Symposium Title: Investigating the Behavioral Phenotype of Fragile X Syndrome: Lifespan Perspectives

Chair: Shannon L. O’Connor

Discussant: Bridgette Tonnsen

Overview: The purpose of this symposium is to highlight the research of graduate students in the field of neurodevelopmental disorders, specifically those studying fragile X syndrome (FXS). The focus of this symposium is on the behavioral phenotype in FXS. FXS is a single gene disorder found in 1: 4,000 males and 1: 8,000 females (CDC, 2015). Although recent work has shed light on the behavioral complications of FXS, much is still unknown. The first three talks will focus on biological and physiological biomarkers and their use in detecting delays and common comorbidities within FXS. The first of these examines the utility of eye-tracking as a biomarker to capture attentional vulnerabilities in preschoolers with FXS. The second talk examines the presence of ADHD symptomology in preschoolers with FXS and whether cardiac indices are linked as potential mechanisms. The third talk presents a novel approach of analyzing acoustic features of early vocalizations and their associations with autism and language outcomes in infants with FXS. The last two talks will focus how maternal characteristics were related to changes in child language outcomes in the context of a parent-implemented language intervention and how genetic status of mothers of children with FXS may contribute to differences in mental health and parenting. Finally, the discussant will shed light on the impact of these talks on the field and the importance of fostering graduate student work.

Paper 1 of 5

Paper Title: Attentional Disengagement as a Potential Biomarker for Preschoolers with FXS

Authors: Carla Wall, Quan Wang, Bridgette Tonnsen and Jane Roberts

Introduction: Early visual attention is critical to shaping learning opportunities. Much work has highlighted atypical attention patterns in young children with neurodevelopmental disabilities including FXS that are linked to co-morbid disorders (Tonnsen, Grefe, Hatton, & Roberts, 2015). Studying early attentional patterns in children with FXS can offer important insight into the presence of comorbid diagnoses like Attention-Deficit/Hyperactivity Disorder (ADHD) or Autism Spectrum Disorder (ASD). In addition, early attention impairments in FXS has been shown to predict later language outcomes, and atypical attention can have cascading effects on other types of learning as well (Kover, McCary, Ingram, Hatton, & Roberts, 2015).

Behavioral measures, such as temperament rating scales, can be useful tools to measure early attention patterns in real-world scenarios. However, parent-rating scales have limitations, particularly in populations with disabilities. Therefore, rating scales can be supplemented by more direct measures of behavior. Biomarkers such eye-tracking can be key to measure low-level differences in attention at young ages in children with FXS. One example of an eye-tracking biomarker that has shown some promise in neurodevelopmental disorders is attentional disengagement, indexed by the gap-overlap paradigm (Elsabbagh et al., 2009). This experiment measures the ability to flexibly shift attention as a function of changes in the visual environment. Although some studies suggest that attentional disengagement is impaired in older individuals with the FXS premutation, little work has examined performance on the gap-overlap task in very young children with the full disorder (Shelton et al., 2014). As such, the present study aims to compare an established behavioral temperament rating of attention shifting to a potential biomarker of attentional disengagement to characterize attentional difficulties in FXS as compared to TD controls using a multi-method approach.

Methods: Data were taken from a longitudinal study of neurodevelopment in children with FXS. The sample included 19 children with FXS (nmales= 14; Mage = 5.11 years), and 21 TD children (nmales = 15; Mage= 4.46 years). Groups did not differ by age (p = .514). Participants viewed the gap-overlap eye-tracking task using the SR Eyelink system. This task included three conditions each with a central fixation followed by a target stimulus on the periphery. In the baseline condition, the target appeared just as the central fixation extinguished; in the gap condition, the central fixation disappeared before the target was presented; and in the overlap

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condition, the central fixation remained on screen when the target appeared. Saccadic reaction times (RT) for gap, overlap, and baseline conditions were recorded. Disengagement was calculated as the difference in RT between the overlap and baseline conditions. Given the small within-group sample and heterogeneity characteristic of at-risk populations, nonparametric methods were utilized to test differences between groups.

