Underconnectivity between voice-selective cortex and reward circuitry in children with autism

Daniel A. Abramsa,1, Charles J. Lynchb, Katherine M. Chengb, Jennifer Phillipsb, Kaustubh Supekara, Srikanth Ryala, Lucina Q. Uddina, and Vinod Menona,b,c,d,1

Departments of aPsychiatry and Behavioral Sciences and bNeurology and Neurological Sciences, cProgram in Neuroscience, and dStanford Institute for Neuro-Innovation and Translational Neurosciences, Stanford University School of Medicine, Palo Alto, CA 94304

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Individuals with autism spectrum disorders (ASDs) often show insensitivity to the human voice, a deficit that is thought to play a key role in communication deficits in this population. The social motivation theory of ASD predicts that impaired function of reward and emotional systems impedes children with ASD from actively engaging with speech. Here we explore this theory by investigating distributed brain systems underlying human voice perception in children with ASD. Using resting-state functional MRI data acquired from 20 children with ASD and 19 age- and intelligence quotient-matched typically developing children, we examined intrinsic functional connectivity of voice-selective bilateral posterior superior temporal sulcus (pSTS). Children with ASD showed a striking pattern of underconnectivity between left-hemisphere pSTS and distributed nodes of the dopaminergic reward pathway, including bilateral ventral tegmental areas and nucleus accumbens, left-hemisphere insula, orbitofrontal cortex, and ventromedial prefrontal cortex. Children with ASD also showed underconnectivity between right-hemisphere pSTS, a region known for processing speech prosody, and the orbitofrontal cortex and amygdala, brain regions critical for emotion-related associative learning. The degree of underconnectivity between voice-selective cortex and reward pathways predicted symptom severity for communication deficits in children with ASD. Our results suggest that weak connectivity of voice-selective cortex and brain structures involved in reward and emotion may impair the ability of children with ASD to experience speech as a pleasurable stimulus, thereby impacting language and social skill development in this population. Our study provides support for the social motivation theory of ASD.

The human voice is a critical communication signal for children. Infants’ engagement by the acoustical features of speech (1) is thought to serve at least two critical developmental functions. First, attraction to the human voice guides early speech perception (2), which in turn underlies subsequent development of language skills (3). Second, speech provides critical emotional value to children (4) and promotes bonding between infants and their parents (5). For example, hearing the adult voice soothes infants during moments of distress (6), and it is thought that this form of vocally mediated comfort constitutes a pleasurable and reinforcing experience during the early stages of development (7). The rewarding and emotional nature of speech has also been documented in studies of older children. In stressful situations, children experience increased oxytocin release upon hearing their mother’s voice (4). Release of this hormone promotes affiliative behaviors and is closely linked with emotion (8) and reward processing (9).

Social impairments are a primary deficit in autism spectrum disorders (ASDs) (10). A common observation in individuals with ASD is a relative indifference to the human voice, a trait noted throughout Kanner’s initial report on autism (11). Kanner writes of one of his patients, “He did not register any change of expression when spoken to,” and of another, “he did not respond to being called or to any other words addressed to him” (11). In contrast to typically developing (TD) children, who are extremely engaged by (12), and sensitive to (13), human vocal stimuli, children with ASD are often oblivious to such stimuli (14, 15). Anecdotal (11) and retrospective (15) accounts, as well as experimental investigations, have shown that children with ASD do not automatically orient to vocal stimuli (16), nor do they show a preference for vocal, compared with nonvocal, sounds (17).

It is not known why children with ASD are often indifferent to human vocalizations. One possibility is that deficits associated with social motivation and cognition (18–20) cause indifference to human vocalizations in ASD. The social motivation theory of ASD posits that deficits in representing the reward value of social stimuli, including speech, impedes children with ASD from actively engaging with these stimuli and consequently impairs social skill development (18). An alternative possibility is that individuals with ASD have a sensory deficit in which abnormal processing of the acoustical features of sound precludes access to brain systems serving human vocalization and speech recognition (21).

Investigations into the neural basis of human voice processing with the use of functional MRI (fMRI) have begun to provide clues regarding the biological basis of speech perception in ASD. For example, adults with ASD fail to activate voice-selective regions of bilateral superior temporal cortex (22) that are reliably activated in neurotypical subjects (22, 23). Beyond this, little is known about brain regions underlying voice processing and their links with distributed systems involved in language, reward, and affective information processing. Critically, to date, no study has examined whether large-scale intrinsic functional connectivity of voice-selective superior temporal sulcus (STS) regions is altered in ASD. This is somewhat surprising given that aberrant brain connectivity is one of the most consistent findings in the autism neuroimaging literature (24, 25).

Functional connectivity MRI has recently emerged as a powerful method for the examination of intrinsic functional relationships across the human brain (26, 27). By identifying specific functional systems impaired in clinical populations, functional connectivity MRI can help constrain our knowledge of distributed circuits that underlie sensory, cognitive, and affective dysfunction in children with neurodevelopmental disorders such as ASDs (28, 29). Moreover, this method is particularly advantageous for studying clinical and developmental populations in that it is free from potential behavioral confounds associated with task-based fMRI studies (30).

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1To whom correspondence may be addressed. E-mail: daa@stanford.edu or menon@stanford.edu.

