Supporting Information

Shultz et al. 10.1073/pnas.1109304108

SI Materials and Methods

Participants. Children were recruited through federally funded Studies to Advance Autism Research and Treatment and Autism Center of Excellence programs based in the Autism Program of the Yale Child Study Center, Yale University School of Medicine. The research protocol was approved by the Human Investigations Committee of the Yale University School of Medicine, and families were free to withdraw from the study at any time.

The toddlers with ASD and typical toddlers were matched on chronological age [toddlers with ASD: M = 2.28 y, SD = 0.55; typical toddlers: M = 2.30 y, SD = 0.56; $t_{(91)}$ = 0.158, P = 0.875] and nonverbal mental age equivalents obtained with the Visual Reception subtest of the Mullen Scales of Early Learning (1) [toddlers with ASD: M = 2.05 y, SD = 0.73; typical toddlers: M = 2.25 y, SD = 0.73; $t_{(91)}$ = 1.294, P = 0.199]. All toddlers were medically screened for visual and auditory function as part of a comprehensive pediatric and genetics protocol that included general physical and neurological examination. For inclusion in the ASD group, children had to fulfill all three of the following conditions: (*i*) meet criteria for either autism or ASD on the Autism Diagnostic Observation Schedule (ADOS) (2) (63% met criteria for autism); (*ii*) meet criteria for autism or ASD on the Autism Diagnostic Interview—Revised (3); and (*iii*) be assigned—independently, by two experienced clinicians on review of all available data, including standardized testing and videotaped material of diagnostic examination—a diagnosis of either autism (59% of the group) or ASD (41% of the group). The ASD group's mean score on the social cluster of the ADOS was 9.5 (SD = 3.75). Although a small subset (n = 5) of typical toddlers had Mullen scores below the normative range, none of the children included in the typical toddler group had either a diagnosis of ASD or any positive family history for ASD.

Data Acquisition and Analysis. The experimental procedures and setting were identical to those described by Jones et al. (4). At the beginning of each session, participants viewed a children's video (e.g., Baby Mozart, Elmo) played on a computer monitor. The computer monitor was mounted within a wall panel, and the audio soundtrack was played through a set of concealed speakers. Toddlers were seated and buckled into a car seat mounted on a pneumatic lift so that viewing height (line-of-sight) was standardized for all children. Viewers' eyes were 30 in (76.2 cm) from the computer monitor, which subtended approximately a 23° × 30° portion of each child's visual field. Lights in the room were dimmed so that only images displayed on the computer monitor could be easily seen. A five-point calibration scheme was used, presenting spinning and/or flashing points of light as well as cartoon animations, ranging in size from 0.5° to 1.5° of visual angle, all with accompanying sounds. The calibration routine was followed by verification of calibration in which more animations were presented at five on-screen locations. Throughout the remainder of the testing session, animated targets (as used in the calibration process) were shown between experimental videos to measure drift in data. In this way, accuracy of the eye-tracking data was verified before beginning experimental trials and was then repeatedly checked between video segments as the testing continued. In the case that drift exceeded 3°, data collection was stopped and the child was recalibrated before further videos were presented. All aspects of the experimental protocol were performed by personnel blinded to diagnostic status of the children. Most aspects of data acquisition and all aspects of coding, processing, and data summary are automated, such that separation between the diagnostic characterization protocol and the experimental protocol was assured.

To analyze blink inhibition as an index of perceived stimulus salience, children were shown a video scene of a boy and girl playing together in a toy wagon (Fig. 1). The video scene was excerpted from Karen Bruso and Mary Richardson's commercially available children's video, *Toddler Takes! Take 1: Toddlers at Play*. The video was presented in full-screen mode with an accompanying audio soundtrack on a 20-in (50.8 cm) computer monitor (refresh rate of 60 Hz noninterlaced). Video frames were eight-bit color images, 640 × 480 pixels in resolution. The video frame rate of presentation was 30 frames per second. The audio soundtrack was a single (mono) channel sampled at 44.1 kHz. The original audio soundtrack contained an instance of adult narrator voiceover; this was removed digitally to make the video scene as naturalistic as possible. The duration of the video was 1 min and 13.6 s. Individual measures of blink rate and blink duration (Fig. 2) were measured during video watching, as opposed to during intertrial intervals (Fig. 3).

