SHORT REPORT

Diminished Respiratory Sinus Arrhythmia Response in Infants Later Diagnosed with Autism Spectrum Disorder

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Indicators of risk for developing Autism Spectrum Disorder (ASD) are difficult to detect within the first year of life. In this study, infants who were originally followed prospectively to examine general developmental risks due to substance exposure interacted with their mother and an unfamiliar experimenter for 2-min episodes at 4 months of age. Electrocardiogram was collected to measure respiratory sinus arrhythmia (RSA) and the session was video recorded for behavioral coding. Two groups of infants were compared: infants with a diagnosed ASD outcome ($N = 8$) and matched controls ($N = 186$). Infants were compared on mean RSA and infant behavioral codes for each 2-min episode. No significant group differences were revealed on RSA or behavior during interactions between the infants and mothers. However, in response to play with a stranger, infants with an ASD outcome had lower RSA ($M = 2.49$, 95% CI [2.30, 2.98]) than controls ($M = 3.12$, 95% CI [3.06, 3.18]). During the interaction with a stranger, lower RSA response was associated with more protesting behaviors ($P < 0.01$), whereas higher RSA response was associated with more social monitoring ($P = 0.001$). Lower RSA suggests that 4-month-old infants later diagnosed with ASD exhibited poorer autonomic regulation during interaction with an unfamiliar adult than did controls. Physiological regulation during interactions with a new social partner may be disrupted early in infancy in children with ASD, as indicated here by lower RSA, and therefore be a potential target for developing early risk screening tools for ASD. ***Autism Res** 2018, 11: 726–731.* © 2018 International Society for Autism Research, Wiley Periodicals, Inc.

Lay Summary: Autism Spectrum Disorder (ASD) emerges within the first years of life; however, it is difficult to identify children who will develop autism before 12 months of age based on behavioral measures. In a study of infants who were followed from birth, infants who were later diagnosed with ASD had poorer physiological regulation during play with a new adult. With additional evidence, poorer physiological regulation may function as an early sign of ASD risk.

Keywords: autism spectrum disorder; infants; respiratory sinus arrhythmia

Introduction

Behavioral symptoms of autism spectrum disorder (ASD) emerge in the late infancy to preschool period but that are difficult to detect within the first year of life [Jones, Gliga, Bedford, Charman, & Johnson, 2014; Szatmari et al., 2016]. Reliable markers of ASD risk are needed, as is an improved understanding of developmental mechanisms that are associated with the onset of observable symptoms. Physiological reactivity to social stimuli is one potential area for developing early markers.

Respiratory sinus arrhythmia (RSA) is a measure of the influence of respiration on heart rate variability thought to reflect parasympathetic involvement. Higher RSA is interpreted as a physiological indicator of emotion regulation and readiness to engage with different environmental stimuli [Grossman & Taylor, 2007]. Finding differences in RSA in infants later diagnosed with ASD would have implications for understanding early developmental processes associated with the development of symptoms and later diagnostic outcomes.

The overall goal of this study was to examine physiological and behavioral responses during challenging social interactions in infants later diagnosed with ASD compared to controls. Infants typically demonstrate a reduction in RSA when their caregiver disengages from social play [Moore & Calkins, 2004; Weinberg & Tronick, 1996]. Because children with ASD are behaviorally less responsive to social partners compared to their

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Table 1. Characteristics of Infants with an Autism Spectrum Disorder (ASD) Outcome and Comparison Group

	ASD ($N = 8$)	Controls ($N = 168$)
Mean gestational age in weeks, mean (SD)	37.63 (4.37)	37.40 (3.22)
Birth weight in grams, mean (SD)	3196.25 (1052.56)	3002.67 (803.94)
Number of drug exposures, mean (SD)	1.75 (0.71)	0.83 (0.93)*
Race/ethnicity, No. (%)		
Black	2 (25%)	117 (69.6%)
White	4 (50%)	31 (18.5%)
Hispanic	2 (25%)	17 (10.1%)
Other	0 (0%)	3 (1.8%)
Low SES, No. (%)	1 (12.5%)	21 (12.5%)
Maternal education, No. (%)		
<12 years	0 (0%)	53 (31.5%)
12 years	4 (50%)	67 (39.9%)
>12 years	4 (50%)	48 (28.6%)
Marital Status, No. (%)		
Married	4 (50%)	51 (30.4%)
Never married	4 (50%)	112 (66.7%)
Divorced	0 (0%)	5 (3%)

*$P < 0.05$.

peers [APA, 2013], our hypothesis was that children with ASD would not demonstrate as much reactivity as controls to the disengagement of their caregiver. Evidence from face and emotion processing research suggests that atypical responses to social stimuli may be specific to unfamiliar social stimuli [Nuske, Vivanti, & Dissanyake, 2013; Pierce & Redcay, 2008]. Due to this evidence, we also hypothesized that infants with ASD would demonstrate lower RSA during an interaction with a stranger than controls, indicating more dysregulation in response to new social partner.

