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1 **Autism sensory dysfunction in an evolutionarily conserved**  
2 **system**

3

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19

20 **Key words**

21 Autism, animal model, *Drosophila*, sensory processing, visual system

22 **Abstract**

23 There is increasing evidence for a strong genetic basis for autism, with many  
24 genetic models being developed in an attempt to replicate autistic symptoms  
25 in animals. However, current animal behaviour paradigms rarely match the  
26 social and cognitive behaviours exhibited by autistic individuals. Here we  
27 instead assay another functional domain – sensory processing – known to be  
28 affected in autism to test a novel genetic autism model in *Drosophila*  
29 *melanogaster*. We show similar visual response alterations and a similar  
30 development trajectory in *Nhe3* mutant flies (total N=72) and in autistic human  
31 participants (total N=154). We report a dissociation between first- and second-  
32 order electrophysiological visual responses to steady-state stimulation in adult  
33 mutant fruit flies that is strikingly similar to the response pattern in human  
34 adults with ASD as well as that of a large sample of neurotypical individuals  
35 with high numbers of autistic traits. We explain this as a genetically driven,  
36 selective signalling alteration in transient visual dynamics. In contrast to  
37 adults, autistic children show a decrease in the first-order response that is  
38 matched by the fruit fly model, suggesting that a compensatory change in  
39 processing occurs during development. Our results provide the first animal  
40 model of autism comprising a differential developmental phenotype in visual  
41 processing.

42

## 43 Introduction

44 Autism spectrum disorder (ASD) has a strong albeit complex genetic basis  
45 with a large number of genes implicated (1–5). A variety of genetic animal  
46 models have been proposed for ASD, including murine models (6–8) and  
47 more recently, fly models (9). However, for an animal model of any  
48 disorder/disease to be useful it needs to fulfill as much face validity as  
49 possible (i.e., exhibit a similar phenotype to humans with the  
50 disorder/disease). This poses a challenge for multifaceted, heterogenic  
51 disorders having symptoms that are difficult to operationalise and measure in  
52 animals. While there have been some attempts at measuring defining  
53 behaviours of ASD in animal models (10), including difficult to assess social  
54 interactions (11), repetitive behaviours (12), and confined interests (13), the  
55 links between human symptoms and equivalent animal behaviours are  
56 tenuous. For example, social symptoms in mice have been evaluated as  
57 defensive behaviour against intruders (11), or as courtship call frequency and  
58 wing extension in fruit flies (9), even though neither behaviour manifests in  
59 humans.

60 In addition to the defining social and behavioural features of ASD, autistic  
61 individuals report a host of sensory symptoms including unusual sensory  
62 interests as well as hyper- and hyposensitivity to intense stimuli such as bright  
63 lights or loud noises (14,15). These human ASD sensory processing  
64 symptoms have been well documented behaviourally (16–18), with  
65 electroencephalography (EEG; 16,17) and neuroimaging (21) and can also be  
66 measured in animals using equivalent methods (22). Functioning in sensory  
67 systems may be better conserved over evolution than more complex  
68 behaviours associated with ASD, therefore we pursued a comparison of  
69 sensory responses in humans with ASD and an *Nhe3* fruit fly model of ASD.

70 A previous study in mice measured visual responses in a related  
71 developmental condition, Rett syndrome, and was able to link decreases in  
72 visual neural responses and poor visual acuity across species (23). However,  
73 it is difficult to generalise these findings to ASD, as human Rett syndrome  
74 lacks the pervasive sensory symptoms characteristic of autism (24). An  
75 advantageous alternative to rodent models are *Drosophila* given the ease in  
76 developing genetic mutations and ability to test many individual animals.  
77 Successful *Drosophila* models of human neurological disorders have so far  
78 been developed for Parkinson's disease (25), fragile X syndrome (26) and  
79 Alzheimer's disease (27). Fruit flies share 75% of human disease-causing  
80 genes (28) and have a visual system exhibiting similar nonlinear neural  
81 properties, including a colour- and luminance-selective module as well as a  
82 motion-selective module (29). The neural dynamics of these modules closely  
83 resemble those of transient and sustained neural populations in humans (30–  
84 32). These factors combine to provide an excellent framework for modelling  
85 changes in early sensory neuronal signalling (32) which may lie behind  
86 atypical sensory processing in autism.

87 In this study we evaluated a genetic *Drosophila* model of human ASD by  
88 measuring comparable visual responses both in autistic humans and in

89 mutant *Drosophila*. In humans, loss-of-function mutations in the gene *SLC9A9*  
90 have been linked to ASD (33). Here we used a *Drosophila* orthologue of  
91 *SLC9A9* – *Nhe3*. A homozygous P-element insertion loss-of-function mutants  
92 (*Nhe3*<sup>KG08307</sup>) and *Nhe3* hemizygotes (*Nhe3*<sup>KG08307</sup>/Df(2L)BSC187) were used  
93 to inhibit *Nhe3* function in fly. The use of two *Nhe3* mutations in different  
94 genetic backgrounds ruled out the possibility of other mutations influencing  
95 the flies' visual responses. To assess the functionality of the visual system in  
96 these species, we measured steady-state visually evoked potentials (ssVEPs)  
97 to temporally-modulated contrast stimuli. During this paradigm a stimulus in  
98 flickered on/off at a particular frequency (for example 12Hz) whilst neural  
99 responses are recorded from the organism. Using Fourier transformation we  
100 then convert time course data into the frequency domain where the amplitude  
101 of different frequency components of the neural responses can be measured.  
102 From there we extract the 1<sup>st</sup> harmonic response (which follows the  
103 stimulation frequency – 12Hz), as well a 2<sup>nd</sup> harmonic response. Second  
104 harmonics are responses generated by the brightening and darkening  
105 transients of the stimulus flicker, thus – 24Hz in the flies and to contrast  
106 onset/contrast offset in human. The first and second harmonics probe  
107 different aspects of the dynamics of the visual system: sustained and transient  
108 neural responses, respectively (34). Previous genetic dissection of the fruit fly  
109 has localised the 1<sup>st</sup> harmonic to photoreceptors and the 2<sup>nd</sup> harmonic to the  
110 lamina (31).

