PRAGMATIC LANGUAGE IN AUTISM AND FRAGILE X SYNDROME:
LINKS WITH PHYSIOLOGICAL AROUSAL AND ANXIETY

Jessica Klusek

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Approved by:

Molly Losh, PhD

Elizabeth R. Crais, PhD

Linda R. Watson, PhD

Heather C. Hazlett, PhD

Gary E. Martin, PhD

Jane E. Roberts, PhD

ABSTRACT

JESSICA KLUSEK: Pragmatic Language in Autism and Fragile X Syndrome:
Links with Physiological Arousal and Anxiety
(Under the direction of Molly Losh)

This dissertation is comprised of three manuscripts focused on delineating pragmatic language profiles in children with autism and fragile X syndrome, and on understanding the potential impact of physiological dysregulation on these profiles. The first manuscript presents a review of the existing literature on physiological arousal in autism and fragile X syndrome, with a focus on the relationship between arousal modulation and social competence. The second two manuscripts present original research: the first consists of a cross-population comparison of pragmatic language in autism and fragile X syndrome; the second extends this line of research by examining cardiac arousal as a mechanism that may play a role of social-communicative impairment in these disorders. These three manuscripts address the extent to which pragmatic language deficits overlap in autism and fragile X syndrome, and whether such deficits are linked with dysfunction of the autonomic nervous system. This work has implications for developing syndrome-specific interventions and ultimately may inform biological pathways that may be common to autism and fragile X syndrome.

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program of research laid the foundation for this dissertation and whose work continues improve the

lives of individuals with developmental disabilities.

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CHAPTER 1

INTRODUCTION

Pragmatic language impairment (i.e., impairment in the appropriate use of language in social contexts) is a universally observed feature of autism (Landa, 2000; Tager-Flusberg, Paul, & Lord, 2005) that is thought to be genetically-mediated (Landa et al., 1992; Losh, Childress, Lam, & Piven, 2008; Piven, Palmer, Landa, Santangelo, & Childress, 1997). Pragmatic language deficits are also seen in fragile X syndrome, the most common known monogenic disorder associated with a diagnosis of autism (Belser & Sudhalter, 2001; Cohen, Pichard, & Tordjman, 2005; Losh, Martin, Klusek, Hogan-Brown, & Sideris, 2012; Martin et al., 2012; Mazzocco et al., 2006; Sudhalter & Belser, 2001). Although pragmatic language deficits have been documented in both autism and fragile X syndrome, it remains unclear whether pragmatic language profiles are shared in these disorders, as only a few cross-population comparisons of pragmatics in autism and fragile X syndrome have been conducted, with inconsistent findings (e.g., Belser & Sudhalter, 2001; Losh et al., 2012; Sudhalter & Belser, 2001; Sudhalter, Cohen, Silverman, & Wolf-Schein, 1990). It is also unclear whether pragmatic deficits are rooted in similar underlying mechanisms. Physiological arousal dysregulation is well-documented in fragile X syndrome, and has been hypothesized to underlie deviant pragmatic language features in the disorder (Belser & Sudhalter, 1995). While some evidence suggests that atypical physiological modulation is also seen in idiopathic autism (Bal et al., 2010; Ming, Julu, Brimacombe, Connor, & Daniels, 2005), few studies have directly examined links between arousal and pragmatic language impairment in autism. This dissertation aimed to clarify physiological overlap in autism and fragile X syndrome, and to explore dysfunctional physiological regulation as a process that might underlie pragmatic language impairment in these disorders. Further delineation of pragmatic language overlap in autism and fragile X syndrome as implications for understanding the

potential role of the fragile X gene, *Fragile X Mental Retardation-1* (*FMR1*), in the pragmatic

language phenotype associated with autism. Additionally, delineation of pragmatic profiles and

associated mechanisms has implications for the development of targeted treatment approaches, given

that pragmatic language impairment has a significant impact on the ability to participate in daily

social interactions (Roberts, Mirrett, Anderson, Burchinal, & Neebe, 2002; Szatmari, Bryson, Boyle,

Streiner, & Duku, 2003).

The first manuscript of this dissertation, *Autonomic Nervous System Function in Autism and*

Fragile X syndrome: Emerging Evidence for Cardiac Vagal Regulation as a Mediator of Social

Behavior, reviews theoretical and empirical evidence for a role of physiological dysregulation in

social deficits seen in autism and fragile X syndrome. Specifically, this manuscript focuses on cardiac

vagal tone as a sensitive index of parasympathetic nervous system functioning, which may be linked

to the core social deficits seen in autism and/or fragile X syndrome. Literature concerning cardiac

arousal in autism and fragile X syndrome is reviewed in detail, followed by a discussion of

implications for future research and clinical practice. The second manuscript of this dissertation,

Pragmatic Language in Boys with Autism and Fragile X Syndrome: A Cross-Population Comparison

of Naturalistic and Standardized Assessments of Pragmatic Language, presents novel research

comparing pragmatic language abilities of boys with idiopathic autism, fragile X syndrome with and

without autism, Down syndrome, and typical development. This paper attempts to address gaps in the

literature by incorporating a multimodal assessment approach in order to comprehensively define

pragmatic language profiles of autism and fragile X syndrome. Expanding upon the first two

manuscripts, the third dissertation article, *Is Pragmatic Language Impairment Related to*

Physiological Arousal Dysregulation in Autism and Fragile X Syndrome?, examines physiological

arousal as a predictor of pragmatic impairment in autism and fragile X syndrome, and explores

associations with clinical symptoms of anxiety (which may also contribute to pragmatic language

deficits). Together, the three manuscripts of this dissertation address the extent to which pragmatic

language and physiological features are shared in autism and fragile X syndrome, which has

implications for the development of targeted interventions, and may inform the role of the *FMR1*

gene in the pragmatic language profile associated with autism.

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CHAPTER 2

AUTONOMIC NERVOUS SYSTEM FUNCTION IN AUTISM AND FRAGILE X SYNDROME: EMERGING EVIDENCE FOR CARDIAC VAGAL REGULATION AS A MEDIATOR OF SOCIAL BEHAVIOR

Summary

Autism is a neurodevelopmental disorder characterized by core deficits in social engagement. Similar social impairments are seen in fragile X syndrome, a genetic condition associated with significantly increased risk for developing autism. An emerging body of literature points to dysfunction in the autonomic nervous system, and specifically dysfunction in parasympathetic cardiac control via the vagal nerve, as a mediator of social behavior in these populations. This paper reviews theoretical and empirical evidence for a role of autonomic dysfunction in social impairment seen in autism and fragile X syndrome, with a focus on cardiac vagal tone as a sensitive, non-invasive psychophysiological marker of autonomic system integrity.

Introduction

Recent literature suggests that social deficits in autism may be linked to underlying dysfunction of the autonomic nervous system (Marshall & Fox, 2006; Porges, 2004). Autonomic imbalance is broadly associated with psychopathology (Beauchaine, Gatzke-Kopp, & Mead, 2007; Field & Diego, 2008; Thayer & Lane, 2007), and studies of typical development suggest that autonomic control plays an important role in the development of social behavior, such as affective expressiveness (Field, Pickens, Fox, Nawricki, & Gonzalex, 1995; Stifter, Fox, & Porges, 1989), empathy (Fabes, Eisenberg, & Eisenbud, 1993; Fabes, Eisenberg, Karbon, Troyer, & Switzer, 1994), attachment (Izard, Porges, Simons, Haynes, & Cohen, 1991), and social approach (Kagan, Reznick, & Snidman, 1987). Therefore, autonomic dysfunction may contribute to social deficits associated with autism. Although social impairment is a core feature of autism that has a significant impact on functional outcomes, thus far attempts to understand the biological basis of such deficits have been hindered by the significant heterogeneity that is seen in the disorder (Abrahams & Geschwind, 2008). Importantly, autonomic system dysfunction has also been documented in fragile X syndrome, which is a well-defined genetic condition associated with autism. The delineation of physiological mechanisms that overlap or diverge in autism and fragile X syndrome may shed light on biological pathways that may be common across etiological subgroups of autism, and which may be linked to defining behavioral features. Parsing out the relationship between autonomic functioning and social behavior in autism and fragile X syndrome has important implications for intervention, as well as broader implications for informing the process by which underlying biological mechanisms give rise to complex social behaviors. This review article examines theoretical and empirical evidence for a role of autonomic dysfunction in the social impairments associated with autism and fragile X syndrome, with a focus on cardiac vagal tone as a sensitive, non-invasive peripheral marker of autonomic nervous system functioning.

The Autonomic Nervous System and Its Functions

The primary function of the autonomic nervous system is to maintain homeostasis, which allows the body to adapt to continuous change while preserving a controlled, functional physiological condition (Cannon, 1929; Porges, 1992). The combined interchange of the sympathetic and parasympathetic subsystems of the autonomic nervous system allows the body to maintain homeostasis. These subsystems work in an antagonistic manner in order to attend to external demands, while supporting the needs of many internal organs and bodily systems. The sympathetic nervous system is responsible for activating the arousal response that prepares the body to respond to environmental stressors. When challenged, the sympathetic system mobilizes the body to respond by increasing metabolic output in preparation for action (Porges, 1992). This includes broad activation of the cardiovascular system, the immune system, and the endocrine glands, and is accompanied by measurable physiological changes such as pupil dilation, release of adrenaline, cessation of digestion, and an increase in heart rate and blood pressure (Lacey, 1967; Porges, 1992). This autonomic defense mechanism, often referred to as the "fight or flight" response, is an adaptive strategy that allows the body to maximize physical reserves in order to protect or defend against danger (Porges, 1995; Thayer & Sternberg, 2006). Therefore, the sympathetic nervous system is responsible for rousing metabolic resources in the face of environmental demands, thus maximizing the body's ability to handle external stressors.

While the sympathetic branch of the autonomic nervous system is responsible for responding to the external environment, the parasympathetic branch responds to the internal needs of the body. The parasympathetic system is associated with growth and restoration, and when not challenged, its primary role is to optimize the function of the internal organs and bodily systems for which it is responsible, which includes the eyes, stomach, intestines, bladder, lungs, and heart (Porges, 1992). The parasympathetic system works in a manner that is antagonistic with the sympathetic system, acting as a restraint or brake to counteract sympathetic activity. When the body is at rest, the parasympathetic system functions to promote a calm physiological state that supports internal needs,

such as slowing heart rate in order to conserve energy (Porges, 1992). However, when the body is challenged, the parasympathetic system responds by releasing the brake to allow for reciprocal increases in sympathetic tone, and accompanying physiological excitation.

The dynamic balance between the parasympathetic and sympathetic systems promotes stability, adaptability, and health (Friedman, 2007; Thayer & Lane, 2000; Thayer & Sternberg, 2006). Chronic imbalance of the autonomic nervous system, when either the sympathetic or parasympathetic subsystem dominates the other, is taxing to the body and increases vulnerability for pathology (Thayer & Lane, 2007; Thayer & Sternberg, 2006). Autonomic dysfunction is found in a variety of medical conditions, such as diabetes (Rosengard-Barland et al., 2009; Singh et al., 1998), obesity (Nagai, Matsumoto, Kita, & Moritani, 2003; Skrapari et al., 2007), and hypertension (Pagani & Lucini, 2001). It is also implicated in a range of psychological disorders, such as panic disorder (Yeragani et al., 1993), anxiety (Friedman, 2007), schizophrenia (Valkonen-Korhonen et al., 2003), anorexia (Mazurak, Enck, Muth, Teufel, & Zipfel, 2011), post-traumatic stress disorder (Sahar, Shalev, & Porges, 2001) and social phobia (Schmitz, Kramer, Tuschen-Caddier, Heinrichs, & Blechert, 2011). Because of its broad association with pathology, dysfunction of the parasympathetic nervous system serves as an index of stress vulnerability and psychophysiological health (Porges, 1992; Porges, 1995; Porges & Furman, 2011), and is hypothesized to play a role in autism (Marshall & Fox, 2006; Porges, 2004).

Autism and Fragile X Syndrome

Autism is a neurodevelopmental disorder that is extremely common, with recent reports indicating prevalence as high as 1 in 88 (CDC, 2012). Although the etiological underpinnings of autism are not fully understood, evidence suggests a large genetic component to autism, with twin studies supporting heritability estimated at 70-80% and multiple genes now identified as conferring risk to autism (Geschwind, 2011; Ronald & Hoekstra, 2011). Additional support for genetic involvement comes from family studies of autism showing elevated sibling and half-sibling recurrence rates (Constantino et al., 2012; Ozonoff et al., 2011) and a milder phenotype among

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unaffected family members, believed to reflect underlying genetic liability (Landa et al., 1992; Losh et al., 2008; Piven et al., 1994). However, the genetic etiology of autism appears highly complex and heterogeneous, involving multiple genetic effects coupled with significant individual variability, which poses a challenge to the identification of susceptibility markers (Bill & Geschwind, 2009). Thus, the diagnosis of autism is presently based off of the aggregation of behavioral symptoms in three core domains of impairment: social reciprocity, communication, and stereotyped behaviors (American Psychiatric Association, 2000). Of these domains, social deficit has been hypothesized to constitute a primary domain of impairment (Charman et al., 2005; Chevallier, Troiani, Brodkin, & Schultz, 2012; Dawson & Bernier, 2007) and is linked with functional outcomes (Landa, 2000; Szatmari et al., 2003).

Individuals with autism show pervasive social deficits across the lifespan. Even during the first year of life children with autism show reduced showing, pointing, and commenting for the purpose of social engagement (Mundy & Stella, 2000). In childhood and adulthood, social impairments in autism may manifest as diminished eye contact, preference for being alone, reduced social motivation and understanding, and deficits in social-communication, such as the ability to engage in conversation (Baron-Cohen, 2000; Volkmar, Paul, Klin, & Cohen, 2005). Because learning is thought to be embedded in social contexts, it is theorized that reduced social participation in autism results in reduced learning opportunities over time, broadly impacting developmental learning (Charman et al., 2000; Fogel, 1993; Hewitt, 1998).

It has been long hypothesized that unusual social response patterns in autism may be rooted in atypical processing of environmental stimuli that is related to physiological dysregulation (Hutt, Hutt, Lee, & Ounsted, 1964; Kootz & Cohen, 1981; Rimland, 1964). For example, individuals with autism have been noted to show behavioral responses that range from extreme passivity to agitation and hyper-reactivity, which is consistent with difficulties in regulating physiological arousal. These difficulties have a clear theoretical link with social impairment, as the inability to efficiently register environmental stimuli would have a broad impact on the ability to engage socially, such as the ability

to process language, to recognize familiar faces, or to tolerate physical touch. Therefore, physiological dysregulation has been hypothesized to underlie the sub-optimal engagement patterns that are seen in autism (Dawson & Lewy, 1989; Lord & McGee, 2001).

Fragile X Syndrome and Overlap with Autism

Fragile X syndrome affects approximately 1 in 2,500 individuals (Fernandez-Carvajal et al., 2009; Hagerman, 2008). The disorder is caused by a mutation in the *Fragile X Mental Retardation-1* (*FMR1*) gene that halts the production of Fragile X Mental Retardation Protein (FMRP), which is needed for brain development and functioning (Hagerman & Hagerman, 2002; Loesch, Huggins, & Hagerman, 2004; Tassone et al., 1999). The neurobehavioral profile of fragile X syndrome includes intellectual disability, communication difficulties, hyperactivity, social deficits, and impairments in executive functions, such as attention and impulse control (Hagerman & Hagerman, 2002; Schneider, Hagerman, & Hessl, 2009). Importantly, approximately 2-8% of cases of autism can be traced back to fragile X syndrome (Hagerman, 2006; Wassink, Piven, & Patil, 2001), making fragile X syndrome the most common known monogenic condition associated with autism (Cohen et al., 2005; Hagerman, 2008). Because the genetic basis of fragile X syndrome is relatively well-understood, fragile X syndrome has been studied as a simplified genetic context that may lend insight into core features that are shared across all etiological subtypes of autism (syndromic or idiopathic), and which may be linked to specific etiological pathways (e.g., Abrahams & Geschwind, 2010; Belmonte & Bourgeron, 2006; Hagerman, Narcisa, & Hagerman, 2011). In other words, the study of autism in fragile X syndrome provides a means for linking a known genetic mutation with core behavioral and biological features associated with autism.

In line with this approach, research has focused on defining behavioral phenotypes that are common in autism and fragile X syndrome, with the eventual goal of identifying shared biological pathways that underlie such phenotypes. Autism symptoms are common in fragile X syndrome, with approximately 30-50% of individuals with fragile X syndrome meeting diagnostic criteria for autistic disorder (Harris et al., 2008; Rogers, Wehner, & Hagerman, 2001), and 60-75% for an autism

spectrum disorder (Clifford et al., 2007; Hall, Lightbody, & Reiss, 2008). Furthermore, almost all individuals with fragile X syndrome, even those who do not meet diagnostic thresholds, exhibit autistic-like behaviors, such as gaze avoidance, social anxiety, and social communication impairment (Bailey et al., 1998; Hagerman & Hagerman, 2002; Hernandez et al., 2009; Kaufmann et al., 2004). However, the nature and origin of autistic symptoms in fragile X syndrome is not clear, as it is theorized that autistic behaviors seen in fragile X syndrome are rooted in fragile X-related anxiety (and associated hyperarousal), and hence do not reflect "true" autism (Cohen, 1995; Cohen, Vietze, Sudhalter, Jenkins, & Brown, 1989). This hypothesis stems from early behavioral observations that children with fragile X syndrome appeared to actively avoid eye contact, whereas children with autism seemed indifferent (rather than avoidant) to social gaze (Cohen et al., 1989). This observation, in conjunction with early skin conductance studies documenting physiological hyperarousal in fragile X syndrome (e.g., Belser & Sudhalter, 1995; Miller et al., 1999), led to the hypothesis that anxiety and associated hyperarousal underlie the social deficits seen in fragile X syndrome (e.g., Cohen, 1995). In support of this hypothesis, several reports have documented associations between anxiety or avoidance behaviors and autism symptoms in fragile X syndrome (Budimirovic et al., 2006; Mazzocco, Kates, Baumgardner, Freund, & Reiss, 1997).

However, a number of cross-population comparison studies have directly compared idiopathic and fragile X-associated autism, with most showing a high degree of behavioral overlap. For example, several studies have reported virtually indistinguishable autism symptom profiles between these groups as measured by gold-standard autism diagnostic tools and other symptom rating scales (Bailey et al., 1998; Dissanayake, Bui, Bulhak-Paterson, Huggins, & Loesch, 2009; Rogers et al., 2001). Similarities are also seen in performance on direct-assessment measures of social-communication ability and theory of mind (i.e., the ability to understand the thought and feelings of others), with performance on these domains showing associations with *FMR1*-related genetic variation (Losh et al., 2012). Furthermore, recent evidence suggests that this behavioral overlap extends to subclinical phenotypic presentations of these disorders; family members with the broader

autism phenotype and the fragile X premutation (who are carriers of the fragile X gene) present with social-communicative and personality features that are similar in quality and severity, and which are distinctive from control participants (Losh et al., 2012). Overall, direct comparison studies of fragile X-associated and idiopathic autism support strong behavioral overlap, which might implicate shared genetic variation in the two disorders, and a potential role of *FMR1*.

Several studies have directly explored this hypothesis by examining associations between FMRP and autism symptom severity, with a consensus that FMRP level is not associated with autism symptomatology after controlling for intellectual ability (Bailey, Hatton, Skinner, & Mesibov, 2001; Harris et al., 2008; Loesch et al., 2007). However, this does not preclude a role of *FMR1* in autism; current hypotheses support a model in which *FMR1* has a synergist effect with other "background" genes, with the presence of the *FMR1* mutation reducing the number of additional background alleles needed to produce autism (Bailey et al., 2001; Harris et al., 2008; Rogers et al., 2001). In line with this hypothesis, several proteins that are known to become dysregulated in the absence of FMRP have also been implicated in molecular studies of autism, such as neuroexin, neuroligin3, neuroligin4, CYFIP, and PTEN (see Hagerman, Hoem, & Hagerman, 2010). This area of research provides promising evidence for a role of *FMR1* in autism at the molecular level, and may contribute to the eventual goal of identifying specific genetic mechanisms that may interact with *FMR1*, as well as to elucidate the complex process by which these underlying genes may lead to behavioral endpoints associated with autism. The study of biological correlates of clinical phenotypes, such as physiological regulatory mechanisms, may contribute to this topic by elucidating intermediate biological processes that may bridge the gap between the phenotype and underlying genotype associated with autism symptomatology.

Given that arousal modulation has been long hypothesized to underlie the behavioral phenotype of both autism and fragile X syndrome (e.g., Cohen, 1995; Hutt et al., 1964; Kootz & Cohen, 1981; Rimland, 1964), and given the strong behavioral overlap in these disorders, the study of

physiological dysregulation (and associated autonomic nervous system dysfunction) in autism and fragile X syndrome may shed light on biological pathways that may be shared in both disorders and which may be linked with *FMR1*-related genetic variation. This article will contribute to this area of research through synthesis of the literature addressing cardiac autonomic dysfunction in autism and fragile X syndrome, and links with social-behavioral deficits. Below, brief background on use of indices of cardiac vagal tone as indicators of autonomic system integrity is provided, including the functions of cardiac vagal tone in typical development. Next, cardiac arousal in autism and fragile X syndrome is reviewed in detail, with emphasis on parasympathetic vagal control as a psychophysiological marker for the core social deficits seen in these disorders. Finally, a summary of findings and concise evaluation of the literature is presented, with a discussion of implications for future research and clinical practice.

Cardiac Vagal Tone as an Index of Parasympathetic Function

Cardiac vagal tone provides a peripheral, non-invasive measure of parasympathetic autonomic activity via the quantification of heart rate variability patterns (discussed in detail below). The tenth cranial nerve, the vagus, provides bidirectional communication between the brain and heart (Porges, 2001). Parasympathetic cardiac responses to environmental change are regulated via vagal efferent pathways, which project to the sinoatrial node of the heart (the heart's natural pacemaker). Activation of vagal efferent fibers slows the firing of the sinoatrial node, which results in a rapid decrease in heart rate (Levy & Warner, 1994; Porges, 2003). In this way, the vagus acts as part of the broader parasympathetic system to regulate cardiac responses to environmental challenge. Thus, cardiac vagal tone can serve as a broad indicator of the ability to respond adaptively to external stressors, and of general autonomic system integrity (Porges, 1992; Porges, 1995; Porges & Furman, 2011).

Several models have been proposed to account for the role of cardiac vagal tone in psycho-emotional health, such as the central autonomic network model (Benarroch, 1997) and the "emotion circuit" model (Damasio, 1998) (see Thayer & Lane, 2000 for detailed discussion). A theory that has

gained traction in recent years is Porges' Polyvagal Theory, which provides a compelling evolutionary framework to support physiological regulation (and the broader autonomic system as managed by the vagal nerve) as a mediator of social behavior (Porges, 1992; Porges, 1995, 2001, 2003, 2007; Porges & Furman, 2011). From an evolutionary perspective, social engagement allowed for communication between friends and foes, and thus was an adaptive behavior that contributed to survival. Porges argues that, in order to facilitate social behavior, mammalian evolution produced an organized nervous system that could fluidly alter between calm and aroused physiological states, thereby facilitating either socio-emotional engagement or mobilization and defense (Porges, 2001). Specifically, Polyvagal Theory emphasizes the role of the vagal nerve as a physiological mediator of social engagement. Porges contends that the dorsal and ventral branches of the vagus, which both terminate in the sinoatrial node of the heart, have evolved to support distinct adaptive behaviors (Porges, 2001). The phylogenetically newer branch, the ventral vagus, has evolved to include myelinated pathways that allow for allow for rapid, transitory responses to environmental changes. Quick, momentary adjustments to the vagal brake via the ventral pathway allow the body to express sympathetic tone without activating the sympathetic-adrenal system, which conserves metabolic resources and allows for efficient, rapid mobilization in response to environmental demands (Porges, 2001, 2007). Polyvagal Theory argues that without the adaptive functions of the phylogenetically newer myelinated vagus, social behaviors would be limited to primitive engagement strategies, such as "flight or fight" sympathetic mobilization, or extreme parasympathetic immobilization (e.g., physiological shut-down) (Porges & Furman, 2011). Thus, the evolution of myelinated vagus permits fine-tuned autonomic regulation via the vagal brake, in the service of adaptive social behavior.

Porges' Polyvagal Theory is in line with an extensive body of research documenting a link between cardiac vagal regulation and environmental engagement throughout the lifespan. In infancy, high baseline vagal tone (i.e., increased parasympathetic activity) is associated with increased environmental reactivity, which is believed to be an adaptive skill that sets the stage for later social interaction by providing more opportunities for interactive learning (Beauchaine, 2001; Calkins &

Fox, 1992; Cicchetti, Ganiban, & Bamett, 1991; Porges, Doussard-Roosevelt, & Maiti, 1994). Infants with elevated vagal tone show more pronounced responses to environmental stimuli that may be either positive (e.g., increased joyful and facial expressions, more frequent vocalization, and increased synchrony in caregiver-infant interactions (Feldman & Eidelman, 2007; Field et al., 1995; Fox & Gelles, 1984; Pickens & Field, 1995; Porter, 2003; Stifter et al., 1989), or negative (e.g., more frequent crying and difficult temperament) (Calkins & Fox, 1992; Fox, 1989; Porges, Doussard-Roosevelt, Portales, & Suess, 1994; Stifter & Fox, 1990). This relationship emerges early in life, with vagal tone predicting behavioral reactivity even among infants as young as one or two days old (Porter, Porges, & Marshal, 1988). Elevated vagal tone during infancy is also a positive predictor of emotional and behavioral outcomes in toddlerhood and childhood (Doussard-Roosevelt, McClenny, & Porges, 2001; Porges et al., 1994).

