



EEG-Based Biomarkers of Neural Circuit Function in Rett Syndrome

Citation

Kranz, Devorah. 2025. EEG-Based Biomarkers of Neural Circuit Function in Rett Syndrome. Doctoral Dissertation, Harvard University Graduate School of Arts and Sciences.

Link

<https://dash.harvard.edu/handle/1/42720740>

Terms of use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material (LAA), as set forth at

<https://harvardwiki.atlassian.net/wiki/external/NGY5NDE4ZjgzNTc5NDQzMGIzZWZhMGFIOWI2M2EwYTg>

Accessibility

<https://accessibility.huit.harvard.edu/digital-accessibility-policy>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#)

HARVARD
Kenneth C. Griffin



GRADUATE SCHOOL
OF ARTS AND SCIENCES

DISSERTATION ACCEPTANCE CERTIFICATE

The undersigned, appointed by the
Division of Medical Sciences
Program in Neuroscience
have examined a dissertation entitled

EEG-Based Biomarkers of Neural Circuit Function in Rett Syndrome

presented by Devorah Kranz
candidate for the degree of Doctor of Philosophy and hereby
certify that it is worthy of acceptance.

Signature: Lauren L. Orefice
Lauren L. Orefice (Jul 31, 2025 13:31:41 EDT)

Typed Name: Dr. Lauren Orefice

Signature: Brielle R. Ferguson
Brielle R. Ferguson (Jul 31, 2025 14:40:49 EDT)

Typed Name: Dr. Brielle Ferguson

Signature: Jeffrey Neul
Jeffrey Neul (Jul 31, 2025 13:29:50 CDT)

Typed Name: Dr. Jeffrey Neul

Signature: Laura Baroncelli
Laura Baroncelli (Aug 1, 2025 16:36:37 GMT+2)

Typed Name: Dr. Laura Baroncelli

Date: July 31, 2025

EEG-Based Biomarkers of Neural Circuit Function in Rett Syndrome

A dissertation presented

by

Devorah Kranz

to

The Division of Medical Sciences

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the subject of

Neuroscience

Harvard University

Cambridge, Massachusetts

July 2025

© 2025 Devorah Kranz
All rights reserved.

EEG-Based Biomarkers of Neural Circuit Function in Rett Syndrome

Abstract

Atypical sensory processing is a key feature of many neurodevelopmental disorders, likely contributing to higher-order deficits in cognition, communication, and behavior. However, the neural mechanisms underlying these abnormalities remain incompletely understood. Rett syndrome (RTT) is one neurodevelopmental disorder, related to autism spectrum disorder and predominantly caused by *de novo* mutations in the X-linked *MECP2* gene. RTT is characterized by an initial period of typical early development followed by rapid regression leading to profound motor, communication, autonomic, and other impairments; disruptions to cognitive function and sensory processing have more recently emerged as core features. Electroencephalography (EEG) at rest, as well as time-locked to sensory stimuli (event-related potentials, or ERPs), offers powerful tools to study the neural dynamics underlying these disruptions, with the potential to bridge neural circuit dysfunction with clinically relevant neurophysiological markers and behavioral outcomes. This dissertation integrates findings from three EEG-based studies in Rett syndrome. First, we aimed to investigate variability in neural responses to sensory stimuli by measuring inter-trial phase coherence (ITPC). We found increased trial-by-trial variability in individuals with RTT in response to both visual and auditory stimuli, correlating with dampened ERPs and atypical sensory processing phenotype. In the second study, we utilized resting-state EEG to investigate thalamocortical contributions to sensory processing dysfunction by measuring

transient beta events (short bursts of beta-frequency activity linked to thalamocortical drive and implicated in tactile processing). We observed elevated transient beta event rates in RTT as well as other related neurodevelopmental disorders, pointing to disruptions within thalamocortical sensory pathways. Finally, we investigated interactions between oscillatory frequencies at rest in RTT by analyzing phase-amplitude coupling, a mechanism of neural coordination that supports a range of cognitive and sensory processes. Results demonstrated significantly increased theta-gamma and alpha-gamma coupling in RTT, and computational modeling further identified dysfunction in VIP+ interneurons as a candidate cellular mechanism underlying this enhanced coupling. Collectively, these findings advance the field's understanding of the neural dynamics underlying altered sensory and cognitive processing in RTT, paving the way for use as functional biomarkers and providing insights that can inform future work.

Table of Contents

Title Page.....	i
Copyright.....	ii
Abstract.....	iii
Table of Contents.....	v
Acknowledgements.....	vii
Chapter 1. General Introduction.....	1
Chapter 2. Variability in sensory processing and evoked potentials in Rett syndrome.....	18
Introduction.....	21
Methods.....	24
Results.....	32
Discussion.....	43
Chapter 3. Transient beta events on EEG in autism and related neurogenetic conditions.....	50
Introduction.....	55
Methods.....	57
Results.....	68
Discussion.....	72
Chapter 4. Altered oscillatory coupling reflects possible inhibitory interneuron dysfunction in Rett syndrome.....	78
Introduction.....	81
Methods.....	86
Results.....	96

Discussion.....	108
Chapter 5. General Discussion.....	115
References.....	122
Supplemental Figures and Tables.....	146
Appendix. Computational Methods.....	159

Acknowledgements

First, I'd like to thank my advisors: Dr. Michela Fagiolini, Dr. April Levin, and Dr. Charles Nelson. I have referred to my advisor trio as the “mentor dream team” many times throughout my PhD. All of you have influenced these projects in unique ways, and they would not be the same without your individual guidance and distinct scientific perspectives. I joined the lab(s) with a very different scientific background, so I thank you for teaching me everything I know about all topics covered in this dissertation. Lastly, thank you for enjoying the science with me at every step and for your unwavering support and kindness throughout the journey of my PhD.

Thank you very much to my dissertation advisory committee: Dr. Lauren Orefice, Dr. Alex Rotenberg, and Dr. Ann Poduri. I deeply appreciate your engagement in my project and your invaluable feedback and insight at every iteration. Thank you as well to my defense committee: Dr. Lauren Orefice, Dr. Jeffrey Neul, Dr. Brielle Ferguson, and Dr. Laura Baroncelli.

Thank you to my lab mates (across all three labs) who have made my PhD experience fun as well as scientifically thought-provoking. Thank you to Fagiolini and Hensch lab members who provided integral feedback at every lab meeting. Special thanks to Klara Szilagyi, Claire Mackay, and Karen Sabol in the Levin Lab for amazing teamwork on collecting data for the project, and a huge thank you to Yael Braverman for training and collaboration on EEG processing techniques and data analysis. Thank you as well to Dr. David Lieberman and the Rett Research Team at BCH.

Thank you to Dr. Nancy Kopell and Dr. Michelle McCarthy for a wonderful and scientifically fruitful collaboration. Michelle, I deeply appreciate your scientific dedication, patience, mentorship, and generosity.

Thank you to the Harvard Program in Neuroscience for these years of education, support, and a wonderful scientific environment, and thank you to my “Covid cohort” who all started PhDs remotely and still managed to be fantastic.

Thank you to Dr. Eve Marder, whose lab I had the wild fortune and privilege of joining as an undergraduate, for igniting my love of neuroscience and paving the way for me in just about every aspect of science and life. Thank you to Dr. Mike Long (and all my former lab-mates in the Long Lab) for amazing post-bac years before my PhD – you taught me, in many ways, how to be a scientist.

Finally, thank you to my friends and family. To my parents for everything you’ve done to get me here, and especially for being thrilled and excited for me at every turn. To my siblings, for support and friendship always. And to my husband – I’m grateful to you for innumerable things, but mostly for your stalwart and calm encouragement throughout this entire journey.

Chapter 1. General Introduction

Sensory processing differences are a key feature of many neurodevelopmental disorders (NDDs), most notably autism spectrum disorder (ASD).¹⁻⁴ Commonly, atypical sensory processing can include hypo-responsivity (seeking out or not responding to sensory stimuli) and hyper-responsivity (avoiding or overly responding to sensory stimuli).^{1,5,6} These differences arise from disruptions in how the nervous system receives, organizes, and interprets incoming sensory information from the environment and the body. Higher-order neural functions require reliable information from lower cortical regions like primary sensory areas to construct accurate representations of the external environment,⁷ and sensory processing is a foundation upon which both advanced cognitive function and social behavior build. Disruptions across sensory modalities can have cascading developmental consequences,⁸⁻¹¹ yet the neural mechanisms underlying sensory processing abnormalities in NDDs remain incompletely understood.

The high heterogeneity of idiopathic ASD—cases in which no clear biological cause has been identified—poses a major challenge to progress in this area, complicating the search for consistent overarching mechanisms and principles governing the underlying pathophysiology. One strategy to address this complexity is to divide the broad and diverse ASD population into biologically defined subgroups. Studying syndromic forms of ASD, such as genetic conditions with a high prevalence of autistic features (examples include Fragile X syndrome, Angelman syndrome, Rett syndrome, Prader-Willi syndrome, Williams syndrome, tuberous sclerosis, SYNGAP1-related disorder, and Phelan McDermid syndrome), offers a powerful approach for identifying the neural mechanisms and circuit-level disruptions underlying sensory processing abnormalities that may generalize to subsets of ASD more broadly. In fact, many of the genes underlying syndromic ASDs are functionally interconnected and converge on similar neural pathways.¹² This dissertation will mainly focus on one such neurogenetic disorder: Rett syndrome.

Rett syndrome and MECP2

Rett syndrome (RTT) is a rare and debilitating neurodevelopmental disorder affecting about 1 in 10,000 individuals, predominantly females. Typically, the disorder first manifests at around 6-18 months of age, with a developmental stagnation and lack of acquisition of new skills followed by a regression of purposeful hand use and spoken language. Other symptoms that emerge can include autism, truncal ataxia, seizures, and autonomic nervous system dysfunctions (including breathing irregularities).^{13–16} Sensory processing abnormalities have more recently been identified as a core feature of this disorder:^{17,18} individuals with RTT have been characterized to have reduced sensitivity to pain^{19,20} and recent data identifies atypical sensory symptoms in RTT overall and across multiple sensory modalities.²¹ However, assessing sensory phenotype and cognitive functioning is difficult given motor problems and challenges in verbal communication, and additional work is needed to build upon these findings.

In 1999, *de novo* pathogenic variants in the X-linked gene encoding for methylCpG binding protein 2 (MeCP2) were discovered to be the genetic basis of RTT.²² Females are mosaic for mutated and wild-type MeCP2 expression because of random X chromosome inactivation—males can be diagnosed with RTT but often present with more severe symptoms or do not survive gestation or infancy.²³ MECP2 mutations are also associated with an increased likelihood of autism spectrum disorder (ASD); many samples of forebrain tissue collected post-mortem from individuals with idiopathic ASD had reduced expression of MeCP2^{24,25} and DNA samples have revealed a higher frequency of mutations in *MECP2* in individuals with ASD.²⁶ Indeed, RTT is often used as a model for ASD because many symptoms overlap, although it can be difficult to diagnose.^{27,28(p2),29,30} Precise levels of MeCP2 are critical, as deviations in either direction—

deficiency or excess—can result in disorder. Currently, due to the phenotypic heterogeneity of *MECP2*-related disorders, which also include *MECP2* duplication disorder, intellectual disabilities, and autism, RTT is diagnosed based on clinical phenotype and followed up with targeted sequencing.¹⁶ However, in over 95% of cases a pathogenic variant of *MECP2* is present,³¹ and disease severity is strongly influenced by the type and location of the mutation as well as by differential patterns of X-chromosome inactivation skewing.^{23,32–37}

MECP2 mutations affect chromatin organization/remodeling, cofactor binding, RNA splicing, and DNA damage repair, and have roles as both transcriptional repressors and activators.^{38–43} The most common *MECP2* mutations found in individuals with RTT are amino acid substitutions R106W, R133C, T158M, R168X, R255X, R270X, R294X, and R306C; other common mutations include C-terminal deletions and large deletions.^{31,34,36} These mutations effect regions across the entire MeCP2 protein, including the methyl-CpG-binding domain, the transcription repression domain, the three AT hooks, the NCoR/SMRT interaction domain, and the C-terminal domain.⁴¹ Phenotype has been shown to be more severe as a result of truncating mutations compared to missense mutations, and when mutations are located earlier than the nuclear localization signal.^{33,35,44,45} *MECP2* mutations in the methyl-CpG binding domain (e.g. T158M, T158A, R106W) disrupt DNA binding and simultaneously destabilize MeCP2 protein; re-expression of MeCP2 T158M rescues several characteristics of the RTT phenotype.^{46–49} Some *MECP2* mutations have also been associated with particular phenotypic presentations (e.g. sleep disturbances, as opposed to overall clinical severity)⁴⁴, but more work is needed to characterize specific effects of each known mutation at both neurophysiological and behavioral levels.

