Physiological Response Patterns During Social Interaction to Predict Internalizing Symptoms in Children with Autism Spectrum Disorder

By

Rachael Anne Muscatello

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Approved:

Christine Konradi, Ph.D.

Blythe A. Corbett, Ph.D.

Katherine Gotham, Ph.D.

Paul Newhouse, M.D.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Chapter I

INTRODUCTION

Stress and its Consequences

The subjective experience of stress is difficult to accurately and objectively define (Levine & Ursin, 1991), though it is frequently conceptualized as the physiological response to some noxious or threatening stimulus. Stress should be differentiated from a similar concept, anxiety, which pertains to feelings of worry or apprehension in the absence of a direct threat (American Psychiatric Association, 2013). Stress, on the other hand, refers to the physiological response (e.g. glucocorticoid or neurotransmitter release) to physical or psychological threat. Psychological, 'processive' (Herman & Cullinan, 1997) stress is likely to occur when the environmental demands are perceived as too demanding or beyond the functioning capacity of the individual (Cohen, Janicki-Deverts, & Miller, 2007) and is often reliant on previous experiences (Herman & Cullinan, 1997). The physiological response can be adaptive, guiding attention to the threatening stimulus in order to remove the source of the stress and restore homeostasis (Levine & Ursin, 1991). However, excess psychological stress and its chronic wear and tear from physiological response (allostatic load; McEwen, 1998) can have severe consequences for physical and psychological well-being.

A number of environmental stimuli may induce a stress response, which will vary in intensity by individual appraisal of the situation and severity of the perceived threat (Lacey & Lacey, 1958; Schneiderman, Ironson, & Siegel, 2005). In many instances, acute responses to threat are adaptive, providing an opportunity for response and coping, followed by a return to

homeostasis. But in conditions of repeated, chronic exposure or especially severe acute stress, responses can lead to substantial long-term damage to health. For example, it is estimated that previous history of a major life stressor is 2.5 times more likely in those who go on to develop depression versus non-depressed controls, and chronic adversities exacerbate these symptoms and impair treatment response (Mazure, 1998). The likelihood of any individual developing a stress-related condition is affected by a number of biological or environment factors (Plotsky, Owens, & Nemeroff, 1998). These factors, such as trauma exposure or social support, can have long-lasting, profound impacts on the psychological stress response and are a substantial source of variability in stress-related physical and mental health outcomes (Schneiderman et al., 2005). For example, young adults reporting higher self-esteem and greater access to positive social support are less likely to experience depressive symptoms associated with increased stress (Cohen, Sherrod, & Clark, 1986). Further, in non-human primates, social bonds prevent elevated physiological stress to novel situations, while social isolation or separation leads to significant increases in stress (see Levine, 1993 for review). Therefore, the broader context of individual factors, especially those such as social support or isolation, is important to consider in any research regarding psychological consequences of severe stress.

The Biological Basis of Stress

The brain, termed the central organ of stress, is especially sensitive to its effects, with multiple brain regions involved in the psychological perception of and physiological response to stress (McEwen, 2013). Most notably are the structures composing the limbic region. The limbic system was first conceptualized by James Papez in order to understand the systems that control emotional behavior (Purves et al., 2008). The modern conceptualization of the limbic system

includes key regions involved in either emotional processing or emotional perception, such as context- and memory-related cues. These structures include the amygdala, medial prefrontal cortex, hypothalamus, cingulate cortex, basal ganglia, hippocampus, and mammillary bodies (Purves et al., 2008). Given the importance of this system in emotional processing, damage to these regions could have significant implications for affect and behavior. Indeed, imaging and postmortem studies in anxiety and depression reveal structural changes of these brain regions. Those with depressive or anxiety-related illnesses have shown hippocampal volume loss (e.g. Sapolsky, 2001; Sheline, Wang, Gado, Csernansky, & Vannier, 1996), prefrontal hypotrophy (e.g. Drevets et al., 1997; Rajkowska, 2000), and hyperactivation of the amygdala (e.g. Peluso et al., 2009; Sheline et al., 2001). These initial structural changes may further contribute to maladaptive stress responses because these regions are involved in the psychological processing of potential threats and are key in regulating stress systems, thereby the limbic system directly effects physiological output. Importantly, the negative stress-related outcomes may not be permanent, because the cycle can be disrupted through intervention and previous neuronal damage reversed (Sapolsky, 2001). For example, there is evidence that antidepressant treatment can increase progenitor cells and hippocampal volume, thus compensating for previous damage from chronic stress exposure (Boldrini et al., 2009). Nonetheless, in order to fully understand the consequences of chronic stress and areas for intervention, it is important to first define the physiological systems involved in these allostatic processes.

The Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal (HPA) axis is the primary neuroendocrine stress system, secreting glucocorticoids from the adrenal glands in response to stress or to maintain

homeostasis via a diurnal rhythm or negative feedback mechanism (Gore, 2008). The primary glucocorticoid and output of the HPA axis in humans is cortisol. Initiation of this neuroendocrine cascade begins at the paraventricular nucleus (PVN) of the hypothalamus, which releases corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) into the pituitary portal (Whitnall, 1993). These hormones will in turn stimulate release of adrenocorticotropic hormone (ACTH) by binding to receptors on corticotropic cells of the anterior pituitary. Subsequently, ACTH binds to receptors in the adrenal cortex, stimulating release of cortisol in humans. Stimulated cortisol feedbacks on this system to modulate further release, binding to glucocorticoid receptors (GRs) on the hippocampus. Activation of these GRs stimulates Gamma-Aminobutyric acid (GABA)-ergic interneurons, resulting in a net inhibition on the PVN of the hypothalamus and preventing additional cortisol release to restore basal levels to homeostasis (Herman & Cullinan, 1997; Jacobson & Sapolsky, 1991).

Stress response in the HPA axis can be conceptualized into two separate pathways, one for physiological threats to homeostasis ('systemic' stressors) and another responsible for responding to perceived threat ('processive' stressors') (Herman & Cullinan, 1997). The systemic stressor pathway does not require higher-order processing, as stressors of this pathway can cause immediate threat to survival. Interoceptive and sensory cues from medullary regions and the lamina terminalis are relayed directly to the PVN to stimulate CRF secretion (Sawchenko et al., 1996). In contrast, perceived threat will involve cognitive appraisal of a potentially threatening situation. The processing of contextual, emotional, and memory-based cues requires a 'limbic-sensitive' circuit mediated by the prefrontal cortex, amygdala, and hippocampus, respectively (Herman & Cullinan, 1997). Due to the importance of limbic regions in determining

and regulating the neuroendocrine stress response, the physiological system is often referred to as the Limbic Hypothalamic Pituitary Adrenal (LHPA) axis.

Limbic regulation of the HPA stress response involves a diverse network of excitatory and inhibitory connections (see Figure 1.1). The hippocampus is commonly cited as an inhibitor of PVN activation (Herman & Cullinan, 1997; Herman et al., 2003), and the medial prefrontal cortex has been shown to influence neuroendocrine response through primarily inhibitory connections (e.g. Diorio, Viau, & Meaney, 1993; Radley, Arias, & Sawchenko, 2006). In contrast, activation of the amygdala will rapidly excite the HPA axis, increasing cortisol output (e.g. Dayas, Buller, & Day, 1999; Herman et al., 2003; Herman & Cullinan, 1997; Ulrich-Lai & Herman, 2009). Notably, these effects do not appear to result from direct connections with the PVN of the hypothalamus. Instead, a relay circuitry exists in which projections from limbic regions synapse onto neurons within the bed nucleus of the stria terminalis (BNST), which then sends GABAergic projections to the PVN (see Herman et al., 2003 for review). Studies in rats and humans show stimulation of the hippocampus decreases HPA output, likely through excitation of the GABAergic neurons of the BNST (Herman, Cullinan, & Watson, 1994; Jacobson & Sapolsky, 1991). Alternatively, excitation of the amygdala, such as during perceived threat or fear, will inhibit the BNST via GABA projections, thus reducing the BNST's inhibition on the PVN, resulting in a summed excitatory effect (Prewitt & Herman, 1998). Moreover, the limbic regions are interconnected, collectively influencing stress response through processing of memory- and context-dependent cues (see Ulrich-Lai & Herman, 2009 for review). The neural control of stress is a multifaceted process involving numerous limbic regions and connected networks, and therefore, any disruption within this complex circuitry could have significant consequences for physical and psychological health.

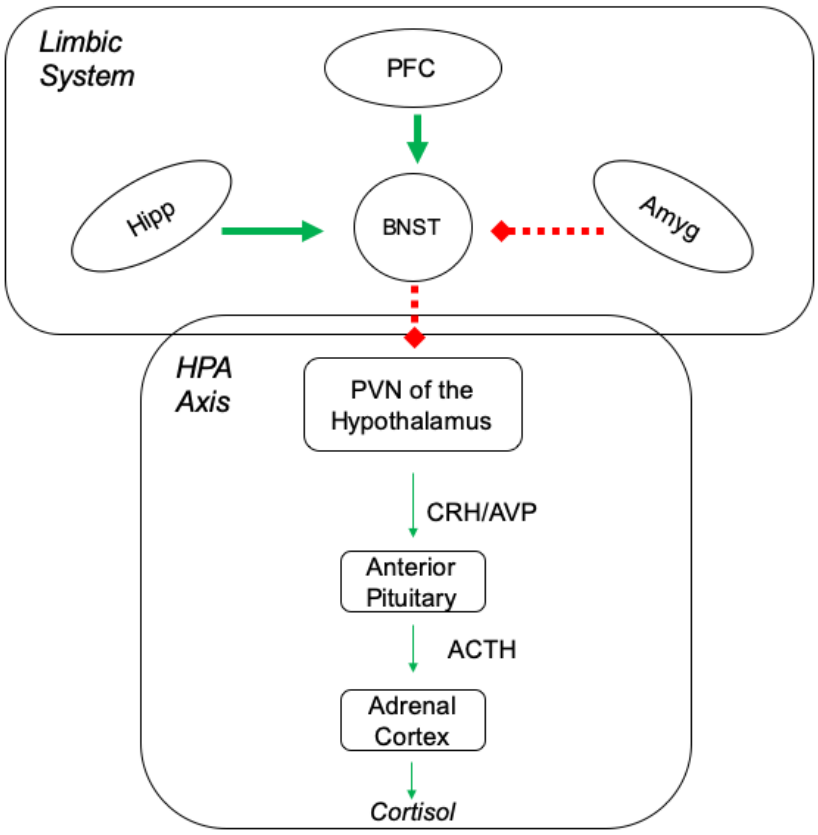

Figure 1.1. Representation of Limbic-Hypothalamic-Pituitary-Adrenal-Axis. Green arrows indicate net excitation; dotted red lines represent net inhibition. *ACTH, adrenocorticotropic hormone; Amyg, Amygdala; BNST, Bed nucleus of the stria terminalis; Hipp, Hippocampus; PFC, Prefrontal cortex; PVN, Paraventricular nucleus.*

Behavioral and Psychological Influences of the HPA axis

Psychological stress is dependent upon the organism's perception of the stimulus or event as threatening. There are four identified situations in which psychological stress has been shown to measurably activate the HPA axis and induce cortisol release, including novelty, unpredictability, social evaluation, and sense of low control (Dickerson & Kemeny, 2004; Mason, 1968). For example, in rodent and non-human primates, exposure to novel, unfamiliar environments consistently leads to significant increases in plasma cortisol (Hennessy & Levine, 1978; Hennessy, Mendoza, Mason, & Moberg, 1995). Additionally, situations in which one has

limited control, such as social rejection due to uncontrollable personal characteristics, promote activation of the HPA axis (Dickerson & Kemeny, 2004). Especially relevant for humans, conditions of social evaluative threat in which an individual is negatively judged by peers, is a key processive stressor. A well-established laboratory-based social evaluative threat paradigm, the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993), consistently induces cortisol elevations in humans (e.g. Buske-Kirschbaum et al., 1997; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004), demonstrating the ability of threats to the social self to activate the HPA axis response. Critically, responses are dependent upon environmental context and higher-order limbic processing. For instance, previous negative experiences, such as peer victimization and bullying, may shape future arousal responses to oft-considered non-threatening peer interactions due to past instances of threat to the social self (Chen, Kong, Deater-Deckard, & Zhang, 2018; Knack, Jensen-Campbell, & Baum, 2011). The entire HPA axis system, including limbic regions involved in memory retrieval and formation or emotional processing and perception, acts in a concerted fashion to ultimately determine the valence of the stimulus and corresponding cortisol response.

While early life exposure to acute stress does not necessarily lead to dysfunction of the HPA axis in adulthood (see Levine, 2005 for review), chronic exposure to multiple, repeated stressors can result in a sustained elevated physiologic response (McEwen, 1998). Chronic exposure to glucocorticoids and glutamate can have neurotoxic effects on limbic regions involved in regulating the HPA axis, due to the high concentration of glucocorticoid (GRs) and mineralocorticoid (MRs) receptors in these areas (De Kloet, Vreugdenhil, Oitzl, & Joëls, 1998; Sapolsky, Krey, & McEwen, 1986). The hippocampus is shown to have an especially high concentration of GRs, and hypersecretion of glucocorticoids will saturate and initiate down

regulation of the receptors on the hippocampus (Sapolsky et al., 1986). As a result, the loss in hippocampal activation will lead to reduced inhibition to the PVN, and thus reduction in the negative feedback mechanism necessary for restoring basal levels and reducing glucocorticoid hypersecretion.

As noted, chronic stress, referred to as repeated exposure to perceived negative events in which one has low control, and resulting dysregulation of the HPA axis, can lead to poor health outcomes including a variety of psychopathological conditions, such as anxiety and depression. Persistent stress and prolonged vigilance characteristic of anxiety disorders has been associated with either elevated evening cortisol (Forbes et al., 2006) or lower overall basal HPA axis regulation (Dieleman et al., 2015). Further, behavioral studies provide evidence for atypical cortisol reactivity in youth (e.g. Booij, Bouma, de Jonge, Ormel, & Oldehinkel, 2013; Dieleman, van der Ende, Verhulst, & Huizink, 2010; Rao, Hammen, Ortiz, Chen, & Poland, 2008) and adults (e.g. Burke, Davis, Otte, & Mohr, 2005) with depression, as well as elevated basal levels (Dahl et al., 1991; Stetler & Miller, 2011; Van den Bergh & Van Calster, 2009; Van den Bergh, Van Calster, Pinna Puissant, & Van Huffel, 2008). A growing number of findings point to dysregulated HPA axis activity as a risk factor for suicidal ideation and attempts (e.g. Giletta et al., 2015; O'Connor, Green, Ferguson, O'Carroll, & O'Connor, 2017). Given this collection of clinical ramifications, continued efforts to target chronic stress and identify risk factors of HPA axis dysfunction will be necessary to ensuring appropriate intervention and enhancing quality of life.

Autonomic Nervous System (ANS)

In addition to the HPA axis, the Autonomic Nervous System (ANS) plays a key role in regulating stress and arousal. The ANS is separated into two branches with primarily opposing functions, the parasympathetic (PNS) and sympathetic nervous systems (SNS). The PNS is described as the 'rest and digest' branch by conserving energy as it slows heart rate (bradycardia), lowers blood pressure, decreases respiration, increases intestinal activity, among other regulatory actions (Purves et al., 2008). In contrast, the metabolically demanding SNS supports 'fight or flight' responses for mobilization to threat, including, but not limited to, increased heart rate and respiration. The primary output of the two systems differ as well, with acetylcholine the primary output of the PNS, while the SNS ultimately releases norepinephrine when activated. Regulation and response of the PNS and SNS can be measured by monitoring changes in visceral organ function, such as changes in heart rate. The sinoatrial node (SA), referred to as the pacemaker of the heart, is dually innervated by both branches of the ANS (Hamill, Shapiro, & Vizzard, 2012; Longhurst & Fu, 2012), and changes in the beat-to-beat heart rate signal (heart rate variability; HRV) can provide insight into individual contributions of each system. In parasympathetically-dominant states, acetylcholine binds to muscarinic receptors of the SA node to slow depolarization and decrease heart rate. However, when the active SNS releases norepinephrine, SA node rhythm and heart rate will increase via beta-adrenergic receptor-mediated second messenger cascade (Berntson et al., 1997). The individual contributions of each branch can be reliably measured through non-invasive cardiac measures of HRV. Respiratory sinus arrhythmia (RSA) represents the high frequency heartbeat intervals that fluctuate with respiration (Berntson, Cacioppo, & Quigley, 1993). RSA increases with greater output of the parasympathetically-mediated vagal nerve, and is sensitive to cholinergic but not

adrenergic blockade, supporting its use as a sensitive marker of PNS functioning (Berntson et al., 1993; Cacioppo, Uchino, & Berntson, 1994). Pre-ejection period (PEP), defined as the time from SA node depolarization to left ventricular ejection (e.g. Sherwood, Allen, Obrist, & Langer, 1986), is sensitive to adrenergic stimulation, but not vagal blockade (e.g. Cacioppo et al., 1994; Sherwood et al., 1986). Therefore, PEP is often considered the preferred index of sympathetic influence on the heart.

Functioning of the ANS is directed by an extensive, interconnected neural network, extending from reflexive or stimulus-specific control at the spinal- and brainstem-level, to more integrated behavioral responses requiring cognitive processing at the forebrain level (Benarroch, 2012). The major brainstem regions directing autonomic output are found within the medullary region and include the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMX) and nucleus ambiguus (NA) (Benarroch, 2012; Porges, 1995). The NTS serves a critical role as a relay station of afferent information from visceral inputs, such as baroreceptor (changes in blood pressure) or cardiac afferents (Benarroch, 1993; 2012). This sensory input, carried from the vagus and glossopharyngeal nerves, relays on to the NTS, which projects to brainstem or forebrain regions for further processing and determination of appropriate physiological response (e.g. Benarroch, 1993; Ulrich-Lai & Herman, 2009).

Several major forebrain regions, collectively known as the Central Autonomic Network (CAN) (Benarroch, 1993), exert additional top-down control over these brainstem regions to further modulate ANS output. The CAN is comprised of the anterior cingulate, insula, ventromedial prefrontal cortex, amygdala, PVN of the hypothalamus, periaqueductal gray matter (PAG), parabrachial nucleus, and other major brainstem regions, such as the NTS, NA, and medulla (Benarroch, 1993). For example, the amygdala can modulate autonomic response to

emotional stimuli through direct connections with the NTS, DMX, and NA (Schwaber, Kapp, Higgins, & Rapp, 1982). Further, the prefrontal cortex (PFC) projects GABAergic inhibitory signals to other major limbic regions (Thayer & Lane, 2000), including the amygdala and hippocampus, thereby positioning itself at the top of the response hierarchy (Ulrich-Lai & Herman, 2009). This coordinated, interconnected system will ultimately culminate in an autonomic response that is either parasympathetic- or sympathetic-dominant. The PNS and SNS outputs reach the sinoatrial of the heart via the vagus nerve, and as such, CAN output is directly linked to and can be indexed by HRV (Thayer & Lane, 2000).

Psychological Health and the Autonomic Nervous System

The ANS has been proposed as a *behavioral regulator*, with the balance between the PNS and the SNS critical to shaping response to changing environmental conditions (Berntson, Norman, Hawkley, & Cacioppo, 2008). In particular, the dynamic ability of the autonomic system to flexibility adapt to changing environment demands is most important for regulating health and behavior. A leading theory on these influences of autonomic arousal and balance is the Polyvagal Theory (Porges, 1995; 2001; 2003b; 2007), which proposes that the myelinated vagus, projecting parasympathetic output from the nucleus ambiguus, evolved as the primary stress response system in mammals (Porges, 1995). Thus, the default physiological state is one in which the PNS maintains a 'vagal brake' on the heart; however, vagal influence on the heart will withdraw in times of stress, allowing for increases in cardiac output without engaging the SNS (Porges, 2001; 2007; Wolff, Wadsworth, Wilhelm, & Mauss, 2012).

Vagal flexibility (Berntson et al., 2008) especially plays an important role in determining social behavior. The Polyvagal Theory (Porges, 1995) relates autonomic functioning to

socialization through the proposed Social Engagement System (Porges, 2001; 2003a; 2005). The parasympathetically-mediated Social Engagement System consists of interconnected brainstem nuclei which regulate the myelinated vagus and other cranial nerves directly involved in regulating muscles of the face and head. These somatomotor components of the system control a number of actions relevant for social behavior, including but not limited to, eye movement (eye contact), vocalization (language), and head turning (social orienting) (Porges, 2005). At the same time, modulation of visceral state from afferent signals and higher-order cortical processing will determine function of the myelinated vagus. Calm, restful visceral states will promote these interconnected social behaviors; however, decreasing influence of the vagus and/or mobilization of the fight or flight system will block the Social Engagement System (Porges, 2007). For example, individuals who demonstrate more cooperativity and sociability tend to have higher PNS regulation (Beffara, Bret, Vermeulen, & Mermillod, 2016; Kogan et al., 2014; Lischke et al., 2018). Additionally, young adults with higher vagal tone are more socially engaged than their peers with lower PNS regulation (Geisler, Kubiak, Siewert, & Weber, 2013). Lastly, it has been demonstrated that increased vagal flexibility (changes in parasympathetic regulation in response to environmental changes) predicts lower SNS activity in supportive social conditions (Wolff et al., 2012). These findings therefore support the core components of the Polyvagal Theory and Social Engagement System (Porges, 2007), suggesting vagal flexibility promotes adaptive and prosocial behaviors.

Several psychiatric conditions, including depression and anxiety, have also been associated with autonomic imbalance and inflexibility. A dynamic equilibrium between the two branches of the ANS is said to be associated with fewer mental health problems (Nederhof, Marceau, Shirtcliff, Hastings, & Oldehinkel, 2015). In contrast, chronic mobilization states,

resulting either from elevated SNS reactivity (e.g. El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008) or reduced PNS/vagal influence (e.g. Beauchaine, 2001; Graziano & Derefinko, 2013), are frequently seen in internalizing conditions. The significance of the ANS in anxiety and depression has been considered in the context of the CAN (e.g. Thayer & Lane, 2000). According to this view, central control of the ANS will determine autonomic and behavioral response. Tonic inhibition from the prefrontal cortex typically prevents a rigid, sympathoexcitatory defensive system driven by the amygdala (Thayer & Brosschot, 2005). However, in anxiety or during worry, the prefrontal cortex is downregulated (Thayer, Friedman, & Borkovec, 1996), allowing for upregulation of the amygdala and sustained activation of mobilization responses. Indeed, patients with generalized anxiety evidence decreased resting state connectivity between the amygdala and prefrontal cortex, and this decreased connectivity is associated with lower PNS regulation (Makovac et al., 2016). The coordinated actions of the autonomic network thus appear to be an important component for regulating social and emotional responses and behavior.

Argument for a Multi-System Approach

The HPA axis and ANS are independent physiological stress systems; however, the systems are also interconnected with overlapping neural circuitry. Understanding how the HPA axis and ANS interact and work together during stress and recovery may be key to elucidating the impact of dysfunction of one or both systems on cognition and behavior, especially in regard to social behavior and emotion regulation. A perceived threat will induce a tightly coordinated response in both systems (e.g. Ulrich-Lai & Herman, 2009). The ANS quickly promotes physiological changes through neurotransmission of acetylcholine or norepinephrine. The HPA

axis, however, acts on a slower time scale, initiating a hormonal cascade eventually culminating in cortisol release. Likewise, the primary outputs of the ANS and HPA axis can influence the activity of the other. Hypothalamic CRH neurons are reciprocally connected to noradrenaline-releasing neurons of the locus coeruleus, and each can stimulate release of the other, and therefore influence activity of the opposing stress system (Stratakis & Chrousos, 1995; Valentino & Foote, 1988). Additionally, the SNS directly innervates the adrenal cortex and stimulates additional cortisol release (Parker, Kesse, Mohamed, & Afework, 1993).

Structurally, a number of limbic regions provide top-down regulatory control over both the HPA axis and ANS (e.g. Ulrich-Lai & Herman, 2009; see Figure 1.2). The prefrontal cortex lies at the top of the network hierarchy, maintaining a tonic inhibition over the HPA axis and SNS through both glutamatergic and GABAergic projections. Specifically, the PFC projects GABA transmission to the central nucleus of the amygdala, inhibiting its sympathoexcitatory effects (e.g. Thayer & Lane, 2009; Thayer & Sternberg, 2006). In contrast, glutamatergic signals from the PFC are projected to the BNST, increasing GABA transmission to the PVN of the hypothalamus and preventing HPA axis activation (see Herman et al., 2003 for review). When the PFC is inactive or downregulated, however, sympathoexcitatory circuitry can dominate. Disinhibition of the amygdala culminates in sympathetic activation through a pathway of NTS inhibition and disinhibition of norepinephrine-release neurons in the ventrolateral medulla and locus coeruleus (e.g. Thayer & Lane, 2009; Ulrich-Lai & Herman, 2009). Simultaneously, inhibition of the NTS from the amygdala downregulates excitatory projections to the NA and DMX, thereby preventing parasympathetic output from the vagus (e.g. Saha, 2005; Smith, Thayer, Khalsa, & Lane, 2017). Additionally, amygdala excitation triggers GABAergic signaling to the inhibitory interneurons on the BNST, culminating in excitation of the PVN and activation

of the HPA axis (Herman & Cullinan, 1997). It should be further noted the amygdala is reciprocally connected with the hippocampus, thus providing a system by which previous memories of threat or safety may influence perception of environments as safe or novel (e.g. LeDoux, 2000; Vaisvaser et al., 2013). Further, there is evidence for hippocampal projections to the medial prefrontal cortex, which may influence signaling to the NTS and thus autonomic tone (Ruit & Neafsey, 1990). The amygdala and PFC share reciprocal connections, as well, allowing for context-dependent conditional behavioral responses to emotional stimuli (Quirk, Likhtik, Pelletier, & Paré, 2003). As demonstrated in Figure 1.2, the HPA axis and ANS interact through a complex neural network, with further detailed connections likely but beyond the scope of this review. Nonetheless, it is evident that functioning of one system is unlikely to occur in isolation without notable impacts on the other system.

