Speech Stimulation during Functional MR Imaging as a Potential Indicator of Autism[1]

Grace Lai, PhD
Harry D. Schneider, MD
Johanna C. Schwarzenberger, MD
Joy Hirsch, PhD

Purpose: To determine the feasibility of applying functional magnetic resonance (MR) imaging as an objective indicator of language disability in autism by using passive speech stimulation.

Materials and Methods: This prospective study was approved by the institutional review board, and informed consent was obtained from the parents or guardians of all subjects. Functional MR imaging was performed during passive presentations of prerecorded speech in 15 control subjects (mean age ± standard deviation, 12.1 years ± 4.3) and 12 language-impaired, age-matched autistic subjects (mean age, 12.4 years ± 4.7). An additional 27 autistic children (mean age, 8.4 years ± 3.1), who underwent imaging while sedated with propofol as part of routine clinical MR evaluations, were also included. Activation maps for each subject were computed by using univariate general linear model analyses. The spread (quantified as number of voxels) and amplitude of the functional MR imaging activation were then quantified within two anatomically specified regions of interest known to be involved with language: the primary auditory cortex (A1) and the superior temporal gyrus (STG). Group differences were compared by using analysis of variance, two-sample *t* tests, and Wilcoxon rank sum tests where appropriate. The threshold for autism was defined as 1 standard deviation below the control mean for subjects imaged in the alert state. A similar threshold was estimated for sedated autistic subjects on the basis of differences between nonsedated and sedated autistic subjects.

Results: Activity in A1 did not differ between autistic and control subjects. However, mean amplitude and spread of activity in the STG differed between autistic and control subjects ($P < .001$). Values for 10 of the 12 (83%) nonsedated autistic subjects decreased at least 1 standard deviation below the control distribution. The threshold derived from sedation-adjusted values of the control group enabled identification of 26 of the 27 (96%) sedated autistic subjects.

Conclusion: Functional MR imaging activation within the STG in response to passive speech stimulation helped differentiate autistic from control subjects, demonstrating the potential utility of functional MR imaging as an objective indicator of language impairment in autism. Future studies may lead to an early and objective indicator for autism with these methods.

[1] From the Functional MRI Research Center (G.L., H.D.S., J.H.), Departments of Neuroscience (G.L., J.H.), Radiology (J.H.), and Psychology (J.H.), Columbia University Medical Center, Neurological Institute, 710 W 168th St, Room B-41, New York, NY 10032; and Department of Anesthesiology, UCLA Medical Center, Los Angeles, Calif (J.C.S.). Received October 23, 2010; revision requested January 5, 2011; revision received February 4; accepted February 14; final version accepted March 25. **Address correspondence to** J.H. (e-mail: *joyhirsch@yahoo.com*).

Autism is a spectrum disorder estimated to affect up to one in 100 children (1,2) in the domains of impaired social interactions, impaired language and communication, and repetitive and restricted behaviors. Despite apparently increasing prevalence, the diagnosis of autism remains limited to parent and clinician observation of missed developmental milestones (3). Because functional magnetic resonance (MR) imaging is performed without added risk or invasiveness relative to standard imaging procedures indicated for medical assessments, functional imaging of impaired systems, including language, may appropriately aid objective indications of language impairment in autistic patients. Recent functional MR imaging studies of language systems in autistic patients indicated group-level patterns of atypical activity in temporal and frontal brain regions known to be involved in language processing (4–10). However, no systematic and widely implementable MR imaging applications have been proposed for individual patients suspected of having or known to have autism.

Tolerance to imaging conditions is a major obstacle to performing functional MR imaging in young children. Thus, functional imaging for diagnostic purposes would necessarily require the use of sedation in most patients. As such, functional MR imaging research related to autism ideally would include data regarding functional MR imaging in the setting of sedation. Sedation has been successfully applied with use of passive stimulation to map language-specific brain areas for neurosurgical planning and clinical assessment (11–13). Furthermore, previous studies in adults and children imaged under sedation indicate that brain responses under propofol sedation are generally consistent with the canonical adult hemodynamic response function (HRF) (12–14), although propofol can alter rates of cerebral blood flow and oxygen extraction (15).

We aim to develop automated and systematic functional MR imaging procedures and measures to document blood oxygen level–dependent (BOLD) activity that would reliably help differentiate language-impaired autistic patients from age-matched control subjects on an individual basis. The purpose of this study was to determine the feasibility of applying functional MR imaging as an objective indicator of language disability in autism by using passive speech stimulation.

Advance in Knowledge

- Functional MR imaging activation in response to passive language stimuli can help differentiate language-impaired autistic subjects from control children with 83% (10 of 12 subjects) specificity and 92% (14 of 15 subjects) sensitivity.