Methods

Participants

Infants were enrolled in the Maternal Lifestyle Study [MLS; Lester et al., 2002]. The goal of the MLS was to evaluate the long-term outcomes of children exposed to cocaine in utero. Within the MLS sample, eight infants later received a diagnosis of ASD and completed some portion of the study protocol at their 4-month visit. Diagnoses were confirmed by clinical judgment and a standardized clinical assessment of autism symptoms in late childhood or adolescence [Lord, Rutter, DiLavore, & Risi, 2000; Rutter, Le Couteur, & Lord, 2003]. A case control comparison group was selected from the MLS sample by matching to each one of the eight infants in the ASD group based on sex, cocaine exposure, socioeconomic status, and gestational age. Of the potential matches, 21 were randomly selected for each infant in the ASD group, resulting in a total comparison group of 168 infants (see Table 1 for participant demographics).

Procedures and Measures

Mother–infant dyads were seen when the infant was 4 months of age (age corrected for prematurity for infants below 37 weeks gestation). Digitized electrocardiography (ECG) recordings were acquired from the standard three electrode placement on the infant's chest and abdomen. The ECG signal was recorded, digitized, and stored on a computer hard drive [Lester & Peucker, 1994]. Physiology and video signals were synced using a Society for Motion Picture and Television Engineers (SMPTE) time code generator.

Face-to-face still-face paradigm. The face-to-face still-face (FFSF) paradigm [Tronick, Als, Adamson, Wise, & Brazelton, 1978] consisted of a 2-min face-to-face play session between the mother and infant (play), followed by a 2-min episode in which the mother was instructed to look at the infant but to not smile, touch, or talk to the infant (still-face), and then a 2-min reunion interaction in which the mother was again instructed to play with her infant (reunion).

Stranger condition. At the end of the session an unfamiliar female examiner engaged the infant in a 2-min play session (stranger).

Behavior coding. The behaviors of the infants and mothers were coded using the Infant and Caregiver Engagement Phases [Weinberg & Tronick, 1998]. The current analyses focused on the infant engagement codes: passive-withdrawn, protest, object-environment, social monitor, and social positive engagement.

Table 2. Means and Standard Deviations of Respiratory Sinus Arrhythmia (RSA) across Condition and within Group

Condition	ASD	Controls	Cohen's D effect size
Play	2.73 (0.56)	3.22 (0.76)	0.73
Still-face	2.55 (0.65)	3.14 (0.83)	0.79
Reunion	2.92 (0.93)	3.23 (0.85)	0.34
Stranger	2.49 (0.51)	3.10 (0.81)	0.90

Coding procedures and reliability (Cohen's kappa range 0.72–0.74) were reported in Tronick and colleagues [2005].

Respiratory sinus arrhythmia. RSA is a measure of the variability in R-R intervals that occur during inspiration and expiration. RSA during the FFSF paradigm was derived from the R-R time series collected from ECG recordings [Porges, 1986; U.S. Patent No. 4,510,944, 1985]. ECG data post-processing was performed using a series of automated algorithms [Conradt et al., 2013].

Statistical Analysis

Descriptive of RSA are reported in Table 2. Within separate general linear mixed models (SAS PROC Mixed, Version 9.4), mean RSA and behavior codes during the still-face and reunion conditions were compared in relation to the play phase of the protocol (i.e., as change from the play session). Covariates were included if they significantly correlated ($P < 0.05$) with RSA or infant behaviors during any of the three phases of the protocol. Due to a significant group difference, the cumulative total substance exposure score was also added to the models of RSA and infant behaviors. Bivariate correlations across the whole group were used to examine relationships between RSA and behavior.

Group differences in RSA and behavioral responses to a play session with a stranger were evaluated using an analysis of variance (ANOVA). The same infant behavioral codes from the session with mother were examined with the stranger. No covariates reached significance ($P < 0.05$) for RSA. Bivariate correlations across the full sample were used to examine relationships between RSA and behavioral measures. One infant in the ASD group did not provide data for the stranger phase of the protocol; therefore, the matched controls for that infant were removed from the analysis. The resulting control group consisted of 147 infants.

