Using high-resolution functional MRI to differentiate impacts of strabismic and anisometropic amblyopia on evoked ocular dominance activity in humans

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Abstract:

We employed high-resolution functional MRI (fMRI) to distinguish the impacts of anisometropia and strabismus (the two most frequent causes of amblyopia) on the evoked ocular dominance (OD) response. Sixteen amblyopic participants (8 females), comprising 8 individuals with strabismus, 7 with anisometropia, 1 with deprivational amblyopia, along with 8 individuals with normal visual acuity (1 female), participated in this study for whom, we measured the difference between the response to stimulation of the two eyes, across early visual areas (V1-V4).

In controls, as expected from the organization of OD columns, the evoked OD response formed a striped pattern that was mostly confined to V1. Compared to controls, the OD response in amblyopic participants formed larger fused patches that extended into downstream visual areas. Moreover, both anisometropic and strabismic participants showed stronger OD responses in V1, as well as in downstream visual areas V2-V4. Although this increase was most pronounced in V1, the correlation between the OD response level and the interocular visual acuity difference (measured behaviorally) was stronger in higher-level visual areas (V2–V4).

Beyond these common effects, and despite similar densities of amblyopia between the anisometropic and strabismic participants, we found a greater increase in the size of V1 portion that responded preferentially to fellow eye stimulation in anisometropic compared to strabismic individuals. We also found a greater difference between the amplitudes of the response to binocular stimulation, in those regions that responded preferentially to the fellow vs. amblyopic eye, in anisometropic compared to strabismic subjects. In contrast, strabismic subjects demonstrated increased correlation between the OD responses evoked within V1 superficial and deep cortical depths, whereas anisometropic subjects did not.

These results provide some of the first direct functional evidence for distinct impacts of strabismus and anisometropia on the mesoscale functional organization of the human visual system, thus extending what was inferred previously about amblyopia from animal models.

Keywords: Amblyopia, Monocular Response, Interocular Visual Acuity Difference, Columnar Organization, High-Resolution FMRI

1. Introduction:

Ocular dominance (OD), the preference for responding to stimulation of one eye over the other, is a prominent characteristic of most neurons in primary visual cortex (V1) (Hubel and Wiesel, 1962). In humans and many non-human mammals, neurons with similar OD preferences are grouped together in ocular dominance columns (ODCs), which form a fundamental architectural feature of V1 (LeVay et al., 1975; Tootell et al., 1988; Sincich et al., 2003; Adams et al., 2007). The development of ODCs depends on balanced binocular visual input at early life stages, also known as the critical period (Hubel et al., 1977; LeVay et al., 1980; Horton and Hocking, 1997). Perturbations to normal binocular visual experience during the critical period impact the selectivity and distribution of ODCs and is associated with amblyopia, a prevalent neurodevelopmental disorder affecting a range of visual functions in one or both eyes (McKee et al., 2003; Maurer and McKee, 2018).

Much of our current understanding of amblyopia and its impact on ODCs is based on electrophysiological and anatomical studies conducted in animal models (Fig. 1). According to these studies, asymmetric binocular vision in early life stages, caused either by misalignment of the eyes (strabismus), differential optics of the eyes (anisometropia), or monocular deprivation, leads to a reduction in the number of V1 neurons that respond binocularly (Crawford and Von Noorden, 1979; Crawford et al., 1996; Smith III et al., 1997b; Kiorpes et al., 1998; Bi et al., 2011). Beyond this common effect, anisometropia and strabismus may impact the evoked OD response in different ways. Specifically, anisometropia, even in milder forms, is associated with a decrease in the number of neurons that respond preferentially to the amblyopic eye. Whereas such a bias is only detectable in strabismic participants with severe amblyopia (Crawford et al., 1996; Kiorpes et al., 1998; Bi et al., 2011). Moreover, strabismus (but not anisometropia) increases the segregation between ODCs with opposing ocular preference (Lowel, 1994; Tychsen et al., 2004).

In humans, fMRI has been used successfully to localize OD bands within primary visual cortex non-invasively (Menon et al., 1997; Cheng et al., 2001; Yacoub et al., 2007; Nasr et al., 2016). Using this technique, further studies suggest amblyopia is associated with a greater number of voxels responding preferentially to the fellow eye compared to the amblyopic eye (Algaze et al., 2002; Goodyear et al., 2002; Liu et al., 2004), and that the OD activity was stronger in amblyopic participants compared to controls (Conner et al., 2007). It was also suggested that amblyopia changes the mechanism of binocular interaction from excitation to suppression (Farivar et al., 2011; Thompson et al., 2019). However, these studies did not clarify whether this effect extends throughout all of V1 or if this effect is limited to regions that response

83   preferentially to the amblyopic eye. Further, these studies did not distinguish the impacts of

84   anisometropia vs. strabismus on the evoked OD response and/or the mesoscale functional

85   organization of V1, presumably due to limited spatial resolution and contrast-to-noise ratio of the

86   neuroimaging techniques available at the time.

87   To address these knowledge gaps, this study used higher spatial resolution fMRI (voxel size

88   = 1 mm isotropic), conducted in a 7T MR scanner. Advanced neuroimaging technologies were

89   used to mitigate the contribution of different nuisance factors (e.g., cardiac and respiratory

90   activities) on signal quality (Polimeni et al., 2015). Additionally, the contrast-to-noise ratio was

91   improved by minimizing the level of unwanted signal blurring without applying any spatial

92   smoothing within cortical layers (Blazejewska et al., 2019; Wang et al., 2022).

93   Using these methods, first we compared the impact(s) of strabismus and anisometropia on

94   the spatial distribution and columnar organization of the evoked OD response in human V1.

95   Notably, while it is known that amblyopia changes the selectivity level of 'horizontal' (i.e.,

96   surface-parallel) connection between ODCs (Tychsen et al., 2004), the impact of amblyopia on

97   'radial' (i.e., perpendicular to the surface) connections between cortical layers remains mostly

98   unknown even in animal models (Horton and Hocking, 1997). Second, we measured the impact

99   of amblyopia on the amplitude of OD responses in V1, and in downstream extrastriate visual

100 areas (V2-V4). Third, we aimed to compare the correlation between the evoked OD response

101 and the interocular visual acuity difference as a measure of amblyopia severity across the

102 human visual system hierarchy. Lastly, we aimed to compare the evoked activity across V1

103 regions to binocular stimulation to test whether the binocular response varies between regions

104 that respond preferentially to the fellow vs. amblyopic eye. Our findings provided the first direct

105 evidence for the differential impact of anisometropia and strabismus on the OD response across

106 different visual areas and confirmed the hypothesized link between the evoked OD response

107 and the interocular visual acuity difference in amblyopia.

108

109 2. Results

110 The OD response was measured in 24 human participants, 16 with amblyopia caused either by

111 strabismus (*n*=8), anisometropia (*n*=7) or deprivational amblyopic (*n*=1), and 8 control

112 participants with normal or corrected-to-normal vision. In addition to data from these individuals,

113 we also measured the OD response in one strabismic (but non-amblyopic) participant whose

114 data are presented separately. To measure the evoked response to stimulation of the eyes,

115 each participant was scanned twice on different days. During these scans, moving random dots

116 were presented to each eye separately (using anaglyphic goggles) in a blocked-design

117 paradigm (see Methods). The OD response was measured for each participant by averaging the
118 activity evoked across these two sessions and calculating the (absolute) difference between the
119 response to stimulation of dominant/fellow vs. non-dominant/amblyopic eye. A subset of
120 subjects (Table 1) also participated in a control test to measure responses to dichoptically
121 presented grating stimuli. Outside the scanner, all participants were tested to measure their
122 visual acuity and stereoacuity, to identify their dominant eye, and to test for suppression and/or
123 diplopia (see Methods).

124

125 **2.1. Age and interocular visual acuity difference**

126 Table 1 shows the participant's demographics and visual testing results. One-way ANOVA

127 (anisometropic vs. strabismic vs. control) did not yield any significant age differences across the

128 three groups ($F_{(2, 23)}=1.11$, $p=0.35$). As expected, a similar analysis applied to the interocular

129 visual acuity difference showed a significant effect of group ($F_{(2, 23)}=8.08$, $p<0.01$) driven by

130 the increased interocular visual acuity difference in both anisometropic and strabismic

131 individuals relative to controls ($p<0.01$; Bonferroni corrected for multiple comparison).

132 Interocular visual acuity difference was similar between the anisometropic and strabismic

133 individuals in our participants ($p=0.89$). Visual acuities of amblyopic ($p=0.29$) and fellow

134 ($p=0.83$) eyes were not different between anisometropic and strabismic participants. Thus, age,

135 interocular visual acuity difference, and visual acuity in the amblyopic and fellow eyes were

136 comparable between anisometropic and strabismic individuals.

137   Monocular suppression and diplopia were more common in strabismic compared to

138 anisometropic participants (Table 1). Also, as expected based on previous studies (Levi et al.,

139 2015), more strabismic individuals demonstrated severely impaired stereoacuity (>500 arc

140 seconds) than anisometropic individuals. All amblyopic individuals had a history of either

141 patching or atropine therapy in childhood.

142

143 **2.2. Head position stability during the fMRI tests**

144 Head motion has a strong impact on the fMRI signal and may influence the level and pattern of

145 evoked fMRI responses which might in turn confound between-group comparisons. Thus, as the

146 first step, we compared the level of head motion between control, strabismic and anisometropic

147 participants. Since all individuals were scanned at least two times on different days, we also

148 tested the consistency of head motion between sessions. One-way repeated measures ANOVA

149 (session (first vs. second)), with a group factor (control vs. strabismic vs. anisometropic

150 individuals), to the measured level of head motion (see Methods) did not yield a significant

151 effect of group (F(2, 21)=0.08, *p*=0.92) or group × session interaction (F(2, 21)=2.57, *p*=0.10) on

152 the degree of head motion. Thus, across the two scan sessions, head motion appears to be

153 comparable across the groups. Head motion was nevertheless included as a nuisance co-

154 variate in all analyses to reduce any residual impact of head motion on our findings.

155

156 **2.3. OD activity mapping**

157 We measured the evoked OD activity for all participants in both deep, middle, and superficial

158 cortical depth levels across visual areas V1-V4 by subtracting the response of the non-dominant

159 eye from the response of the dominant eye. Fig. 2A shows the evoked OD activity in a control

160 participant (Participant #1) across deep, middle and superficial layers. Consistent with post-

161 mortem anatomical studies in humans (Adams et al., 2007) and non-human primates (Hubel et

162 al., 1976; Tootell et al., 1988; Sincich et al., 2003) with normal vision, the cortical topography of

163 the evoked OD response was organized into mostly-parallel stripes. These striped patterns

164 were similarly detected across cortical depths, reflecting the columnar organization of V1 ODCs

165 (Tootell et al., 1988). In both hemispheres, these stripes were predominantly limited to the

166 regions of V1 (r<10˚), representing the central retinotopic visual field that were stimulated during

167 the scans. This pattern was consistently observed in all control participants in each hemisphere

168 (Fig. 3).

169   In all controls, we detected a fused activity patch close to the dorsal portion of V1-V2 border

170 that responded preferentially to the contralateral eye (Fig. 3). Notably, this cortical region

171 represents the inferonasal visual field occluded by the head coil resulting in monocular

172 representation by the other eye. Also as expected, we did not detect representation of the blind

173 spot and/or temporal monocular crescent, because these regions are represented more

174 peripherally (r>15˚) outside the stimulus borders and scan coverage (Tootell et al., 1998; Awater

175 et al., 2005; Adams et al., 2007; Nasr et al., 2020).

