SOCIAL INFLUENCES ON AUTONOMIC AROUSAL

IN AUTISM SPECTRUM DISORDERS

by

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ABSTRACT

The purpose of this study was to gain new understanding of autonomic nervous system (ANS) dysfunction in familiar and unfamiliar social situations in children with autism spectrum disorder (ASD). Both Children with ASD and typically developing peers viewed three sets of stimuli on a computer screen: 1) a screensaver (initial and final baseline), 2) objects moving to music (attention), and 3) narratives produced by both a caregiver and a stranger (familiar and unfamiliar social situations). Physiological measures of heart rate and skin conductance were acquired to assess ANS functioning. It was expected that 1) ANS activity would differ between children with ASD and typically developing peers at baseline, 2) differences in ANS activity between the two groups would be greater in the attention vs. the baseline task, and 3) differences in ANS activity between the two groups would be greater in the unfamiliar vs. the familiar tasks. Results showed that sympathetic, but not parasympathetic, arousal was greater for children with ASD as compared to typically developing children, but these measures did not differ across tasks. Results are interpreted to suggest that children with ASD perceived the experimental conditions as more challenging as compared to children who are typically developing.

DEDICATION

This thesis is dedicated to the memory of Granddaddy. He loved education nearly as much as he loved me. His last words of encouragement to me were this: no matter how tough school gets, "you're tougher."

LIST OF ABBREVIATIONS AND SYMBOLS

ADOS	*Autism Diagnostic Observation Schedule*
ANOVA	Analysis of variance
ANS	Autonomic nervous system
ASD	Autism spectrum disorders
ECG	Electrocardiogram
EEG	Electroencephalogram
ERP	Event-related potentials
HP	Heart period
IBI	Inter-beat interval
fMRI	Functional magnetic resonance imaging
Leiter-3	*Leiter International Performance Scale- Third Edition*
PDD-NOS	Pervasive developmental disorder, not otherwise specified
RSA	Respiratory sinus arrhythmia
RSB	Repetitive and stereotyped behaviors

SC	Skin conductance
SCR	Skin conductance responses
TYP	Typical

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INTRODUCTION

Characteristics of autism

The American Psychiatric Association characterizes autism spectrum disorder (ASD) by three social indicators: 1) impaired social interaction, 2) expressive language delay, and 3) stereotypical and repetitious behaviors (American Psychiatric Association, 2000). ASD is extremely heterogeneous, with great variability of symptoms and behaviors present among individuals with the disorder (Paul & Norbury, 2012). Repetitive and stereotyped behaviors (RSB) might often occur in challenging or socially unfamiliar situations. Such behaviors might be produced as a way to regulate emotion due to autonomic nervous system (ANS) dysfunction. The present study attempted to investigate ANS activity and its potential relation to social responding in the school-aged population.

Social Behavior

Social impairments of children with ASD include 1) responding to or recognizing emotion, particularly facial expressions, 2) establishing joint attention with another person, an important skill for conversation, and 3) the presence of repetitive behaviors when emotionally aroused.

Recognizing Emotion in Others

One social impairment associated with ASD is attending to and understanding facial expressions (e.g., Dawson, Webb, and McPartland, 2005). A number of studies have used electroencephalogram (EEG) and event-related potentials (ERP) to investigate whether young children and adults with autism process emotions represented in facial expressions differently from typically developing individuals (Grice, et al., 2001; Webb, Dawson, Bernier, and Panagiotides, 2006; Khorrami, Tehrani-Doost, and Esteky, 2013). ERP is a technique used to

extract neural responses from an EEG signal that are related to some event or stimulus (Luck, 2005). In an EEG study, Grice, et al. (2001) reported that patterns of brain activity did not differ for people with autism for faces oriented upright or inverted, as was the case for typical controls. This suggests that people with ASD process faces differently than typically developing individuals. In an ERP study, Webb, Dawson, Bernier, and Panagiotides (2006) found that children with ASD between the ages of 3 and 4 years exhibited ERP responses to facial stimuli with greater latency compared to children with developmental delays and typically developing children. This also suggests that cognitive processes involved in the processing of emotion may also be impaired in children with ASD. In a more recent ERP study, Khorrami, Tehrani-Doost, and Esteky (2013) had individuals with ASD and typically developing controls between the ages of 9 and 17 years view stimuli that consisted of pictures of faces and cars. Similar to Webb et al. (2006), facial stimuli were visually presented in either an upright or inverted manner. Findings showed that the upright faces were processed faster than other stimuli for both groups, but people with ASD were slower to process stimuli overall. The authors suggested that people with autism have difficulty processing information in the environment, particularly more complex stimuli such as faces.

Functional magnetic resonance imaging (fMRI) has also been used to investigate the emotion processing ability of individuals with ASD. Functional magnetic resonance imaging creates an image of brain activity from the magnetic characteristics of blood as it flows throughout the brain (Fox & Raichle, 2007). In one fMRI study by Kleinhans, et al. (2010), adults with ASD demonstrated atypical brain activity in the areas of the brain responsible for emotional face processing and for perceiving faces (e.g., the fusiform gyrus). In another fMRI study, Pierce et al. (2004) investigated socially familiar versus socially unfamiliar faces in people

with ASD. They reported that when adults with ASD were presented with faces that were socially familiar or personally significant (e.g., relatives or coworkers), their neural activity was similar to that of adults without ASD. This finding suggests that, although individuals with ASD are associated with impaired processing of social stimuli, this impairment may be less pronounced when processing stimuli that are more familiar. Studies using fMRI thus provide more evidence that social impairments in people with ASD may be associated with a neurobiological factor.

Joint Attention

Impairments in recognizing facial stimuli can have a negative impact on establishing joint attention, a shared behavior important for conversation. To this end, social interaction of children with ASD has been investigated in key ways. First, a number of studies have investigated eye contact. For example, Kaartinen et al. (2012) presented facial stimuli to children with ASD and typically developing controls between the ages of 8 and 16 years. Some of the faces were oriented directly ahead (i.e., direct gaze), some were oriented sideways (i.e., indirect gaze), and others had closed eyes. The experimenters measured autonomic arousal via skin conductance. They reported heightened autonomic arousal in children with ASD when presented with a stimulus consisting of direct gaze as compared to indirect gaze. For children with ASD, Kaartinen et al. reported a correlation between increased skin conductance responses to direct gaze versus indirect gaze and social impairments (e.g., gesturing and non-verbal play).

Eye gaze is a critical skill in establishing join attention in play or other activities involving social interaction. Eye gaze in children with ASD has therefore been a subject of recent research. Dawson, et al. (2004) investigated attention to social stimuli compared to non-social stimuli in a study involving children with ASD, children who were developmentally

delayed, and typically developing children. Compared to the other two groups, children with autism made fewer attempts to initiate joint attention with an examiner and were less likely to respond to examiner's attempts to initiate joint attention with them. In addition, children with ASD were less likely to orient to social and non-social auditory stimuli, such as humming, calling their name, snapping of the fingers, or the patting of hands on the thigh. Dawson, et al. reported that a combination of joint attention and social orientation was reliable in distinguishing children with ASD from children without ASD.

Impairments in joint attention have been linked to delays in early social interaction. Rutherford, Young, Hepburn, and Rogers (2007) investigated joint attention and pretend play skills in children with ASD, children with developmental disorders, and typically developing children. Pretend play in this study was defined as substituting one object for another, pretending properties of an object were present, pretending that objects were present, and pretending that a doll or toy was animated. They reported that joint attention was a reliable predictor in all three groups of the development of pretend play. Thus, impairments in the processing of complex social stimuli, like human faces, can lead to difficulties in establishing joint attention with other people, which might negatively impact the development of social interaction skills.