Although MeCP2 is expressed widely throughout the body, its levels are 5 to 10-fold higher in neurons compared to other cell types; neuron-only mutations of *Mecp2* render mice as impaired

as those with the mutation expressed across tissue types, indicating that neuronal dysfunction is required for RTT phenotype.⁵⁰ MeCP2 is expressed from early embryonic stages to adulthood and affects the brain throughout the life cycle: its function affects prenatal neurogenesis, development of neuronal circuitry, synaptic plasticity, and even maintenance of adult neural function such as sensory processing.^{38,40,51–60} Overall, MeCP2 is involved in more than sixty neural pathways including dendritic spine morphology and activity-dependent gene expression.^{61–64}

Circuit dysfunction in RTT: excitatory/inhibitory balance

In addition to clinical studies, the use of an extensive gene-editing toolkit in mice—which possess the conserved *Mecp2* gene—has yielded substantial insights into circuit dysfunction in Rett syndrome.⁶⁵ Most animal models of RTT involve either hemizygous males (*Mecp2*^{−/y}; resulting in a total knockout of the *Mecp2* gene) or heterozygous females (*Mecp2*^{+/-}; mosaic due to X chromosome inactivation), which have good validation, recapitulating many core aspects of the RTT phenotype.⁶⁶ Hemizygous males exhibit early and robust symptoms, while heterozygous females are more aligned with the X-inactivation and mosaicism of most RTT patients but exhibit later symptom onset and greater phenotypic variability. In addition to these germline models, studies have employed a range of conditional *Mecp2* mouse lines that enable spatially or temporally restricted manipulation of *Mecp2* expression—including cell type-specific or region-specific deletions and rescue/reactivation strategies using Cre-lox systems.^{67–69} These models enable investigation of the role of *Mecp2* in defined neural populations and developmental windows. Other lines carry patient-relevant point mutation knock-ins that model specific pathogenic variants observed in individuals with RTT.^{47,70–73} Phenotypically, overall disease severity is commonly assessed using a standardized scoring system based on inertia, gait, hind-

limb clasping, tremor, and irregular breathing, and is often complemented by additional relevant behavioral assays (e.g. of motor function, sociability, learning, etc.).^{74,75}

At the core of neural circuit function is the capacity for synaptic communication, which enables the integration and processing of external stimuli. A proper balance between excitatory and inhibitory (E/I) signaling is essential for maintaining circuit stability and information flow.^{76,77} Disruption of this balance has emerged as a defining feature of RTT, with numerous *in vivo* and *in vitro* studies identifying E/I abnormalities across multiple cortical and subcortical regions. Importantly, the effects of *Mecp2* loss on E/I balance vary depending on the type of mutation and the specific brain region and cell population. For example, using specific biotin tagging of MeCP2, two specific *MECP2* mutations were revealed to be differentially regulated in excitatory versus inhibitory cortical neurons.⁴⁹

Overall, in both *Mecp2*^{-y} and *Mecp2*^{+/-} mice and across multiple cortical areas, circuits consistently exhibit a shift toward inhibition.^{78-80,80,81} This shift likely arises from a convergence of alterations, including delayed maturation of excitatory synapses and a decrease in the firing of excitatory pyramidal (PYR) neurons,⁸²⁻⁸⁴ as well as the overall functional and structural upregulation of parvalbumin-positive inhibitory interneurons (PV+INs) and inhibitory circuits which normally provide fast-spiking inhibition to PYR neurons.^{78,85-89} Among neural cell types, GABAergic inhibitory interneurons seem to be particularly vulnerable to *Mecp2* dysfunction. MeCP2 is more abundantly expressed in inhibitory interneurons than in excitatory PYR cells, and selective removal from GABAergic neurons is sufficient to reproduce many features of the RTT phenotype.⁹⁰ *Mecp2*-deficient mice display upregulation and accelerated maturation of PV+ circuits (whereas somatostatin-expressing (SOM+) neurons are downregulated) with increased perineuronal net engulfment and enhanced synaptic complexity at the soma of PYR neurons,^{86(p20)}

premature NMDA receptor maturation in PV+ interneurons compared to slower maturation in excitatory PYR cells in visual cortex,⁸⁹ and excessive excitatory inputs converging onto PV+ cells, including thalamocortical inputs.^{87,88} Furthermore, the density of calretinin+ cells and PV+ cells are increased at early post-natal stages (whereas SOM+ cells, for example, are not affected).⁹¹ Vasoactive intestinal peptide-expressing interneurons (VIP+INs), which synapse onto both PYR cells and other interneurons, disinhibiting PYR neurons,^{92,93} have also recently emerged as an especially relevant class of GABAergic neurons in RTT circuit dysfunction: selective deletion of *Mecp2* from VIP+INs results in altered overall firing rates and a loss of state-dependent modulation of cortical activity.⁹⁴

Functionally, these changes produce substantial and distinct consequences, and selective deletion of *Mecp2* from specific GABAergic interneuron subtypes produces different, nonoverlapping phenotypes. Loss from PV+ neurons disrupts experience-dependent plasticity^{78,95} and leads to a broad spectrum of RTT symptoms including sensory, motor, social, and cognitive deficits, whereas loss in SOM+ neurons primarily results in seizures and stereotypies.⁹⁶ Loss of *Mecp2* from VIP+INs (but not from PV+ or SOM+ IN populations) results in a reversal of normal social preferences and deficits in marble burying (a task implicated in anxiety and OCD-like behaviors), but seizure and mortality rates are unaffected.⁹⁴ Deletion of *Mecp2* in glutamatergic neurons, on the other hand, leads to a more mild (but distinct) phenotype compared to deletion from inhibitory neurons, including tremor and increased anxiety.⁶⁸ Deletion in forebrain excitatory neurons postnatally produces motor and social deficits,⁹⁷ and in the prefrontal cortex, loss of *Mecp2* from PYR neurons leads to lower rates of neural transients during social activity, as well as overall hypoactivity and lack of social preference.^{98,99}

These region- and cell-type-specific effects extend to primary sensory areas. In visual cortex, in addition to upregulation of inhibitory PV+ circuitry, an attenuation of both spontaneous and visually evoked neural firing was found, as well as a regression in visual acuity.^{78,86} Furthermore, experience-dependent plasticity is impacted: in *Mecp2*^{-y} mice, the critical period for ocular dominance plasticity was earlier than in wildtype, opening and closing precociously and disrupting binocular matching of orientation tuning.⁸⁵ Interestingly, in auditory cortex, conditional deletion of MeCP2 is sufficient to produce impairment in maternal gathering behavior usually performed in response to pup distress calls.⁸¹ Importantly, basic auditory function was preserved, and this behavior was correlated with heightened expression of PV+ and perineuronal nets; in wildtype mice, maternal experience suppresses the PV response in auditory cortex, enabling response of PYR neurons, but this is abolished in *Mecp2*^{+/-} mice.¹⁰⁰ In the somatosensory cortex, brain slice recordings from *Mecp2*^{-y} mice reveal reduced spontaneous PYR activity, diminished miniature excitatory postsynaptic currents, and a general shift toward enhanced inhibition.⁸³ *Mecp2*^{-y} mice also have increased PNN density in primary somatosensory cortex, with disrupted lateralization patterns following maternal experience.¹⁰¹ Behaviorally, *Mecp2*^{-y} mice exhibit altered tactile and thermal sensitivity.¹⁰²

In the hippocampus, *MeCP2* deficiency leads to disrupted excitatory synaptic function and network hyperexcitability through both cell-autonomous and circuit-level mechanisms. At the single-cell level, glutamatergic neurons in the hippocampus lacking MeCP2 have a lower excitatory synaptic response (due to lower synaptic density),¹⁰³ and hippocampal cultures exhibit decreased spontaneous excitatory synaptic transmission (mEPSC) frequency, even when *MeCP2* is deleted post-synaptogenesis.⁸² In contrast, in *Mecp2*^{-y} mice, CA3 PYR neurons exhibit increased spontaneous EPSC amplitude and frequency alongside reduced inhibitory

synaptic input, pointing to an emerging network hyperactivity.^{104,105} Thus, while loss of *MeCP2* weakens excitatory connectivity cell-autonomously, broader circuit-level dysfunction of inhibitory circuits in the hippocampus may contribute to overall hyperexcitability. These synaptic and circuit disruptions align with impairments in hippocampus-dependent spatial, contextual, and social memory in *Mecp2* mutant mice.¹⁰⁶

Beyond cortical and hippocampal networks, loss of *MeCP2* also affects a variety of other neural systems and cell populations differentially. In *Mecp2*^{-/-} mice, hindbrain circuits exhibit elevated expression of the immediate early gene *c-Fos* (*which is* associated with synaptic hyperexcitability), while *it* is reduced in forebrain regions.¹⁰⁷ Furthermore, region-specific deletion of *Mecp2* in the brainstem and spinal cord causes autonomic abnormalities affecting heart rate and breathing.¹⁰⁸ In the thalamus, beginning only after synaptic development during the later experience-dependent phase of remodeling retinogeniculate synapses, retinal inputs fail to strengthen and synaptic plasticity is disrupted in *Mecp2*^{-/-} mice.¹⁰⁹ Deletion of *Mecp2* in various neuromodulatory systems also produces dissociable behavioral phenotypes: selective deletion in cholinergic neurons impairs recognition memory,¹¹⁰ deletion from dopaminergic and noradrenergic neurons results in deficits to locomotor activity, and loss in serotonergic neurons results in increased aggressive behavior.¹¹¹ Strikingly, targeted deletion of *Mecp2* exclusively from peripheral somatosensory neurons impairs presynaptic inhibition from primary sensory neuron afferents in the spinal cord to the central nervous system and results in tactile hypersensitivity.¹¹² Altered tactile responses arise whether deletion is performed in development or adulthood, but if done during development, mice also develop aberrant anxiety-like behaviors as well as impaired socialization, highlighting sensory processing as a building block for and contributor to higher-order phenotypic deficits such as affective and social behaviors.

Together, these findings underscore the central role of MeCP2 in regulating excitatory/inhibitory balance and shaping synaptic function and circuit wiring across diverse brain regions and cell types. Disruption of these mechanisms likely contributes to the emergence and progression of atypical sensory processing as well as broader cognitive impairments in RTT throughout development.

EEG-based tools

While animal models have provided critical insights into the cellular and circuit-level consequences of *MeCP2* dysfunction, probing these mechanisms in humans presents a substantial challenge. Electroencephalography (EEG) provides a unique opportunity to study neural activity in both animal models and humans using a shared methodology. By capturing large-scale electrical activity generated by summed coordinated postsynaptic potentials, EEG can provide a powerful measure of circuit-level etiopathology and large-scale neural dynamics.¹¹³ Its cross-species applicability makes EEG particularly well-suited for identifying translational biomarkers that reflect circuit-level abnormalities in disorders like RTT. In this context, EEG can link clinical features—such as behavioral phenotypes or potential neural signatures observed in individuals with RTT—to the pathophysiological mechanisms identified in preclinical models.¹¹⁴

Using EEG, one can study neural oscillations—rhythmic fluctuations in excitability of large populations of neurons. These rhythms emerge from reciprocal interactions between excitatory and inhibitory neurons,^{77,115} and brain-wide dynamics rely heavily on coordination of and by neural oscillations to facilitate communication between distant cortical regions and support cognitive processing.¹¹³ Neural oscillations are characterized by frequency, amplitude, and phase, and can be transformed into frequency bands with standard frequencies ranging from below 1 Hz

to over 100 Hz, including delta (<4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-30 Hz), and gamma (>30 Hz).¹¹³ EEG rhythms at these different frequencies have been implicated in sensory perception, memory, and cognition in both typical development and NDDs.^{114,116} Given the widespread alterations caused by MeCP2 dysfunction across diverse brain regions and functions, studying these oscillations provides a powerful way to investigate neural dynamics at the level of large-scale brain networks in RTT.^{13,117}

Resting/Spontaneous EEG

EEG can be analyzed in both spontaneous and time-locked contexts, offering complementary perspectives. Resting-state EEG captures ongoing neural activity in the absence of discrete events. Clinical EEG at rest in RTT has long revealed visually observed abnormalities such as diffuse slowing of activity and epileptiform discharges that generally emerge by age three.^{118–121} Slowing of background EEG tends to persist throughout the disorder, while the presence of epileptiform activity does decline with age. More recently, studies have also utilized quantitative analyses of resting EEG in RTT; a common analytic approach is spectral power analysis, used to examine the power of each individual frequency present in the signal (i.e. its contribution to ongoing activity). Studies have revealed evidence of region-specific spectral power disturbances in 57 individuals with RTT and 37 TD controls, including increases in delta and theta power, reductions in alpha power, and alterations in the 1/f spectral slope.^{122,123} In *Mecp2*^{-y} mice, decreases in alpha and gamma power have also been reported (*unpublished data*). Notably, several studies have also used resting EEG-based biomarkers to track treatment efficacy and disease progression.¹²⁰ For example, a trend toward more typical spectral powers was reported in EEG of girls with RTT after just five days of cognitive training.¹²⁴ Additionally, alpha asymmetry (greater

alpha power at right vs left frontal electrodes), which is an index of anxiety and depression,¹²⁵ has been used to evaluate the effects of mecamsermin treatment in a RTT clinical trial.¹²⁶ These results demonstrate that quantifiable abnormalities in EEG can be sensitive to treatment and position them as valuable biomarkers.

Evoked potentials

Event-related or evoked potentials (ERPs) provide a noninvasive means of probing neural responses to sensory stimuli. By time-locking the brain's response to a discrete external stimulus (e.g., an auditory tone or visual stimulus), ERPs capture the millisecond-by-millisecond time course of information transmission through the brain, resulting in a series of voltage deflections in the ongoing EEG. ERPs can be reliably elicited in response to passively presented sensory stimuli even in the absence of active engagement or overt behavior, making ERPs particularly well-suited for studying individuals with RTT who often experience severe impairments in motor function and communication. Moreover, ERP features are increasingly recognized as clinically meaningful: in typically developing (TD) children, ERP abnormalities correlate with lower cognitive performance as measured by the Mullen Scales of Early Learning, and in individuals with RTT, ERP abnormalities have been shown to correlate with disease severity.¹²⁸

ERPs consist of amplitude changes over time, and they are most often characterized by polarity: positive 'P' or negative 'N' peaks. The latency of these peaks can be thought of as the timing of a cognitive process that is modulated by the stimulus, and the amplitude of the peaks refers to the degree of activation or strength of that process. Often, ERPs are measured at electrode locations on the scalp (for example, VEP is usually measured from electrodes above primary visual cortex, sometimes called 'Oz'), and are traditionally resolved by averaging many single trials. ERP

abnormalities have been well-documented across multiple neurodevelopmental disorders with atypical sensory processing and some overlapping features with RTT, including ASD,^{129(p201),130,131} Down Syndrome,¹³² Prader-Willi syndrome,¹³³ Fragile X syndrome,^{134,135} Angelman Syndrome,¹³⁶ Phelan McDermid syndrome,¹³⁷ CDKL5 deficiency disorder¹³⁸ and others, highlighting that despite differing genetic etiologies, ERPs can serve as a common endophenotype across disorders.

