Published in final edited form as:

Res Dev Disabil. 2012 ; 33(6): 2255–2264. doi:10.1016/j.ridd.2012.06.011.

Differential Brain Responses to Cries of Infants with Autistic Disorder and Typical Development: An fMRI Study

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Abstract

This study used fMRI to measure brain activity during adult processing of cries of infants with autistic disorder (AD) compared to cries of typically developing (TD) infants. Using whole brain analysis, we found that cries of infants with AD compared to those of TD infants elicited enhanced activity in brain regions associated with verbal and prosodic processing, perhaps because altered acoustic patterns of AD cries render them especially difficult to interpret, and increased activity in brain regions associated with emotional processing, indicating that AD cries also elicit more negative feelings and may be perceived as more aversive and/or arousing. Perceived distress engendered by AD cries related to increased activation in brain regions associated with emotional processing. This study supports the hypothesis that cry is an early and meaningful anomaly displayed by children with AD. It could be that cries associated with AD alter parent-child interactions much earlier than the time that reliable AD diagnosis normally occurs.

Autistic Disorder (AD) is a neurodevelopmental disorder of children's communicative and social skills as well as their motor repertoire. With an eye toward intervention and treatment, interest has now focused on the earliest stages of communication and social development in AD and how child characteristics altered by AD might influence children's social relationships, especially with their caregivers.

Murdock, Cost, and Tieso (2007) suggested that alterations to communication in AD can vary from absence of verbal communication to pragmatic deficits in otherwise fluent speech. Because of the significance of AD in development, it is important to understand compromises to very early communication in AD; in infants, this means facial expression and vocalization. Some research has focused on the facial expressions of children with AD during the first years of life (Cassel et al., 2007), but fewer studies have been conducted on vocal development, especially crying, in children with AD (Bieberich and Morgan, 1998; Esposito and Venuti, 2008, 2010). Human infants display a range of affiliative behaviors to elicit care from mothers or other potential caregivers. Among the first channels of communication newborns and infants use to express their needs is to cry (Barr et al., 2000;

Cecchini et al., 2007; Irwin, 2003; Gingras et al., 2005). Cry is a critical feature of infants' first communications and is a vital early social signal in human development, but cry is also a well-researched physiological and psychopathological indicator (Acebo and Thoman, 1992, 1995; LaGasse et al., 2005; Rautava et al., 2007). Functionally, infant crying involves two agents: the infant who cries and the caregiver who listens to the crying, interprets it, and acts on that interpretation. Crying elicits physiological reactions in adults, such as increases in heart rate (Huffman et al., 1998) and endocrine responses (specifically, higher levels of prolactin; Fleming et al., 2002) that prompt care behaviors to reduce infant distress (Bowlby, 1969; Gustafson et al., 2000; LaGasse et al., 2005). Crying also activates the central nervous systems of both infant and caregiver, and this activation reinforces states of reciprocal attention. For these reasons crying is considered a core complex behavioral mechanism that regulates the infant-caregiver relationship, and has generated a large research literature on parents' and others' reactions.

Adult responses to infant cry reflect morphological characteristics of the cry itself (Gustafson and Green, 1989; Ziefman, 2003), caregiver characteristics (Frodi, 1995), and cultural norms (Barr et al., 1991). Here, we focused on specific prominent morphological characteristics.

Morphological characteristics, the acoustic features, of infant cry, include a variety of parameters that contribute to regulating adult responses (Gustafson and Green, 1989; Ziefman, 2003). However, cry pitch (related to frequency) has been identified as the most influential factor governing caregiver perceptions and responses (Lester and LaGasse, 2008); notably, higher-frequency cries are regularly perceived as more aversive and distressed than lower-frequency cries (Dessureau et al., 1998; Zeskind and Lester, 2001). Duration of pauses within an episode of cry is another important acoustic variable. Zeskind and colleagues (1992) modified the length of the expiratory phase and pauses in infant crying episodes. Listeners regarded shorter pauses as more activating and informative and also more distressed. Generally, the morphological characteristics of child cry elicit alertness, distress, and uneasiness (Zeskind et al., 1992).

