Development of the pupillary light reflex from 9 to 24 months: association with common autism spectrum disorder (ASD) genetic liability and 3-year ASD diagnosis

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Background: Although autism spectrum disorder (ASD) is heritable, the mechanisms through which genes contribute to symptom emergence remain unclear. Investigating candidate intermediate phenotypes such as the pupillary light reflex (PLR) prospectively from early in development could bridge genotype and behavioural phenotype. **Methods:** Using eye tracking, we longitudinally measured the PLR at 9, 14 and 24 months in a sample of infants (N = 264) enriched for a family history of ASD; 27 infants received an ASD diagnosis at 3 years. We examined the 9- to 24-month developmental trajectories of PLR constriction latency (onset; ms) and amplitude (%) and explored their relation to categorical 3-year ASD outcome, polygenic liability for ASD and dimensional 3-year social affect (SA) and repetitive/restrictive behaviour (RRB) traits. Polygenic scores for ASD (PGS_{ASD}) were calculated for 190 infants. **Results:** While infants showed a decrease in latency between 9 and 14 months, higher PGS_{ASD} was associated with a smaller decrease in latency in the first year (β = −.16, 95% CI = −0.31, −0.002); infants with later ASD showed a significantly steeper decrease in latency (a putative 'catch-up') between 14 and 24 months relative to those with other outcomes (typical: β = .54, 95% CI = 0.08, 0.99; other: β = .53, 95% CI = 0.02, 1.04). Latency development did not associate with later dimensional variation in ASD-related traits. In contrast, change in amplitude was not related to categorical ASD or genetics, but decreasing 9- to 14-month amplitude was associated with higher SA (β = .08, 95% CI = 0.01, 0.14) and RRB (β = .05, 95% CI = 0.004, 0.11) traits. **Conclusions:** These findings corroborate PLR development as possible intermediate phenotypes being linked to both genetic liability and phenotypic outcomes. Future work should incorporate alternative measures (e.g. functionally informed structural and genetic measures) to test whether distinct neural mechanisms underpin PLR alterations. **Keywords:** Autism spectrum disorder; neurodevelopment; infancy; pupillary light reflex.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised by social communication impairments, repetitive/restricted behaviours, and sensory anomalies (American Psychiatric Association, 2013). ASD is not typically diagnosed until behavioural symptoms become clear at 2–3 years (Ozonoff et al., 2015). Underpinning the gradual emergence of recognisable ASD symptoms are environmental and genetic factors (Bai et al., 2019), hypothesised to funnel onto a set of common mechanisms critical in early development (Jones, Gliga,

Bedford, Charman, & Johnson, 2014). Measuring early intermediate phenotypes using longitudinal prospective studies with infants with a family history of ASD (who have a 10%–20% likelihood of developing ASD; Ozonoff et al., 2011) may reveal these early mechanisms.

One candidate intermediate phenotype is the pupillary light reflex (PLR), the reflexual pupil constriction in response to increased optical luminance (Daluwatte, Miles, Sun, & Yao, 2015). A simple four-neuron ocular-motor circuit mediates this reflex (McDougal & Gamlin, 2015) and is innervated by a range of brain regions [e.g. prefrontal cortex (Becket Ebitz & Moore, 2017), cerebellum (Ijichi, Kiyohara, Hosoba, & Tsukahara, 1977), and locus coeruleus

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(Bast, Poustka, & Freitag, 2018; Lynch, 2018)]. Two measurable components of the PLR can be linked to distinct mechanisms. PLR latency (constriction onset time) is likely regulated by neural signal transduction efficiency (Dinalankara, Miles, Takahashi, & Yao, 2017), whereas PLR amplitude is highly, but not exclusively, dependent on cholinergic and norepinephrine neurotransmission efficiency (Heller, Perry, Jewett, & Levine, 1990; Loewenfeld, 1999; Lynch, 2018; de Vries, Fouquaet, Boets, Naulaers, & Steyaert, 2021) – neural processes implicated in early ASD (Abreu-Villaça, Filgueiras, & Manhães, 2011; Ben-Bashat et al., 2007). Indeed, preliminary evidence indicates altered PLR during infancy associates with later ASD, although results are mixed. As a group, infants with ASD family history may show smaller constriction but no differences in latency (Kercher et al., 2020); others have reported faster latency relative to controls at 9 months (Nyström, Gredebäck, Bölte, & Falck-Ytter, 2015). Prospectively, PLR amplitude at 9 months is larger in infants with versus without later ASD and positively correlates with symptom severity (Nyström et al., 2018). One step forward is to study developmental trajectories rather than static cross-sectional points, as has proved informative for other putative ASD markers (e.g. Elsabbagh et al., 2013; Jones & Klin, 2013). Both PLR latency and amplitude change over early development, with Kercher et al. (2020) reporting latency decreased (becomes faster) from 6 to 24 months while amplitude increased (becomes stronger); ASD family history did not alter these developmental trajectories. However, Nyström et al. (2018) demonstrated amplitude increased from 9 to 14 months in infants with typical development but decreased in those with later ASD – no analysis was reported on latency development. These findings highlight PLR development as potentially fruitful in understanding early ASD development.

High heritability estimates indicate strong genetic influences on ASD (Tick, Bolton, Happé, Rutter, & Rijsdijk, 2016), which includes the additive loading of many common small effect variants (Bai et al., 2019). Investigating associations between developing candidate intermediate phenotypes like infant PLR and individual polygenic scores for ASD (PGS_{ASD}) – weighted sums of ASD-associated common genetic variants (Euesden, Lewis, & O'Reilly, 2015) – could highlight developmental pathways through which common genetic liability manifests into later symptomology. To date, the relation between infant PLR and individual PGS_{ASD} has not been investigated (de Vries et al., 2021).