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Here we examine intrinsic functional circuitry of voice-selective temporal cortex in TD children and children with ASD to test competing models of social and communication impairments in ASD. Models that view ASD symptomatology as a deficit in social motivation and cognition would predict abnormal connectivity between the posterior STS (pSTS) and the reward circuit, including the ventral tegmental area (VTA), nucleus accumbens (NAc), orbitofrontal cortex (OFC) (31), and the amygdala (19, 20). In contrast, models based on sensory processing deficits in ASD (21) would predict that a key component of pSTS connectivity would include abnormal connectivity with primary auditory cortical regions, which are critical for acoustical processing of speech (32). We investigated voice-selective regions of left- and right-hemisphere pSTS (23) based on their putative roles in speech comprehension. Bilateral pSTS are critical for a number of speech and language-related processes (33) whereas right-hemisphere pSTS is also associated with the analysis of the emotional content of speech (34). Based on the social motivation theory of ASD (18), we hypothesized that children with ASD would show aberrant intrinsic connectivity of voice-selective pSTS throughout brain regions implicated in reward and emotion, including the OFC, NAc, insula, and amygdala (31). Such findings would support a role for motivational and affective factors, rather than low-level sensory abnormalities, in speech-related communication deficits in children with ASD.

Results

pSTS Functional Connectivity in TD Children and Children with ASD.

To understand the basic functional circuitry associated with human voice-selective areas in children, we first examined functional connectivity of the pSTS separately within the TD and ASD groups (Fig. 1, Left). Results indicate that the left-hemisphere pSTS has significant connectivity with superior temporal gyrus (STG) and STS bilaterally in TD children and children with ASD, with connectivity extending posteriorly into bilateral angular gyrus (AG) in the ASD group and anteriorly into mid-STG, STG, and planum temporale in the TD group. TD and ASD groups also showed significant connectivity with ventrolateral prefrontal cortex; however, in the TD group, this connectivity was limited primarily to left-hemisphere OFC, whereas, in the ASD group, connectivity was present bilaterally in pars triangularis (Brodmann area 45). TD children also showed significant connectivity with the basal ganglia, including the NAc, putamen and ventral caudate; however, there was no significant connectivity with the basal ganglia in children with ASD. Similarly, TD children showed significant connectivity between the left-hemisphere pSTS and visual cortical structures, including bilateral occipital pole and left-hemisphere linguistic gyrus; however, the left-hemisphere lateral occipital cortex was the only occipital lobe structure that showed significant connectivity with the left-hemisphere pSTS in the ASD group.

Next, we examined within-group functional connectivity for the right-hemisphere pSTS seed. Results show that TD children have extensive connections between right-hemisphere pSTS and several frontal, temporal, and parietal cortical regions; however, in children with ASD, connectivity was limited to a relatively small region of superior temporal cortex (Fig. 1, Right). Specifically, TD and ASD groups showed significant connectivity with right-hemisphere mid-STSS; however, in the TD group, connectivity extended anteriorly along the STS and was also present in similar left-hemisphere superior temporal cortex. TD individuals also showed significant connectivity in left-hemisphere OFC, bilateral ventromedial prefrontal cortex (vmPFC), AG, and subcortical structures, including right-hemisphere thalamus, caudate, and putamen.

Aberrant pSTS Connectivity in Children with ASD.

We then examined group differences in connectivity patterns between children with ASD and TD children for the left-hemisphere pSTS seed. Results for the TD>ASD contrast showed a specific and striking pattern of ASD-related underconnectivity between the left-hemisphere pSTS and distributed structures of the dopaminergic reward pathway (Fig. 2). ASD-related underconnectivity was evident in bilateral VTA of the brainstem, the NAc and putamen of the basal ganglia, vmPFC, as well as the left-hemisphere caudate, anterior insula, and OFC. One structure outside of the reward pathway, the left-hemisphere supplementary motor area, also showed ASD underconnectivity. Group differences in connectivity strength were driven by relatively strong positive connectivity in TD subjects and significantly reduced positive connectivity in the ASD subjects (Fig. 2, Lower). Importantly, ASD underconnectivity was not evident in any regions in parietal, occipital, or temporal cortex, including auditory cortical structures. No voxels in the brain showed significant differences for the ASD>TD contrast, indicating that there was no ASD hyperconnectivity between left-hemisphere pSTS and other brain structures.

We then examined differences between TD and ASD groups in intrinsic connectivity patterns for voice-selective right-hemisphere pSTS regions. Results for the TD>ASD contrast from the first of these two right-hemisphere pSTS functional connectivity analyses showed no ASD underconnectivity with any cortical structures; however, results for the second pSTS seed showed widespread ASD underconnectivity across a range of cortical structures (Fig. 3). These structures included bilateral hippocampus, precentral and superior frontal gyri, left-hemisphere OFC, mid-STS, and middle frontal gyrus, and right-hemisphere amygdala, vmPFC, and crus I of the cerebellum. Again, group mean results showed that group differences in connectivity strength were driven by relatively strong positive connectivity in TD subjects and significantly reduced positive connectivity in ASD subjects (Fig. 3, Lower). Importantly, there was no ASD underconnectivity between either of the right-hemisphere pSTS seeds and the VTA or structures of the basal ganglia. Consistent with left-hemisphere pSTS connectivity.
reward network, including the VTA, nucleus accumbens (NAc), insula, and anterior insula, vmPFC, and OFC (37), as well as the amygdala, a critical structure for processing emotional content in speech (38). Furthermore, the strength of functional connectivity between voice-selective cortex and reward centers in the brain predicts standardized scores of communication abilities in children with ASD. Children with ASD showed similar patterns of connectivity between bilateral PAC and superior temporal cortex as TD children, at a reduced threshold ($P < 0.05$, height). For the right-hemisphere pSTS seed, children with ASD showed significantly reduced connectivity in the right-hemisphere amygdala, bilateral hippocampus and precentral gyrus and left-hemisphere OFC, as before, and additional clusters in left-hemisphere amygdala and AG, bilateral temporal pole, and the cerebellum (SI Text).

