Respiratory Sinus Arrhythmia and Restricted Repetitive Behaviors in Autism Spectrum Disorder

Emma Elizabeth Condy

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Psychology

Bruce H. Friedman, Committee Chair

Martha Ann Bell

Angela Scarpa

May 3, 2016

Blacksburg, VA

Keywords: autism spectrum disorder, respiratory sinus arrhythmia, repetitive behaviors

Respiratory Sinus Arrhythmia and Restricted Repetitive Behaviors in Autism Spectrum Disorder

Emma Elizabeth Condy

ABSTRACT

In addition to social communication deficits, restricted repetitive behaviors (RRBs) are a key diagnostic feature of autism spectrum disorder (ASD). Two theories regarding the etiology of RRBs in ASD have been proposed: the hyper-arousal theory, and the hypo-arousal theory. Both of these theories posit the autonomic nervous system (ANS) as being dysfunctional in ASD, resulting in the occurrence of RRBs. Many studies investigating ANS activity in ASD have focused solely on its relation to social functioning. The few that have addressed RRBs have had inconclusive findings. Not only do the current theories and studies simplify ANS activity to a measure of baseline arousal levels through vague measures such as heart rate (HR) and skin conductance response (SCR), but the literature has also framed the theories as mutually exclusive. This study used respiratory sinus arrhythmia (RSA) patterns in children with and without an ASD diagnosis as an indicator of ANS functioning to analyze its relationship to the manifestation of RRBs. Baseline RSA and RSA reactivity were found to predict RRB severity and exploratory analyses revealed that these measures were associated with specific subgroups of RRBs. These results are discussed in regards to the current behavioral literature on RRBs and the benefits of finding biomarkers for these behaviors.

Respiratory Sinus Arrhythmia and Restricted Repetitive Behaviors in Autism Spectrum Disorder

Emma Elizabeth Condy

GENERAL AUDIENCE ABSTRACT

In addition to social communication deficits, restricted repetitive behaviors (RRBs) are a key diagnostic feature of autism spectrum disorder (ASD). Two theories regarding the cause of RRBs in ASD have been proposed: the hyper-arousal theory, and the hypo-arousal theory. Both of these theories posit the autonomic nervous system (ANS) as being dysfunctional in ASD, resulting in the occurrence of RRBs. Many studies investigating ANS activity in ASD have focused solely on its relation to social functioning, another deficit seen in ASD. The few that have addressed RRBs have had inconclusive findings. Not only do the current theories and studies simplify ANS activity to a measure of baseline arousal levels, but the literature has also framed the theories as mutually exclusive. This study used respiratory sinus arrhythmia (RSA), a measure of ANS functioning, to analyze its relationship to the manifestation of RRBs in children with and without an ASD diagnosis. Various aspects of this measure were found to predict RRB severity, indicating that ANS activity is related to the occurrence of RRBs. Furthermore, exploratory analyses revealed that these measures were associated with specific types of RRBs. These results are discussed in regards to the current behavioral literature on RRBs and the benefits of finding biomarkers for these behaviors.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANS	Autonomic nervous system
ASD	Autism spectrum disorder
CAN	Central autonomic network
CI	Circumscribed interests
ECG	Electrocardiogram
HR	Heart rate
HRV	Heart rate variability
IBI	Inter-beat interval
IS	Insistence on Sameness
KBIT-2	Kaufman Brief Intelligence Scale, Second Edition
lnHFHRV	Natural log-transformed high frequency heart rate variability
PNS	Parasympathetic nervous system
RBS-R	Repetitive Behavior Scale – Revised
RMB	Repetitive motor behavior
RMSSD	Root mean squared successive differences
RRB	Restricted repetitive behavior
RSA	Respiratory sinus arrhythmia
RSA-W	Respiratory sinus arrhythmia withdrawal
SC	Skin conductance
SCR	Skin conductance response
SNS	Sympathetic nervous system
SRS-2	Social Responsiveness Scale, Second Edition
TD	Typically developing

Respiratory Sinus Arrhythmia and Restricted Repetitive Behaviors in Autism Spectrum Disorder

1.0. Introduction

Autism spectrum disorder (ASD) is defined by two symptoms: social communication/interaction deficits, and restricted repetitive behaviors (RRBs) (American Psychiatric Association, 2013). RRBs are understudied in the ASD literature even though they have been associated with increased stress on caregivers (Harrop, McBee, & Boyd, 2016) and distressing to children affected by the disorder (Gabriels et al., 2005). These symptoms are highly heterogeneous, with presentations of ASD looking vastly different between individuals diagnosed with the disorder, making them difficult to address therapeutically. This heterogeneity is particularly evident in the manifestation of restricted repetitive behaviors. The DSM-5 criteria alone reveal the highly variable nature of these behaviors in the description of the different behaviors that fall within the RRB domain. This heterogeneity is further supported by a variety of research which has shown that profiles of RRBs are widely varied between individuals with ASD (Carcani-Rathwell, et al., 2006; Lam, Bodfish, & Piven, 2008; Bishop et al., 2013). Although the presence of RRBs is observed during early stages of typical development (Thelen, 1981) and RRBs are often present in other neurological and psychiatric disorders, these behaviors are significantly more pervasive in autism spectrum disorders (Bodfish, Symons, Parker, & Lewis, 2000). Due to their role as a key feature of the disorder as well as their highly variable nature, RRBs could be a valuable tool in parsing apart behavioral phenotypes of ASD.

In this study, RRB severity is addressed in respect to its differential pattern of manifestation across individuals with ASD diagnosis and their typically developing peers. The study addresses the differences in autonomic functioning between children with ASD and typically developing children, as well as the relationship between RRBs and the activity of the

autonomic nervous system (ANS), including ANS baseline, reactivity, and recovery.

1.1. Behavioral Profiles of Restricted Repetitive Behaviors

The restricted repetitive behaviors that are presented in autism spectrum disorder are highly heterogeneous and can be assessed in a variety of ways. The three most popular forms of RRB assessment are the Repetitive Behavior Scale-Revised (RBS-R; Lam & Aman, 2007), the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & Le Couteus, 1994), and the Repetitive Behavior Questionnaire/Interview (RBQ/RBI; Honey, Rodgers, & McConachie, 2012). These tools can be helpful not only in assessing the severity and quality of RRBs in individuals with ASD, but also in creating subgroups of individuals with similar phenotypic presentations of RRBs. These assessments have been compared and were used in the studies discussed below to assess their concordance and subscale validity (Honey et al., 2012). In doing so, the goal was to help classify RRBs into subgroups within ASD. Identifying phenotypic patterns of RRBs in individuals with ASD could help identify biological components of the behaviors (Bishop et al., 2013), and potentially aid in identifying additional genes at play in ASD (Happe, Ronald, & Plomin, 2006). Multiple studies have begun to delve into this area in attempt to parse apart different RRB profiles in ASD.

Certain RRB profiles are associated with demographic characteristics (e.g., age and IQ). For instance, older individuals with ASD often have less severe and less frequent RRBs than younger individuals with ASD; however, there are no qualitative differences in RRBs as a function of age (i.e., there was not a specific type of RRB – such as motor movements, rituals, etc. - associated with age). These results suggest that RRB subtype remains heterogeneous across the lifespan (Esbensen et al., 2008). Conversely, another study found that younger children with autism engage in more simple motor behaviors, whereas older children engaged in more complex

behaviors, indicating that age may be related to RRB type (Militerni et al., 2002). Similarly, there have also been conflicting findings regarding IQ and RBS-R type and severity. While some have found no significant effect of intellectual disability (ID) on RRB severity (Esbensen et al., 2008) others have identified a negative correlation between total RBS-R score and non-verbal IQ (NVIQ; Gabriel et al., 2005). However, when individuals with pervasive developmental disorder (PDD) or pervasive developmental disorder and mental retardation (PDD+MR) were compared to individuals with mental retardation alone (MR), those with a PDD or PDD+MR diagnosis are more likely to display RRBs than MR alone (Carcani-Rathwell et al., 2006). Although RRBs are present in a variety of developmental disabilities (Thelen, 1981), these results show that these behaviors might occur more frequently in ASD. Differences in IQ have also been associated with different phenotypic RRB profiles in individuals with ASD, such that low IQ was associated with more of the sensory focused behaviors (Militerni et al., 2002; Carcani-Rathwell et al., 2006). The disparity between these studies reveals contradictory conclusions in the literature regarding RRBs and their relation to categorical characteristics of individuals with ASD.

The relationship between clinical features of ASD and RRBs has been analyzed in hope of uncovering a pattern of RRB manifestation within the disorder. ASD assessments have been shown to have a weak positive correlation with the number of RRBs expressed by individuals with ASD (Militerni et al, 2002), indicating that that RRBs may inform us about the severity of ASD. Furthermore, adaptive functioning skills and communication ability have also been associated with RBS-R severity scores (Gabriels et al., 2005). The associations between RRB severity and clinical features of ASD are important in considering behavioral subtypes of RRBs. Though these studies did not indicate differences in qualitative aspects of RRBs in relation to ASD symptoms, doing so would be a logical progression of this research. Due to the

heterogeneity of RRBs, creating clusters of RRB types could be a valuable mode of inquiry in relating RRB occurrence to clinical features of ASD.

In dividing restricted repetitive behaviors into commonly occurring clusters and analyzing their patterns of manifestation, researchers may be able to more effectively identify specific subgroups of RRBs in ASD. Two RRB subgroups in the literature are the sensory motor symptom group (SMS, which included repetitive movement & sensory behaviors) and a cognitive rigidity symptom group (CRS; Carcani-Rathwell et al., 2006). ID severity tends to be associated with SMS behaviors, but not CRS behaviors. The difference in the association with ID between the subtypes suggests that CRS may be the more ASD specific form of RRB. Similar subgroups have been identified through the use of exploratory factor analysis on items from the Autism Diagnostic Interview-Revised (ADI-R; Lam, Bodfish, & Piven, 2008), which identified three factors within RRBs: repetitive motor behaviors (RMB), insistence on sameness (IS), and circumscribed interests (CI). Again, like the SMS behaviors in the previous study, RMBs were found to be directly correlated with categorical variables, such as IQ, age, social/communication impairments. However, IS was only correlated with social/communication impairment, a key feature of ASD. These findings are consistent with Carcani-Rathwell et al. (2006) in that the cognitive rigidity symptoms, such as insistence on sameness, seemed to be more characteristic of ASD. By narrowing down the types and severity of RRBs specific to ASD, a more thorough assessment of RRB subgroups can be done. This is necessary in order to identify specific, homogeneous behavioral phenotypes of ASD. Doing so will aid in creating useful, evidence based groupings of individuals with similar symptomatology, which could be beneficial clinically and empirically.

1.2. Hypothesized Arousal Profiles of Restricted Repetitive Behaviors

Two primary theories regarding the presence of restricted repetitive behaviors and social dysfunction in ASD implicate arousal as a key component in the manifestation of these behaviors. While these theories are important to discuss due to their historical significance and how they inform the way RRBs have been approached in the literature, it is important to note that they are based on a dated notion of arousal. The hypo- and hyper-arousal theories of ASD have each been supported by various studies; however, neither theory has overtaken the other in the literature.