111 As the visual systems of humans and fruit flies are difficult to compare  
112 anatomically, the visual responses obtained here were produced by  
113 functionally equivalent human and fruit fly neural substrates. In each organism  
114 we assessed the same functional mechanism - contrast transduction. This  
115 computation in the fly is performed at the level of photoreceptors and lamina,

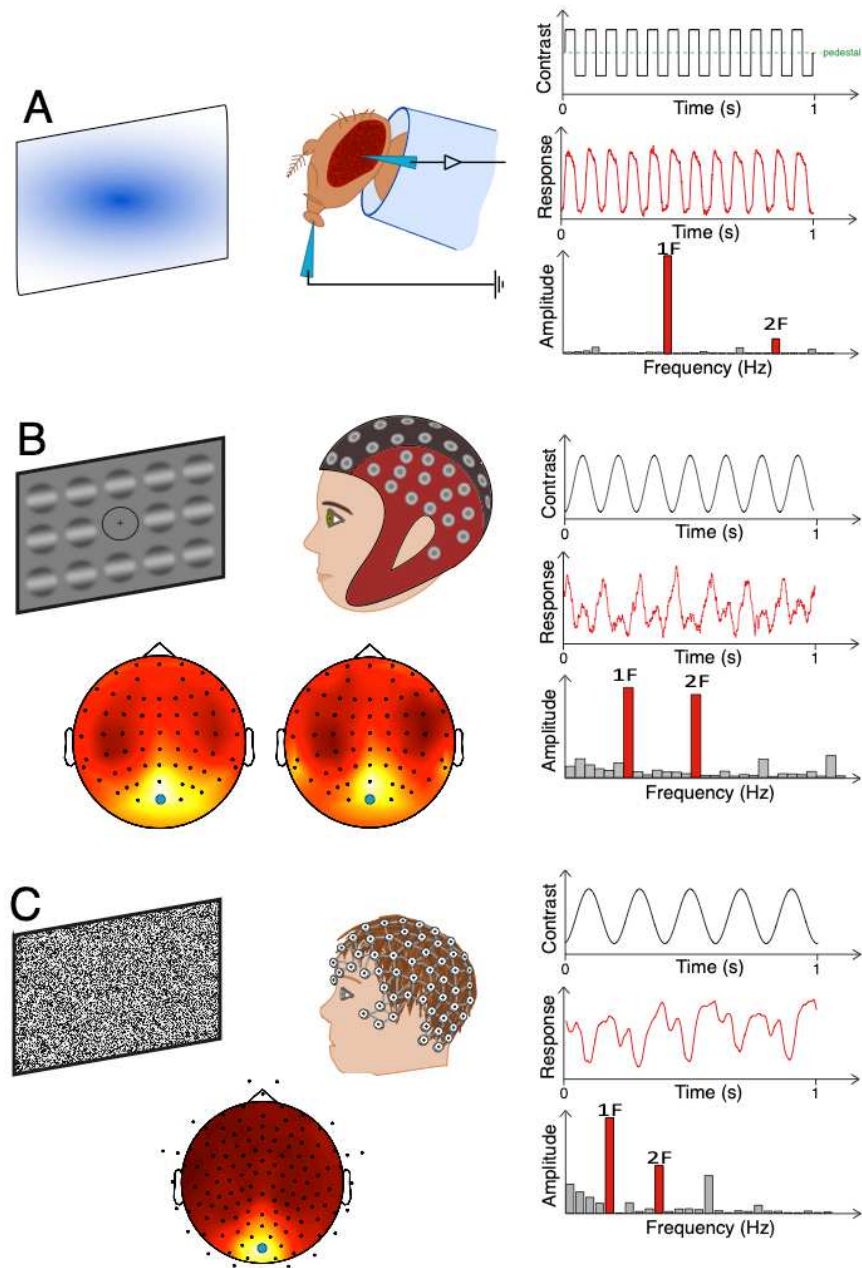
116 whereas in humans the same computation is performed in the retina and in  
117 early visual cortex (V1). A similar cross-species computational equivalence in  
118 the face of vastly different neural substrates has been shown previously for  
119 motion perception: third order correlations required for motion perception were  
120 found in the lamina of the fly and areas V1 and MT in humans (35).

121 Furthermore, to investigate the progression of ASD sensory atypicalities over  
122 the course of development, we also measured visual responses at two stages  
123 of fruit fly maturation and acquired similar responses from autistic children and  
124 adults. Finally, as the ASD phenotype is complex and non-binary, we  
125 validated our sensory model with a large sample of neurotypical participants  
126 with high and low numbers of autistic traits.

## 127 **Results**

128 **Increased sustained/transient response ratio in *Nhe3* fruit flies.** Using a  
129 steady-state visual evoked potential (ssVEP) paradigm (25) (see *Fig 1*) we  
130 measured *Drosophila* visual responses to flickering stimuli via an electrode on  
131 the fly's eye. Wild type, eye-colour matched flies (a cross between isogenic  
132 and Canton-S) were used as controls (+). Twelve flies from each genotype

133 were tested at three days (when the flies are young; total n=36) and at 14  
 134 days post eclosion (older; total n=36). First harmonic (12Hz) and second  
 135 harmonic (24Hz) response amplitudes were derived by fast Fourier transform  
 136 (see *Methods*). Although the first harmonic responses of mutant and wild-type  
 137 flies were the same, the second harmonic response was significantly reduced  
 138 in the *Nhe3* mutants (*Fig 2a, 2b*).

