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Early Behavioral and Physiological Predictors of Autism in 12-month-old Siblings of Children with Autism

Nicolas Poupore, Abigail L. Hogan, Ph.D., and Jane Roberts, Ph.D.

University of South Carolina – Columbia
Early Behavioral and Physiological Predictors of Autism in 12-month-old Siblings of Children with Autism

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder defined by social communicative deficits and a pattern of repetitive interests and behaviors (American Psychiatric Association [APA], 2013). The prevalence of ASD is high, impacting 1 in 68 children; boys are 4.5 times more likely to be diagnosed with ASD than girls (Christensen et al., 2016). Even though the exact etiological mechanisms that cause ASD are unknown, many studies have found that both genetic and environmental factors are involved in increasing risk (Geschwind, 2011; Hallmayer et al., 2011).

While there currently is no cure for ASD, many studies have shown that early identification and intervention are crucial to improving the long-term outcomes of a child diagnosed with ASD (Ben-Itzchak & Zachor, 2007; Dawson et al., 2010). Studies have shown that a diagnosis of ASD at two years of age is reliable and stable (Kleinman et al., 2008; Lord et al., 2006). However, the median age for a diagnosis of ASD is almost four years (Christensen et al., 2016). This obvious chasm between the research and clinical practice has led many researchers to try to develop improved screening measures to detect children at a young age who are at high risk for ASD. One test that has gained strength is the Autism Observation Scale for Infants (AOSI), which particularly focuses on younger siblings of a child with ASD (ASIB; Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008). These children have been found to have a 20% chance of developing ASD (Ozonoff et al., 2011). The AOSI is a semi-structured play observation designed to identify early behavioral markers of ASD in children aged from 6 to 18 months (Bryson et al., 2008). While the AOSI has shown to be effective in research for identifying these markers, it is not quite sensitive enough to be used clinically as a
diagnostic screening measure, especially in highly verbal children (Bryson & Zwaigenbaum, 2014). Another limitation to using this test clinically is the extensive training required to become reliable in administering and scoring the exam. Due to the nature of scoring behavioral measures, there is the possibility of different interpretations of similar behavior. Even though the inter-rater reliability was found to be excellent at 12 months for AOSI marker scores and total scores (.92 and .93) and the test-retest reliability was found to be acceptable at 12 months (.61 and .68, for total scores and total marker counts, respectively), these numbers suggest intensive training and practice for clinicians is needed in order for this exam to be used as a definitive clinical measure (Bryson & Zwaigenbaum, 2014). Lastly, another limitation to using this clinically is that behavior is the most distal marker of a pathology or disorder. While behaviors are incredibly important and used extensively for identifying disorders, finding and using physiological markers that are causing these behavioral changes as a screening mechanism can be more accurate and sensitive in identifying those at risk of the disorder.

With many behaviors of early ASD being well quantified, other research has focused on physiological markers that could potentially be causing these atypical behaviors. One area that has developed stems from the Polyvagal Theory that was developed and proposed by Dr. Stephen W. Porges. In short, this theory explains and defines an unmyelinated and myelinated tract of the vagus nerve, and that this interaction with the sympathetic nervous system shows the evolutionary development of the autonomic nervous system and gives a biological framework to explain positive social behavior (Porges, 2001). Respiratory sinus arrhythmia (RSA) has been proposed as in important marker of this physiological regulation, which in turn is critical for emotional, cognitive, and behavioral regulation (Porges, 1986; Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996). Further research into RSA has shown that it is an effective
physiological biomarker for the function of the parasympathetic nervous system (PNS; Katona & Jih, 1975). Taking this work, RSA changes have been studied to see if they are correlated or causal to any behaviors. Decreased RSA in children developing children has been theoretically shown to linked to social-communicative problems (Neuhaus, Bernier, & Beauchaine, 2014). Another physiological marker studied is RSA Suppression, which is the difference between the RSA at a basal state and the RSA at a reactive state. Decreased RSA Suppression has been shown in some studies to be related to decreased physiological, emotional, and behavioral regulation (Porges et al., 1996). Another study has shown that poor RSA Suppression is a significant predictor of an atypical developmental trajectory of stranger fear in infants (Brooker et al., 2013). These studies suggest that studying both static and reactive RSA can lend important information on the functioning of the PNS which could be influencing the atypical behaviors seen in infants.