The hypo-arousal theory of repetitive behaviors proposes that RRBs are caused by a chronic state of under-arousal, as defined by decreased ANS activity, in individuals with ASD. The function of RRBs in autism is to heighten arousal by increasing sensory input. The prevalence of self-injurious behaviors in ASD is then attributed to this, in that these acts are effective at heightening arousal (DesLauriers & Carlson, 1969).

While there is some evidence for this theory (Hirstein, Iversen, & Ramachandran, 2001; Hubert, Wicker, Monfardini, & Deruelle, 2009), the literature also supports the hyper-arousal hypothesis of RRBs, which suggests that individuals with autism experience an elevated level of arousal which results in RRBs. This hypothesis states that oversensitivity and poor habituation to stimuli in the environment causes an over-aroused state (Hutt et al., 1964; Hutt & Hutt, 1965). Theoretically, RRBs allow individuals with ASD to self-soothe, reducing heightened arousal. A few studies have taken the hyper-arousal hypothesis into account when addressing RRBs. For instance, Joosten et al. (2009) analyzed motivational differences surrounding RRBs in individuals with ASD compared to those with intellectual disability (ID). Five sources of motivation were measured: gaining attention, access to tangible objects, escape from people/activities, sensory stimulation, and anxiety reduction. Each RRB that occurred over a 6

week period was coded for motivation sources by the participant's teacher and the experimenter. Overall, motivation for engaging in RRBs differed between the ASD+ID and ID groups. Anxiety reduction was found to be a greater intrinsic motivator than sensory seeking in the ASD+ID group than the ID group. This relationship was reversed in the ID group. Since anxiety is often associated with changes in autonomic state such as increased heart rate and shortness of breath (Sharma, Sagar, Deepak, Mehta, & Balhara, 2011), an excited autonomic state was hypothesized to be relieved by RRB engagement in the ASD group. Though this is a simplistic characterization of ANS activity, the findings implicate a differential etiological factor for the presence of RRBs in ASD compared to ID. Further investigation of these motivational sources that includes autonomic measures of arousal could lead to valuable distinctions regarding arousal profiles in individuals with ASD and developmental disabilities. Many studies have attempted to pit the hyper-arousal theory and hypo-arousal theory against each other; however, their findings have conflicted across various autonomic measures. More research and replication is needed in this area (Rogers & Ozonoff, 2005).

The importance of continuing to investigate the arousal hypotheses surrounding RRBs is evident in Cunningham & Schreibman's (2008) review, which focused on understanding the function of RRBs. In contrast, the DSM-5 (American Psychiatric Association, 2013) does not consider the function of RRBs for diagnostic purposes. RRBs are grouped as one class of behaviors even though they are qualitatively different between individuals, as noted by studies that categorize these behaviors into subgroups. RRBs are also not specific to ASD: they appear across developmental disorders and even in typically developing children (Cunningham & Schreibman, 2008). For this reason, the function of these behaviors is key in uncovering unique variations in individuals. Not only is this important for diagnostic applications, but also for

treatment and behavior intervention protocols.

When looked at comprehensively, the literature has been inconclusive in determining a single pattern of autonomic arousal that might contribute to RRBs present in ASD. Furthermore, the language used in much of the literature is vague in its definition of arousal. Arousal is not a unidimensional concept, as the literature in this area seems to suggest, and to speak about it in such terms is a primitive approach. It has long been known that different aspects of arousal, such as behavioral arousal, electrocortical arousal, and autonomic arousal, are dissociable (Lacey, 1967). These disassociations indicate multiple facets of arousal, making it inappropriate to talk about arousal as a single construct. By expanding the scope of measures to include ANS reactivity and recovery, a fuller profile is established than baseline alone. In doing so, more details of autonomic functioning and activity are captured.

1.3. Autonomic functioning patterns in ASD

Generally speaking, profiles of autonomic activity are significantly different in individuals with autism compared to those of their typically developing peers. Most studies of autonomic activity in ASD have focused on resting baseline levels. A variety of measures have been utilized in these studies, including heart rate (HR), heart rate variability (HRV), and skin conductance (SC). Comparing these studies is difficult due to the variety of measurement techniques and experimental paradigms, resulting in inconclusive findings regarding a specific pattern of autonomic functioning in ASD (Rogers & Ozonoff, 2005).

HRV refers to the differential lengths of interbeart intervals (IBIs) present within the electrocardiogram (ECG) signal (Task Force, 1996). These IBIs are dependent on the activity of the sinoatrial (SA) node of the heart, which is concurrently regulated by the two components of the autonomic nervous system (ANS): the sympathetic nervous system (SNS) and the

parasympathetic nervous system (PNS). The SNS acts as an excitatory mechanism and signals to increase HR, and the PNS is inhibitory, acting through the vagus nerve to decrease activity of the SA node. Certain HRV metrics, such as high frequency HRV and the root mean successive squared difference (RMSSD), specifically reflect the vagal influence on the heart (Berntson et al., 1997; Porges, 2007) and thus act as indices of PNS influence on cardiac activity.

ASD studies using HR and HRV as autonomic measures have been inconclusive at best. HR and HRV have been used to assess mean differences in autonomic functioning between children with autism and children with other developmental disabilities (DD). In one early study, children with autism who were exposed to week long changes in "environment load" (manipulated through increase/decrease in the strength of sensory input from their teacher) showed higher HRV, as quantified through RMSSD (a measure of vagal influence on the heart), compared to a control group of children with other developmental disorders (Graveling & Brooke, 1978). It was concluded that individuals with autism were generally "less aroused" at baseline than controls; however, this conclusion is questionable due to limitations of the study. Not only were conditions not counterbalanced, but the environment manipulation was not properly controlled. Furthermore, HRV was not well understood in 1978, leaving the study open to issues of experimenter bias and weakening the validity of the measures. Nonetheless, differences between children with ASD and children with DD were evident.

Other studies using cardiac measures in children have also found baseline differences between an ASD group and typically developing (TD) control groups (Goodwin et al., 2006; Ming et al., 2005). In these studies, children with ASD had higher baseline HR than TD controls. "Cardiac vagal tone" was used as an index of variation in IBIs collected from an ECG and is considered an indicator of parasympathetic activation. Significantly lower "cardiac vagal tone"

and cardiac sensitivity to baroflex, as well as higher mean arterial pressure and diastolic blood pressure were found in children with autism compared to TD children (Ming et al., 2005). These results indicate that children with ASD display lower "cardiac vagal tone" (i.e., parasympathetic baseline activity) than TD children along with elevated sympathetic tone (The term "cardiac vagal tone" is problematic as the measurement of this construct is not well defined in the literature. It is addressed differently by different researchers, with some quantifying it through baseline RSA measures, while others quantify it through RSA reactivity measures, and occasionally through a specific quantification technique, namely Porges's moving polynomial method (Beauchaine, 2001). For this reason, the term cardiac vagal control will be used for the remainder of this paper). Furthermore, cardiac reactivity in children with ASD and age- and sex-matched TD controls has been found to differ. HR during stressful situations, such as a loud noise, a challenging task, or exercise (Goodwin et al., 2006), showed a change 20% of the time in ASD children, whereas the control group showed a change 66% of the time. However, since the ASD group maintained a higher baseline HR than the control group, the difference in HR reactivity may have been due to a "general state of autonomic defensiveness" (p. 108) in the ASD group. This study is of particular importance due to the additional information regarding autonomic responsivity to stressors. In showing that individuals with ASD differ from controls in both baseline and reactivity, greater variation in autonomic profiles is evident. The collective findings indicate a possibility of an autonomic signature for ASD discerning them from TD (Goodwin et al., 2006; Ming et al., 2005) and DD children (Graveling & Brooke, 1978),.

Skin conductance response (SCR) has also been used to assess autonomic functioning in ASD. In accord with literature on autonomic arousal in ASD, these studies have also detected differences in individuals with ASD compared to TD peers. These studies have shown

differential reactivity to social stimuli, such as smaller SCRs in response to viewing emotional faces in individuals with ASD compared to controls (Hubert et al., 2009), and heightened SCR to direct gaze compared to averted gaze in ASD (Kylliainen & Hietnam, 2006). These studies present contrasting views of the role of hypo vs. hyperarousal in response to social stimuli in ASD. Furthermore, in using social stimuli, a layer of complexity is introduced to the design since neither social processing nor autonomic functioning are well understood in ASD. Studying baseline autonomic activity and responsivity to non-social stressors in individuals with ASD would eliminate many confounds allowing these patterns to be more readily assessed.

While these studies provide evidence of autonomic irregularities in ASD, a distinctive, common pattern of autonomic activity associated with ASD is not evident in the current literature. Most studies aimed at assessing autonomic activity in ASD have compared mean levels at baseline using a single baseline physiological measure. In doing so, important details about autonomic patterns are lost. While it is valuable to establish overarching autonomic differences in individuals with ASD compared to controls, very little insight about how this relates to the presence of deficits seen in ASD is afforded by these designs.

1.4. Restricted Repetitive Behaviors and Autonomic Activity in ASD

Very few studies have simultaneously examined autonomic activity and RRBs in ASD. However, one such study found that hyper-responsive autonomic reactions (as measured by SCR) were often followed by self-stimulation activities in children with ASD, and hypo-responsive individuals tended to engage in more self-injurious behavior (Hirstein, Iversen, & Ramachandran , 2001). These findings indicate differential intradiagnostic patterns in ANS activity. Differential patterns within ASD are supported across multiple measures of ANS activity, as evidenced by the inconclusive findings in the field.

Heart rate has also been used to categorize physiological profiles of stereotyped behaviors in children with ASD (average age 60 months; Willemsen-Swinkels et al., 1998). RRBs were coded in terms of both stereotypic motor behaviors and mood state. HR was analyzed surrounding the occurrence of each stereotypic behavior; different HR profiles were found for the various moods associated with these behaviors. Future research that includes all RRBs covered in the DSM 5 and various ASD assessments (RBS-R, ADI-R, RBQ/RBI), in addition to a more comprehensive profile of physiological measures may more clearly delineate distinct patterns of autonomic activity associated with various RRB profiles.

These findings reveal abnormal baseline patterns in ASD, but also show a range of autonomic functioning patterns and potential RRB correlates within the disorder. By continuing in this direction and using more nuanced measures of autonomic activity profiles and their associated behaviors could be identified, uncovering valuable information about clinical features and etiological factors associated with ASD.

1.5. Heart Rate Variability and Behavioral Associations in ASD

Higher basal HRV often has been associated with greater behavioral flexibility and better coping strategies in typically developing populations. Conversely, decreased resting HRV is connected to poor emotion regulation, coping strategy deficits, and a variety of psychopathologies such as generalized anxiety disorder and panic disorder (Appelhans & Leucken, 2006). The *neurovisceral integration model* proposes that these two branches of the autonomic nervous system (ANS) are controlled by the central autonomic network (CAN), which consists of a dynamic network of forebrain, midbrain, and hindbrain structures (Benarroch, 1993; Thayer & Friedman, 2004; Thayer, Hansen, Saus-Rose, & Johnsen, 2009). The CAN controls emotional, physiological, and behavioral responding through a number of

feedback loops that regulate the system. HRV can serve as a peripheral indicator of the functionality of the CAN, with high resting HRV indicating contextually appropriate inhibition and subsequently increased emotional regulation, attention control, and behavioral flexibility. HRV theories and associated findings that relate psychopathology to HRV support its utility as an indicator of aberrant, perseverative behaviors. For this reason, HRV could be a valuable tool for assessing RRBs in ASD.