**pSTS Connectivity Is Related to Symptom Severity in Children with ASD.** The final goal of this work was to examine whether connectivity strength between voice-selective pSTS and structures of the reward system and amygdala (Fig. 4) were predictive of ASD symptom severity on standardized measures of communication abilities. Results from binary logistic regression analysis showed that connectivity strength between the left-hemisphere pSTS and multiple regions of the reward pathway was predictive of communication subtests scores of the Autism Diagnostic Observation Schedule (ADOS; $P = 0.008$) and Autism Diagnostic Interview (ADI; $P = 0.003$).

**Discussion**

ASD has long been associated with abnormal processing of the human voice (11). Consistent with the social motivation theory of autism (18), we show that high-functioning, verbally fluent children with ASD (Table 1) have reduced intrinsic brain connectivity between voice-selective cortical regions and a distributed reward processing system that includes the VTA, NAc, anterior insula, vmPFC, and OFC (37), as well as the amygdala, a critical structure for processing emotional content in speech (38). Furthermore, the strength of functional connectivity between voice-selective cortex and reward centers in the brain predicts standardized scores of communication abilities in children with ASD. Children with ASD showed similar patterns of connectivity between bilateral PAC and superior temporal cortex as TD children, at a reduced threshold ($P < 0.05$, height). For the right-hemisphere pSTS seed, children with ASD showed significantly reduced connectivity in the right-hemisphere amygdala, bilateral hippocampus and precentral gyrus and left-hemisphere OFC, as before, and additional clusters in left-hemisphere amygdala and AG, bilateral temporal pole, and the cerebellum (SI Text).

**Between-Group Functional Connectivity Differences Examined with “Scrubbing” Procedures.** To investigate whether group differences in pSTS functional connectivity were influenced by group differences in subject movement (36), we used the scrubbing method on individual subjects’ resting state data (36) and repeated left- and right-hemisphere pSTS functional connectivity analyses. Consistent with the initially reported findings (Fig. 2), scrubbed results for the left-hemisphere pSTS seed show significantly reduced ASD connectivity in bilateral NAc and left-hemisphere OFC and anterior insula (SI Text). Connectivity with the VTA was also evident, albeit results, no voxels in the brain showed significant differences for the ASD>TD contrast, indicating that there was no ASD hyperconnectivity between bilateral pSTS and other brain structures.

Our next goal was to examine whether reward and affect-related ASD underconnectivity was specific to voice-selective auditory regions of pSTS or, alternatively, whether ASD underconnectivity was also evident between primary auditory cortex (PAC) and these downstream brain structures, thereby representing a more general auditory connectivity phenomenon. To examine this question, we performed two additional functional connectivity analyses in which the seed regions were bilateral PAC (35). Results from this analysis show that TD and ASD groups had comparable connectivity between PAC and all downstream brain structures identified in the pSTS connectivity analysis (SI Text). Independent-samples $t$ tests performed on $\beta$-values from TD and ASD PAC connectivity analyses failed to reach statistical significance at the $P < 0.01$ level for all left-hemisphere (SI Text) and right-hemisphere (SI Text) connections, and only one connection was significant at the $P < 0.05$ level (right-hemisphere Te1.0 region to left-hemisphere precentral gyrus; $P = 0.0498$).

![Fig. 2. Between-group functional connectivity results for left-hemisphere voice-selective cortex. Group differences for the TD>A SD contrast indicated ASD underconnectivity between left-hemisphere pSTS and structures of the reward network, including the VTA, nucleus accumbens (NAc), insula, and OFC. No voxels showed significant connectivity for the ASD>TD contrast. The seed used in this analysis was a 6-mm sphere centered in left-hemisphere pSTS at MNI coordinates [−63, −42, 9] (23). Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. Mean connectivity differences between TD children and children with ASD are plotted in the bar graphs for six left-hemisphere and four right-hemisphere regions (error bars represent SEM). SFG, superior frontal gyrus; SMA, supplementary motor area.](image)

![Fig. 3. Between-group functional connectivity results for right-hemisphere voice-selective cortex. Group differences for the TD>A SD contrast indicated ASD underconnectivity between right-hemisphere pSTS and an array of cortical regions. No voxels showed significant connectivity for the ASD>TD contrast. The seed used in this analysis was a 6-mm sphere centered at MNI coordinates [57, −31, 5] (23). Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. Mean connectivity differences between TD children and children with ASD are plotted in the bar graphs for four left-hemisphere and three right-hemisphere regions (error bars represent SEM). Ent, entorhinal cortex; Hipp, hippocampus; mSTS, mid-superior temporal sulcus; SFG, superior frontal gyrus; SMA, supplementary motor area.](image)
Social Motivation Theory and Reward Circuitry in ASD. The etiology of the pronounced social deficits in ASD remains elusive, and several hypotheses have been proposed to explain these deficits (39–41). The social motivation theory states that impaired salience and reward value attributed to faces and vocal stimuli has a causal effect on social skill development in children with ASD (18). The reward circuit consists of a distributed set of brain regions that includes the midbrain VTA, NAc of the basal ganglia, anterior cingulate cortex, vmPFC, and the OFC (37), and activity in this pathway is known to modulate auditory cortical representations (42). Previous task-related fMRI studies have reported impaired function in these brain structures in individuals with ASD. For example, it has been shown that children with ASD exhibit reduced activation of the reward pathway, including the NAc and OFC, while viewing smiling faces (43). Moreover, reduced activation in reward regions has also been shown for nonsocial stimuli (44), supporting a more general reward-related impairment in ASD. Our findings provide support for the social motivation theory by showing diminished intrinsic connectivity between voice-selective cortex and most of the brain regions previously implicated in the social motivation theory, including the NAc, OFC, vmPFC, and amygdala (31). These results demonstrate that abnormal reward-related processes are not limited to visual social stimuli (43, 44) and that auditory voice-selective brain regions that are important for social information processing are also affected in ASD.

