

Larger pupil dilation to nonsocial sounds in infants with subsequent autism diagnosis

Maja Rudling,¹  Pär Nyström,² Sven Bölte,^{3,4} and Terje Falck-Ytter^{1,3,5}

¹Development and Neurodiversity Lab, Department of Psychology, Uppsala University, Uppsala, Sweden; ²Uppsala Child and Babylab, Department of Psychology, Uppsala University, Uppsala, Sweden; ³Department of Women's and Children's Health, Center of Neurodevelopmental Disorders (KIND), Centre for Psychiatry Research, Karolinska Institutet, Stockholm, Sweden; ⁴Curtin Autism Research Group, School of Occupational Therapy, Social Work and Speech Pathology, Curtin University, Bentley, WA, Australia; ⁵Swedish Collegium for Advanced Study (SCAS), Uppsala, Sweden

Background: Studies of infants with an elevated likelihood of autism spectrum disorder can identify basic developmental processes that are associated with subsequently emerging clinical symptoms. Atypical responsiveness to sounds in infancy is such a potential early marker of autism. Here, we used pupillometry to quantify reactivity to social and nonsocial sounds in infants with a subsequent diagnosis. Previous research suggest that pupil dilation reflects attentional alerting, and link it to the locus coeruleus norepinephrine system. **Methods:** We measured pupil dilation responses to child-directed speech and the sound of running water; sounds infants often hear in their everyday life. The final sample consisted of 99 ten-month-old infants (52 girls), of whom 68 had an elevated likelihood of autism and 31 were typically developing low-likelihood infants. At follow-up (36 months of age), 18 children in the elevated-likelihood group were diagnosed with autism. **Results:** Compared to infants without diagnosis, the infants who were subsequently diagnosed with autism had larger pupil dilation when listening to nonsocial sounds, while reactivity to speech was strikingly similar between groups. In the total sample, more pupil dilation to the nonsocial sound was associated with higher levels of autistic symptoms. We also found that on a trial-by-trial basis, across all conditions and groups, more pupil dilation was associated with making fewer gaze shifts. **Conclusions:** This study did not find evidence of atypical pupillary reactivity to child-directed speech early in life in autism. Instead, the results suggest that certain nonsocial sounds elicit atypically strong alerting responses in infants with a subsequent autism diagnosis. These findings may have important theoretical and clinical implications. **Keywords:** Autism spectrum disorder; infancy; auditory attention; pupil dilation.

Introduction

An essential task for any cognitive system is to adaptively regulate its responsiveness to new sensory input given the constraints of the situation (Eldar, Cohen, & Niv, 2013). Conversely, the consequences of atypical reactivity to basic sensory signals, particularly if it is present during early brain development, could be far-reaching (Rubenstein & Merzenich, 2003). Autism Spectrum Disorder (*autism* for simplicity in this article) is a common, heritable, and heterogeneous neurodevelopmental condition defined by difficulties with social communication and interaction along with restricted and repetitive behaviours and interests. Sensory hypo- and hypersensitivity have been included as autism criteria in DSM-5 (American Psychiatric Association, 2013), and both animal modelling and genetic work suggest that differences in basic sensory systems could be early emerging and possibly causal in autism (Mercati et al., 2017; Rubenstein & Merzenich, 2003).

One such difference in basic sensory systems could be infant reactivity to sounds. Several studies indicate that young children with autism show less preference and attentional orienting to social sounds and speech compared to typically developing and

developmentally delayed children (Baranek et al., 2013; Ceponiene et al., 2003; Klin, 1991). There are also more recent indications of atypical processing of nonsocial sounds in autism (Cui, Wang, Liu, & Zhang, 2017; O'Connor, 2012; Schwartz, Shinn-Cunningham, & Tager-Flusberg, 2018). For example, infants who later receive an autism diagnosis have shown atypical habituation to repeated sounds (Kolesnik et al., 2019). Furthermore, using Functional Near Infrared Spectroscopy, Lloyd-Fox et al. (2018) found cortical *hypo-activation* for social sounds and *hyper-activation* for nonsocial sounds in a small group of infants with subsequent autism diagnosis. However, the literature regarding reactivity to sounds in early autistic development is not consistent, and to our knowledge, it has not previously been studied using pupillometry.

In this study, we used pupillometry to assess how the basic attentional system of infants who later receive an autism diagnosis is engaged by social and nonsocial sounds. Although the size of the pupil is largely influenced by luminance, pupil fluctuations also reflect ongoing psychological processes such as arousal and alertness to stimuli (Laeng, Sirois, & Gredebäck, 2012). At the neural level, pupil size has been used as a marker of activity in the locus coeruleus (LC), a key structure for regulation of norepinephrine activity and gain (responsivity) of

Conflict of interest statement: No conflicts declared.

processing throughout the brain (Aston-Jones & Cohen, 2005; de Barbaro, Clackson, & Wass, 2017; Eldar et al., 2013; Reimer et al., 2016). In turn, LC activity has been associated with psychological processes related to attention orienting, sensory processing and selectivity (Mather, Clewett, Sakaki, & Harley, 2016). These processes have been implicated in autism, and atypical LC activity has been proposed as one underlying mechanism of autistic development (Bast, Poustka, & Freitag, 2018). Thus, pupil dilation represents a noninvasive measure of the child's reactivity to incoming stimuli with a clear link to underlying neurophysiology, which can be assessed in preverbal infants without any need for instruction.

In light of the above-mentioned behavioural hypo-reactivity reported for certain social sounds, we tested the hypothesis that infants with a subsequent autism diagnosis would show a weaker pupil dilation effect in response to speech compared to infants who did not develop autism. In line with previous reports (e.g. Falck-Ytter, Nyström, Gredebäck, Gliga, & Bölte, 2018) we focussed our analysis on the first year of life, at ten months of age, before behavioural symptoms of autism are typically observable. We contrasted the speech condition with a nonsocial sound condition, consisting of recordings of water running from a tap. This stimulus was chosen because, like the speech stimuli, it is continuous and the two sounds can be matched in volume and length. Further, like speech, the sound of water running from a tap is frequently heard in infants' everyday lives and may occur when infants are engaged in other activities like exploring the visual environment, similar to the setup in our experiment. Lastly, with one clear nonsocial contrast to our social sound condition, we could create a short, infant friendly experiment, which could be followed up with more detailed manipulations in the future (see e.g. Pierce, Conant, Hazin, Stoner, & Desmond, 2011).