There are a variety of widely used HRV metrics; selection of an appropriate measure depends on the construct of interest. For instance, HRV can be used to estimate cardiac vagal control under the appropriate conditions. There are two primary classes of metrics that are used to assess HRV: variance-based and frequency-based (Appelhans & Luecken, 2006). Variance-based metrics indicate the mean amount of variation (in milliseconds) between the IBIs present in the ECG. Such measures are held to index PNS mediated HRV; however, most only provide a general overview of HRV. One exception is the root mean squared successive differences (RMSSD), which is a variance-based metric that provides specific information regarding vagal activity. Frequency based metrics provide a more comprehensive profile of HRV. By dividing HRV into frequency bands, a power spectrum is created that reflects all of the variation present within the ECG. The high frequency (HF) band (0.15–0.4 Hz) is interpreted as PNS mediation of SA node activity; however, the autonomic origins of low frequency (LF; 0.04-0.15 Hz) activity are debated (Malik et al., 1996; Appelhans & Leucken, 2006).

Vagal influences can be identified from the HRV signal through frequency analysis due to the differential rates of influence of the PNS and SNS on the SA node (Appelhans & Leucken, 2006). While SNS influence takes approximately 4 seconds to reach the SA node, it only takes ~0.5-1 second for the influence of the PNS to take effect. The disparity in activation time is what

makes frequency analysis of HRV possible. Frequency analysis provides metrics of many components of HRV. One metric of particular interest is the quantification of respiratory sinus arrhythmia (RSA). RSA is a physiological phenomenon that can be measured through spectral analysis based on the respiration frequency band (0.12-0.40 Hz; Allen, Chambers, & Towers, 2007). RSA specifically indexes vagal influence on the heart, providing a measure of PNS activity.

RSA is a useful biomarker of adaptive functioning in both clinical and typically developing populations of children (Graziano & Derefinko, 2013). In a meta-analysis of 44 studies, baseline RSA and RSA withdrawal (RSA-W) were used to assess four domains of adaptive functioning (e.g., externalizing problems, internalizing problems, social problems, and cognitive/academic problems). RSA-W refers to the change in RSA between a baseline task and a challenging task. A challenging task would typically elicit an increase in RSA-W. When these variables were evaluated in children under 18 years old, lower externalizing problems (EP), lower internalizing problems (IP), and fewer cognitive/academic problems (CP) were associated with higher RSA-W levels. Furthermore, typically developing (TD) children had higher baseline RSA levels than the clinical/at-risk children. When the baseline differences were controlled for higher levels of RSA-W were still found in TD children compared to clinical/at-risk children. Furthermore, increased RSA-W in response to a challenging social situation has been associated with less EP, IP, and better self-regulation ability (Hastings et al., 2008). These findings indicate that decreased baseline RSA and dampened RSA-W are associated with a variety of behavioral problems and clinical disorders. For this reason, baseline RSA and RSA responsivity can be valuable biomarkers in assessing behavioral dysregulation across a variety of diagnoses.

HRV measures have been used to investigate social functioning in ASD, but the literature

is limited. When RSA was collected while children watched videos of familiar or unfamiliar people reading them a children's book, children with ASD showed differential responses to familiar versus unfamiliar faces compared to TD controls (Van Hecke et al., 2009). The ASD group had lower baseline RSA than TD controls. RSA decreased in response to unfamiliar faces in the ASD group, whereas the control group maintained similar RSA levels across the familiar and unfamiliar conditions, indicating poor autonomic regulation in response to unfamiliar people. The response to unfamiliar people observed in the ASD subjects can be directly related to the social deficits seen in ASD. However these findings do not address the pervasive social functioning issues seen in ASD as social deficits are also present during interactions with familiar people. Similar studies have found that higher baseline RSA has been linked to better social functioning and language abilities in children with ASD (Patriquin, Scarpa, Friedman, & Porges, 2013). Baseline RSA was compared to participant's scores on the Social Responsiveness Scale (SRS-2) and was found to be positively correlated with social functioning ability; however, the relationship did not reach significance. Higher baseline RSA was significantly related to stronger receptive language skills. These results indicate differentiation of symptom severity within ASD as it relates to HRV. Furthermore, the direct relationship between social functioning and RSA is complementary to theories implicating RSA as a factor in behavioral flexibility and emotion regulation abilities, as these resources must be used to navigate the social difficulties associated with ASD.

1.6. Research Domain Criteria and RRBs

ASD is a heterogeneous disorder, making a definite behavioral profile difficult to identify. The Research Domain Criteria (RDoC) was recently developed by the National Institute of Mental Health (NIMH) to address concerns about the diagnostic reliability of guidelines in the

DSM and ICD. The establishment of RDoC highlights the need for assessing disorders on multiple pathophysiological levels (Cuthbert & Kozak, 2013; Insel et al., 2010). The variety of behaviors included under "restricted repetitive behaviors" in the ASD diagnosis is an example of where the DSM is highly unspecified. RRBs in particular are potentially valuable in parsing behavioral and physiological phenotypes present within the disorder, as they are highly diverse (Lam, Bodfish, & Piven, 2008; Esbensen et al., 2009).

In order to examine differential RRB phenotypes, behavioral measures as well as biological correlates, such as physiological profiles, should be used to establish quantitative differences between groups. Identifying physiological profiles of certain behaviors could also be helpful in further validation of current RRB behavioral scales. Furthermore, establishing patterns of autonomic activity through physiological measurements could afford deeper understanding of why individuals with ASD engage in RRBs. According to the neurovisceral integration model (Thayer & Friedman, 2004; Thayer & Lane, 2009), disinhibition of feedback loops within the sympathetic nervous system which are typically under tonic inhibition can lead to perseverative behaviors. This imbalance is reflected and measured through HRV, as it is vagally mediated, and is posited to reflect activity of the sympathetic nervous system (Porges, 2003). Many psychological disorders (such as generalized anxiety disorder) are associated with a reduction in HRV (Friedman, 2007; Appelhans & Leucken, 2006). The model proposes that these disorders arise from the malfunction of inhibitory circuits in the prefrontal cortex, resulting in a positive feedback loop that creates maladaptive, inflexible behaviors.

Studies assessing RRBs in relation to HRV are virtually nonexistent. Since RRBs are a perseverative behavior and may arise from a lack of behavioral flexibility or appropriate coping strategies, they are a great exemplar of behavior thought to be associated with decreased PNS

mediated HRV. Assessing the RSA profiles (i.e., basal and reactivity RSA) surrounding various subgroups and severities of RRBs in ASD could address the behavioral outcomes of reduced PNS activity suggested by the neurovisceral integration model, but also aid in uncovering a biobehavioral mechanism of the dysfunctions in ASD.

1.7. Aims

The RRBs essential to an ASD diagnosis are a pronounced representation of the rigid, perseverative behaviors that are referred to in the neurovisceral integration model. In the present study, RRBs are considered a product of inflexibility and inhibitory failure within the CAN. By examining the relationship between RRBs and HRV, the functionality of the CAN in relation to these behaviors can be assessed. This study aimed to analyze the autonomic profiles (specifically RSA) associated with the expression of RRBs. Autonomic activity was established by examining HRV during a vanilla baseline condition, as well as two stress conditions which were each followed by vanilla recovery periods. By collecting HRV across these conditions, multiple parameters of HRV were assessed as a function of RRB severity. By comparing HRV patterns of children with ASD and typically developing children, unique profiles of autonomic functioning between groups, as well as within the ASD group were uncovered. Establishing differences in HRV as a function of RRB severity scores was the primary interest of this study.

The following hypotheses were addressed through this study:

1. Children with ASD have lower baseline HRV than typically developing controls.
2. Reduced baseline HRV is predictive factor of greater RRB severity in both children with ASD and TD children.
3. Reduced HRV withdrawal from the baseline to stressor conditions is predictive of greater RRB severity in both children with ASD and TD children.

4. Reduced HRV recovery between the stressor conditions and recovery periods is predictive of greater RRB severity in both children with ASD and TD children.

2.0. Method

2.1. Participants

Participants were recruited through the Virginia Tech Center for Autism Research (VTCAR) Registry and through the aid of local organizations. Parents who listed their child on the registry were contacted with a flyer describing the study and containing the researcher's contact information. The flyer was also sent to VTCAR's recruitment resources email list, which includes a variety of autism action groups and parent organizations in the New River Valley. These recruitment measures also addressed the recruitment of control participants, as the flyers were present in forums at which both the parents of ASD affected children and unaffected children will be present. Flyers were also posted on community bulletin boards and online forums (e.g., Craigslist) to recruit participants for the ASD and control groups.

When parents contacted the researcher for participation they were informed of the general procedure of the study and asked if they thought their child will be amenable to wearing the heart rate monitor. If they did not, they were not scheduled for a laboratory session; however, none of the parents who contacted the lab reported that this would be an issue. Incentives were offered and consisted of either a $20 Visa gift card or $20 in cash. These incentives were funded in part by the Virginia Tech Center for Autism Research's Graduate Student Award and the Virginia Tech Graduate Research Development Program. Any parent was allowed to accompany their child to the lab session and was asked to report their relationship to the child, as well as the child's medical history and any current medications during the lab visit. Participants were excluded if they have any history of cardiovascular disease, known genetic disorders, or

neurological disorders. Informed parental consent and permission and child assent were obtained for all children who participated in the study.

Participants in the study (N=29) were children between the ages of 5-10 years old with an ASD diagnosis (n = 13; age in years, M =7.43, SD = 1.95). A group of age-matched (n = 16, M = 6.94, SD = 1.06), typically developing peers was also included in the study as a control group. A majority of participants in the ASD group were male (males = 11; females = 2; 18% female), which was expected, as the prevalence rates for males range from 3.2–7.1 times as high as females (Autism and Developmental Disabilities Monitoring Network, 2006). Gender was matched for as best as possible in the control group, with our control group consisting of 13 males and 3 females (23% female). In the ASD group, 5 participants had a comorbid communication or language disorder, 5 participants had a comorbid attention deficit hyperactivity disorder diagnosis, and 3 had a comorbid anxiety disorder diagnosis. In the control group, 1 participant was reported to have an anxiety disorder diagnosis. Furthermore, while 6 children in the ASD group and 1 child in the TD group were on some sort of medication, none were medications that would interfere with cardiac measures. The means and standard deviations of other demographic variables for these groups, such as IQ and questionnaire scores are summarized in Table 1.

All participants in the experimental group were required to have a previously existing ASD community diagnosis. Parents were asked to bring documentation of this diagnosis to the lab visit; however, of the 13 ASD participants, only 5 parents remembered to bring in this paperwork. For this reason, ASD diagnoses were also verified through scores above the cutoff on the SRS-2. One participant in the ASD group had a community diagnosis and brought the appropriate paperwork confirming their diagnosis, but did not reach cutoff scores on the SRS.

