Symposium Title: Benefits and Challenges of Biomarker Research: Lessons Learned from Studies of Peripheral and Neural Indicators in IDD

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Overview: Increasing attention has focused on the potential value of integrating biomarkers into research to advance our understanding of mechanistic factors associated with impairment. Biomarkers hold particular promise in the field of intellectual and developmental disabilities (IDD) by circumventing inherent limitations in the capacity to understand and communicate experiences that characterize many persons with IDD. Given the critical role of early detection and intervention to reduce impairment in persons with IDD, biomarkers can also signal biological vulnerability to later emerging symptoms or disorders and to leverage effective treatment, providing a level of measurement that is not possible with behavioral markers alone. Despite the promise and value of biomarkers, there are also many challenges and pitfalls associated with these methods. Among these include sample bias related to behavioral compliance, acquisition of normative data, and post-collection processing procedures. There can also be an “objectivity” bias in biomarker research which assumes that biological markers are more objective, thus more valid, than behavioral measures. In this series of talks, we provide the theoretical underpinnings and core methodological procedures along with the benefits and challenges of three oft-utilized biomarkers in the IDD field: heart rate variability, electroencephalography, and brain imaging. We will provide data from studies employing these measures to illustrate lessons learned regarding the benefits and challenges of biomarker research.

Paper 1 of 3

Paper Title: Heart Rate Variability in Fragile X Syndrome and Its Relationship to Anxiety

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Introduction: Atypical physiological arousal has been associated with a number of maladaptive outcomes in fragile X syndrome (FXS); however, empirical support linking arousal to specific traits or disorders is scarce. Anxiety disorders are present in over 80% of males with FXS, and they are some of the most frequent disorders associated with elevated arousal despite little research documenting this relationship. Evidence linking atypical arousal to social anxiety or other impairments can inform treatment efforts including behavioral and psychopharmacological techniques to reduce arousal. Heart activity is an ideal biomarker to examine arousal in FXS as it can be collected using non-invasive and easily tolerable techniques and it represents arousal in “real time” which facilitates linkages with behavior. In this talk, we will present a study to increase our understanding of the trajectory of atypical physiological arousal and its relationship to adverse outcomes in FXS. The aim of this study is to characterize multiple cardiac indicators and their relationship to behavioral indices of behavioral regulation in males with FXS from infancy through preschool-age and their relationship to symptoms of social anxiety within an experimental paradigm. We use two contexts to examine these relationships including a baseline and an experimental press for social fear and anxiety.

Methods: Longitudinal baseline heart activity data on 42 males with FXS (M CA at initial assessment=27 months; 125 total observations) and 41 TD males (M CA at initial assessment=12 months; 155 total observations), aged 4 months to 9 years was examined. Longitudinal data during the “stranger” task in 25 males with FXS (49 observations, M CA at initial assessment = 26.67
months) and 33 TD males (105 observations M CA at initial assessment = 12.42 months), aged 5 months to 8 years. The stranger task is drawn from the laboratory assessment battery and is designed to measure fear to the approach of a novel social partner. Behavioral responses of the participants are coded offline including escape (e.g., moving or turning away) and gaze (e.g., target of attention).

Results: Group differences in age-related changes over time in inter-beat interval (IBI) and respiratory sinus arrhythmia (RSA) were estimated between FXS and TD controls using multilevel modeling for Baseline and stranger conditions. For the resting baseline period, both IBI and RSA model results indicated significant increases in RSA (p<.001) and IBI (p<.001) over time, as well as significant age-by-group interactions (RSA p=.0025; IBI p=.0031). Age-related trends indicated that the FXS group showed a decelerated increase in both IBI and RSA relative to the TD group. Age-by-group interactions were such that infants with FXS experienced an early shift in RSA at approximately 14 months and IBI in approximately 18 months (see Figures).

For the Stranger task, both IBI and RSA model results indicated significant increases in RSA ($b = .04, p < .0001$) and IBI ($b = 2.84, p < .0001$) over time. Additionally, the age-by-group interactions were significant (RSA: $b = -.02, p < .05$; IBI: $b = -1.37, p < .01$), suggesting that the FXS group showed a decelerated increase in both IBI and RSA relative to the TD group.

Correlations between Stranger physio and behavioral fear were examined. In the FXS group, Escape behaviors were correlated with IBI, $r = -.71, p < .01$, and gaze avoidance, $r = -.50, p < .05$. These relationships were not observed in the TD group.

Discussion: Given the high prevalence and significant impairment of anxiety in FXS, increased understanding of its emergence and mechanistic underpinnings has great value to refine the phenotype and direct both timing and targets for treatment. Results of this study demonstrate that physiological arousal follows a non-linear trend with initial levels characteristic of hypo-arousal during infancy followed by a shift to hyper-arousal in the second year of life. These atypical arousal levels are related to increased expression of behavioral inhibition that signal early features of social anxiety.

Figure 1. Age-Related-Trends in IBI
Paper Title: Electroencephalographic Markers of Atypical Development in Infants with Tuberous Sclerosis Complex

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Background: Tuberous sclerosis complex (TSC) is one of the most commonly-occurring single-gene disorders associated with autism spectrum disorder (ASD) and intellectual disability (ID). Recent work from our group has demonstrated that, at the behavioral level, infants with TSC/ASD can be distinguished from infants with TSC/noASD by 12 months (McDonald et al, 2016), and that by 3 years, infants with TSC/ASD show striking overlap in social communication profiles to those of toddlers with non-syndromic ASD (Jeste et al, 2015). In animal models, mutations in the TSC1/2 gene, through downstream effects on the mTOR pathway, have been linked to alterations in synaptic development and neuronal connectivity. Electroencephalography (EEG) can be used to examine changes in functional brain network development in these infants and may shed light on the neural mechanisms underlying atypical development in these high risk infants. In this study, we aimed to identify electrophysiological markers associated with the development of ASD in infants with TSC. Specifically, we asked whether developmental trajectories of EEG power (as a marker of neural synchrony) and coherence (as a measure of neural connectivity) distinguish infants with TSC/ASD from infants with TSC/noASD in the first year of life.

