Temporal whole field sawtooth flicker without a spatial component elicits a myopic shift following optical defocus irrespective of waveform direction in chicks

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Purpose. Myopia (short-sightedness) is the commonest visual disorder and greatest risk factor for sight threatening secondary pathologies. Myopia and hyperopia can be induced in animal models by rearing with optical lens defocus of opposite sign. The degree of refractive compensation to lens-induced defocus in chicks has been shown to be modified by directionally drifting sawtooth spatio-temporal luminance diamond plaids, with Fast-ON sawtooth spatio-temporal luminance profiles inhibiting the myopic shift in response to negative lenses, and Fast-OFF profiles inhibiting the hyperopic shift in response to positive lenses. What is unknown is whether similar sign-of-defocus dependent results produced by spatio-temporal modulation of sawtooth patterns could be achieved by rearing chicks under whole field low temporal frequency sawtooth luminance profiles at 1 or 4 Hz without a spatial component, or whether such stimuli would indiscriminately elicit a myopic shift such as that previously shown with symmetrical (or near-symmetrical) low frequency flicker across a range of species. **Methods.** Hatchling chicks ($n =$ 166) were reared from days five to nine under one of three defocus conditions (No Lens, +10D lens, or -10D lens) and five light conditions (No Flicker, 1Hz Fast-ON/Slow-OFF sawtooth flicker, 4Hz Fast-ON/Slow-OFF sawtooth flicker, 1Hz Fast-OFF/Slow-ON sawtooth flicker, or 4Hz Fast-OFF/Slow-ON sawtooth flicker). The sawtooth flicker was produced by light emitting diodes (white LEDs, 1.2 – 183 Lux), and had no measurable dark phase. Biometrics (refraction and ocular axial dimensions) were measured on day nine. **Results.** Both 1Hz and 4Hz Fast-ON and Fast-OFF sawtooth flicker induced an increase in vitreous chamber depth that was greater in the presence of negative compared to positive lens defocus. Both sawtooth profiles at both temporal frequencies inhibited the hyperopic shift in response to +10D lenses, whilst full myopic compensation (or over-compensation) in response to -10D lenses was observed. **Conclusions.** Whole field low temporal frequency Fast-ON and Fast-OFF sawtooth flicker induces a generalized myopic shift, similar to that previously shown for symmetrical sine-wave and square-wave flicker. Our findings highlight that temporal modulation of retinal ON/OFF pathways per se (without a spatial component) is insufficient to produce strong sign-of-defocus dependent effect.

Temporal Whole Field Sawtooth Flicker without a Spatial Component Elicits a Myopic

Shift Following Optical Defocus Irrespective of Waveform Direction in Chicks

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17   **Abstract**

18   **Purpose.** Myopia (short-sightedness) is the commonest visual disorder and greatest risk factor

19   for sight threatening secondary pathologies. Myopia and hyperopia can be induced in animal

20   models by rearing with optical lens defocus of opposite sign. The degree of refractive

21   compensation to lens-induced defocus in chicks has been shown to be modified by directionally

22   drifting sawtooth spatio-temporal luminance diamond plaids, with Fast-ON sawtooth spatio-

23   temporal luminance profiles inhibiting the myopic shift in response to negative lenses, and Fast-

24   OFF profiles inhibiting the hyperopic shift in response to positive lenses. What is unknown is

25   whether similar sign-of-defocus dependent results produced by spatio-temporal modulation of

26   sawtooth patterns could be achieved by rearing chicks under whole field low temporal frequency

27   sawtooth luminance profiles at 1 or 4 Hz without a spatial component, or whether such stimuli

28   would indiscriminately elicit a myopic shift such as that previously shown with symmetrical (or

29   near-symmetrical) low frequency flicker across a range of species.

30   **Methods.** Hatchling chicks ($n = 166$) were reared from days five to nine under one of three

31   defocus conditions (No Lens, +10D lens, or -10D lens) and five light conditions (No Flicker,

32   1Hz Fast-ON/Slow-OFF sawtooth flicker, 4Hz Fast-ON/Slow-OFF sawtooth flicker, 1Hz Fast-

33   OFF/Slow-ON sawtooth flicker, or 4Hz Fast-OFF/Slow-ON sawtooth flicker). The sawtooth

34   flicker was produced by light emitting diodes (white LEDs, 1.2 – 183 Lux), and had no

35   measurable dark phase. Biometrics (refraction and ocular axial dimensions) were measured on

36   day nine.

37   **Results.** Both 1Hz and 4Hz Fast-ON and Fast-OFF sawtooth flicker induced an increase in

38   vitreous chamber depth that was greater in the presence of negative compared to positive lens

39   defocus. Both sawtooth profiles at both temporal frequencies inhibited the hyperopic shift in

40   response to +10D lenses, whilst full myopic compensation (or over-compensation) in response to

41   -10D lenses was observed.

42   **Conclusions.** Whole field low temporal frequency Fast-ON and Fast-OFF sawtooth flicker

43   induces a generalized myopic shift, similar to that previously shown for symmetrical sine-wave

44   and square-wave flicker. Our findings highlight that temporal modulation of retinal ON/OFF

45   pathways per se (without a spatial component) is insufficient to produce strong sign-of-defocus

46   dependent effect.