176   Fig. 2B and 2C illustrate the evoked OD activity in a strabismic (Participant #13; interocular

177 visual acuity differences = 0.50 logMAR) and an anisometropic participant (Participant #17;

178 interocular visual acuity differences = 0.42 logMR), respectively, with comparable levels of

179 interocular visual acuity difference. Compared to controls, OD activity was stronger and formed

180 larger, fused patches at all three cortical depth levels and extended downstream to visual areas

181 V2-V4 (see also Figs. 4 and 5).

182   As demonstrated in Fig. 4, in most strabismic individuals, we found larger regions that

183 responded preferentially to the fellow eye within the hemisphere contralateral relative to fellow

184 eye. However, participant #12 was the exception to this trend. Representation of the two eyes

185 appeared to be more balanced in the hemisphere ipsilateral relative to the fellow eye (further

186 analysis in Section 2.5). The fused activity patch close to the dorsal portion of V1-V2 border was

187 readily apparent in 4 strabismic individuals (Participants #10, #12, #13 and #16), as in controls.

188   Among the participants categorized as having strabismic amblyopia, participants #9 and #14

189 had only a small difference in interocular visual acuity at the time of testing (≤0.12 logMAR;

190 Table 1). Both individuals had a history of strabismus surgery and patching in childhood.

191 Despite the small interocular visual acuity difference, both individuals showed signs of diplopia

192 on Worth 4-dot testing, with reduced stereoacuity in participant #9 but not in participant #14. In

193 both cases, we found an elevated OD response in V1, especially in the hemisphere

194 contralateral to the fellow eye, similar to the other strabismic individuals. This result suggests

195 that imbalanced ocular dominance may persist despite recovery of monocular visual acuity in

196 the amblyopic eye, consistent with behavioral evidence for impaired *dichoptic* amblyopic eye

197 visual acuity despite resolved interocular visual acuity differences after patching treatment

198 (Birch, 2013; Birch et al., 2022).

199   In anisometropic individuals (Fig. 5), OD activity bias in favor of the fellow eye was

200 detectable bilaterally in almost all subjects. There was no apparent difference between the two

201 hemispheres. Among the four anisometropic individuals who did not show monocular

202 suppression (Table 1), participants #17 and #22 showed a strong bias in favor of the fellow eye,

203 but in participants #18 and #20 this bias was comparatively weaker. In contrast to strabismic

204 individuals and controls, the activity patch along the V1-V2 border was less apparent in

205 anisometropic individuals, likely due to strong bias in favor of the fellow eye.

206   Notably, the individual with deprivational amblyopia (participant #21) showed strong OD

207 activity bias in favor of the fellow eye in both hemispheres, as in anisometropic individuals, even

208 though the (unilateral; left eye) cataract was removed when the participant was a child, and the

209 stimuli were perceived with best correction. Here again, this activity bias propagated into

210 downstream visual areas. Considering the similarity between this individual's OD pattern and

211 those of anisometropic participants, we included this subject in the anisometropic group in the

212 following analyses.

213

214 **2.4. Reproducibility of the OD response maps across sessions**

215 To compare the reproducibility of these maps across the three groups, we measured the

216 correlation between OD activity maps evoked during the first and second scan sessions. This

217 measurement was conducted separately for the activity evoked within the deep and superficial

218 cortical layers and for the ipsilateral and contralateral hemispheres relative to the fellow eye. As

219   we have shown previously (Nasr et al., 2016), OD activity maps remained highly correlated

220   across sessions (Fig. 6). Two-way repeated-measures ANOVA (hemisphere and cortical depth,

221   with a group factor), did not yield an effect of group ($F_{(2, 21)}=0.42$, $p=0.66$)) or an interaction

222   between group and the other independent variables ($p>0.14$). The same result was found in

223   areas V2-V4, suggesting that activity maps were reproducible to the same extent for the three

224   groups across visual areas.

225

226   **2.5. Overrepresentation of the fellow eye in amblyopic participants**

227   Previous studies in human (Goodyear et al., 2002; Liu et al., 2004) and non-human primates

228   (Smith III et al., 1997b; Kiorpes et al., 1998) have suggested an increased representation of the

229   fellow eye in amblyopic compared to control participants. Consistent with these reports, we

230   found an increase in the size of the V1 region that responded preferentially to the fellow eye in

231   amblyopic participants compared to controls across deep and superficial cortical depth levels

232   (Table 2). This effect also tended to be larger in anisometropic compared to strabismic

233   individuals. Two-way repeated-measures ANOVA (hemisphere and cortical depth, with a group

234   factor) yielded a significant effect of group ($F_{(2, 21)}=5.74$, $p=0.01$), and a significant group $\times$

235   hemisphere interaction ($F_{(2, 21)}=3.86$; $p=0.04$), but no group $\times$ cortical depth interaction ($F_{(2,}$

236   $_{21)}=0.64$; $p=0.53$) on the size of the V1 portion that responded preferentially to the fellow eye.

237   Post-hoc analyses showed that in strabismic participants, this effect was significantly larger in

238   the hemisphere contralateral compared to ipsilateral relative to the fellow eye ($p=0.03$). We did

239   not find such a difference in either anisometropic ($p=0.35$) or control ($p=0.56$) participants.

240   Notably, all measurements were normalized relative to the size of V1 area that was stimulated.

241

242   **2.6. The impact of amblyopia on the amplitude of the OD response**

243   In addition to the change in the size of V1 portion that responded preferentially to the fellow eye,

244   there was an increase in the amplitude of the evoked OD response in amblyopic compared to

245   control participants (Fig. 7). A two-way repeated-measures ANOVA (as above) revealed a

246   significant effect of group ($F_{(2, 21)}=11.91$, $p<10^{-3}$). Post-hoc analysis further showed that the

247   evoked OD response in V1 was significantly larger in strabismic ($p<10^{-3}$) and anisometropic

248   ($p<10^{-5}$) participants compared to controls without a significant difference between strabismic

249   and anisometropic participants ($p=0.22$). Thus, in line with previous studies in humans (Conner

250   et al., 2007) and non-human primates (Crawford and Von Noorden, 1979; Crawford et al., 1996;

251   Smith III et al., 1997b; Kiorpes et al., 1998; Bi et al., 2011) studies, amblyopia increased the

252   amplitude of the OD response in human V1 in our fMRI data.

253   Importantly, the heightened OD response extended beyond V1 into downstream visual

254   areas V2, V3, V3A and V4 (Fig. 7A and B). Despite a gradual decrease in the OD response

255   amplitude from V1 through V4, the significantly stronger OD response in amblyopic individuals

256   compared to controls was preserved across all tested areas (p<0.01). As in V1, the amplitude of

257   the OD response remained comparable between strabismic vs. anisometropic participants

258   (p>0.10). These results suggest that the impact of amblyopia on the OD response amplitude

259   propagated to downstream visual areas, irrespective of amblyopia subtype.

260   Beyond this effect, we found a moderate correlation between the interocular visual acuity

261   difference (as in the scans; see Table 1) and OD response amplitudes across visual areas V1-

262   V4 (r>0.43; p<0.01). This correlation was considerably stronger in areas V2-V4 (r = 0.55 – 0.70)

263   compared to V1 (r = 0.47), especially in deeper cortical depth levels, despite the decrease in the

264   overall level of evoked OD response (Fig. 7C and D; Table 3). This correlation was similarly

265   detected in contralateral and ipsilateral hemispheres and across superficial and deep cortical

266   depth levels. To compare these correlation values more directly, we generated a linear multiple

267   regression model using the interocular visual acuity difference as the dependent parameter and

268   the OD activity evoked within V1-V4 (averaged between the two hemispheres) as the

269   independent parameter. As demonstrated in Table 3, we found a stronger standardized beta

270   value for V4 compared to V1 activity in both superficial and deep cortical depth levels. Thus,

271   while the impact of amblyopia on the amplitude of the OD response was stronger in V1

272   compared to downstream visual areas, the correlation between OD response and interocular

273   visual acuity difference was stronger in higher-level visual cortical areas such as V3 and V4.

274

275   **2.7. Contributions of residual strabismus**

276   As reported in Table 1, the strabismic participants show some residual misalignments between

277   the two eyes, despite prior surgical correction. To test whether mild strabismus, in the absence

278   of amblyopia, may lead to the stronger OD responses we observed in individuals with strabismic

279   amblyopia, we scanned a non-amblyopic individual with mild strabismus (separate from the

280   other 8 controls (Participant #25; Table 1)). This participant showed normal, balanced visual

281   acuities, no evidence of suppression or diplopia and showed measurable stereoacuity (70

282   seconds of arc).

283   As demonstrated in Fig. 8A, in this participant, the overall pattern of the OD response in V1

284   and downstream visual areas was distinguishable from that in individuals with strabismic

285   amblyopia; instead, it more closely resembled the results in the controls (Fig. 2 and 3).

286   Specifically, the size of the region that showed response preference to dominant eye stimulation

287   within the contralateral (46.72%) and ipsilateral (43.03%) hemisphere (relative to the dominant

288   eye) remained small compared to the individuals with strabismic amblyopia (Table 2). Similar

289   results were detected within the superficial cortical levels. Thus, strabismus per se, in absence

290   of amblyopia, is not the main cause of increased OD response in our participants. Notably, data

291   from this participant were not used in any other analyses.

292

2.8. Contributions of uncorrected visual acuity

294   Among the participants, two strabismic (Participants #12 and #15) and one anisometropic

295   (Participant #22) individual could not be tested with their best optical correction. Even though

296   this deviation had a relatively small impact on the level interocular visual acuity difference (Table

297   1; <0.11 logMAR), we tested whether this deviation from the best corrected visual acuity was

298   the main source of increased OD activity in these individuals. In separate scan sessions, one

299   control individual (Participant #6) was tested again with increased visual acuity difference by

300   instructing the participant not to wear their prescribed contact lenses. Visual acuity worsened

301   without correction by 0.76/1.00 (Right/Eye visual acuity) and the interocular visual acuity

302   difference increased from 0.06 to 0.24 logMAR, as in the 3 participants with amblyopia who

303   could not be tested with their best corrected visual acuity.

304   Fig. 8B shows the evoked OD response in this individual, measured within the deep cortical

305   depth levels. While increased the level of bias in favor of the dominant eye, the level of evoked

306   OD activity only increased 0.04% (fMRI signal level) and 0.12% in the contralateral and

307   ipsilateral hemispheres, respectively. Moreover, OD activity in this participant was comparably

308   weaker, compared to the OD activity detected on average in amblyopic individuals, in both

309   contralateral (0.99%) and ipsilateral (0.82%) hemispheres. Similar results were detected in the

310   superficial cortical depth levels. Thus, the increased OD activity in the three individuals with

311   amblyopia who were unable to wear their best correction is only marginally attributable to the

312   absence of optical correction.