The relationship between vagal tone and pro-social behavior in childhood and adulthood has been studied in two ways -- studies of tonic vagal activity and studies of vagal reactivity to a particular stimulus (see Table 2.1). At rest, elevated vagal tone marks efficient maintenance of homeostasis, greater awareness, and increased capacity for self-regulation and engagement with the environment (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996). Children with high resting vagal tone are less behaviorally inhibited than their low vagal tone peers, and are more likely to initiate and engage in social interaction (Fox & Field, 1989; Kagan et al., 1987). These children also have higher scores on developmental assessments (Fox & Porges, 1985), greater capacity for sustained attention (Suess, Porges, & Plude, 1994), and less maladaptive behaviors (El-Sheikh, Harger, & Whitson, 2001; Katz & Gottman, 1995, 1997). Furthermore, a substantial body of research links high vagal tone to pro-social behavior in childhood, including emotional expression, self-regulation, empathy, and overall social competence (Blair & Peters, 2003; Calkins, 1997; Calkins & Keane, 2004; Eisenberg et al., 1996; Fabes et al., 1993; Fabes et al., 1994). This relationship extends into adulthood, with documented associations between vagal tone and complex social regulatory behaviors such as adaptive processing of threatening social stimuli (Miskovic & Schmidt, 2010);

(Park, Van Bavel, Vasey, & Thayer, 2012) and the regulation of facial affect (Demaree, Robinson,

Everhart, & Schmeichel, 2004).

Table 2.1. Cardiac Indices of Physiological Arousal

Cardiac Index	Measurement	Relation to the Autonomic Nervous System
Heart rate (general arousal)	Inter-beat-interval	Reflects both parasympathetic and sympathetic activity
Vagal tone	RSA or descriptive measures of heart rate variability	Parasympathetic tone
Vagal reactivity	Change in RSA/heart rate variability from baseline	Adaption of parasympathetic tone in response to environmental change

In contrast to studies of tonic vagal estimates, studies of vagal reactivity examine change in

vagal tone from baseline levels following the introduction of an environmental stressor. Reduction in

vagal activity from baseline, or vagal suppression, allows the body to switch from attending to

internal homeostatic needs to respond to external demands that might require sustained attention and

behavioral arousal (Lovallo, 2005; Porges, 1995, 2001; Porges et al., 1996). Greater vagal

suppression in response to cognitive or attention-demanding challenges is an adaptive response that

predicts enhanced social and emotional competence in other situations (e.g., Calkins & Keane, 2004;

Gentzler, Santucci, Kovacs, & Fox, 2009; Graziano, Keane, & Calkins, 2007; Stifter & Corey, 2001).

On the other hand, vagal suppression *during* social interaction is likely to represent a maladaptive

response associated with hypervigilance and the perception of threat, and is associated with poorer

social outcomes (Heilman et al., 2008). For example, preschoolers who show increased vagal

suppression during interaction with a stranger are rated as having increased anxiety, depression, and

internalizing problems (Heilman et al., 2008). Along the same lines, infants who show patterns of

vagal activity characterized by greater suppression during social interaction show more negative

affective signaling and difficulty calming motor movements (Bazhenova, Plonskaia, & Porges, 2001).

Thus, a vast body of research supports individual differences in vagal tone and reactivity as a

physiological marker of social adaptive behavior in typical development. In this review evidence is

examined to extend this relationship to the study of autism and fragile X syndrome, which are disorders characterized by atypical social engagement.

Respiratory Sinus Arrhythmia as an Index of Vagal Tone

Parasympathetic vagal control of the heart is commonly estimated from Respiratory Sinus Arrhythmia (RSA), which indexes the variability in heart rate during cycles of respiration (Bernston et al., 1997; Eckberg, 1983; Katona & Jih, 1975; Porges, 2007). Because the heart is innervated by both parasympathetic and sympathetic projections to the sinoatrial node, simple measures of heart rate (i.e., inter-beat-interval) reflect both parasympathetic and sympathetic activity (Bernston, Cacioppo, & Quigley, 1993). RSA, however, can be used to sensitively index parasympathetic vagal activity (Porges, 2007). At rest, heart rate varies with respiratory parameters, quickening upon inhalation and slowing upon exhalation. These cyclical patterns of heart rate variability are linked with sympathetic and parasympathetic influences; expiratory slowing of heart rate is mediated by the parasympathetic system via the vagus, whereas inspiratory quickening of the heart reflects transitory release of the vagal brake (Bernston et al., 1993; Berntson et al., 1994). Therefore, the beat-to-beat variability in heart rate patterns that occurs with spontaneous breathing allows for the estimation of vagal cardiac influences (Porges & Byrne, 1992). Specifically, greater variability in the rise and fall of heart rate (i.e., greater amplitude RSA) indexes greater vagal cardio-inhibitory influences on the heart, and increased parasympathetic control.

Quantification of RSA: Although detailed review of methods for quantifying RSA is beyond the scope of this article, a general overview of this literature is presented below to facilitate understanding of RSA as an index of vagal tone. Most methods for quantifying RSA fall under the categories of time-domain or frequency-domain methods. Time-domain methods calculate variability in heart rate using either statistical or geometric analyses of the electrocardiogram signal (Billman, 2011). The most basic time-series method for estimating RSA is through descriptive statistics of the heart rate variability, such as through calculation of standard deviation, successive mean difference, or the log variance of successive inter-beat intervals over a specified length of time (e.g., Eckberg,

1983; Fouad, Tarazi, Ferrario, Fighaly, & Alicandri, 1984; Grossman & Svebak, 1987). Descriptive measures of heart rate variability provide only a rough estimate of RSA. They capture variability that is attributable to both periodic and random sources, and RSA accounts for only about half of the variance in these estimates (Billman, 2011; Grossman, van Beek, & Wientjes, 1990). These approaches are also easily influenced by the inadequate artifact correction in the time series (Malik et al., 1993).

Other more advanced statistical approaches have been applied to extract variability while better controlling for factors extraneous to RSA. For example, Grossman's peak-valley technique controls for respiratory parameters through statistical manipulation (Grossman et al., 1990). Non-linear geometric approaches have also been utilized, which map the patterns of inter-beat-interval length to provide graphical depictions of variability (Malik, Farrell, Cripps, & Camm, 1989; Mayer-Kress et al., 1988). Although these time-series estimation techniques provide a measure of total variability, they are limited in their ability to distinguish individual contributors to variability (Billman, 2011). Frequency-domain methods for estimating RSA address this limitation by decomposing the heart rate time signal into component frequencies, either through the use of Fourier transformations or with autoregressive modeling (Denver, Reed, & Porges, 2007; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The extraction of high frequency peaks (> .15 Hz) from the time series signal can provide an estimate of parasympathetic vagal tone; evidence from pharmacological blockade studies suggests that the high frequency range reflects parasympathetic input, whereas sympathetic influences are present in the low (< .04 Hz) and mid (0.04- 0.15 Hz) frequency bands (Akselrod et al., 1981; Appel, Beger, Saul, Smith, & Cohen, 1989; Bigger, 1997). Another widely used quantification method, Porges' moving polynomial algorithm, integrates both time and frequency domain approaches to extract heart rate variability within a specified frequency band (Bohrer & Porges, 1982; Porges & Bohrer, 1990). Similar to other frequency-domain methods, this approach de-trends data to remove variance outside of the desired frequency band, but because of its use of a "moving filter," it has the

added ability to estimate heart rate variability from non-stable baselines, such as during exercise (Billman, 2011). Although most non-descriptive methods for indexing RSA are highly correlated, there is some evidence to suggest that the Porges' moving polynomial method most closely upholds statistical assumptions and is the most sensitive to pharmacological blockade studies (Lewis, Furman, McCool, & Porges, 2012). For more detailed review of RSA quantification methods, see Billman, 2011; Denver et al., 2007; Grossman & Taylor, 2007; Lewis et al., 2012.

It should be noted that although pharmacological blockade studies have detected significant associations between progressive drug-induced cardiac vagal withdrawal and RSA (e.g., Fouad et al., 1984; Grossman & Kollai, 1993; Hayano et al., 1991; Pyetan, Toledo, Zoran, & Akselrod, 2003), the relationship between RSA and vagal tone is imperfect. Correlations between RSA and vagal tone range from .5-.91 (Grossman, Karemaker, & Wieling, 1991; Grossman & Kollai, 1993; Hayano et al., 1991; Jennings & McKnight, 1994). It appears that respiratory parameters (rate and tidal volume) may confound RSA estimates in certain situations (see Grossman & Taylor, 2007), although, there remains some debate as to whether it is necessary or useful to control for respiratory characteristics when measuring RSA (see Denver et al., 2007; Porges, 2007).

These unresolved issues underline that RSA is an indirect estimate of cardiac vagal tone, and it should not be interpreted as a quantitative measure of the extent of vagal efferent nerve firing (see Grossman & Taylor, 2007). Nonetheless, RSA provides a rare glimpse into autonomic functioning that could not otherwise be afforded using non-invasive techniques. RSA has been widely used as a marker for biopsychological vulnerability, and has been successful at differentiating healthy individuals from those with psychopathology (Beauchaine et al., 2007; Field & Diego, 2008; Thayer & Lane, 2007). Therefore, studying RSA as an index of vagal tone is a promising method for exploring physiological processes that may underlie social deficits in autism and fragile X syndrome. In the following section cardiac indices of autonomic regulation in autism and fragile X syndrome are reviewed, with a focus on links to social engagement. While these studies have only measured vagal tone indirectly (through quantification of RSA), the term "vagal tone" is adopted throughout this

review for the sake of consistency. Given that most RSA quantification techniques provide roughly equivalent estimates, measurement details are only provided for those studies that have used descriptive statistics to index RSA, which are thought to provide a less precise estimate of RSA and vagal tone.

Cardiac Indices of Autonomic Nervous System Function in Autism and Fragile X Syndrome

Taken as a whole, physiological dysregulation is a well-replicated feature of fragile X syndrome. This includes general patterns of hyperarousal as indexed by elevated heart rate, as well as atypical vagal tone and vagal reactivity, which is consistent with broader dysfunction of the autonomic nervous system. While atypical patterns of physiological regulation have also been documented in autism, findings have proven less consistent than those in fragile X syndrome. For instance, while some studies have found elevated heart rate and diminished vagal tone, others studies have found arousal and parasympathetic vagal control to be on par with developmental or age-based expectations. Discrepant findings in the autism literature could simply reflect the heterogeneity of this population. Unlike fragile X syndrome, which is cause by a single genetic mutation, the causes of autism are multifactorial, and clinical expression varies widely (Betancur, 2011; Geschwind & Levitt, 2007). Methodological considerations might also account for inconsistent findings.

First, the majority of studies in have included small samples, and it is likely that some null findings are due to underpowered statistical tests. Power analyses were not reported in the most of studies, so it is difficult to discern to what extent limited power obscured effects; sample sizes are provided in the review below to facilitate interpretation, as well as power analyses when available. Secondly, there have been significant advances in autism diagnostic methods and techniques for indexing cardiac activity since the earliest investigations of physiological responses in autism, which date back to the early 1970's. Reviewed below, these early reports should be interpreted with some caution given the differences in methods. This is less of a concern in deciphering the studies of fragile X syndrome, as most investigations were conducted within the last two decades.

Third, studies of autism have focused on a wide range of age and ability levels, which likely

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accounts for some inconsistencies across findings. Although vagal activity is known to increase with age (Alkon et al., 2003; Bar-Haim, Marshall, & Fox, 2000; Longin, Gerstner, Schaible, Lenz, & Konig, 2006; Porges et al., 1994; Sahni et al., 2000), it is unclear whether this vagal maturation is purely linked to physical changes that occur with development, or if it is also influenced by developmental factors such as growth in cognitive or language ability. Because developmental influences on cardiac indices of autonomic functioning are not well understood, it is unclear how different participant characteristics and matching procedures (e.g., chronological versus mental-age matching of groups) across studies might lead to different conclusions. There also exists considerable inconsistency in experimental conditions, particularly across studies of autism. For example, physiological activity has been measured in conditions ranging from solitary unstructured time to social-interactive challenges, with little replication of experimental context across studies. Heart activity is known to vary according to context, and even small changes in environmental context might elicit divergent responses (Alkon et al., 2003).

Finally, gender effects should be considered, given that males and females show systematic differences in heart activity (Beauchaine, 2001; Saab, 1992). In autism, most studies have examined only males, or have included only a very small number of females. There have been no investigations of cardiac regulation specifically among females with autism. Therefore, gender effects in autism are not be discussed in this review, as there is little available evidence on this topic. However, some emerging research has addressed physiological arousal among females with the fragile X syndrome, and these findings are considered.

General Arousal Indexed by Heart Rate

Heart rate, or inter-beat-interval, indexes general arousal levels; when the body is stressed, heart rate generally increases and becomes more stable (Porges & Raskin, 1969). Although investigations of heart rate (without measurement of RSA) cannot contribute to our understanding of specific areas of breakdown within the autonomic system (given that heart rate is influenced by both sympathetic and parasympathetic activity), studies of heart rate are nonetheless informative in

understanding general arousal patterns.

Autism: Many early investigations of autism measured heart rate in response to sensory stimuli, in an attempt to validate theoretical accounts that atypical sensory responses in autism were rooted in physiological mechanisms (e.g., Hutt et al., 1964; Rimland, 1964). For example, Bernal and Miller (1970) examined heart rate and skin conductance during the presentation of flashing lights and auditory stimuli among 20 children who had been diagnosed with the autism subtype of childhood schizophrenia. Similar heart rate was detected in autism as compared to typical controls, although electrodermal measures suggested reduced sympathetic response in autism. In another early investigation, MacColloch and Williams (1971) examined heart rate of 19 children with autism who were residential patients in a mental hospital. The heart rate of the children with autism did not differ from that of children with typical development or children with intellectual disability who were of a similar age. Graveling and Brooke (1978) also did not detect differences in heart rate between a small sample of children autism (*n* = 5) and a group of children with intellectual disability, as measured during arousal-provoking changes to classroom activities.

These early findings are consistent with several more recent reports that found general arousal of individuals with autism to be similar to that of controls. Corona et al. (1998) examined heart rate of 20 preschool-aged children with autism as compared to an intellectual disability group that was in age, cognition, and language ability. No differences were detected in the heart rate of the two groups during a baseline condition, in which the children played quietly with toys. Similar patterns were detected by Sigman and colleagues (2003) in a study of the social responses of children with autism (*n* = 22) during interactions with a stranger and with their mother. In comparison to an intellectual disability group (who were matched on language, chronological age, and mental age), the children with autism showed similar mean heart rate, despite observable differences in behavior during the interactions (such as reduced vocalizations in the autism group). Finally, Althaus and colleagues (1999) did not detect differences in resting heart rate between two groups of 18 children

with Pervasive Developmental Disorder-Not Otherwise Specified who did or did show signs of hyperactivity and typical children similar chronological ages.

Increased heart rate has also been widely reported in studies of autism. In an early report, Cohen and Johnson (1977) reported elevated heart rate among ten children with autism compared to typical controls during a series of activities (watching a video, reading a book, listening to a story), although group differences were not tested statistically. In a larger study, James and Barry (1980) investigated cardiac responses of a large sample of 40 children with autism during the presentation of visual and auditory stimuli. Results indicated that the children with autism had elevated heart in comparison to typically developing children, but that did not differ in comparison to children with intellectual disability. More recently, Denver (2004) detected increased heart rate in 20 children and adolescents with autism during a series of laboratory assessments that involved word repetition and watching video clips. Goodwin et al. (2006) and Woodard et al. (2012) also found elevated heart rate in small samples of children with autism compared to typical controls during a range of potentially stressful tasks (such as sensory stimulation). Several studies have also found heart rate in autism to be elevated during calm baseline conditions (Bal et al., 2010; Mathewson et al., 2011; Watson, Roberts, Baranek, Mandulak, & Dalton, 2012; Woodard et al., 2012).

One study of heart rate reactivity has also detected reduced responses in autism. Jansen et al. (2003) examined the heart rate of 10 children with autism in the time preceding a public speaking task, and found that, unlike typical control children, the children with autism failed to increase heart rate in anticipation of the task. However, the heart rate of the children with autism did not differ from controls during a physical stress test (bicycle exercise), suggesting that the physiological differences were related to failure in the autism group to physiologically anticipate psychosocial stress. Studies including a range of experimental conditions, such as the Jansen et al. (2003) study, are informative as they permit the differentiation of condition-dependent cardiac responses from chronic patterns of under- or over-arousal. In another study including a range of conditions, Groden et al. (2005) exposed

a small group of ten adolescents and adults with autism to a range of conditions that were meant to elicit stress responses of varying intensity. Unexpectedly, only 10% of the sample showed increased arousal in response to the investigator-identified "unpleasant event" (the introduction of an unfamiliar staff person), whereas the "relaxing activity" (sitting alone with unstructured time) elicited significant increases in heart rate for 50% of the participants (Groden et al., 2005). These studies illustrate that cardiac arousal is not a static trait; rather, it fluctuates in response to the environment, sometimes in unexpected ways. Investigations into heart rate during select, isolated conditions can only provide a fragmented understanding of cardiac activity that is not sufficient for identifying patterns that may vary systematically across environmental conditions.

Although evidence is inconsistent, the majority of findings point towards cardiac arousal that is either typical or elevated in autism; few studies have provided evidence of chronic under-arousal, although the Jansen et al. (2003) study discussed above suggests that heart rate reactivity in autism may be reduced in response to select stimuli. Given that majority of studies that have not detected elevated heart rate in autism were in comparison with individuals with intellectual disability (e.g., Corona et al., 1998; Graveling & Brooke, 1978; James & Barry, 1980; Sigman et al., 2003), it appears that developmental level may play a role in general arousal. Studies comparing individuals with autism and those with typical development more consistently point towards elevated general arousal in autism. Differences in experimental conditions may also account for some discrepancy across findings, as the various conditions used may have elicited responses of varying intensities. Much of the extant literature is also characterized by low sample sizes, which makes it difficult to determine whether the failure to detect group differences may have been related to limited statistical power.

Fragile X Syndrome: Elevated heart rate during resting or quiet play conditions has been detected among infant, school-aged, and adolescent males with fragile X syndrome in comparison to typically developing chronological-age matched peers (Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Heilman, Harden, Zageris, Berry-Kravitz, & Porges, 2011; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001; Roberts, Tonnsen, Robinson, & Shinkareva, 2012). Males with fragile X

syndrome also show elevated heart rate during challenging conditions, such as during word repetition tasks (Heilman et al., 2011), conversation with an examiner (Hall et al., 2009), and cognitive testing (Boccia & Roberts, 2000; Roberts et al., 2001). Only one report failed to detect increased general arousal among males with fragile X syndrome. In a longitudinal investigation of a small sample of young boys with fragile X syndrome (n = 12), Roberts, Hatton et al. (2012) did not find differences in heart rate during quiet toy play as compared to chronological-age and mental-age matched typically developing children. However, the small sample size of this study may have limited the ability to detect small differences between groups, and group means follow a trend of increased heart rate among children with fragile X syndrome. Overall, then, elevated cardiac arousal appears to be a well-replicated, defining feature of the physiological profile of males with fragile X syndrome.

Much less is known about arousal among females with fragile X syndrome, as only two reports exist examining heart activity among females with the disorder. In the first of these reports, Keysor and Mazzocco (2002) examined physiological arousal in 13 females with fragile X syndrome (aged 13-22 years), 11 females with Turner syndrome, and 14 typically developing females of similar chronological age. The heart rate of the females with fragile X syndrome did not differ from either groups during baseline or a series of cognitive tasks, although the females with fragile X syndrome did show higher baseline arousal as indexed by skin conductance measures. A second report by Hall and colleagues (2009) substantiates these findings, in which 24 females with fragile X syndrome (aged 5-19 years) did not differ in heart rate from sex-matched typically developing siblings during rest and during conversation with an examiner. Thus, it appears that unlike their male counterparts, females with fragile X syndrome may not present with elevated cardiac arousal, although additional research is needed given the small number of studies conducted to date.

Vagal Tone

Static measures of vagal tone measure parasympathetic control of the heart that maps the integrity of the autonomic nervous system and broader psychophysiological health (Porges, 1992; Porges, 1995; Porges & Furman, 2011). Resting vagal tone is thought to mark efficient maintenance

of homeostasis, greater awareness, and increased capacity for self-regulation and engagement with the environment (Porges et al., 1996).

Autism: Several studies have detected diminished vagal tone among children and adults with autism. Bal and colleagues (2010) examined resting vagal tone among school-aged children with autism ($n = 17$) and a group of typically developing children ($n = 36$) who were similar on chronological age and IQ; results revealed lower vagal tone among the children with autism. Mathewson et al. (2011) also detected lower vagal tone in 15 high-functioning adults with autism as compared to age and IQ-matched typically developing individuals, both during rest and during performance on an emotional Stroop task (participants named the color of faces while ignoring their emotional expression). Reduced vagal tone in autism samples has also been detected in other conditions, such as watching video clips (Van Hecke et al., 2009) and during a word repetition/video watching tasks (Denver, 2004) as compared to typical peers. Notably, vagal tone appears to be the most diminished in those children who show other symptoms of autonomic dysfunction (Ming et al., 2005). Ming et al. (2005) examined vagal tone among 15 children with autism and 17 healthy controls, using a device that indexes cardiac parasympathetic tone through real-time measurement of the brainstem activity. The children with autism had significantly lower resting vagal tone than controls. When the autism group was divided into subgroups according to the presence of other symptoms of autonomic dysfunction (e.g., sleep disturbance, gastrointestinal problems), the symptomatic subsample had the most significant reductions in vagal tone, which was significantly lower than both controls and the asymptomatic autism group (Ming et al., 2005). These results suggest that vagal tone is related to more pervasive symptoms of autonomic dysfunction in autism, which provides additional support for reduced vagal control as a physiological marker of autonomic nervous system dysfunction in autism.

On the other hand, several studies have not found vagal tone to differ between autism and comparison groups. Watson et al. (2012) examined vagal activity collected during passive viewing of

live or recorded stimuli among 20 young children with autism spectrum disorder (29-42 months) and chronological age-matched controls. The groups did not differ on vagal tone, although power analyses indicated low statistical power that may have prevented the detection of differences; group means indicated lower vagal estimates in the autism group (Watson et al., 2012). Althaus and colleagues (1999) also did not detect differences in baseline vagal tone between 36 school-aged boys and girls with autism (diagnosed specifically with Pervasive Developmental Disorder-Not Otherwise Specified) and typically developing children that did not differ in age or IQ. The children with autism were divided into subgroups of 18 children who showed symptoms of hyperactivity and 18 children who did not. Neither subgroup of children with autism differed from the controls in vagal activity during resting (analyses comparing the full autism sample to controls were not reported). Levine et al. (2012) also did not find differences in resting vagal tone between 19 children with high-functioning autism in comparison to 11 non-autistic children who were similar in age and IQ. However, five children in the non-autistic group were siblings of children with autism, which may have led to increased similarity between the two groups, given that siblings of children with autism are at increased risk for showing phenotypic characteristics of autism themselves (Bailey, Palferman, Heavey, & Le Couteur, 1998; Szatmari et al., 2000). Finally, a report by Toichi and Kamio (2003) found vagal activity in autism to be on par with mental age-based expectations, as differences were not detected between a group of 20 adolescents and young adults autism as compared to age and IQ-matched controls with typical development or non-specific intellectual disability.

Some early reports using descriptive statistics to index heart rate variability found vagal tone to be elevated among children with autism. MacColloch and Williams (1971) examined vagal tone in 19 children with autism who were residential patients in a mental hospital. Compared to ten children with typical development and nine "non-autistic subnormals" of a similar age, the children with autism had increased heart rate variability during unstructured alone time. Graveling and Brooke (1978) also detected elevated heart rate variability during a series of arousal-provoking manipulations

to classroom activities among a small sample of low-functioning children with autism ($n = 5$) as compared to an intellectual disability comparison group. Similar findings were reported by Hutt and colleagues (1975), in a study of nine hospitalized children with autism during free play and puzzle tasks; the children with autism showed higher variability in heart rate in comparison to typically developing children. Finally, Zahn et al. (1987) detected higher vagal tone among adult males with autism ($n = 14$) than typical controls during rest and passive listening to pure-tone sounds. It is difficult to compare the results of these early studies to more recent work, given that autism diagnostic criteria and technology for estimating vagal tone has changed considerably over the last several decades. Additionally, some of these studies examined responses to arousal-provoking experimental conditions without reference to a baseline, which makes it difficult to determine whether the elevated vagal activity might more accurately represent a failure to initiate task-related vagal suppression (as opposed to tonic vagal activity).

Overall, while there is some inconsistency across studies, evidence suggests that parasympathetic vagal control is reduced in at least a subset of individuals with idiopathic autism. The discrepancy across studies might reflect heterogeneity in the autonomic profiles of individuals with autism, a possibility that is supported by the findings of Ming et al. (2005), who detected the most diminished vagal tone among the subset of individuals who showed other signs of autonomic dysfunction. Additional factors that should be considered in future work include functioning level of the participants, such as language and cognitive level. These factors have not been systematically examined in relation to vagal estimates, although emerging work suggests that individuals with autism who have better language ability show increased vagal tone (e.g., Patriquin, Scarpa, Friedman, & Porges, 2011; Watson, Baranek, Roberts, David, & Perryman, 2010, reviewed in detail below). Therefore, differences in the verbal abilities of the samples might account for some conflicting results. Psychotropic medication use in autism should also be considered as a factor that might introduce variability, given its well-known effect on cardiovascular activity (e.g., O'Brien & Oyebode, 2003; Rechlin, 1995; Silke, Campbell, & King, 2002). In fact, a recent report by

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Mathewson et al. (2011) found that antipsychotic medication use, but not autism diagnostic status, was a significant predictor of heart rate and vagal tone in a sample of adults with autism. Comparison to typical controls showed that vagal tone was reduced only among those individuals with autism who were taking antipsychotic medications, whereas medication-free individuals did not differ from controls. Although medication use is a clear confound for both studies of autism and fragile X syndrome, it has not been systematically accounted for across studies. While some investigations have controlled for medication use with statistical techniques (e.g., Woodard et al., 2012), most investigations have controlled for medication use by excluding those individuals who take antipsychotic medications. Given that ~55% of children with autism and ~75% of individuals with fragile X syndrome use psychotropic medications (Mandell et al., 2008; Morgan, Roy, & Chance, 2003; Valdovinos, Parsa, & Alexander, 2009), it is probable that the exclusion of these individuals impacts the generalizability of findings. Specifically, this practice might limit samples to only mildly-affected individuals (i.e., those who did not need pharmaceutical intervention), who may show more typical autonomic responses. Furthermore, all patterns of physiological arousal, whether they are organic or pharmacologically-induced, are likely to have functional effects on other systems and behaviors. Systematic investigation of the behavioral and biological effects of psychotropic medication use in autism and fragile X syndrome is needed to clarify the significance of this confound.