In the present study, we measured PLR longitudinally at 9, 14, and 24 months from 264 individuals, 139 of which overlapped with Nyström and colleagues' (2018) sample. We aimed to expand on previous work by exploring the early developmental trajectory of the PLR amplitude and latency across

the first and second postnatal years in infants with and without a family history of ASD. Specifically, we tested to what degree the change in mean (a) latency and (b) amplitude, from 9 to 14 months and 14 to 24 months, was associated with: (1) 3-year ASD outcome, (2) PGS_{ASD}, and (3) 3-year dimensional (a) social affect and (b) repetitive behaviours. We predicted amplitude development would significantly associate with categorical ASD outcome, polygenic liability, and dimensional ASD traits; we could not make specific predictions regarding latency developmental changes pertaining to 3-year ASD outcome, PGS_{ASD}, and 3-year dimensional ASD traits (social affect and repetitive behaviours) due to limited previous work.

Methodology
Sample

Participants ($N = 264$) were recruited into the British Autism Study of Infant Siblings (BASIS, $N = 143$) or Studying Autism and Attention Deficit Hyperactivity Disorder Risks programme (STAARS, $N = 121$). Infants had either an older full sibling with a community ASD diagnosis ($N = 195$) or no ASD family history ($N = 69$); 139 of these infants (54.72%) were included in Nyström et al. (2018). Infants were born full-term (36+ weeks) and had no ASD-associated genetic syndrome nor visual, auditory or other disability. NHS Research Ethics Committees granted ethical approval (06/MRE02/73, 08/H0718/76 [BASIS] and 13/LO/0751 [STAARS], 15/LO/0468 [DNA collection, extraction and analysis]). Informed written consent was provided by the parent(s).

Measures and procedure

Pupillary light reflex (PLR). The PLR was induced by one of two stimuli that transitioned from a black to white slide (see Figure 1A–C). For the BASIS cohort, stimulus one (Gliga et al., 2015) was presented 32 times at 9/14 months and 16 times at 24 months; a Tobii T120 eye tracker (sampling rate = 60 Hz; Tobii Technology AB, Danderyd, Sweden) measured pupil diameter (millimetres to 2 decimal places). Following previous work (Nyström et al., 2018), to control for differing PLR inducing light levels across the white slide in stimulus one, only trials where the first look was within the centre 5% of the white slide (see Figure 1B) were included. Stimulus two was presented to the STAARS cohort 12 times at all timepoints; a Tobii TX300 eye tracker (sampling rate = 120 Hz; Tobii Technology AB, Danderyd, Sweden) measured pupil size (millimetres to 14 decimal places). Stimuli were interspersed throughout a longer eye-tracking battery. Ambient room lighting and testing setup were held constant within each protocol. Infants passively watched the

stimuli monitor while sat on their caregiver's lap at an average distance of 60.61 cm (59.67 cm–61.81 cm). Differences across protocols were accounted for in the statistical models via random effects (see below).

We preprocessed raw pupil data using a processing pipeline similar to Nyström and colleagues (2018; see Appendix S1 for further details). This removed artefacts and extract PLR latency (constriction onset time; ms) and amplitude (maximum constriction amplitude relative to average pupil size before latency; %). Latency was defined as the minimum acceleration from 110–570ms after white slide onset. Amplitude was calculated using the Fan, Miles, Takahashi, and Yao (2009) formula (Figure 1C) such that higher numbers represent greater constriction. All trials were visually inspected for validity. Infants required three or more valid trials per timepoint for inclusion; percentage of missing PLR trials (Missingness; see Appendix S1: Table S1 and Figure S1) was included as a covariate in all analyses. The median latency and amplitude per individual per timepoint were calculated and included in models as dependent variables.

Clinical diagnosis at 3 years. Experienced clinical researchers ascertained consensus 3-year DSM-5 ASD diagnosis (American Psychiatric Association, 2013; see Appendix S2 for more details). Those who did not reach criterion for ASD diagnosis but had above threshold Autism Diagnostic Observational Schedule (ADOS; Gotham, Pickles, & Lord, 2009; Lord et al., 2000, 2012) and/or The Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, Le Couteur, & Free Hospital, 1994) scores or low (<−1.5 SD) scores on one or more of the Mullen Scales of Early Learning (MSEL; Mullen, 1995) subscales were categorised as 'Other' at 3 years.

Dimensional traits at 3 years. ADOS-G (BASIS; Lord et al., 2000) and ADOS-2 (STAARS; Lord et al., 2012) Calibrated Severity Score (ADOS-CSS; Gotham et al., 2009) Social Affect (SA) and Repetitive/Restricted Behaviour (RRB) scores operationalised 3-year dimensional domain-specific ASD traits (Appendix S2 and Table S2 for summary). Higher scores indicated heightened trait levels.

