Supplementary Information

Autism group diagnostic information

All children included in the study met the diagnosis of provisional autism (Autistic disorder (AD) or Autism Spectrum Disorder (ASD)) from the autism diagnostic observation scales (ADOS) (1) at the time of fMRI data acquisition. All children additionally met criteria for autism on the autism diagnostic interview (ADI-R), except for one (ASD2) who was nonverbal and missed the nonverbal cut-off by 1 point. He received a score in the Autism range on the ADOS both at 26 and 36 months of age and was given an ASD diagnosis (See Table S1). All children received the ADI-R at age 3 years or older except for 3 participants for whom an ADI-R was obtained at 30 months. One of these children received an ADOS, but not an ADI-R, at 36 months and obtained an Autism diagnosis at that age. All three children received an autism severity score in the 'severe' range, as determined from the childhood autism rating scales (CARS); thus it is unlikely that an ADI-R several months later would reveal a change in diagnosis. All children were screened for major neurological disorders and Fragile X.

Table S1. Diagnostic Information

		ADI-R Scores					ADOS Scores			
ID	**Age at scan**	**Age at ADI-R**	**Social**	**Communication**	**Restricted & Repetitive Behaviors**	**Total**	**Age at ADOS**	**Communication**	**Social**	**Total**
ASD1	25.7	55.17	16	13*	7	36	23.97	6	13	19
ASD2	26.5	30.12	9	5	8	22	26.10	5	8	13
ASD3	29.7	47.38	10	10	10	30	28.37	5	8	13
ASD4	30.3	29.88	16	9	3	28	29.42	7	12	19
ASD5	30.3	52.01	25	14	5	44	22.5(53.2)	6(2)	14(9)	20(11)
ASD6	31.8	38.76	14	11	5	30	26.70	5	10	15
ASD7	32.1	31.17	18	10	4	32	30.97	8	12	20
ASD8	36.2	37.35	14	8	3	25	37.55	5	12	17
ASD9	41.0	41.00	20	9	6	35	40.83	10	11	21
ASD10	41.9	55.99	16	19*	8	43	42.15	4	8	12
ASD11	46.5	45.04	21	11	9	41	48.59	6	14	20
ASD12	46.9	57.07	16	14*	5	35	49.51	8	10	18
n=12	34.9(7.4)	43.4(10.2)	16.3(4.5)	11.1(3.6)	6.1(2.3)	33.4(6.9)	33.9(9.5)	6.3(1.7)	11.0(2.2)	17.3(3.2)

ADI-R Communications scores are Non-verbal except for the 3 verbal ASD subjects (denoted by an '*'). Age is given in months

ADOS scores were obtained 8 months prior to the MRI scan for participant ASD5. ADOS scores from a second visit, at 53 months, are also given in parentheses.

Stimulus Design

Both forward speech conditions were 20 second excerpts from a children's story. The F:s condition was taken from a story designed for children between 1-3 years of age while the F:c story was taken from a book of children's prose. The forward speech stories differed on several parameters including sentence length and word frequency; however, in the current analyses we collapsed across these conditions to examine the response to forward speech (F). The forward speech stories were read in a neutral voice with typical intonation patterns of story telling, but not 'motherese'. The backward speech condition was created by temporally reversing the F:s passage. This condition thus provided an acoustic control for the forward speech condition but contained no semantic information. All stimuli were recorded by the same female speaker using Cool Edit 2000 software (http://www.adobe.com) and normalized across conditions. Each condition was repeated three times in a semi-counterbalanced order for a total of 6 minutes of data acquisition.

Data Acquisition

Prior to the scan parents were mailed a CD of scanner noise and earplugs for practice prior to the scan night. Children and parents arrived at the scanner between 9 and 10 PM, depending on the typical bedtime of the child. In addition to earplugs and headphones, towels were used to pad the sides of the head to dampen acoustic noise from the scanner and minimize head motion. Earplugs remained in the ears during the entire data acquisition. Data acquisition typically began about 10-15 minutes after the child had fallen asleep on the scanner bed.

Motion correction

Data points were considered to have uncorrectable levels of head motion if the distance between consecutive volumes was greater than 0.4 mm. Distance was estimated from calculating the sum of the square root of the sum of squares of the translational (x, y, z) and rotational (roll, pitch, yaw) motion parameter values. In the ASD group, data points were uncorrectable and removed from the analyses for two participants (5% of the run for both). Data from 1 MA control (10% of the run) and 2 CA controls (2% and 6% of the run) were also removed from the analyses.