Repetitive Behaviors

Finally, social impairments associated with ASD can also include repetitive behaviors. The types of repetitive behaviors exhibited by children with ASD include both behavioral and verbal behaviors. Repetitive behaviors can be described as perseveration of vocalizations or other behaviors such as hand or arm movements (Arora, 2012). Children with autism spectrum disorders, when compared with typically developing children and children with developmental delay, typically display repetitive and stereotyped behaviors (RSB) more frequently and for a

longer amount of time (Watt, Wetherby, Barber, Morgan, 2008). Watt, et al. studied 50 children with ASD, 50 typically developing children and 25 children with developmental delays to compare RSB between groups. Although typically developing children and children with developmental delays can exhibit these behaviors, Watt, et al. suggested that the increase in frequency and duration of these behaviors in children with ASD could be a significant indicator of difference between the groups. They also noted that that RSB with objects could be used to measure autism symptom severity in children with communication delays who were 3 years of age. Barber, Wetherby, and Chambers (2008) completed a follow-up to the Watt et al. study and reported that young children with ASD displayed more repetitive and stereotyped behaviors than their typically developing peers. These behaviors included rocking and flipping, swiping, spinning, rubbing, rolling, clutching, and moving objects, rubbing their body, and stiffening.

Summary

Factors such as recognizing complex social stimuli such as faces, establishing joint attention, and the presence of repetitive and stereotyped behaviors, are all characteristic of ASD. It is unclear, however, why social development is atypical for children with ASD. Better understanding of the autonomic nervous system could provide some answers to understanding the source of these social impairments.

Autonomic Nervous System (ANS)

Sympathetic and parasympathetic activity

The primary function of the autonomic nervous system (ANS) is to regulate automatic behaviors and processes, such as those related to fight and flight, as well as homeostasis (Porges, 2001). The overall functioning of the ANS can be assessed through measures of the branches of the ANS, the sympathetic and parasympathetic nervous systems, and their influence on the

activity of the heart. The sympathetic nervous system mediates fight or flight behaviors in response to challenge or threat (Dickerson & Kemeny, 2004). On the other hand, the parasympathetic nervous system primarily functions to inhibit sympathetic activity, which is thought to be a necessary condition for social engagement (Porges, 2007). The sympathetic-adrenal system mediates sympathetic influences on the heart, in which cardiac activity may increase in the presence of environmental stress or challenge (Porges, 2007). Cranial nerve X, the vagus nerve, mediates parasympathetic influence on the heart (Berntson et al., 1997). This "vagal" input on the heart arises from the nucleus ambiguus in the brainstem, and it plays an important role in the development of social communication. Thus, atypical development of this parasympathetic or vagal input on the heart could contribute to developmental impairments in social engagement.

Respiratory Sinus Arrhythmia (RSA)

Parasympathetic influence on the heart can be indexed by respiratory sinus arrhythmia (RSA), which is the magnitude of increase and decrease in heart period (the inverse of heart rate) during inspiration and expiration in spontaneous breathing (Porges, 1995). When a person is resting or is relaxed, the magnitude or amplitude of RSA is relatively high, and thus provides a vagal "brake" on the heart to facilitate appropriate ANS functioning for social engagement or social interaction (Porges, 2007).

Respiratory sinus arrhythmia (RSA) can be used to assess parasympathetic regulation of the heart, and is commonly used in the area of psychopathology on tasks that present social or cognitive challenges. In such studies, emotion regulation can be measured by decreases in RSA from baseline to challenge tasks, or the magnitude of RSA during baseline (Calkins & Dedmon, 2000; Porges et al., 1996; Stifter & Fox, 1990). Many studies have reported decreases in RSA

after stressful or emotional stimuli are presented (Bar-Haim, Fox, VanMeenen, & Marshall, 2004; Calkins & Keane, 2004; Suess, et al., 1994; Weber, ver der Molen, & Molenaar, 1994). Such change indicates that the sympathetic nervous system is engaging to meet challenging situations through the release of the vagal "brake". In addition, RSA at baseline is often used to assess the extent of a person's capability of regulating sympathetic responding (Calkins, 1997; Calkins & Dedmon, 2000; Calkins, Graziano, & Keane, 2007; Porges, et al., 1996).

Physiological aspects of ASD

Although the vagal "brake" may be engaged when a person is resting and feeling relaxed, for example, during social interaction, the vagal "brake" may be disengaged for any mobilization required to meet threats in the environment (Porges, 1995). Such threats can take the form of either social or physical challenges. For some individuals with social impairments, release of the vagal "brake" may occur in socially unfamiliar or threatening circumstances, potentially interfering with social engagement or social communication.

Children for whom vagal regulation of the heart is dysfunctional or atypical, such as children with ASD, regulation of emotional arousal in novel or challenging situations can be problematic, the result of which might be impaired social communication (Porges, 2007). In the event that the vagal "brake" releases during a socially challenging situation, communicative effectiveness can be compromised. In other words, if a person's autonomic nervous system does not adapt appropriately to social stimuli, communication might be impaired (Doussard-Roosevelt, Montgomery, & Porges, 2003; Graziano, Keane, & Calkins, 2007; Porges, 2001). Therefore, investigating ANS activity, particularly parasympathetic influence on the heart, of children with ASD in socially familiar and unfamiliar situations, can provide better understanding of social impairments associated with ASD.

Studies suggest that autonomic nervous system (ANS) dysfunction may be associated with the presence of ASD (e.g., MacCullough & Williams, 1971). Within the autism spectrum, it is possible that dysfunction of parasympathetic regulation of the sympathetic nervous system could contribute to difficulty regulating emotional arousal, particularly in socially or socially unfamiliar situations. In addition, repetitive behaviors in children with ASD might be utilized to compensate for ANS regulatory dysfunction. Thus, ANS dysfunction could potentially have a causal influence on the presence of repetitive behaviors, if the child with autism is attempting to compensate for dysfunctional levels of autonomic arousal. Findings from this investigation could therefore be beneficial in informing intervention approaches for children with ASD.

A few recent studies have examined emotion regulation through measures of RSA in children with ASD. In one study, Van Hecke et al. (2009) examined sympathetic and parasympathetic influences on the heart during socially familiar or socially unfamiliar conditions. Conditions consisted of viewing stories told by a caregiver and a stranger. They found that adolescents with ASD had lower overall RSA compared to typically developing children, and that RSA was significantly lower in the socially unfamiliar vs. the socially familiar condition, a pattern that was not found in typically developing children. This suggests that the adolescents with ASD perceived the socially unfamiliar as threatening, but the typically developing children did not.

In another study, Bal et al. (2010) reported that children with ASD exhibited lower overall amplitude of RSA and a faster heart rate compared to typically developing children. Children viewed pictures of neutral faces that changed slowly into six different emotions, and with the ASD group, children with higher RSA were faster to recognize emotions and more accurate in recognizing which emotion was being displayed. Finally, Patriquin, Scarpa,

Friedman, and Porges (2011) compared RSA to social behaviors in 4-7 year old typically developing children and children with ASD. They found that overall RSA amplitude was associated with the spontaneous production of conventional gestures and joint attention. Results of such studies indicate that physiological differences could contribute to social impairment in children with ASD.

In summary, previous research appears to support the idea that ANS activity is different for children with ASD compared to typically developing children, and that parasympathetic influence on the heart as indexed by RSA could be particularly important in distinguishing children with ASD from typically developing children. Social situations that people with ASD perceive as challenging could trigger physiological responses within an impaired autonomic nervous system, contributing to impairments in social behaviors such as joint attention and potentially leading to the production of repetitive and stereotyped behaviors as a compensatory mechanism.

Interventions applied in treatment of autism

Due to the heterogeneous nature of ASD, many different intervention approaches have been utilized to treat the variety of characteristics associated with ASD. Some of the primary methods include scripts, imitation, Social Stories™, and peer mediation. Other intervention approaches may potentially involve physiology.

Scripts

Introducing and then fading scripts is a useful tool for increasing social communication. It can allow for an increased number of initiations of social communication, but can also allow for generalization of that script into additional words and phrases not already provided (Krantz & McClannahan, 1993). Scripts consist of pre-determined conversational statements, and can

facilitate children with autism participating in appropriate conversation about stimuli. In a study by Krantz and McClannahan (1998), participants with ASD were able to use scripts to open a conversation with a familiar adult teacher. In another study, Sarokoff, Taylor and Poulson (2001) found that even as scripts faded, that conversation was maintained about present stimuli and was able to generalize to new stimuli.

Imitation or modeling

Another intervention approach utilizes imitation or modeling. A clinician can utilize a model of appropriate verbal or behavioral output for a child with ASD (Paul & Norbury, 2012). An effective approach is video modeling, in which a child with ASD watches videos of another person's behavior in various situations, and then applies that to their own behavior (Paul & Norbury, 2012). The child with ASD can discuss with a clinician what occurred in the video, and analyze various attributes of behaviors in the video (Charlop & Milstein, 1989). Studies have shown that this technique can lead to faster generalization of daily behaviors in children with ASD as compared to live demonstrations of these behaviors (Charlop-Christy, Le, & Freeman, 2000).

