Improving Anxiety Assessment in Autism: A Potential Use for Heart Rate Variability and Heart Rate

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ABSTRACT

Anxiety is an area of documented challenge for people with Autism Spectrum Disorder (ASD). Despite this, some studies state that those with ASD and language deficits have lower reported anxiety than those without language deficits. This effect may be due to the complicated task of appropriately evaluating anxiety in those with compromised language. Using biomarkers of anxiety, such as reduced Heart Rate Variability (HRV) and increased Heart Rate (HR), may improve anxiety assessment but more research is necessary. Specifically, it would be helpful to understand if the relationship between HRV/HR and anxiety is moderated by language abilities in children with ASD, and whether HRV/HR can discriminate between anxiety and other emotions, like anger, in children with ASD. This study examined the relationship between baseline HRV/HR, language ability, and different emotional states (i.e., anxiety and anger) in a sample of 23 children with ASD. It was hypothesized that receptive language would moderate the relationship between decreased HRV/increased HR and increased anxiety but not the relationship between decreased HRV/increased HR and increased anger. Multiple regression analyses indicated that HRV and HR were not significant predictors of anxiety or anger, and language was not a significant moderator. Future studies may wish to take new approaches to determining the role of language in the relationship between HRV/HR and anxiety.
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Anxiety is an area of documented challenge for people with Autism Spectrum Disorder (ASD). Despite this, some studies state that those with ASD and language deficits have lower reported anxiety than those without language deficits. This effect may be due to the complicated task of appropriately evaluating anxiety in those with compromised language. Using biomarkers of anxiety, such as reduced Heart Rate Variability (HRV) and increased Heart Rate (HR), may improve anxiety assessment but more research is necessary. Specifically, it would be helpful to understand if the relationship between HRV/HR and anxiety is moderated by language abilities in children with ASD, and whether HRV/HR can discriminate between anxiety and other emotions, like anger, in children with ASD. This study examined the relationship between baseline HRV/HR, language ability, and different emotional states (i.e., anxiety and anger) in a sample of 23 children with ASD. It was hypothesized that receptive language would moderate the relationship between decreased HRV/increased HR and increased anxiety but not the relationship between decreased HRV/increased HR and increased anger. Multiple regression analyses indicated that HRV and HR were not significant predictors of anxiety or anger, and language was not a significant moderator. Future studies may wish to take new approaches to determining the role of language in the relationship between HRV/HR and anxiety.
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Introduction

About 1 in 68 children is diagnosed with Autism Spectrum Disorder (ASD; CDC 2009). ASD is a developmental disorder which is comprised chiefly of deficits in communication and social skills and the presence of restricted interests and repetitive behaviors (Christensen, 2016). Although these are the primary deficits in ASD, individuals with ASD face struggles and challenges across many different areas of functioning. One such area of functioning is that of emotion regulation, particularly anxiety. Many studies suggest that people with ASD have more struggles with regulating their emotions than do typically developing individuals (Davis et al., 2011; DeLussey, Herrington, Bradstreet, Guy & Souders, 2014; Garfinkel, Tiley, O'Keeffe, Harrison, Seth, & Critchley, 2016; Laurent & Rubin, 2004; Mazefsky et al., 2013). Increased anxiety in this population is one such manifestation of this difficulty. Kerns et al. (2014) found that the presence of an anxiety disorder in their ASD sample, however, was associated with stronger expressive and receptive language abilities. It is possible that anxiety is also heightened in those with ASD and language impairment, but perhaps this is not captured by current assessment tools. A possible way to improve this assessment for minimally verbal children with ASD (MV-ASD) may be to examine the use of biomarkers of anxiety to augment standard anxiety assessment procedures, such as heart rate (HR) and heart rate variability (HRV), which have been shown to be related to anxiety (i.e., increased HR and reduced HRV).

There are challenges to this measurement, however; namely, that HR and HRV may be related to many different states and conditions. Heightened HR and reduced HRV, for example, have been found in ASD itself (Daluwatte, 2013; Ming et al., 2005) and have also been
associated with angry behaviors such as reactive aggression (Scarpa, Haden, & Tanaka, 2010).

If HRV and HR are ever to be used as biomarkers for anxiety in ASD for those with MV-ASD, it would be important to understand more about the relationships between HRV, HR, language and anxiety within the ASD population. Additionally, it would be important to examine differential relationships of HR/HRV with other emotional states such as anger. To begin this line of research, the current study examined receptive language as a moderator between HR and HRV and the emotions of anger and anxiety.

**Difficulty with Anxiety Assessment in ASD**

Consistent with the findings that children with ASD often have difficulties with emotion regulation, anxiety disorders are one of the most common comorbidities with ASD. About 40% of children with ASD also experience anxiety concerns in the form of a traditional anxiety disorder (Kerns et al., 2014). Additionally, it is reported that between 11% and 84% of children with ASD experience some form anxiety that interferes with their daily life (White, Oswald, Ollendick & Scahill 2009).

Based on these findings, it seems important that there be reliable and valid measures for the assessment of anxiety and the diagnosis of anxiety disorders in ASD. Unfortunately, this does not always seem to be the case. Although Kerns et al. (2014) found a positive association between anxiety disorder and stronger expressive and receptive language abilities, it could simply be the case that stronger language capabilities facilitate the detection of an anxiety disorder. Interestingly, one study demonstrated that in TD children and in children with Pervasive Developmental Disorder- Not Otherwise Specified (PDD-NOS), poorer expressive language was correlated with increased anxiety (Davis et al., 2011). However, in this same study, poorer communication ability was correlated with decreased anxiety in children diagnosed with
autistic disorder PDD-NOS is a pervasive developmental disorder listed in the DSM-IV with features of autism that did not meet the full DSM-IV criteria. In the newest iteration of the DSM (i.e., the DSM-5) PDD-NOS has been subsumed into the ASD category (APA, 2013). It is unclear why the findings differ between PDD-NOS and ASD in this study, since PDD-NOS is now encompassed within the diagnosis of ASD in the DSM-5; more research may need to be done in this area. This seemingly contradictory result may be explained by inferring that TD children and children with PDD-NOS in this study were still able to express anxiety in some way despite decreased overall communication ability, while children with the diagnosis of autistic disorder were not. The authors imply more severe communication difficulties in the autistic disorder group, stating that the communication deficits and increased likelihood of alexithymia present in autism may make it difficult in this population to evaluate anxiety symptoms as they are currently defined. It is also possible that some components of anxiety are more recognizable to parents than others (i.e., running away is more recognizable than a child feeling scared).

This concern would be easier to address if all the ASD population had similar communication abilities or difficulties. However, expressive and receptive language and social communication are often notable deficits of children with ASD, and the presentation of this deficit is extremely variable. It is estimated that 30% of the total ASD population acquire very little language or never acquire any language at all (Davis et al., 2011); yet, there is surprisingly little work done on the relationship between language, ASD, and anxiety. One study reported findings in which groups with ASD and greater language abilities showed higher anxiety than control groups (Gillott, Furnis & Walter, 2001); however, this study only looked at children with no language impairments. Therefore, no conclusions may be drawn about children with language
impairment. Still others assert that greater levels of anxiety could potentially be a function of characteristics associated with ASD, including communication deficits such as reduced receptive and expressive language (Wood & Gadow, 2010). Still another study compared groups of children classified as having either “Autism”- the diagnosis more likely to be associated with reduced receptive and expressive language- or “Aspergers”- the diagnosis associated with no language delay- as per DSM-IV criteria and found no difference in caregiver reported levels of anxiety between the two groups (Kim, Szatmari, Bryson, Streiner, & Wilson, 2000).

These variable findings demonstrate that currently the results are very mixed as to whether children with MV-ASD experience more, less or the same amount of anxiety as their more verbal peers with ASD. However, considering the documented struggles with increased anxiety this population is known to experience and the challenges that compromised language (whether expressive or receptive) has on ability of clinicians to accurately diagnose anxiety, it seems important that more research be directed towards accurate assessment of anxiety in children with MV-ASD. This paper will consider lower language ability as a potential risk factor for anxiety due to the nature of the difficulties created with understanding communication and communicating with others.