Auditory-evoked potentials in RTT

Of all sensory modalities, auditory processing has been studied most comprehensively in RTT. Although verbal communication is severely impaired, the degree of auditory processing retained in RTT has required further examination. Many initial studies focused the auditory brainstem response (ABR), and most report normal ABR in RTT patients^{139–141} as well as mouse models.^{47(p201)} Auditory information travels up the nervous system from the ear to the brainstem to the thalamus and finally to the auditory cortex, which can be measured as the auditory evoked potential (AEP). AEP has long been shown to be atypical in RTT, from early studies^{141–143} to more recent ones.^{17,128,144–146} A total absence of N1 and P2 components has been reported in some individuals with RTT,¹⁴² as well as significantly delayed latencies of both components.¹⁴¹ Similarly, both latency and amplitude of N1-P2 complex were found to be significantly altered in RTT participants compared to TD controls.^{17,146} Recently, a standardized presentation of tones was used to elicit AEP, with RTT participants exhibiting significantly lower P1-N1 and N1-P2 amplitudes compared to TD, and lower amplitudes correlated with higher disease severity (but latency was not significantly different between groups).¹²⁸

AEP has also been measured in *Mecp2* models. Mice carrying a substrate of the *Mecp2* mutation T158A exhibit both delayed and diminished AEPs in response to white noise after

starting to display symptoms.⁴⁷ Importantly, no differences in hearing sensitivity were measured by ABR, indicating that the ERP abnormalities are a result of disruptions to cortical processing. Interestingly, forebrain GABAergic neurons were sufficient to produce these altered AEP waveforms,⁶⁷ and the AEP response was rescued by increasing T158M MeCP2 protein levels,⁴⁸ which is exciting for both future treatments and the validation that this gives to ERPs as useful functional biomarkers in RTT.

Some studies have also presented variable auditory stimuli while recording AEP to evaluate higher-order auditory processing.^{141,143,146} These studies utilize an oddball paradigm, where an infrequent tone is presented amongst a string of more frequent tones, and then a mismatch response is calculated; in TD individuals, the deviant auditory stimulus elicits a response with enhanced amplitude. These enhanced responses, although clearly retained in RTT participants, are significantly delayed and protracted in duration.¹⁴⁶ Furthermore, a mismatch response in girls with RTT is elicited by a change in stimulus duration, but only if stimuli are presented at a fast rate—if stimuli are presented at a slower rate, the enhanced response to changed duration disappears. In other words, although individuals with RTT can detect deviations in auditory duration, the ‘memory span’ for duration is severely curtailed.¹⁷ Interestingly, using a slightly differing paradigm, auditory responses in anesthetized *Mecp2*^{+/-} rats were found to be hyperexcitable given sounds presented with a long inter-stimulus interval but hypoexcitable given a short interstimulus interval, and hyperexcitable given sounds presented at high intensities but hypoexcitable given low intensities.¹⁴⁷

A recent study examined the difference in auditory responses of children with RTT and MECP2 duplication syndrome to their own name vs a random name. While children with MECP2 duplication syndrome exhibit more positive evoked potentials for their own name compared to

other names, no such discrimination is observed in participants with RTT; elevated AEP amplitude in patients with MECP2 duplication syndrome is also associated with higher social functioning.¹⁴⁸ Girls with RTT have also been reported to show AEPs differentiating between words and non-words at right temporal sites instead of the more canonical left sites.¹⁴⁹

Visual evoked potentials in RTT

Visual evoked potentials (VEPs) are usually elicited using a reversing pattern (often a checkerboard at low spatial frequency and high contrast) and also exhibit substantial abnormalities in RTT. This robust response matures within the first 6 months of age¹⁵⁰ whereas the morphology of the AEP changes in adults compared to children.¹⁵¹ In one of the earliest studies examining VEP in RTT, 10 RTT girls were shown this stimulus—although they reported altered waveforms, the data did not reach statistical significance.¹⁵² Later, an oddball task was utilized to investigate how individuals with RTT process novel visual stimuli; TD controls exhibited larger VEP responses to novel stimuli compared to frequent stimuli, while no clear discrimination between the groups could be made in the responses of RTT participants.¹⁴³ More recently, altered VEP waveforms were shown in *Mecp2*^{+/-} mice, as well as consistent differences in multiple components of the VEP from 34 girls with RTT.¹⁵³ Individuals with RTT were shown to have substantially decreased N1-P1 amplitude, an increase in N2 latency, and a strong increase in P1-N2 time. These findings are impacted by disease stage and mutation severity. In addition to examining the standard VEP, varying spatial frequencies were also presented (albeit in a smaller group) to evaluate visual acuity: RTT participants present with reduced sensitivity, with a dominant spatial frequency significantly lower than TD individuals. These results have been further validated, with significantly lower N1-

P1 and P1-N2 amplitudes in 41 RTT participants compared to TD participants; the degree of amplitude change correlated with disease severity.¹²⁸

Somatosensory evoked potentials in RTT

Much less is known about somatosensory processing in humans with RTT. Early research shows delayed latencies in N13 and N20 components of somatosensory evoked potentials (SSEPs), as well as prolonged interpeak intervals in the N13-N20 and N20-P30 complexes, but no amplitude changes.^{154–156} Interestingly, other early studies have focused on “giant” cortical SSEPs in N20-P30 or P30-N35 complexes^{155,157,158}; giant SSEPs are found in many individuals with myoclonus, which is also a symptom of RTT. Similarly, SSEPs of 9 girls with RTT and 6 children with myoclonus epilepsy revealed giant SSEPs in most individuals of both groups.¹⁵⁷ Studies in this area have been limited by technique, power, lack of TD controls, and high clinical variability, and further work is needed to elucidate if tactile sensation is affected similarly to vision and audition, or whether it perhaps trends in a different direction.

Looking forward

The Autism Biomarkers Consortium has defined biomarkers as a measurable characteristic of a biological process or response; they differ from other clinical assessments in that they focus on objectively quantifiable measures and can also expose mechanism.^{131,159} EEG-based biomarkers are entering clinical trials more frequently and are considered increasingly important for future diagnostics, evaluating of disease progression, and quantifying response to therapeutic interventions.¹⁶⁰ Ideally, objective biological markers of central nervous system function would replace clinical assessments as the efficacy readout due to greater sensitivity and less bias, and

there is therefore a pressing need for biomarkers of neurodevelopmental disorders. Studying Rett syndrome, a rare monogenic disorder with relatively little heterogeneity, allows for the in-depth, controlled, and generalizable study of principles that can apply to other disorders.

Outside of traditional ERP analyses, spectral power, and symmetry studies, there are a range of other quantitative analyses available using EEG that may be best suited to answer functional, circuit-level questions. This dissertation will utilize three such techniques—inter-trial phase coherence, transient beta events, and phase-amplitude coupling—to probe underlying neural mechanisms and circuit alterations that could give rise to atypical sensory and cognitive processing in this disorder.

Chapter 2. Variability in sensory processing and evoked potentials in Rett syndrome

Devorah Kranz,^{1,2} Klara Szilagyi,¹ Claire MacKay,¹ Karen Sabol,¹ David Lieberman,^{1,2} Charles Nelson,^{1,2} April R Levin,^{*1,2} Michela Fagiolini^{*1,2}

*Co-last authors

Abstract

Rett syndrome (RTT) is a rare neurodevelopmental disorder caused primarily by pathogenic variants in the *MECP2* gene and characterized by severe cognitive, motor, and autonomic impairments. Atypical sensory processing is increasingly recognized as a key feature of RTT, with both hypo- and hyper-responsivity to sensory stimuli reported. These seemingly opposing sensory processing phenotypes frequently co-occur in related neurodevelopmental disorders including autism spectrum disorder and can significantly affect quality of life. However, objective and quantifiable biomarkers that capture variability in sensory processing remain limited. Event-related potentials (ERPs), which capture time-locked neural responses to sensory stimuli, have previously revealed delayed or attenuated auditory and visual responses in RTT, though the cause of these impairments is not fully understood. Inter-trial phase coherence (ITPC), which quantifies trial-by-trial variability within individuals' neural responses to sensory stimuli, offers important analysis of ERPs and has emerged as a promising functional biomarker of atypical sensory responsivity in neurodevelopmental disorders where response consistency cannot be assumed. In this study, we investigated neural response variability as a possible contributor to dampened ERPs and atypical sensory responsivity in 32 individuals with RTT and 28 typically developing (TD) controls. We found that hypo- and hyper-responsivity to sensory stimuli were both significantly elevated in individuals with RTT and strongly associated with one another. ITPC

was significantly lower in RTT across both visual and auditory modalities, correlating with reduced ERP amplitudes. Reduced ITPC in visual-evoked potentials were also associated with elevated visual responsivity and behavioral symptom severity. These findings suggest that variability in neural responses may contribute to both diminished ERPs and ultimately atypical sensory responsivity phenotype in RTT, informing the development of functional biomarkers which may be applicable across neurodevelopmental disorders.

Author affiliations

1. Boston Children's Hospital, Boston, MA 02115
2. Harvard Medical School, Boston, MA 02115

Correspondence to:

Dr. Michela Fagiolini, michela.fagiolini@childrens.harvard.edu, Center for Life Sciences, 3 Blackfan St, Boston, MA 02115

Dr. April Levin, april.levin@childrens.harvard.edu, 2 Brookline Place, BCH 3433, Brookline, MA 02445

Keywords

Rett syndrome; Neurogenetic disorders; Sensory processing; Sensory paradox; Variability; EEG; ERP; Auditory-evoked potential; Visual-evoked potential; Inter-trial phase coherence

Introduction

Rett syndrome (RTT) is a rare neurodevelopmental disorder affecting about 1 in 10,000 individuals predominantly caused by de novo pathogenic variants in the X-linked *MECP2* gene.²² These mutations are also associated with an increased likelihood of autism spectrum disorder (ASD); RTT is often used as a model for ASD because many symptoms overlap, although ASD can be hard to diagnose in Rett given cognitive and motor challenges.³⁰ Primarily affecting females, individuals with RTT initially experience a typical period of development followed by rapid and regressive loss of purposeful hand use and verbal language. Cognitive, motor, and autonomic dysfunctions including epilepsy, irregular or absent gait, and breathing irregularities are additional common symptoms.^{13,161} More recently, differences in sensory processing—a key feature of many neurodevelopmental disorders (NDDs), especially autism spectrum disorder (ASD)^{1–3}—have also been identified as a core feature of RTT.^{17,18}

These atypical sensory processing can include hypo-responsivity (seeking out or not responding to sensory stimuli) and hyper-responsivity (avoiding or overly responding to sensory stimuli). Both hypo- and hyper-responsivity to sensory stimuli have been shown^{1,2,5,6} and frequently co-occur^{6,162,163} within individuals with ASD, suggesting that atypical sensory responsivity may therefore reflect underlying variability in sensory processing rather than distinct or opposing sensory profiles. These atypical responses to sensory stimuli can have cascading developmental consequences, impacting social interactions, cognitive functioning, and overall quality of life.^{8–10} In RTT, similar differences in sensory responsivity has been reported, suggesting increased sensory hyper-responsivity, hypo-responsivity, and seeking behaviors.²¹ However, additional work is needed to examine the relationship between these various aspects of sensory

processing, understand how intra-individual variability in sensory responses may contribute to atypical sensory processing, and develop biomarkers that reflect this variability.

Electroencephalography (EEG), and particularly event-related potentials (ERPs)—offer powerful tools to investigate the neurophysiological underpinnings of sensory processing deficits in RTT.^{128,143,153,164} By time-locking the brain's response to an external stimulus (such as the punctate presentation of a visual or auditory stimulus), ERPs capture the msec-by-msec time course of information transmission through the brain, resulting in a series of voltage deflections in the ongoing EEG. ERPs are measured on scalp electrodes and consist of amplitude changes over time that are characterized by polarity: positive 'P' or negative 'N' peaks. ERPs can be reliably elicited in response to passively presented stimuli requiring no patient-report or behavioral response, making them very suitable for studying individuals with RTT and other neurodevelopmental disorders. Furthermore, there is growing evidence that ERP abnormalities correlate with lower scores on the Mullen Scales of Early Learning in TD individuals¹²⁷ and overall clinical severity in RTT.^{128,153} ERP abnormalities have been well-documented across multiple neurodevelopmental disorders with strong features of sensory processing deficits and some overlapping features with RTT, including ASD,^{129(p201),130,131} Down Syndrome,¹³² Prader-Willi syndrome,¹³³ Fragile X syndrome,^{134,135} Angelman Syndrome,¹³⁶ Phelan McDermid syndrome,¹³⁷ CDKL5 deficiency disorder¹³⁸ and others.

Auditory evoked potentials (AEPs) have consistently revealed abnormalities in RTT,^{17,128,141–146} most prominently characterized by the altered amplitude of components such as P1-N1 and N1-P2 which associate with clinical severity.^{17,128,146} Knockin mice recapitulating a specific *Mecp2* mutation exhibit delayed and reduced AEPs without any alterations in auditory brainstem response, indicating that the ERP abnormalities are a result of disruptions to cortical

processing.⁴⁷ Studies of higher-order auditory processing, assessed through paradigms such as oddball tasks (where detection of infrequent tones elicits a mismatch response with enhanced amplitude), also reveal delayed and prolonged responses in RTT.^{141,143,146} Additionally, individuals with RTT show impaired auditory discrimination abilities, particularly in response to stimulus duration changes when presented slowly, suggesting disrupted auditory memory span.^{17,148} Visual evoked potentials (VEPs), typically elicited by reversing patterns—often a checkerboard or grid at low spatial frequency and high contrast—also exhibit substantial abnormalities in RTT. This robust response matures quickly within the first 6 months of age¹⁵⁰, whereas the morphology of the AEP changes in adults compared to children.¹⁵¹ Altered VEP waveforms have been shown in *Mecp2* heterozygote female mice, and in individuals with RTT, significant differences in P1 amplitude and N2 latency have been observed, correlating with disease severity and progression.^{128,153} Despite extensive evidence of impaired evoked potentials in RTT in both the visual and auditory domains, the cause of these disrupted neural responses still requires elucidation.