Before and after the video, a centering cue was presented on an otherwise blank screen to draw the attention of viewers to a common fixation location. The centering cue was 1.5° in visual angle with alternating blue and white sections, rotating in time to a chiming sound. During presentation of the centering cue, 91.4% of the children were compliant in looking at the cue; there were no between-group differences in the proportion of children who were compliant (z = 1.12, P = 0.24).

Eye-tracking data were acquired and analyzed as described by Jones et al. (4). Visual fixation patterns were measured with eye-tracking equipment using hardware and software created by ISCAN, Inc. The eye-tracking technology was video-based, using a dark pupil/corneal reflection technique with eye movement data collected at the rate of 60 Hz. Analysis of eye movements and coding of fixation data were performed with in-house software written in MATLAB (MathWorks). The first phase of analysis was an automated identification of nonfixation data, comprising blinks, saccades, and fixations directed away from the stimuli presentation screen.

Blinks were identified by an automated algorithm measuring occlusion of the pupil by rate of change in pupil diameter and by vertical displacement of the measured pupil center. The blink detection algorithm was supplemented by simultaneous video recording in all participants and verified by manual coding of the video data in 10% of participants' data. The algorithm was also verified by simultaneous video and electromyography (EMG) recording in one adult viewer. In comparison with video recordings, the algorithm accurately detected 95.0% of all blinks identified by manual coding of video images. In comparison with EMG recordings, the algorithm accurately detected 96.4% of blinks recorded by EMG. Events identified by the algorithm as blinks but shorter than 166.7 ms or longer than 566.7 ms were excluded from analysis in accordance with previous studies of blink duration (5, 6) and in agreement with visual inspection of the video images (blinks in Fig. 4, which appear longer than 566.7 ms, are actually multiple blinks separated by brief fixations, obscured by the plot resolution). Duration measurements comparing blinks detected by the algorithm and blinks detected by EMG were different by less than 10 ms (i.e., less than the sampling detection threshold of the eye-tracker). Saccades were identified by eye velocity using a velocity threshold of 30° per second (7). Off-screen fixations, when a participant looked away from the video screen, were identified by fixation coordinates to locations beyond the screen bounds. Throughout all viewing data, the proportion of nonfixation data (saccades + blinks + off-screen fixations) was not significantly different between the ASD (M = 24.25%, SE = 1.2) and typical (M = 24.7%, SE = 1.5) groups [$t_{(91)} = 0.22$, $P = 0.82$].

Ratings of Affective and Physical Events. Ten adults rated the affective content of the video scene in a two-stage process. First, the entire video was divided into 15 segments, and viewers were asked to rank the segments from most affective to least affective. Interrater coefficient of concordance for these rankings was highly significant (Kendall's W = 0.879, $X^2 = 123.02$, df = 14, $P < 0.0001$) (8). The eight segments ranked most highly were then used to identify precise timing of the affective events. To do so, adult raters examined each of the eight most affective segments frame-by-frame and selected the time point at which the affective event began and the time point at which the affective event ended. The SE of start and end times across all raters was 152 ms. Start and end times for each affective segment were averaged across the 10 raters, resulting in eight affective events. Physical events were defined as all time points in which the wagon door was moving (with start and end points set by the start and stop of the door's motion).

Instantaneous Blink Rate. Instantaneous blink rate was computed as a density function (9); related methods can be found in the study by Paulin (10). Data for each individual were recorded as 60-Hz time series. Binary values indicating whether a given individual was blinking or not were recorded at each point in the time series (0 for not blinking and 1 for blinking, with a contiguous sequence of 1's indicating a complete blink with duration equal to the length of that contiguous sequence). At each time, t, in the time series, instantaneous blink rate was calculated according to the following equation:

$$bpm(t) = \frac{1}{\Delta t} \times \frac{n_b(t)}{N_v(t)}$$

where $bpm(t)$ is the instantaneous blink rate (blinks per minute) at time t, Δt is the sampling interval (1/60 s for 60-Hz sampling, converted to minutes as 1/3,600 min), $n_b(t)$ is the sum of blinks (i.e., summed across individuals) occurring at time t, and $N_v(t)$ is the total number of viewers either blinking or looking at the screen at time t. Finally, the instantaneous blink rate density function was smoothed with a Gaussian window (300 ms at full-width half-maximum) selected to match the mean individual blink duration (11).

Note that in a free-viewing experiment, $N_v(t)$ should exclude any participant looking away from the screen at time t. Also note that n_b is a fractional count of total blinks: A single blink lasting 300 ms, measured in 60-Hz samples, would span 18 samples in the time series and would be counted as 1/18 of a blink at each time t.

