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## Brief Report: Atypical Expression of Distress During the Separation Phase of the Strange Situation Procedure in Infant Siblings at High Risk for ASD

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### Abstract

Previous studies have provided preliminary evidence that disruptions in cry acoustics may be part of an atypical vocal signature of autism early in life. We examined the acoustic characteristics of cries extracted from the separation phase of the strange situation procedure in a sample of toddler of younger siblings of a child with autism spectrum disorder-autism spectrum disorders (ASD) (high risk, HR) and a low risk (LR) group. Cry samples derived from vocal recordings of 15-month-old HR ( $n = 13$ ) and LR infants ( $n = 14$ ) were subjected to acoustic analyses. HR toddlers, compared to those with LR, produced cries that were shorter and had a higher fundamental frequency ( $F_0$ ). Three HR toddlers later classified with an ASD at 36 months (autistic disorder in all cases) produced cries that had among the highest  $F_0$  and shortest durations. Taken together these results indicate that toddlers at high risk for ASD (and those with an ASD) express atypical patterns of distress in response a social stressor. Implications for early diagnosis and parenting are discussed.

### Keywords

Cry; Autism spectrum disorders; Fundamental frequency; Strange situation; Behavior

## Introduction

To better understand social communication during early development, a growing literature is assessing the vocal production of children with Autism Spectrum Disorders (ASD). ASD affects communicative and social skills, and crying constitutes the child's first vocal communicative system and remains central to parent-child communication through toddlerhood (e.g. Wood and Gustafson 2001). Using both prospective and retrospective methodologies, a number of researchers have focused on the expression of distress via crying in children with ASD and the younger siblings of children with ASD, high-risk siblings (Biebe-rich and Morgan 1998; Esposito and Venuti 2009, 2010; Esposito et al. 2011; Oller et al. 2010; Sheinkopf et al. 2000, 2012). In the current prospective study, we examined differences in the acoustic characteristics of cries extracted from the separation phase of the Strange Situation Procedure (SSP; Ainsworth and Wittig 1969; Ainsworth et al. 1978) in a sample of toddler at high risk for ASD and a typically developing (TD) group.

There is evidence that disruptions in cry acoustics may reveal an atypical vocal signature of autism in early life. Using a prospective design, Sheinkopf et al. (2012) reported differences in acoustic features of infant cry vocalizations in infants at high risk for autism during naturalistic home recordings at 6 months. They found that high-risk infants produced cries with higher and more variable fundamental frequency (F0) than low-risk infants. Furthermore, high-risk infants later classified with ASD at 36 months had higher F0 values than those who were not later classified with ASD. Esposito and Venuti (2010) reported findings congruent with those of Sheinkopf et al. applying acoustic analysis to epochs of crying selected from retrospective home videos of children with ASD, TD or developmental delay (DD) at 5 and 18 months. The authors showed that children later diagnosed with ASD at 18 months had cries with higher levels of F0 than other children. In addition, children with later ASD did not show a decrease in F0 between the first and second year that was evident in both TD and DD children. Both Sheinkopf et al. and Esposito and Venuti characterized the acoustical characteristics of crying in the development of ASD. However, both reports analyzed spontaneous crying in non-standardized social contexts, potentially limiting the reproducibility of findings.

The literature suggests that ASD distress calls are perceived differently than other cries and may alter parent-infant interaction. Esposito et al. (2012, 2013) found that the cries of children with ASD—which were characterized by atypical acoustical features—were judged to be more distressing than cries of typically developing (TD) children by adults in two diverse cultures (Italian and Japanese). Finally, in a fMRI study using whole brain analysis in adults with and without caregiving experience, Venuti et al. (2012) found that the cries of infants with ASD, compared to those of typically developing (TD) infants, elicited increased activity in brain regions (left inferior frontal gyrus/anterior insula) associated with the emotional processing of aversive and arousing stimuli, suggesting that ASD cries may be perceived as more aversive and/or arousing than other cries. Finally, in a retrospective study, Esposito and Venuti (2009) found that maternal reactions to the crying of one-year-olds later diagnosed with autism were qualitatively different from the mothers' responses to cries of the comparison children. Mothers of children with ASD reacted to their children's cries with increased verbalizations, while mothers of matching control children were more likely to soothe their children using tactile stimulation (e.g. patting or stroking) or vestibular stimulation (e.g. rocking or swaying).