This participant was still included in all analyses as having an ASD diagnosis. Furthermore, control participants were required to be below cutoff scores on an ASD screener to confirm that they were not at risk for ASD.

2.2. Power analysis

Effect sizes for the association between RSA and RRBs in ASD were calculated through the G*Power applet (Erdfelder, Faul, Lang, & Buchner, 2007) with information from Van Hecke et al. (2009). A correlation of -0.46 between our predictor (baseline RSA) and outcome measure (in Van Hecke et al., RRB severity on the SRS) was found. Based on this value, an effect size (f^2 =0.2683917) was calculated for a linear regression model using one predictor (RSA). Using this effect size, power of 0.80, and an $\alpha = 0.05$, the applet suggests a total sample size of 32. With these power analyses, the present study aimed for a minimum total sample size of 32, with approximately 16 participants per group. Due to difficulties with recruitment of children with ASD, the current size of the ASD group is 13.

2.3. Cognitive Assessment

All participants underwent the Kaufman Brief Intelligence Test, Second Edition (KBIT-2; Kaufman & Kaufman, 1990) to assess their level of cognitive functioning. The KBIT-2 is not a comprehensive intelligence test; however, it is an appropriate screening tool for intelligence, provides verbal and nonverbal scores, and can be used in children ages 4 and up, making it suitable for this study (Bain & Jaspers, 2010). It has standardized scores (*M*=100, *SD*=15) and ranked percentiles by age. The KBIT-2 took approximately 20 minutes to complete. Since subgroups and severities of RRBs are correlated with IQ (Lam, Bodfish, Piven, 2008) using IQ cutoffs would have limited the range of RRBs in the sample. For this reason, IQ cutoffs were not used in the study.

2.4. Questionnaires

Parents were asked to fill out the Restricted Repetitive Behavior Scale-Revised (RBS-R) to assess their child's RRB profile, the Short Sensory Profile (SSP), and the Social Responsiveness Scale (SRS-2) to evaluate other ASD symptomatology. The SSP total score was used to co-vary out sensory sensitivity in the relevant regression analyses.

2.5. Materials

2.5.1. Apparatus. The BioHarness 3 Data Logger and Telemetry Physiology Monitoring system was used to record electrocardiogram (ECG; Zephyr Technology, Annapolis, MD). The signals were acquired through the BioHarness (sampling rate: 1000 Hz) and were collected and cleaned through AcqKnowledge 4.4 software. In AcqKnowledge, the 'find cycle' function was used to do a cursory detection of R-spikes in the ECG signal. Each file was then visually inspected and any mislabeled R-spikes were manually removed or inserted. The 'find rate' function was then used to measure the IBIs (ms) within each epoch. The IBI files were then analyzed using Kubios HRV v2.0 (Tarvainen, Niskanen, Lipponen, Ranta-ahol, & Karjalainen, 2009).

2.5.2. Electrocardiogram. A variety of HRV measures were derived from the ECG data. All HRV analyses were performed with Kubios HRV v2.0 (Tarvainen, Niskanen, Lipponen, Ranta-Ahol, & Karjalainen, 2009). In the temporal domain, the root mean squared successive difference (RMSSD) was calculated to assess HRV. RMSSD removes slower trends from ECG data, leaving the faster, vagally mediated modulations to serve as a measure of cardiac vagal control (Friedman et al., 2002). In the frequency domain, respiratory sinus arrhythmia (RSA) was derived through spectral analysis based on the fast Fourier transform, which calculated the power density (ms^2) of the high frequency band associated with respiration in the ECG signal

(Grossman et al., 1990). While in adults this frequency band is between 0.15–0.4 Hz, the present study used a range of 0.24-1.04 Hz, as this is more appropriate in a sample of young children (Berntson, Quigley, & Lozano, 2007). A natural log transformation was then used on these data to remove positive skew, as is typical in high frequency HRV analysis (Ellis et al., 2008). This measure will be denoted as lnHFHRV. Respiratory data was considered after the fact to ensure that participants were breathing within the estimated range and did not differ between groups or conditions.

2.6. Procedure

2.6.1. Parental questionnaires. Once in the lab, the parent was informed about the measures and equipment being used in the study. They were then be given an informed consent form to read and sign. Verbal assent was obtained from all child participants in the study. Parents were then asked to fill out the child history form, RBS-R (43 items; Cronbach's α=.976), SSP (38 items; Cronbach's α=.980), and SRS-2 (65 items; Cronbach's α=.988) while sitting in the laboratory during their child's data collection session. It took them approximately 25 minutes to fill out these forms.

2.6.2. Cognitive assessment. Once consent and assent were obtained, child participants completed the KBIT-2 with the researcher. The KBIT-2 took approximately 20 minutes to administer. After completing the KBIT-2, participants were then fitted with the BioHarness. With aid from the child's parent, the experimenter put the BioHarness on the child. The BioHarness was worn right under the ribs and under the shirt. The child then acclimated to the presence of the telemetry strap. Acclimation to the presence of the strap consisted of a two minute period during which the child was provided with a mildly amusing video on the computer monitor ('Animal Babies (Animal Atlas)';

https://www.youtube.com/watch?v=7NnL8WORZY4) to occupy them while their parent sat in a separate chair behind them.

2.6.3. Vanilla baseline. After the acclimation period was complete, the child continued to sit in front of a computer monitor watching the same 'Animal Babies' video and the vanilla baseline period commenced. A vanilla baseline was collected for three minutes during which the child continued to quietly sit and watch the video provided by the experimenter. Their parent remained in the room during data collection, except for one three minute experimental condition.

2.6.4. Experimental conditions. Conditions were chosen based on their suitability for participants of varying intellectual functioning and their ability to elicit a stress response. These conditions were drawn from a study by Goodwin et al. (2006) where they were used to assess heart rate as a measure of sympathetic activity in individuals with ASD. The first condition was a sensory stressor ('loud noise') that lasted three minutes. During the 'loud noise' condition a vacuum cleaner was running outside of the lab while the participant was seated in the lab and continued watching the same baseline video with their parent sitting in a separate chair behind them. This condition was then followed by a recovery period of three minutes, with conditions akin to the vanilla baseline period. The second condition was an anticipatory/uncertainty stressor ('unstructured time') that also lasted three minutes. During the 'unstructured time' condition the parent left the room and the child was only told that their parent would be back shortly. Again, this condition was followed by a three minute recovery period consisting of the same conditions as the vanilla baseline. Each participant was exposed to the 'loud noise' condition and the 'unstructured time' condition once during their visit. The condition order was counterbalanced between participants to control for ordering effects. The vanilla baselines, experimental

conditions, and recovery periods resulted in an 18 minute physiological data collection session.

3.0. Results

Prior to HRV analysis, respiration rate was assessed through a one-way repeated measures ANOVA to ensure that respiration rate did not differ across conditions. There was no difference in respiration rate across conditions ($F(5,125)=.626$, $p=.68$). Furthermore, a series of Mann-Whitney U tests revealed no differences in respiration rate between the TD and ASD group in any condition (Table 2). Similarly, there were no correlations between BMI and the HRV measures used in the analyses. Non-parametric t-tests were used for this and for the remainder of the group comparison analyses since the groups had small and unequal sizes (Lincoln, 1952). Since there were no differences in respiration rate between groups or conditions, and no relationship between BMI and the HRV measures, respiration rate and BMI were not controlled for in subsequent analyses.

Participants' cardiac vagal activity was assessed by deriving RMSSD and lnHFHRV from their ECG signal. These measures were calculated for each participant's vanilla baselines, as well as each task condition and recovery periods. Differences between the preceding vanilla baseline and task condition calculated for each task provided two measures of both lnHFHRV withdrawal and RMSSD withdrawal, and the differences between each recovery period and task condition provided two measures of both lnHFHRV recovery and RMSSD recovery. Withdrawal scores were calculated by subtracting the baseline period measurement from the task period measurement, and recovery scores were calculated by subtracting the recovery period measurement from the task period measurement. These measures were separately calculated for each condition in case the two conditions differentially affected children with ASD and TD children. Baselines were acquired for every participant; however, due to signal noise, task and

recovery data, and subsequently reactivity and recovery scores, were only acquired for part of the ASD sample (n=10).

To check whether physiological data for each condition (baseline, task, and recovery) could be collapsed into composite values, a series of one-tailed non-parametric independent samples and dependent samples t-tests were used to analyze any between like-condition differences. Wilcoxon signed-rank tests revealed no differences in RMSSD or lnHFHRV between the two baseline periods, two task periods, two recovery periods, two withdrawal scores, or two recovery scores (Table 3). For this reason, the conditions were collapsed across by taking the mean of the measurements for corresponding epochs, providing a single measure of RMSSD for baseline, task, recovery, withdrawal score, and recovery score and a single measure of lnHFHRV for baseline, task, recovery, withdrawal score, and recovery score for each participant. A series of one-tailed Mann-Whitney U tests were then performed in place of a repeated measure ANOVA to assess between-group differences in these measures. The means, standard deviations, and results of these analyses can be found in Table 4. Analyses addressing the specific hypotheses of the present study were then conducted on the composites for each epoch.

The first hypothesis was evaluated through one-tailed Mann-Whitney U tests. There were no statistically significant differences in baseline RMSSD or baseline lnHFHRV between children with ASD and their typically developing peers. However, differences in baseline RMSSD (U=68.00, Z=-1.58, p=.057, d=.56) and baseline lnHFHRV (U=70.0, Z=-1.49, p=.067, d=.71) trended towards significance and had medium and large effect sizes, respectively. With a larger sample size these tests may have reached significance.

The remaining hypotheses were tested through a series of multiple linear regression

analyses. The regression equations for these analyses are listed in Table 5, and the results of the lnHFHRV analyses and RMSSD analyses are summarized in Table 6 and Table 7, respectively. Diagnosis was included as a dummy coded predictor in all regression equations to account for a main effect of diagnosis on RRB severity. Furthermore, correlation analyses were performed to check whether IQ and age were significantly related to our outcome variable. These analyses revealed a significant relationship between IQ and RRB severity, $r(27) = -.38$, $p = .04$; however, there was no relationship between IQ and age. For this reason, IQ was added to the subsequent regression equations as a covariate.

First, a comparison model (Model 0) was assessed to compare the variance explained by IQ alone so that this could then be compared to the models that included the HRV predictors. IQ explained 12% of the variance in RRB severity, ($F(1, 27) = 4.66$, $p = .04$, $R^2 = .15$, $Adj\text{-}R^2 = .12$).

To assess the second hypotheses, the next set of regression analyses assessed whether baseline HRV measures predicted RRB severity. Two regressions were performed to evaluate both baseline HRV measures (baseline RMSSD and baseline lnHFHRV). Regression analyses revealed that IQ and baseline lnHFHRV explained a significant amount of variance in RRB severity ($F(2, 26) = 12.08$, $p < .001$, $R^2 = .48$, $Adj\text{-}R^2 = .44$), with baseline lnHFHRV predicting an additional 32% of variance in RRB severity compared to IQ alone. Similar results were found for baseline RMSSD, ($F(2,26) = 7.94$, $p = .002$, $R^2 = .38$, $Adj\text{-}R^2 = .33$), with baseline RMSSD predicting an additional 21% of variance in RRB severity compared to IQ alone. Within their respective equations, both baseline lnHFHRV ($\beta = -11.37$, $t(26) = -4.10$, $p < .001$) and baseline RMSSD ($\beta = -0.29$, $t(26) = -3.12$, $p = .004$) predicted RRB severity score. These results support the hypothesis that a significant portion of variance in the outcome variable (RRB severity) can be explained by the predictor (baseline lnHFHRV or baseline RMSSD) when controlling for IQ.