Materials and Methods

Subjects

For this prospective study, we analyzed single-subject data acquired between 2008 and 2010 within a group study designed to investigate the neural mechanisms that underlie language impairment in autistic subjects. Data were analyzed from 15 nonautistic control children (mean age ± standard deviation, 12.13 years ± 4.34; age range, 4.19–17.78 years) and 12 autistic subjects (mean age, 12.40 years ± 4.70; age range, 7.01–22.47 years) who were imaged while alert. The control group included five girls (mean age, 12.28 years ± 5.47; age range, 5.05–17.51 years) and 10 boys (mean age, 12.05 years ± 4.00; age range, 4.19–17.78 years), and the autistic subjects included two female (mean age, 12.015 years ± 5.14; age range, 8.38–15.63 years) and 10 male (mean age, 12.481 years ± 4.90; age range, 7.01–22.47 years) subjects. An additional 27 autistic subjects (mean age, 8.62 years ± 3.14; age range, 5.41–17.93 years) who underwent routine clinical MR imaging evaluations (structural and functional) while under propofol sedation were also evaluated. Those subjects included two girls (mean age, 8.24 years ± 2.84; age range, 6.23–10.24 years) and 25 boys (mean age, 8.38 years ± 3.12; age range, 5.41–17.93 years). The parents or guardians of all subjects gave permission to include their images in this study in accordance with procedures approved by the institutional review board and Health Insurance Portability and Accountability Act guidelines. Detailed subject information is provided in Tables 1–3. Groups were matched for age and maternal education. The study initially included 17 control subjects and 16 nonsedated autistic subjects; however, two control and four autistic subjects were excluded because of excess head movement.

Autistic subjects were recruited by means of physician referral, and control subjects were recruited by posting flyers around the hospital. Autistic subjects met inclusion criteria for this study if

Implications for Patient Care

- The results of this study suggest that objective imaging techniques may be used to differentiate language-impaired autistic subjects from control subjects with normal language development.
- Additional observations with subjects under light propofol sedation, which is often necessary when imaging young children and those with developmental delay, suggest that these methods may also apply to sedated patients.

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Abbreviations:
A1 = primary auditory cortex
ADI-R = Autism Diagnostic Interview–Revised
ANOVA = analysis of variance
BOLD = blood oxygen level–dependent
HRF = hemodynamic response function
ROI = region of interest
STG = superior temporal gyrus

Author contributions:
Guarantors of integrity of entire study, G.L., J.H.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, all authors; clinical studies, all authors; statistical analysis, G.L., J.H.; and manuscript editing, G.L., J.C.S., J.H.

Potential conflicts of interest are listed at the end of this article.

they were diagnosed with autism according to criteria from the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (16) and the Autism Diagnostic Interview–Revised (ADI-R). With the ADI-R, autism is diagnosed when the subject's score is higher than a specified minimum on all three sections (social: >10; language: >8; repetitive behavior: >3). The course of medical treatment was not altered for subjects included in this study. Control subjects met inclusion criteria for the study if they were developmentally normal, if they did not have siblings on the autism spectrum, and if they performed at expected academic and social levels, as confirmed by scholastic performance at grade level and parent reports. Control and autistic subjects were excluded from this study if they had comorbid neurologic or developmental disorders or any contraindications to MR imaging. In addition, control subjects were excluded if they were diagnosed with any psychiatric conditions or if they were taking any psychiatric medications. All autistic subjects were evaluated by the referring physician (H.D.S., with 13 years of experience) and referred only if they met all inclusion and exclusion criteria.

Functional MR Imaging Stimulation

The duration of functional MR image acquisition was 2 minutes 29 seconds. A 24-second baseline period was followed by four 15-second presentations of speech stimulation alternating with 15-second baseline epochs. A 24-second initial baseline period was used because the first three acquisitions (9 seconds total) would be discarded before image processing owing to the signal artifact at the beginning of each run before steady-state imaging has been reached. Two images were acquired within a total of 4 minutes 38 seconds. Prerecorded parents' voices were used as stimuli, and voices were presented passively via MR imaging–safe headphones. Similar techniques using passive stimulations are used for neurosurgical planning and other evaluation purposes (11,17).

For all recordings, parents were instructed to talk about the same topics (ie, being in the imaging unit, recent events, plans after imaging). Familiar voices are used to increase task compliance in younger and autistic children. Audio stimuli were power-normalized across subjects to ensure similar acoustic properties across subjects. Two independent raters (not authors) judged whether the 15-second clips of voice recordings from parents of 10 randomly selected autistic subjects could be differentiated from those from parents of 10 randomly selected control subjects. Both raters judged the subject's diagnosis with only 55% accuracy (11 of 20 subjects) and a 43% correspondence (nine of 20 subjects). Close-to-chance levels of performance indicate that narratives from parents of autistic subjects did not differ perceptibly from those of parents of control subjects.

Imaging Procedures

Alert subjects.—To minimize head movement and distractibility, a familiar video was shown on mute by means of a rear-projection screen or MR imaging–compatible goggles throughout the imaging examination. Comparisons across stimulus conditions (speech vs baseline) reveal activity related to the auditory stimulus and not the video that played continuously during both auditory and baseline epochs.

Sedated subjects.—Subjects imaged under conventional clinical conditions with use of sedation underwent imaging for neurologic assessment as ordered by their referring physician. Anesthesia was induced via a mask with sevoflurane in an oxygen–nitrous oxide mixture to facilitate placement of an intravenous line. Once the intravenous line was placed, patients were transitioned to an intravenous-based anesthetic with propofol. The initial propofol dose was adjusted to render the patient motionless but able to maintain his or her airway without an endotracheal tube. The condition of sleep produced with a steady-state propofol dose was studied for one cycle of functional MR imaging testing. With use of the absence of movement artifacts at either end-tidal CO_2 or pulse oximetry tracings, propofol dose was reduced in 50 mcg/kg/min increments by using a previously described technique (11). Functional MR imaging resumed at this lower anesthetic concentration. Imaging was stopped if gross patient movement occurred. Anesthesia was administered by J.C.S., an attending anesthesiologist with 10 years of experience.