**Results:** For the eye tracking gap-overlap task, the median disengagement times for each group were $\theta_{\text{FXS}} = 119.5$ ms, and $\theta_{\text{TD}} = 74.3$ ms. Results of the Independent-Samples Mann-Whitney U test suggest that the groups differ, with the FXS group having longer latency to disengage ($U = 111.0, p = .034$). Median CBQ attention shifting scores for each group were $\theta_{\text{FXS}} = 4.27$, and $\theta_{\text{TD}} = 4.53$. Results of the Independent-Samples Mann-Whitney U test suggest that the groups do not differ on this behavioral measure ($U = 112.0, p = .187$). A non-significant Spearman rank-order correlation also provided further support that these measures are independent ($\rho = 0.128, p = .535$).

**Discussion:** Atypical patterns in attention are a hallmark feature of FXS compared to TD individuals, and there is a need for valid and useful ways to detect attentional difficulties in children with neurodevelopmental disorders. Eye-tracking has emerged as a means to detect subtle, low-level differences in attention present in FXS that are not captured by broad-based parent rating scales. Future work should focus on earlier ages to track the emergence of these differences and their potential cascading effect on learning. In addition, the relation between difficulty with disengaging attention and other outcomes, like the presence of comorbid diagnoses should also be examined. Given mounting interest in neurodevelopmental pathways to common and divergent phenotypes in childhood disorders, the examination of early visual attention as a biomarker in young children with FXS offers an important avenue for current research.

**References/Citations:**

**Paper 2 of 5**

**Paper Title:** Heart Activity Associations with ADHD Symptomatology in Preschoolers with Fragile X

**Authors:** Shannon L. O’Connor¹, Elizabeth Will¹, Abigail L. Hogan¹, and Jane Roberts¹

**Introduction:** Attention Deficit/Hyperactivity Disorder (ADHD) is the most commonly diagnosed behavioral disorder in children, with approximately 11% of the general population diagnosed with ADHD. Symptoms of ADHD, such as inattention, hyperactivity and impulsivity, are elevated in children with fragile X syndrome (FXS). Heart activity is dysregulated in infants and children with FXS (Klusek, Roberts, & Losh, 2015) and accounts for severity of comorbid autism symptomatology in infants (Roberts, Hatton, Long, Anello, & Colombo, 2012). Multiple studies have shown that heart rate variability (HRV) is related to ADHD in the general population and can differentiate children with and without ADHD in resting and stress related states (Bunford et al., 2017; Imeraj et al., 2011; Musser et al., 2011). To date, no research has investigated symptom rates of ADHD in preschoolers with FXS or examined heart activity as an underlying mechanism for ADHD symptomatology in FXS. The present study aims to use multiple indicators of heart activity, including inter-beat interval (IBI), and respiratory sinus arrhythmia (RSA) to determine the extent to which they serve as underlying mechanisms of ADHD symptomatology in preschoolers with FXS contrasted to typically developing (TD) preschoolers.
Methods: Participants included 16 boys with FXS ($M = 49.83$ months, $SD = 11.37$), and 18 TD boys ($M = 44.76$ months, $SD = 9.16$). The Child Behavior Checklist 1½-5 ADHD-DSM subscale raw scores (CBCL; Achenbach, 2001) were used to assess the severity of ADHD symptoms. Measures of heart rate (IBI and RSA) were obtained from a baseline index collected while children watched a neutral movie. For the purposes of this study, IBI served as an indicator of overall heart activity, and RSA as an indicator of vagal tone/parasympathetic input.

Results: Two regression models (i.e., RSA model and IBI model) were estimated to determine group differences and influences of heart activity on ADHD symptomatology. RSA and IBI were centered at the mean, and ADHD symptomatology was regressed on group and RSA (Model 1), and group and IBI (Model 2). Chronological age was entered as a covariate in both models. Model 1 predictors accounted for approximately 34% of the variance in ADHD symptomatology ($R^2 = 0.337$), which was a significant effect ($F(3, 30) = 5.08; p = .006$). Parameter estimates indicated that significant group differences in ADHD symptomatology were driving this effect ($b = -1.49; p = .011$) and not RSA or age. The FXS group scored approximately 1.5 standard points higher on ADHD symptoms compared to the TD group. Model 2 (IBI model) was also significant and accounted for the same portion of the variance in ADHD outcomes ($R^2 = .336; F(3, 30) = 5.06; p = .006$); however, IBI was not significantly associated with ADHD outcomes.