Introduction

47

48 Most species are born with hyperopic eyes that, with normal visual experience, increase in size

49 during early development to achieve emmetropia (i.e., no refractive error) (Norton & Siegwart Jr

50 1995; Rabin et al. 1981; Stone et al. 1995; Troilo & Wallman 1991; Wildsoet 1997). This

51 emmetropization process is controlled by complex gene-environment interactions (Fan et al.

52 2014; Morgan & Rose 2005; Verhoeven et al. 2013). Epidemiological studies have demonstrated

53 that exposure to environmental risk factors (such as spending limited time outdoors (Rose et al.

54 2008) or doing prolonged near-work (Huang et al. 2015)) can predispose the eye to excessive

55 growth resulting in myopia (short-sightedness). Myopia is now the most common ocular disorder

56 (Schneider et al. 2010) with rapidly increasing prevalence and severity worldwide (Lee et al.

57 2013; Seet et al. 2001), making the development of treatments to control ocular growth a health

58 and socioeconomic priority.

59

60 Ocular growth has been extensively studied in animals, where myopia can be induced by rearing

61 with negatively powered defocusing lenses affixed over the eye or form deprivation via lid suture

62 or plastic occluders (Hodos & Kuenzel 1984; Irving et al. 1992; Schaeffel et al. 1988; Wallman

63 et al. 1978; Wiesel & Raviola 1977), and hyperopia (long-sightedness) can be induced by

64 positive lenses (Irving et al. 1992; Schaeffel et al. 1988). Ocular growth and refractive shifts in

65 these animal models can also be mediated by pharmacological manipulations of retinal ON/OFF

66 pathway activity (Crewther & Crewther 1990; Crewther et al. 1996; Ehrlich et al. 1990; Smith et

67 al. 1991). Suppression of ON-pathway activity by L-α-aminoadipic acid in chicks and kittens

68 decreases axial growth in normally developing animals (Crewther & Crewther 1990; Crewther et

69 al. 1996; Crewther & Crewther 2003). Conversely, OFF-pathway suppression by D-α-

70 aminoadipic acid increases axial growth in normally developing chicks (Crewther & Crewther

71 1990), and inhibits optically-induced growth changes (Crewther & Crewther 1990; Crewther et

72 al. 1996; Crewther & Crewther 2003). More recent gene knockout studies have demonstrated an

73 increased susceptibility to FD in mice with ON-pathway defects (Chakraborty et al. 2015; Pardue

74 et al. 2008), consistent with the effects of ON-pathway suppression in the chick FD model

75 (Crewther & Crewther 1990). Mice with an OFF-pathway defect did not develop myopia in

76 response to FD (although this latter result may reflect the genetic background) (Chakraborty et

77 al. 2014).

78

79   Although pharmacological and genetic studies have provided strong evidence implicating retinal

80   ON/OFF pathways in ocular growth and refractive change, fewer studies have investigated the

81   effects of visual stimuli designed to preferentially activate ON- or OFF-systems. One of the first

82   studies was a notable paper by Schwahn & Schaeffel 1997, (Schwahn & Schaeffel 1997) who

83   raised chicks in flickering light of different temporal frequencies (12 and 6 Hz) and different

84   duty cycles (4-75% ratio of light to dark periods) produced by rotating chopper disks. Not

85   surprisingly, it was found that the extent to which FD and defocus-induced myopia could be

86   suppressed was related to the length of the duty cycle, suggesting that myopia is related to dark

87   duration and OFF pathway activation. Interestingly, there was no observable suppression of

88   hyperopia.

89

90   In 2002, Crewther and Crewther (Crewther & Crewther 2002) reared chicks in an environment

91   lit with directionally moving sawtooth diamond plaids based on the human Brücke-Bartley

92   Effect whereby a moving Fast-ON/Slow-OFF plaid has a perceptual darkening effect, and a Fast-

93   OFF/Slow-ON spatiotemporal luminance profile creates a brightening effect (Cavanagh & Anstis

94   2018; Cavanagh & Anstis 1986). Chicks reared with Fast-ON sawtooth plaid illumination

95   compensated fully to positive, but not to negative lenses. Conversely, chicks reared with Fast-

96   OFF sawtooth plaid illumination compensated fully to negative, but not positive, lenses

97   (Crewther & Crewther 2002). More recently, Riddell et al (Riddell et al. 2016) examined the

98   electrophysiological basis of the Brücke-Bartley Effect in toad eye cups recordings and showed a

99   sustained direct-current (DC) trans-tissue potential to drifting gratings. As in the human

100 experience of moving sawtooth gratings, the sustained DC potential effect was greater for Fast-

101 OFF (brightening effect) compared to Fast-ON sawtooth (darkening effect), suggesting that the

102 asymmetries in DC potential originate in the photoreceptoral response to Fast-ON and Fast-

103 OFFprofiles. Thus, the ability of this paradigm to inhibit growth in a sign-of-defocus dependent

104 manner that allows for potential localisation of structural origins, suggests that environmental

105 modulation of the spatio-temporal activity of the ON/OFF pathways could represent a promising

106 non-invasive treatment avenue for refractive errors using therapies aimed at the outer retina, as

107 suggested by Crewther (Crewther 2000).