With both behavioral risk markers and physiological risk markers being separately defined, this study specifically wants to bring these two factors together to study their relationship in an effort to establish a more effective screening measure for ASD that combines behavior and physiology as well as add to the growing body of research of the neurological and physiological mechanisms of ASD. This study examined 12-month ASIBs because there have been many studies showing noticeable behavioral differences and changes just before 12 months in children later diagnosed with ASD. Some examples of behavioral changes at 12 months include not babbling, not showing objects to caregivers, lack of gestures, and not following an adult’s pointed finger (Plauché Johnson, 2008; Watson & Crais, 2013; Zwaigenbaum et al., 2009). This relationship between behavioral and physiological risk markers in 12-month old ASIBs to our knowledge has not been studied.
This study included two primary objectives. First, we aimed to see if the ASIBs demonstrate atypical physiological regulation relative to low risk controls (LRCs) and if ASD behavioral risk markers are related to physiological regulation at 12 months of age. We hypothesized that the ASIBs will have atypical physiological control in both RSA and RSA suppression compared to the LRCs and that the behavioral ASD risk markers are correlated to these atypical physiological regulations. Second, we aimed to determine if physiological regulation differs in the ASIBs that do and do not end up with an ASD diagnosis, and if there is a relationship between the ASD behavioral signs and physiological regulation in all three groups. We predicted that all three groups would differ on RSA and RSA suppression, with the ASIBs who did not end up with ASD (ASIB-NonASD) showing an intermediate profile and the ASIBs who were diagnosed with ASD (ASIB-ASD) exhibiting the most divergent profile from the LRCs. These physiological profiles would parallel the behavioral risk markers between the three groups.

Methods

Procedures

Participants were recruited as part of a larger longitudinal study at the University of South Carolina – Columbia (USC) that focused on the early emergence of ASD in high-risk infants. Infants were assessed at the USC laboratory or at their home, based on the needs of the family. When possible, the measures were typically administered in a standard order and at similar times of the day (mid-morning) to account for the effects of circadian rhythms on physiology and behavior. Parents of the infants were recruited using flyers, list-serves, and word of mouth. The families were compensated for their time and travel expenses. Parents provided informed written consent.
Participants

The study included 34 ASIBs and 33 LRCs. All ASIBs were verified by the presence of an older sibling that was diagnosed with ASD. Participants were considered low risk if they were born full term and had no history or suspicion of developmental delay from a parental report and research assessment results. Low risk controls that later developed ASD at 24 months were excluded from the study. Descriptive statistics for participant demographics are shown in Table 1.

For the second research question, which included ASD diagnostic outcomes at 24 months, the ASIB group was split into two groups: 5 ASIBs-ASD and 24 ASIBs-NonASD. This question, which excluded LRCs that did not have 24-month outcomes, included 26 LRCs. See Table 2 for the participant demographics.

Measures

**Cognitive Level.** The Mullen Scales of Early Learning (MSEL; Mullen, 1995) was administered at the 12-month assessment for an index of general cognitive abilities. The MSEL is for children from 0 to 68 months of age. It includes the five subscales of Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language. An Early Learning Composite (ELC) score is derived from all subscales except Gross Motor and is calculated with a mean of 100 and a standard deviation of 15. Internal reliability is .75 to .83 for subscales, and .91 for the ELC (Mullen, 1995).

**Autism Observation Scale for Infants.** At the 12-month assessment, the Autism Observation Scale for Infants (AOSI; Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008) was used to identify and measure early ASD symptoms and behaviors. The AOSI is a semi-structured play observation designed to detect signs of ASD in infants between the ages of
6 and 18 months. There is a Total Score ranging from 0-50 across 16 items and a Marker Score ranging from 0-16. A Total Score of 9 or higher and a Marker Score of 7 or higher are indicators of elevated ASD risk (Bryson et al., 2008; Zwaigenbaum et al., 2005). The AOSI has test-retest reliability of .61 for Total Score and .68 for Marker Score (Bryson et al., 2008), and strong sensitivity (84%) and specificity (98%) for ASD at 12 months (Zwaigenbaum et al., 2005). For the larger longitudinal study, item-level inter-rater agreement (81%) was checked for 20% of administrations.