Fragile X Syndrome: Several investigations of males with fragile X syndrome have detected decreased vagal tone as compared to typically developing children during resting conditions (Boccia & Roberts, 2000; Hall et al., 2009; Heilman et al., 2011; Roberts et al., 2001; Roberts, Boccia, Hatton, Skinner, & Sideris, 2006), as well as during attention-demanding tasks such as toy play (Roberts et al., 2012), arm restraint (Roberts et al., 2012), conversation with an examiner (Hall et al., 2009), word repetition tasks (Heilman et al., 2011), and cognitive assessment (Boccia & Roberts, 2000; Roberts et al., 2001). These studies encompass a wide range of age groups, suggesting that

dampened parasympathetic activity is a feature that is seen consistently in males with fragile X syndrome throughout developmental periods.

Recent evidence suggests that autism symptoms in fragile X syndrome are linked to individual differences in parasympathetic tone. Roberts, Tonnsen et al. (2012) examined vagal activity among 31 males with fragile X syndrome (aged 8-40 months), who were divided into subgroups according to the presence of autism symptoms. Group comparisons showed that infants with co-occurring autism had significantly lower vagal tone than age-matched controls, whereas those children with fragile X without autism had moderately reduced vagal tone that did not differ significantly from either controls or the group of children with fragile X syndrome with autism. Furthermore, lower vagal tone predicted the emergence of later autism symptoms among the infants and toddlers with fragile X syndrome, providing compelling evidence for a relationship between autonomic dysregulation and autism symptomatology within the context of fragile X syndrome.

The only two studies to examine vagal tone in females with fragile X syndrome produced conflicting results. In the first, no differences were detected in the tonic vagal activity of females with fragile X syndrome (n = 13; aged 12-22 years) as compared to females with typical development and Turner syndrome during rest and a cognitive stressor task (Keysor et al., 2002). A later study including a larger sample of 24 females (aged 5-19 years) detected diminished vagal tone in comparison to typical developing female siblings during rest and conversation (Hall et al., 2009). Additional research specifically focusing on females with fragile X syndrome might help tease apart the extent to which physiological profiles in fragile X syndrome are related to the specific effects of *FMR1,* as opposed to general patterns of intellectual disability. Because females have a second X chromosome that continues to produce normal levels of FMRP, they are generally less affected than males with the disorder (Hagerman et al., 1992; Rousseau et al., 1994). They are also less likely to have comorbid autism (Clifford et al., 2007; Hall et al., 2008), which may be linked to the variation in physiological profiles. Additional focus on physiological arousal in females with fragile X syndrome might shed light on the relationship between physiological arousal, cognitive impairment, and autism

symptomatology. Thus, further research is needed to determine whether vagal activity is reduced in females with fragile X syndrome, although it appears that diminished parasympathetic vagal control is a robust feature among males with fragile X syndrome.

Vagal Reactivity

Whereas tonic vagal activity reflects an individual's steady-state parasympathetic functions and the efficient maintenance of homeostasis, vagal reactivity (or the change in vagal tone from baseline in response to a stressor) reflects the capacity to organize metabolic resources to meet external demands (Porges et al., 1996). Reductions in vagal tone from baseline, or vagal suppression, allow the body to respond to environmental change with increased attention and arousal (Lovallo, 2005; Porges, 1995, 2001; Porges et al., 1996).

Autism: Only a handful of studies have examined vagal responsivity in autism. In the first of these, Althaus and colleagues (1999) investigated change in vagal activity during a visual memory search task in 36 school-aged children with autism, who were divided into subgroups according to whether they showed signs of hyperactivity. Compared to typically developing children who were of similar age and IQ, the children with autism showed less vagal suppression (i.e., less reduction in vagal tone from baseline levels) in response to a visual memory task than typically developing children, with the most pronounced differences seen among the subgroup of children with autism who also showed symptoms of hyperactivity. Interestingly, the groups did not differ in levels of resting vagal tone, perhaps suggesting that atypical parasympathetic activity in the autism group was limited to the process of responding to increased environmental demand, as opposed to chronic reductions in parasympathetic control. Toichi and Kamio (2003) also detected a lack of vagal suppression among 20 adolescents and young adults with autism in response to an arithmetic stressor task, whereas age and IQ-matched controls decreased vagal activity in accordance with the increased task demands. The groups did not differ in baseline vagal tone. Notably, further examination of individual response patterns revealed significant individual variability that was not captured in the group-level analysis. While all of the control participants decreased vagal tone in response to the stressor, half of the

individuals with autism actually showed *increases* in vagal tone, suggesting that a subgroup of individuals with autism found the "challenging" arithmetic task to be less demanding than the resting baseline condition. This study illustrates how autonomic activity may vary according to individual participant characteristics as well as to experimental context. Because individuals with autism may exhibit idiosyncratic responses to select stimuli, careful consideration of the cognitive, attentional, and emotional significance of a given experimental condition is key to the interpretation of findings. Thus far, a wide range of experimental conditions have been employed in the study of autism with little replication, which makes it difficult to separate apart cohort effects from effects related to the context from which heart activity was measured. The use of standardized stressor protocols such as the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993) or the Laboratory Temperament Assessment Battery (Goldsmith & Rothbart, 1988), would greatly facilitate the interpretation of study results, given that these widely tested in typical development and have a well-documented effect on autonomic activity.

One other study examined the effect of different social stimuli on vagal reactivity in autism. Van Hecke et al. (2009) examined responses of 8-12 year old children with autism ($n = 19$) to a series of videos that included non-social stimuli (objects moving to classical music), familiar social stimuli (video of the caregiver reading a story), and non-familiar social stimuli (video of an unfamiliar person reading a story). While typically developing children of a similar age did not show any reactionary changes in vagal tone across these conditions, the children with autism showed significant reductions in vagal tone in response to the unfamiliar social stimuli. These results suggest that the children with autism may have perceived the unfamiliar person as threatening and reacted physiologically to this threat with excessive reductions in parasympathetic tone. This report is consistent with recent studies of typical development that demonstrate vagal withdrawal during social interaction is a maladaptive response associated with poorer social outcomes (e.g., Bazhenova et al., 2001; Heilman et al., 2008). Therefore, it appears that atypical patterns of vagal reactivity in autism may be seen in response to both cognitive and social stimuli. Studies of vagal reactivity in response to live social stimuli are

needed to replicate and extend the findings of Van Hecke and colleagues. Evidence that individuals

with autism are less able to modulate physiological arousal during social situations might provide a

clearer link between the behavioral deficits seen in social contexts and underlying physiological

mechanisms.

Fragile X Syndrome: Several reports indicate that vagal suppression is either reduced or

absent among males with fragile X syndrome in response to cognitively-challenging tasks (i.e., IQ

testing or other cognitive stressor tasks, such as arithmetic challenges), whereas typically developing

boys of similar chronological ages show clear vagal suppression in response to task demands (Boccia

& Roberts, 2000; Roberts et al., 2001; Roberts et al., 2006). In a recent study, Heilman and colleagues

(2011) examined vagal reactivity among 12 males with fragile X syndrome (aged 6-23 years) and 21

typically developing males of similar ages, in response to a word repetition task. While the typically

developing boys suppressed vagal tone in response to the increased task demands, the boys with

fragile X syndrome responded with atypical increases in parasympathetic vagal tone, indicating a

failure to release the vagal brake in response to challenge. In the one study that investigated vagal

reactivity among females with fragile X syndrome ($n = 13$) in response to tasks involving mental

arithmetic, divided attention, and risk-taking stressor tasks, vagal suppression of the females with

fragile X syndrome did not differ from typically developing controls or females with Turner

syndrome (Roberts, Mazzocco, Murphy, & Hoehn-Saric, 2008).

Whereas males with fragile X appear to exhibit atypical vagal reactivity in response to

cognitive challenge, *social* challenges do not appear to elicit atypical vagal response. Hall and

colleagues (2009) examined vagal reactivity among 26 males with fragile X syndrome at baseline and

in response to an unstructured conversation with an examiner with regular prompts to make eye

contact (considered a social stressor). Similar responses were observed among the boys with fragile X

syndrome and their typically developing brothers (who served as a control group), with no reactionary

changes in vagal tone across conditions, perhaps suggesting that the conversational task was not

sufficiently stress-inducing to prompt parasympathetic modulation in either group. This study also

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examined vagal reactivity in females with fragile X syndrome, and did not detect differences between the females and their typical sisters. However, the groups did differ in vagal reactivity that was measured by descriptive measures of heart rate variability (as opposed to RSA); whereas the typically developing females showed increases in heart rate variability in response to conversation, the females with fragile X syndrome maintained baseline levels of heart rate variability throughout the protocol. This might suggest that typically developing females found the conversational task to be more calming that those females with fragile X syndrome (as evidence by increased parasympathetic tone), although it is curious that this finding was not reflected in the measures of vagal activity as indexed by RSA, which is accepted to be a more sensitive index of parasympathetic control than heart rate variability. It is unclear how the use of unaffected siblings as a comparison group may have influenced the findings of this study. Although comparing the performance of siblings helps control for confounds associated with environmental variables (such as social economic status or child rearing practices), siblings of individuals with fragile X syndrome may vary systematically from individuals of the general population. Fragile X families often struggle with increased medical and psychiatric problems (Bourgeois et al., 2009), and therefore unaffected siblings might experience a unique family environment that leads to outcomes that differ from that of the general population. Studies of other physiological regulators, such as the stress hormone cortisol, have documented significant family effects (Hessl et al., 2001). Taken together, findings appear to support diminished or absent vagal suppression among males with fragile X syndrome under conditions of cognitive challenge. Though social challenge tasks do not appear to elicit such patterns, only one study has addressed this question. There is little evidence to support atypical vagal reactivity among females with fragile X syndrome, although the research in this area is still limited.

Behavioral and Genetic Correlates of Cardiac Arousal

Autism: A great deal of individual variation exists in the physiological responses of individuals with autism (e.g., Groden et al., 2005). This variability is consistent with the broader behavioral and biological heterogeneity observed in autism (Betancur, 2011; Geschwind & Levitt,

2007). In an attempt to understand the sources of such variability, a number of studies have examined the behavioral correlates of physiological arousal. Some early investigations reported associations between increased heart rate *during* repetitive motor mannerisms (Sroufe, Stuecher, & Stutzer, 1973), and decreased heart rate *following* repetitive motor mannerisms (Hutt et al., 1975), suggesting that stereotyped behaviors may be used by individuals with autism as an arousal regulatory mechanism. Other sensory-related behaviors, however, do not appear to be related to physiological arousal. A small study by Woodard et al. (2012) exposed eight young children with autism to a series of potentially aversive sensory sensation (pungent odors, loud sounds, etc.) and did not find any relationship between hyper- or hypo-sensitive behavioral reactions and general arousal indexed by heart rate.

More recently, studies have focused on the relationship between heart rate and social behavior in autism. Jansen and colleagues (2006) detected an association between heart rate and the severity of autism symptoms. More significant increases in heart rate in response to a public speaking task were correlated with greater severity of social and communication impairment on the Autism Diagnostic Interview-Revised (Lord, Rutter, & Le Couteur, 1994) in a small sample of ten adults with autism. While this finding does link arousal responses with impaired social functioning, given that the typically developing controls showed increases in heart rate during the public speaking task, it is unclear why a "more normal" heart rate response was associated with increased autism symptoms. In another study by Jansen and colleagues (2003) that focused on a children with autism (*n* = 10), heart rate during public speaking was not related to scores on the Autism Diagnostic Interview-Revised, or with caregiver-reported behavioral/emotional difficulties on the Child Behavior Checklist (Achenbach, 2001). Both of these studies were limited by small sample sizes, which might account for the discrepant findings. Additionally, vagal tone was not measured, which may have provided a clearer picture of parasympathetic versus sympathetic influences on the observed arousal patterns and their relation to behavioral symptoms.

Patriquin et al. (2011) examined behavioral correlations of both heart rate and vagal tone in a group of 23 children with autism. Faster baseline heart rate was related to deceased use of communicative gestures during a play-based observational assessment. Heart rate was not, however, significantly associated with broader measures of social behavior, such as the subscales of the Social Responsiveness Scale (SRS; Constantino, 2005), which measures social symptoms of autism. Exploration of correlations with vagal tone revealed additional relationships. Higher baseline vagal tone was associated with increased acts of sharing and more frequent use of communicative gestures during play-based assessment. An association was also detected between vagal tone and receptive language (measured with the Peabody Picture Vocabulary Test-III; Dunn & Dunn, 1997), with higher vagal tone related to better language ability. Neither heart rate nor vagal tone was significantly associated with parent-reported measures of social behavior on the SRS, although trends followed the same direction as the other relationships (i.e., decreased heart rate and increased vagal tone associated with better outcomes). Van Hecke et al. (2009) also found vagal tone to be related to social outcomes in a study of 19 children with autism. Vagal tone was positively associated with better parent-reported social skills on the Social Skills Rating System (Gresham & Elliott, 1990). Correlations with the SRS were also examined and no significant relationships were detected, although patterns were consistent with increases in social impairment as vagal tone decreases. Finally, Watson et al. (2010) found that vagal tone predicted later social outcomes for young children with autism. In a prospective study of 15 young boys with autism who had language age equivalent scores of less than 24 months upon study entry, higher vagal tone during social contexts accounted for significant variance in social-communication adaptive skills and expressive language one year later (Watson et al., 2010). In sum, emerging evidence supports vagal tone as a mediator of social behavior in autism. Specifically, vagal tone appears to be particularly linked with communication ability, as several studies have now shown vagal tone to be related to vocabulary development, the use of communicative gestures, and social-communication skills in autism (see Table 2.2 for a summary of correlates in autism).

Table 2.2. Correlates of Vagal Activity in Autism and Fragile X Syndrome

Cardiac Index	Correlates in Autism	Correlates in Fragile X syndrome
Vagal tone	Sharing and use of communicative gestures (Patriquin et al., 2011)	Autism symptom severity (Roberts et al., 2012)
	Receptive and expressive language (Patriquin et al., 2011; Watson et al., 2010)	FMRP, in females (Hall et al., 2009)
	Social-communication (Watson et al., 2010)	
	General social skills (Van Hecke et al., 2009)	
	Performance on emotional identification task (Bal et al., 2010)	

Two studies of autism have documented an association between vagal activity and performance on emotion recognition tasks. Bal et al. (2010) found that faster identification of facial emotions was associated with increased baseline vagal tone in a sample of 33 children with autism. Mathewson et al. (2011) found somewhat conflicting results in a study of performance on an emotional Stroop task, in which participants named the colors of faces while inhibiting attention to their neutral or emotional expression. Adults with high-functioning autism ($n = 15$) had slower response times that adult controls, suggesting that the autism group took longer to process the content of the faces. The autism group was divided into subgroups according to psychotropic medication use, and correlations between cardiac activity and task performance were examined. A positive association was detected between baseline vagal tone and biased attention to happy faces in the medicated autism group, which might suggest that increased parasympathetic vagal control is related to greater attention to positive facial expressions in autism. No other associations were detected with baseline vagal activity, but additional associations emerged between task performance and vagal suppression in response to the Stroop task. In the medication-free autism group, greater vagal suppression was associated with slower performance on the Stroop task; the opposite pattern was seen in the typical control group, with increased suppression relating to faster performance. The authors suggested that

this finding might have resulted from excessive vagal suppression in some individuals with autism, which could have led to arousal levels that were elevated beyond a range that would facilitate task performance. There are several alternative explanations that might account for these findings. First, low participant numbers in the autism subgroups may have led to spurious findings; groups consisted of seven or eight individuals with autism after the participants were divided by medication status. Alternatively, it may be that while both individuals with autism and controls exhibited vagal suppression to the task, the reductions in vagal tone might represent different underlying processes across groups. The Stroop task involved both a cognitive and social components (i.e., rapid naming of the color of the face while simultaneously inhibiting the emotional content associated with the stimuli), making it difficult to decipher whether the vagal responses reflected emotionally- or cognitively- mediated processes. It is possible that the vagal suppression in the autism group reflected increased vigilance to social stimuli, as was suggested by the findings of Van Hecke and colleagues (2009), discussed above. In the other hand, vagal suppression in the control group may have reflected cognitively-mediated adaption to task demands, which resulted in faster performance on the color recognition task. Replication in a larger sample and with further exploration of the social versus cognitive demands of the task might clarify the meaning of these results.

Finally, some research has explored direct associations between physiological arousal and clinical measures of anxiety, with the hypothesis that anxiety might be a mediating factor in the relationship between physiological dysfunction and social outcomes in autism. That is, the inability to modulate arousal may cause an individual to remain anxious and "on edge" during social situations, which prevents optimal social engagement. Mathewson et al. (2011) found that self-reported trait anxiety was not correlated with baseline levels of heart rate or vagal tone in 15 high-functioning adults with autism. This is consistent with findings from Jansen et al. (2006), which found the heart rate responses to ten high-functioning adults with autism to be unrelated to subjective appraisal of stress during a public speaking task. Despite rating the task to be equally stress-provoking as did controls, the adults with autism showed significantly less elevation of heart rate in response to the

task. These studies suggest that the perception of stress is not tied to physiological increases in arousal in autism.

Fragile X Syndrome: The presence of autism symptoms is among the features examined most frequently in relationship to physiological activity in fragile X syndrome. In the first of these studies, Roberts et al. (2001) found that children with fragile X syndrome who showed autism symptoms had faster mean heart rate and lower vagal estimates than those children who did not show signs of autism, although this trend was not tested statistically. Consistent with this finding, Roberts, Hatton et al. (2012) detected a trend-level association between the severity of autism symptoms and impaired cardiac orienting responses in a small sample of infants with fragile X syndrome ($n = 12$), suggesting that infants with fragile X syndrome who show the most autism symptoms are the least physiologically reactive to the environment. In a study of a larger sample of 31 infants and toddlers with fragile X syndrome, Roberts, Tonnsen et al. (2012), found deficits in vagal tone were particularly pronounced among those infants and toddlers with fragile X syndrome who also showed signs of autism. Developmental interactions were detected, indicating that vagal tone and autism symptoms were inversely related among the children with fragile X syndrome who were 22 months of age and older, while decreased vagal tone was not associated with autistic behavior in younger infants and toddlers. In other words, reduced vagal activity did not emerge as a correlate of autism symptoms in fragile X syndrome until toddlerhood. On the other hand, faster heart rate was associated with fewer autism symptoms at 10 months but more autism symptoms at 37 months. This study underscores the importance of considering developmental patterns in the emergence of physiologically-mediated behaviors, and highlights the need for longitudinal research designs to delineate the relationship between autism symptoms and physiological functions over time.

Other studies have examined the relationship between physiological profiles and anxiety, in order to explore the hypothesis that social difficulties in fragile X stem from broader patterns of anxiety (particularly, social anxiety) that are tied to physiological dysfunction (Cohen, 1995). Hall and colleagues (2009) examined the specific associations between arousal and eye gaze in 50 boys

and girls with fragile X syndrome, as gaze aversion is hypothesized to stem from anxiety associated with social contact (Cohen et al., 1989; Cornish, Turk, & Levitas, 2007). Hall et al. (2009) found no relationship between heart rate or vagal tone and gaze avoidance during social interaction with an examiner, indicating that gaze aversion was not rooted in the inability to modulate arousal. Keysor and Mazzocco (2002) also examined broader relationships between anxiety and physiological responsivity in 13 adolescent females with fragile X syndrome who were relatively high-functioning (IQ's ranged from 59 to 125). The females were asked to rate their perception of anxiety prior to and following a cognitive stressor task; results showed that self-reported anxiety level was not correlated with heart rate. This finding is consistent with studies of autism, which did not find a relationship between anxiety and cardiac measures of arousal (e.g., Jansen et al., 2006; Mathewson et al., 2011, discussed above), as well as with evidence from studies of individuals with anxiety disorders (Kelly, Brown, & Shaffer, 1970; McLeod, Hoehn-Saric, Zimmerli, de Souza, & Oliver, 1990; Tyrer & Alexander, 1980). Finally, an investigation by Baranek and colleagues (2008) examined physiological features in relation to specific hyper- and hypo-reactive sensory processing behaviors in fragile X syndrome, which may be broadly related to anxiety in that extreme aversion to a particular sensory stimuli might invoke anxious, hyper-reactive behaviors. Caregiver-reported and direct-assessment measures of hyper- and hypo-responsive sensory processing were collected for 13 young boys with fragile X syndrome. Findings indicated that neither heart rate nor vagal tone was a significant predictor of the boys' sensory profiles. Together, these findings do not support an association between specific anxiety-linked behaviors in fragile X syndrome and physiological measure of heart rate of vagal tone. These studies did not examine vagal reactivity, and perhaps associations would have emerged with this physiological index as it may better capture task-dependent physiological responses. Alternatively, it may be that cardiac physiological arousal (and broader autonomic system integrity) in fragile X syndrome is more closely tied to broader, pervasive social impairment (such as the presence of autism) than to individual, context-dependent behaviors.

Finally, there has been some interest in the relationship between cardiac vagal control and Fragile X Mental Retardation Protein (FMRP), which is deficient in fragile X syndrome and believed to underlie the neurocognitive phenotype of the disorder (Hagerman & Hagerman, 2002; Loesch et al., 2004; Tassone et al., 1999). FMRP is needed for the modification and elimination of synaptic structures; in its absence neural plasticity is affected, disrupting normal brain development and functioning (Reiss & Dant, 2003; Schneider et al., 2009). FMRP is also acts as a regulator for the translation of other proteins, and its deficiency has broad consequences on the normal expression of other genes (Hagerman et al., 2010). It is through this role as a regulator of "background genes" that FMRP is thought to be involved in autism, as many of the proteins that are regulated by FMRP are also implicated in autism (see Belmonte & Bourgeron, 2006; Hagerman et al., 2010). Thus, FMRP has a broad impact on neural pathways that influence cognitive and emotional development, and FMRP deficiency is associated with social-behavioral deficits in fragile X syndrome, such as social withdrawal and anxiety/depression, social adaptive impairment, and language delay (Bailey, Hatton, Tassone, Skinner, & Taylor, 2001; Hessl et al., 2001). Understanding the impact of FMRP deficiency (and more generally, the impact of the *FMR1* mutation) on physiological arousal could help map *FMR1*-related molecular effects to broader autonomic nervous system functioning, thereby establishing a link between genetic etiology, mediating biological processes, and eventual behavioral endpoints. While it is unclear what neural mechanisms regulate cardiac vagal tone, involvement of several cortical regions has been suggested, including the amygdala, prefrontal cortex, and hypothalamus (see Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). Some of these regions, such as the amygdala, have also been implicated in fragile X syndrome (Gothelf et al., 2008; Hazlett et al., 2009), which might suggest a link between FMRP's role in brain development and physiological dysregulation. This possible link was not supported by two investigations that explored associations between FMRP and physiology in boys with fragile X syndrome, however, in that neither detected a relationship with either heart rate of vagal tone. This might suggest that physiological dysregulation

in fragile X syndrome is linked with factors other than FMRP (Hall et al., 2009; Roberts et al., 2001), although additional research is warranted given the restricted range of FMRP that is seen in the boys in both of these samples, which may have obscured associations. When examining females with fragile X syndrome, who show a wider range of FMRP levels than males, Hall et al. (2009) did detect a relationship between FMRP and vagal tone as indexed by descriptive measures of heart rate variability (with higher levels of FMRP predicting patterns of heart rate variability that were the most similar to that of controls). However, the significance of this relationship is unclear, as FMRP was not associated with vagal tone indexed by RSA, which is known to better control for respiratory confounds than heart rate variability. Future research including larger participant samples might clarify these findings. Exploration with other molecular variants associated with the *FMR1* mutation (such as activation ratio, mRNA, and CGG repeat length) might also clarify the role of the fragile X gene in autonomic dysfunction.

Summary of Findings and Key Considerations for Future Research

Findings reviewed here suggest physiological dysfunction that may be common to both autism and fragile X syndrome. This is evidenced by overall patterns of hyperarousal (i.e., faster heart rate) in both autism and fragile X syndrome as compared to typically developing controls. Reduced vagal tone, indicative of dampened parasympathetic activation, is well-documented in fragile X syndrome and is also seen an at least a subset of individuals with autism. Finally, atypical patterns of vagal reactivity in response to cognitive load are seen in both disorders, suggesting reduced capacity of the parasympathetic system to mobilize adaptive resources. Consistent with the Polyvagal Theory, as well as a large body of research supporting a link between vagal modulation and social behaviors in typical development, this growing evidence base indicates that a number of social behaviors in autism and fragile X syndrome are linked with vagal control, such as social-communication, receptive and expressive language, general social ability, and autism symptom severity. This evidence provides an exciting new perspective for understanding the process by which underlying biological processes give rise to complex social behaviors in autism and fragile X syndrome.

