

Idiosyncratic pupil regulation in autistic children

Isabel Bleimeister^{1,2*}, Inbar Avni^{3,2,*}, Michael Granovetter⁴, Gal Meiri^{5,2}, Michal Ilan^{1,2,5} Analya Michaelovski^{6,2}, Idan Menashe^{7,2}, Marlene Behrmann⁴, Ilan Dinstein^{1,2,3}

1. Psychology Department, Ben Gurion University of the Negev, Beer Sheva, Israel 84105
2. Azrieli National Centre for Autism and Neurodevelopment Research, Ben Gurion University of the Negev, Beer Sheva, Israel
3. Cognitive and Brain Sciences Department, Ben Gurion University of the Negev, Beer Sheva, Israel
4. Department of Ophthalmology, University of Pittsburgh, Pittsburgh, U.S.A 15213
5. Pre-school Psychiatry Unit, Soroka Medical Center, Beer Sheva, Israel 84105
6. Child Development Institute, Soroka Medical Center, Beer Sheva, Israel 84105
7. Public Health Department, Ben-Gurion University, Beer Sheva, Israel 84105

* Equal contribution as first author

Correspondence: Isabel Bleimeister isb30@pitt.edu

1 **Abstract**

2 Recent neuroimaging and eye tracking studies have suggested that children with autism spectrum
3 disorder (ASD) may exhibit more variable and idiosyncratic brain responses and eye movements
4 than typically developing (TD) children. Here we extended this research for the first time to
5 pupillometry recordings. We successfully completed pupillometry recordings with 103 children (66
6 with ASD), 4.5-years-old on average, who viewed three 90 second movies, twice. We extracted
7 their pupillary time-course for each movie, capturing their stimulus evoked pupillary responses.
8 We then computed the correlation between the time-course of each child and those of all others
9 in their group. This yielded an average inter-subject correlation value per child, representing how
10 similar their pupillary responses were to all others in their group. ASD participants exhibited
11 significantly weaker inter-subject correlations than TD participants, reliably across all three
12 movies. Differences across groups were largest in responses to a naturalistic movie containing
13 footage of a social interaction between two TD children. This measure enabled classification of
14 ASD and TD children with a sensitivity of 0.82 and specificity of 0.73 when trained and tested on
15 independent datasets. Using the largest ASD pupillometry dataset to date, we demonstrate the utility of a new
16 technique for measuring the idiosyncrasy of pupil regulation, which can be completed even by young
17 children with co-occurring intellectual disability. These findings reveal that a considerable
18 subgroup of ASD children have significantly more unstable, idiosyncratic pupil regulation than TD
19 children, indicative of more variable, weakly regulated, underlying neural activity.

20 Introduction

21 Pupillometry studies in children with ASD have generated considerable interest in recent
22 years (1). Pupil size is regulated by the pupillary light reflex (PLR) (2) and by Norepinephrine (NE)
23 release from the Locus Coeruleus (LC) (3), which also regulates arousal, attention, and
24 exploration/exploitation behaviors (4,5). Hence, potential differences in pupillary responses
25 between ASD and typically developing (TD) individuals could indicate underlying physiological
26 differences in LC-NE and/or PLR function (6).

27 Several pupillometry studies have reported results from comparisons of relatively small
28 ASD and TD samples (20-30 per group). Studies measuring baseline pupil diameter during stable
29 luminance have reported mixed results with some reporting larger pupil size in ASD relative to TD
30 (7,8) while others have reported the opposite (9), and, yet others report no difference across
31 groups (1,10,11). In contrast, pupil dilation responses to target/novel stimuli with identical
32 luminance (e.g., in oddball tasks) is consistently weaker in ASD (7,8,10), particularly in tasks with
33 higher attentional load (e.g., one-back memory task with added distractors) (11). Smaller pupil
34 dilation suggests weaker LC-NE activity in ASD. Similarly, pupil constriction responses to increased
35 luminance were weaker (i.e., weaker PLR) (12,13) and delayed in time (1) in children and adults
36 with ASD relative to controls. But interestingly, abnormally stronger PLR was reported in 9–10-
37 month-old toddlers who developed ASD at later ages, suggesting different abnormalities during
38 early versus late ASD development (14).

39 Taken together, the studies above suggest that pupillary responses in ASD children and
40 adults are muted, with LC-NE generating weaker pupil dilations during attention demanding tasks
41 or in response to novel/surprising stimuli, and PLR generating weaker pupil constrictions in
42 response to increased luminance. Because these differential pupillary responses generated by LC-
43 NE (10) and PLR (12,13) are apparent even during passive viewing of sensory stimuli in the
44 absence of a task, they may serve a useful role in distinguishing between ASD and TD individuals in
45 situations where task performance is not possible (e.g., non-verbal children). Hence, there is clear
46 motivation to develop task-free, experimental protocols with child-friendly, engaging stimuli for
47 this purpose.

48 Previous studies have demonstrated the high ecological validity of using naturalistic movies
49 to study brain function with fMRI (15), especially in young children (16–18). Of particular interest
50 are studies demonstrating that ASD individuals exhibit significantly more variable and idiosyncratic
51 brain responses than TD individuals when observing movies (19,20). These studies quantified
52 idiosyncrasy by measuring inter-subject correlation (inter-SC), which revealed that cortical activity
53 was more strongly correlated across TD individuals observing the same movie than across ASD
54 participants. ASD participants exhibited more idiosyncratic and unique cortical responses with

55 larger between-subject variability. In a recent study we applied the same inter-SC technique to
56 recordings of gaze position during natural viewing of movies. We demonstrated that ASD children
57 also exhibited weaker inter-SC magnitudes, gazing at movies less consistently than TD children and
58 exhibiting more idiosyncratic gaze patterns (21).

59 Here, we extend this research further by applying the inter-SC approach for the first time
60 to pupillometry data in ASD children. Importantly, we established the largest pupillometry dataset
61 to date from ASD children using an experimental design with 3 different movies, each presented
62 twice, which enabled us to assess the reliability of findings across movies and presentations.

63

64 **Methods**

65 A subset of the eye-tracking recordings examined in the current study were analyzed previously to
66 compare gaze patterns across ASD and TD children in a separate study (21). Here, we extracted
67 and analyzed pupillometry data from these and additional recordings.

68 **Participants**

69 We initially recruited 121 children for the current study through the Azrieli National Centre for
70 Autism and Neurodevelopment Research, between 2016 and 2019. Of these, 81 were diagnosed
71 with ASD according to DSM-V criteria (mean age: 4.46 ± 1.91 years; age range: 1.09-10.07 years; 64
72 male, 17 female) and 40 were TD (mean age: 4.28 ± 2.10 years; age range: 1.03-10.03 years; 26
73 male, 14 female). One TD child was removed for having a Social Responsiveness Scale (SRS) score
74 greater than the clinical cut-off (22,23). In addition, data from 15 ASD and an additional 2 TD
75 children were excluded due to low eye tracking quality (see details below), yielding a final sample
76 of 103 children (Table 1). The study was approved by the Soroka Medical Center Helsinki
77 committee and the Ben Gurion University Internal Review Board committee. Written informed
78 consent was obtained from all parents.