Social Stories™

Social Stories™ (Gray & Garand, 1993) are often used to increase social skills in children with ASD. In one study conducted with preschool age children, Crozier and Tincani (2007) reported that two out of the three participants had an increase of socially appropriate behavior and a decrease of socially inappropriate behavior after implementing the stories. In another study that focused on the use of Social Stories™ in three school-age children with ASD, Delano and Snell, (2006) reported that the use of these stories increased positive behaviors and led to generalization of these behaviors into the classroom setting. However, one of the three

participants with ASD in their study did not show generalization from the intervention into the classroom.

Peer mediation

Another intervention approach in the treatment is peer-mediation, which focuses on teaching people with ASD new and appropriate social skills and helping them generalize these new skills in a variety of settings (Sperry, Neitzel, & Engelhardt-Wells, 2010). The goals of peer-mediated intervention are to teach typically developing peers how to interact with peers with ASD, increase the interaction time within the two groups, and increase interactions across activities (Sperry, et al., 2010). The intervention also aims to decrease adult support, such as prompting and cueing behaviors, and to improve the peers' the quality of interactions with each other.

Summary

In summary, social impairment in children with ASD may be associated with dysfunction of the autonomic nervous system (ANS). Complex social stimuli, such as human faces, might trigger release of the vagal brake. This could result in an inappropriate activation of the sympathetic nervous system, creating a physiological obstacle for social engagement and social communication, resulting in social impairments. Understanding potential limitations of ANS functioning in children with ASD can lead to modified or new intervention approaches used in the treatment of autism spectrum disorders. This study aimed to provide some insight into ANS functioning in children with ASD in the context of complex social stimuli.

AIMS OF RESEARCH

1) The first aim was to evaluate ANS activity of children with ASD in the absence of challenge by comparing their baseline measures of skin conductance and sympathetic and parasympathetic components of heart rate with typically developing children.
2) The second aim was to evaluate ANS activity in children with ASD in a socially familiar context by assessing change in measures of skin conductance and sympathetic and parasympathetic components of heart rate in a socially familiar vs. non-social condition relative to typically developing children.
3) The third aim was to evaluate ANS activity in children with ASD in a socially unfamiliar context by assessing change in measures of skin conductance and sympathetic and parasympathetic components of heart rate from socially familiar vs. socially unfamiliar situation relative to typically developing children.

METHOD

Participants

A total of eighteen children with autism spectrum disorder and typically developing peers (the control group) participated in this study. Eight of these were children with autism spectrum disorder, and ten were typically developing children. Data from one typically developing child was not used because of equipment failure. Data from one other typically developing child was not used because no ASD match was available. Thus, equal number of children with ASD and typically developing children were used for the study. Gender and age breakdown can be seen in Table 1. The study was approved by the Institutional Review board at the University of Alabama (see Appendix 8).

Table 1. Gender, Age and Cognitive Ability Means and Standard Deviations for Both Children With ASD and Typically Developing Children.

Group	Gender		Months		Leiter	
	M	F	M	SD	M	SD
ASD	8	0	108.13	20.12	116.38	12.21
TYP	7	1	104.38	17.45	106.00	19.32

All participating children were between six years, zero months and ten years, eleven months of age. Both children with ASD and the typically developing peers completed the *Leiter International Performance Scale- Third Edition* (Leiter-3), which measures the non-linguistic, cognitive capabilities of the children (Roid, Miller, Pomplun, & Koch, 2013). Group means on the Leiter can be seen in Table 1. All participants with ASD had a diagnosis meeting standard diagnostic criteria, placing them on the autism spectrum (i.e., the ADOS; Lord et al., 1999).

Seven of the children were confirmed to have ASD via the *Autism Diagnostic Observation Schedule* (ADOS), and one participant was confirmed to have pervasive developmental disorder, not otherwise specified (PDD-NOS).

Children with ASD and typically developing children were both recruited through an advertisement placed at the University of Alabama Autism Spectrum Disorders Clinic (Appendix 1). The advertisement was placed on a bulletin board within the clinic, as well as on a window at the front desk. Families of children within the intended age range who had expressed interest in being involved in participation in research were contacted from a database at the University of Alabama Autism Spectrum Disorders Clinic.

Families expressing an interest in participating in the study were asked to meet at the Speech and Hearing Clinic at the University of Alabama. The study was described in detail to parents and caregivers upon arrival. Caregivers were then presented with a consent form (Appendix 8) allowing permission for participation from their child and for audio/video recording. Since all participants were at least 6 years of age, the children in the study also gave assent (Appendix 8). Caregivers were then assured than any information obtained, including all data and audio-video recordings, was to be kept secure in a room that was under lock and key, and that data could only accessed by study investigators. Participants were also compensated with a $40.00 gift card to a local business.

Procedures

Participation required approximately ninety minutes. Leiter-3 administration required approximately 45 minutes, and data acquisition required approximately 30 minutes. A checklist for setting up the lab and presenting the experimental portions of the study is included in Appendix 7. The *Leiter International Performance Scale- Third Edition* was administered to

participants in a room across the hall from the lab where stimulus presentation and physiologic data acquisition was to occur. All children with ASD and the typically developing children were scored for the cognitive portion of the test, which provides a measure of the cognitive functioning of the participant. The Leiter-3 is a nonverbal assessment, and therefore any potential expressive language deficits would not impact performance.

Two stories were made available for use in this study, including *The Three Little Pigs* and *The Three Bears*. Two other books, *Green Eggs and Ham* by Dr. Seuss or *Where the Wild Things Are* by Maurice Sendak, were available if the session required additional narratives. The child's caregiver was audio-video recorded reading one of the two stories while the Leiter-3 simultaneously was being administered to the participant. This recording of the caregiver was used as one of the stimuli during the experimental portion of the study.

The narrative was presented to the caregivers on PowerPoint slides. A camera was placed on a computer monitor approximately 24 inches away (see Illustration 1). The PowerPoint slides did not have accompanying pictures on them, and the slides automatically moved to the next slide after exactly nine seconds. Therefore, the presentations did not require the caregiver to push any buttons, and each narrative presentation was the same standard length. Prior to recording, the caregiver was instructed to read with emphasis, as if they were reading a story to a child. They were instructed to put extra emphasis on words that were bolded, as a means of controlling how the stories would be narrated.

After the Leiter-3 had been administered and the caregiver had finished recording the story, participants were led to an adjacent lab containing a booth (where the caregiver had previously recorded the story). The booth had a rocket ship theme to provide a more friendly and playful atmosphere for participants. Participants were asked if they wanted to play a game where

they could pretend to drive a spaceship. A script was used to introduce participants to the electrodes that would be applied to their skin (Appendix 6). The participants were reassured that the electrodes were non-invasive and would not cause them any physical discomfort. If the participant appeared worried about the electrodes, the experimenter would then place the electrodes on her own skin, or allow the child to place the electrodes on her skin, to demonstrate that it did not cause discomfort.

Illustration 1. Internal View of the Booth.

Next, caregivers were led out of the room. To control for social context in the experiment, it was ideal that the parent not be present in the booth with the participant. However, if the participant became too upset to be in the lab without their caregiver, the caregiver was asked to come back into the lab, but not into the booth. If the child continued to remain upset

with the caregiver in the room, the session could be discontinued. No sessions were discontinued during the course of the study due to agitation of participants. The door to the booth was left open in order to prevent any additional stress on the participant, as it was deemed that keeping the door to the booth closed would worry some participants and interfere with accurate data acquisition.

After participants were seated in the booth, they were prepared for data acquisition. First, participants' skin was cleaned with electrode gel, which was applied only to the areas where the electrodes were to be placed. Next, hypoallergenic electrodes were placed on the skin to acquire skin conductance and heart rate data. For skin conductance, electrodes were placed on the fingertips of the forefinger and middle finger of the left hand. Participants were instructed to keep their hand palm-up throughout the experiment to prevent interference from the table. For heart rate (i.e., electrocardiogram), electrodes were placed superior to the clavicle on the right side of the body and at the lower ribcage (12^{th} rib) on the left side of the participant's body. Both heart rate and skin conductance were acquired continuously throughout all four conditions of the experiment. There was some difficulty getting participants in this young age group to sit still for the amount of time required. Frequent reminders had to be made to keep their palms facing up or to keep as still as possible.