Morphological characteristics of cries vary with child health and psychopathological status (Lester and LaGasse, 2008; Trevarthen and Daniel, 2005). For example, the cries of children with visceral colic are characterized by their high frequency and are typically perceived as more distressed. Neurological insult typically leads to overall higher levels of the fundamental frequency (f_0). Most pertinent to the present study, analyses of acoustic features of cries of children with AD, compared to those of TD children, reveal a number of differences. Episodes of cry in children with AD have higher fundamental frequencies (Esposito and Venuti, 2008, 2010; Esposito et al., 2012). Atypical functioning of the brainstem and limbic system (both areas are compromised in children with AD; Amaral et al., 2008) has been invoked to explain anomalies found in the cry of children with AD (Bieberich and Morgan, 1998).

Previous behavioral research has focused on how parents perceive crying of children with AD compared to TD children (Venuti et al., 2004; Esposito and Venuti, 2008, 2009a, 2010a, b). In a clinical interview study, parents of children with AD often mentioned their negative feelings with respect to crying and that AD cries were not understandable in the sense that parents could not easily identify their cause (Esposito and Venuti, 2008). In a "Listen-and-Response" experiment designed to assess effects of the atypical structure of crying episodes characteristic of children with AD, adults also reported experiencing increasingly negative mental states (i.e., anxiety) when listening to AD cries and less negative mental states when listening to cries of TD children (Esposito and Venuti, 2008). A follow-up study confirmed

that the higher fundamental frequencies acoustic characteristic of cries of children with AD accounted for the mental state uneasiness in listeners (Esposito and Venuti, 2010).

Given the evolutionary significance of infants' cries, recent work has turned to investigate the loci of adult brain responses to cries of TD infants using neuroimaging techniques. Studies that compare cry to non-vocal stimuli, such as noise, have reported preferential activation of the thalamus, medial prefrontal cortex, right orbitofrontal cortex and cingulate cortex, midbrain, hypothalamus, dorsal and ventral striatum, and the lateral septal region (e.g., Loberbaum et al., 2002). Results of these studies are heterogeneous likely due to their wide methodological variation. When cry is compared to other emotional vocalizations, activity in the cingulate cortex, insula, amygdala, ventral prefrontal cortex, and temporo-parietal cortex is reported (e.g., Seifritz et al., 2003). Studies investigating responses to own versus other infant cry in parents have revealed preferential activation of midbrain, basal ganglia, cingulate, amygdala, and insula (Swain et al., 2003) as well as prefrontal cortex and hypothalamic regions (Swain et al., 2004). Overall, infant cries appear to activate brain areas associated with parenting care, processing of aversive and alarming stimulation, and empathy. Thus, neuroimaging data support the idea that cry is a key component of early parent-child transactions and a crucial infant communicative signal that activates a variety of care-related responses in adults (Sroufe, 2000; Trevarthen, 2003; Tronick, 2005).

With these several literatures in mind, notably evidence of atypical features of AD cry, and adult brain and behavior sensitivities to infant cry, the present study aimed to evaluate brain activity during adults' processing of cries of infants with AD compared to cries of TD infants. Guided by this literature, we developed two main hypotheses. First, we expected increased activation of brain areas involved in auditory processing in response to cries of infants with AD compared to those of TD children; second, we expected increased activation in areas involved in processing of aversive and/or alarming stimuli in response to cries of infants with AD compared to those of TD children.

With respect to our first hypothesis, we expected increased activation at both primary and secondary processing levels. Specifically, in accord with AD cries' atypical acoustic features, we expected cries of children with AD would activate the primary auditory cortex (BA41,42) more than cries of TD children as activation in this area is largely modulated by basic acoustic properties of the stimuli such as f_0 (Woods et al., 2009). More importantly, we expected that, compared to cries of TD children, cries of children with AD would elicit enhanced activity in the secondary auditory cortex (BA 21, 22) which is involved in responding to behaviorally relevant complex sounds (Hart et al., 2003) and in bilateral inferior frontal areas that have been reported as relevant to processing emotional prosody (Wildgruber et al., 2006). These expectations reflect behavioral evidence from previous studies of the difficulties adults report in understanding and interpreting AD cry.