79 Most of the participating ASD children (52 out of 66) completed the Autism Diagnostic
80 Observation Schedule – 2nd edition (ADOS-2) (24). In addition, 46 of the 66 ASD children
81 completed either a Wechsler Preschool and Primary Scale of Intelligence (25) or the Bayley
82 Cognitive Scales test (26). TD children did not complete ADOS-2 or cognitive tests, but all parents
83 of the TD children, except in three instances, completed the Social Responsiveness Scale (SRS)
84 (22).

85

86

87 **Table 1: Participant characteristics**

Variable	Mean	SD	Range
ASD group (n=66, 53 males)			
Age at eye tracking (months)	57.70	22.83	21-127
ADOS-2 Total CSS (n=52)	6.69	2.65	1-10
ADOS-2 SA CSS (n=52)	6.42	2.59	1-10
ADOS-2 RRB CSS (n=52)	7.38	2.47	1-10
Cognitive Scores (n=46)	80.39	16.80	49-117

89 **TD group (n=37, 24 males)**

Age at eye tracking (months)	53.30	22.43	15-123
SRS scores (n=34)	34.21	12.68	11-57

90 **Data acquisition**

91 Participants were seated approximately 60cm from the display screen and the left eye pupil
92 diameter was recorded using an EyeLink 1000+ head-free eye tracking system with a sampling rate
93 of 500 Hz (SR Research Inc. Canada). The participants' head position was tracked using a sticker
94 placed on their forehead. An infrared camera, located below the display screen, measured pupil
95 size. For each participant, the eye tracker was calibrated prior to data collection: the participant
96 made saccades to each of five stimuli presented sequentially on the screen, and gaze accuracy was
97 then validated to be <2 degrees. Additional validations of calibration accuracy were performed
98 after each movie and re-calibration was performed if error >2 degrees. Experiment Builder and
99 Data Viewer (SR Research Inc. Canada) were used to construct the experiment and visualize the
100 data.

101 **Experimental design**

102 Once calibration was successfully completed, participants were shown three different movie clips,
103 each presented twice. Each movie was 1.5 minutes long and the total duration of the experiment
104 was roughly 10 minutes. The first movie segment, from the Pixar animation "Jack-Jack Attack",
105 showed the adventures of a babysitter taking care of an infant with supernatural powers. The
106 second movie segment, taken from the Walt Disney animation "The Jungle Book", contained a
107 segment in which Mowgli meets the Monkey King who sings and dances while interacting with

108 other monkeys. The third movie contained an un-cut home-video with two sisters (2 and 5 years
109 old) interacting socially in a typical, messy room containing everyday objects.

110 **Pre-processing and data cleaning**

111 The Eyelink 1000+ records the pupil area as the number of pixels within the image area identified
112 as the pupil, which is equivalent to the angular area of the pupil (27). We identified and removed
113 data segments where the eye tracker lost track of the children's pupil due to eye blinks and off-
114 screen gazes. This included segments with timepoints where the pupil size equaled zero, was
115 larger than 1200 pixels, or where the pupil size changed faster than 5 pixels per ms – all of which
116 are physiologically implausible. Movies where more than 40% of the data was removed, were
117 entirely excluded from further analysis. We removed 197 of 474 (41.6%) movies observed by ASD
118 children and 43 of 234 (18.4%) movies observed by TD children. This yielded the final sample
119 described above of 66 ASD children and 37 TD children who contributed at least one movie to the
120 analyses.

121 Of the remaining movies, we removed segments according to the criteria described above such
122 that $16.3 \pm 8.6\%$ and $10.9 \pm 9.0\%$ of the data were removed from recordings of ASD and TD children,
123 respectively (note that, below, we take the amount of removed data into account in the statistical
124 analysis of the data). We also excluded the first second of each movie to minimize potential
125 stimulus onset responses. Removed time-points were set to NaN values and the remaining
126 segments of analyzed data were smoothed using a Gaussian filter with a width of 250 samples
127 (i.e., half a second). Finally, pupil diameter was computed from angular pupil area using the
128 following formula:

129
$$d = \alpha L \phi$$

130 Where d is the pupil diameter, α is a scaling factor deduced empirically by measuring an artificial
131 pupil with known area at a distance of 60cm using our setup (15mm lens, $\alpha=0.248$), L is the
132 distance between the participant and the screen (i.e., 60cm), and ϕ is the visual angle of the pupil,
133 which is the square root of the angular pupil area measured by the eye tracker (see Eyelink
134 documentation and Hayes and Petrov, 2016).

135 **Data Analysis**

136 All analyses were performed with custom written code in MATLAB (Mathworks Inc., USA). We
137 extracted time-courses of pupil diameter in mm units for each movie, separately for first and
138 second presentations, per participant. We computed the inter-subject correlation (inter-SC) per
139 movie presentation by correlating the pupil time-courses across all pairs of participants within
140 each group (ASD or TD). We then computed the mean correlation for each child and all other
141 children in their group, yielding a mean inter-SC value per participant. We also computed an intra-

142 subject correlation (intra-SC) value by correlating the pupil time-courses across the two
143 presentations of each movie, per participant.

144 **Statistical Analyses**

145 To determine whether there were significant differences in the percent of excluded data across
146 groups, we performed a univariate ANOVA analysis with movie type (Jack-Jack Attack, Jungle
147 Book, Naturalistic) as the dependent variable and diagnostic group (ASD and TD) as the between-
148 subjects factor. To evaluate whether there were significant differences in tonic pupil size, variance,
149 inter-SC, and intra-SC across groups, we performed univariate ANCOVAs for each movie with
150 diagnostic group (ASD and TD) as the between-subjects factor, and percent of valid data and age
151 as covariates. Correlations between pupil diameter and behavioral measures (ADOS, cognitive
152 scores) were assessed with Pearson's correlation coefficient. Finally, we derived receiver operating
153 characteristic (ROC) analyses for the classification of ASD and TD children using the mean inter-SC
154 measure as calculated for each movie per first/second presentation. We computed the Youden
155 index to determine the optimal threshold for separating ASD and TD groups according to the inter-
156 SC values from the first movie presentation and then tested the accuracy of the threshold for
157 separating ASD and TD individuals using the inter-SC values from the second movie presentation.