Image Acquisition and Preprocessing

Alert subjects were imaged with a unit devoted to research, and sedated subjects were imaged at a clinical site within the same hospital by using a comparable unit of the same brand and model with use of the same imaging parameters. At both locations, a 1.5-T unit (Twin Speed; GE Medical Systems, Milwaukee, Wis) was used and functional MR images were acquired by using an echo-planar T2*-weighted gradient-echo sequence (repetition time = 3000 msec, echo time = 51 msec, 83° flip angle). Twenty-seven contiguous transverse sections covering the entire brain were acquired along the anterior commissure–posterior commissure plane, with a 192 × 192-mm field of view imaged on a 128 × 128 grid, yielding an in-plane resolution of 1.56 × 1.56 mm and a section thickness of 4.5 mm. High-spatial-resolution structural images were acquired by using a three-dimensional

Table 1

Characteristics of Control Subjects

Subject No./Sex	Dominant Hand	Age at Imaging (y)
1/M	Right	13.55
2/M	Right	14.62
3/M	Right	17.78
4/M	Right	11.10
5/M	Left	16.84
6/M	Right	4.19
7/F	Right	15.93
8/F	Right	15.02
9/M	Right	9.87
10/F	Right	5.05
11/F	Right	7.90
12/M	Right	13.07
13/F	Right	17.51
14/M	Right	9.64
15/M	Right	9.84
Mean ± standard deviation	...	12.13 ± 4.34

spoiled gradient-echo sequence (124 sections, 256 × 256, 220-mm field of view), with a total imaging time of 10 minutes 38 seconds.

Functional MR images were processed by using software (FSL 4.1; FMRIB Software Library, available at *www.fmrib.ox.ac.uk/fsl/*). Preprocessing consisted of brain extraction, motion correction, spatial smoothing (Gaussian kernel, full width at half maximum = 5 mm), high-pass filtering (cutoff = 60 seconds), and prewhitening. Preprocessed images were normalized to standard Montreal Neurologic Institute coordinate space.

Regions of Interest

We measured the spread and amplitude of BOLD activation within anatomically defined regions of interest (ROIs) for the primary auditory cortex (A1) and superior temporal gyrus (STG), a region associated with sentence comprehension (18–20) with preserved activation during propofol sedation (11,13,21,22). Spread of activation within each ROI was defined as the number of voxels exceeding a threshold of $z > 1.6$ ($P < .05$, uncorrected). Amplitude was defined as the mean z score of all voxels in the specified ROI. ROIs were defined anatomically for A1 and STG. ROIs were computer-generated in normalized space by using the Harvard-Oxford atlas probability distributions (*http://www.cma.mgh.harvard.edu/*) and multiplied by each subject's normalized second-level functional MR image.

Estimation of Custom HRFs

HRFs were estimated for each subject by using FLOBS, a three-function basis set included in the FSL software package. Active voxels within an anatomically defined region of A1 were selected to reconstruct the fitted HRF shape by using parameter estimates for the basis functions. This technique has been used previously to estimate custom HRFs for individual subjects (23).

Differentiation between Autistic and Control Subjects

To quantify the separation between individual autistic and control subjects, spread and amplitude measures were transformed to the number of standard deviations away from the mean of the control group. Subjects with standard deviation–transformed measures of less than 1.0 from the control mean were considered positive for autism, and those with values above this threshold were considered negative for autism. With the criterion for diagnosis of autism as −1.0 standard deviation from the control mean, specificity, sensitivity, and positive and negative predictive likelihood ratios were computed for the test. Because images of sedated healthy control subjects were not acquired, we derived a diagnostic test for sedated subjects by using observations of the effect of sedation in autistic subjects. We subtracted the difference between means of the sedated and nonsedated autistic subjects from the control (nonsedated) mean, and an estimated diagnostic threshold for sedated subjects was taken as 1 standard deviation (the standard deviation of the nonsedated control group) below this adjusted mean.

Statistical Analyses

All statistical analyses were performed by G.L. (with 6 years of experience) and supervised by J.H. (with more than 17 years of experience) by using FSL software for image analysis and SPSS software (SPSS, Chicago, Ill) for behavioral and individual subject analyses, as described below.

Behavioral Analysis

Mean ADI-R scores on each of three subsections (language and communication, reciprocal social interactions, and restricted, repetitive, and stereotyped behaviors and interests) were compared between sedated and nonsedated subjects by using two-sample t tests.

Image Analysis

HRFs.—We compared the means of the latency to peak and the latency of the poststimulus minimum of the fitted HRF curves by using a nonparametric Wilcoxon rank sum test given observed nonnormal distribution of peak latency values across subjects. Mean amplitudes across 500-msec windows between 0 and 14 seconds were computed for each subject. Group comparisons at each interval were based on two-tailed independent sample t tests, where $P < .05$ was considered to indicate a significant difference.