ERPs are traditionally resolved by averaging many single trials, where interpretations often assume that within individuals, the response to repeated presentations of an identical stimulus will remain identical (with a fixed latency and polarity). Therefore, averaging the signal across trials would enhance the clarity of the neural response by reducing background noise (even oscillatory EEG processes) that are considered irrelevant to stimulus processing.^{165,166} While this may largely hold true in neurotypical populations, it might not accurately reflect the underlying neural dynamics in neurodevelopmental disorders with variable sensory responsivity like ASD or Rett syndrome.¹¹⁴ In such populations, averaging in this way could inadvertently reduce neural signals if they are temporally unaligned. In other words, although important insights can be gleaned from

amplitude and latency measures averaged across trials solely in the time-domain, critical information contained within intra-individual trial-by-trial variability may be lost. It has indeed been shown that in typically developing (TD) individuals, ERP components emerge largely from consistent phase alignment of neural oscillations across trials, especially within lower frequency bands such as alpha.^{167–170}

Time-frequency analyses, and in particular inter-trial phase coherence (ITPC), can quantify this evoked oscillatory phase-locking across trials, measuring the variability or consistency of the neural response on from totally random phase distribution to perfect phase synchronization. Higher ITPC reflects stable neural responses with closely aligned phases, whereas lower ITPC indicates greater variability. Studies have demonstrated reduced task-related ITPC in ASD,^{171–175} and in RTT, recent data examining variability and signal processing techniques showed high levels of variability across multiple metrics including ITPC in auditory-evoked potentials.¹⁴⁴ However, ITPC in visual-evoked potentials has not yet been studied in RTT, and it remains unclear whether this variability is related to any phenotypic measures of sensory processing.

Here, we characterized sensory responsivity phenotype in individuals with Rett syndrome, and measured variability in neural responses to auditory and visual sensory stimuli by quantifying ITPC in evoked potentials and comparing RTT to typical development (TD). We aimed to test three main hypotheses: (1) that individuals with RTT would have co-occurring hypo- and hyper-responsivity to sensory stimuli (2) that individuals with RTT would exhibit lower ITPC compared to TD in both AEP and VEP (2) that this neural response variability would be associated with reduced ERP amplitude as well as sensory responsivity.

Materials and Methods

Participants

Data were acquired at BCH as part of the IDDRC Rett Syndrome Biomarkers Study (RSBS). The experimental protocol was approved and overseen by the Institutional Review Board at Boston Children's Hospital.

Participants were individuals with Rett Syndrome (RTT; n=36), aged 1-45 years old, and typical development (TD; n=28), aged 2-23 years old. While many prior studies in RTT have focused on a narrower age range, we chose this wider range to capture the full spectrum of the disorder and examined the effects of age on our analyses as described below. Participants were recruited through hospital-based Rett clinics, a research registry, clinical referrals, community sources, and word of mouth. Informed consent was obtained from all guardians or participants above 18 years old, and assent from all participants developmentally able.

In the RTT group, inclusion criteria included being 12 months to 50 years old at time of enrollment and a diagnosis of RTT with a previously identified pathogenic or likely pathogenic variant in the *MECP2* gene. TD participants were recruited to be approximately age and sex-matched to the RTT participants and were included if they had no underlying genetic diagnoses or prior medical conditions associated with increased likelihood of ASD or intellectual disability. In both groups, the primary language of participants and caregivers was English. Exclusion criteria for the RTT group included taking an investigational drug as part of another research study within 30 days prior to study enrollment.

Demographic and other characteristics are reported in Table 2.1.

Table 2.1 Participant Information

	Diagnostic Group	
	RTT (n = 32)	TD (n = 28)
Clinical Severity Score		
<10	3	
10->19	16	
20->29	11	
30->39	2	
Clinical Global Impressions of Severity		
<4	1	
4	13	
5	17	
>5	1	
Mutation Severity		
Severe	18	
Intermediate	10	
Mild	4	
Rett Diagnosis		
Typical	20	
Atypical	12	
Seizure History		
No	11	
Yes	21	
Sex		
Female	32	26
Male	0	2
Age		
	Range: 1-45	Range: 2-23
	Median: 9	Median: 7
	IQR: 16.25	IQR: 6.25
Usable VEP		
Yes	31	28
No	1	0
Usable AEP		
Yes	27	26
No	0	0

Phenotypic and clinical measures: sensory processing

Parents or caregivers completed detailed medical history questionnaires and phenotypic assessments during the visit (or at home to be sent back within 6 months of the visit date). Sensory processing phenotype was assessed for both groups using the Child Sensory Profile 2 (SP2), a standardized parent-report questionnaire that measures sensory processing patterns in everyday life.^{1,176} We used the Child SP2 for all participants in the study regardless of age; the Child SP2 has only been validated for individuals in the developmental age up to 14 years old, but given the developmental level of our RTT participants, we used it outside of the normative range. Parents were asked to rate their child's response to a sensory experience on a scale ranging from 'Almost Never' (1) to 'Almost Always' (5). An overall hyper-responsivity score was calculated by summing the raw score of all questions in the Avoiding and Sensitivity quadrants, and an overall hypo-responsivity score was calculated by summing the raw score of all questions in the Registration and Seeking quadrants.⁶ Visual responsivity and auditory responsivity scores were then analyzed. In all cases, higher scores indicate more sensory difficulties. We use the term "responsivity" for these phenotypic measures throughout, as in He, et al., 2023.¹⁷⁷ Note that the SP2 was introduced later in the study, and not all participants with ERP recordings also have SP2 data; we report on SP2 results from 29 individuals with RTT and 25 typically developing individuals, and out of these, we recorded VEP from 24 RTT and 25 TD participants, and AEP from 24 RTT and 23 TD participants.

Phenotypic and clinical measures: clinical severity and mutations

Clinical severity in RTT was assessed using two measures completed by clinicians: the Clinical Severity Scale (CSS)³⁶ and Clinical Global Impressions of Severity (CGI-S).^{178,179}

Clinical scores were typically obtained on the same day as the ERPs, and in the case the visits did not occur on the same day, the scores from the closest clinical visit were used. Clinicians also categorized Rett diagnoses as either atypical vs typical and logged seizure frequency (none, daily, weekly, or monthly).

Behavioral symptom severity was also assessed using the Rett Syndrome Behavior Questionnaire (RSBQ), a questionnaire completed by caregivers developed to capture the broad neurobehavioral phenotype of RTT, including general mood, breathing problems, hand behaviors, repetitive face movements, body rocking and expressionless face, nighttime behaviors, fear/anxiety, and walking/standing.^{180–182} Higher total RSBQ scores reflect greater caregiver-reported severity and frequency behavioral abnormalities, whereas lower scores indicate milder symptom burden. We quantified the total scale score (max 90), which provides a quantifiable index of neurologic and behavioral symptomatology and has been increasingly used to monitor clinical progression or treatment response.^{126,183–186}

Mutations were categorized as severe, intermediate, or mild based on genotype/phenotype correlations established in previous literature.^{32,33,35–37} Four participants had mutations classified as mild severity, including p.Arg133Cys (n=1), p.Arg294X (n=2), and p.Arg306Cys (n=1). Eighteen participants had mutations classified as severe severity, including p.Arg168X (n=1), p.Arg267X (n=1), p.Arg255X (n=7), p.Arg270X (n=3), p.Arg106Trp (n=1) early frameshift truncations (e.g., p.Thr99Asnfs4, *p.Gly16Glu*fs22; n=2), large deletions (Xq28 deletions; n=2), and p.Glu258Glyfs58 (n=1). Ten participants had mutations classified as intermediate severity, including middle-range truncations (*p.Leu386fs*, *p.Pro388His*fs5; n=3), p.Thr158Met (n=3), p.Phe157Leu (n=1), p.Pro152Ala (n=1), p.Lys305Asn (n=1), and p.Pro302Leu (n=1). Clinical scores and mutation severity are reported in Table 2.1.

EEG data collection

Participants were seated 65 cm from a monitor in a dimly lit, sound-attenuated, electrically shielded room. Participants either sat independently in a chair or stroller, or in a caregiver's lap (in which case the caregiver was instructed to avoid interacting or speaking with their child). A behavioral assistant sat in the room with the participant to keep them as calm as possible and redirect their attention to the monitor if necessary. Continuous EEG data were recorded via a Net Amps 400 amplifier using the 128-channel Hydrocel Geodesic Net (Magstim Electrical Geodesic, Inc., Eugene, OR). Data were sampled at 1,000 Hz with reference to the electrode "Cz". Stimuli were presented using Presentation software, with the following parameters:

VEP: 400 trials of a reversing black and white checkerboard, where the check size was .5 cycles per degree and each trial lasted 500ms for a reversal rate of 2 Hz.^{128,153} Progression of stimulus delivery was contingent on the participant attending to the stimulus; monocular eye gaze was measured using an EyeLink 1000 Plus and if the participant looked away the monitor paused until gaze was redirected.

AEP: 250 trials of 500 Hz sinusoidal tones 300ms duration played at 60dB.¹²⁸ The interstimulus interval was jittered between 1 and 3 seconds. The movie *WALL-E* was played on a tablet in front of the monitor in black and white and without any sound or time-locked stimuli to keep participants calm and focused (in 3 cases, a video of choice was played in black and white and without sound instead to soothe the participant). This is standard procedure for obtaining high-quality EEG and ERP in individuals with neurodevelopmental disorders.^{134,187}

EEG data pre-processing

EEG data preprocessing was conducted using the Batch EEG Automated Processing Platform.¹⁸⁸ Within BEAPP, data were artifact detected and corrected using the Harvard Automated Processing Pipeline for Electroencephalography (HAPPE) 3.0, a MATLAB-based EEG processing pipeline optimized for data collected from young children and/or study participants with neurodevelopmental disorders.¹⁸⁹ Data were first bandpass filtered using a high pass (1 Hz) and low pass (30 Hz) filter and resampled to 250 Hz. HAPPE applied line noise removal at 60 Hz via CleanLine's multi-taper method and then automatically detected and rejected bad channels, movement, muscle, and eye blink artifact with wavelet-enhanced independent component analysis (ICA) and ICA with Multiple Artifact Rejection Algorithm (MARA).^{190,191} Following artifact removal, bad channels were interpolated using spherical interpolation and data were re-referenced to an average reference. Data were then segmented into epochs from 400ms before and 500ms after the onset of each stimulus. Segments were rejected based on HAPPE's recommended amplitude cut off (40 μ V) and joint-probability rejection criteria, and then 185 (VEP) or 200 (AEP) randomly selected segments were chosen for each participant for subsequent analysis.

EEG rejection criteria

Participants were excluded if they had too few EEG segments of usable data or fell outside of 3 standard deviations from the mean on the following HAPPE quality control parameters: percent good channels, mean retained artifact probability, median retained artifact probability, percent of independent components rejected as artifact, and percent of EEG signal variance retained after artifact removal. Based on the above criteria, out of 59 participants with VEPs, 1 was rejected, and out of 53 participants with AEPs, none were rejected (Table 2.1). Note that the

reason there are fewer AEPs recorded is because the AEP paradigm was introduced later in the study.

Event-related potentials

For each participant independently, average amplitudes for all trials were calculated by setting a time window for each component and measuring the highest (for “P” components) or lowest (for “N” components) voltage deflection during that window.

VEP: All analyses were performed on data from one electrode over the occipital scalp (“Oz”) except for color maps in Fig. 2.2B-C, which display voltage deflections in all 128 electrodes across the scalp at each component timepoint. The window for the N1 was defined as 50-75ms, and the window for the P1 was defined as 75-125ms.

AEP: All analyses were performed on data from one electrode over the frontal-central scalp (“Fz”) except for color maps in Fig. 2.2G-H, which display voltage deflections in all 128 electrodes across the scalp at each component timepoint. The window for the P1 was defined as 75-125ms, and the window for the N1 was defined as 125-175ms.

Inter-trial phase coherence (ITPC)

For each participant, Morlet wavelet convolution was utilized to transform EEG data into the time-frequency domain. ITPC was then calculated as the circular coherence (mean resultant vector length) across phase values extracted at each time-frequency point, where the length of the average vector indicates the degree of coherence and its direction indicate the dominant phase.¹⁹²

The formula is given as:

$$\text{ITPC}(t, f) = \left| \frac{1}{N} \sum_{n=1}^N e^{i\theta_n(t,f)} \right|$$

where $\theta_n(t,f)$ represents the phase angle at a given trial n , time t , and frequency f . ITPC values range from zero (totally random phase distribution) to one (perfect phase synchronization).

To visualize the difference between groups, ITPC was averaged across participants and displayed using a color map for each ERP, with the analyzed frequency range spanning from 8-30. For statistical analysis using individual values, ITPC was averaged across a given time window (e.g. P1) across frequencies to obtain one value (this method is based on prior work).^{135,144}

Statistical analyses

A series of independent samples t-tests compared phenotypic measures and ERP characteristics (amplitude or ITPC in various components) between groups. In cases where sample size or variance varied widely (e.g. mutation severity or seizure frequency) Welch's correction was applied to all pairwise comparisons. For within-group analyses, paired t-tests were used. To evaluate associations between variables, linear regression analyses were computed, and False Discovery Rate (FDR) was used to correct for multiple comparisons and applied to p-values. Spearman correlation was computed separately for RTT and TD groups. All statistical analyses were performed in MATLAB.

Results

Atypical responsivity to sensory stimuli in RTT

We first sought to evaluate sensory processing phenotype in Rett syndrome using the SP2, a parent-reported questionnaire that measures sensory processing patterns in everyday life. We computed an overall hypo- and hyper-responsivity scores to sensory stimuli for each participant, summing Registration and Seeking quadrants and Avoiding and Sensitivity quadrants, respectively.⁶ TD participants in our study typically fell within the expected standardized range of 40-60 in both hypo- and hyper-responsivity¹ (Fig. 2.1A-B). The RTT group exhibited much higher scores compared to TD—indicating more sensory challenges—in both hypo-responsivity (scores ranging from 43-168) and hyper-responsivity (scores ranging from 44-115) ($P < .001$ in both cases) (Fig. 2.1A-B).