Discussion: These findings suggest that symptoms of ADHD are clearly evident in preschool aged males with FXS. Although preschoolers with FXS demonstrated significantly higher levels of ADHD symptomatology compared to TD preschoolers, baseline physiological arousal may not account for these differences. However, future work should consider whether heart rate variability during tasks with greater demands on attentional control may account for differences in ADHD symptomatology between FXS and TD children. Additionally, future work should investigate what other factors may contribute to symptomatology differences in ADHD in preschoolers with FXS and TD children such as eye tracking, or direct behavioral measurement.

References/Citations:

Paper Title: Acoustic Properties of Early Vocalizations in Infants with Fragile X Syndrome

Authors: Lisa Rague, Amanda Seidl, Bridgette Tonnsen

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Introduction: Fragile X syndrome (FXS) is a neurogenetic disorder and the leading known single-gene cause of autism spectrum disorder (ASD). Social communication is a core area of deficit in ASD and is often an area of weakness in FXS, as well (Finestack, Richmond, & Abbeduto, 2009). Establishing measures that are sensitive to markers of social communication impairments very early in development is crucial for routing high-risk children to appropriate and targeted treatments. A growing literature suggests that features of early vocalizations – such as volubility, complexity, pitch and duration of vocalizations – predict later language abilities and ASD outcomes in children at high risk for ASD (Patten et al., 2014), suggesting these markers may provide insight into early topography of social communication deficits and help identify children in need of treatment. However, vocalization features and their relationship to later outcomes have yet to be examined in FXS. In the only known study of early vocalization features in FXS, 9- to 12-month-old infants had lower volubility and less complex vocalizations than age-matched TD peers (Belardi et al., 2017). Notably, this FXS sample only included infants who were not later diagnosed with ASD; thus, the relationship of volubility and complexity with ASD status in FXS remains unclear. The present study takes a novel approach by analyzing acoustic features of early vocalizations in infants with FXS to explore associations of these features with later language abilities and ASD outcomes.

Methods: Participants include 15 nine-month-old infants with FXS ($M_{age} = 9.36$ months, $SD_{age} = 0.78$ months). The Autism Observation Scale for Infants (Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008) was used as a standardized child-examiner interaction from which infant vocalizations were coded. Vocalizations were coded in an acoustic coding software (Praat; Boersma & Weenink, 2016) by a team of trained coding pairs. Five vocalization variables were then extracted using Praat: volubility (rate of speech vocalizations per minute), complexity (rate of consonant usage), average vocalization duration, average vocalization pitch, and pitch range. ASD symptom severity was measured using the ADOS Social Affect Calibrated Severity Score (ADOS SA CSS), expressive language abilities were measured using the Mullen Expressive Language age equivalent (MSEL EL AE), and nonverbal mental age (NV MA) was calculated by averaging the Mullen Visual Reception and Fine Motor age equivalents.

Results: Spearman semi-partial correlations of vocalization variables with ADOS SA CSS controlling for NV MA suggested that ASD symptoms were not strongly related to vocalization variables (magnitude of $r’s = .01-.14$; all $p’s > .05$). Associations of vocalization variables with expressive language abilities were slightly stronger. Spearman semi-partial correlations controlling for NV MA indicated the strongest relationships between expressive language and volubility ($r = .42$, $p = .14$) and vocalization duration ($r = .39$, $p = .17$). Final regression analyses will evaluate relative contributions of later language abilities and ASD outcomes on vocalization variables in an expanded sample ($n = 30$; data collection complete, coding ongoing).

Discussion: This study applies a novel method of examining early social communication in FXS by analyzing the acoustic properties of early vocalizations and their associations with later language abilities and ASD outcomes. Preliminary results suggest that pre-verbal vocalization features may have the ability to act as early markers of later language abilities in FXS, independent of social communication impairments associated with ASD features. With further study in expanded cohorts, this work may inform early identification and intervention targets for infants with FXS at high-risk for social communication deficits.