With respect to our second hypothesis, based on published behavioral studies showing that AD cries are perceived as more aversive and elicit more negative feelings compared to TD cries, we expected to find increased activity in brain structures involved in processing negative feelings typically induced by aversive and arousing stimuli, such as amygdala (Anderson and Phelps, 2001; Zald, 2003) and insula (Caria et al., 2010; Reiman et al., 1997; Wager et al., 2003). The increased activation in areas involved in processing of aversive and/or alarming stimuli in response to cries of children with AD compared to those of TD children would replicate and corroborate those of previous behavioral studies on subjective responses to cries of children with AD and would, for the first time, extend them to an objective neurophysiological level.

Before testing our two main hypotheses, AD cries were compared to white noise to verify that they elicited brain activation consistent with that reported in the literature about typical cry. Specifically, we expect that cries of children with AD activated temporo-parietal regions implicated in sound and voice processing as well as brain areas involved in parenting behaviors and, more generally, with processing of emotional stimuli, including thalamus, putamen, and insula (Swain et al. 2007).

Because crying is a fundamental biosocial phenomenon that reflects the status of the nervous system and influences early parent-child interactions (Lester, 1984; Lester et al., 1995, LaGasse et al., 2005), we hoped that the results of this study would shed light on the early communicative signals of children with AD, a developmental disorder of striking and increasing international incidence (Elsabbagh et al. 2012) whose initial ontogenetic stages are still largely unknown (Esposito et al. 2009a).

Methods

Participants

Twenty-one healthy adults (12 females: M age = 31.86 years, SD = 5.17; and 9 males: M age = 35.38 years, SD = 4.63; 11 primiparous parents of TD children (6F/5M), 1 non primiparous father and 9 non-parents (6F/3M; 20 right-handed, 1 left-handed) were recruited through the University of Trento webpage and local advertisements. Advertisement did not provide details of the study. Inclusion criteria were age between 18 and 40 years, ethnically homogeneous of European heritage, and, for parents, own children older than 3 years. Exclusion criteria were neurological or psychiatric disorders, substance abuse/dependence, psychotropic medications, and pregnancy. We planned to have an experimental group of about 20 participants as conventional fMRI studies require $N = 16-20$. Prior to the experiment, candidates were screened by a neurologist to check compatibility with MRI scan. Occupation and level of education varied in the sample, but most participants had attended (33%) or completed (38%) university. All participants gave written informed consent for participation, and experimental procedures were approved by the ethical committee for experiments involving humans at the University of Trento.

Acoustic Stimuli

A total of 20 acoustic excerpts of natural cry episodes from infants were used. Excerpts were extracted from 16 home videos of unedited cry bouts of 16 firstborns taken at 13 months of age. They belonged to one of two groups of children: autistic disorders (AD; $n = 8$, 4 boys/4 girls) and typically developing (TD; $n = 8$, 4 girls/4 boys). Children with AD received a clinical diagnosis at the age of ~ 3 years from a child psychiatrist according to DSM-IV-R criteria, confirmed by ADI-R, ADOS-G. Cries of infants with a diagnosis of Pervasive Developmental Disorder not otherwise specified (PDD-Nos) or Asperger Syndrome were not included. To prevent the presence in the stimulus sample of cases of autism in comorbidity with other psychopathologies, children with AD had to be free of other medical conditions (e.g., seizures, Fragile syndrome, etc.) and had no other known visual or hearing impairments. TD children were part of a longitudinal research project on typical development and did not present any significant medical or developmental concerns as confirmed by their normal scores on the Child Behavior Checklist (Achenbach and Rescorla, 2001). A research assistant who was unaware of the purposes of the study and blind to children's group membership gleaned audio records of the two groups of 13-month-old children from a large pool of home videos. This set of videos was collected by the Observation and Functional Diagnosis Laboratory at University of Trento (Esposito and Venuti, 2008, 2009a, b, Esposito and Venuti, 2010, 2011). The home videos (e.g., segments from family play situations) did not vary systematically between groups.