Group comparisons.—For statistical modeling, first-level general linear model analysis was performed by using FEAT, which is part of the FSL software package. One regressor modeled the stimulus-on periods with a weight of +1 and baseline periods with a weight of 0 for each subject and each run. A second-level fixed-effects analysis was performed for the average effect across the two functional runs. Group analyses were performed by using FLAME 1, FSL's mixed effects analysis. Contrasts between control subjects versus sedated autistic subjects and between nonsedated versus sedated autistic subjects were run within A1 and STG ROIs.

Spread and amplitude of functional MR imaging signals.—Repeated-measures analysis of variance (ANOVA) was performed to test main effects and interactions between groups (control subjects vs nonsedated autistic subjects, nonsedated autistic subjects vs sedated autistic subjects), regions (STG and A1), and hemispheres (left, right). Significant interactions were followed by post hoc paired comparisons by using two-tailed two-sample t tests for between-group comparisons and two-tailed paired t tests for within-group comparisons. Significance levels (P values) were determined with Bonferroni adjustment for multiple comparisons, and $P < .05$ was considered to indicate a significant difference.

Results

Behavior

All autistic subjects scored in the high range of impairment on all three ADI-R subsections (reciprocal social interaction: mean, 21.18 ± 1.66; language and communication: mean, 18.87 ± 2.62; restricted, repetitive, and stereotyped behavior: mean, 6.00 ± 1.15) (Tables 2, 3). ADI-R scores were not measured in healthy control subjects, all of whom would presumably score zero on all

Table 2

Characteristics of Nonsedated Autistic Subjects

Subject No./Sex	Dominant Hand	Age at Imaging (y)	ADI-R Score		
			Social	Language	Repetitive Behavior
1/M	Right	16.72	20	17	6
2/M	Ambidextrous	7.01	22	18	6
3/M	Right	10.85	22	17	6
4/M	Right	22.47	21	22	8
5/F	Right	8.38	21	20	5
6/M	Left	9.10	21	18	8
7/M	Right	16.56	19	22	6
8/M	Right	9.21	19	20	5
9/M	Right	13.39	19	19	6
10/F	Right	15.65	17	16	5
11/M	Right	7.41	17	12	4
12/M	Right	12.09	24	21	6
Mean	...	12.40 ± 4.70	20.17 ± 2.08	18.50 ± 2.84	5.92 ± 1.16

Table 3

Characteristics of Sedated Autistic Subjects

Subject No./Sex	Dominant Hand	Age at Imaging (y)	ADI-R Score			Propofol Level (mcg/kg/min)
			Social	Language	Repetitive Behavior	
1/M	Ambidextrous	6.37	24	20	6	220
2/M	Left	5.81	23	21	5	200, 230
3/M	Left	7.36	21	17	6	175
4/M	Right	7.28	22	20	6	200
5/M	Left	10.90	20	18	5	200
6/M	Right	7.39	19	21	6	200
7/M	Left	7.99	21	26	9	150
8/M	Left	6.34	22	17	6	200
9/M	Ambidextrous	6.18	22	17	5	200
10/M	Ambidextrous	5.41	23	19	5	175
11/M	Right	6.81	22	26	9	300
12/M	Right	9.59	23	20	5	225
13/M	Ambidextrous	7.37	22	18	6	200
14/M	Right	6.44	23	18	5	200
15/F	Left	6.23	23	20	5	200
16/M	Ambidextrous	9.41	22	19	6	200
17/M	Right	7.69	21	19	7	200
18/M	Right	14.18	22	19	6	125, 150
19/M	Right	7.52	22	17	5	210
20/M	Right	8.82	23	20	5	200
21/M	Right	8.33	23	20	5	250
22/M	Right	6.60	20	15	6	200
23/F	Right	10.24	23	21	8	175
24/M	Right	6.25	21	17	6	200
25/M	Right	15.21	21	18	7	175
26/M	Right	17.93	21	17	6	200
27/M	Right	6.24	24	19	6	...
Mean	...	8.37 ± 3.05	21.96 ± 1.22	19.22 ± 2.47	6.00 ± 1.14	...

sections. ADI-R scores between sedated and nonsedated subjects with regard to language (P = .3168) or repetitive behaviors (P = .99) did not differ significantly. Sedated subjects were more impaired in the social domain ($P < .001$). Significant correlations were not observed between ADI-R scores and functional MR imaging measures.

HRF

There were no differences in the shape and amplitude of the group-average fitted HRFs between autistic and control subjects who underwent imaging while alert (Fig 1a) and no statistical differences in time to peak (control subjects = 4.55 seconds, autistic subjects = 4.77 seconds; P = .92). Although the average latency to peak was about 1 second later for the sedated group relative to the nonsedated group (nonsedated subjects = 4.77 seconds, sedated subjects = 5.64 seconds), comparisons between nonsedated and sedated autistic subjects also revealed no differences (P = .13) (Fig 1b). The latency at which the poststimulus minimum occurred was also not significantly different (P = .29). Peak amplitude of the group average curves was 4.20 seconds for control subjects, 4.35 seconds for nonsedated autistic subjects, and 5.05 seconds for sedated subjects. A time to peak of 5 seconds is compatible with the normal adult HRF and consistent with estimates from sleeping infants (24). There were also no significant amplitude differences between control subjects and nonsedated autistic subjects or between nonsedated and sedated autistic subjects.