Results

Mother–Infant FFSF

Model results are reported in Table 3. The significant main effect of the still-face phase indicated a decrease in RSA from the play phase to the still-face phase,

Table 3. Parameter Estimates of RSA during Mother-Infant Face-to-Face Still-Face Paradigm

	Estimate	SE	t	df	*P*
Fixed effects					
Intercept (play)	3.23	0.36	9.01	171	<0.0001
Still-face	−0.10	0.05	−2.01	346	0.04
Reunion	0.01	0.05	0.22	346	0.82
Controls	0.35	0.27	1.27	171	0.20
Race	−0.17	0.07	−2.33	171	0.02
Education	−0.09	0.07	−1.22	171	0.22
Total exposures	0.08	0.06	1.33	171	0.18
	Estimate	SE	Z	p	
Random effects					
Intercept	0.44	0.05	8.08	<0.0001	

RSA, respiratory sinus arrhythmia.

($\beta = -0.10$; 95% CI [−0.198, −0.002]; $P = 0.04$). There was no significant difference in RSA between the play and reunion phases. When the model was rerun with the still-face condition as the reference category, estimates indicated that RSA increased from the still-face ($\beta = 3.14$; 95% CI [2.78, 3.50]; $P < 0.001$) to the reunion phase ($\beta = 3.25$; 95% CI [3.20, 3.30]; $P = 0.03$) in both groups. The main effect of group did not reach significance ($\beta = 0.35$, 95% CI [−0.18, 0.88]; $P = 0.20$). The interactions between group and conditions did not generate significant effects or improve model fit ($\chi^2 = 2.7$, $P = 0.25$), and, therefore, were not included in the model. None of the models comparing infant behaviors during the FFSF paradigm resulted in significant group differences. The level of RSA during the play phase was negatively correlated with object-environment ($r_{(173)} = -0.17$, $P = 0.03$) and positively correlated with social monitoring ($r_{(173)} = 0.16$, $P = 0.04$). Children who looked more at other environmental stimuli besides their mothers tended to have lower RSA, whereas infants who engaged in social monitoring behaviors tended to have higher RSA levels, indicating better regulation. Social monitoring was also positively correlated with RSA during the still-face phase ($r_{(176)} = 0.17$, $P = 0.02$), indicating that social monitoring was associated with higher RSA during the stressful phase of the task (Fig. 1).

Stranger Condition

RSA during the stranger condition was significantly different between the groups, $F_{(1,\ 151)} = 4.16$, $P = 0.04$. Mean RSA during the stranger condition was lower in the ASD group ($M = 2.49$, 95% CI [2.12, 2.86]) than the control group ($M = 3.10$, 95% CI [2.96, 3.24]). Total drug exposures did not reach significance as a covariate within the model ($P = 0.64$). None of the ANCOVAs of infant behavior revealed significant group differences. Infant protest was negatively correlated with RSA ($r_{(154)} = -0.24$, $P = 0.003$) and infant social monitoring

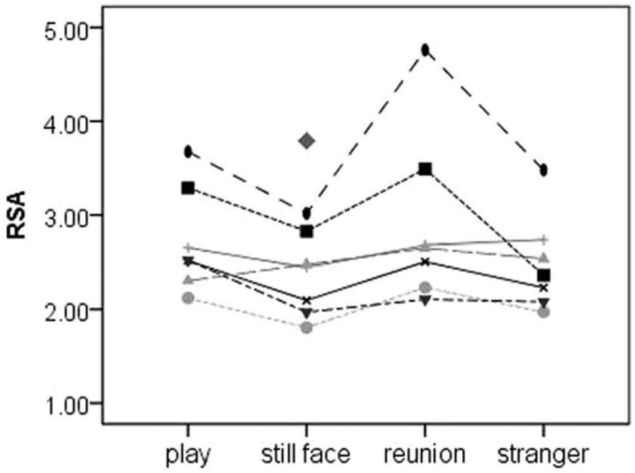

Figure 1. Respiratory sinus arrhythmia (RSA) response of individuals within the autism spectrum disorder (ASD) group across conditions.