**Clinical Best Estimate Diagnosis.** The AOSI and Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) were administered and scored by research reliable project staff consisting primarily of doctoral students, post-doctoral fellows, and Ph.D.-level investigators. Adapted from standard procedures (Lord et al., 2006, 2012), a clinical best estimate (CBE) diagnosis of ASD was determined based on a review of data collected at the 24-month visit. These diagnoses were determined by a multidisciplinary team that included a licensed psychologist and licensed speech-language pathologist; both were research reliable on the ADOS-2.

**Heart Activity.** Heart activity data were collected during a baseline condition and during the AOSI. During baseline, participants watched a Baby Einstein video for 3 minutes. Movie watching was chosen as the baseline task because it was thought that the infants would not be able to complete a more traditional baseline task (sitting alone quietly); this approach parallels several other physiology studies of developmental disabilities (e.g. Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Roberts et al., 2011; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001). Electrocardiogram data was collected with an Alive Wireless Heart Monitor (Alive Technologies, Copyright 2005–2009), with either two electrodes that were placed on the
participant’s chest or with an elastic Polar belt that contained electrode receptors (the belt was presented as an option for participants who refused the electrodes). Data were sampled at a rate of 300 times per second. Data were edited with CardioEdit software (Brain-Body Center, University of Illinois at Chicago) by a reliable research assistant. Editing consisted of visually inspecting the electrocardiogram signal to identify false heart periods (e.g., faulty R wave detection) and manual adjustment of artifacts using integer arithmetic. Estimates for RSA and interbeat interval (IBI) were extracted. IBI is measured as the time in milliseconds between successive R waves. Heart rate (HR) is calculated from IBI using the fact that there are 60,000 milliseconds in one minute; the equation is \( \text{HR} = 60,000 / \text{IBI} \). To find RSA, sequential heart periods are sampled at 250-ms epochs, and data is detrended with a 21-point moving polynomial algorithm (Porges & Bohrer, 1990). Data is then bandpass filtered to extract variance associated with spontaneous breathing parameters (0.24–1.04 Hz), and the variance is transformed to its natural logarithm to yield an estimate of RSA. The average RSA and HR for each condition, measured from 30-s epochs, were used in analyses. RSA Suppression was computed by subtracting AOSI RSA from Baseline RSA.

**Statistical Analysis Plan**

In order to determine whether ASIBs exhibit atypical physiological regulation (e.g., RSA), ASIBs and LRCs were compared using independent samples \( t \)-tests on the following variables: AOSI Total Score, AOSI Marker Score, Baseline RSA, Baseline HR, AOSI RSA, AOSI HR, and RSA suppression. In order to determine whether infants’ RSA varied dependent on context, we used paired samples \( t \)-tests to examine differences in RSA in the Baseline and AOSI conditions. Pearson correlations were then utilized to examine relationships between AOSI scores and physiological variables.
In looking at whether ASIBs exhibited atypical physiological regulation based on their ASD outcome, ASIBs-ASD, ASIBs-NonASD, and LRCs were compared using one-way analyses of variance (ANOVA) for the variables described above. To see if differences were exhibited in RSA in the Baseline and AOSI conditions, paired samples t-tests were utilized. Pearson correlations were then used to study the relationship between AOSI scores and physiological variables.

**Results**

Independent-samples t-tests were conducted to compare AOSI Total Scores and AOSI Marker Scores in LRCs and ASIBs. For AOSI Total Score, there was a marginally significant difference in the LRCs and ASIBs, \( t(65) = -1.70, p = 0.09 \) (see Figure 1). On AOSI Marker Score, groups differed significantly, \( t(65) = -2.29, p < .05 \) (see Figure 2). Groups did not differ on any physiological variables (see Table 3 for details).

