**Symposium Title:** Investigating and Integrating Biomarkers and Behavior in Rett Syndrome

**Chair:** Frank Symons¹

**Discussant:** Jeffrey Neul,²

**Overview:** Investigating and integrating biomarkers into behavioral research in neurodevelopmental disorders is fraught with peril but loaded with promise. Rett syndrome (RTT), a rare neurodevelopmental disorder caused by MECP2 mutations (and related variants), is among the leading IDD-relevant syndromes for which novel or repurposed compounds are being tested as therapeutics. There is an urgent need to identify and clarify classes of biomarkers that can serve as clinically-relevant endpoints. There is an equally urgent need to understand behavior with respect to context and learning as well as genotype-phenotype patterns. In this symposium, we present a body of work representing basic and applied discovery focused on testing innovative investigative tools/approaches. Talks will highlight aspects of a new tool or novel application/analysis of an existing tool/approach to knowledge discovery in RTT specific to biomarkers and/or behavior in relation to clinically relevant phenotypic features of RTT.

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**Paper 1 of 6**

**Paper Title:** Therapeutic Potential of Mglu7 Modulation in Rett Syndrome and Related Neurodevelopmental Disorders

**Authors:** Rocco G. Gogliotti², Nicole M. Fisher², Branden J. Stansley², Sheryl A. Vermudez², Rebecca K. Senter², Jeffrey Adams², Rocio Zamorano², Adam G. Walker², Anna L. Blobaum², Darren W. Engers², Corey R. Hopkins², J. Scott Daniels², Zixiu Xiang², Carrie K. Jones², Craig W. Lindsley², P. Jeffrey Conn², Colleen M. Niswender²

**Introduction:** Rett syndrome (RTT) and MECP2 Duplication syndrome (MDS) are neurodevelopmental disorders that result in impairments in cognitive function and are caused by mutations in or duplications of the *Methyl-CpG-Binding Protein 2* (*MECP2*) gene, respectively. The cognitive impairments seen in mouse models of RTT and MDS correlate with alterations in long-term potentiation (LTP) at Schaffer Collateral (SC)-CA1 synapses in the hippocampus. Metabotropic glutamate receptor 7 (mGlu7) is the predominant mGlu receptor expressed presynaptically at SC-CA1 synapses in adult mice and its activation is required for the generation of LTP [1]. The requirement for mGlu7 activation in LTP induction, the molecular correlate of learning and memory, further suggests that precise levels of mGlu7 activity are required for proper cognitive function; in concert with this hypothesis, mice lacking mGlu7 display impairments in cognition assays. These LTP and behavioral studies in animals fit nicely with emerging data indicating that primary GRM7 mutations are a cause of severe intellectual disability [2, 3], suggesting translational relevance regarding changes in mGlu7 function as a potential underlying cause of cognitive deficits in disorders with an intellectual disability component. This project aims to validate the therapeutic potential of modulating mGlu7 activity in models of cognitive impairment using small molecule therapeutics.

**Methods:** We used positive and negative allosteric modulators (PAMs and NAMs) with mGlu7 activity in mouse models of RTT and MDS to test the hypothesis that modulating mGlu7 would rescue synaptic plasticity defects, as assessed using electrophysiology, as well as behavioral deficits observed in fear conditioning, cognition, and social interaction tasks. We also assessed effects of various compounds in reversing apneas, which affect a large percentage of RTT patients and represent a clinical objective endpoint that could be used in eventual clinical trials to assess compound efficacy.