From the pool of about 120 home videos, a different research assistant selected crying episodes with the best acoustic quality. To ensure that the cries used in this study were representative of the typical range of cry sounds for the two groups (AD and TD), the cry sounds were digitized and analyzed using the Praat acoustic analysis software (Boersma and Weenink, 2005). A long-term average spectrum (LTAS) was employed to provide spectral information for each cry episode. LTAS is used to discriminate cry characteristics of different categories of children (Lin and Green, 2007). For all episodes of crying, the First Spectral Peak (FSP) of the LTAS was obtained. FSP is the frequency value (in Hz) of the first amplitude peak across the LTAS. It is an estimate of the average fundamental frequency (f_0) of the episode of crying (Lin and Green, 2007). The FSPs of cry episodes used in this study were for AD $M = 637.87$ ($SD = 66.56$) and for TD $M = 526.87$ ($SD = 60.33$), a significant difference, $F(1,16)=18.8$, $p<.001$. This result is consonant with previous findings that cries of children with AD at 13 months of age have higher f_0 (e.g., Esposito and Venuti, 2010 a, b, 2011). Cries of children with AD at 13 months of age also have shorter pauses (AD $M = 1.9$ s, $SD = 2.1$; TD $M = 4.7$ s, $SD = 1.3$), $F(1,15)=3.36$, $p = .01$). The durations of the original cry bouts across groups were analyzed as well; no difference emerged across groups (AD $M = 27.3$ s, $SD = 12.9$; TD $M = 31.1$ s, $SD = 10.5$), $F(1,16)=2.06$, *ns*. Audio clips of white noise (WN), controlled for f_0 and duration, were used as additional comparison stimuli. All acoustic files were edited using computer software to normalize them for volume and duration (10 s) and to remove all background noise.

fMRI protocol

During functional scanning, participants passively listened to the acoustic stimuli presented binaurally at ~ 75 dB SPL using Serene Sound (Resonance Technologies, Northridge, CA) headphones, with stereo quality sound (40 Hz to 40KHz frequency response) and passive scanner noise attenuation (30 dB). Subjects underwent a single fMRI run in which stimuli were presented in a blocked design. Acoustic stimuli of each category (TD, AD and WN) lasted 10 sec, with an inter-stimulus interval of 14 sec during which no stimuli was presented. The stimulus categories were presented in the following fixed order: TD, WN, AD, WN repeated for a total of ten times. The ten cry stimuli in each category (10 TD and 10 AD) were different, and they were pseudo-randomized between participants. Each individual cry was presented once to reduce habituation effects.

fMRI data acquisition

Participants underwent MRI scanning at 4 Tesla in a MedSpec Biospin MR scanner (Bruker Ettlingen, Germany) and an 8-channel birdcage head coil. Mild external head restraint was used to minimize head movement during scanning. Before collecting functional images, a high-resolution T1 weighted image of the whole brain (MPRAGE: 176 slices, GRAPPA acquisition with an acceleration factor of 2, FOV=256×256 mm², voxel size = 1×1×1 mm, TI = 1020 ms TE = 4.18 ms, TR = 2700 ms) was acquired for the purpose of spatial coregistration. Whole-brain functional data were acquired using echoplanar imaging, sensitive to BOLD contrast (34 slices, tilted 18° from intracommisural plane, FOV=192×192 mm, voxel size = 3×3×3 mm, slice gap = 15%, flip angle (FA), 73°, TE = 33 ms, TR = 2 s per volume). We performed an additional scan to measure the point-spread function (PSF) of the acquired sequence, which served for distortion correction that is expected with high-field imaging. The experimental session consisted of 489 whole brain images per participant; these included four dummy scans at the start of each time series to allow for T1 equilibration. The total experiment lasted about 20 min.

fMRI data analysis

To correct for distortions in geometry and intensity in the EPI images, we applied distortion correction on the basis of the PSF data acquired before the EPI scans (Zeng and Constable,