Polygenic scores for ASD (PGS$_{ASD}$). A total of 190 infants had PGS$_{ASD}$ and PLR data for at least one timepoint. PGS (an aggregate of trait-related effect sizes of single nucleotide polymorphisms [SNPs] distributed throughout the genome derived from independent genome-wide association studies) was constructed for infants and other family members using PRSice-2 (Choi & O'Reilly, 2019). The full sample and methods are fully described in Harrison et al. (under review; see Appendix S3). SNP linkage
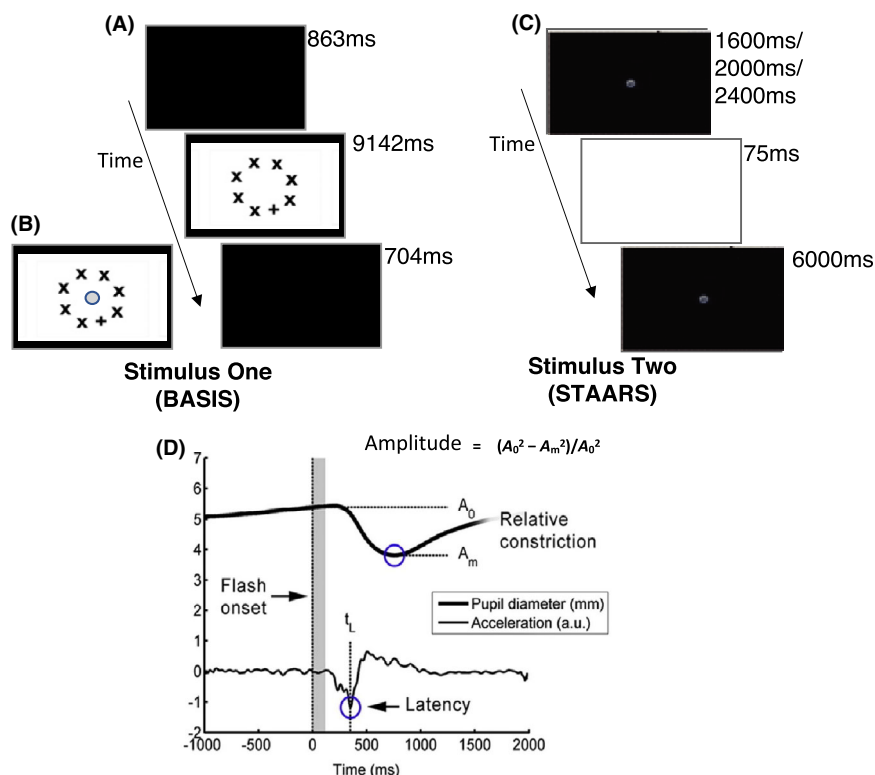

Figure 1 (A) Stimulus one presented to the BASIS cohort (Gliga et al., 2015) alongside, (B) stimulus one's PLR inducing stimuli slide demonstrating the approximate location of the centre area of interest (centre ~5% of white slide). (C) Stimulus two presented to the STAARS cohort. (D) Pupil size (mm) and acceleration (a.u.) traces demonstrating relative constriction formula components (Hellmer & Nyström's, 2017). *A*0 = average pupil diameter in the window 100 ms before constriction onset (latency). *A*m = minimum pupil diameter in 170–1450 ms relative to *A*0. ms = milliseconds

disequilibrium pruning based on *p*-value informed clumping (pairwise LD $r^2 < .1$ within 250 kb of the index SNP) was performed on the target dataset. Two principal components calculated with the PC-AiR function of the 'GENESIS' R-package (Gogarten et al., 2019) were included as covariates to account for relatedness in the sample (kinship coefficient threshold for relatedness = 0.125). For this study, we calculated a high-resolution best-fit PGS$_{ASD}$ using an independent sample's GWAS summary statistics (Grove et al., 2019; 18,381 cases, 27,969 controls, SNP heritability = 0.118) and target phenotype of later ASD/No ASD. The best-fit PGS$_{ASD}$ was obtained at a GWAS *p*-value threshold of .021 (Nagelkerke's $R^2 = 0.023$, $p = .0042$, 8,294 SNPs) and standardised ($M = 0$, $SD = 1$). See Figure S2 for final best-fit PGS$_{ASD}$ across 3-year outcome group.

Statistical analysis

In total, we conducted 8 separate linear multilevel models to examine mean changes in PLR amplitude and latency between the model reference timepoint (14 months) and earlier/later timepoints (9/24 months) depending on (1) 3-year ASD outcome [ASD, Typical Development (TD) or Other], (2) PGS$_{ASD,}$ and (3) 3-year ASD domain trait level [ADOS-CSS (a) SA and (b) RRB]. We followed the experiment-wise procedure of multiple testing correction whereby all tests leading to the same conclusion should be corrected for multiplicity (Bender & Lange, 2001). Therefore, we did not apply multiple comparison corrections to the models as each model asked a specific question in which a specific conclusion would be drawn (results alongside Bonferroni corrected confidence intervals, and *p*-values for all models are reported in relevant appendices referenced below).

We included random intercepts of participant (to account for within-subject correlated variance) and protocol (to account for different stimuli, eye tracker and sampling rate used across cohorts). The percentage of missing trials (Missingness, see Appendix S1) and baseline pupil size [the average pupil diameter in the 100 ms window before PLR latency (i.e. Constriction onset time)] were covariates in all models. Mean distance from the screen was considered as a covariate, though did not alter results (referenced in relevant appendices). Final model specifications and the sample size for each model are reported in Table 1. Models were implemented using scaled continuous variables for comparable coefficients across models in R (R Development Core Team, 2011); model construction: 'lmerTest' R-package (Kuznetsova, Brockhoff, & Christensen, 2017); confidence intervals: 'lme4' R-package (Bates, Mächler, Bolker, & Walker, 2015); continuous variable scaling: 'base' R-package *scale*() function (R Development Core Team, 2011). Restricted estimated maximum likelihood was used

to handle missing data, and unstructured variance-covariance matrix was specified. Assumptions were checked through visual evaluation of residuals.