**Results:** We found that loss of *MECP2* causes decreased mGlu7 protein expression in cortex and cerebellar samples from RTT patients, as well as within the brains of mice modeling RTT [4]. Our studies showed that positive allosteric modulation of mGlu7

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activity with two distinct PAMs identified at Vanderbilt, VU0422288 and VU0155094, restored LTP in MeCP2-deficient mice. VU0422288, a brain penetrant compound, also improved contextual fear learning, novel object recognition, and social memory. Furthermore, VU0422288 administration decreased apneas in MeCP2-/- mice, suggesting that mGlu7 may represent a therapeutic target for multiple aspects of the RTT phenotype. We then extended our studies to MDS. Mouse models of RTT and MDS show opposing changes in presynaptic activity at SC-CA1 synapses, suggesting an opposing change in a presynaptic regulator of neuronal function in these two disease contexts. Based on these results, we hypothesized that genetic reduction of mGlu7, or a small molecule mGlu7 negative allosteric modulator (NAM), might rescue deficits in MDS mice. Surprisingly, cognitive and anxiety phenotypes found in MDS animals were not impacted by genetic decreases of mGlu7 expression or administration of an mGlu7 NAM. In contrast, the PAM VU0422288 corrected these deficits, suggesting that shared underlying circuitry changes in these two disease models may explain this finding.

Discussion: We have found therapeutic potential for mGlu7 potentiation in models of both RTT and MDS. We are now moving into the study of primary GRM7 mutations and correlating GRM7 single nucleotide polymorphisms with neurodevelopmental disorders in clinical populations. We postulate that mGlu7 PAMs may be useful in treating cognitive deficits in multiple forms of intellectual disability.

We acknowledge the generous donation of samples from RTT patient families, and we thank the University of Maryland Brain and Tissue Bank and the Harvard Brain Tissue Resource center for their assistance in obtaining this valuable resource. We also acknowledge the following funding sources: Rettysyndrome.org, a Weatherstone Fellowship and a Treatment Award from Autism Speaks, the PhRMA Foundation, NIH grants T32AG000260, T32MH065215, T32GM007628, F32MH111124, U54MH084659, R01NS031373, R01MH062646, R21MH102548, R01MH102548, and CDMRP grant PR160102.

References/Citations:

• Gogliotti, R.G., et al., mGlu7 potentiation rescues cognitive, social, and respiratory phenotypes in a mouse model of Rett syndrome. Sci Transl Med, 2017. 9(403).

Paper 2 of 6

Paper Title: Quantifying Data Quality in Eye-Tracking Research with Rett Syndrome

Authors: Breanne J. Byiers¹, Kirsten Dalrymple¹, Jed Elison¹

Introduction: Eye tracking has been used to evaluate a range of behaviors and processes in a variety of populations. Currently, few studies systematically examine the accuracy and precision of eye tracking data quality. It has been shown that issues with data quality can affect estimates of several key variables in eye-tracking research, which can lead to inaccurate reporting of experimental or between-group differences (e.g., Wass, Forssman, & Leppanen, 2014). In addition to the potential data quality effects that exist in most eye tracking studies, individuals with Rett syndrome (RTT) may introduce artefacts due to excessive

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movement, difficulty following verbal instructions, and ophthalmologic problems, such as strabismus, that may affect eye tracking data quality. Therefore, objective measures of data quality are needed to improve the quality and replicability of eye tracking research in this population.

**Methods:** Six individuals with Rett syndrome (age range = 2:11 to 26:7 years) and five healthy female controls (age range = 5:4 to 21:10 years) have participated in this study to date. All participants completed a task designed to evaluate the accuracy and precision of the eye tracking data collected. The task involved an animated ball that appears in five different locations on the screen. For each stimulus presentation, accuracy was computed as the Euclidean distance (in pixels) from the location of the stimulus and the gaze data. Precision of the gaze measures was calculated as the root mean square (RMS) of successive X and Y gaze locations. Between-group differences in each of these variables were examined.

**Results:** Distance measures from the RTT group ranged from 63.23 to 108.975 pixels (mean = 78.12, SD = 21.30), and were consistently and significantly higher than those from the control group (min = 25.49, max = 48.83, mean = 35.10, SD = 7.72; t(9) = 4.258, p = .002). Precision measures for the X coordinates did not differ significantly between groups (RTT: mean = 7.26, SD = 5.11; control: mean = 4.89; SD = 1.79; t(9) = -0.982, p = .352), whereas variability for the Y coordinates was significantly greater in the RTT group (RTT mean = 10.02, SD = 3.39; control: mean = 5.84, SD = 1.38; t(9) = -2.564, p = .030).

