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1 Full field electroretinogram in autism spectrum disorder

2

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19

20 **Abstract**

21 **Purpose**

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

24 **Methods**

25 Dark adapted (DA) ERGs were acquired to a range of flash strengths, (-4.0 to 2.3 log phot cd.s.m⁻²), including
26 and extending the ISCEV standard, from two subject groups: (ASD) N=11 and (Control) N=15 for DA and
27 N=14 for light adapted (LA) ERGs who were matched for mean age and range. Naka-Rushton curves were
28 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
29 (V_{max} , K_m and n) were compared between groups. Scotopic 15 Hz flicker ERGs (14.93Hz) were recorded to 10
30 flash strengths presented in ascending order from -3.0 to 0.5 log Td.s to assess the slow and fast rod pathways
31 respectively. LA ERGs were acquired to a range of flash strengths, (-0.5 to 1.0 log phot cd.s.m⁻²). Photopic 30
32 Hz, flicker ERGs, oscillatory potentials (OPs) and the responses to prolonged 120 ms ON- OFF stimuli were
33 also recorded.

34 **Results**

35 For some individuals the DA b-wave amplitudes fell below the control 5th centile of the controls with up to four
36 ASD participants (36%) at the 1.5 log phot cd.s.m⁻² flash strength and two (18%) ASD participants at the lower
37 -2 log phot cd.s.m⁻² flash strength. However, across the thirteen flash strengths there were no significant group
38 differences for b-wave amplitude's growth (repeated measures ANOVA $p=0.83$). Nor were there any significant
39 differences between the groups for the Naka-Rushton parameters ($p>0.09$). No group differences were observed
40 in the 15Hz scotopic flicker phase or amplitude ($p>0.1$), DA ERG a- wave amplitude or time to peak ($p>0.26$).
41 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
42 single ISCEV LA 0.5 log phot cd.s.m⁻² ($p<0.001$) was lower in the ASD group. Repeated measures ANOVA for
43 the LA series was also significantly ($p=0.01$) different between groups. No group differences were observed for
44 the LA a-wave, b-wave time to peak or the photopic negative responses (phNR) ($p>0.08$) to the single flash
45 stimuli although there was a significant interaction between group and flash strength for the b-wave amplitude
46 (corrected $p=0.006$). The prolonged 120 ms ON-responses were smaller in the ASD group (corrected $p=0.003$),
47 but the OFF response amplitude ($p>0.6$) and ON and OFF times to peaks ($p>0.4$) were similar between groups.
48 The LA OPs showed an earlier bifurcation of OP2 in the younger ASD participants, however no other
49 differences were apparent in the OPs or 30Hz flicker waveforms.

50

51 **Conclusion**

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

55 **Keywords:**

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

57 **Introduction**

58

59 Autism is a neurodevelopmental disorder of unknown aetiology with a prevalence of ~1:100 and a predilection
60 for affecting males [1,2]. The term autism or autistic spectrum disorder (ASD) is characterised by impairments
61 in reciprocal social interaction, imagination and language development. With early intervention and diagnosis
62 [3] these difficulties can be helped, but ASD remains a lifelong condition that impacts upon an individual's and
63 family's quality of life [4,5].

64

65 ASD individuals display morphological differences of the cerebral cortex [6,7] and Genome Wide Association
66 Studies have linked ASD with variations in genes associated with neural development and synaptic transmission
67 [8-11]. One important gene network links the metabolic glutamate receptor (mGluR) family and autism [12]. Of
68 this family the mGluR6 receptors are recognised as being critical for the ERG b-wave generation [13].

69 Alterations in CNS development, final organisation and neurotransmitter signalling in ASD may manifest
70 therefore as altered retinal signalling [14,15]. The retinal signalling pathways are broadly divided between
71 vertical excitatory pathways, comprising photoreceptors, bipolar cells and ganglion cells and horizontal
72 inhibitory pathways formed by amacrine and horizontal cells. Glutamate is the major excitatory neurotransmitter
73 and γ -amino butyric- acid (GABA) and dopamine the main inhibitory neurotransmitters. Altered
74 neurotransmitter receptor functions for these neurotransmitters have also been implicated in the ASD phenotype
75 [16-18] and may additionally contribute to differences in retinal signalling and responses.

76

77 The ERG is a non-invasive clinical tool whose waveform can be related to specific neural pathways,
78 neurotransmitters and their receptors [15,19]. Should the ERG detect differences in retinal signalling in ASD it
79 could become a useful monitor of novel drugs targeting the CNS [20,21]; for example emerging therapeutic
80 agents for ASD are targeting metabotropic glutamate gene networks and receptor groups [21]. Retinal studies of
81 patients with schizophrenia and their children lend support to this hypothesis [22-25]. Schizophrenia and ASD
82 have common genetic variants [26,27] and individuals with schizophrenia exhibit autism like traits [28].

83 Individuals with a high genetic risk of schizophrenia have reduced dark adapted (DA) b-wave amplitudes at the
84 plateaux of the Naka-Rushton function [23]. This parallels a report of small amplitude scotopic ERG b-waves in
85 ASD published 25 years ago, [29,30]. More recently, these findings were confirmed in schizophrenic
86 individuals, who also showed reduced mixed rod-cone b-wave amplitude and reduced light adapted (LA) b-
87 wave at the peak of the photopic hill [22]. Given the genotypic and phenotypic overlap between ASD and
88 schizophrenia, it is of interest to see if these ERG profiles are found in ASD.