The third hypothesis was assessed through another set of multiple regression analyses, this time using lnHFHRV withdrawal and RMSSD withdrawal as predictors of RRB severity. Again, IQ was included as a covariate. Furthermore, since one of the task conditions involved a sensory stressor ('loud noise'), scores on the SSP were assessed in relation to RRB severity scores. There was a significant relationship between SSP total score and RRB severity ($r(27)=-.86$, $p<.001$), so SSP total score was added to these equations as a covariate. A new comparison model assessed the variance in RRB severity explained by IQ and SSP score. The resulting model was significant overall, ($F(2,26)=61.86$, $p<.001$, $R^2=.83$, $Adj\text{-}R^2=.81$). This model was then compared to the models which included RSA withdrawal measures. In the first equation (where RMSSD withdrawal was used as a predictor) the overall model was significant; however, RMSSD withdrawal did not predict a significant amount of variance in RRB severity. Instead, in this model the variance in RRB severity was explained by the covariates. However, in the second equation in which lnHFHRV withdrawal was used as a predictor, the overall model was significant ($F(3,23)=65.58$, $p<.001$, $R^2=.90$, $Adj\text{-}R^2=.88$) and lnHFHRV withdrawal predicted variance in RRB severity ($\beta=16.15$, $t(23)=4.48$, $p<.001$). The change in *adjusted*-R^2 revealed that lnHFHRV withdrawal predicted an additional 7% of the variance in RRB severity compared to IQ and SSP scores alone. These results support the hypothesis that lnHFHRV withdrawal explains variance in the outcome variable (RRB severity) when controlling for IQ and SSP scores.

Finally, the fourth hypothesis was also assessed through two multiple linear regressions. Again, IQ and SSP were included as covariates in these equations. These equations explained a significant amount of variance in RRB severity; however, neither RMSSD recovery score nor lnHFHRV recovery score predicted RRB severity. Instead, all of the variance was being

explained by the covariates in these equations. These results do not support the hypothesis that HRV recovery score explains variance in the outcome variable (RRB severity) when controlling for IQ and SSP scores.

3.1. Exploratory analyses

Exploratory analyses were conducted to analyze whether baseline RSA was differentially related to RRB subtypes and social communication deficits. The RBS-R sameness subscale scores, RBS-R stereotyped motor movement subscale scores, and SRS-2 social communication index scores were assessed in relation to baseline lnHFHRV and baseline RMSSD. The results from these correlations can be found in Table 8. A significant relationship was found between RBS-R sameness subscale scores and both baseline lnHFHRV ($r(27)=-.63$, $p=.001$) and baseline RMSSD ($r(27)=-.53$, $p=.003$); however, they did not relate to the RBS-R stereotyped movement subscale scores. The SRS-2 social communication index scores also correlated with baseline lnHFHRV ($r(27)=-.40$, $p=.03$) and baseline RMSSD ($r(27)=-.39$, $p=.04$).

4.0. Discussion

The proposed hypotheses and subsequent analyses in this study were consistent with the neurovisceral integration model and the Polyvagal theory (Porges, 2007), as well as the literature summarized by Graziano & Derefinko (2013), in that low baseline vagal control was shown to predict less adaptive behaviors in children with and without an ASD diagnosis. While the present study did not replicate the between group differences in baseline vagal control between children with ASD and their typically developing peers often seen in the literature, with a larger sample size it is possible that these analyses would become statistically significant. However, the second hypothesis regarding the capacity for baseline RSA to predict maladaptive behavior (in this case RRB severity) was supported by the study. Not only do the findings support the second

hypothesis, but also provide new information about the utility of RSA and ANS measurement in the field of RRB research. While RSA has been studied in relation to social skills in ASD, few studies have examined its relationship with the other half of an ASD diagnosis. This is perplexing since much of the early literature on RRBs was focused on their relation to differences in ANS activity. The results from this study provide evidence for a link between aberrant baseline PNS activity and RRBs.

Though the hypotheses regarding baseline measures of RSA were supported, RSA withdrawal results were mixed in this study. RSA withdrawal has been found to be adaptive in stressful situations, allowing for flexible behavior (Graziano & Derefinko, 2013). Due to the inflexibility associated with RRBs, it was expected that dampened RSA withdrawal would be predictive of more severe RRBs. However, it is important to note that the physiological response to stress can either manifest as withdrawal or augmentation, and thus it is more appropriate to refer to this variable as 'RSA reactivity.' The present study predicted that RSA reactivity in the form of dampened withdrawal would be predictive of more severe RRBs since dampened withdrawal is traditionally proposed to be the aberrant physiological response in the literature. For this reason, RSA reactivity in the form of augmentation during the stressor task was unexpected. Increased RSA is typically associated with positive mood states and not stress. However, there is some debate in the field as to what psychological processes RSA reactivity actually indicates. RSA withdrawal in the presence of a stressor is often framed as an adaptive reaction as it indicates the activation of the fight-or-flight response (Beauchaine, 2001; Porges, 1995), whereas RSA augmentation would be indicative of a more relaxed/positive emotional state. In reality the difference between RSA withdrawal and RSA augmentation is not well understood, though it has been suggested that there may be an important distinction between

them and that differences in these responses may be context dependent (Graziano & Derefinko, 2013). The RSA reactivity findings in this study indicated that RSA augmentation during the stressor was associated with more severe RRBs. There is evidence that RSA augmentation is suggestive of increased demand on self-regulatory efforts as opposed to positive emotional experience (Butler et al., 2006). For instance, children from families with a history of domestic violence are more likely to display RSA augmentation in response to interpersonal conflict (Katz, 2007). These results suggested that a hypervigilance to conflict leads to RSA augmentation during the stressor as a function of increased attention and active coping efforts. From this perspective, increased RSA during the stressors in the present study may be signaling greater self-regulatory or coping efforts in these children. In that context, it makes sense that increased RSA/self-regulatory effort to a stressor would predict RRB severity, as RRBs are the result of an inflexible behavior and self-regulatory deficits. Furthermore, the ability for RSA reactivity to predict RRB severity in the present study highlights the importance of assessing multiple facets of ANS activity. Oftentimes researchers only focus on baseline measures of RSA, but this finding reinforces the importance of assessing the reactivity of the system to change and stress. It is particularly important to incorporate this when studying clinical populations, as they often experience excessive amounts of anxiety or stress compared to a typically developing population, making reactivity even more relevant.

4.1. Limitations

The primary limitation of the current study was the unequal sample sizes between the ASD and TD group. This was due in part to difficulties recruiting children with an ASD diagnosis to participate in the study. Furthermore, though 15 children with an ASD diagnosis were brought into the lab, baseline data was only acquired for 13 of these participants and full

data was only acquired for 10. Much of the loss of data was due to signal noise issues that were encountered when using the BioHarness. While there are benefits to using a wireless heart rate monitor in a younger population, movement artifact was more severe in the signal acquired from the BioHarness compared to signals collected through wired ECG systems. The increased movement artifact made getting full data challenging. Researchers using cardiac measures should be mindful of this trade-off when designing future studies so as to avoid data acquisition issues.

The unequal sample sizes also affected some of the study's analyses. While the findings supported the hypotheses regarding baseline RSA and RSA reactivity in relation to RRB severity, there were limitations that primarily influenced the analyses evaluating the first hypothesis regarding group differences at baseline between children with and ASD diagnosis and their TD peers. The small and unequal sample sizes of these two groups required the use of non-parametric t-tests to assess differences in the groups' RSA measures (Lincoln, 1952); however, unequal sample sizes in combination with unequal variance between groups has been shown to influence the power of the statistical tests. Specifically, the Mann-Whitney U test is shown to be less powerful when the smaller sample is associated with larger variance (Zimmerman, 1987), as is the case with the baseline RSA measures in this study. Less powerful tests correspond to increased Type II error (falsely failing to reject the null hypothesis), which might contribute to the null finding for the first hypothesis. Furthermore, baseline RSA measures appeared to be lower in the ASD group compared to the TD group as evidenced by moderate to large effect sizes in baseline RMSSD (d=.56) and baseline lnHFHRV (d=.71), and trended toward significance (p=.057 and p=.068, respectively). A larger sample with equal group sizes may have resulted in a significant test and supported the study's first hypothesis, which would have been consistent with the finding that children with ASD have lower baseline RSA compared to their

TD peers shown in multiple studies (Van Hecke et al., 2009; Bal et al., 2010; Klusek, Roberts, & Losh, 2015).

Another limitation of this study is the lack of variation in the sample, particularly in regard to IQ. The ASD group had a mean IQ within the average intelligence range (*M*=101.92, *SD*=21.39; average range: 85-115), and the TD group was only slightly above average (*M*=116.38, *SD*=11.67; above average range: 116-130). In the entire sample, only four participants fell in the below average IQ range and none fell into the lower extreme range (Figure 1). Ideally a sample with greater variation in IQ scores would have been attained. This is particularly important as a majority of individuals with ASD typically fall within the lower extreme/intellectual disability IQ range (55%), with only 28% of individuals with ASD falling within the average intelligence range and 3% in the above average range (Charman et al., 2010). In light of that distribution, it is evident that the sample in this study is not representative of the full breadth of functioning seen in children with ASD. A wide-ranging sample is useful for multiple reasons, but is particularly important when investigating RRBs as certain types of RRBs have been shown to be associated with lower IQ (Carcani-Rathwell et al., 2006; Lam et al., 2008). A more representative sample could have been achieved by recruiting participants with certain characteristics to diversity the sample, such as an ASD only group and an ASD with intellectual disability group, or by attaining a larger sample size. While the findings of the study are promising, it is important that future research looks at cardiac vagal control and RRBs in individuals across a spectrum of functioning.

4.2. Future Directions and Applications

The results of the present study provide support for further investigating the role of cardiac vagal control in relation to RRBs. One way in which this research could be extended

would be through incorporating a wider array of diagnoses while using a similar methodological approach. While this study used a transdiagnostic approach by including children with ASD and TD children, the addition of other populations that display RRB-like behaviors could be informative. RRBs are commonly observed in individuals with intellectual disabilities (Thelen, 1981). Furthermore, obsessive compulsive disorder is characterized by compulsive thoughts, which relate to constructs such as IS and CI, as well as repetitive motor behaviors. These motoric repetitions are also the primary feature of tic disorders (American Psychiatric Association, 2013). The inclusion of individuals with intellectual disability, obsessive compulsive disorder, and tic disorders in addition to those with ASD would help determine whether cardiac vagal control measures predict RRB severity transdiagnostically. If this is the case, this would provide evidence for a common mechanism behind this class of behaviors. That being said, there is the possibility of finding that these measures do no consistently predict RRBs across diagnoses, and instead relate to particular RRB subgroups that are differentially presented across these disorders. Similarly, another important future direction is the examination of RRB subgroups in relation to cardiac vagal measures within ASD. As discussed previously, RRB subgroups within ASD have been well-established behaviorally. The investigation of a biological marker related to these differing subgroups is the next step in this research. The exploratory analyses presented in this study provide support for differential biological markers for RRB subgroups, as the measures used in this study significantly correlated with IS behaviors but not with RMBs. Distinct biological markers for different RRB subgroups could subsequently lead to unique biological mechanisms for these behavioral subgroups. Such information could have major implications for how different types of RRBs are addressed during treatment.