313

2.9. The impact of amblyopia on the evoked response to binocular visual stimulation

315   In separate blocks, we also measured the evoked response to concurrent stimulation of both

316   eyes. We compared binocular response amplitudes between regions preferring the

317   dominant/fellow eye with those of the non-dominant/amblyopic eye for each group. As

318   demonstrated in Fig. 9, results of this test revealed two important phenomena: First, there was

319   no apparent difference between the level of response evoked within the V1 regions that

320   responded preferentially to the fellow eye in amblyopic individuals compared to the V1 region

321   that responded preferentially to the dominant eye in the controls. Second, in controls, binocular

322   responses were comparable between V1 regions that responded preferentially to the dominant

323   vs. non-dominant eye, whereas in amblyopic participants, evoked responses to binocular visual

324   stimulation were stronger in V1 regions that responded preferentially to the fellow eye than

325   those for the amblyopic eye. This effect appeared to be stronger at the superficial cortical depth,

326   in the hemisphere contralateral to the dominant/fellow eye, and in anisometropic compared to

327   strabismic individuals. Three-way repeated measures ANOVA (Hemisphere, Preferred-Eye, and

328   Cortical Depth, with a group factor) yielded significant Group × Preferred-Eye ($F_{(2, 20)}=9.99$,

329    $p<10^{-3}$), Group × Preferred-Eye × Layer ($F_{(2, 20)}=6.17$, $p<0.01$), and Group × Preferred-Eye ×

330   Cortical Depth × Hemisphere ($F_{(2, 20)}=8.41$, $p<0.01$) interactions for evoked binocular

331   responses. A post hoc test to compare the response in anisometropic vs. strabismic individuals

332   directly also showed a significant Group × Preferred-Eye × Cortical Depth ($F_{(1, 14)}=33.47$,

333    $p<10^{-3}$) interaction whereas the other effects remained non-significant after correction for

334   multiple comparisons. These results suggest that anisometropic, compared to strabismic,

335   amblyopia is associated with a stronger decrease in the level of binocular activity within V1

336   regions that respond preferentially to the amblyopic eye.

337

338   **2.10. The impact of amblyopia on the columnarity of OD response**

339   Our knowledge of the impact of amblyopia on the vertical connections between cortical layers is

340   limited to qualitative observations (Horton and Hocking, 1997). We tested the extent that

341   amblyopia affects the functional link between deep and superficial cortical layers by comparing

342   the correlation between activity maps evoked within deep and superficial cortical depths across

343   the three groups.

344   As demonstrated in Fig. 10, we found an increased correlation between OD activity maps in

345   deep and superficial cortical depth levels (i.e. inter-level correlation) of V1 in strabismic

346   individuals compared to the two other groups. One-way repeated-measures ANOVA

347   (hemisphere with a group factor) showed a significant effect of group ($F_{(2, 21)}=8.32$, $p<0.01$)

348   without any group × hemisphere interaction ($F_{(2, 21)}=0.29$, $p=0.75$) for inter-level correlation

349   values in V1. A post-hoc test showed that the magnitude of inter-level correlation was stronger

350   in strabismic compared to anisometropic ($p<0.01$) and control ($p<0.01$) participants. Despite the

351   extension of the OD response into the downstream visual areas (see above), application of this

352   analysis to the evoked activity within V2-V4 did not yield a significant effect of group in any of

353   those regions ($p>0.17$). This suggests that the impact of amblyopia on the columnarity of OD

354   response is limited to primary visual cortex using these methods.

355   To test the reproducibility of this effect, first we repeated our tests for individual scan

356   sessions, conducted on separate days, rather than the averaged activity maps. Again, two-way

357   repeated measures ANOVA (hemisphere and session, with a group factor) showed a significant

358   effect of group (F(2, 21)=7.74, $p<0.01$) without any significant group × session interaction (F(2,

359   21)=1.21, p=0.32) for inter-level correlation values, suggesting that the impact of amblyopia on

360   the columnarity of the OD response was reproducible across scan sessions.

361   Second, in a randomly selected subset of our participants, consisting of 5 control and 4

362   strabismic individuals (Table 1), we tested whether this enhanced inter-level correlation is also

363   seen in responses to dichoptically presented drifting gratings (rather than random dots). Briefly,

364   we measured the level of OD activity evoked by gratings presented to fellow/dominant vs.

365   amblyopic/non-dominant eye and then measured the correlation between the evoked OD

366   activity across V1 at deep and superficial cortical depths. Despite fewer individuals participating

367   in this test, we found significantly stronger inter-level correlation in strabismic compared to

368   control participants (F(1, 7) = 11.09; p=0.01) and a significant group × hemisphere interaction

369   (F(1, 7)=11.12, p=0.01). Thus, the enhanced inter-level correlation in the strabismic individuals

370   was reproducible across stimulus types.

371

372   3. Discussion

373   By measuring the evoked activity in response to monocular and binocular stimuli, using high-

374   resolution fMRI collected in an ultra-high field scanner, we showed direct evidence for the

375   distinct impacts of anisometropia versus strabismus on the fMRI activity evoked within the

376   human visual cortex. Specifically, we showed that the expanded representation of the fellow eye

377   is more pronounced in anisometropic compared to strabismic participants, especially in the

378   hemisphere ipsilateral relative to the fellow eye. Moreover, compared to strabismus,

379   anisometropia has a stronger impact on the activity evoked during binocular stimulation within

380   V1 regions that respond preferentially to the amblyopic eye. Strabismic amblyopia has a

381   stronger impact on the level of correlation between the OD response evoked within V1 deep and

382   superficial layers compared to anisometropic amblyopia. These findings were observed in

383   anisometropic and strabismic participants with amblyopia of similar severity.

384

385   3.1. Consistency with findings based on animal models

386   Pronounced expansion of fellow eye representation in anisometropic compared to strabismic

387   participants is consistent with single unit recordings in non-human primate V1 (Kiorpes et al.,

388   1998; Bi et al., 2011). According to these studies, the number of neurons that respond

389   preferentially to the fellow and amblyopic eye remains comparable in milder forms of strabismic

390   amblyopia, whereas there is a relative increase of neurons responding preferentially to the

391   fellow eye even in milder forms of anisometropic amblyopia.

392   The decreased binocular responses in our amblyopic participants is also consistent with

393   previous reports that amblyopia may change the mechanism of interaction between the input

394   from the two eyes (Smith III et al., 1997a; Kumagami et al., 2000; Bi et al., 2011; Farivar et al.,

395   2011; Thompson et al., 2019). Here we showed that this decreased binocular activity is limited

396   to V1 regions that respond preferentially to the amblyopic eye, at least by fMRI, suggesting that

397   binocular integration is differentially impaired in V1 regions according to the ocular preference.

398   Our finding that strabismus is associated with an increase in the level of correlation between

399   the OD activity in deep vs. superficial cortical depths is novel. To the best of our knowledge, no

400   previous electrophysiological study had measured such a correlation in their participants

401   directly. This finding is in line with anatomical studies in V1 of strabismic animals suggesting

402   increased segregation between ODCs with opposite ocular preference (Shatz et al., 1977;

403   Lowel, 1994; Tychsen et al., 2004). Moreover, according to animal studies, shrinkage of ODCs

404   in layer 4 after monocular deprivation is associated with decreased cytochrome oxidase activity

405   of blobs that fall in register with the shrunken columns, suggesting a change in vertical

406   connections spanning cortical layers (Horton and Hocking, 1997). However, this effect has

407   never been tested in vivo in anisometropic and/or strabismic participants.

408

409   **3.2. Amblyopia impacts beyond V1**

410   Since the original studies by Hubel and colleagues (Hubel et al., 1976), most amblyopia studies

411   in animals have focused their efforts on understanding the impact of amblyopia on primary

412   visual cortex. While this impact is expected to extend to downstream areas, only a few studies

413   have examined this phenomenon in extrastriate visual cortex. Among them, Bi et al. reported

414   that the increased OD response caused by strabismus extends to V2 (Bi et al., 2011). However,

415   this extension was only detected in animals with severe amblyopia, suggesting a link between

416   downstream extrastriate extension and visual impairment.

417   Consistent with that report, here we show that the correlation between the level of OD

418   response and the interocular visual acuity difference, a functional measure correlated with

419   ocular dominance shift, increased from V1 to downstream visual areas such as V3 and V4. This

420   increase in correlation was detected despite the decrease in the OD response amplitude,

421   suggesting that canonical propagation of OD deficits in amblyopia reflects functional visual

422   impairment and highlighting the clinical relevance of downstream visual areas for future studies
423   of evoked activity in the amblyopic brain.
424

425   **3.3. Amblyopia impacts on visual attention**
426   It could be argued that the reported correlation between the OD response and the interocular
427   visual acuity difference is a result of amblyopia's impact on the participant's attention control
428   mechanism, influencing both measurements concurrently. Degraded visual attention in
429   amblyopia has been previously reported (Ho et al., 2006; Hou et al., 2016; Verghese et al.,
430   2019). To reduce the influence of attentional bias that may confound OD responses and their
431   correlation with visual acuity, two separate steps were taken: First, the OD response was
432   measured while the participant's attention was directed to an orthogonal task (i.e., shape
433   change detection for the fixation object) separate from the stimuli used to elicit the OD
434   measurement. Second, the fixation stimuli were presented dichoptically to reduce the potential
435   impacts of biased attention in favor of the fellow eye. Thus, altered visual attention is unlikely to
436   solely account for the strong OD response correlation with the visual acuity deficit in amblyopia.
437

438   **3.4. The potential underlying mechanism for the increased OD response**
439   Convergent evidence from both humans and non-human primates show that amblyopia is
440   associated with an increase in the level of OD response in early visual areas. However, the
441   mechanism underlying this phenomenon remains unclear. In several mammalian species, it is
442   widely accepted that monocular deprivation in the first few weeks of life leads to a drastic
443   decrease in afferent input originating from the amblyopic eye to V1 (Hubel et al., 1976; LeVay et
444   al., 1980; Horton and Hocking, 1997). Given the typical developmental age of naturally
445   occurring amblyopia (Shaw et al., 1988; Keech and Kutschke, 1995), disruption of binocular
446   vision would not be expected to change the number of thalamocortical afferent inputs to V1.
447   Consistent with those expectations, anatomical studies in humans (Horton and Stryker, 1993;
448   Horton and Hocking, 1996) and animals (Horton et al., 1997) with naturally occurring amblyopia
449   have reported intact ODC patterns in V1. Thus, the increased OD response among amblyopic
450   human participants is unlikely to be attributable to decreased number of afferent connections
451   originating from the amblyopic eye.
452   Amblyopia is also linked to changes in connections between ODCs. Anatomical studies
453   have shown that strabismus and anisometropia are respectively associated with stronger and
454   weaker segregation between ODCs (Shatz et al., 1977; Lowel, 1994; Tychsen et al., 2004).
455   Horton and Hocking also showed evidence for a change in connections between layers 4 and

456   2/3 (the site of binocular convergence) after monocular deprivation (Horton and Hocking, 1997).

457   However, the direction of this change (i.e. increased or decreased connectivity) remains

458   unclear. Altered horizontal and/or radial connection between the ocular dominance columns

459   may influence the ocular preference of V1 neurons and increase the OD response in amblyopic

460   compared to non-amblyopic individuals. Longitudinal developmental studies are required to

461   clarify the critical period for these effects and to test their correlation with the severity and

462   distinct visual deficits of amblyopia.

