Autism sensory dysfunction in an evolutionarily conserved system

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Key words

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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.

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Introduction

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

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

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

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

3

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*^{KG08307}) and *Nhe3* hemizygotes *(Nhe3*^{KG08307}/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 1st harmonic response (which follows the
103　stimulation frequency – 12Hz), as well a 2nd 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 1st harmonic to photoreceptors and the 2nd 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.

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*).

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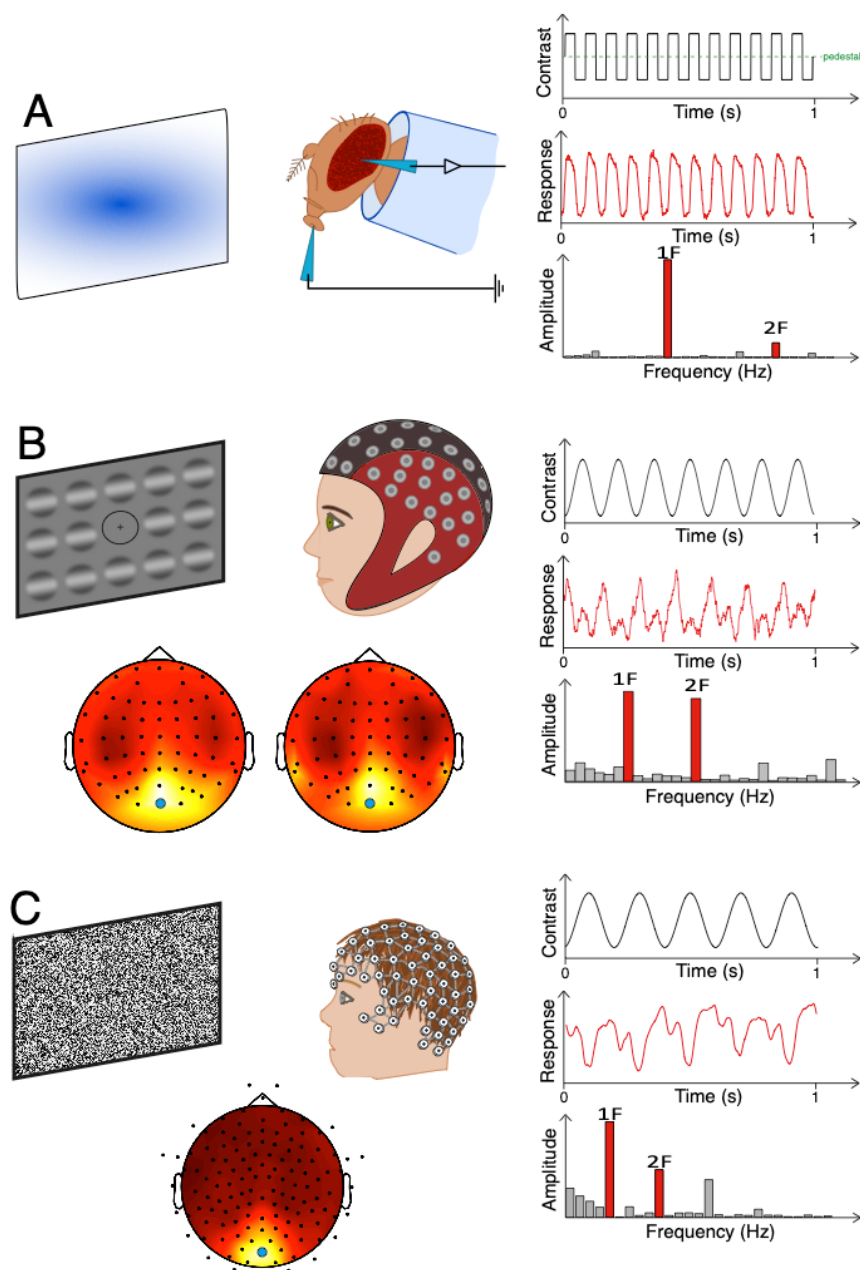
Fig 1. Human and *Drosophila* steady-state electrophysiology methods.
Panel A left illustrates the experimental set up for fruit fly electrophysiology
(see *Drosophila electroretinography* for more details). Panel A right shows the
square wave stimulus trace flickering at 12Hz (top), example
electrophysiological responses over time (middle) and Fourier-transformed

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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 1st 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.