Importantly, evidence for overlapping autonomic profiles in autism and fragile X syndrome is key to understanding how biological pathways may be common across etiological subgroups of autism, and may be associated with behavioral and genetic variation associated with the *FMR1* gene. The evidence reviewed here is broadly suggestive of shared physiological mechanisms in autism and fragile X syndrome, although cross-population studies directly comparing carefully matched groups of individuals with autism and fragile X syndrome are needed to determine whether physiological pathways are common to these disorders, and whether they are linked to similar behavioral endpoints. Even if only evident in a subgroup of individuals with autism, such findings would provide a clear roadmap for investigations of the causes of autism. Efforts to identify reliable and valid biological markers for autism are hindered by the significant etiological heterogeneity seen in the disorder (Bill & Geschwind, 2009). As a disorder that can be traced back to defect in a single gene, *FMR1*, fragile X syndrome can help reduce "genetic noise" in the study of autism. Given the significant behavioral overlap that is seen in autism and fragile X syndrome, the study of autism in relation to fragile X may assist in the identification of biological pathways that lead to common phenotypic endpoints. Specifically, the delineation of physiological profiles common to autism and fragile X syndrome can lend insight into the role of autonomic dysfunction in producing the core social-behavioral deficits of these disorders, and how these features may be linked back to the neurobiological pathways associated with *FMR1*. Eventually, such research might help uncover key systems that could be amenable to pharmaceutical or behavioral interventions.

Implications for Theoretical Frameworks of Autism and Fragile X Syndrome

A remaining question is how empirical evidence of physiological regulation in autism and fragile X syndrome fits into existing theoretical accounts. Arousal modulation has been long hypothesized to underlie the behavioral phenotype of both autism and fragile X syndrome (e.g., Cohen, 1995; Hutt et al., 1964; Kootz & Cohen, 1981; Rimland, 1964). Broadly, it has been hypothesized that sub-optimal engagement may be rooted in atypical processing of environmental stimuli caused by hypo- and/or hyper-arousal. For example, lack of orienting to name-call in autism

might reflect under-arousal and resultant inability to process incoming auditory information. While only two studies have directly examined physiological profiles in relation to behavioral hyper- or hypo-reactivity, neither investigation found physiological arousal level to be related to the sensory processing features of children with autism or fragile X syndrome (Baranek et al., 2008; Woodard et al., 2012). Thus, there does not appear to be a direct relationship between arousal level and sensory processing, although further research is needed to replicate these findings in larger samples, across developmental periods, and in relation to measures of vagal reactivity.

Another prominent theory, particularly with respect to fragile X syndrome, posits that social deficits are rooted in anxiety, which is tied to underlying hyperarousal (Cohen, 1995). Studies of both autism and fragile X syndrome have explored this hypothesis by examining the relationship between physiological arousal and measures of anxiety, with overall findings failing to support such a relationship (Jansen et al., 2006; Keysor et al., 2002; Mathewson et al., 2011). While further investigation is needed, findings from these studies are consistent with studies of individuals with anxiety that have found that the perception of anxiety is not necessarily tied to physiological arousal (Kelly et al., 1970; McLeod et al., 1990; Tyrer & Alexander, 1980).

An alternative explanation might be that physiological arousal is linked specifically to social anxiety, as opposed to patterns of general or task-related anxiety. Few studies have directly explored this hypothesis, although research examining physiological arousal during social interaction might provide some clues. Studies of autism that have included social interaction tasks have not found heart rate to be elevated in autism as compared to other developmental disabilities (Corona et al., 1998; Sigman et al., 2003), although it is unknown whether results may have differed in comparison to a typically developing group. Similarly, Groden et al. (2005) found that the "social stressor" of interacting with an unfamiliar staff person only elicited increased arousal in one of ten participants with autism. Thus, social interaction does not appear to elicit significant physiological stress in individuals with autism, which might suggest that atypical social behaviors do not stem from hyperarousal in social contexts. Yet, these reports relied on measures of heart rate and did not

incorporate vagal tone or reactivity, which better index parasympathetic activity. One study of autism did detect significant reductions in vagal tone in autism in response to a video of an unfamiliar person, which might reflect hypervigilance to unfamiliar social stimuli (Van Hecke et al., 2009). No studies of autism have measured vagal activity in response to live social stimuli, which might replicate and extend the finding of Van Hecke and colleagues. A single study of fragile X syndrome has specifically examined physiological activity during a social context, with the finding that individuals with fragile X syndrome showed similar heart rate and vagal activity responses to social interaction as did their typically developing siblings (Hall et al., 2009). Furthermore, neither heart rate nor vagal tone was associated with gaze avoidance behavior during the interaction, suggesting that social avoidant behaviors did not stem from the inability to modulate physiological arousal.

From these few studies, it appears that social interaction is not a specific elicitor of physiological stress responses in autism or fragile X syndrome, although it is unknown whether the social tasks utilized were sufficiently stressful to invoke stress, as anxiety was not directly measured. Or perhaps the structure of the research protocol led to reduced arousal, whereas arousal might have been seen in more ecologically valid social settings, such during social interaction at school. Additional research is needed to tease apart the specific relationship between physiological arousal, anxiety, and social behavior. Given that anxiety is exceedingly common in both autism and fragile X syndrome (Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998), better understanding its interaction with physiological processes has implications for intervention.

Although it is reasonable to expect faster heart rate and vagal withdrawal when the body is challenged (including contexts that are perceived as threatening, thereby invoking anxiety), it may be that the anxiety hypothesis is too narrow a framework to account for how disruption in the physiological system creates vulnerability to social impairment. The anxiety/hyperarousal hypothesis emphasizes immediate, context-dependent responses of the physiological system and largely ignores the role of physiological regulation (particularly, the role of the vagus) as a broader indicator of

overall psychobiological health. It may be that vagal function better serves as an index of general adaptive capacity, as opposed to a measure of reactive, context-dependent behavior. This may explain why vagal activity has been fairly consistently linked to broad social-developmental outcomes in autism and fragile X syndrome (such as overall communication ability or the severity of autism symptoms), while associations with task-dependent behavioral responses (such as eye contact or reactivity to sensory stimulation) have been less consistently detected (e.g., Hall et al., 2009; Woodard et al., 2012)

Accounting for Heterogeneity

Although evidence suggests that atypical patterns of physiological arousal are seen in some individuals with autism, findings are inconsistent, particularly with regards to tonic heart rate and vagal tone estimates. In some respects, the inconsistency across studies is not surprising given the significant clinical and etiologic heterogeneity in autism (Betancur, 2011; Geschwind & Levitt, 2007). Considering the wide range of clinical presentation observed in the disorder, variable findings in autonomic response patterns in autism are not unexpected. The examination of continuously distributed autism symptoms (as opposed to dichotomous group comparisons) may also assist in accounting for the spectrum of behaviors that are seen in autism. This approach yielded promising results in a study by Roberts and colleagues (2012), who detected developmental shifts in the relationship between heart rate, vagal tone, and continuously distributed autistic behaviors. Other studies have addressed heterogeneity through the investigation of clinically-defined subgroups of autism, who might be more likely to share underlying physiological features. For example, individuals with autism who show clinical signs of attention deficits (Althaus et al., 1999), and symptoms of general autonomic dysfunction (Ming et al., 2005) show greater impairment in parasympathetic vagal control than those who do not share these symptoms. The delineation of physiological profiles among clinical subgroups of autism can eventually inform which individuals might be most responsive to interventions targeted at strengthening the physiological system.

Another approach for parsing apart heterogeneity is to define autism subgroups at the physiological level, with the assumption that individuals who share physiological profiles are more likely to represent an etiologically homogenous group than those who share behavioral profiles. Such an approach may be useful for placing individuals into more homogeneous subgroups, which may increase power in genetic linkage studies of autism (Abrahams & Geschwind, 2008; Almasy & Blangero, 2001; Happe, Ronald, & Plomin, 2006). Additional evidence of physiological dysregulation in autism and within extended family members might support physiological dysfunction as a vulnerability marker of underlying genetic liability to autism, or an "endophenotype" (Gottesman & Gould, 2003). Endophenotypes are thought to be more closely related to underlying pathways than are full diagnostic categories and thus may be useful in elucidating gene-behavior relationships within the context of complex neuropsychological disorders such as autism (Almasy & Blangero, 2001; Gottesman & Gould, 2003; Leboyer et al., 1998). Measures of physiological regulation are ideal candidate endophenotypes for autism, as they are non-invasive, reliable indices that tap biological functions.

Developmental Influences

Thus far, most studies of physiological activity in autism and fragile X syndrome have been limited to investigation of a single point in time, with little ability to infer developmental patterns or prospective features that might predict later impairment. Longitudinal studies are needed to fully understand the impact of autonomic dysfunction across development, a point that is underscored by recent evidence supporting developmental interactions in the relationship between vagal tone and the emergence of autism symptoms (with vagal activity not emerging as a correlate of autism until toddlerhood) (Roberts et al., 2012, reviewed in detail above). Furthermore, a recent study by Heilman et al. (2011) found atypical patterns of age-related decreases in vagal tone in a cross-sectional sample of children and young adults with fragile X syndrome, supporting the possibility for developmental patterns in physiological development that are specific to fragile X syndrome, as vagal tone is known to increase with age in typical development (Alkon et al., 2003; Bar-Haim et al., 2000; Longin et al.,

2006; Porges et al., 1994; Sahni et al., 2000). Few studies have examined longitudinal physiological

profiles, and it is s unclear how physiological regulation evolves over time in individuals with

developmental disabilities and how the precise timing of physiological events might influence

behavior. Understanding developmental influences on cardiac arousal and vagal control might help

identify those individuals who are at greatest risk for autonomic dysfunction, and eventually for

identifying developmental periods that are prime for intervention.

       Although studies of typical development show that parasympathetic tone increases with age

(Alkon et al., 2003; Bar-Haim et al., 2000; Longin et al., 2006; Porges et al., 1994; Sahni et al., 2000),

it is unclear whether this relationship is moderated by cognitive development (or other cognitively-

linked processes, such as language ability). At this point, the interaction between cognitive ability and

autonomic function is largely undefined, although it wouldn't be unexpected for these variables to be

linked, considering the association between vagal activity and select cognitive processes such as

attention (e.g., Hansen, Johnsen, & Thayer, 2003; Suess et al., 1994). Related to this point is the

selection of comparison groups. Matching on chronological age often leads to mismatch in other

relevant developmental areas, such intellectual or language level. On the other hand, the use of a

younger, mental age-matched typically developing comparison group may lead to confounds

associated with age-related physiological maturation. For instance, Roberts and colleagues (2012)

found reduced vagal activity in boys with fragile X syndrome at 12 months as compared to age-

matched typically developing children, whereas vagal activity of the boys at 18 months did not differ

from younger, mental-age matched typically developing children. These divergent findings

underscore the importance of the careful group matching procedures, and also suggest an influence of

general developmental level on vagal activity, which has received relatively little empirical attention.

The inclusion of developmentally delayed comparison groups helps to avoid these confounds, by

allowing for matching on both developmental and chronological age. However, this approach has also

raises questions regarding the physiological "typical-ness" of developmentally delayed comparison

groups, as autonomic dysfunction has been suggested in a number of other developmental disabilities,

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including Down syndrome, Rett syndrome, Prader-Willi syndrome, and cerebral palsy (e.g., DiMario, Dunham, Burleson, Moskovitz, & Cassidy, 1994; Figueroa et al., 2005; Iellamo et al., 2005; Julu et al., 2001; Zamunér et al., 2011). The choice of comparison group undoubtedly has a significant impact on experimental results and should be carefully considered in interpreting differences across studies.

Beyond the Autonomic Nervous System: Integrating Evidence Across Multiple-Systems

The autonomic nervous system is only one of many coordinated subsystems that contribute to the overall maintenance of an adaptive physiological state. For example, the neuroendocrine system (the hypothalamic-pituitary-adrenal axis) and the immune system (inflammation) are also involved in adaptive responsivity to changing conditions. Although the exact process by which different regulatory mechanisms work together is not completely understood, it is likely that physiological regulation is achieved through the active interplay of the different regulatory subsystems. In certain situations, it may be most efficient for the body to respond with activation of one or several of these subsystems, as opposed to wide-spread activation of all regulatory mechanisms (McEwen, 1998). Therefore, narrow focus on the autonomic nervous system is likely to result in an incomplete understanding of the body's capacity for adaptive response. While some studies have adapted a broader approach through concurrent assessment of multiple regulatory subsystems, the results of these studies are not easily interpreted without a better understanding of the dynamic interaction between these subsystems. For example, Jansen et al. (2006) examined stress responses of individuals with autism using indices of cardiac activity as well as measures of the stress hormone cortisol, which is an index of hypothalamic-pituitary-adrenal axis functioning. Although decreased cardiac arousal in the individuals with autism was suggestive of atypical autonomic response, cortisol levels did not support dysfunction of the neuroendocrine system. Further delineation of the interactions among regulatory subsystems is needed to provide a comprehensive account of physiological regulation as a mediator of behavior in autism and fragile X syndrome.

Implications for Intervention

Given the literature supporting autonomic dysfunction as a potential mechanism underlying social dysfunction in autism and fragile X syndrome, a natural next step is to identify behavioral or pharmaceutical interventions that can correct autonomic profiles in these disorders. Thus far, pharmaceutical trials of oxytocin and lithium did not have a measureable impact on cardiac vagal control in fragile X samples, although much is still unknown regarding the requisite dosage and timing of pharmaceutical interventions (Hall, Lightbody, McCarthy, Parker, & Reiss, 2012; Heilman et al., 2011). One study did detect positive treatment effects on heart rate modulation following stimulant medication in a sample of boys with fragile X syndrome (Roberts et al., 2011). To date, no studies of autism have examined the impact of pharmaceutical interventions on arousal.

Although no behavioral intervention studies directed at physiological targets have been conducted in autism or fragile X syndrome, evidence from other populations suggests that behavioral interventions may be effective in treating vagal parasympathetic deficits. For example, at-risk infants who are treated with massage or skin-to-skin contact show elevated vagal tone and increased periods of alertness as compared to control infants (Feldman & Eidelman, 2003; Lee, 2005). This is consistent with studies of autism that indicate improved social and language skills following massage treatment, although links with vagal regulation are only theoretical, as no studies to date have directly assessed the effects of massage therapy on vagal activity in autism (Silva, Schalock, Ayres, Bunse, & Budden, 2009). It appears that traditional behavioral interventions may also result in improvements in autonomic functioning; enhanced vagal regulation has been detected in toddlers following play-based therapy (Bagner et al., 2009; Graziano, Bagner, Sheinkopf, Vohr, & Lester, 2012). Studies of adults also support the potential for improving autonomic regulation with behavioral interventions, which have documented enhanced parasympathetic control following acupuncture and controlled relaxation interventions (Chambers & Allen, 2002; Miu, Heilman, & Miclea, 2009). This of research is promising and deservers further attention.

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Conclusion

Although there is some inconsistency across studies of autism that merits further investigation, a trend emerges in the extant literature to support overlapping profiles of physiological dysregulation in autism and fragile X syndrome. This is evidenced by patterns of hyperarousal, dampened parasympathetic tone, and atypical parasympathetic response to environmental change. Consistent with the Porges' Polyvagal Theory and evidence from typical development, an expanding body of evidence supports physiological health as a mediator of social outcomes in autism and fragile X syndrome, including adaptive skills, receptive and expressive language, overall social ability, the emergence of autism symptoms over time. This evidence provides an exciting new perspective for understanding the process by which underlying biological systems give rise to complex social behaviors. The intersection of physiological pathways in autism and fragile X syndrome, a neurodevelopmental disability that can be traced to a single genetic defect, provides a starting-point for determining how physiological regulatory mechanisms may interact with environmental and genetic factors to give rise to the behavioral phenotype associated with autism. Such research has implications for understanding underlying autonomic system dysfunction as a mechanisms leading to social-behavioral impairment, which might eventually informing the pathophysiological basis of autism and fragile X syndrome and the development of targeted interventions.

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CHAPTER 3

PRAGMATIC LANGUAGE IN BOYS WITH AUTISM AND FRAGILE S SYNDROME: A
CROSS-POPULATION COMPARISON OF SEMI-NATURALISTIC AND STANDARDIZED
PRAGMATIC ASSESSMENTS

Summary

Impaired pragmatic language (i.e., language used in social contexts, such as conversation) is a hallmark feature of both autism and fragile X syndrome (FXS), the most common known monogenic condition associated with autism. However, few cross-population comparisons of autism and FXS have been conducted, and it is unclear whether pragmatic language profiles in these disorders overlap. This study used semi-naturalistic and standardized assessment methods to comprehensively characterize the pragmatic language skills of 34 boys with idiopathic autism, 38 boys with FXS and comorbid autism, and 10 boys with FXS without autism, as compared to 20 boys with Down syndrome and 20 boys with typical development who were of a similar language age. Results supported similar severity of pragmatic language deficits in both of the autism groups (idiopathic autism and fragile X-associated autism). The presence of autism had a significant impact on the pragmatic language abilities of boys with FXS as assessed in the semi-naturalistic conversational context. Some different patterns emerged across the two pragmatic assessment tools, with more robust group differences observed in pragmatics assessed from the conversational context. These findings have implications for pragmatic assessment and intervention, as well as for understanding the potential role of the fragile X gene, *Fragile X Mental Retardation-1*, in the pragmatic language phenotype of autism.

Introduction

Pragmatic language refers to the use of language to communicate meaning in social contexts (Bates, 1976; McTear & Conti-Ramsden, 1992; Prutting, 1982). Examples of these skills include the selection of conversational topics fitting the situation, and the ability to modify language in order to match the expectations and knowledge base of the communication partner. Without appropriate pragmatic language use, communicative intent is obscured and social interchange becomes ineffective. Individuals with pragmatic language deficits are unable to optimally participate in social contexts, which is thought to reduce learning opportunities and lead to downstream effects on development (Chapman, 2000; Dickinson & McCabe, 1991; Fogel, 1993; Hewitt, 1998; McTear & Conti-Ramsden, 1992; Yoder & Warren, 1993). Thus, pragmatic language ability is a critical skill that has a broad impact on social functioning and learning. Evidence indicates that social-communicative ability is a significant predictor of outcomes in autism (Szatmari et al., 2003), fragile X syndrome (FXS) (Roberts et al., 2002), and a number of other developmental disorders (Coplan & Weeks, 2009; Leonard, Milich, & Lorch, 2011; Szatmari et al., 2003).

Pragmatic language deficits are central to both autism and fragile X syndrome (FXS)-- a disorder that is associated with elevated risk for autism and is caused by a single genetic mutation on the X chromosome (Cohen et al., 2005). It is unclear, however, whether pragmatic language profiles are similar in autism and FXS, as few cross-population comparison studies have been conducted. Delineation of syndrome-specific pragmatic profiles in autism and FXS will clarify the extent to which core phenotypes of these disorders overlap, which has implications for targeting intervention strategies and will contribute to understanding of common phenotypic endpoints in autism and FXS that may stem from shared neurobiological pathways. Below, autism and FXS are briefly described as disorders of a neurogenic basis, followed by a review of pragmatic language impairment in these disorders and discussion of assessment considerations.

Autism

Autism is a serious, lifelong disability that affects approximately 1 in 88 children (CDC, 2012). The diagnosis of autism is determined behaviorally by the presence of social and communication impairments, as well as repetitive or restricted behaviors (American Psychiatric Association, 2000). Pragmatic language impairment is a central feature of autism, as all individuals with autism show deficits in the social use of language (Landa, 2000; Tager-Flusberg et al., 2005). Converging evidence from twin, family, and molecular-genetic studies of autism supports a large genetic component in the etiology of autism (Devlin & Scherer, 2012; Miles, 2011). However, while autism is highly heritable, it is also clinically and etiologically heterogeneous, which has slowed the identification of genetic markers for the disorder (Geschwind, 2011; Ronald & Hoekstra, 2011). The study of autism within the context of associated genetic conditions, such as FXS, has been proposed as a method for reducing etiological complexity in the search for autism genes (e.g., Abrahams & Geschwind, 2010; Belmonte & Bourgeron, 2006; Hagerman et al., 2011). Such an approach provides a simplified genetic context from which to identify core features that are shared across etiological subtypes of autism, and which may be linked to an identifiable genetic cause.

FXS

FXS occurs in as many as 1 in 2,500 individuals (Fernandez-Carvajal et al., 2009; Hagerman, 2008), and is the most common known genetic disorder associated with autism (Cohen et al., 2005). Unlike autism, the genetic basis of FXS is relatively well-understood; it is caused by an expanded number of Cytosine-Guanine-Guanine (CGG) nucleic acid repeats on the *Fragile X Mental Retardation-1* (*FMR1)* gene of the X chromosome (Hagerman & Hagerman, 2002). When the CGG expansion exceeds 200 copies, the *FMR1* gene methylates (shuts down) and stops producing Fragile X Mental Retardation Protein (FMRP), which is a protein that is needed for brain development and functioning (Bassell & Warren, 2008; Irwin, Galvez, Weiler, Beckel-Mitchener, & Greenough, 2002; Weiler & Greenough, 1999). Because FMRP normally acts as a translator for other proteins, its

absence has widespread consequences for the normal functions of other genetic pathways. Many protein systems that become dysregulated in the absence of FMRP have also been implicated in autism, and it is through this interaction with other genes that the *FMR1* mutation is thought to increase risk for autism (Hagerman, Au, & Hagerman, 2011; Hagerman et al., 2010).

Some evidence of autism-like features is seen in almost all individuals with FXS (Hagerman et al., 2010). Assessment using gold-standard autism diagnostic tools shows that 30-50% of individuals with FXS meet diagnostic criteria for autistic disorder (Harris et al., 2008; Rogers et al., 2001), and 60-75% meet criteria for an autism spectrum disorder (Clifford et al., 2007; Hall et al., 2008). While it is clear that autism and FXS share significant behavioral overlap, the nature of autism in FXS is controversial. It has been hypothesized that idiopathic and fragile X-associated autism stem from divergent underlying mechanisms, with fragile X-specific anxiety or intellectual disability underlying the autism phenotype of FXS (Cohen, 1995; Cohen et al., 1989; Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010). While this theory has not been supported by cross-population comparison studies failing to detect unique autism symptom profiles in idiopathic and fragile X-associated autism (Bailey et al., 1998; Dissanayake et al., 2009; Rogers et al., 2001), few studies have conducted a more fine-grained analysis of specific autism-associated features in autism and fragile X syndrome, which might reveal syndrome-specific behavioral profiles.

The present study adopted this approach through a focused investigation of pragmatic language ability in autism and FXS. Pragmatic impairment is seen universally in autism and is also a well-documented feature of the FXS phenotype (Hagerman, 2002; Hagerman, 2002; Keysor & Mazzocco, 2002; Landa, 2000; Sudhalter & Belser, 2001; Tager-Flusberg et al., 2005). Because subclinical pragmatic difficulties are seen in relatives of individuals with autism as part of the broad autism phenotype, pragmatic impairment is hypothesized to represent a genetically-mediated trait that marks vulnerability to autism (Landa et al., 1992; Losh et al., 2008; Piven et al., 1997). Premutation carriers of the *FMR1* gene also show subclinical pragmatic features that are similar in quality and severity with those seen in the broad autism phenotype, suggesting a role of *FMR1* in social-

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communication features associated with the autism and the broad autism phenotype (Losh et al., 2012). Thus, the study of pragmatic language features that may overlap or diverge in autism and FXS is promising method for identifying phenotypic commonalities in autism and FXS that may stem from biological disruptions associated with *FMR1*. Moreover, careful characterization of pragmatic language skills in autism and FXS, including exploration of the impact of autism severity on these skills, will inform the use of autism-tailored social-communication interventions for treating individuals with FXS.

Pragmatic Language in Autism and FXS

Impairments in pragmatic aspects of language are observed across with the entire autism spectrum, regardless of functioning level (Landa, 2000; Tager-Flusberg et al., 2005). During conversation, individuals with autism struggle with turn-taking (Capps, Kehres, & Sigman, 1998; Paul et al., 1987) and have difficulty maintaining a given conversational topic (Adams, Green, Gilchrist, & Cox, 2002; Tager-Flusberg & Anderson, 1991). The conversational contributions of individuals with autism are characterized by unusual word choice (Ghaziuddin & Leonore, 1996), perseveration (Ross, 2002), irrelevant details (Paul, Orlovski, Marcinko, & Volkmar, 2009), and unclear references (Fine, Bertolucci, Szatmari, & Ginsberg, 1994). Communicative repair is also affected, with difficulties in adequately responding to the clarification requests of others (Geller, 1998; Volden, 2004). The narrative abilities (i.e., storytelling skills) of individuals with autism also show atypical pragmatic features, such as inappropriate or irrelevant statements (Diehl, Bennetto, & Young, 2006; Loveland, McEvoy, & Tunali, 1990) and ambiguous references (Norbury & Bishop, 2003). When narrating, individuals with autism fail to provide causal explanations of the characters' actions and emotions (Capps, Losh, & Thurber, 2000; Diehl et al., 2006; Losh & Capps, 2003; Tager-Flusberg, 1995), have difficulty narrating causal relationships when describing their own experiences (Losh & Capps, 2003), fail to communicate complex emotions (Losh & Capps, 2006), and have difficulty adopting the perspectives of others in their narratives (García-Pérez, Hobson, & Lee, 2008).

Pragmatic language difficulties are a hallmark feature of the autism phenotype.

Like autism, FXS is characterized by atypical pragmatic language use, such as impaired use of communicative repair strategies (Abbeduto et al., 2008), word and topic perseveration (Belser & Sudhalter, 2001; Martin et al., 2012; Roberts et al., 2007; Sudhalter et al., 1990; Wolf-Schein et al., 1987), and difficulty with conversational topic maintenance (Roberts et al., 2007; Sudhalter & Belser, 2001; Sudhalter et al., 1990; Wolf-Schein et al., 1987). Impaired narrative processing and formulation have also been documented in FXS (Estigarribia et al., 2011; Simon, Pennington, Taylor, & Hagerman, 2001). Importantly, recent evidence shows that pragmatic language impairment in FXS is associated with *FMR1*-related molecular variation (specifically, CGG repeat length and percent methylation), which provides a direct link between *FMR1* and pragmatic impairment (Losh et al., 2012). Although it is clear that individuals with FXS show pragmatic deviance, the impact of autism status in FXS on such deficits is less well understood, as most investigations have not accounted for autism comorbidity. Some emerging evidence suggests that autism comorbidity has an added detrimental effect on social communication abilities in FXS; research indicates that boys with FXS and co-occurring autism exhibit more off-topic conversational turns during spontaneous conversation (Roberts et al., 2007), increased perseveration (Martin et al., 2012), and are reported by their caregivers to produce more stereotyped language (McDuffie et al., 2010) than their non-autism counterparts. They also perform more poorly on standardized measures of pragmatic language than boys with FXS without autism (Losh et al., 2012).