Studies in humans provide evidence for these functional interconnections and their significance for stress and overall health. As physiological responses are often emotion-specific (e.g. Thayer & Lane, 2000), the coordinated functioning of the HPA axis and ANS could have noteworthy psychological implications in cases of chronic stress or disruption within the shared neural circuitry. Though relatively under-investigated, the importance of studying the interactive nature of the two primary stress systems has been highlighted through proposed models by which patterns of activation may affect behavior. The Additive model (Bauer, Quas, & Boyce, 2002), proposes that reciprocal arousal between the HPA axis and SNS is optimal, while excessively low or high overall arousal will contribute to behavioral risks. In contrast, the Interactive model (Bauer et al., 2002) emphasizes a balanced system in which response of one system mirrors the other, thereby asymmetrical activation (i.e. high HPA axis and low SNS) is associated with increased psychopathologies (e.g., internalizing disorders). A few investigations in youth found

that individuals with hyperarousal of both the HPA axis and the SNS also had the highest rates of stress and internalizing or externalizing behaviors, providing support for the Additive model (El-Sheikh et al., 2008; Nederhof et al., 2015; Rotenberg & McGrath, 2016). Overall, however, relatively limited evidence exists as to the nature of the combined physiological stress response. In consideration of enhanced identification of risk profiles for psychopathologies linked via examining a physiological system in isolation, future efforts should be dedicated to furthering our understanding of the functional connections between the HPA axis and ANS and the consequences for behavior and health.

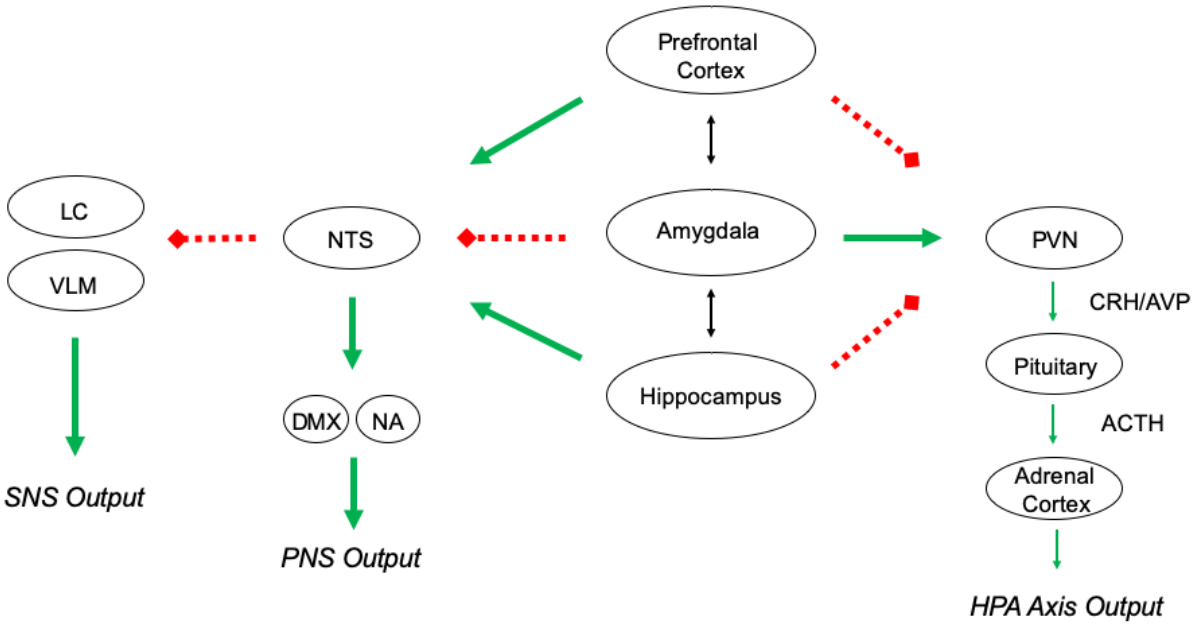

Figure 1.2. Simplified representation of overlapping neurocircuitry of HPA axis and ANS. Green arrows indicate net excitation; dotted red lines represent net inhibition. *ACTH, Adrenocorticotropic hormone; AVP, Arginine vasopressin; CRH, Corticotrophin-releasing hormone; DMX, Dorsal motor nucleus of the vagus; LC, Locus coeruleus; NA, Nucleus ambiguous; NTS, Nucleus tractus solitarius; PVN, Paraventricular nucleus; VLM, Ventrolateral medulla.*

The Physiology of Stress and Behavior: Autism Spectrum Disorder (ASD) as a Model

Autism spectrum disorder (ASD) is a neurodevelopment disorder defined by deficits in social communication and interaction, as well as restricted, repetitive patterns of behavior and interests (American Psychiatric Association, 2013). Therefore, individuals with ASD often have significant difficulty engaging with others in reciprocal social exchanges and responding to novel situations. National prevalence rates are currently estimated at every 1 in 59 children, a 15% increase from 2016 (Baio et al., 2018). Males are currently 4 times more likely to be diagnosed than girls. In addition to the core diagnostic criteria, individuals with ASD are at elevated risk for a number of comorbid conditions, including but not limited to epilepsy, sleep disorders, attention deficit/hyperactivity disorder, anxiety disorders, and depression (e.g. Accardo & Malow, 2015; Simonoff et al., 2008). Comorbid rates of depression and anxiety are especially high, with lifetime anxiety prevalence recently estimated at 42% (Hollocks, Lerh, Magiati, Meiser-Stedman, & Brugha, 2019), and percentage of individuals diagnosed with a depressive disorder estimated at 40% by adulthood (Hollocks et al., 2019; Hudson, Hall, & Harkness, 2019), far surpassing global rates (Mayes, Calhoun, Murray, Ahuja, & Smith, 2011).

Many youth with ASD experience significant stress during social interactions (Corbett, Schupp, Simon, Ryan, & Mendoza, 2010; Corbett et al., 2014; Lopata, Volker, Putnam, Thomeer, & Nida, 2008; Schupp, Simon, & Corbett, 2013) and find these experiences to be anxiety-inducing (Bellini, 2006). An avenue of increased interest is focused on the likely connections between social functioning, psychological stress, internalizing symptoms, and physiological arousal in ASD. A diagnostic challenge in autism is that assessment of internalizing symptoms or psychological stress often rely on self- or parent-report, which can be challenging in a population that may have difficulty verbalizing emotions (e.g. Losh & Capps,

2006) or having limited insight into their internal states (e.g. Hill, Berthoz, & Frith, 2004). Thus, use of more objective measures could contribute to enhanced understanding of biological markers of psychological conditions and provide a means to more clearly define the interactions between social functioning, mood, and physiology in ASD. By recognizing the apparent role of physiological stress systems in influencing social and emotional behavior and dysregulation (see Benevides & Lane, 2015; Taylor & Corbett, 2014 for review), ASD may serve as an ideal pathological model to elucidate the interconnections between physiological stress, social functioning impairment, and internalizing symptoms in humans.

Evidence exists for dysfunction of the HPA axis in youth with ASD, including an atypical diurnal rhythm or altered stress reactivity (see Taylor & Corbett, 2014 for review). A number of studies have shown elevated evening cortisol in youth with ASD (Corbett, Schupp, Levine, & Mendoza, 2009; Muscatello & Corbett, 2018; Putnam, Lopata, Thomeer, Volker, & Rodgers, 2015; Tomarken, Han, & Corbett, 2015; Tordjman et al., 2014), which appears to be related to changes in daily activities and routine throughout the day (Corbett et al., 2009). Additionally, individuals with ASD present with atypical cortisol responses to a variety of stressors, to include nonsocial stressors. For example, when exposed to a novel medical procedure (i.e., mock-MRI scanner), children with ASD evidenced heighted stress responsivity (Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006). Not surprisingly, individuals with ASD often respond with heightened stress under certain social contexts. For example, during a playground interaction with TD peers (Corbett et al., 2010), many children with ASD show elevated HPA reactivity, providing evidence they may perceive these benign social interactions to be stressful (Corbett et al., 2010; 2014; Schupp et al., 2013). In contrast, many ASD youth do not show a physiological response to a social evaluative stressor consistently shown to increase

HPA axis reactivity in TD individuals (Edmiston, Blain, & Corbett, 2017a; Jansen et al., 2006; Lanni, Schupp, Simon, & Corbett, 2012; Levine et al., 2012). Similarly, increasing evidence supports autonomic dysfunction in individuals with ASD, though the nature of this dysfunction still remains somewhat uncertain. Several studies have noted reduced baseline parasympathetic regulation relative to TD individuals (Bal et al., 2010; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Vaughan Van Hecke et al., 2009); however, others report no differences in resting PNS function (Kushki, Brian, Dupuis, & Anagnostou, 2014; Levine et al., 2012; Neuhaus, Bernier, & Beauchaine, 2016; Watson, Roberts, Baranek, Mandulak, & Dalton, 2012). The few studies to date to examine cardiac measures of baseline sympathetic arousal have found no differences between youth with or without ASD (Edmiston, Muscatello, & Corbett, 2017b; Schaaf, Benevides, Leiby, & Sendecki, 2015). Given the hypothesized connection with autonomic arousal and social engagement (e.g. Porges, 2007), understanding atypical functioning of the ANS may be particularly relevant for youth with ASD (Porges, 2005).

Autism is a disorder most clearly characterized by difficulties in the social domain. As humans, we live in an inherently social world, with peer connections and social support consistently cited as protective factors against a number of physical and psychiatric diseases (see House, Landis, & Umberson, 1988; Thoits, 2011; Uchino, 2006 for review). As such, the physiological stress response to peer interactions and its potential detriments to social behavior has and continues to be an area of prime focus in research of ASD. A handful of studies have found youth with ASD who experience HPA hyperreactivity during social interaction to engage in significantly less cooperative play with peers (Corbett et al., 2014; Schupp et al., 2013). Further, children and adolescents with ASD are reported to experience reduced parasympathetic regulation in response to a number of social stressors (Edmiston, Jones, & Corbett, 2016;

Neuhaus, Bernier, & Beauchaine, 2014; Vaughan Van Hecke et al., 2009), which is often associated with social problems in these youth (Edmiston et al., 2016; Vaughan Van Hecke et al., 2009). However, it may also be that for certain youth with ASD, some elevation in physiological responsivity and arousal is adaptive and necessary for social engagement (Corbett, Blain, Ioannou, & Balser, 2017). Examination of the connections and balance between physiological response and social functioning is critical. In the process, specific patterns of arousal may be identified as optimal in which the body is best primed to interact with the social environment.

Since comorbid rates of anxiety and depression are especially high in individuals with ASD (e.g. Hollocks et al., 2019), their connections with physiology should also be considered. Previous studies examining the relation between stress and affective symptoms in ASD is somewhat limited, especially in regard to depressive disorders. Nonetheless, children with ASD and high anxiety have been reported to experience significant ANS dysfunction, such as states of heightened sympathetic arousal, during a series of social cognitive and attentional tasks (Kushki et al., 2013; Panju, Brian, Dupuis, Anagnostou, & Kushki, 2015). Conversely, blunted parasympathetic functioning during social interactions has been associated with more internalizing and withdrawn/depressed symptoms (Neuhaus et al., 2014). The literature to date is somewhat mixed as not all previous findings have supported a hyperarousal model. Specifically, blunting of the HPA axis has been related to both anxiety and depressive symptomology in youth with ASD (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014; Hollocks, Pickles, Howlin, & Simonoff, 2016; Sharpley, Bitsika, Andronicos, & Agnew, 2016) and other studies have failed to find any associations between internalizing symptoms and physiology (Cai, Richdale, Dissanayake, & Uljarević, 2019; Kushki et al., 2014; Lanni et al., 2012; Muscatello & Corbett, 2018; Tomarken et al., 2015). These inconsistencies may be due in part to the complex

interactions between physiological systems. A more complete approach which considers the interactions of the HPA axis and both branches of the ANS will likely be far more informative in describing the contributions of physiological dysregulation to internalizing conditions than would studying either system in isolation.

Conclusion

The HPA axis and ANS stress responses are heavily implicated in social and emotional functioning. While they are independent systems, the HPA axis and ANS circuity share a significant amount of overlap, and it is these shared connections that may have implications for risk of internalizing symptoms. As previous findings have shown, children with ASD frequently display dysfunction of the HPA axis and ANS responses to stress, such as social interactions with peers. In addition to difficulties in social function, individuals with ASD often experience elevated anxiety and depression. Considering this collection of physiological dysfunction, social impairments, and psychiatric comorbidities, there likely exists a complex, interconnected system (see Figure 1.3) by which all of these symptoms interact within ASD to affect overall psychological functioning.

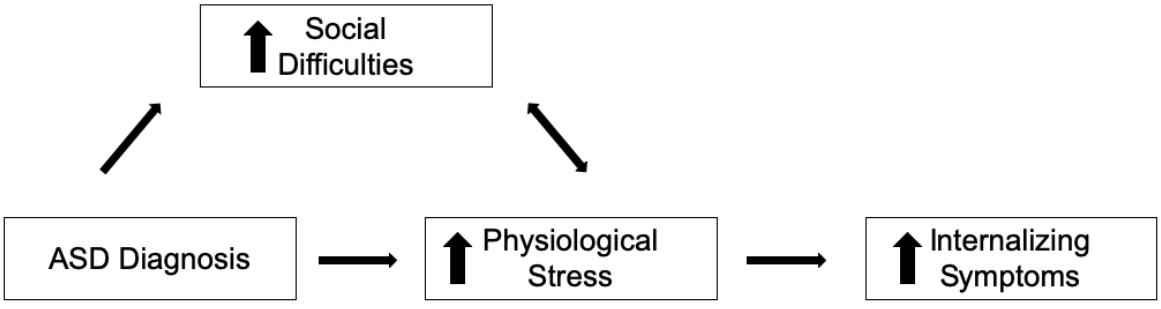

Figure 1.3. Proposed Interaction between Social functioning, Stress, and Internalizing Symptoms in ASD.

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Chapter II

EXPLORING MULTI-SYSTEM PHYSIOLOGICAL PROFILES AT REST AND THE ASSOCIATION WITH DEPRESSIVE SYMPTOMS IN AUTISM SPECTRUM DISORDER

Introduction

Depression is one of the most common mental health conditions worldwide. Recent estimates from the National Institute of Mental Health indicate 7.2% of adults and a staggering 14.4% of adolescents in the United States report a major depressive episode in the past 12-months (SAMHSA, 2019). Investigations into potential biological mechanisms of depression have identified physiological arousal systems as possible underlying factors. Two primary arousal systems, the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS), may substantially influence psychological functioning, leading to significant behavioral consequences. Moreover, though they are independent systems, the HPA axis and ANS are interrelated with considerable neural overlap, which serves to maintain an appropriate response to environmental demands and to adaptively regulate behavior and emotion based upon the current context. Vulnerable populations, including individuals with autism spectrum disorder (ASD), are at an especially increased risk of being diagnosed with a depressive disorder in their lifetime, with comorbid rates of depression reported at 20.2% for adolescents with ASD (Greenlee, Mosley, Shui, Veenstra-VanderWeele, & Gotham, 2016), with rates increasing to 40% by adulthood (Hudson, Hall, & Harkness, 2019). Given the implication of the HPA and ANS systems in depression in the general population, these systems may also contribute to the

complex association between ASD symptomatology and high prevalence of internalizing disorders. Therefore, investigations into the interactions of these systems in ASD are warranted.

The HPA axis is a neuroendocrine stress system that releases the human glucocorticoid, cortisol, from the adrenal glands (Herman & Cullinan, 1997). The cascade is initiated by release of corticotropin-releasing hormone (CRH) from the hypothalamus, followed by adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary to ultimately signal the release of cortisol. Secretion is modulated by three main functions of the HPA axis—diurnal regulation, response to stress, and negative feedback to restore basal levels. Limbic forebrain brain regions, including the prefrontal cortex, amygdala, and hippocampus, also modulate regulation by initiating or inhibiting the HPA axis cascade (Herman & Cullinan, 1997).

The autonomic nervous system (ANS) of the peripheral nervous system is divided into two branches—the sympathetic (SNS) and parasympathetic (PNS) nervous systems. The two branches act in a relatively reciprocal fashion, with the PNS defined as the 'rest and digest' branch, lowering heart rate, blood pressure, and increasing digestion, among other functions. In contrast, the SNS mediates the more metabolically demanding 'fight or flight' response, such as increased heart rate, respiration, and blood pressure. The ANS has been proposed as a primary behavioral regulator, with the *balance* between the PNS and the SNS being crucial in mediating that regulation (Berntson, Norman, Hawkley, & Cacioppo, 2008). Similar to regulation of the HPA axis, centrally mediated brain regions such as the prefrontal cortex and amygdala send downstream signals to the ANS brainstem output regions, thereby providing a top-down influence on autonomic control (Benarroch, 1993; 2004).

A number of studies have cited atypical levels of salivary cortisol, a reliable measure of HPA axis function (Kirschbaum & Hellhammer, 1989), in depressed patients relative to healthy

controls (see Stetler & Miller, 2011 for review). For instance, in a large cohort of adults, those with current or remitted major depressive disorder showed evidence of hypercortisolism, to include elevated evening cortisol or cortisol awakening response (Vreeburg et al., 2009). Several studies in children and adolescents have also reported elevated cortisol, especially in the evening, in those with clinically-significant levels of depressive symptoms or with a depression diagnosis (Dahl et al., 1991; Van den Bergh & Van Calster, 2009; Van den Bergh, Van Calster, Pinna Puissant, & Van Huffel, 2008). However, others have reported atypically low cortisol (hypocortisolism) in depressive disorders, especially in chronic, severe cases (e.g. Badanes, Watamura, & Hankin, 2011; Bremmer et al., 2007; Heim, Ehlert, & Hellhammer, 2000). Similarly, reduced parasympathetic tone (e.g. Rottenberg, Clift, Bolden, & Salomon, 2007; Thayer, Smith, Rossy, Sollers, & Friedman, 1998; Yaptangco, Crowell, Baucom, Bride, & Hansen, 2015) and/or sympathetic predominance (Schumann, Andrack, & Bär, 2017) may distinguish depressed individuals from healthy controls. Nonetheless, others report no relation between autonomic function and mood (Byrne et al., 2010). Despite some mixed findings regarding specific associations, it is apparent that a dynamic physiological system capable of reacting and adapting to environmental demands is critical to psychological health (Friedman, 2007).

While the HPA axis and ANS are independent systems, they share substantial neural overlap, especially at the level of frontolimbic regions involved in top-down regulation of physiological output. As both have individually been implicated in psychiatric conditions, identifying interactions between the systems and the patterns of multiple physiological responses likely improves identification of risk factors associated with psychiatric conditions such as depression (Bauer, Quas, & Boyce, 2002). It has been shown that during periods of rest, the

prefrontal cortex maintains tonic inhibition over sympathoexcitatory circuitry (e.g. HPA axis, SNS) via inhibition of the amygdala and downstream projections to the nucleus tractus solitarius (NTS) and paraventricular nucleus (PVN) of the hypothalamus, key regions involved in ANS and HPA signaling (e.g. Benarroch, 1993; Thayer & Brosschot, 2005; Ulrich-Lai & Herman, 2009). Ultimately, the PNS is upregulated, promoting a calm visceral state while inhibiting the excitatory SNS and HPA systems (e.g. Friedman, 2007; Porges, 2007). If the prefrontal cortex is hypoactive, however, such as in depression (e.g. Davidson, Pizzagalli, Nitschke, & Putnam, 2002; Koenigs & Grafman, 2009), the amygdala is disinhibited, leading to SNS and HPA activation, as well as PNS suppression (e.g. Thayer & Lane, 2009). Therefore, if there is dysfunction anywhere within these interrelated systems, the entire network could be altered, and different patterns of activation between systems will likely differentially affect behavioral outcomes (Bauer et al., 2002).

In a study of second- and third-grade children with typical development, high baseline parasympathetic regulation was shown to be protective against internalizing symptoms, even in the presence of elevated basal cortisol, whereas those with low PNS regulation and high HPA activity had the highest levels of anxiety (El-Sheikh, Arsiwalla, Hinnant, & Erath, 2011). Other recent research in typically developing school-aged children has found that symmetrical hyperarousal of the SNS and HPA axis is correlated with increased internalizing and externalizing symptoms (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008) or perceived stress (Rotenberg & McGrath, 2016), which supports an ‘additive’ model of arousal (Bauer et al., 2002). Research regarding interactions between the ANS and HPA axis remains relatively scarce, despite the unique advantages such investigations may provide in defining more precise at-risk physiological profiles. Therefore, identifying disruption within these systems could have

clinical implications for earlier and more precise diagnosis, especially for populations shown to be more susceptible to depression or anxiety.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in two core diagnostic domains—social communication and restricted, repetitive behaviors and interests (American Psychiatric Association, 2013). Of significant concern, many individuals with ASD are diagnosed with a comorbid internalizing disorder, with depression rates surpassing those of the global population (Mayes, Calhoun, Murray, Ahuja, & Smith, 2011). Moreover, individuals with ASD appear more likely to experience dysfunction within the HPA axis and/or ANS relative to typically developing (TD) peers, both at rest and during social and non-social stressors (see Benevides & Lane, 2013; Taylor & Corbett, 2014 for review). Youth with ASD have shown greater variability of the HPA axis (Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006; Corbett, Mendoza, Wegelin, Carmean, & Levine, 2008; Corbett, Schupp, Levine, & Mendoza, 2009), as well as elevated cortisol in the evening (Corbett et al., 2009; Muscatello & Corbett, 2018; Tomarken, Han, & Corbett, 2015). Parasympathetic regulation is often reduced in individuals with ASD relative to TD peers (Bal et al., 2010; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Vaughan Van Hecke et al., 2009), and this blunting is further associated with social difficulties (Edmiston, Jones, & Corbett, 2016; Patriquin, Scarpa, Friedman, & Porges, 2013; Vaughan Van Hecke et al., 2009). However, there is likely substantial heterogeneity in physiological function and dysfunction, as others have found no differences between individuals with ASD or TD in resting state autonomic functioning (Edmiston, Muscatello, & Corbett, 2017; Kushki, Brian, Dupuis, & Anagnostou, 2014; Levine et al., 2012; Neuhaus, Bernier, & Beauchaine, 2016; Watson, Roberts, Baranek, Mandulak, &

Dalton, 2012) nor do they tend to differ in basal afternoon cortisol levels (e.g. Corbett et al., 2006; 2008; Tomarken et al., 2015).

Given the likely presence of physiological dysfunction in ASD, as well as the high rates of depressive disorders, elucidating any relationship between physiology and internalizing symptoms in youth with ASD could serve to identify markers for risk of psychiatric comorbidities and to identify targets for intervention. While there is some previous evidence for associations between HPA and/or ANS dysfunction and anxiety in ASD (Corbett, Blain, Ioannou, & Balser, 2017; Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014; Hollocks, Pickles, Howlin, & Simonoff, 2016; Jansen, Gispen-de Wied, van der Gaag, & van Engeland, 2003; Kushki et al., 2013; Neuhaus, Bernier, & Beauchaine, 2014; Panju, Brian, Dupuis, Anagnostou, & Kushki, 2015), limited research exists regarding the correlation between physiological dysfunction and the high rates of depression in this population. One study of school-aged males with and without ASD found that across the full sample, lower parasympathetic regulation was associated with more internalizing symptoms and withdrawn/depressed symptoms (Neuhaus et al., 2014). Another study in females with ASD found that girls with a lower cortisol awakening response (opposite to the predicted response) were more likely to self-report symptoms of major depressive disorder (Sharpley, Bitsika, Andronicos, & Agnew, 2016). Yet other studies have cited no associations with internalizing symptoms and physiological response in ASD (Cai, Richdale, Dissanayake, & Uljarević, 2019; Kushki et al., 2014; Lanni, Schupp, Simon, & Corbett, 2012; Muscatello & Corbett, 2018; Tomarken et al., 2015).

The inconsistencies in previous research may be due in part to more complex relationships in physiological systems. Investigating HPA axis and ANS interactions has the

potential to convey greater information regarding internalizing disorder susceptibility compared to examining either system in isolation. To our knowledge, no study has investigated these interactions in ASD and their associations with internalizing symptoms. The current study argues that since the systems are not entirely independent, examining their interactions has the potential to better explain and predict differences in behavior and affect than the traditional approach of investigating the HPA and ANS separately. Based upon previous findings in the ASD literature (e.g. Benevides & Lane, 2013), it was hypothesized that children with ASD would exhibit a hyperarousal profile, especially within the ANS, including lower PNS and greater SNS regulation at rest relative to typically developing (TD) controls. There were no a priori hypotheses regarding the within-diagnosis interactions and directionality of associations with depression given the exploratory nature of the study.