158

159 **Results**

160 Initial analyses demonstrated that there were significantly more excluded/invalid data in
161 recordings of ASD versus TD children in the first presentation of the Jack-Jack Attack ($F(1)=16.86$,
162 $p=0.0001$, $\eta^2=0.18$) and Jungle Book ($F(1)=10.21$, $p=0.002$, $\eta^2=0.12$) movies. We, therefore,
163 included the percent of valid data as a covariate in all further analyses. We also added age as a
164 covariate to ensure that potential differences across groups were not attributable to this variable.
165 The percent of valid data did correlate positively with the age of the ASD children ($r(64)=0.23$,
166 $p=0.059$) but not with their ADOS-2 (ADOS-2 SA: $r(50)=-0.18$, $p=0.19$; ADOS-2 RRB: $r(50)=-0.23$,
167 $p=0.095$; ADOS-2 Total: $r(50)=-0.19$, $p=0.19$) or cognitive ($r(44)=0.061$, $p=0.69$) scores. The ability
168 to contribute valid data in this eye tracking study was, therefore, not significantly associated with
169 the cognitive abilities or core ASD symptom severity of the ASD children.

170

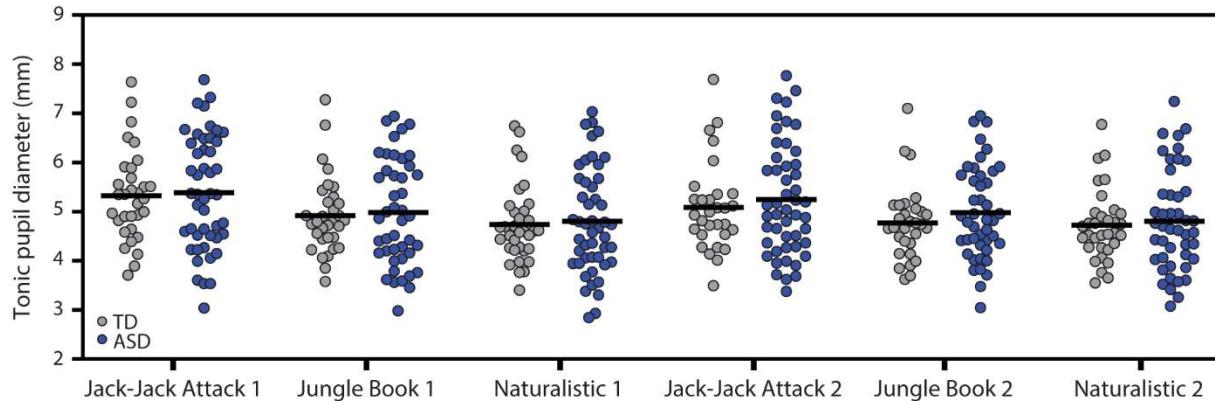
171 **No group difference in tonic pupil size**

172 Tonic pupil size, estimated as the mean pupil diameter across all included timepoints of each
173 movie, revealed similar values across ASD and TD participants in all movies and in both
174 presentations (Figure 1). ANCOVA analyses, per movie, demonstrated no significant differences in
175 tonic pupil size across groups for any of the movies ($F(1)<0.65$, $p>0.42$, $\eta^2<0.009$), with no effect of

176 age ($F(1)<1.22, p>0.27, \eta^2<0.017$) or percent of valid data ($F(1)<0.86, p>0.36, \eta^2<0.012$). Moreover,
177 the tonic pupil size of individual children in both groups was highly reproducible and significantly
178 correlated across the two presentations of each movie, reflecting the high intra-subject reliability
179 of this measure (TD: $r>0.93, p<0.001$; ASD: $r>0.94, p<0.001$; Figure 1 B-D).