Critically, brain connectivity between voice-selective pSTS and brain structures implicated in reward and affective processes was predictive of the social communication scores of the ADOS and ADI. These findings suggest that aberrant brain connectivity associated with the reward pathway may be a primary mechanism underlying weakness in perceiving speech as a socially meaningful and rewarding stimulus in children with ASD (17, 45). Although the present results cannot provide information about the causal relationship between brain connectivity of voice-selective cortex and the ability to perceive speech as a rewarding and socially meaningful stimulus, we suggest that this model represents a parsimonious and plausible explanation for this auditory behavioral phenotype. Moreover, the significant relationship between impaired reward circuitry and social communication symptom severity are central predictions of the social motivation theory (18, 31).

Brain Circuitry Underlying Prosody and Emotional Information in ASD. Germane to the study of speech processing in ASD, strong empirical evidence has accumulated from behavioral studies showing that individuals with ASD have pronounced deficits for extracting prosodic information from speech, which conveys emotional state information regarding the speaker through intonation and rhythm (46). Importantly, it has been shown that, in TD individuals, the processing of prosodic information is performed in right-hemisphere temporal cortex (34) and right-hemisphere amygdala (47). Connectivity results from the present study provide evidence regarding the neural basis for impaired prosodic speech processing in ASD. Specifically, our results show weak intrinsic coupling between right-hemisphere voice-selective cortex and the amygdala. We hypothesize that this disconnection may play a role in impeding access of auditory-based information, such as prosodic cues, to regions of the brain necessary for emotional learning and memory (48).

Our results also address more general hypotheses linking ASD with amygdala dysfunction. The amygdala has long received attention from ASD researchers because of its established role in social behavior (49), and abnormal amygdala function has been hypothesized to contribute to social deficits in ASD (20). Specifically, it has been proposed that the amygdala is critical for identifying emotional information from complex visual stimuli such as mental state information that can be detected from the eye region of an individual (50). Consistent with previous findings in the visual domain (51), results from our study provide support for the hypothesized role of the amygdala in autism by showing abnormal functional connectivity of the amygdala in children with ASD. Critically, our findings extend this hypothesis by linking amygdala dysfunction with auditory-based social processing.

Implications for Models of Auditory and Speech Processing in Individuals with ASD. Auditory perception in individuals with ASD is poorly understood and includes a number of paradoxical observations. For example, many children with ASD experience an increased sensitivity to the loudness of sounds (52), yet they often display insensitivity to the human voice, one of the most common sounds in their environment (11, 45). A model for considering different stages of voice perception in TD adults was proposed by Belin et al. According to this model, speech is first subjected to a low-level acoustical analysis, followed by voice structural analysis, then, in parallel, vocal content, affect, and speaker recognition units are processed (53). This is a useful model for considering auditory and speech information processing in ASD given the variety of auditory deficits reported in this population. Because all levels of auditory processing described in the model of Belin et al. have been implicated in behavioral (11, 45, 54) and neurobiological...
investigations of ASD, it is plausible that a relatively early stage of this hierarchy is impaired in ASD, thereby negatively impacting all higher levels. Considering the present results linking voice-selective cortex with the reward system, we propose that weak connectivity between the voice structural analysis module in the model of Belin et al. and the reward system is specifically impaired in ASD, negatively impacting all higher-level speech related processes. In support of this hypothesis, we found no evidence for underconnectivity between PAC and reward and affective brain circuitry in children with ASD (SI Text), which suggests that a reward-related connectivity deficit does not impact the acoustical analysis module of this model. Beyond voice structural analysis, the pSTS has also been more broadly implicated in the processing of communicative intent (57), and, from this perspective, the present results may reflect a weakness in connectivity between brain structures that facilitate the recognition and extraction of communicative significance inherent to vocal stimuli and structures of the reward pathway.

### Conclusion

We demonstrate that childhood ASD is associated with underconnectivity between voice-selective posterior temporal cortical regions and reward circuitry, providing important insights into the behavioral and clinical phenotype of abnormal speech and language processing observed in the disorder. Critically aberrant brain connectivity was associated with the severity of social communicative deficits in children with ASD. Our findings shed light on the neurobiological bases of one of the core deficits in ASD by identifying key dysfunctional circuits associated with human voice processing. Taken together, our study provides support for the social motivation theory of ASD.

### Materials and Methods

#### Participants

The Stanford University Institutional Review Board approved the study protocol. Parental consent and the child’s assent were obtained for all evaluation procedures, and children were paid for their participation in the study. Participants were recruited locally from schools and clinics near Stanford University. All children were required to have a full-scale intelligence quotient (IQ) >70, as measured by the Wechsler Abbreviated Scale of Intelligence (SB). A group of 20 children who met ASD criteria on module 3 of the ADOS (59) or criteria for autism on the ADI-Revised (60) were matched for full-scale IQ, age, and sex with a group of 20 TD children (Table 1) using a previously described algorithm (29). Importantly, children in the ASD sample are considered “high-functioning” and had fluent language skills and above-average reading skills (Table 1). Nevertheless, these children are generally characterized as having communication impairments, especially in the area of reciprocal conversation. One control participant was excluded from the analysis as a result of issues related to data quality. As a result, the final group consisted of 20 children with ASD and 19 TD children. These data were used in recent publications from our group (29, 61) and are publicly available (http://icon_1000.projects.nitrc.org/indi/abide/).