Although atypical distress expressions are thought to influence interaction between children with ASD and their caregivers, these distress expressions are typically elicited in laboratory contexts or in opportunistic social contexts. The purpose of the current research is to investigate the acoustic characteristics of cries elicited in a standardized social interaction

context, the Strange Situation Procedure (SSP; Ainsworth and Wittig 1969; Ainsworth et al. 1978). The SSP is a gold-standard measure of infant attachment security which consists of a series of separations and reunions with the caregiver designed to activate the infant's attachment behavioral system. Infants often become distressed during separations from the caregiver, and the SSP has been used to assess infant affect and emotional reactivity (Belsky et al. 2001; Gaensbauer et al. 1983; Shiller et al. 1986). The SSP is a standardized yet naturalistic way to study distress reactions both in typical populations and in at-risk populations such as toddlers at high risk for ASD.

We selected epochs of crying from the second separation episode of the SSP when the parent leaves the infant alone. We considered these to be epochs of socially-induced crying because the infant cries in reaction to the parent's departure. The study hypothesis is that the acoustic signature of the cries of HR toddlers will differ from those of LR toddlers during the standardized separation from the parent during the SSP.

## Method

### Participants

Informed consent was obtained from parents prior to participation in the research procedures. Participants ( $n = 27$ ) were enrolled in a larger longitudinal study investigating the early social and emotional development conducted in the Miami metropolitan area. The toddlers belonged to either a high-risk group (HR,  $n = 13$ ) or low-risk group (LR,  $n = 14$ ). High-Risk Siblings had one (only two of them had two) older siblings with a community diagnosis of an ASD (i.e. Autistic Disorder, Asperger Syndrome, or PDD-NOS) that was confirmed via a DSM-IV-TR -based clinical diagnosis (American Psychiatric Association [APA], 2004) from a licensed psychologist with ASD experience and administration of the ADOS (Lord et al. 1999). Low-Risk Siblings had no reported history of ASD in their first degree relatives, and all of their older siblings received a cutoff score lower than 9 (indicating no evidence of ASD) on the Social Communication Questionnaire (SCQ; Berument et al. 1999). Low-risk and high-risk groups did not differ significantly on either the age of the older siblings (older brother age: HR  $M = 3.8$ yo  $SD = 2.1$ ; LR  $M = 3.8$ yo  $SD = 2.60$ ), or the number of older siblings (HR  $M = 1.6$   $SD = 1.02$  range = 1–4; LR  $M = 1.3$   $SD = .46$ ).

Children were excluded from the study if they had a gestational age below 37 weeks, or major birth complications. Additional inclusion criteria were: (1) administration of the SSP, and (2) audio recordings of cries for acoustic measurements that did not contain background noises that would interfere with the acoustic analysis (e.g. adult talk, sounds from toys, or other environmental noises). The mean age of SSP administration was 15 months. The SSP was conducted with mothers, and mother's age was not statistically different in the two groups (HR  $M = 35.1$  years  $SD = 5.56$ ; LR  $M = 34.6$  years  $SD = 4.49$ ). At 36 months, diagnostic assessments for Autism Spectrum Disorder (ASD) were conducted based on administration of the ADOS and a DSM-IV-TR-based clinical diagnosis by a licensed psychologist experienced in ASD. Three toddlers from the HR group met criteria for an ASD; autistic disorder was the clinical best estimate diagnosis for all three of these toddlers.

In a group that overlapped with the current sample, Haltigan et al. (2010) found that high-risk siblings were just as likely as low-risk siblings to be securely attached, but were less likely to approach mother strongly and immediately. We asked whether possible risk group differences in acoustic parameters might be due to differences in attachment status between the groups. There was no statistically significant difference in the distribution of secure and insecure toddlers between the two risk groups (HR: 8 secure/5 insecure; LR 11 s/3u;  $\chi^2 = .29$ , *ns*). There were also no differences between risk groups in the attachment status

distribution using the Belsky-Rovine split (hypoactivating = A1–B2 vs hyperactivating = B3– C2) emerged (HR: 6 A1–B2/7 B3–C2; LR 6 A1–B2/8 B3– C2;  $\chi^2 = .05, ns$ ).

## Procedure

Infant-mother attachment security was assessed at 15-months using the Strange Situation Procedure (SSP; Ainsworth and Wittig 1969; Ainsworth et al. 1978). The SSP is a 25-minute procedure, which contains brief episodes of increasing stress for the infant, including two mother-infant separations and reunions. Cries—expressed vocalization of distress—were extracted from the second separation episode of the SSP, during which the infant is left alone (the stranger, who is part of the SSP, is not in the room). All infant cries started within the first 30 s of the separation.