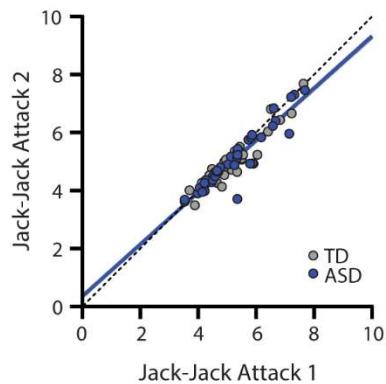
A

Tonic pupil size by group and movie



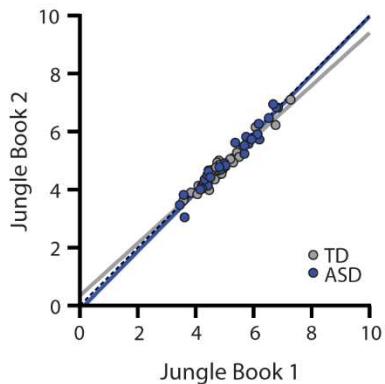
B

Tonic pupil size



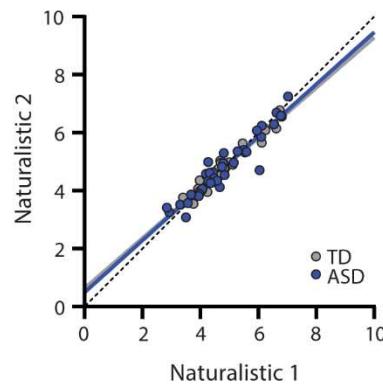
C

Tonic pupil size



D

Tonic pupil size



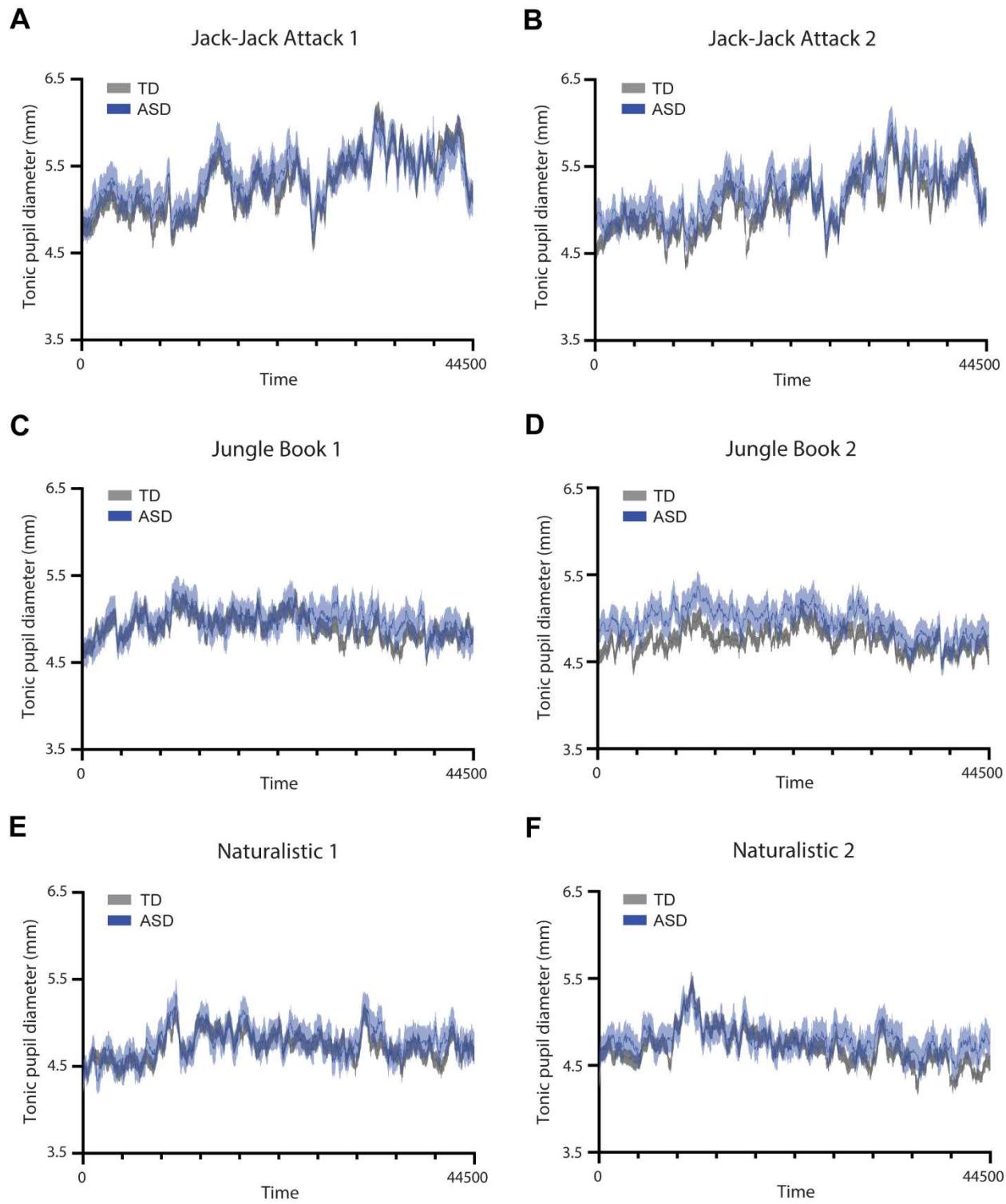
180
181 **Figure 1:** Tonic pupil diameter (mm) in each of the six movies. A. Scatter plot demonstrating tonic
182 pupil diameter for each individual, across both groups, per movie. Black line: mean tonic pupil
183 diameter per group. There were no significant differences across groups. B-D. Scatter plots
184 demonstrating stability of tonic pupil diameter per participant across two presentations of each
185 movie. Each point represents a single child. Solid lines: Least squares fit. Dotted line: unity line.
186 Blue: ASD, Gray: TD.

187

188 **Reproducible stimulus-evoked pupillary time-courses**

189 Each movie elicited a unique pupillary time-course, generated by its unique visual content. The
190 mean pupillary time-courses were highly similar across ASD and TD groups per movie (Figure 2)

191 with strong correlations across groups in the Jack-Jack Attack (first presentation: $r=0.93$, $p<0.001$;
192 second presentation: $r=0.91$, $p<0.001$), Jungle Book (first presentation: $r=0.65$, $p<0.001$; second
193 presentation: $r=0.76$, $p<0.001$), and Naturalistic (first presentation: $r=0.79$, $p<0.001$; second
194 presentation: $r=0.81$, $p<0.001$) movies. Correlations were also strong across the two presentations
195 of the Jack-Jack Attack (ASD: $r=0.89$, $p<0.001$; TD: $r=0.94$, $p<0.001$), Jungle Book (ASD: $r=0.59$,
196 $p<0.001$; TD: $r=0.7$, $p<0.001$), and Naturalistic (ASD: $r=0.51$, $p<0.001$; TD: $r=0.62$, $p<0.001$) movies.
197 In contrast, correlations across the different movies were weak and sometimes negative such that,
198 on average, the correlations in both groups were close to zero (TD: $r=0.03$, $p<0.001$; ASD: $r=0.06$,
199 $p<0.001$). While all correlation coefficients were significant due to the large number of degrees of
200 freedom (pupillary time-courses had 44,500 samples), all within-movie correlations were of large
201 effect size ($r>0.5$) while between-movie correlations were of negligible effect size ($r\leq0.06$). Taken
202 together, these results demonstrate movie-evoked pupillary time-courses were highly
203 reproducible across presentations and unique to each movie in data from both groups of
204 participants (Figure 2).



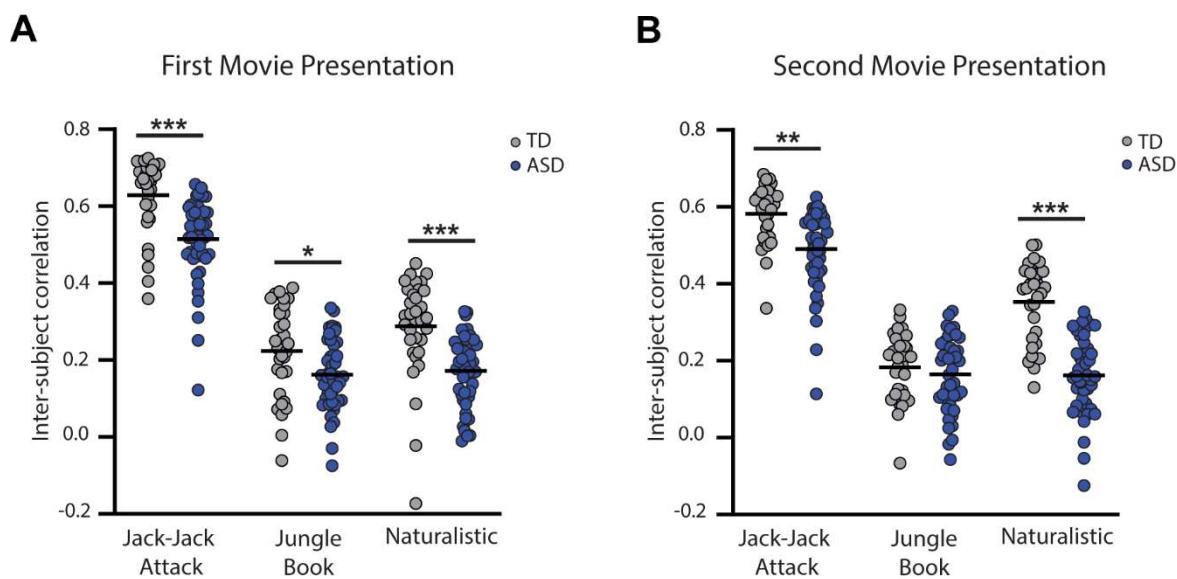
205
206 **Figure 2:** Time-courses of pupil diameter from each presentation of the three movies. A, C, E: First
207 presentation. B, D, F: Second presentation. Solid line: average pupil diameter across participants
208 per group. Shaded area: standard error of the mean. Blue: ASD. Gray: TD.

209

210 **Pupillary inter-subject correlations (inter-SC) are consistently weaker in ASD**

211 We computed the pair-wise correlation between the pupillary time-course of a given child and
212 each of the other children in the child's group, and then computed the mean correlation across all
213 pairs, yielding an inter-SC value per child for each movie presentation (Figure 3). This value
214 represents the similarity of stimulus-evoked pupillary changes between each child and all others in
215 their group.