463

464   **3.5. Limitations**

465   Despite recent advances in neuroimaging technologies (Polimeni et al., 2015; Blazejewska et

466   al., 2019; Wang et al., 2022) that enabled us to map the OD response with relatively high spatial

467   resolution (1 mm), our techniques may still have missed even-smaller OD patches, especially

468   within the more peripheral portions of V1 (Adams et al., 2007). This caveat limits the

469   interpretation of OD maps (Figs. 2-5). For instance, a relatively large patch that shows a uniform

470   preference for one eye may contain small patches that are inaccessible due to limitations in

471   spatial resolution.

472   Another limitation is that fMRI indirectly measures neuronal responses based on the

473   concentration of deoxy-hemoglobin and blood flow. It has been shown that the existence of pial

474   veins has a significant impact on increasing the level of evoked response and blurring the

475   activity pattern in more superficial cortical layers (Koopmans et al., 2010; Polimeni et al., 2010;

476   De Martino et al., 2013; Nasr et al., 2016). Existence of diving veins (Duvernoy et al., 1983) may

477   also increase the level of correlation between deep and superficial depths. To the best of our

478   knowledge, no previous study has shown evidence that amblyopia impacts vascularization of

479   visual cortex. Nevertheless, the existence of diving veins may have influenced our estimation of

480   the impact of amblyopia on the columnarity of OD response. Thus, any interaction between

481   amblyopia and cortical depth must be assessed carefully and re-examined using fMRI

482   sequences less sensitive to vascularization (Yacoub et al., 2007; Huber et al., 2015; Akbari et

483   al., 2023). Unfortunately, these methods (e.g., spin echo and/or vascular space occupancy

484   (VASO)) have low contrast-to-noise sensitivity that limits their application for assessing the

485   mesoscale organization of the human brain.

486   Lastly, due to the small size of the head coil used in 7T scanners, we were not able to use

487   accessories designed for lower field (e.g. 3T) scanners to correct visual acuity in those

488   individuals who exclusively wore glasses, to stimulate more of the peripheral visual field (r<10°),

489   and/or to monitor eye movements. While the impact of microsaccades and/or fixation instability

490 on the fMRI signal is expected to be small, and our control experiments suggested that lack of

491 optic correction and strabismus are unlikely to be responsible for the increased OD response in

492 amblyopic subjects, these limitations prevented us from including individuals who required high

493 degrees of optical correction and/or those who showed larger eye misalignments.

494

3.6. Conclusion

496 Despite its high prevalence in humans, our understanding of how amblyopia impacts the

497 mesoscale organization of the visual system has been based primarily on animal models. In this

498 study, high-resolution fMRI has documented the impact of amblyopia on the evoked OD

499 response with functional correlates and drawn distinctions between the impact of anisometropia

500 and strabismus on cortical responses.

501

4. Methods

4.1. Participants

504 Twenty-five human participants (10 females), aged 19–56 years old, participated in this study

505 (Table 1). This included 7 anisometropic, one deprivational and 8 strabismic participants with

506 amblyopia. We also included 8 individuals with normal (n=6) or correct-to-normal (n=2) visual

507 acuity, as controls. One extra participant with mild strabismus (but no amblyopia) also

508 participated in our study. The data from this individual is demonstrated separately. All

509 participants had radiologically intact brains and no history of neuropsychological disorders.

510   During the main experiments, three amblyopic individuals could not wear their prescribed

511 eye-glasses due to safety concerns with MRI compatibility (Table 1). To test the impact of this,

512 one control participant underwent an additional control experiment during which the participant

513 was tested without their contact lenses.

514   All experimental procedures conformed to NIH guidelines and were approved by

515 Massachusetts General Hospital protocols. Written informed consent was obtained from all

516 participants prior to all experiments.

517

4.2. Ophthalmological assessment

519 Outside the scanner, participants were tested by an optometrist (J.S.) with extensive experience

520 with amblyopic individuals. During these tests, participants' visual acuity (ETDRS retro luminant

521 chart (Precision Vision)) was measured with pinhole (i.e. best corrected) and without pinhole (as

522 in fMRI scans). The stereoacuity was measured using Randot stereo test (Stereo Optical). We

523 identified the participant's dominant eye (Miles Test) and tested for suppression or diplopia
524 (Worth 4 Dot).

525

526 **4.3. MRI experiments**

527 Participants were scanned in an ultra-high field 7T scanner (whole-body system, Siemens
528 Healthcare, Erlangen, Germany) for the functional experiments. All participants were also
529 scanned in a 3T scanner (Tim Trio, Siemens Healthcare) for structural imaging.
530   During the fMRI experiments, stimuli were presented via an LCD projector (1024 × 768 pixel
531 resolution, 60 Hz refresh rate) onto a rear-projection screen, viewed through a mirror mounted
532 on the receive coil array. MATLAB 2021a (MathWorks, Natick, MA, USA) and the
533 Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) were used to control stimulus presentation.
534 The participants were instructed to look at a centrally presented fixation object (radius = 0.15°)
535 and to do either a shape-change for the fixation target (circle-to-square or vice versa) during the
536 OD measurements or a random dot-detection during the retinotopic mapping. These tasks were
537 conducted without any significant difference across experimental conditions (p>0.10).

538

539 **4.3.1. Response to monocular and binocular visual stimulation based on moving random**
540 **dots**

541 All participants completed 2 separate scan sessions. In each session, we stimulated the
542 participant's fellow (dominant) and amblyopic (non-dominant) eyes in different blocks (i.e.,
543 block-design; 24 s per block). The stimuli were sparse (5%) moving random red (50% of blocks)
544 and green (the rest of blocks) dots (0.09° × 0.09°; 56 cd/m^2), presented against a black
545 background. In separate blocks, we also measured the response to binocular presentation of
546 the simultaneous stimulation of both eyes (with zero disparity) in all participants except for one
547 control.
548   Participants viewed the stimuli through custom made anaglyph spectacles (with red and
549 green filters) mounted to the head coil. During the blocks, dots were oscillating horizontally (-
550 0.22° to 0.22°; 0.3 Hz). Stimuli extended 20° × 26° in the visual field. Each experimental run
551 began and ended with 12 s of uniform black. The sequence of blocks was pseudo-randomized
552 across runs (14 blocks per run) and each participant participated in 12 runs. Filter laterality (i.e.,
553 red-left vs. red-right) was counter-balanced between sessions and across participants.

554

555 **4.3.2. Response to monocular visual stimulation based on moving gratings**

556   To test whether the strabismic amblyopia impact on the columnarity of the OD response was

557   detectable based on stimuli other than random dots, in this experiment participants were

558   presented with gratings (2.25 cycle/degree). Red and green gratings were presented in different

559   blocks (24 s per block) and participants viewed the stimuli through custom anaglyph spectacles

560   mounted on the head coil. To avoid adaptation, gratings were oscillating left-to-right (-0.22° to

561   0.22° (0.3 Hz)). Stimuli were presented against a black background, extending 20° x 26° in the

562   visual field. The orientation of gratings varied randomly between blocks.

563   Each experimental run began and ended with 12 s of uniform black. The sequence of blocks

564   was pseudo-randomized across runs (7 blocks per run) and each participant participated in 2

565   runs. Filter laterality (i.e., red-left vs. red-right) was counter-balanced across participants.

566

567   **4.3.3. Retinotopic mapping**

568   For all participants the border of retinotopic areas were defined retinotopically (Sereno et al.,

569   1995). Stimuli were based on a flashing radial checkerboard, presented within retinotopically

570   limited apertures, against a gray background. These retinotopic apertures included wedge-

571   shaped apertures radially centered along the horizontal and vertical meridians (polar

572   angle = 30°). These stimuli were presented to participants in different blocks (24 s per block).

573   The sequence of blocks was pseudo-randomized across runs (8 blocks per run) and each

574   participant participated in at least 4 runs.

575

576   **4.4. Imaging**

577   Functional experiments (see above) were conducted in a 7T Siemens whole-body scanner

578   (Siemens Healthcare, Erlangen, Germany) equipped with SC72 body gradients (70 mT/m

579   maximum gradient strength and 200 T/m/s maximum slew rate) using a custom-built 32-channel

580   helmet receive coil array and a birdcage volume transmit coil. Voxel dimensions were nominally

581   1.0 mm. We used single-shot gradient-echo EPI to acquire functional images with the following

582   protocol parameter values: TR=3000 ms, TE=28 ms, flip angle=78°, matrix=192×192, BW=1184

583   Hz/pix, echo-spacing=1 ms, 7/8 phase partial Fourier, FOV=192×192 mm, 44 oblique-coronal

584   slices, acceleration factor R=4 with GRAPPA reconstruction and FLEET-ACS data (Polimeni et

585   al., 2015) with 10° flip angle. The field of view included occipital cortical areas V1, V2, V3 and

586   the posterior parts of V4v and V4d.

587   Structural (anatomical) data were acquired in a 3T Siemens TimTrio whole-body scanner,

588   with the standard vendor-supplied 32-channel head coil array, using a 3D T1-weighted

589   MPRAGE sequence with protocol parameter values: TR=2530 ms, TE=3.39 ms, TI=1100 ms,

590   flip angle=7°, BW=200 Hz/pix, echo spacing=8.2 ms, voxel size=1.0 × 1.0 × 1.33 mm^3,

591   FOV=256 × 256 × 170 mm^3.

592

593   **4.5. General data analysis**

594   Functional and anatomical MRI data were pre-processed and analyzed using FreeSurfer and

595   FS-FAST (version 7.11; http://surfer.nmr.mgh.harvard.edu/) (Fischl, 2012).

596

597   **4.5.1. Structural analysis**

598   For each participant, inflated and flattened cortical surfaces were reconstructed based on the

599   high-resolution anatomical data (Dale et al., 1999; Fischl et al., 1999; Fischl et al., 2002). Then,

600   during this reconstruction process, the standard pial surface was generated as the gray matter

601   border with the surrounding cerebrospinal fluid or CSF (i.e. GM-CSF interface). The white

602   matter surface was also generated as the interface between white and gray matter (i.e., WM-

603   GM interface). To enable intra-cortical smoothing (see below), we also generated a family of 9

604   intermediated equidistant surfaces, spaced at intervals of 10% of the cortical thickness, between

605   WM-GM and the GM-CSF interface surfaces. To improve the co-registration of functional and

606   structural scans, all surfaces were unsampled (Wang et al., 2022).

607

608   **4.5.2. Functional analysis**

609   The collected functional data were first unsampled (to 0.5 mm isotropic) and then corrected for

610   motion artifacts. For each participant, functional data from each run were rigidly aligned (6 DOF)

611   relative to their own structural scan using rigid Boundary-Based Registration (Greve and Fischl,

612   2009). This procedure enabled us to compare data collected for each participant across multiple

613   scan sessions.

614   To retain the spatial resolution, no tangential spatial smoothing was applied to the imaging

615   data acquired at 7T (i.e., 0 mm FWHM). Rather we used the more advanced method of radial

616   (intracortical) smoothing (Blazejewska et al., 2019) – i.e., perpendicular to the cortex and within

617   the cortical columns. For deep cortical depths, the extent of this radial smoothing was limited to

618   WM-GM interface and the adjacent 2 surfaces right above it (see above) – i.e., the bottom 30%

619   of the gray-matter thickness starting from the WM-GM interface. For the superficial cortical

620   depths, the extent of this procedure was limited to GM-CSF interface and the adjacent 2

621   surfaces right below it. For the middle cortical layers, used only for presentation (Fig. 2), the

622   extent of this procedure was limited to the three middle reconstructed cortical surfaces.