Methods

Participants

Initially, 100 children were enrolled in the study; however, thirteen participants were excluded due to incomplete physiological data from equipment malfunction or excessive artifact in the ECG or impedance signal (TD: n=4, ASD: n=9, χ_2=2.21, p=0.14). Thus, a total of 87 children, ages 10-13 years, with ASD (N=41) or typical development (TD; N=46) were included in the analyses. The two groups had roughly equal ratios of males to females, with 14 females in the TD group and 11 in the ASD group. Age did not significantly differ between the two groups (Table 2.1). Participants were recruited from the community using university-wide research announcements, other autism-related studies, research registries, local pediatric and ASD diagnostic clinics, and social media. Participants were part of a larger, longitudinal study of

pubertal development (Corbett, 2017). All children had to have an intelligence quotient (IQ) ≥ 70, based on the Wechsler Scale of Abbreviated Intelligence, 2nd edition (WASI-2, Wechsler, 1999) in order to complete self-report questionnaires as part of the larger study. Diagnosis of ASD was based on DSM-5 criteria (American Psychiatric Association, 2013), and established by (1) previous diagnosis by psychiatrist, psychologist, or clinician with autism expertise, (2) current clinical judgement, and (3) corroborated by the Autism Diagnostic Observation Schedule, 2nd edition (Lord et al., 2012), administered by research reliable personnel.

Inclusion and exclusion criteria were reviewed at phone screening, prior to enrollment. Typically developing participants were excluded if they had a biological sibling with ASD. Parents also completed the social communication questionnaire (SCQ; Rutter, Bailey, & Lord, 2003) as a screening measure for the assessment of ASD symptoms. TD participants were required to have a total score <10 in order to be included in the study. In an effort to be more representative of the population of youths with ASD, the sample did not require medication-naïve participants. However, those on medications that may directly affect the HPA axis (e.g., corticosteroids; Granger, Hibel, Fortunato, & Kapelewski, 2009) or the ANS (e.g., stimulants; Shibao & Okamoto, 2012) were excluded from the study. Fourteen participants in the ASD group were on medications, including selective-serotonin reuptake inhibitors (n=5), melatonin (n=5), and antihistamines (n=10), or others (n=4). Some participants were on multiple medications. Three participants in the TD group were on antihistamines for daily allergy prevention. All regression analyses controlled for medication status.

The current study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The Vanderbilt Institutional Review Board

approved all study procedures. Informed written consent and verbal assent was obtained from a parent/guardian and the child, respectively, prior to inclusion in the study.

Table 2.1. Demographics and Dependent Variables.

	ASD		TD				
	M	**SD**	**M**	**SD**	*t*	p	Cohen's *d*
Age	11.49	1.05	11.36	1.08	-0.57	0.54	0.12
IQ**	101.68	17.43	118.85	12.95	5.16	<0.001	1.12
SCQ**	17.29	7.81	2.48	2.23	-11.73	<0.001	2.58
ADOS	12.68	4.62	--	--	--	--	--
BMI Percentile*	73.24	21.17	50.00	33.32	-3.53	0.001	0.76
CBCL Affective (Raw)**	5.61	4.12	1.63	2.11	-5.57	<0.001	1.21

IQ, Intelligence Quotient; SCQ, Social Communication Questionnaire; ADOS, Autism Diagnostic Observation Schedule; BMI, Body Mass Index; CBCL, Child Behavior Checklist; ASD, Autism Spectrum Disorder; TD, Typically Developing; ***p<0.01; **p<0.001**

Procedures

The current study required two visits to a university laboratory. Visit 1 consisted of diagnostic and psychological testing to confirm eligibility, while parents completed the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001). During the first visit, participants also underwent a physical examination to measure height and weight. Body mass index (BMI) was calculated using the standard formula (lb./in2) x 703 then age- and sex-adjusted using the CDC growth charts for children and adolescents (2 through 19 years; https://www.cdc.gov/healthyweight/bmi/calculator.html). Detailed procedures for the physical exam are described elsewhere (Corbett, Muscatello, Tanguturi, McGinn, & Ioannou, 2019). At

the second visit, participants underwent physiological data collection as part of a larger study of stress and social functioning.

Child Behavior Checklist (***CBCL;*** Achenbach & Rescorla, 2001). The CBCL is a parent-report questionnaire to assess problem behaviors. Scores are calculated as total raw scores, which are then converted to T-scores based on established national norms. The measure includes several *DSM-oriented scales*, with high scores suggesting diagnoses to be considered for further evaluation. The Affective Problems subscale includes items consistent with a diagnosis of dysthymia or major depressive disorder and was a primary dependent variable in analyses. The clinical cut-off is a t-score ≥ 70. The measure has shown excellent sensitivity (0.93) and good specificity (0.74) for the Affective Problems subscale's overall diagnostic accuracy in youth with ASD (Magyar & Pandolfi, 2017). Raw scores were used in analyses, as recommended in the CBCL manual (Achenbach & Rescorla, 2001).

Physiological Measures

HPA Axis: Salivary Cortisol

HPA axis regulation was measured through the collection of salivary cortisol. Collecting cortisol through saliva is noninvasive and was collected in the lab using well-established methods (Corbett et al., 2008). Samples were provided by passive drool approximately 40 minutes after arrival to the lab to allow for ample acclimation time. All visits began between 2pm – 3pm to control for the diurnal rhythm of cortisol.

Cortisol Assay

Prior to assay, samples were stored at -20°C. Salivary cortisol assay was performed using a Coat-A-Count® radioimmunoassay (RIA) kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) modified to accommodate lower levels of cortisol in human saliva relative to plasma. Saliva samples were thawed and centrifuged at 3460 rpm for 15 min to separate the aqueous component from mucins and other suspended particles. All samples were duplicated. The coated tube from the kit was substituted with a glass tube into which 100ml of saliva, 100ml of cortisol antibody (courtesy of Wendell Nicholson, Vanderbilt University, Nashville, TN), and 100ml of ^{125}I-cortisol were mixed. After incubation at 4ºC for 24h, 100ml of normal rat serum in 0.1% PO4/ EDTA buffer (1:50) and precipitating reagent (PR81) were added. The mixture was centrifuged at 3460 rpm for 30 min, decanted, and counted. Serial dilution of samples indicated a linearity of 0.99. Interassay coefficient of variation was 1.03%.

ANS: Respiratory Sinus Arrhythmia (RSA) and Pre-ejection Period (PEP)

Cardiac autonomic measures were collected using MindWare Mobile Impedance Cardiograph units (MindWare Technologies LTD, Gahanna, OH) for synchronized electrocardiography (ECG) and respiration data using a seven-electrode configuration. A color cartoon was provided to all participants, illustrating where electrodes would be placed, and all children had the opportunity to place an electrode on their hand prior to placement in order to increase participant comfort with the protocol. Baseline collection consisted of a five-minute rest period, with all participants sitting quietly and not engaging in any other tasks.

Parasympathetic regulation was indexed using respiratory sinus arrhythmia (RSA), a measure of high frequency heart rate variability, indicating variation in timing between

successive heart beats in association with respiration. RSA was derived in accordance with the guidelines by the Society for Psychophysiological Research committee on heart rate variability (Berntson et al., 1997; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and calculated on a minute-by-minute basis. ECG signal was sampled at 500 Hz and analyzed using the Heart Rate Variability Software Suite provided by MindWare Technologies (MindWare Technologies LTD, Gahanna, OH). RSA was quantified as the integral power within the respiratory frequency band (0.15 to 0.42 Hz). The respiration signal was processed by impedance cardiography and displayed to ensure that the values were within the designated respiratory frequency band. Of the total analyzed data, 0.4% were excluded due to excessive motion artifact or arrhythmia.

Sympathetic arousal was measured using impedance cardiography, a measurement of the mechanical activation of the heart, and can be quantified via pre-ejection period (PEP), which represents the interval from electrical stimulation to mechanical opening of the aorta. PEP was processed using MindWare Technologies Impedance Cardiography Analysis Software (MindWare Technologies, LTD, Gahanna, OH) and calculated as the distance (in ms) from the ECG Q-point of the QRS complex to the B point of the impedance waveform, which corresponds with the time from ventricular depolarization to aortic valve opening (Sherwood et al., 1990). PEP was ensemble-averaged for each one-minute epoch by the MindWare software, and B-point was calculated at 55% of the R-Z interval (time to dZ/dt peak) (Lozano et al., 2007). The QRS complex and dZ/dt signal was confirmed by visual inspection.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp, 2019). Independent sample *t*-tests were conducted to test for differences between ASD and TD groups on demographic and diagnostic variables, including age, IQ, BMI, SCQ, and the CBCL. If assumption of equal variance was not met, the Welch degree of freedom transformation was used. Effect sizes were reported as Cohen's *d.* For internalizing symptoms, participants were characterized as reaching clinically significant depressive symptoms is CBCL T-scores ≥ 70. Differences in number of participants falling in clinically significant or non-significant categories were examined using chi-square.

Cortisol values were positively skewed and thus log-10 transformed to achieve normality; RSA and PEP were normally distributed. Between-group differences in physiological measures were tested using analysis of covariance, controlling for age and BMI percentile. Partial η_2 was calculated to indicate effect size.

Hierarchal regression with bootstrapping for robust estimators (Efron & Tibshirani, 1993) and bias-corrected and accelerated confidence intervals (to account for the non-normal distribution of the outcome variable) assessed the contributions of cortisol, RSA, and PEP interactions in predicting parent-reported affective symptoms. Separate models were conducted for the ASD and TD groups. Medication use and BMI percentile were controlled in the first step of the model, and then the main effects of cortisol, RSA, and PEP were added in the second step. All possible two-way interaction terms between physiological variables were individually added to the base model in a third step to determine the extent to which each interaction contributed additional variance to the prediction of affective symptoms. Significant interactions were further examined using the PROCESS v3.4 macro (Hayes, 2013) to plot regression lines at high (+1

SD), mean, and low (-1 SD) values of the interacting variables (Aiken & West, 1991). All control and independent variables were mean centered prior to analyses (and prior to creating the interaction terms) to avoid multicollinearity effects.

Results

Demographic and diagnostic information between groups is presented in Table 2.1. There were no differences between the groups in age; however, the TD group did have higher average IQ than the ASD group. Nonetheless, the mean score for the ASD group fell well within the average range for IQ.

In regard to physical health status, youth with ASD presented with higher average BMI (age- and gender-adjusted percentiles), as measured by physical examination, relative to the TD youth, thus all regression models controlled for BMI percentile along with medication use.

Regarding internalizing symptoms, relative to parents of TD children, parents of children with ASD reported that their sons/daughters had significantly more affective problems on the CBCL. Participants were further categorized according to whether or not they met clinical cutoffs for affective problems ($T \geq 70$). More youth with ASD met the clinically significant cutoff for affective problems relative to TD youth (39% ASD vs. 6.5% TD, $p<0.001$, Fisher's exact test).

Results of between group comparisons for RSA, PEP, and cortisol are illustrated in Figure 2.1. After controlling for BMI percentile, youth with ASD and TD did not differ in baseline cortisol levels ($F(1,83)=0.01$, $p=0.94$, Partial $\eta^2<0.01$, Figure 2.1a), suggesting no significant differences in HPA axis regulation during a resting period. Similarly, the groups showed no statistically significant differences in baseline RSA ($F(1,84)=2.55$, $p=0.11$, Partial

η_2=0.03; Figure 2.1b) or PEP (F(1,84)=0.63, p=0.43, Partial η_2=0.01; Figure 2.1c). Therefore, youth with ASD and TD had comparable parasympathetic and sympathetic regulation during a resting state.

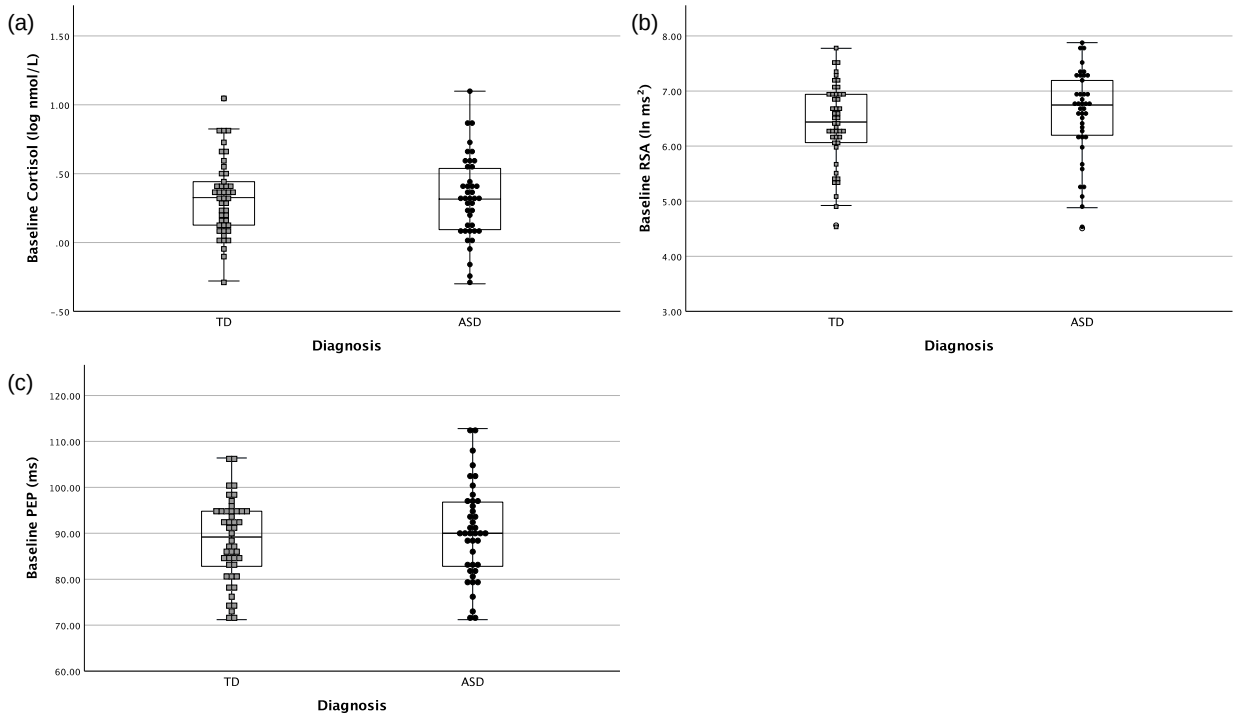

Figure 2.1. Mean Baseline Physiological Levels for Children with ASD and TD. There were no significant differences between children with ASD and TD in (a) baseline log cortisol, (b) baseline respiratory sinus arrhythmia, or (c) baseline pre-ejection period.

When modeling predictors of depressive symptoms in the ASD group, after controlling for medication use and BMI percentile, addition of the main effects for RSA, PEP, and cortisol did not account for significant additional model variance. Separate models were run for each two-way interaction in order to test the individual contribution of each interaction (see Table 2.2). In the ASD group, the RSA*PEP interaction contributed an additional 11% of variance to

the base model, while the cortisol*RSA and cortisol*PEP interactions did not contribute statistically significant additional variance ($p>0.05$).

When further examining the RSA*PEP interaction using simple slopes, it was shown that only at high levels of PEP (+1 SD) was there a significant negative relationship between RSA and depressive symptoms (b=-2.60, p=0.02, 95% CI [-4.75, -0.44]; Figure 2.2). Further, Johnson-Neyman analysis (Hayes & Matthes, 2009; Johnson & Fay, 1950) confirmed the threshold of significance at a mean-centered PEP value of 3.13, and the effect for RSA and depression became stronger and more negative as PEP increased. Therefore, children with ASD and highest levels of PEP (less sympathetic arousal) had significantly fewer depressive symptoms with increasing parasympathetic regulation. At low and mean values of PEP, there was a nonsignificant relationship between RSA and CBCL affective symptoms (all $p>0.05$; see Figure 2.2).

Regression models in the TD group revealed neither the physiological main effects (RSA, cortisol, PEP) nor any of the interactions contributed significant additional variance to the overall model (see Table 2.3). However, the model coefficients for PEP and the RSA*PEP interaction were weakly significant. Nonetheless, Johnson-Neyman analysis found no points at which PEP moderated the relationship between RSA and affective symptoms ($p>0.05$), nor were the slopes statistically significant at any level of PEP or RSA (see Figure 2.3), suggesting a lack of association between PNS functioning and depression in TD youth at varying levels of SNS arousal or insufficient power to detect possible relationships within the current sample.

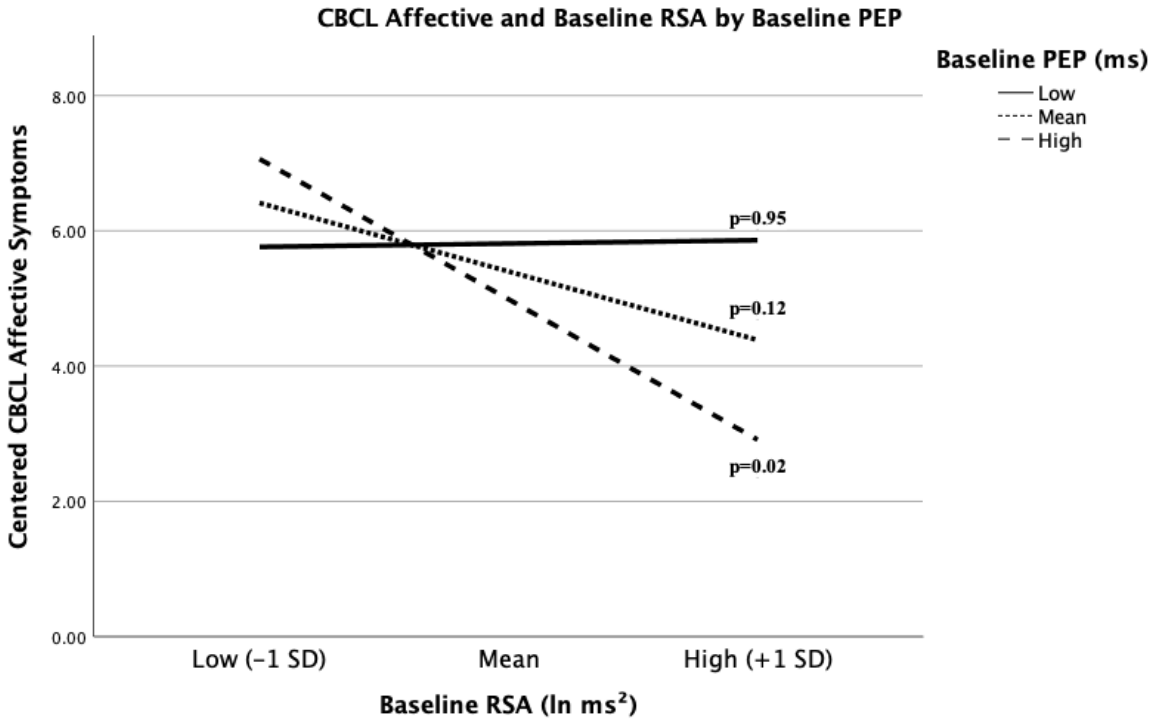

Figure 2.2. RSA and PEP Interact to Predict Depressive Symptoms in ASD. High PEP moderates the negative association between RSA and affective symptoms in youth with ASD. High and low values for PEP and RSA are equivalent to +/- 1 SD from the mean. P-values for each slope are provided.

Table 2.2. Regression Models Predicting Scores on Affective Subscale of CBCL in ASD

	Model 1		**Model 2**		**Model 3**					
					3a		**3b**		**3c**	
Variable	***b***	***p*a**	***b***	***p*a**	***b***	***p*a**	***b***	***p*a**	***b***	***p*a**
Medications	-0.82	0.25	-0.67	0.76	-0.80	0.29	-0.83	0.22	-0.64	0.41
BMI	0.02	0.51	0.01	0.59	0.01	0.76	0.02	0.40	0.01	0.62
Cortisol	--	--	-2.18	0.24	-0.36	0.86	-2.05	0.26	-2.20	0.28
RSA	--	--	-1.18	0.17	-0.71	0.42	-0.90	0.23	-1.25	0.16
PEP	--	--	0.01	0.88	0.02	0.76	-0.02	0.70	-0.01	0.86
Cortisol*RSA	--	--	--	--	-6.67	0.13	--	--	--	--
RSA*PEP	--	--	--	--	--	--	**-0.15***	**0.01***	--	--
Cortisol*PEP	--	--	--	--	--	--	--	--	0.22	0.47

	Model 1	**Model 2**	**Model 3a**	**Model 3b**	**Model 3c**
R^2	0.06	0.13	0.45	0.49	0.37
ΔR^2	0.06	0.06	0.07	**0.11***	0.01
$\Delta F_{(df)}$	$1.29_{(2,38)}$	$0.86_{(3,35)}$	$3.17_{(1,34)}$	$\mathbf{4.80_{(1,34)}}$*	$0.46_{(1,34)}$

RSA, Respiratory Sinus Arrhythmia; PEP, Pre-ejection Period; BMI, Body Mass Index; ***p*-value < 0.05**;
a*p*-values based on bootstrapping with 1000 bootstrap samples

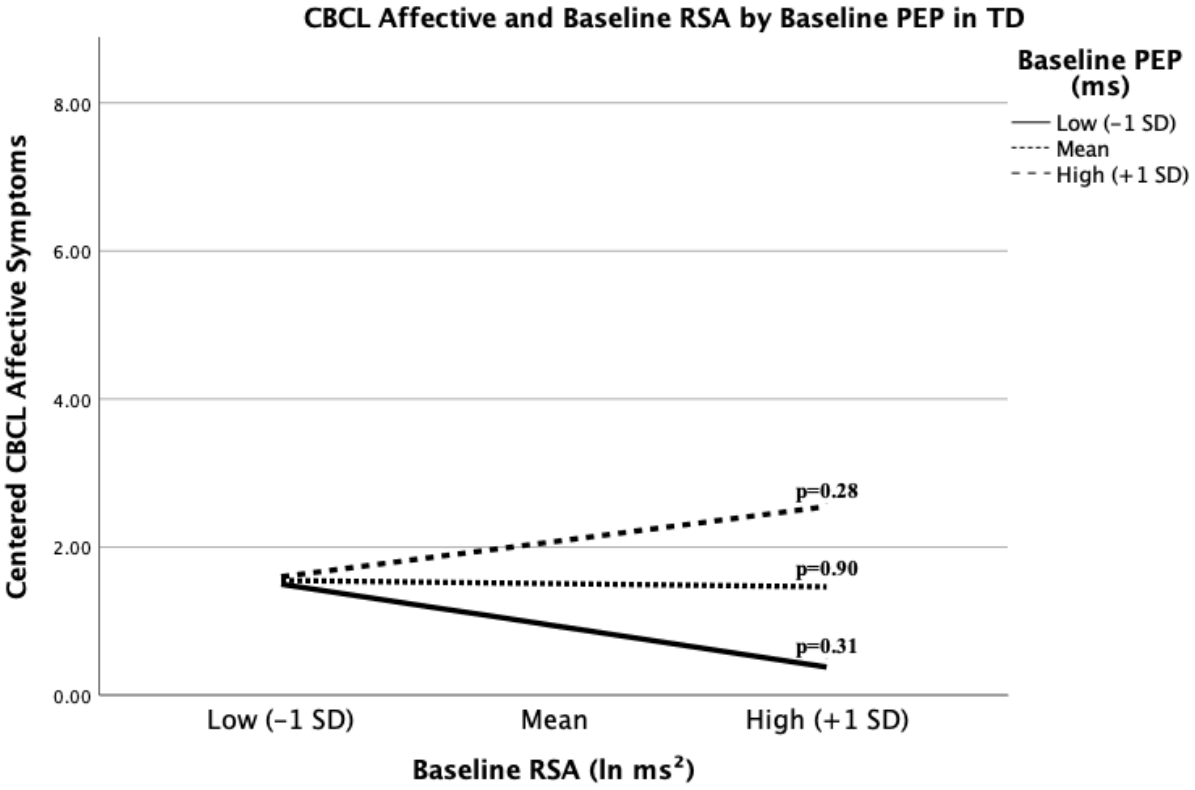

Figure 2.3. Illustration of RSA and Depressive Symptom Associations by level of PEP in TD. The relationship between RSA and affective symptoms in with TD is not significant at any value of PEP. High and low values for PEP and RSA are equivalent to +/- 1 SD from the mean. P-values for each slope are provided.

Table 2.3. Regression Models Predicting Scores on Affective Subscale of CBCL in TD group.