We next asked whether it was possible that the same participants had sensory challenges in both domains (i.e. whether the same participants were reported to be both hyper- and hypo-responsive to sensory stimuli, as opposed to two subgroups within the RTT group where some participants were hypo-responsive and some hyper-responsive). Strikingly, these measures were highly positively correlated with each other in both RTT ($r=.52$; $P<.01$) and TD ($r=.74$; $P<.001$) (Fig. 2.1C).

We then focused on two sensory modalities that have been studied in RTT and could be reliably measured using ERP techniques^{128,153}: audition and vision. Similarly to above, the RTT group had significantly higher scores in both visual (Fig. 2.1D) and auditory (Fig. 2.1E) responsivity ($P<.001$ in both cases). Importantly, these scores reflected sensory modality-specific questions coded for both hypo- and hypersensitivity.

These results highlight a compelling “Sensory Paradox,” in Rett syndrome, where a single individual can exhibit both hypo- and hyper-responsivity to sensory stimuli. We therefore set out

to test whether neural response variability, as measured by ITPC, could help explain the Sensory Paradox.

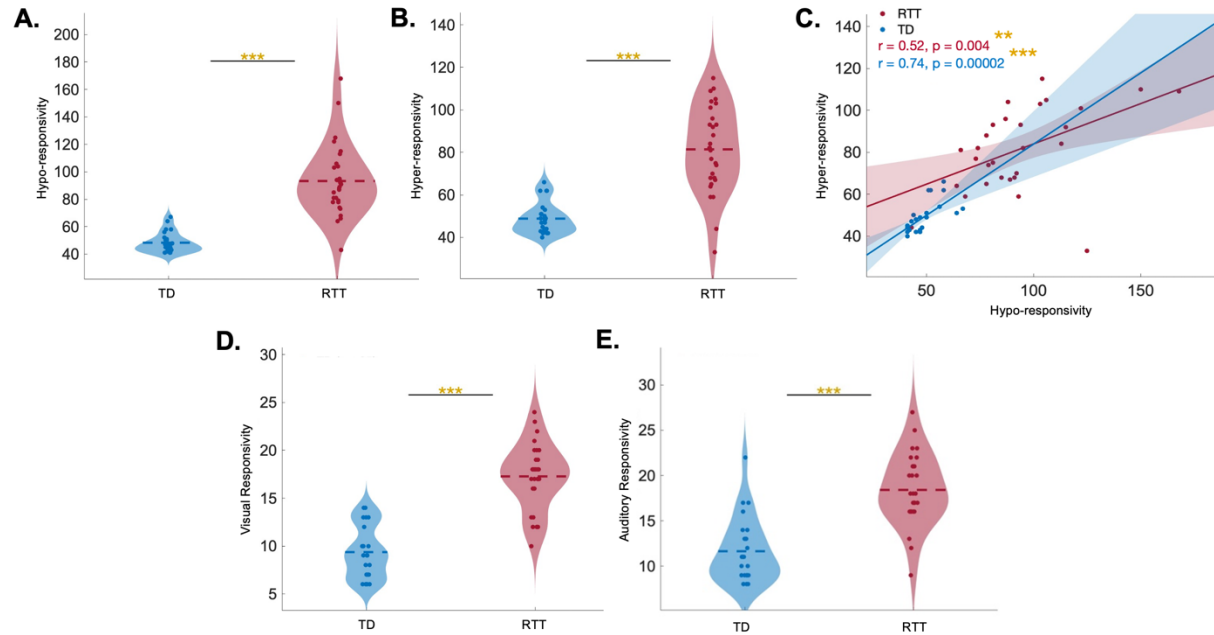


Figure 2.1 Atypical responsivity to sensory stimuli in RTT. Circles represent individual participant scores on the Sensory Profile 2. TD participants (n=25) are shaded in blue and RTT participants (n=29) are shaded in red. Statistical significance is indicated as follows: *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$. (A, B) Violin plots of overall hypo- and hyper-responsivity scores for each group. Violin widths represent kernel density estimates; dashed horizontal lines indicate group means. (C) Association between overall hypo- and hyper-responsivity for each group. Shaded areas indicate 95% confidence intervals around linear regression lines. (D, E) Violin plots of sensory modality-specific responsivity scores for each group (visual in C, auditory in D). Violin widths represent kernel density estimates; dashed horizontal lines indicate group means.

ERP amplitudes are dampened in RTT

We first replicated previous data showing atypical ERPs in Rett syndrome compared to TD in both visual- and auditory-evoked potentials. We plotted the grand average VEP waveform (Fig. 2.2A) in the standard time domain as a function of voltage. Color maps demonstrating voltage in all electrodes across the scalp are displayed to demonstrate the chosen region-of-interest at the two

components chosen for analysis: N1 (Fig. 2.2B) and P1 (Fig. 2.2C). We extracted the mean amplitude at each component for every participant and found that as expected, individuals with RTT had significantly reduced VEP amplitude compared to TD in both the N1 ($P<.05$, Fig. 2.2D) and P1 ($P<.01$, Fig. 2.2E). The grand average AEP waveform was similarly plotted (Fig. 2.2F), with scalp topographies at component timepoints: P1 (Fig. 2.2G) and N1 (Fig. 2.2H). Although a clear difference between groups is visible, group differences in extracted P1 (Fig. 2.2I) and N1 (Fig. 2.2J) amplitude alone did not reach statistical significance.

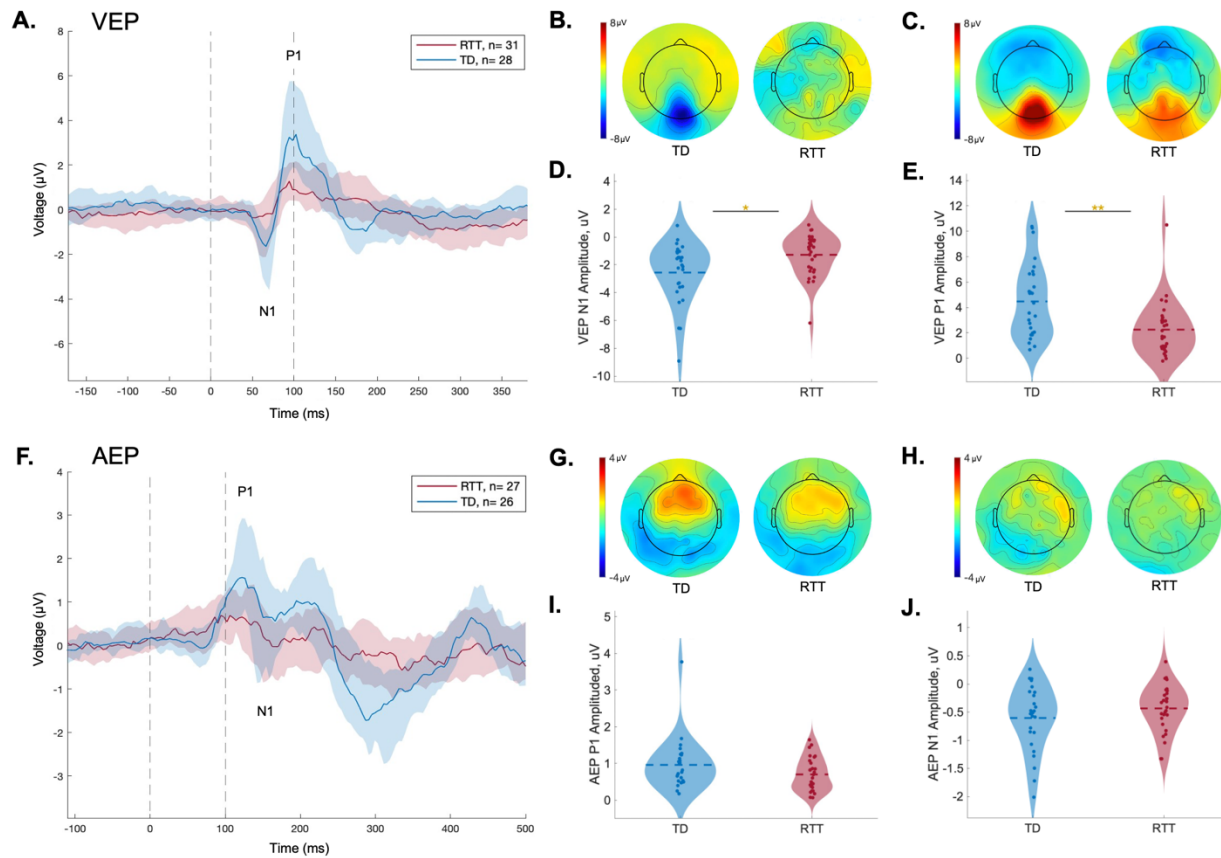


Figure 2.2. Diminished ERP amplitudes in RTT. ERPs in response to visual and auditory stimuli are shown for RTT (red) and TD (blue) participants. For VEP panels (A–C), RTT $n=31$ and TD $n=28$; for AEP panels (D–F), RTT $n=27$ and TD $n=26$. Statistical significance is indicated as follows: *** indicates $P<0.001$, ** indicates $P<0.01$, and * indicates $P<0.05$. (A) Grand average VEP waveforms, analyzed at occipital electrode Oz. N1 and P1 components are indicated. Shaded

areas represent the 25th to 75th percentile range across participants. Vertical dashed lines indicate stimulus onset (0ms) and 100ms post-stimulus. **(B, C)** Topographic color maps display voltage in all 128 electrodes at each component. **(D, E)** Violin plots of corresponding VEP N1 and P1 amplitudes at Oz, where circles represent individual participants. Violin widths represent kernel density estimates; dashed horizontal lines indicate group means. **(F)** Grand average AEP waveforms, analyzed at frontal-central electrode Fz. P1 and N1 components are indicated. Shaded areas represent the 25th to 75th percentile range across participants. Vertical dashed lines indicate stimulus onset (0ms) and 100ms post-stimulus. **(G, H)** Topographic color maps display voltage in all 128 electrodes at each component. **(I, J)** Violin plots of corresponding AEP P1 (E) and N1 (F) amplitudes at Fz, where circles represent individual participants. Violin widths represent kernel density estimates; dashed horizontal lines indicate group means.

It has been well-documented that the morphology of the AEP changes with development in both TD^{151,193} and RTT,^{128,144} and when separated by age (above or below 10 years old), significant differences emerged in the AEP P1 and N1 amplitude for the >10 year old subset (Fig. S2.1A-D). To further clarify the impact of age on ERP amplitude in our dataset, we performed linear regression analyses: VEP P1 (but not N1) amplitude was significantly associated with age, declining as age increased in both groups (RTT: $r = -.42$, $P < .05$, TD: $r = -.41$, $P < .05$) (Fig. S2.2A-B), as was AEP P1 amplitude in the RTT group (RTT: $r = -.46$, $P < .05$) (Fig. S2.2C). In the TD group, AEP N1 amplitude increased with age (TD: $r = -.58$, $P < .01$) (Fig. S2.2D). These results are consistent with previous work.^{128,145,153} We chose to analyze all ages together to preserve statistical power for further analyses and avoid diluting group-level effects (as in Brima et al, 2024). Where relevant, we included age as a covariate to account for its influence.

Inter-trial phase coherence is lower in RTT and correlates with ERP amplitude

To test whether increased neural response variability could contribute to the atypical sensory processing and diminished ERP amplitudes in individuals with RTT, we next analyzed ITPC in both visual and auditory-evoked potentials. ITPC measures the phase coherence across

single trials for each participant; low ITPC indicates high variability in the phase-locked response, and high ITPC indicates low variability, or a more stable response.