623   A standard hemodynamic model based on a gamma function was fitted to the fMRI signal to

624   estimate the amplitude of the BOLD response. For each individual participant, the average

625   BOLD response maps were calculated for each condition (Friston et al., 1999). Finally, voxel-

626   wise statistical tests were conducted by computing contrasts based on a univariate general

627   linear model, and the resultant significance maps were projected onto the participant's

628   anatomical volumes and reconstructed cortical surfaces.

629

630   **4.6. Region of interest (ROI) analysis**

631   To test the impacts of amblyopia on the OD response, ROIs including deep and superficial

632   depths of areas V1, V2, V3, V3A, and V4, defined for each participant based on their own

633   structural and retinotopic mapping (see above).

634   To test the impact of amblyopia on the evoked response to binocular stimulation, V1 surface

635   was divided into two ROIs based on the ocular preference of the vertices, defined during the

636   monocular tests. These ROIs were defined independently for deep and superficial cortical

637   depths.

638   Notably, no hemisphere was excluded from any ROI analyses and all vertices within each

639   ROI were used in the analyses.

640

641   **4.7. Statistical data analysis**

642   Three independent parameters included group (anisometropic vs. strabismic vs. control

643   participants), hemisphere (ipsilateral vs. contralateral relative to the dominant/fellow eye) and

644   cortical depth level (deep vs. superficial). To test the impact of these parameters, we used either

645   one-way or two-way repeated-measures ANOVA with a group factor. Since this analysis is

646   particularly susceptible to the violation of sphericity assumption, caused by the correlation

647   between measured values, when necessary (determined using a Mauchly test), results were

648   corrected for violation of the sphericity assumption, using the Greenhouse-Geisser method. All

649   post-hoc analyses were conducted after Bonferroni correction for multiple comparisons.

650

651   **4.8. Data availability statement**

652   Data and codes will be shared upon request.

653

Table 1 – Demography and ophthalmologic assessment of the participants

Participant #	Group α	Age	Gender	Age at Diagnosis	RE Visual Acuity (pinhole)	LE Visual Acuity (pinhole)	RE Visual Acuity (as in fMRI scans)	LE Visual Acuity (as in fMRI scans)	Dominant Eye	Suppression Worth 4 Dots	Stereoacuity (arc seconds)	Strabismus at 110/400 cm (PD)	Strabismus Type μ	Control Exp. 1 γ	Control Exp. 2 Θ
1	C	30	M	N/A			+0.00	+0.00	RE	None	40	None	N/A	Y	Y
2	C	20	M	N/A			-0.16	-0.14	RE	None	30	None	N/A	Y	Y
3	C	40	M	N/A			+0.00	+0.00	RE	None	25	None	N/A	N	N
4	C	43	M	N/A			-0.18	-0.04	RE	None	20	None	N/A	Y	Y
5	C	27	M	N/A			+0.00	+0.00	RE	None	70	None	N/A	Y	N
6	C	23	F	N/A			+0.04	-0.02	RE	None	30	None	N/A	Y	N
7	C	31	M	N/A			+0.02	+0.00	LE	None	70	None	N/A	Y	Y
8	C	35	M	N/A			-0.26	-0.22	LE	None	20	None	N/A	Y	Y
9	S	40	F	<1	-0.06	+0.06	-0.06	+0.08	RE	Diplopia	>500	25/16	XT	Y	Y
10	S	56	M	5	-0.20	+0.06	-0.20	+0.06	RE	LE	>500	16/16	ET	Y	N
11	S	28	M	2	+0.00	+0.60	+0.00	+0.60	RE	LE	>500	14/10	ET	Y	N
12	Sβ	19	M	3	**+0.09**	**+0.30**	+0.52	+0.84	LE	RE	>500	12/10	ET	Y	N
13	S	28	M	6	+0.48	-0.02	+0.48	-0.02	LE	RE	>500	25/18	ET	Y	Y
14	S	26	F	6	+0.00	-0.06	+0.00	+0.02	LE	Diplopia	50	4/4	ET	Y	N
15	Sβ	31	F	3	**+0.26**	**+0.04**	+0.44	+0.32	LE	RE	>500	10/8	ET	Y	Y
16	S	26	F	2	+0.46	-0.06	+0.46	-0.06	LE	RE	>500	10/8	ET	Y	Y
17	A	31	F	5	-0.22	+0.20	-0.22	+0.20	RE	None	>500	None	N/A	Y	N
18	A	23	M	5	-0.08	+0.30	-0.08	+0.30	RE	None	400	None	N/A	Y	N
19	A	35	M	11	-0.04	+0.26	-0.04	+0.26	RE	LE	200	None	N/A	Y	N
20	A	20	F	6	+0.02	+0.16	+0.06	+0.32	RE	None	40	None	N/A	Y	N
21	D	29	M	4	-0.26	+0.10	-0.26	+0.10	RE	LE	100	None	N/A	Y	N
22	Aβ	26	M	8	**+0.00**	**+0.17**	+0.32	+0.40	RE	Diplopia	200	None	N/A	Y	N
23	A	19	F	5	+1.00	-0.08	+1.00	-0.08	LE	RE	>500	None	N/A	Y	N
24	A	24	F	8	+0.64	-0.10	+0.64	-0.10	LE	RE	>500	None	N/A	Y	N
25	CS	23	F	N/A	-0.06	-0.02	-0.06	-0.02	LE	None	70	16/16	XT	N	N

655   **α:** Groups definition (**C:** Control, **S:** Strabismic Amblyopia, **A:** Anisometropic Amblyopia, **D:** Deprivational

656   Amblyopia, **CS:** Control with strabismus (but without amblyopia))

657   **β:** Participants who were tested without correction to normal visual acuity

658   **γ:** Response to binocular visual stimulation

659   **Θ:** Response to dichoptic presentation of gratings

660   **μ: ET:** Esotropia**, XT:** Exotropia

661   **Table 2 –** The size of V1 portion that responded preferentially to

662   the fellow/dominant eye (mean ± S. D.).

663

664

	Deep Cortical Layers	**Superficial Cortical Layers**
Control	55.37% ± 15.38%	56.23% ± 15.54%
Strabismic	66.80% ± 11.95%	67.31% ± 12.15%
Anisometropic	79.30% ± 15.30%	78.53% ± 11.83%

668

669 * All measurements were normalized relative to the size of V1

670

671

672   **Table 3 –** The correlation between the interocular visual acuity difference

673   and OD activity evoked across V1-V4

674

|	**Deep Layers**					**Superficial Layers**				
	V1	**V2**	**V3**	**V3A**	**V4**	**V1**	**V2**	**V3**	**V3A**	**V4**
Correlation Value	0.48	0.62	0.65	0.57	0.67	0.53	0.69	0.77	0.69	0.66
Lower Confidence Interval	0.10	0.30	0.33	0.22	0.36	0.16	0.39	0.52	0.40	0.36
Upper Confidence Interval	0.74	0.82	0.83	0.79	0.84	0.77	0.85	0.89	0.86	0.84
Standardized Regression Coefficient	0.62	0.63	0.30	0.40	1.27	0.50	0.71	2.76	0.16	1.95

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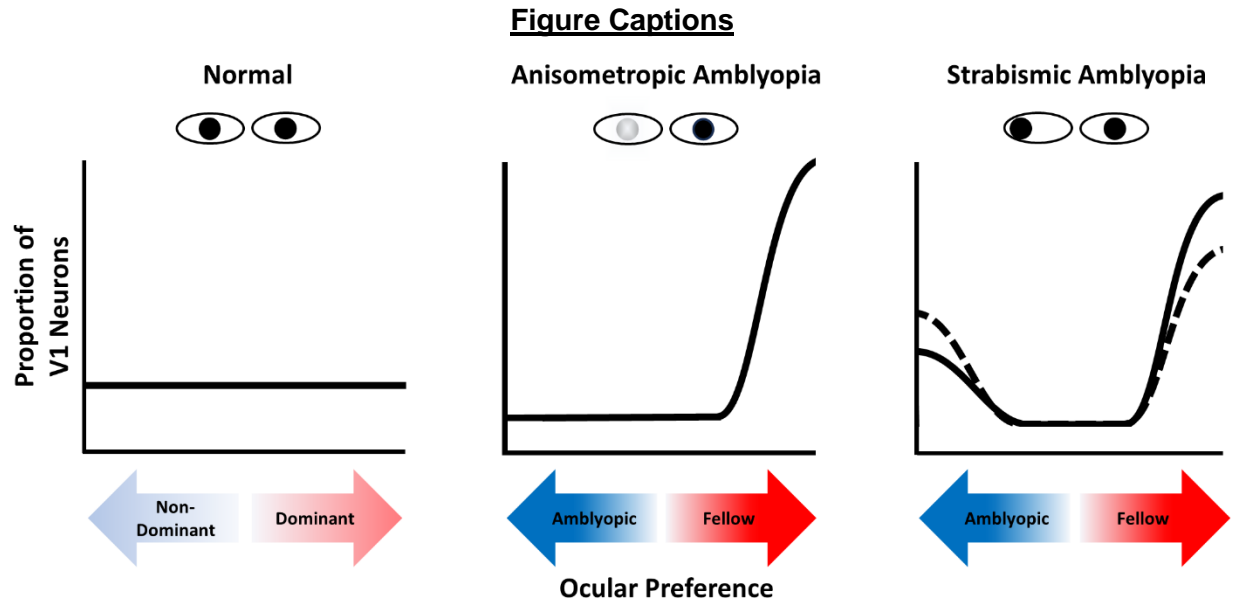
678

679 **Figure 1** – Schematic representation of the relative impact of anisometropic and strabismic
680 amblyopia on the ocular preference of V1 neurons in non-human primates. Individuals with
681 normal binocular vision and no amblyopia (**left**) have a uniform preference for either eye, with
682 some neurons favoring the dominant or non-dominant eye and others showing varying degrees
683 of binocular preference. Amblyopic individuals (regardless of cause) show a decrease in the
684 total number of binocular neurons in V1 (Crawford and Von Noorden, 1979; Crawford et al.,
685 1996; Smith III et al., 1997b; Kiorpes et al., 1998; Bi et al., 2011), while the distribution varies
686 with type: In anisometropic amblyopia (**middle**), this effect is accompanied with a decrease in
687 the number of V1 neurons that respond preferentially to the amblyopic eye, even in those with
688 milder forms of amblyopia. In strabismic individuals with milder forms of amblyopia (**right -
689 dashed line**), amblyopic eye-preferring neurons remain frequently detectable across V1,
690 whereas in more severe forms (**right - solid line**), these neurons are less frequently observed.
691
692