89

90 Individuals with ASD often show peculiarities in visual tasks, one of the strongest is their ability to find a
91 specific shape or object within a hidden pattern [31] as well as displaying superior visual search strategies
92 [32,33]. Their orientation discrimination thresholds are superior for detecting simple-first luminance defined
93 grating patterns over more complex- second order texture defined grating patterns [34]. Cortical

104 electrophysiological findings [35] also support evidence for abnormal motion perception in ASD [36]. Whilst,
105 the higher level visual tasks have been extensively studied: for reviews see Dakin and Frith (2005) [37] and
106 Simmons et al (2009) [38], there have been fewer studies into the primary visual sense in ASD; and in particular
107 the retinal response to luminance. This is despite evidence for increased pitch, tone [39,40] and decreased odour
108 [41] discrimination in ASD supporting altered sensory processing in ASD. A recent study has found reduced
109 pupillary constriction in ASD suggesting a difference in light sensitivity in this population [42] which is also
110 supported by decreased peripheral visual field sensitivity in ASD [43]. Given the differences in sensory
111 discrimination that are observed in ASD [44], the ERG may be able to discern if the retinal response to light
112 contributes to these differences.

113

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

115

116 **Methods**

117

118 **Participants**

119

120 A total of 11 ASD participants and 15 typically developing (Control) participants performed DA full field ERGs
121 and 14 LA full field ERGs. The mean age and range of the control participants group were matched with the
122 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
123 ± 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
124 female, p=0.96).

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

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

139

140 **ERG Recordings**

141

142 ISCEV protocols for full field ERG recording were followed [53]. All eyes were fully dilated with 1.0%
143 tropicamide. DTL Plus electrodes, (Diagnosys LLC, MA10854, USA) were placed across the limbus and
144 referenced to Ag/AgCl gel sticker electrodes placed at each outer canthus and a ground electrode placed on the
145 forehead. Each participant was dark adapted for 20 minutes before binocular stimulation to an ascending range
146 of flash strengths with a colordome stimulator Diagnosys Espion² (Diagnosys, Oxford, UK). DA full field ERGs
147 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
148 ms white flashes generated by LEDs (colour temperature 6,500K) or xenon tube as required (200 phot cd.s.m⁻²)
149 presented scotopically. ERGs were acquired in a time window of 250 ms, that included a 20 ms pre-stimulus
150 interval and typically several trials were averaged for each flash strength. Additionally, DA scotopic 15 Hz
151 flicker ERGs (14.93Hz) were elicited to 10 flash strengths presented in ascending order from -3.0 to 0.5 log Td.s
152 testing slow and fast rod pathways respectively [54]. Flicker ERGs to each of these stimuli were acquired within
153 a 402ms time window containing six periods and response magnitude, and phase were determined by Fourier
154 analysis. Response waveform significance was estimated [55]. For the LA full field ERGs, participants were
155 light adapted for 10 minutes (30 cd.m⁻²) before binocular stimulation to an ascending range of flash strengths -
156 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
157 250 ms, including a 20 ms pre-stimulus interval with several trials averaged for the flash strengths. Additionally,
158 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
159 presented for 120 ms at LA 43 cd.m⁻² were acquired in a 345 ms time window, which included a 15 ms pre
160 stimulus interval. LA OPs were extracted by filtering between 100 and 300Hz from the 0.5 log phot cd.s.m⁻²
161 single flash.

162

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

165

166 **ERG Analysis**

167

168 The ERG traces were analysed in accordance with the ISCEV standard [53]. Traces were examined off-line for
169 both eyes' recordings and individual traces removed manually if there were blink or movement artefacts. The
170 ERG from the eye with the larger amplitude for each individual was used in the final statistical analysis.
171 Amplitudes and time to peak for the a- and b- waves were measured from the averaged traces following artefact
172 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

173 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
174 higher flash strengths. The phNR was measured from the b-wave peak to the subsequent trough.

175

176 **Naka-Rushton Parameters**

177

178 The Naka-Rushton function describes the change in retinal response with increasing flash luminance. It is a
179 logistic growth function and is expressed by equation 1. The parameter V (μV) represents the b-wave amplitude
180 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
181 the semi-saturation constant at which the flash strength elicits a response equal to 0.5V_{max}. The dimensionless
182 constant n of defines the slope of the function. The parameters are believed to represent three aspects of retinal
183 function with K_m associated with retinal sensitivity, n with retinal homogeneity and V_{max} with retinal
184 responsiveness [56].

185

$$186 \quad V = [V_{max} \cdot I / (K_m + I)]^n \quad \dots\dots\dots 1$$

187

188 The parameters for the Naka-Rushton function were derived from regression line analysis of the transformed
189 fitted raw data [56]. To derive the three Naka-Rushton parameters b-wave amplitudes were plotted against flash
190 strength and a natural log curve fitted and K_m calculated from equation 2 generating the first transformed
191 function from which K_m could be derived. The value of V_{max} was set to 1% greater than the highest amplitude
192 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
193 function only included values up until the first plateau derived from the rod driven pathway [56]. One
194 participant, ASD69 (M, 54.3 yo; ADOS =10) showed an atypical drop at flash strength -1.0 log phot cd.s.m⁻²
195 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
196 used to estimate the parameters.

197

$$198 \quad V_{max}/2 = a \cdot \ln(K_m) + c \quad \dots\dots\dots 2$$

199

200 With the values of V_{max} and K_m derived a second linear function was plotted using the calculated V_{max} value and
201 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
202 value n in the Naka Rushton equation: equation 3.