Cross-Population Comparison Studies Pragmatic Language of Autism and FXS

While many studies have detected pragmatic deficits in autism and FXS, only a handful of studies have directly compared social-communicative features across these populations, with mixed results. A few early investigations examined specific pragmatic features in autism and FXS as they occurred in spontaneous or elicited language samples. While it is difficult to draw definitive conclusions from this work given the inconsistent handling of autism comorbidity in FXS, this literature provides some indication of qualitative differences in the pragmatic profiles of autism and

FXS. For example, one research group analyzed the spontaneous conversation of males with idiopathic autism and FXS (autism status not reported) to find that males with FXS showed more repetitive speech (Belser & Sudhalter, 2001) and tangential language (Sudhalter & Belser, 2001) than males with autism. In another investigation, Sudhalter et al. (1990) compared spontaneous language of individuals with autism and individuals with FXS (who did not have autism), and found that the autism group exhibited increased echolalia, whereas the FXS group showed more frequent perseveration. These studies provide hints that pragmatic violations in autism and FXS may differ qualitatively, although additional research is needed to tease apart the impact of autism comorbidity on the pragmatic profile of individuals with FXS.

To our knowledge, only one study has compared pragmatic language in autism and FXS using standardized assessment tools. Losh et al. (2012) compared the pragmatic performance of boys with idiopathic autism, FXS with autism, and FXS without autism using the Pragmatic Judgment subtest of the Comprehensive Assessment of Spoken Language (CASL-PJ; Carrow-Woolfolk, 1999), which is a standardized, direct-assessment measure of pragmatic language use. The teacher version of the Children's Communication Checklist-2 (CCC-2; Bishop, 2006) was also administered as a secondary measure of communication skill. Findings showed that the boys with idiopathic autism and FXS with comorbid autism performed similarly on the CASL-PJ, and significantly worse than the boys with FXS without autism (who performed similarly to Down syndrome and typical comparison groups). Conversely, on the CCC-2, the autism, FXS with autism, and FXS without autism groups were all rated to have similar overall severity of pragmatic impairment. These findings highlight the need to integrate information across multiple sources in the assessment of pragmatic language, as different patterns emerged between direct-assessment and teacher-reported standardized measures.

In reviewing the extant literature on pragmatic language in autism and FXS, two key questions remain. First, the role of autism in pragmatic language deficits in FXS remains unclear, as this has been inconsistently accounted for in prior studies. Secondly, no studies have incorporated both standardized and naturalistic assessments of pragmatic language, and it unclear how the use of

different assessment methods may impact findings. Prior studies using semi-naturalistic measures suggest that qualitative differences might distinguish the pragmatic profiles of autism and FXS, whereas standardized measures doe not appear to distinguish these groups. Research including both standardized and semi-naturalistic assessment methods can provide a more comprehensive picture of pragmatic ability. Below, the specific advantages and disadvantages of standardized and naturalistic assessment are discussed in relation to the study of autism-associated developmental disorders.

Assessment of Pragmatic Language

The context-dependent nature of pragmatic language poses a number of unique methodological challenges, and best practices for pragmatic assessment have been discussed at great length in the literature (e.g., Adams, 2002; Prutting & Kittchner, 1987). In the assessment of other language domains, such as semantics or phonology, norm-referenced tools have traditionally played an integral role in determining the presence of impairment. In line with this tradition, several standardized direct-assessment pragmatic tools have been developed, which are widely used in both clinical and research settings. There are several advantages of standardized assessment tools: they tend to be quick and relatively simple to administer, the structured format facilitates a controlled testing environment, and most standardized tools are norm-referenced (allowing for comparison of performance relative to peers). On the other hand, the de-contextualized form of standardized tools may limit generalizability to performance in real-life settings. Standardized pragmatic language assessments attempt to measure spontaneous social-communicative ability from elicited, highly structured contexts that have clearly-defined social expectations, and do not require real-time responses. For example, norm-referenced assessment techniques glean information from contrived contexts, such as answering questions about pictorial stimuli or responding to hypothetical scenarios. These contexts differ greatly from real-life communicative situations and may overestimate pragmatic skill. This is particularly problematic for the study of individuals with autism, who are known to perform better in contexts that are structured (Clark & Rutter, 1981), and in contexts that do not require complex information processing or interpretation of contextual information (Loukusa et al.,

2007; Williams, Goldstein, & Minshew, 2006). Therefore, standardized assessment might overlook pragmatic deficits that are apparent in more ecologically valid communicative contexts.

For these reasons, researchers have long supported naturalistic measures as the gold-standard for pragmatic language assessment (Adams, 2002; Hyter, 2007; McTear & Conti-Ramsden, 1992; Prutting & Kittchner, 1987; Roth & Spekman, 1984). However, naturalistic assessment tools are not without weaknesses. While a wide number of conversational rating systems have been proposed (e.g., Adams & Bishop, 1989; Adams et al., 2002; de Villiers, Fine, Ginsberg, Vaccarella, & Szatmari, 2007; Prutting & Kirchner, 1983; Rice, Sell, & Hadley, 1990; Yont, Hewitt, & Miccio, 2000), most have focused on a narrow set of conversational behaviors that cannot provide a comprehensive picture of pragmatic competence. Also, the detailed coding schemes of many naturalistic measures tend to be time-intensive and can be difficult to implement reliably across different research and clinical settings. In response to these concerns, there has been a call for the development of efficient naturalistic tools that are able to sample a wide range of pragmatic behaviors (e.g., Adams, 2002). This study adds to this literature through the use of a new rating scale, the Pragmatic Rating Scale-School Age (PRS-SA; Landa, 2011), which was designed specifically for capturing pragmatic features associated with autism. This scale samples a wide range of pragmatic behaviors, and can be scored from a number of ecologically-valid communicative contexts.

Rationale for Present Study

This study builds upon prior research examining the overlap of autism and FXS through cross-population comparison of pragmatic language in autism and FXS, and through examination of the role of autism in these features. Complementary standardized and semi-naturalistic assessment techniques were employed in order to characterize precise, ecologically-valid pragmatic profiles that may allow for better delineation of syndrome-specific phenotypes. The aims of this study were:

1. To determine whether boys with idiopathic autism, FXS, Down syndrome, and typical development differ in performance on semi-naturalistic and standardized assessments of pragmatic language skills.

2. To explore the impact of autism severity on pragmatic language ability. This aim was approached in two ways:

    a. Through group comparisons of pragmatic ability, accounting for autism comorbidity in FXS categorically.

    b. Through the examination of continuously-distributed autism traits as a unique predictor of pragmatic impairment in autism and FXS.

3. To explore the congruence between standardized and semi-naturalistic pragmatic assessment methods, through comparison of two pragmatic assessment tools: the Pragmatic Judgment subtest of the Comprehensive Assessment of Spoken Language (Carrow-Woolfolk, 1999) and the Pragmatic Rating Scale- School Age (Landa, 2011).

Methods

Participants

Participants included 34 boys with idiopathic autism (autism spectrum disorder only; ASD-O), 38 boys with FXS and comorbid ASD (FXS-ASD), 10 boys with FXS without ASD (FXS-only; FXS-O), 20 boys with Down syndrome (DS), and 20 typically developing (TD) boys of a similar language age. Children with DS were included to help determine whether pragmatic language features might be better attributed to general intellectual disability than specific processes of autism or FXS. The mean chronological age of the disability groups was 11.47 (SD 3.20, range 3.18-17.90), and the mean age of the TD boys was 4.82 (SD 1.00, range 3.54-6.69); see Table 3.2 for further detail. Only boys participated in the study because females with FXS are generally less affected than males, and less likely to have autism (Clifford et al., 2007; Hagerman & Hagerman, 2002; Hall et al., 2008).

Study participants were drawn from a larger pool of children participating in an ongoing longitudinal study of pragmatic language in FXS, which has been described previously (see Losh et al., 2012). Participants were selected from the larger sample if they had completed the autism,

cognitive, and vocabulary assessments of interest (described below). Given the longitudinal design of

the larger study, in some instances an individual participant had available data from several different

time points. In these cases, the time point was selected that best facilitated group-level matching on

vocabulary, according to a raw score composite of the Peabody Picture Vocabulary Test-III (PPVT;

Dunn & Dunn, 1997) and the Expressive Vocabulary Test (EVT; Williams, 1997). Groups were

matched on receptive and expressive language in order to examine pragmatic deficits above and

beyond what could be attributed to general language ability. To supplement participant numbers, an

additional three children with ASD-O were recruited from a related study of language in autism (PI:

Losh, NIDCD 1R01DC010191-01). Standardized pragmatic language data for forty-two children

have been previously reported by Losh et al. (2012). The groups did not differ in race, household

income, or maternal education level (*ps* > .171); see Table 3.1.

Table 3.1. Demographic Characteristics

	ASD	FXS-All	FXS-ASD	FXS-O	DS	TD
Race %						
Caucasian	88.2	83.3	81.6	90.0	80.0	75.0
African American	8.8	--	--	--	15.0	5.0
Asian	--	6.3	7.0	--	--	--
Multi-racial	2.9	2.1	2.6	--	--	2.1
Not Reported	--	8.3	7.9	10.0	5.0	10.0
Income %						
<20*k*	5.9	--	--	--	--	--
20*k*-39*k*	14.7	4.2	5.3	--	--	10.0
40*k*-59*k*	5.9	8.3	10.5	--	--	10.0
60*k*-79*k*	17.6	6.3	7.9	--	5.0	20.0
>80*k*	26.5	50.0	50.0	50.0	35.0	30.0
Not Reported	29.4	31.3	26.3	50.0	60.0	30.0
Maternal Education Level %						
High School	20.6	20.8	18.4	--	--	15.0
Associate	8.8	14.6	15.8	10.0	10.0	10.0
Bachelor	35.3	20.8	23.7	10.0	10.0	30.0
Master	17.6	10.6	20.0	15.0	15.0	12.5
Doctorate	2.9	10.4	10.5	10.0	5.0	--
Not Reported	14.7	27.1	21.1	50.0	60.0	30.0

The boys with ASD-O had better receptive language ability than all groups except FXS-O,

with no other differences between groups. The boys with ASD-O also had higher expressive

vocabulary level than the boys with FXS-ASD and TD, but did not differ from boys with FXS-O or

DS. The ASD-O boys had significantly higher nonverbal mental age than all other groups, as measured by the Leiter International Performance Scale-Revised (Leiter-R; Roid & Miller, 1997) or the Performance IQ scale of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), described below. See Table 3.2 for group characteristics. All structural language variables, nonverbal mental age, and chronological age were controlled for in analysis.

Table 3.2. Group Characteristics

	Group					
	ASD-O $n = 34$	FXS-All $N = 48$	FXS-ASD $n = 38$	FXS-O $n = 10$	DS $n = 20$	TD $n = 20$
	M (SD) Range	M (SD) Range	M (SD) Range	M (SD) Range	M (SD) Range	M (SD) Range
Chronological age	9.61 (3.05)[a] 3.18-14.56	11.99 (2.95)[b] 6.46-17.82	12.23 (2.91)[b] 6.58-12.23	11.07 (3.06)[a,b] 6.47-16.38	12.90 (2.75)[b] 8.38-17.90	4.82 (1.00)[c] 3.54-6.69
Nonverbal mental age[1]	7.75 (3.62)[a] 2.33-19.67	5.13 (0.60)[b] 3.50-6.67	5.13 (0.60)[b] 3.50-6.67	5.16 (0.64)[b] 4.00-6.00	5.66 (1.23)[b] 4.33-9.58	5.27 (1.16)[b] 2.58-7.50
Receptive vocabulary age[2]	7.44 (2.99)[a] 1.75-14.50	6.53 (1.49)[a] 3.50-9.33	6.15 (1.51)[a] 3.50-9.33	6.96 (1.29)[a] 5.17-9.00	5.93 (2.06)[a] 2.42-10.92	6.07 (1.29)[a] 3.75-8.67
Expressive vocabulary age[3]	6.92 (2.77)[a] 2.58-15.58	5.55 (1.44)[b] 3.58-9.92	5.47 (1.41)[a,b] 3.85-9.92	6.03 (1.56)[a,b] 4.17-8.25	5.89 (1.36)[a,b] 3.42-8.33	5.67 (1.37)[a,b] 3.33-8.83
Mean length of utterance	4.80 (1.55)[a] 1.81-9.33	3.67 (1.02)[b,c] 1.80-7.30	3.46 (0.85)[c] 1.80-6.05	4.46 (1.27)[a,b,c] 2.89-7.30	2.22 (0.85)[c] 1.91-5.08	4.88 (0.59)[a] 4.12-6.06

Note: [1]Age equivalent of the Leiter-R Full Scale IQ or WASI Performance IQ; [2]PPVT age equivalent [3]EVT age equivalent. Means in the same row with different superscripts differ significantly at $p < .05$

All participants were regularly using phrases of at least three words, and English was the primary language spoken at home. Pure-tone hearing thresholds were screened at 500, 1000, 2000 and 4000 Hz with a MAICO MA 40 audiometer; children were excluded for failing the screener at 30 dB in the better ear. The boys with FXS had a diagnosis of the full mutation. Autism was ruled out in the DS and TD groups using the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DeLavore, & Risi, 2001), described below. Four boys with DS were initially recruited but later dropped from the study after scoring above diagnostic cutoffs for autism spectrum disorder. All boys in the TD group scored within normal limits (within 1.5 SD of the mean) on the standardized vocabulary and cognitive measures (described below). Recruitment was based in the Eastern and Midwestern regions of the United States. Participants were ascertained through advertisement at

genetic clinics, parent support groups, physician's offices, and through the Research Participant

Registry Core of the Carolina Institute for Developmental Disabilities at the University of North

Carolina at Chapel Hill.

Procedures

Assessments were administered as part of a broader research protocol, which lasted

approximately 4-6 hours (including time for breaks). Testing took place in a university-affiliated

research laboratory, the child's school, or in a quiet room in the child's home. Consent was obtained

in accordance with the Institutional Review Boards of the University of North Carolina at Chapel Hill

and Northwestern University.

Characterization of Autism: The Autism Diagnostic Observation Schedule (ADOS; Lord et

al., 2001) was administered to confirm autism status in the ASD-O group, and in order to determine

autism comorbidity among the boys with FXS. The ADOS involves direct-observation of social-

communicative and restricted/repetitive behaviors during a play-based semi-structured interaction

between the participant and an examiner. The ADOS was coded by examiners who had achieved

reliability either through direct training with the developers of the ADOS or through intra-lab

reliability in accordance with the standards of the instrument developers. All boys in the autism

groups (ASD-O and FXS-ASD) met diagnostic criteria for "autism" or "autism spectrum" on the

revised diagnostic algorithm of the ADOS (Gotham et al., 2008; Gotham, Risi, Pickles, & Lord,

2007). Eleven of the children with FXS had been administered the ADOS at three independent time

points, through participation in related longitudinal studies of language and speech characteristics that

followed the same cohort of boys at younger or older ages (Roberts et al., 2007; Zajac, Harris,

Roberts, & Martin, 2009). All available diagnostic information was considered in determining autism

status in attempts to determine the best-estimate diagnosis. Four boys scored as autism/spectrum at

3/3 time points and three boys scored as autism/spectrum at 2/3 times points; these boys were

characterized as FXS-ASD. Two boys scored as non-spectrum at 3/3 time points and two scored as

non-spectrum at 2/3 time points; these boys were assigned to the FXS-O group. The best-estimate

diagnosis agreed with concurrent ADOS classification for all but one of the boys, who had met diagnostic criteria for autism at his current assessment, but had scored below thresholds for autism spectrum at two earlier assessments. Concurrent ADOS diagnostic information was used for the remaining participants. The ADOS was also used as a continuous measure of autism symptoms; severity scores were computed in accordance with Gotham, Pickles, & Lord (2009). For those participants who had been administered the ADOS several times, severity scores from all available time points were averaged to compute a best-estimate autism severity score. This included the 11 boys with FXS discussed above, as well as six boys with DS and one with boy with TD.

Measurement of Cognitive Ability: Nonverbal cognitive ability was assessed using the Brief IQ Composite of the Leiter International Performance Scale-Revised (Leiter-R; Roid & Miller, 1997). Leiter-R data were unavailable for three participants with ASD-O. For these participants, the Performance IQ scale of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was used as a substitute measure of nonverbal intelligence. Age equivalent scores were used in analysis.

Measurement of Structural Language Skills: Receptive language was measured with either the Peabody Picture Vocabulary Test-III or Peabody Picture Vocabulary Test-IV (PPVT; Dunn & Dunn, 1997, 2007). The Expressive Vocabulary Test (EVT; Williams, 1997) was used as a measure of expressive language. Age equivalent scores were computed for each measure from published norms. In addition to standardized language measures, mean length of utterance in morphemes (MLU) was computed using transcription conventions as outlined by Systematic Analysis of Language Transcripts (SALT; Miller & Chapman, 2008). The ADOS served as the language sample for the transcripts. Fifty-five intelligible child conversational turns from "play" contexts and 55 from "non-play" contexts (e.g., conversation) were transcribed. This strategy was employed to ensure that the context of the language sample was comparable across groups. The language samples were transcribed by trained research assistants who had achieved morpheme agreement of 80% or higher as compared to a "gold-standard" transcript for two language samples from each diagnostic group. MLU

was calculated on novel, intelligible, non-routine utterances, as per SALT conventions. Seven percent of the transcripts were randomly selected and transcribed by an independent transcriber; morpheme-to-morpheme reliability was calculated at 78% agreement.

Standardized Assessment of Pragmatic Language: The Pragmatic Judgment subtest of the Comprehensive Assessment of Spoken Language (CASL-PJ; Carrow-Woolfolk, 1999) was used as a standardized measure of the knowledge and use of pragmatic language. In the CASL-PJ, participants are told short stories about children in various social situations and are scored on their ability to provide a pragmatically appropriate response explaining what the children should do or say in each scenario. The CASL-PJ is normed on individuals aged 3-21 years, and is a reliable index of pragmatic language ability, with test-retest reliability coefficients ranging from .66-.85 across age groups. Age-equivalent scores were used in analysis. One participant with DS obtained a raw score of "0" on this measure, and data for this participant were considering missing. CASL-PJ data was unable to be collected for four participants with ASD-O and three with FXS-ASD, due to time constraints when testing in the field.

Semi-naturalistic Assessment of Pragmatic Language: The Pragmatic Rating Scale-School Age (PRS-SA; Landa, 2011) was used to rate pragmatic language behaviors during semi-structured social interaction. The ADOS, which generally lasts 40-60 minutes, was used as a semi-structured conversational context from which to rate pragmatic language skills. The ADOS is an ideal context for sampling conversation because the semi-structured format provides continuity across administrations but is flexible in following the child's lead (Tager-Flusberg et al., 2009). Ninety-three children were administered the ADOS module 3 (for verbally fluent individuals), and 33 were administered module 2 (for use with individuals who have phase speech). There was a roughly equal distribution of both modules across diagnostic groups, and there were no significant differences between the PRS-SA scores of children who had been administered a module 2 versus module 3.

The PRS-SA assesses 34 features related to pragmatic language, such as verbosity, social inappropriateness, scripting, and redundancy. Items are rated for severity on a scale of "0" to "2"

according to operational definitions of each trait. Items are summed to produce a total score, with a higher score indicating greater severity of pragmatic language difficulties. The PRS-SA also provides five theoretically-derived subdomain scores, which were explored in this study with factor analysis (described in *Data Reduction*). All PRS-SA ratings were conducted by the first author (JK), who had achieved reliability with the developer of the PRS-SA. The coder was blind to the diagnosis of 86% of participants; it was not possible to maintain blinding to all participants, as the coder had assisted with recruitment and testing for the study. Fifteen percent of the sample was randomly selected and second-scored by an independent, blind rater who had also achieved coding reliability with the developer of the PRS-SA. Inter-rater reliability was as follows: ICC (3, 2): .91 for the overall sample (.74 for ASD-O, .83 for FXS-ASD, .79 for FXS-O, .89 for DS, and .84 for TD). ICC values of 0.40-0.75 represent "fair" to "good" agreement, and values greater than .75 signify "excellent" agreement (Fleiss, Levin, & Paik, 2004; Landis & Koch, 1977).

Data Reduction and Analysis

First, factor analysis was conducted to explore empirical evidence to support the use the five theoretically-derived pragmatic subdomains of the PRS-SA. Evidence strongly suggested that the PRS-SA items represented a single pragmatic domain, and thus analyses are presented only for the PRS-SA total score. Briefly, exploratory factor analysis was conducted with PASW Statistics 18 (IBM). The model was fit under weighted least squares estimation with a geomin rotation. Items were treated as categorical variables, and polychoric correlations are used to produce the asymptotic covariance matrix for analysis (Joreskog, 1994). Examination of the scree plot showed a distinct leveling after the first factor; eigenvalues for the first eight factors were greater than one, with a significant drop from the first to second factor (6.10 to 3.01). A one-factor model provided the most theoretically meaningful constructs. Thus, results strongly suggested that a one-factor model was the best fit for the data. A confirmatory factor analytic model was then conducted in Mplus (Muthen & Muthen, 2006) to determine whether the data might converge on the pre-identified subscales. Like the exploratory model, the confirmatory factor analysis was fit under weighted least squares estimation

with a geomin rotation, with polychoric correlations to handle the categorical items. The model using the pre-identified subscales as factors failed to converge, and even the preliminary solution indicated very high between factor correlations, implying that the one factor solution was sufficient.

For the group comparisons on pragmatic language, two separate sets of analyses were run in order to account for autism symptoms in the FXS group either categorically or continuously. First, group differences on the pragmatic language variables were examined with autism status in the FXS group considered as a categorical trait (i.e., the group was divided into FXS-ASD and FXS-O subgroups, as described previously). For these models, multivariate analysis of covariance (MANCOVA) was used to test whether the mean PRS-SA and CASL-PJ scores differed by group, controlling for chronological age, nonverbal mental age (Leiter-R/WASI) and structural language (EVT, PPVT, MLU). Planned pair-wise comparisons were conducted to test for specific group differences. False discovery was controlled for by adjusting at the level of the omnibus F-test, using the Benjamini-Hochberg correction procedure (Benjamini & Hochberg, 1995).

The second set of analyses took a continuous approach to account for autism, using a series of linear regression analyses to explore ADOS severity score as a unique predictor of pragmatic language ability in the ASD-O and FXS-All groups. Chronological age, nonverbal mental age, structural language, and autism severity were entered in a stepwise fashion into two different regression models predicting PRS-SA and CASL-PJ performance. Chronological age was entered into the model first, followed by Leiter-R/WASI, PPVT, EVT, MLU, and then by ADOS severity score. These analyses were conducted only in the ASD-O and FXS groups because of the limited range of autism severity scores in the DS and TD groups. Data were first examined for skewedness, kurtosis, and heteroscedasticity; no corrections were necessary.

Finally, in order to examine the relationship between the semi-naturalistic and standardized pragmatic language measures, simple Pearson correlations were conducted between the PRS-SA and CASL-PJ.

Results

Group Comparisons on Pragmatic Language

MANCOVA revealed a significant effect of group on the PRS-SA and CASL-PJ scores [$V = 11.35$, $F(8, 206) = 11.33$, $p < .001$]. Univariate analysis testing the specific effect of group on PRS-SA showed a significant overall effect for group [$F(4, 112) = 35.13$, $p < .001$]. Post-hoc comparisons of PRS-SA performance indicated that the difference between the ASD-O and FXS-ASD groups approached significance ($p = .064$). Both ASD-O and FXS-ASD showed greater pragmatic impairment than all other groups ($ps < .002$). The FXS-O and DS groups did not differ on PRS-SA total score ($p = .116$). While FXS-O showed greater impairment than TD ($p = .002$), the DS group did not differ from TD in pragmatic skills ($p = .117$). Group comparisons are presented in Figure 3.1.

Figure 3.1. Group Comparisons on PRS-SA Total Score
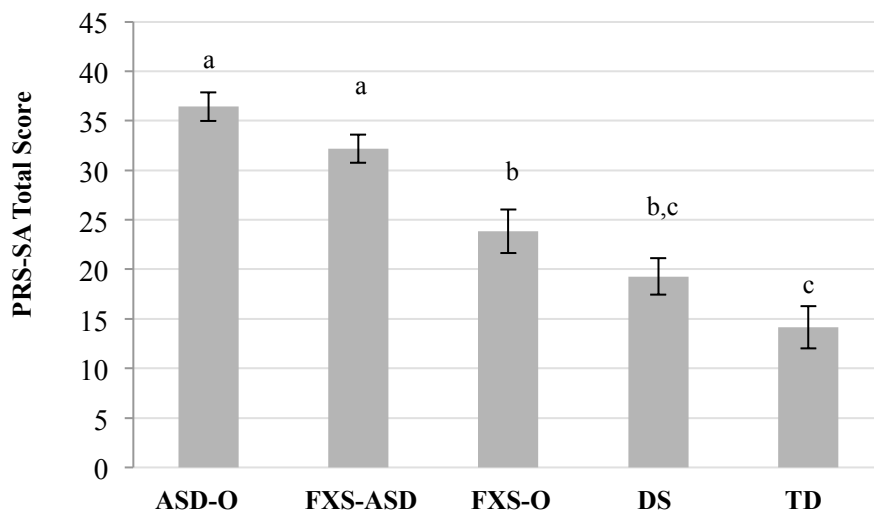
Note: Covariate-adjusted means, controlling for chronological age, nonverbal mental age, receptive and expressive vocabulary, and mean length of utterance. Groups sharing the same letter did not differ significantly ($p < .05$). Higher scores indicate greater impairment.