**Language and The Polyvagal Theory’s Social Engagement System**

When considering a theoretical framework, it seems important to consider one that will connect both of these constructs- communication/language and anxiety- with underlying physiological processes. One theoretical explanation of HRV and HR as potential biomarkers of anxiety that also incorporates communication is Porges’ *Polyvagal Theory* (Porges, 2001; 2007). Concisely, this theory suggests that different portions of the vagus nerve control different aspects of the nervous system related to social communication, approach and avoidance behaviors.
(Porges, 2007). Specifically, it states that three different physiological circuits support three different systems of behavior -- social communication, mobilization and immobilization (Porges, 2007, 2001). In this theory the mobilization system is related to the sympathetic nervous system, while the parasympathetic nervous system is divided into two branches each related to a different portion of the vagus nerve. The ventral vagal pathway connects to the eyes, ears and other regions in the face used for social communication and this system is most engaged when the environment is perceived to be safe, thus an appropriate setting to engage in social behavior, and is called the social communication system. In the context of the polyvagal theory, children with ASD are often thought of as having a compromised social communication system; therefore, there should be some indication of this deficit in the corresponding physiological system.

According to Porges, the Social Engagement System is comprised of portions of the cortex and brainstem, which innervate both somatomotor and visceromotor components via the myelinated portion of the vagus (Porges, 2001). In this model, cranial nerves control facial muscles essential for social communication such as the eyes (needed for eye contact), the middle ear muscles (needed to discern speech from background noise), and the larynx and pharynx (needed for expressive language). The myelinated vagus is also implicated as a cranial nerve that regulates HRV/HR by allowing for quick changes in HR to respond to internal and external demands (Applehans & Luecken, 2006).

As an example of support for this theory and its relationship to language in the literature, Patriquin, Lorenzi, & Scarpa (2013) found that reduced resting Respiratory Sinus Arrhythmia (RSA), was a significant predictor of the likelihood of parents reporting that their child was nonverbal. RSA is a specific kind of vagally mediated HRV which takes into account the rate of respiration. This makes RSA a good index of parasympathetic regulation of heart rate. As such,
it is possible that language difficulties would be related to anomalies in HRV as an index of the integrity of the social engagement system.

Children with anxiety may show physiological markers in the systems of mobilization (the fight or flight response) and immobilization (freezing in response to a threat), which according to polyvagal theory exist to regulate responses to threat (Scarpa, 2015).

**HRV and HR as Potential Biomarkers for Anxiety in ASD**

Autonomic features such as HRV and HR have been hypothesized to be biomarkers for the presence of anxiety or even of the elevated levels of anxiety present in anxiety disorders (Chalmers, Quintana, Abbott & Kemp, 2014; Pittig, Arch, Lam & Craske, 2013; Yeragani et al., 1993). It has been shown that compared to controls, people with anxiety disorders have significantly lower baseline HRV (Chalmers, Quintana, Abbott, & Kemp, 2014; Penninx, van Dyck, de Geus, Eco, & Licht, 2009; Pittig, Arch, Lam, & Craske, 2013). Increased HR is also indicative of many different heightened emotional states, one of which is anxiety (Watkins, Grossman, Krishnan, & Sherwood, 1998). However, it has also been shown that there may be some HR anomalies present as a function of ASD. For example, some studies find that those with ASD have a higher baseline HR than typical populations (Daluwatte et al., 2013; Kushki, Brian, Dupuis, & Anagnostou, 2014; Ming, Julu, Brimacombe, Connor, & Daniels, 2005).

Increased HR is associated both with increased sympathetic and decreased parasympathetic nervous system activity, while HRV is partially vagally-mediated and is sometimes interpreted to reflect overall cardiac vagal activity (Penninx, van Dyck, de Geus & Licht 2009). More specifically, Respiratory Sinus Arrhythmia (RSA) refers to HRV that occurs as a function of respiration and is mediated by the vagus nerve, which is under parasympathetic influence. Many studies that examine the relationship of HRV to anxiety and ASD measure HRV
with RSA. (Beauchaine, 2015; Benevides & Lane, 2015). Moreover, most studies use resting RSA due to the increased influence of the vagus nerve during resting state (Benevides & Lane, 2015).

In contrast to some studies that have examined cardiac variables solely in relation to anxiety, Beauchaine (2015) noted relationships to anxiety and ASD as well as several other disorders, and refers to RSA as a transdiagnostic marker of emotional dysregulation. The general finding of reduced baseline HRV also has been shown across several different anxiety disorders including Panic Disorder, Generalized Anxiety Disorder, and Social Phobia (Friedman 2007; Pittig, Arch, Lam & Craske 2013).

This body of research suggests that baseline HRV and HR may be promising biomarkers for anxiety in ASD, and some studies have even gone so far as to claim that results are demonstrative of this promise. For example, one study stated that reduced baseline HRV may be a promising biomarker for the many difficulties that may underlie problems with social approach behaviors, such as impaired stress regulation (Alvares et al., 2013). Another study referred to HRV as “transdiagnostic,” but characterized it as a transdiagnostic biomarker of “worry” specifically (Chalmers, Quintana, Abbott, & Kemp, 2014). In order to make this claim more powerful, however, it seems important to not only examine the literature surrounding anxiety and autonomic biomarkers, but to also look at the literature related to other characteristics of ASD and autonomic activity. For example, some studies have shown that HRV is not specific to anxiety (Neuhaus, Bernier, & Beauchaine, 2014; Patriquin, Lorenzi, Scarpa, Calkins, & Bell 2015), and that HRV anomalies, like HR anomalies, may be present as a feature of ASD itself (Daluwatte, 2013). Additionally, HRV and HR face challenges in terms of a paucity of knowledge with respect to its interactions with language ability. Few studies have been
conducted to specifically test whether or not HRV and HR may discriminate between anxiety and other maladaptive states and behaviors.

**Goals of the Current Study**

In order to consider the use of HRV/HR as biomarkers of anxiety in MV-ASD it would be helpful to have a better understanding of the relationship between baseline HRV/HR, anxiety and language. Significant relationships between any of these variables will likely impact the interpretation of HRV/HR anomalies in populations with language difficulties who are being assessed for anxiety. It would also be helpful to better understand whether baseline HRV/HR can discriminate between different maladaptive states and symptoms or whether they are better suited as a transdiagnostic measure and may need to be combined with other measures as part of an assessment protocol. While many studies have shown decreased HRV and increased HR are related to increased anxiety it has been suggested that these are reflective of broad autonomic dysfunction, and this would make them of little use as an independent measure for specific symptomology. If HRV/HR are to be used as a biomarker for anxiety in ASD, it seems important to understand within an ASD sample if HRV and HR will show any discriminative relationships between elevated anxiety and elevations of other maladaptive behaviors such as reactive anger or social withdrawal. Specifically, the scale that was be compared to anxiety in this study was “disruptive/anti-social,” which includes irritability and reactive aggressive behaviors, and served as a proxy measure for anger.

**Hypotheses**

While some studies suggest that HRV/HR anomalies are broad markers of emotional difficulties or transdiagnostic (Neuhaus, Bernier, & Beauchaine, 2014), others suggest that they may be able to mark specific elevations in the domain of anxiety (Alvares, 2014; Chalmers,
Quintana, Abbott, & Kemp, 2014). This leaves uncertainty in the research as to the nature of these measures and how they may best fit in to the future of assessment.

In this study we hypothesized that, if the existing research of HR/HRV as a marker of anxiety is supported, there would be differences between HRV/HR as a predictor of anxiety vs. anger. Additionally, language ability would play a moderating role in anxiety, but not in anger.

In this study it was hypothesized that:

I. Decreased baseline HRV would be predictive of increased anxiety (i.e., significant main effect of HRV on anxiety) but this relationship would not exist for anger; and

II. The strength of this relationship between HRV and anxiety would increase as receptive language decreased (i.e., significant moderating effect of receptive language on the relationship between HRV and anxiety, but not for anger).

III. Increased baseline HR would be predictive of increased anxiety (i.e., significant main effect of HR on anxiety), but this relationship would not exist for anger; and

IV. The strength of this relationship between HR and anxiety would increase as receptive language decreased (i.e., significant moderating effect of receptive language on the relationship between HR and anxiety, but not for anger).

Methods

Power Analyses

Moderation was tested by using multiple regression analyses with three predictor variables, based on the methods of Cohen, Cohen, West, and Aiken (2003). Power analyses were conducted to determine minimum sample size needed to detect small (Cohens $f^2 = .02$, medium
(Cohens $f^2 = .15$), or large (Cohens $f^2 = .35$) effects, with power = .8 and $p = .05$. A sample size of $n = 311$ was required to detect a small effect, $n = 43$ to detect a medium effect, and $n = 20$ to detect a large effect. The current sample size was sufficient for detecting a large effect. Due to the small sample size, effect size ($R^2$ value) will be noted throughout where $R^2 > .02$ = small effect, $R^2 > .15$ = medium effect, $R^2 > .35$ = large effect.