216 ANCOVA analyses, with age and percent valid data included as covariates, revealed that inter-SC
217 values were significantly lower in the ASD group in both presentations of Jack-Jack Attack (first
218 presentation: $F(1)=11.95$, $p<0.001$, $\eta^2=0.14$; second presentation: $F(1)=9.63$, $p=0.003$, $\eta^2=0.11$),
219 both presentations of the Naturalistic movie (first presentation: $F(1)=16.28$, $p<0.001$, $\eta^2=0.17$;
220 second presentation: $F(1)=56.49$, $p<0.001$, $\eta^2=0.43$), and the first presentation of the Jungle Book
221 movie ($F(1)=6.26$, $p=0.015$, $\eta^2=0.08$). There were no significant differences in the second
222 presentation of the Jungle book movie ($F(1)=0.68$, $p=0.41$, $\eta^2=0.01$).



223

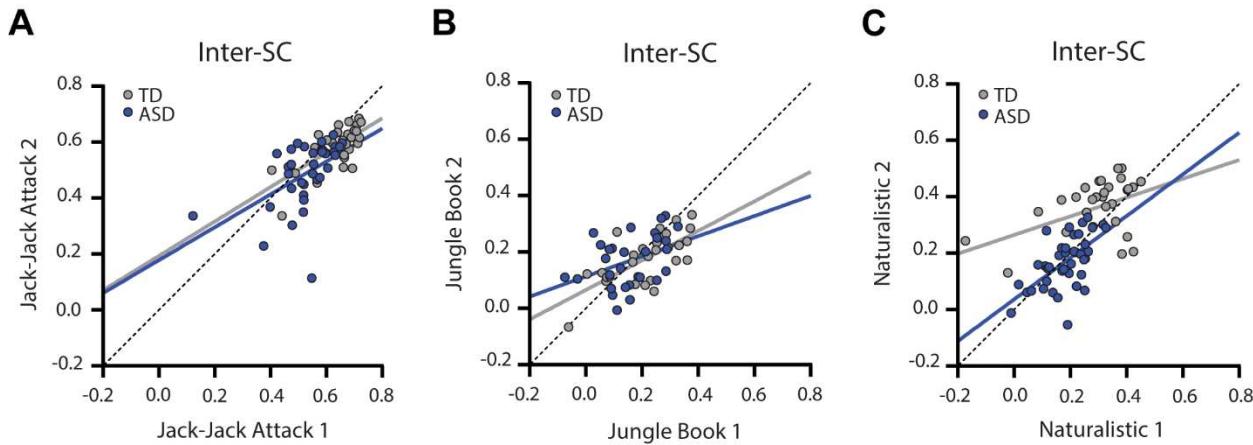
224 **Figure 3:** Comparison of inter-subject correlation values across groups for each movie and
225 presentation. A: First presentation. B: Second presentation. Blue: ASD, Gray: TD. Asterisks:
226 Significant differences across groups according to ANCOVA tests (* $p<0.05$, ** $p<0.01$, ***
227 $p<0.001$).

228

229 **Pupillary inter-SC are reliable across presentations**

230 Individual inter-SC magnitudes were significantly correlated across presentations of the Jack-Jack
231 (ASD: $r(34)=0.51$, $p=0.0016$; TD: $r(27)=0.70$, $p<0.001$), Jungle Book (ASD: $r(27)=0.40$, $p=0.032$; TD:

232 $r(25)=0.69, p<0.001$), and Naturalistic (ASD: $r(37)=0.62, p<0.001$; TD: $r(28)=0.44, p=0.015$) movie
233 presentations. This demonstrates that the inter-SC measure exhibited relatively high test-retest
234 reliability in both ASD and TD children.



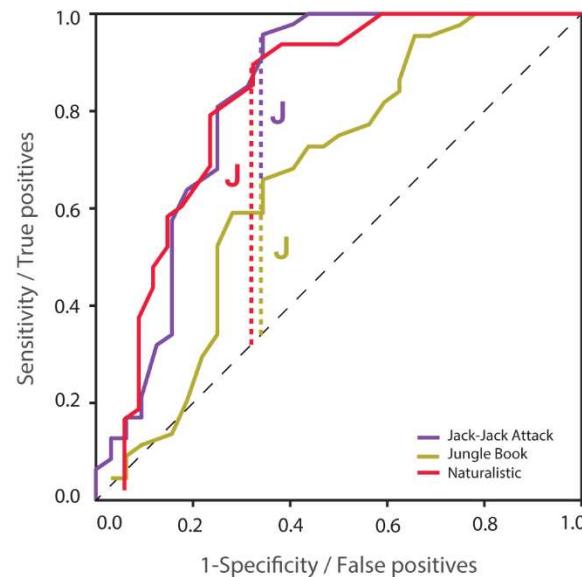
235
236 **Figure 4:** Scatter plots of inter-SC values demonstrating their correlation across the two
237 presentations of Jack-Jack Attack (A), Jungle Book (B), and Naturalistic (C) movies. Blue: ASD, Gray:
238 TD. Solid lines: Least squares fit. Dotted line: unity line.

239
240 **Classification of ASD and TD children according to their inter-SC**
241 Given the group differences described above, we performed an ROC analysis to quantify the ability
242 of each movie to classify ASD and TD individuals according to their inter-SC values (Figure 5). We
243 compared classification across movies using the Youden Index (J) (28), which identifies the inter-SC
244 value (i.e., classification threshold) that yields the highest sum of sensitivity and specificity
245 (optimal point on the ROC). The optimal inter-SC values when analyzing the first presentation of
246 the Jack-Jack Attack, Jungle Book, and Naturalistic movies were 0.64, 0.2, and 0.27, yielding
247 sensitivity/specificity values of 0.96/0.66, 0.66/0.66, and 0.9/0.68, respectively.

248 Applying these inter-SC thresholds to data from the second movie presentation (i.e., independent
249 sample) yielded sensitivity/specificity values of 1/0.2, 0.52/0.5, and 0.82/0.73, respectively. Taken
250 together, these results suggest that the Naturalistic movie yielded the most reliable between-
251 group classification accuracy.