Group Functional MR Imaging Contrasts

BOLD responses were analyzed within the anatomically defined ROIs for STG and A1 (Fig 2a, left). Contrasts classified as "control > autism (alert)" indicated greater activity within STG (but no difference in A1) in the control group compared with nonsedated autism group, even at the most lenient threshold of $P < .05$ (Fig 2a, middle). Contrasts classified as "nonsedated autism > sedated autism" indicated greater activation within both STG and A1 in the nonsedated autism group compared with sedated autism group ($P < .05$) (Fig 2a, right).

Spread: Number of Voxels

A group (control, autistic subjects) × region (STG, A1) × hemisphere (left, right) repeated-measures ANOVA indicated significant main-effect differences for group and region (Table 4) and a group × region interaction ($F_{[1,11]}$ = 18.176, $P < .001$) for spread of the BOLD signal within each ROI. Paired comparisons revealed larger spread for control subjects relative to nonsedated autistic subjects in STG bilaterally ($P < .001$ for both left and right hemispheres) but not in A1 ($P < .99$) (Fig 2b). A similar ANOVA comparing nonsedated and sedated autistic subjects indicated significant main effects for group and region (Table 4) as well as a group × region interaction ($F_{[1,11]} = 4.931$, $P < .048$). Paired comparisons revealed larger spread for nonsedated autistic subjects relative to sedated subjects in bilateral STG ($P < .03$) and A1 (left hemisphere: $P < .049$; right hemisphere: $P < .011$) (Fig 2a).

Amplitude of Signal Strength: Mean *z* Score

Results for comparisons of signal amplitude were similar to those observed for spread. A group (control, autistic subjects) × region (STG, A1) × hemisphere (left, right) repeated-measures ANOVA indicated significant main-effect differences for group and region (Table 4) and a significant group × region interaction ($F_{[1,11]}$ = 20.19, $P < .001$). Paired comparisons revealed greater mean amplitude in control subjects relative to autistic subjects in bilateral STG ($P < .001$) but not in A1 ($P < .99$) (Fig 2b). A similar ANOVA between nonsedated and sedated autistic subjects indicated significant main effects for group and region (Table 4). Paired comparisons revealed greater activation in bilateral STG (left hemisphere: $P < .04$; right hemisphere: $P < .05$)

Figure 1

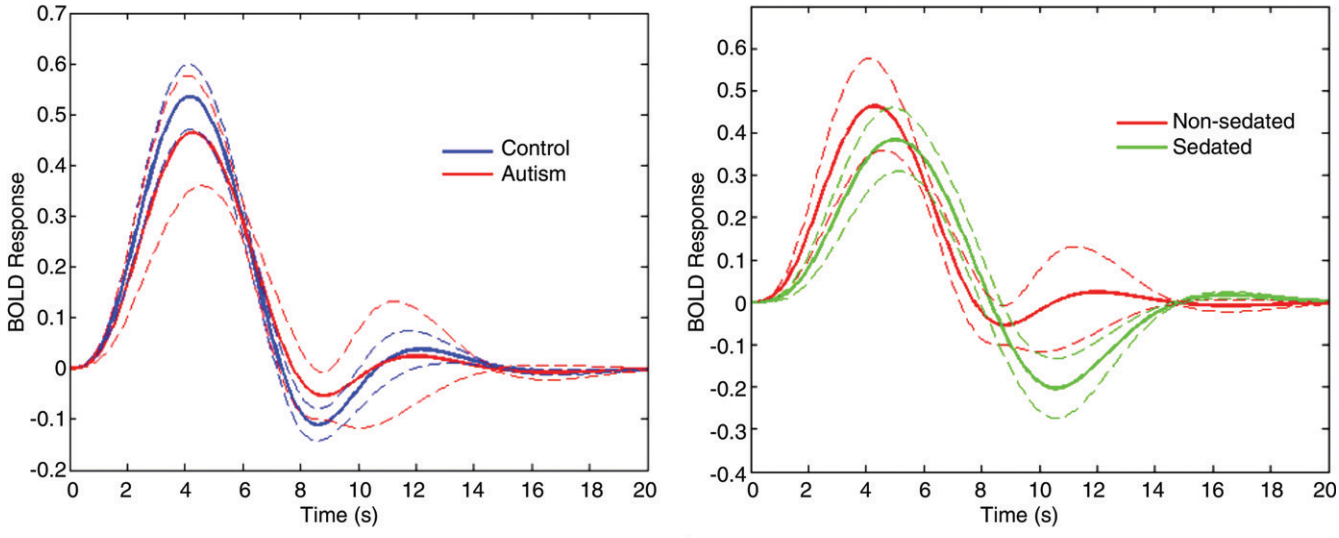

Figure 1: Group-averaged fitted HRFs for **(a)** nonsedated control and autistic subjects and **(b)** nonsedated and sedated autistic subjects. Dotted lines = 1 standard deviation above and below the average curve.

and right A1 ($P < .02$), but not in left A1 ($P < .07$), in nonsedated relative to sedated subjects (Fig 2c).