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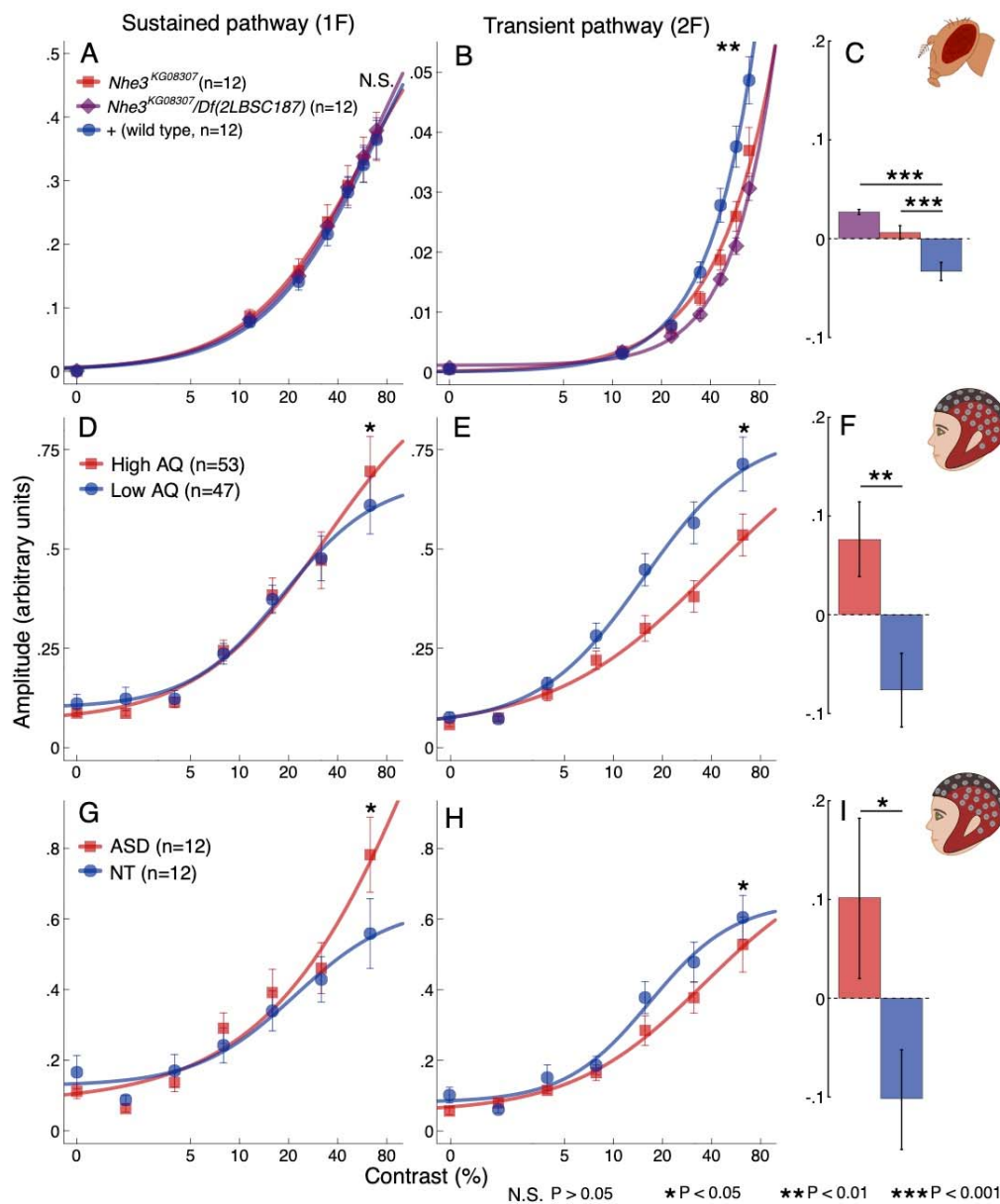
Fig 2. Older ASD-mimic flies and autistic humans show reduced visual responses in the transient component. Contrast response functions for adult *Nhe3* mutant flies (*Nhe3*^{KG08307} homozygotes, red squares and