**Participants**

The archival data reported here are derived from a larger protocol designed to assess participant’s autonomic responses to different auditory stimuli (music vs. an audiobook) (Patriquin, Scarpa, Friedman, Porges, 2013). The laboratory sessions described below were conducted at a university clinic in Southwest Virginia. A phone screen was conducted with parents prior to the session to determine eligibility. In order to participate it was required that children be able to tolerate the sensation of the LifeShirt® on their skin for the 38 minutes of physiological data collection. Additionally, it was required that children’s abdomen’s measure at least 20 inches around and that their chest measure at least 21 inches around in order to ensure a proper fit of the LifeShirt®.

Participants consisted of 23 children ($n$ males = 18, $n$ females = 5). Children were ages 4 years 3 months to 7 years 9 months and had a prior DSM-IV diagnosis of an ASD (i.e., Autism, $n = 12$; Asperger’s Disorder, $n = 10$; PDD-NOS, $n = 1$) and no severe tactile hypersensitivities (because of the use of adhesive electrodes on the skin). Participants were sampled from the Southwest Virginia area and were recruited through the Virginia Tech Autism Clinic, electronic autism listservs, emails sent out to local autism organizations, and parent support groups. Each received $20 compensation for completing the experimental session, although some participants did not receive compensation as funding was received after their participation.

**Measures**
The following measures were used to obtain demographic information and assess for sensory issues, communication and language skills, fear, anxiety, and autonomic responses. All measures were from a larger existing data set that includes other measures not described here.

Demographic Survey (Appendix A). This demographic survey obtained information about the parent (e.g., education level, relation to the child, income), the child (e.g., age, race, education level), the child’s current diagnosis, and the child’s current symptoms.

Developmental Behavior Checklist, Parent Version (DBC-P; Einfeld & Tonge, 1992, 1995, 2002). The DBC is a questionnaire consisting of 96 Likert scale items. Each description of behavior was scored either 0 = “not true as far as you know,” 1 = “somewhat or sometimes true,” or 2 = “very true or often true.” The DBC was completed by parents reporting for individuals with development and/or intellectual disabilities, aged 4 - 18 years old, for problems over a six-month period. T-scores were used for each of the 6 different scales. These subscales include “anxiety” (α=0.762), “disruptive/anti-social” (α=0.905), “self-absorbed” (α=0.855), “communication disturbance” (α=0.806), and “social relating” (α=0.732). All scales showed acceptable internal consistency. This measure has shown good reliability and validity (Einfeld & Tongue,1995; 2002). In the original paper it is recommended that the cut-off for each of the subscales be determined by the either the sensitivity or specificity called for the by the treatment setting. For maximum sensitivity, a cut-off of 34 is recommended. For maximum specificity a cut-off score of 68 is recommended (Einfeld & Tongue,1995). This study utilized a cut-off score of 65, as this is one standard deviation from the mean and is often used as a clinical cut-off. About half of the children were above the clinical cut-off for anxiety (N=11) and anger (N=11) (although these were not the same 11 children).
*Peabody Picture Vocabulary Test, Third Edition* (PPVT-III; Dunn & Dunn, 1997). The PPVT-III measures receptive language skills (understanding and comprehension of spoken words) in a format that is conducive to measuring language skills in children with ASD. The examiner orally presented a stimulus word with a set of pictures, and the participant selected the picture that best represented the words’ meaning. The PPVT-III is significantly correlated to the Vocabulary Comprehension Index on the *Wechsler Intelligence Scale for Children*, Third Edition, $r = 0.75$, $p < .01$ (Tannenbaum, Torgesen, & Wager, 2006). The PPVT-III score was calculated by subtracting the number of errors the child makes from a total ceiling score. The raw score was converted into a standard score. The standard score ($M = 100$, $SD = 15$), which is a measurement of relative standing among chronologically aged peers. The PPVT has been used in experimental studies to assess receptive vocabulary in children with autism (e.g., Badawi, 2006) and has demonstrated good psychometric validation (Williams & Wang, 1997). The scores obtained from the PPVT and used in these analyses are standard scores.

*LifeShirt!* (Vivometrics). The *LifeShirt!* is an ambulatory physiological monitoring system. In the current study, the *LifeShirt!* was used to measure autonomic functioning (i.e., HR, HRV, and respiration). The study required that the participants must be without severe tactile hypersensitivities since adhesive electrodes were used to monitor cardiac functioning. There are many benefits of using *LifeShirt!* over typical laboratory physiology measurement systems. Specifically, the *LifeShirt!* is worn like a vest or shirt and hides electrodes, electrode wires, and respiration straps. Especially for children with ASD, this system of measurement was a more familiar and comfortable way to collect data. Additionally, the *LifeShirt!* provides a strong ability to measure ambulatory autonomic response (Heilman & Porges, 2007). This allowed the child to move freely during the semi-structured play and listening periods of the study. Heilman
and Porges (2007) compared the LifeShirt® to the Biopac MP35 (Biopac Systems, Goleta, CA, USA), a frequently used physiological monitoring system; they found that the LifeShirt! is both accurate in the detection of R-waves and in the timing of R-R intervals (Heilman & Porges, 2007). For the purposes of this study, HR was measured in beats per minute (bpm) and HRV was measured using baseline Respiratory Sinus Arrhythmia (RSA). As stated in the introduction, RSA is a measure of HRV that takes respiration into account and there are many metrics for quantifying RSA. While RSA is a good index of parasympathetic regulation of heart rate, it should be noted that this does not necessarily generalize to all parasympathetically mediated functions. Data were analyzed using the CardioBatch software (Brain-Body Center, University of Illinois at Chicago). CardioBatch is a program that utilizes algorithms, specifically the Porges-Bohrer method, to extract the heart rate variance within the frequencies of spontaneous breathing to determine the amplitude of RSA (Porges, 1985; Porges & Bohrer, 1990). For more details of the calculation of RSA refer to Patriquin, Lorenzi, & Scarpa, 2013 and Patriquin, Scarpa, Friedman & Porges, 2013. Several past studies have utilized Porges method which filters out slower vacillations and leaves only the fast frequencies that are considered to be vagal (Applehans & Luecken, 2006).

Procedure

Upon arrival at the clinic parents were given an informed consent form. The study and consent details were explained to the parent and consent to participate was obtained. Child assent was obtained from the child when possible in regard to the child’s verbal abilities. After consent the parent was given the above stated questionnaires to complete while their child completed the audio task. The parent was able to watch their child from behind a mirror.
Before completing the audio task, the LifeShirt® was carefully put on the child. The child then watched a 3-minute baseline video. This video consisted of a 3-minute section of National Geographic’s Animal Holiday, a nature video intended for children 4 - 10 years old. After completing this baseline task, the child completed an audio task, in which the child was randomly assigned to listening to either self-selected songs on an Apple iPod or to an excerpt of a self-selected audiobook on an Apple iPod. During the duration of the audio task, the experimenter remained in the room and the child colored quietly at a table. After the audio task children completed a recovery task consisting of 3 minutes of watching National Geographic’s Animal Holiday. After this, the LifeShirt® was removed and any questions the parent or child had were answered. The total session time was approximately 90 minutes.

Results

Descriptive Statistics. Descriptive statistics were conducted to examine each of the relevant variables (see Table 1). The results of these analyses indicate that the mean for this sample on the DBC anxiety subscale (n=65.91, SD=26) was above the determined clinical cut-off demonstrating that overall this sample has significant parent-reported anxiety. Additionally, the PPVT scores (n=88.36, SD=27.37) indicate that overall this sample had language in the average range, although there was some variability. This suggests that in terms of the distribution of language scores this sample was well suited to this research question.

Correlations. Bivariate correlations were conducted between all demographic variables and variables of interest (see Table 2). There was a significant correlation between age and PPVT score (r=.51, p<.05), as well as between age and baseline HR (r=-.46, p<.05). For this reason, age was included as a covariate in the regression analyses. Additionally, there was a significant
correlation between baseline HR and PPVT score ($r=-.49$, $p<.05$). Finally, the DBC subscale scores for anxiety and anger were significantly correlated ($r=.50$, $p<.05$).

**Hypothesis testing.** Resting RSA and HR data were collected over a 3-minute baseline period and averaged over the three minutes. Prior to hypothesis testing, all variables of interest were centered to reduce collinearity.