Results
Sample

A sample breakdown and characteristics are displayed in Table 2 (and Table S3). See Appendix S4 (including Tables S4 and S5) for comparisons of sample characteristics across 3-year outcome.

ASD outcome

Separate linear multilevel models (LMM) with random intercepts (participant and protocol) were conducted to explore associations between 3-year ASD outcome and 9- to 14-/14- to 24-month changes in amplitude ($N = 247$) and latency ($N = 252$). Figure 2 displays unstandardised estimated marginal means (EMMs) over visit timepoints. Full reports of fixed and random effect estimates and pairwise comparisons are presented in Appendix S5: Tables S6–S14.

Table 1 Model specifications for each model displaying model predictor of interest, dependent variable, model syntax and sample size (overall and divided by 3-year outcome group)

Model predictor	Dependent variable	Model syntax	N (3-year outcome)
Outcome	Amplitude	PLR ~ ASD Outcome[a] + Visit[b] + ASD Outcome[a]*Visit[b] + Likelihood[c]** +	247 (ASD = 27, Other = 49, Typical = 171)
	Latency	Missingness + Baseline Pupil Size + (1|Participant) + (1|Protocol)	252 (ASD = 27, Other = 50, Typical =175)
PGS$_{ASD}$	Amplitude	PLR ~ PGS$_{ASD}$ + Visit[b] +	187 (ASD = 19)
	Latency	PGS$_{ASD}$*Visit[b] + Missingness + Baseline Pupil Size + (1|Participant) + (1|Protocol)	190 (ASD = 19)
Dimensional Traits (ADOS SA or RRB)	Amplitude	PLR ~ADOS + Visit[b] + ADOS-2-CSS*Visit[b] +	243 (ASD = 26***)
	Latency	Missingness + Baseline Pupil Size + (1|Participant) + (1|Protocol)	248 (ASD = 26***)

(1\|Variable) = random intercept. Superscript letters denote the reference category in the model for the relevant fixed effect variable: [a]ASD; [b]14 month; [c]Elevated Likelihood.
*Interaction.
**Likelihood added as covariate.
***See Appendix S2 Note A.

Table 2 Sample size and characteristics describing, mean age, Mullen Early Learning Composite score (M-ELC) across 3-year outcome group for each visit timepoint for individuals with PLR latency data

Likelihood (outcome)	Total N (m)	9 months			14 months			24 months		
		N (m)	Age M (SD)	M-ELC M (SD)	N (m)	Age M (SD)	M-ELC M (SD)	N (m)	Age M (SD)	M-ELC
TL (TD)	64 (35)	45 (22)	9.93 (1.04)	101.64 (17.09)	49 (26)	15.04 (0.82)	92.81 (16.9)	57 (31)	25.28 (1.09)	113.37 (17.85)
EL (TD)	111 (51)	91 (41)	9.52 (1.04)	103.78 (17.37)	93 (43)	15.18 (0.94)	90.26 (15.54)	86 (38)	26.07 (1.6)	103.45 (17.39)
EL (O)	50 (28)	40 (22)	9.37 (0.97)	96.95 (16.91)	42 (25)	15.12 (0.94)	86.83 (16.48)	43 (23)	25.6 (1.66)	94.92 (22.48)
EL (ASD)	27 (22)	22 (18)	9.24 (1.1)	100.05 (15.82)	19 (14)	14.97 (0.94)	79.11 (12.31)	23 (19)	25.81 (2.19)	79.36 (19.14)
Other*	12 (7)	7 (4)	10.03 (1.11)	95.71 (13.01)	5 (2)	14.94 (0.89)	81.8 (12.07)	7 (4)	25.46 (2.08)	102.86 (17.93)

Likelihood: TL, Typical; EL, Elevated. Outcome: TD, Typical; O, Other. M, Mean; (m), male; M-ELC, Mullen Early Learning Composite score (see Appendix S2); SD, standard deviation.
Other* participants withdrew before 3-year assessment, but were included in the PGS analysis as typically developing due to 24-month assessment (Appendix S3).

Amplitude. Though EMMs indicate the direction of 9- to 14-month amplitude change to differ across outcome (9- and 14-month unstandardised EMMs, respectfully, for: ASD = 37.52%, 35.53%; typical = 35.95%, 37.64%; other = 35.34%, 35.73%), interaction estimates revealed no significant difference in these changes between those with ASD and other ($\beta = -.27$, $SE = 0.25$, 95% CI = -0.77, 0.22) or typical ($\beta = -.41$, $SE = 0.22$, 95% CI = -0.85, 0.02) development.

Across the cohort, EMMs indicate a decreasing 14- to 24-month amplitude change (14- and 24-month unstandardised EMMs, respectfully, for: ASD = 35.53%, 28.11%; typical = 37.64%, 31.89%; other = 35.73%, 30.50%). Between 14 and 24 months, amplitude significantly decreased for those with ASD ($\beta = -.85$, $SE = 0.21$, 95% CI = -1.25, -0.44), a change that did not significantly differ from those with other ($\beta = .25$, $SE = 0.25$, 95% CI = -0.25, 0.75) or typical ($\beta = .17$, $SE = 0.22$, 95% CI = -0.26, 0.61) development.

Latency. Across the cohort, EMMs indicate a small decreasing pattern of latency change between 9 and 14 months (9- and 14-month unstandardised EMMs, respectfully, for: ASD = 339.69 ms, 334.35 ms; typical = 331.52 ms, 323.70 ms; other = 337.81 ms, 325.47 ms). For those with ASD, this decrease was not significant ($\beta = .15$, $SE = 0.26$, 95% CI = -0.21, 0.78) and was not significantly different from the 9- to 14-month change in the Other ($\beta = .20$, $SE = 0.26$, 95% CI = -0.32, 0.71), or Typical ($\beta = .07$, $SE = 0.23$, 95% CI = -0.38, 0.52) development groups.