**Discussion:** These results support the notion that between-group differences in data quality may affect results of eye tracking research when comparing data collected from individuals with RTT and healthy comparison samples. Additional work is needed to determine the degree to which the observed differences in data quality may affect key eye tracking variables, such as fixation time and eye movement latency. Further, strategies for improving the calibration process and other factors that affect data quality among individuals with RTT and other intellectual and developmental disabilities should be evaluated.

**References/Citations:**

**Paper Title:** Non-Word Memory in Rett Syndrome and Rett-Related Disorders

**Authors:** Sarika U. Peters², Dorita Jones³, Alexandra P. Key³

**Introduction:** Mutations in the methyl CpG-binding protein 2 gene (MeCP2) were first identified as a primary cause of Rett syndrome (RTT) in females (Amir et al., 1999). MECP2 Duplication syndrome is a related X-linked genomic disorder (Ramocki et al., 2009), primarily affecting boys although some girls are now being identified, and it accounts for 1-2% of all cases of X-linked intellectual disability (ID; Ramocki et al., 2009). The MeCP2 protein is highly expressed in the brain, and many studies have demonstrated that even slight over- or underexpression results in alterations to brain development and functioning. The phenotype of RTT, which is associated with underexpression of MeCP2 protein, is characterized by regression, loss of purposeful hand skills and replacement with stereotyped hand movements/hand-washing motions, limited speech, dyspraxia, and abnormal muscle tone (Percy et al., 2010). The phenotype of MECP2 Duplication syndrome, associated with overexpression of MeCP2 protein, is characterized by infantile hypotonia, ID, autism, progressive neurological declines, choreiform movements, and recurrent respiratory infections (Ramocki et al., 2009). Despite significant advances at the level of basic research that have progressed to treatment trials, sensitive biomarkers of higher-level cognitive and language processing deficits in RTT and RTT-related disorders remain elusive. To test whether EEG could be used to dissociate capacity for auditory learning and memory, we assessed how children with RTT and MECP2 duplication syndrome became familiarized with a repeatedly presented spoken non-
word, testing whether differentiation of familiarized vs. novel non-word stimuli could be detected with event-related potentials (ERP).

**Methods:** Participants were between the ages of 4-12 years, and were presented with a total of 100 Nonword trials (50 = word was said once, and 50= repeated word). We hypothesized that repeated non-words would elicit more positive parietal responses than novel words.

**Results:** 32 typically developing participants, 7 with MECP2 Duplication syndrome, and 13 with RTT provided usable data. The results show that the TD group and the MECP2 duplication group show the expected sign of incidental memory for repeated > once presented nonwords at the parietal scalp at 250-500ms (t=-2.55; p=.04 in MECP2 dup, t=-3.970; p<.001 in TD). RTT participants did not resemble the typical or MECP2 Duplication group, and results were not significant at any time window. Better discrimination was related to better receptive language in RTT and MECP2 Duplication (using the PPVT), but increased behavioral severity (irritability, hyperactivity) as measured by the Aberrant Behavior Checklist.

**Conclusion:** This passive auditory memory task differentiated between Rett-related neurogenetic disorders, and performance was related to receptive language and behavioral severity. This task could have applicability to other nonverbal, lower-functioning populations. Different neural signatures may relate to levels of MeCP2 expression.