**Anxiety and HRV** (Table 3): A four-step hierarchical multiple regression was conducted with baseline RSA and PPVT score as the independent variables and DBC anxiety subscale score as the dependent variable. Child age in years was entered at step 1 of the regression to control for age, baseline RSA was entered at step two, PPVT score at step three, and their interaction term at step four. The overall model was not significant, and the effect was small ($F= 1.02$, $p=.61$, $R^2=.14$).

At step one of the hierarchical multiple regression, child age did not contribute significantly to the regression model, $F (1,21) = 2.11$, $p=.16$, $R^2=.10$, and accounted for 9.5% of the variation in anxiety score. Introducing baseline RSA explained no additional variation in anxiety score, $F (2,21) = 1.01$, $p=.38$, $R^2=.10$. Adding PPVT score to the regression model explained no additional variation in anxiety, $F (3,21) = .63$, $p =.60$, $R^2=.10$. Finally, the addition of interaction terms to the regression model explained an additional 4.3% of the variation in anxiety; however, this change in $R^2$ was not significant, $F (5,21) = .68$, $p = 0.61$, $R^2=.14$.

Although the results of this analysis were not significant, the direction of the interaction was examined as an exercise to inform future research questions because the interaction effect size was close to medium. This analysis was conducted by dividing the sample in half, creating a high and low language group, and examining the slopes that resulted from graphing the two sets of data points. For the high-language group, the slope of the regression line was negative suggesting
that resting HRV decreased as anxiety increased. However, for the low-language group the slope of the regression line was positive suggesting that for these children resting HRV actually increased as anxiety increased (Figure 1).

**Anger and HRV (Table 4):** Similarly, a four-step hierarchical multiple regression was conducted with baseline RSA and PPVT score as the independent variables and DBC disruptive subscale score as the dependent variable. Child age in years was entered at step 1 of the regression to control for age, baseline RSA was entered at stage two, PPVT score at step three, and their interaction term at step four. The overall model was not significant, and the effect was medium \( (F= .93, \ p=.47, \ R^2=.18) \).

Results indicated that child age did not contribute significantly to the regression model, \( F (1,21) = 1.82, \ p=.19, \ R^2=.08 \), and accounted for 8.4% of the variation in anger score. Introducing the baseline RSA explained an additional 1% of variation in anger score and this change in \( R^2 \) was not significant, \( F (2,21) = 0.92, \ p=.41, \ R^2=.09 \). Adding PPVT score to the regression model explained an additional 6.2% of the variation in anger and this change in \( R^2 \) was also not significant, \( F (3,21) = 1.08, \ p =.38, \ R^2 = .15 \). Finally, the addition of interaction terms to the regression model explained an additional 3.2% of the variation in anger, and this change in \( R^2 \) square was not significant, \( F (4,21) = .93, \ p= 0.46, \ R^2 =.18 \).

Although the results of this analysis were not significant, the direction of the interaction was examined as an exercise to inform future research questions because the effect size of the interaction was medium. This analysis was conducted by dividing the sample in half, creating a high and low language group, and examining the slopes that resulted from graphing the two sets of data points. For the high language group, the slope of the regression line was positive, suggesting that for children with more language, resting HRV increased as anger increased. For
the low-language group the slope of the regression line was negative, suggesting that for children with less language, resting HRV decreased as anger increased (Figure 2).

**Anxiety and HR** (Table 5): The same analyses were repeated using HR instead of HRV. A four-step hierarchical multiple regression was conducted with baseline HR and PPVT score as the independent variables and DBC anxiety subscale score as the dependent variable. Child age in years was entered at step 1 of the regression to control for age, baseline HR was entered at step two, PPVT score at step three, and their interaction term at step four. The overall model was not significant, and the effect was small (F= .69, p=.65, R^2=.13).

The hierarchical multiple regression determined that at step one, child age did not contribute significantly to the regression model, F (1,21) = 2.11, p=.16, R^2=.10, and accounted for 10% of the variation in anxiety score. Introducing the baseline HR explained no additional variation in anxiety score, F (2,21) = 1.01, p=.38, R^2=.10. Adding PPVT score to the regression model explained an additional variation in anxiety F (3,21) =.64, p=.60, R^2=.10. Finally, the addition of interaction term to the regression model explained an additional 3% of the variation in anxiety and this change in R^2 square was not significant, F (4,21) = .63, p=.64, R^2=.13.

**Anger and HR** (Table 6): Similarly, a four-step hierarchical multiple regression was conducted with baseline RSA and PPVT score as the independent variables and DBC disruptive subscale score as the dependent variable. Child age in years was entered at step 1 of the regression to control for age, baseline HR was entered at step two, PPVT score at step three, and their interaction term at step four. The overall model was not significant, and the effect was medium (F= .76, p=.42 R^2=.19).

The hierarchical multiple regression determined that at step one, child age did not contribute significantly to the regression model, F (1,21) = 1.82, p=.19, R^2=.08, and accounted for 8% of
the variation in disruptive scale score. Introducing baseline HR explained no additional variation in anger score, $F(2,21) = .87, p=.43, R^2=.08$. Adding PPVT score to the regression model explained an additional 7.2% of the variation in anger and this change in $R^2$ was also not significant, $F(3,21) =, p=.37, R^2=.16$. Finally, the addition of interaction terms to the regression model explained an additional 4% of the variation in anger and this change in $R^2$ square was not significant, $F(4,21) = 1.02, p=.42, R^2=.19$.

Although the results of this analysis were not significant, because the effect size was medium the direction of the interaction was examined as an exercise to inform future research questions. This analysis was conducted by dividing the sample in half, creating a high and low language group, and examining the slopes that resulted from graphing the two sets of data points. For the high-language group the slope of the regression line was negative, suggesting that resting HR increased as anger decreased. For the children with low-language, the slope of the regression line was positive suggesting that as anger increased resting HR also increased (Figure 3).

**Discussion**

ASD is a developmental disorder which, besides several core deficits, is associated with struggles and challenges across many different areas of functioning. One such area of functioning is that of emotion regulation, specifically anxiety. Several studies have suggested that people with ASD have more struggles with regulating their emotions than do typically developing individuals (Davis et al., 2011; DeLussey, Herrington, Bradstreet, Guy & Souders, 2014; Garfinkel, Tiley, O'Keeffe, Harrison, Seth, & Critchley, 2016; Laurent & Rubin, 2004; Mazefsky et al., 2013). Increased anxiety in this population is one such manifestation of this difficulty. However, there is conflicting evidence as to the relationship of language ability and anxiety in ASD (Kerns et al., 2014). This study sought to examine this relationship with the use
of physiological data that may facilitate the identification of potential biomarkers for anxiety. In our study, HRV and HR did not significantly predict anxiety or anger. Further, receptive language did not significantly moderate the relationship between anxiety and HRV, anxiety and HR, anger and HRV or anger and HR.

There could be several explanations for the lack of significant findings in this study, despite previous research suggesting the hypothesized relationships. First, the low power of this study was a major limitation of the analyses and likely effected the findings. It is worth noting that while none of the results of the analyses were statistically significant, results in the four models ranged from small to medium effect sizes. As the power in this study was only sufficient to detect a large effect, these results suggest that some of the moderate effects in our sample may not have been detectable with this level of power.

Besides the limitation of low power there is significant research to support that children with ASD may experience more anxiety than their same age peers (White et al., 2013), this is consistent with the mean of this sample, which is in the clinical range. However, the role of language deficits complicates this picture. This study’s hypothesis was predicated on the notion that lack of expressive and receptive language is a risk factor for anxiety. However, if this is not the case, and instead if the mixed findings in the literature are solely a result of reporting difficulty or inconsistency in children with expressive and receptive language deficits, then the reporting biases could explain the non-significance of these findings. In other words, if parents simply cannot recognize or report emotions in their children, then it would not be possible to detect differences between parent-reported anxiety.

Analyses performed with anger as the predictor had slightly larger effect sizes; however, overall, there did not seem to be any major statistical differences when the analyses were
conducted with anger as the outcome variable instead of anxiety. There are several possible explanations for this lack of distinction between anger and anxiety. Similar to above, an explanation may be parents may have had difficulty reporting on anxiety vs. anger and reported many of the same behaviors in both categories, for example a “meltdown” could be attributed to anger or anxiety by a third-party observer. Although this sample size was too small to conduct an exploratory factor analysis, some literature that suggests that the anger and anxiety constructs may not be separate in a population this young (Achenbach, 2009).