203

$$204 \quad V/V_{max} = n[(1+K_m)^{-1}] + c \quad \dots\dots\dots 3$$

205

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

211

212 **Statistics**

213

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

220

221 **Results**

222

223 **Dark Adapted ERG**

224

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

227

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

228

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

232

233 **a-wave**

234

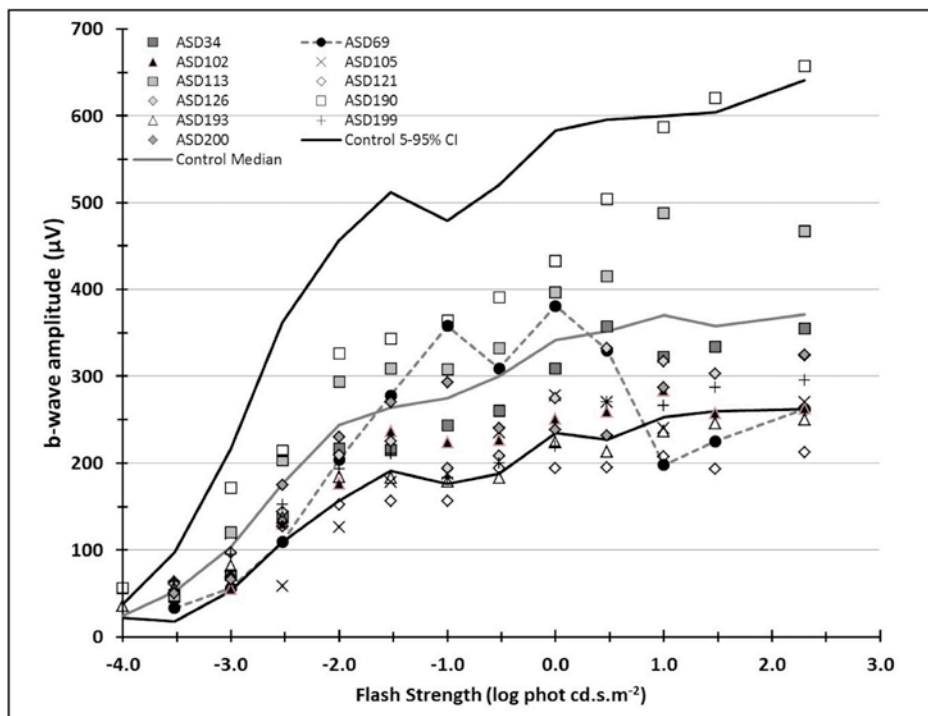
235 The a-waves of the control and ASD group were not significantly different in their amplitudes or time to trough.
 236 The 1.0 and 2.3 log phot cd.s.m⁻² ERGs were used for these inter-group comparisons as the a-wave is well
 237 defined and timing unambiguous at higher flash strengths. The median ASD amplitude and time to trough were
 238 296µV (157-451µV) and 9ms (8-13ms) and for the control group 316µV (220-482µV) and 8ms (7-10ms) at 2.3
 239 log phot cd.s.m⁻². There were no significant group differences for time to trough (p>0.56) nor amplitude
 240 (p>0.47) indicating no differences in phototransduction in the DA state.

241
 242 **b-wave**

243
 244 The b-wave amplitudes at low flash strengths were not significantly different between groups (p>0.13), although
 245 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
 246 control group (157 µV) with b-wave amplitudes of 126 (ASD105) and 152 µV (ASD 121). The range of these
 247 control data for the ISCEV standard flash ERGs were comparable to published data recorded from 53 healthy
 248 25-50 year olds [52] (See Fig 1). Following the rod dominated lower limb the b-waves of the ASD group had a
 249 higher proportion (4/11 or 36%) that fell below the control 5th centile at flash strengths of 1.0 and 1.5 log phot
 250 cd.s.m⁻².

251
 252 There was a slight delay in the time to peak of the b-waves at higher flash strengths (corrected p=0.04 and
 253 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-
 254 wave amplitudes in the ASD group compared to the control group. Figure 1 shows the full luminance response
 255 range from -4.0 to 2.3 log phot cd.s.m⁻².

256



257
 258 **Fig 1** The full DA luminance response series for the ASD and control group demonstrating that some of the
 259 ASD participants responses were outside the 5% confidence interval of the control group. ASD69 (filled circles)