To determine whether infants’ RSA values varied across Baseline and AOSI contexts, paired-samples t-tests were run for each group. For the ASIB group, RSA decreased by an average of 0.39 (SD = 0.64) from Baseline to AOSI, \( t(14) = 2.33, p < .05 \). For the LRC group, RSA differed by 0.37 (SD = 0.67), \( t(21) = 2.57, p < .05 \). Figure 3 depicts the suppression of RSA from Baseline to AOSI for both groups.

Pearson correlation coefficients were computed to assess the relationship between the behavioral markers and the physiological markers. In the LRC group, there was a marginal negative correlation between AOSI Total Score and AOSI RSA, \( r = -0.37, n = 23, p = .09 \), as well as a marginal negative correlation between AOSI Marker Score and AOSI RSA, \( r = -0.36, n = 23, p = .10 \). An opposite relationship was seen in the ASIB group, where there was a marginal positive correlation between AOSI Total Score and AOSI RSA, \( r = 0.44, n = 15, p = .10 \). Also in
the ASIB group, a marginal negative correlation was observed between AOSI Total Score and RSA Suppression, $r = -.46$, $n = 15$, $p = .09$, and a negative correlation was observed between AOSI Marker Score and RSA Suppression, $r = -.52$, $n = 215$, $p < .05$ (see Figure 4). There were no other significant correlations between the behavioral markers and the physiological markers (see Table 4 for details).

Next, the ASIBs were separated by ASD outcome, and one-way Analyses of Variance (ANOVAs) were employed to investigate group differences in AOSI scores and physiological regulation. For AOSI Total Score, a significant effect of group emerged, $[F(2, 52) = 5.74, p < .01]$. Post-hoc comparisons using the Bonferroni correction indicated that the mean score for the LRC group was significantly different than the ASIB-ASD group ($M = 10.60$, $SD = 3.98$). However, the ASIB-NonASD group ($M = 6.58$, $SD = 3.87$) did not significantly differ from the LRC and ASIB-ASD groups (see Figure 5). A one-way ANOVA was conducted to compare the group effects on AOSI Marker Score in the three groups. There was a significant group effect on AOSI Marker Scores for the three groups $[F(2, 52) = 10.55, p < 0.001]$. Post hoc comparisons indicated that the mean score for the LRCs ($M = 2.96$, $SD = 2.12$) was significantly different than the ASIBs-NonASD ($M = 4.38$, $SD = 1.81$) and the ASIB-ASDs ($M = 7.20$, $SD = 1.92$). It also indicated that the mean score for the ASIBs-NonASD was significantly different than the ASIBs-ASD (see Figure 6). A one-way ANOVA was conducted to compare the group effects on AOSI RSA. There was a marginally significant group effect on AOSI RSA for the three groups $[F(2, 30) = 3.30, p = .05]$. Post hoc comparisons indicated that the mean score for the ASIBs-NonASD ($M = 3.72$, $SD = .64$) was marginally different than the ASIBs-ASD ($M = 4.55$, $SD = .54$). The groups did not differ on any other physiological variables (see Table 5 for details).
Next, paired-samples were run for the three groups, to investigate whether RSA values varied across Baseline and AOSI contexts. For the ASIB-ASD group, RSA decreased by an average of 0.00 (SD = 0.31) from Baseline to AOSI, \( t(4) = 0.00, p = 1.00 \). For the ASIB-NonASD group, RSA decreased by an average of 0.54 (SD = 0.72) from Baseline to AOSI, \( t(8) = 2.27, p = .05 \). For the LRC group, RSA differed by 0.39 (SD = 0.65), \( t(18) = 2.62, p < .05 \). Figure 7 depicts the suppression of RSA from Baseline to AOSI in each group.

Pearson correlation coefficients were computed to assess the relationship between the behavioral markers and the physiological markers for the three groups. No significant correlations were present between the two different types of variables; see Table 6 for details.

**Discussion**

ASD is a highly prevalent disorder that is still not fully understood in terms of the neurobiological mechanisms. Moreover, while the screening measures for high-risk children have greatly improved, they are not quite sensitive enough to be used clinically. Improving on these screening measures could improve the paradigm of most children being diagnosed with ASD at around age 4. With many studies showing the stability and accuracy of diagnosis at age 2, this would add additional time for these children to receive the proper interventions and therapies, which could greatly improve outcomes. By looking at both behavioral and physiological markers at 12 months, this will both help to identify biomarkers for screening and diagnosis as well as an increased understanding into the mechanisms of ASD which can lead to further targeted treatments.