Although the findings were not significant, medium effect sizes were probed as an exercise to inform future research questions. In the first analyses examining the relationship between anxiety and resting HRV, the results were as expected for the children with higher receptive language, with resting HRV decreasing as anxiety increased. However, the children with lower-receptive language had the opposite of the expected effect, with resting HRV increasing as anxiety increased.

In the analysis examining the effect of anger on resting HRV, in the high language group the slope of the regression line was positive, suggesting that resting HRV increased as anger increased. This is contrary to what existing research would suggest. For the low-language group the slope of the regression line was negative, suggesting that for children with less language resting HRV decreased as anger increased. Had this result been significant, it would be supported by the research that implicates HRV as a transdiagnostic biomarker, or at least of the research suggesting that HRV is not solely related anxiety.

Finally, in the analysis examining the effect of anger on resting HR, for the high-language group the slope of the regression line was negative, suggesting that resting HR increases as anger decreases. For the children with low-language, the slope of the regression line was positive
suggesting that as anger increased resting HR also increased. Of these two results, only the second, if significant, would be in line with what would be suggested by the research on HR as a transdiagnostic biomarker.

Overall, had these effect sizes been significant, their interpretation would be variable. It is important to note, that of all analyses run the ones in which anger predicted HRV/HR had the largest effect sizes, which seems contradictory to the idea that HRV/HR are best conceptualized as a biomarker of anxiety. With this in mind, future research utilizing larger sample sizes may wish to concentrate their efforts on HRV/HR as a marker of anger or, consistent with the suggestion of Beauchaine (2015), HRV/HR as a transdiagnostic marker of distress.

Although the patterns in this paper were not replicated with anxiety, there is still a large body of research suggesting the hypothesized relationships with anxiety. Consequently, there may be two important take aways from this. One may be that, in light of existing evidence in the literature, future researchers may wish to continue researching the relationship of HRV and HR to anxiety but be mindful that the relationship of these variables to anger should not be ignored in these research projects. The second is that it is notable that if the findings of this paper would have been significant, then at least in this sample, HRV/HR are related to anger expressions more so than anxiety expressions in children with low language. This notion might also align with the idea that angry behavior is easier for parents to see and identify.

Had these interactions been significant there would also have been interesting implications for the role of language in this model. All of the slopes examined had opposite directions, which, had the results been significant, would have suggested a potential moderating role of language in the relationship of anxiety and anger to HRV/HR. It is also noticeable that in this sample there was a significant and large, though insignificant, correlation between HRV and PPVT score and
HR and PPVT score, respectively. This finding has already been examined and discussed further in Patriquin, Lorenzi, & Scarpa (2013) and Patriquin, Scarpa, Friedman & Porges (2013), however, it does lend support to the connection between language and HR/HRV and suggests that more studies should consider examining this relationship. Additionally, this finding suggests the issue of clinical vs. statistical significance. Despite the large correlation between HR and receptive language, the finding was not statistically significant. This may be important to keep in mind for interpretation of future results in studies of this sample size as large correlations, or effect sizes, may have some clinical significance even when not shown statistically. Future research utilizing a larger sample size may wish to examine this relationship again or potentially new models to explain the role of language. For example, it may be interesting to utilize a curvilinear regression to see if there is a non-linear relationship between these three variables. This may be especially important as connection between language and HR/HRV could lend to support and contribute to the evidence base surrounding the polyvagal theory (Porges, 2007).

**Limitations**

The chief limitation of this study is the small sample size which was only sufficient to detect a large effect. For this reason, the smaller effects indicated in our sample may have been undetectable. Additionally, the study would have benefitted from a measure of expressive as well as receptive language.

**Future Directions**

These results suggest several possible directions for future research. Although the results of this study were not significant there is still a large body of research suggesting the hypothesized relationships. Given the limitations of this data set, more research should be dedicated to this topic in the future that addresses some of the limitations of this study.
Additionally, future research may wish to take different approaches to furthering our understanding of what anxiety looks like in MV-ASD. While this study was not successful in clarifying the role of language in the relationship between anxiety and ASD, it is still an important and relevant question and there are many novel approaches to studying this question that were not explored in this study. Additionally, in this sample only about half of the children were above the clinical cut-off for anxiety (N=11) and anger (N=11) (although these were not the same 11 children). Future studies may wish to seek to recruit at the outset a sample of children with clinically significant anxiety or anger difficulties and examine how results in these populations may look different than in this sample.
References


Table 1
Descriptive Statistics

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<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
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<td>124.3</td>
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<td>Receptive Language</td>
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<td>88.36</td>
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Table 2
Correlations Between Descriptive Variables and Target Variables

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Note: *\(p < .05\), **\(p < .01\), ***\(p < .001\).
\(1=\) Peabody Picture Vocabulary Test; \(2=\) Respiratory Sinus Arythmia (ln(ms)2); \(3=\) Developmental Behavior Checklist
Table 3  
*Regression of Anxiety on HRV*

<table>
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*Note. * $p < .05$, ** $p < .01$, *** $p < .001$.  
$R^2>.02$=small effect, $R^2>.15$=medium effect, $R^2>.35$=large effect 
$1=$ Respiratory Sinus Ahythmia (ln(ms)$^2$); $2=$ Peabody Picture Vocabulary Test*
Table 4
Regression of Anger on HRV

<table>
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<th>R²</th>
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<td>RSA¹</td>
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F-value .93

Note. * p < .05, ** p < .01, *** p < .001.
R²>.02=small effect, R²>.15=medium effect, R²>.35=large effect
1= Respiratory Sinus Arythmia (ln(ms)²); 2= Peabody Picture Vocabulary Test

Table 5
Regression of HR on Anxiety

<table>
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F-value .63

Note. * p < .05, ** p < .01, *** p < .001.
R²>.02=small effect, R²>.15=medium effect, R²>.35=large effect
1= Heart Rate (bpm); 2= Peabody Picture Vocabulary Test
Table 6  
Regression of HR on Anger

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*Note.  * $p < .05$, ** $p < .01$, *** $p < .001$.
$R^2$>.02=small effect, $R^2$>.15=medium effect, $R^2$>.35=large effect
1= Heart Rate (bpm) ; 2= Peabody Picture Vocabulary Test
Figure 1: Anxiety and HRV Interaction

Group
- low
- high

Low: $R^2$ Linear = 0.109
High: $R^2$ Linear = 0.006

Equations:
- Low: $y = 46.29 + 0.23x$
- High: $y = 74.63 - 0.03x$
Figure 2: Anger and HRV Interaction

Group
- Low
- High

Equations:
- Low: \( y = 72.45 + 0.05x \)
- High: \( y = 59.42 - 0.11x \)

\( R^2 \) for Linear:
- Low: 0.034
- High: 0.024
Figure 3: Anger and HR Interaction

Group
- low
- high
- low
- high

low: R² Linear = 0.019
high: R² Linear = 0.002

y = 84.98 - 0.08x
y = 25.84 + 0.25x
Appendix A

Parental Information Form for:

The Effect of Music on Autonomic Response in Children with Autism Spectrum Disorders – Phone Interview

I. Purpose of this Research Project
The purpose of this study is to research the physiological effects of music on children with Autism Spectrum Disorders (ASDs). Since ASDs are characterized primarily by a lack of social understanding and social development, we hope to find one potential explanatory mechanism (i.e., autonomic response—heart rate and heart rate variability) of these deficits. Research has suggested that children with ASDs have elevated physiological state to unfamiliar people and therefore we are using music or a book-on-tape to soothe an elevated physiological state. Through this soothed state, social engagement behaviors will hopefully increase. For this study we are collecting data from twenty-four children aged 4-7 years old, all previously diagnosed with ASDs.

II. Procedures
During the phone interview, the experimenter will confirm your child’s diagnosis, size of their abdomen, and administered a Short Sensory Profile.

III. Risks
There are no more than minimal risks related to the phone interview.

IV. Benefits
There are no direct or indirect benefits to you or your child to participate in the phone interview.

V. Extent of Anonymity and Confidentiality
All data related to you and your child will be de-identified with an identification number, including all phone interview data. All consent forms will be stored in a locked cabinet separate from all other data. In addition, during the laboratory session, the researchers will videotape my child doing the play tasks. The video of your child and all other data (e.g., Short Sensory Profile from the phone interview) will be labeled with his/her participant identification number and will be placed in a locked cabinet separate from the consent form so the data can not be identified.