A total of 159 epochs of crying were extracted from audio to video recordings of the second separation episode. All the recordings were made in the same assessment room with an overhead omnidirectional microphone. A research assistant who was blind to the status of the children extracted all epochs of crying in which there was not audible background noise in the second separation episode of the SSP. All selections were independently judged as appropriate by an expert audio analyst. Cries were broken into epochs if there was a pause between cry utterances of greater than 5 s. An average of 4.5 epochs of cry from each participant were analyzed (no differences emerged for the number of epochs for each group at each time point, see Table 1).

## Acoustic Analysis

After controlling for sound quality, digital signal processing and the acoustic measurements were accomplished using Praat (Boersma and Weenink 2005). The sampling rate was 44,100 Hz, and the signal was low pass filtered at 10,000 Hz (Rautava et al. 2007). The minimum and maximum F0 values were initially set to 200 and 700 Hz, respectively. These values are consistent with data on typical infants' cries (Rothganger 2003) and no cry epochs were excluded because all fundamental frequencies were within the 200–700 Hz band. A long-term average spectrum (LTAS) was employed to provide spectral information for the crying epochs. The LTAS has been helpful in discriminating the acoustic characteristics of different categories of infant cries (Lin and Green 2007). For all the epochs 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 (F0) of the epochs of crying (Lin and Green 2007). Besides the F0, the Maximum Pitch (F0 Max, highest level of the FSP), variability of Pitch (F0 Range, range of F0 across the cry epoch), and the duration (average time in seconds) was also analyzed. All these measures were extracted for the entire sequence of each child's crying (excluding the inter-utterances interval), and for the child's first cry utterance. Within an epoch, two coders selected the boundaries between utterances in order to exclude intervening non-crying intervals. If a toddler had multiple cries the mean of the cries' variables (*F0, F0 Max, F0 Range, Duration*) was used for the analysis of the group mean.

## Analyses

Prior to data analysis, univariate and multivariate distributions of the variables (*F0, F0 Max, F0 Range, Duration*) for the whole sequence of crying as well as the first utterance, were examined for normalcy and homogeneity of variance. Variables were normally distributed. One-way ANCOVAs, controlling for Age was performed on all the variables (*F0, F0 Max, F0 Range, Duration*).

## Results

### Whole Cry

Descriptive and inferential statistics are reported in Table 1. For the variable *F0*, an effect for *group* emerged. HR toddlers had a higher *F0* than LR toddlers. No significant differences for the two groups emerged for the variables *F0 Max* and *F0 Range*. For the variable *Duration* a significant effect for *group* emerged. HR toddlers had shorter cry *durations* than LR toddlers.

### First Utterance

Descriptive and inferential statistics are reported in table 1. For the variable *F0* and *F0 Max*, an effect for *group* emerged. HR toddlers had higher *F0* and higher *F0 Max* than LR toddlers. No significant differences for the two groups emerged for the variable *F0 Range*. For the variable *Duration* a significant effect for *group* emerged. HR toddlers had longer cry *durations* than LR toddlers.

### Subject Level Analyses of Whole Cry: High Risk with ASD Versus High Risk Without ASD

Individual values of *F0*, *F0 Max* and *Duration* for the overall cries of all toddlers (LR, HR with no ASD, and HR with ASD) are shown in Fig. 1. Nonparametric comparisons of HR toddlers with ( $n = 3$ ) and without an ASD diagnosis ( $n = 10$ ) are provided to complement the descriptive data in the figure. The three toddlers with an ASD diagnosis all had a score of 6 at the ADOS severity score at 30 months evaluation, were in the ASD group at the 36 months ADOS assessment, and had a diagnosis of autistic disorder. All of the three had only one older sibling with ASD. On the Mullen Scales of Early Learning Assessment at 36 months, their age equivalent motor ( $M = 21.1\text{mo}$ ;  $SD = 4.3$ ), and linguistic (receptive:  $M = 19.0\text{mo}$ ;  $SD = 4.0$ ; expressive:  $M = 24.3\text{mo}$ ;  $SD = 10.5$ ) development was below the typical range. Wilcoxon rank sum test with continuity correction indicated that the HR toddlers with a later diagnosis of ASD had significantly higher *F0* than the HR toddlers with no diagnosis of ASD ( $U = 28$ ,  $p < .05$ ). Wilcoxon rank sum test with continuity correction showed that the HR toddlers with a later diagnosis of ASD had higher *F0 Max* than the HR toddlers with no diagnosis of ASD ( $U = 28$ ,  $p < .05$ ). The tendency of the toddlers with ASD outcomes to have shorter cry *Duration* than other HR toddlers was not statistically significant ( $U = 4$ ,  $p = .07$ ).