Methods: These data were collected as part of a multisite, prospective study of infants with TSC (n=40) and typically developing (TD) infants (n=32) across the first three years of life (PI Jeste and Nelson). Baseline EEG was recorded from infants at 9, 12, 18, 24 and 36 months using a high-density system (EGI Inc.). EEG data processing was performed per previously published protocols from our lab (i.e. Dickinson et al, 2017). ASD diagnosis at 24 and 36 months was determined using the Autism Diagnostic
Observation Schedule and clinical best estimate. We assessed overall development using the Mullen Scales of Early Learning, and we collected clinical information regarding epilepsy and medication status across development.

**Results:** Mixed-effects models revealed differences in developmental trajectories of both EEG alpha (7-12 Hz) power and alpha phase coherence between (i) TSC and TD infants, and (ii) TSC/ASD and TSC/noASD. TSC infants had reduced whole-brain alpha power across early development (from 9-36mo) compared to TD infants (p<.001). From 12 months, alpha power trajectories differentiated TSC/ASD infants from TSC/noASD, with TSC/ASD infants showing the lowest levels of whole-brain alpha power (p<.01). We also identified significant differences in alpha phase coherence across early development between TSC/ASD and TSC/noASD infants from 12 months (t(24)=2.07, p<.05). These differences were identified despite comparable rates of seizures, epilepsy severity, and medication exposure between the TSC/ASD and TSC/noASD groups.

**Conclusions:** We identified differences in brain function between infants with TSC/ASD and TSC/noASD from 12 months of age, well before the age at which an ASD diagnosis may be established in this population. Our findings suggest that EEG can identify patterns of brain function that underlie, and possibly precede, the development of ASD in TSC. In particular, group differences in alpha power and coherence trajectories suggest alterations in white matter development in TSC/ASD. These findings highlight the need for further prospective studies mapping early brain function in TSC from within the first year of life, to identify whether differences in developmental trajectories may precede even the earliest emerging behavioral signs of ASD. In this presentation, we will discuss both challenges and opportunities in EEG studies in early development and consider these findings in the broader context of studies of biomarkers of atypical development in high risk infants, including those with a familial risk for ASD.

**References/Citations:**

**Paper Title:** Early Brain Development in Infants at High Risk for Autism

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**Introduction:** Autism spectrum disorder (ASD) is characterized behaviorally by deficits in social communication and the presence of ritualized and repetitive behaviors. While there have been attempts to identify ASD as early as possible, the average age for a clinical diagnosis still falls around the age of 3-4 years. However, there are efforts underway to look at neurobiobehavioral characteristics, specifically features of brain morphology and development, to help detect and predict ASD outcomes. The NIH funded an Autism Center of Excellence (ACE) Network, the Infant Brain Imaging Study (IBIS), to prospectively examine brain development in a sample of infants at high risk for autism. This presentation will discuss some of the latest IBIS findings using neuroimaging measures and discuss how they have contributed to the search for biomarkers for ASD.
**Methods:** In a collection of studies presented (Hazlett et al., 2017; Emerson et al., 2017; Shen et al., 2017), we examined data from participants in a longitudinal study that included 318 infants at high familial risk for ASD (HR), of which 70 met clinical best-estimate criteria for ASD (HR-ASD) and 248 did not meet criteria for ASD (HR-neg) at 24 months of age, and 117 infants at low familial risk (LR) for ASD, who also did not meet criteria for ASD at 24 months. Infants were evaluated at 6, 12 and 24 months of age with detailed behavioral assessments and high-resolution brain magnetic resonance imaging (MRI), to prospectively investigate brain and behavioral trajectories during infancy. The analyses described below were conducted on different subsets from the IBIS sample.

**Results:** We first examined whether brain measures of surface area and cortical thickness at 6 and 12 months of age can be used to accurately identify those infants who later meet criteria for ASD at 24 months of age. The classification approach successfully distinguished the HR-ASD group from the HR-neg group (94% accuracy, 88% sensitivity, 95% specificity, and 81% positive predictive value (PPV)). Examination of functional connectivity data in another subset of the IBIS dataset applied at 6 months correctly predicted 9 of 11 infants who received an ASD diagnosis at 24 months (100% PPV and 81.8% sensitivity). Lastly, a study examining extra-axial cerebrospinal fluid (CSF) at 6 months found increased volume could predict later ASD outcome at 24 months of age (with 69% accuracy, 66% sensitivity, 68% specificity).

**Discussion:** In a sample at high-risk for ASD, neural biomarkers have been identified that show early detection of ASD is possible using measures derived from neuroimaging. These biomarkers show good specificity and sensitivity to predict ASD outcomes in this population at an age that precedes the clinical diagnostic symptoms. These findings hold promise that physiological and/or morphological biomarkers can aid in early identification. However, they are not without challenges and limitations for practical implementation, and the discussion will focus on highlighting some of these points.

**References/Citations:**