**References:**

**Paper 4 of 6**

**Paper Title:** Epidermal and Vascular Innervation in Rett Syndrome

**Authors:** Frank Symons¹, Chantel Barney², Breanne Byiers¹, Brian McAdams¹, Shawn Foster¹, Timothy Feyma², Gwen W Wendelschafer-Crabb³, & William R Kennedy¹²

**Introduction:** Rett syndrome (RTT), a rare neurodevelopmental disorder occurring primarily in females (1:10-15,000 female live births), is most often caused by loss-of-function mutations in the X-linked methyl-CpG-binding protein 2 gene (MECP2). Clinical investigation of the periphery has been extremely limited despite numerous neurologically-relevant diagnostic (blunted pain response) and clinical features (cold extremities) suggesting peripheral involvement and dysfunction. To begin investigating peripheral effects in RTT, we compared 3 mm epidermal punch biopsy specimens between girls with and without RTT.

**Methods:** A prospective case comparison series design was used. Lab assays were performed under blinded conditions. Four adolescent female RTT patients recruited consecutively through a pediatric rehabilitation and specialty care hospital formed a convenience sample and were compared against an archived approximate age-, sex-, body-site matched comparison sample of healthy adolescent females (N=8, ages 12-18). All parents provided informed consent and all families approached consented. The planned primary outcome variable was the epidermal nerve fiber density (ENFd) value (enf/mm)¹.

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Results: The four participating RTT patients were all Caucasian and ranged in age from 12-19 yrs old. Healthy gender- and body-site matched controls were selected from an archived source representing a comparable age range. Confocal imaging revealed atypical looking epidermal nerve fiber (ENF) innervation, large Merkel cells, atypical arteriole innervation, elongated mast cells, and densely innervated hair follicles compared with healthy female control individuals. The average ENF density value estimates for the RTT sample was 42.0 ENF/mm (SD = 22.0, range = 12.2 – 60.3) and for the control sample was 27.3 ENF/mm (SD = 9.7; range = 15.3 - 41.1) \[t = -1.72, p = 0.058\].

Discussion: Our findings should alert clinical and scientific investigative groups to the importance of peripheral function in RTT. The numerous co-occurring markers for peripheral involvement may point to a primary cause of many of the syndrome’s features. The clinical management and diagnostics of RTT patients requires an interdisciplinary approach that includes considering central and peripheral mechanisms. This is the first documented observation of the atypical arteriole patterns and warrants further investigation given the ‘cold hands/feet’ phenotype, a pathognomonic sign of RTT.

References/Citations:


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Paper Title: Testing Two Observational System Approaches to Measure Behavioral Reactivity during Modified Quantitative Sensory Testing in Rett Syndrome

Authors: Alyssa Merbler1, Breanne Byiers1, Chantel Barney2, & Frank Symons1

Introduction: There are documented issues concerning somatosensory and nociceptive function in Rett syndrome (RTT), and the current body of literature has reports of both pain insensitivity (Hagberg, 2002)1 and evidence of pain expression (Symons et al. 2013; Barney et al., 2015)2,3. The current ‘gold standard’ for determining the presence of pain remains verbal self-report, and the complex communication issues make it difficult to characterize pain and/or somatosensory function. There is a need, then, to determine ways to evaluate the sensory (tactile/nociceptive) function to further investigate pain in RTT. One potential tool is the Pain and Discomfort Scale4, a coding system which scores vocal, facial, gross motor, and physiological-related behaviors associated with pain and discomfort for individuals who are non-verbal, and has been used to measure behavioral reactivity in various developmental disability populations, including RTT3. The purpose of this study was to develop a coding system based on this tool to successfully capture pain- and discomfort-related reactivity as a potential sensitive, clinic-friendly outcome measure for clinical trials.

Methods: We conducted a modified quantitative sensory test (mQST) in a convenience sample of 20 girls and women with clinically diagnosed RTT (M age = 15.9 years old), comprised of six calibrated tactile and nociceptive stimuli applied to the hands and feet5. Behavioral reactivity was quantified using a modified version of the Pain and Discomfort Scale (PADS). Two methods of scoring were used to determine what level of detail was needed to fully capture reactivity while still maintaining the ability to train coders to a high reliability standard. These 20 videotaped mQST protocols were scored as 1. Individual behaviors or 2. Scored as behavioral classes (e.g. vocal). Descriptive statics were calculated to characterize patterns of responding scored by both methods, and total scores between the two methods were compared using Pearson correlation.