VI. Compensation
You will not receive compensation for my participation in the phone interview.

IX. Parent/Guardian’s Permission
You were explained confidentiality procedures of this study for the phone interview data and you verbally consented to participate in the phone interview and administration of the Short Sensory Profile

Should you have any questions about the protection of human research participants regarding this study, you may contact:

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Graduate Student

Angela Scarpa, Ph.D.
Associate Professor
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ascarpa@vt.edu

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540-998-3414
mpatriq@vt.edu

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Office of Research Compliance
2000 Kraft Drive Suite 2000 (0497)
Blacksburg, VA 24060
540-231-4991  moored@vt.edu
### Classification by Part of Speech for the Peabody Picture Vocabulary Test, Fourth Edition (PPVT-IV), Form A

**Directions:** Circle the item numbers of the noun and adjective PPVT-IV items administered. For incorrect responses, draw a line through the open box in the right of the item number. Specify the correct category chosen. Then, write the totals by category for *F*Taken and *F*Incorrect in the summary box to the right.

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### Comparison of PPVT-III and PPVT-IV Scores Over Time

The GSV (growth scale valued score) is designed for measuring change over time. Like a raw score, the GSV score is an indication of absolute, not relative, performance. If an examinee's vocabulary increases, his or her GSV score will increase. The GSV scale is like a yardstick, and placing GSV scores over time can reveal how the examinee's vocabulary performance has changed.

The standard score serves a different purpose, which is to tell how the examinee's score compares with the average score for a particular age. It is a helpful supplement to the GSV when examining change. If an examinee's standard score on the same or both occasions is less than the examinee's vocabulary performance has increased in the average one or two years. If the standard score declines from the first testing to the next, the examinee may have improved in vocabulary knowledge (as shown by higher GSV), but the rate of growth was below average.

Refer to Appendix G (Use for PPVT-IV Manual) for further information on interpreting change as GSV scores.

### Using GSV Scores to Measure Change

The GSV provides a meaningful way to characterize change in performance over time. Complete the following steps:

1. Write a sequence number next to the GSV score of each PPVT-III and PPVT-IV administration you created in this page, starting with 1 for the earliest administration.
2. Mark the points on the GSV standard corresponding to each GSV score.
3. Note the corresponding administration sequence number next to each mark on the GSV standard.

---

**Pearson**

PsychCorp

Psychological Corporation

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Chicago, IL 60610

www.PearsonAssessments.com

www.psychcorp.com

www.pearsonpsychometrics.com

Worldwide: 01296 623500

North America: 1 800 227 9777

Technical Support: 1 866 614 0520

Customer Service: 1 800 942 4242

(Reference: 10371)

PPVT is an abbreviation of the Peabody Picture Vocabulary Test. PVVT is a registered trademark of the publisher, Pearson. The PPVT-III, 4th Edition is a trademark of the publisher, Pearson. PFVT-IV is a trademark of the publisher, Pearson. The GSV is a trademark of the publisher, Pearson.
Appendix C

DEVELOPMENTAL BEHAVIOUR CHECKLIST
(DBC-P)

Some children with developmental delay have problems with their emotions and behaviour. These can sometimes be a problem for their carers.

By completing this checklist, you will help us learn more about these problems. This will assist us to know how the person might respond to help.

Subject #: ________________

Date Completed: ____________

Is the Child: (please circle) Unable to see/unable to hear
Unable to use arms/legs
Unable to speak/speaks very little
Subject to other serious medical condition

Please describe:

What does he/she do best?

What do other people like about him/her?

What are his/her favourite activities?

Is there anything you feel he/she does as well or better than others?

Have you sought help for any behaviour or emotional problems, apart from slow development, of the child or teenager in your care? Yes/No

If so, from whom?

Office Use Only

Developmental Level (circle one only)

<table>
<thead>
<tr>
<th>Profound</th>
<th>Severe</th>
<th>Modest</th>
<th>Mild</th>
<th>Unknown</th>
<th>Contact Person:</th>
</tr>
</thead>
</table>

| Page 2 | | | | |
| Page 3 | | | | |
| Page 4 | | | | |
| Total | | | | |

Please continue over the page →

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Instructions ©1981 T.M. Achenbach. modified, with permission

Revised subscales 2002
Many of the following behaviours may not apply to the child or teenager in your care. For each item that does describe the person in your care, now or within the past six months, please circle the 1 if the item is very true or often true. Circle 1 if the item is somewhat or sometimes true of your child. If the item is not true of your child circle the 0.

0 = not true as far as you know 1 = somewhat or sometimes true 2 = very true or often true

If your child is unable to perform an item, circle the 0. For example, if your child has no speech, then for the item “Talks too much or too fast” circle the 0.

Underline any you are particularly concerned about

<table>
<thead>
<tr>
<th>Office Use Only</th>
<th>Please Circle</th>
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<tbody>
<tr>
<td>1. ☒ 0 1 2</td>
<td>Appears depressed, downcast or unhappy</td>
</tr>
<tr>
<td>2. ☒ 0 1 2</td>
<td>Avoids eye contact. Won’t look you straight in the eye.</td>
</tr>
<tr>
<td>3. ☐ 0 1 2</td>
<td>Aloof, in his/her own world.</td>
</tr>
<tr>
<td>4. ☒ 0 1 2</td>
<td>Abusive. Swears at others.</td>
</tr>
<tr>
<td>5. ☒ 0 1 2</td>
<td>Arranges objects or routine in a strict order.</td>
</tr>
<tr>
<td></td>
<td>Please describe:</td>
</tr>
<tr>
<td>6. ☒ 0 1 2</td>
<td>Bangs head.</td>
</tr>
<tr>
<td>7. ☒ 0 1 2</td>
<td>Becomes over-excited.</td>
</tr>
<tr>
<td>8. ☒ 0 1 2</td>
<td>Bites others.</td>
</tr>
<tr>
<td>9. ☒ 0 1 2</td>
<td>Cannot attend to one activity for any length of time, poor attention span.</td>
</tr>
<tr>
<td>10. ☐ 0 1 2</td>
<td>Chews or mouths objects, or body parts.</td>
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<td>11. ☒ 0 1 2</td>
<td>Cries easily for no reason, or over small upsets.</td>
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<tr>
<td>12. ☒ 0 1 2</td>
<td>Covers ears or is distressed when hears particular sounds.</td>
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<td>Please describe:</td>
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<tr>
<td>13. ☒ 0 1 2</td>
<td>Confuses the use of pronouns e.g. uses “you” instead of “I”.</td>
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<tr>
<td>14. ☐ 0 1 2</td>
<td>Deliberately runs away.</td>
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<tr>
<td>15. ☒ 0 1 2</td>
<td>Delusions: has a firmly held belief or idea that can’t possibly be true.</td>
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<td>Please describe:</td>
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<tr>
<td>16. ☐ 0 1 2</td>
<td>Distressed about being alone.</td>
</tr>
<tr>
<td>17. ☒ 0 1 2</td>
<td>Doesn’t show affection.</td>
</tr>
<tr>
<td>18. ☒ 0 1 2</td>
<td>Doesn’t respond to others’ feelings, e.g. shows no response if a family member is crying.</td>
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<tr>
<td>19. ☒ 0 1 2</td>
<td>Easily distracted from his/her task, e.g. by noises.</td>
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<tr>
<td>20. ☒ 0 1 2</td>
<td>Easily led by others.</td>
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<tr>
<td>21. ☐ 0 1 2</td>
<td>Eats non-food items e.g. dirt, grass, soap.</td>
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<tr>
<td>22. ☐ 0 1 2</td>
<td>Excessively distressed if separated from familiar person.</td>
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<tr>
<td>23. ☐ 0 1 2</td>
<td>Fears particular things or situations, e.g. the dark or insects.</td>
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<td>Please describe:</td>
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<td>24. ☐ 0 1 2</td>
<td>Facial twitches or grimaces.</td>
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<td>25. ☐ 0 1 2</td>
<td>Flicks, taps, twirls objects repeatedly.</td>
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<td>26. ☐ 0 1 2</td>
<td>Fussy eater or has food fads.</td>
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<tr>
<td>27. ☐ 0 1 2</td>
<td>Goes for food. Will do anything to get food e.g. takes food out of garbage bins or steals food.</td>
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<tr>
<td>28. ☐ 0 1 2</td>
<td>Gets obsessed with an idea or activity.</td>
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<td>Please describe:</td>
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<tr>
<td>29. ☐ 0 1 2</td>
<td>Grinds teeth.</td>
</tr>
<tr>
<td>30. ☐ 0 1 2</td>
<td>Has nightmares, night terrors or walks in sleep.</td>
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</tbody>
</table>