108

109   What remains to be seen is whether the same inhibitory effects on refractive compensation

110   produced by spatio-temporal modulation of sawtooth patterns, can be achieved using whole field,

111   low temporal frequency sawtooth luminance profiles at 1 or 4 Hz without a spatial component, or

112   whether such stimuli would indiscriminately elicit a myopic shift such as that previously shown

113   to result from symmetrical (or near-symmetrical) low frequency flicker across a range of species

114   (Britton et al. 2014; Cheng et al. 2004; Cremieux et al. 1989; Crewther et al. 2006; Di et al.

115   2013a; Di et al. 2013b; Di et al. 2014; Li et al. 2016; Luo et al. 2017; Pan et al. 2017; Wang et al.

116   2013; Yu et al. 2011; Zhi et al. 2013).

117   In an attempt to resolve this question, Crewther et al. (Crewther et al. 2006) investigated the

118   effect of whole field, low frequency flicker and found a myopic shift for all lens conditions with

119   a stimulus comprised of an asymmetric light pulse similar to that of a Slow ON/Fast OFF

120   sawtooth and 1:2 ratio light:dark cycle. However, due to the extended dark period in the stimulus

121   profile created by the use of a halogen globe, the stimulus utilized in this study failed to replicate

122   a true temporal illuminance-based Brücke-Bartley effect with a reversible sawtooth profile as

123   produced in the 2002 study by the same lab, preventing an examination of the effect of

124   continuous full field light dark transitions on directional growth following suppression of either

125   the ON or OFF pathways.

126

127   Thus, the present study aimed to re-investigate the effects of whole field low frequency Fast-ON

128   and Fast-OFF sawtooth flicker on refractive compensation to defocusing lenses in chick using

129   the white light emitting diodes (LEDs) LEDS. LEDS are a more precise system for controlling

130   temporal luminance modulation and able to deliver reversible asymmetric light profiles (Fast-

131   ON/Slow-OFF and Fast-OFF/Slow-ON) that closely mimicked the temporal aspects of the

132   spatiotemporal sawtooth plaid paradigm (Crewther & Crewther 2002).

133

134   **Materials & Methods**

135   ***Animals and Rearing***

136   A total of 166 male hatchling chicks (Leghorn/New Hampshire) were obtained from a local

137   hatchery and raised from day five to nine in a light (12/12 hour day/night cycle) and temperature

138    $(30 \pm 0.5°C)$ controlled enclosure (height: 0.5 m, length: 1.0 m, width: 0.75m). On day five,

139 chicks were fitted with either a monocular +10D or -10D lens, or left as No Lens controls. These

140 defocusing goggles were made from modified human PMMA contact lenses (8.1 mm in

141 diameter) attached to Velcro© and affixed to the periocular feathers of the right eye. Within

142 each lens condition, chicks were randomly assigned to one of five light profiles: normal light

143 (referred to herein as No Flicker [NF]), 1Hz Fast-ON/Slow-OFF flicker, 4Hz Fast-ON/Slow-

144 OFF flicker, 1Hz Fast-OFF/Slow-ON flicker, or 4Hz Fast-OFF/Slow-ON flicker. All light

145 profiles followed a 12/12 hour day/night cycle.

146

147 The five light profiles were delivered using LEDs mounted to the roof of the enclosure. Ambient

148 light was maintained constantly at 183 lux during the 12 hour day cycle (No Flicker) or

149 modulated (using the same lamp) at frequencies of either 1 Hz or 4 Hz where light levels varied

150 between a minimum of 1.2 lux to a maximum of 183 lux, with an average light level of 97 lux.

151 Ambient lighting in the No Flicker condition was maintained at the maximum (rather than the

152 mean) of the flicker conditions to facilitate comparison with our previous study with a 'no

153 flicker' condition of 180 lux (Crewther et al. 2006). For the flicker conditions, the LEDs

154 produced a whole field sawtooth temporal luminance profile with a strict linear rise or fall in

155 luminance achieved through feedback control circuitry connected to a DC power supply with

156 computer input to implement waveform and frequency (for specifications please refer to

157 Supplementary Information). These white LEDs work through a Blue LED driving a fluorescent

158 material, ensuring that there is absolutely no change in the spectral distribution with brightness.