In addition to this main analysis, we addressed two other questions. First, because the locus coeruleus norepinephrine system (LC-NE) modulates visual attention at short timescales in infants (de Barbaro et al., 2017), we assessed whether within-subject fluctuation of pupil dilation was reliably associated with gaze shift behaviour. Second, we tested if age moderated any potential group effect on pupil dilation by analysing pupil data from the same infants during their second year of life, at 14 and 18 months of age.

Methods

Participants

The experiment was part of a prospective sibling study (Early Autism Sweden, EASE, <http://www.smasyskon.se>), in which infant siblings of children with autism were followed into early childhood. The probability of being diagnosed with autism is increased in the case of older biological siblings with the

condition (Ozonoff et al., 2011; the recurrence rate is around 20%), hence the denotation 'elevated likelihood'.

Participants were recruited mainly from the greater Stockholm area. Infants in the elevated-likelihood groups had an older sibling with an autism diagnosis (verified through interview with parents and inspection of medical records), and were recruited via the project's website, advertisement, and clinical units. Infants in the low-likelihood group were recruited from birth records and advertisements. They were typically developing, had at least one older typically developing sibling, and there were neither any parental autism-specific concerns about the infant, nor any first or second degree family members with a history of autism. The infants included in the study were reported by their parents to have no known medical conditions such as epilepsy, no known genetic syndrome associated with autism, nor any other known medical conditions affecting brain development, no visual or auditory impairment, and were born full-term (after week 36).

At 36 months of age, a comprehensive diagnostic evaluation of the infants' developmental- and adaptive abilities and autistic symptoms were assessed by experienced clinicians, using gold standard instruments and procedures: ADOS-2 (Lord, Luyster, Gotham, & Guthrie, 2012), ADI-R (Rutter, LeCouteur, & Lord, 2003), MSEL (Mullen, 1995), and the Vineland Adaptive Behaviour Scales (Sparrow, Cicchetti, & Balla, 2005). Categorical diagnostic assessments were based on DSM-5 (American Psychiatric Association, 2013).

The final sample consisted of 99 ten-month-old infants. We compared three groups: Infants with elevated likelihood of autism who subsequently received an autism diagnosis (EL-AUT; $n = 18$, 8 girls), infants with elevated likelihood of autism *without* a subsequent autism diagnosis (EL-noAUT; $n = 50$, 30 girls), and infants with low likelihood of autism without a subsequent autism diagnosis (LL; $n = 31$, 14 girls). In the final sample, 26% of the infants with elevated likelihood received an autism diagnosis. The three groups did not differ significantly in terms of socioeconomic status (operationalised as parents' highest educational level) or general development level (Table 1). One infant in the LL group received an autism diagnosis and was excluded.

Ethical considerations

The study was approved by the Regional Ethical Board in Stockholm, Sweden and conducted in accordance with the 1964 Declaration of Helsinki. Written informed consent was provided by the parents.

Experimental stimuli

We used corneal reflection eye-tracking to measure pupillary responses to auditory stimulation occurring during a visual task (Figure 1A–C). Two types of auditory stimuli were embedded in an eye-tracking experiment that also assessed visual preference for biological motion (as reported in Falck-Ytter et al., 2018). The visual stimulus was used as an attention grabber, to increase likelihood of obtaining useful eye-tracking data from the infants.

Specifically, while observing the visual stimulus, the infants intermittently heard the voices of two different females speaking to them using an infant-directed tone of voice (social sound condition) and two recordings of water running from a tap (nonsocial sound condition; Figure S1, Videos S1 and S2). The visual stimulus lasted for 15 s, and the infants watched eight such videos. Each video was accompanied by one sound from the social sound condition and one sound from the nonsocial sound condition, and the order of the sounds within the video was counterbalanced within subjects across trials. The first sound started 3,000 ms after the trial start and the second

Table 1 Participant characteristics by group

	EL-AUT	EL-noAUT	LL	Group comparisons ^a
<i>N</i> total (girls)	18 (8)	50 (30)	31 (14)	
10 months measures				
Age in days	<i>M</i> = 309, <i>SD</i> = 7	<i>M</i> = 313, <i>SD</i> = 13	<i>M</i> = 310, <i>SD</i> = 14	$F(2, 96) = 0.75, p = .474$
SES (1–5) ^b	<i>Mdn</i> = 5, <i>IQR</i> = 3, 5	<i>Mdn</i> = 5, <i>IQR</i> = 3, 5	<i>Mdn</i> = 5, <i>IQR</i> = 5, 5	$H(2) = 4.34, p = .114$
MSEL total ^c	<i>M</i> = 95.6, <i>SD</i> = 14.8	<i>M</i> = 101.5, <i>SD</i> = 13.3	<i>M</i> = 102.9, <i>SD</i> = 12.2	$F(2, 96) = 1.81, p = .170$
MSEL NVIQ ^c	<i>M</i> = 113.6, <i>SD</i> = 12.0	<i>M</i> = 117.8, <i>SD</i> = 16.2	<i>M</i> = 123.0, <i>SD</i> = 12.3	$F(2, 96) = 2.62, p = .078$
MSEL VIQ ^c	<i>M</i> = 85.6, <i>SD</i> = 22.7	<i>M</i> = 93.6, <i>SD</i> = 20.6	<i>M</i> = 90.6, <i>SD</i> = 19.6	$F(2, 96) = 1.00, p = .372$
36 months measures				
ADOS-2 ^d	<i>Mdn</i> = 7, <i>IQR</i> = 6, 8	<i>Mdn</i> = 2, <i>IQR</i> = 2, 4	<i>Mdn</i> = 2, <i>IQR</i> = 1, 4	$H(2) = 38.60, p < .000$
Vineland ^e	<i>M</i> = 80.1, <i>SD</i> = 8.1	<i>M</i> = 91.9, <i>SD</i> = 7.5	<i>M</i> = 97.7, <i>SD</i> = 9.2	$F(2, 95) = 25.24, p < .000$

EL-AUT, Elevated likelihood – autism diagnosis; EL-noAUT, Elevated likelihood – no autism diagnosis; LL, low likelihood.