2002). The fMRI time series data were analyzed using SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>) on a Matlab platform (Mathworks Inc.). Echoplanar images were corrected for head movement, and the T1 image was coregistered to the mean of realigned images. Functional images were then normalized to the Montreal Neurological Institute (MNI) stereotaxic standard space and then spatially (9-mm full-width half-maximum Gaussian kernel) and temporally (cut-off period 256 s) smoothed. For each participant, an analytic design matrix was constructed, modeling onsets and duration of each trial as epochs convolved with a hemodynamic response function. The three stimulus types (AD, TD, WN) were modeled as separate regressors and interrogated to derive contrast images for second-level (group) mixed-effect analysis using a general linear model (GLM). For each participant, contrast images of AD cries versus WN and AD versus TD cries were created. These images were then entered into a second-level (random-effects) analysis to allow inferences across participants that generalize to the population. One sample t -tests on the contrast images imported from the first-level analysis were performed to assess group effects across all participants. The t -tests indicated whether observed differences between AD cry and WN, and AD cry and TD cry, differed significantly from zero (Holmes and Friston, 1998). Threshold significance for functional imaging data was set at $p < 0.01$, corrected using a cluster-wise ($k = 10$) false discovery rate (FDR) correction for multiple comparisons (Chumbley and Friston, 2009). A whole brain analysis was performed.

Results

fMRI data

AD cry versus WN comparison—AD cry compared to WN elicited activity in several cortical and subcortical regions of both hemispheres. Specifically, bilateral activity was detected in superior temporal gyri and poles, inferior frontal gyri (pars triangularis and opercularis), middle temporal gyri, thalamus, and precentral gyri. Additionally, we found preferential activity in the left Heschl gyrus, superior frontal gyrus, insula, putamen, and supplementary motor area ($p < 0.01$, FDR corrected; Table 1, Fig. 1).

AD cry versus TD cry comparison—AD cries activated the superior temporal and inferior frontal gyri bilaterally (BA 21, 22, 41, 42). Moreover, activity in the right superior frontal gyrus (BA 9) was observed. The maximum activation was observed in a cluster with a peak in the left superior temporal gyrus extending to the supramarginal gyrus (BA 40). The cluster of activity in the left inferior frontal gyrus included the anterior insula ($p < 0.01$, FDR corrected; Table 2, Fig. 2). No clusters were significantly activated by TD compared to AD cries. Assessments of participant gender and parental status showed no differences when comparing male and female participants, or parents and non-parents, in the contrasts of interest (using a p -value corrected for multiple comparisons).

Behavioral data

To further test our hypothesis that AD cries compared to TD cries elicit increased activity in brain structures involved in processing aversive or arousing stimuli, following the scanning session participants rated on a 4-point Likert-type scale (ranging from *not at all* to *extremely*) their feeling of distress when hearing each cry. Repeated-measures ANOVA (RM-ANOVA) on these scale scores used cry type (AD vs. TD) as a repeated factor. A main effect of cry type emerged. Participants reported feeling more distress when they heard AD cries ($M = 2.77$, $SD = .59$) compared to TD cries ($M = 2.07$, $SD = .59$), $F(1, 19) = 29.43$. We then computed for each participant: (1) the change of activation in the left inferior frontal gyrus/anterior (MNI coordinates: $-39, 27, -6$) during AD cries versus TD cries (AD minus TD BOLD percent signal change) and (2) the mean difference in ratings of distress for the

two types of cries (AD minus TD distress rating). The Pearson correlation between these two measures was positive and significant ($r=0.51$, $df=19$, $p=0.01$; Fig. 3).

Additionally, participants rated all cries on a similar Likert-type scale for their desire to care for the child. With this rating we wanted to assess whether AD and TD cries elicit different care responses. No RM-ANOVA main effect emerged for the desire-to-care response to AD cry ($M=2.63$, $SD=.62$) compared to TD cry ($M=2.62$, $SD=.67$), $F(1, 19)=.01$, ns . Both cry types elicited equivalent ratings of participants' desire to care for a crying infant.