Permutation Testing. To test whether instantaneous blink rate was significantly modulated during the video watching, we used permutation testing (12). In each of 1,000 iterations, the binary times series blink data for each child (0 = not blinking, 1 = blinking) were permuted by circular shifting (13), following the equation:

$$b_{j,c}(t) = b_j(t - s_j, \text{modulo } T)$$

written as

$$b_{j,c}(t) = b_j(\langle t - s_j \rangle_T),$$

which, for $s_j \geq 0$, equals

$$b_{j,c}(t) = \begin{cases} b_j[t - s_j], & s_j < t \leq T \\ b_j[T - s_j + t], & 0 \leq t \leq s_j, \end{cases}$$

where b_j is the measured blink time series data for each participant, j; $b_{j,c}$ is the circular-shifted blink time series data for the same participant j; t is a time point in the time series defined over the interval $0 \leq t \leq T$; T is the total duration of the stimulus (in the present case, the duration of the entire movie shown to participants); and s_j is the size of the circular shift, in the same units of time as t, for each participant j. The size of the circular shift for each participant was drawn independently from a random number generator with uniform distribution, with possible values ranging from $-T$ to T. After circular shifting, for each iteration, i, instantaneous blink rate was calculated as previously described:

$$bpm_i(t) = \frac{1}{\Delta t} \times \frac{n_{b_c}(t)}{N_{v_c}(t)}$$

In this way, in each iteration, durations of blinks and interblink intervals were preserved for each individual but the timing of each blink was made random in relation to both the actual time line of video content and in relation to the timing of other participants' blinking. By this approach, in the permuted data, the mean blink rate of participants during the entire task remains unchanged (and task-specific) but the timing of when instantaneous blink rate is increased or decreased is made random.

We repeated this permutation process in 1,000 iterations and then measured the statistical distribution of blink rate across all iterations at each point in the time series. At each time point across all iterations, the fifth percentile of permuted data was used as a nonparametric threshold for identifying time points of significant blink inhibition. This enabled the comparison of actual patterns of eye blinking to randomized, chance patterns of eye blinking, enabling us to test the null hypothesis that the timing of eye blinks was unrelated to scene content.

We found that the blink rate for typical toddlers was significantly inhibited (exhibiting values less than the 0.05 threshold of shuffled data) during 8.8% of video viewing time and that the blink rate for the ASD group was significantly inhibited during 7.0% of viewing time. We tested the difference between observed blink rates and permuted data for each group by two-sample Kolmogorov–Smirnov tests, finding significant differences for each ($D = 0.22$, $P < 0.001$ for typical toddlers and $D = 0.28$, $P < 0.001$ for toddlers with ASD).

Fig. S1 shows graphs of the empirical cumulative distribution functions comparing actual data with permuted data. These plots show both an increase in low blink rates (the gap between actual data and permuted data at the left end of abscissa) as well as an increase in high blink rates (gap between actual data and permuted data at the right end of abscissa).

1. Mullen E (1995) *Mullen Scales of Early Learning: AGS Edition* (AGS, Circle Pines, MN).
2. Lord C, et al. (1999) *Autism Diagnostic Observation Schedule—WPS (ADOS-WPS)* (Western Psychological Services, Los Angeles, CA).
3. Rutter M, LeCouteur A, Lord C (2003) *The Autism Diagnostic Interview, Revised* (Western Psychological Services, Los Angeles, CA).
4. Jones W, Carr K, Klin A (2008) Absence of preferential looking to the eyes of approaching adults predicts level of social disability in 2-year-old toddlers with autism spectrum disorder. *Arch Gen Psychiatry* 65:946–954.
5. VanderWerf F, Brassinga P, Reits D, Aramideh M, Ongerboer de Visser B (2003) Eyelid movements: Behavioral studies of blinking in humans under different stimulus conditions. *J Neurophysiol* 89:2784–2796.
6. Schellini SA, Sampaio AA Jr., Hoyama E, Cruz AA, Padovani CR (2005) Spontaneous eye blink analysis in the normal individual. *Orbit* 24:239–242.
7. Leigh R, Zee D (1999) *The Neurology of Eye Movements* (Oxford Univ Press, Oxford).
8. Kendall SM (2005) *Rank Correlation* (John Wiley & Sons).
9. Gerstner W, Kistler W (2002) *Spiking Neuron Models. Single Neurons, Populations, Plasticity* (Cambridge Univ Press).