Talairach registration

We conducted pilot studies to determine the degree of anatomical co-registration for the central sulcus and pars opercularis in a group of 10 toddlers, 10 3-year olds, and 10 adults (23-27 years). We found superior anatomical alignment when images were placed in Talairach space for the younger child groups than for the adults. These data are published as supplementary material in Redcay *et al.*, 2008 (2). Additionally, we examined alignment in Talairach space of these same regions in a group of autistic toddlers and also found good anatomical alignment, with no significant differences in the degree of variance between autistic toddlers, typical toddlers, and adult controls. These data are not published. Thus, we concluded that anatomical co-registration is appropriate for young typically developing children and children with autism. However, due to the differences in brain size and shape between adults and young children, the Talairach coordinates may not reflect the same anatomical region as that in adults. For this reason, we utilized atlas-based anatomical landmark identification to report regions of activation. We do report Talairach coordinates in the tables but these should be used with caution when extrapolating to the adult brain.

Response to Forward Speech vs. Rest within groups

Both the CA-matched and ASD groups showed activity in bilateral superior temporal regions [(CA: left STG (63,18,3), t=5.39; right STG (-49,21,7), t=4.51)(ASD: left STG (54,5-2), t=4.51; right STG (-64,21,7), t=5.71)] in response to forward speech versus rest. The MA-group also utilized superior temporal cortices but this activation did not reach significance at the threshold of p<.01, cluster-corrected at p<.05. In a previous paper, we note the increased variability in the toddler group in superior temporal cortices (2). At p<.01, corrected, the MA-matched group recruited regions within left middle and medial frontal cortex (left middle frontal gyrus (31,-54,3), t=5.47; left medial frontal cortex (10,-46,6), t=4.19), left angular gyrus ((t=44,61,42), t=6.45)), and extrastriate cortex (left cuneus (14,100,4), t=5.46; right cuneus (-19,89,25), t=3.94). For a full list of activations within each group, see Table 2.

Correlation Analyses

Correlational analyses were focused to the ASD group as that group had notable variability in clinical and behavioral scores. Regions showing a significant correlation between receptive language age equivalent and activity to forward speech are given in Table S2.

Table S2. Correlations in the ASD Group with BOLD Response to Forward speech

Region	Side	Talairach coordinates (x,y,z)	t-value
Receptive Language Age Equivalent			
Frontal			
Medial Frontal Gyrus	R	(10,47,19)	5.79
Inferior Frontal Gyrus	R	(51,20,23)	5.56
Temporal			
Superior Temporal Sulcus & Middle Temporal Gyrus	R	(47,-25,3)	4.77
Parietal			
Posterior Cingulate Cortex	R	(5,-51,27)	3.72
Precuneus	R	(5,-62,42)	5.94
Posterior Cingulate Cortex	L	(-11,-45,18)	5.28
Occipital			
Cuneus	L	(-20,-85,7)	6.66
CARS Autism Severity Score			
Frontal			
Inferior & Middle Frontal Gyrus	R	(38,15,35)	-6.79
Medial & Superior Frontal Gyrus	R	(11,56,-1)	-4.76
Superior & Middle Frontal Gyrus	L	(-22,11,58)	-5.29
Temporal			
Superior Temporal Sulcus & Middle Temporal Gyrus	R	(54,-24,-5)	-5.74
Superior Temporal Sulcus & Middle Temporal Gyrus	L	(-55,-26,-9)	-6.18
Superior Temporal Gyrus	L	(-61,-16,2)	-6.33
Parietal			
Supramarginal Gyrus & Inferior Parietal Lobule	R	(51,-45,45)	-6.86

The peak t-value and Talairach coordinate is given for each region showing a significant correlation between behavior and BOLD response to forward speech

A post-hoc analyses of language-related brain activation and receptive language age-equivalent in the CA group revealed no significant regions of correlation at $p<.005$, corrected. Within the ASD group, partial correlation analyses were run to determine whether the regions showing a significant relationship between brain activation to forward speech and receptive language age would still show this relationship when Visual Reception age-equivalent (VR Age) was held constant. All correlations remained significant at $p<.05$, however, only R STS, R medial frontal gyrus, L cuneus, and L posterior cingulate remained significant at $p<.01$ (See Table S3.) Receptive language age-equivalent and visual reception age-equivalent scores were significantly correlated in the ASD group as well ($r(12) = .882$, $p<.0001$), making it difficult to tease apart effects of language skill from other cognitive abilities.