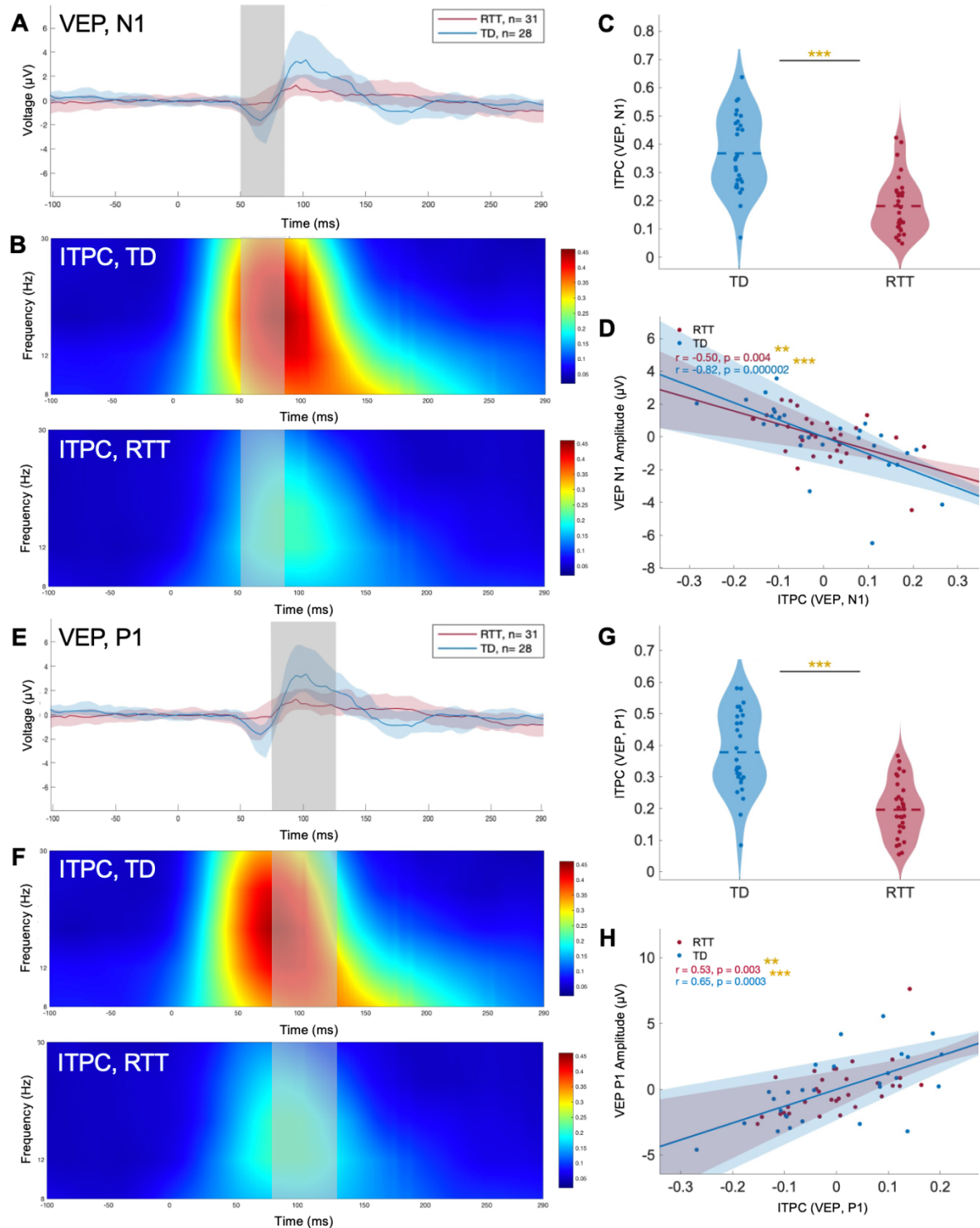


Figure 2.3. Reduced ITPC in RTT: visual-evoked potentials. ERPs were analyzed at occipital electrode Oz. RTT participants are shown in red (n=31), TD participants in blue (n=28). Statistical significance is indicated as follows: *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$. **(A)** Grand average VEP waveforms for each group, where the gray shaded box indicates the N1 time window; blue and red shaded areas represent the 25th to 75th percentile range across participants. **(B)** Top: time–frequency plot shows group-averaged ITPC values for TD. Bottom: time–frequency plot shows group-averaged ITPC values for RTT. **(C)** Violin plot comparing corresponding ITPC in the VEP N1 between groups, where circles represent individual participants. ITPC for each participant was averaged across the shaded N1 time window and 8–30 Hz frequencies. Violin widths reflect kernel density estimates; horizontal dashed lines indicate group means. **(D)** Association between VEP N1 ITPC and VEP N1 amplitude for each group. Shaded areas indicate 95% confidence intervals around linear regression lines; age was included as a covariate and regressed out. **(E-H) All figures and analyses are repeated for the P1 component of the VEP.**

For both the VEP and AEP, we first plotted ITPC dynamics in a time-frequency figure across the entire ERP and from 8-30 Hz, averaging across groups. In TD participants, ITPC was low until after stimulus onset (at timepoint 0) and then increased, peaked, and went down as the effects of the stimulus faded. Visually, it is immediately apparent that although present, ITPC was much lower in RTT compared to TD across many frequencies in both sensory modalities. We then quantified ITPC for each individual and compared RTT to TD, focusing on the same two ERP components as above and averaging across frequencies (as in Brima et al, 2024). For each component, we also asked whether ITPC in that time window (as shown in Fig. 2.3 and 2.4, left) was associated with the observed amplitude (as shown in Fig. 2.2). Note that to account for effects of age on ERPs as described above, age was included as a covariate and regressed out in the linear regression analyses.

The N1 component of the VEP is indicated in the VEP waveform in Fig. 2.3A, with corresponding ITPC shown for each group in Fig. 2.3B. RTT participants had significantly lower ITPC in the VEP N1 compared to TD ($P < .001$) (Fig. 2.3C), and ITPC was strongly correlated with

VEP N1 amplitude in the RTT group ($r=-.50$, $P<.01$) and TD group ($r=-.82$, $P<.001$) (Fig. 2.3D). Similarly, The P1 component of the VEP is indicated in the VEP waveform in Fig. 2.3E, with corresponding ITPC in Fig. 2.3F. RTT participants had significantly lower ITPC in the VEP P1 compared to TD ($P<.001$) (Fig. 2.3G), and ITPC was once again strongly correlated with VEP P1 amplitude in the RTT group ($r=.53$, $P<.01$) and TD group ($r=.65$, $p<.001$) (Fig. 2.3H).

The P1 component of the AEP is indicated in the AEP waveform in Fig. 2.4A, with corresponding ITPC shown for each group in Fig. 2.4B. RTT participants had significantly lower ITPC in the AEP P1 compared to TD ($P<.01$) (Fig. 2.4C), and ITPC was strongly correlated with AEP P1 amplitude in the RTT group ($r=.67$, $P<.001$) but not in the TD group (Fig. 2.4D). The N1 component of the AEP is indicated in the AEP waveform in Fig. 2.4E, with corresponding ITPC in Fig. 2.4F. RTT participants had significantly lower ITPC in the AEP N1 compared to TD ($P<.01$) (Fig. 2.4G), but ITPC was not associated with AEP N1 amplitude in either group (Fig. 2.4H).

These results indicate that higher neural response variability, as measured by ITPC, may contribute to the diminished ERP amplitudes that have been reported in RTT.

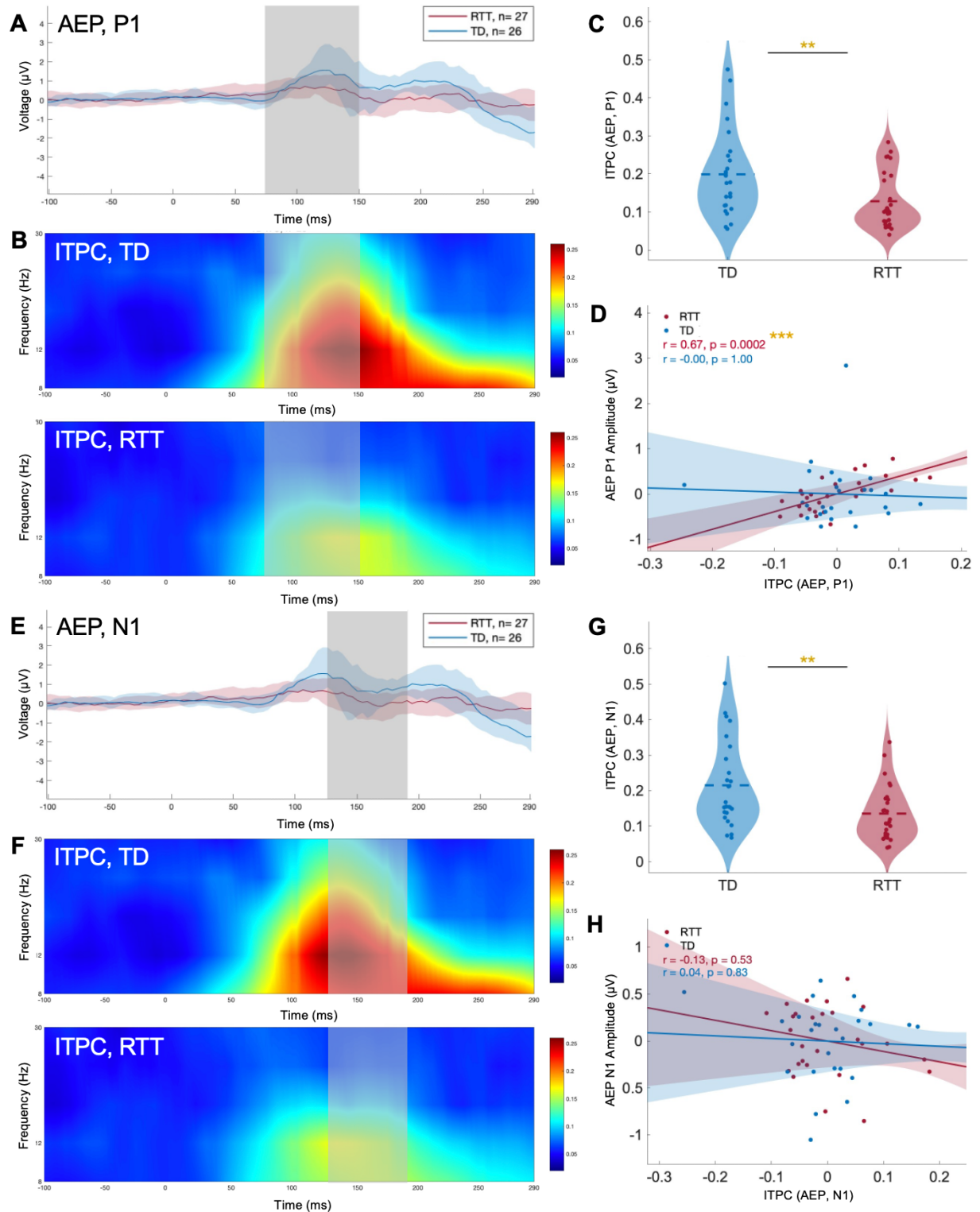


Figure 2.4. Reduced ITPC in RTT: auditory-evoked potentials. ERPs were analyzed at frontal-central electrode Fz. RTT participants are shown in red ($n=27$), TD participants in blue ($n=26$). Statistical significance is indicated as follows: *** indicates $P<0.001$, ** indicates $P<0.01$, and * indicates $P<0.05$. (A) Grand average AEP waveforms for each group, where the gray shaded box

indicates the P1 time window; blue and red shaded areas represent the 25th to 75th percentile range across participants. **(B)** Top: time–frequency plot shows group-averaged ITPC values for TD. Bottom: time–frequency plot shows group-averaged ITPC values for RTT. **(C)** Violin plot comparing corresponding ITPC in the AEP P1 between groups, where circles represent individual participants. ITPC for each participant was averaged across the shaded P1 time window and 8–30 Hz frequencies. Violin widths reflect kernel density estimates; horizontal dashed lines indicate group means. **(D)** Association between AEP P1 ITPC and AEP P1 amplitude for each group. Shaded areas indicate 95% confidence intervals around linear regression lines; age was included as a covariate and regressed out. **(E-H) All figures and analyses are repeated for the N1 component of the AEP.**

ITPC in visual-evoked potentials is associated with visual responsivity and behavioral symptom severity, and impacted by mutation severity in RTT

We next tested whether ITPC was associated with sensory responsivity as measured by the SP2 and shown in Figure 2.1. Indeed, lower ITPC in both components of the VEP were associated with higher visual responsivity scores in the RTT group (N1 ITPC: $r=-.42$, $P<.05$; P1 ITPC: $r=-.43$, $P<.05$) (Fig. 2.5A-B). ITPC was not associated with auditory responsivity in either component of the AEP (Fig. 2.5C-D).

Sensory processing is a foundation upon which advanced cognitive function, social behavior, and many other skills build, and deficits could contribute to some of the broader impairments in Rett syndrome.^{8–10,112} Therefore, we also assessed the relationship between ITPC and several other outcome measurements which could be affected by neural response variability. Clinical severity (as measured by the clinician-completed CSS and CGI-S) did not correlate with ITPC in any ERP component tested. Lower ITPC was, however, significantly correlated with higher behavioral symptom severity in RTT (quantified using a single aggregate score from the parent-reported RSBQ) in the N1 ($r=-.37$, $P<.05$) and P1 ($r=-.37$, $P<.05$) of the VEP (Fig. S2.3A-B), but not in any component of the AEP (Fig. S2.3C-D).

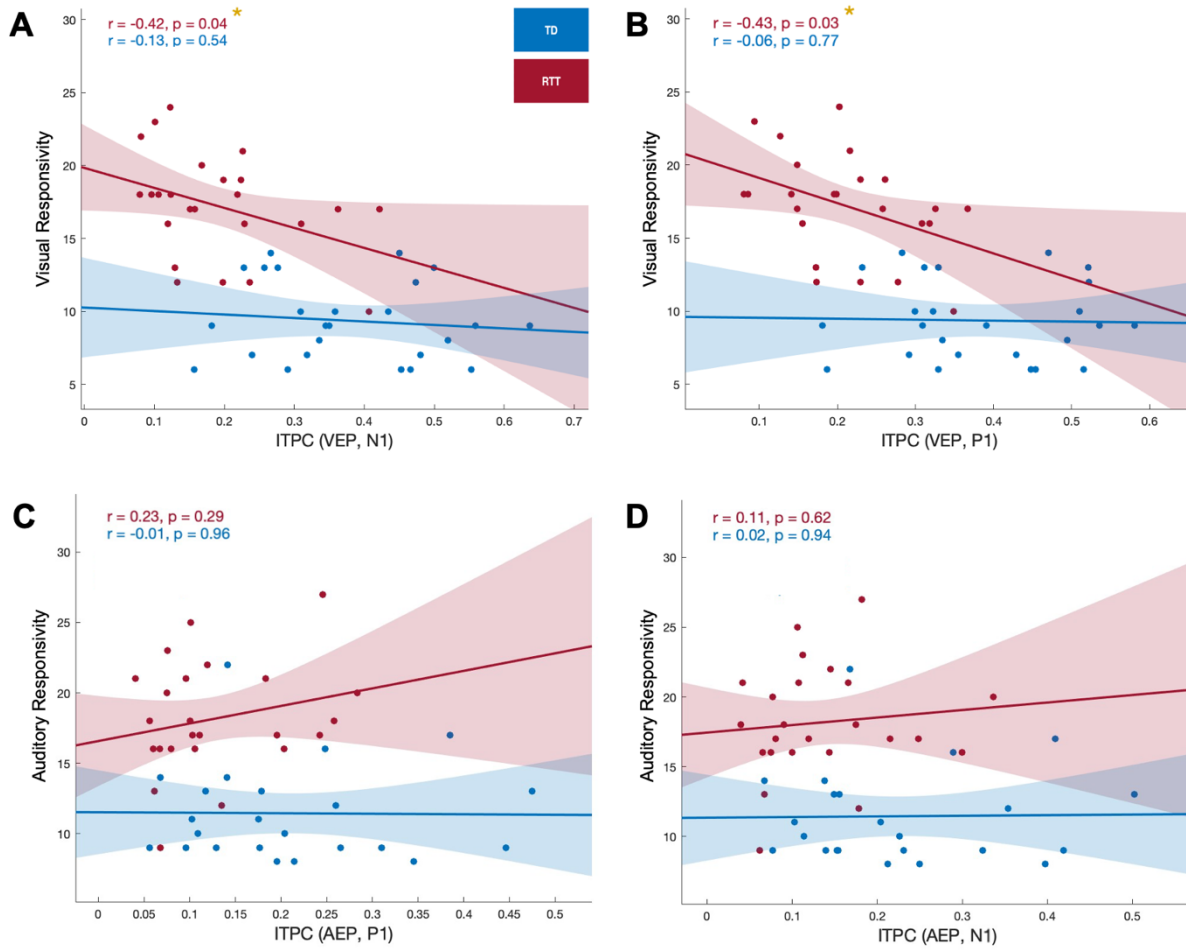


Figure 2.5. VEP phase coherence is associated with visual responsivity in RTT. Association between ITPC in VEP components and visual responsivity (RTT n=24, TD n=25) (A-B), and between ITPC in AEP components and auditory responsivity (RTT n=24, TD n=23) (C-D). Shaded areas indicate 95% confidence intervals around linear regression lines. Statistical significance is indicated with r and P values, where: *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$.