Follow-up univariate analyses of CASL-PJ performance also showed a significant overall effect for group [$F(4, 113) = 2.89$, $p = .040$]. Pair-wise group comparisons indicated that ASD-O had significantly lower (i.e., more impaired) CASL-PJ scores than TD ($p = .006$) and FXS-O ($p = .015$)

but did not differ from FXS-ASD ($p = .279$) or DS ($p = .224$). No other significant group differences were detected. Group comparisons are presented in Figure 3.2.

Figure 3.2. Group Comparisons on CASL-PJ
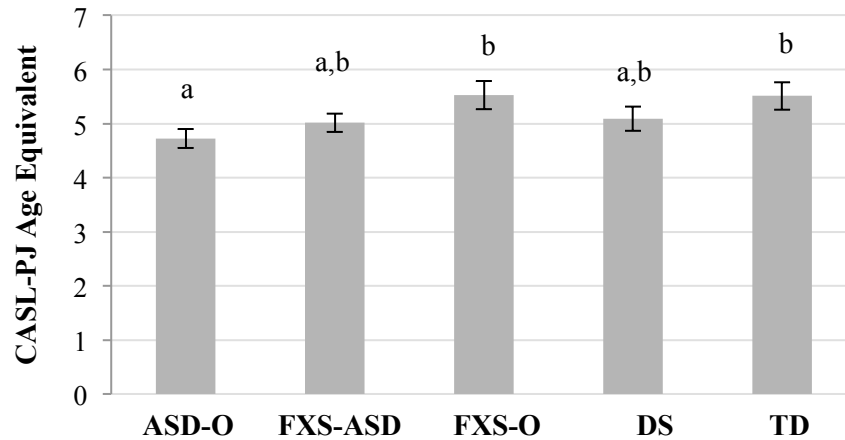
Note: Covariate-adjusted means, controlling for chronological age, nonverbal mental age, receptive and expressive vocabulary, and mean length of utterance. Groups sharing the same letter did not differ significantly ($p < .05$). Lower scores indicate greater impairment.

Autism Severity as a Unique Predictor of Pragmatic Language Ability

Predictors of PRS-SA: As depicted in Table 3.3, chronological age was not a significant predictor of PRS-SA performance in either ASD-O or FXS groups. The addition of nonverbal mental age and structural language to the model did not account for significantly greater variance in PRS-SA score in either group. Autism severity was a significant unique predictor of PRS-SA performance in both the ASD-O and FXS group (see Table 3.3). After accounting for chronological age, mental age, and structural language, autism severity accounted for 27% of the variance in the PRS-SA score in ASD-O, and 34% of the variance in FXS.

Table 3.3. Regression Coefficients Depicting Predictors of PRS-SA in ASD-O and FXS

			B (SE)	*β*	R^2	$R^2\Delta$	*F*Δ
	Step 1	Constant	31.92 (3.40)		.03	.03	0.80
		Chronological Age	0.36 (0.40)	0.16			
ASD-O	Step 2	Constant	33.89 (5.66)		.20	.18	1.49
		Chronological Age	0.92 (0.47)	0.41			
		Leiter-R/WASI	-0.25 (0.66)	-0.14			

			B (SE)	β	R^2	ΔR^2	F
		PPVT	-1.38 (1.22)	-0.62			
		EVT	0.69 (0.99)	0.28			
		MLU	0.04 (0.95)	0.01			
	Step 3	Constant	21.30 (5.86)		.47	.27	13.05**
		Chronological Age	0.81 (0.40)	0.36*			
		Leiter-R/WASI	0.07 (0.55)	0.04			
		PPVT	-1.86 (1.02)	-0.83			
		EVT	0.75 (0.83)	0.31			
		MLU	-0.34 (0.80)	-0.08			
		ADOS Severity	2.01 (0.57)	0.55**			
	Step 1	Constant	29.90 (5.05)		.01	.01	0.04
		Chronological Age	0.09 (0.41)	0.03			
	Step 2	Constant	32.77 (11.65)		.19	.19	2.36
		Chronological Age	0.18 (0.44)	0.07			
		Leiter-R/WASI	1.77 (2.46)	0.13			
		PPVT	-2.94 (1.32)	-0.54*			
		EVT	2.11 (1.43)	0.34			
FXS-All		MLU	-1.66 (1.47)	-0.20			
	Step 3	Constant	12.51 (9.81)		.52	.34	28.13***
		Chronological Age	-0.47 (0.36)	-0.17			
		Leiter-R/WASI	3.07 (1.93)	-0.22			
		PPVT	-3.04 (1.02)	-0.56**			
		EVT	2.46 (1.11)	0.40*			
		MLU	0.28 (1.20)	0.03			
		ADOS Severity	2.28 (0.43)	0.67***			

Note: Leiter-R/WASI = Leiter International Performance Scale- Revised/ Wechsler Abbreviated Scale of Intelligence; PPVT = Peabody Picture Vocabulary Test; EVT = Expressive Vocabulary Test; MLU = mean length of utterance; ADOS = Autism Diagnostic Observation Schedule. *$p < .05$, **$p < .01$, ***$p < .001$

Predictors of CASL-PJ: Chronological age was not a significant unique predictor of CASL-PJ performance in either ASD-O or FXS groups. The combined influence of mental age and structural language ability accounted for significant unique variance in CASL-PJ scores in both ASD-O and FXS (coefficients are reported in Table 3.4). In ASD-O, mental age and structural language accounted for 77% of the variance in CASL-PJ beyond chronological age. In the FXS group, these variables uniquely accounted for 64% of the variance in CASL-PJ. Autism severity did not account for significant variance in CASL-PJ in the ASD-O group, after accounting for chronological age, mental age, and structural language. In FXS, autism severity uniquely accounted for 5% of the variance in CASL-PJ beyond the effects of chronological age, mental age, and structural language (see Table 3.4).

Table 3.4. Regression Coefficients Depicting Predictors of CASL-PJ in ASD-O and FXS

			B (SE)	β	R^2	$R^2\Delta$	$F\Delta$
	Step 1	Constant	3.71 (1.22)		.07	.07	1.98
		Chronological Age	0.17 (0.12)	0.26			
	Step 2	Constant	1.15 (0.91)		.83	.77	27.16***
		Chronological Age	-0.16 (0.07)	-0.24*			
		Leiter-R/WASI	0.01 (0.10)	0.01			
		PPVT	0.70 (0.19)	0.91**			
		EVT	0.09 (0.17)	0.10			
ASD-O		MLU	0.03 (0.16)	0.02			
	Step 3	Constant	1.67 (1.09)		.84	.01	0.78
		Chronological Age	-0.15 (0.07)	-0.23*			
		Leiter-R/WASI	-0.01 (0.10)	-0.02			
		PPVT	0.71 (0.19)	0.92**			
		EVT	0.08 (0.18)	0.09			
		MLU	0.06 (0.16)	0.04			
		ADOS Severity	-0.09 (0.10)	-0.08			
	Step 1	Constant	3.20 (0.87)		.08	.08	3.82
		Chronological Age	0.14 (0.07)	0.29			
	Step 2	Constant	-0.70 (1.31)		.73	.64	22.85***
		Chronological Age	0.01 (0.05)	0.01			
		Leiter-R/WASI	-0.03 (0.27)	-0.01			
		PPVT	0.48 (0.14)	0.50**			
		EVT	0.34 (0.15)	0.31			
FXS-All		MLU	0.22 (0.15)	0.16			
	Step 3	Constant	0.67 (1.29)		.78	.05	8.71**
		Chronological Age	0.05 (0.04)	0.10			
		Leiter-R/WASI	-0.12 (0.25)	-0.05			
		PPVT	0.49 (0.12)	0.52***			
		EVT	0.30 (0.14)	0.28*			
		MLU	0.09 (0.14)	0.07			
		ADOS Severity	-0.15 (0.05)	-2.95**			

Note: Leiter-R/WASI = Leiter International Performance Scale- Revised/ Wechsler Abbreviated Scale of Intelligence; PPVT = Peabody Picture Vocabulary Test; EVT = Expressive Vocabulary Test; MLU = mean length of utterance; ADOS = Autism Diagnostic Observation Schedule. *$p <$ .05, **$p <$ .01, ***$p <$ .001

Relationship between Pragmatic Language Assessments

A significant, though moderately weak, association was detected between the PRS-SA total score and the CASL-PJ age equivalent score in the overall sample ($r = $ -.26, $p = $ .006), indicating that performance on the CASL-PJ decreased as the severity of pragmatic language violations on the PRS-SA increased. Within-group correlations revealed similar patterns, with significant associations between PRS-SA and CASL-PJ in the ASD-O group ($r = $ -.40, $p = $ .033), and the correlation approaching significance in FXS-ASD ($r = $ -.33, $p = $ .065). Associations in the FXS-O, DS, and TD

groups followed a similar trend although correlations were not significant (*ps* >.130). Correlations are

presented in Table 3.5.

Table 3.5. Correlations between CASL-PJ and PRS-SA

	PRS-SA					
	Full Sample	ASD-O	FXS-ASD	FXS-O	DS	TD
CASL-PJ	-.26*	-.40*	-.32	-.30	-.36	-.20
n	114	30	35	10	19	20

Note: PRS-SA = Pragmatic Rating Scale- School Age; CASL-PJ = Comprehensive Assessment of Spoken Language, Pragmatic Judgment subtest. **p* < .05

Discussion

This study detected similar severity of pragmatic language deficits in boys with idiopathic

autism and FXS with autism, which builds on growing evidence for shared behavioral profiles in

idiopathic autism and autism associated with the *FMR1* mutation. While the pragmatic performance

of boys with FXS with and without autism did not differ on standardized assessment, evaluation of

pragmatic ability in a semi-naturalistic setting revealed clearly divergent profiles, suggesting that

autism comorbidity plays a significant role in these boys' abilities to communicate in real-life social

contexts. The results of this study support the utility of multimodal pragmatic language assessment in

clinical practice, and highlight the need to consider autism comorbidity in the evaluation and

treatment of individuals with FXS. Support for phenotypic overlap in autism and FXS has

implications for the eventual identification of causal pathways that may be shared in these disorders,

shedding light on *FMR1* as a candidate gene for autism.

By documenting similar pragmatic profiles in ASD-O and FXS-ASD on two pragmatic

assessment tools, this study adds to evidence of common phenotypic profiles in idiopathic autism and

autism associated with the *FMR1* mutation. In support of pragmatic language impairment as a central

feature of autism, results of this study indicate that the core pragmatic language deficits that are seen

in idiopathic autism extend to syndromic forms of the disorder (namely, autism associated with FXS).

Because pragmatic impairment is core to autism, it may a promising trait for identifying gene-

behavior or brain-behavior relationships implicated in the disorder. The incorporation of FXS, a

single-gene disorder, into the study of pragmatic language in autism may help identify autism-associated behaviors that are linked to the neurobiological effects of the *FMR1* mutation.

The PRS-SA proved to be an effective tool for differentiating small differences in pragmatic language skills across groups. While the CASL-PJ was successful at distinguishing children with ASD-O from TD controls, it was unable to differentiate the pragmatic performance of the other groups. Only a modest correlation was detected between the CASL-PJ and the PRS-SA (*r* = -.26). Performance on the two assessment tools was predicted by different skills---together, cognition and structural language skills accounted for 73-83% of variance in CASL-PJ performance after accounting for age, whereas these variables accounted for about 20% of the variability in PRS-SA scores with the FXS and ASD-O groups. This stark contrast suggests that performance on the CASL-PJ is highly influenced by cognition and general language abilities (or, possibly by general test-taking skills that would be needed to perform favorably on standardized assessments in general). Nevertheless, the CASL-PJ was able to differentiate performance of the ASD-O group from TD after controlling for cognitive and language confounds, which supports its ability to capture frank pragmatic language violations. Overall, this study illustrates the importance of incorporating multiple measures in the assessment of pragmatic language. Particularly, semi-naturalistic assessment is a robust tool that may capture subtle pragmatic differences that are overlooked by standardized tools. Clinically, the PRS-SA may be useful for measuring treatment gains related to pragmatic language, given that it was more sensitive to small pragmatic differences than standardized assessment. A further advantage of the PRS-SA is that it can be administered as often as needed with virtually no risk of the learning effects.

Findings from the semi-naturalistic assessment showed that autism comorbidity had a substantial influence on the pragmatic abilities of individuals with FXS. This is consistent with a body of evidence indicating that individuals with FXS with comorbid autism show greater cognitive, social, and adaptive impairments than their non-autistic counterparts (e.g., Bailey, Hatton, Mesiboy, Ament, & Skinner, 2000; Dissanayake et al., 2009; Hernandez et al., 2009; Kau et al., 2004; Loesch

et al., 2007; Philofsky, Hepburn, Hayes, Hagerman, & Rogers, 2004; Rogers et al., 2001). This finding is significant, as it may shed light on the nature of autism in FXS—individuals with FXS-ASD continued to show greater impairment even after controlling for mental age and structural language ability. This finding is not consistent with hypothesis that autism features in FXS stem from intellectual disability (e.g., Hall et al., 2010; Loesch et al., 2007), as it suggests that pragmatic language deficits in FXS are similar to those seen in idiopathic autism and occur independently of intellectual disability. Clinically, these findings underscore the importance of considering autism comorbidity in the evaluation and treatment of individuals with FXS. Autism is highly likely to co-occur in FXS and has a detrimental effect on developmental outcomes; all individuals with FXS should be evaluated for autism. Presently, it is unknown whether interventions designed for individuals with idiopathic autism are effective for individuals with fragile X-associated autism. However, it is likely that the treatment of individuals with FXS-ASD would warrant different intensity and type of services that FXS-O, given the divergent behavioral profiles of these groups. Research investigating the efficaciousness of autism-specific treatments for individuals with FXS will be important for determining best clinical practices for this population.

The impact of autism on the pragmatic language profile of FXS varied according to the metric chosen to represent autism status. While autism had a clear impact on the PRS-SA outcomes with both dichotomous and continuous autism characterization, only the continuous metric revealed an impact of autism on the CASL-PJ scores in FXS. This is consistent with a report by McDuffie et al. (2012), who found that autism was related to expressive and receptive language skills in FXS only when accounted for continuously. The examination of continuously-distributed autism traits is an approach that has also been adopted in studies of autism, as it yields greater information than a traditional categorical approach and enhances statistic power to detect effects (Constantino, 2011)

There are several limitations to the present study. First, a single diagnostic tool, the ADOS, was used to characterize autism. Best-practices dictate the use of multiple sources of information in determining the presence of autism. The incorporation of other autism diagnostic tools, such as the

Autism Diagnostic Interview-Revised (Lord et al., 1994), might have allowed for more precise characterization of autism. Secondly, the PRS-SA ratings were based off of a single interaction with a highly trained examiner. Perhaps the examination of social-communication skills in other contexts, such as during conversation with a peer, would have revealed different results. The extension of this work to other communicative contexts might provide a more complete picture of the pragmatic language abilities of these children. Furthermore, this study only included boys and it is unclear whether the findings of this study would generalize to females with autism or FXS. It should also be noted that all study participants were using phrase speech, and thus participants represented a select subgroup of individuals; results may not generalize to the communication abilities of nonverbal individuals with autism or FXS. Finally, the results of this study are based on the presentation of these disorders at a single point in time, with limited ability to account for developmental patterns or prospective features that may predict pragmatic impairment. Longitudinal studies are needed to understand the emergence of pragmatic language deficits in autism and FXS across development, which might help identify developmental periods that are most optimal for intervention.

In conclusion, the results of this study provide support for overlapping social-communication deficits in idiopathic and fragile X-associated autism, regardless of whether standardized or semi-naturalistic assessment tools are utilized. This finding may be informative for future studies aimed at uncovering the pathogenesis of autism, as shared pragmatic deficits in autism and FXS might imply a role of *FMR1* in the communication phenotype of autism. Evidence from this study also suggests that autism comorbidity has a significant impact on the social-communicative abilities of children with FXS, which has implications for considering autism status in the evaluation and treatment of this population.

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CHAPTER 4

IS PRAGMATIC LANGUGAE IMPAIRMENT RELATED TO PHYSIOLOGICAL AROUSAL DYSREGULATION IN AUTISM AND FRAGILE X SYNDROME?

Summary

Pragmatic language deficits (i.e., impaired social language) are common in autism spectrum disorders (ASD) and fragile X syndrome (FXS). This study explored the hypothesis that pragmatic impairments in ASD and FXS are rooted in the inability to regulate physiological arousal. Participants included 33 boys with idiopathic ASD and 31 boys with FXS, aged 4-17 years. Cardiac indices of arousal (collected at rest and during conversation with an examiner) were examined in relation to pragmatic skills. Associations between arousal, anxiety, autism severity, and receptive/expressive language were also explored. Results showed that boys with FXS were hyperaroused in comparison to ASD in both conditions, although no group differences were detected in respiratory sinus arrhythmia (RSA), an index of parasympathetic vagal tone. Conversation did not elicit increased arousal in either group. In ASD, higher resting RSA predicted better pragmatic language, and several associations were observed between physiological activity and structural language. Several trend-level associations were observed between cardiac arousal and pragmatic, receptive, and expressive language in FXS. Anxiety was associated with increased physiological reactivity in FXS but not ASD. These findings suggest that physiological modulation may play a role in pragmatic language development, and supports further investigation of interventions targeting breakdowns in the autonomic nervous system in ASD and FXS. Findings also provide some support for divergent physiological profiles in ASD and FXS, which has implications for understanding the potential role of *FMR1* (the FXS gene) in pathophysiological basis of ASD.

Introduction

Fragile X syndrome (FXS) is a monogenic disorder associated with significantly increased risk for autism spectrum disorder (ASD) (Cohen, Pichard, & Tordjman, 2005). Pragmatic language impairment is seen in both ASD and FXS (Landa, 2000; Losh, Martin, Klusek, Hogan-Brown, & Sideris, 2012; Sudhalter & Belser, 2001), yet it is unknown whether such impairment stems from similar underlying mechanisms in these disorders. It has been hypothesized that physiological hyperarousal (and associated anxiety) in FXS leads to sub-optimal social performance, causing the atypical social communication features that are seen in the disorder (e.g., Belser & Sudhalter, 1995). The present study investigated the role of physiological dysregulation in pragmatic language deficits in ASD and FXS, through group comparisons of physiological activity during a social-communicative context and by examining physiological regulation as a predictor of pragmatic impairment. Further understanding of arousal, a biophysiological marker for stress, as a mechanism underlying the social-communicative phenotypes of FXS and ASD has implications for the development of targeted interventions, and may lend insight into shared biological pathways in ASD and FXS that may be traced back to *Fragile X Mental Retardation-1* (*FMR1*), the FXS gene.

Genetic Basis of ASD and FXS

ASD is characterized by atypical social and communication development, along with repetitive and restricted behavioral patterns (American Psychiatric Association, 2000). Affecting approximately 1 in 88 individuals, ASD is seen at epidemic levels and there is an urgent need to understand the etiological basis of the disorder (CDC, 2012). Evidence supports a strong genetic component in the etiology of ASD, although the exact genetic underpinnings still remain undefined (Devlin & Scherer, 2012; Miles, 2011). The genetic basis of ASD is thought to be heterogeneous and extremely complex, with many different gene-gene and gene-environment interactions leading to the common phenotypic endpoint of ASD (Abrahams & Geschwind, 2008). Single gene disorders, such as FXS, are implicated in about 10% of cases of ASD (Betancur, 2011). The study of ASD within the context of associated genetic conditions provides a better-understood genetic paradigm for studying

ASD, which may provide a starting-point for pinning-down pathophysiological mechanisms (Abrahams & Geschwind, 2008; Hagerman, Hoem, & Hagerman, 2010; Persico & Bourgeron, 2006).

While the etiological basis of ASD is multifaceted and largely undefined, FXS can be traced back to a single genetic cause-- a trinucleotide expansion on the *FMR1* gene (Pieretti et al., 1991). This expansion silences the gene and halts the production of Fragile X Mental Retardation Protein (FMRP), which is a protein that is highly expressed in the brain and is thought to play a role in synaptic development (Hagerman & Hagerman, 2002; Loesch, Huggins, & Hagerman, 2004; Tassone et al., 1999). Deficiency in FMRP is thought to underlie the neurobehavioral profile of FXS, which includes intellectual disability, language impairment, social difficulties, anxiety, hyperactivity, and deficits in executive functions (Abbeduto, Brady, & Kover, 2007; Baumgardner, Reiss, Freund, & Abrams, 1995; Hagerman, 2002; Mazzocco, Pennington, & Hagerman, 1993; Reiss & Dant, 2003). Strikingly, 50-75% of individuals with FXS meet criteria for ASD, and those who do not reach diagnostic thresholds nevertheless show symptoms consistent with ASD, such as reduced eye gaze and repetitive behaviors (Clifford et al., 2007; Hagerman et al., 1986; Hall, Lightbody, & Reiss, 2008; Harris et al., 2008). This significantly elevated risk for ASD suggests that the *FMR1* mutation may play a role in the development of autistic symptoms, either by disrupting the normal functions of other "background" genes that are involved in ASD, or through toxicity in mRNA that occurs as a result of gene silencing (Belmonte & Bourgeron, 2006; Hagerman, Au, & Hagerman, 2011; Hagerman et al., 2010). For example, FMRP assists in the translation of several proteins that are dysregulated in idiopathic ASD (e.g., neuroexin, neuroligin3, neuroligin4, CYFIP, PTEN) and the absence of FMRP in FXS has a widespread impact on the expression of other genes (see Hagerman et al., 2010). Therefore, the presence of the *FMR1* mutation may lower the threshold of interacting genetic effects needed to produce ASD; the study of FXS provides a known genetic context from which to examine ASD that may facilitate the identification of genetic/molecular pathways involved in ASD.

Although behavioral studies of ASD and FXS show that the autism symptom profiles in idiopathic and FXS-associated ASD are virtually indistinguishable (Dissanayake, Bui, Bulhak-Paterson, Huggins, & Loesch, 2009; Rogers, Wehner, & Hagerman, 2001), it is unknown whether these traits stem from similar etiological underpinnings, as only a handful of studies have directly compared neurobiological characteristics in these disorders. Dysfunction of the autonomic nervous system and the ability to modulate physiological arousal is well-documented in FXS and is hypothesized to underlie social deficits seen in the disorder, such as impaired pragmatic language performance (Belser & Sudhalter, 1995; Cohen, 1995; Miller et al., 1999). Although arousal dysregulation has also been proposed as a mechanism that may underlie social deficits in idiopathic ASD (e.g., Dawson & Lewy, 1989; Hutt, Hutt, Lee, & Ounsted, 1964; Rimland, 1964), few studies have directly compared physiological profiles in ASD and FXS, limiting our understanding of the autonomic nervous system dysfunction as a biophysiological marker that is shared in ASD and FXS, and which may underlie social-communication deficits in these disorders.

Pragmatic Language in ASD and FXS

Pragmatic language impairment is a central feature of ASD that is evident across the entire autism spectrum, regardless of language or adaptive functioning level (Landa, 2000; Tager-Flusberg, Paul, & Lord, 2005). It is hypothesized that pragmatic deficits in ASD are linked to underlying etiological mechanisms, as subclinical pragmatic differences are well-documented in relatives of individuals with ASD as part of the broad autism phenotype (Landa et al., 1992; Losh, Childress, Lam, & Piven, 2008; Piven, Palmer, Landa, Santangelo, & Childress, 1997) and show patterns suggestive of intra-familial transmission (Klusek, Losh, & Martin, in press). Pragmatic language deficits are also seen in FXS (Belser & Sudhalter, 2001; Losh et al., 2012; Martin et al., 2012; Mazzocco et al., 2006; Sudhalter & Belser, 2001) and, importantly, have been shown to relate to *FMR1*-related genetic variation (Losh et al., 2012). Recent evidence shows that pragmatic language difficulties also extend to the *FMR1* premutation, with carriers of *FMR1* showing pragmatic language features that are similar in rate and quality to those seen in relatives of individuals with ASD (Losh et

al., 2012). Together, these studies suggest that *FMR1* may be involved in the pragmatic language profile associated with ASD.

Although pragmatic language deficits appear to be shared in ASD and FXS, different underlying mechanisms have been proposed to cause pragmatic language deficits in these disorders. In ASD, a number of neurocognitive theories have been proposed to account for pragmatic language impairment including impaired social-cognition, executive functioning, and central coherence (Martin & McDonald, 2003). On the other hand, theories of pragmatic language impairment in FXS have primarily focused on dysfunctional physiological arousal regulation as a cause of pragmatic language deviance, as dysfunctional arousal modulation is a consistently documented feature of FXS (Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001; Roberts, Tonnsen, Robinson, & Shinkareva, 2012). In this model, the inability to modulate arousal causes an individual to remain anxious and "on edge" during social situations, presenting as anxiety. Over time, individuals who are unable to regulate physiological responses may withdrawal and avoid social situations, further impacting social development by limiting opportunities to learn skills through interaction with others (Rubin & Burgess, 1991). Given than physiological regulatory deficits have also been documented in ASD (Bal et al., 2010; Mathewson et al., 2011; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Van Hecke et al., 2009), this study explored physiological dysregulation as an alternative model to account for social-communication deficits in both FXS and ASD.