^aOne-way analysis of variance F-tests (age, MSEL, Vineland) or Kruskal-Wallis H-test (SES, ADOS-2 due to non-normal distribution).

^bSocioeconomic status based on parents' education level (1–5).

^cMullen Scales of Early Learning composite scores of the total scale (MSEL total), the nonverbal IQ scale (MSEL NVIQ), and the verbal IQ scale (MSEL VIQ).

^dADOS-2 total calibrated severity scores.

^eVineland Adaptive Behaviour Scales summed adaptive behavior composite.

sound started 10,000 ms after the trial start. The duration of each sound was always 3,000 ms (Figure 1A). The 3,000 ms delay of the first sound was to give the infants time to actively engage with the visual stimulus and to distinguish the pupil dilation response to sounds from the pupillary light reflex (Figure 1A). Both the social and the nonsocial sounds were played at a relatively low volume (<65 peak dB) to decrease the risk of startle responses.

The visual stimulus consisted of two animations with point-light displays of human motion (moving dots representing the major joints of a human body; Qualisys, Göteborg, Sweden; Figure 1C). One point-light animation was played upright (head up) and one inverted (head down), with left-right counterbalancing across trials. Both animations were played forwards in time (Falck-Ytter et al., 2018). The pupil is strongly influenced by luminance, but the small black dots on a homogeneous white background creates a rather constant luminosity. Thus, exactly where on the screen an infant looked had negligible effect on the amount of light that reached the eye in this experiment. Further, we have previously shown that the three groups had very similar looking behaviour to these types of stimuli (Falck-Ytter et al., 2018).

Procedure

The families visited the lab for a full day with several assessments and experiments, with the possibility of breaks when needed. During the 10-min eye-tracking session (which also included stimuli linked to other experiments) the infant was placed in their parent's lap, ~60 cm from the eye-tracker screen, in a moderately lit room. A five-point calibration was conducted before eye-tracking recording, by directing the infants' attention to a series of positions on the screen using moving stimuli. The calibration procedure was repeated if necessary. Parents were instructed to sit still and to not influence the infant's looking behaviour during the experiment.

Data collection, processing and analysis

Pupil and gaze data were collected using eye-trackers Tobii 1750 and Tobii TX300 (Tobii, Danderyd, Sweden; Appendix S1). Pupil and gaze data were preprocessed in Matlab (Mathworks Inc., CA, USA) using the Timestudio framework (Nyström, Falck-Ytter, & Gredebäck, 2016) and

custom written scripts. Decisions regarding processing of data were made prior to any statistical analyses.

Statistical analyses were performed using IBM SPSS Statistics Version 25 (IBM Corp., Armonk, NY, USA). Significance tests were two-tailed ($\alpha = .05$). All measures were examined for outliers (z -score > ± 3 from group mean, and visual data inspection) and met the assumptions of normality (nonsignificant Shapiro-Wilk's test and inspection of Q-Q plots, Figure S2–S5) and homogeneity of variance (nonsignificant Levene's tests), unless otherwise stated.

Pupil dilation to sounds at ten months of age

We used pupil measurement of the right eye (Bala, Whitchurch, & Takahashi, 2020). Processing of the pupil data consisted of interpolation of gaps of less than six samples (20 ms) and a moving average filter of 10 samples (33 ms). Trials were segmented into the full period that the sound was played (3,000 ms), plus a baseline interval of 500 ms prior to sound onset. We operationalised pupil dilation as the median pupil dilation during the response segment (3–6 s and 10–13 s from visual stimuli start) minus the median pupil dilation during the corresponding baseline segments (2.5–3 s and 9.5–10 s from visual stimuli start; Figure 1A; Mathôt, Fabius, van Heusden, & Van der Stigchel, 2018). We excluded trials with <25% data in baseline- and measurement segments, and participants with <2 trials from each condition. Two participants in the EL-AUT group and two in the EL-noAUT group were excluded by this procedure. One participant in the EL-noAUT group was excluded due to having outlier values of mean pupil dilation ($z = -4.63$ in the nonsocial condition).

To test the main hypothesis, an ANOVA was performed (group as between-subjects and condition as within-subjects variables). A one-way ANOVA was used to analyse group differences within each condition to follow up potential interaction effects, followed by Bonferroni-corrected t -tests to assess differences in pupil dilation in significant conditions between the EL-AUT group, and the EL-noAUT and LL groups, respectively.

In the condition that significantly differed between groups we calculated Spearman's Rho correlation (due to non-normality) between pupil dilation at ten months of age and autistic symptoms at 36 months of age: summed algorithm scores (PH21–47) of ADI-R, and calibrated severity scores of ADOS-2 (total), ADOS-2 Social Affect scale, and ADOS-2