Across the cohort, EMMs indicate a decreasing pattern of latency change between 14 and 24 months which was much larger for those with ASD (14- and 24-month unstandardised EMMs, respectfully, for: ASD = 334.35 ms, 312.30 ms; typical = 323.70 ms, 320.78 ms; other = 325.47 ms, 322.29 ms). Between 14 and 24 months, latency decreased significantly more in infants with later ASD ($\beta = -.62$, $SE = 0.22$, 95% CI = -1.04, -0.19) compared to those with typical ($\beta = .54$, $SE = 0.23$, 95% CI = 0.08, 0.99) or other ($\beta = .53$, $SE = 0.26$, 95% CI = 0.02, 1.04) development. Pairwise comparisons revealed no significant differences in latency between 14 and 24 months for typical ($\beta = -.11$, 97.5% CI = -0.33, 0.11) or other ($\beta = -.15$, 97.5% CI = -0.56, 0.25) development.

ASD polygenic score

Separate LMMs (with participant and protocol random intercepts) were conducted to explore associations between PGS_{ASD} and 9- to 14-/14- 24-month amplitude ($N = 187$) and latency ($N = 190$) development. Figure 3 displays LMM interactions plots. Full fixed and random effect estimates lists are reported in Appendix S5: Table S15–S20.

Amplitude. No significant associations were reported between PGS_{ASD} and amplitude change (9 to
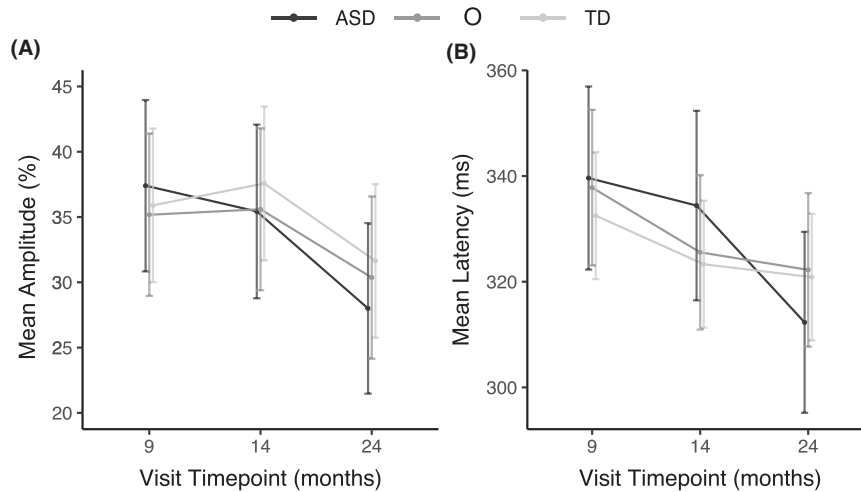
Figure 2 Association of 3-year ASD outcome with mean PLR (A) amplitude (%) and (B) latency (ms) across visit timepoints. ASD, ASD outcome; ms, milliseconds; O, Other Development; TD, Typical Development. Error bars = *SE*
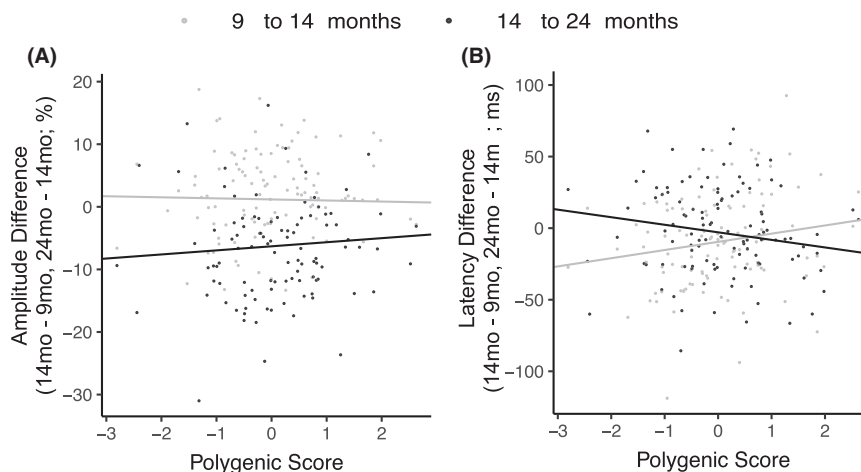

Figure 3 Mean difference scores from 9 to 14 months and 14 to 24 months against PGSASD for PLR (A) amplitude (%) and (B) PLR latency (ms). Lines = unstandardised interaction estimates obtained with LMM

14 months: $\beta = .02$, $SE = 0.08$, 95% CI = $-0.14, 0.18$; 14 to 24 months: $\beta = .07$, $SE = 0.08$, 95% CI = $-0.09, 0.23$).

Latency. Within the subset with PGS scores available, latency significantly decreased between 9 and 14 months ($\beta = .27$, $SE = 0.08$, 95% CI = $0.11, 0.43$). Higher PGS$_{ASD}$ was associated with a smaller decrease in latency between 9 and 14 months ($\beta = -.16$, $SE = 0.08$, 95% CI = $-0.31, -0.002$). Within this subset of infants, latency did not significantly change between 14 and 24 months ($\beta = -.08$, $SE = 0.08$, 95% CI = $-0.23, 0.08$); PGS$_{ASD}$ was not significantly associated with individual differences in change profile (though effect sizes were similar to those at 9- to 14-month PGS-associated change, $\beta = -.14$, $SE = 0.08$, 95% CI = $-0.29, 0.01$).