Please be sure you have answered all items

Continue next page ➔
-3-  

0 = not true as far as you know  1 = somewhat or sometimes true  2 = very true or often true  
Underline any you are particularly concerned about

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| 44. | 0 1 2 | Likes to hold or play with an unusual object, e.g. string, twigs; overly fascinated with something, e.g. water.  
Please describe: |
| 45. | 0 1 2 | Loss of appetite. |
| 46. | 0 1 2 | Masturbates or exposes self in public. |
| 47. | 0 1 2 | Mood changes rapidly for no apparent reason. |
| 48. | 0 1 2 | Moves slowly, underactive, does little, e.g. only sits and watches others. |
| 49. | 0 1 2 | Noisy or boisterous. |
| 50. | 0 1 2 | Overactive, restless, unable to sit still. |
| 51. | 0 1 2 | Oversensitive. |
| 52. | 0 1 2 | Overbreathes, vomits, has headaches or complains of being sick for no physical reason. |
| 53. | 0 1 2 | Overly attention-seeking. |
| 54. | 0 1 2 | Overly interested in looking at, listening to or dismantling mechanical things e.g. lawnmower, vacuum cleaner. |
| 55. | 0 1 2 | Poor sense of danger. |
| 56. | 0 1 2 | Prefers the company of adults or younger children. Doesn't mix with his/her own age group. |
| 57. | 0 1 2 | Prefers to do things on his/her own. Tends to be a loner. |
| 58. | 0 1 2 | Preoccupied with only one or two particular interests.  
Please describe: |
| 59. | 0 1 2 | Refuses to go to school, activity centre or workplace. |
| 60. | 0 1 2 | Repeated movements of hands, body, head or face e.g. handflapping or rocking. |
| 61. | 0 1 2 | Resists being cuddled, touched or held. |
| 62. | 0 1 2 | Repeats back what others say like an echo. |
| 63. | 0 1 2 | Repeats the same word or phrase over and over. |
| 64. | 0 1 2 | Smells, tastes, or licks objects. |
| 65. | 0 1 2 | Scratches or picks his/her skin. |
| 66. | 0 1 2 | Screams a lot. |

Please be sure you have answered all items  
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<td>67.</td>
<td>0 1 2</td>
<td>Sleeps too little. Disrupted sleep.</td>
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<td>68.</td>
<td>0 1 2</td>
<td>Stares at lights or spinning objects.</td>
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<td>69.</td>
<td>0 1 2</td>
<td>Sleeps too much.</td>
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<td>70.</td>
<td>0 1 2</td>
<td>Soil outside toilet though toilet trained. Smears or plays with faeces.</td>
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<td>71.</td>
<td>0 1 2</td>
<td>Speaks in whispers, high pitched voice, or other unusual tone or rhythm. Please describe:</td>
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<td>72.</td>
<td>0 1 2</td>
<td>Switches lights on and off, pours water over and over; or similar repetitive activity. Please describe:</td>
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<td>73.</td>
<td>0 1 2</td>
<td>Steals.</td>
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<td>74.</td>
<td>0 1 2</td>
<td>Stubborn, disobedient or unco-operative.</td>
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<td>75.</td>
<td>0 1 2</td>
<td>Shy.</td>
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<td>76.</td>
<td>0 1 2</td>
<td>Strips off clothes or throws away clothes.</td>
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<td>77.</td>
<td>0 1 2</td>
<td>Says he/she can do things that he/she is not capable of.</td>
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<td>78.</td>
<td>0 1 2</td>
<td>Stands too close to others.</td>
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<td>79.</td>
<td>0 1 2</td>
<td>Sees, hears, something which isn't there. Hallucinations. Please describe:</td>
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<td>80.</td>
<td>0 1 2</td>
<td>Talks about suicide.</td>
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<td>81.</td>
<td>0 1 2</td>
<td>Talks too much or too fast.</td>
</tr>
<tr>
<td>82.</td>
<td>0 1 2</td>
<td>Talks to self or imaginary people or objects.</td>
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<td>83.</td>
<td>0 1 2</td>
<td>Tells lies.</td>
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<td>84.</td>
<td>0 1 2</td>
<td>Thoughts are unconnected. Different ideas are jumbled together with meaning difficult to follow.</td>
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<td>85.</td>
<td>0 1 2</td>
<td>Tense, anxious, worried.</td>
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<td>Throws or breaks objects.</td>
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<td>87.</td>
<td>0 1 2</td>
<td>Tries to manipulate or provoke others.</td>
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<td>88.</td>
<td>0 1 2</td>
<td>Underreacts to pain.</td>
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<td>89.</td>
<td>0 1 2</td>
<td>Unrealistically happy or elated.</td>
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<td>90.</td>
<td>0 1 2</td>
<td>Unusual body movements, posture, or way of walking. Please describe:</td>
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<td>91.</td>
<td>0 1 2</td>
<td>Upset and distressed over small changes in routine or environment. Please describe:</td>
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<td>92.</td>
<td>0 1 2</td>
<td>Unnates outside toilet, although toilet trained.</td>
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<td>93.</td>
<td>0 1 2</td>
<td>Very busy.</td>
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<td>94.</td>
<td>0 1 2</td>
<td>Wanders aimlessly.</td>
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<tr>
<td>95.</td>
<td>0 1 2</td>
<td>Whines or complains a lot. Please write in any problems your child has that were not listed above</td>
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</table>
| 96. | 0 1 2         | Overall, do you feel your child has problems with feelings or behaviour, in addition to problems with development? If not, please circle the 0. If so, but they're minor, please circle the 1. If they're major problems, please circle the 2.  

Please be sure you have answered all items. Are there any other comments you would like to make?

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THANK YOU
Appendix D

Parent Subject Consent Form for:

The Effect of Music on Autonomic Response in Children with Autism Spectrum Disorders

I. Purpose of this Research Project
The purpose of this study is to research the physiological effects of music on children with Autism Spectrum Disorders (ASDs). ASDs are characterized primarily by a lack of social understanding and social development; we are investigating one potential explanatory mechanism (i.e., autonomic response—heart rate and heart rate variability) of these deficits. Research suggests that children with ASDs have an elevated physiological state to unfamiliar people and therefore we are using music or a book-on-tape to soothe an elevated physiological state. Through this soothed state, social engagement behaviors should increase. For this study we are collecting data from twenty-four children aged 4-7 years old, all previously diagnosed with ASDs.

II. Procedures
For this experimental session you will sign and agree to the participation of your child in the study at the Virginia Tech Autism Clinic. Then, you will be asked to fill out a measure of ASD severity for your child, fear, temperament, and anxiety scales for my child and a demographic questionnaire. After you have been explained the questionnaires, your child will be brought into the experiment room. You will be allowed to watch the experimenter and your child interact throughout the entire session behind a one-way window while you complete the questionnaires. It will take approximately 30 – 40 minutes to complete all questionnaires.

III. Risks
There are no more than minimal risks related to this study and your participation. However, electrode removal may be uncomfortable (similar to the pain of removing a band-aid) for your child. The experimenter, in removing these electrodes, will make particular caution. The experimenter will warn your child at the beginning of the study of the potential discomfort associated with the electrodes and that it may “hurt like a band-aid” when they are removed. In addition, before removal the experimenter will ask if your child would like to remove the electrodes him/herself, have his/her parent remove them, or have the experimenter remove them. Additionally, electrode gel can cause a localized allergic reaction where the electrode was placed.

IV. Benefits
There are no direct or indirect benefits to you. There are societal benefits of understanding the anxiety, fear, temperament, ASD severity correlates to your child’s social engagement and physiology. No promise or guarantees of benefits have been made to encourage you to participate.

V. Extent of Anonymity and Confidentiality
All data related to you and your child will be de-identified with an identification number. All consent forms will be stored in a locked cabinet separate from all other data. In addition, the video of your child and all other data will be labeled with his/her participant identification number and will be placed in a locked cabinet separate from the consent form. The only people with access to this cabinet will be designated research personnel. The video will be viewed by two trained undergraduate research assistants in order to code the structured play situation that your child engaged in. They will be given no identifying information about your child, only your child’s participant identification number. It is possible that the Institutional Review Board (IRB) may view this study’s collected
data for auditing purposes. The IRB is responsible for the oversight of the protection of human subjects involved in research. Data will be destroyed 2 years past publication of the results. If you or your child express any child abuse, or the intent to harm or kill yourselves or someone else, the researchers are legally obliged to inform an authority.