Results: When examining total scores for each participant from the two scoring methods, there was an overall correlation of 0.71 (p = 0.0004). Both scoring systems led to similar patterns of behavior class scores and reactivity across the six stimuli.

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Additionally, both systems captured variability in response patterns across participants. The two methods varied in the amount of time needed to train coders, as scoring by individual behaviors took several months longer to reach 90% reliability.

**Discussion:** Overall, both methods quantified pain- and discomfort-related behavior in RTT. Both methods used were moderately correlated and showed similar patterns of responses during the mQST, indicating that scoring classes of behavior may be sufficient to fully describe pain- and discomfort-related reactivity in RTT. An advantage to the ‘scoring classes of behavior’ method is that it significantly less time (several months) for coders to reach a high reliability standard. The approach also reduces the time needed to code one participant’s protocol, saving both time and financial resources. Although there are increasing advances in automated facial coding related to pain expression, automated methods also present challenges and lack the inclusion of vocal, gross motor, and physiological signs of reactivity. With the move from mouse to human and the increasing number of clinical trials in RTT, a valid and reliable pain- and discomfort- based scoring method may provide a sensitive endpoint, as well as a way for further research to investigate somatosensory and nociceptive function in RTT.

**References/Citations:**


**Paper 6 of 6**

**Paper Title:** Telehealth-Supported Assessment and Intervention to Improve Communication Outcomes in Rett Syndrome

**Authors:** Jennifer J. McComas¹, Brittany Pennington¹, Nigar Noor¹, Shawnie Girtler¹, Jessica Simacek¹, & Adele Dimian¹,

**Introduction:** Rett syndrome (RTT) is a severe X-linked neurodevelopmental disability affecting an estimated 1 in 10,000 girls resulting in profound multiple disabilities across motor, communication, and cognition domains. Most individuals with RTT regress to the point of losing the ability to walk, talk, and use their hands functionally. Given the severity and multiple nature of the disabilities associated with RTT, it is not surprising that parents of children with RTT prioritize communication among their top concerns. Although parents frequently report they believe their daughters use idiosyncratic behaviors (e.g., eye pointing, facial expressions, gestures, body movements) to communicate, they also report that the lack of an efficient communication system has a substantial negative effect on the quality of life for their child with RTT. Despite scientific advances in the biological and genetic factors related to RTT, empirical research demonstrating effective communication interventions for individuals with RTT is virtually absent from the literature. The aim of this research is to develop a reinforcement-based intervention model addressing the complex communicative needs of individuals diagnosed with Rett syndrome.

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**Methods:** To date, six female participants between the ages of 21 mo and 9 years, have completed the study procedures. Each engaged in an idiosyncratic response that the family reported as communicative (e.g., eye gaze, crying). Single-subject experimental designs were used to examine the effect hypothesized reinforcers on (a) idiosyncratic and (b) augmentative alternative communicative (AAC) behavior in at least one context (e.g., snack time, play time) for with each participant. Direct observation of target behavior was conducted during 3-5 min sessions, on average 4 sessions per day, two days per week across an average of 18 weeks (4.5 months) via live telehealth sessions in which an experimenter coached the parent to implement all analysis procedures.

**Results:** In all cases, a functional relationship was identified between the target behavior (in some phases, the idiosyncratic response and in other phases, the AAC response) and the reinforcer. Illustrative data will be presented graphically.

**Discussion:** Our findings to date indicate that, for our sample of participants, individuals with RTT syndrome engage in idiosyncratic responses that serve a function and that they are able to learn a more formal, recognizable communicative response (AAC) that serves the same function.

**References/Citations:**