In this study, we used both behavioral, measured by using the AOSI, and physiological, measured by recording HR and RSA, biomarkers to see if significant differences could be detected at 12-months of age in ASIBs. While our study agreed with much of the current
research that ASIBs do have some atypical behaviors compared to LRCs, the most important findings were seen when we divided the ASIB group based on 24-month outcome of ASD. By doing this, a step-wise pattern appeared in both behavior and physiology. The ASIB-NonASD showed an intermediate profile in AOSI scores and RSA Suppression, and the ASIB-ASD showed the most divergent profiles in both areas. However, significant correlation data did not appear in the three groups, but when the ASIBs were not grouped based on 24-month outcomes, the ASIB group had a negative correlation with AOSI Marker Score and RSA Suppression.

These findings are of critical importance for both screening and mechanism research. This study shows that there are noticeable and quantifiable biomarkers at 12 months of age for if a child is at a high-risk for ASD at 24 months. Being able to identify children at this stage could allow for therapies to be implemented very early in development, which could drastically improve outcomes for these children. With a majority of neurodevelopment happening by 3 years of age (Gilmore et al., 2007; Nowakowski, 2006), different therapies during this time should greatly improve their social and communicative skills. More importantly, it further adds to the evidence that there are subclinical differences in the ASIBs compared to LRCs. While these children may never be diagnosed with any developmental disorder, there is still the potential of behavioral therapies being beneficial for these children. It also suggests that their PNS or other neurobiological mechanisms are partially compromised and should be addressed. Moreover, this study shows that something is happening physiologically either before or at 1 years of age in terms of how ASD develops. The irregularity of RSA suppression in both the ASIB-NonASD and ASIB-ASD groups suggest that there is a problem in either the development or functioning of the parasympathetic nervous system. This inappropriateness of the reactivity and control of the PNS to a stressor situation could be one of the factors behind the behavioral
differences seen in both groups of ASIBs. The support of this comes from the significant negative correlation results in the ASIB group between RSA Suppression and AOSI Marker scores. While there are many factors between these two variables that need to be further researched, there seems to be some interaction between atypical RSA Suppression and AOSI Marker Scores.

Two interesting trends were noticed in the correlation data. The first was the marginally positive significant correlation between RSA and AOSI Total Score in the ASIB group. This trend is opposite from what we expected, as much of the research has shown that lower RSA can lead to emotional, social, and communicative problems. This area should be further researched to see if RSA has a different effect in the ASIBS compared to LRCs. The other area of interest was that the three groups did not reveal any significant correlations. While for behavior and physiology, a step-wise pattern was revealed, the correlation data did not show any relationship between these two variables. In looking at this, the issue seems to be one of power. By separating the ASIB group, not enough participants were left in each group to reveal significant results. More research should be focused on this area to confirm any correlations that may not be present due to low power.

There are a few limitations to the study. First is small sample size of ASIB-ASD group. A larger group of these children would allow for further conclusions to be drawn from this group. Another potential issue with this group is the absence of girls in the ASIB-ASD group. While there are a higher proportion of ASD boys than girls, the group should contain girls diagnosed with ASD to better represent the population. Secondly is the use of the AOSI as a social or reactive stressor. While in typical situations, this type of test is seen as social, children later diagnosed with ASD may not view this as a social stressor. It could be argued that they are not
repressing their RSA due to their failed understanding of the situation. However, if this was the case, then that would suggest another problem of these children not properly recognizing social situations. On the contrary, these children may recognize the social stressor, but may not be able to properly respond to it, potentially leading to atypical behaviors. Parsing out these differences is an area of further research that would lead to a greater understanding of ASD as a social-communicative disorder.