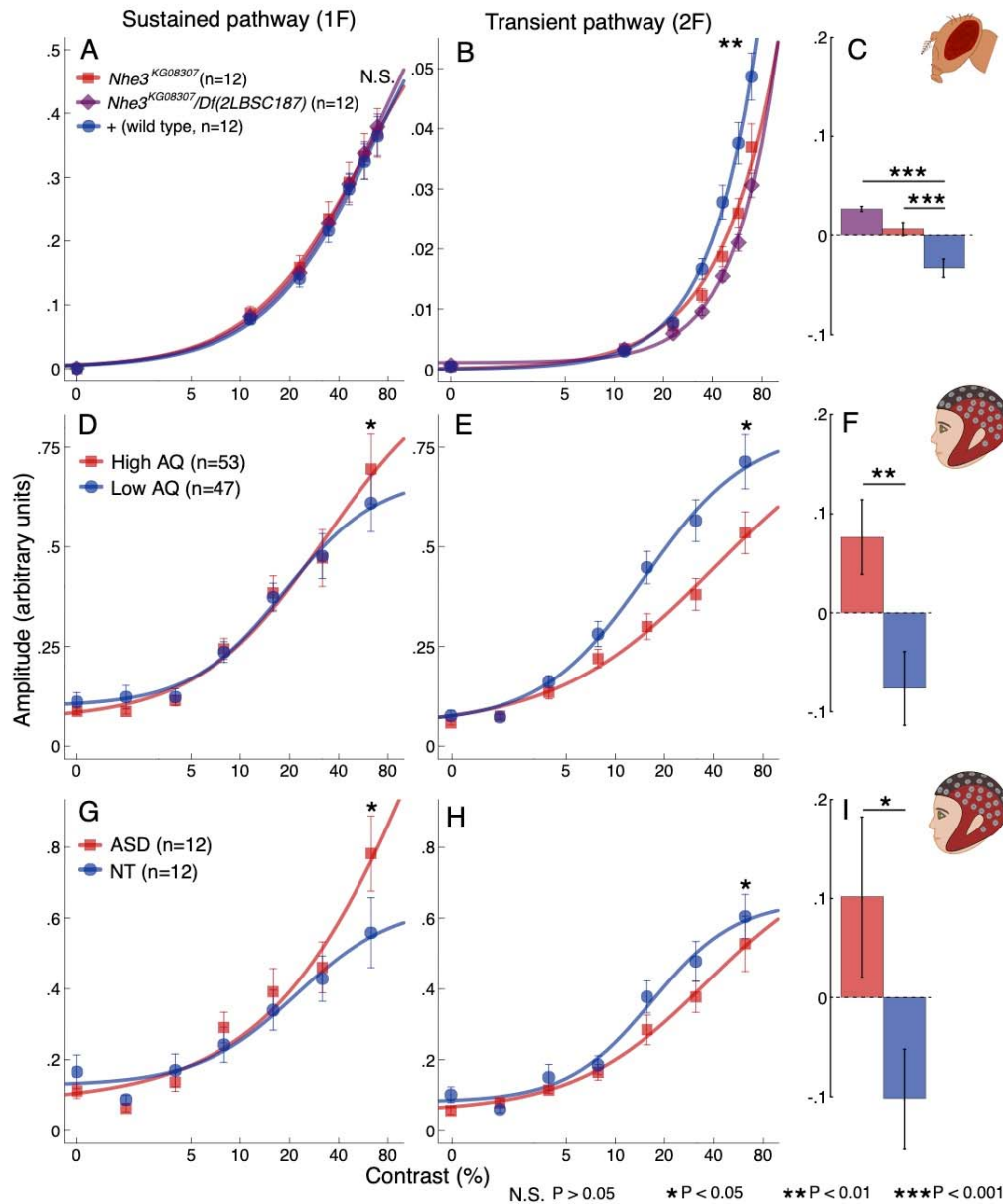


139

140 **Fig 1. Human and *Drosophila* steady-state electrophysiology methods.**

141 Panel A left illustrates the experimental set up for fruit fly electrophysiology  
 142 (see *Drosophila electroretinography* for more details). Panel A right shows the  
 143 square wave stimulus trace flickering at 12Hz (top), example  
 144 electrophysiological responses over time (middle) and Fourier-transformed

145 response amplitudes in the frequency domain (bottom). Panel B left illustrates  
 146 the experimental set up for adult participants, who were presented with a grid  
 147 of sinusoidal gratings flickering at 7Hz whilst ssVEPs were recorded with a  
 148 64-channel EEG cap (top). SSVEPs were measured from occipital electrode  
 149 Oz (blue circle) where the highest 1<sup>st</sup> harmonic amplitude was centred (AQ  
 150 adults – bottom left, ASD adults – bottom right). Panel B right shows the  
 151 stimulus trace (top), example responses in the time domain (middle) and in  
 152 the frequency domain (bottom). Panel C shows equivalent experimental set  
 153 up, stimulus and response traces for the children’s dataset.



154

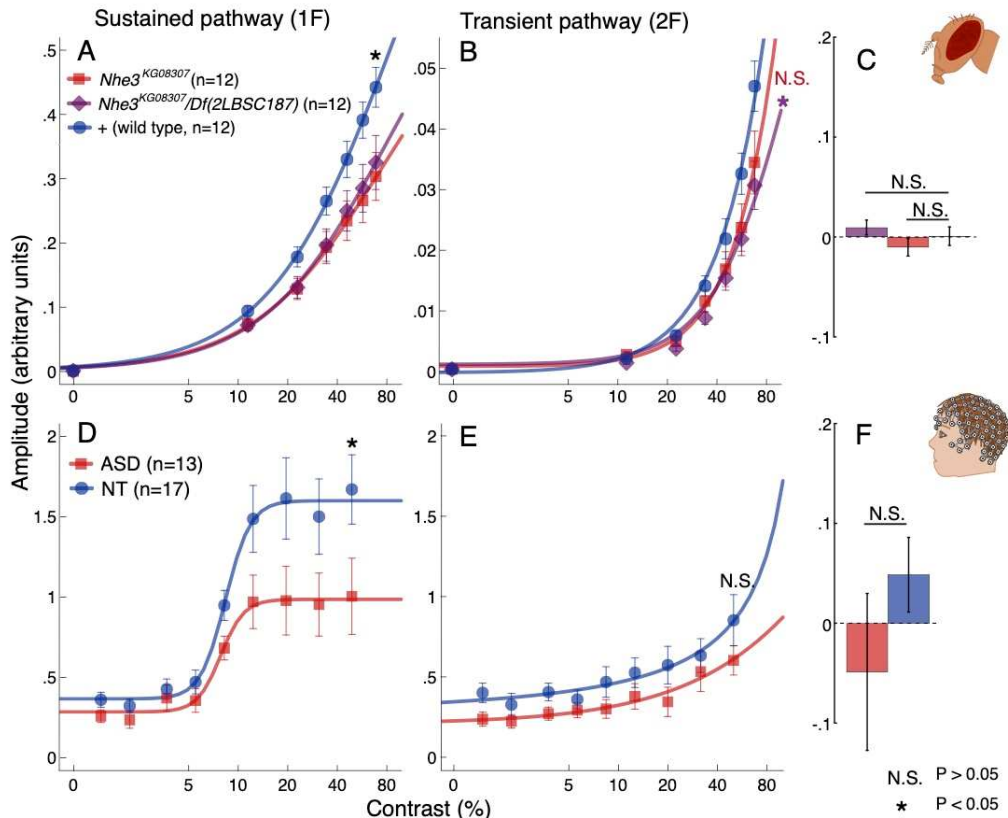
155 **Fig 2. Older ASD-mimic flies and autistic humans show reduced visual**  
 156 **responses in the transient component.** Contrast response functions for  
 157 adult *Nhe3* mutant flies (*Nhe3*<sup>KG08307</sup> homozygotes, red squares and