	Model 1		**Model 2**		**Model 3**					
					3a		**3b**		**3c**	
Variable	***b***	***p*a**	***b***	***p*a**	***b***	***p*a**	***b***	***p*a**	***b***	***p*a**
BMI	0.004	0.66	0.004	0.63	0.004	0.66	0.005	0.48	0.003	0.74
Cortisol	--	--	2.11	0.08	2.04	0.09	2.20	0.09	2.15	0.09
RSA	--	--	0.12	0.76	0.09	0.83	0.05	0.86	0.11	0.76
PEP	--	--	**0.07***	**0.04***	**0.07***	**0.04***	**0.08***	**0.04***	0.07	0.07
Cortisol*RSA	--	--	--	--	-0.45	0.79	--	--	--	--
RSA*PEP	--	--	--	--	--	--	**0.09***	**0.05***	--	--
Cortisol*PEP	--	--	--	--	--	--	--	--	-0.08	0.56

	Model 1	**Model 2**	**Model 3a**	**Model 3b**	**Model 3c**
R^2	0.003	0.15	0.15	0.20	0.16
ΔR^2	0.003	0.15	0.001	0.05	0.01
$\Delta F_{(df)}$	$0.14_{(1,43)}$	$2.24_{(3,40)}$	$0.06_{(1,39)}$	$2.41_{(1,39)}$	$0.28_{(1,39)}$

RSA, Respiratory Sinus Arrhythmia; PEP, Pre-ejection Period; BMI, Body Mass Index;***p-value < 0.05;**
ap-values based on bootstrapping with 1000 bootstrap samples

Discussion

The current study aimed to examine HPA axis and ANS regulation in children with and without ASD, as well as the impact of physiological dysregulation on depressive symptoms in youth. The investigation uniquely sought to identify patterns of interaction between these physiological systems, which may especially contribute to elevated rates of depression in ASD. It was hypothesized that resting states characterized by greater sympathetic and HPA activation, as well as reduced parasympathetic regulation, would be more prevalent in ASD. Contrary to hypotheses, no differences were seen between the two groups on any of the physiological variables; however, as expected, children with ASD demonstrated significantly elevated depressive symptoms. Additionally, interactions between physiological systems uniquely predicted depressive symptoms, especially in children with ASD, which provided preliminary insight into the interrelated nature of these physiological systems and their potential role in effecting emotional states.

Prevalence of depression in youth with ASD has recently been reported to be as high 20.2% for adolescents with ASD (Greenlee et al., 2016). Our sample of 10-13-year-old youth similarly displayed elevated levels of depression compared to TD youth, as reported by their parents. When identifying those reaching clinically significant thresholds on the parent-report measures, children with ASD met the clinical threshold 39% of the time, while only 6.5% of TD children met the clinical significance threshold. This early age of onset for depression in youth with ASD is notable, as psychiatric symptoms may contribute to poor future outcomes, including difficulties with employment and poor adaptive skills (e.g. Kraper, Kenworthy, Popal, Martin, & Wallace, 2017; Walsh, Lydon, & Healy, 2014). Moreover, depressive symptoms may exacerbate tendencies toward social withdrawal and social isolation (Ghaziuddin, Ghaziuddin, & Greden,

2002; Greenlee et al., 2016). Loneliness and social isolation are identified risk factors for suicidal behavior (Hedley, Uljarević, Foley, Richdale, & Trollor, 2018), with suicidality risk significantly higher in ASD relative to the general population (Cassidy et al., 2014). Clearly, early diagnosis and treatment is necessary to help avoid these negative consequences. Future studies with larger samples might illuminate associations between elevated depressive scores in children and adolescents with ASD and future maladaptive outcomes, as well as to highlight the impact of earlier diagnosis and intervention of depression in this population.

One concern regarding the diagnosis of depression in ASD is that individuals with ASD may exhibit deficits in expressive affective states and language thereby impeding their ability to effectively communicate symptoms (Losh & Capps, 2006). Physiological functioning, which has gained interest in its utility as a biological marker for ASD, may also be used in identifying risk or likelihood of being diagnosed with a depressive disorder in this population. However, as previously mentioned, the existing literature is unclear regarding the extent to which physiology and psychology are related in ASD, with several instances of conflicting results. Youth with ASD have been shown to demonstrate hyperarousal in the HPA axis (e.g. Muscatello & Corbett, 2018; Tomarken et al., 2015; Tordjman et al., 2014) and ANS (e.g. Kushki et al., 2014; Vaughan Van Hecke et al., 2009); however, others have cited no differences between youth with ASD and TD (Bitsika, Sharpley, Andronicos, & Agnew, 2015; Corbett et al., 2006; Watson et al., 2012). Similarly, some report associations between physiological arousal and internalizing symptoms (Neuhaus et al., 2014; Sharpley et al., 2016), while others cite no relationships (e.g. Tomarken et al., 2015). These contrasting findings suggest identifying patterns of interaction between the HPA axis and ANS may be more informative than examining individual systems when assessing associations with internalizing profiles.

In the current study, the main effects of each individual system revealed little information regarding the risk for internalizing disorders, as groups did not differ in baseline physiological response, nor systems singularly associated with depressive symptoms. However, upon looking at the interactions of the systems within-diagnosis, more distinct patterns of risk emerged. Autonomic balance, or the relationship between PNS and SNS regulation, was associated with depressive symptom risk in children with ASD. Specifically, youth with a predominantly reciprocal parasympathetic response system (i.e. increased PNS with decreased SNS; Berntson et al., 2008) were noted to have the lowest levels of depressive symptoms. This tendency towards a lower baseline arousal pattern, with elevated inhibition from the PNS and removal of excitation of the SNS, may be suggestive of less chronic stress and/or be protective against the wear and tear (allostatic load; McEwen, 1998) associated with chronic stress exposure. In this case, regions often implicated in depression (i.e. prefrontal cortex, amygdala, hippocampus; McEwen, 2003) would be less likely to experience neuronal damage associated with excitotoxicity. Caution should be taken in these interpretations, however, as these results are preliminary and only correlational, not causational. Future longitudinal studies with larger samples sizes may provide more information regarding the time course and chronicity of these physiological and behavioral changes to elucidate the directionality of relationships. Additionally, studies assessing stress reactivity may reveal different patterns of adaptive or maladaptive arousal, as previously symptoms of depression, such as loneliness, have been associated with a blunted (hypoarousal) cardiovascular and endocrinological stress response (e.g. Brown, Gallagher, & Creaven, 2018). Notwithstanding, the current findings offer initial support for the added benefits of investigating interactions across the HPA axis and ANS, rather than their singular, isolated functions, when assessing their role in regulating behavior in ASD.

Other factors are important to consider when interpreting these findings. The study focused on a narrow age range of 10-13-year old children, yet incidence of depression is known to increase with age, with a particular risk during the adolescent period of development (Gotham, Brunwasser, & Lord, 2015). Importantly, the current sample included five children being actively treated with SSRIs and up to 39% reporting clinically significant depressive symptoms on behavioral report. Longitudinal studies (Corbett, 2017) following youth from childhood through the adolescent period may reveal important changes in symptom profile through development and the ways in which physiological systems interact and relate with mental health status. While results suggested a possible interaction with RSA and PEP in the TD group, no significant relationships were seen between physiology and affect within these youth, despite previous research finding inter-relations across systems and with internalizing symptoms (e.g. El-Sheikh et al., 2008; 2011). The current TD sample was smaller than previous studies and without psychiatric incidence, with only 6.5% of the sample falling in the clinically significant range for symptoms on the affective subscale on the CBCL. Therefore, future research with larger samples comparing across ASD and TD should also consider a more variable TD group, possibly also including individuals with a diagnosed depressive disorder as a third comparison group.

The investigation is strengthened by the novel examination of the interrelated actions of the HPA axis and ANS in predicting depressive symptoms in children with ASD, thereby helping to fill an important gap in our understanding of the connection between physiological arousal and affect. The study, however, is not without limitations. First, the sample was relatively limited by its narrow age range. Second, inclusion of only individuals without cognitive impairment reduces interpretations of findings to only a subset of the ASD population.

Third, the sample size is comparable to many empirical studies in ASD, but a larger sample with greater power to handle the interaction analyses may have resulted in stronger or additional findings. This is especially emphasized as there were likely three-way interactions between HPA axis, ANS, and diagnostic group, as seen by the differential findings within the ASD vs. TD group; however, we did not have sufficient power to statistically evaluate these higher-order interactions. Fourth, the current study utilized methods collected in the lab on a single day in an effort to have all three measures reflect arousal at approximately the same point in time, which is not uncommon amongst physiological studies (e.g., El-Sheikh et al., 2008; 2011). Nevertheless, more comprehensive methods, such as at-home collection of diurnal cortisol rhythm and 24-hour ambulatory monitoring of heart rate variability, as done in recent studies (Rotenberg & McGrath, 2016), may yield different results with a more complete profile of regulation. Lastly, the current study focused on baseline, resting physiological functioning. Future studies will want to investigate these interactions and associations in response to a stressor, as previous research suggests critical differences in physiological reactivity in youth with ASD, such as in response to social stress (see Benevides & Lane, 2013 for review).

Conclusions

The current study aimed to examine the interactions between the HPA axis and ANS and their associations with depression. The results extend previous findings on physiological dysregulation in children with ASD, identifying preliminary evidence for unique multi-system interactions, which may predict parent-reported symptoms of depression. While children with ASD displayed significantly more depressive symptoms, physiological arousal did not differ between diagnostic groups; however, distinct interactions, especially within the opposing

branches of the ANS, were evident. As depressive disorders continue to be a prevalent concern for many youth and young adults with ASD, accurate diagnosis and early intervention are critical to effectively manage and improve quality of life. Our findings underscore the importance and benefits of examining arousal across multiple systems to more accurately identify these physiological profiles associated with psychological risk and behavioral outcomes in ASD, which may eventually aid in earlier diagnosis of comorbid symptom profiles and inform treatment to facilitate more favorable outcomes for those with these symptoms.

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Chapter III

FRIENDLY PEER INTERACTION INDUCES ATYPICAL STRESS AND AROUSAL IN CHILDREN WITH AUTISM SPECTRUM DISORDER

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder estimated to affect 1 in 59 children (Baio et al., 2018), defined by symptoms across two core diagnostic domains-impairments in social interaction and communication, and restrictive and repetitive patterns and behaviors (American Psychiatric Association, 2013). Individuals with ASD have significant difficulty engaging with others and responding to novel social situations, and often find peer interactions to be stressful (Corbett, Schupp, Simon, Ryan, & Mendoza, 2010; Corbett et al., 2014; Lopata, Volker, Putnam, Thomeer, & Nida, 2008; Schupp, Simon, & Corbett, 2013). Despite such challenges, children must interact with peers nearly every day—in the classroom, on the playground, and in the community.

In addition to homeostatic maintenance, the hypothalamic-pituitary-adrenal (HPA) axis is prominently involved in stress response to physical ('systemic') and psychological ('processive') stressors (Herman & Cullinan, 1997). Dynamic changes in cortisol reactivity can be reliably and non-invasively measured through saliva (Kirschbaum & Hellhammer, 1989), thus providing an efficient tool for assessing HPA axis reactivity to a variety of stressors. Additionally, the stress response is modulated by regions of the limbic system, including but not limited to, the prefrontal cortex, hippocampus, and amygdala (Herman & Cullinan, 1997). Specifically, the amygdala and prefrontal cortex are likely involved in the processing and interpreting of

emotional- and context-dependent cues, thus determining stress response based on whether a stimulus is deemed threatening or safe (e.g. Ulrich-Lai & Herman, 2009).

The four features of psychological stress reliably shown to activate the HPA axis include novelty, unpredictability, social evaluation, and a sense of low control (Dickerson & Kemeny, 2004). For example, a common laboratory-based stress protocol, the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), is a social evaluative threat paradigm in which participants are required to give a speech in front of unsupportive 'judges' exhibiting neutral affect. The protocol has been shown to consistently induce cortisol elevation in children and adults (e.g. Buske-Kirschbaum et al., 1997; Kudielka & Kirschbaum, 2005; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004), presumably due to the evaluative nature of the protocol. Situations perceived as uncontrollable and evaluative may be particularly stressful for humans (Dickerson & Kemeny, 2004). Therefore, instances of social rejection, especially resulting from uncontrollable personal characteristics, would presumably be sources of stress for many individuals. The HPA axis response appears to be moderated by the psychological perception of the stimulus as stressful. The perception of a public speaking task as stressful has been shown to be positively related to the accompanying physiological response, especially in adolescents (Evans et al., 2013), thus highlighting the significant role of psychological processing in determining stress reactions.

In ASD, an extensive literature points to dysfunction of HPA axis regulation and response. There is likely disruption of the diurnal rhythm, as evidenced by elevated evening cortisol in children and adolescents with ASD (Corbett, Mendoza, Wegelin, Carmean, & Levine, 2008; Corbett, Schupp, Levine, & Mendoza, 2009; Muscatello & Corbett, 2018; Putnam, Lopata, Thomeer, Volker, & Rodgers, 2015; Tomarken, Han, & Corbett, 2015; Tordjman et al., 2014).

Further, many individuals demonstrate atypical arousal response patterns to a variety of stressors. For example, the TSST frequently fails to elicit a cortisol response in youth with ASD (Corbett, Schupp, & Lanni, 2012; Jansen et al., 2000; Lanni, Schupp, Simon, & Corbett, 2012; Levine et al., 2012), suggesting they do not perceive the task as stressful. Indeed, it has been shown that perception of neutral affect mediates the stress response on the TSST in children with and without ASD (Corbett, Muscatello, & Baldinger, 2019a). In contrast, play-based interaction paradigms often lead to increased stress responses in youth with ASD relative to TD peers. During a peer interaction paradigm with two novel peers on a playground, children with ASD were found to have elevated cortisol responses, while TD children did not perceive this activity as stressful (Corbett et al., 2010; 2014; Schupp et al., 2013). In a direct comparison between these two paradigms, children with ASD showed greater stress to social interaction with peers and diminished stress to the TSST, which was opposite of same-age typically developing children (Corbett et al., 2012). These stress responses are further modified by age, as older youth with ASD show significantly more cortisol elevation relative to younger children (Corbett et al., 2010; Schupp et al., 2013). In summary, youth with ASD have heightened reactivity to relatively benign social interactions but do not respond to prototypical social evaluative stressors, reflecting an apparent alteration in the limbic-hypothalamic-pituitary-adrenal circuit.

A second stress response system, often implicated in response to social stressors, is the autonomic nervous system (ANS). The ANS is separated into two branches with primarily opposing functions, the parasympathetic (PNS; ‘rest and relax’) and sympathetic nervous systems (SNS; ‘fight or flight’). The sinoatrial (SA) node, or pacemaker, of the heart is dually innervated by the PNS and SNS, and heart rate variability (HRV) utilizes this dual innervation to serve as a useful marker of changes in PNS and SNS activity (Task Force of the European

Society of Cardiology, 1996). Specifically, high frequency HRV, most often measured as respiratory sinus arrhythmia (RSA), indexes PNS influences. In contrast, the pre-ejection period (PEP), a metric of time from electrical stimulation to mechanical opening of the aortic value, serves as a measure of pure SNS function. These metrics are further influenced by developmental maturation, as ANS function changes as children age and advance through puberty (Benevides & Lane, 2015).

The ANS is a complex network in which efferent signals originating from medullary brainstem regions (Benarroch, 2012; Porges, 1995) affect functioning of peripheral visceral organs, including the heart. The dually innervated SA node is said to be under tonic parasympathetic inhibition via the myelinated vagal nerve (Porges, 1995; 2001). This 'vagal brake' regulates behavior through maintenance and balance of PNS influence to the heart, thus allowing for changes in heart rate as parasympathetic regulation changes in response to changing environmental stimuli (Porges, 1995; 2001; 2007). Therefore, in the presence of a stressor, removal of the 'vagal brake' can allow for increases in heart rate and respiration without engaging the metabolically demanding SNS (Wolff, Wadsworth, Wilhelm, & Mauss, 2012). The parasympathetically-mediated Social Engagement System (Porges, 2001; 2003b; 2007) is active during these calm visceral states of vagal flexibility, thus promoting activation of the interconnected craniofacial nerves and their associated motor behaviors. However, in cases of more severe threat, the SNS will activate, presumably inhibiting parasympathetic systems and blocking the Social Engagement System while initiating the fight or flight response to respond to the stressor.

It has been noted that many of the behaviors associated with the Social Engagement System, including eye gaze, language and vocalization production, and emotional expression

(Porges, 2001; 2003b; 2007) are often impaired in a number of neurological conditions, most notably, autism spectrum disorder (Porges, 2003a; 2005). The autonomic system is likely dysregulated in ASD, evidenced by reductions in resting PNS regulation relative to TD peers (Bal et al., 2010; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Vaughan Van Hecke et al., 2009) as well as numerous studies which cite atypical PNS and SNS reactivity (e.g. Althaus, Mulder, Mulder, Aarnoudse, & Minderaa, 1999; Edmiston, Jones, & Corbett, 2016; Neuhaus, Bernier, & Beauchaine, 2016; Toichi & Kamio, 2003). In a study of school-aged children, those with ASD demonstrated lower RSA during interactions with unfamiliar peers; moreover, the reduction in RSA was associated with more social problems and problem behaviors (Vaughan Van Hecke et al., 2009). A similar reduction in parasympathetic regulation, along with sympathetic hyperarousal, was seen in ASD children, compared to TD controls, when interacting with a familiar partner (Neuhaus et al., 2016). In the context of social play, higher resting PNS regulation has been associated with more gestures and sharing behavior in young children with ASD during play with an adult actor (Patriquin, Scarpa, Friedman, & Porges, 2013). Therefore, social difficulties in ASD may be, in part, explained by failures of the parasympathetically-mediated vagal nerve to efficiently regulate the *Social Engagement System*, where PNS withdrawal and/or SNS hyperarousal inhibits the facial nerves and associated motor neurons responsible for many social behaviors (Porges, 2005).

Studying across multiple physiological systems (HPA axis and ANS) can provide an opportunity to more thoroughly examine stress response to social interactions in ASD. Moreover, the use of a naturalistic stressor more accurately mirrors participants' daily lives and increases salience (Adam, 2006). One study found that during a friendly social interaction with a novel peer (Trier Social Stress Test-Friendly (TSST-F); Wiemers, Schoofs, & Wolf, 2013), children

and adolescents with ASD youth did not demonstrate a stress response in the HPA axis or PNS (Corbett et al., 2019a). However, between-group analyses were limited due to a moderate sample size, and SNS functioning was not considered. Further, in a similar Peer Interaction Paradigm (PIP; Corbett et al., 2010), in which children engaged in a social encounter with peers of similar age and sex, youth with ASD show enhanced HPA activation (e.g. Corbett et al., 2014). It is important to note that stress responsivity in ASD has been shown to be variable (Corbett et al., 2012) and presumably related to age and context (Corbett et al., 2010; 2012; Schupp et al., 2013). It is also plausible that response may vary across stress and arousal systems such that investigating both the HPA axis and ANS together will more completely elucidate the complex relationship between stress and social functioning in ASD.

The current study sought to extend previous studies by examining physiological stress response to a friendly social encounter (TSST-F) in youth with and without ASD. Specifically, we measured HPA axis and ANS responses over time—from baseline, throughout the interaction, until post-interaction—to examine whether youth with ASD showed a unique pattern of response relative to TD peers. Given previous research in similar peer interaction paradigms (e.g. Corbett et al., 2014), as well as noted developmental effects on HPA and ANS regulation and responsivity (e.g. Beauchaine, 2001; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009), we hypothesized youth with ASD, especially older children, would show heightened stress and arousal to the current social interaction paradigm. Additionally, we expected findings would be consistent with the Polyvagal Theory and Social Engagement System (e.g. Porges, 2007), such that social difficulties in ASD would be associated with a dysregulated physiological state. Specifically, we predicted that children with ASD would show: (1) heightened cortisol responsivity, lower RSA, and elevated SNS arousal compared to TD children, (2) stress and

arousal responses would be modified by age, and (3) HPA and ANS responsivity would be modified by severity of behaviorally reported social symptoms.

Methods

Participants

Participants included 100 children 10-to-13-years of age, with ASD (n=50, mean age=11.48) or typical development (n=50, mean age=11.35). Gender was matched between groups, with 14 females in each group. As part of a longitudinal study of pubertal development (Corbett, 2017), families were enrolled from the community within a 200-mile radius through research registries, university-wide announcements, autism- and child-development clinics, and social media. An estimated 42% of children with ASD have been reported to take at least one psychotropic medication (Mire, Nowell, Kubiszyn, & Goin-Kochel, 2014), thus the current study did not exclude those on medications in order to be more representative of the overall ASD population. However, participants on medications that may directly affect the HPA axis (e.g. corticosteroids, Granger, Hibel, Fortunato, & Kapelewski, 2009) or ANS (e.g., stimulants, Shibao & Okamoto, 2012) were excluded. In total, 18 children with ASD were on medications at the time of the study, primarily antihistamines, melatonin, or selective-serotonin reuptake inhibitors (SSRIs). Three TD participants were taking antihistamines at the time of enrollment (see Appendix Table 3A for detailed information on medications).

Diagnostic Criteria

All participants were required to have an estimated IQ $\geq$ 70, as measured by the Wechsler Scale of Abbreviate Intelligence (WASI-II, Wechsler, 1999), in order to ensure ability to

complete self-report forms associated with the larger longitudinal study (Corbett, 2017). Parents completed the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003), a screening questionnaire for identifying symptoms of ASD. In order to be included in the study, TD youth had to score <10 on the SCQ as rated by their parents. Additionally, TD participants could not have a biological sibling with ASD. Diagnosis of ASD was based on DSM-5 criteria (American Psychiatric Association, 2013), and established by (1) previous diagnosis by psychiatrist, psychologist, or clinician with autism expertise, (2) current clinical judgement, and (3) corroborated by the Autism Diagnostic Observation Schedule, 2nd edition (Lord et al., 2012), administered by research-reliable personnel.

Procedures

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The Vanderbilt Institutional Review Board approved all study procedures. In compliance with the Institutional Review Board, informed written consent and verbal assent was obtained from all parent/guardians and children, respectively, prior to inclusion in the study. The study was completed across two visits to a university research lab. Diagnostic and cognitive measures were administered at visit 1. Parents also completed the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001) and Social Responsiveness Scale (SRS-2; Constantino & Gruber, 2012). At visit 2, participants were exposed to the social interaction protocol- the Trier Social Stress Test-Friendly (TSST-F, Wiemers et al., 2013) and completed all physiological data collection

Trier Social Stress Test-Friendly

The Trier Social Stress Test-Friendly (Wiemers et al., 2013) is an alternative form of the original TSST (Kirschbaum et al., 1993), which has been shown to elicit a physiological stress response from social evaluative threat. The TSST-F, however, consists of a more "friendly" protocol, in which participants describe him or herself and/or a favorite book, movie, hobbies, or other interest in front of a novel peer of the same sex that shows encouragement (smiles, nods shows interest, follow-up questions). The "friendly" TSST, unlike the original TSST, produces no increase in cortisol in typically developing individuals (Wiemers et al., 2013; Wiemers & Wolf, 2015) and parallels other peer interaction paradigms (Corbett et al., 2010). The TSST-F paradigm consists of a 5-minute preparation period, 10-minute social interaction, and 5-minute debrief/recovery period. Physiological data was collected continuously throughout the interaction (see Figure 3.1). The 20-minute protocol requires reciprocal social interaction with a novel trained peer, conceptualized to be a more potent stressor for children with ASD especially during adolescence.

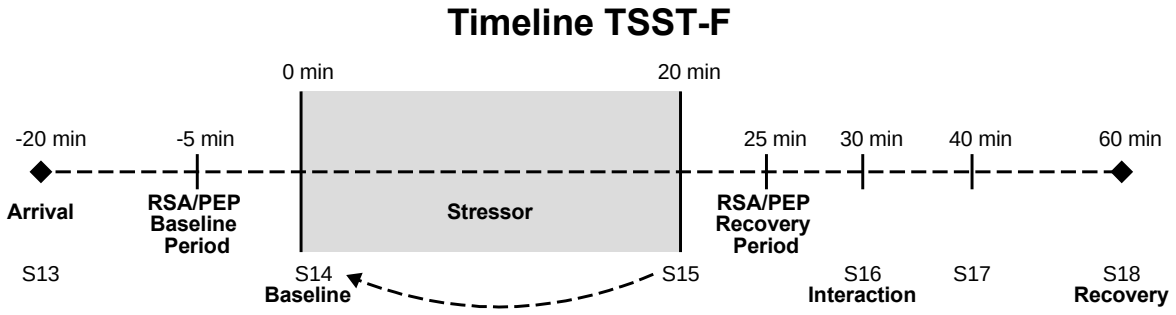

Figure 3.1. Trier Social Stress Test-Friendly Timeline. S13= Cortisol Arrival, S14= Cortisol Baseline, etc.

Dependent Measures

Social Symptoms and Perceived Anxiety

The *Child Behavior Checklist* (*CBCL;* (Achenbach & Rescorla, 2001) is a parent-report measure of behavioral and emotional problems in children ages 6-18 years. Scores are determined using a Likert scale rating from 0 ("Not True") to 2 ("Very Often True"). The CBCL has demonstrated good-to-excellent reliability in ASD, with individual scale reliabilities ranging from 0.69 to 0.94, including a reliability of 0.84 for the Social Problems domain (Pandolfi, Magyar, & Dill, 2012). Due to the a priori hypotheses regarding social symptoms and physiology, we specifically examined the Social Problems subscale. Previously, youth with ASD demonstrated significantly elevated scores on the Social Problems subscale relative to controls (Mazefsky, Anderson, Conner, & Minshew, 2011). Raw scores were used in analyses, as recommended in the CBCL manual (Achenbach & Rescorla, 2001).