159 Figure 1 shows the sawtooth wave profile recorded using a PowerLab (AD Instruments,

160 Melbourne, Australia) via a light probe situated in the animal enclosure.

161

162   [Figure 1]

163

164 ***Biometric Analysis***

165 On day nine, chicks were anaesthetized (Ketamine 45mg/kg: Xylazine 4.5mg/kg i.m.) and both

166 eyes were refracted by retinoscopy (Keeler, Vista Diagnostic Instruments) and axial dimensions

167 were obtained from the average of at least three A-Scan ultrasonography traces (A-Scan III,

168 TSL: Teknar, Inc. St Louis, 7 MHz probe). Each trace from the A-Scan provided peaks

169 indicating the length of the eye (anterior pole of the cornea to inner limiting membrane of the

170   retina), the distance from the posterior pole of the lens to the inner limiting membrane of the

171   retina and the depth of the vitreous chamber (all measured in millimeters); subtracting the lens to

172   retina measurement from the axial length gave a measure of the depth central to the axis of the

173   anterior chamber. All procedures were conducted in strict accordance with La Trobe University

174   Animal Ethics Committee guidelines (Approval Number 08/30) and adhered to the NHMRC of

175   Australia and ARVO Statements for the use of Animals in Ophthalmic and Vision Research.

176

177   ***Data Analysis***

178   To control for differences in eye size within groups, refractive and biometric measures were

179   converted to raw score differences between experimental and fellow eyes. To assess whether

180   changes in ocular size were due to the experimental manipulation a 3 (lens condition × frequency

181   × light profile) way ANOVA was utilized. Student Newman Keuls or Games-Howell post hoc

182   tests were performed as appropriate.

183

184   **Results**

185   Table 1 reports the raw scores for refraction and axial length (mean and standard error of the

186   mean) for each experimental group.

187

188   [Table 1]

189

190   Figure 2 illustrates the mean differences (Experimental – Fellow Eye) in biometric measures

191   across groups.

192

193   [Figure 2]

194

195   As can be seen in Figure 2a, all chicks wearing -10D lenses developed myopia under all light

196   conditions. Hyperopic refractive compensation was also seen in eyes reared with +10D lenses

197   under all flicker conditions. Fast OFF flicker at a frequency of 1 Hz produced the most notable

198   impact on refraction, with eyes reared with -10D lenses under this condition displaying

199   approximately 2 D more myopia than other -10 D lens groups. Fast OFF flicker also resulted in

200   the greatest suppression of hyperopia in eyes wearing +10D lenses. Chicks reared without a lens

201 were largely plano under all light conditions. Simple main effects analysis revealed a significant

202 effect of light profile ($F_{1, 149} = 10.62$, p = .0001), lens ($F_{2, 149} = 453.65$, p < .0001) and frequency

203 ($F_{2, 149} = 13.32$, p < .0001). Significant interactions were also observed between lens and light

204 ($F_{2, 149} = 3.89$, p = .023), lens and frequency ($F_{2, 149} = 3.98$, p = .021), light and frequency ($F_{1, 149}$

205 = 13.17, p = .0001), and lens and light and frequency ($F_{2, 149} = 3.28$, p = .04).

206

207 Post hoc analysis comparing the effect of flicker frequency on refractive state revealed that eyes

208 reared with -10D lenses were significantly more myopic under Fast OFF light conditions with 1

209 Hz flicker, in comparison to 4 Hz and no flicker (NF) conditions (see Table 2). No difference in

210 refraction was found between flicker frequencies with a Fast ON sawtooth profile for eyes reared

211 with -10D lenses. Under Fast OFF sawtooth conditions, chicks wearing +10D lenses were

212 significantly less hyperopic when raised with 1 Hz flicker compared to 4 Hz and no flicker

213 conditions (see Figure 2a). Chicks reared under Fast OFF light were significantly less hyperopia

214 than all other chicks wearing +10 D lenses. Refractions were significantly different between all

215 lens groups when compared within frequency and sawtooth conditions. Comparisons between

216 the effect of the sawtooth profile on refraction revealed that Fast OFF with 1Hz flicker resulted

217 in significantly greater myopia than Fast ON + 1 Hz or NF light conditions with -10D lenses, and

218 significantly less hyperopia with +10D lens-wear. A summary of post hoc analyses is presented

219 in Supplementary Table 1.

220

221 [Table 2]

222

223 An overall increase in axial length in response to -10D lenses was observed, with eyes reared

224 under Fast OFF flicker at 1 Hz showing the longest eyes (Figure 2b). Fast OFF flicker at 1 Hz

225 also resulted in longer axial length with +10D lens-wear in comparison to NF control, as did Fast

226 ON flicker at 1 Hz. No Lens chicks showed slight increases in length for all conditions. As

227 expected, all other +10D lens groups showed an overall decrease in axial length compared to No

228 Lens groups. Simple main effects analysis revealed a significant effect of lens ($F_{2, 149} = 131.00$,

229 p < .0001) and frequency ($F_{2, 149} = 13.53$, p < .0001). A significant interaction effect was

230 obtained for lens and frequency ($F_{2, 149} = 4.46$, p = .013). Post hoc analysis confirmed the

231 observation that 1Hz flicker for both sawtooth profiles led to longer axial lengths for +10D lens

232 conditions.