Given established genotype-phenotype relationships^{32,33,35–37}, we then asked whether in the RTT group, the type of *MECP2* mutation (categorized as mild, intermediate, or severe) impacted ITPC in any ERP component. Pairwise comparisons revealed no significant difference between mutation severities in ITPC in either the P1 or N1 of the AEP (Fig. S2.4A-B). Interestingly, the TD group was only significantly different from the severe *MECP2* mutation group in both the P1

and N1 components ($P < .01$) but not the mild or intermediate groups, indicating that participants with severe mutations may have driven the differences between RTT and TD in AEP ITPC (note that most participants in our dataset had severe mutations, so this was the largest group) (Fig. S2.4A-B). There was a significant difference between mild and severe mutations ($P < .01$) and between mild and intermediate mutations ($P < .05$) in ITPC in the N1 of the VEP, and between mild and severe mutations ($P < .05$) in ITPC in the P1 of the VEP, with surprisingly higher ITPC in more severe mutations (Fig. S2.4C-D). The TD group was significantly higher than all three categories of *MECP2* mutations in ITPC in both the N1 and P1 of the VEP ($P < .001$ in all three cases) (Fig. S2.4C-D). Rett diagnosis (atypical vs typical) and seizure frequency did not significantly impact ITPC.

Lastly, ITPC was not significantly associated with age in either component of the VEP for either group (Fig. S2.5A-B). However, we found ITPC to increase with age in the P1 component of the AEP in both the RTT group ($r = .49$, $P < .01$) and the TD group ($r = .79$, $P < .001$), and in the N1 component of the AEP in the TD group ($r = .75$, $P < .001$) (Fig. S2.5C-D).

Together, these results indicate that increased variability in neural responses to visual stimuli could contribute to atypical visual responsivity in Rett syndrome, as well as other cognitive and behavioral outcomes.

Discussion

Atypical responsivity to sensory stimuli is a key feature of many neurodevelopmental disorders including Rett syndrome, significantly affecting cognitive functioning, social behavior, and overall quality of life.^{8–10,112} In this study we characterized responsivity to sensory stimuli and

recorded the visual- and auditory-evoked potential from infants, children and adults with and without Rett syndrome. We found elevated hypo- and hyper-responsivity to sensory stimuli in RTT compared to TD, and these two measures were highly correlated with each other. Furthermore, auditory and visual responsivity were both elevated in RTT. This observed “Sensory Paradox” phenotype, where one individual can be both hypo- and hyper-responsive to sensory stimuli—even those in the same sensory modality—drove us to investigate whether neural response variability could be playing a role in the dampened ERP amplitudes previously observed in RTT and eventually behavior. To investigate this, we first replicated previous work showing attenuated amplitude in both the N1 and P1 of the VEP in RTT as well as age-dependent differences in AEP P1 and N1 amplitude. ITPC analysis revealed striking differences between RTT and TD in phase coherence within both auditory and visual ERPs, with response consistency significantly reduced in RTT in all components analyzed. We found that ITPC was strongly associated with ERP amplitude in both the N1 and P1 of the VEP, and the P1 of the AEP in the RTT group. ITPC in both components of the VEP was correlated with higher visual responsivity in participants with Rett syndrome, as well as behavioral symptom severity. Mutation severity also impacted ITPC in both components of the VEP.

It is important to consider the possible sources of increased variability in sensory processing within RTT cortical circuitry. Whether the brain is hyper- or hypo-responsive to a given stimulus depends on a variety of factors, such as arousal state, attention, or environmental context. On a moment-to-moment basis, these factors alter how the brain filters, amplifies, or dampens sensory information. At the circuit level, both hyper- and hypo-responsivity may emerge from disruptions in the balance between excitatory and inhibitory (E/I) activity in cortical networks—a hallmark of many neurodevelopmental disorders including RTT. In Rett syndrome, mutations in the *MECP2*

gene are known to lead to widespread dysregulation of synaptic transmission and plasticity. E/I balance is not static within an individual; it changes dynamically depending on the state of the circuit at a given time.¹⁹⁴

Multiple studies have demonstrated that GABAergic inhibitory interneurons are preferentially effected by loss of *Mecp2*.^{90,94} In *Mecp2* knockout mice, it has been shown in the visual cortex that parvalbumin-positive (PV+) interneurons exhibit increased connectivity and activity,⁸⁶ and that they receive stronger excitatory input from the thalamus.⁸⁷ Revealingly, specific deletion of *Mecp2* from forebrain GABAergic neurons was sufficient to produce AEP deficits, and a rescue of the AEP was possible by restoring *Mecp2* expression to PV+ or somatostatin-positive (SOM+) interneurons.⁶⁷ Altogether, increased inhibitory tone (and less active excitatory pyramidal neurons) is apparent, but more work is needed to understand how and which of these circuit dynamics—both local and thalamocortical—result in lower ITPC in ERPs in RTT, and how both hyper- and hypo-responsivity to sensory stimuli is achieved. Future studies using mouse models that selectively manipulate GABAergic neuron function—either through cell-type specific restoration of *Mecp2* or pharmacological modulation of inhibitory signaling—could directly test whether enhancing inhibitory-excitatory balance is sufficient to normalize ERP phase coherence.

Surprisingly, participants with *MECP2* mutations categorized as more severe had significantly higher ITPC—indicating less variability—in both components of the VEP compared to more mild mutations. This raises interesting questions about how specific *MECP2* mutations differentially impact neural circuit function and response consistency. Future work could employ mouse models carrying distinct patient-derived *MECP2* mutations to systematically examine how genotype influences phase coherence in ERPs, and to probe the underlying cellular and circuit-

level mechanisms—such as differing changes to synaptic dynamics and E/I balance—that may drive variation in ITPC across mutation types.

Additionally, given the wide age range in our sample, longitudinal studies are needed to assess whether for a single individual with RTT, ITPC deficits emerge early and remain stable, or whether variability increases with disease progression. Such work would also help establish the potential for ITPC as a functional biomarker that could help track disease progression and/or treatment efficacy, ultimately helping to bridge the gap between molecular pathology and clinical phenotype in RTT.

Interestingly, we found some evidence for a sensory-modality specific effect: although phase coherence was significantly affected in both the VEP and AEP, reduced ITPC was only associated with sensory responsivity and other outcome measures in VEP components (decreasing with higher visual responsivity and higher behavioral symptom severity). Moreover, ITPC in the AEP and ITPC in the VEP were not correlated with each other (Fig. S2.6). It is possible that individuals with RTT syndrome rely more heavily on visual processing compared to auditory processing, or it may be that visual and auditory circuits are differentially vulnerable to loss of *MECP2*. For example, in the visual system, *Mecp2* knockout mice demonstrate a regression in visual acuity and decreased activity in visual cortex,⁷⁸ including disruptions to critical periods in ocular dominance experience-dependent plasticity.⁸⁵ In *Mecp2* heterozygous rats, spontaneous firing in auditory cortex is similarly reduced, but stimulus-evoked responses become hyperexcitable (though significantly delayed).¹⁴⁷ Further work is needed to parse out differences in cortical processing deficits and resulting behavior between sensory modalities.

Moreover, higher-order cognitive processes often begin with the integration of information across multiple sensory modalities, and growing evidence suggests that neural oscillations in

specific frequency bands support this process by integrating multi-modality information and coordinating activity across distributed brain regions.¹⁹⁵ While we focused on one sensory modality at a time, future work could extend these findings by incorporating multi-modality stimuli or paradigms that probe integrative sensory processing. It is also possible that ITPC is modulated in Rett syndrome based on factors such as stimulus saliency, required cognitive load, or arousal state, and future work could systematically vary these factors to assess their influence on phase consistency. Some examples include utilizing speech vs nonspeech sounds (which has been done in ASD),^{173,196} varying acoustic complexity¹⁹⁷, or presenting faces with variable emotions (which reliably elicits differential ERPs in TD infants).¹⁹⁸ Additionally, even without relying on verbal communication, paradigms that contrast passive and active engagement (e.g., visual tasks using eye tracking) could clarify the effects of attentional modulation on ITPC in RTT.

In conclusion, taken altogether, our data demonstrate a Sensory Paradox in Rett syndrome, with co-occurring hypo- and hyper-responsivity to sensory stimuli, and uncover neural response variability (measured here using ITPC) as a potential underlying contributor to dampened ERPs, atypical sensory responsivity, and overall symptom severity in RTT. Given that atypical sensory processing is a common feature across many neurodevelopmental conditions (including both syndromic and idiopathic ASDs), these findings may also have broader implications for understanding shared neurophysiological mechanisms, developing generalizable biomarkers of neural response variability, and informing trans-diagnostic therapeutic strategies.

Data Availability

Data is available upon reasonable request.

Author Contributions

DK, AL, and MF conceptualized the study. DK wrote and prepared the initial manuscript draft. DK, CM, and KS collected clinical data. DK performed the analysis. DK, AL, MF, and CN interpreted the analytic findings. All authors contributed to interpretation of the results and critical revision of the manuscript.

Acknowledgements

Thank you very much to all the participants in these studies and their families, especially those who travelled and extended themselves to contribute to research in Rett syndrome. Thank you to the Rett Syndrome Angels (originally Rett Syndrome Association of Massachusetts) for their incredible support. Thank you to the Rett syndrome clinics and to all members of the labs of Dr.'s April Levin, Michela Fagiolini, Charles Nelson, and Takao Hensch for your invaluable feedback and contributions. This work could not have been completed without all of you.

Funding

This work was supported partially by: P50HD105351 - Project 1 - Michela Fagiolini

Competing interests

The authors report no competing interests.

Chapter 3. Transient Beta Events on EEG in Autism and Related Neurogenetic Conditions

Gerardo Parra,^{1*} Klara Szilagyi,^{1,2*} Yael Braverman,^{1*} Devorah Kranz,^{1,3} E. Martina Bebin,⁴ Raphael A. Bernier,⁵ Jonathan A. Bernstein,⁶ Elizabeth Berry-Kravis,⁷ Joseph D. Buxbaum,^{8,9,10,11} Katarzyna Chawarska,¹² Emma E. Condry,¹³ Laura Cornelissen,¹⁴ Geraldine Dawson,^{15,16,17} Abigail Dickinson,¹⁸ James Dziura,^{19,20} Charis Eng,^{21,22,23,24} Susan Faja,²⁵ Jennifer H. Foss-Feig,^{8,9} Antonio Y. Hardan,²⁷ Ellen Hanson,^{2,25} Shafali Jeste,^{27,28} Linnea Joffe-Nelson,^{1,25} Alexander Kolevzon,^{8,9,29} Darcy A. Kreuger,^{30,31} Balu Krishnan,³² Robert Law,^{1,2} David N. Lieberman,¹ Claire MacKay,^{1,2} Julian A. Martinez-Agosto,^{33,34,35} Adam J. Naples,¹² Charles A. Nelson,²⁵ Hope Northrup,³⁷ Ernest Pedapati,^{30,31,38} Annapurna Poduri,¹ Vineet Punia,³⁹ Rajsekar R. Rajaraman,⁴⁰ David M. Ritter,^{30,31} Celine Saulnier,⁴¹ Frederick Shic,^{42,43} Latha Valluripalli Soorya,⁷ Catherine A. Sugar,^{44,45} Kyle E. Takach,^{1,46} Audrey Thurm,¹³ Sara J. Webb,⁴² Kimberly Wiltout,¹ Damla Şentürk,⁴⁴ Stephanie Jones,^{47,48} Michela Fagiolini,^{1,49,50} James C. McPartland,^{12,51} Mustafa Sahin,^{1,2} and April R. Levin,^{1,2} on behalf of the Developmental Synaptopathies Consortium and the Autism Biomarkers Consortium for Clinical Trials.

*These authors contributed equally to this work.

Abstract

Traditionally, the search for biomarkers relevant to autism spectrum disorder (ASD) relies on current phenotypic or genetic definitions. Transient beta events (TBE) during baseline neural oscillations reflect thalamocortical function, which bridges genotype to phenotype. Compared to typically developing controls, we found elevated TBE-rate in some children with idiopathic ASD and a majority of children with three neurogenetic conditions associated with atypical

neurodevelopment. TBE-rate thus offers promise as a stratification biomarker with divergent and convergent properties across ASD and neurogenetic conditions, respectively.