In this case, you will be informed of the need to do so, and am encouraged to contact with the researchers the Psychological Services Center (540-231-6914) or other counseling agency. However, any outside counseling sought by the you for yourself or your child will be at your expense, as neither the researchers, nor Virginia Tech, have money set aside for such purposes.

VI. Compensation
You will not receive compensation for my participation in this study.

VII. Freedom to Withdraw
This project has been explained to you and you have been allowed to ask questions about it. You understand that you do not have to fill out the questionnaires or participate in any way if you do not want to and no one will treat us badly. You can stop part way through or withdraw at any time, if you choose.

VIII. Parent (or Guardian) /Subject's Responsibilities
You voluntarily agree to participate in this study. You are willing to answer questions asked on the questionnaires. You understand, however, you may withdraw at any point and you do not have to answer any questions that you are not comfortable answering.

IX. Parent/Guardian’s Permission
You have read the Parent Subject Consent Form and the conditions of this project. You have had all your questions answered. You hereby acknowledge the above and give your voluntary consent for yourself to participate in this study.

Parent/Guardian Signature: ___________________________ Date: _________________

Should I have any questions about the protection of human research participants regarding this study, I may contact:

Michelle Patriquin, B.S.
Graduate Student
Department of Psychology
540-998-3414
mpatriq@vt.edu

David Moore
Chair, Virginia Tech IRB
Office of Research Compliance
2000 Kraft Drive
Suite 2000 (0497)
Blacksburg, VA 24060
540-231-4991 moored@vt.edu

Angela Scarpa, Ph.D.
Associate Professor
Department of Psychology
540-231-2615
ascarpa@vt.edu

David W. Harrison, Ph.D., Chair
Human Subjects Committee
Psychology Department
Virginia Tech
540-231-4422
dwh@vt.edu
Appendix E

Parental Permission for child to participate in:

The Effect of Music on Autonomic Response in Children with Autism Spectrum Disorders

I. Purpose of this Research Project

The purpose of this study is to research the physiological effects of music on children with Autism Spectrum Disorders (ASDs). ASDs are characterized primarily by a lack of social understanding and social development; we are investigating one potential explanatory mechanism (i.e., autonomic response—heart rate and heart rate variability) of these deficits. Research suggests that children with ASDs have an elevated physiological state to unfamiliar people and therefore we are using music or a book-on-tape to soothe an elevated physiological state. Through this soothed state, social engagement behaviors should increase. For this study we are collecting data from twenty-four children aged 4-7 years old, all previously diagnosed with ASDs.

II. Procedures

For this experimental session your child will be video-taped throughout the entire experimental session, except for adhesion and removal of the LifeShirt®. This experimental session takes place at the Virginia Tech Autism Clinic and will last approximately 90-minutes. The procedures of the experimental session are as follows:

The following are the procedures for the study, for your child. He/she will be:

- Verbally assented.
- Brought into the experiment room
- Given a measure of receptive vocabulary for 10-15 minutes.
- Assisted by the experimenter in putting on the LifeShirt®. The LifeShirt® is a physiological monitor that fits like a vest and measures heart rate and respiration. The electrodes used with the LifeShirt® have a mild adhesive designed particularly for younger children.
- Instructed to watch at 5-minute nature video created for children 4-10 years old entitled, National Geographic’s Animal Holiday.
- Participating in a 10-minute structured play session with the experimenter.
- Selecting 5 songs or 1 book-on-tape that they would like to listen to during a listening period of 12-minutes.
- Listening to the music while they do whatever they would like in the experiment room (e.g., play with toys, do a puzzle, sit).
- Participating in another 10-minute structured play session with the experimenter.
- Watching another portion of National Geographic’s Animal Holiday.
- Helped by the experimenter in removing the LifeShirt® and electrodes.

III. Risks

There are no more than minimal risks related to this study. However, electrode removal may be uncomfortable (similar to the pain of removing a band-aid). The experimenter, in removing these electrodes, will make particular caution. The experimenter will warn your child at the beginning of the study of the potential discomfort associated with the electrodes and that it may “hurt like a band-aid” when they are removed. In addition, before removal the experimenter will ask if your child would like to remove the electrodes him/herself, have his/her parent remove them, or have the experimenter remove them. Additionally, electrode gel can cause a localized allergic reaction where the electrode was placed. All other aspects of the study should be familiar to your child and will hopefully be enjoyable.
IV. Benefits
There are no direct or indirect benefits to your child or you. There are societal benefits of understanding the physiological mechanisms that may be associated with social deficits in children with ASDs. No promise or guarantees of benefits have been made to encourage your child or you to participate.

V. Extent of Anonymity and Confidentiality
All data related to you and your child will be de-identified with an identification number. All consent forms will be stored in a locked cabinet separate from all other data. In addition, the video of your child and all other data will be labeled with his/her participant identification number and will be placed in a locked cabinet separate from the consent form. The only people with access to this cabinet will be designated research personnel. The video will be viewed by two trained undergraduate research assistants in order to code the structured play situation that my child engaged in. They will be given no identifying information about your child, only your child’s participant identification number. It is possible that the Institutional Review Board (IRB) may view this study's collected data for auditing purposes. The IRB is responsible for the oversight of the protection of human subjects involved in research. Data will be destroyed 2 years past publication of the results. If you or your child express any child abuse, or the intent to harm or kill ourselves or someone else, the researchers are legally obliged to inform an authority. In this case, you will be informed of the need to do so, and am encouraged to contact with the researchers the Psychological Services Center (540-231-6914) or other counseling agency. However, any outside counseling sought by the you for yourself or your child will be at your expense, as neither the researchers, nor Virginia Tech, have money set aside for such purposes.

VI. Compensation
You will not receive compensation for your child’s participation in this study.

VII. Freedom to Withdraw
This project has been explained to you and your child and we both have been allowed to ask questions about it. You understand that your child or you do not have to fill out the questionnaires or participate in any way if you do not want to and no one will treat us badly. You or your child can stop part way through or withdraw at any time, if you choose.

VIII. Parent (or Guardian) /Subject’s Responsibilities
You voluntarily agree for your child to participate in this study. Your child may participate in a structured-play, watch a video, have his/her physiology monitored, and listen to music or books-on-tape. You understand, however, that you or your child may withdraw at any point and you both are not required to complete any of these activities.

IX. Parent/Guardian’s Permission
You have read the Parental Permission Form and conditions of this project. You have had all my questions answered. You hereby acknowledge the above and give your voluntary consent for your and your child to participate in this study.

Parent/Guardian Signature: ______________________________________ Date:
________________________________________

Should I have any questions about the protection of human research participants regarding this study, I may contact:

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Appendix F

Child Verbal Assent Document/Script:
To be verbally reviewed with the child participant prior to any data collection and/or the child entering the experiment room.

The Effect of Music on Autonomic Response in Children with Autism Spectrum Disorders

I. Purpose of this Research Project
“Some children have a hard time meeting people or children they don’t know. This may be because their hearts beat way to fast and they can only feel their hearts beat and can’t talk to and or play with other person or child. So, we will be measuring how your heart beats to talking and playing with a person you don’t know. You will also get to watch a movie and listen to either music or a book on tape that you pick out so we get to know how your heart beats to that, too!”

II. Procedures
“To measure your heart, we will use something that looks like a lifevest. Have you ever used one of those in the water before? It is pretty neat! This vest is different from a lifevest—it has little stickers that attach to it, that then attach to your skin. Two stickers are placed by your heart and one is placed on your stomach. These stickers help us measure your heart—pretty cool, huh! You will wear this vest for about an hour while we play and when you listen to music or a book-on-tape. Your [mom/dad/grandma/grandpa/etc] will be watching you the whole time through a window we have in this room [point to room], they will be here the whole time. When we take off the electrodes, they may be a little sticky and it can feel like a band-aid to take off. But, [experimenter name] will let you take them off yourself if you want, or your [mom/dad/grandma/grandpa/etc] can help you take them off.”

VII. Freedom to Withdraw
“If you do not want to do anything in the study just let [experimenter name] know, and you don’t have to do it! You only should do what you want to do, and if you are scared or would like your [mom/dad/grandma/grandpa/etc] to come in the room, just let [experimenter name] know.

IX. Child’s Verbal Assent
“Do you have any questions about what I told you? Do you want to do the study?” [Experimental session will be commenced only if child agrees to participate]