References/Citations:
**Paper Title:** Maternal Psychological Profiles and Child Language Outcomes in a Parent-Implemented Spoken Language Intervention for Fragile X Syndrome

**Authors:** Sarah Nelson\(^5,6\), Lauren Bullard\(^5,6\), Andrea McDuffie\(^5\), Leonard Abbeduto\(^5\)

**Introduction:** Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability and is further characterized by delays in multiple domains of spoken language (Abbeduto, Brady, & Kover, 2007). Phenotypic characteristics associated with FXS often make it difficult for parents to engage their affected sons in sustained and productive conversational interactions. Additionally, given that FXS is an intergenerational disorder, mothers of children with FXS are also affected, either as premutation carriers or by the full mutation. Premutation carriers frequently experience symptoms of anxiety and depression (Bourgeois et al., 2011). Thus, characteristics of individuals with FXS, along with the mental health symptoms of premutation carriers, could constrain the development of warm and nurturing relationships between a mother and child (Warren, Brady, Sterling, Fleming, & Marquis, 2010). Thus, we examined how maternal characteristics were related to changes in child language outcomes in the context of a parent-implemented language intervention (PILI; McDuffie et al., 2017) that depends critically on successful parent-child interactions. To address this, we asked the following research questions: (1) What are the profiles of anxiety, depression, parenting stress, and parenting competence in mothers at pre-treatment? and (2) How are changes in child spoken language related to aspects of maternal mental health and parenting at pre-treatment?

**Methods:** Participants included 19 boys with FXS between the ages of 10 and 17 and their biological mothers. Dyads were randomly assigned to a treatment-as-usual comparison group (N=9) or a treatment group (N=10). The treatment group received an intervention in which mothers were taught to use language facilitation strategies to support their child’s spoken language within the context of a shared storytelling interaction. Dyads enrolled in the intervention received 12 weekly coaching, feedback, and homework sessions via distance teleconferencing (i.e., Skype). All dyads completed three shared storytelling samples during the two weeks prior to, and following, the intervention. These samples were transcribed, coded, and analyzed for the presence of maternal strategy use and child language outcomes (e.g., vocabulary, syntax, and story-related talking). Additionally, mothers completed a battery of questionnaires to collect information about maternal mental health status and parenting. Given that premutation carriers are at an increased risk for anxiety and depression, those domains of mental health were assessed using the Symptom Checklist-90-Revised (SCL-90-R). In order to measure parenting stress, mothers completed the Parenting Stress Index (PSI-SF-4). Lastly, mothers completed the Parenting Sense of Competence scale (PSOC).

**Results:** Overall, the mothers in our sample had relatively high levels of depression and anxiety, average levels of parenting stress, and a moderate to high sense of parenting competence. Specifically, 26% of our mothers scored in the clinical range for depression, which is about three times higher than the average rate of depression among women in the United States. Sixteen percent of our mothers were in the clinical range for anxiety, whereas roughly 3% of adults in the US have generalized anxiety disorder, with women twice as likely as men to have a diagnosis. The comparisons of our sample to the general US population are based on data provided by the NIMH. In regard to parenting stress, 21% of our mothers reported clinically significant levels on the subscale of parenting distress, 5% on the parent-child dysfunctional interaction subscale, and 26% on the difficult child subscale. Lastly, for parenting competence, 42% of our mothers reported moderate parental competence and 58% reported high parental competence.

To address our second question, we conducted a series of one-tailed Pearson correlations between maternal mental health and stress measures and child growth in language in the context of the intervention. Multiple comparisons were accounted for using Bonferroni corrections. Although domains of the SCL-90-R and the PSOC did not correlate with measures of child spoken language for the comparison group, child vocabulary and story-related talking were significantly and negatively associated with maternal depression (\(p < .025\)) and child vocabulary was significantly and positively associated with parent sense of competence (\(p < .05\)) in the treatment group. Measures of parenting stress were not correlated with child spoken language for the treatment
group, but child vocabulary and story-related talking were significantly and negatively associated with the difficult child subscale for the comparison group ($p < .017$).

**Discussion:** Mental health and parenting stress of mothers were related to improvements in child spoken language over a 12-week intervention. For the mothers in the treatment group, depression and parenting sense of competence were related to changes in child vocabulary and story-related talking, such that mothers with lower rates of depression and those with higher parenting competence had children who demonstrated greater change in their spoken language over the course of the intervention. Alternatively, for mothers in the non-treatment comparison group, only characteristics related to the child (i.e., the difficult child subscale of the PSI) were related to changes in child spoken language. These findings provide preliminary evidence of ways in which maternal characteristics might influence child language outcomes in the context of a parent-implemented intervention. These findings suggest that maximizing the impact of a parent-implemented language may require a concurrent focus on maternal psychological states.

