M, Gagne AM, Paradis ME, Jomphe V, Roy MA, Merette C et al. (2010) Retinal response to light in young nonaffected offspring at high genetic risk of neuropsychiatric brain disorders. Biol Psychiatry 67 (3):270-274. doi:10.1016/j.biopsych.2009.08.016 [doi]

24. Warner R, Laugharne J, Peet M, Brown L, Rogers N (1999) Retinal function as a marker for cell membrane omega-3 fatty acid depletion in schizophrenia: a pilot study. Biol Psychiatry 45 (9):1138-1142. doi:S0006-3223(98)00379-5 [pii]

25. Balogh Z, Benedek G, Kéri S (2008) Retinal dysfunctions in schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32 (1):297-300. doi:DOI: 10.1016/j.pnpbp.2007.08.024

627 26. Pathania M, Davenport EC, Muir J, Sheehan DF, Lopez-Domenech G, Kittler JT (2014) The autism and
628 schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the
629 stabilization of mature spines. Translat Psychiatry 4:e423. doi:10.1038/tp.2014.36

630 27. Singh SM, Castellani C, O'Reilly R (2010) Autism meets schizophrenia via cadherin pathway.
631 Schizophrenia Research 116 (2-3):293-294. doi:10.1016/j.schres.2009.09.031

632 28. Matsuo J, Kamio Y, Takahashi H, Ota M, Teraishi T, Hori H et al. (2015) Autistic-like traits in adult
633 patients with mood disorders and schizophrenia. PloS one 10 (4):e0122711. doi:10.1371/journal.pone.0122711

634 29. Realmuto G, Purple R, Knobloch W, Ritvo ER (1989) Electroretinograms (ERGs) in four autistic probands
635 and six first-degree relatives. Can J Psychiatry 34 (5):435-439

636 30. Ritvo ER, Creel D, Realmuto G, Crandall AS, Freeman BJ, Bateman JB et al. (1988) Electroretinograms in
637 autism: a pilot study of b-wave amplitudes. Am J Psychiatry 145 (2):229-232

638 31. Shah A, Frith U (1983) An islet of ability in autistic children: a research note. J Child Psychol Psychiatry 24
639 (4):613-620. doi:doi:10.1111/j.1469-7610.1983.tb00137.x

640 32. Plaisted K, O'Riordan M, Baron-Cohen S (1998) Enhanced visual search for a conjunctive target in autism: a
641 research note. J Child Psychol Psychiatry 39 (5):777-783

642 33. Baldassi S, Pei F, Megna N, Recupero G, Viespoli M, Igliozzi R et al. (2009) Search superiority in autism
643 within, but not outside the crowding regime. Vis Res 49 (16):2151-2156. doi:DOI: 10.1016/j.visres.2009.06.007

644 34. Bertone A, Mottron L, Jelenic P, Faubert J (2005) Enhanced and diminished visuo-spatial information
645 processing in autism depends on stimulus complexity. Brain 128 (10):2430-2441. doi:10.1093/brain/awh561

646 35. Constable PA, Gaigg SB, Bowler DM, Thompson DA (2012) Motion and pattern cortical potentials in adults
647 with high-functioning autism spectrum disorder. Doc Ophthalmol 125(3):219-227. doi:10.1007/s10633-012-
648 9349-7

649 36. Koldewyn K, Whitney D, Rivera SM (2010) The psychophysics of visual motion and global form
650 processing in autism. Brain 133 (2):599-610. doi:10.1093/brain/awp272

651 37. Dakin S, Frith U (2005) Vagaries of visual perception in autism. Neuron 48 (3):497-507. doi:DOI:
652 10.1016/j.neuron.2005.10.018

653 38. Simmons DR, Robertson AE, McKay LS, Toal E, McAleer P, Pollick FE (2009) Vision in autism spectrum
654 disorders. Vis Res 49 (22):2705-2739. doi:DOI: 10.1016/j.visres.2009.08.005

655 39. Bonnel A, Mottron L, Peretz I, Trudel M, Gallun E, Bonnel A-M (2003) Enhanced pitch sensitivity in
656 individuals with autism: a signal detection analysis. J Cognitive Neuroscience 15 (2):226-235.
657 doi:doi:10.1162/089892903321208169

658 40. Bonnel A, McAdams S, Smith B, Berthiaume C, Bertone A, Ciocca V et al. (2010) Enhanced pure-tone
659 pitch discrimination among persons with autism but not Asperger syndrome. Neuropsychologia 48 (9):2465-
660 2475. doi:10.1016/j.neuropsychologia.2010.04.020

661 41. Tonacci A, Billeci L, Tartarisco G, Ruta L, Muratori F, Pioggia G et al. (2015) Olfaction in autism spectrum
662 disorders: A systematic review. Child Neuropsychol:1-25. doi:10.1080/09297049.2015.1081678