Differentiation between Autistic and Control Subjects

Plots of voxel count (spread, Fig 3a) and *z* score (amplitude, Fig 3b) of individual control subjects and autistic subjects for STG (y-axis) against A1 (x-axis) visually confirm the separation between groups for STG but not for A1. Because there were no significant differences between hemispheres in all groups (Table 4), these results were collapsed across hemispheres.

The extent to which differences in the spread and amplitude of activation in STG helped differentiate autistic from control subjects can be quantified by the degree of overlap across the groups (Fig 4). The percentage of sedated and nonsedated autistic subjects and control subjects with values that fell within 1–4 standard deviations from the control mean are shown for spread (Fig 4a) and amplitude (Fig 4b). For both spread and amplitude, 83% of nonsedated autistic subjects had values below 1 standard deviation of the control mean, whereas only 7% of control subjects had values that fell in that region. We used this observation to establish a diagnostic boundary for autism as 1 standard deviation below the control mean (Fig 3) for both measures. For nonsedated subjects, the sensitivity (proportion of true-positive findings) of this test was 83% (10 of 12 subjects, confidence interval: ±21.1%) and the specificity (proportion of true-negative findings) was 93% (14 of 15 subjects; confidence interval: ±12.6%). The positive likelihood ratio (number of true-positive to false-positive findings) was 12.5, and the negative likelihood ratio (number of true-negative to false-negative findings) was 5.6.

Because of the unavailability of sedated healthy volunteer children, the sensitivity of a similar test for sedated autistic subjects was estimated by adjusting the diagnostic threshold by the effect of sedation between nonsedated and sedated autistic subjects (Fig 4). The sensitivity of a test based on this adjusted threshold for sedated autistic subjects was 85% (23 of 27 subjects; confidence interval: ±11.3%) for spread and 96% (26 of 27 subjects; confidence interval: ±7.12%) for amplitude.

Figure 2

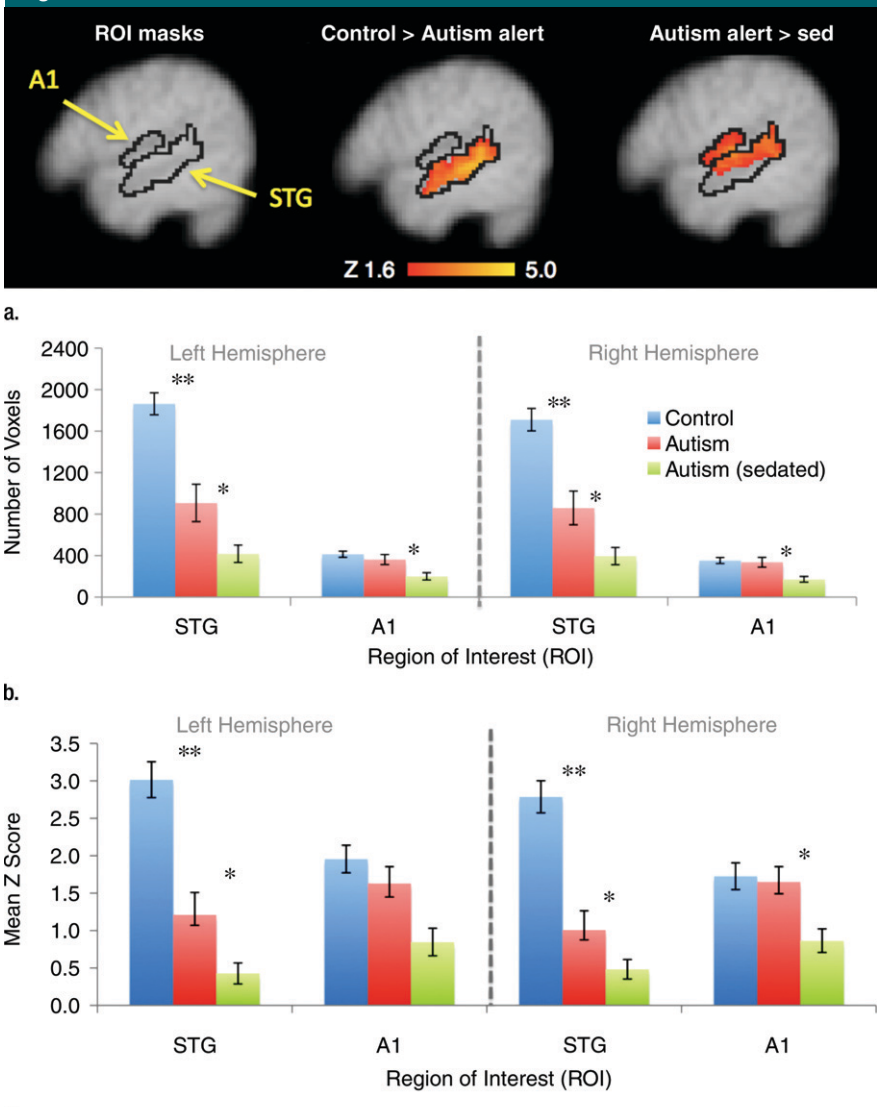

Figure 2: **(a)** MR image (left) illustrates anatomic ROIs for A1 and STG. Activation maps (middle and right) demonstrate greater activity within STG, but not A1, in the control group compared with the nonsedated autism group (control > autism alert, middle image) and greater activation within both STG and A1 in the nonsedated compared with the sedated autism group (autism alert > sed, right image). **(b, c)** Bar charts show **(b)** spread and **(c)** amplitude of activation of individual subjects within ROIs (two-sample *t* tests; ** = $P <$ .01, * = $P <$ .05, with Bonferroni adjustment). *sed* = sedated.