**References/Citations:**


**Paper 5 of 5**

**Title:** Risk and Resilience in Mothers with Fragile X Syndrome: An Exploratory Study

**Authors:** Heather Fielding7, Steven F. Warren8

**Introduction:** This study explored possible sources of risk and resilience in mothers with Fragile X Syndrome (FXS). FXS is a rare neurodevelopmental disorder caused by a CGG repeat on the X chromosome. Individuals who have more than 200 repeats have the full mutation (FM), and those who have between 55 and 200 repeats have the premutation (PM). Females with the PM and those with the FM report higher rates of anxiety disorders than controls (Franke et al., 1998). Women with the FM have lower IQs than women with the premutation. Women with the PM or the FM often have children with the FM. Raising a child with FXS likely contributes to increased stress and parenting difficulties. McCarthy et al. (2006) propose that the pre- or full mutation may itself impact parental stress due to difficulties with anxiety and affective disorders. Higher levels of depression have been reported for PM than FM mothers (Bailey et al., 2008). The aims of this study were to characterize FM mothers in comparison to demographic-matched PM mothers, and to identify factors beyond genetic status that may contribute to differences in mental health and parenting between pre- and full mutation mothers.

**Methods:** 55 mothers of a child with full mutation FXS were visited. Three of these mothers also had the full mutation. Each full mutation mother was matched with two premutation mothers based first on age, then marital status, number of children, income, and education yielding a total sample of 9 mothers. During the visit, the mothers were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D), the Profile of Mood States Tension/Anxiety and Anger/Hostility scales (POMS-TA and -HA), and the Parenting Stress Index (PSI). IQ was previously determined (Brady et al., 2014). Each mother-child dyad was recorded for 15 minutes doing three activities (snack, craft, and book reading) for five minutes each. From this data, maternal warmth, affect, and other aspects of responsibility were ascertained. Children were assessed for autism symptomology at the time.
of the observation using the Childhood Autism Rating Scale (CARS), and for adaptive and problem behavior using the Vineland Adaptive Behavior Scales (VABS) and the Childhood Behavior Checklist (CBCL), respectively.

**Results:** CGG repeat length was significantly correlated with depression, $r(9) = -0.68, p < .05$, IQ, $r(9) = -0.70, p < .05$, and warmth, $r(9) = -0.71, p < .05$, such that FM mothers had lower depression, IQ and warmth than PM mothers. Depression and tension/anxiety were significantly correlated with IQ, $r(9) = 0.67, p = .05$; $r(9) = 0.79, p < .05$, respectively, such that higher IQ was associated with higher depressive and anxious symptoms. Anger/Hostility was correlated with child behavior problems $r(9) = 0.71, p < .05$, autism symptomology score, $r(9) = 0.81, p < .01$, and total maternal stress, $r(9) = 0.80, p < .05$. Additionally, higher maternal stress was associated with increased child behavior problems, $r(9) = 0.78, p < .05$, and autism symptomology, $r(9) = 0.77, p < .05$.

**Discussion:** Parenting a child with a developmental disability, such as FXS, puts mothers at higher risk for increased stress levels. Within our sample of PM and FM mothers, those whose children had higher levels of autism symptomology and more problem behaviors had higher parenting stress and anger/hostility. Mothers of more difficult children are more susceptible to mental health disorders due to the increased stress that they experience. However, there are also specific risks that come with the mother having PM or FM FXS herself. While previous studies have reported higher levels of anxiety in women with FXS than controls, our FM mothers reported lower levels of anxiety than matched PM mothers. Like Bailey et al. (2008) we found lower depressive symptoms in FM mothers than in PM mothers. It is hypothesized that anxiety and depression may be mediated by IQ in FM mothers, such that these mothers are less susceptible to the parenting stresses of raising a child with a developmental disorder. It should be noted that FM mothers’ children had comparatively fewer autistic symptoms, which may factor into their relative resilience. Additionally, two of the FM mothers had daughters, but it is unclear whether this is a source of resilience, as these mothers were matched with PM mothers who also had daughters. Full mutation mothers also demonstrated relatively less warmth than the PM mothers, suggesting that FXS status may impact this element of parenting. These preliminary observations suggest several hypotheses to be explored with a much larger sample of pre- and full mutation mothers.

**References/Citations:**