The hypotheses of this study linking RSA to inflexible behaviors like RRBs were built on

principles from the neurovisceral integration model, which proposes an entire central autonomic network related to PNS function. Since the results of this study support the concepts put forth by this model, the proposed pathways within this network should be further investigated in relation to RRBs. In fact, some imaging studies have already found structural and functional links between RRBs and neural structures within the central autonomic network. One such structure is the anterior cingulate, which has been found to be associated with these behaviors such that decreased anterior cingulate activity is associated with more severe RRBs (Thakkar et al., 2008; Shafritz et al., 2008). By taking a multilevel approach and investigating these structures in conjunction with RSA in relation to these behaviors, this network could potentially help parse ASD into more homogenous subtypes not only for research purposes but also to inform effective clinical interventions for varying subtypes and severities of RRBs. Additionally, it is unknown whether or not differential patterns of RRBs are present in ASD versus those seen in other developmental disabilities (Rogers & Ozonoff, 2005); however, establishing a biological profile and function of these behaviors in the ASD (where they are most commonly exhibited) could be helpful in addressing their occurrence in other diagnoses.

The relationship between these variables should also be further investigated to determine whether or not an approach such as HRV-focused biofeedback could be beneficial in decreasing RRB severity in individuals with ASD. HRV biofeedback has shown promise in addressing issues across a variety of diagnoses and symptoms (Lehrer & Gevirtz, 2014). For instance, HRV biofeedback has been shown to be a relatively easy, useful supplement to traditional approaches in the treatment of anxiety disorders (Reiner, 2008) and to effectively decrease depressive symptoms in as little as four biofeedback sessions (Karavidas, 2008). Other biofeedback techniques, such as neurofeedback, have shown promising results in decreasing ASD

symptomatology, such as social communication deficits and stereotyped behaviors (Coben et al., 2010). These findings indicate that individuals with ASD could potentially engage in HRV biofeedback training. Research on HRV biofeedback would also establish whether cardiac vagal control is part of a causal mechanism contributing to the RRBs observed in ASD, which would not only demonstrate the benefits of HRV biofeedback as a therapeutic strategy, but also provide confirmation of a biological mechanism of RRBs that could be investigated in future research.

4.3. Summary and Conclusion

RRBs are considered a key diagnostic criterion for ASD in the DSM 5 (American Psychiatric Association, 2013); however, they are underrepresented in the literature. Their highly heterogeneous manifestation in ASD (Lam, Bodfish, & Piven, 2008; Bishop et al., 2013), indicates intradiagnostic differences that could be important in identifying ASD subgroups. The present study provided evidence that cardiac vagal control measures are predictive of RRB severity, which had not been previously investigated in the literature. The exploratory analyses in this study provide a particularly interesting springboard for future directions in the field of psychophysiological research in relation to RRBs, as it appears that there may be patterns of ANS activity that correspond with behaviorally established RRB subtypes, namely IS and RMBs (Bishop et al., 2013; Lam, Bodfish, & Piven, 2008).While RBS-R Sameness subscale scores (corresponding the IS subgroup of RRBs) were related to both measures of baseline RSA, the RBS-R stereotyped movement subscale score (corresponding to the RMB subgroup of RRBs) was not. This differential relationship is promising evidence of a differential biological mechanism for these RRB subtypes.

References

Allen, J.J.B., Chambers, A.S., & Towers, D.N. (2007). The many metrics of cardiac chronotropy: A pragmatic primer and a brief comparison of metrics. *Biological Psychology, 74*, 243-262.

Appelhans, B.M., & Luecken, L.J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of General Psychology, 10,* 229-240.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing.

Autism and Developmental Disabilities Monitoring Network Surveillance Year 2006 Principal Investigators, Centers for Disease Control and Prevention. (2006). Prevalence of autism spectrum disorders - Autism and Developmental Disabilities Monitoring Network, United States, 2006. *MMWR Surveillance Summary, 58,* 1–20.

Bain, S. K., & Jaspers, K. E. (2010). Test Review: Review of Kaufman Brief Intelligence Test: Kaufman, AS, & Kaufman, NL (2004). "Kaufman Brief Intelligence Test." Bloomington, MN: Pearson, Inc. *Journal of Psychoeducational Assessment, 28*, 167-174.

Bal, E., Harden, E., Lamb, D., Van Hecke, A.V., Denver, J.W., & Porges, S. W. (2010). Emotion recognition in children with autism spectrum disorders: Relations to eye gaze and autonomic state. *Journal of Autism and Developmental Disorders, 40*, 358 –370.

Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13*, 183-214.

Benarroch, E. (1993). The central autonomic network: Functional organization, dysfunction, and perspective. *Mayo Clinic Proceedings, 68*, 988-1001.

Berntson, G.G., Bigger, J.T., Eckberg, D.L., Grossman, P., Kaufmann, P.G., Malik, M., Nagaraja, H.N., Porges, S.W., Saul, J.P., Stone, P.H., & Van Der Molen, M.W. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology, 34*, 623-648.

Berntson, G.G., Quigley, K.S., & Lozano, D. (2007). Cardiovascular Psychophysiology. In Cacioppo, J. T., Tassinary, L. G., & Berntson, G. (Eds.), *Handbook of Psychophysiology (182-210)*. New York: Cambridge University Press.

Bishop, S.L., Hus, V., Duncan, A., Huerta, M., Gotham, K., Pickles, A., Kreiger, A., Buja, A., Lund, S., & Lord, C. (2013). Subcategories of restricted and repetitive behaviors in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 43*, 1287-1297.

Bodfish, J.W., Symons, F.J., Parker, D.E., & Lewis, M.H. (2000). Varieties of repetitive behavior in autism: Comparisons to mental retardation. *Journal of Autism and Developmental Disorders, 30,* 237-243.

Butler, E.A., Wilhelm, F.H., & Gross, J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology, 43*, 612-622.

Carcani-Rathwell, I., Rabe-Hasketch, R., & Santosh, P.J. (2006). Repetitive and stereotyped behaviours in pervasive developmental disorders. *Journal of Child Psychology and Psychiatry, 47*, 573-581.

Charman, T., Pickles, A., Simonoff, E., Chandler, S., Loucas, T., & Baird, G. (2010). IQ in children with autism spectrum disorders: data from the Special Needs and Autism Project. *Psychological Medicine, 41*, 619-627.

Coben, R., Linden, M., & Myers T.E. (2010). Neurofeedback for autistic spectrum disorder: a

review of the literature. *Applied Psychophysiology and Biofeedback, 35,* 83-105.

Cunningham, A.B., & Schreibman, L. (2008). Stereotypy in autism: The importance of function. *Research in Autism Spectrum Disorders, 2,* 469-479.

Cuthbert, B.N., & Kozak, M.J. (2013). Constructing constructs for psychopathology: the NIMH Research Domain Criteria. *Journal of Abnormal Psychology, 122,* 928-937.

DesLauriers, A.M., & Carlson, C.F. (1969). *Your child is asleep: Early infantile autism.* Homewood, IL: Dorsey Press.

Ellis, R. J., Sollers, J. J., Edelstein, E. A., & Thayer, J. F. (2008). Data transforms for spectral analyses of heart rate variability. *Biomedical Sciences Instrumentation, 44,* 392-397.

Esbensen, A.J., Seltzer, M.M., Lam, K.S.L., & Bodfish, J.W. (2008). Age-related differences in restricted repetitive behaviors in autism spectrum disorders. *Journal of Autism and Developmental Disorders, 39,* 57- 66.

Faul, F., Erdfelder, E., Lang, A.G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39,* 175-191.

Friedman, B.H., Allen, M.T., Christie, I.C., & Santucci, A.K. (2002). Validity concerns of common heart-rate variability indices. *IEEE Engineering in Medicine & Biology, 21,* 35-40.

Friedman, B.H. (2007). An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biological Psychology, 74,* 185-199.

Gabriels, R.L., Cuccaro, M.L., Hill, D.E., Ivers, B.J., & Goldson, E. (2005). Repetitive Behaviors in autism: relationships with associated clinical features. *Research in Developmental Disabilities, 26,* 169-181

Goodwin, M.S., Groden, J., Velicer, W.F., Lipsitt, L.P., Baron, M.G., Hofmann, S.G., & Groden, G. (2006). Cardiovascular arousal in individuals with autism. *Focus on Autism and Other Developmental Disabilities, 21*, 100-123.

Graveling, R.A., & Brooke, J.D. (1978). Hormonal and cardiac response of autistic children to changes in environmental stimulation. *Journal of Autism and Childhood Schizophrenia, 8*, 441-453.

Graziano, P., & Derefinko, K. (2013). Cardiac vagal control and children's adaptive functioning: a meta-analysis. *Biological Psychology, 94*, 22-37.

Grossman, P., Van Beek, J., Wientjes, C. (1990). A comparison of three quantification methods for estimation of respiratory sinus arrhythmia. *Psychophysiology, 27*, 702-714.

Happe, F., Ronald, A., & Plomin, R. (2006). Time to give up on a single explanation for autism. *Nature Neuroscience, 9,* 1218-1220.

Harrop, C., McBee, M., & Boyd, B.A. (2016). How are child restricted and repetitive behaviors associated with caregiver stress over time? A parallel process multilevel growth model. *Journal of Autism and Developmental Disorders, 46*, 1773-1783.

Hirstein, W., Iversen, P., & Ramachandran V.S. (2001). Autonomic responses of autistic children to people and objects. *Proceedings of the Royal Society B: Biological Sciences, 268*, 1883-1888.

Honey, E., Rodgers, J., & McConachie, H. (2012). Measurement of restricted and repetitive behaviour in children with autism spectrum disorder: Selecting a questionnaire or interview. *Research in Autism Spectrum Disorders, 6,* 757-776.

Hubert, B.E., Wicker, B., Monfardini, E., & Deruelle, C. (2009). Electrodermal reactivity to emotion processing in adults with autism spectrum disorders. *Autism, 13*, 9-19.

Hutt, C., & Hutt, S.J. (1965). Effects of environmental complexity on stereotyped behaviours of children. *Animal Behaviour, 13,* 1-4.

Hutt, C., Hutt, S.J., Lee, D., & Ounsted, C. (1964). Arousal and childhood autism. *Nature, 204,* 908-909.

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D.S., Quinn, K., Sanislow, C., & Wang, P. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *American Journal of Psychiatry, 167,* 748-751.

Joosten, A.V., Bundy, A.C., & Einfeld, S.L. (2009). Intrinsic and extrinsic motivation for stereotypic and repetitive behavior. *Journal of Autism and Developmental Disorders, 39,* 521 - 531.