158 *Nhe3*<sup>KG08307</sup> /Df(2L)BSC187, purple diamonds) were similar at the first  
159 harmonic (a one-way ANOVA showed no effect of group  $F_{2,33} = 0.05$ ,  $P =$   
160  $0.95$ , panel A) but responses were reduced for P/P (simple contrast,  $P=0.025$ )  
161 and P/Df mutants compared to controls at the second harmonic (simple  
162 contrast  $P = 0.001$ ; ANOVA group effect  $F_{2,33} = 6.71$ ,  $P < 0.01$ ; panel B).  
163 Ratios between frequencies ( $\frac{1F-2F}{1F+2F}$ ) were significantly higher for P/P ( $P <$   
164  $0.001$ ) and for P/Df ( $P < 0.0001$ ) than for the control genotype (C). First  
165 harmonic responses were also similar for the high AQ and low AQ groups  
166 (panel D) and for autistic and neurotypical adults (panel G). However, second  
167 harmonic responses were reduced for both adults with high AQ (panel E) and  
168 autistic adults compared to controls (panels H). The ratio between harmonics  
169 was also higher in both experimental groups compared to controls (panels F  
170 and I,  $P = 0.005$  and  $P = 0.04$ , respectively). Curved lines are hyperbolic  
171 function fits to the data. Frequency ratios are baselined in respect to the mean  
172 over groups of each comparison for display purposes. Error bars in all panels  
173 represent  $\pm$ SEM.

174 To quantify this functional dissociation whilst controlling for overall  
175 responsiveness of the visual system, we calculated a normalised ratio  
176 between first (1F) and second (2F) harmonics ( $\frac{1F-2F}{1F+2F}$ ) and averaged over the  
177 highest contrast conditions (where the response rises above the noise floor,  
178 see *Methods*). This allowed us to measure the differences between sustained  
179 and transient responses whilst normalising for overall responsiveness of the  
180 visual pathway. The ratio was significantly higher in both mutant strains than  
181 in the controls (ANOVA,  $F_{2,33} = 20.53$ ,  $P < 0.0001$ , both paired contrasts  $P <$   
182  $0.001$ ; *Fig 2c*). These data suggest an impairment in the post-receptor  
183 neural structures (downstream of the photoreceptors) of the older mutant flies  
184 (36).

185 Interestingly, unlike the older flies, the young 3-day-old flies showed a  
186 reduced response at both frequencies (see *Fig 3a,b*) relative to controls.  
187 Importantly, there was no effect of genotype on the ratio between harmonics  
188 ( $F_{2,33} = 1.38$ ,  $P = 0.27$ ; *Fig 3c*). These results suggest a deficit in the  
189 sustained visual module of young mutant flies. These differences between  
190 visual responses at two stages of life suggest a change in visual processing  
191 over the course of development.



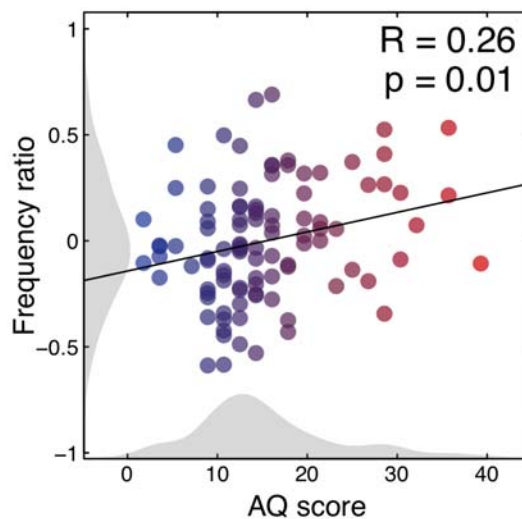


192

193 **Fig 3. Young ASD-mimic flies and autistic children show reduced visual**  
 194 **responses in the sustained component.** Young fruit flies showed reduced  
 195 responses at the first harmonic ( $F_{2,33} = 3.73$ ,  $P = 0.035$ ; panel A) with P/P and  
 196 P/Df flies showing a significant difference from control flies (respectively,  $P =$   
 197  $0.016$  and  $P = 0.040$ ). There was also a significant effect of genotype at the  
 198 second harmonic ( $F_{2,33} = 3.39$ ,  $P = 0.046$ , panel B). P/Df flies showed a  
 199 significant difference from control flies ( $P = 0.018$ ), however, P/P showed a  
 200 non-significant difference from controls ( $P = 0.064$ ). The flies had normal  
 201 frequency ratios (panel C). Autistic children also showed reduced first  
 202 harmonic ( $t_{28} = 2.065$ ,  $P = 0.048$ ; panel D) but not second harmonic  
 203 responses ( $t_{28} = 1.26$ ,  $P = 0.22$ ; panel E) and had frequency ratios similar to  
 204 that of control children ( $t_{28} = 1.21$ ,  $P = 0.24$ ; panel F). Curved lines are  
 205 hyperbolic function fits to the data. Frequency ratios are baselined in respect  
 206 to the mean over groups of each comparison for display purposes. Error bars  
 207 in all panels represent  $\pm$ SEM.