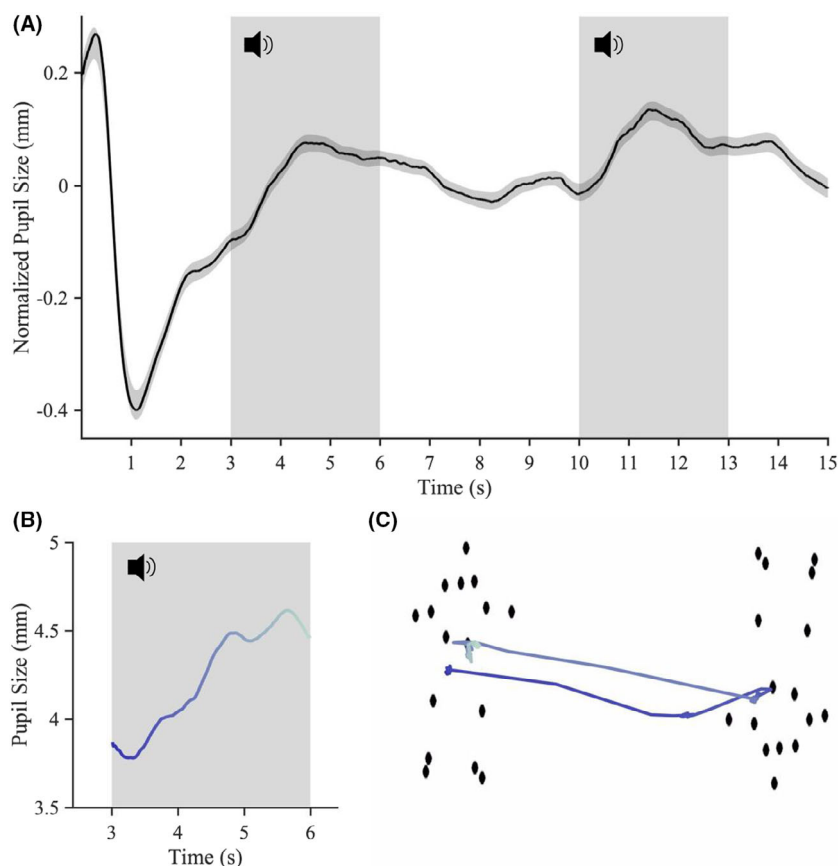


Figure 1 Methods overview. (A) Mean pupil size aggregated across all infants and all stimuli (social and nonsocial sound conditions). Time windows with auditory stimulation are marked with grey rectangles and loud-speaker symbols. Each video contained a social and a nonsocial sound; the order was counterbalanced across trials within subject (social first versus nonsocial first). The shaded area represents standard error of the mean. Data are normalised by subtracting each infant's average from that infant's time series. (B) Pupil size for one participant in one auditory trial. (C) Visual stimulus: biological motion (left) and inverted display (right). Coloured line represents gaze behaviour of the same participant and trial as in B. Time is represented by colour (time axis in B). Pupil data are smoothed for illustrative purposes

Restrictive and Repetitive Behaviour scale. We performed this analysis on the whole sample and on the elevated-likelihood group separately (Appendix S1).

Additional analyses

Association between pupil dilation and gaze shift behaviour. Gaze shift behaviour was operationalised as number of gaze shifts per second within a 4,000 ms time window starting 1,000 ms after sound onset, to account for potential lags in arousal effects on eye movements relative to the pupil response. The mean gaze data from both eyes were preprocessed with a 100 ms moving median filter, including only data where the gaze was on the screen. After visual inspection of gaze velocity and x-coordinates over time, a fixed threshold for eye movement velocity was set to 150°/s. This threshold allowed for identification of most clear gaze shifts while avoiding capture of noise and artefacts and was determined prior to statistical analysis. Because gaze shift frequency initially correlated with amount of missing data, we added a criterion that gaze shifts were discarded if they started within 33 ms from missing data. The relation between gaze shift frequency and pupil dilation was significant irrespective of the application of this criterion.

To analyse the relation between pupil dilation and gaze shift frequency on an individual level, we first estimated the relation between pupil dilation and gaze shift frequency within each

participant. This was done by calculating a regression coefficient for each infant (using data from each trial), with gaze shift frequency as dependent variable and pupil dilation as independent variable. To test if this effect was significantly different from zero, these individual standardised regression coefficients were submitted to a one-sample t-tests. Only trials that were included in the main pupil dilation analysis were used. Prior to regression, trials were removed if the gaze shift frequency exceeded ± 3 z-scores from the group mean. This resulted in nine removed trials based on pupil dilation, and five removed trials based on gaze shift frequency, but no participant was excluded due to this procedure.

Longitudinal analysis of pupil dilation into the second year of life.

The sample used for the longitudinal analysis consisted of 110 infants with data from at least one age point (95 infants at 14 months of age, 98 infants at 18 months of age; no infants were excluded due to outlier values at these ages). Fitting of a linear mixed model was done using restricted maximum likelihood estimation and forward variable selection to find the model with best fit (lowest Akaike Information Criteria (AIC)). The final model (AIC = -711.24) included mean pupil dilation (across trials) as the outcome variable, with group (3), condition (2), and age (3) as fixed effect factors. Condition and age were nested as repeated measures within each subject, using first order autoregressive covariance structure with heterogeneous variance, and subject level

random slopes of age were included with fixed intercept and unstructured co-variance structure.

Results

Pupil dilation to sounds at ten months of age

In terms of pupil dilation, we found a significant interaction between group and condition ($F(2, 96) = 4.11, p = .019, \eta_p^2 = .08$; Figure 2) indicating that group differences were different for the two types of sounds. Additionally, we observed a main effect of sound ($F(1, 96) = 7.77, p = .006, \eta_p^2 = .08$), but no main effect of group ($F(2, 96) = 2.47, p = .090, \eta_p^2 = .05$). A one-way ANOVA of group effects performed for each condition separately showed that there were significant group differences in the nonsocial sound condition ($F(2, 96) = 5.54, p = .005, \eta^2 = .10$), but not in the social sound condition ($F(2, 96) = 0.07, p = .936, \eta^2 = .001$). Infants who subsequently received an autism diagnosis dilated their pupils more in response to nonsocial sounds ($M = 0.22$ mm, $SD = 0.11$, 95%CI [0.17, 0.28]) compared to both the infants in the EL-noAUT group ($M = 0.15$ mm, $SD = 0.13$, 95%CI [0.11, 0.18]; $t(66) = 2.33, p^{\text{bonferroni}} = .046$, Hedges' $g = 0.64$) and the LL group ($M = 0.09$ mm, $SD = 0.17$, 95%CI [0.02, 0.15]; $t(47) = 3.02, p^{\text{bonferroni}} = .008$, Hedges' $g = 0.89$). See full report in Tables S1 and S2. More pupil dilation in response to nonsocial sounds was associated with more severe autistic symptoms at 36 months of age, assessed with the ADOS-2 calibrated severity scores ($r_s = .28, p = .005, n = 97$), the calibrated severity scores of ADOS-2 social affect scale ($r_s = .25, p = .013$) and the ADOS-2 restrictive and repetitive behaviour scale ($r_s = .30, p = .003$), as well as the ADI-R summed algorithm score ($r_s = .34, p = .002, n = 80$). Although pupil dilation was enhanced in infants with subsequent autism diagnosis for the nonsocial sounds, it is notable that all groups showed pupil dilation in both conditions (Figure 2).