10. Paulin MG (1992) Digital filters for firing rate estimation. *Biol Cybern* 66: 525–531.
11. Parzen E (1962) On estimation of a probability density function and mode. *Ann Math Stat* 33:1065–1076.
12. Good P (2000) *Permutation Test: A Practical Guide to Resampling for Testing Hypotheses* (Springer, New York).
13. Oppenheim AV, Schafer RW (1975) *Digital Signal Processing* (Prentice Hall, Englewood Cliffs, NJ).

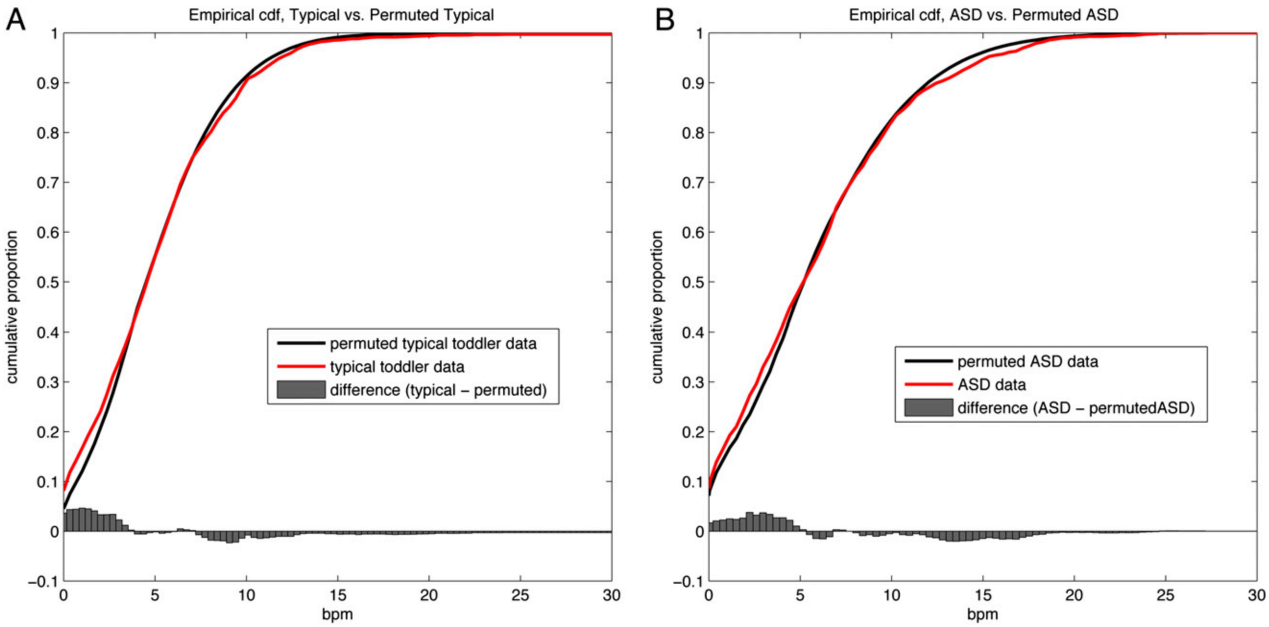

Fig. S1. Empirical cumulative distribution function (cdf) comparing actual data with permuted data. (*A*) Empirical cdf for typical toddler blink data and permuted typical toddler data. (*B*) Empirical cdf for ASD toddler blink data and permuted ASD toddler data. For both groups, in the comparison of actual data relative to permuted data, empirical cdfs indicate an increase in low blink rates (the gap between actual data and permuted data at the left end of plots) as well as an increase in high blink rates (gap between actual data and permuted data at the right end of plots).

Table S1. Participant characterization

	Toddlers with ASD	Typical toddlers	*t* values	*P* values
N	41	52		
Sex	36 M, 5 F	33 M, 19 F		
Age, y	2.28 (0.55)	2.30 (0.56)	0.158	0.875
Nonverbal function*	2.05 (0.73)	2.25 (0.73)	1.294	0.199
ADOS	9.5 (3.75)			

Data are given as mean (SD). ADOS, score for Social domain; F, female; M, male.
*Nonverbal function corresponds to age-equivalent scores (in years) as obtained in the Visual Reception subtest of the Mullen Scales of Early Learning.