208 **High autistic trait population show similar ssVEPs to *Nhe3* flies.** To  
 209 assess the relevance of the *Nhe3* model to the human ASD phenotype we  
 210 used a comparable and similarly sensitive ssVEP paradigm in human  
 211 participants. One hundred neurotypical participants with putative autistic traits  
 212 measured using the Autism Spectrum Quotient (AQ) questionnaire (37) were  
 213 tested with the ssVEP paradigm. Visual responses were recorded from an  
 214 occipital electrode (Oz, located at the back of the head over the visual cortex)  
 215 to grating stimuli flickered at 7Hz. Seven contrast conditions (each repeated  
 216 eight times) were presented in a randomised order. First and second

217 harmonic ssVEP responses were again derived via Fourier analysis. The  
218 evoked response data were averaged separately over participants split by  
219 their median (median = 14) AQ score: high (n = 53, AQ mean = 20.57, SD =  
220 6.66) and low (n = 47, AQ mean = 9.47, SD = 3.08) AQ (high AQ implying  
221 many autistic traits). The second harmonic was notably reduced in the high  
222 AQ group, similarly to mutant fruit flies (*Fig 2d, 2e*). In addition, the first  
223 harmonic response was slightly increased in the high AQ group. A two-way  
224 ANOVA showed the interaction between group and frequency to be significant  
225 ( $F_{1,98} = 6.17$ ,  $P = 0.015$ ). The high AQ group also had a significantly higher  
226 frequency ratio than the low AQ group ( $t_{98} = 2.86$ ,  $P < 0.01$ , *Fig 2f*). Moreover,  
227 a regression analysis showed that AQ scores correlated with the frequency  
228 ratio, with high AQ scores being predictive of higher ratios ( $R = 0.26$   $F_{1,98} =$   
229  $6.87$ ,  $P = 0.01$ ; see *Fig 4*). This result shows a relationship between the  
230 amplitude of the second harmonic response and the severity of the subclinical  
231 ASD phenotype, however, this effect cannot be directly generalised to clinical  
232 autism as the AQ is not diagnostic of full-blown ASD.



233

234 **Fig 4. Positive relationship between the number of autistic traits and**  
235 **first/second harmonic ratio.** Scatterplot showing a significant positive  
236 relationship between AQ scores and frequency ratios in the 100 neurotypical  
237 adult dataset indicating a gradual increase in response differences with the  
238 number of reported autistic traits. The black line indicates the regression line  
239 of best fit. Shaded grey areas show histograms of AQ scores and frequency  
240 ratios. Blue-red colour transition indicates number of AQ traits with  
241 participants split by median into low and high AQ groups as presented in *Fig*  
242 *2*.

243 **Adult autistic individuals show a similar pattern of responses as mature**  
244 ***Nhe3* flies.** We assessed the ssVEP difference between harmonics in clinical  
245 ASD by testing 12 typical-IQ autistic adults (diagnosis confirmed with the  
246 Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), Lord et  
247 al., 2000) and 12 age- and gender-matched controls using the same human  
248 ssVEP paradigm. The pattern of data again mimicked that of the previous  
249 adult dataset: there was a significant interaction between group and frequency

250 ( $F_{1,22} = 5.85$ ,  $P = 0.02$ ; *Fig 2g, 2h*), with the difference in second harmonic  
251 responses replicating that of the high AQ individuals and older mutant fruit  
252 flies. The ratio between harmonics was again significantly larger in the ASD  
253 group than in the control group ( $t_{22} = 2.13$ ,  $P = 0.04$ ; *Fig 2i*).

#### 254 **Young *Nhe3* fly responses are similar to autistic children's responses.**

255 Considering the striking similarity between the adult human datasets and the  
256 adult fruit fly model, it is reasonable to ask if similarities also exist between  
257 human children and young ASD-mimic flies. Specifically, our fly model  
258 predicts that the visual system of autistic children should show reduced  
259 responses at both the first and second harmonics. To examine this, we  
260 recorded from 13 autistic children (5 – 13 years old) and 17 neurotypical age-  
261 and gender-ratio-matched controls using an ssVEP contrast-sweep paradigm.  
262 Artifact rejection was employed to control for movement and blinking in both  
263 groups. The stimulus in each sweep trial increased continuously in contrast  
264 from 0% to 50% in logarithmic steps. Data were binned into 9 contrast levels  
265 before being Fourier transformed to compute response amplitudes.

266 As predicted by the model, the ASD group showed reduced amplitudes of the  
267 1F, sustained response ( $t_{28} = 2.07$ ,  $P = 0.04$ ; *Fig 3d, 3e*) which was not found  
268 in the autistic adults, individuals with high AQ or older mutant fruit flies. A two-  
269 way ANOVA also revealed a significant group effect over both frequencies  
270 ( $F_{1,28} = 4.23$ ,  $P = 0.049$ ). Unlike adults, children exhibited no difference in  
271 frequency ratios between the groups ( $t = 1.41$ ,  $P = 0.17$ ; *Fig 3f*). Although  
272 children showed reduced amplitudes in the sustained response as predicted  
273 by the *Drosophila* model, the amplitude reduction observed in the fruit fly  
274 second harmonic responses was in the same direction, but was not  
275 statistically reliable in the children ( $t_{28} = 1.26$ ,  $P = 0.219$ ). This may be due to  
276 difficulty in measuring the relatively smaller F2 response in children.