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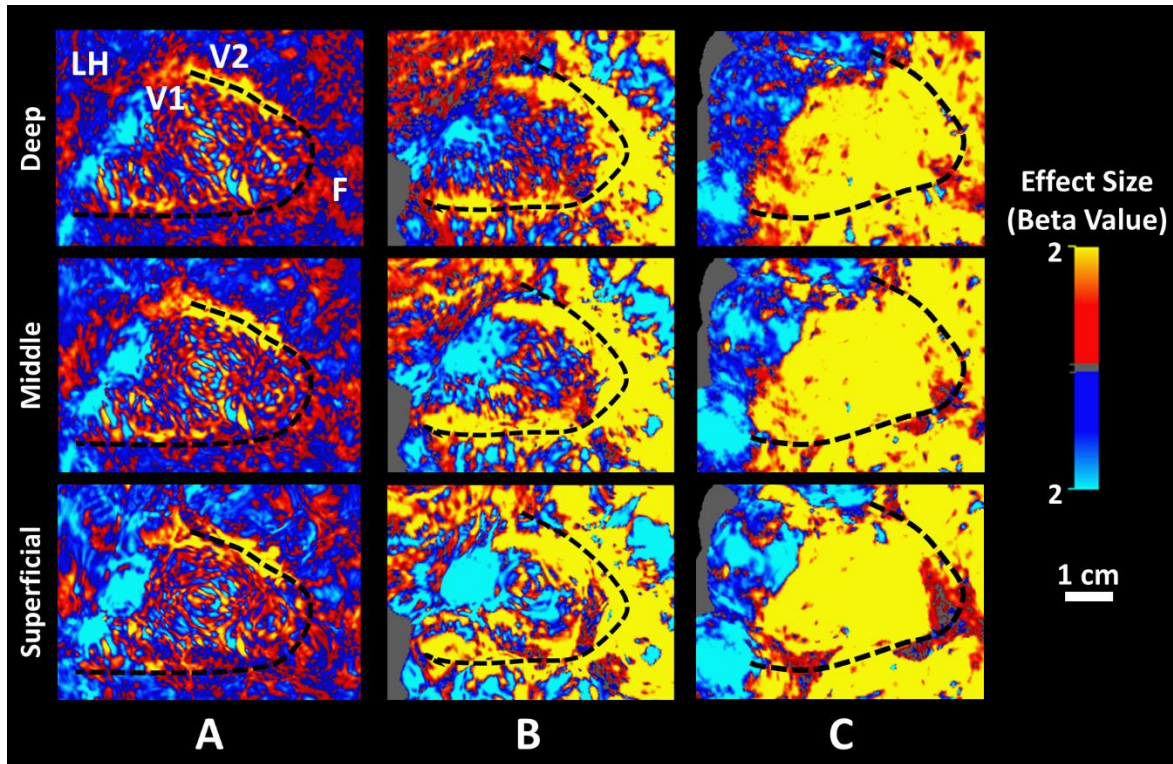
Figure 2 – The OD response evoked by contrasting the response, evoked within the left hemisphere (LH), to stimulation of dominant/fellow (red to yellow) vs. non-dominant/amblyopic (blue to cyan) eye across cortical depth levels, measured during two separate scan sessions. Panels **A-C** show the unthresholded activity maps detected within deep (top), middle and superficial (bottom) cortical depths, in the left hemisphere of a control (Subject #1; Table 1), a strabismic (Subject #13), and an anisometropic (Subject #17) subject, respectively. In the control participants, the OD activity formed mostly parallel stripes that were mostly confined to V1 borders. In the amblyopic participants, especially the anisometropic individual, OD stripes were less pronounced, and the evoked activity extended well beyond the V1 border. This phenomenon was apparent comparably detectable across cortical depths. In all panels, activity maps are overlaid on the subject's own reconstructed cortical surface. The V1-V2 border (black dashed line) is also defined for each subject based on their own retinotopic mapping. The foveal direction is shown with letter F in top-left panel.
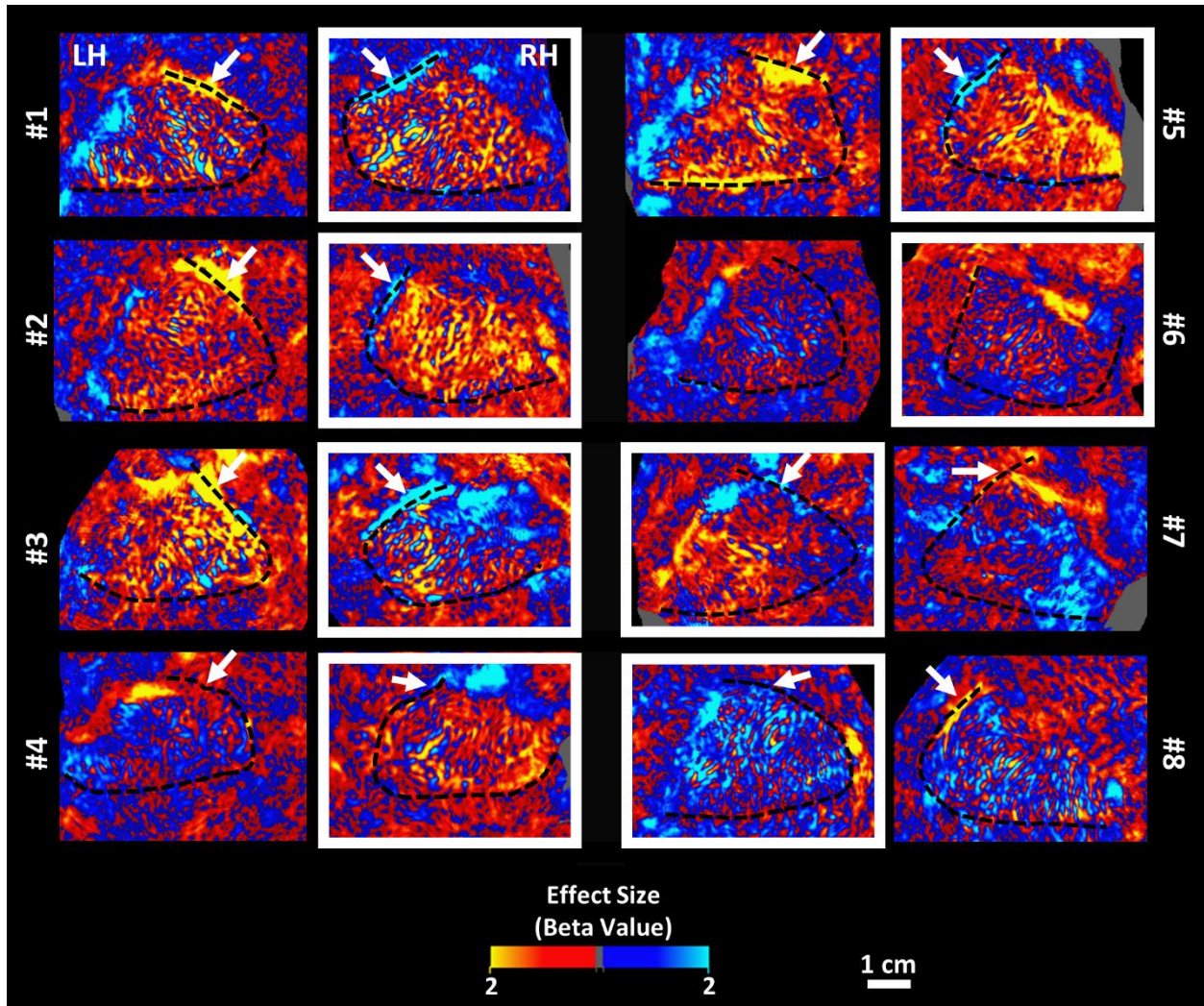
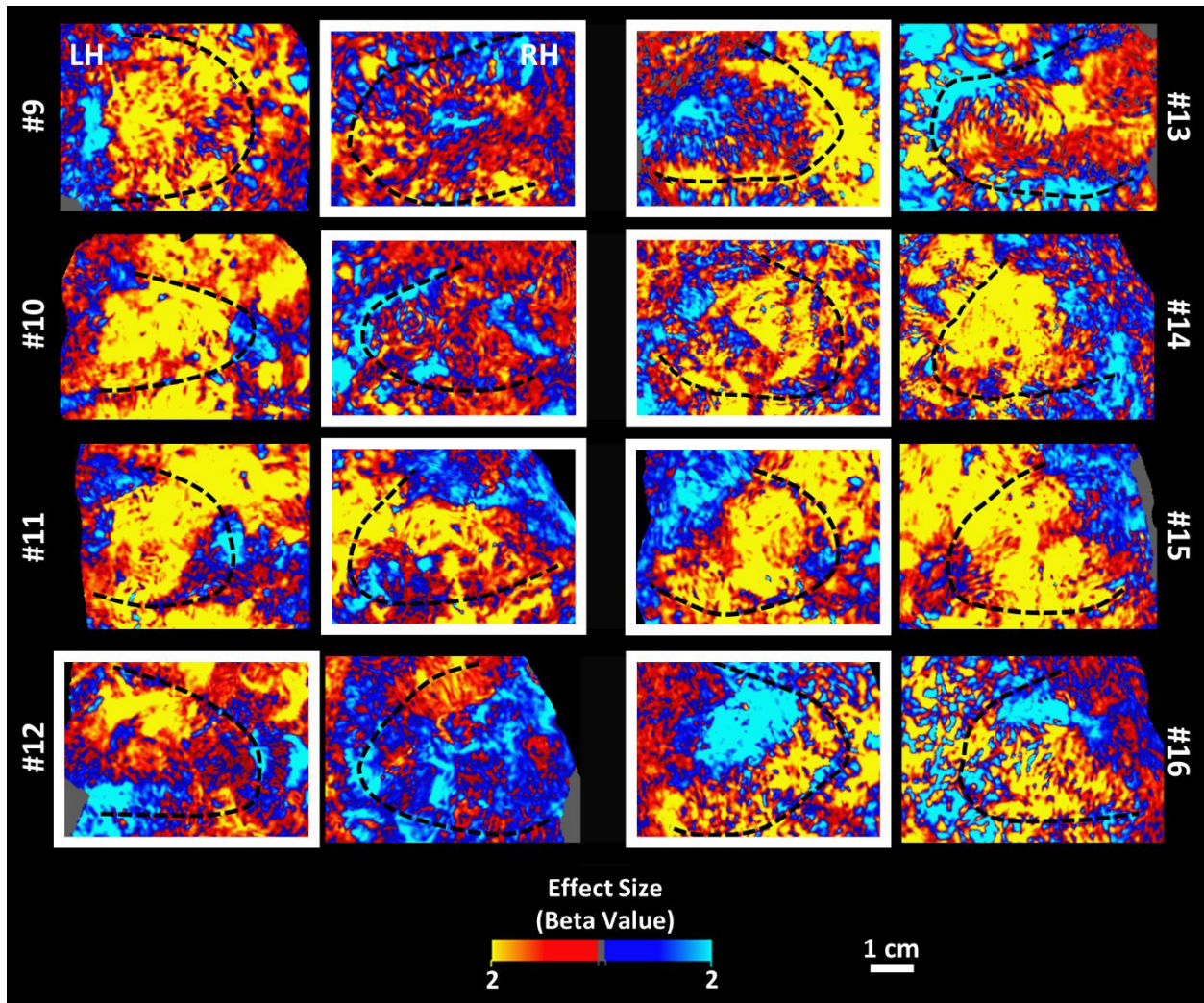**Figure 3 –** The OD activity mapping in 8 control participants, collected from deep cortical depths. In all participants, the striped pattern was apparent within V1. The response amplitude decreased sharply outside the V1 border. For each subject, the white box indicates the hemisphere ipsilateral relative to the dominant eye. The white arrowheads show the large activity patch along the dorsal portion of V1-V2 that responded preferentially to the contralateral eye. This patch was detectable in almost all participants except for participant #6. Other details are the same as in Fig. 2.