663 42. Daluwatte C, Miles JH, Sun J, Yao G (2014) Association between pupillary light reflex and sensory
664 behaviors in children with autism spectrum disorders. Research in developmental disabilities 37C:209-215.
665 doi:S0891-4222(14)00502-2 [pii]

666 43. Milne E, Scope A, Griffiths H, Codina C, Buckley D (2013) Brief report: preliminary evidence of reduced
667 sensitivity in the peripheral visual field of adolescents with autistic spectrum disorder. J Autism Dev Disord 43
668 (8):1976-1982. doi:10.1007/s10803-012-1730-6

669 44. Lane AE, Molloy CA, Bishop SL (2014) Classification of children with autism spectrum disorder by
670 sensory subtype: a case for sensory-based phenotypes. Autism Res 7 (3):322-333. doi:10.1002/aur.1368

671 45. Stockman A, Sharpe LT, Ruther K, Nordby K (1995) Two signals in the human rod visual system: a model
672 based on electrophysiological data. Vis Neurosci 12 (5):951-970. doi:S0952523800009500

673 46. Stockman A, Sharpe LT, Zrenner E, Nordby K (1991) Slow and fast pathways in the human rod visual
674 system: electrophysiology and psychophysics. Journal of the Optical Society of America 8 (10):1657-1665

675 47. Sharpe LT, Fach CC, Stockman A (1993) The spectral properties of the two rod pathways. Vis Res 33
676 (18):2705-2720. doi:0042-6989(93)90230-T

677 48. Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L et al. (1989) Autism diagnostic
678 observation schedule: a standardized observation of communicative and social behavior. J Autism Dev Disord
679 19 (2):185-212

680 49. Diagnostic and statistical manual of mental disorders (DSM-IV-TR) (2000). American Psychiatric
681 Association, Washington DC, USA

682 50. The Psychological Corporation (2000) Wechsler Adult Intelligence Scale. London, UK

683 51. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E (2001) The Autism-Spectrum Quotient
684 (AQ): Evidence from Asperger syndrome/high-functioning autism, males and females, scientists and
685 mathematicians. J Autism Dev Disord 31 (1):5-17

686 52. Neveu MM, Dangour A, Allen E, Robson AG, Bird AC, Uauy R et al. (2011) Electroretinogram measures in
687 a septuagenarian population. Doc Ophthalmol 123 (2):75-81. doi:10.1007/s10633-011-9282-1

688 53. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R et al. (2015) ISCEV Standard
689 for full-field clinical electroretinography (2015 update). Doc Ophthalmol130 (1):1-12. doi:10.1007/s10633-014-
690 9473-7

691 54. Scholl HP, Langrová H, Weber BH, Zrenner E, Apfelstedt-Sylla E (2001) Clinical electrophysiology of two
692 rod pathways: normative values and clinical application. Graefe's archive for clinical and experimental
693 ophthalmology 239 (2):71-80

694 55. Meigen T, Bach M (1999) On the statistical significance of electrophysiological steady-state responses. Doc
695 Ophthalmol 98 (3):207-232

696 56. Evans LS, Peachey NS, Marchese AL (1993) Comparison of three methods of estimating the parameters of
697 the Naka-Rushton equation. Doc Ophthalmol 84 (1):19-30

698 57. Kim YS, Leventhal BL (2015) Genetic Epidemiology and Insights into Interactive Genetic and
699 Environmental Effects in Autism Spectrum Disorders. Biol Psychiatry 77 (1):66-74. doi:S0006-3223(14)00827-
700 0

701 58. Vardi T, Fina M, Zhang L, Dhingra A, Vardi N (2011) mGluR6 transcripts in non-neuronal tissues. J
702 Histochemistry and Cytochemistry 59 (12):1076-1086. doi:10.1369/0022155411425386

703 59. Zielinski BA, Prigge MB, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS et al. (2014) Longitudinal
704 changes in cortical thickness in autism and typical development. Brain 137 (Pt 6):1799-1812.
705 doi:10.1093/brain/awu083

706 60. Lange N, Travers BG, Bigler ED, Prigge MB, Froehlich AL, Nielsen JA et al. (2014) Longitudinal
707 Volumetric Brain Changes in Autism Spectrum Disorder Ages 6-35 Years. Autism Res 8(1):82-93.
708 doi:10.1002/aur.1427

709 61. Enoch MA, Rosser AA, Zhou Z, Mash DC, Yuan Q, Goldman D (2014) Expression of glutamatergic genes
710 in healthy humans across 16 brain regions; altered expression in the hippocampus after chronic exposure to
711 alcohol or cocaine. Genes, brain, and behavior 13 (8):758-768. doi:10.1111/gbb.12179

712 62. Hanna MC, Calkins DJ (2007) Expression of genes encoding glutamate receptors and transporters in rod and
713 cone bipolar cells of the primate retina determined by single-cell polymerase chain reaction. Mol Vis 13:2194-
714 2208. doi:v13/a249

715 63. Egan MF, Straub RE, Goldberg TE, Yakub I, Callicott JH, Hariri AR et al. (2004) Variation in GRM3
716 affects cognition, prefrontal glutamate, and risk for schizophrenia. PNAS 101 (34):12604-12609.
717 doi:10.1073/pnas.0405077101

718 64. Kawakubo Y, Suga M, Tochigi M, Yumoto M, Itoh K, Sasaki T et al. (2011) Effects of metabotropic
719 glutamate receptor 3 genotype on phonetic mismatch negativity. PloS one 6 (10):e24929.
720 doi:10.1371/journal.pone.0024929

721 65. Green DG, Kapousta-Bruneau NV (1999) A dissection of the electroretinogram from the isolated rat retina
722 with microelectrodes and drugs. Vis Neurosci 16 (4):727-741.