Table S3. Bivariate and Partial Correlation Coefficients between Receptive Language Age and BOLD Response to Forward Speech

Region	Side	Pearson's r-value	Partial Correlation r-value with VR Age constant
Receptive Language Age Equivalent			
Frontal			
Medial Frontal Gyrus	R	.892***	.739**
Inferior Frontal Gyrus	R	.890***	.719*
Temporal			
Superior Temporal Sulcus & Middle Temporal Gyrus	R	.889***	.768**
Parietal			
Posterior Cingulate Cortex	R	.846***	.692*
Precuneus	R	.851***	.714*
Posterior Cingulate Cortex	L	.863***	.740**
Occipital			
Cuneus	L	.956***	.858***

r-values are given for correlations of receptive language age-equivalent scores and activity to forward speech in the ASD group for bivariate correlations and partial correlations with Visual Reception (VR) age equivalent scores

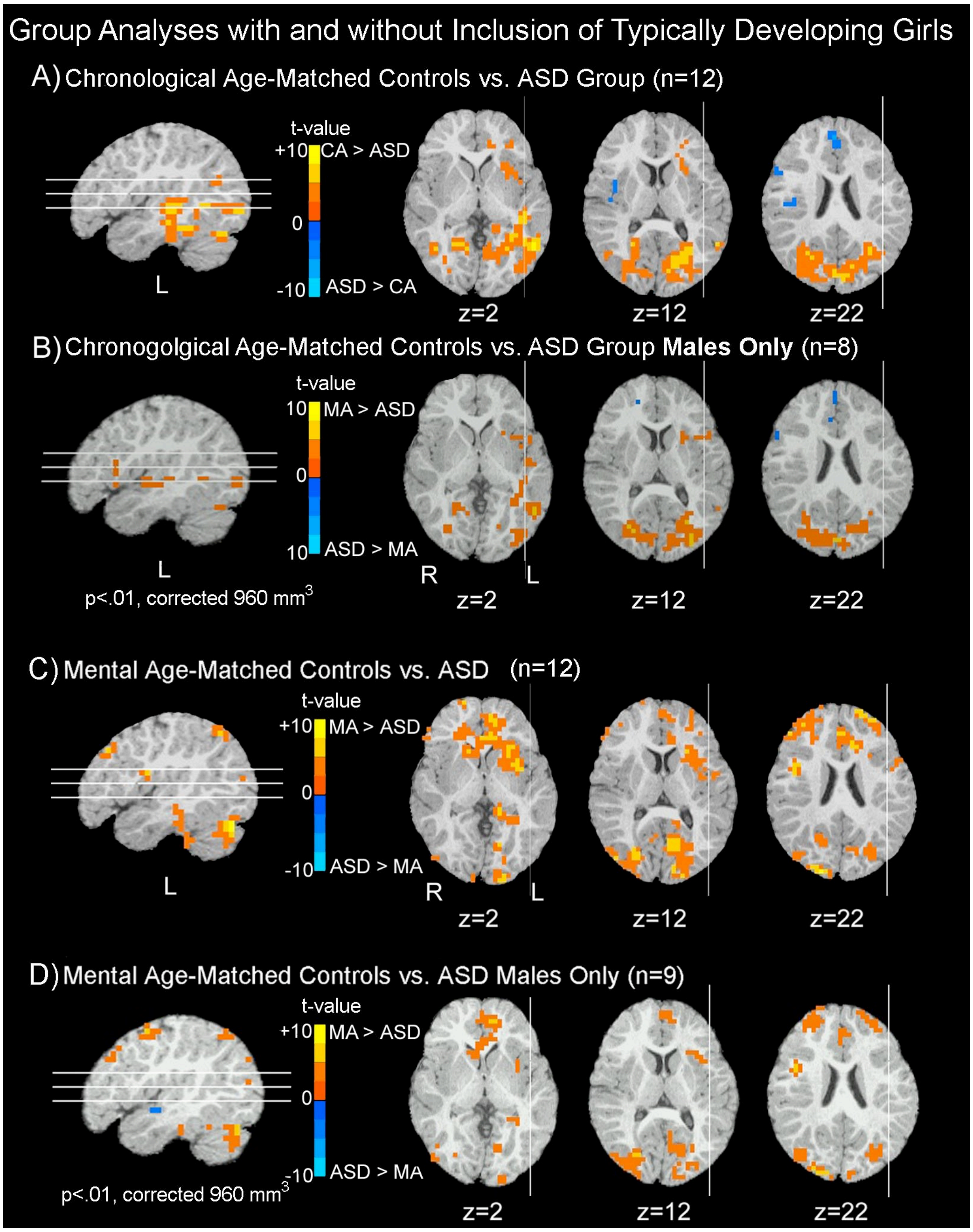
Speech-specific contrast

While this pattern of deviant laterality in ASD was clear in the forward speech vs. rest comparison, the speech-specific contrast did not reveal such a distinction (Fig 4). Rather, the ASD group recruited bilateral superior temporal gyri while CA-matched controls only showed significantly greater activity to forward as compared to backward speech in right superior temporal areas. Left lateral temporal areas were also significantly active at $p<.01$ in the CA group, but these clusters did not reach a cluster size minimum of 960 mm^3. Interestingly, the response to backward speech in the CA group also recruited bilateral superior temporal areas, particularly within the left hemisphere: a pattern which was not seen in either the MA or ASD groups. Backward speech, like forward speech, does contain fast auditory transitions and conveys some phonetic information, which may have led to the similar levels of activation to backward speech within superior temporal cortex in the CA controls. In fact, one study of adults which did not require explicit semantic or lexical judgment found no activation difference between words and reversed words (3).

In a previous study utilizing the same speech paradigm and many of the same control participants as in the CA group, we did find bilateral superior temporal activation to be greater to forward than to backward speech (2). In the current study, 4 of the 12 participants in the CA group were younger than those of the previous study suggesting age may play a role in the neural discrimination between forward and backward speech within superior temporal lobe during sleep. Further studies of narrower age ranges and different nonspeech control stimuli are warranted to disentangle this apparent discrepancy between left superior temporal activation to forward as compared to backward speech in the previous study but not in this study. Nonetheless, the laterality analyses of speech as compared to rest suggest a trend towards a left hemispheric bias for speech in controls, and a right hemisphere bias in autism.