## 277 **Discussion**

278 We found sensory processing alterations in our *Drosophila* model of ASD that  
279 were consistent with similar response alterations in human data at two stages  
280 of development. Our steady-state electrophysiology data showed a selective  
281 depression in second harmonic visual responses in autistic adults, individuals  
282 with high levels of autistic traits and *Nhe3* mutant fruit flies, suggesting that  
283 this response alteration is specific to the autistic phenotype in mature  
284 individuals of both species. These differences were also present when we  
285 calculated 1<sup>st</sup>/2<sup>nd</sup> harmonic ratios in order to control for changes in overall  
286 visual sensitivity. This suggests that the transient component of visual  
287 processing is selectively affected. Autistic children and young *Nhe3* flies  
288 showed an alteration in sustained visual processing, not present in the adults.  
289 The *Nhe3* fruit fly model of autism was predictive of these sustained visual  
290 response alterations both in children and in adults (atypical in early life,  
291 normal in later life), suggesting a fundamental and pervasive change in visual  
292 processing occurs during development in ASD. Although the human *Nhe9* is  
293 only one gene implicated in ASD, its ortholog in fruit flies was able to produce  
294 a measureable sensory processing effect, which has a close counterpart in  
295 human ASD.

296 We replicated the response alterations of autistic adults in neurotypical  
297 individuals with high AQ: this group had visual responses consistent with  
298 those of autistic participants diagnosed ASD suggesting common visual  
299 response properties between samples. This was unsurprising as previous  
300 research has found that AQ scores in the general population are highly  
301 correlated ( $R = 0.77$ ) with sensory processing difficulties, as measured by the  
302 Glasgow Sensory Questionnaire (39), indicating that high AQ individuals  
303 exhibit milder forms of sensory difficulties.

304 The intact first harmonic response in adult flies and humans indicates normal  
305 functioning of mechanisms which give rise to the sustained response.  
306 Conversely, the reduced second harmonic response as well as the increased  
307 ratio between harmonics suggest a modification in the transient dynamics of  
308 the visual system. In fly, the first harmonic has been associated with  
309 sustained photoreceptor polarisation and the second harmonic with second-  
310 order lamina cells (31). In human, an association has been made between  
311 simple cell and sustained responses to pattern onset and between complex  
312 cells and transient responses at both stimulus onset and offset (40). Although  
313 simple cells exhibit some transient response properties as well (40,41), the  
314 intact first harmonic of adults suggests that their response modification is  
315 specific to human complex cells that only generate even-order response  
316 components. This early, cell-type-specific deficit may explain previous findings  
317 of atypical neural dynamics of spatial frequency processing in ASD in the face  
318 of normal sensitivity thresholds (20,42).

319 Mechanistically, lower 2<sup>nd</sup> harmonic responses could either be generated by  
320 disturbances in non-linear transduction of visual signals or by subsequent  
321 temporal processing. As the 2<sup>nd</sup> harmonic, by definition, has a higher temporal  
322 frequency, a bandpass temporal filter shifted towards lower frequencies would  
323 attenuate signals at this frequency more compared to the 1<sup>st</sup> harmonic. There  
324 is at present no consistent evidence for lowered temporal  
325 resolution/prolonged integration in human ASD. One study found no  
326 difference between autistic and neurotypical participants (Kwakye et al 2011),  
327 one study found finer/higher temporal resolution (Falter et al., 2012) and  
328 another coarser/lower temporal resolution (de Boer-Schellekens, 2013). The  
329 possible role of temporal integration time could be tested in future work by  
330 using a lower stimulus frequency (such that the 2<sup>nd</sup> harmonic would now equal  
331 the current 1<sup>st</sup> harmonic frequency) and by observing whether the difference  
332 between harmonics disappears. An absence of a difference would indicate  
333 that temporal filtering is affected in ASD, whereas a persistently reduced 2<sup>nd</sup>  
334 harmonic would indicate a difference in the non-linearity.

335 The differences in sustained and transient modules observed in our *Nhe3*  
336 model mimics the alteration of neural dynamics in autistic adults. *Nhe3* affects  
337 the exchange of sodium and hydrogen ions in cell membranes directly  
338 affecting neural signalling (33,43). Differential expression of *Nhe3* and other  
339 genes in ASD, which has been observed in other parts of the brain (33,44)  
340 may extend to differential expression in colour and motion modules in the  
341 *Drosophila* visual system. As *Nhe3* (*SLC9A9* in humans) is only a single gene  
342 in a multifaceted genetic etiology of autism, it is likely that the expression of

343 several genes in human autism affects simple and complex cell dynamics,  
344 producing similar effects at the neural population level. Furthermore, such  
345 abnormality in gene expression in other parts of autistic brains, as well as  
346 environmental influences and gene-environment interactions, may give rise to  
347 a wide range of cognitive and social differences in childhood and adulthood.

348 Our data indicate little or no over-responsivity in the visual responses that are  
349 predicted by excitation/inhibition (E/I) imbalance theories (45,46) and  
350 consistent with measurements of some previous studies (47,48). However, it  
351 is possible that an E/I imbalance in autism stemming from GABA-ergic  
352 mechanism differences affects different neuron types or processing pathways  
353 in distinct ways and to different extents. It is also possible that E/I imbalance  
354 in sensory cortical areas in autistic individuals compensates for lower sensory  
355 signals (such as the second harmonic response here) in childhood.  
356 Regardless, cell-type based processing modifications may explain previous  
357 inconsistencies in studies of sensory symptoms in ASD that did not  
358 differentiate the relevant neural dynamics (17). Furthermore, the current  
359 results can provide an amended explanation to the magnocellular (M  
360 pathway) dysfunction hypothesis (16). As it is difficult to isolate the M pathway  
361 by changing stimulus properties (34), the paradigms previously used to  
362 investigate magnocellular dysfunction in ASD may have been selectively  
363 activating responses of transient components rather than the M pathway, in  
364 particular (16,49).

365 Developmentally, the observed lessening of the response modifications in  
366 both species with increasing age is in accordance with previous findings  
367 showing reduction or complete rescue of neuroanatomical differences present  
368 in early ASD childhood over the course of maturation (50). Previous  
369 longitudinal research has also shown that symptom severity in individuals  
370 diagnosed with ASD in childhood decreases over time (51,52). McGovern &  
371 Sigman (52) found that 48 adolescents, who were diagnosed with ASD as  
372 children, showed marked improvement in social interaction,  
373 repetitive/stereotyped behaviours and other symptoms, with two no longer  
374 meeting criteria for ASD under ADI-R criteria, and four under ADOS criteria.  
375 This might be explained by a change in neural processing during  
376 development, which would likely affect both complex behavioural and simpler  
377 sensory outcomes.