The experimenter was seated at a desk outside of and adjacent to the booth. The door to the booth was left open to minimize distress of the participant, but a curtain was pulled shut so that the participant could not turn and see the experimenters (Illustration 2). The experimenter was able to view and hear the participant from outside the booth while the participant was completing the experimental tasks through a window to the booth. This allowed the experimenter to intervene if the participant was in distress or if an electrode became displaced from its position

on the participant's skin. The experimenter seated outside the booth collected physiological data and presented stimuli to the participant from two separate computers.

Illustration 2. External View of the Booth.

Stimuli consisted of an initial baseline task, a non-social, attention-engaging task, a pre-recorded socially familiar task, a pre-recorded socially unfamiliar task, and a final baseline task. After all stimuli had been presented, the experimenter saved the audio-video recording of the child and the physiological signals to a computer. The experimenter then removed the wires

connected to the electrodes. If the child was worried about removal of the electrodes, the experimenter offered to let the participant leave the facility with the electrodes still on the skin of the participant so that the parent could remove them. The $40 gift cards were then presented to either the participants or their caregivers, depending on the wishes of the caregiver. Finally, the experimenter asked the caregiver if they had any questions before they left the lab.

Experimental Design

The experimental design was a sequence of tasks consisting of stimuli that were either socially familiar vs socially unfamiliar or non-social in nature. These included baseline tasks in which a screensaver of pictures of space were presented, a non-social or attention task of pictures moving to music, a socially familiar task of a story being told by a caregiver, and a socially unfamiliar task of a story being told by a female stranger (Table 2). Baselines were presented first and last, and the three experimental tasks followed the initial baseline task, and these tasks were counterbalanced across participants. To confirm that participants paid attention to the stories, after each of the stories, five simple questions were asked regarding the content of the stories (see Appendices 2-5).

Table 2. Example of the Order of Presentation of Stimuli.

Example of Order of Presentation of Stimuli	
Task 1 – Baseline	View blank screen
Task 2 – Non-social condition	Video of music with graphics
Task 3 – Socially familiar	Narrative presented via video by caretaker
Task 4 – Socially unfamiliar	Narrative presented via video by stranger
Task 5 - Final	Gaze at blank screen to return to baseline

Signal Processing

The Biopac MP150 system (Biopac Sytems, Inc.) was used to acquire raw physiological signals, including an electrocardiogram (ECG) and skin conductance (SC). Raw signals were digitized at 1250 Hz, and high frequency noise or low frequency drift relative to the raw signal was filtered. Data reduction occurred across all five four-minute conditions, from which the sympathetic and parasympathetic components of heart rate and skin conductance levels were measured.

Skin conductance

High frequency noise related to the raw, tonic signal was low-pass filtered at 0.5 Hz to remove high frequency noise. This filtering functioned to remove any signal artifacts due to movement, which are typically higher than 1.0 Hz. The phasic component of this filtered signal was derived by using a 0.05 high-pass filter, thus revealing departures from baseline. Finally, peaks within the phasic signal that exceeded 0.5 microSiemens were counted within each task. These are referred to as skin conductance responses (SCR), and were reported for this study as SCR per minute.

Electrocardiogram

The raw ECG signals were first band-pass filtered with high-pass filter of 0.5 Hz to remove low frequency drift and a 35 Hz low-pass filter remove high frequency noise. An automated procedure in the Biopac AcqKnowledge software (Biopac systems, Inc.) was used to derive the inter-beat interval (IBI) series, or heart period series (i.e., the time between successive heart beats). CardioEdit software (Brain-Body Center, University of Illinois at Chicago, 2007) enabled correction of false and missed R-wave detection within the IBI series. Measurements of

respiratory sinus arrhythmia (RSA) and heart period (HP) were produced from the IBI files using CardioBatch software (Brain-Body Center, University of Illinois at Chicago, 2007).

Respiratory sinus arrhythmia (RSA) is calculated according to the methodology developed by Porges and Bohrer (Porges & Bohrer, 1990). This calculation uses a 21-point polynomial to detrend periodicities in the IBI series that are slower than the frequency of a person's spontaneous breathing. A band-pass filter was then applied to the IBI series to extract the variance at the frequency of respiration for children (i.e., 0.24 and 1.04 Hz). From this, the approximation of RSA was expressed as the natural log of this variance in units of $\ln(ms)^2$. To insure reliability of artifact correction, a second researcher coded approximately 20% of all data. Values of RSA between the two researchers were within 0.05 $\ln(ms)^2$ of each other.

Data analysis

Each physiological measure (i.e., RSA, HP, and SCR) was used as a dependent variable in a series of analysis of variance (ANOVA) models in which Group and Task were included as factors. First, dependent measures were evaluated across Tasks and between Groups to assess magnitude of ANS activity. Second, differences for each dependent measure relative to baseline were used to evaluate magnitude of change between 1) baseline and attention tasks, 2) attention and socially familiar tasks, and 3) socially familiar and socially unfamiliar tasks. Magnitude of ANS activity was interpreted as physiological capacity to respond to challenge. Magnitude of change in ANS was interpreted as physiological regulation or adaptation to challenge. Finally, between-group differences in age in months and score on the Leiter-3 were evaluated at baseline using t-tests.

RESULTS

Results are divided into three sections. First, comparisons are made between groups to evaluate potential differences in the age of the participants and their scores on the Leiter-3. Second, magnitude of ANS activity was evaluated between the two groups and across the five tasks for each of the three dependent measures (i.e. HP, RSA, SCR). Finally, magnitude of change in ANS activity was evaluated between the two groups for 1) baseline vs. attention, 2) attention versus socially familiar, and 3) socially familiar vs. socially unfamiliar.

Between-group comparisons

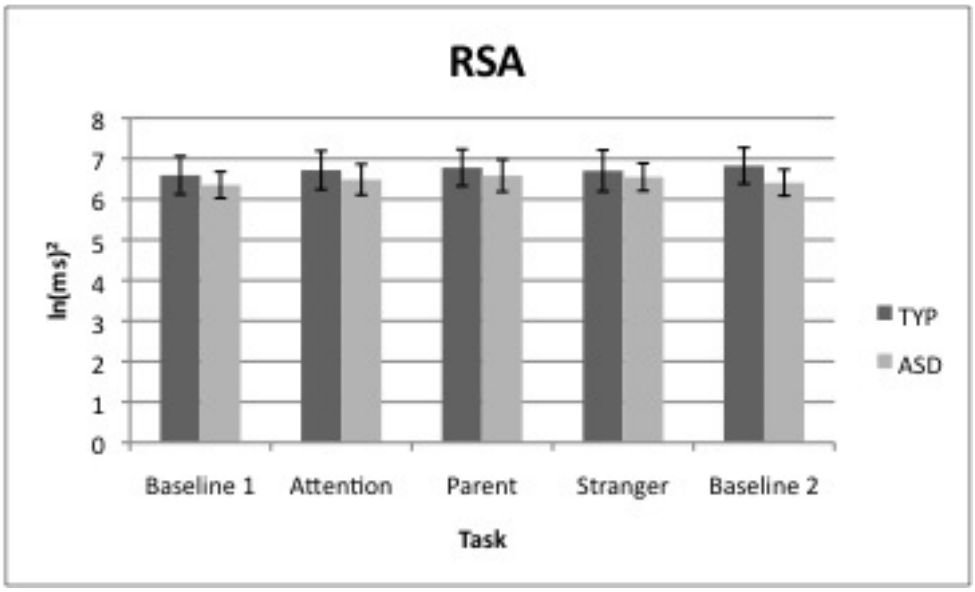
First, Welch t-tests assuming unequal variances showed that the children with ASD and TYP kids did not differ in terms of age in months: $t(13.73) = 1.297$, $p = .223$, or for the score on the Leiter-3, $t(11.82) = 0.398$, $p = .670$. These results confirm that groups were matched in terms of age as well as in terms of non-linguistic cognitive skills.