Discussion

This study found that activation within language-sensitive brain regions during passive speech stimulation at functional MR imaging enabled the differentiation of language-impaired autistic subjects from age-matched control children. We identified two measures, signal spread and amplitude, by using anatomically defined ROIs applied to individual patterns of activation. A standard atlas was chosen to define two ROIs (A1 and STG) to provide a systematic and automated method (25) transferable to standard

clinical practice. Both spread and amplitude of activation within STG were greater for control children relative to autistic subjects who underwent imaging while alert, whereas A1 showed no difference. Differences in activation in STG, but not A1, suggest intact auditory processing but disrupted linguistic comprehension at higher processing stages.

Our findings are consistent with those from previous functional MR imaging studies that used passive stimulation paradigms such as those used herein and reported decreased activation in the temporal lobe in autistic subjects (10,26,27). Structural MR imaging studies have also reported atypical laterality of temporal lobe brain volume in groups of autistic adults and children (28,29). The present study extends previous reports of group-level differences and suggests that individual functional MR imaging measures of spread and amplitude can be used to differentiate autistic from nonautistic subjects. In practice, only one measure (spread or amplitude) would be sufficient. Future studies are required to determine optimal diagnostic criteria.

A major obstacle to imaging children in functional studies includes task compliance, tolerance of the imaging environment, and maintenance of a steady head position for a sufficient duration of time. To image children while they were alert, we used a "silent video" to overcome these obstacles. However, patients who could not tolerate the imager environment even under these optimized circumstances and for whom a conventional image was indicated were imaged under clinical management and propofol sedation, a technique that offers an effective alternative to enable functional imaging in young children (11–14). If functional MR imaging is to be applied as a diagnostic tool for use in children with developmental delay, sedation would likely be necessary in most cases.

We found overall decreases in spread and amplitude of signal in both temporal regions (A1 and STG) in sedated autistic subjects relative to nonsedated autistic subjects, a result that is consistent with those from functional MR imaging studies in sedated adults and children (14,21,22,30). We did not include healthy subjects imaged under sedation as controls for the sedated subjects. Patients without autism or developmental delay undergoing MR imaging under sedation may serve as a possible control group in future studies. At present, a test derived from adjusted threshold values based on the effect of sedation between nonsedated and sedated autistic subjects showed similarly high sensitivity estimates for the diagnosis of sedated autistic children. Differential patterns of brain activation have also been previously described in children with language delay imaged under sedation (14). The observation that the time to peak of the HRF is similar between autistic subjects and control children imaged alert and between autistic subjects imaged alert and under sedation indicates that sedation does not differentially affect this fundamental property of the BOLD signal. These findings are consistent with those from previous studies that optimally modeled functional MR imaging responses to language stimuli by using the canonical HRF in sleeping and sedated children with and without developmental delay (12,13,24).

Table 4

ANOVA: Two Group × Two Region × Two Hemisphere Comparison

Parameter	$F_{(1,11)}$	*P* Value
Spread: no. of voxels		
Control vs nonsedated autistic subjects		
Group	13.909	.003*
Region	307.833	<.001*
Hemisphere	3.479	.89
Group × region	18.176	.001*
Group × hemisphere	0.387	.55
Region × hemisphere	0.689	.42
Group × region × hemisphere	0.202	.66
Nonsedated vs sedated autistic subjects		
Group	11.631	.006*
Region	21.958	.001*
Hemisphere	0.351	.57
Group × region	4.931	.05*
Group × hemisphere	0.053	.82
Region × hemisphere	0.006	.94
Group × region × hemisphere	0.133	.72
Amplitude: mean *z* score		
Control vs nonsedated autistic subjects		
Group	13.15	.004*
Region	20.116	.001*
Hemisphere	3.709	.08
Group × region	20.192	.001*
Group × hemisphere	0.265	.62
Region × hemisphere	1.855	.20
Group × region × hemisphere	0.014	.91
Nonsedated vs sedated autistic subjects		
Group	10.427	.008*
Region	11.846	.006*
Hemisphere	0.183	.677
Group × region	0.198	.665
Group × hemisphere	0.354	.564
Region × hemisphere	0.363	.559
Group × region × hemisphere	0.819	.385

* Statistically significant.

Limitations to our study include the question of age and how these findings would apply to younger children, who are at an age at which an objective medical diagnostic procedure would be most useful for the purpose of early intervention. The mean age of our sedated subjects was less than that of our nonsedated subjects. However, it is unlikely that observed differences between sedated and nonsedated subjects in the present study are due to age, as previous studies in typically developing infants as young as 3 months have shown activation in STG to passive language stimulation (24,31). Furthermore, limitations associated with the quantification of functional MR imaging signals include the choice of an appropriate threshold because activation patterns will change depending on threshold. At present, we opted to use a most liberal threshold of significance, $P < .05$ ($z > 1.6$), for comprehensive assessment.