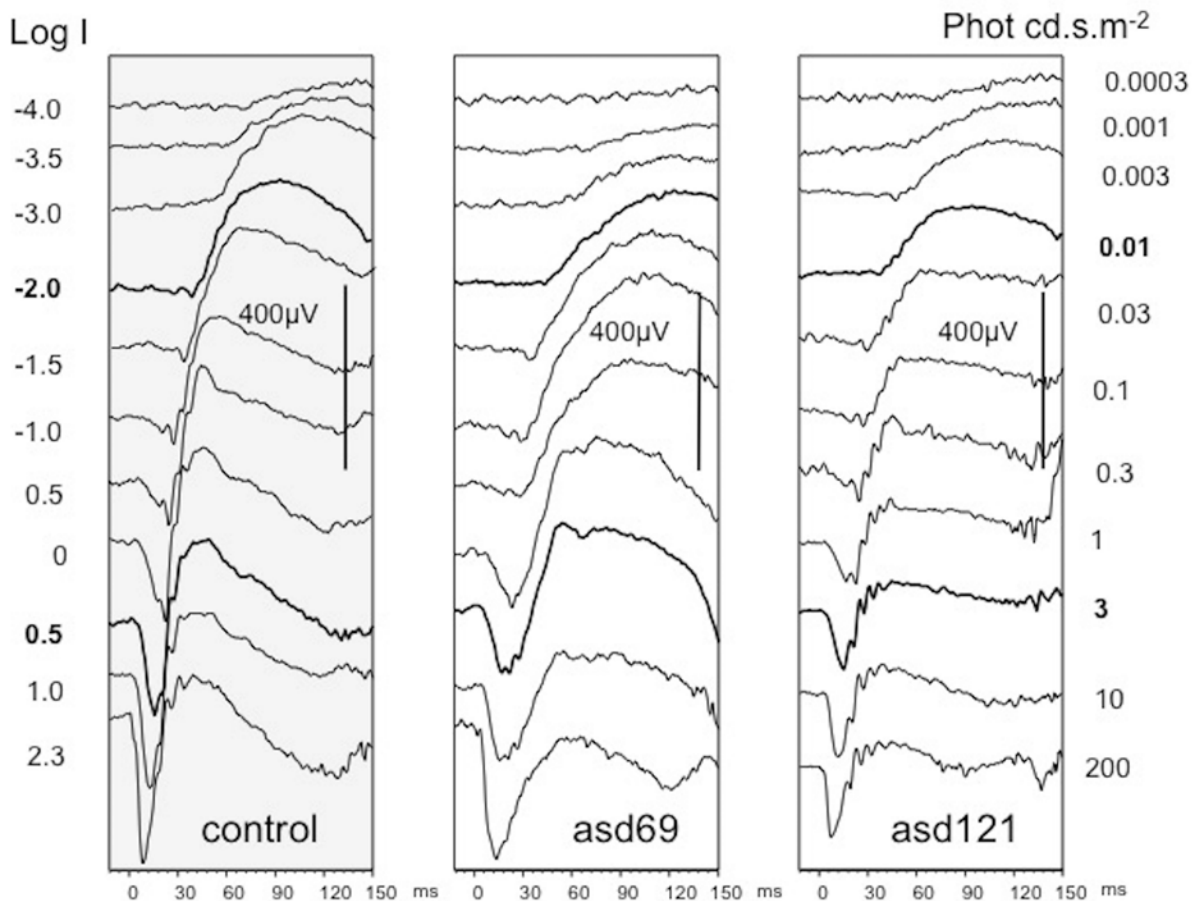
260 showed a dip in the luminance response function at $-0.5 \log \text{phot cd.s.m}^{-2}$ (dotted line). The control 5-95% CI
261 (black solid line) and the control median (solid grey line) shown.

262

263 Atypical Electroretinograms

264

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

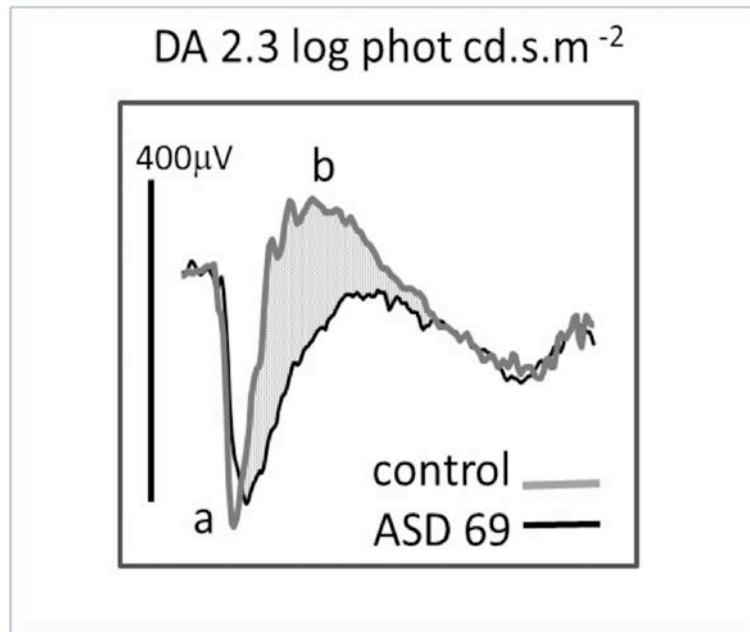


274

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

279

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



284
 285 **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
 286 illustrate the selective reduction in the b-wave compared to the a-wave. (a: a-wave; b: b-wave).

287

288 **b:a ratios**

289

290 Group b:a ratios for ASD participants and controls to ISCEV dark adapted 0.5 log phot cd.s.m⁻² were not
 291 significantly different: median b:a ratio ASD 1.79, range 1.38-2.48 compared with controls 1.75, range 1.44-
 292 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
 293 1.10-1.76 compared with controls 1.53, range 1.26-1.80 (p=0.05).

294

295 **Naka Rushton**

296

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

302

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

307 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)
 308 nor the 1log phot cd.s.m⁻² [ASD 286 (207-587 μV); Control 370 (253-600 μV p=0.13).