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Figure 4 – The OD activity mapping in 8 strabismic participants, collected from deep cortical depths. Compared to controls (Fig. 3), the amplitude of OD response was larger. Moreover, the OD response extended beyond the V1-V2 borders into downstream visual areas. This effect was accompanied by an extension of those regions that responded preferentially to the fellow eye. This overrepresentation was more pronounced in the hemisphere contralateral relative to the fellow eye. Other details are the same as in Figs. 2 and 3.
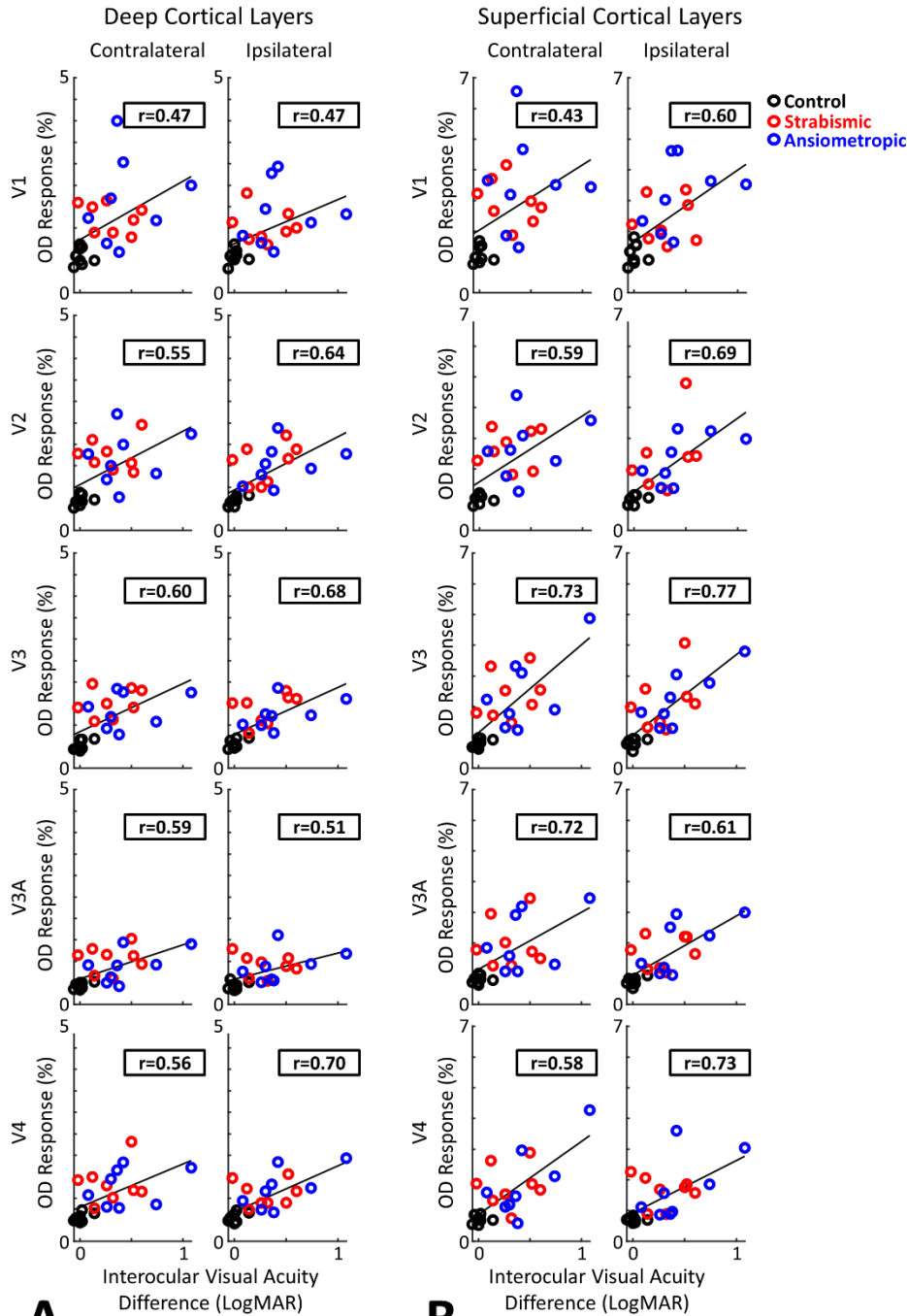
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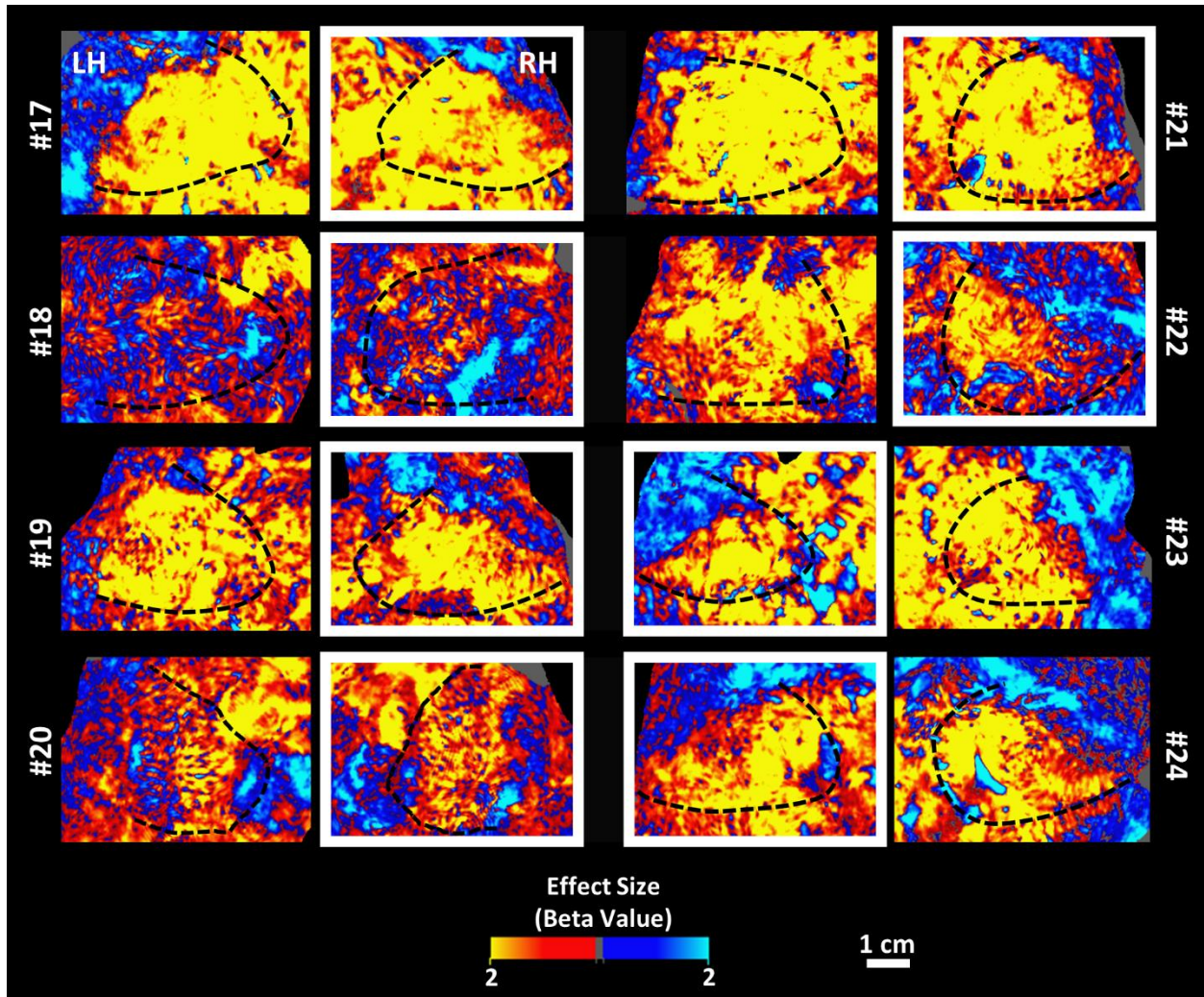
Figure 5 – The OD activity mapping in 8 anisometropic participants, collected from the deep cortical depths. As in the strabismic participants (Fig. 4), the amplitude of OD response is larger relative to controls and the OD response extended beyond the V1-V2 borders. There was an overrepresentation of the fellow eye, as seen in strabismic participants. However, in contrast to the strabismic participants, this phenomenon was detected bilaterally without any apparent difference between the two hemispheres. Other details are the same as in Figs. 2 and 3.
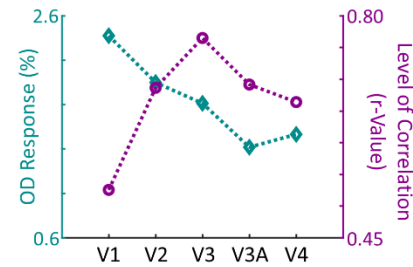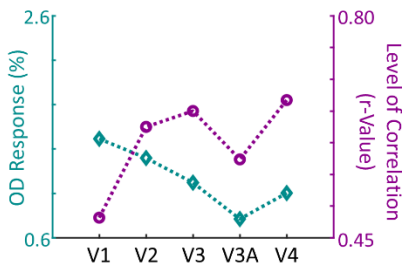
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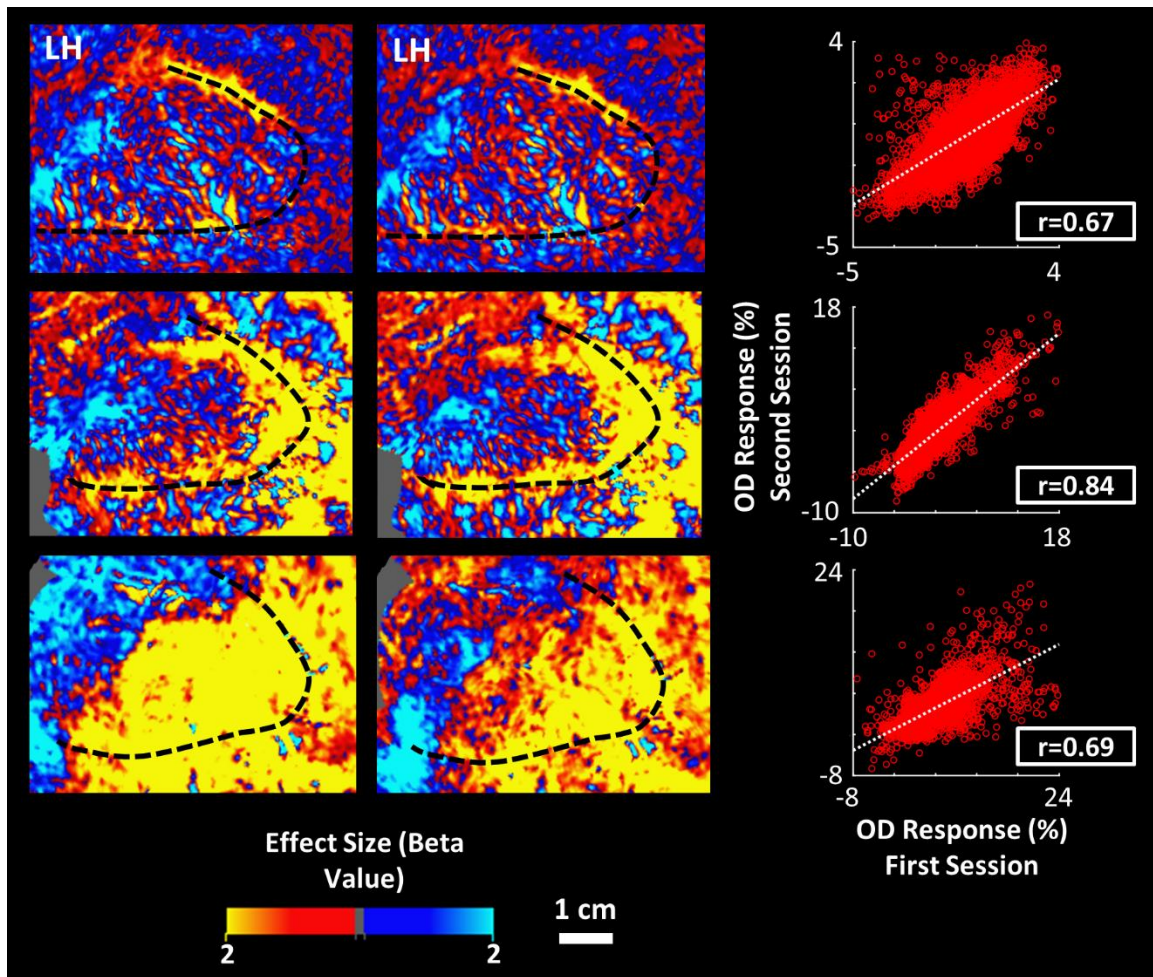
Figure 6 – Reproducibility of the OD maps across scan session. The activity maps show the OD
response evoked within the left hemisphere of the same control (top), strabismic (middle) and
anisometropic (bottom) participants, as in Fig. 2, across two separate sessions (see Methods).
The scatter plots highlight the correlation ($p<10^{-3}$) between the OD response evoked with V1
across the two sessions. Each data point represents activity in one vertex from the
reconstructed cortical surface mesh.