The use of functional MR imaging to determine physiologic differences between autistic and control subjects may be investigated in future studies to differentiate between autism and other developmental disorders such as specific language impairment. Given the concern over the extent to which these disorders are etiologically distinct (32–34), an objective biomarker to differentiate between these disorders might be used to shape the diagnostic criteria. Because our study did not include subjects with abnormalities other than autism, our findings cannot be used to differentiate autism from other causes of developmental delay. Nonetheless, our findings complement those of a recent study in which MR imaging and pattern recognition techniques were used to identify subtle differences in neuroanatomic features among autistic subjects, control subjects, and those with attention deficit/hyperactivity disorder (35) and support the potential use of neurophysiologic measurements for diagnosis. Furthermore, although correlations between the degree of behavioral impairment and functional MR imaging measures were not observed in the present study, possibly due to limited variability in our sample, future studies including

Figure 3

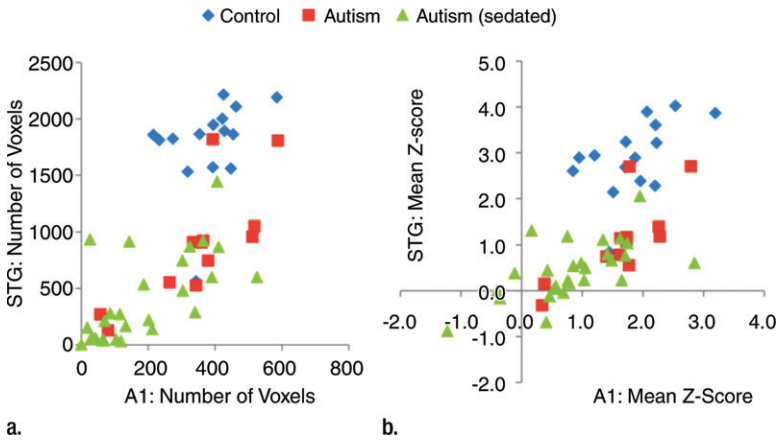

Figure 3: Scatterplots show individual measures for **(a)** spread and **(b)** amplitude in STG and A1. Control and autistic (nonsedated and sedated) subjects are separated along the STG (y) axis and not along the A1 (x) axis. Values are averaged across hemispheres.

Figure 4

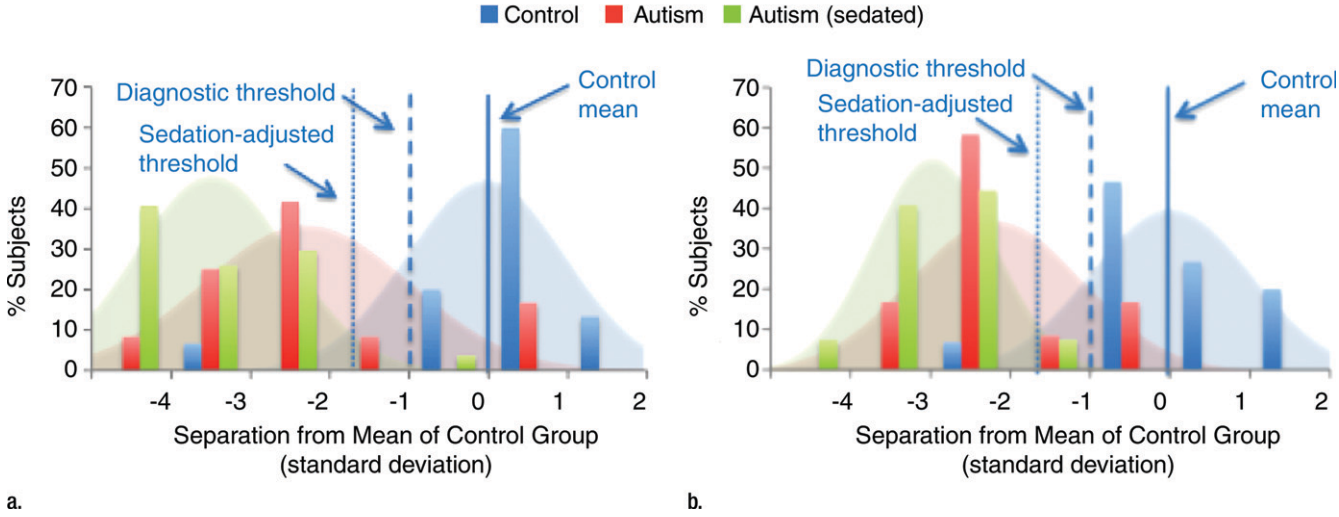

Figure 4: Graphs show the percentage of subjects whose values for **(a)** spread and **(b)** amplitude fell within 1–3 standard deviations from the control mean (solid lines). The diagnostic threshold for nonsedated autistic subjects was set to 1 standard deviation below the control mean (dashed lines). A similar threshold was estimated for sedated autistic subjects on the basis of differences between nonsedated and sedated autistic subjects (dotted lines).

patients with varying levels of disability along the autism spectrum may help establish a relationship between these measures. If so, functional MR imaging may serve as a neural measure of change in function following medical or neuropsychologic intervention.

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