309

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

314

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

319

	V_{max} (μV)	$\text{Log}_{10}(K_m)$	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

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

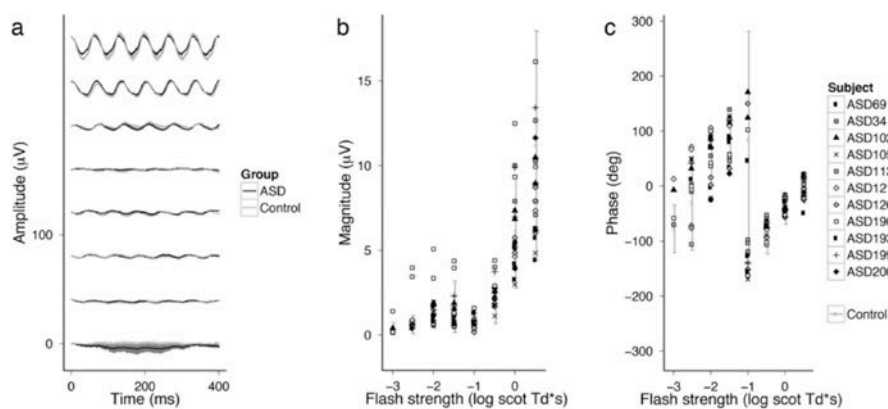
323

324 15 Hz slow and fast rod pathways

325

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

330



331

332 **Fig 4a** Group averages for amplitude of the 15Hz flicker and phase of the response showing no significant

333 group differences. **Fig 4b** plots the amplitude of the flicker response for the ASD individuals that exhibit a

334 typical response profile compared to the controls. **Fig 4c** shows a similar phase reversal as the slow and fast rod

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

337

338 **Light Adapted ERG**

339

340 The group results for amplitudes and time to peaks for the phNR, the a- and b-waves are shown in table 3 for the
 341 ISCEV standard 0.5 log phot cd.s.m⁻² single flash. Independent samples for the 5 flash strengths using found no
 342 significant interaction between the a-wave parameters of amplitude or time to trough (p>0.08) and group.

343 Similarly the phNR amplitude and time to trough were non-significant (p>0.07). However, there was a
 344 significant interaction between groups and flash strengths for the b-wave amplitude (corrected p=0.006) across
 345 the flash strength series. Follow up Mann-Whitney test for the b-wave amplitude of the 0.5 log phot cd.s.m⁻²
 346 revealed a significantly (corrected p<0.001) lower b-wave amplitude: median (95% CI) [ASD 94 (87-186 μ V);
 347 Control 137 (80-234 μ V)]. No group differences were observed in the time to peak of this response (p=0.72).

348 Figure 5 shows the group data (median with 95% CI) for the LA ERGs across the flash strengths of -0.5 to 1.0
 349 log phot cd.s.m⁻².

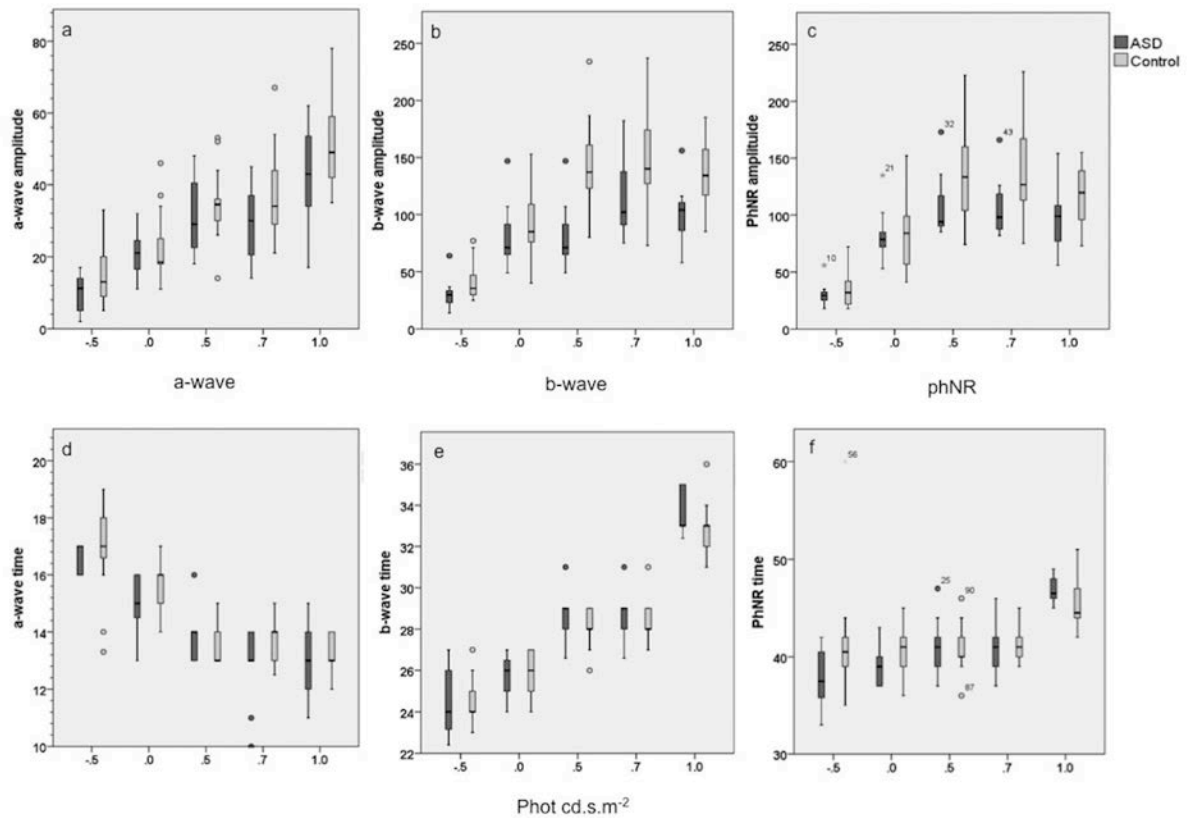
350

0.5 log phot cd.s.m ⁻² single flash	ASD		Control		p-value
	median	5 th to 95 th percentile	median	5 th to 95 th percentile	
a-wave amplitude (μ V)	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 (μ V)	94	87-186	137	80-234	<0.001*
b-wave time to peak (ms)	29	27-31	28	27-29	0.72
phNR amplitude (μ V)	94	85-173	134	74-223	0.07
phNR time to peak (ms)	41	37-47	40	36-46	0.30