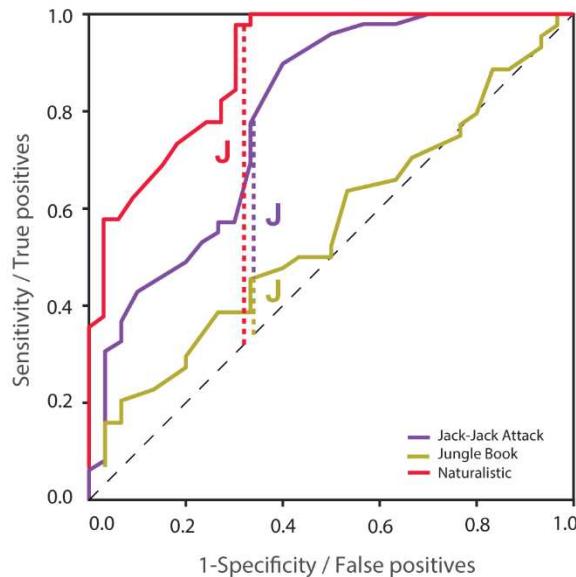
A

ROC curve first movie presentation



B

ROC curve second movie presentation



252

253 Figure 5: ROC analyses using inter-SC to classify ASD and TD participants. A: First presentation. B:
254 Second presentation. Vertical dashed lines represent the optimal classification threshold as
255 determined using Youden's J statistic for the Jack-Jack Attack (purple), Jungle Book (yellow), and
256 Naturalistic (red) movies. Optimal classification thresholds were determined with data from the
257 first presentation (left panel) and their accuracy was tested with data from the second
258 presentation (right panel). Black dashed line: unity line.

259

260 **Discussion**

261 Our results reveal that ASD children exhibit pupillary responses that are significantly more
262 idiosyncratic (i.e., vary from one individual to another) than those of TD children, consistently
263 across three different movies (Figure 3). The group-average pupillary time-courses were highly
264 correlated across ASD and TD groups (Figure 2) demonstrating that each movie elicited a unique
265 and reliable stimulus-driven pupillary time-course, on average. However, pupillary time-courses of
266 ASD children diverged from the mean (i.e., weaker inter-SC) to a larger extent than those of TD
267 children. Individual magnitudes of inter-SC were strongly correlated across presentations (Figure
268 4) demonstrating that idiosyncrasy (how different one is from the group) is a reproducible
269 individual characteristic.

270 Inter-SC differences were most pronounced in the pupillary responses to a naturalistic un-cut
271 home-video of two TD children engaged in a social interaction. Extracting a simple inter-SC
272 threshold based on ROC analysis and Youden Index using data from the first presentation of this

273 movie, enabled us to classify ASD and TD children with 82% sensitivity and 73% specificity in held-
274 out independent data from the second movie presentation (Figure 5). These results highlight the
275 potential clinical utility of the inter-SC measure for identifying many ASD individuals when using
276 pupillometry recordings during movies with naturalistic social interactions.

277 These results indicate that pupil size regulation by LPR and LC-NE mechanisms is more variable
278 (i.e., less reliable) in children with ASD than in TD controls. Such an interpretation would be
279 consistent with previous hypotheses proposing that sensory neural responses in the visual,
280 auditory, and tactile domains may be more variable in some ASD individuals (29,30).

281 ***Idiosyncrasy rather than consistent group differences***

282 A growing body of literature demonstrates that ASD individuals exhibit idiosyncratic behavioral
283 and physiological responses that differ from TD responses. Examples include idiosyncratic fMRI
284 activation time-courses in response to movies (19,20), idiosyncratic resting-state fMRI functional
285 connectivity (31–33), idiosyncratic gaze patterns when observing movies (21,34), as well as other
286 behavioral idiosyncrasies (35,36). These studies suggest that rather than exhibiting consistently
287 weaker or stronger behavioral or physiological responses, ASD individuals tend to differ from one
288 another to larger extents and in unique ways. This means that, on average, measures may be
289 similar to those of TD individuals, yet ASD individuals are scattered further away from the mean.

290 Quantifying individual idiosyncrasy, as calculated here with inter-SC, reveals that the majority, but
291 not all ASD participants exhibit large idiosyncrasy. Our results demonstrated that idiosyncratic
292 pupillometry responses to the Naturalistic movie (defined by a classification threshold of inter-
293 SC<0.27) were apparent in 82% of the ASD participants and only 27% of the TD participants (see
294 ROC analysis). For comparison, a previous fMRI study reported that about one third of ASD
295 participants exhibited distinctive idiosyncratic brain activations when observing a movie (20). In
296 the context of the current study, the high selectivity and specificity for classifying ASD/TD group
297 membership suggests that quantification of pupillometry idiosyncrasy may be useful for
298 identifying a large group of ASD children with poorer LPR and LC-NE regulation.

299 Importantly, the approach outlined here does not require cooperation or compliance with an
300 explicit task, which may be difficult for the more severe or younger ASD individuals, thereby
301 extending its utility and generalizability to the broader ASD population. Future studies could assess
302 whether idiosyncrasy magnitudes are similarly apparent across multiple behavioral and
303 physiological domains and multiple sensory modalities. This could reveal potential generalized
304 behavioral and neural idiosyncrasy or specific idiosyncrasy in particular domains, in distinct
305 subgroups.

306

307 ***Pupillometry abnormalities in ASD***

308 A variety of studies have reported that ASD participants may exhibit different pupillometry
309 abnormalities indicative of underlying hyper- or hyporegulation by LC-NE and/or LPR circuits. One
310 hypothesis is that ASD participants may exhibit larger tonic pupil size indicative of stronger LC-NE
311 tonic activity, potentially associated with hyper-arousal and increased behavioral flexibility (5).

312 While some studies with relatively small samples (<32 ASD participants in each) have reported
313 significantly larger tonic pupil size in ASD (7,8), others have reported the opposite (9) or no
314 differences across groups (1,10,11). Our results, with a somewhat larger sample and with multiple,
315 repeated measurements, suggest that there is indeed no significant difference in baseline pupil
316 size across groups (Figure 1).

317 Another hypothesis is that ASD individuals may exhibit weaker pupil dilations to stimuli with
318 cognitive, attentional, or social load that may indicate poor phasic LC-NE modulation. Phasic
319 increases in LC-NE dopamine innervation are important for increasing arousal during task
320 demanding periods and achieving optimal performance (5). Several studies have reported that
321 pupillary responses in ASD individuals are weaker to visual stimuli in the context of a spatial
322 attention task (8), an odd-ball task (7), a one-back memory task (11), or when stimuli include social
323 information (37,38).

324 A third hypothesis suggests that ASD individuals may exhibit weaker pupil constriction in response
325 to luminance increases (i.e., weaker PLR). Several studies have indeed reported weaker (12,13)
326 and delayed (1) PLR responses in children and adults with ASD. Surprisingly, there have also been
327 reports of abnormally strong PLR responses in 9–10-month-old toddlers who developed ASD at
328 later ages(14).

329 Taken together, the last two hypotheses suggest attenuated pupillometry time-courses in ASD
330 with weaker PLR associated constrictions and weaker LC-NE associated dilations. Our pupillometry
331 results do not seem to show differences, on average, in the pupillary response time-courses across
332 ASD and NT groups, in any of the movies (Figure 2). Pupillary time-courses were highly correlated
333 across groups, demonstrating that the temporal structure of pupillary responses was overall highly
334 similar across groups. Nevertheless, our study was not designed to separate PLR and LC-NE
335 responses or isolate specific stimulus events that would be expected to generate a pupillary
336 response. Hence, our results do not offer strong evidence for the existence of specific LC-NE and
337 LPR differences across groups or lack thereof. Further studies are necessary to test hypotheses
338 regarding potential atypicalities in each of these neural mechanisms in ASD.