Physiological Arousal in ASD and FXS

Dysfunction of the physiological system is thought to interfere with the ability to engage with the external environment, leading to reduced capacity for adaptive social engagement (Porges & Furman, 2011). The present study focused on cardiac indices of physiological arousal, as they provide a non-invasive measure of parasympathetic autonomic functioning. Specifically, we focused on measures of heart rate (a measure of general arousal, reflecting both sympathetic and parasympathetic input) and of respiratory sinus arrhythmia (RSA), which indexes parasympathetic control of the heart

via the vagal nerve (Bernston et al., 1997; Eckberg, 1983; Katona & Jih, 1975; Porges, 2007). The vagus works as part of the "rest and restore" parasympathetic system; when the body is at rest, the vagus works as a "brake" to counteract sympathetic ("fight or flight") excitation, slowing heart rate and creating a calm physiological state. In the face of external stress, the vagal brake releases (i.e., vagal tone reduces) to allow for mobilization and sympathetic excitation (Porges, 1992; Porges, 1995, 2001; Porges & Furman, 2011). According to Porges' Polyvagal Theory, efficient vagal control allows the body to achieve a physiological state that optimizes either social participation or social defense (Porges, 1992; Porges, 1995; Porges & Furman, 2011). In support of this theory, a vast body of literature links increased resting vagal tone with enhanced social outcomes in typical development, including greater sympathetic responding, increased feelings of social connectedness, and better overall social skills (Blair & Peters, 2003; Calkins & Keane, 2004; Fabes, Eisenberg, & Eisenbud, 1993; Fabes, Eisenberg, Karbon, Troyer, & Switzer, 1994; Kok & Fredrickson, 2010). Vagal reactivity (i.e., change in vagal tone from baseline in response to a stimulus) is also linked with social behavior; toddlers who show the greatest vagal increases in response to social interaction have better receptive and expressive language abilities and more sophisticated play skills (Suess & Bornstein, 2000). On the other hand, children who exhibit vagal withdrawal in response to social interaction (indicating physiological defensiveness and hypervigilance) show increased anxiety, depression, and internalizing problems (Heilman et al., 2008).

FXS: Physiological dysfunction is a hallmark feature of FXS. Faster heart rate, marking increased arousal, is consistently seen in individuals with FXS as compared to typically developing peers (Boccia & Roberts, 2000; Hall et al., 2009; Heilman, Harden, Zageris, Berry-Kravitz, & Porges, 2011; Roberts et al., 2001; Roberts et al., 2012). Dampened parasympathetic tone is also well-documented in FXS, both at rest and during other conditions such as conversation, arm restraint, and cognitive assessment (Boccia & Roberts, 2000; Hall et al., 2009; Heilman et al., 2011; Roberts et al., 2001; Roberts, Boccia, Hatton, Skinner, & Sideris, 2006; Roberts et al., 2012). Although deficient physiological regulation is hypothesized to be related to communicative impairment in FXS (e.g.,

Belser & Sudhalter, 1995; Belser & Sudhalter, 2001), few studies have directly examined this hypothesis. In one preliminary report examining skin responses in two males with FXS, Belser and Sudhalter (1995) found increased electrodermal responsivity (indicating greater arousal) was associated with more perseverative speech. Specifically with regards to cardiac measures of arousal, Hall et al. (2009) examined the associations between heart rate, vagal tone, and gaze avoidance in 50 children with FXS, and found no relationship between cardiac activity and the extent of gaze aversion during conversation with an examiner. However, evidence does indicate that physiological regulation in FXS is more broadly associated with symptoms of ASD. In a sample of 31 infants and toddlers with FXS, Roberts et al. (2012) found that vagal tone was the most reduced in children with FXS who showed symptoms of autism (when the FXS group was divided into autism subgroups, only those children with co-occurring autism had significantly lower vagal tone than controls). Furthermore, vagal tone predicted the severity of autism symptoms across developmental periods, with reduced vagal tone linked with more severe autistic traits in toddlerhood but not infancy (Roberts et al., 2012). Therefore, while much is still unknown about the relationship between physiological activity and social-communication in FXS, prior research suggests that physiological profiles in FXS may be broadly linked to autism-associated features, and possibly to more specific social-communicative deficits.

   ASD: Like in FXS, parasympathetic vagal activity has been found to be reduced in children and adults with ASD (Bal et al., 2010; Mathewson et al., 2011; Ming et al., 2005; Van Hecke et al., 2009), albeit less consistently (Althaus, Mulder, Mulder, Aarnoudse, & Minderaa, 1999; Levine et al., 2012; Toichi & Kamio, 2003). Studies of heart rate are similarly conflicting, as several reports have detected increased heart rate in comparison to typical and developmental disability comparison groups (Bal et al., 2010; Denver, 2004; Goodwin et al., 2006; James & Barry, 1980; Mathewson et al., 2011), while other reports have found heart rate in ASD to be similar to that of controls (Althaus et al., 1999; Bernal & Miller, 1970; MacCulloch & Williams, 1971). Nonetheless, it appears that

individual differences in cardiovascular activity may mediate communicative outcomes in ASD. That is, individual physiological differences may account for variability in communication profiles of persons with ASD. In a study of 23 children with ASD, Patriquin et al. (2011) found that lower baseline heart rate was associated with increased use of communicative gestures during play-based assessment, and baseline vagal tone was linked to better receptive vocabulary skills (Patriquin et al., 2011). Van Hecke et al. (2009) reported that higher vagal tone was related to better parent-reported social skills in a study of 19 children with ASD. Furthermore, vagal tone has been shown to prospectively predict later communication skills in ASD. In a study of 15 young boys with ASD, Watson et al. (2010) found that vagal tone during a social listening task (while listening to child-directed speech) accounted for significant variance in social-communication and expressive language outcomes one year later.

In sum, physiological regulation may play a role in the core pragmatic language deficits that are seen in ASD and FXS, although few studies have directly explored this hypothesis. Furthermore, no studies to our knowledge have directly compared cardiac physiological profiles in ASD and FXS, limiting our ability to determine whether physiological pathways are shared in ASD and FXS, and whether they are be linked with similar behavioral endpoints, such as pragmatic language. This study addressed these gaps in the literature by examining cardiac indicators of physiological arousal in ASD and FXS at rest and during a pragmatic context (conversation). Relations between arousal and pragmatic language were explored, as well as associations with the clinical presentation of anxiety (which is thought to be tied to physiological dysregulation).

Links between Physiological Arousal and Anxiety

The terms "arousal" and "anxiety" are often used interchangeably in studies of ASD and FXS, as both features are thought to reflect vulnerability to stress. The two mechanisms have strong theoretical ties, and anxiety is conceptualized as a key component in the relationship between physiological dysregulation and social outcomes. However, few studies of ASD or FXS have

examined the relationship between arousal and anxiety empirically, and those that did examine these relationships have not detected an association between cardiac physiological indices and anxiety. Briefly, Mathewson et al. (2011) found that self-reported anxiety was not correlated with baseline levels of heart rate or vagal tone in 15 high-functioning adults with ASD. Jansen et al. (2006) also found the heart rate responses of ten adults with ASD to be unrelated to self-reported stress during a public speaking task. Finally, Keysor and Mazzocco (2002) found that self-reported anxiety level of adolescent females with FXS ($n = 13$) was not related to their heart rate during a cognitive stressor task. While this research suggests that anxiety is not tied to cardiac physiological arousal in ASD or FXS, additional research is needed to replicate these findings in larger samples, and to incorporate measures of physiological reactivity (which might better tap immediate, context-dependent responses than tonic measures of vagal tone or heart rate). Given that clinically significant symptoms of anxiety are seen in as many as 53-85% of individuals with FXS (Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Merenstein et al., 1996), and 11-84% of individuals with ASD (van Steensel, Bögels, & Perrin, 2011; White, Oswald, Ollendick, & Scahill, 2009), further understanding of the relationship between physiological activity and anxiety is important for the development of targeted anxiety treatments. This line of research will also inform theories of how physiological dysfunction might bring about social-communication deficits in ASD and FXS.

Study Rationale and Hypotheses

The present study addressed the hypothesis that physiological dysregulation (and associated social anxiety) in FXS and ASD is linked with sub-optimal performance in social-communicative contexts. Pragmatic language impairment is a defining feature of both ASD and FXS, although it is unclear whether such deficits stem from similar underlying mechanisms in ASD and FXS, which has implications for targeted intervention. Given that physiological dysregulation is hallmark to FXS (Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001; Roberts, Tonnsen, Robinson, & Shinkareva, 2012), it has been hypothesized that deviant pragmatic language features in FXS stem from inefficient arousal modulation, which leads to anxiety

and sub-optimal social performance (e.g., Belser & Sudhalter, 1995). The present study addressed this hypothesis by examining cardiac arousal dysmodulation as a predictor of pragmatic language deficits in FXS. Given that physiological dysfunction is also seen in ASD (Bal et al., 2010; Mathewson et al., 2011; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Van Hecke et al., 2009), we extended this hypothesis to the study pragmatic language deficits in idiopathic ASD. No studies have directly compared cardiac activity in ASD and FXS, which has implications for understanding the role of the *FMR1* gene in the autonomic profiles in these disorders. Through cross-population comparison of physiological reactivity in FXS and ASD during a pragmatic (i.e., conversational) context, this study directly examined cardiac arousal as a biophysiological pathway that may be shared in FXS and ASD, and which may directly relate to core social-communicative deficits that are seen in these disorders. Such research eventually may inform the use of interventions designed to normalize autonomic dysfunction in the treatment of social-communicative impairments in FXS and ASD. The aims of this study are:

1. To determine whether cardiac physiological activity differs in ASD and FXS during resting and conversational contexts, and to explore the impact of autism on these physiological profiles.

    Hypothesis 1a: Children with ASD and FXS do not differ in heart rate or vagal tone in either resting or conversational conditions.

    Hypothesis 1b: Both children with ASD and children with FXS show significant increases in heart rate from baseline to conversation (suggesting increased arousal), but vagal tone does not differ across conditions (indicating a failure to modulate parasympathetic activity to support social engagement).

    Hypothesis 1c: Autistic traits negatively impact physiological activity in both ASD and FXS, as marked by increased heart rate, decreased vagal tone, heart rate reactivity, and dampened vagal reactivity.

2. To examine physiological activity as a predictor of pragmatic language impairment in ASD and FXS.

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Hypothesis: Increased heart rate, lower vagal tone, increased heart rate change (reflecting heightened arousal in response to conversation), and reduced vagal change across conditions (reflecting poor parasympathetic adaptation to the conversational task) predict poorer pragmatic language performance in both children with ASD and in children with FXS, independent of receptive/expressive language skills.

3. To explore the relationship between behavioral symptoms of anxiety, physiological activity, and pragmatic language in FXS and ASD.

Hypothesis 3a: The severity of anxiety is significantly associated with the change in heart rate and vagal tone (measuring reactivity), but is not associated with static estimates of heart rate or vagal tone.

Hypothesis 3b: Pragmatic language impairment increases with increased severity of anxiety.

Method

Participants

Thirty-three school-aged boys with idiopathic ASD and 31 boys with full mutation FXS participated in the study. Participants represented a subgroup of children who were participating in a larger study of pragmatic language in FXS and ASD (Losh, Martin, Klusek, Hogan-Brown, & Sideris, 2012). Participants were chosen for inclusion in the present study if they had completed the autism and physiological assessment protocols (described below). An additional seven children with ASD were recruited from a related study of language in ASD in order to supplement participant numbers (PI: Losh, NIDCD 1R01DC010191-01). Only boys participated in the study because girls with FXS are generally less severely affected and show more heterogeneous profiles (Hagerman, 2004). As part of the inclusion criteria of the larger study, all participants spoke English as their primary language and were regularly using phrase speech (i.e., using sentences of three or more words). Hearing was screened at 500, 1000, 2000 and 4000 Hz with a MAICO MA 40 audiometer; participants who failed screening at 30 dB in the better ear were excluded. Recruitment was focused in the Eastern and Midwestern regions of the United States, through local advertisement and through

the Research Participant Registry Core of the Carolina Institute for Developmental Disabilities at the University of North Carolina at Chapel Hill. The groups did not differ in race, household income, or maternal education ($ps > .123$); see Table 4.1 for demographic characteristics.

Table 4.1. Demographic Characteristics

	ASD	FXS
Race %		
Caucasian	78.8	83.9
African American	9.1	--
Asian	--	9.7
Multi-racial	3.0	3.2
Not Reported	9.1	3.2
Income %	6.1	--
<20*k*	15.2	3.2
20*k*-39*k*	3.0	6.5
40*k*-59*k*	21.2	9.7
60*k*-79*k*	24.2	48.4
>80*k*	30.3	32.3
Not Reported		
Maternal Education Level %		
High School/ GED	21.2	9.7
Associate	12.1	16.1
Bachelor	33.3	19.4
Master	15.2	16.1
Doctorate	3.0	9.7
Not Reported	15.2	29.0

Group characteristics are presented in Table 4.2. The ages of the participants ranged from 4.08-17.82 years. On average, the ASD group was younger than the FXS group. Chronological age was controlled for statistically in group comparisons, as cardiac activity matures with age (Alkon et al., 2003; Bar-Haim, Marshall, & Fox, 2000; Sahni et al., 2000). Nonverbal mental age of the ASD group was higher than that of the FXS group, as measured by the Leiter International Performance Scale-Revised (Leiter-R; Roid & Miller, 1997) or the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). The groups did not differ on receptive vocabulary ability on the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997, 2007), although the ASD group did show higher expressive language skills on the Expressive Vocabulary Test (EVT; Williams, 1997) and higher mean length of utterance (see *Procedures*). The pragmatic language skills of the groups were similar, as measured by the Pragmatic Judgment subtest of the Comprehensive Assessment of Spoken

Language (CASL-PJ; Carrow-Woolfolk, 1999) and the Pragmatic Rating Scale- School Age (PRS-

SA; Landa, 2011), described below.

Table 4.2. Group Characteristics

	ASD n = 33	FXS n = 31
	M (SD) Range	M (SD) Range
Chronological age (years)	9.61 (3.05)[a] 4.08-14.56	12.36 (2.66)[b] 6.46-17.82
Leiter-R/WASI	8.96 (4.18)[a] 3.67-19.67	5.19 (0.66)[b] 3.50-6.67
PPVT	8.23 (3.97)[a] 2.58-22.00	6.50 (1.48)[a] 3.92-9.33
EVT	7.75 (3.93)[a] 3.17-19.75	5.57 (1.33)[b] 3.58-8.25
MLU	5.04 (1.67)[a] 1.81-9.33	3.61 (0.87)[b] 1.80-5.56
CASL-PJ	5.76 (2.95)[a] 2.58-16.00	4.95 (1.47)[a] 2.42-7.67
PRS-SA	33.42 (7.51)[a] 16.00-49.00	31.11 (8.84)[a] 12.00-47.00
Medication %		
Antidepressant	3	3
Stimulant	12	10
Antipsychotic	--	6
Antianxiety	--	3
More than one	15	13

Note: Leiter-R/WASI = Leiter International Performance Scale- Revised/ Wechsler Abbreviated Scale of Intelligence; PPVT = Peabody Picture Vocabulary Test; EVT = Expressive Vocabulary Test; MLU = mean length of utterance; ADOS = Autism Diagnostic Observation Schedule; PRS-SA = Pragmatic Rating Scale- School Age; CASL-PJ = Comprehensive Assessment of Spoken Language, Pragmatic Judgment subtest. Means in the same row with different superscripts differ significantly at $p < .05$.

A similar percentage of boys from both groups were taking psychoactive medications at the

time of assessment (30% boys with ASD and 35% with FXS). Due to missing data, the medication

status of five boys with ASD and 14 boys with FXS was unknown; the remaining participants were

not reported by their caregivers to be using medications. Medication use is summarized in Table 4.2.

Evidence suggests that psychoactive medication use may influence heart activity, although the extent of influence varies according to dosage and individual characteristics, such as weight and metabolic functioning (O'Brien & Oyebode, 2003; Rechlin, 1995; Silke, Campbell, & King, 2002). Given that a third of the sample was taking medications and broad exclusionary criteria would significantly reduce sample size, we took an approach consistent with that of Hall et al. (2009); all participants were included regardless of medication use and follow-up analyses were conducted to determine the extent that medication use may have influenced results.

Procedures

All procedures were approved by the Institutional Review Boards of the University of North Carolina at Chapel Hill and of Northwestern University. Testing took place in either a research laboratory, or in a quiet room in the participant's home or school, according to caregiver preference. Assessments were conducted within the context of a broader protocol, which generally lasted four to six hours. Caregivers were given the option of completing the assessments over the course of several days. In general, the structured language and cognitive assessments (e.g., PPVT, Leiter-R) were administered at the beginning of the protocol, followed by less structured tasks. This administration order allowed participants some time to "warm up" by starting with assessments that required simple non-verbal responses (pointing). However, the protocol was flexible and examiners were permitted to modify the order of assessments and the frequency of breaks according to the child's needs.

Characterization of ASD: Clinical diagnoses of ASD were confirmed with the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DeLavore, & Risi, 2001). Autism comorbidity in the FXS group was also determined using the ADOS, so that relations between autism and arousal in FXS could be explored. All examiners were trained to reliability administer and code the ADOS either through direct training with the developers of the ADOS or through intra-lab reliability conducted in accordance with the recommendations of the instrument developers. The "autism spectrum" cut-offs of the revised diagnostic algorithms were used to determine the presence of ASD (Gotham et al., 2008; Gotham, Risi, Pickles, & Lord, 2007). Nine of the boys with FXS had

been administered the ADOS by our research group at three or more time points, through participation in a related longitudinal study (see Roberts et al., 2007). In order to use a best-estimate diagnosis for these boys, all available information was considered in determining autism status (boys who met criteria for ASD in the majority of assessments were determined to have comorbid ASD). Autism severity scores were also computed as described by Gotham, Pickles, and Lord (2009). An average severity score was used for the boys who had multiple available ADOS scores. Twenty-three of the boys with FXS met criteria for ASD.

Cognitive Assessment: The Brief IQ Composite of the Leiter International Performance Scale-Revised (Leiter-R; Roid & Miller, 1997) was used to assess nonverbal cognition. Leiter-R scores were not available for the seven boys with ASD who were recruited as supplemental participants from the related language study, and the Performance IQ scale of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was used as a substitute measure of nonverbal cognitive ability for these participants.

Receptive and Expressive Language Assessments: The Peabody Picture Vocabulary Test-III or the Peabody Picture Vocabulary Test-IV (PPVT; Dunn & Dunn, 1997, 2007) were employed as a receptive language measure. Expressive language was measured with the Expressive Vocabulary Test (EVT; Williams, 1997). Age equivalent scores for the PPVT and EVT were used in analysis. Three boys with ASD were missing PPVT data, and two were missing EVT. Mean length of utterance in morphemes (MLU) was also computed as a general index of expressive language development (Brown, 1973; Scarborough, Rescorla, Tager-Flusberg, Fowler, & Sudhalter, 1991). Systematic Analysis of Language Transcripts (SALT; Miller & Chapman, 2008) was used to compute MLU from 110 intelligible child conversational turns that occurred during the ADOS. Turns were sampled equally from play and non-play contexts of the ADOS, in order to ensure a similar sampling context across groups. Language samples were transcribed by research assistants who had achieved morpheme-to-morpheme agreement of 80% or higher for eight language samples as compared to

"gold-standard" transcripts. MLU was calculated using novel, intelligible, non-routine utterances, according to SALT conventions.

Pragmatic Language Assessments: The Pragmatic Judgment subtest of the Comprehensive Assessment of Spoken Language (CASL-PJ; Carrow-Woolfolk, 1999) was administered as a measure of the knowledge and use of language in social contexts. In this standardized assessment, participants answer questions about what should be said or done in various social situations. Age-equivalent scores were used in analysis. These data were missing for six participants with ASD and two participants with FXS due to time constraints.

The Pragmatic Rating Scale- School Age (PRS-SA; Landa, 2011) was also administered as a semi-naturalistic measure of pragmatic language ability. The PRS-SA consists of 33 items that sample a range of pragmatic features, such as the ability to initiate topics, the provision of necessary background information, or the use of appropriate rate and volume of speech. Items are scored on a scale of "0" to "2" (with a higher score indicating greater pragmatic language difficulty) and are tallied to obtain a total score. The PRS-SA was scored from video, based on interaction that occurred during the ADOS. Six children from each group were administered the ADOS module 2 (for use with individuals who have phase speech) and the remaining children were administered the ADOS module 3 (for verbally fluent individuals); there were no significant differences between the PRS-SA scores of children who had been administered a module 2 versus module 3. The first author (JK), who had achieved reliability with the developer of the PRS-SA, coded all of the samples. The coder was blind to the diagnosis of 16/33 participants with ASD and 23/31 participants with FXS (the coder had assisted in participant recruitment and testing and it was not possible to maintain blinding to all participants). Twenty percent of the sample was randomly selected and second-scored by an independent rater who was blind to all diagnoses and who had also achieved reliability with the developer of the PRS-SA. Intra-class correlations were computed to determine inter-rater reliability [ICC (3, 2)], and overall reliability was .96 (.91 for ASD, and .91 for FXS).

Physiological Assessment: Heart activity data were collected during a baseline and a conversational condition. During baseline, participants watched ten minutes of an animated children's film on a portable movie player. The examiner remained in the room during the baseline condition but did not engage with the participant. Immediately following the baseline period, participants conversed with an examiner about the movie clip or about any other topic of interest for ten minutes. Examiners maintained the conversation by commenting, asking questions, and bringing up topics of possible interest to the participant. No specific demands were placed on the participant during the conversational condition, other than that they remain seated and try not to touch the electrodes (the participant was not specifically redirected if they failed to maintain eye contact, etc.). Electrocardiogram data was collected with an Alive Wireless Heart Monitor (Alive Technologies, Copyright 2005-2009), either via two electrodes that were placed on the participant's chest or with an elastic Polar belt that contained electrode receptors (participants who refused to wear the electrode were given the option of wearing the belt). Data were sampled at a rate of 300 times per second. Data collection for 12 boys with FXS and three boys with ASD (not included in sample *n*s) was attempted but not completed due to random equipment malfunction, excessive number of movement/recording artifacts (> 20%), or uncooperativeness.

Electrocardiogram data from the last five and a half minutes of baseline and the first eight minutes of conversation were included in analysis. Data were edited with CardioEdit software (Brain-Body Center, University of Illinois at Chicago) by a research assistant who had achieved reliability with the creators of the software. Data were first visually inspected to identify invalid heart periods (e.g., faulty R-wave detection) and artifacts were adjusted manually with integer-arithmetic. Estimates for respiratory sinus arrhythmia (RSA) and inter-beat-interval (IBI) were extracted with CardioBatch (Brain-Body Center, University of Illinois at Chicago), as described by Porges (1985). Specifically, IBI was measured as the time in ms between successive R-waves in the electrocardiogram signal. To extract RSA, CardioBatch samples sequential heart periods at 250 ms epochs to create equal-interval time series values. Data is then de-trended with a 21-point moving

polynomial algorithm (Porges & Bohrer, 1990). De-trended data is bandpass filtered to extract variance associated with spontaneous breathing parameters (0.24-1.04 Hz), and the bandpassed variance is transformed to it its natural logarithm to yield an estimate of RSA. RSA and IBI were measured during 30 s epochs for each condition, and the averages within each condition were used in analyses. Change scores measuring reactivity were computed by subtracting the conversational estimates from the baseline, for IBI and RSA (with positive RSA change scores indicating the extent vagal reduction, and positive IBI change scores reflecting increase in heart rate). IBI is inversely associated to heart rate-- as IBI increases (marking a longer time interval between successive heart beats), heart rate becomes slower. IBI is a non-specific index of arousal that reflects both sympathetic and parasympathetic influences on the heart; heart rate becomes faster (and IBI becomes shorter) under stress (Bernston, Cacioppo, & Quigley, 1993; Porges & Raskin, 1969). RSA is an index of vagal tone; higher resting vagal tone marks greater parasympathetic input to the heart and awareness of the environmental (Porges, 1995).

Assessment of Clinical Symptoms of Anxiety: Symptoms of clinical anxiety were assessed with the Child Behavior Checklist-1 ½ -5 years (CBCL; Achenbach & Rescorla, 2000), which was completed by the primary caregiver. The DSM-IV-oriented anxiety subscale was used, which corresponds to the diagnostic criteria for anxiety outlined in the Diagnostic and Statistical Manual of Mental Health Disorders (American Psychological Association, 1994). This subscale contains 10 items that are rated on a two-point scale, yielding a possible range of 0-20 for the anxiety subscale total raw score. The preschool version of this assessment was administered, as the items for younger children were more closely aligned with the mental ages of the study participants. Raw scores were used in analysis for two reasons: first, a number of participants were outside of the age range of the normative sample. Secondly, the test publishers recommend the use of raw scores for research purposes, as the normative *t*-scores are truncated at 50 (Achenbach & Rescorla, 2000). These data were missing for seven participants with ASD and 11 participants with FXS.

Data Analysis

First, descriptive statistics were computed to examine the physiological variables. Then, group differences in physiological activity were tested using a repeated measures analysis of covariance (ANCOVA), with condition (baseline, conversation) as a within-participant factor and RSA and IBI as outcomes, co-varying for chronological age. Spearman correlations were used to explore the relationships among the physiological and language variables. Chronological and mental age were not controlled for in these correlations because neither chronological age nor mental age was significantly associated with any of the physiological variables ($ps > .081$). Follow-up regression analyses were conducted to test more specific predictive relationships between physiological activity and pragmatic language, and possible mediation by receptive/expressive language ability. Given that structural language ability includes the social use of language (McTear & Conti-Ramsden, 1992), mediation modeling allowed for the influences of structural language to be parsed apart from the relationship between physiological activity and pragmatics. Mediation was tested according to the procedures outlined by Baron and Kenny (1986). Specifically, the following conditions must be met to support a mediating relationship: a) the independent variable predicts the mediator, b) the independent variable predicts the dependent variable, and c) the mediator predicts the dependent variable. If these conditions are met, mediation holds if the independent variable does not have a significant effect on the dependent variable after controlling for the mediating variable. Mediation was tested with a series of linear regression models. In order to test structural language as a potential mediator, a composite score of the EVT and PPVT was created by totaling the raw scores for each test.