Discussion

The present study aimed to assess whether cries of infants later diagnosed with AD elicit specific brain responses in adults compared to otherwise matched cries of TD infants. Cries of children with AD have specific acoustic features and represent one of the earliest anomalies that specifically characterize Autistic Disorder (Esposito and Venuti, 2010). Based on previous behavioral studies indicating that adults report comprehension difficulties and more negative emotional experiences when listening to cries of children with AD compared to those of TD children (Esposito and Venuti, 2008), we hypothesized, first, that cries of children with AD would elicit activity in brain areas implicated in auditory processing and interpretation of the human voice and, second, in brain regions mediating emotional responses to aversive and arousing stimuli. To test these hypotheses we applied fMRI during passive listening of 10 s of continuous cry episodes of infants later diagnosed with AD and cries of TD children and with white noise. Prior to the fMRI experiment we analyzed the acoustic characteristics of the selected cry episodes and verified that AD cries possess atypical features, such as higher fundamental frequency and shorter pauses. Studies of neurological responses to infant cry are of moment for several reasons. Infant crying facilitates bonding, and this bonding is instrumental to infant survival. Infant survival is facilitated because, when hearing the infant cry, the caregiver is alerted and can respond to the infant's primary needs (Murray, 1979) as well as the infant's affective, social, and cognitive requirements. Moreover, caregivers' behavioral responsiveness to infant cry has historically been conceived to play an important role in affective, social, cognitive, and language development in the child (Ainsworth et al., 1978; Bowlby, 1969).

Compared to white noise, cries of infants with AD elicited brain responses in line with previous neuroimaging studies conducted on cries of TD children (Lorberbaum et al., 1999, 2002; Seifritz et al., 2003; Swain et al., 2003). Specifically, cries of children with AD activated temporo-parietal regions implicated in sound and voice processing as well as brain areas involved in parenting behaviors and, more generally, with processing of emotional stimuli, including thalamus, putamen, and insula (Swain, Lorberbaum, Kose, and Strathearn, 2007). Additionally, activity in supplementary motor cortex and precentral gyri emerged, suggesting preparation to care behaviors (Nachev et al., 2008).

Supporting our first main hypothesis, we found that cries of children with AD compared to those of TD children specifically activate brain regions critical for second level acoustic processing in addition to regions associated with the analysis of basic acoustic features. Cries of children with AD preferentially activated the primary auditory cortex (BA 41, 42) as well as the secondary auditory cortex (BA 21, 22 including Wernicke's area), together with the inferior frontal gyrus bilaterally (including Broca's area) and the left supramarginal gyrus. Whereas activity in the primary auditory cortex is largely activated by basic acoustic properties of stimuli (such as frequency), the secondary auditory cortex responds to behaviorally relevant complex sounds (Hart et al., 2003). Moreover, activation of the superior temporal cortex and IFG in the right and left hemispheres has been found to subserve comprehension of emotional prosody (Leitman, Wolf, Ragland, Laukka,

Loughead, Valdez, et al., 2010; Wildgruber, Ackermann, Kreifelts, and Ethofer, 2006). In particular, the right IFG plays a critical role in processing emotional information and evaluating the affective salience of speech (Rota et al., 2009; Friederici and Alter, 2004). Moreover, the bilateral superior temporal cortex is specifically associated with voice discrimination during changing auditory conditions which require sustained auditory attention to target stimuli (Ikeda et al., 2010). The left supramarginal gyrus is also implicated in voice processing; specifically, preferential activity of the left supramarginal gyrus occurs when speech comprehension is made more difficult at the syntactic, semantic, or perceptual levels (Price, 2010) and during reading.

Cries of infants later diagnosed with AD compared to white noise also activated the right superior frontal gyrus (dorso-medial prefrontal cortex, BA9) that is implicated in cognitive abilities such as executive function (Cohen et al., 1996) and working memory (Townsend et al., 2010). Nakai and colleagues (2005) reported that discrimination of human speech is associated with activation in BA9 together with Wernicke's area and Broca's area. Preferential activation in this area during listening to AD cries may reflect efforts to attend to a vocal communicative signal with atypical features.

In short, it seems that listening to the cries of children with AD calls for deeper and more effortful auditory attention and comprehension, and in particular comprehension of "emotional content" which may be compromised in the cries of infants with AD. The augmented recruitment of brain areas involved in human voice processing may be attributable to altered acoustic patterns that typify the cries of children with AD and render them difficult to interpret. These findings accord with behavioral studies that have reported difficulty in decoding the causes of AD cries vis-à-vis TD cries by healthy parents (Esposito and Venuti, 2008). Cries of children with AD have been described as "inexplicable" by parents (Esposito and Venuti, 2008).