Karavidas, M. (2008). Heart rate variability biofeedback for major depression. *Biofeedback, 36,* 18-21.

Katz, L.F. (2007). Domestic violence and vagal reactivity to peer provocation. *Biological Psychology, 74,* 154-164,

Kaufman, A.S., & Kaufman, N.L. (1990). *Manual for the Kaufman Brief Intelligence Test*. Circle Pines, MN: American Guidance Service.

Klusek, J., Roberts, J.E., & Losh, M. (2015). Cardiac autonomic regulation in autism and Fragile X Syndrome: A review. *Psychological Bulletin, 141,* 141-175.

Kylliäinen, A., & Hietanen, J.K. (2006). Skin conductance responses to another person's gaze in children with autism. *Journal of Autism and Developmental Disorders, 36,* 517-525.

Lacey, J.I. (1967). Somatic response patterning and stress: Some revisions of activation theory. In M.H. Appley & R. Trumbull (Eds.), *Psychological Stress: Issues in Research*. Appleton, NY: Century-Crofts, pp. 14-37.

Lam, K. S. L. and M. G. Aman (2007). The Repetitive Behavior Scale-Revised: independent validation in individuals with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 37,* 855-866.

Lam, K.S.L., Bodfish, J.W., & Piven, J. (2008). Evidence for three subtypes of repetitive behavior in autism that differ in familiarity and association with other symptoms. *Journal of Child Psychiatry, 49,* 1193-1200.

Lehrer, P.M., & Gevirtz, R. (2014). Heart rate variability biofeedback: how and why does it work? *Frontiers in Psychology, 5,* 756.

Lincoln, M. (1952). Non-parametric statistics for psychological research. *Psychological Bulletin, 49,* 122-143.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders,* 24, 659-685

Malik, M., Bigger, J.T., Camm, A.J., Kleiger, R.E., Malliani, A., Moss, A.J. et al. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European society of cardiology and the North American society of pacing and electrophysiology. *Circulation, 93,* 1043–1065.

Militerni, R., Bravaccio, C., Falco, C., Fico, C., & Palermo, M.T. (2002). Repetitive behaviors in autism. *European Child & Adolescent Psychiatry, 11,* 210-218.

Ming, X., Julu, P.O., Brimacombe, M., Connor, S., & Daniels, M.L. (2005). Reduced cardiac parasympathetic activity in children with autism. *Brain & Development, 27,* 509-516.

Patriquin, M.A., Scarpa, A., Friedman, B.H., & Porges, S.W. (2013). Respiratory sinus arrhythmia: A marker for positive social functioning and receptive language skills in

children with autism spectrum disorder. *Developmental Psychobiology, 55*, 101-112.

Porges, S.W. (1995). Orienting in a defensive world: Mammalian modifications of our evolutionary heritage. A Polyvagal Theory. *Psychophysiology, 32*, 301-318.

Porges, S.W. (2003). The Polyvagal Theory: Phylogenetic contributions to social behavior. *Physiology and Behavior, 79*, 503-513.

Porges, S.W. (2007). The polyvagal perspective. *Biological Psychology, 74*, 116-143.

Reiner, R. (2008). Integrating a portable biofeedback device into clinical practice for patients with anxiety disorders: Results of a pilot study. *Applied Psychophysiology and Biofeedback, 33*, 55-61.

Rogers, S.J., & Ozonoff, S. (2005). Annotation: What do we know about sensory dysfunction in autism? A critical review of the empirical evidence. *Journal of Child Psychology and Psychiatry, 46*, 1255-1268.

Shafritz, K.M., Dichter, G.S., Baranek, G.T., & Belger, A. (2008). The neural circuitry mediating shifts in behavioral response and cognitive set in autism. *Biological Psychiatry, 63*, 974-980.

Sharma, R. K., Sagar, R., Deepak, K. K., Mehta, M., & Balhara, Y. P. S. (2011). Clinical and autonomic functions: a study of childhood anxiety disorders. *Annals of Saudi Medicine, 3*, 250-257.

Sherwood, A., Allen, M.T., Fahrenberg, J., Kelsey, R.M., Lovallo, W.R., & van Doornen, L.J.P. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology*, 27, 1-23.

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., & Karjalainen, P. A. (2009). Kubios HRV—a software for advanced heart rate variability analysis. In *4th*

European Conference of the International Federation for Medical and Biological Engineering, 1022-1025.

Thakkar, K.N. Polli, F.E., Joseph, R.M., Tuch, D.S., Hadjikhani, N., Barton, J.J.S., & Manoach, D.S. (2008). Response monitoring, repetitive behavior and anterior cingulate abnormalities in autism spectrum disorder (ASD). *Brain, 232,* 2464-2478.

Thayer, J.F., & Friedman, B.H. (2004). A neurovisceral integration model of health disparities in aging. In N.B. Anderson, R.A., Bulatao, and B. Cohen (Eds.), *Critical perspective on racial and ethnic differences in health in late life*. Washington, D.C.: The National Academies Press, pp. 567-603.

Thayer, J.F., Hansen, A.L., Saus-Rose, E., & Johnsen, B.H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Annals of Behavioral Medicine, 37,* 141-153.

Thayer, J.F., & Lane, R.D. (2009). Claude Bernard and the heart–brain connection: Further elaboration of a model of neurovisceral integration. *Neuroscience & Biobehavioral Reviews, 33,* 81-88.

Thelen, E. (1981). Rhythmical behavior in infancy: An ethological perspective. *Developmental Psychology, 17,* 237-257.

Van Hecke, A. V., Lebow, J., Bal, E., Lamb, D., Harden, E., Kramer, A., Denver, J., Bazhenova, O., & Porges, S. W. (2009). Electroencephalogram and heart rate regulation to familiar and unfamiliar people in children with autism spectrum disorder. *Child Development, 80,* 1118-1133.

Willemsen-Swinkels, S.H.N., Buitelaar, J.K., Dekker, M., and van Engeland, H. (1998).

Subtyping stereotypic behavior in children: the association between stereotypic behavior, mood, and heart rate. *Journal of Autism and Developmental Disorders*, 28, 547-557.

Zimmerman, D.W. (1987). Comparative power of student T Test and Mann-Whitney U Test for unequal sample sizes and variances. *The Journal of Experimental Education, 55*, 171-174.

Table 1.

Participant demographics. Means (M) and standard deviations (SD) of demographic variables by diagnostic group.

	ASD (*n* = 13)		TD (*n* = 16)	
	M	SD	M	SD
Age (in months)	89.23	23.05	83.25	12.77
KBIT-2 Full Scale IQ	101.92	21.39	116.38	11.67
RBS-R Total	42.00	20.44	1.56	3.01
SSP Total	114.85	30.46	174.81	11.13
SRS-2 Total	79.92	11.08	44.94	4.71

Note. M=Mean. SD = Standard deviation.

Table 2.

Mann-Whitney U tests of respiration rate (RR) between groups in six epochs.

	Mean Rank		*Z*	*p*
	ASD	TD		
Baseline 1 RR	16.21	13.22	-.95	.35
Task 1 RR	15.27	13.13	-.69	.51
Recovery 1 RR	14.35	12.97	-.45	.66
Baseline 2 RR	14.63	14.41	-.07	.95
Task 2 RR	16.25	13.19	-.98	.35
Recovery 2 RR	17.25	12.44	-1.53	.13

Note. Task 1 = 'unstructured time' condition. Task 2 = 'loud noise' condition. RR is measured in breaths per minute.

Table 3.

Wilcoxon signed-rank tests analyzing lnHFHRV (ms^2) and RMSSD (ms) across corresponding epochs.

	Task 1 Block		Task 2 Block			
	M	*SD*	*M*	*SD*	*Z*	*p*
lnHFHRV Baseline	7.26	1.32	7.20	1.15	-1.32	.19
lnHFHRV Task	7.42	1.08	7.27	1.05	-1.66	.098
lnHFHRV Recovery	7.64	.96	7.47	1.06	-.63	.53
lnHFHRV Withdrawal	.093	.62	.042	.54	-.55	.58
lnHFHRV Recovery Score	.12	.60	.20	.65	-.83	.41
RMSSD Baseline	78.36	41.24	77.31	44.00	-.26	.79
RMSSD Task	82.03	42.47	76.23	38.77	-1.87	.061
RMSSD Recovery	88.74	43.12	84.06	41.54	-.014	.99
RMSSD Withdrawal	1.89	18.17	-1.320	15.68	-.40	.69
RMSSD Recovery Score	3.21	28.20	7.83	17.88	-1.40	.16

Note. M = Mean. *SD* = Standard deviation. Task 1 = 'unstructured time' condition. Task 2 = 'loud noise' condition.

Table 4.

One-tailed Mann-Whitney U comparing collapsed lnHFHRV (ms^2) and RMSSD (ms) in different epochs between groups.

	ASD			TD					
	M	*SD*	*n*	*M*	*SD*	*n*	*Z*	*d*	*p*
lnHFHRV Baseline	6.70	1.50	13	7.57	.86	16	-1.49	.71	.068
lnHFHRV Task	6.91	1.25	11	7.63	.84	16	-1.23	.67	.11
lnHFHRV Recovery	7.23	1.21	10	7.76	.77	16	-1.42	.52	.077
lnHFHRV Withdrawal	.16	.63	11	.058	.30	16	-.099	.21	.46
lnHFHRV Recovery Score	.21	.47	10	.13	.13	16	-.34	.18	.37
RMSSD Baseline	64.63	40.60	13	86.87	39.47	16	-1.58	.56	.057
RMSSD Task	66.40	39.43	11	87.90	39.64	16	-1.48	.54	.069
RMSSD Recovery	80.14	43.70	10	90.63	9.54	16	-.84	.26	.20
RMSSD Withdrawal	-.032	8.40	11	-3.03	16.30	16	-.44	.23	.33
RMSSD Recovery Score	10.16	5.71	10	2.73	4.57	16	-.84	.41	.20

Note. M=Mean. *SD*=Standard deviation. *n*=group sample size. *d*=Cohen's d (effect size).

Table 5.

Regression analysis equations.

Hypothesis	Regression equation	Conditions
Model 0	$\hat{Y}=b_0+b_1(IQ)$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when all predictors are 0 b_1 = change in RRB severity when IQ increases by one unit
Hypothesis #2	$\hat{Y} = b_0+b_1(\text{Baseline HRV})+b_2(IQ)$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when predictors are 0 b_1 = change in RRB severity when baseline RSA increases by one unit b_2 = change in RRB severity when IQ increases by one unit
Hypothesis #3	$\hat{Y}=b_0+b_1(\text{HRV withdrawal})+ b_2(IQ)+b_3(\text{SSP Score})$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when predictors are 0 b_1 = change in RRB severity when RSA reactivity increases by one unit b_2 = change in RRB severity when IQ increases by one unit b_3 = change in RRB severity when SSP score increases by one unit
Hypothesis #4	$\hat{Y}=b_0+b_1(\text{HRV recovery score})+b_2(IQ)+b_3(\text{SSP Score})$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when predictors are 0, b_1 = change in RRB severity when RSA recovery score increases by one unit b_2 = change in RRB severity when IQ increases by one unit b_3 = change in RRB severity when SSP score increases by one unit

Table 6.