#### Data Acquisition and Preprocessing

For the resting-state fMRI scan, participants were instructed to keep their eyes closed and remain still for the duration of a 6-min scan. Whole-brain functional images were acquired on a 3-T Signa scanner (GE Healthcare). Details are provided in SI Materials and Methods.

#### Region of Interest Selection

Coordinates for the pSTS regions of interest (ROIs) were chosen based on a previous study that showed cortical regions selective for vocal stimuli compared with acoustical control conditions in neurotypical adults (23). Results from this study showed that left- and right-hemisphere pSTS are selective for the human voice compared with a number of control sounds, including environmental sounds, scrambled voices, and amplitude modulated noise. This previous study reported coordinates for any of the ROIs examined. Between-group functional connectivity maps were calculated by using independent-samples t tests on individual subjects’ functional connectivity contrast images. Between-group maps were thresholded at P < 0.01 uncorrected for height and a voxel cluster extent of 100 (corresponding to P < 0.01 for height and P < 0.01 for extent). Although our analysis and interpretation focuses on between-group functional connectivity differences that directly compare children with ASD and TD children, for the sake of completeness, we have also presented within-group functional connectivity maps (Fig. 1), which were generated by using one-sample t tests of individual functional connectivity contrast images. Within-group functional connectivity maps were thresholded at P < 0.000001 uncorrected for height and 100 voxels for extent. Details are provided in SI Materials and Methods.

#### Functional Connectivity Analysis with Scrubbing Procedures

To ensure that our findings are not severely confounded by participant motion, we performed additional analyses in which we applied the data-scrubbing method proposed by Power et al. (36). Details are provided in SI Materials and Methods.

#### Brain-Behavior Regression Analysis

To investigate whether the degree of connectivity between pSTS ROIs and brain structures identified in the between-group analysis predicts communication symptom severity in ASD, we used binary logistic regression with the first case as the control group. The strength between ROIs identified in the functional connectivity analysis by identifying the voxel with peak group connectivity differences within reward-related structures and the amygdala (Table S1). The time series for these point-ROIs were extracted for each subject, and Pearson correlation coefficients for each subject were calculated for the 10 connections specified in Fig. 4. We then used binary logistic regression to model the relationship between the independent variables, which were Fisher-transformed Pearson correlation coefficients describing connectivity strength between pSTS and brain structures in the reward pathway and amygdala. The reason for performing a regression analysis with the use of binary rather than continuous ADI and ADOS values is that the distributions for these subtests in our sample are narrow (Table 1) and are better suited for a classification-based approach. Therefore, the ADI and ADOS analyses examined whether connectivity strength between pSTS and brain structures in the reward pathway and amygdala could predict group membership in the “more severe” or “less severe” ASD group based on binary scores on ADI and ADOS communications subscales. We used a median split to group subjects in either the more severe or less severe ASD groups. SPSS software (IBM) was used for all regression analyses.

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Supporting Information

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SI Results
Given the finding of strong autism spectrum disorder (ASD) underconnectivity between left-hemisphere posterior superior temporal sulcus (pSTS) and the distributed reward circuit (Fig. 2), we sought to examine whether abnormal connectivity of reward circuitry extended beyond those involved in processing of the human voice (1). To address this, we examined group differences in resting-state connectivity of the left-hemisphere nucleus accumbens (NAc), a key region within the reward pathway highlighted in our main results (Fig. 2). To avoid circularity, we identified the left-hemisphere NAc as defined by the Harvard–Oxford probabilistic structural atlas (2) using a probability threshold of 25%. Results from this analysis revealed ASD underconnectivity between left-hemisphere NAc and several brain structures, including bilateral pSTS and left-hemisphere mid–middle temporal gyrus of temporal cortex, bilateral medial and lateral occipital cortex, bilateral precuneus, right-hemisphere supramarginal gyrus, left-hemisphere central operculum of parietal cortex, precentral gyrus, and posterior cingulate cortex (Fig. S8). Only the anterior medial cerebellum showed ASD overconnectivity for the NAc seed (Fig. S8, Lower). A striking result of this analysis is the overlap between underconnected regions of bilateral pSTS identified in the NAc connectivity analysis and the pSTS seeds used in the original analysis. It is also worth noting that ASD underconnectivity of left-hemisphere NAc was not evident in any region of prefrontal cortex despite its many connections with this area of cortex (3). Taken together, the results provide evidence that, in addition to weak brain connectivity between the reward pathway and voice-selective auditory cortex, children with ASD also have aberrant connectivity between reward circuitry and brain regions involved in other sensory modalities. Additional research is needed to reconcile these findings with NAc hyperconnectivity reported in a previous study of children with ASD (4).

SI Materials and Methods

Data Acquisition. Functional MRI. Functional images were acquired on a 3-T Sigma scanner (General Electric) by using a custom-built head coil. Participants were instructed to stay as still as possible during scanning, and head movement was further minimized by placing memory-foam pillows around the participant’s head. A total of 29 axial slices (4.0-mm thickness, 0.5-mm skip) parallel to the anterior/posterior commissure line and covering the whole brain were imaged by using a T2*-weighted gradient-echo spiral in-out pulse sequence (5) with the following parameters: repetition time, 2,000 ms; echo time, 30 ms; flip angle, 80°; one interleave. The field of view was 20 cm, and the matrix size was 64 × 64, providing an in-plane spatial resolution of 3.125 mm. Reduction of blurring and signal loss arising from field inhomogeneities was accomplished by the use of an automated high-order shimming method before data acquisition.