ASD dimensional domain traits

Separate LMMs (with participant and protocol random intercepts) were conducted to explore associations between 3-year ADOS-CSS (social affect [SA]

and repetitive behaviours [RRB]) and 9- to 24-month amplitude ($N = 243$) and latency ($N = 248$) development. Figure 4 displays LMM interactions plots. Full fixed and random effect estimates lists are reported in Appendix S5: Table S21–S26.

Amplitude. Overall, amplitude became larger from 9 to 14 months (SA: $\beta = -.32$, $SE = 0.11$, 95% CI = $-0.54, -0.10$; RRB: $\beta = -.32$, $SE = 0.13$, 95% CI = $-0.57, -0.07$); less change significantly associated with higher ADOS-CSS scores (SA: $\beta = .08$, $SE = 0.03$, 95% CI = $0.01, 0.14$; RRB: $\beta = .05$, $SE = 0.03$, 95% CI = $0.004, 0.11$). Over the cohort, amplitude significantly decreased from 14 to 24 months (SA: $\beta = -.81$, $SE = 0.11$, 95% CI = $-1.03, -0.58$; RRB: $\beta = -.61$, $SE = 0.13$, 95% CI = $-0.86, -0.35$); ADOS-CSS scores did not associate with this change (SA: $\beta = .60$, $SE = 0.03$, 95% CI = $-0.01, 0.12$; RRB: $\beta = -.01$, $SE = 0.03$, 95% CI = $-0.07, 0.04$).

Latency. Latency did not change between 9 and 14 months (SA: $\beta = .19$, $SE = 0.11$, 95% CI = -0.03,
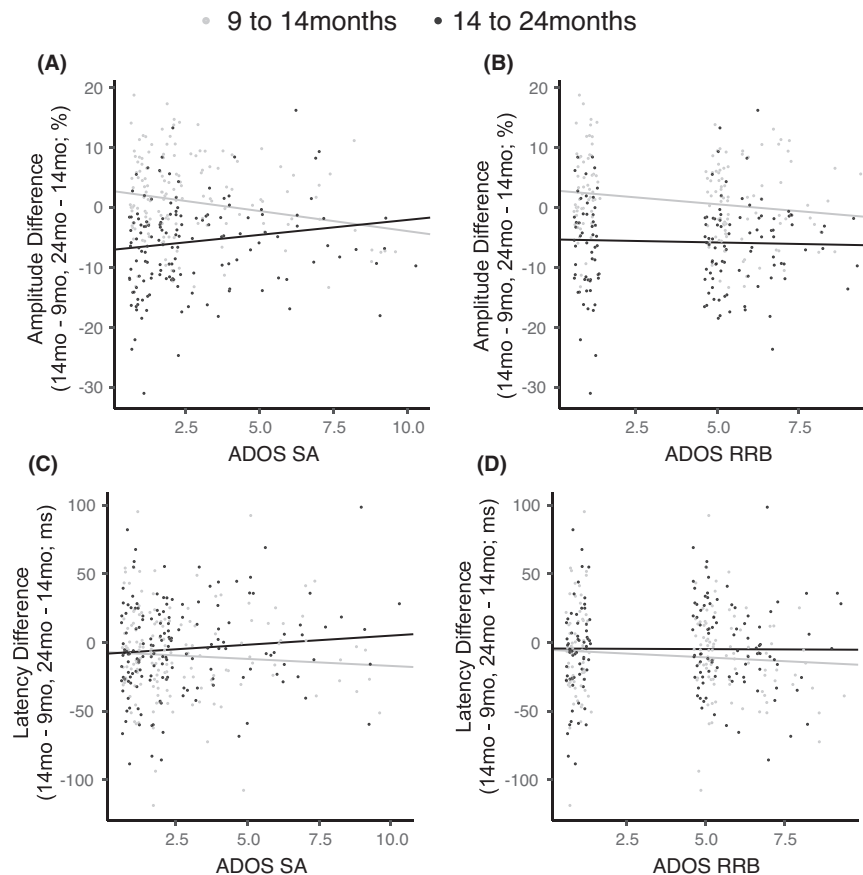
Figure 4 Mean PLR differences from 9 to 14 months and 14 to 24 months against 3-year ADOS severity scores. Mean PLR amplitude difference (%) against (A) social affect and (B) repetitive/restricted behaviours. Mean PLR latency difference (ms) against (C) Social Affect and (D) Repetitive/Restricted Behaviours. RRB, Repetitive/Restricted Behaviours; SA, Social Affect. Lines = unstandardised interaction estimates obtained with LMM

0.42; RRB: $\beta = .15$, $SE = 0.13$, 95% CI = -0.10, 0.40), and individual variation was not associated with ADOS-CSS scores (SA: $\beta = .03$, $SE = 0.03$, 95% CI = -0.04, 0.10; RRB: $\beta = .03$, $SE = 0.03$, 95% CI = -0.02, 0.08). Latency decreased between 14 and 24 months (SA: $\beta = -.23$, $SE = 0.11$, 95% CI = -0.45, -0.01; RRB: $\beta = -.12$, $SE = 0.13$, 95% CI = -0.37, 0.13); again, this was not linked to ADOS-CSS scores (SA: $\beta = .04$, $SE = 0.03$, 95% CI = -0.03, 0.10; RRB: $\beta = -.003$, $SE = 0.03$, 95% CI = -0.06, 0.05).