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Deep Cortical Layers

Contralateral   Ipsilateral

r=0.47 r=0.47

r=0.55 r=0.64

r=0.60 r=0.68

r=0.59 r=0.51

r=0.56 r=0.70

A

Superficial Cortical Layers

Contralateral   Ipsilateral

r=0.43 r=0.60

r=0.59 r=0.69

r=0.73 r=0.77

r=0.72 r=0.61

r=0.58 r=0.73

B

Interocular Visual Acuity Difference (LogMAR)

C

D

746

747   **Figure 7 –** The amplitude of the OD response was measured in both deep (**A**) and superficial

748   (**B**) cortical depths of V1-V4. Across all areas, the level of OD response was higher in the

749   amblyopic participants compared to the controls, without a significant difference between the

750   anisometropic and the strabismic individuals. To avoid signal cancelation, the ROI analysis was

751   applied to the absolute value of OD response. Panels **C** and **D** show that, in both deep and

752   superficial depths, the average OD response decreased in downstream visual areas relative to

753   V1. However, the correlation between OD response and the interocular visual acuity difference

754   increased from V1 to V2 to V3. Each point in these panels represents the average data from

755   both hemispheres. Notably, the correlation values were calculated based on all participants.

756   However, exclusion of controls did not change the overall results.

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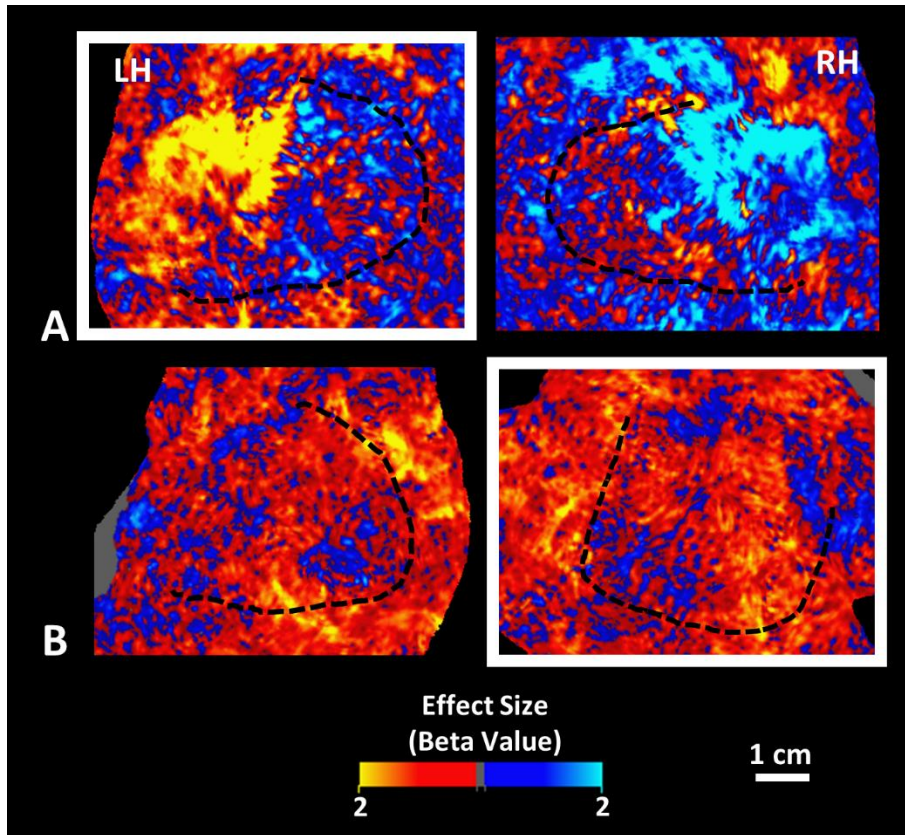
Figure 8 – The OD activity mapping in non-amblyopic strabismic and anisometropic
participants. Panel **A** shows the OD response in one non-amblyopic strabismic individual
(participant #25; Table 1), collected from the deep cortical depth levels. The size of region that
showed an OD bias in favor of the dominant eye remained close to what we found in control
individuals (Table 2) in the contralateral (46.72%) and ipsilateral (43.03%) hemisphere (relative
to the dominant eye). Panel **B** shows the OD response in one control subject (participant #6),
after increasing the interocular visual acuity difference in favor of their dominant eye (from 0.06
to inducing 0.24 logMAR) by instructing the participant not to wear their contact lenses. Despite
the increased level of interocular visual acuity difference, the evoked OD activity remained
weaker compared to those detected in the amblyopic anisometropic individuals (Fig. 5). Other
details are the same as Figs. 2-6.
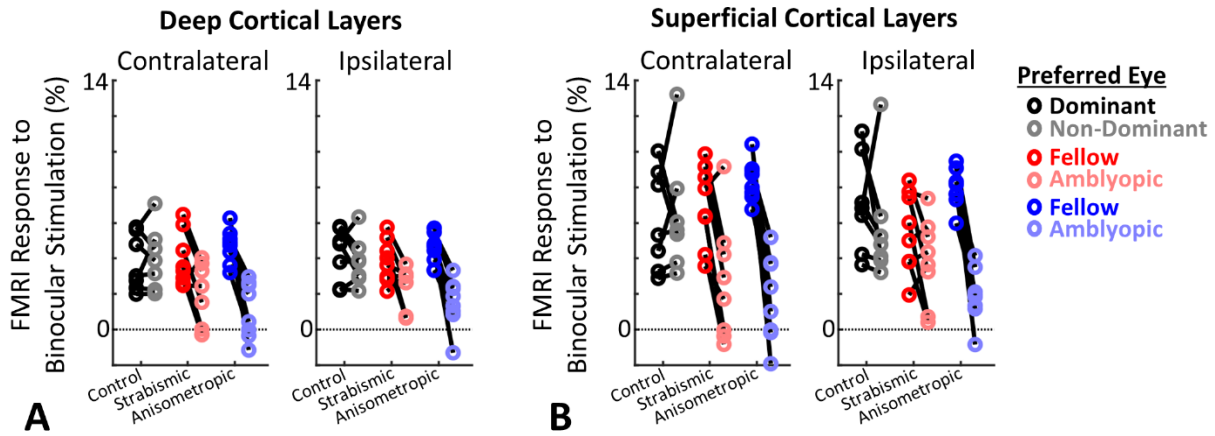
Figure 9 – Activity evoked during binocular stimulation in V1 regions that responded preferentially to the dominant/fellow vs. non-dominant/amblyopic eye. Panels **A** and **B** show the activity evoked in deep and superficial cortical depth levels, respectively. In both depth levels and hemispheres, the level of activity evoked in V1 regions that responded preferentially to the dominant eye remained comparable across the three groups. Whereas, in V1 region that responded preferentially to non-dominant eye, binocular stimulation evoked a weaker response in anisometropic compared to strabismic and controls. This effect was more apparent in more superficial rather than deep cortical depths, and in contralateral rather than ipsilateral hemispheres (relative to the dominant eye). In all panels, each dot pair represents one individual subject.
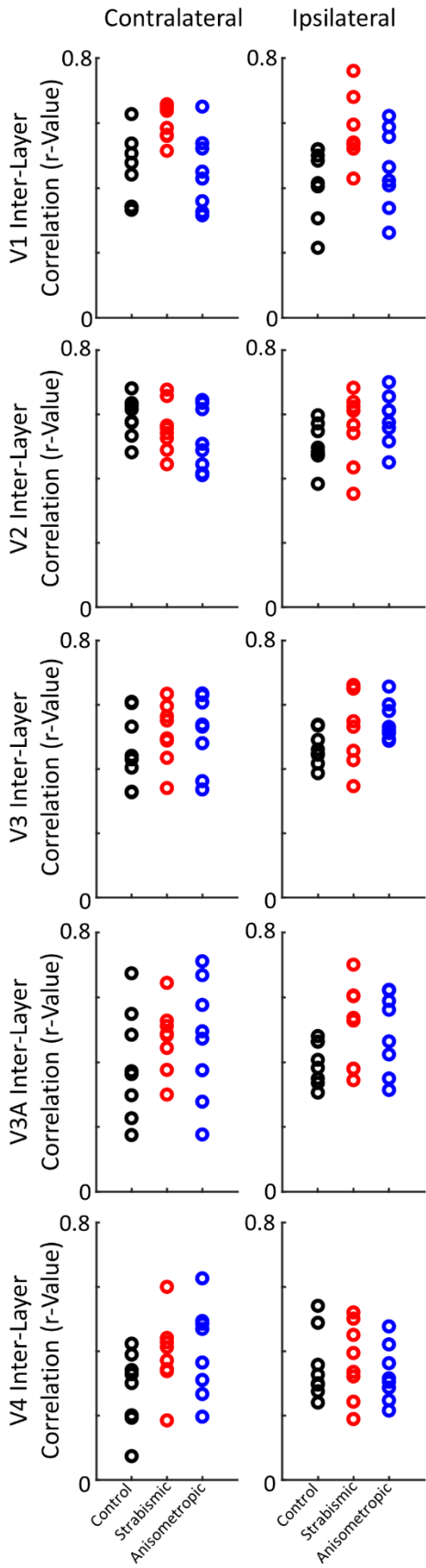
787   **Figure 10 –** The level of correlation between the pattern of OD response evoked within deep

788   and superficial cortical depths, across areas V1-V4. In area V1, but not the other visual areas,

789   strabismic participants show a higher correlation compared to controls and anisometropic

790   individuals. In each graph, each data point shows the data from one individual subject.

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