Gender-Matched Group Analyses

Behavioral evidence suggests that girls show earlier maturation in language development than males with gender accounting for 1-5% of the variance in early vocabulary measures (4-6). Furthermore, a longitudinal study of brain anatomy revealed gender-related differences in the shape and height of developmental trajectories between ages 3 to 27 years. For example, cortical and subcortical gray matter volumes peak earlier in females than males (10.5 years for females versus 14.5 years for males) (7). The above behavioral and anatomical evidence of gender-related differences suggests patterns of brain function may also differ between typically developing boys and girls. The few functional imaging studies (ERP and fMRI) examining language processing between infancy and late childhood have found either no effect or a very small effect size for brain functional differences between boys and girls ((8-10), but see (11)). Nonetheless, given the known behavioral and anatomical effects of gender in this age group, we conducted a post-hoc group comparison with male-only groups (n=8 for the CA comparison; n=9 for the MA comparison). These are presented in Supplementary Information Figure S1 and Table S4. Differences between the ASD and control groups were largely consistent with those reported in the larger group analysis of males and females. Specifically, in comparison to the MA group, the ASD group showed a pattern of reduced activation within the same frontal, occipital, and cerebellar regions. Furthermore, in comparison to the CA group, the ASD group recruited greater medial and right hemisphere frontal regions. In sum, while future studies would benefit from gender-matched control groups, the inclusion of females in the control group does not appear to drive the major findings of the current paper.



<u>Supplementary Figure S1 Legend</u>

Figure S1: Group Analyses with (A&C) and without (B&D) Typically Developing Girls. ANOVAs were run to examine the BOLD response to forward speech between ASD and control groups with both girls and their corresponding chronological and age-matched ASD boys removed from the analyses. The original group analyses for the CA (A) and MA (C) groups as compared to the full n=12 ASD group are given for reference. As seen in Figure S1 and Table S4, in the CA vs. ASD males-only comparison (B), results are consistent with the exclusion of girls to those of the full sample (A). Likewise, in the MA vs. ASD males-only comparison (D), very similar regions show greater activation to speech in the MA controls as compared to the ASD as those seen in the full sample comparison (C).