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158   *Nhe3^{KG08307} /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 ±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-receptoral
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.
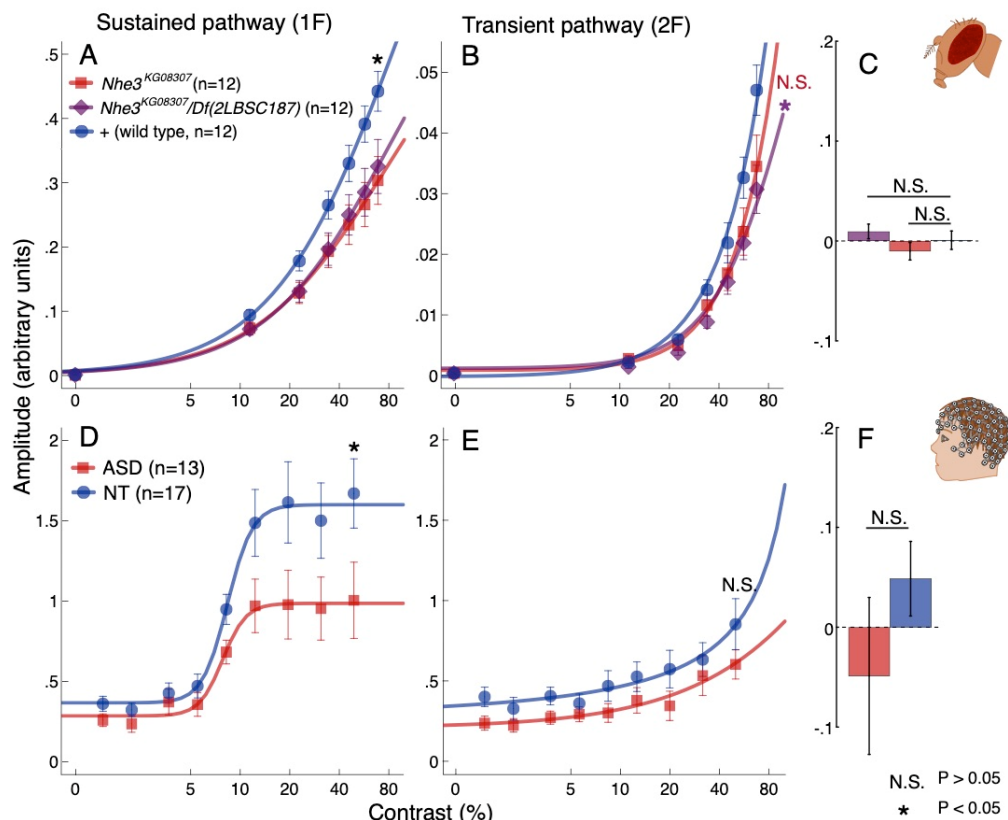
Fig 3. Young ASD-mimic flies and autistic children show reduced visual responses in the sustained component. Young fruit flies showed reduced responses at the first harmonic ($F_{2,33}$ = 3.73, P = 0.035; panel A) with P/P and P/Df flies showing a significant difference from control flies (respectively, P = 0.016 and P = 0.040). There was also a significant effect of genotype at the second harmonic ($F_{2,33}$ = 3.39, P = 0.046, panel B). P/Df flies showed a significant difference from control flies (P = 0.018), however, P/P showed a non-significant difference from controls (P = 0.064). The flies had normal frequency ratios (panel C). Autistic children also showed reduced first harmonic (t_{28} = 2.065, P = 0.048; panel D) but not second harmonic responses (t_{28} = 1.26, P = 0.22; panel E) and had frequency ratios similar to that of control children (t_{28} = 1.21, P = 0.24; panel F). Curved lines are hyperbolic function fits to the data. Frequency ratios are baselined in respect to the mean over groups of each comparison for display purposes. Error bars in all panels represent ±SEM.

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

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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.
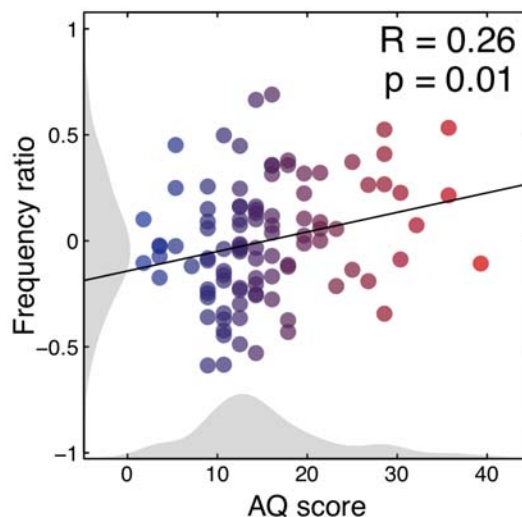
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234   **Fig 4. Positive relationship between the number of autistic traits and**
235   **first/second harmonic ratio.** Scatterplot showing a significant positive
236   relationship between AQ scores and frequency ratios in the 100 neurotypical
237   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 1st/2nd 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.

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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 2nd harmonic responses could either be generated by
320 disturbances in non-linear transduction of visual signals or by subsequent
321 temporal processing. As the 2nd 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 1st 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 2nd harmonic would now equal
331 the current 1st 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 2nd
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.

Methods

400

Drosophila stocks

401

402　Two *Drosophila melanogaster* genotypes were used as ASD models. The
403　*Nhe3* loss-of-function P-element insertion (*Nhe3*^{KG08307} homozygotes)
404　mutation was homozygous *P{SUPor-P}Nhe3*^{KG08307} (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*^{KG08307} */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_2 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 4th and 9th hour of the daylight
416　cycle.

Drosophila electroretinography

417

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).

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^2 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

14

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^2. 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.

Competing interests

543 Authors have no competing interests.

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
549 from all authors.

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