Associations between the physiological variables and anxiety (CBCL-anxiety subscale) were explored with Spearman correlations. Group differences on the CBCL-anxiety subscale were also tested, using ANCOVA and including chronological age as a covariate. To explore the possibility that physiological patterns might differ in individuals with FXS with and without comorbid ASD, group

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comparisons on the physiological variables were repeated, comparing the subset of individuals with FXS who met criteria for autism spectrum disorders (FXS-ASD) to the idiopathic ASD group. Separate analyses were not conducted in the subgroup of FXS without autism, given the limited sample size in this group ($n = 8$), although physiological patterns were examined descriptively. The impact of autism was also explored by examining the Spearman correlations between the physiological variables and continuously-distributed autism symptoms (ADOS severity score) within the full FXS group and within ASD. Finally, in order to determine the extent to which the detected patterns may have been influenced by medication, all analyses were repeated after excluding participants who were taking psychotropic medications.

Results

Aim 1: To Determine Whether Cardiac Activity Differs in ASD and FXS

Descriptive statistics for the physiological variables are presented in Table 4.3. Examination of individual patterns of physiological activity showed that 20/33 (61%) of the participants with ASD and 23/31 (74%) of the participants with FXS increased RSA from baseline to conversation. The majority of participants from both groups decreased IBI (i.e., increased heart rate) from baseline to conversation— 26/33 (79%) of participants with ASD and 28/31 (90%) of participants with FXS).

Table 4.3. Descriptive Statistics of Physiological Variables across Conditions and Groups

Condition	Group	Physiological Index			
		Inter-Beat-Interval		Respiratory Sinus Arrhythmia	
		Observed *M (SE)*	Adjusted *M (SE)*	Observed *M (SE)*	Adjusted *M (SE)*
Baseline	ASD	699.13 (137.31)	711.08 (21.22)	4.18 (1.38)	4.19 (0.26)
	FXS	665.66 (102.59)	652.94 (21.94)	4.06 (1.43)	4.06 (0.26)
Conversation	ASD	651.70 (79.71)	661.78 (14.04)	4.58 (1.23)	4.52 (0.21)
	FXS	626.89 (85.29)	616.16 (14.52)	4.62 (1.60)	4.69 (0.22)
Change (Baseline-Conversation)	ASD	47.43 (83.51)	49.30 (12.21)	-0.40 (1.13)	-0.33 (0.19)
	FXS	38.77 (44.50)	36.79 (12.62)	-0.57 (1.04)	-0.63 (0.20)

Note: Adjusted means depict estimates controlling for chronological age.

Group Comparisons Across Conditions: ANCOVA revealed a significant main effect for condition on RSA [F (1, 61) = 4.45, p = .039], with higher RSA estimates during conversation (reflecting adaptive increases in parasympathetic tone in conversation). The effect of group and its interaction with condition were non-significant (ps > .210), indicating that RSA did not differ across both groups, and that groups showed similar levels of RSA across conditions. A significant group effect was detected for IBI [F (1, 61) = 4.26, p = .043], with FXS showing lower IBI (i.e., faster heart rate) than ASD. Condition and its interaction with group did not have a significant effect on IBI (ps > .477), indicating that heart rate did not increase during conversation in either group, and FXS showed higher heart rate than ASD in both conditions.

Associations with Autism Severity: Autism severity was not associated with any of the physiological variables in ASD (ps > .286). In FXS, autism severity was significantly associated with RSA during the conversation (r = -.37, p < .040), with lower vagal tone associated with increased autism severity.

Group Comparisons of ASD-subgroups: Group comparisons were conducted to test differences on the physiological variables between the idiopathic ASD group and the subgroup of children with FXS who had comorbid ASD (FXS-ASD). Results were identical to the group comparisons conducted with full FXS group. Specifically, a significant main effect for condition was detected for RSA [F (1, 53) = 5.67, p = .021], with higher RSA estimates during conversation. The effects of group and its interaction with condition were non-significant (ps > .242). Similar to the analyses including the full FXS sample, a significant group effect was detected for IBI [F (1, 53) = 4.73, p = .034], with FXS showing lower IBI. The effects of condition and its interaction with group on IBI were non-significant (ps > .560).

Although the small number of participants with FXS without comorbid ASD (FXS-only, FXS-O) prevented group differences from being tested statistically, physiological patterns were examined descriptively in FXS-O, FXS-ASD, and ASD groups (see Figure 4.1). Overall, IBI in the FXS-O subgroup was higher than that of FXS-ASD (indicating slower heart rate in FXS-O), and

more similar to the IBI estimates seen in ASD-O. While mean RSA at baseline in FXS-O was relatively similar to that of the ASD-O and FXS-ASD groups, RSA during conversation in FXS-O was higher than that of either the ASD-O or FXS-ASD group.

Figure 4.1. Mean IBI and RSA estimates in ASD and FXS with and without Comorbid ASD
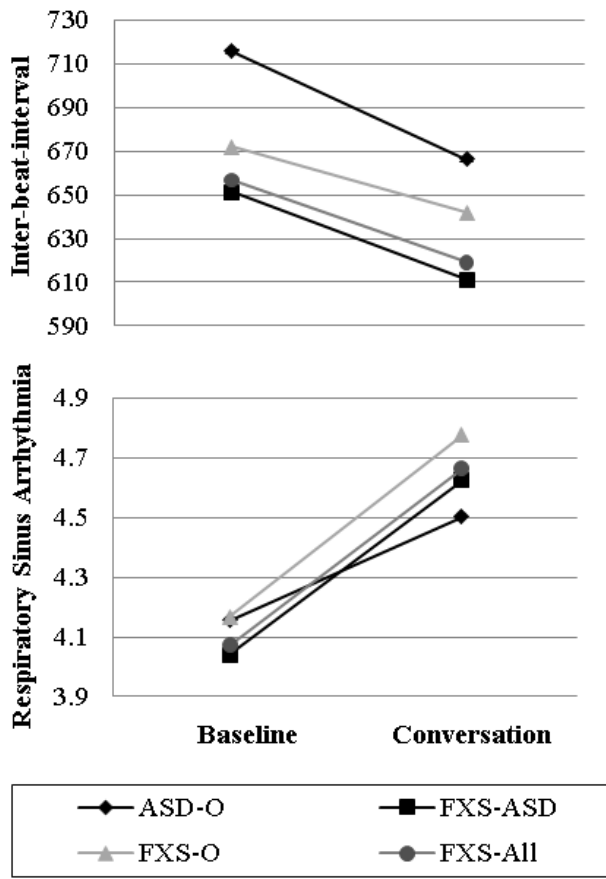
Note: Means adjusted for chronological age.
Lower IBI reflects faster heart rate.

Aim 2: To Examine Physiological Activity as a Predictor of Pragmatic Language Impairment

  Exploratory Associations with Pragmatic Language: Correlations between physiological activity and the pragmatic language variables are presented in Table 4.4. In ASD, PRS-SA scores were negatively associated with conversational RSA, indicating that higher vagal tone in the conversational context was linked to better pragmatic language ability in a semi-naturalistic social

interaction. In ASD, performance on the CASL-PJ was associated with IBI baseline and change scores (with better performance on the standardized pragmatic assessment linked with lower baseline heart rate, and with greater elevation in heart rate from baseline to conversation). In FXS, several trend-level associations were detected. The correlation between IBI change and PRS-SA approached significance ($p = .056$), supporting a trend for worse pragmatic ability as participants with FXS showed greater elevation in heart rate from baseline to conversation. A marginal association was also detected between RSA change score and CASL-PJ in FXS ($p = .067$), indicating lower language ability with dampened increases in vagal tone from baseline to conversation.

Exploratory Associations with Structural Language: Correlations between physiological activity and receptive/expressive language were conducted, to determine which relationships should be examined more closely as potential mediators. In ASD, IBI change score was significantly associated with PPVT and EVT performance, with skills increasing as participants showed greater elevation in heart rate from baseline to conversation. Baseline IBI was also marginally associated with PPVT performance in ASD ($p = .090$). No significant associations were detected in the FXS group, although marginal associations were observed between RSA change score and PPVT ($p = .061$) and EVT ($p = .081$), suggesting a trend for lower skills as participants showed less pronounced vagal increase from baseline to conversation. Correlations are reported in Table 4.4.

Table 4.4. Relationship between Physiological Activity and Language

| | Language Variable | | | | | |
	PRS-SA	CASL-PJ	PPVT	EVT	MLU	PRS-SA	CASL-PJ	PPVT	EVT	MLU
	\multicolumn{5}{c}{ASD}				\multicolumn{5}{c}{FXS}				
Baseline IBI	.05	.43*	.32†	.29	-.09	-.09	.04	.12	.03	.12
Baseline RSA	-.19	.29	.20	.28	.02	.19	-.20	-.26	-.11	-.14
Conversation IBI	-.05	.19	.15	.12	-.24	-.29	.04	.03	.23	.04
Conversation RSA	-.51**	.17	.06	.08	.12	.04	.04	-.10	.10	.12
Change IBI	-.01	.61**	.43*	.39*	.13	.35†	-.17	.08	-.09	.05
Change RSA	-.12	.31	.18	.22	-.12	.06	-.34†	-.32†	-.34†	-.20

Note: IBI= inter-beat interval; RSA= respiratory sinus arrhythmia; PRS-SA= Pragmatic Rating Scale- School Age; CASL-JP= Comprehensive Assessment of Spoken Language, Pragmatic Judgment subtest; EVT= Expresive Vocabulary Test; MLU= mean length of utterance. *$p < .05$, **$p < .01$, $^\dagger p < .09$

Physiological Activity as a Predictor of Pragmatic Language: Following-up on the exploratory correlations detected in ASD, linear regression models were run to test physiological activity as a predictor of pragmatic language, with structural language as a potential mediator of this relationship. Conversational RSA was a significant predictor of PRS-SA in ASD [R^2 = .40, $F\Delta$ (1, 31) = 5.76, p = .023]. However, conversational RSA did predict structural language [R^2 = .02, $F\Delta$ (1, 29) = 0.55, p = .464]; conditions for mediation were not upheld (Baron & Kenny, 1986). In other words, the relationship between conversational RSA and PRS-SA in ASD was not mediated by receptive/expressive language skills.

Next, mediation between baseline IBI and CASL-PJ score in ASD was tested. Linear regression showed that baseline IBI was a significant predictor of CASL-PJ in ASD [R^2 = .48, $F\Delta$ (1, 25) = 7.48, p = .011]. However, baseline IBI was not a significant predictor of structural language in ASD [R^2 = .11, $F\Delta$ (1, 29) = 3.44, p = .074], and thus conditions for mediation were not upheld-- structural language did not mediate the relationship between baseline IBI and CASL-PJ performance in ASD.

Finally, structural language was tested as a mediator of the association between IBI change score and pragmatic language on the CASL-PJ in ASD. Conditions for mediation were upheld: IBI change score was a significant predictor of structural language [R^2 = .19, $F\Delta$ (1, 29) = 6.99, p = .013] and pragmatic language on the CASL-PJ [R^2 = .35, $F\Delta$ (1, 25) = 13.41, p = .001]. Structural language significantly predicted CASL-PJ [R^2 = .82, $F\Delta$ (1, 25) = 110.10, p < .001]. After partialing out the variance associated with structural language, IBI change was no longer a significant predictor of pragmatic language on the CASL-PJ (see Table 4.5), which supports receptive/expressive language as a mediator of the relationship between IBI change and CASL-PJ performance in ASD.

Table 4.5. Regression Coefficients Testing Structural Language as a Mediator of the Relationship between IBI Change and CASL-PJ score in ASD

		B (SE)	β	R^2	$R^2\Delta$	$F\Delta$
Step 1	Constant	-1.63 (0.75)		.82	.82	110.10***

	PPVT/EVT Composite	0.05 (0.01)	0.90			
Step 2	Constant	-1.78 (0.87)		.82	.01	0.12
	PPVT/EVT Composite	0.05 (0.01)	0.93			
	IBI Change	-0.01 (0.01)	-0.04			

Note: PPVT/EVT = Peabody Picture Vocabulary Test/ Expressive Vocabulary Test;
***$p < .001$

Aim 3: To Explore the Relationship between Physiological Activity and Anxiety

Group comparisons indicated that the ASD and FXS groups did not differ on the CBCL-anxiety subscale [F (1, 46) = 0.01, p = .941]. Anxiety was not significantly associated with any of the receptive, expressive, or pragmatic language variables in either group (ps > .136), and no relationships were detected between anxiety and autism severity (ps > .700). While anxiety was not associated with any of the physiological variables in ASD (ps > .452), the severity of anxiety in FXS was associated with change scores for RSA (r = .51, p = .022) and IBI (r = .50, p = .024). These associations indicate that FXS participants who showed more substantial increases in arousal and dampened increases in parasympathetic vagal tone from baseline to conversation were rated by their caregivers as showing greater symptoms of anxiety.

Impact of Medication Status on Physiological Activity

To determine the extent to which the detected patterns may have been influenced by mediation use, analyses were repeated after excluding participants who were taking psychotropic medications (leaving 23 boys with ASD and 20 boys with FXS). Similar patterns were detected, with only minor fluctuations in p-values that appeared to result from a reduced sample size. Specifically, group comparisons on RSA showed identical results as comparisons in the full sample. Although the group effect for IBI was no longer significant (p = .118), group means followed a similar direction. Correlations between the physiological and language variables were similar in direction and significance in the ASD group, with the exception that the associations between IBI change score and EVT and PPVT were no longer significant (p = .191; p = .250, respectively). The marginal associations between RSA change and the PPVT, EVT, and CASL-PJ that were previously detected in FXS remained similar in strength and significance (ps <.091). The association between IBI in

conversation and the PRS-SA was now significant in FXS ($r = -.43, p = .049$). The association between autism severity and conversational RSA in FXS no longer reached significance, although the direction and strength of the relationship was similar ($r = -.34, p = .132$). The associations between IBI and RSA change and anxiety in FXS were also no longer significant ($ps > .405$), although it is important to note that the sample size for this particular correlation was drastically reduced ($n = 12$), given that a number of children with FXS were missing CBCL data. Overall, results appeared to follow a similar pattern when excluding participants who were taking medications, and the fluctuations in p-values appeared to be due to loss of statistical power rather than to differences related to medication status.

Discussion

This study examined physiological arousal as a potential neurobiological marker of social-communication impairment in children with ASD and FXS. While similar levels of parasympathetic activity were detected in ASD and FXS, the children with FXS had heightened general arousal level in comparison to children with ASD at rest and during conversation. Conversation with an examiner did not elicit increased arousal in either the ASD or FXS groups as a whole, although arousal reactivity was linked to anxiety in FXS. Vagal tone and heart rate were significant predictors of pragmatic language ability in ASD, adding to emerging support for a role of autonomic nervous system dysfunction in communicative impairment in ASD.

Group Differences in Physiological Activity

To our knowledge, this is the first report to directly compare cardiac indices of arousal in ASD and FXS. As a single-gene disorder that is associated with significantly elevated risk for ASD, the study of FXS is thought to reduce "genetic noise" to speed the identification of pathophysiological mechanisms implicated in ASD. The finding of identical patterns of cardiac vagal tone across ASD and FXS groups supports parasympathetic neural control as an intermediate biological process that is shared in these disorders. Though parasympathetic tone did not differ across groups, the boys with FXS did show elevated general arousal relative to ASD (which appeared to represent a chronic

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physiological condition in FXS, as heart rate was consistently elevated and did not fluctuate across experimental conditions). The fact that heart rate was not associated with autism severity in FXS might suggest that hyperarousal is an FXS-specific process that occurs independently of autism. Given than parasympathetic tone did not differ across groups, it may be that the elevated arousal in FXS was driven by increased sympathetic tone relative to the ASD group. This interpretation is consistent with evidence from skin conductance studies showing elevated sympathetic tone in FXS (Miller et al., 1999; Roberts, Mazzocco, Murphy, & Hoehn-Saric, 2008). While this study cannot directly address this hypothesis, future research might include measures of both parasympathetic and sympathetic tone to pinpoint specific areas of breakdown in the autonomic system in FXS. Some questions also remain as to whether the observed physiological patterns represented atypical processes, as this study did not include a control group. Specifically, we cannot rule out the possibility that arousal in both groups was atypically elevated, with the FXS group showing the most extreme profiles. It is also unknown whether the shared parasympathetic profiles in ASD and FXS would have differed from controls-- this seems likely given that atypical vagal control is a consistently documented feature of FXS (Boccia & Roberts, 2000; Hall et al., 2009; Heilman et al., 2011; Roberts et al., 2001; Roberts et al., 2006) that is also seen in ASD (Bal et al., 2010; Ming et al., 2005; Van Hecke et al., 2009).

Conversation as an Elicitor of Arousal in ASD and FXS

With regards to the physiological measures, a notable finding was that unstructured conversation with an examiner did not elicit increased general arousal in either ASD or FXS groups. This is consistent with a report by Sigman et al. (2003), who found that young children with ASD did not increase heart rate during interactions with a stranger or with their mother. However, it conflicts with a report by Hall et al. (2009) that found increased heart rate in FXS in response to a conversational stressor task. This discrepancy may be related to differences in experimental condition; the conversational task of the Hall et al. study involved regular prompts to maintain eye contact and explicitly used direct questions to initiate topics, which may have created greater pressure

for conversational participation. The more naturalistic interaction used in this study did not elicit a physiological stress response. In fact, both groups showed increased vagal tone in response to conversation, suggesting that the children adapted physiologically to meet the social demands of the conversational interaction (although without comparison to a control group it unclear whether the observed responses may have been dampened in comparison to that of typically developing individuals). Overall, the observed physiological patterns suggest that naturalistic conversation does not elicit hyperarousal in either ASD or FXS. This finding might imply that atypical social behaviors of these disorders are not related to socially-induced hyperarousal, at least during unstructured conversation with an examiner. This does not, however, preclude a relationship between general physiological health and social engagement; it merely implies that social interaction itself does not appear to be a catalyst for increased arousal.

Links between Physiology and Pragmatic Language

An important finding of this study is that parasympathetic vagal tone during conversation predicted pragmatic language ability in ASD. It is interesting that pragmatic language was specifically related to vagal tone in the conversational context, but not at baseline. This finding is similar to that of Watson et al. (2010), who found that vagal tone during a social context (but not while watching a non-social video) accounted for significant variance in parent-reported social-communication outcomes of young children with ASD. As suggested by Watson et al. (2010), children with ASD who show higher vagal tone in social contexts may present with a physiological state that is more optimal for engaging with social stimuli. Consistent with transactional theories of social learning, increased social engagement is thought to lead to greater opportunities for social learning over time, including the learning of pragmatic conversational rules (Chapman, 2000; Dickinson & McCabe, 1991; Fogel, 1993; Hewitt, 1998; McTear & Conti-Ramsden, 1992; Yoder & Warren, 1993). Perhaps resting vagal tone was not related to pragmatic language outcomes because social learning might depend specifically on the ability to make physiological adjustments to adapt to social demands.

Although no significant associations between physiology and pragmatic language were detected in FXS, a number of trend-level relationships did emerge between heart rate/vagal tone and pragmatic language, suggesting that physiological modulation may also be related to pragmatic ability in FXS. These findings, particularly those within the ASD group, add to growing evidence to support autonomic flexibility as a mediator of social-communicative ability. The identification of neurophysiological markers associated with social dysfunction in ASD and FXS has implication for the development of interventions targeted at correcting breakdowns at the physiological level. Vagal tone appears to respond to non-invasive treatments such as massage therapy or breathing exercises in typical development (Chambers & Allen, 2002; Feldman & Eidelman, 2003; Lee, 2005; Miu, Heilman, & Miclea, 2009), and some evidence suggests that pharmaceutical intervention may improve arousal modulation in FXS (Roberts et al., 2011). The results of this study support further investigation of treatments targeting autonomic system dysfunction as a means to improve communication outcomes in ASD and FXS.

While significant associations between physiological activity and language were detected in ASD, these relationships within the FXS group only approach significance. One explanation might be that physiological arousal is not associated with language in FXS, although this seems unlikely given the number of trend-level associations that were detected. Alternatively, the lack of significant associations may have been related to the reduced variability in the language scores in FXS as compared to ASD. When considering the marginal associations that were detected in FXS, differential patterns begin to emerge across the disability groups. Specifically, performance on the structured language measures was associated greater increase in arousal in ASD, but with greater increases in parasympathetic tone (generally associated with *decreased* arousal) in FXS. Given that baseline arousal of the groups differed, perhaps the groups relied on different regulatory processes to achieve an arousal level that was most optimal for test-taking (with either too much or too little arousal negatively impacting performance). While the ability to increase arousal was important for the performance of the children with ASD, it appears that performance in the already hyperaroused

FXS group was related to the ability to suppress arousal via the vagal brake. Therefore, these associations might provide preliminary evidence that physiological regulation is related to language ability in both ASD and FXS, but with perhaps different influences of the parasympathetic and sympathetic systems across groups.

The exploratory associations between physiological activity and language ability revealed patterns that appeared to be related to language assessment method. In general, associations with the three structured language measures grouped together, with differential patterns detected between cardiac activity and performance on the semi-naturalistic language measures (i.e., MLU and PRS-SA). For example, IBI change was associated with PPVT, EVT, and CASL-PJ performance, but not with scores on the PRS-SA or MLU (despite the fact that these measures tap similar language domains as the structured measures). These patterns may be related to the different demands involved in structured versus naturalistic language assessments-- the structured measures may rely more heavily on cognitively mediated test-taking skills (such as attention), whereas naturalistic assessments may be more influenced by the ability to respond to the social demands of the language-sampling context. Perhaps specific physiological functions allowed for enhanced focus during attention-demanding structured assessments, but yet were not helpful in meeting the complex social demands of the naturalistic language-sampling context. These results suggest that structured versus naturalistic assessment methods might differentially related to physiological activity, highlighting the importance of using a multi-modal assessment approach. Better understanding of task-related effects on physiological activity might clarify some discrepancies in the extant literature, and may also inform the design and interpretation of future research.

Effects of Autism Severity on Physiological Activity

Conversational vagal tone was associated with autism severity in FXS, with patterns suggesting that children with FXS who showed less symptoms of autism were more physiologically prepared for engagement during the conversational condition. This is consistent with a report by Roberts et al. (2012), which found that vagal dysfunction in infants and toddlers with FXS was

associated with autism symptoms. It is curious that vagal tone was not related to autism severity in the ASD group. One explanation might be that the narrow range of autism severity scores in ASD limits power to detect covariance (by definition, the ASD group only included children with ADOS severity scores within the higher range). This interpretation is supported by the fact that vagal tone was associated with autism-associated pragmatic deficits on the PRS-SA, which is a tool that allows for a greater range of scores than the ADOS severity score. Alternatively, the finding that autism symptoms were associated with parasympathetic activity in FXS but not ASD might provide preliminary support for divergent physiological underpinnings of autistic behaviors in idiopathic and fragile X-associated ASD. This area of research warrants further attention, as understanding how independent biophysiological mechanisms may lead to the common presentation of autism will be informative for the development of pharmaceutical or behavioral interventions targeting these pathways.

Links with Anxiety

Although both groups showed average increases in parasympathetic tone from baseline to conversation, there was great individual variability in responses; nearly a third of children from both groups *decreased* vagal tone in conversation, which is consistent with mobilization and physiological hypervigilance. In FXS, individual physiological response patterns were associated with psychological vulnerability; children who showed the greatest increases in arousal and dampened increases in parasympathetic tone were rated as the most anxious by their caregivers. It is unclear why these relationships were not observed in the ASD group. Both groups were equally as likely to show symptoms of anxiety, so the group differences do not appear to be driven by differences in general anxiety level across the groups. This syndrome-specific pattern might suggest different mechanisms underlying anxiety in ASD and FXS, with physiological regulation implicated in FXS but not ASD. The finding that physiological modulation is not linked to anxiety in the ASD group has implications for understanding the process by which physiological dysregulation leads to impaired social

performance-- if not through anxiety, physiological modulation may be linked with social-communicative outcomes through its involvement in cognitive processes such as attention or memory (Hansen, Johnsen, & Thayer, 2003). In support of this hypothesis, behavioral symptoms of anxiety were not associated with the pragmatic language measures in ASD, further suggesting that social-communicative deficits in this group were no underpinned by anxiety. However, relationships may have been obscured by the general anxiety measure employed in this study; the CBCL captures trait anxiety, and it is possible that physiological responsivity may be more closely tied to measures of trait anxiety. While further research is needed, these results support the need for investigation of interventions targeting autonomic dysfunction to reduce anxiety in FXS.

Limitations and Directions

A limitation of this study is the lack of a control group, which limits the ability to determine whether the detected physiological responses were "atypical" in comparison to the general population. Other limitations include the relatively small sample size, which may have limited statistical power to detect effects. Because of the difficulty in recruiting children with low-incidence disabilities such as FXS, it was not possible to exclude children who were taking psychoactive medications, which might have influenced findings. Arguably, the inclusion of all children, regardless of medication status, might lead to findings that are more generalizable to the larger population, given that 50-75% of children with ASD and FXS use psychotropic medications (Mandell et al., 2008; Morgan, Roy, & Chance, 2003; Valdovinos, Parsa, & Alexander, 2009). Future research including larger, well-matched participant samples and comparison to controls might enhance understanding of physiological underpinnings of language in ASD and FXS. The present study was also restricted to examination of a single time point; longitudinal research is needed to understand how physiological characteristics may be related to language outcomes across time, which might inform developmental periods that might be most responsive to intervention.

Conclusion

To our knowledge, this is the first study to directly compare cardiac physiological profiles in ASD and FXS, with the finding that boys with FXS were hyperaroused in comparison to boys with ASD, but did not differ in vagal parasympathetic activity. Importantly, unstructured conversation with an examiner did not elicit increased arousal in either group, which might suggest that social impairments in ASD and FXS do not stem from socially-induced hyperarousal. However, individual differences in the ability to modulate arousal predicted pragmatic language ability in ASD (with trend-level associations also observed in FXS). This research highlights the promise of further investigation of autonomic nervous system dysfunction as a mechanism underlying of communicative impairment in ASD and FXS. Such research will be informative for developing interventions directly targeting underlying causes of impairment, and for understanding biophysiological mechanisms that may overlap in ASD and FXS, and which may be linked to *FMR1*-related genetic effects.

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