There were no significant group differences in pupil dilation during the baseline period (Kruskal-Wallis $H(2) = 4.38, p = .112$; EL-AUT: $M = 3.89$ mm, $SD = 0.61$ mm; EL-noAUT: $M = 3.58$ mm, $SD = 0.47$ mm; LL: $M = 3.78$ mm, $SD = 0.57$ mm). Nor were there any group differences in amount of valid pupil data measured as percent recorded pupil data out of the total possible amount within each trial (Kruskal-Wallis $H(2) = 1.18, p = .553$; EL-AUT: $M = 82.92\%$, $SD = 13.91\%$; EL-noAUT: $M = 85.65\%$, $SD = 13.40\%$; LL: $M = 82.39\%$, $SD = 15.73\%$). Kruskal-Wallis tests were used because the baseline and amount of valid pupil data were not normally distributed. Adding MSEL nonverbal IQ score as a covariate in the main analysis did not change the interaction effect between condition and group on pupil dilation.

In principle, group differences in pupil dilation to sounds could be linked to differences in visual

preferences. To evaluate this, preference for the upright biological motion, defined as the looking time to the upright animation relative to looking time to the whole screen, was calculated on raw gaze data without fixation filter. We found no group differences for this measure ($F(2, 96) = 0.167, p = .847, \eta^2 = 0.003$).

Additional analyses

Association between pupil dilation and gaze shift behaviour. A one-sample t-test showed that the average within-infant regression coefficient of the pupil dilation by gaze shift frequency function was negative and different from zero. This means that trials with more pupil dilation tended to include fewer gaze shifts ($t(98) = -2.12, p = .036, d = 0.21$; M (regression coefficient) = $-0.08, SD = 0.38, 95\%CI [-0.15, -0.01]$). The group differences were not significant ($F(2, 96) = 1.26, p = .288, \eta^2 = .03$).

Longitudinal analysis of pupil dilation into the second year of life. The analysis using data from 10-, 14-, and 18-months visits yielded a significant interaction effect between condition and group ($F(2, 566) = 3.32, p = .037, \eta^2 = .01$), similar to the 10-months results. There was no three-way interaction effect of age*condition*group ($F(4, 566) = 0.79, p = .535, \eta^2 = .01$) nor other effects involving the age factor (Tables S3–S5). Thus, we did not find evidence for significant moderation of group differences by age.

Discussion

Contrary to our hypothesis, we observed no difference between the groups in terms of pupil dilation to child-directed speech. Rather, we found that infants who are subsequently diagnosed with autism show enhanced reactivity to the nonsocial sound of running water, with medium to large effect sizes. Thus, while there is decreased orienting and attention towards social sounds and speech early in life in autism (Baranek et al., 2013; Ceponiene et al., 2003; Klin, 1991), pupil reactivity to speech appears to be typical at 10 months of life.

The group difference in pupil responses to the nonsocial sounds supports the view that there are basic atypicalities of auditory processing in autism that are not specifically linked to social stimuli (Mercati et al., 2017; Schwartz et al., 2018). This is consistent with previous work suggesting increased cortical reactivity to repeated tones (Kolesnik et al., 2019) and higher cortical activation to nonsocial sounds in left lateralised temporal regions in a small group of infants with subsequent autism compared to typically developing infants (Lloyd-Fox et al., 2018). Interestingly, Lloyd-Fox et al. (2018) also found reduced cortical responsivity to vocal sounds for the infants with subsequent autism, which may

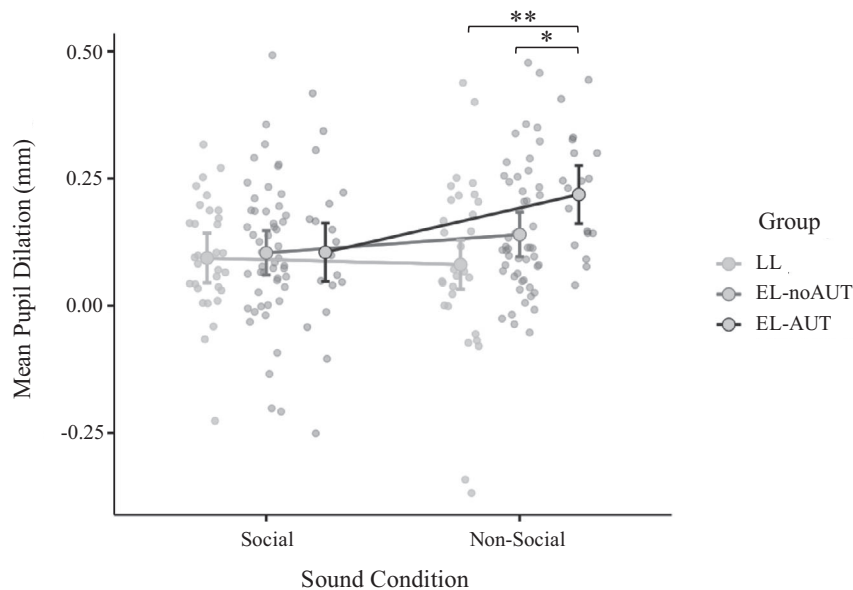


Figure 2 Pupil dilation to sounds relative to baseline interval 500-0 ms prior to sound onset, by group and condition. Error bars represent 95% CI, * $p < .05$, ** $p < .01$, Bonferroni corrected. Dots represent individual data points

seem contrary to our results. However, methodological differences in regards to stimuli, measures, and involved brain areas may explain these differences in findings.