Discussion

The pupillary light reflex (PLR), a candidate ASD intermediate phenotype, could illuminate developmental pathways linking genes to behaviour. We investigated associations between PLR (latency and amplitude) changes in the first and second years of life and ASD outcome, domain trait levels and ASD polygenetic liability (Findings are summarised in Appendix S6: Table S27–S28). Overall, PLR constriction magnitude (amplitude) increased from 9 to 14 months and decreased thereafter; latency (constriction onset time) decreased with time. From 9 to 14 months, higher PRS_{ASD} associated with slower

latency decreases; 14- to 24-month latency then decreased rapidly for those with later ASD. Latency change was not associated with 3-year dimensional trait measures; relatively smaller 9- to 14-month amplitude increases associated with Social Affect (SA) and Repetitive/Restrictive Behaviours (RRB), but not categorical ASD or PGS_{ASD}. Overall, in the first year, changes in amplitude associate with dimensional ASD traits at 3 years while stagnating latency development associated with polygenic liability, followed by a more rapid decrease in latency in the second year that was associated with categorical ASD outcome. Given our growing understanding of the PLR's neural underpinnings, examining its development may yield insights into early mechanistic pathways to emerging ASD.

Amplitude

Across the cohort, pupillary constriction amplitude between 9 and 14 months varied between outcome, though not significantly. Within this time window, relatively smaller changes in amplitude associated with higher SA and RRBs. These development patterns corroborate previously reported increasing amplitude in the first year in typically developing

infants (Kercher et al., 2020; Nyström et al., 2018). In a partially overlapping sample, Nyström et al. (2018) also demonstrated infants with later ASD showed decreasing 9- to 14-month amplitude, which significantly differed from the typically developing group's trajectory. Although effects are in the same direction in the present report (Figure 2), results are nonsignificant. We did not observe trait, outcome group or polygenic effects in the second year contributing novel findings to the field. Collating our work with previous investigations, infants with later traits show an initially stronger PLR constriction that weakens over the first year, in contrast to typically developing infants who show the opposite profile.

The association between early amplitude and later ASD traits may represent the emergence of higher-level cognitive processes like executive attention. Amplitude is modulated by acetylcholine (Loewenfeld, 1999) and norepinephrine (Lynch, 2018); key neurotransmitters involved in early attention regulation (Bast et al., 2018; Colombo, 2001). Indeed, PLR constriction can be induced semantically (Mathôt, Dalmaijer, Grainger, & Van der Stigchel, 2014) and by stimulating prefrontal brain regions regulating attention (Ebitz & Moore, 2017). Developmental changes in amplitude may reflect developmental shifts from early arousal regulated attention to more endogenous forms of attention control (Colombo, 2001). The weaker changes between 9 and 14 months in infants with later ASD (Nyström et al., 2018) or those with higher trait levels (present study) may reflect altered neurotransmitter systems supporting executive attention. Indeed, altered developmental changes in attention between 9 and 14 months have previously been linked to later ASD (Elsabbagh et al., 2013) and executive functioning (Hendry et al., 2018). Our findings could be interpreted through the AMEND model of ASD development (Johnson, Charman, Pickles, & Jones, 2021) whereby later-developing processes (e.g. executive attention) modify or compensate for perturbation in earlier-emerging sensory/motor functions; a reduction in the action of developmental modifiers is hypothesised to contribute to ASD traits. Further investigations should be done to test the relationship between attention and PLR development and its putative reflection of a key neurocognitive modifier system.

Latency

While the cohort overall showed decreasing latency across the first two years, infants with higher PGS_{ASD} showed relatively slower 9- to 14-month decreases, while infants with later ASD had a steeper 14- to 24-month latency decrease. These findings alongside the pattern in Figure 2B suggests an ASD-associated stagnated latency development that is potentially driven by earlier action of polygenic factors. This stagnation is also demonstrated by other candidate ASD intermediate phenotypes during infancy [e.g. look duration at faces (Hendry et al.,

2018), and motor skills (Harris, 2017)]. Latency depends on neural transduction efficiency, potentially reflecting white matter maturation (Kercher et al., 2020), a neural feature during early development associated with later ASD (Wolff et al., 2012) and other early features of ASD [e.g. visual orienting (Elison et al., 2013) and motor development (Parikh et al., 2020)]. This profile of early stagnation followed by a later 'catch-up' to apparently typical latency at 2 years (see also Dinalankara et al., 2017) may point to an adaptive or modified developmental pathway triggered by earlier atypicalities (Johnson, 2017; Johnson et al., 2021). Future work should determine long-term stability of the 'catching up' profile and consider potential modifiers.

While developmental changes in latency related to categorical ASD outcome, they were not significantly related to dimensional variation in SA and RRB. We operationalised ASD traits using calibrated severity scores obtained from the ADOS (an observational assessment with an unfamiliar examiner). As such, this measure may underestimate infrequent behaviours or overestimate social difficulties in shy children. Consequently, diagnosis is made on additional observational assessments and developmental history questionnaires (e.g. the ADI). Latency may relate more strongly to these other aspects of diagnosis or measures capturing broader development. Alternatively, latency changes may reflect differences in basic sensory processing in early development that trigger an adaptive process contributing to behavioural symptoms of ASD (Johnson, 2017). Under this view, there may be no linear map between early latency and later dimensional behaviours because the latter emerges through the response of a complex dynamical system to early perturbation (Turkheimer et al., 2021).