339

340

341 ***Limitations***

342 While our study utilized a highly ecological design that enabled inclusion of ASD children across
343 the spectrum including those with intellectual disability, it had several important limitations. First,
344 we did not design our stimuli or analyze it post-hoc to separate pupillary responses associated
345 with PLR versus LC-NE mechanisms. It is likely that naturalistic, complex stimuli contain multiple
346 transitions in both low-level visual features (e.g., luminance and contrast) as well as high-level
347 content features (e.g., narrative complexity, novelty, social and emotional valence). Hence, our
348 study measured pupillary changes that were the product of both PLR and LC-NE regulation.
349 Second, data loss was clearly an issue in the current study. While all 103 children included in the
350 study successfully watched at least one movie, most participating children did not successfully
351 watch all 6 movies. Since our experimental design included considerable redundancy with multiple
352 movies and presentations, we believe that our results are reliable and conclusive despite partial
353 data collection from many children. Nevertheless, this raises an important limitation of eye
354 tracking studies with ASD children. While previous studies have reported success rates as high as
355 95% in collecting eye tracking data from ASD children (39), these were collected from ASD children
356 without intellectual disability. We believe that improving stimuli and acquisition conditions to
357 maximize data collection from young children with ASD and intellectual disability should be an
358 important goal of future studies.

359 ***Conclusions***

360 Most children with ASD exhibit pupillometry time-courses that differ from those of TD children.
361 This suggests that pupil regulation differs in ASD children in idiosyncratic ways, which can be
362 quantified using inter-SC, including in children with ASD and intellectual disability. Beyond the
363 clinical value of this measure for identifying individuals with ASD, it suggests that PLR and LC-NE
364 circuits do not operate in a uniform fashion across ASD individuals. Rather than attenuated or
365 excessive circuit responses, we speculate that these circuits may exhibit larger variability in ASD
366 individuals, perhaps due to excitation/inhibition imbalances that have been implicated as a
367 potential underlying mechanism (40,41). Further studies delineating PLR and LC-NE responses
368 while using naturalistic stimuli that can be used with large samples of ASD children, including
369 those with intellectual disability, are highly warranted.

370

371

372 Acknowledgements

373 This research was supported by Fulbright U.S. Student Program to IB, Israeli Science Foundation
374 (1150/20), Israeli Ministry of Science and Technology, and Azrieli Foundation grants to ID,
375 National Institute of General Medical Sciences grants T32GM008208 to IB and MCG,
376 T32GM081760 to MCG, and a Simons Foundation Autism Research Initiative grant to MB. MB
377 acknowledges support from P30 CORE award EY08098 from the National Eye Institute, NIH, and
378 unrestricted supporting funds from The Research to Prevent Blindness Inc, NY, and the Eye &
379 Ear Foundation of Pittsburgh. The content is solely the responsibility of the authors and does
380 not necessarily represent the official view of the Fulbright Program, the National Institute of
381 General Medical Sciences, or the National Institutes of Health. Finally, the authors thank the
382 participants and their families for making this research possible.

383

384 Disclosures

385 **Conflict of Interest Statement:** Behrman is a founder of Precision Neuroscopics, a company
386 developing medical technologies with a focus on equity and inclusion in healthcare. All other
387 authors declare no competing financial interests.

388

389 References

- 390 1. de Vries L, Fouquaet I, Boets B, Naulaers G, Steyaert J (2021): Autism spectrum disorder and
391 pupillometry: A systematic review and meta-analysis. *Neurosci Biobehav Rev* 120: 479–508.
- 392 2. Belliveau AP, Soman AN, Dossani RH (2023): Pupillary Light Reflex. *StatPearls*. Retrieved
393 September 10, 2023, from <https://www.ncbi.nlm.nih.gov/books/NBK537180/>
- 394 3. Joshi S, Li Y, Kalwani RM, Gold JI (2016): Relationships between Pupil Diameter and Neuronal
395 Activity in the Locus Coeruleus, Colliculi, and Cingulate Cortex. *Neuron* 89: 221–234.
- 396 4. Maness EB, Burk JA, McKenna JT, Schiffino FL, Strecker RE, McCoy JG (2022): Role of the locus
397 coeruleus and basal forebrain in arousal and attention. *Brain Res Bull* 188: 47–58.
- 398 5. Aston-Jones G, Cohen JD (2005): An integrative theory of locus coeruleus-norepinephrine
399 function: adaptive gain and optimal performance. *Annu Rev Neurosci* 28: 403–450.
- 400 6. Lynch G (2018): Using Pupillometry to Assess the Atypical Pupillary Light Reflex and LC-NE
401 System in ASD. *Behavioral sciences (Basel, Switzerland)* 8.
402 <https://doi.org/10.3390/BS8110108>
- 403 7. Kim Y, Kadlaskar G, Keehn RMN, Keehn B (2022): Measures of tonic and phasic activity of the
404 locus coeruleus-norepinephrine system in children with autism spectrum disorder: An
405 event-related potential and pupillometry study. *Autism Res* 15: 2250–2264.
- 406 8. Bast N, Boxhoorn S, Supér H, Helfer B, Polzer L, Klein C, et al. (2023): Atypical Arousal
407 Regulation in Children With Autism but Not With Attention-Deficit/Hyperactivity Disorder
408 as Indicated by Pupillometric Measures of Locus Coeruleus Activity. *Biol Psychiatry Cogn
409 Neurosci Neuroimaging* 8: 11–20.

410 9. Müller N, Baumeister S, Dziobek I, Banaschewski T, Poustka L (2016): Validation of the Movie
411 for the Assessment of Social Cognition in Adolescents with ASD: Fixation Duration and Pupil
412 Dilation as Predictors of Performance. *J Autism Dev Disord* 46: 2831–2844.

413 10. Zhao S, Liu Y, Wei K (2022): Pupil-Linked Arousal Response Reveals Aberrant Attention
414 Regulation among Children with Autism Spectrum Disorder. *J Neurosci* 42: 5427–5437.

415 11. Granovetter MC, Burlingham CS, Blauch NM, Minshew NJ, Heeger DJ, Behrman M (2020):
416 Uncharacteristic Task-Evoked Pupillary Responses Implicate Atypical Locus Ceruleus
417 Activity in Autism. *J Neurosci* 40: 3815–3826.

418 12. Fan X, Miles JH, Takahashi N, Yao G (2009): Abnormal transient pupillary light reflex in
419 individuals with autism spectrum disorders. *J Autism Dev Disord* 39: 1499–1508.