351

352 **Table 3.** Results of time to peak of the a- and b-waves and the photopic negative response (phNR) of the full
 353 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
 354 amplitude was significantly lower in the ASD group (corrected p<0.001). * indicates Bonferroni corrected p-
 355 value for multiple tests.

356



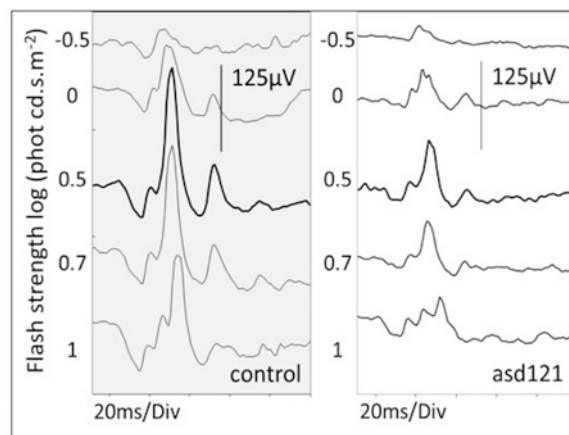
357

358 **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
 359 centile range. A significant difference (corrected p=0.006) between groups was observed for the b-wave
 360 amplitude (fig 1b) across the luminance series. At 0.5log phot cd.s.m⁻² the ASD group's b-wave amplitude was
 361 significantly lower than the control group's (p<0.001).

362

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

365



366

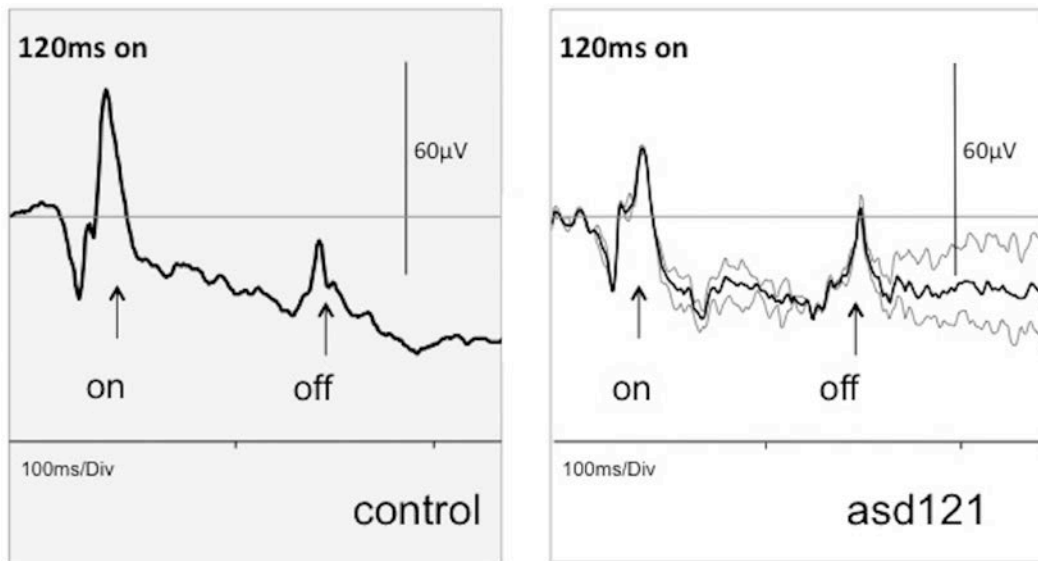
367 **Fig 6** A typical series of photopic ERGs from flash intensities (-0.5 to 1.0 log phot cd.s.m⁻²) for ASD121 and
 368 age matched control participant showing the reduction in the b-wave across the flash series. The bold trace
 369 highlights the ISCEV standard 0.5 log phot cd.s.m⁻² single flash.