Magnitude of ANS activity

Analysis of variance (ANOVA) was used to evaluate the magnitude of ANS activity between the two groups and across the five tasks. First, using RSA as the dependent variable (Figure 1), no main effect was found for group, $F(1,56) = 0.711$, $p = .403$, task, $F(3,56) = 0.014$, $p = .998$, and the interaction between group and task did not approach significance, $F(3,54) = 0.038$, $p = .990$.

Second, using HP as the dependent variable (Figure 2), a main effect was found for group, $F(1,56) = 9.624$, $p = .003$, but not for task, $F(3,56) = 0.236$, $p = .871$, and the interaction between group and task did not approach significance, $F(3,54) = 0.024$, $p = .995$.

Figure 1. Respiratory Sinus Arrhythmia as the Dependent Variable.



Finally, using SCR frequency as the dependent variable (Figure 3), a main effect was found for group, $F(1,56) = 4.901$, $p = .031$, but not for task, $F(3,56) = 0.720$, $p = .545$, and the interaction between group and task did not approach significance, $F(3,54) = 0.180$, $p = .910$.

Figure 2. Heart Period as the Dependent Variable.

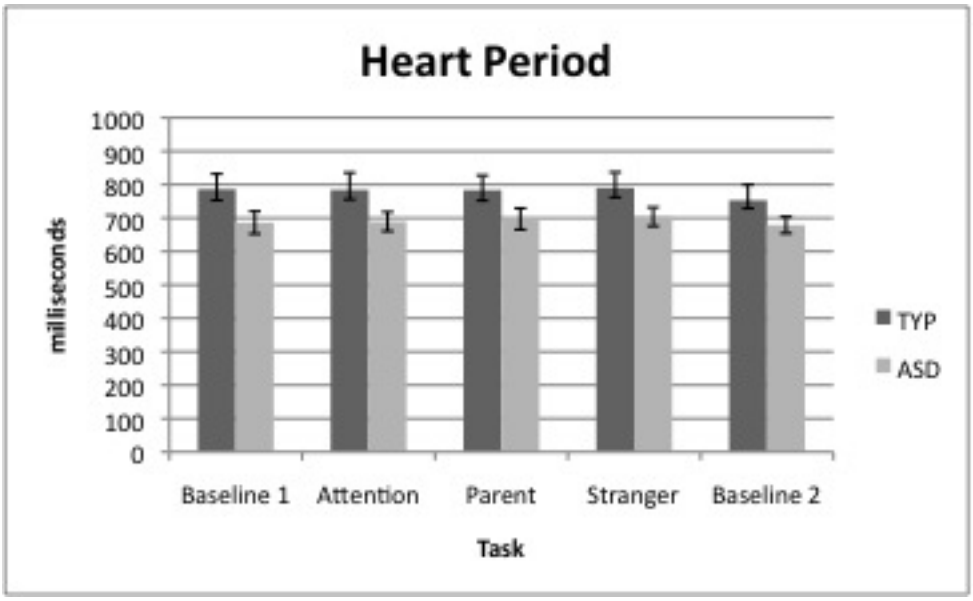
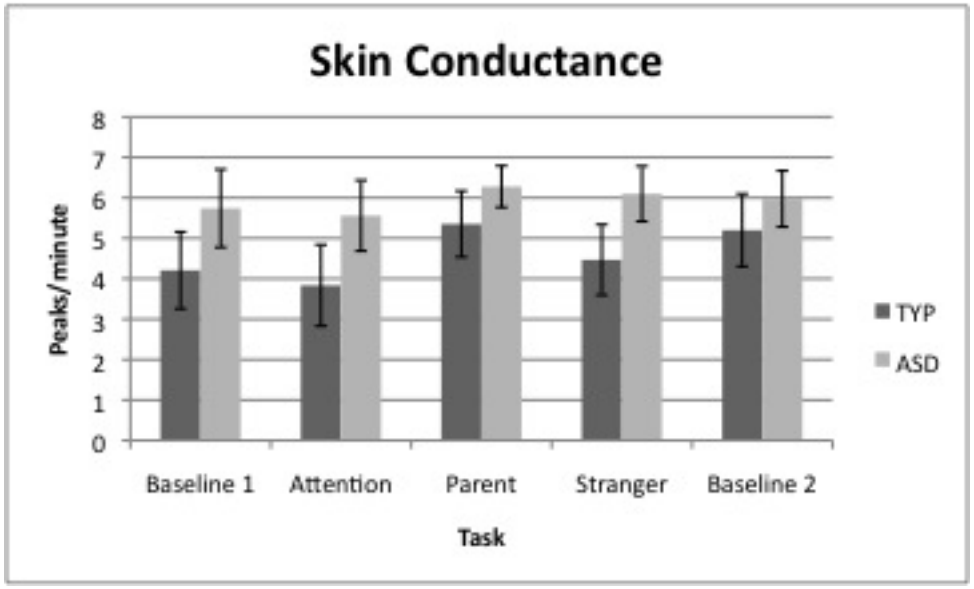


Figure 3. Skin Conductance as the Dependent Variable.



In summary, magnitude of ANS activity did not differ across the five experimental tasks for either group, but the frequency of both HP and SCR significantly differed between the two groups. This means that magnitude sympathetic activity was greater for the participants with ASD than for typically developing controls, but parasympathetic activity did not significantly differ.

Magnitude of change in ANS activity

To evaluate change in magnitude of ANS activity, differences between the first baseline task and all other experimental conditions were used as dependent variables. This was intended to account for the level of ANS activity associated with the experimental context, from which change could be evaluated (Wilder, 1957). Comparisons made between the 1) second baseline task and the attention task, 2) the attention task and the socially familiar task, and 3) the socially familiar task and the socially unfamiliar task.

First, RSA, HP, and SCR were evaluated between the baseline and the attention tasks. For the comparison between the baseline and the attention task, there was no significant

difference between the group or across tasks, and there was no significant interaction between group and tasks, for any of the three dependent measures (Table 3).

Table 3. RSA, HP, and SCR Between Baseline and Attention Tasks.

	Group	Task	Group/Task Interaction
RSA	F(1,28) = 0.534 *p* = .471	F(1,28) = 0.202 *p* = .888	F(1,28) = 0.653 *p* = .426
HP	F(1,28) = 1.359 *p* = .253	F(1,28) = 2.477 *p* = .127	F(1,28) = 0.619 *p* = .438
SCR	F(1,28) = 0.131 *p* = .720	F(1,28) = 1.298 *p* = .264	F(1,28) = 0.370 *p* = .548

For the comparison between the attention and socially familiar tasks, there was no significant difference between the group or across tasks, and there was no significant interaction between group and tasks, for any of the three dependent measures (Table 4).

Table 4. RSA, HP, and SCR Between Attention and Socially Familiar Tasks.

	Group	Task	Group/Task Interaction
RSA	F(1,28) = 0.049 *p* = .826	F(1,28) = 0.440 *p* = .512	F(1,28) = 0.022 *p* = .884
HP	F(1,28) = 0.615 *p* = .440	F(1,28) = 0.980 *p* = .756	F(1,28) = 0.135 *p* = .716
SCR	F(1,28) = 0.077 *p* = .783	F(1,28) = 2.261 *p* = .144	F(1,28) = 0.288 *p* = .560

Finally, for the comparison between the socially familiar and socially unfamiliar tasks, there was no significant difference between the group or across tasks, and there was no

significant interaction between group and tasks, for any of the three dependent measures (Table 5).

Table 5. RSA, HP, and SCR Between Socially Familiar and Unfamiliar Tasks.

	Group	Task	Group/Task Interaction
RSA	F(1,28) = 0.246 *p* = .624	F(1,28) = 0.185 *p* = .670	F(1,28) = 0.025 *p* = .875
HP	F(1,28) = 0.987 *p* = .329	F(1,28) = 0.212 *p* = .649	F(1,28) = 0.000 *p* = 1
SCR	F(1,28) = 0.160 *p* = .692	F(1,28) = 0.711 *p* = .406	F(1,28) = 0.314 *p* = .580

In summary, the magnitude of change in ANS activity did not change between children with ASD or typically developing kids among any of the contrasts.