Future directions for the research can be looked at in the two areas mainly discussed: screening and understanding of the mechanisms. For screening, we want to look at if combining these two measures of behavior and RSA suppression could be potentially used clinically to label children as high-risk in order to get them services earlier in their development. Particularly, seeing if the RSA suppression would increase the sensitivity of the AOSI would be the chief concern of our further research. In terms of looking at the neurological mechanisms, it would be of importance to see how the RSA suppression and behavioral markers interact. It could be studied to see if this lack of RSA suppression causes some or all of the atypical behavioral markers. In looking at this the other way, since ASD has been shown to be mainly a polygenetic disease, to see if there are any mutations in the genome related to the development of the PNS. This could help to further define the pathway from genetics, functioning of the physiology, to the phenotype in terms of behavior.

Overall, this study shows that there is a noticeable step-wise physiological and behavioral pattern of profiles between ASIBs-ASD, ASIBs-NonASD, and LRCs. With these profiles shown, these can be used in both improving screening and diagnostic measures along with the neurobiological mechanisms of ASD in infants. This helps fill the gap of research in how physiological and behavioral markers are present and interact in 12-month ASIBs.
References


Brooker, R. J., Buss, K. A., Lemery-Chalfant, K., Aksan, N., Davidson, R. J., & Goldsmith, H. H. (2013). The development of stranger fear in infancy and toddlerhood: normative development, individual differences, antecedents, and outcomes. Developmental Science, 16(6), n/a-n/a. https://doi.org/10.1111/desc.12058


Randomized, Controlled Trial of an Intervention for Toddlers With Autism: The Early Start Denver Model. *Pediatrics, 125*(1).


Figure 1. Total Scores on the AOSI. Groups had marginally significantly different scores. $t(65) = -1.70, p = .09$
Figure 2. Marker Score on the AOSI. Groups had significantly different scores. \( t(65) = -2.29, p < .05 \)
Figure 3. RSA Suppression from Baseline to AOSI. For the ASIB group, RSA significantly decreased by an average of 0.39 (SD = 0.64), $t(14) = 2.33, p < .05$. For the LRC group, RSA significantly differed by 0.37 (SD = 0.67), $t(21) = 2.57, p < .05$. 

For the ASIB group, RSA significantly decreased by an average of 0.39 (SD = 0.64), $t(14) = 2.33, p < .05$. For the LRC group, RSA significantly differed by 0.37 (SD = 0.67), $t(21) = 2.57, p < .05$. 

* $p < .05$
Figure 4. Correlation between AOSI Marker Score and RSA Suppression in the ASIB Group. A negative correlation was observed between the two variables, $r = -.52$, $n = 21$, $p < .05$. 

$R^2 = 0.2695$
Figure 5. Total Score on the AOSI. Groups with different letters had significantly different scores. $F(2,52) = 5.74, p < .01$
Figure 6. Marker Score on the AOSI. Results from ANOVA suggest that groups differed on AOSI Marker Score, $F(2,52) = 10.55$, $p < .001$. Groups with different superscripts differed significantly, $ps < .05$. 

**Figure 6.** Marker Score on the AOSI. Results from ANOVA suggest that groups differed on AOSI Marker Score, $F(2,52) = 10.55$, $p < .001$. Groups with different superscripts differed significantly, $ps < .05$. 

***$p < .001$
Figure 7. RSA Suppression from Baseline to AOSI. Paired samples t-tests were used to examine RSA suppression within each group. For the ASIB-ASD group, RSA decreased by an average of 0.00 (SD = 0.31), t(4) = 0.00, p = 1.00. For the ASIB-NonASD group, RSA marginally significantly decreased by 0.54 (SD = 0.72), t(8) = 2.27, p = .05. For the LRC group, RSA significantly decreased by 0.39 (SD = 0.65), t(18) = 2.62, p < .05.
Table 1.

*Participant Demographics*

<table>
<thead>
<tr>
<th></th>
<th>ASIB (n = 34)</th>
<th>LRC (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months) – M (SD)</td>
<td>12.70 (.80)</td>
<td>12.38 (.60)</td>
</tr>
<tr>
<td>Gender – n (%) males</td>
<td>26 (76%)</td>
<td>26 (79%)</td>
</tr>
<tr>
<td>Mullen Early Learning Composite – M (SD)</td>
<td>98.33 (14.53)</td>
<td>100.25 (12.87)</td>
</tr>
</tbody>
</table>

*Note.* Participants were divided based on presence of an older sibling with ASD.
Table 2.