370

371 **ON and OFF response**

372

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

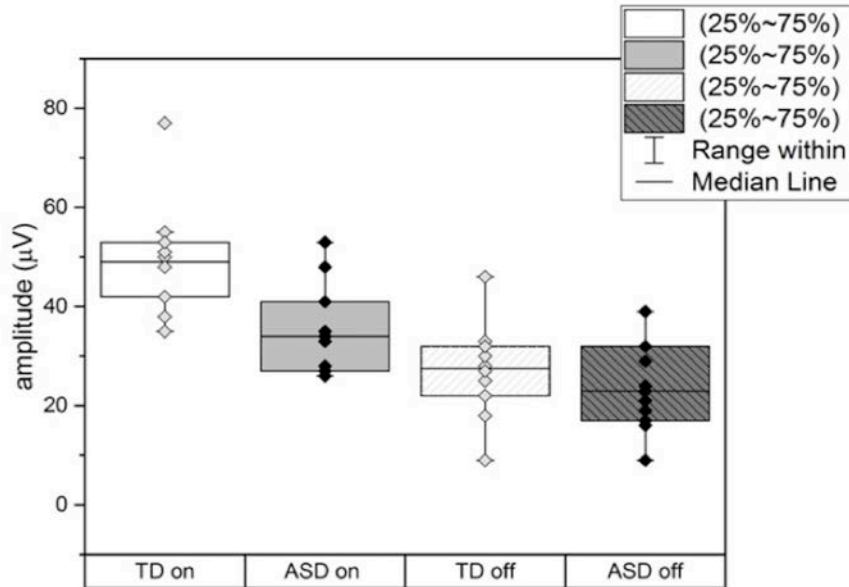


379

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

383

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



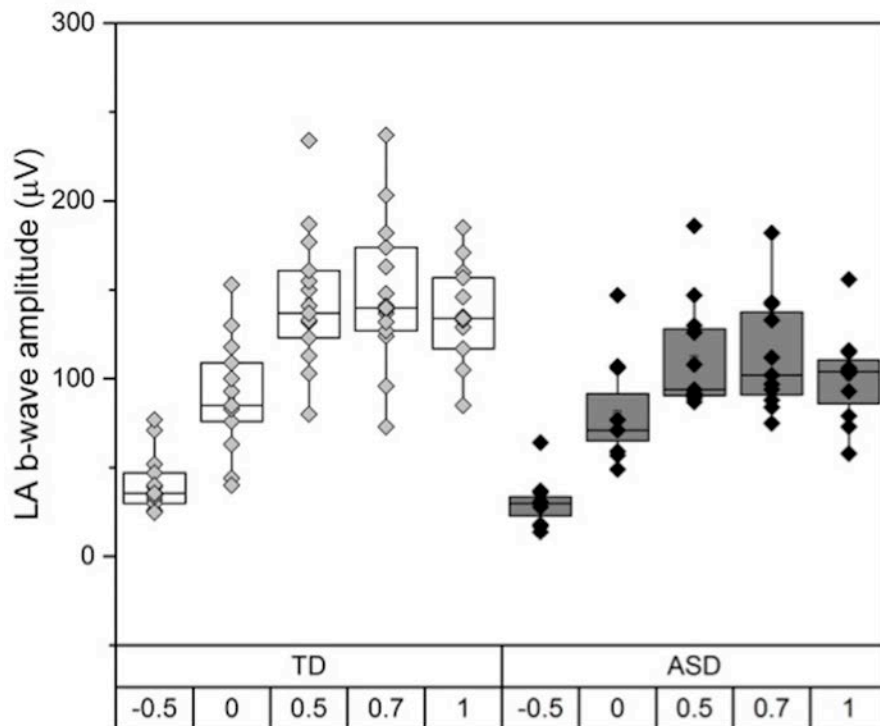
389
 390 **Fig 8.** The ON and OFF amplitudes between groups. The ASD group had a significantly reduced ON response
 391 ($p=0.003$) with no differences between groups for the OFF response. Box plots show the 25-75 % centiles the
 392 horizontal black line the median.
 393

	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

394
 395 **Table 4.** Parameters for the 120ms extended flash which enables the ON and OFF bipolar cell contribution to
 396 the photopic ERG to be separated. A significant group difference was present for the b-wave at light onset
 397 (corrected $p=0.003$). * indicates Bonferroni corrected p-value for multiple tests.
 398

399 Photopic Hill

400
 401 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-
 402 wave amplitude was analysed using a 5 responses x 2 (Group [ASD, Control]) repeated measures ANOVA. This
 403 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
 404 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
 405 peak with the control group, but the ASD group did not establish a clear peak until approximately ~ 1 log phot
 406 cd.s.m^{-2} . The V_{max} b-wave amplitudes for the ASD group were; median (CI) [102 (75-182) and for control 140
 407 (73-277)] which was non-significant ($p=0.06$). The a-wave amplitude at the fixed point V_{max} peak of the
 408 photopic hill was also not different between groups ($p=0.15$).



409

410 **Fig 9.** The photopic hill represented by the median with 5-95% CI. for each group. The median represented by
 411 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 median
 412 peak for the ASD group occurs at ~ 1.0 log phot cd.s.m⁻² with a noticeable flattening of the photopic hill.
 413 Repeated measures ANOVA revealed a significant (p=0.01) difference between groups although no significant
 414 difference was observed at the peak of the hill (fixed V_{max} p=0.06).