DISCUSSION

Overall, results show that 1) children with ASD displayed a significantly greater magnitude of sympathetic activity when compared to typically developing kids, as reflected in lower HP (i.e., greater heart rate with smaller time frames between each beat) and higher SCR frequency, but that 2) there was no difference across tasks differing by attention and social familiarity. The discussion is comprised of three sections. First, parasympathetic regulation of the heart is discussed in relation to the Specific Aims of the study. Second, ANS activity of children with ASD as compared to typically developing children is discussed relative to the main findings of this study. Third, potential reasons for why a significant difference in magnitude of change in RSA was not found between the groups are discussed. Finally, clinical implications of the study are discussed.

Parasympathetic activity

According to Porges (e.g., Porges, 2007), parasympathetic influence on the heart can be used to assess autonomic nervous system (ANS) regulation. Parasympathetic influence on the heart can be indexed by respiratory sinus arrhythmia (RSA). RSA is the increase and decrease in heart rate associated with inspiration and expiration. Parasympathetic influences on the heart typically inhibit sympathetic influences on the heart while a person is resting or participating in social engagement, essentially creating a vagal "brake" on heart rate. However, when a person experiences a challenging or threatening situation, this vagal brake on heart rate can be released, allowing the sympathetic activity influencing the heart to rise in activity to meet that challenge. The magnitude of RSA when not faced with challenge or threat can be interpreted as the capacity to adapt to challenging or threatening situations. The magnitude of change in the amplitude of RSA can be interpreted as a measure of physiological regulation. Thus, both greater amplitude of

RSA prior to experiencing a challenging situation and greater decreases in RSA when experiencing a challenging or threatening situation would be expected for well-regulated individuals.

Children with autism spectrum disorder (ASD) appear to exhibit atypical ANS activity (MacCulloch & Williams, 1971). ASD is a developmental disorder characterized by impaired social interaction, expressive language delay, and stereotypical and repetitious behavior (American Psychiatric Association, 2000). Recent studies examining activity of the ANS in children with ASD have reported overall lower levels of RSA for children with ASD, and associations between measures of RSA and social impairment (e.g., Bal et al., 2010; Patriquin et al., 2011; Van Hecke, et al., 2009). Difficulty regulating emotions because of potentially dysfunctional (ANS) activity may require other means of regulation, particularly in situations of social novelty or social complexity, potentially leading to repetitive and stereotypical behaviors (RSB) or escape or withdrawal from the social situation that is perceived as threatening.

The purpose of this thesis was to assess ANS activity, particularly parasympathetic influences on the heart, in children with ASD across situations differing in social complexity. To restate the Specific Aims:

1. The first aim was to evaluate ANS activity of children with ASD in the absence of challenge by comparing their baseline measures of skin conductance and sympathetic and parasympathetic components of heart rate with typically developing children.
2. The second aim was to evaluate ANS activity in children with ASD in a socially familiar context by assessing change in measures of skin conductance and sympathetic and parasympathetic components of heart rate in a socially familiar vs. attention condition relative to typically developing children.

3. The third aim was to evaluate ANS activity in children with ASD in a socially unfamiliar context by assessing change in measures of skin conductance and sympathetic and parasympathetic components of heart rate from socially familiar vs. socially unfamiliar situation relative to typically developing children.

ANS activity

Results showed that children with ASD exhibited relatively greater sympathetic arousal when compared to typically developing children, as indicated by significantly lower HP (i.e., greater heart rate) and increased frequency of SCR. One possible reason for this difference is that children with ASD may perceived the experimental situation as more challenging relative to typically developing children, and responded with a greater amount of sympathetic arousal (Dickerson & Kemeny, 2004). Therefore, there may have been no true "baseline" associated with the experiment. Rather, children with ASD may have perceived the experimental situation to be more challenging than typically developing children. This is not surprising if one considers that being in any socially familiar or unfamiliar situation may be perceived as more challenging or threatening by children with ASD.

Another possible reason for this elevated sympathetic influence on the heart is that parasympathetic influences on the heart were functioning differently for children with ASD. According to the polyvagal model, an increase in sympathetic influence on the heart is enabled by release of the "vagal brake" (Porges, 2007). However, children with ASD were not found in this study to have significantly lower RSA when compared to typically developing children, although a trend for lower RSA for ASD children is evident in Figure 1. This finding is inconsistent with Van Hecke et al. (2009) and Bal et al. (2010), who reported that children with ASD had significantly lower RSA overall. One potential reason for this difference is that,

although children in this study were all on the autism spectrum, they may have been relatively higher functioning than children in these earlier studies, and may therefore have been responding more similarly to typically developing children.

However, consistent with Bal et al. (2010), children with ASD in this study exhibited faster heart rate compared to typically developing children. Although, according to the polyvagal theory (Porges, 2007), elevated heart rate is enabled by lower parasympathetic influence on the heart, this pattern was not evident among children with ASD in the present study. One possible reason for this is that regulation of the heart is qualitatively different for children with ASD, and so different patterns of parasympathetic vs. sympathetic activity are evident. More research is needed to explore this idea further.

Magnitude of change in ANS activity

No change in ANS activity across tasks was found for either children with ASD or typically developing children in the present study. Although stimuli were constructed similarly to those in the Van Hecke et al. (2009) study, in the present study experimental manipulations did not bring about the intended affect. Specifically, it was predicted that children with ASD would have reduced RSA in the socially unfamiliar versus the socially familiar conditions, but not typically developing children. However, findings did not support this prediction. Rather, it appears that participants in both groups did not find any of the experimental tasks challenging.

One can speculate that perhaps social aspects differed in each experimental context, and this had a greater impact on ANS activity than the stimuli themselves. In the Van Hecke, et al. (2009) study, typically developing children were excluded if they were found to have a sibling with ASD. In the present study, typically developing children with siblings with ASD were included. Having sibling relations among participants in the study could have resulted in ANS

activity being more similar between the groups, as they have genetic similarities that could potentially impact ANS activity.

Another possibility that could have influenced results is to what extent the parent or caregiver was nearby. In the present study, the participant did not see or hear the caregiver between Leiter-3 administration and the conclusion of the experiment, apart from when they viewed their caregiver reading the story during the socially familiar task. Rather, the child participated alone in a booth. However, in the Van Hecke, et al. (2009) study, caregivers filled out questionnaires while seated behind the child in the experimental space. This may have potentially had the effect of increasing social complexity, leading to a decrease in RSA for ASD children.

Another possible reason for why changes in ANS activity were not detected among tasks in the study relates to aspects of the testing room. In the present study, the room was a small booth in a lab that had some decoration to resemble a rocket ship. Although some participants found the room to be inviting, others did not seem to be interested, which could have impacted their overall level of comfort in the testing room and thus overwhelmed important aspects of the stimuli. In the Van Hecke, et al. (2009) study, participants were seated in a chair in the lab, which they had previously been introduced to via pictures before entering. It is also possible that the chair in which children were seated was not comfortable, or that being in the small booth was not comfortable for the children. Thus, although efforts were made to make the room inviting to participants, overall discomfort in the booth may have overwhelmed factors related to the stimuli.

A final possibility concerns the stimuli themselves. The experimental procedure might not have allowed observation of differences that are actually present. Perhaps the stories chosen

for this study were more suitable for children younger than the age range of children in this study. In the present study, *The Three Bears* and *The Three Little Pigs* were narrated by caregivers and by one stranger. Perhaps other stories would have been more age appropriate for this group of children. Children might also have been already familiar with the stories, reducing task complexity for the children with ASD. There is potential that the stimuli were not socially robust. The children were not required to interact with anyone in real time during data acquisition, which would have increased the social complexity of the experiment and could have impacted arousal of the participants. Instead, they were simply required to attend to the presentation of pre-recorded stimuli involving subjects that were socially familiar or unfamiliar. This lack of social complexity could have reduced the response of the children to social stimuli. Additionally, some caregivers might have prepared some of the participants with basic information about what they would encounter during the experimental session.

Though the Van Hecke, et al. (2009) study, upon which the present study was based, used participants who were between the ages of eight and twelve years, the present study used younger participants who were between six and ten years of age. It is important to understand the causal influences to ASD as it is a developmental disorder. Therefore, investigating children with ASD as close to the onset of symptoms as possible is important. As the child with ASD develops throughout childhood and into the adolescent and teenage years, environmental factors become more salient, which can potentially obscure causal factors of the disorder. The most ideal age range in which to study ASD is the point in the child's development in which social impairments are first observed. Another aspect of this would be to use participants who are not as high functioning, in order to provide a more accurate measure of the physiological aspects of ASD.