Consistent with our second hypothesis, cries of children with AD compared to those of TD children activated the left inferior frontal gyrus/anterior insula which have been found to mediate brain responses to aversive and arousing stimuli. Our participants subjectively rated cries of children with AD as more distressing than those of TD children. The anterior insular region has also been found to activate during awareness (Singer et al., 2004; Singer et al., 2006) or viewing (Jackson et al., 2006; Lamm et al., 2007; Morrison et al., 2004) of another individual's pain. Previous neuroimaging studies of TD children's cries revealed that the insula is activated by child cry in general and by own children's cry in particular (Lorberbaum, 2002; Seifritz, 2003; Swain et al., 2003, 2004). More generally, the insula is associated with subjective perceptions of emotional states (Craig, 2002, 2003) and awareness of emotionally salient stimuli (Craig, 2009; Critchley et al., 2004). Further evidence of the role of the left insula in mediating negative emotions comes from an investigation by Caria and colleagues (2010) who, using neurofeedback, demonstrated that increased activity of insula led to perceiving aversive stimuli as more unpleasant. Additionally, the insular cortex has been suggested as being an arousal center, participating in emotional responses to potentially distressing cognitive or interoceptive sensory stimuli (Reiman et al., 1997). The insular cortex is also implicated in sound detection, non-verbal processing, and auditory temporal processing (Bamiou et al., 2003; Levitin et al., 2003; Lorberbaum et al., 2002; Seifritz et al., 2003, Swain et al., 2003, 2004).

In short, it appears that, compared to cries of TD infants, cries of infants later diagnosed with AD elicit more negative feelings and are perceived as more aversive and/or arousing. Behavioral data acquired after the fMRI experiment help to confirm this interpretation. The altered acoustic characteristics of cries of AD infants, and especially their high fundamental frequencies and shorter pauses, may help to account for participants' negative emotional

reactions, as higher-frequency cries are often perceived as more aversive and distressed than lower-frequency cries (Lester and LaGasse, 2008). Moreover, the difficulty in interpreting AD cries may engender feelings of uneasiness. That said, participants' subjective ratings indicated that cries of children's with AD and TD elicited equivalent levels of desire to care for the crying child. Although atypical and more distressing, AD cries appear to activate adults' intentions to reduce infant distress with the same intensity as TD cries.

This study has two notable limitations. First, a stronger test of the specificity of brain responses to AD cries might involve comparison of cries of children with other developmental disorders. However, previous behavioral studies have shown that acoustic features (high pitch sounds, aspiration/expiration, pause) of cries of children with some developmental delays do not differ from those of cries of TD children but do differ from those of children with AD (Esposito and Venuti, 2009b). To limit the duration of the participants in the scanner in present experiment, and to reduce risks associated with habituation effects, we did not include additional types of child cry. Although we cannot draw conclusions about the specificity to AD, we believe that the results deepen our understanding of communicative behavior in a disorder whose earliest developmental stages are still largely unknown. Second, our sample included a mix of females and males, parents and non-parents, and this sample composition modifies the generalizability of our findings. On the one hand, we found no gender or parenthood status effects, and therefore believe that our findings may be broadly generalizable. In this first study of neurobiological responses to cries of children with AD, we aimed to include both adults with and without previous parenting experience because they represent the range of caregivers of children with AD. On the other hand, Seifritz and colleagues (2003) showed that parenthood modulated amygdala response to cry of unfamiliar TD children but did not influence activity in other brain areas involved in cry processing. Clearly, larger numbers of participants may modify these conclusions.

In terms of future work, several other possible paths are laid out by these initial findings. Endocrine responses are often activated while listening to child cries (higher levels of prolactin, for example, in Fleming et al. 2002). We have not considered them in this study. Theoretically, we would expect a role of endocrine responses on brain activation. For this reason we aim to include assessments of different hormones in association with brain area activation in future studies of typical and atypical cry perception.