Recent data from rhesus monkeys has demonstrated that the link between pupil dilation and phasic activity in the LC is especially robust during auditory stimulation (Joshi, Li, Kalwani, & Gold, 2016). Such phasic activity in the LC is linked to norepinephrine activity in the cortex (Larsen & Waters, 2018; Reimer et al., 2016). Against this background, our findings suggest that certain nonsocial sounds may elicit atypical norepinephrine activity in infants with subsequent autism diagnosis. If these atypical responses generalise to other environmental stimuli, it could have cascading consequences for learning and development (Aston-Jones & Cohen, 2005; Mather et al., 2016; Sara, 2009). It has also been suggested that norepinephrine interacts with glutamate in sensory areas to enhance perceptual selectivity and memory for salient stimuli, amplifying activation of prioritised representations while inhibiting nonprioritised representations (Mather et al., 2016). Thus, atypical activation of the LC-NE during auditory stimulation could contribute to different cortical specialisation in autism compared to typical development (Bast et al., 2018). It should be emphasised that atypical activation of the LC-NE does not necessarily imply that the atypical functioning originates in this system. However, the well-established link between pupil dilation and the LC-NE indicates that this system is involved. Our results could thus indicate early attentional prioritisation of certain aspects of the environment in early autistic development, potentially leading to increased specialisation towards those aspects at the cost of other sources of information (for a related

theoretical discussion, see Johnson, Charman, Pickles, & Jones, 2021).

Our study was designed to provide a first test of phasic pupillary responses to social versus nonsocial sounds in infants with elevated likelihood of autism. Therefore, its scope is limited in terms of delineating why the infants with subsequent autism seem to react stronger to the nonsocial sounds, while performing similarly in the social sound condition. It is possible that the EL-AUT group is more distracted by task-irrelevant sounds when engaging with the visual stimuli (Granovetter et al., 2020; Lawson, Mathys, & Rees, 2017). If the infants perceive the sound of running tap water as more task-irrelevant than human speech during observation of biological motion, this may explain the increased response to the nonsocial sounds in that group. Similarly, it is possible that the EL-AUT group reacts stronger to the nonsocial sounds due to attenuated habituation to distracting sounds (Guiraud et al., 2011; Jamal, Cardinaux, Haskins, Kjelgaard, & Sinha, 2020). The two experimental conditions were matched on overall volume and length. Therefore, the specific group difference linked to the nonsocial sound condition must be explained by other properties of that condition.

Our data suggests that some common nonsocial sounds elicit atypically high levels of pupil dilation in infants subsequently diagnosed with autism, while speech does not. Beyond this, it is difficult to draw specific conclusions. For example, the typically developing infants showed no difference in pupil dilation between the two conditions (Figure 2), suggesting that the sound types were equally arousing for them. However, it is possible that these infants reacted to different aspects of the sounds in each condition. Correspondingly, while the three groups

showed similar pupil dilation in the social sound condition, we cannot know if they reacted to the same aspects of the stimuli. Such negative results should be interpreted with particular caution, also for statistical reasons.

The group difference in pupil dilation was specific to the nonsocial sounds, and the order of the two sound conditions was counterbalanced within subject in terms of first versus last sound. Consequently, it is unlikely that the observed effect can be attributed to either differential recovery from the pupillary light reflex (in the beginning of the visual stimuli; Figure 1A), differences in base level tonic pupil dilation, or to general differences in the ability to build up expectations about the timing of the sounds. These explanations would namely predict a group effect that is not specific to one condition (see Appendix S1, Table S6, and Figures S6–S11 for supporting analyses).

The observed within-subject association between more pupil dilation and less gaze shifts is in line with pupil dilation as an indirect measure of norepinephrine activation, as this system is thought to modulate exploration of the environment (Aston-Jones & Cohen, 2005; Bast et al., 2018; Eldar et al., 2013). It is notable that in monkeys, such trial to trial fluctuation in pupil dilation has a particularly strong relation to firing in the LC (Joshi et al., 2016). While this result was not specific to autism, it generally supports the view that increased pupil dilation to sounds may affect information intake on short timescales.

Although previous research has indicated that group differences in infants and toddlers are often moderated by age (e.g. Hazlett et al., 2017; Nyström, Thorup, Bölte, & Falck-Ytter, 2019), we found no support for this in our study. While this could imply that group differences in responsivity to nonsocial sounds are stable across early development, it needs to be further studied before any such conclusion can be drawn.

Our conclusions should be considered in light of some limitations. First, although comparable to most studies on infants with an elevated likelihood of autism (e.g. Falck-Ytter et al., 2018; Jones & Klin, 2013), the sample size is limited, particularly in the EL-AUT group. Autism is heterogeneous, and a larger sample would be necessary to reliably evaluate potential moderating factors of the observed effect. Second, because the elevated-likelihood sample consists of younger siblings of children with autism, the generalisability to other autistic samples may be limited. Third, the nonsocial sound condition was developed as a control stimulus and we only test one type of nonsocial sound. Therefore, we are limited in generalising conclusions to other nonsocial sounds. Future research should systematically manipulate more fine-grained aspects of auditory stimuli and test the scope of the current findings

across more sound types. Fourth, the auditory stimuli were played while the infants watched point-light displays of biological motion. While this embedding cannot explain the group by condition effect, due to experimental counterbalancing, it is conceivable that the results have limited generalisability outside this context. It is notable, however, that we found no indication that the infants with subsequent autism diagnosis looked at the visual stimuli in a different way than the other groups, suggesting that the effect of sounds arose despite comparable visual engagement and attention. Finally, the change of eye-tracker during the course of the study is methodologically suboptimal. However, adding eye-tracker apparatus as a covariate did not change the significant interaction effect of our main analysis (Appendix S1).

The findings of this study provide leads on early behavioural intervention strategies that could be evaluated in clinical research with minimal side effects. Specifically, our results suggest that during social interaction with infants with an elevated likelihood of autism, one may promote development by minimising task irrelevant background sounds. This principle is well known in intervention for older children with autism, but to our knowledge it has not been emphasised in prodromal intervention trials so far. Finally, in contrast to most other early signs of autism, pupil dilation measures can easily be translated to animal models, an important step for advancing our understanding of molecular-genetic mechanisms (Loth et al., 2016).

Conclusions

Our study suggests that infants who subsequently receive an autism diagnosis respond to certain nonsocial sounds with increased alertness compared to infants without autism. Atypical reactivity to everyday environmental sounds is likely to have cascading effects on development. Future studies of early signs of autism should therefore evaluate pupillary reactivity across a larger range of sounds. Our results suggest that reactivity to sounds affects gaze behaviour at short timescales (seconds) and is related to autism at longer timescales (years).