Limitations

The ASD outcome subgroup was relatively small ($N = 27$), limiting capture of the full ASD spectrum and reducing generalisability. Additionally, data amalgamation from different stimuli, eye trackers and sampling rates [to which latency is susceptible (Bergamin & Kardon, 2003)] limit our study, though latency is averaged across trials within individuals to improve resolution [as suggested by previous investigations (Bergamin & Kardon, 2003)] and statistically accounted for using random intercepts and previously reported (Nyström et al., 2018). Although we conducted eight models, we did not apply multiple comparison corrections as we followed experiment-wise procedure of multiple testing correction whereby all tests leading to the same conclusion should be corrected for multiplicity (Bender & Lange, 2001). Each model in our analysis asked a specific question regarding the relationship between PLR development and ASD. We acknowledge some would consider this too lenient and have provided corrected confidence

intervals and *p*-values for multiple comparisons within each PLR parameter in Appendix S5. Additionally, the procedure for the development of the PGS$_{ASD}$ is under review and only accounted for 2% of the phenotypic variance in our sample; therefore, findings may alter with future iterations of the PGS and more robust scores as larger GWAS are generated. Future investigations should consider samples with ranging severities and co-occurring conditions aiming for homogenous protocols with higher sampling frequencies to improve latency sensitivity (Bergamin & Kardon, 2003) and future potentially more robust PGS. Our stimuli only stimulated retinal rods and cones, limiting inferences on the complete PLR circuit. Cyan blue light sensitive and dopaminergic, intrinsically photosensitive retinal ganglion cells (ipRGC) also induce the PLR (Hattar, Liao, Takao, Berson, & Yau, 2002; Pickard & Sollars, 2012). Future investigations should include stimuli targeting ipRGC (Hellmer & Nyström, 2017) to clarify specificities associating PLR and ASD.

Summary
The present study demonstrates PLR development associates with later ASD (categorically and dimensionally), with PLR latency development relating to ASD polygenic liability. This supports PLR as a candidate intermediate phenotype for investigating pathways between genes and phenotype. PLR atypicalities varied over developmental time, reiterating the importance of longitudinal investigations in early development. Future work should incorporate alternative measures (e.g. functionally informed structural and genetic measures) to test whether distinct neural mechanisms underpin PLR alterations and explore the degree to which findings might reflect compensatory or adaptive processes that follow an earlier period of disruption (Johnson et al., 2021). Taken together, the potential for cross-developmental and translational insights yielded by simple measures like the PLR makes it an excellent candidate for future large-scale investigation.

Supporting information
Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1. PLR stimuli and processing.
Appendix S2. 3-year Clinical outcome.
Appendix S3. DNA Processes and Polygenic Score Calculations.
Appendix S4. Sample characteristics.
Appendix S5. Model results.
Appendix S6. Overview of findings.
Table S1. Mean number of trials included and percentage of trials missing during processing for each PLR parameter for each visit.

Table S2. 3-year sample characteristics of individuals with PLR from at least one timepoint from 9 to 24 months.
Table S3. Results from ANOVA and Tukey pairwise comparisons examining group-wise differences in age across outcome at each timepoint.
Table S4. Results from ANOVA and Tukey pairwise comparisons examining group-wise differences in M-ELC across outcome at each timepoint.
Table S5. Sample size and characteristics describing, age, Mullen Early Learning Composite score (M-ELC) across 3-year outcome group for each visit timepoint for individuals with PLR Amplitude data.
Table S6. Standardised and unstandardised fixed effect estimates for association between outcome and PLR Amplitude obtained using linear mixed effect models.
Table S7. Standardised and unstandardised random Effect variance and standard deviation for association between outcome and PLR Amplitude obtained using linear mixed effect models.
Table S8. Estimated marginal means obtained using LMM on the relation between PLR Amplitude and 3-year ASD outcome over visits.
Table S9. Standardised and unstandardised fixed effect estimates for association between outcome and PLR Amplitude obtained using linear mixed effect models including mean distance from screen as covariate.
Table S10. Standardised and unstandardised fixed effect estimates for association between outcome and PLR Latency obtained using linear mixed effect models.
Table S11. Standardised and unstandardised random Effect variance and standard deviation for association between outcome and PLR Latency obtained using linear mixed effect models.
Table S12. Estimated marginal means obtained using LMM on the relation between PLR Latency (ms) and 3-year ASD outcome over visits.
Table S13. Standardised and unstandardised pairwise comparisons of Latency within outcome group across 14 to 24 months.
Table S14. Standardised and unstandardised fixed effect estimates for association between outcome and PLR Latency obtained using linear mixed effect models including mean distance from screen as covariate.
Table S15. Standardised and unstandardised fixed effect estimates for association between ASD polygenic score and PLR Amplitude obtained using linear mixed effect models.
Table S16. Standardised and unstandardised random effect variance and standard deviation for association between ASD polygenic score and PLR Amplitude obtained using linear mixed effect models.
Table S17. Standardised and unstandardised fixed effect estimates for association between ASD polygenic score and PLR Amplitude obtained using linear mixed effect models including mean distance from screen as covariate.
Table S18. Standardised and unstandardised fixed effect estimates for association between ASD polygenic score and PLR Latency obtained using linear mixed effect models.
Table S19. Standardised and unstandardised random Effect variance and standard deviation for association

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Key points

- Infant pupillary light reflex (PLR) is the reflexual pupil constriction in response to light.
- Decreasing 9- and 14-month PLR constriction amplitude associates with increased social difficulties and repetitive behaviours.
- Stagnated 9- to 14-month PLR latency development associates with polygenic liability for ASD; steeper 14- to 24-month decreases associates with ASD outcome.
- Infant PLR development may be used to uncover neural mechanisms to emerging ASD.

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