Regression analyses for lnHFHRV (ms^2) metrics.

	Model 0		**Model 1**		**Model 2**		**Model 3**	
Predictor	*B*	*SE*	*B*	*SE*	*B*	*SE*	*B*	*SE*
IQ	-.52*	.24	-.50*	.19	-.30*	.093	-.34*	.11
Baseline lnHFHRV			-11.37*	2.78				
SSP Total					-.54*	.045	-.55*	.054
lnHFHRV Reactivity					16.15*	3.60		
lnHFHRV Recovery Score							-5.94	3.85
F	4.66*		12.08*		65.58*		39.72*	
Adjusted R^2	.12		.44		.88		.82	

Note. *p<0.05

Table 7.

Regression analyses for RMSSD (ms) metrics.

	Model 0		**Model 1**		**Model 2**		**Model 3**	
Predictor	*B*	*SE*	*B*	*SE*	*B*	*SE*	*B*	*SE*
IQ	-.52*	.24	-.54*	.21	-.42*	.12	-.36*	.11
Baseline RMSSD			-.29*	.093				
SSP Total					-.52*	.060	-.54*	.056
RMSSD Reactivity					.18	.16		
RMSSD Recovery Score							-.14	.11
F	4.66*		7.94*		33.81*		38.09*	
Adjusted R^2	.12		.33		.79		.82	

Note. *p<0.05

Table 8.

Pearson's correlation (r) of RBS-R and SRS-2 subscale scores with baseline RSA metrics.

Symptom score	**Baseline lnHFHRV (ms^2)**	**Baseline RMSSD (ms)**
RBS-R Stereotyped motor behavior subscale	-.36	-.28
RBS-R Sameness subscale	-.63**	-.53**
SRS-2 Social Communication Index	-.40*	-.39*

Note. *p<0.05, two-tailed, **p<0.01, two-tailed

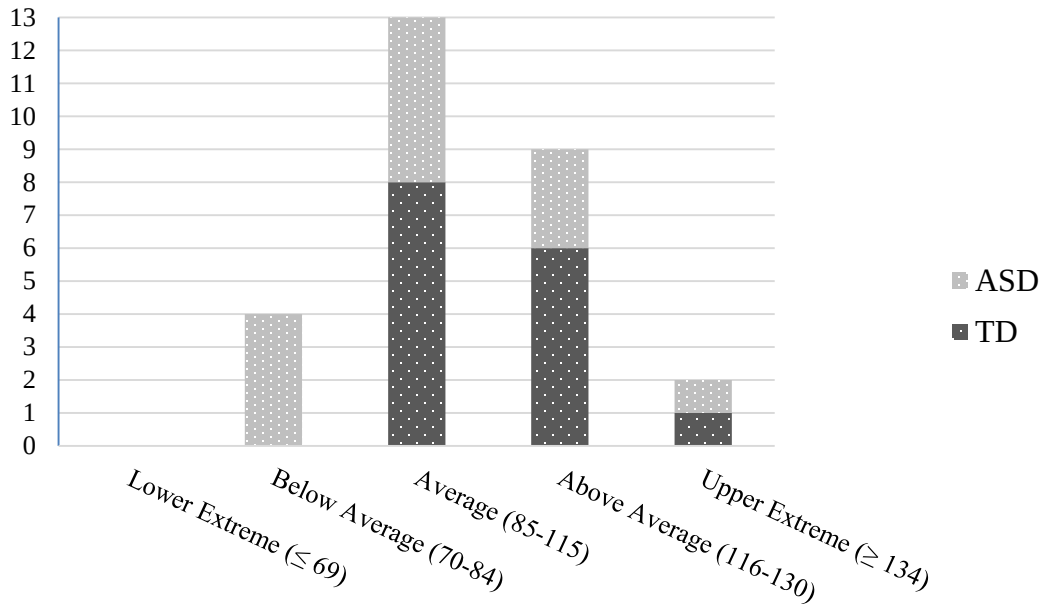

Figure 1. *Histogram of KBIT-2 composite score IQ classifications for ASD and TD groups.*

Appendix A

Child History Form

Child's Name:_____ Today's Date:_____

Age:_____ Gender: M___ F___

Country of Birth: _____

Race: Asian _____ Black_____ Hispanic_____ White_____ Other_____

Forms completed by:_____

Relationship to child (please circle one):

- Biological Mother
- Biological Father
- Adoptive Mother
- Adoptive Father
- Foster Mother
- Foster Father
- Other: _____

Child's Medical History

What is your child's current height? _____ Current weight? _____

Is your child: Right handed __ Left handed__ Mixed handedness__ D/K__

Medical conditions:

___Meningitis

___Heart Disease

___Seizures

___Bone disease

___Cancer

___Encephalitis

___Heart murmur

___Leukemia

___Muscle disease

___Measles

___Asthma

___Hydrocephalus

___Anemia

___Kidney problems

___Mumps

___Diabetes

___Cerebral palsy

___Arthritis

___Tuberculosis

___Chicken pox

___None of the above

Hospitalizations: Has you child ever had any medical hospitalizations: Yes___ No___

Age: Length of stay: Reason for hospitalization:

_____

_____

Medications: If your child is currently taking any medications, please specify the name of the medications and the following: dosage & time of day medicine is taken.

None _____

ADHD medications _____

Anti-depressant medications_____

Anti-anxiety medications_____

Anti-seizure medications_____

Allergy/Asthma medications_____

Other medications_____

_____

Has your child ever had a diagnosis other than autism? Yes___ No___

If yes, what was the diagnosis? _____

_____

Child's Psychiatric/Emotional History

Has child previously been diagnosed (by anyone) with (please circle all that apply):

Autistic disorder	Yes	No
Asperger syndrome	Yes	No
Pervasive Developmental Disorder (PDD-NOS)	Yes	No
Autism spectrum disorder	Yes	No
Any form of intellectual disability (e.g., MR or ID)	Yes	No
Communication disorder (i.e., language disorder, speech sound disorder, childhood fluency disorder)	Yes	No
Social (pragmatic) communication disorder	Yes	No
Generalized anxiety disorder	Yes	No
Separation anxiety disorder	Yes	No
Obsessive compulsive disorder	Yes	No
Social anxiety disorder or social phobia	Yes	No
Selective mutism	Yes	No
Specific phobia	Yes	No
Panic disorder	Yes	No
Posttraumatic stress disorder	Yes	No
Any other anxiety disorder	Yes	No
Oppositional anxiety disorder	Yes	No
Conduct disorder	Yes	No
Attention deficit hyperactivity disorder (ADHD)	Yes	No
Depression/Dysthymia	Yes	No
Disruptive mood dysregulation disorder	Yes	No

Does child have any other psychiatric, neurodevelopmental, or medical diagnosis?	Yes	No
If yes, please specify diagnosis:		

Family Medical History

Do any members of the family have a medical or psychological problem? Yes___ No___

Examples include, but are not limited to:

Genetic/inherited diseases (condition that runs in the family) Yes___ No___

Autism/Pervasive Developmental Disorder Yes___ No___

Seizures, starting spells or "fits" Yes___ No___

Developmental delay or mental retardation Yes___ No___

Schizophrenia or depression Yes___ No___

If yes was checked for any of the above, list this person's name and briefly describe:

Name:_____ Relation to child:_____

Concern_____

Name:_____ Relation to child:_____

Concern_____

Appendix B

Supplementary Analyses

Table 5.

Regression analysis equations.

Hypothesis	Regression equation	Conditions
Model 0	$\hat{Y}=b_0+b_1(\text{diagnosis})+b_2(\text{IQ})$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when all predictors are 0 b_1 = change in RRB severity when diagnosis increases by one unit b_2 = change in RRB severity when IQ increases by one unit
Hypothesis #2	$\hat{Y} = b_0+b_1(\text{Baseline HRV})+b_2(\text{diagnosis})+b_3(\text{IQ})$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when predictors are 0 b_1 = change in RRB severity when baseline RSA increases by one unit b_2 = change in RRB severity when diagnosis increases by one unit b_3 = change in RRB severity when IQ increases by one unit
Hypothesis #3	$\hat{Y}=b_0+b_1(\text{HRV withdrawal})+b_2(\text{diagnosis})+b_3(\text{IQ})+b_4(\text{SSP Score})$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when predictors are 0 b_1 = change in RRB severity when RSA reactivity increases by one unit b_2 = change in RRB severity when diagnosis increases by one unit b_3 = change in RRB severity when IQ increases by one unit b_4 = change in RRB severity when SSP score increases by one unit
Hypothesis #4	$\hat{Y}=b_0+b_1(\text{HRV recovery score})+b_2(\text{diagnosis})+b_3(\text{IQ})+b_4(\text{SSP Score})$	$\hat{Y}$ = predicted RRB severity, b_0 = predicted value of RRB severity when predictors are 0, b_1 = change in RRB severity when RSA recovery score increases by one unit b_2 = change in RRB severity when diagnosis increases by one unit b_3 = change in RRB severity when IQ increases by one unit b_4 = change in RRB severity when SSP score increases by one unit

Table 6.

Regression analyses for lnHFHRV (ms^2) metrics.

	Model 0		**Model 1**		**Model 2**		**Model 3**	
Predictor	*B*	*SE*	*B*	*SE*	*B*	*SE*	*B*	*SE*
Diagnosis	39.40	5.73*	32.43*	5.10	9.01	6.17	5.02	7.79
IQ	-.07	.16	-.14	.13	-.22*	.10	-.30*	.12
Baseline lnHFHRV			-6.87*	1.89				
SSP Total					-.44*	.08	-.49*	.11
lnHFHRV Reactivity					15.79*	3.53		
lnHFHRV Recovery Score							-5.28	4.04
F	29.93*		33.73*		52.14*		29.10*	
Adjusted R^2	.67		.78		.89		.82	

Note. *p<0.05

Table 7.

Regression analyses for RMSSD (ms) metrics.

	Model 0		**Model 1**		**Model 2**		**Model 3**	
Predictor	*B*	*SE*	*B*	*SE*	*B*	*SE*	*B*	*SE*
Diagnosis	39.40*	5.73	34.80*	5.40	11.50	8.22	6.27	7.72
IQ	-.07	.16	-.13	.15	-.32*	.14	-.31*	.13
Baseline RMSSD			-.17*	.061				
SSP Total					-.39*	.11	-.47*	.11
RMSSD Reactivity					.19	.15		
RMSSD Recovery Score							-.12	.11
F	29.93*		27.38*		26.90*		28.29	
Adjusted R^2	.67		.74		.80		.81	

Note. *p<0.05

Table 8.

Partial correlation (r) of RBS-R and SRS-2 subscale scores with baseline RSA metrics when controlling for diagnosis.

Symptom score	**Baseline lnHFHRV (ms^2)**	**Baseline RMSSD (ms)**
RBS-R Stereotyped motor behavior subscale	-.15	-.11
RBS-R Sameness subscale	-.61**	-.53**
SRS-2 Social Communication Index	-.20	-.34

Note. *p<0.05, two-tailed, **p<0.01, two-tailed