The *State-Trait Anxiety Inventory for Children* (*STAIC;* (Spielberger, 1973) is a self-report measure of anxiety, completed by participants, in which an individual describes how he/she is currently feeling (state) and how he/she usually feels (trait). Previous studies have found youth with ASD are able to identify anxiety following stressors (Corbett, Blain, Ioannou, & Balser, 2017; Lanni et al., 2012; Simon & Corbett, 2013), including reporting elevated state anxiety following a social interaction (Corbett et al., 2019a). Because the current study was particularly focused on social anxiety in context, the State anxiety was a primary outcome, in order to assess participants' perceived anxiety immediately following the peer interaction.

The *Social Responsiveness Scale* (*SRS-2*; Constantino & Gruber, 2012) is a parent-report questionnaire developed to identify severity of ASD symptoms across several domains, including Social Awareness, Social Motivation, Social Cognition, Social Communication, and Restricted

and Repetitive Behaviors. Domain and total scores are presented as standardized T scores. The SRS shows high sensitivities (0.74 to 0.80) and specificities (0.69 to 1.00) for ASD (Bölte, Westerwald, Holtmann, Freitag, & Poustka, 2011). Analyses included SRS Total scores in order to examine total range of ASD-related symptoms.

Salivary Cortisol

Salivary cortisol was collected using well-established methods (Corbett et al., 2008) via passive drool. Participants were also instructed not to eat or drink prior to visiting the lab and to notify the lab if they became sick in order to have the visit rescheduled once they resumed healthy status. Twenty minutes following arrival to the lab, the initial baseline sample was collected. Salivary cortisol has a 20-min lag time for detection; thus, the first sample represents HPA axis regulation at immediate arrival. A total of six samples were collected throughout the visit to capture cortisol regulation and responsivity from baseline through the interaction to recovery (see Figure 3.1). All visits began between 2pm – 3pm to control for the diurnal rhythm of cortisol.

Prior to assay, samples were stored at -20°C. Salivary cortisol assay was performed using a Coat-A-Count® radioimmunoassay (RIA) kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) modified to accommodate lower levels of cortisol in human saliva relative to plasma. Saliva samples were thawed and centrifuged at 3460 rpm for 15 min to separate the aqueous component from mucins and other suspended particles. All samples were duplicated. The coated tube from the kit was substituted with a glass tube into which 100ml of saliva, 100ml of cortisol antibody (courtesy of Wendell Nicholson, Vanderbilt University, Nashville, TN), and 100ml of ^{125}I-cortisol were mixed. After incubation at 4ºC for 24h, 100ml of normal rat serum in

0.1% PO4/ EDTA buffer (1:50) and precipitating reagent (PR81) were added. The mixture was centrifuged at 3460 rpm for 30 min, decanted, and counted. Serial dilution of samples indicated a linearity of 0.99. Interassay coefficient of variation was 1.03%.

Heart Rate Variability

Cardiac autonomic measures were collected using MindWare Mobile Impedance Cardiograph units (MindWare Technologies LTD, Gahanna, OH) for synchronized electrocardiography (ECG) and respiration data collection using a seven-electrode configuration. Participants were told they would be wearing 'stickers' throughout the protocol, and a color cartoon was provided to illustrate the location of the electrodes. Participants were given the opportunity to place an electrode on their hand prior to placement, and a five-minute acclimation period followed electrode placement to allow children time to become comfortable with the sensory aspects of the protocol. All 100 study participants agreed to complete the heart rate collection and were able to comfortably tolerate the electrode placement.

Resting ANS regulation was acquired using a five-minute baseline collection period in which participants were instructed to sit quietly without engaging in other tasks. During the social interaction, cardiac measures were collected continuously, calculated on a minute-by-minute basis, and averaged into five-minute epochs for each major period of the paradigm—Baseline, Prep, TSST-F Interaction (Parts 1 and 2), and Recovery (see Figure 3.1).

Parasympathetic regulation was indexed using respiratory sinus arrhythmia (RSA) and derived in accordance with the guidelines set forth by the Society for Psychophysiological Research committee on heart rate variability (Berntson et al., 1997; Task Force of the European Society of Cardiology, 1996). ECG signal was sampled at 500 Hz and analyzed using the Heart

Rate Variability Software Suite provided by MindWare Technologies (MindWare Technologies LTD, Gahanna, OH). RSA was quantified as the integral power within the respiratory frequency band (0.12 to 0.42 Hz), and respiration was monitored by impedance cardiography (Ernst, Litvack, Lozano, Cacioppo, & Berntson, 1999). The respiration signal was displayed to ensure that the values were within the designated frequency band. Respiratory frequency was confirmed to lie within the high frequency/RSA band (0.12-0.42 Hz) for all participants. Of the total collected data, 1.0% were excluded due to excessive motion artifact or cardiac arrhythmias. RSA was measured in ln (ms^2).

Pre-ejection period (PEP) was collected using impedance cardiography and represents the interval from electrical stimulation to mechanical opening of the aorta. PEP was processed with MindWare Technologies Impedance Cardiography Analysis Software (MindWare Technologies, LTD, Gahanna, OH) and calculated as the distance (in ms) from the ECG Q-point of the QRS complex to the B point of the impedance waveform, which corresponds with the time from ventricular depolarization to aortic valve opening (Sherwood et al., 1990). PEP was ensemble-averaged for each one-minute epoch by the MindWare software, and B-point was calculated at 55% of the R-Z interval (time to dZ/dt peak) (Lozano et al., 2007). The QRS complex and dZ/dt signal was confirmed by visual inspection (RAM). Due to equipment malfunction or excessive artifact in the impedance signal, 14 participants had incomplete PEP data (TD, n=6, ASD, n=8, $\chi^2(1)=0.33$, p=0.56). An additional 2.0% of total data was excluded due to values less than 70 ms, which falls below physiological norms (Blascovich, Vanman, Mendes, & Dickerson, 2011) and is suggestive of equipment or measurement error.

Statistical Analysis

Demographic, diagnostic, and inclusion variables were compared between ASD and TD groups using independent sample *t*-tests. The Welch degree of freedom approximation was used to correct for violations of homogeneity of variance. Because cortisol levels were positively skewed, values were log 10 transformed prior to analyses. Following log transformation, three extreme outliers remained (> 3 SD), thus these were winsorized to 3 SD above the mean. RSA and PEP were normally distributed and free of extreme outliers.

Linear mixed models examined cortisol, RSA, and PEP response throughout the TSST-F, including diagnosis, time, age, diagnosis*time interactions, and a random effect for subject in the base model. Time was modeled continuously, calculated from five time points— baseline, prep, TSST-F first half, TSST-F second half, and recovery— with baseline modeled as time zero. Time was treated as a quadratic term. An additional model with an age*diagnosis interaction was run to test for hypothesized age effects. Finally, exploratory models were investigated with CBCL Social Problems, SRS Total Problems, and STAIC State Anxiety as potential covariates or effect modifiers.

Results

Demographics

Age did not differ between ASD and TD groups (see Table 3.1). While there was a significant difference based on IQ, the ASD group was within the average range of functioning. As expected, children with ASD were rated by their parents as having significantly more social symptoms on the CBCL and SRS. The ASD group also self-reported greater anxiety after the TSST-F relative to TD youth. Within the ASD group, those with and without medications were

assessed to confirm there were no differences based on medication status. No differences were seen in any of the demographic or outcome variables (all p>0.05).

Table 3.1. Demographic and Dependent Variables.

	ASD		TD				
	M	**SD**	**M**	**SD**	*t*	df	p
Age	11.48	1.06	11.35	1.05	-0.62	97.99	0.54
IQ**	100.28	17.83	120.20	13.33	6.33	90.74	<0.001
ADOS	12.69	4.73	--	--	--	--	--
CBCL Social Problems (Raw)**	7.20	4.23	1.88	2.47	-7.68	78.98	<0.001
STAIC (*State*)*	31.18	7.15	28.62	4.73	-2.11	85.04	0.04
SRS Total (T Score)**	68.46	10.40	47.08	6.21	-12.48	80.01	<0.001

IQ, Intelligence Quotient; ADOS, Autism Diagnostic Observation Schedule; CBCL, Child Behavior Checklist; STAIC, State-Trait Anxiety Inventory for Children; SRS, Social Responsiveness Scale; ASD, Autism Spectrum Disorder; TD, Typically Developing; ***p<0.05; **p<0.001**

Cortisol Responsivity

The base model of cortisol, diagnosis, time, and age significantly improved fit compared to a trivial model with constant cortisol ($\chi_2(4)=99.88$, p<0.001). Adding interaction terms for diagnosis with linear and quadratic effects of time improved fit at trend-level significance ($\chi_2(2)=5.22$, p=0.07). Wald test showed the interaction between diagnosis and time was a significant fixed effect (Table 3.2), such that children with ASD did not demonstrate significant change in cortisol throughout the interaction (b=-0.01, t(199.05)=-1.55, p=0.12). In contrast, TD children demonstrated a significant decline in cortisol over time (b=-0.02, t(199.02)=-5.27, p<0.001; Figure 3.2). To test the hypothesized age effect, an age*diagnosis interaction term was

added to the base model, but the interaction was not a significant main effect ($\chi_2(1)=0.007$, p=0.93; Table 3.3).

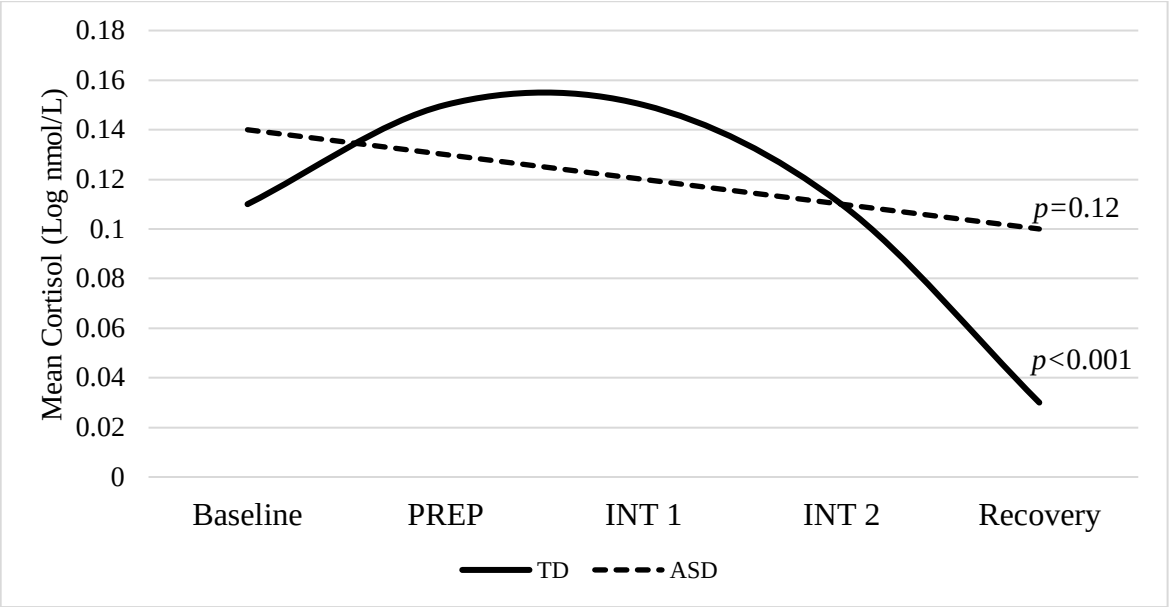

Figure 3.2. Trajectory of Change in Cortisol over Time Differs in ASD and TD Youth. Average cortisol plotted for TD and ASD during TSST-F. Youth with ASD had a non-significant change in cortisol over time, while TD youth significantly decreased in cortisol.

Exploratory models with the CBCL showed no main effect for Social Problems ($\chi_2(1)=0.121$, p=0.73); however, there was a significant interaction with Social Problems and time ($\chi_2(2)=9.946$, p=0.007), suggesting the change in cortisol over time differed according to severity of social problems. Models for STAIC state anxiety or SRS Total score were not a significant improvement over the base model with diagnosis, time, and age (all p>0.05; see Appendix Table 3B).

Table 3.2. Model Estimates of Physiological Variables Change over Time Conditioned on Diagnosis.

Variable	**Estimate**	***SE***	**df**	***t***	***p***	**(95% CI)**
Cortisol						
Intercept	0.11	0.30	100.56	0.37	0.71	(-0.48, 0.70)
Diagnosis	0.01	0.06	138.52	0.27	0.79	(-0.10, 0.13
Time*	0.06	0.02	398.13	2.79	0.005	(0.02, 0.10)
Time$_2$**	-0.02	0.005	398.08	-4.82	<0.001	(-0.03, -0.01)
Age	0.02	0.02	100.00	0.79	0.43	(-0.03, 0.07)
Diagnosis*Time*	-0.07	0.03	398.08	-2.29	0.02	(-0.13, -0.01)
Diagnosis*Time$_2$*	0.02	0.01	398.08	2.22	0.03	(0.002, 0.03)
RSA						
Intercept**	5.82	0.89	99.61	6.57	<0.001	(4.06, 7.58)
Diagnosis	0.02	0.18	139.96	0.14	0.89	(-0.32, 0.38)
Time**	0.37	0.07	394.14	5.54	<0.001	(0.24, 0.51)
Time$_2$**	-0.08	0.02	394.09	-4.89	<0.001	(-0.11, -0.05)
Age	0.04	0.08	98.95	0.56	0.57	(-0.11, 0.20)
Diagnosis*Time	-0.08	0.10	394.09	-0.87	0.39	(-0.27, 0.10)
Diagnosis*Time$_2$	0.01	0.02	394.06	0.29	0.77	(-0.04, 0.05)
PEP						
Intercept*	70.06	11.01	90.34	6.36	<0.001	(48.19, 91.93)
Diagnosis	1.72	2.08	104.69	0.83	0.41	(-2.41, 5.85)
Time	0.34	0.54	340.01	0.64	0.52	(-0.71, 1.40)
Time$_2$	-0.10	0.13	339.44	-0.82	0.41	(-0.36, 0.15)
Age	1.66	0.96	90.12	1.73	0.09	(-0.25, 3.57)
Diagnosis*Time	0.18	0.77	339.72	0.23	0.81	(-1.34, 1.70)
Diagnosis*Time$_2$	-0.10	0.18	339.31	-0.55	0.58	(-0.46, 0.26)

***p<0.05; **p<0.001**

Table 3.3. Model Estimates of Physiological Variable Change by Diagnosis and Age.

Variable	**Estimate**	***SE***	**df**	***t***	***p***	**(95% CI)**
Cortisol						
Intercept	0.15	0.42	100.17	0.37	0.71	(-0.68, 0.99)
Diagnosis	-0.08	0.59	99.99	-0.13	0.89	(-1.26, 1.10)
Time	0.02	0.01	398.08	1.65	0.10	(-0.005, 0.06)
Time$_2$**	-0.02	0.004	398.09	-4.55	<0.001	(-0.02, -0.01)
Age	0.02	0.04	100.05	0.49	0.62	(-0.05, 0.09)
Diagnosis*Age	0.004	0.05	100.00	0.09	0.93	(-0.10, 0.11)
RSA						
Intercept**	3.86	1.22	99.12	3.16	0.002	(1.44, 6.28)
Diagnosis*	3.92	1.72	98.95	2.28	0.02	(0.50, 7.35)
Time**	0.34	0.05	394.09	6.97	<0.001	(0.24, 0.43)
Time$_2$**	-0.08	0.01	394.06	-6.63	<0.001	(-0.10, -0.05)
Age*	0.22	0.11	98.95	2.08	0.04	(0.01, 0.43)
Diagnosis*Age*	-0.35	0.15	98.95	-2.34	0.02	(-0.65, -0.05)
PEP						
Intercept**	62.26	15.00	89.97	4.15	<0.001	(32.47, 92.06)
Diagnosis	18.37	22.02	90.14	0.83	0.41	(-25.38, 62.13)
Time	0.43	0.39	339.78	1.11	0.27	(-0.33, 1.19)
Time$_2$	-0.15	0.09	339.36	-1.68	0.09	(-0.34, 0.03)
Age	2.36	1.31	89.89	1.79	0.08	(-0.25, 4.97)
Diagnosis*Age	-1.48	1.92	90.18	-0.77	0.44	(-5.29, 2.33)

***p<0.05; **p<0.001**

RSA Responsivity

The initial base model for diagnosis, time, and age to predict RSA was significantly improved relative to the trivial model with constant RSA ($\chi_2(4)=46.55$, $p<0.001$). Addition of diagnosis and time interaction terms were not significant ($\chi_2(2)=4.41$, $p=0.11$), suggesting the change in RSA over time does not differ between youth with ASD and TD. Next, an additional model including an interaction term for diagnosis by age was compared to the initial base model and was a significant improvement ($\chi_2(1)=5.35$, $p=0.01$; see Table 3.2). The diagnosis*age interaction was shown to be a significant fixed effect (Table 3.3), suggesting the association with age and RSA differed according to diagnosis. Specifically, when separate models were conducted based on diagnosis, it was shown that youth with ASD had a blunted, non-significant change in RSA for increasing age (b=-0.13, $t(49.97)$=-1.10, p=0.28), while the TD children showed a significant positive slope between age and RSA (b=0.22, $t(49.00)$=2.42, p=0.02; Figure 3.3).

Adding the social problems domain of the CBCL to the base model significantly improved fit ($\chi_2(1)=8.63$, $p=0.003$); however, CBCL social problems*time effects were not significant predictors of RSA ($\chi_2(2)=3.61$, $p=0.16$). Thus, mean RSA differed based on severity of scores on the Social Problems domain, but the change in RSA over time (slope) did not. Models for STAIC state anxiety or SRS Total score were not a significant improvement over the base model with diagnosis, time, and age (all $p>0.05$; see Appendix Table 3C).

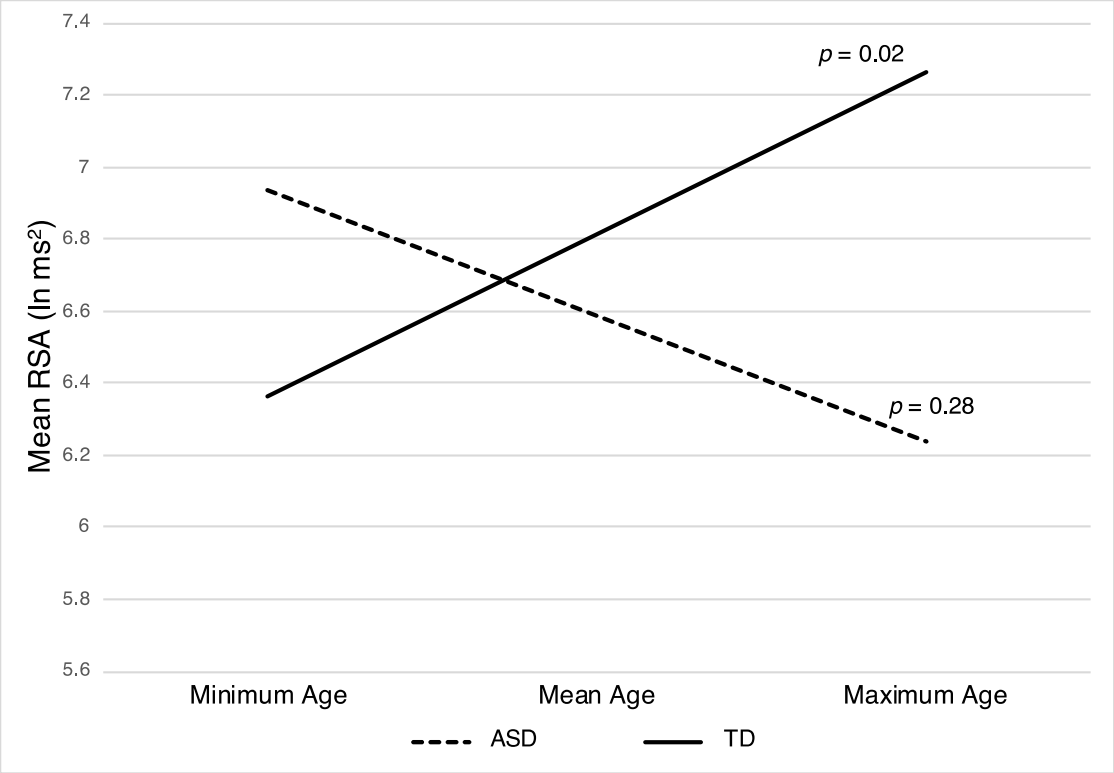

Figure 3.3. Trajectory of Change in RSA by Age Differs in ASD and TD Youth. Average RSA plotted for TD and ASD at minimum, mean, and maximum age. Youth with ASD had a non-significant negative association between RSA and age, while TD youth significantly increased in RSA as age increased.

PEP Responsivity

The hypothesized base model of diagnosis, time, and age improved fit relative to a model with constant PEP ($\chi_2(4)=9.501$, p=0.05). Addition of diagnosis and time interaction terms were not significant ($\chi_2(2)=1.345$, p=0.51). A second model including an interaction term for diagnosis by age was not significant ($\chi_2(1)=0.591$, p=0.44). See Table 3.2 and Table 3.3 for detailed model results. Further models with social symptoms were non-significant in predicting PEP (all p>0.05).

Discussion

The primary objective of the current study was to determine whether youth with ASD showed differential physiological responses to a naturalistic social interaction task. Results revealed a complex profile of stress and arousal in youth with ASD, in which physiological system, age, and social symptoms may all contribute to physiological responses related to social interactions with peers. The HPA axis and PNS appeared especially sensitive to diagnostic differences, with ASD youth characterized by relative inflexibility and lack of adaptability of these systems. In contrast, the SNS did not demonstrate sensitivity to the task or differences associated with ASD symptoms. Age differences were also apparent, in which older children with ASD were especially sensitive to the interaction revealing atypical physiological responses, particularly in the PNS. Youth with ASD may be in a chronically mobilized state with an inability to adapt to changing environmental stimuli and demands, which can increase risk for stress-related conditions (e.g. Chalmers, Quintana, Abbot, & Kemp, 2014; Koenig, Kemp, Beauchaine, Thayer, & Kaess, 2016; Rottenberg, 2007; Thayer, Friedman, & Borkovec, 1996; Yaptangco, Crowell, Baucom, Bride, & Hansen, 2015), further emphasizing the important implications of defining and identifying HPA and ANS dysfunction in ASD youth.

Previously, individuals with ASD have been reported to show a greater stress response to social engagement with peers (Bishop-Fitzpatrick, Mazefsky, Minshew, & Eack, 2015; Corbett et al., 2010; 2014; Spratt et al., 2012). Partially consistent with our hypotheses, children with ASD in the current study differed in the change in cortisol response across the interaction task. However, both TD and ASD groups demonstrated elevated cortisol levels at the beginning of the task, suggesting some level of anticipatory stress. Yet while the TD group gradually decreased in HPA responsivity throughout the interaction, youth with ASD remained relatively elevated. Our

findings are consistent with previous literature, in which both ASD and TD individuals exhibit an initial stress response to novelty (Corbett et al., 2012; Taylor, Muscatello, & Corbett, 2018). In observations of social interaction, however, TD youth demonstrate a relatively rapid recovery while youth with ASD maintain elevated stress responses (Corbett et al., 2012). In typical development, the HPA axis is structured to flexibly adapt to novel situations no longer perceived as stressful. In contrast, many youths with ASD may continue to perceive these social situations as stressful, fail to adapt to novelty (Kanner, 1943), or fail to recover from heightened activation, resulting in persistent cortisol elevation and thus, over time, increased risk for chronic stress-related conditions (e.g. McEwen, 1998; 2003).

In contrast to cortisol, RSA and PEP responsivity over time did not differ by diagnostic group. In regard to PEP, the SNS is often considered a second line of defense, only activated during more severe conditions of stress (Wolff et al., 2012). The PNS is more flexible, facilitating autonomic responses to dynamic conditions via changes in vagal tone, or suppression and activation of the 'vagal brake' (e.g. Porges, 2007). Therefore, the PNS as measured by RSA, would be expected to change in response to a wider variety of stimuli. While changes in RSA to the social interaction did not differ in ASD, there were notable interactions with age suggesting developmental factors may be contributing to PNS function. Specifically, overall RSA did not increase with age in the ASD group, which would be expected according to the developmental trajectory of the PNS (e.g. Beauchaine, 2001; Shader et al., 2018) and observed age effects in the TD group. Since in the ASD group there was no observed change in RSA as a function of age, it suggests a blunted response in older ASD youth in RSA over time. Such an interaction would be consistent with previous studies of HPA axis responsivity in ASD, which show that older children with ASD have significantly elevated stress responses to social play relative to younger

children with ASD and same-aged TD peers (Corbett et al., 2010; Schupp et al., 2013). While the current sample was underpowered to test this hypothesized three-way interaction, Figure 3.4 suggests similar trends are observed in RSA, with older ASD youth demonstrating reduced PNS regulation to social interaction compared to younger children with ASD and same-aged TD peers. While it is possible that these differences may arise from an inherent atypicality in the physical development of the ANS as individuals with ASD age, there is more likely an alternative explanation, such as previous social experiences (e.g. bullying) shaping future social anxiety (e.g. Weiss, Cappadocia, Tint, & Pepler, 2015) and contributing to these age effects in ASD.