Structural MRI. For each subject, a high-resolution T1-weighted spoiled-grass gradient-recalled inversion-recovery 3D MRI sequence was acquired to facilitate anatomical localization of functional data. The following parameters were used: inversion time, 300 ms; repetition time, 8.4 ms; echo time, 1.8 ms; flip angle, 15°; 22-cm field of view; 132 slices in coronal plane; 256 × 192 matrix; two excitations; and acquired resolution, 1.5 × 0.9 × 1.1 mm.

Functional MRI Preprocessing. A linear shim correction was applied separately for each slice during reconstruction by using a magnetic field map acquired automatically by the pulse sequence at the beginning of the scan. Functional MRI data were then analyzed by using SPM8 analysis software (www.fil.ion.ucl.ac.uk/spm). Images were realigned to correct for motion, corrected for errors in slice timing, spatially transformed to standard stereotaxic space [based on the Montreal Neurologic Institute (MNI) coordinate system], resampled every 2 mm by using sinc interpolation, and smoothed with a 6-mm full-width half-maximum Gaussian kernel to decrease spatial noise before statistical analysis. Translational movement in millimeters (x, y, z) and rotational motion in degrees (pitch, roll, yaw) was calculated based on the SPMS parameters for motion correction of the functional images in each subject.

Region of Interest Selection. Coordinates for the pSTS regions of interest (ROIs) were chosen based on a previous study that showed cortical regions selective for vocal stimuli compared with acoustical control conditions in neurotypical adults (1). Peak coordinates described in that study for the contrast of vocal stimuli minus control sounds were transformed from Talaraich to MNI space by using the tal2mni function in Matlab (http://imaging.mrc-cbu.cam.ac.uk/ing/downloads/MNI2tal/tal2mni.m). The coordinates for the left-hemisphere pSTS peak were [−63, −42, 9], and the coordinates for the two right-hemisphere pSTS peaks were [57, −31, 5] and [46, −46, 4]. The ROIs used in the functional connectivity analysis were 6-mm spheres centered on these three coordinates. To examine whether the size of ROIs affected between-group connectivity results, 8-mm spheres centered at left- and right-hemisphere pSTS peaks were used as seeds in supplementary analyses (Figs. S4 and S5). Coordinates for the low-level auditory cortical analyses were taken from a study showing cytoarchitectonically dissociable regions of auditory cortex (6). The Te1.0 region was defined as the core koniocortical field in this work. Coordinates for bilateral Te1.0 served as our primary auditory cortical (PAC) ROIs. ROIs were extracted by using the anatomy toolbox of SPM software, and the center of mass for the PAC ROIs was calculated in SPM. For the left-hemisphere PAC, coordinates for Te1.0 were [−48, −20, 6], and, for the right-hemisphere PAC, coordinates for Te1.0 were [50, −15, 5]. The ROIs used in the functional connectivity analysis were 6-mm spheres centered on these coordinates. We also performed a follow-up functional connectivity analysis by using the left-hemisphere NAc as the seed region (SI Results and Fig. S8). To avoid circularity with the pSTS seed analysis (Fig. 2), we identified the left-hemisphere NAc as defined by the Harvard–Oxford probabilistic structural atlas (2) using a probability threshold of 25%. This method is consistent with a previous study of intrinsic connectivity of the NAc (7).

Functional Connectivity Analysis. For each ROI, a resting-state time series was extracted by averaging the time series of all voxels within it. The resulting ROI time series was then used as a covariate of interest in a linear regression whole-brain analysis. A global time series, computed across all brain voxels, along with six motion parameters, were used as additional covariates to remove confounding effects of physiological noise and participant movement. Because of the unresolved controversies in the field surrounding the use of global signal regression in resting-state functional connectivity analyses (8–12), we conducted additional analyses in which we removed white matter and cerebrospinal fluid (CSF) confounds, as an alternative to global signal regression (8). We regressed the mean time series of a 2-mm ROI located in the center of the left lateral ventricle (MNI coordinates [−24 −44 8]) and left superior corona radiata (MNI coordinates [−24 −40 4]) in place of the global signal correction used in our primary analysis.

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As can be seen from Figs. S6 and S7, the majority of our original results were unaltered by the removal of white matter and CSF confounds. No new ASD->typically developing (TD) effects are present after using this alternative analysis. The ASD and TD groups did not significantly differ in motion \((P > 0.7)\) or have average RMS movement >0.35 mm. To demonstrate the robustness of our findings against potential movement confounds, we performed additional supplementary analyses. We computed correlations between movement parameters and brain connectivity values, and found that there was no significant correlation between mean brain connectivity values and RMS of displacement for any of the ROIs examined. Group-level connectivity maps were generated by using one-sample \(t\) tests of individual functional connectivity contrast images. Within-group functional connectivity maps were thresholded at \(P < 0.000001\) uncorrected for height and 100 voxels for extent. Between-group functional connectivity maps were thresholded at \(P < 0.01\) uncorrected for height and a voxel cluster extent of 100 (corresponding to \(P < 0.01\) for height and \(P < 0.01\) for extent).