233

234 Consistent with axial length measures, Figure 2c shows an overall increase in vitreous chamber

235 depth in response to -10D lenses for all light conditions, and a notable decrease in vitreous

236 chamber depth in response to +10D lenses for all light conditions except for Fast OFF and Fast

237 ON 1 Hz flicker in comparison to No Lens groups. No Lens chicks showed a slight increase in

238 vitreous chamber depth. Simple main effects analysis revealed a significant effect of lens ($F_{2, 149}$

239 = 158.01, p < .0001) and frequency ($F_{2, 149}$ = 13.37, p < .0001), and a significant interaction

240 between lens and frequency ($F_{2, 149}$ = 4.47, p = .013). Post hoc analysis again confirmed the

241 observation that 1Hz flicker for both sawtooth profiles led to longer vitreous chamber depths for

242 +10D lens conditions.

243

244 As expected, a great degree of variability was observed in anterior chamber depth in response to

245 lens wear (Figure 2d). A significant interaction between lens and frequency ($F_{2, 149}$ = 5.88, p =

246 .004) was found. Post hoc tests showed that 4 Hz flicker for both Fast OFF and Fast ON profiles

247 led to shallower anterior chamber depths for +10D lens conditions.

248

249 **Discussion**

250 Previous studies have shown that low frequency sine-wave and square-wave flicker induces a

251 generalized myopic shift in a range of species (Britton et al. 2014; Cheng et al. 2004; Cremieux

252 et al. 1989; Di et al. 2013a; Di et al. 2013b; Di et al. 2014; Li et al. 2016; Luo et al. 2017; Yu et

253 al. 2011; Zhi et al. 2013). In the present study, we demonstrate that whole field temporal Fast

254 ON and Fast OFF sawtooth flicker with no spatial component or dark phase induces a general

255 increase in vitreous chamber depth and a subsequent myopic shift that is greater in the presence

256 of defocus (particularly positive defocus), and greater at lower temporal frequencies (i.e., 1Hz

257 versus 4Hz). The largest effects occurred for 1Hz Fast OFF flicker, which strongly inhibited

258 refractive compensation to positive lenses and induced over-compensation to negative lenses.

259 Although this latter finding is in agreement with our previous study of spatiotemporal sawtooth

260 flicker (Crewther & Crewther 2002), on the whole, these results suggest that whole field

261 temporal modulation of retinal ON/OFF pathways does not produce the strong sign-of-defocus

262   dependent effects previously demonstrated for spatiotemporal ON/OFF stimuli (Crewther &
263   Crewther 2002).

264

265   As light levels can affect responses to lens and occluder wear (Norton & Siegwart 2013), further
266   research is needed to rule out a contribution from differing mean illuminance between the
267   temporal sawtooth in the present study and the spatiotemporal sawtooth in our previous study (97
268   and 387 lux, respectively), and between the no flicker and flicker conditions in the present study
269   (183 and 97 lux, respectively). Past research has shown that chicks reared under very dim
270   lighting (≤50 lux) for weeks to months develop longer axial lengths and a more myopic
271   refraction (Bercovitz et al. 1972; Cohen et al. 2011; Cohen et al. 2012; Lauber & Oishi 1987).
272   However, shorter duration studies have shown no difference in refractive development following
273   4 days of occluder wear under 50 lux versus 500 lux lighting (Ashby et al. 2009), or following 7
274   days of wearing neutral density filters (0.5-1 log unit attenuation) versus clear filters under 500
275   lux lighting (Feldkaemper et al. 1999). These latter findings suggest that the contribution of
276   mean illuminance to refractive outcomes across conditions is likely to be minimal. That said, a
277   comparison of the present findings with those of our past study investigating near-symmetrical
278   flicker (Crewther et al. 2006) is not inconsistent with a contribution from mean luminance in
279   driving refractive change. This past study investigated the effects of flicker with a gradual rise
280   and slightly sharper decline (approximating a Fast-OFF profile), and variable duration dark
281   phase (longest for 1Hz flicker and shortest for 4Hz flicker) (Crewther et al. 2006). In this
282   previous study, the 1Hz flicker condition (which had the lowest mean luminance) caused the
283   strongest inhibition of compensation to positive lenses, and all frequencies caused a similar
284   degree of over-compensation to negative lenses. The refractive shifts observed in the present
285   study, where there was no measurable dark phase and thus a slightly higher mean luminance,
286   were similar but less extreme. The results of these two studies are also interesting in the context
287   of previous research showing a correlation between the degree of myopia suppression by flicker
288   and the duration of the dark phase at high frequencies (with longer dark phases conferring
289   greater suppression) (Schwahn & Schaeffel 1997). Our previous study (Crewther et al. 2006)
290   demonstrated equivalent over-compensation to negative lenses despite large differences in the
291   duration of the dark phase at 1, 2, and 4Hz. These results demonstrate that the correlation

292 between the flicker dark phase and the degree of myopia induction does not hold at low temporal

293 frequencies when the mean illuminance is not controlled.