### High Risk Without ASD Versus Low Risk

Parametric comparisons were used to compare HR toddlers without an ASD diagnosis ( $n = 10$ ) and LR toddlers ( $n = 14$ ). For the variable *F0*, an effect for *group* emerged. HR toddlers without an ASD diagnosis had higher *F0* than LR toddlers (HR > LR;  $F_{(1,23)} = 7.61$ ,  $p < .01$ ). No significant differences for the two groups emerged for the variables *F0 Max* and *F0 Range*. For the variable *Duration*, HR toddlers without an ASD diagnosis had somewhat longer cry *durations* than LR toddlers, but this difference did not reach statistical significance (HR > LR;  $F_{(1,23)} = 3.26$ ,  $p = .06$ ).

## Discussion

The purpose of this research was to investigate the acoustic characteristics of the cries of toddlers at high risk of ASD. Cries were elicited in a standardized social context, the separation phases of the SSP. In accord with our initial hypothesis we found group differences in several acoustical features (*F0* and *F0 maximum*) of infant cries. As in previous reports (Sheinkopf et al. 2012), the crying of children at risk of ASD had a higher mean *F0* than the crying of comparison children. Analyses of the first utterance of crying

also indicated a higher fundamental frequency of crying in the HR group, as well as a higher maximum fundamental frequency. The first cry utterance of HR toddlers was longer than that of LR toddlers; however the total duration of HR toddlers' cries was briefer (had a shorter overall duration) than that of LR toddlers.

HR toddlers classified with ASD at 36 months produced cries that had a higher F0 than other HR toddlers. HR toddlers that were not classified with ASD at 36 months, produced cries that had a higher fundamental frequency than LR toddlers. HR toddlers that were not classified with ASD at 36 months, produced cries that were marginally shorter than LR toddlers. In synthesis, toddlers with HR showed differences compared with the LR group, and within the HR group, those toddlers with an outcome of ASD represented the most extreme cases. These results are in agreement with the view that HR group share some common variance (genetic and/or environmental) and they all belong to a broader autism phenotype.

The onset of crying is thought to be under direct neural control (specifically of the brain stem and limbic system; LaGasse et al. 2005), after which infants tend to move into a more rhythmic crying pattern, which tends to be more homogeneous. Thus, the study of early cry utterances may be particularly informative to gain insights on the link between the anomalies at the central nervous level and at the social level. The current findings of *F0* differences in the first utterance of crying suggest poor central control (at least the level of brain stem, limbic system and hypothalamus) of crying in toddlers at high-risk of ASD.

There is a similarity between the absence of group differences in the variability of the fundamental frequency (*F0 Range*) in the current study and the results of Sheinkopf et al. (2012). Sheinkopf et al. made audio–video recordings in the infants' homes during daily activities. Cries were then categorized as either pain-related or non-pain-related based on videotape coding of the context. They did not find range differences in the non-pain cries, the cries that were likely most similar to the social cries elicited in the SSP in the current study.

With respect to duration we found that HR toddlers tended to have a shorter total duration of crying than LR toddlers. In addition, HR toddlers who had ASD outcomes, had among the shortest overall cry durations of the sample. Together with previous reports (Esposito and Venuti 2008, 2009), this result underscores short duration of overall crying to be a characteristic of children with ASD and at high risk for ASD. However, the duration of the first utterance of crying was longer among HR than comparison toddlers. It is likely that the subsequent utterances of HR toddlers were longer than those of comparison toddlers. A pattern of longer utterances with shorter overall duration of crying suggests that the cries of ASD toddlers have fewer pauses than those of comparison toddlers. This is noteworthy from a parental practice perspective because a recent cross-cultural study (Esposito et al. 2012) indicated that cry episodes with shorter pauses are perceived as aversive by caregivers.

An innovative feature of the current research is the use of the separation episodes of the SSP as an elicitor of crying. These episodes are a replicable method of eliciting crying, and elicited cries in our view represent a better experimental procedure than non-elicited (naturally occurring) cries because all subjects responded to the same, known stimulus (LaGasse et al. 2005). Early medical procedures (e.g. vaccination injections) are an alternate stimulus in early infancy. A unique advantage of the separation episodes of the SSP is that the crying elicitor is social rather than physical. Thus the cry elicitor pertains to the general category of behavior in which children with ASD are likely to manifest difficulties (Grzadzinski et al. 2012).