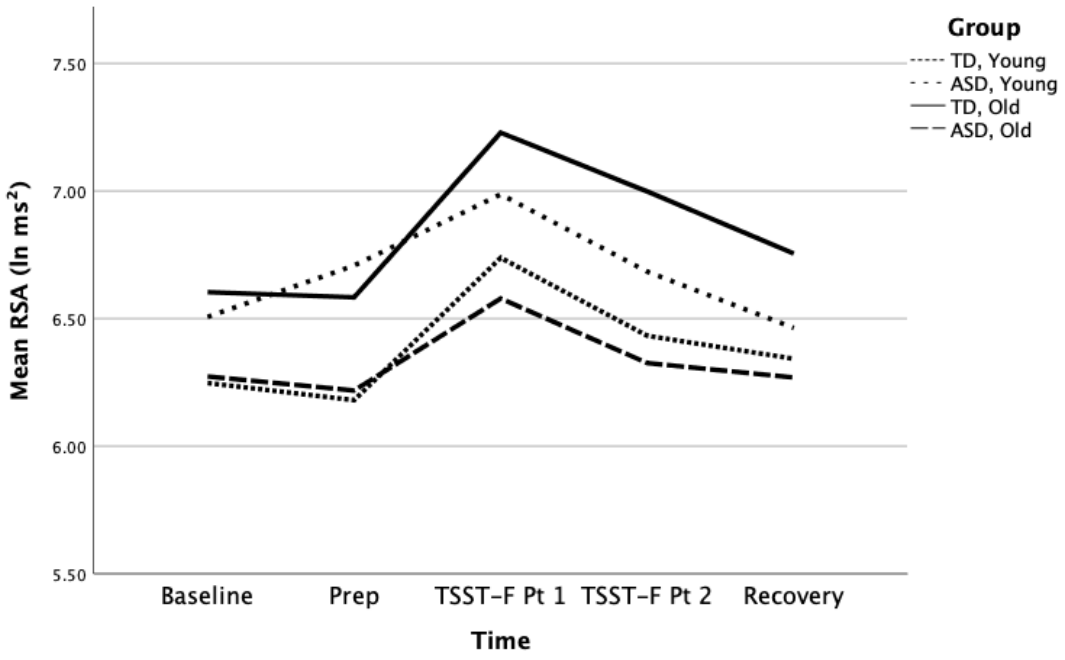

Figure 3.4. Projected RSA Response Profiles by Age and Diagnosis. Distribution of response profiles suggest a three-way diagnosis by age by time interaction, but this was not tested statistically. Age dichotomized based on median split for the purpose of illustrating possible relationships.

However, if such was the case, it may be expected that a similar profile would be observed in the cortisol, yet no age effects were noted for the HPA axis, which was inconsistent with our hypotheses. According to the argument that previous negative social experiences can shape future stress responses and increase social anxiety (Bellini, 2006), one would expect that older youth with ASD would have higher cortisol throughout the TSST-F, while younger children might show a pattern more similar to TD children in which cortisol decreases over time. In consideration of differences in the current findings compared to previous studies (Corbett et al., 2010; Schupp et al., 2013), alternative explanations must be considered.

First, previous studies (Corbett et al., 2010; Schupp et al., 2013) were conducted in prepubescent children, while the current study did not exclude based on pubertal status. Puberty is a time of notable physical and emotional transition that affects HPA functioning and responsivity (Kiess et al., 1995; Marceau, Dorn, & Susman, 2012; Walker, Walder, & Reynolds, 2001). While age is often used as a proxy for pubertal development, it is not a perfect correlate, and thus future studies should utilize more reliable measures of pubertal status (Corbett, Muscatello, Tanguturi, McGinn, & Ioannou, 2019b) when investigating age or pubertal effects on stress responsivity. Additionally, the current study was unique relative to previous research (Corbett et al., 2010; Schupp et al., 2013) in its inclusion of female participants. Females are historically underrepresented in ASD research (Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015) given the higher ratio of males with ASD (American Psychiatric Association, 2013). Further, sex differences in physiological systems have been demonstrated (e.g. Dart, Du, & Kingwell, 2002; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999); therefore, future research should strive to investigate more comparable numbers of males and females in

order to identify possible moderating effects of sex in ASD and the unique physiological profiles.

The TSST-F was designed to be a relatively benign engagement protocol, meant to emulate a naturalistic face-to-face conversation with another peer. In the context of the Polyvagal Theory and Social Engagement System (Porges, 2001; 2003b; 2007), this non-stressful situation should promote calming physiological responses and inhibition of mobilization behaviors, which in turn would promote behaviors associated with social engagement. Those who do not demonstrate the expected increase in vagal tone or have an increase but to a lesser degree, may be in a more mobilized state favoring hyperarousal and inhibiting social engagement. Variability and flexibility of these arousal systems is necessary for maintaining dynamic, adaptive relationships with the environment (Berntson, Norman, Hawkley, & Cacioppo, 2008; Friedman, 2007; Thayer & Lane, 2000). Therefore, decreased variability, which is reflective of limited adaptability, is often associated with pathological conditions, and may represent a state of persistent vigilance of preparation for threat mobilization (Thayer & Lane, 2000).

The current study further examined the contributions of social symptoms in predicting stress response. In both the HPA axis and PNS, severity of social problems affected stress and arousal response, regardless of diagnostic status. Specifically, increased number of parent-reported social problems was associated with an increase in cortisol response and lower overall mean RSA. Indeed, heightened HPA axis responsivity has been associated with social avoidance (Roelofs, Elzinga, & Rotteveel, 2005). As a case in point, social anxiety disorder has been characterized by elevated stress responsivity and diminished social approach (Roelofs et al., 2009). Our findings in the PNS are consistent with previous literature, such that RSA has frequently been associated with impairments in social functioning (e.g. Shahrestani, Stewart,

Quintana, Hickie, & Guastella, 2014), especially in children and adolescents with ASD (Bal et al., 2010; Edmiston et al., 2016; Neuhaus et al., 2016; Neuhaus, Bernier, & Beauchaine, 2014; Vaughan Van Hecke et al., 2009).

While youth with ASD reported more anxiety following the interaction, self-reported state anxiety did not predict any of the physiological responses to the task. These findings are consistent with other recent work investigating perceived anxiety to social interaction (Corbett et al., 2019a). It is important to note that the lack of an association between physiological arousal and perceived anxiety suggest distinct systems. Despite the lack of an association, it must be underscored that anxiety symptoms are prevalent in ASD, estimated to affect between 20-80% (Simonoff et al., 2008; van Steensel, Bögels, & Perrin, 2011; White, Oswald, Ollendick, & Scahill, 2009). Moreover, chronic, atypical physiological arousal has been frequently cited in a number of anxiety conditions (e.g. Badanes, Watamura, & Hankin, 2011; Chalmers et al., 2014; Van den Bergh, Van Calster, Pinna Puissant, & Van Huffel, 2008). Therefore, heightened responsivity to benign stimuli, though maybe not immediately associated with perceived anxiety, may contribute to persistent anxious tendencies (e.g., trait anxiety) and development of anxiety conditions, especially as youth with ASD age.

Limitations and Future Directions

Despite the rigorous approach and compelling findings across multiple physiological systems, the current study has limitations. First, although the sample was comparable to many other studies in ASD, we lacked sufficient power to examine higher order interactions, such as three-way interactions with diagnosis, social functioning, and physiology, which may have further elucidated biobehavioral profiles in youth with and without ASD. Second, social

symptoms were solely measured via parent-report questionnaire reflecting general functioning whereas previous studies of the HPA axis response examined observable social behavior during the interaction (Corbett et al., 2010; 2014; Schupp et al., 2013). Expanded studies should similarly integrate behavioral observation in order to more precisely identify whether HPA and ANS functioning is directly associated with social engagement behaviors. Finally, while the HPA axis, PNS, and SNS were all measured, these systems do not operate in isolation but are interconnected. Thus, considering their interactions within individuals will likely increase insight into unique physiological responses in ASD and their relationships with social behavior beyond studying a single system examined in isolation.

Conclusion

The current study supports a growing literature linking atypical physiological reactivity in ASD during relatively benign social situations. The results uniquely demonstrate evidence for inflexibility across multiple arousal systems, in which the HPA axis and ANS in individuals with ASD are less efficient in responding and adapting to changes in environmental stimuli. As children are confronted with frequent social encounters with peers, the implications for atypical physiological arousal, such as chronic activation, to these daily occurrences are numerous. Chronic stress might increase susceptibility to a number of conditions, including gastrointestinal problems (e.g. Ferguson et al., 2016)) or internalizing disorders (e.g. McEwen, 1998; 2003), while impaired social engagement behaviors may increase social isolation and loneliness (Ghaziuddin, Ghaziuddin, & Greden, 2002; Greenlee, Mosley, Shui, Veenstra-VanderWeele, & Gotham, 2016), thereby increasing risk for depression or suicidality (e.g. Hedley, Uljarević, Foley, Richdale, & Trollor, 2018). Future research should aim to explain these complex

relationships between physiology, social functioning, and emotion, as well as the contributions of age and development, in order to define physiology as a marker of physical and behavioral health risk in children and adolescents with ASD.

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Appendix

Appendix Table 3A. Medication List for ASD and TD Participants.

Medication	ASD	TD
SSRIs	7	0
Melatonin	6	1
Antihistamine	8	2
Other	5	0
Anxiolytic	1	0
Triptan	1	0
Anti-nausea	1	0
Anti-convulsant	2	0
More than one medication	7	1

Note: Participants can fall into more than one category. *SSRI, Selective-Serotonin Reuptake Inhibitor.*

Appendix Table 3B. Model Estimates of Cortisol Change over Time Conditioned on Symptom Severity.

Variable	**Estimate**	***SE***	**df**	***t***	***p***	**(95% CI)**
STAIC-State						
Intercept	-0.06	0.35	105.53	-0.17	0.86	(-0.75, 0.63)
Time	0.04	0.08	398.06	0.59	0.55	(-0.11, 0.20)
Time$_2$	-0.03	0.02	398.16	-1.81	0.07	(-0.07, 0.003)
Age	0.03	0.03	100.01	1.02	0.31	(-0.02, 0.08)
Diagnosis	-0.04	0.05	99.99	-0.80	0.43	(-0.15, 0.06)
STAIC-State	0.004	0.005	135.98	0.87	0.39	(-0.005, 0.01)
STAIC-*Time	-0.001	0.002	398.08	-0.27	0.79	(-0.006, 0.004)
STAIC*Time$_2$	0.001	0.001	398.20	0.91	0.36	(-0.001, 0.002)
SRS Total						
Intercept	0.003	0.33	103.63	0.01	0.99	(-0.65, 0.66)
Time*	0.12	0.06	398.07	2.06	0.04	(0.005, 0.24)
Time$_2$*	-0.04	0.01	398.04	-2.68	0.01	(-0.07, -0.01)
Age	0.02	0.02	100.00	0.77	0.44	(-0.03, 0.07)
Diagnosis	-0.07	0.11	100.05	-0.63	0.53	(-0.28, 0.15)
SRS Total T	0.002	0.003	109.33	0.75	0.46	(-0.004, 0.01)
SRS*Time	-0.002	0.001	398.04	-1.69	0.09	(-0.003, 0.000)
SRS*Time$_2$	0.000	0.000	398.03	1.55	0.12	(-0.001, 0.001)

STAIC, State-Trait Anxiety Inventory for Children; SRS, Social Responsiveness Scale. ***p*<0.05**

Appendix Table 3C. Model Estimates of RSA Change over Time Conditioned on Symptom Severity.

Variable	**Estimate**	***SE***	**df**	***t***	***p***	**(95% CI)**
STAIC-State						
Intercept*	6.31	1.05	104.79	6.01	<0.001	(4.23, 8.40)
Time	0.27	0.24	394.12	1.11	0.27	(-0.20, 0.74)
Time$_2$	-0.04	0.06	394.07	-0.72	0.47	(-0.15, 0.07)
Age	0.03	0.08	98.94	0.40	0.69	(-0.12, 0.19)
Diagnosis	-0.07	0.16	98.97	-0.41	0.68	(-0.40, 0.26)
STAIC-State	-0.01	0.01	137.31	-0.69	0.49	(-0.04, 0.02)
STAIC-*Time	0.002	0.01	394.16	0.30	0.76	(-0.01, 0.02)
STAIC*Time$_2$	-0.001	0.002	394.09	-0.63	0.53	(-0.005, 0.002)
SRS Total						
Intercept*	6.14	0.98	103.02	6.24	<0.001	(4.20, 8.09)
Time*	0.48	0.19	394.10	2.59	0.01	(0.12, 0.85)
Time$_2$*	-0.09	0.04	394.06	-2.06	0.04	(-0.18, -0.004)
Age	0.05	0.08	98.96	0.62	0.54	(-0.10, 0.20)
Diagnosis	0.20	0.32	98.98	0.62	0.53	(-0.43, 0.83)
SRS Total T	-0.01	0.01	109.07	-0.74	0.46	(-0.03, 0.01)
SRS*Time	-0.002	0.003	394.07	-0.81	0.42	(-0.01, 0.003)
SRS*Time$_2$	0.000	0.001	394.05	0.36	0.72	(-0.001, 0.002)

STAIC, State-Trait Anxiety Inventory for Children; SRS, Social Responsiveness Scale. ***p<0.05**

Chapter IV

EXAMINING SOCIAL, EMOTIONAL, AND PHYSIOLOGICAL PROFILES IN YOUTH WITH AND WITHOUT AUTISM SPECTRUM DISORDER

Introduction

Persistent deficits in social communication and interaction are a hallmark diagnostic feature of autism spectrum disorder (ASD) (American Psychiatric Association, 2013), significantly impacting their interactions with peers. It has been proposed that many of these social difficulties stem from low social motivation or desire to interact with others (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012). Accordingly, infrequent social orienting (e.g. eye contact), reduced social seeking (e.g. initiation or joint attention), and less social maintenance (e.g. reputation management) are said to directly lead to social cognitive deficits characteristic of ASD (Baron-Cohen, 1995). However, autism is an inherently heterogenous condition, and many youths with ASD demonstrate clear social interest and motivation (Corbett et al., 2014; Wing & Gould, 1979) and seek meaningful friendships (Bauminger, Solomon, Aviezer, Heung, Brown, et al., 2008a; Bauminger, Solomon, Aviezer, Heung, Gazit, et al., 2008b; Kasari, Locke, Gulsrud, & Rotheram-Fuller, 2011). For decades, the socioemotional and behavioral profile of individuals with ASD has been a point of interest. Indeed, Wing and Gould (1979) in their seminal paper attempted to classify children with ASD into subgroups based on their social interaction style. The classification resulted in three subgroups: aloof (the child does not seek social interaction or respond to the approaches of others), passive (the child does not initiate social interaction, but responds appropriately to initiatives made by others) and active-but-odd (the child actively seeks

interactions with others, but in an odd or atypical way). Regardless of the specific behavioral phenotype, individuals with autism are less likely to be included in social activities such as play and more likely to experience social exclusion (Humphrey & Symes, 2011; Symes & Humphrey, 2010).

Play is a key developmental training ground for the development of social skills and the formation of peer relationships (Ginsburg, 2007; Piaget, 1952). Play-based studies of children and adolescents with ASD show that overall, these youth engage in less cooperative play (Corbett et al., 2014; Corbett, Schupp, Simon, Ryan, & Mendoza, 2010; Schupp, Simon, & Corbett, 2013) and verbal bouts (Corbett et al., 2014) with peers relative to typically developing youth. When individuals do engage, they often find these interactions to be especially stressful (Corbett et al., 2010; Lopata, Volker, Putnam, Thomeer, & Nida, 2008) and possibly contribute to feelings of social anxiety (Bellini, 2006). Moreover, children with ASD report greater difficulty forming and maintaining positive peer relationships (Hill & Frith, 2003), while often experiencing more instances of peer victimization and bullying (see Maïano, Normand, Salvas, Moullec, & Aimé, 2016 for review). A history of negative social encounters may further exacerbate social anxiety (Bellini, 2006), hinder peer interaction (Schupp et al., 2013), and therefore promote social isolation (Bellini, 2006; Tantam, 2000). Lack of social engagement may in turn prevent age-appropriate social skill development (Rubin & Burgess, 2001), further increase the likelihood of poor social experiences thereby prompting a cycle of isolation and rejection. Ultimately, these cascading impacts lead to increased social withdrawal (Bellini, 2006) and set the stage for a number of health risks, such as internalizing conditions (e.g. Hedley, Uljarević, Foley, Richdale, & Trollor, 2018; Tebartz van Elst, Pick, Biscaldi, Fangmeier, & Riedel, 2013).

Whether by reduced social interest or social exclusion, children with ASD tend to be more socially isolated than peers (Bauminger, Shulman, & Agam, 2003; Humphrey & Symes, 2011). In the general population, social withdrawal and isolation are significant risk factors for internalizing conditions; namely, anxiety and depression (e.g. Cacioppo & Cacioppo, 2014; Cacioppo, Hawkley, & Thisted, 2010; Hall-Lande, Eisenberg, Christenson, & Neumark-Sztainer, 2007). For example, one study in which feelings of loneliness were induced via hypnosis found that loneliness led to significant changes in personality and socioemotional characteristics, including increased feelings of anxiety. These findings suggest the emotional response triggered by loneliness leads to individuals feeling more anxious (Cacioppo, Hawkley, Ernst, Burleson, Berntson, Nouriani, et al., 2006a). Loneliness is frequently associated with depressive symptoms (e.g. Cacioppo et al., 2010; Cacioppo, Hawkley, Ernst, Burleson, Berntson, Nouriani, et al., 2006a; Cacioppo, Hughes, Waite, Hawkley, & Thisted, 2006b; Heinrich & Gullone, 2006). Similarly, social withdrawal may also be symptomatic or contributary to depression (American Psychiatric Association, 2013). It has been shown that social withdrawal in early childhood can be predictive of later development of symptoms of loneliness and depression (Rubin, Hymel, & Mills, 1989). In consideration of the aforementioned literature, it is not surprising that depression is a significant concern in individuals with ASD, with a recent meta-analysis estimating pooled lifetime prevalence at 40% by adulthood (Hudson, Hall, & Harkness, 2019), relative to about 10% of the general population (SAMHSA, 2019). It is estimated that 50% of adults with ASD have at least one anxiety disorder and as many as 50-70% have an episode of major depression in their lifetime (Hofvander et al., 2009; Lugnegård, Hallerbäck, & Gillberg, 2011).

The picture for children and adolescents with ASD and internalizing disorders is just as alarming. In fact, an estimated 40% of youth with ASD have an anxiety disorder (van Steensel,

Bögels, & Perrin, 2011), while approximately 20% may have depressive symptoms (Greenlee, Mosley, Shui, Veenstra-VanderWeele, & Gotham, 2016). As with adults with ASD, there is often a strong link to internalizing symptoms and social connectedness. Many youths with ASD report more loneliness and have a desire for increased peer interactions relative to their typically developing peers (Bauminger & Kasari, 2000; White & Roberson-Nay, 2009). These children and adolescents also report significantly more difficulties in peer relationships compared to their typically developing peers (e.g. Bauminger et al., 2003; Hill & Frith, 2003). Recent research strongly indicates that loneliness and social connectedness mediate the association between internalizing symptoms and autistic traits (Stice & Lavner, 2019). Thus, it is imperative to better understand plausible predictive factors in children and adolescents with ASD in an effort to curb the impact of these debilitating comorbid conditions.

A relatively understudied but emerging area of importance to better understand social functioning and internalizing symptoms in ASD, is the link to underlying physiological arousal and stress. Previous research has shown that physiological functioning may play a role in the social and emotional profile of children with ASD (see Benevides & Lane, 2015; Taylor & Corbett, 2014 for review). As previously described in detail (Chapters 2 and 3), the direct measurement of physiological stress systems, including the hypothalamic pituitary adrenal (HPA) axis and autonomic nervous system (ANS) provides an objective, noninvasive marker of arousal. Further, both the HPA axis and ANS interact with or influence social behavior (e.g. Dickerson & Kemeny, 2004; Porges, 2003; 2007) and psychiatric well-being (e.g. Schumann, Andrack, & Bär, 2017; Stetler & Miller, 2011). Thus, utilization of physiological measures of stress and arousal provides an effective means to elucidate the reciprocal relationship between

arousal and social functioning and the possible influences on internalizing behaviors, especially in vulnerable populations such as individuals with ASD.

In the current study, we aimed to characterize social communicative behaviors in children with and without ASD during a friendly social interaction with a novel peer. Operationally defined behaviors of social motivation, skill, and interest were compared between the two groups, with predicted deficits for the ASD group. Further, we hypothesized that youth with ASD would demonstrate more severe internalizing symptoms as reported by their parents and by self-report. Given the previously defined relationship between social support and prevalence of anxiety/depression (e.g. Cacioppo et al., 2010; Cacioppo & Cacioppo, 2014; Hall-Lande et al., 2007), the association between behavior and internalizing conditions was examined. We also examined the extent to which physiological stress and arousal was related to behavior and affect. It was hypothesized that children who engaged in fewer social behaviors would be more anxious and depressed, and they would demonstrate elevated physiological stress and arousal.

Methods

Participants

Participants included 100 children, ages 10 to 13 years of age, with ASD (n=50) or typical development (TD, n=50). Participants were gender-matched, with 14 females in each group, and age did not significantly differ (see Table 3.1 for sample demographics). Participants were enrolled as part of a larger longitudinal study of pubertal development from a catchment radius of 200 miles (Corbett 2017) and were recruited from university-wide announcements, autism- and child-development clinics, and social media.

Inclusion criteria were reviewed prior to enrollment. Participants were required to have an intelligence quotient (IQ) ≥ 70, based on the Wechsler Scale of Abbreviated Intelligence, 2nd edition (WASI-2, Wechsler, 1999) in order to complete self-report questionnaires. Parents of all participants completed the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003), and TD participants were required to score <10 in order to be included in the study. None of the TD participants had a biological sibling with ASD. Those on medications directly affecting the HPA axis (e.g. corticosteroids; Granger, Hibel, Fortunato, & Kapelewski, 2009) or the ANS (e.g. stimulants; Shibao & Okamoto, 2012) were excluded. ASD participants were required to meet diagnostic standards according to DSM-5 criteria (American Psychiatric Association, 2013), established by (1) previous diagnosis by psychiatrist, psychologist, or clinician with autism expertise, (2) current clinical judgement, and (3) corroborated by the Autism Diagnostic Observation Schedule, 2nd edition (Lord et al., 2012), administered by research-reliable personnel.

All study procedures were approved by the Vanderbilt Institutional Review Board. Following thorough explanation of the study, parents provided informed written consent and children gave verbal assent prior to inclusion in the study. The research was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Participation required two research visits to the University. The first visit included collection of diagnostic, cognitive, and behavioral assessments. At the second visit, participants completed the social interaction paradigm and physiological procedures described below.

Trier Social Stress Test-Friendly Version

The Trier Social Stress Test-Friendly (TSST-F; Wiemers, Schoofs, & Wolf, 2013) is an alternative form of the original Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993), a social evaluative threat paradigm consistently shown to elicit a physiological stress response. The TSST-F, however, was established as a more "friendly" protocol, in which peers show interest and provide encouragement and does not lead to any increase in physiological stress in TD adults (Wiemers et al., 2013; Wiemers & Wolf, 2015). In the current study, participants were instructed to share about themselves while meeting with a same-aged, gender-matched, typically developing research confederate. In order to avoid impinging on the natural environment, research personnel were not in the room for the conversation but remained outside the door. Interactions were recorded using Sony HDR CX-440 camera with built-in audio. The 20-minute paradigm parallels our peer interaction paradigm (Corbett et al., 2010) and requires social interaction with a novel peer, which may be a significant stressor for children with ASD.

The interaction paradigm consisted of the participant (child with either ASD or TD) and one gender- and age-matched research confederate. All research confederates were thoroughly trained in advance and instructed to show interest, demonstrate positive body language (e.g., face the participant, smile), and ask follow-up questions to keep the conversation going; however, confederates were not to speak for greater than 50% of the time to ensure participants had ample opportunity to initiate and lead the conversation. In advance of completing the TSST-F, participants were informed that they would have the opportunity to share about themselves with another child. Each participant had 5-min to prepare what they wanted to discuss to include what they liked to do, their favorite hobbies, books, or movies, or anything else they wanted the other child to know about them. Following the 5-min preparation period, participants were escorted to

the TSST-F room for the 10-minute social interaction. After 10 minutes, research personnel re-entered the TSST-F room, initiating a 5-minute debriefing in which they were given the opportunity to share the experience with the research member, which was followed by the recovery period (see Figure 3.1).