294

295 Previous physiological research in monkey (Khan et al. 2005) and toad eye cup (Riddell et al.

296 2016) indicates that temporal and spatiotemporal sawtooth modulation produces complex post-

297 receptoral ON/OFF pathway asymmetries, with push-pull interactions evident between ON- and

298 OFF- bipolar cell contributions to Fast ON and Fast OFF waveforms in both paradigms (Khan et

299 al. 2005; Riddell et al. 2016). Although both paradigms mediate ON/OFF pathway activity in a

300 polarity-specific manner, their effects on gross trans-retinal potentials are vastly different.

301 Spatiotemporal sawtooth produces a sustained trans-retinal potential increase (presumably

302 resulting from the summation of potentials from different local retinal regions), that has

303 asymmetries at the photoreceptor-RPE level as well as post-receptorally (Riddell et al. 2016).

304 The important difference between the Crewther and Crewther's 2002 (Crewther & Crewther

305 2002) stimuli and stimuli utilised in the current study is the removal of the spatial aspects of each

306 local diamond stimulus. The current stimulation procedure is a full field sawtooth ON to OFF, or

307 OFF to ON temporal modulation of the environment. The neural response to whole field

308 temporally modulated light is an 'entire retina' photoreceptor response that results in a global

309 change in retinal transmembrane potential (Khan et al. 2005). In comparison, the neural response

310 to the spatial plaid components of the diamond pattern is expected to allow relatively localized

311 spatial and temporal buffering of the change of potential across the retina (Riddell et al. 2016).

312 Such differences in their effects can be conceptualized in terms of spatial buffering of ions. Each

313 change in illuminance, whether temporal only or spatiotemporal, results in a large efflux of

314 sodium ions into the outer segments and potassium out in the subretinal space during darkness

315 and reversal of this process during light onset (See (Bialek & Miller 1994; Gallemore et al. 1997;

316 Hamann 2002; Li et al. 1994; Steinberg 1985; Steinberg et al. 1985). It is proposed that the

317 reduced effect 4 Hz flicker on eye growth is related to the refractory period required to modulate

318 directional change in the buffering of ion distributions in conjunction with the magnitude of the

319 change in transretinal potential caused by the flicker stimulus. Thus, the full field stimulation of

320 the current 2018 study forces ionic changes in the radial dimension, reducing the processes to a

321 1-dimensional situation across the retina. In comparison, the 2002 spatiotemporal diamonds

322 could allow buffering in the direction tangential to the retinal layers, for example, in the

323 subretinal space, or indeed within individual RPE cells to produce a directional growth signal in

324 response to signed defocus. Further investigation is warranted to determine the nature of the

325 refractory period for ionic spatial buffering and the impact of spatiotemporal versus temporal

326 frequency signals in driving signed compensation.

327

328 **Conclusions**

329 The present study demonstrated that both whole field low temporal frequency fast ON and fast

330 OFF flicker induce a generalized myopic shift in chicks wearing defocusing lenses, but do not

331 affect refractive development in chicks not wearing lenses. These results are highly similar to our

332 previous findings using near-symmetrical flicker approximating a Fast OFF profile (Crewther et

333 al. 2006), as well as being broadly concordant with the myopic shift shown following exposure

334 to symmetrical flicker in chicks (Britton et al. 2014), mice (Yu et al. 2011), guinea pigs (Cheng

335 et al. 2004; Di et al. 2013a; Di et al. 2013b; Di et al. 2014; Li et al. 2016; Luo et al. 2017; Zhi et

336 al. 2013), and cats (Cremieux et al. 1989). Most importantly, this study highlights the fact that

337 temporal luminance modulation *per s*e is not adequate to induce signed directional refractive

338 compensation. Rather, signed modulation of refractive compensation requires a directional

339 spatiotemporal component which likely effects relative adaptive brightness in local regions

340 across the retina.

341

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347 al. 2010).

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349

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506

Mean (±S.E) refraction and axial length of experimental eyes (EE) and fellow eyes (FE) for each lens, light profile and frequency condition.

			-10 D					No Lens					+10 D			
		n	EE RE (D)	FE RE (D)	EE AL (mm)	FE AL (mm)	n	EE RE (D)	FE RE (D)	EE AL (mm)	FE AL (mm)	n	EE RE (D)	FE RE (D)	EE AL (mm)	FE AL (mm)
Fast OFF	1 Hz	10	-12.6 ±1.04	.30 ±.15	9.32 ±.07	8.81 ±.06	9	-.56 ±.47	-.14 ±.28	8.81 ±.06	8.75 ±.05	9	0.75 ±1.15	.17 ±.12	8.92 ±.12	8.85 ±.06
	4 Hz	11	-7.18 ±1.68	.14 ±.30	9.03 ±.07	8.66 ±.05	9	-.28 ±.41	.11 ±.29	8.65 ±.06	8.60 ±.06	11	5.56 ±.39	.52 ±.24	8.43 ±.05	8.66 ±.04
No Flicker		10	-8.4 ±.29	1.25 ±.21	9.20 ±.05	8.74 ±.05	19	.64 ±.25	1.01 ±.22	9.60 ±.04	9.55 ±.04	11	9.68 ±.29	1.30 ±.18	8.45 ±.07	8.75 ±.05
Fast ON	1 Hz	11	-8.43 ±1.04	.32 ±.23	9.23 ±.10	8.70 ±.08	11	.89 ±.33	.95 ±.34	8.76 ±.04	8.68 ±.04	12	5.62 ±1.17	.17 ±.31	8.80 ±.10	8.81 ±.06
	4 Hz	11	-8.70 ±1.43	.10 ±.27	9.13 ±.10	8.73 ±.06	10	.60 ±.58	.62 ±.21	8.71 ±.07	8.65 ±.06	12	6.38 ±1.20	.75 ±.22	8.50 ±.04	8.70 ±.06

Summary of within light profile condition and within frequency condition post hoc interactions.