Table S4. Between Group Comparisons for Males Only

Region	Side	BA	Talairach Coordinates (x,y,z)	t-value
MA (n=9) > ASD (n=9)				
Frontal				
Anterior Cingulate Cortex	L	24	(-6,31,3)	3.67
Anterior Cingulate Cortex	R	24	(14, 39, -5)	3.92
Medial Frontal Cortex	L	32	(-9, 47, 2)	5.50
Superior Frontal Gyrus	R	9	(22, 46, 26)	5.52
Superior Frontal Gyrus	L	9	(-28,43,26)	4.83
Superior Frontal Gyrus	R	6	(18,-1,55)	3.73
Superior Frontal Gyrus	R	10	(20,59,16)	4.31
Cingulate Gyrus	R/L	31	(1,2,31)	4.01
Orbitofrontal Gyrus	L	11	(-19,23,-16)	5.12
Middle Frontal Gyrus	L	9	(-18,55,20)	4.49
Middle Frontal Gyrus	R	9	(30,40,30)	5.20
Precentral Gyrus	L	4	(-46,-1,49)	5.49
Precentral Gyrus	R	4	(41,-1,27)	4.36
Insula				
Insula	L		(-34,11,6)	4.12
Temporal				
Parahippocampal Gyrus	R	36	(21,-28,-16)	3.64
Parahippocampal Gyrus	L	30	(-22,-42,-1)	4.03
Inferior Temporal Gyrus	L	20	(-46,-33,-20)	4.39
Parietal				
Superior Parietal Lobule	L	7	(-9,-44,43)	3.64
Superior Parietal Lobule	R	7	(11,42,43)	5.97
Angular Gyrus	L	39	(-35,-73,38)	3.78
Precuneus	L	7	(-13,-65,35)	4.82
Occipital				
Cuneus	R	19	(18,-89,26)	6.11
Cuneus	L	18	(-5,-69,14)	3.59
Subcortical				
Cerebellum	L		(-37,-70,-20)	7.00
Cerebellum	R		(22,-43,-18)	4.16
Caudate	R		(12,19,-1)	3.61
ASD (n=9) > MA (n=9)				
Temporal				
Superior Temporal Gyrus	L	38	(-34,-4,-17)	-4.75
Parietal				
Postcentral Gyrus	R	3	(34,-32,55)	-3.56
Postcentral Gyrus	L	3	(-35,-29,55)	-4.09
Insula				
Insula	L	47	(-38,-5,-5)	-6.88
Insula	R	47	(44,-1,-6)	-4.03

Region	Side	BA	Talairach Coordinates (x,y,z)	t-value
CA (n=8) > ASD (n=8)				
Frontal				
Inferior Frontal Gyrus	L	47	(-42,12,10)	5.52
Cingulate Gyrus	L	31	(-10,3,36)	4.00
Insula				
Insula	L		(-30,12,0)	4.50
Temporal				
Superior Temporal Gyrus	L	22	(-46,-10.-1)	4.90
Middle Temporal Gyrus	L	37	(-50,-55,3)	5.05
Fusiform Gyrus	L	37	(-33,-49,-13)	4.03
Parahippocampal Gyrus	L	36	(-28,-45,3)	3.60
Parietal				
Superior Parietal Lobule	R	7	(26,-72,39)	5.13
Superior Parietal Lobule	L	7	(-26,-57,42)	5.10
Posterior Cingulate	L	30	(-22,-65,11)	4.24
Cingulate Gyrus	L	31	(-11,-22,39)	4.68
Occipital				
Lingual Gyrus	R	19	(17,-54,-1)	4.47
Cuneus	L	18	(-10,-82,16)	4.61
Cuneus	L	19	(-10,-81,35)	5.31
Subcortical				
Cerebellum	R		(33,-54,-21)	4.66
Cerebellum	L		(-33,-70,-17)	6.69
ASD (n=8) > CA (n=8)				
Frontal				
Anterior Cingulate Gyrus	R	32	(6,26,20)	-3.20
Medial Frontal Gyrus	R	32/9	(6,42,19)	-5.08
Inferior Frontal Gyrus	R	44/9	(49,15,31)	-4.13
Cingulate Gyrus	R	31	(10,-2,25)	-4.78
Middle Frontal Gyrus	L	6	(-20,11,47)	-4.83

The peak t-value and Talairach coordinate is given for each region or Brodmann Area (BA) showing significant activity.

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