378 One possible mechanism that would explain the developmental change is that  
379 the atypical nature of neural signalling (such as ion balance in the case of  
380 *Nhe3*), changes over time. In flies, reduced *Nhe3* expression may reduce the  
381 rate at which sodium ions and protons are exchanged across the cell  
382 membrane. At least in mosquito, this exchanger is found in the gut, and  
383 Malpighian tubules (the fly equivalent of the kidney) (53). Failure to properly  
384 regulate ionic balance in young adult flies might affect the sodium  
385 concentration, or proton levels in the body and brain, and affect the speed and  
386 intensity of action potentials. Later in life, the normal balance may be restored.  
387 A similar reduction in efficacy of *SLC9A9*, linked to ASD, may also be present  
388 and explain the homology. In this respect, we note that another transporter,  
389 the potassium/chloride exchanger, has been linked to epilepsy in young

390 people: with age the kcc/KCC2 eventually achieves a normal ionic balance  
391 and proper inhibitory GABA signalling (54).

392 The *Nhe3* model may facilitate further research on the development of ASD in  
393 young brains as well as the development of early biomarkers and treatments.  
394 Consistency between the fly and human datasets at both ages indicates a  
395 modification of a fundamental sensory mechanism comprising two  
396 components that have been conserved over 500 million years of evolution.  
397 The conservation of the phenotype and mechanisms from fly to human opens  
398 up the option to utilise the unrivaled genetic tractability of the fly to dissect the  
399 molecular mechanisms underpinning the disorder.

## 400 **Methods**

### 401 ***Drosophila* stocks**

402 Two *Drosophila melanogaster* genotypes were used as ASD models. The  
403 *Nhe3* loss-of-function P-element insertion (*Nhe3*<sup>KG08307</sup> homozygotes)  
404 mutation was homozygous *P{SUPor-P}Nhe3*<sup>KG08307</sup> (Bloomington Drosophila  
405 Stock Center (BDSC) 14715). The deficiency was Df(2L)BSC187 (BDSC  
406 9672). To avoid second site mutations in the P-element stock, we used the  
407 hemizygote *Nhe3*<sup>KG08307</sup> /Df(2L)BSC187 as a second experimental genotype.

408 For our control cross we mated the lab stock of *Canton-S* (*CS*) flies with those  
409 with isogenic chromosomes 2C and 3J (55). All tested flies had dark red eyes.  
410 All genotypes were raised in glass bottles on yeast-cornmeal-agar-sucrose  
411 medium (10g agar, 39g cornmeal, 37g yeast, 93.75g sucrose per litre).  
412 They were kept at 25°C on a 12 hour light-dark cycle. Male flies were  
413 collected on CO<sub>2</sub> the day after eclosion and placed on Carpenter (1950) (56)  
414 medium in the same environmental conditions for either 3 days or 14 days.  
415 Flies were tested approximately between the 4<sup>th</sup> and 9<sup>th</sup> hour of the daylight  
416 cycle.

### 417 ***Drosophila* electroretinography**

418 Steady-state visual evoked potentials (SSVEPs) were obtained from the fruit  
419 flies (25,31). Flies were recorded in pairs in a dark room. They were placed in  
420 small pipette tips and secured in place with nail varnish. One glass saline-  
421 filled electrode was placed inside the proboscis of the fly and another on the  
422 surface of the eye. A blue (467nm wavelength) LED light (Prizmatix FC5-LED)  
423 with a Gaussian spectral profile (FWHM 34nm) was placed in front of the flies  
424 together with a diffuser screen and used for temporal contrast stimulation.  
425 Flies were dark adapted for at least two minutes and then tested for signal  
426 quality with six light flashes. Steady-state stimulation lasted 12 min and  
427 comprised seven contrast levels (0 – 69% in linear steps) each with five  
428 repetitions. The frequency of the light flicker was 12Hz. Each trial (contrast  
429 level repetition) was 11 s. The order of the contrast conditions was  
430 randomised. The stimulation and the recording from the fly was controlled by

431 in-house MATLAB scripts (scripts can be found in  
432 <https://github.com/wadelab/flyCode>).

### 433 **Adult EEG**

434 One-hundred neurotypical adult participants (32 males, mean age 21.87,  
435 range 18 – 49, no reported diagnosis of ASD, reportedly normal or corrected  
436 to normal vision) took part in the autism spectrum quotient (AQ) measurement  
437 study. The AQ is an instrument used for quantifying autistic traits in the  
438 neurotypical population and has been shown to have high face validity and  
439 reliability in these populations (37). Due to time constraints we used an  
440 abridged version of the AQ questionnaire which consists of 28 questions  
441 rather than the typical 50 (AQ-Short, (57)). Scores were then scaled to fit the  
442 conventional AQ scale. Each participant completed the AQ questionnaire on a  
443 computer in the laboratory. The participants were then median split (median =  
444 14) into high and low AQ groups.

445 For the autistic adult ssVEP study, 12 typical-IQ autistic participants and 12  
446 gender- and age- matched controls (11 males, mean age 23.53, range 18 –  
447 39, reportedly normal or corrected to normal vision) took part. ASD diagnosis  
448 was confirmed with the Autism Diagnostic Observation Schedule, second  
449 edition (ADOS-2). Although IQ was not explicitly measured in this study, all  
450 adults had normal speech and a high level of independence (the majority  
451 were university students). The absence of ASD diagnosis in the neurotypical  
452 participants was also confirmed with ADOS-2 (none of the control participants  
453 met criteria for ASD). All participants in the study gave informed consent and  
454 were debriefed on the purpose of the study after the experiment. The  
455 experiments were approved by the Department of Psychology Ethics  
456 Committee at the University of York.

457 Steady-state VEPs were recorded using an ANT Neuro system with a 64-  
458 channel Waveguard cap. EEG data were acquired at 1kHz and were recorded  
459 using ASALab, with stimuli presented using MATLAB. The timing of the  
460 recording and the stimulation was synchronised using 8-bit low-latency digital  
461 triggers. All sessions were performed in a darkened room, testing lasted 45-  
462 60min with approximately 20min set up time.