Behavioral Coding

Analysis of the observational data was completed using Observer XT Version 10 software (*Noldus Observer XT Version 10*, 2010). Analyses utilized a standard ethological approach, based on previously established methods for determining frequency and duration (Corbett et al., 2014). Operationalized behaviors were derived from the Peer Interaction Paradigms (PIP; Corbett et al., 2010; 2014; Schupp et al., 2013) and social evaluative threat protocols (TSST; Edmiston, Blain, & Corbett, 2017), with modifications to account for the differences in the current interaction scenario relative to previously used methods. Raters established and maintained reliability at or above Cohen's Kappa K=0.80. Codes included duration of poorly modulated vs well-modulated eye contact and reciprocal, non-reciprocal verbal bouts vs. silence. Reciprocity was defined as conversation bout by the participant with the peer for the purpose of shared enjoyment and which made sense in the context of the conversation. Example of nonreciprocal conversation would include focused and intense interest on a preferred topic with little regard for the peer's attempts to change the topic. The majority of participants did not demonstrate nonreciprocal verbal bouts, thus the variable was not included in analyses. Number of gestures and questions were coded as frequency behaviors. Overall conversation quality was defined using a 7-point Likert scale for rating rapport and total involvement, derived from the Contextual Assessment of Social Skills (Ratto, Turner-Brown,

Rupp, Mesibov, & Penn, 2011). Operational definitions of behaviors and descriptions of quality ratings are outlined in Table 4.1.

Table 4.1. Operationalized Social Behaviors.

Behavior		Definition
Eye Contact	Well-Modulated	Maintain clear, appropriate, socially modulate eye gaze.
	Poorly Modulated	Does not maintain socially regulated eye contact. May be looking down, away from peer, staring, or other abnormal modulation.
Verbal Bout	Reciprocal	Bout directed to the peer, relevant to the conversation.
	Silent	Participant is not engaged in a verbal bout.
Gestures		Any movement (typically with hands) to express an idea or meaning.
Ask Questions		Participant asks peer a question to engage in conversation.
Conversation Quality (Ratto et al., 2011)	Involvement	Participant indicates he/she is interested and involved in the conversation and what the peer is saying. (Rated on a 7-point Likert scale).
	Rapport	Summary of rapport, give-and-take. Considers extent to which one person had to initiate and maintain the conversation. (Rated on a 7-point Likert scale).

Dependent Measures

Child Behavior Checklist (*CBCL;* Achenbach & Rescorla, 2001). The CBCL is a parent-report measure of problem behaviors that may indicate psychiatric problems in children. Frequency of behaviors are rated on a Likert scale ranging from 0 (“Not True”) to 2 (“Very Often True”). Primary outcome variables included *DSM*-Oriented subscales, Affective Problems

and Anxiety Problems. Raw scores were used in analyses, as recommended in the CBCL manual (Achenbach & Rescorla, 2001).

Multidimensional Anxiety Scale, 2nd Edition (*MASC-2;* March, 2013). The MASC-2 includes parent- and self-report versions consisting of 50 items to assess anxiety symptoms in children. Scores are generated for Total, Generalized Anxiety Index, Social Anxiety, Obsession and Compulsions, Physical Symptoms, and Harm Avoidance. Raw scores are converted to T-scores based on gender- and age-norms. Total and Social Anxiety scale T scores were included as outcome variables.

Child Depression Inventory, 2nd Edition (*CDI-2;* Kovacs, 2011). The CDI is a self-report questionnaire for children ages 7-17, in which participants rate symptoms of depression, scored on a Likert scale from 0 (none) to 2 (definite). Scores are subdivided into four subscales- Negative Mood/Physical Symptoms, Negative Self-esteem, Interpersonal Problems, and Ineffectiveness. Raw scores are converted into T scores based on gender- and age-norms. The primary outcome variable was CDI total T score.

Heart Rate Variability

Cardiac autonomic measures were collected using MindWare Mobile Impedance Cardiograph units (MindWare Technologies LTD, Gahanna, OH) for synchronized electrocardiography (ECG) and impedance cardiography (IMP) using a seven-electrode configuration. All study participants agreed to complete the heart rate collection and were able to comfortably tolerate the electrode placement. Heart rate data was collected during a five-minute baseline rest period and throughout the TSST-F protocol, as described in detail in Chapter 3.

Heart rate measures were calculated on a minute-by-minute basis and averaged within each period of the protocol- Baseline, Preparation, TSST-F Interaction, and Recovery (see Figure 3.1).

Respiratory sinus arrhythmia (RSA) indexed parasympathetic influences on the heart. RSA indicates variation in timing between successive heart beats in association with respiration and was derived in accordance with guidelines set forth by the Society for Psychophysiological Research committee on heart rate variability (Berntson et al., 1997; Task Force of the European Society of Cardiology, 1996). ECG signal was sampled at 500 Hz and analyzed using the Heart Rate Variability Software Suite provided by MindWare Technologies (MindWare Technologies LTD, Gahanna, OH). RSA was quantified as the integral power within the respiratory frequency band (0.15 to 0.42 Hz), with respiration processed by impedance cardiography to ensure values were within the designated respiratory frequency band.

Pre-ejection period (PEP) represents the interval from electrical stimulation to mechanical opening of the aorta. PEP was processed using MindWare Technologies Impedance Cardiography Analysis Software (MindWare Technologies, LTD, Gahanna, OH) and calculated as the distance (in ms) from the ECG Q-point of the QRS complex to the B point of the impedance waveform, which corresponds with the time from ventricular depolarization to aortic valve opening (Sherwood et al., 1990). The QRS complex and dZ/dt signal was confirmed by visual inspection. See Chapter 3 for expanded details regarding physiological data collection and analysis.

Cortisol Sampling Protocol

Salivary cortisol can be measured reliably and non-invasively utilizing small amounts of saliva (Kirschbaum & Hellhammer, 1989) and was collected using well-established methods

(Corbett, Mendoza, Wegelin, Carmean, & Levine, 2008). Basal salivary cortisol was collected in the home over 3 days, 4 times per day; however, these data are not part of the current study. As described in Chapter 3, samples were provided by passive drool, and cortisol values are representative of stress response 20-min prior to collection time. A total of six samples were collected throughout the visit (See Figure 3.1 for timing of sample collection). All visits began between 2pm – 3pm to control for the diurnal rhythm of cortisol.

Prior to assay, samples were stored at -20°C. Salivary cortisol assay was performed using a Coat-A-Count® radioimmunoassay (RIA) kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) modified to accommodate lower levels of cortisol in human saliva relative to plasma. Saliva samples were thawed and centrifuged at 3460 rpm for 15 min to separate the aqueous component from mucins and other suspended particles. All samples were duplicated. The coated tube from the kit was substituted with a glass tube into which 100ml of saliva, 100ml of cortisol antibody (courtesy of Wendell Nicholson, Vanderbilt University, Nashville, TN), and 100ml of ^{125}I-cortisol were mixed. After incubation at 4ºC for 24h, 100ml of normal rat serum in 0.1% PO4/ EDTA buffer (1:50) and precipitating reagent (PR81) were added. The mixture was centrifuged at 3460 rpm for 30 min, decanted, and counted. Serial dilution of samples indicated a linearity of 0.99. Interassay coefficient of variation was 1.03%.

Data Analysis

Behavioral and internalizing variables were compared between groups using Wilcoxon Rank-sum test. Estimated effect sizes (*r*) were calculated as the z-score for the test statistic divided by the square root of the total N (Rosenthal, 1991). Physiological stress response during the TSST-F was compared between groups using analysis of covariance, controlling for baseline

physiological values. Specifically, group differences were examined for overall RSA during TSST-F, overall PEP, and Cortisol samples S16 and S17 (see Figure 3.1). For correlation analyses, difference scores were calculated for cortisol, RSA, and PEP in which baseline levels were subtracted from arousal during or immediately following the TSST-F (e.g. Cortisol S16 – Average Afternoon Cortisol; RSA Interaction – RSA Baseline). Of note, more positive difference scores for cortisol and RSA indicate elevated reactivity or regulation of the HPA axis and PNS, respectively. For PEP, more negative difference scores indicate greater SNS reactivity (lower PEP = more SNS arousal). Spearman rho correlations were conducted between behavior, internalizing, and physiological variables.

Results

Demographic characterization of the sample is presented in Table 3.1. Diagnostic outcomes, namely anxiety and depression symptoms, are presented in Table 4.2. TD children had significantly higher estimated IQ; however, the ASD group fell well within the average range of functioning. Cortisol, RSA, and PEP were not significantly correlated with IQ (all p>0.05).

Table 4.2. Between Group Internalizing Outcome Variables.

	ASD Mean (SD)	TD Mean (SD)	W	*p*	*r*
CBCL Affective	5.66 (4.23)	1.54 (2.06)	3318.5	<0.001	0.55
CBCL Anxiety	5.22 (2.68)	1.48 (1.78)	3457.0	<0.001	0.65
MASC-P Total	65.90 (13.32)	47.74 (7.24)	3498.0	<0.001	0.67
MASC-P SA	58.64 (11.42)	47.46 (7.22)	3251.0	<0.001	0.50
MASC-S Total	60.22 (12.23)	52.36 (9.43)	2913.5	0.005	0.32
MASC-S SA	54.78 (10.90)	49.56 (8.82)	2787.5	0.018	0.24
CDI Total	58.02 (11.60)	49.26 (6.27)	3002.5	0.003	0.39

W= Wilcoxon W; p-values calculated by Wilcoxon ranked sum test. *r* = Estimated effect size.
***p*<0.05, ****p*≤0.001**

Social Interaction Behaviors

The TSST-F behavioral results are listed in Table 4.3. Both groups significantly differed in all duration variables, including Poorly Modulated (and Well-modulated) Eye Contact, as well as Reciprocal Verbal Bouts and time in which they were silent (all $p < 0.05$). Specifically, children with ASD were actively engaged 54.0% of the time compared to TD children who spent 72.5% of the interaction engaged in reciprocal verbal interaction with the confederate (see Table 4.3). The ASD group showed well-modulated eye contact only 68.3% of the time and showed poorly modulated eye contact 31.7% of the time. In contrast, most children with TD maintained well-modulated eye contact at 89.3%. The between-group differences remain when controlling for IQ by robust regression with bootstrapping and bias-corrected confidence-intervals.

The two groups did not differ in the frequency of behaviors related to reciprocal social communication (all $p > 0.05$), including Asks Questions and Gestures. For example, the ASD group asked slightly more questions on average (4.8) relative to the TD group (3.9). Additionally, both groups demonstrated similar numbers of gestures. Children with ASD tended to gesture 9.1 times during the course of the interaction, while TD children gestured only slightly more on average at 10.0 times per interaction.

Regarding overall conversation quality, the TD group was rated as showing higher Involvement and better Rapport with the research confederate compared to the ASD group. While children with ASD were mostly engaged in the conversation, the interactions were often odd, stilted, awkward, and/or uncomfortable (mean Involvement score 4.4). In contrast, TD youth were rated on average as being appropriately engaged throughout and generally worked to keep the conversation going (mean Involvement score 5.5). Similarly, the ASD group demonstrated significantly lower Rapport ratings, with an average score of 4.2 (slightly awkward

or uncomfortable interaction). TD youth, however, averaged higher Rapport at a rating of 5.2 (conversation is polite and appropriate, but not clearly comfortable). The between-group differences remain when controlling for IQ by robust regression with bootstrapping and bias-corrected confidence-intervals.

Table 4.3. Descriptive Statistics Behavioral Variables for TSST-F.

	ASD Mean (SD)	TD Mean (SD)	W	*p*	*r*
Eye Contact					
Well-Modulated**	68.33 (39.35)	89.31 (27.59)	1910.0	0.001	-0.34
Poorly-Modulated**	31.37 (39.35)	10.69 (27.59)	2785.0	0.001	0.33
Verbal Bouts					
Reciprocal**	53.98 (22.71)	72.47 (17.01)	1783.0	<0.001	-0.42
Silent*	40.59 (24.43)	27.42 (17.08)	2760.0	0.006	0.27
Questions	4.85 (7.49)	3.94 (7.15)	2511.5	0.321	0.10
Gestures	9.06 (9.19)	9.96 (7.20)	2205	0.223	-0.12
Involvement**	4.37 (1.62)	5.46 (1.23)	1943.0	<0.001	-0.36
Rapport**	4.20 (1.54)	5.18 (1.08)	2006.0	0.001	-0.32

W= Wilcoxon W; p-values calculated by Wilcoxon ranked sum test. *r* = Estimated effect size.
***p*<0.05, ****p*≤0.001**

Social Behaviors and Internalizing Symptoms

Associations between social behaviors and internalizing symptoms were examined using Spearman rho correlations. Several associations were identified with both parent- and self-reported symptoms of depression and anxiety, and findings are presented in Table 4.4. Parent-reports of child internalizing symptoms were negatively associated with behaviors reflecting positive social engagement. For example, elevated depressive symptoms measured on the CBCL Affective scale were related to less time spent in reciprocal engagement, as well as lower overall Involvement and Rapport. Anxiety symptoms were similarly negatively associated with positive

social behaviors. In contrast, as would be expected, depressive and anxious symptoms were positively associated with the amount of time silent during the interaction. Moreover, results from self-reports partially supported the parent-reports, as child-reported anxiety was negatively associated with Involvement and Rapport, but not duration of Verbal Bouts. In contrast, child's self-reported depressive symptoms on the CDI-2 did not significantly correlate with any of the social behaviors, including Verbal Bouts, Eye Contact, and Conversation Quality. Overall, however, the findings, especially from parent-reports, support the hypothesized association between affective dysregulation and social withdrawal.

Table 4.4 Spearman Correlations for Behavioral and Affective Outcomes.

	Well-Mod EC	Poor-Mod EC	Reciprocal Verbal Bout	Silent	Involvement	Rapport
Parent-Report						
CBCL Affective	-0.08	0.08	**-0.23***	**0.23***	**-0.20***	**-0.21***
CBCL Anxiety	-0.14	0.14	**-0.28***	0.16	**-0.21***	**-0.23***
MASC-P	-0.16	0.16	**-0.29***	0.19	-0.16	-0.14
MASC-SA	-0.07	0.07	-0.13	0.11	-0.07	-0.02
Self-Report						
MASC-S	**-0.31***	0.31	-0.17	**0.20***	**-0.30***	**-0.33****
MASC-SA	-0.14	0.14	-0.08	0.08	**-0.22***	**-0.28***
CDI	-0.18	-0.18	-0.13	0.05	-0.13	-0.10

CBCL, Child Behavior Checklist; CDI, Child Depression Inventory; MASC-P/S, Multidimensional Anxiety Scale for Children- Parent/Self; MASC-SA, Multidimensional Anxiety Scale for Children- Social Anxiety Subscale; Well-Mod EC, Well-Modulated Eye Contact; Poor-Mod EC, Poorly-Modulated Eye Contact. ***p*<0.05, ****p*≤0.001**

Physiological Arousal

For the TSST-F, there were no significant differences between the groups in cortisol response either during (S16) ($F(1,97)=0.63$, $p=0.43$) or immediately after (S17) ($F(1,97)=0.64$,

p=0.42) the interaction while controlling for average afternoon cortisol levels (Figure 4.1). Similarly, neither RSA (F(1,95)=2.15, p=0.15; Figure 4.2) nor PEP (F(1,80)=0.18, p=0.67; Figure 4.3) during the TSST-F were significantly different between groups.

Relationships with arousal and social measures were examined for possible associations between physiology and observable behaviors during social interaction. Cortisol, RSA, and PEP were corrected for baseline values by creating difference scores, in which baseline regulation was subtracted from arousal during interaction. (e.g. Cortisol S16 – Average Afternoon Cortisol; RSA Interaction – RSA Baseline). Spearman correlations revealed a weak negative correlation with PEP and time silent (r(83)= -0.22, p=0.05), such that lower PEP values (elevated SNS arousal) were associated with more time silent. All other associations between arousal and behavior were not significant (all p>0.05).

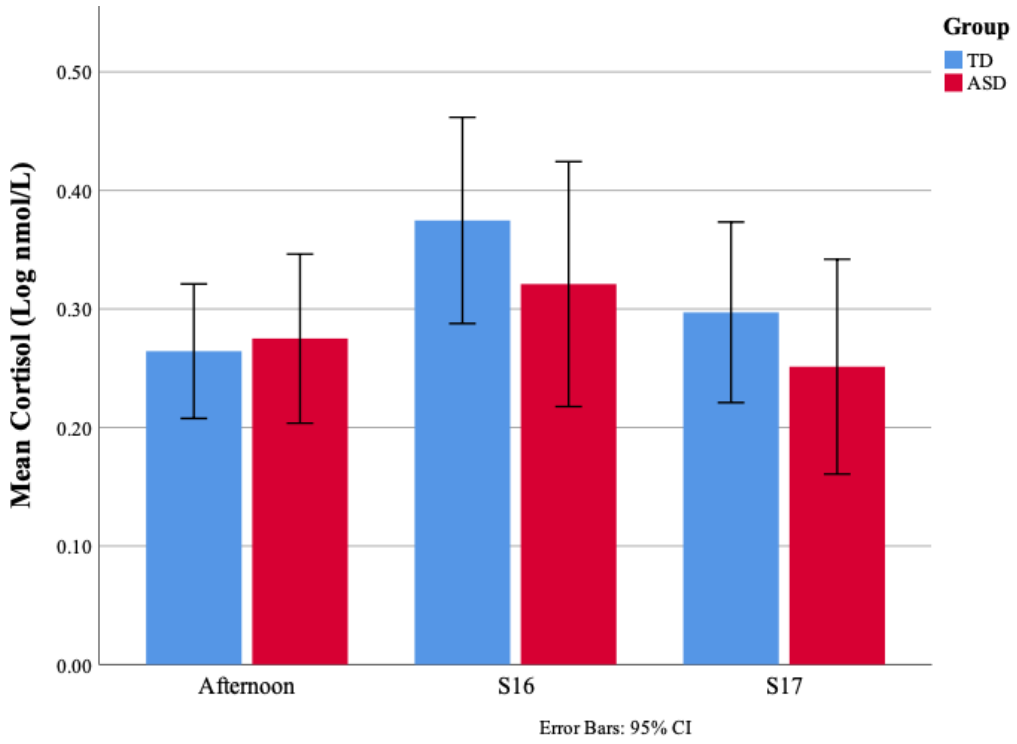

Figure 4.1. Mean Afternoon Cortisol, During the TSST-F (S16) and Immediately after the TSST-F (S17) for Children with ASD and TD.

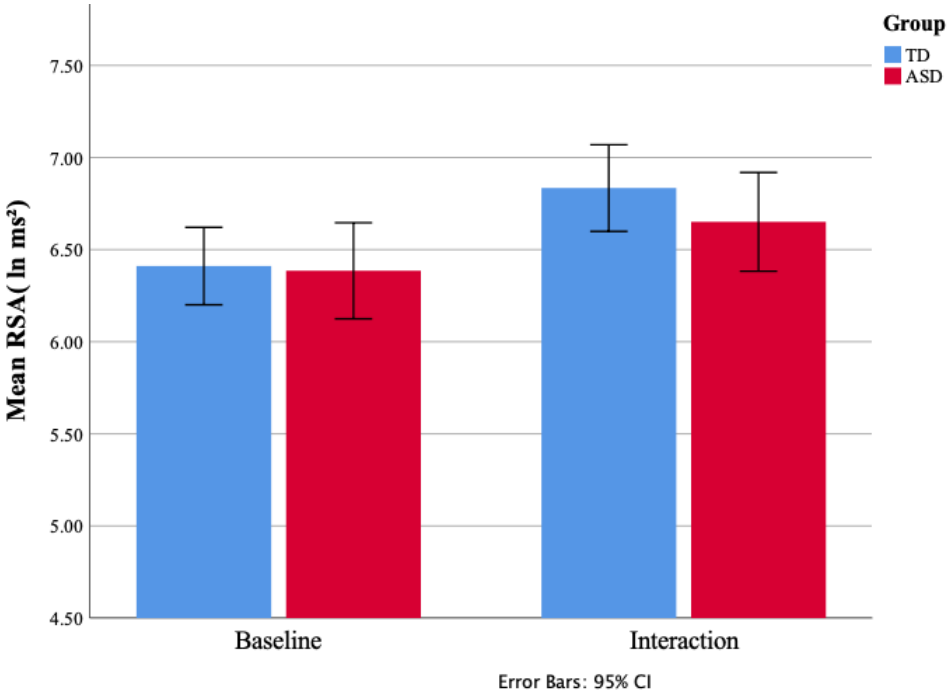

Figure 4.2. Mean Respiratory Sinus Arrhythmia at Baseline and During the TSST-F for Children with ASD and TD.

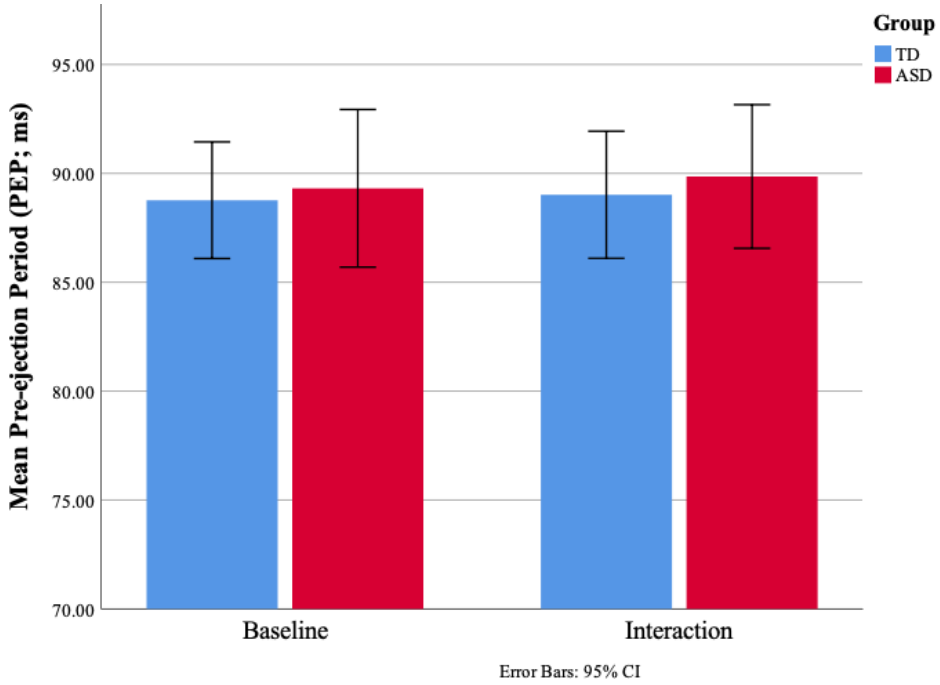

Figure 4.3. Mean Pre-ejection Period at Baseline and During the TSST-F for Children with ASD and TD.

Discussion

The purpose of the current study was to examine social communication deficits in children with ASD through observation and quantification of operationalized behaviors during a friendly peer interaction. As hypothesized, youth with ASD consistently demonstrated fewer behaviors associated with social engagement, including less time engaged in verbal interaction and eye contact, as well as more time spent withdrawn or silent. Furthermore, children with ASD were rated as showing less overall conversation involvement and rapport with the research confederate relative to their TD peers. Our findings are consistent with previous literature showing that during social interactions, many individuals with ASD engage less with peers, demonstrate less overall involvement, and exhibit fewer prosocial behaviors (Corbett et al., 2010; 2014; Humphrey & Lewis, 2008; Ratto et al., 2011; Schupp et al., 2013).

Social impairments and negative peer experiences often contribute to development of symptoms of social anxiety (Bellini, 2006). Moreover, isolation and withdrawal are significant risk factors for depression (e.g. Cacioppo et al., 2010). Therefore, we aimed to examine potential relationships between observed behavior and internalizing symptoms in the current sample of youth with and without ASD. As hypothesized, children with ASD were rated by their parents as having significantly more symptoms of depression and anxiety compared to TD children. This is consistent with previous research indicating that a high percentage of children with ASD evidence symptoms of depression or anxiety based on parental report (Mayes, Calhoun, Murray, Ahuja, & Smith, 2011). Similarly, ASD children self-reported their own anxiety and depression to a greater extent than did the TD group.

It was hypothesized that internalizing symptoms as reported by parents would be associated with observed social behaviors. In fact, many of the social interaction behaviors were

shown to be associated with symptoms of anxiety and depression. Specifically, youth who were reported to be more depressed and anxious by their parents engaged in less verbal interaction with the peer and were less involved in the conversation overall. These findings provide evidence for a predicted socioemotional profile such that individuals with higher internalizing symptoms showed reduced social engagement or isolation and less social interest.

When children self-reported their symptoms, similar patterns emerged regarding anxiety, in that those with elevated anxiety tended to be less socially engaged. Interestingly, however, only the child self-reports of social anxiety were significantly correlated with social involvement and rapport, while parent-reports did not reach statistical significance. Previous research has shown that , children with ASD are able to accurately report trait anxiety in relation to a social stressor (Simon & Corbett, 2013). Thus, these youth may be reliable reporters of internal states related to feelings of anxiety or nervousness, especially in certain social situations. Previously, significant emphasis has been placed on the use of physiological markers of affective conditions (e.g. Beauchaine, 2015). However, our results suggest for high-functioning youth with ASD, these biological markers may not reliably map on to internal states, nor may they be more precise indicators of anxiety compared to child's own self-report. Taken together, our findings emphasize the need to consider multiple informants when assessing internalizing symptoms in youth, including, but not necessarily limited to, self-report, parent-report, and biological markers.