	Sawtooth Profile			Frequency	
Fast OFF/Slow ON	-10D Condition	1 Hz < (4 Hz No Diff. NF)	1 Hz	-10D Condition	Fast OFF < (Fast ON No Diff. NF)
	No Lens Condition	1 Hz No Diff. NF No Diff. 4 Hz		No Lens Condition	Fast OFF No Diff. Fast ON. Fast OFF No Diff. NF
	+10D Condition	1 Hz < 4 Hz < NF		+10D Condition	Fast OFF < (Fast ON No Diff. NF)
Fast ON/Slow OFF	-10D Condition	1 Hz No Diff. NF No Diff. 4 Hz	4 Hz	-10D Condition	Fast OFF No Diff. Fast ON. Fast OFF < NF
	No Lens Condition	1 Hz No Diff. NF No Diff. 4 Hz		No Lens Condition	Fast OFF No Diff. Fast ON. Fast OFF No Diff. NF
	+10D Condition	(1 Hz No Diff. 4 Hz) < NF		+10D Condition	(Fast OFF No Diff. Fast ON) < NF

1   *Note*: < denotes more negative refraction (i.e. more myopic). NF = No Flicker. No Diff = No significant difference between conditions.

Light probe recordings of Fast ON/Slow OFF (Blue) and Fast OFF/Slow ON (Red) sawtooth flicker at a frequency of (a) 1 Hz, and (b) 4 Hz.
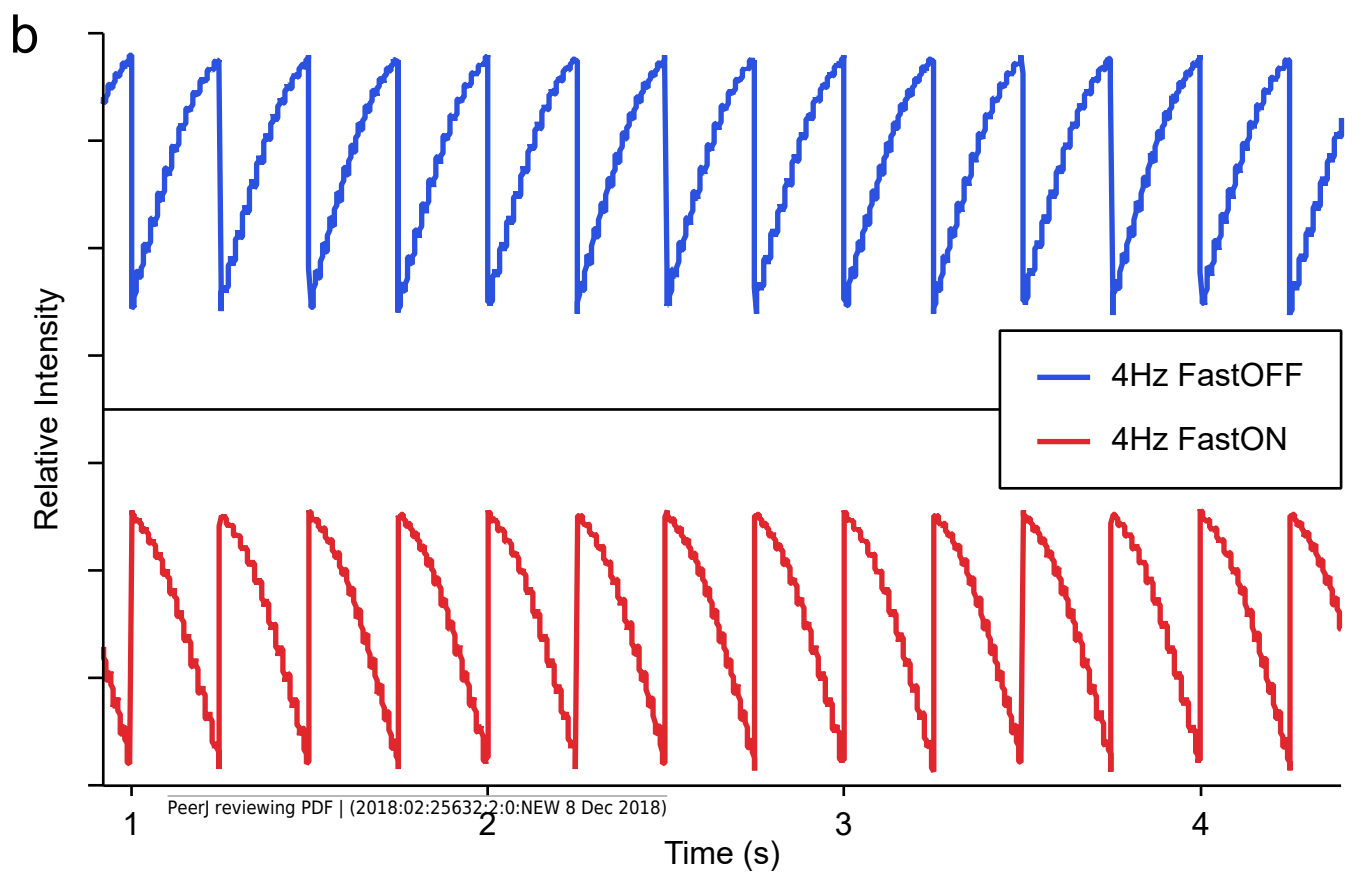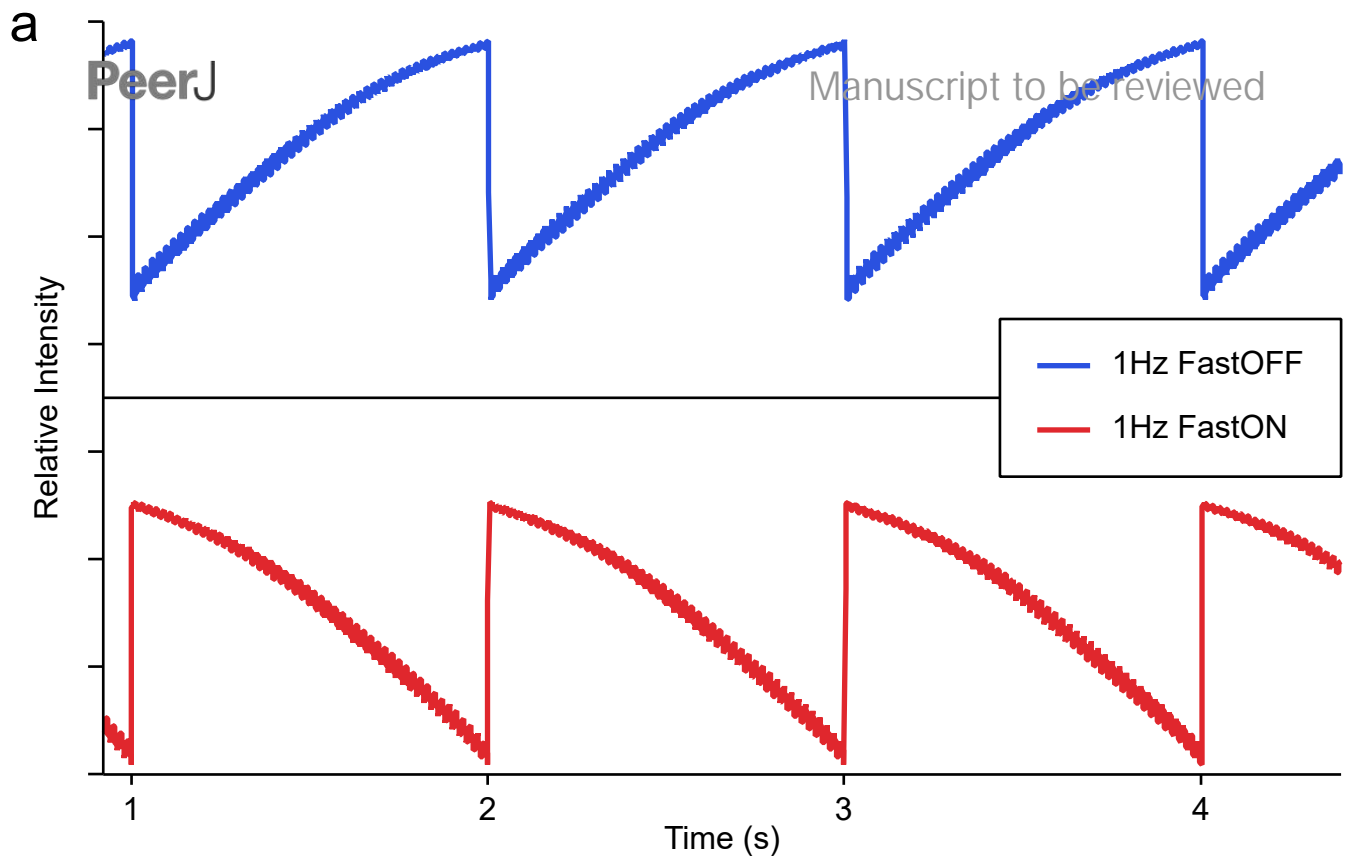
Figure 2

Mean difference (± Standard Error S.E) in biometric measures between experimental and fellow eyes across lens and light conditions.

(a) Refractive difference (diopters), **(b)** axial length difference (mm), **(c)** vitreous chamber difference (mm), and **(d)** anterior chamber difference (mm). Asterisks indicate significant differences between condition for post hoc analysis.