*Participant Demographics*

<table>
<thead>
<tr>
<th></th>
<th>ASIB-ASD (n = 5)</th>
<th>ASIB-NonASD (n = 24)</th>
<th>LRC (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months) – $M \ (SD)$</td>
<td>12.31 (.41)</td>
<td>12.73 (.78)</td>
<td>12.33 (.47)</td>
</tr>
<tr>
<td>Gender – n (%) males</td>
<td>5 (100%)</td>
<td>18 (75%)</td>
<td>20 (77%)</td>
</tr>
<tr>
<td>Mullen Early Learning Composite – $M \ (SD)$</td>
<td>97.6 (15.45)</td>
<td>98.30 (15.87)</td>
<td>101.6 (12.74)</td>
</tr>
</tbody>
</table>

*Note.* ASIBs were divided on 24-month outcomes of ASD.
Table 3.

**Behavioral and Physiological Differences between Groups**

<table>
<thead>
<tr>
<th>Variables</th>
<th>ASIBs M (SD)</th>
<th>LRCs M (SD)</th>
<th>Test Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOSI Total Score</td>
<td>6.56 (4.24)</td>
<td>4.85 (4.00)</td>
<td>t(65) = -1.70</td>
<td>.09</td>
</tr>
<tr>
<td>AOSI Marker Score</td>
<td>4.41 (2.27)</td>
<td>3.15 (2.22)</td>
<td>t(65) = -2.30</td>
<td>.03</td>
</tr>
<tr>
<td>Baseline RSA</td>
<td>4.48 (1.00)</td>
<td>4.49 (1.07)</td>
<td>t(48) = .02</td>
<td>.98</td>
</tr>
<tr>
<td>Baseline HR</td>
<td>124.41 (12.77)</td>
<td>126.50 (10.98)</td>
<td>t(48) = .62</td>
<td>.54</td>
</tr>
<tr>
<td>AOSI RSA</td>
<td>4.00 (.69)</td>
<td>4.20 (.65)</td>
<td>t(36) = .92</td>
<td>.37</td>
</tr>
<tr>
<td>AOSI HR</td>
<td>128.51 (8.67)</td>
<td>128.72 (11.25)</td>
<td>t(36) = .06</td>
<td>.95</td>
</tr>
<tr>
<td>RSA Suppression</td>
<td>.39 (.64)</td>
<td>.37 (.67)</td>
<td>t(35) = -.07</td>
<td>.94</td>
</tr>
</tbody>
</table>

Note. Groups did not have any significant differences on any of the physiological variables.
Table 4.

*Correlations between Behavioral and Physiological Markers*

<table>
<thead>
<tr>
<th></th>
<th>Baseline HR</th>
<th>Baseline RSA</th>
<th>AOSI HR</th>
<th>AOSI RSA</th>
<th>RSA Suppression</th>
</tr>
</thead>
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<tr>
<td>ASIB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AOSI Total Score</td>
<td>.19</td>
<td>-.08</td>
<td>.04</td>
<td>.44†</td>
<td>-.46†</td>
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<tr>
<td>AOSI Marker Score</td>
<td>.22</td>
<td>-.18</td>
<td>.01</td>
<td>.39</td>
<td>-.52*</td>
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<tr>
<td>LRC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AOSI Total Score</td>
<td>.00</td>
<td>-.04</td>
<td>.10</td>
<td>-.37†</td>
<td>-.06</td>
</tr>
<tr>
<td>AOSI Marker Score</td>
<td>-.02</td>
<td>-.08</td>
<td>.05</td>
<td>-.36†</td>
<td>-.09</td>
</tr>
</tbody>
</table>

*Note.† p < .10; * p < .05. In the LRC group, there was a marginal negative correlation between AOSI Total Score and AOSI RSA, r = -.37, n = 23, p = .09, and a marginal negative correlation between AOSI Marker Score and AOSI RSA, r = -.36, n = 23, p = .10. In the ASIB group, there was a marginal positive correlation between AOSI Total Score and AOSI RSA, r = .44, n = 15, p = .10, a marginal negative correlation was observed between AOSI Total Score and RSA Suppression, r = -.46, n = 15, p = .09, and a negative correlation was observed between AOSI Marker Score and RSA Suppression, r = -.52, n = 15, p < .05.*
### Table 5.