Group mean connectivity between seed ROIs and brain regions identified in the whole-brain analysis were calculated to examine the basis for TD->ASD group differences (bar graphs in Figs. 1 and 2). The reason for this analysis is that group connectivity differences can result from a number of different factors. For example, both TD and ASD groups could show negative connectivity between the seed regions and their targets, and significant TD->ASD group differences could be driven by greater negative connectivity in subjects with ASD. Significant group differences were inherent to this ROI analysis as they are based on results from the whole-brain connectivity analysis (13); however, results provide important information regarding the magnitude and sign of connectivity results in both TD and ASD groups.

To calculate group mean connectivity within specific ROIs, we identified the voxel with maximum TD->ASD group difference from the pSTS functional connectivity analyses within each brain ROI as defined by the Harvard–Oxford probabilistic structural atlas (2) using a probability threshold of 25%. For identification of the ventral tegmental area of the midbrain, we used Duvernoy’s Atlas of the Brainstem (14) and confirmed overlap of our connectivity map with several previously published ventral tegmental area coordinates (15, 16). We then extracted the \(\beta\)-value from individual subjects’ contrast maps from the pSTS functional connectivity analyses using the coordinates for each of these ROIs. The mean and SEs were calculated for the TD and ASD groups, and independent-sample \(t\) tests were performed on these values.

**Functional Connectivity Analysis Using Scrubbing Procedures.** Recent work has shown that small group differences in subject movement can result in significant artifacts in functional connectivity results, with increased movement artificially reducing the strength of long-range brain connections (17). This finding is particularly concerning for autism research because reduced long-range brain connectivity represents a prominent neural theory of autism (18), and individuals with autism typically display greater subject movement during functional MRI (fMRI) data collection compared with control subjects (19). To ensure that our findings are not severely confounded by participant movement, we performed additional analyses in which we applied the “data scrubbing” method proposed by Power et al. (17). Volumes with frame-wise displacement greater than 0.5 mm and derivative variance greater than 0.5% of blood oxygen level-dependent signal were identified and excluded by using the parameters proposed previously (17).

Fig. S1. Between-group functional connectivity results for bilateral PAC. (Upper) Left-hemisphere PAC connectivity was comparable between TD and ASD groups in brain structures that previously showed group differences in the left-hemisphere pSTS analysis (Fig. 2). Mean connectivity differences between TD children and children with ASD are plotted in the bar graphs for six left-hemisphere and four right-hemisphere regions identified in the left-hemisphere pSTS connectivity analysis (error bars represent SEM). The seed region used in this analysis was a 6-mm sphere centered in left-hemisphere PAC at MNI coordinates \([-48, -20, 6]\) (6). Group differences for the connections displayed in the bar graphs failed to reach significance at the \(P < 0.05\) level. (Lower) Right-hemisphere PAC connectivity was comparable between TD and ASD groups in brain structures that previously showed group differences in the right-hemisphere pSTS analysis (Fig. 3). Mean connectivity differences between TD children and children with ASD are plotted in the bar graphs for four left-hemisphere and three right-hemisphere regions identified in the right-hemisphere pSTS connectivity analysis (error bars represent SEM). The seed region used in this analysis was a 6-mm sphere centered in right-hemisphere PAC at MNI coordinates \([50, -15, 5]\) (6). Group differences for the connections displayed in the bar graphs failed to reach significance at the \(P < 0.01\) level; however, one connection was significant at the \(P < 0.05\) level (left-hemisphere precentral gyrus; \(P = 0.0498\)). Hipp, hippocampus; OFC, orbitofrontal cortex; SMA, supplementary motor area; VTA, ventral tegmental area.
Fig. S2. Between-group functional connectivity results for left-hemisphere speech-selective cortex using scrubbed fMRI data. After controlling for participant motion using the scrubbing procedure (17), group differences for the TD>ASD contrast continued to indicate ASD underconnectivity between left-hemisphere pSTS and structures of the reward network, including the NAc, insula, and orbitofrontal cortex. The seed used in this analysis was a 6-mm sphere centered in left-hemisphere pSTS at MNI coordinates [−63, −42, 9] (1). Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels.