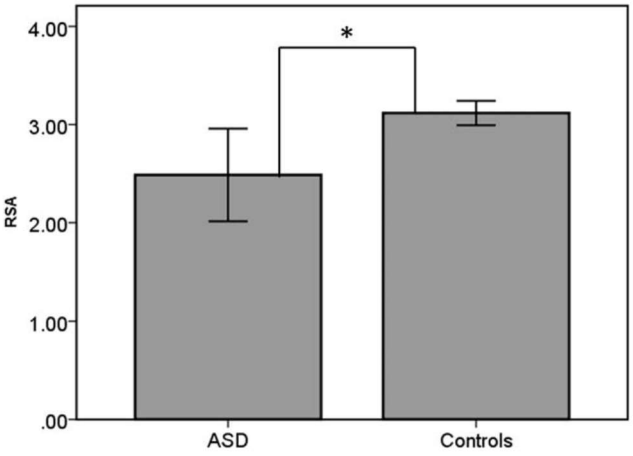

Figure 2. RSA response during the stranger condition. $^{*}P < 0.05$. Error bars represent standard error.

was positively correlated with RSA ($r_{(154)} = 0.25$, $P = 0.001$). Infants with lower RSA exhibited more negative protesting behaviors, whereas infants with higher RSA engaged in more social monitoring behaviors.

Discussion

During social play with a stranger, infants with an ASD outcome had significantly lower RSA than controls, indicating poorer regulation. The RSA finding suggests that the interaction with a novel adult was more stressful for the infants in the ASD group; that they were less physiologically prepared to engage with the adult play partner than controls. Across the FFSF paradigm, infants with an ASD outcome had lower RSA than controls as evidenced by moderate effect sizes, however, these differences did not reach statistical significance within our model. Although there were not significant group differences in behavioral measures, moderate though significant correlations indicated that lower RSA was associated with more negative behaviors and higher RSA was associated with more social behaviors (Fig. 2).

The patter of group differences in physiological response to unfamiliar versus familiar people is consistent with findings in older populations [Nuske, Vivanti, & Dissanyake, 2014; Vaughan Van Hecke, et al., 2009]. These RSA findings are also consistent with theory linking individual differences in RSA to variation in social functioning [Patriquin et al., 2014] and specifically to social-communication deficits seen in children with ASD [Patriquin et al., 2013; Vaughan Van Hecke et al., 2009]. This finding also indicates that infants with later ASD diagnoses show differences in physiologic responses to social challenge in infancy, prior to the onset of observable behavioral symptoms. The positive correlation between social monitoring and RSA suggests that social monitoring during stressful social situations, in this case the still-face and the introduction of a new person, is a regulatory strategy. If replicated, this relationship indicates that social monitoring behaviors could be an important early intervention target.

This study has several strengths, including a unique sample for studying ASD symptoms in infancy. The majority of the extant literature examining symptoms in infancy is based on infants at genetic risk of developing ASD, specifically infant siblings of children with ASD. In this paper, we report on a sample of infants enrolled at birth and followed prospectively, but none were selected on the basis of ASD family history. We utilized case control methods for unbiased selection of controls from the larger longitudinal cohort. Diagnostic outcomes were confirmed in late childhood and adolescence using validated clinical measures together with expert clinician diagnostic ratings. These procedures helped ensure that the ASD cases were well identified.

The results from this work should be interpreted with some limitations in mind. While this study contributes to the literature with a unique prospective cohort, this is also a complex sample. To account for this, groups were carefully matched. Despite matching on cocaine exposure (the main exposure of interest in the parent project), groups differed on exposure to other substances (e.g., tobacco, alcohol). To account for this, amount of exposure was included within analyses as a covariate. MLS was a large cohort; however, only a small number of participants were identified with an ASD outcome (1.01% vs. 1.47% in the general population [Center for Disease Control, 2014]), and not all ASD cases in MLS had data available on the assessments used in the current study. Although the small sample size limits our ability to pursue variability analyses, wide differences in variability could account for our finding of

nonsignificant mean differences. The small sample also only included males with ASD. Increasing evidence suggests that ASD symptoms may present differently in males and females, including potential difference in infancy [Chawarska, Macari, Powell, DiNicola, & Shic, 2015].

Conclusions

Our finding of diminished RSA during interaction with a novel adult indicates that social responsiveness may be affected by ASD even in very early development, adding to our understanding of early developmental mechanisms in ASD. These findings raise the possibility that biomarkers of social responsiveness can be used to refine developmental targets of treatment, including what to treat and when to implement interventions. Although our finding highlights a promising mechanism to target for the development of biomarkers, substantial further research is needed to elucidate these issues and to determine whether such biomarkers have sufficient specificity for clinical use [Anderson, 2015].

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