420 13. Daluwatte C, Miles JH, Christ SE, Beversdorf DQ, Takahashi TN, Yao G (2013): Atypical
421 pupillary light reflex and heart rate variability in children with autism spectrum disorder. *J
422 Autism Dev Disord* 43: 1910–1925.

423 14. Nyström P, Gliga T, Jobs EN, Gredebäck G, Charman T, Johnson MH, et al. (2018): Enhanced
424 pupillary light reflex in infancy is associated with autism diagnosis in toddlerhood. *Nat
425 Commun* 9. <https://doi.org/10.1038/S41467-018-03985-4>

426 15. Finn ES, Bandettini PA (2021): Movie-watching outperforms rest for functional connectivity-
427 based prediction of behavior. *Neuroimage* 235.
428 <https://doi.org/10.1016/J.NEUROIMAGE.2021.117963>

429 16. Cantlon JF, Li R (2013): Neural activity during natural viewing of Sesame Street statistically
430 predicts test scores in early childhood. *PLoS Biol* 11.
431 <https://doi.org/10.1371/JOURNAL.PBIO.1001462>

432 17. Vanderwal T, Eilbott J, Kelly C, Frew SR, Woodward TS, Milham MP, Castellanos FX (2021):
433 Stability and similarity of the pediatric connectome as developmental measures.
434 *Neuroimage* 226. <https://doi.org/10.1016/J.NEUROIMAGE.2020.117537>

435 18. Lerner Y, Scherf KS, Katkov M, Hasson U, Behrman M (2021): Changes in Cortical
436 Coherence Supporting Complex Visual and Social Processing in Adolescence. *J Cogn
437 Neurosci* 33: 2215–2230.

438 19. Hasson U, Avidan G, Gelbard H, Vallines I, Harel M, Minshew N, Behrman M (2009): Shared
439 and idiosyncratic cortical activation patterns in autism revealed under continuous real-life
440 viewing conditions. *Autism Res* 2: 220–231.

441 20. Byrge L, Dubois J, Tyszka JM, Adolphs R, Kennedy DP (2015): Idiosyncratic Brain Activation
442 Patterns Are Associated with Poor Social Comprehension in Autism. *The Journal of
443 Neuroscience* 35: 5837.

444 21. Avni I, Meiri G, Bar-Sinai A, Reboh D, Manelis L, Flusser H, et al. (2020): Children with autism
445 observe social interactions in an idiosyncratic manner. *Autism Res* 13: 935–946.

446 22. Constantino JN, Gruber CP (2012): Social Responsiveness Scale-Second Edition (SRS-2).
447 *Western Psychological Services*.

448 23. Moody EJ, Reyes N, Ledbetter C, Wiggins L, DiGuiseppi C, Alexander A, et al. (2017):
449 Screening for Autism with the SRS and SCQ: Variations across Demographic,
450 Developmental and Behavioral Factors in Preschool Children. *J Autism Dev Disord* 47: 3550–
451 3561.

452 24. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, *et al.* (2000): The autism
453 diagnostic observation schedule-generic: a standard measure of social and communication
454 deficits associated with the spectrum of autism. *J Autism Dev Disord* 30: 205-223.

455 25. Wechsler, D (2012): Wechsler Preschool and Primary Scale of Intelligence—Fourth Edition
456 (WPPSI-IV).

457 26. Albers, CA, Grieve, AJ (2007): Review of *Bayley Scales of Infant and Toddler Development—*
458 *Third Edition*. *Journal of Psychoeducational Assessment* 25: 180–190.

459 27. Hayes TR, Petrov AA (2016): Mapping and correcting the influence of gaze position on pupil
460 size measurements. *Behav Res Methods* 48: 510-27.

461 28. Youden WJ (1950): Index for rating diagnostic tests. *Cancer* 1: 32–35.

462 29. Dinstein I, Heeger DJ, Behrmann M (2015): Neural variability: friend or foe? *Trends Cogn Sci*
463 19: 322–328.

464 30. Sohal VS, Rubenstein JLR (2019): Excitation-inhibition balance as a framework for
465 investigating mechanisms in neuropsychiatric disorders. *Mol Psychiatry* 24: 1248–1257.

466 31. Hahamy A, Behrmann M, Malach R (2015): The idiosyncratic brain: distortion of
467 spontaneous connectivity patterns in autism spectrum disorder. *Nat Neurosci* 18: 302–309.

468 32. Nunes AS, Peatfield N, Vakorin V, Doesburg SM (2019): Idiosyncratic organization of cortical
469 networks in autism spectrum disorder. *Neuroimage* 190: 182–190.

470 33. Benkarim O, Paquola C, Park B yong, Hong SJ, Royer J, Vos de Wael R, *et al.* (2021):
471 Connectivity alterations in autism reflect functional idiosyncrasy. *Commun Biol* 4.
472 <https://doi.org/10.1038/S42003-021-02572-6>

473 34. Keles U, Kliemann D, Byrge L, Saarimäki H, Paul LK, Kennedy DP, Adolphs R (2022): Atypical
474 gaze patterns in autistic adults are heterogeneous across but reliable within individuals.
475 *Mol Autism* 13. <https://doi.org/10.1186/S13229-022-00517-2>

476 35. Pegado F, Hendriks MHA, Amelynck S, Daniels N, Steyaert J, Boets B, Op de Beeck H (2020):
477 Adults with high functioning autism display idiosyncratic behavioral patterns, neural
478 representations and connectivity of the “Voice Area” while judging the appropriateness of
479 emotional vocal reactions. *Cortex* 125: 90–108.

480 36. Cavallo A, Romeo L, Ansuini C, Poddia J, Battaglia F, Veneselli E, *et al.* (2018): Prospective
481 motor control obeys to idiosyncratic strategies in autism. *Sci Rep* 8.
482 <https://doi.org/10.1038/S41598-018-31479-2>

483 37. Polzer L, Freitag CM, Bast N (2022): Pupillometric measures of altered stimulus-evoked
484 locus coeruleus-norepinephrine activity explain attenuated social attention in preschoolers
485 with autism spectrum disorder. *Autism Research* 15: 2167–2180.

486 38. Bast N, Banaschewski T, Dziobek I, Brandeis D, Poustka L, Freitag CM (2019): Pupil Dilation
487 Progression Modulates Aberrant Social Cognition in Autism Spectrum Disorder. *Autism Res*
488 12: 1680–1692.

489 39. Shic F, Naples AJ, Barney EC, Chang SA, Li B, McAllister T, *et al.* (2022): The autism
490 biomarkers consortium for clinical trials: evaluation of a battery of candidate eye-tracking
491 biomarkers for use in autism clinical trials. *Mol Autism* 13: 1–17.

492 40. Nelson SB, Valakh V (2015): Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism
493 Spectrum Disorders. *Neuron* 87: 684–698.

494 41. Sohal VS, Rubenstein JLR (2019): Excitation-inhibition balance as a framework for
495 investigating mechanisms in neuropsychiatric disorders. *Mol Psychiatry* 24: 1248.