*Behavioral and Physiological Differences between Groups*

<table>
<thead>
<tr>
<th>Variables</th>
<th>ASIB-NonASDs M (SD)</th>
<th>ASIB-ASD M (SD)</th>
<th>LRCs M (SD)</th>
<th>Test Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOSI Total Score</td>
<td>6.58 (3.87)</td>
<td>10.60 (3.98)</td>
<td>4.54 (3.80)</td>
<td>F(2,52) = 5.74</td>
<td>.01</td>
</tr>
<tr>
<td>AOSI Marker Score</td>
<td>4.38 (1.81)</td>
<td>7.20 (1.92)</td>
<td>2.96 (2.13)</td>
<td>F(2,52) = 10.55</td>
<td>.00</td>
</tr>
<tr>
<td>Baseline RSA</td>
<td>4.27 (1.02)</td>
<td>4.55 (.50)</td>
<td>4.61 (1.04)</td>
<td>F(2,40) = .58</td>
<td>.56</td>
</tr>
<tr>
<td>Baseline HR</td>
<td>124.94 (13.19)</td>
<td>129.13 (11.81)</td>
<td>126.33 (11.24)</td>
<td>F(2,40) = .24</td>
<td>.79</td>
</tr>
<tr>
<td>AOSI RSA</td>
<td>3.72 (.64)</td>
<td>4.55 (.54)</td>
<td>4.28 (.67)</td>
<td>F(2,30) = 3.30</td>
<td>.05</td>
</tr>
<tr>
<td>AOSI HR</td>
<td>128.92 (8.91)</td>
<td>128.16 (12.14)</td>
<td>127.84 (11.78)</td>
<td>F(2,30) = .03</td>
<td>.97</td>
</tr>
<tr>
<td>RSA Suppression</td>
<td>.54 (.72)</td>
<td>.00 (.31)</td>
<td>.39 (.65)</td>
<td>F(2,30) = 1.19</td>
<td>.32</td>
</tr>
</tbody>
</table>

*Note.* Groups did not have any significant differences in any physiological measures, except for AOSI RSA, which was marginally significant between groups.
Table 6. 

Correlations between Behavioral and Physiological Markers

<table>
<thead>
<tr>
<th></th>
<th>Baseline HR</th>
<th>Baseline RSA</th>
<th>AOSI HR</th>
<th>AOSI RSA</th>
<th>RSA Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASIB-ASD</td>
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</tr>
<tr>
<td>AOSI Total Score</td>
<td>.16</td>
<td>-.56</td>
<td>.53</td>
<td>-.45</td>
<td>-.17</td>
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<tr>
<td>AOSI Marker Score</td>
<td>-.08</td>
<td>-.62</td>
<td>.28</td>
<td>-.48</td>
<td>-.17</td>
</tr>
<tr>
<td>ASIB-NonASD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AOSI Total Score</td>
<td>-.03</td>
<td>.14</td>
<td>-.47</td>
<td>.47</td>
<td>-.26</td>
</tr>
<tr>
<td>AOSI Marker Score</td>
<td>.03</td>
<td>-.05</td>
<td>-.22</td>
<td>.10</td>
<td>-.38</td>
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<td>LRC</td>
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<td></td>
</tr>
<tr>
<td>AOSI Total Score</td>
<td>.08</td>
<td>-.17</td>
<td>-.03</td>
<td>-.34</td>
<td>-.28</td>
</tr>
<tr>
<td>AOSI Marker Score</td>
<td>.05</td>
<td>-.22</td>
<td>-.07</td>
<td>-.31</td>
<td>-.28</td>
</tr>
</tbody>
</table>

Note. *p < .10; *p < .05. Groups did not have any significant correlations.