Fig. S3. Between-group functional connectivity results for right-hemisphere speech-selective cortex using scrubbed fMRI data. After controlling for participant motion using the scrubbing procedure (17), group differences for the TD>ASD contrast continued to indicate ASD underconnectivity between right-hemisphere pSTS and brain structures serving reward, emotional, memory, and motor functions. The seed region used in this analysis was a 6-mm sphere centered in right-hemisphere pSTS at MNI coordinates [57, −31, 5] (1). Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. AG, angular gyrus; Hipp, hippocampus; mSTS, mid-superior temporal sulcus; MTG, middle temporal gyrus; TP, temporal pole.
Fig. S4. Between-group functional connectivity results for left-hemisphere speech-selective cortex using an 8-mm sphere as the seed region. In our main analysis, we used a 6-mm sphere for the left-hemisphere pSTS seed to ensure anatomical specificity for voice-selective regions of cortex (1). Here, we investigated whether increasing the seed region to 8-mm spheres affected TD>ASD connectivity results. Results show nearly identical results as those shown in the main results (Fig. 2). Specifically, results show decreased connectivity of left-hemisphere pSTS in children with ASD in the distributed reward circuit, including bilateral ventral tegmental area (VTA), NAc, ventromedial prefrontal cortex (vmPFC), left-hemisphere dorsal caudate, anterior insula, and orbitofrontal cortex. ASD underconnectivity was also evident in motor planning regions including bilateral MCC and supplementary motor area, as well as right-hemisphere middle temporal gyrus (MTG), bilateral superior frontal gyrus (SFG), and lateral occipital cortex (LOC). Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. MCC, mid-cingulate cortex; OFC, orbitofrontal cortex; SMA, supplementary motor area.
Fig. S5. Between-group functional connectivity results for right-hemisphere speech-selective cortex using an 8-mm sphere as the seed region. In the main analysis, we used a 6-mm sphere for the right-hemisphere pSTS seed to ensure anatomical specificity for voice-selective regions of cortex (1). Here, we investigated whether increasing the seed region to 8-mm spheres affected TD>ASD connectivity results. Again, results show nearly identical results as those shown in the main results (Fig. 3). Specifically, results show ASD underconnectivity of right-hemisphere amygdala and supplementary motor area as well as bilateral mid-superior temporal sulcus (mSTS), ventromedial prefrontal cortex (vmPFC), precentral gyrus, and hippocampus. Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. Ent, entorhinal cortex; ITG, inferior temporal gyrus; Hipp, hippocampus; OFC, orbitofrontal cortex; SFG, superior frontal gyrus; SMA, supplementary motor area.
Fig. S6. Between-group functional connectivity results for left-hemisphere speech-selective cortex after removing white matter and CSF confounds. In the main analysis, we performed global signal regression, which is a common approach for resting-state functional connectivity analyses. However, there is concern that this method may induce artifactual negative correlations. To address this concern, we conducted additional analyses (SI Materials and Methods, Functional Connectivity Analysis) whereby we removed white matter and CSF confounds as an alternative to global signal regression (8). Results show that the majority of brain regions identified by using global signal regression (Fig. 2) were also identified after removing white matter and CSF confounds. Specifically, results show ASD underconnectivity in several structures of the reward circuit, including bilateral putamen, left-hemisphere NAc, dorsal caudate, anterior insula, and orbitofrontal cortex. Results also show ASD underconnectivity in visual processing regions, including right-hemisphere lateral occipital cortex, lingual gyrus, and fusiform gyrus. Consistent with results from global signal regression, no brain regions were identified for the ASD>TD contrast. Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. FG, fusiform gyrus; LOC, lateral occipital cortex; OFC, orbitofrontal cortex.
Fig. S7. Between-group functional connectivity results for right-hemisphere speech-selective cortex after removing white matter and CSF confounds. Similar to Fig. S6, we have conducted additional analyses (SI Materials and Methods, Functional Connectivity Analysis) whereby we removed white matter and CSF confounds as an alternative to global signal regression (8). Results show that the majority of brain regions identified by using global signal regression (Fig. 3) were also identified after removing white matter and CSF confounds. Specifically, results show ASD underconnectivity in right-hemisphere amygdala, mid-superior temporal gyrus, sulcus, and hippocampus, as well as bilateral orbitofrontal cortex and left-hemisphere NAc. Results also show ASD underconnectivity in visual processing regions, including right-hemisphere lateral occipital cortex, lingual gyrus, and fusiform gyrus, as well as bilateral precentral gyrus. Consistent with results from global signal regression, no brain regions were identified for the ASD>TD contrast. Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. Ent, entorhinal cortex; FG, fusiform gyrus; Hipp, hippocampus; LOC, lateral occipital cortex; mMTG, mid-middle temporal gyrus; mSTS, mid-superior temporal sulcus; OC, occipital cortex; OFC, orbitofrontal cortex.
Between-group functional connectivity results for left-hemisphere NAc. We performed this analysis to examine group differences in connectivity patterns originating from the reward circuit. Results show ASD underconnectivity between left-hemisphere NAc and several brain structures, including bilateral pSTS and left-hemisphere mMTG of temporal cortex, bilateral medial and lateral occipital cortex, bilateral precuneus, right-hemisphere supramarginal gyrus, left-hemisphere central operculum of parietal cortex, precentral gyrus, and posterior cingulate cortex. Only a small region of the anterior medial cerebellum showed ASD overconnectivity for the NAc seed (Lower). Images are thresholded at $P < 0.01$ for voxel height and an extent of 100 voxels. LOC, lateral occipital cortex; MFG, middle frontal gyrus; mMTG, mid-middle temporal gyrus; OC, occipital cortex; PCC, posterior cingulate cortex; PO, parietal operculum; SMG, supramarginal gyrus.
Table S1. Brain connections entered into brain-behavior regression analyses

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left hemisphere pSTS</td>
<td>−63, −42, 9</td>
</tr>
<tr>
<td>Left hemisphere caudate</td>
<td>−18, 4, 20</td>
</tr>
<tr>
<td>Left hemisphere insula</td>
<td>−28, 18, −10</td>
</tr>
<tr>
<td>Left hemisphere NAc</td>
<td>−12, 18, −8</td>
</tr>
<tr>
<td>Left hemisphere putamen</td>
<td>−24, 14, −8</td>
</tr>
<tr>
<td>Left hemisphere OFC</td>
<td>−30, 24, −12</td>
</tr>
<tr>
<td>Right hemisphere pSTS</td>
<td>57, −31, 5</td>
</tr>
<tr>
<td>Right hemisphere VTA</td>
<td>2, −22, −20</td>
</tr>
<tr>
<td>Right hemisphere caudate</td>
<td>14, 22, −6</td>
</tr>
<tr>
<td>Right hemisphere NAc</td>
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<tr>
<td>Right hemisphere putamen</td>
<td>16, 14, −10</td>
</tr>
<tr>
<td>Right hemisphere amygdala</td>
<td>30, −4, −24</td>
</tr>
</tbody>
</table>

OFC, orbitofrontal cortex; VTA, ventral tegmental area.