Using a social elicitor we found that 15-month-olds at high risk for ASD had shorter cry utterances that had higher fundamental frequencies than low-risk toddlers. Strikingly, those HR toddlers later diagnosed with ASD had amongst the most extreme values in the sample. This concordance between the risk group and diagnosed children suggest that disturbed cry patterns, although not a core communication deficit in children with ASD, may provide an early marker of risk for later autism (Sheinkopf et al. 2012) and suggest further research to differentiate among characteristics that may be valuable as early indicators (i.e. present only in ASD) and characteristics that may simply be signs of risk (i.e. present in the HR group). Although the current sample size highlights the needs for independent replication, these findings add evidence for a general pattern of higher mean fundamental frequency and shorter durations of cry utterances among children with ASD (Sheinkopf et al. 2012; Esposito et al. 2011). These cry characteristics appear to be distressing to adult listeners (Esposito et al. 2012) and may, in turn, render adequate parental responses more difficult (Trevvarthen et al. 1998). Understanding the stability of these cry characteristics and their potential impact on the parent–child relationship will require continued longitudinal follow-up of high risk siblings in their family context beyond the age of first diagnosis.

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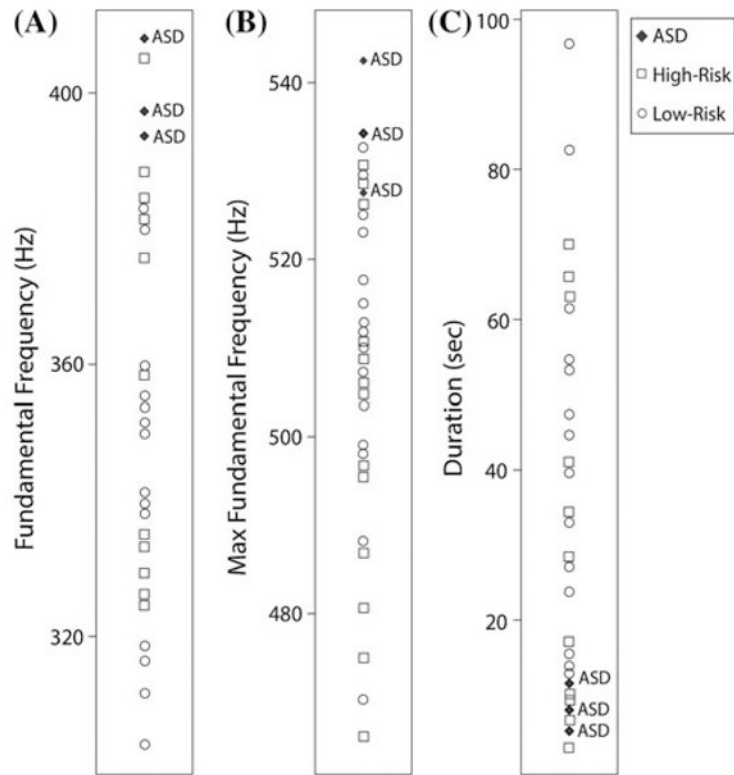
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**Fig. 1.** Individual data by risk group for overall cry elicited by separation **a** fundamental frequency ( $F_0$ ); **b** maximum fundamental frequency ( $F_0$  Max); **c** duration. Individuals with 36-month classification of autism spectrum disorder (ASD) are noted (autistic disorder in all cases)

**Table 1**  
**Descriptive and inferential statistics are for all the variables (Number of Epochs of cries, F0, F0 Max, F0 Range, and Duration) for the whole epoch of crying and for the first utterance**

	High-risk (N = 13)		Low-risk (N = 14)		F
	M	SD	M	SD	
Gender	4F/9 M		7F/7 M		
Age	15.46	1.12	15.21	0.80	ns
No. of epochs	2.50	2.28	2.71	0.99	ns
<i>Whole cry</i>					
F0	370.54	30.48	329.96	26.84	16.82**
F0 Max	519.06	17.65	501.90	29.03	ns
F0 Range	26.66	31.25	31.14	23.41	ns
Duration	22.01	22.72	46.58	27.85	7.62*
<i>First utterance</i>					
F0	360.48	52.72	318.61	28.18	7.90*
F0 Max	483.16	47.25	435.90	66.14	6.12*
F0 Range	59.56	68.86	50.23	38.05	ns
Duration	2.16	0.93	1.42	0.73	7.43*

Univariate analyses were conducted for age and gender. Age ranged from 15 to 18 months across both groups. Inferential statistics for Number of Epochs, F0, F0 Max, F0 Range, and Duration from ANCOVAs, controlling for Age and Gender

\*  $p < .05$ ;

\*\*  $p < .01$