Supporting information

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

Appendix S1. Supporting methods and results.

Figure S1. Waveform and spectrogram of the four auditory stimuli used in the study.

Figure S2. Q-Q plots of pupil dilation variables for the EL-AUT group, divided on condition (columns) and age (rows).

Figure S3. Q-Q plots of pupil dilation variables for the EL-noAUT group, divided on condition (columns) and age (rows).

Figure S4. Q-Q plots of pupil dilation variables for the LL group, divided on condition (columns) and age (rows).

Figure S5. Q-Q plots of gaze shift frequency at 10 months of age divided on the three groups.

Figure S6. Histogram of percent missing pupil data per trial for the EL-AUT group.

Figure S7. Histogram of percent missing pupil data per trial for the EL-noAUT group.

Figure S8. Histogram of percent missing pupil data per trial for the LL group.

Figure S9. Mean number of valid pupil dilation trials by group and condition.

Figure S10. Box- and whisker plots of slope coefficients of the (within subject) trial number * pupil dilation function, non-social sound condition only.

Figure S11. Mean pupil size in baseline segment by group and condition.

Table S1. Group * Condition ANOVA of pupil dilation 10 months.

Table S2. Estimated marginal means.

Table S3. Longitudinal analysis: Tests of fixed effects.

Table S4. Longitudinal analysis: Descriptive statistics – Mean (SD).

Table S5. Longitudinal analysis: Estimates of fixed effect parameters.

Table S6. Median and inter-quartile range (IQR) of percent missing data per pupil dilation trial divided on group, and number of valid trials per group.

Video S1. Recording of the visual and auditory stimuli as was presented to the infants in the study.

Video S2. Recording of the visual and auditory stimuli as was presented to the infants in the study.

Acknowledgements

The authors would like to thank the participating children and families and the EASE team members. This study was funded by the Swedish Research Council (2018-06232), Riksbankens Jubileumsfond in collaboration with the Swedish Collegium for Advanced Study (Pro Futura), and the Knut and Alice Wallenberg Foundation; and the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 777394. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and AUTISM SPEAKS, Autistica, SFARI. The work leading to these results was also supported by funds from the European Commission (H2020 project CANDY; Grant No. 847818). S.B. discloses that he has in the last 5 years acted as an author, consultant or lecturer for Medice and Roche. He receives royalties for textbooks and diagnostic tools from Hogrefe (ADOS-2, ADI-R, SRS-2, SCQ), Kohlhammer, and UTB. The authors have declared that they have no competing or potential conflicts of interest.

Correspondence

Maja Rudling, Department of Psychology, Uppsala University, Box 1225, 751 42 Uppsala, Sweden; Email: maja.rudling@psyk.uu.se

Key points

- Reduced sensitivity to social stimuli in infancy may be an early marker of autism but few studies have assessed infant responsivity to sounds.
- Pupil dilation is a measure of attentional responsiveness which is linked to arousal systems in the brain that facilitates perceptual prioritisation.
- We found that infants with subsequent autism show stronger pupil dilation to the nonsocial sounds than typically developing infants, whereas for the social sounds there was no group difference.
- The results of this study suggests that infants with subsequent autism react with stronger attentional alerting responses to certain everyday nonsocial sounds.
- The results could have implications for early identification and intervention in autism.

References

- American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders* (5th edn). Arlington, VA: Author.
- Aston-Jones, G., & Cohen, J.D. (2005). An integrative theory of locus coeruleus-norepinephrine function: Adaptive gain and optimal performance. *Annual Review of Neuroscience*, 28, 403–450.
- Bala, A.D.S., Whitchurch, E.A., & Takahashi, T.T. (2020). Human auditory detection and discrimination measured with the pupil dilation response. *Journal of the Association for Research in Otolaryngology*, 21, 43–59.
- Baranek, G.T., Watson, L.R., Boyd, B.A., Poe, M.D., David, F.J., & McGuire, L. (2013). Hyporesponsiveness to social and nonsocial sensory stimuli in children with autism, children with developmental delays, and typically developing children. *Development and Psychopathology*, 25, 307–320.
- Bast, N., Poustka, L., & Freitag, C.M. (2018). The locus coeruleus-norepinephrine system as pacemaker of attention – A developmental mechanism of derailed attentional function in autism spectrum disorder. *European Journal of Neuroscience*, 47, 115–125.
- Ceponiene, R., Lepisto, T., Shestakova, A., Vanhala, R., Alku, P., Naatanen, R., & Yaguchi, K. (2003). Speech-sound-selective auditory impairment in children with autism: They