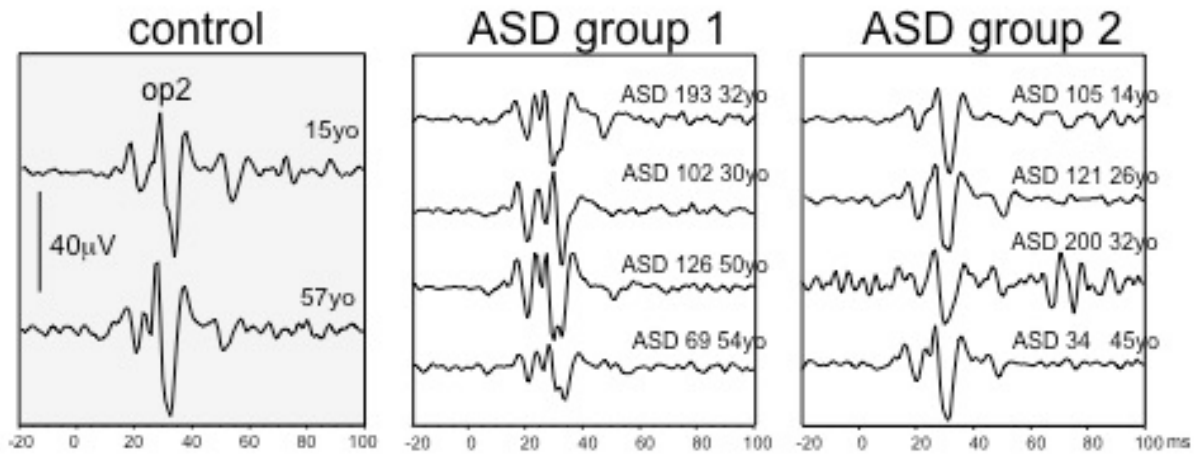
415

416 **Oscillatory Potentials and 30Hz**

417

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

422



423
 424 **Fig 10.** The morphological differences in the photopic OPs. The ASD group falls into two groups, group 1
 425 shows a bifurcation of the OP2. Control examples of the limits of the participant age range are shown.

426
 427 **Discussion**

428
 429 The findings of our exploratory study of the scotopic luminance response function in ASD individuals support
 430 the earlier work of Ritvo et al [30]. They found scotopically balanced red and blue full field flash ERG b-waves
 431 to be smaller from individuals with ASD compared to controls and attributed this to the glutamate rod driven
 432 ON- bipolar pathway[30]. We also found ASD DA b-waves to be low amplitude, but a smaller proportion 2/11
 433 (18%) fell below normal range compared with 13/28 (48%) in Ritvo's study [30]. The different rates may be
 434 due in part to the lower power of this exploratory report but also the severity of ASD. The individuals with ASD
 435 in our study represent the higher functioning end of the ASD spectrum whilst Ritvo et al assessed a range of low
 436 functioning ASD individuals. If neurological behaviour and retinal function are linked then we may not expect
 437 to see as a higher proportion showing marked b-wave differences. A larger sample would provide the
 438 opportunity to see if specific autistic traits measured by the ADOS [48] or AQ [51] were associated with
 439 changes in the ERG. However, the new findings reported here are the first to investigate the ERG responses
 440 across a wide range of stimulus strengths and the low b-wave amplitudes under LA conditions points to a
 441 difference in the cone-bipolar synapse.

442
 443 The linked gene networks associated with ASD provide evidence for a role of altered neurotransmitter signalling
 444 affecting the behaviour of ASD individuals [11,57], and the metabotropic glutamate pathway has been
 445 specifically implicated in the pathogenesis of ASD [11,12]. In high functioning adults with ASD we found
 446 reduced DA b-wave amplitude to the $-2.0 \log \text{phot cd.s.m}^{-2}$ flash in two ASD individuals. This represented 18%
 447 of the group, falling below the control 5th centile lower amplitude of $172\mu\text{V}$. Although mGluR6 receptors are
 448 strongly expressed in the ON-bipolar cells, the expression of mGluR6 is *not* widespread in the cortex [58].
 449 Cortical changes in ASD have been well documented [59] in ASD with particular differences evident in the
 450 frontal cortex and superior temporal sulcus [60] which are cortical regions that are not rich in mGluR6 [61].
 451 However, Hanna & Calkin's (2007) [62] study of the expression profile of glutamate receptors and transporters
 452 in primate rod bipolar cells found mGluR3 receptors in 74% of cells. Given that mGluR3 is strongly expressed

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

455

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

463

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

475

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

484

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

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

500

501 The normal LA a-wave parameters in the ASD group contrast with schizophrenia where reduced a-waves
502 [22,24,25] have been reported. The LA a-wave has a contribution from the OFF-bipolar cells [71] which implies
503 the OFF-bipolar cell pathway is not affected in ASD. The ASD group did not establish a clear photopic hill peak
504 until $\sim +0.3 \log \text{phot cd.s.m}^{-2}$ higher than control. The shape of the photopic hill appeared to be more sigmoidal
505 which may be in response to a loss of the logistic growth component that is derived from the ON-bipolar
506 pathway [72] and consistent with the findings of reduced cone-ON bipolar contribution in ASD. One weakness
507 of our study is that we did not extend the LA flash strength series to $> 1 \log \text{phot cd.s.m}^{-2}$ and cannot fully
508 analyse the characteristics of the photopic hill into its component parts [72,73]. Overall the ERG findings in
509 ASD were not as striking as those observed in schizophrenia with the main similarity being a reduced b-wave
510 under LA conditions [22,23].

511

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

517

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

524

525 A possible mechanism could be downstream components of the metabotropic signalling cascade such as G_α and
526 $G_{\beta\gamma}$ that regulate the non-selective cation channel transient receptor potential melastatin (TRPM1) channel
527 in the ON-bipolar cell that when gated open results in depolarisation [77]. SNPs associated with mGluR5 and
528 $G_0\alpha$ and phospholipase- β have recently been postulated as possible markers for identifying ASD [78]. Deletion
529 of the $G_0\alpha$ subunit abolishes the DA and LA b-wave in mouse [79,80] and therefore potentially a partial loss of
530 function in $G_0\alpha$ may contribute to the diminished b-waves observed in the ASD group. Knock out mice for
531 $G\beta(3)$ exhibit a more pronounced loss of the LA b-wave amplitude compared to the DA b-wave which is
532 similar to the pattern of results observed in this study population [81]. Therefore, whilst $G\beta(3)$ knock-out mice

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

536

537 **Conclusion**

538

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

547

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549

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554

555 **Conflict of Interest**

556

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

562

563

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564

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