In conclusion, the present study identifies brain regions activated by cries of AD infants. These regions may underlie why adults perceive this crucial infant communication signal as less intelligible and more distressing. The differential neurobiological responses in adults activated by cries of infants with AD versus TD may also underpin adult interactions with infants with AD much earlier than reliable AD diagnosis.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, NICHD. The fMRI experiment was performed at the Center for Mind/Brain Sciences (CiMeC) of the University of Trento. We thank Paola Rigo for assistance.

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Research Highlights

1. Cries of infants with autistic disorder compared to those of typically developing infants elicit enhanced activity in brain regions involved in verbal and prosodic processing.
2. Cries of children with autistic disorder elicit increased activity in the left inferior frontal gyrus/anterior insula.
3. Autistic cries may elicit more negative feelings and may be perceived as more aversive and/or arousing.

Figure 1. AD cry > Noise

Note. SPM t -maps of adults brain activations in response to cries of AD infants compared to noise. ($p < 0.001$, uncorrected for visualization). SFG = Superior frontal gyrus. SMA = Supplementary motor area. Th = Thalamus. IFG/AI = inferior frontal gyrus/anterior insula. STG = Superior temporal gyrus

Figure 2. AD cry > TD cry

Note. SPM t -maps of adults brain activations in response to cries of AD infants compared to TD infants. ($p < 0.001$, uncorrected for visualization). SFG = Superior frontal gyrus. IFG/AI = inferior frontal gyrus/anterior insula. IFG = inferior frontal gyrus. STG = Superior temporal gyrus

Figure 3. Correlation of distress rating and activity in IFG/AI

Note. The inter-individual significant positive Pearson correlation between (1) the change of activation in the left inferior frontal gyrus/anterior insula (MNI coordinates: $-39, 27, -6$) during AD versus TD cries and (2) the mean difference in the rating of distress for AD versus TD cries ($r=0.51$, $p=0.01$).

Table 1

AD cry > noise

Location	Side	Coordinates(MNI)	Brodmann Area (BA)	t value
Superior temporal gyrus	L	-51 -6 0	22	20.02
Superior temporal gyrus	R	60 -9 -3	21	18.40
Heschl gyrus	L	-39 27 12	41	14.55
Superior temporal pole	R	51 -3 -12	38	13.91
Middle temporal gyrus	R	63 -36 3	22	13.38
Superior temporal gyrus	R	63 -33 12	42	12.97
Superior temporal pole	L	-45 6 -12	38	10.85
Inferior frontal gyrus (pars triangularis)	R	51 27 3	45	10.55
Inferior frontal gyrus (pars triangularis)	L	-48 27 9	45	8.16
Inferior frontal gyrus (pars opercularis)	R	54 15 18	44	7.61
Middle temporal gyrus	L	-66 -45 9	22	7.53
Inferior frontal gyrus (pars opercularis)	L	-39 30 -6	45	7.45
Superior frontal gyrus	L	-3 57 39	9	7.41
Insula	L	-45 18 0	47	7.33
Putamen	L	-18 3 9		6.75
Thalamus	R	12 -12 6		6.70
Supplementary motor area	L	-3 15 66	6	6.60
Precentral gyrus	L	51 -3 48	6	6.50
Thalamus	L	-9 -12 6		6.33
Precentral gyrus	R	-48 3 54	6	5.66

Note. Significant BOLD activations during processing of cries of infants with AD compared to white noise

AD cry > TD cry

Table 2

Location	Side	Coordinates(MNI)			Brodmann Area (BA)	t value
Superior temporal gyrus	L	-42	-30	3	21	7.43
Superior temporal gyrus	R	45	-33	9	41	6.84
Superior frontal gyrus	R	-3	54	39	9	6.62
Superior temporal gyrus	R	48	-18	-9	22	6.38
Superior temporal gyrus	R	57	-36	18	42	6.01
Supramarginal gyrus	L	-54	-36	27	40	5.93
Inferior frontal gyrus (pars triangularis)	L	-54	18	24	44	5.86
Inferior frontal gyrus (pars orbitalis)	R	54	33	-6	47	5.62
Inferior frontal gyrus (pars orbitalis)/anterior insula	L	-39	27	-6	47	4.41

Note. Significant BOLD activations during processing of cries of infants with AD compared to cries of TD infants.