However, social impairments that are associated with ASD can limit the recruitment of participants and limit the acquisition of data.

Physiological approaches to treatment

An important motivation for investigating ANS activity in children with ASD is to better understand why such social impairments might arise the first place. Because children with ASD are widely considered to exhibit social impairments, it is clinically beneficial to understand any underlying physiological factors contributing to this (Van Hecke, et al., 2009). For example, better understanding of these physiological factors could lead to the modification of interventions or the creation of new interventions that account for the physiological regulation associated with children with ASD. Additionally, investigating autonomic nervous system activity in children with ASD across different social contexts may lead to better understanding of the physiological conditions in which social impairments manifest. Future research could investigate strategies used to maintain autonomic arousal in children with ASD. For example, what role and function might RSB have in autonomic arousal? In the long run, better understanding of physiological conditions might lead to the creation and implementation of more effective treatments.

CONCLUSIONS

Social impairments of children with ASD are potentially related to dysfunctional physiological regulation. For example, difficulty regulating physiological arousal could lead to impairments in approaching or withdrawing from social interaction, and repetitive and stereotypical behaviors could be utilized as compensatory regulatory mechanisms. Although the nature of the relationship between social impairments and the processing of environmental information is unclear, the present study contributes to a growing body of literature investigating physiological regulation in children with ASD. In the present study, physiological differences were found between children with ASD and typically developing children. However, there was no difference between groups in terms of the magnitude of parasympathetic influences on the heart, or change in the parasympathetic influence on the heart between the socially familiar and socially unfamiliar conditions. Future research into this physiology could not only improve our understanding of why social impairments exist in children with ASD, but it could shed light onto how we can provide more effective intervention for this population.

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APPENDICES

Appendix 1

The following information will be presented to the potential study participants' caregivers.

"Anthony P. Buhr, Ph.D., and Rachel Saffo, Ph.D., are currently recruiting families to participate in a study seeking to better understand social factors in autism spectrum disorders. The study will require approximately 90 minutes of your time, and you will be compensated a value of $40. The study will require one visit to the University of Alabama Speech and Hearing Center.

In the study, you will tell a story according to pictures from a children's book. Your child will later watch this story, as well as another story be another parent. While your child watches these stories, we will get information about your child's heart rate and skin conductance. This information will help us understand how your child responds to familiar and unfamiliar people. Would you be interested in taking part in this study?

If you would like to participate in the study, please contact Dr. Anthony P. Buhr at 205-348-1413, or send an email to speechlabama@gmail.com."

Appendix 2

The questions for The Three Little Pigs are as follows:

1) What did the little pigs build?
2) What tried to eat the three little pigs?
3) What did the big bad wolf do to the houses?
4) Did the three little pigs escape from the big bad wolf?
5) Can you tell me one thing about the story?

Appendix 3

The questions for The Three Bears are as follows:

1) Who went into the bear family's home?
2) Whose bed did the little girl sleep in?
3) What did the little girl do when she woke up?
4) Did the bears come home while the little girl was asleep?
5) Can you tell me one thing about this story?

Appendix 4

The questions for *Green Eggs and Ham* by Dr. Suess are as follows:

1) In the beginning of the story, who likes to eat green eggs and ham?
2) What does he want his friend to eat?
3) At the beginning of the story, does Sam I Am's friend want to eat green eggs and ham?
4) Does Sam I Am's friend ever eat green eggs and ham?
5) At the end of the story, does Sam I Am's friend like green eggs and ham?

Appendix 5

The questions from *Where the Wild Things Are* by Maurice Sendak are as follows:

1) What is the little boy's name?
2) What grows in Max's room after he is sent to bed without eating?
3) What creatures live in the land where Max sailed?
4) Who do the Wild Things choose to be their king?
5) Where is Max at the end of the story?

Appendix 6

"We are going to play a game. In this game, you get to pretend you are in a spaceship! Isn't that neat? Inside of this spaceship, you get to look at some things on the computer. But to be in this spaceship, you MUST wear your space gear." (*Show them the electrode equipment.)* "If you take your space gear off, then the spaceship game is over."

Appendix 7:

Autonomic Regulation and ASD Study Checklist

Study ID: _____

Date: _____

		Check
1)	**Computer setup**	
a.	Turn on MacBook Pro	____
b.	Turn on Mac Mini (boot up in Mac mode by holding option key)	____
c.	Confirm that monitors in booth show display	____
d.	Confirm that speaker in booth turned on (plug into mac mini)	____
2)	**Biopac setup**	
a.	Turn Biopac MP150 system on	____
b.	Confirm that both ECG and GSR cords wires are connected	____
c.	On Macbook desktop, in ASD folder, open ASD.gtl	____
d.	Save file with initials/date/task (e.g., AB_1.1.13)	____
e.	Confirm that Biopac MP150 system is recognized	____
f.	Press *Start* in AcqKnowledge software to confirm signals	____
3)	**Audio/video setup**	
a.	On Mac Mini, open video capture software	____
b.	Confirm that image shows in video capture software window	____
c.	Capture a few seconds of audio-video as a test	____
d.	Confirm test recording (audio + video) on monitor in booth	____
4)	**Stimulus presentation**	
a.	In ASD folder on MacBook Pro, open counterbalance spreadsheet	____
b.	In next available space enter subject initials (e.g., AB_1.1.13)	____
c.	Confirm stimulus order presentation (e.g., A, B, C, etc.)	____
d.	On Mac Mini open narrative presentation for *caregiver*	____
e.	On Mac Mini open narrative audio-video file of *stranger*	____
f.	On Mac Mini open file of classical music set to movements	____
5)	**Preparing participant for data acquisition**	
a.	Bring caregiver and child into lab.	____
b.	Confirm consent from caregiver and ask for signature	____
c.	Confirm assent from participant using assent form	____
d.	Take child to another room to administer testing	____
e.	Seat caregiver in booth for audio-video recording	____
f.	Save audio-video recording to ASD study folder	____
6)	**Preparing participant for data acquisition**	
a.	Take parent to another room (where child is)	____
b.	Bring child into booth. Seat in front of monitor & present script*	____

c. Apply SkinPrep gel (nuprep: neck and side. White bottle: SCR) ____
d. Attach electrodes for acquisition of ECG and GSR; White for neck, red for torso ____
e. Confirm signals in AcqKnowledge software ____

7) Steps to take before running participant (*circle only one***)**

a. Close door to booth ____
b. Child becomes agitated, keep the door open and remind of script* ____
c. Child still agitated, bring parent into the room and remind of script* ____
d. Child still agitated, take a short break and try again* ____
e. Child still agitated, discontinue session ____

8) Running participant

a. Press *Start* in Acqknowledge software to begin recording ____
b. Present first baseline (blank monitor) ____
c. Present first experimental condition ____
d. Present second experimental condition ____
e. Present third experimental condition ____
f. Present second baseline ____

9) Ending experiment

a. Press *Stop* in Acqknowledge software to stop recording ____
b. Go file to Save (or Command-S) to save file ____
c. Stop audio-video capture (will save automatically) ____
d. Remove electrodes from participant ____
e. Use alcohol pad to clean fingers and GSR electrodes ____
f. Throw used ECG electrodes into waste basket ____

10) Conclusion

a. Ask caregiver if they have any questions ____
b. Move webcam videos file to appropriate folder ____
c. Close software and shut down computers ____

Event Hot Keys

F1 = Baseline 1
F2 = Experimental 1
F3 = Experimental 2
F4 = Experimental 3
F5 = Baseline 2
F7 = Movement Artifact

Appendix 8

Appendix 1: Consent Form

UNIVERSITY OF ALABAMA
HUMAN RESEARCH PROTECTION PROGRAM

Social Influences on Autonomic Arousal in Autism Spectrum Disorder

Anthony P. Buhr, Assistant Professor, Communicative Disorders
Rachel Saffo, Assistant Professor, Communicative Disorders

You (and your child, spouse, partner, etc.) are being asked to participate in a research study. You are being asked to give permission for your child, for whom you are a guardian, to take part in this research study. This study is called "Social Influences on Autonomic Arousal in Autism Spectrum Disorder". The study is being conducting by Anthony P. Buhr and Rachel Saffo, both assistant professors at the University of Alabama.