463 Stimuli were presented on a ViewPixx display (VPixx Technologies Inc.,  
464 Quebec, Canada) with a mean luminance of 51cd/m<sup>2</sup> and a refresh rate of  
465 120Hz. Stimuli were 0.5 cycle/deg sine-wave gratings enveloped by a raised  
466 cosine envelope. Gratings subtended 3 degrees of visual angle and were tiled  
467 in a 17x9 grid. The participants fixated on a circle in the middle of the screen  
468 and performed a fixation task (two-interval-forced-choice contrast  
469 discrimination) to maintain attention. All participants were able to perform the  
470 task at above chance levels. There were seven contrast conditions for the  
471 flickering gratings (0%, and 2 - 64% in logarithmic steps, where  $C_{\%} =$   
472  $100(L_{max}-L_{min})/(L_{max}+L_{min})$ ,  $L$  is luminance) and eight repetitions. Stimuli  
473 flickered on/off sinusoidally at 7Hz. Trials were presented in random order in  
474 four testing blocks with short breaks in between. Each trial was 11 seconds  
475 long and contained gratings of a random spatial orientation to avoid

476 orientation adaptation effects. These trials were intermixed with orthogonal  
477 masking trials that are not presented as part of this study. Data were taken  
478 from the occipital electrode Oz.

#### 479 **Child EEG**

480 Thirteen children with a diagnosis of ASD and 20 neurotypical controls  
481 matched on gender ratio (10 and 12 males respectively) and average age  
482 (mean age 9.31 and 8.94 respectively, range 5 – 13) completed the study.  
483 Three of the neurotypical children were tested but excluded due to having  
484 autistic siblings (17 participants were included). All children were in  
485 mainstream local schools (if they were old enough) and did not have other (or  
486 any – in the case of the neurotypical group) reported history of serious  
487 medical, psychiatric, or neurological conditions.

488 Steady-state EEG data were acquired with a 128-channel HydroCell  
489 Geodesic Sensor Net (Electrical Geodesics Inc.). Data were digitised at  
490 432Hz and band-pass filtered from 0.3Hz to 50Hz and were recorded using  
491 NetStation 4.3 Software. Highly noisy data were excluded by removing  
492 repetitions with amplitudes that were four standard deviations away from the  
493 group mean (for each contrast level and harmonic individually). There were 10  
494 repetitions in total, however, two autistic and one neurotypical child only  
495 completed 8 repetitions.

496 Increasing contrast sweep ssVEPs were used. Stimuli for this experiment  
497 were presented on an HP1320 CRT monitor with 800x600 pixel resolution,  
498 72Hz refresh rate and mean luminance of 50cd/m<sup>2</sup>. Stimuli were random  
499 binary noise patterns of two luminance levels that increased in contrast in 9  
500 logarithmic steps (0% – 50%) of 1 second each. Each trial contained a  
501 prelude at the initial value of the sweep and a postlude at the final sweep  
502 value, lasting 12 seconds in total. Stimuli flickered at 5.12Hz. Data from the  
503 middle 9 seconds during the sweep were binned according to contrast steps.  
504 Methodological differences between the adult and child datasets were due to  
505 different conventions being used by the two laboratories in which data were  
506 collected.

#### 507 **Data analysis**

508 A Fast Fourier transform (in MATLAB) was used to retrieve steady-state  
509 response amplitudes at the stimulation frequency (12Hz for fruit flies, 7Hz for  
510 adult participants and 5.12Hz for children) and at the second harmonic (24Hz,  
511 14Hz and 10.24Hz respectively). Fourier transforms were applied to 10 s of  
512 each trial (first 1s discarded; total trial length was 11s) for the fruit fly and the  
513 adult participant datasets and to 1 second binned data for the children's  
514 dataset. Contrast response functions were obtained by coherently averaging  
515 the amplitudes over repetitions for each contrast level within a participant.  
516 Group/genotype scalar means over response amplitude (discarding phase  
517 angle) were then calculated for each contrast across participants/flies.



518 Two-way (harmonic x group) ANOVAs were performed on amplitudes at the  
519 highest contrast level to investigate the interactions and group effects in all  
520 human datasets where only two groups were compared. To identify at which  
521 harmonic the autistic children showed a decreased response, two  
522 independent samples t-tests were also conducted. One-way ANOVAs with  
523 simple planned contrasts were conducted to assess the genotype differences  
524 in fruit fly first and second harmonic responses separately as that aided the  
525 interpretability of the results between the three genotypes.

526 To investigate the dissociation between first and second harmonic responses  
527 a scaled ratio  $\frac{1F-2F}{1F+2F}$  (where 1F is the first and 2F is the second harmonic) was  
528 calculated for each participant/fly and each contrast condition. To increase the  
529 power of statistical analyses and to decrease the type I error rate, the ratios  
530 were then averaged over the contrast conditions that had first harmonic  
531 amplitudes significantly above the baseline response (0% contrast condition).  
532 For fruit flies this was six conditions (11.5 – 69%), for adult participants this  
533 was four conditions (8 – 64%) and for children this was five conditions (8.5 –  
534 50%). This procedure resulted in a single frequency-ratio index for each  
535 participant/fly. One-way ANOVAs with simple planned contrasts (comparing  
536 mutant genotypes with the control genotype) were conducted on the fly  
537 frequency ratios for each age separately. Independent t-tests were used to  
538 compare frequency ratios in all human datasets between groups. Additionally,  
539 a linear regression was conducted on the adult AQ measurement dataset to  
540 assess the predictive power of AQ scores on the ratios between frequencies.  
541 All statistical tests were two-tailed.

## 542 **Competing interests**

543 Authors have no competing interests.

## 544 **Authors' contributions**

545 All authors contributed to conceiving and designing of experiments; G.V., F.P.  
546 collected data; G.V., D.H.B., A.M.N. performed statistical analyses; G.V.,  
547 A.R.W., A.M.N., D.H.B. interpreted the results with contributions from all  
548 authors; G.V. wrote the manuscript with A.M.N, D.H.B. and A.R.W. with input  
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