Another aim of the study was to explore the relationship between physiological stress and social behavior. It was predicted that children with ASD would have elevated stress and arousal, such as heightened HPA axis or SNS activation or reduced PNS regulation. It was also expected that children with elevated arousal would be less socially engaged, showing fewer positive social behaviors such as verbal interactions or rapport. However, aside from a weak correlation with

sympathetic arousal and amount of time silent, arousal and behavior were not significantly related, which did not support the hypotheses. While many children with ASD have shown elevated cortisol during play with peers (Corbett et al., 2010; 2014; Lopata et al., 2008; Schupp et al., 2013), the current sample did not differ between ASD and TD groups on any of the physiological measures- cortisol, RSA, or PEP. The TSST-F may not be a potent enough stressor to induce a physiological change. It is important to recognize that social stress and enhanced arousal may be highly specific to the social context and demands. For example, in the PIP children are required to engage in solicited interactive play with novel confederate children on a playground. The TSST-F requires the participant to engage in an interactive conversation. While the two paradigms are similar in that they both include peer confederates and novel social exposures, they are different in the physical context and interactive demands. It may be the case that interactions in larger, less defined spaces with two or more peers may be more likely to lead to the hypothesized stress response between children with and without ASD. Further, stress differences in youth with ASD may best be observed as change in response over time (see Chapter 3) instead of as static mean values. In such cases, it would be important to assess the extent to which observable social behavior contributes to variability in the overall stress response patterns in ASD.

It is well-established that HPA axis and ANS dysfunction is often implicated in psychiatric conditions such as depression and anxiety (Schumann et al., 2017; Stetler & Miller, 2011). Previous research has often focused on the association between elevated or atypical stress response to a variety of stressors in individuals evidencing high rates of comorbid internalizing disorders (e.g. Booij, Bouma, de Jonge, Ormel, & Oldehinkel, 2013; Burke, Davis, Otte, & Mohr, 2005). Nevertheless, the current study found no difference in arousal response to social

interaction. Despite the lack of association, the children clearly demonstrated significant deficits in social behavior. Further, low conversational involvement was related to both depression and anxiety in youth with and without ASD. Our findings suggest a connection between social functioning and affect, which is not necessarily dependent upon physiological functioning. Thus, while recent pharmacological interventions which target physiological functioning have shown some promise (Sagar-Ouriaghli, Lievesley, & Santosh, 2018; Zamzow et al., 2014), the current study results also support the continued use of behavioral interventions, which target the impaired social functioning directly (Corbett et al., 2019; Kasari et al., 2016; Laugeson, Frankel, Mogil, & Dillon, 2009; Solomon, Goodlin-Jones, & Anders, 2004). Given our findings of a link between social behavior and mood, pursuit of targeted behavioral interventions to improve both social functioning and associated affect is an area ripe for future research. For example, social skills programs that directly target enhancement of conversational skills which are peer-mediated may also contribute to reductions in anxiety and depression.

Limitations

The current study was strengthened by the use of direct observation of social behavior and detailed behavioral coding in well-characterized groups of children with ASD and TD. Further, assessment of physiological arousal was extensive, considering the HPA axis and both branches of the ANS. However, the study is not without limitations. While the social interaction task represented a naturalistic face-to-face conversation with a peer, children often interact with more than one child at a time, and thus a paradigm requiring interaction with multiple peers may yield different results. Stress response for the current study was only examined at a single time point during the interaction. Future studies investigating arousal responses over time, along with

interactions between physiological systems, can further elucidate biobehavioral profiles in children with ASD during social interactions. Lastly, while the study benefitted from the use of multiple informants for reporting of internalizing symptoms (parent- and self-), official diagnosis of anxiety and/or depression was not independently confirmed by a clinician.

Conclusions

Psychiatric diagnoses, including anxiety and depression, are often comorbid in individuals with ASD. There are likely a number of contributors to these comorbidities in ASD, and social impairments are no exception. Withdrawal from peers, irrespective of physiological response, is related to elevated depressive and anxious symptoms. Future longitudinal studies have the potential to elucidate the directionality of these relationships in ASD to identify specific socioemotional profiles, determining whether social functioning predicts later mood disorders, and if development of affective symptoms is associated with later worsening of social engagement.

Findings reveal that children with ASD demonstrate less social engagement in a novel, naturalistic and friendly social conversation paradigm. Moreover, these behaviors are associated with greater internalizing symptoms. Humans are inherently social creatures, with social bonds being critical from birth, when mother-infant attachment can greatly define early development (Ainsworth, 1969; Bowlby, 1969; 1973). Throughout the lifespan, positive social bonds are cited as protective factors against a number of physical and psychiatric conditions. In contrast, social isolation may put one at risk for a range of poor outcomes. Most notably, social isolation is directly related with feelings of loneliness (e.g. Cacioppo et al., 2010), which in turn is a substantial risk factor for depressive disorders and anxiety (e.g. Cacioppo, Hughes, Waite,

Hawkley, & Thisted, 2006b) The aforementioned findings underscore the importance of observing child interactions with peers as a lens into the role that internalizing symptoms may play in the social world of youth with ASD.

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Chapter V

CONCLUDING REMARKS

Summary of Project Aims

Autism spectrum disorder (ASD) is characterized by core deficits in social communication and interaction (American Psychiatric Association, 2013), and impaired engagement with the social world highlights a primary focus for ASD research. The current series of studies examined stress and arousal responses to positive social interactions in youth with and without ASD. We aimed to determine the extent to which physiological systems respond atypically to social interactions in youth with ASD. We also expected physiological response to further affect social behaviors, thereby suggesting a bidirectional relationship between social functioning and stress/arousal. Additionally, as stress and arousal can contribute to risk for anxiety and depression (e.g. McEwen, 1998), the ultimate contributions of physiological response patterns on internalizing symptoms were explored in the current sample of ASD and TD youth. The collection of expected social and physiological functioning differences in ASD, along with elevated anxious and depressive symptoms, suggest there may be an interconnected system of biology and behavior to influence clinical outcomes (i.e. internalizing diagnoses; see Figure 1.3 for hypothesized interaction model).

Summary of Findings

The results presented in the preceding studies provide evidence for altered stress and arousal responses to a friendly social interaction task in youth with ASD. The approach was

unique relative to most previous literature in ASD in its inclusion of multiple physiological systems; namely, the HPA axis and parasympathetic and sympathetic branches of the ANS. Using this multi-system approach, we first found that the interactions between systems, particularly within opposing branches of the ANS, provided important information regarding depressive symptoms in ASD (Chapter 2). More specifically, the findings provided evidence to support the utility of examining multiple systems in assessing possible risk of depressive symptoms in ASD, above and beyond the contributions of a single system in isolation. Secondly, direct comparison of stress and arousal systems in ASD and TD youth provided evidence for atypical physiological functioning, especially in the HPA axis and PNS. We implemented a novel friendly social interaction task, the Trier Social Stress Test Friendly, to demonstrate that while resting state HPA and ANS regulation did not differ between ASD and TD groups, there were notable differences in response to social exposure (Chapters 2 and 3). Specifically, ASD youth were characterized by a relative inflexibility of the HPA axis and PNS, with reductions in parasympathetic regulation (low RSA) as well as persistently elevated HPA axis response (high cortisol). In addition to these physiological atypicalities, ASD youth further demonstrated fewer operationalized behaviors associated with age-appropriate social interaction (e.g., eye contact, verbal bout). However, social behavior was not related to physiological response (Chapter 4). Instead, associations were found between behavior and affective symptoms (e.g. elevated depression and lower rapport or social interest), identifying an additional and critical link between social functioning and internalizing comorbidities that could have significant clinical implications, irrespective of physiological arousal.

A number of additional factors likely contributed to key findings and need to be discussed in the context of clinical and future research implications. In particular, it is important

to acknowledge the developmental effects of age, and likely puberty, in contributing to physiological responses related to social interactions. The current studies provide evidence for significant age differences, in which older youth with ASD are particularly inclined to demonstrate hyper-responsive stress and arousal profiles during social interactions. Questions remain, however, regarding the extent to which factors of pubertal development, such as changes in sex-specific steroid hormones, affect physiology, behavior, and emotion in individuals with ASD across the lifespan.

Our findings additionally support the notion that the social experiences themselves can further influence physiology and affect. Future studies are necessary to determine how atypical stress and arousal responses may inhibit socialization in ASD. Additionally, it will be important to understand to what extent social history (e.g. social isolation, peer victimization) shapes these physiological responses. The sum of our findings highlight an interconnected network between physiological systems, social behaviors and experiences, and internalizing symptoms to determine unique biobehavioral profiles in ASD (Figure 5.1). The future significance of these findings and the potential implications for translation to clinical settings is further discussed below.

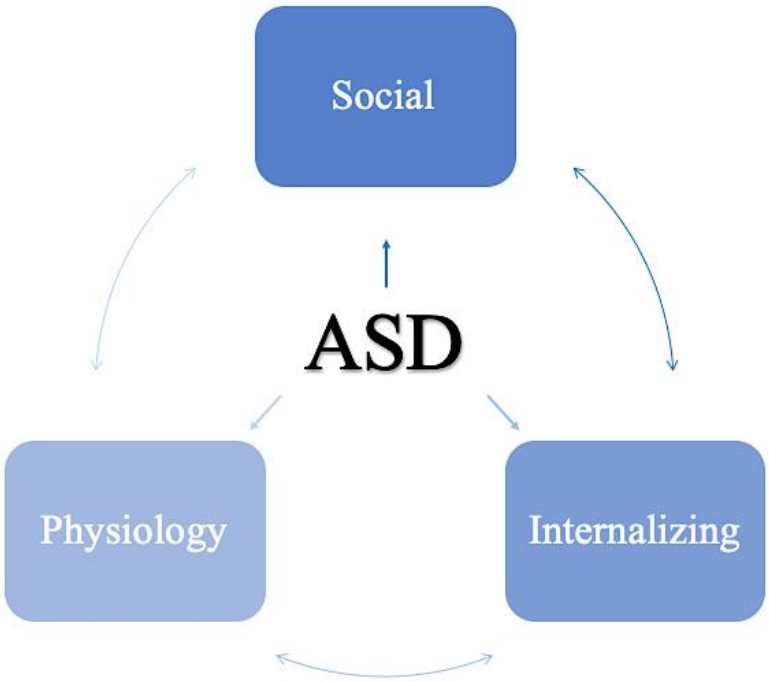

Figure 5.1. Modified Interaction Model between Social functioning, Stress, and Internalizing Symptoms in ASD.

Translation and Clinical Implications

Balance of Physiological Systems

The majority of stress research has historically focused on either the HPA axis or ANS; however, these systems are highly coordinated and interconnected. Given their coordinated relationship, researchers have hypothesized that understanding the interactive profiles of these systems will provide greater insight in psychological conditions, such as internalizing disorders (Bauer, Quas, & Boyce, 2002). Various interaction patterns are feasible and have been discussed in previous research. For example, the model of Neurovisceral Integration describes a network of interconnected brain regions, which interact to regulate ANS and, through downstream signaling, HPA regulation along with behavioral and cognitive processes (e.g. Friedman, 2007; Thayer &

Lane, 2000). Further, activation of the HPA axis and ANS has been hypothesized to fit an Additive Model in which symmetrically over-active or under-active systems convey greatest mental health risk (Bauer et al., 2002). Similarly, models have been proposed describing excessive active asymmetries as conveying greatest risk (Interactive Model, Bauer et al., 2002). Finally, the balance of the two branches of the ANS has been discussed in the model of Autonomic Space, describing autonomic balance as falling in two-dimensional space as opposed to a one-dimensional, reciprocal response axis (Berntson et al., 1994; Berntson, Norman, Hawkley, & Cacioppo, 2008).

Regardless of the specifics of each hypothesized model, all emphasize the importance of examining interactions between the related systems. Our findings support these arguments, demonstrating for example that interactions between branches of the ANS is predictive of depressive symptoms in ASD despite neither the PNS nor the SNS being significantly related to depression when examined in isolation (Chapter 2). Along these lines, specific responder profiles likely exist to define subgroups within ASD. For example, youth with and without ASD have been classified as cortisol responders or non-responders following social interactions (e.g. Corbett, Schupp, Simon, Ryan, & Mendoza, 2010; Corbett et al., 2014; Schupp, Simon, & Corbett, 2013) and psychosocial stress (e.g. Pruessner et al., 2008). Differing responder status may also be associated with changes in behavior (Corbett et al., 2014; Schupp et al., 2013) or differential brain activation (Pruessner et al., 2008). Similarly, according to the model of Autonomic Space (Berntson et al., 1994; 2008), individuals can be classified as either balanced or reactive autonomic responders, which is further divided into four response quadrants—reciprocal parasympathetic, coactivation, reciprocal sympathetic, and co-inhibition. Collectively, an extensive range of responder profiles is possible when considering both the HPA axis and

ANS. While the current study was underpowered to investigate subgroups, we hypothesize that distinct response profiles within ASD may be *predictive* of later behavioral problems and others may be identified as more *protective* against future internalizing disorders throughout development.

Age and Pubertal Development

Pubertal onset and maturation is a time of significant biological and psychological changes, including alterations in gonadal hormone release (Dahl, 2004; Spear, 2000). Changing hormonal levels will influence functioning of the HPA axis and ANS (e.g. Coupal et al., 2019; Gunnar & Vazquez, 2006; Roberts & Lopez-Duran, 2019), and therefore, puberty is often marked by distinct changes in regulation and responsiveness of these systems (e.g. Coupal et al., 2019; Kiess et al., 1995; Marceau, Dorn, & Susman, 2012). Moreover, the adolescent period, defined primarily by chronological age (Spear, 2000; Steinberg, 2005), may be characterized as a time of increased stress reactivity (Lupien, McEwen, Gunnar, & Heim, 2009) as well as increased prevalence of anxiety and depression (Paus, Keshavan, & Giedd, 2008). For youth with ASD, the pubertal transition and adolescent period remains a relatively understudied area of research (Picci & Scherf, 2015). Nevertheless, our findings, in concert with previous reports of age and/or puberty effects on stress and arousal (e.g. Beauchaine, 2001; Corbett et al., 2010; Romeo, 2013; Schupp et al., 2013) highlight the need to define pubertal effects on biobehavioral profiles in ASD.

Few studies have directly compared pubertal timing or development with stress and arousal responses to social interactions. Such studies are necessary, as age is not a perfect proxy for pubertal development. Previous research in ASD have found that while age is a significant

predictor of pubertal development stage, it is not the sole predictor (Corbett, Muscatello, Tanguturi, McGinn, & Ioannou, 2019b). Further, when assessing diurnal regulation of the HPA axis, pubertal stage significantly accounted for variance in evening cortisol values, even after accounting for any effects of age (Muscatello & Corbett, 2018). As such, use of more precise pubertal staging techniques is critical in efforts to delineate the pubertal effects on biology and behavior in ASD (Corbett, Muscatello, Tanguturi, McGinn, & Ioannou, 2019b).

The results presented here provide further evidence of developmental changes in stress responses, which could hypothetically have downstream effects on behavior and affect. Nevertheless, much like most previous research (e.g. Corbett et al., 2010), age was the primary metric used to index developmental maturation. It remains to be seen whether use of pubertal staging methods such as physical examination would yield similar or different findings. Moreover, while there is evidence that atypical physiological patterns persist from childhood into adolescence (Muscatello & Corbett, 2018), no study to date has mapped the trajectory of stress and arousal, along with corresponding social and affective behaviors, as children with and without ASD enter and transition through the adolescent period. Longitudinal studies are currently underway to address these important questions as to how a child's physiology, behavior, and experiences may predict later outcomes.

Social Support

Interlinked with the noted effects of age are the contributions of previous social experiences and support on stress, arousal, and psychological health. As previously discussed, social bonds are critical for mental health, as isolation, withdrawal, and loneliness are consistently linked to increased incidence of internalizing symptoms (e.g. Cacioppo, Hawkley, &

Thisted, 2010). Similarly, we found elevated symptoms of anxiety and depression were associated with limitations in various social behaviors, such as reduced verbal interaction and eye contact (Chapter 4). Social impairments characteristic of ASD are likely to predispose youth to failed peer interactions, and over time older youth with ASD may become more aware of these social challenges and the effects on relationships (Knott, Dunlop, & Mackay, 2006; Kuusikko et al., 2008; Lopata, Volker, Putnam, Thomeer, & Nida, 2008). It is well-established that youth with ASD are more likely to experience peer victimization and bullying (Humphrey & Lewis, 2008; Zeedyk, Rodriguez, Tipton, Baker, & Blacher, 2014). This collection of social victimization, failed interactions, and increased awareness into one's own deficits has been speculated to lead to enhanced social anxiety and stress (Bellini, 2006; Kuusikko et al., 2008). Subsequently, anxiety can then further drive social isolation and elevated stress and arousal in these youth, thereby creating a cycle of poor social experience, anxiety, and stress.

It is important to highlight that physiological responses are also influenced by positive peer interactions. A widely discussed moderator of stress response is social companionship (Hennessey 2009). This 'social buffering' has been noted to reduce the HPA axis and ANS stress response, as well as dampen behavioral stress responses, in animal models (e.g. Gunnar, Gonzalez, & Levine, 1980; Gust, Gordon, Brodie, & McClure, 1994) and in human studies (e.g. Adams, Santo, & Bukowski, 2011; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995). For example, we presented evidence for reductions in stress (HPA) in TD youth after an initial anticipatory response when interacting with a friendly, supportive peer (Chapter 3). For youth with ASD, social skills interventions utilizing peers as 'interventionists (i.e. peer-mediated interventions) show evidence for effectively enhancing social participation in more generalized, naturalistic settings (e.g. Corbett, Ioannou, Key, Coke, Muscatello, Vandekar, et al., 2019a;

Corbett et al., 2016; Kamps et al., 2002; Mason et al., 2014) while reducing anxiety (Corbett, Blain, Ioannou, & Balser, 2017; Corbett, Ioannou, Key, Coke, Muscatello, Vandekar, et al., 2019a). Peer-mediation techniques may, over time, provide similar stress-reductive benefits for youth with ASD; however, future research is necessary to determine the extent to which peer mediation moderates stress and arousal to social interaction paradigms in ASD.

Translational Therapeutic Interventions

Numerous behavioral (see Weitlauf et al., 2014 for review) interventions have been developed and tested to treat the core social impairments in ASD (e.g. Corbett et al., 2016; Corbett, Ioannou, Key, Coke, Muscatello, Vandekar, et al., 2019a; Kasari et al., 2016; Laugeson, Ellingsen, Sanderson, Tucci, & Bates, 2014; Solomon, Goodlin-Jones, & Anders, 2004) and comorbid internalizing symptoms (e.g. McNally Keehn, Lincoln, Brown, & Chavira, 2013; White, Ollendick, Scahill, Oswald, & Albano, 2009; White et al., 2018; Wood et al., 2015). Nevertheless, ASD is a heterogenous condition and no single treatment has been shown to be universally effective. Thus, research in ASD is constantly identifying new biobehavioral targets for treatment, enhanced diagnostic techniques, and improved interventions to treat these debilitating symptoms.

Many of our daily experiences center around social interactions, and absence of positive social relationships puts one at risk for a range of negative health outcomes (e.g. House, Landis, & Umberson, 1988; Uchino, 2006). As such, many interventions seek to identify unique ways to treat social skill impairments in ASD. In addition to behavioral approaches, pharmaceutical interventions are being studied. Relatively recent pilot work has investigated the effects of propranolol, a beta-antagonist which blocks sympathetic actions in the periphery during social

conversation in ASD (Zamzow et al., 2016). The use of a sympathetic antagonist may seek to target the presumed connection between autonomic functioning and social engagement; however, pilot studies to this point suggest propranolol's positive effects on social functioning are due to its actions in the central nervous system, not the periphery (Narayanan et al., 2010). Further, while the current studies identified altered physiological stress and arousal response in ASD, these response patterns were not directly correlated with social behavior. While evidence suggests functioning of physiological systems may be a potential marker of social dysfunction in ASD, its utility as a treatment target remains unclear and requires more research.

A second critical focus of our findings with far-reaching implications, is the link between social functioning and internalizing conditions. The prevalence of anxiety and depressive disorders in ASD surpasses rates of the global population (Mayes, Calhoun, Murray, Ahuja, & Smith, 2011). These internalizing symptoms are often associated with core social impairments of ASD (e.g. Bellini, 2004), though the directionality of the relationship remains in question (Duvekot, van der Ende, Verhulst, & Greaves-Lord, 2018; White et al., 2018). In addition to more traditional treatments for anxiety or depression, such as cognitive-behavioral therapy (see White et al., 2018 for review), there is evidence for improvement in internalizing symptoms following social skills interventions (Corbett et al., 2017; Hillier, Fish, Siegel, & Beversdorf, 2011; Schiltz et al., 2018). Based on our findings, interventions which promote positive peer interactions may be most effective in improving not only social communication but also symptoms of anxiety and depression. For example, an empirically supported social skills training for youth with ASD (PEERS®; Laugeson et al., 2014; Laugeson, Frankel, Mogil, & Dillon, 2009) includes training sessions on general social skills as well as how to handle social rejection. In fact, participants in the PEERS® program were recently shown to report fewer depressive

symptoms and decreased suicidality (Schiltz et al., 2018). Furthermore, participants of a peer-mediated theatre intervention (SENSE Theatre®; Corbett et al., 2016; Corbett, Ioannou, Key, Coke, Muscatello, Vandekar, et al., 2019a) reported significantly less anxiety when interacting with novel peers and demonstrated an increase in cooperative play following the 10-week program (Corbett et al., 2016). According to the findings presented here, peer-mediated interventions with positive, supportive interactions with peer models show promise. We suspect such interventions, powered by social buffering, may aid in disrupting the cycle of social impairments, internalizing symptoms, and physiological arousal in ASD.

Suggestions for Future Directions

A number of future directions may be taken from the presented findings. First, effects of age and pubertal development contribute to increased stress and arousal which are also associated with social and emotional functioning. While we were able to draw conclusions regarding the relationships between physiological, social impairments, and internalizing symptoms, the directionality of these associations is unclear. Therefore, it will be important for future research to examine the longitudinal trajectories of biobehavioral profiles in ASD, especially through critical transition periods such as puberty. It may be possible to identify youth before or during the pubertal transition who are most at risk for anxiety or depression by their biobehavioral subgroup. Our findings indicate that children who show imbalance between ANS functioning evidence increased symptoms of depression. Therefore, this index may be apparent during the prepubescent period alerting the need for more careful monitoring and possible preventative intervention. Alternatively, despite the persistent march to identify biomarkers, the findings show that established behavioral indicators, such as limited eye contact and social

withdrawal, remain key indices of early warning signs of possible internalizing symptoms and must not be ignored. Taken together, the findings support the effort to examine the links between physiological, social and emotional markers in well-defined larger samples of youth with ASD.

Secondly, possible sex differences in social, behavioral and biological systems continues to be a prime area for future research in ASD. While the ratio of males to females in ASD is often reported at 4:1 (American Psychiatric Association, 2013), more recent estimates suggest the ratio may be closer to 3:1, males to females (Loomes, Hull, & Mandy, 2017). With the strong male bias in ASD, females have been historically underrepresented in ASD research (Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015). Importantly, the HPA axis and ANS are shown to differ between sexes, likely due to sex-specific hormones (e.g. Dart, Du, & Kingwell, 2002; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Additionally, sex differences are often noted in anxiety and depression prevalence (e.g. Sterba, Prinstein, & Cox, 2007; Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). Therefore, studies which consider these sex-specific effects will be essential for elucidating biobehavioral profiles in ASD.

Finally, it is important to acknowledge the wide range of social experiences and situations that impact the daily life of a child with ASD, which may contribute to or ameliorate physiological stress or arousal. The extent to which we are able to recreate such social scenarios (e.g., social play, reciprocal conversation) into ecologically valid research paradigms that capture the social stress and anxiety experienced in the real world is an ongoing pursuit. It is apparent that there are differences between previous (e.g. PIP, Corbett et al., 2010; TSST, Kirschbaum, Pirke, & Hellhammer, 1993) and current social stress protocols (TSST-F, Wiemers, Schoofs, & Wolf, 2013) used in social stress research in ASD. Specifically, the peer interaction paradigm (PIP, Corbett et al., 2010) is a social interaction with two novel peers conducted during outdoor

play on a playground. In contrast, the social paradigm used in the current studies, the TSST-F (Wiemers et al., 2013), consists of a social conversation with one peer in a room. It is highly plausible that the discrete task demands contributed to the differences in the findings. Therefore, future studies may attempt to directly compare stress, arousal and social patterns between the PIP and TSST-F to examine what aspects of social experience are particularly stress-inducing.

Summary and Conclusions

Social experiences can be a source of positive peer interactions and attachments, but they can also be anxiety-provoking and stressful for many youth with ASD. The purpose of the current studies was to examine social communicative impairments in ASD, along with physiological stress and arousal response to social interactions. We aimed to determine whether the severity of social impairments and stress responsivity would predict internalizing symptoms in this population. The present findings provide evidence for a complex and dynamic interplay between social communication, physiological functioning, and internalizing behaviors in ASD. This interconnected system highlights the importance of monitoring social communicative skills and associated behavioral physiological responses in children and adolescents with ASD in order to efficiently intervene to protect emotional well-being and promote the development of social functioning.

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