Is the researcher being paid for this study?
Neither Dr. Buhr nor Dr. Saffo is being paid for conducting this study.

Is this research developing a product that will be sold?
No product is being developed as part of this study.

Does the investigator have any conflict of interest in this study?
Neither Dr. Buhr nor Dr. Saffo has a conflict of interest from conducting this study.

What is this study about? What is the investigator trying to learn?
This study is being conducted to explore the impact of social influences on the functioning of emotion regulation in children with autism spectrum disorder (ASD). The study seeks to understand how stories told by a stranger and a parent impact emotional arousal. We will place sensors on your child's fingertips to measure slight sweat changes and on your child's ribcage to monitor heart rate. This is one way that we can tell if your child finds the stories exciting or stimulating. The results of this study will add to what is already known about ASD.

Why is this study important or useful?
This study is important because it will add to knowledge regarding emotion regulation in children with autism spectrum disorder (ASD). Results of this study will help researchers, speech-language pathologists, and other professionals as they strive to provide better treatment practices for children with ASD.

Why have I been asked to be in this study?
You have been asked to be in this study because your child is within the range of 6 years 0 months to 9 years 11 months. Participation on your part is completely voluntary, and if you decide to quit at any point, you are able to do so.

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CONSENT FORM APPROVED: 10-25-13
EXPIRATION DATE: 3-22-14

How many people will be in this study?
A total of 36 children, along with their caregivers, will participate in the study. Of this total, eighteen children will be with autism spectrum disorder (ASD) and 18 without ASD.

What will I be asked to do in this study?
If you agree to be in this study, you will be asked to do several things. First, you will be asked to come to the University of Alabama Speech and Hearing Clinic on a single occasion. In the clinic, we will audio/video record you reading one of four books, Where the Wild Things Are by Maurice Sendak, Green Eggs and Ham by Dr. Suess, Three Little Pigs, and The Three Bears. These recordings will be presented to your child and to at least one other participant in the study. We will also audio/video record your child watching the story you tell as well as a story told by another parent or caregiver. Finally, we will attach hypoallergenic electrodes to your child's skin at the fingertips and near the ribs so that we can monitor your child's heart rate and skin conductance to measure emotion.

How much time will I spend being this study?
You will only make one visit to the University of Alabama Speech and Hearing Clinic. This visit should last no longer than 90 minutes.

Will being in this study cost me anything?
There will be no costs to you to participate in this study, other than your time spent participating in the study.

Will I be compensated for being in this study?
You will be reimbursed with a $40 gift card for your time and effort in this study.

Can the investigator take me out of this study?
The investigator will not take you out of the study unless your child becomes distressed.

What are the risks (dangers or harms) to me if I am in this study?
There are not any foreseeable risks associated with this study.

What are the benefits (good things) that may happen if I am in this study?
There are no direct benefits to you for participating in the study, but you will be contributing to researcher knowledge about autism spectrum disorder (ASD) and how children with ASD might be impacted in certain social situations.

What are the benefits to science or society?
The benefit of this study to society is a better understanding of autism spectrum disorder (ASD). A benefit to science is better understanding of the link between social factors and emotion in children with ASD.

How will my privacy be protected?
All personal information you choose to share about your child, including audio/video recordings, will be stored on a secure hard drive, located in a locked room in the Speech and Hearing Clinic

at the University of Alabama, and only accessible to the primary investigator. Recordings will be erased from the hard drive within 10 years of the conclusion of the study.

How will my confidentiality be protected?
Any personal information collected from you and your child will be stored in a database that is only identifiable by a number.

What are the alternatives to being in this study? Do I have other choices?
The alternative to being in this study is to decline to participate.

What are my rights as a participant in this study?
Taking part in this study is voluntary. It is your free choice. You can refuse to be in it at all. If you start the study, you can stop at any time.

The University of Alabama Institutional Review Board ("the IRB") is the committee that protects the rights of people in research studies. The IRB may review study records from time to time to be sure that people in research studies are being treated fairly and that the study is being carried out as planned.

Who do I call if I have questions or problems?
If you have questions, concerns, or complaints about the study right now, please ask them. If you have questions, concerns, or complaints about the study later on, please call the primary investigator (Dr. Anthony P. Buhr) at (205-348-1413).

If you have questions about your rights as a person in a research study, call Ms. Tanta Myles, the Research Compliance Officer of the University, at 205-348-8461 or toll-free at 1-877-820-3066.

You may also ask questions, make suggestions, or file complaints and concerns through the IRB Outreach website at http://osp.ua.edu/site/PRCO_Welcome.html or email the Research Compliance office at participantoutreach@bama.ua.edu.

After you participate, you are encouraged to complete the survey for research participants that is online at the outreach website, or you may ask the investigator for a copy of it and mail it to the University Office for Research Compliance, Box 870127, 358 Rose Administration Building, Tuscaloosa, AL 35487-0127.

I have read this consent form. I have had a chance to ask questions. I agree to take part in it. I will receive a copy of this consent form to keep.

_____
Signature of Research Participant Date

_____
Signature of Investigator Date

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CONSENT FORM APPROVED: 10-25-13
EXPIRATION DATE: 3-22-14

Appendix 3: Audio/video Recording Consent

Audio/Video Recording Consent

Audio/video recordings of you and your child will be obtained in this study. Audio/video recordings of your child will allow us evaluate behaviors during your child's participation, and audio/video recordings of you telling a story will be used to present to your child as well as other participants in this study. These recordings will be stored on a secure hard drive, located in a locked room in the Speech and Hearing Clinic at the University of Alabama, and only accessible to the primary investigator. These recordings may also prove to be valuable for future research about autism spectrum disorder (ASD). However, recordings will be erased from the hard drive within 10 years of the conclusion of the study.

I understand that part of my participation in this research study will be audio/video recorded and I give my permission to the research team to record speech and language. I understand these recordings will be stored in a secure location.

☐ **Yes,** my participation in "Social Influences on Autonomic Arousal in Autism Spectrum Disorder" can be audio/video recorded, and I give my permission to use these recordings for future research

☐ **Yes,** my participation in "Social Influences on Autonomic Arousal in Autism Spectrum Disorder" can be audio/video recorded, but I prefer these recordings **not** be used for future research.

Signing below constitutes your agreement to be audio/video recorded. You will be provided with a copy of this form.

_____ _____

Printed Name Date

_____

Signature

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CONSENT FORM APPROVED: 10-25-13
EXPIRATION DATE: 3-22-14

Appendix 2: Assent form

The following assent script for the study "*Social Influences on Autonomic Arousal in Autism Spectrum Disorder*" will be presented to children whose parents have chosen to participate in the study.

"Hi __(child's name)__. My name is __(researcher's name)__. I am from the University of Alabama. I am trying to understand some important things about how kids listen to stories. You are ___(*within* 6 and 10)__ years old, and thought you might like to be in my study. All I want you to do is watch some stories on a computer. I will put some stickers on your skin, and we'll make a movie of you watching the stories. After that, I want you to answer some questions about the stories. Your __(mom or dad)__ will be reading one of the stories to you. I also want you to know that you can stop watching the stories at any time. Do you have any questions for me? If you don't have any more questions for me, would you like to be in my study?"

YES NO

_____ DATE_____

Signature of Person Obtaining Assent

UNIVERSITY OF ALABAMA IRB
CONSENT FORM APPROVED: 10-25-13
EXPIRATION DATE: 3-22-14

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Office for Research

Institutional Review Board for the
Protection of Human Subjects

October 23, 2013

THE UNIVERSITY OF
ALABAMA
RESEARCH

Anthony P. Buhr, Ph.D.
Department of Communicative Disorders
College of Arts & Sciences
The University of Alabama

Re: IRB Protocol # 13-005-ME
"Social Influences on Automatic Arousal in Autism Spectrum Disorder"

Dr. Buhr:

The University of Alabama IRB has received the revisions requested by the full board on 10/15/13. The board has reviewed the revisions and the change in your protocol has been approved.

Please remember that your approval period expires one year from the date of your original approval, March 22, 2013, not the date of this revision approval.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants.

Good luck with your research.

Sincerely,

John C. Higginbotham, Ph.D., MPH
Medical IRB Chair
The University of Alabama

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