- can perceive but do not attend. *Proceedings of the National Academy of Sciences*, *100*, 5567–5572.
- Cui, T., Wang, P.P., Liu, S., & Zhang, X. (2017). P300 amplitude and latency in autism spectrum disorder: A meta-analysis. *European Child and Adolescent Psychiatry*, *26*, 177–190.
- de Barbaro, K., Clackson, K., & Wass, S.V. (2017). Infant attention is dynamically modulated with changing arousal levels. *Child Development*, *88*, 629–639.
- Eldar, E., Cohen, J.D., & Niv, Y. (2013). The effects of neural gain on attention and learning. *Nature Neuroscience*, *16*, 1146–1153.
- Falck-Ytter, T., Nyström, P., Gredebäck, G., Gliga, T., & Bölte, S. (2018). Reduced orienting to audiovisual synchrony in infancy predicts autism diagnosis at 3 years of age. *Journal of Child Psychology and Psychiatry*, *59*, 872–880.
- Granovetter, M.C., Burlingham, C.S., Blauch, N.M., Minshew, N.J., Heeger, D.J., & Behrmann, M. (2020). Uncharacteristic task-evoked pupillary responses implicate atypical locus coeruleus activity in autism. *Journal of Neuroscience*, *40*, 3815–3826.
- Guiraud, J.A., Kushnerenko, E., Tomalski, P., Davies, K., Ribeiro, H., & Johnson, M.H. (2011). Differential habituation to repeated sounds in infants at high risk for autism. *NeuroReport*, *22*, 845–849.
- Hazlett, H.C., Gu, H., Munsell, B.C., Kim, S.H., Styner, M., Wolff, J.J., ... & Piven, J. (2017). Early brain development in infants at high risk for autism spectrum disorder. *Nature*, *542*, 348–351.
- Jamal, W., Cardinaux, A., Haskins, A.J., Kjelgaard, M., & Sinha, P. (2020). Reduced sensory habituation in autism and its correlation with behavioral measures. *Journal of Autism and Developmental Disorders*, *51*, 3153–3164.
- Johnson, M.H., Charman, T., Pickles, A., & Jones, E.J.H. (2021). Anterior Modifiers in the Emergence of Neurodevelopmental Disorders (AMEND)—A systems neuroscience approach to common developmental disorders. *Journal of Child Psychology and Psychiatry*, *62*, 610–630.
- Jones, W., & Klin, A. (2013). Attention to eyes is present but in decline in 2–6-month-old infants later diagnosed with autism. *Nature*, *504*, 427–431.
- Joshi, S., Li, Y., Kalwani, R.M., & Gold, J.I. (2016). Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. *Neuron*, *89*, 221–234.
- Klin, A. (1991). Young autistic children's listening preferences in regard to speech: A possible characterization of the symptom of social withdrawal. *Journal of Autism and Developmental Disorders*, *21*, 29–42.
- Kolesnik, A., Begum Ali, J., Gliga, T., Guiraud, J., Charman, T., Johnson, M.H., & Jones, E.J.H. (2019). Increased cortical reactivity to repeated tones at 8 months in infants with later ASD. *Translational Psychiatry*, *9*, 1–11.
- Laeng, B., Sirois, S., & Gredebäck, G. (2012). Pupillometry: A window to the preconscious? *Perspectives on Psychological Science*, *7*, 18–27.
- Larsen, R.S., & Waters, J. (2018). Neuromodulatory correlates of pupil dilation. *Frontiers in Neural Circuits*, *12*, 1–9.
- Lawson, R.P., Mathys, C., & Rees, G. (2017). Adults with autism overestimate the volatility of the sensory environment. *Nature Neuroscience*, *20*, 1293–1299.
- Lloyd-Fox, S., Blasi, A., Pasco, G., Gliga, T., Jones, E.J.H., Murphy, D.G.M., ... & Johnson, M.H. (2018). Cortical responses before 6 months of life associate with later autism. *European Journal of Neuroscience*, *47*, 736–749.
- Lord, C., Luyster, R., Gotham, K., & Guthrie, W. (2012). *Autism diagnostic observation schedule, second edition (ADOS-2) manual (Part II): Toddler module*. Torrance, CA: Western Psychological Services.
- Loth, E., Spooen, W., Ham, L.M., Isaac, M.B., Auriche-Benichou, C., Banaschewski, T., ... & Murphy, D.G.M. (2016). Identification and validation of biomarkers for autism spectrum disorders. *Nature Reviews Drug Discovery*, *15*, 70–73.
- Mather, M., Clewett, D., Sakaki, M., & Harley, C.W. (2016). Norepinephrine ignites local hotspots of neuronal excitation: How arousal amplifies selectivity in perception and memory. *Behavioral and Brain Sciences*, *39*, 1–75.
- Mathôt, S., Fabius, J., van Heusden, E., & Van der Stigchel, S. (2018). Safe and sensible baseline correction of pupil-size data. *Behavior Research Methods*, *50*, 94–106.
- Mercati, O., Huguet, G., Danckaert, A., André-Leroux, G., Maruani, A., Bellinzoni, M., ... & Bourgeron, T. (2017). CNTN6 mutations are risk factors for abnormal auditory sensory perception in autism spectrum disorders. *Molecular Psychiatry*, *22*, 625–633.
- Mullen, E.M. (1995). *Mullen Scales of Early Learning (AGS ed.)*. Circle Pines, MN: American Guidance Service.
- Nyström, P., Falck-Ytter, T., & Gredebäck, G. (2016). The TimeStudio Project: An open source scientific workflow system for the behavioral and brain sciences. *Behavior Research Methods*, *48*, 542–552.
- Nyström, P., Thorup, E., Bölte, S., & Falck-Ytter, T. (2019). Joint attention in infancy and the emergence of autism. *Biological Psychiatry*, *86*, 631–638.
- O'Connor, K. (2012). Auditory processing in autism spectrum disorder: A review. *Neuroscience and Biobehavioral Reviews*, *36*, 836–854.
- Ozonoff, S., Young, G.S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., ... & Stone, W.L. (2011). Recurrence risk for autism spectrum disorders: A baby siblings research consortium study. *Pediatrics*, *128*, 488–495.
- Pierce, K., Conant, D., Hazin, R., Stoner, R., & Desmond, J. (2011). Preference for geometric patterns early in life as a risk factor for autism. *Archives of General Psychiatry*, *68*, 101–109.
- Reimer, J., McGinley, M.J., Liu, Y., Rodenkirch, C., Wang, Q., McCormick, D.A., & Tolia, A.S. (2016). Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. *Nature Communications*, *7*, 1–7.
- Rubenstein, J.L.R., & Merzenich, M.M. (2003). Model of autism: Increased ratio of excitation/inhibition in key neural systems. *Genes Brain & Behavior*, *2*, 255–267.
- Rutter, M., Le Couteur, A., & Lord, C. (2003). *Autism Diagnostic Interview – Revised (ADI-R)*. Los Angeles, CA: Western Psychological Services.
- Sara, S.J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature Reviews Neuroscience*, *10*, 211–223.
- Schwartz, S., Shinn-Cunningham, B., & Tager-Flusberg, H. (2018). Meta-analysis and systematic review of the literature characterizing auditory mismatch negativity in individuals with autism. *Neuroscience and Biobehavioral Reviews*, *87*, 106–117.
- Sparrow, S.S., Cicchetti, D.V., & Balla, D.A. (2005). *Vineland adaptive behavior scales: Second edition (Vineland II), survey interview form/caregiver rating form*. Livonia, MN: Pearson Assessments.

Accepted for publication: 17 August 2021