723 66. Friedburg C, Allen CP, Mason PJ, Lamb TD (2004) Contribution of cone photoreceptors and post-receptoral
724 mechanisms to the human photopic electroretinogram. J Physiol 556 (Pt 3):819-834.
725 doi:10.1113/jphysiol.2004.061523

726 67. Park JC, Cao D, Collison FT, Fishman GA, Jason McAnany J (2015) Rod and cone contributions to the
727 dark-adapted 15-Hz flicker electroretinogram. Doc Ophthalmol 130 (2):111-119. doi:10.1007/s10633-015-9480-
728 3

729 68. Yang X-L (2004) Characterization of receptors for glutamate and GABA in retinal neurons. Progress in
730 Neurobiology 73 (2):127-150

731 69. Viswanathan S, Frishman LJ, Robson JG, Harwerth RS, Smith EL, 3rd (1999) The photopic negative
732 response of the macaque electroretinogram: reduction by experimental glaucoma. Invest Ophthalmol Vis Sci 40
733 (6):1124-1136

734 70. Li B, Barnes GE, Holt WF (2005) The decline of the photopic negative response (PhNR) in the rat after
735 optic nerve transection. Doc Ophthalmol 111 (1):23-31. doi:10.1007/s10633-005-2629-8

736 71. Bush RA, Sieving PA (1994) A proximal retinal component in the primate photopic ERG a-wave. INVEST
737 OPHTHALMOL VIS SCI 35 (2):635-645

738 72. Hamilton R, Bees MA, Chaplin CA, McCulloch DL (2007) The luminance-response function of the human
739 photopic electroretinogram: a mathematical model. Vis Res 47 (23):2968-2972. doi:S0042-6989(07)00206-4

740 73. Rufiange M, Dassa J, Dembinska O, Koenekoop RK, Little JM, Polomeno RC et al. (2003) The photopic
741 ERG luminance-response function (photopic hill): method of analysis and clinical application. Vis Res 43
742 (12):1405-1412. doi:DOI: 10.1016/S0042-6989(03)00118-4

743 74. Dimopoulos IS, Freund PR, Redel T, Dornstauder B, Gilmour G, Sauve Y (2014) Changes in rod and cone-
744 driven oscillatory potentials in the aging human retina. Invest Ophthalmol Vis Sci 55 (8):5058-5073.
745 doi:10.1167/Invest Ophthalmol Vis Sci.14-14219

746 75. Ring M, Gaigg SB, Bowler DM (2015) Object-location memory in adults with autism spectrum disorder.
747 8(5) 609-619 Autism Res. doi: 10.1002/aur.1478

748 76. Ring M, Gaigg SB, Bowler DM (2015) Relational Memory Processes in Adults with Autism Spectrum
749 Disorder. Autism Res. doi:10.1002/aur.1493

750 77. Shen Y, Rampino MA, Carroll RC, Nawy S (2012) G-protein-mediated inhibition of the Trp channel
751 TRPM1 requires the Gbetagamma dimer. PNAS 109 (22):8752-8757. doi:10.1073/pnas.1117433109

752 78. Skafidas E, Testa R, Zantomio D, Chana G, Everall IP, Pantelis C (2014) Predicting the diagnosis of autism
753 spectrum disorder using gene pathway analysis. Mol Psychiatry 19 (4):504-510. doi:10.1038/mp.2012.126

754 79. Dhingra A, Jiang M, Wang TL, Lyubarsky A, Savchenko A, Bar-Yehuda T et al. (2002) Light response of
755 retinal ON bipolar cells requires a specific splice variant of $G\alpha_O$. J Neurosci 22 (12):4878-4884. doi:22/12/4878

756 80. Dhingra A, Lyubarsky A, Jiang M, Pugh EN, Jr., Birnbaumer L, Sterling P et al. (2000) The light response
757 of ON bipolar neurons requires $G\alpha_O$. J Neurosci 20 (24):9053-9058. doi:20/24/9053 [pii]

758 81. Dhingra A, Ramakrishnan H, Neinstein A, Fina ME, Xu Y, Li J et al. (2012) $G\beta_3$ is required for normal light
759 ON responses and synaptic maintenance. J Neurosci 32 (33):11343-11355. doi:10.1523/JNEUROSCI.1436-
760 12.2012