Author Affiliations:

1. Department of Neurology, Boston Children's Hospital/Harvard Medical School, Boston, MA, USA
2. Rosamund Stone Zander Translational Neuroscience Center, Boston Children's Hospital, Boston, MA, USA
3. Program in Neuroscience, Division of Medical Sciences, Graduate School of Arts and Sciences, Harvard Medical School, Boston, MA, USA
4. University of Alabama School of Medicine, Birmingham, AB, USA
5. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, USA
6. Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA
7. Rush University Medical Center, Chicago, IL, USA
8. Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY, USA
9. Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA
10. Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
11. Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA

12. Child Study Center, School of Medicine, Yale University, New Haven, CT, USA
13. Neurodevelopmental and Behavioral Phenotyping Service, National Institutes of Health, Bethesda, MD, USA
14. Department of Anesthesiology, Critical Care & Pain Medicine, Boston Children's Hospital/Harvard Medical School, Boston, MA, USA; Current Address: Eisai Inc., Clinical Evidence Generation – Neurology, Nutley, NJ, USA
15. Duke Institute for Brain Sciences, Duke University, Durham, NC, USA
16. Duke Center for Autism and Brain Development, Duke University, Durham, NC, USA
17. Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC, USA
18. Semel Institute of Neuroscience and Human Behavior, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
19. Yale Center for Clinical Investigation, Yale University, New Haven, CT, USA
20. Yale Center for Analytical Sciences, Yale University, New Haven, CT, USA
21. Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA
22. Center for Personalized Genetic Healthcare, Medical Specialties Institute, Cleveland Clinic, Cleveland, OH, USA
23. Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, OH, USA
24. Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA
25. Division of Developmental Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

26. Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA
27. Department of Neurology, University of Southern California Keck School of Medicine, Los Angeles, CA, USA.
28. Department of Pediatrics, University of Southern California Keck School of Medicine, Los Angeles, CA, USA
29. Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA
30. Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
31. Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
32. Neurological Institute, Epilepsy Center, Cleveland Clinic, Cleveland, OH, USA
33. Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
34. Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
35. Department of Psychiatry, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
36. Department of Human Genetics and Department of Pediatrics, University of California, Los Angeles, CA, USA
37. Department of Pediatrics, McGovern Medical School, University of Texas Health Science Center at Houston (UTHealth) and Children's Memorial Hermann Hospital, Houston, TX, USA

38. Department of Psychiatry, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
39. Epilepsy Center, Cleveland Clinic, Cleveland, OH, USA
40. Division of Pediatric Neurology, Department of Pediatrics, UCLA Mattel Children's Hospital, David Geffen School of Medicine, Los Angeles, CA, USA
41. Neurodevelopmental Assessment and Consulting Services, Decatur, GA, USA
42. Center for Child Health, Behavior and Development, Seattle Children's Research Institute, Seattle, WA, USA
43. Department of Pediatrics, University of Washington, Seattle, WA, USA
44. Department of Biostatistics, University of California, Los Angeles, CA, USA
45. Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, CA, USA
46. Stritch School of Medicine, Loyola Medical School, Maywood, IL, USA
47. Department of Neuroscience, Brown University, Providence, RI, USA
48. Center for Neurorestoration and Neurotechnology, Providence VA Medical Center, Providence, RI, USA
49. Hock E. Tan and K. Lisa Yang Center for Autism Research at Harvard University, Boston, MA, USA
50. International Research Center for Neurointelligence (IRCIN), University of Tokyo Institutes for Advanced Study, Tokyo, Japan
51. Yale Center for Brain and Mind Health, Yale University, New Haven, CT, USA

Introduction

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental condition¹⁹⁹ often associated with atypical sensory responsivity.^{200,201} Precise characterization of sensory responsivity is complicated by the vast genotypic and phenotypic heterogeneity in ASD, as well as the reliance on subjective clinician and self- or parent-report instruments to quantify sensory features. Studying ASD alongside distinct neurogenetic conditions with increased rates of ASD may help stratify biologically meaningful subgroups within ASD and characterize shared underlying mechanisms affecting sensory responsivity.

Atypical tactile responsivity is common in autistic individuals and animal models of ASD.^{202–209} In humans, these sensory differences can impede daily activities, increase likelihood for co-occurring anxiety, depression and attention problems, and negatively affect quality of life.^{208,210–214}

Brief increases in high power electroencephalography (EEG) cortical activity in the beta-band, referred to as transient beta events (TBEs), are one measure reflecting tactile responsivity. TBEs in sensorimotor cortex have been well-studied in animal models, computational circuits, and neurotypical humans.^{215–219} Across-species studies have suggested that TBEs arise from a broad burst of excitatory synaptic drive from lemniscal thalamus to the proximal dendrites of cortical pyramidal neurons in the infragranular layers that is simultaneous with a faster and stronger ~50ms burst of drive from nonlemniscal thalamus to distal pyramidal neuron dendrites in supragranular layers.^{215,217} These synaptic drives create transient events (or bursts) of high-power activity in the 15-29 Hz (beta) frequency range that have a characteristic waveform and typically last less than 150 ms.^{17,20} TBEs are functionally distinct from more traditionally studied averaged broad-band beta power.²²⁰

TBEs measured across the somatosensory cortex are reliable predictors of tactile perception.^{17–19} Higher TBE rates before a tactile stimulus predict a suppression of perception, and a TBE occurring just before a tactile stimulus suppresses sensory perception, whereas a TBE simultaneous with a tactile stimulus facilitates perception.^{216,217} This is particularly relevant to ASD, where differences in tactile processing can take the form of hyper- or hypo-responsivity.²⁰⁶ TBEs measured during resting EEG, an accessible and scalable methodology, thus offer a compelling window into neurophysiological mechanisms that underlie differences in tactile processing. Despite this promising lens through which to investigate sensory processing, no study has characterized TBE rates (TBE-rates) in ASD or related conditions.

Materials and Methods

Participants

Study participants were drawn from two studies of idiopathic ASD and three additional studies comprising five neurogenetic conditions commonly associated with ASD. Data and ethnicity are per report from the parent or legal guardian of each participant, using classification terms provided by the research team. The definition of “usable” EEG data is below in the section on “EEG preprocessing”, and quantification of EEGs excluded are described in Supplementary Table 3.7.

Autism Biomarkers Consortium for Clinical Trials (ABC-CT)

Data were collected from six to eleven year old TD and ASD children longitudinally—two visits, six weeks apart—from five sites: Boston Children’s Hospital; University of California, Los

Angeles; University of Washington; Duke University; and Yale University. ASD diagnoses were confirmed using rigorous standardized diagnostic measures (Autism Diagnostic Observation Schedule, Second Edition (ADOS-2 and DSM-5 criteria by reliable clinicians)).²²¹ Study details have been previously described.^{159,222} 117 TD and 262 ASD participants provided usable EEG data at the first timepoint (T1), and of these 108 TD and 247 ASD participants provided usable EEG data at the six-week timepoint (T2). Exclusionary criteria included neurological conditions (including epilepsy), known genetic conditions causally related to ASD, or full scale IQ that fell outside of 60-150 on the Differential Ability Scales, Second Edition (DAS-II).²²³ Medication was allowed given a stable regimen of at least eight weeks preceding enrollment. The protocol was approved and overseen by a central IRB at Yale University (HIC#: 1509016477; FWA00002571). Written informed consent from all guardians, and assent for all participants, was obtained after procedures were fully explained and the opportunity to ask questions offered.

Sensory Processing and Adaptation (SPA) Study

Data were collected from three- and four-year-old TD and ASD children at Boston Children's Hospital at a single time point. 26 TD and 26 ASD participants provided usable EEG data. Group placement was confirmed by the ADOS-2²²¹ conducted by a research reliable examiner, as well as structured developmental history. Mullen Scales of Early Learning²²⁴ were conducted to confirm that social communication challenges could not be better accounted for by cognitive or language delays. Exclusionary criteria included a first-degree relative with ASD for the TD group, deafness, blindness, inability to complete procedures in English, and motor challenges that preclude participation in testing. The protocol was approved and overseen by the IRB at Boston Children's Hospital (IRB-P00029639). Informed consent from all guardians—and

assent for all participants developmentally able to assent—was obtained after procedures were fully explained and the opportunity to ask questions offered.

Developmental Synaptopathies Consortium (DSC)

Data were collected from two- to 11-year-old children with TD, Phelan McDermid Syndrome (PMS), Tuberous Sclerosis Complex (TSC), and *PTEN* Hamartoma Tumor Syndrome (PHTS) at one time point from seven sites (Boston Children’s Hospital; University of California, Los Angeles; Cincinnati Children’s Medical Hospital, Cleveland Clinic, National Institute of Health and Icahn School of Medicine at Mount Sinai). 22 TD, 25 TSC, 41 PMS and 12 PHTS provided usable EEG data. Exclusionary criteria included taking an investigational drug as part of another research study within 30 days prior to study enrollment, behaviors that could prevent EEG lead placement and data collection, and inability to comply with study procedure/assessments. Additional exclusions for typically developing controls were uncorrected auditory or visual impairments. Autism diagnostic confirmation was provided by Clinician Best Estimate informed by gold-standard measures including the Autism Diagnostic Interview-Revised (ADI-R),²²⁵ ADOS-2³⁰ (Childhood Autism Rating Scale-2, CARS-2,²²⁶ for telehealth visits during the COVID-19 pandemic), DSM-5 Checklist,²⁰¹ medical history, and clinical certainty ratings. In cases where data was unavailable or invalid (e.g., due to extremely low cognition), information on autism diagnosis was marked as “missing.” The protocol was approved and overseen by a central IRB. Informed consent from all guardians, and assent for all participants where cognitively and developmentally feasible, was obtained after procedures were fully explained and the opportunity to ask questions offered.

SYNGAP1-Related Disorder (SYNGAP1)

Data were collected from two- to 11-year-old *SYNGAP1* participants at one timepoint from two sites: Boston Children's Hospital and University of California, Los Angeles. Nine *SYNGAP1* participants provided usable EEG data. Exclusion criteria centered on whether the *SYNGAP1* variant could be validated by the Citizen *SYNGAP1* Natural History registry. Autism diagnosis was based on parent report of whether the child had previously received an autism diagnosis from a clinician. The protocol was approved and overseen by a central IRB at Boston Children's Hospital (IRB-P00039324). Informed consent from all guardians, and assent for all participants developmentally able to assent, was obtained after procedures were fully explained and the opportunity to ask questions offered.

RTT Syndrome Biomarkers Study (RTT)

Data were collected from one- to 25-year-old TD and RTT participants at Boston Children's Hospital. 15 TD and 15 RTT participants provided usable data. In the RTT group, inclusion criteria included a diagnosis of RTT with a previously identified pathogenic or likely pathogenic variant in the methyl-CpG binding protein 2 (*MECP2*) gene. Exclusionary criteria included taking an investigational drug as part of another research study within 30 days prior to study enrollment. Additional exclusion criteria for the TD group included no diagnoses or medical conditions associated with increased risk of ASD or intellectual disability. ASD diagnostic status was not included in this study as limited motor skills in many of the participants precluded accurate assessment of developmental and cognitive level which in turn precluded assessment of whether social communication skills were impaired out of proportion to cognitive skills, as would be necessary to determine ASD diagnostic status. The protocol was approved and overseen by the

IRB at Boston Children's Hospital (IRB-P00038294). Informed consent from all guardians, and assent for all participants developmentally able to assent, was obtained after procedures were fully explained and the opportunity to ask questions offered.

Demographics and clinical characteristics for all cohorts can be found in Supplementary Tables 3.1 and 3.2.

Phenotypic Data Collection

Sensory Profile 2

A subset of participants (SPA_ASD n = 22; SPA_TD n = 20; Supplementary Table 3.5) completed the Sensory Profile 2 Caregiver questionnaire (SP2),¹⁷⁶ a norm-referenced questionnaire that evaluates a child's sensory processing across home, school, and community contexts. For the current study, three summary scores were extracted: the overall Touch Subscale, and Tactile Hyper-Responsivity and Tactile Hyper-Responsivity scores. As previously described⁵, the Tactile Hyper-Responsivity score was the sum the following items (each scored on a Likert scale from 1 to 5): "Shows distress during grooming," "Becomes irritated by wearing shoes or socks," "Shows an emotional or aggressive response to being touched," "Becomes anxious when standing close to others," and "Rubs or scratches a part of the body that has been touched." The tactile Hypo-Responsivity score was the sum of the following items: "Touches people or objects to the point of annoying others," "Displays need to touch toys, surfaces, or textures," "Seems unaware of pain," "Seems unaware of temperature changes," "Touches people and objects more than same-aged children," and "Seems oblivious to messy hands or face." Of note, some studies have called this "Hyper-Reactivity"⁵ but we use the term "Hyper-Responsivity" to better reflect behavioral responsivity as recommended by He et al.¹⁷⁷

Nonverbal Intelligence Quotient (NVIQ)

To assess non-verbal cognitive ability, participants in the ABC-CT, SPA, and DSC cohorts were administered either the Mullen Scales of Early Learning,²²⁴ the Differential Ability Scales, 2nd edition (DAS-II),²²³ or the Stanford Binet-5 (SB-5).²²⁷ NVIQ was compiled based on the test given: for the Mullen and the SB-5, the NVIQ was taken; for participants given the DAS-II, the nonverbal reasoning standard score was taken. No formal cognitive assessments were done as part of the RTT study (due to concerns that motor abilities may preclude accurate assessment) or *SYNGAP1* study.

EEG Data Collection

EEG data were collected in the resting condition using similar protocols across all studies, as described below. Given the variable abilities of study participants to follow instructions, “resting” EEG is defined here as data collected during an awake quiet state with eyes open and without any time-locked stimulus or task.

ABC-CT: EEG data were collected in full lighting, using a 128-channel Hydrocel Geodesic Net (Electrical Geodesic, Inc., Eugene, OR) and NetStation software (Geodesic, Inc) with a sampling rate of 1000 Hz. Children watched an approximately three minute video, comprising six soundless videos akin to screensavers. Each video played forward for 15 seconds and then in reverse for 15 seconds. The six videos were played in three blocks of two videos each at 30 frames per second. Video display was limited to 7×9.3 cm to minimize eye movement during task completion. A behavioral assistant sat in the room with the participant to direct the child to pay attention to the screen.

SPA: EEG data were collected in dim lighting, and methods were otherwise identical to those described for the ABC-CT cohort.

DSC: EEG data were collected in dim lighting, and for the Cleveland Clinic site, was recorded on Brain Vision system with Brain Vision 64 channel cap. Methods were otherwise identical to ABC-CT.

RTT: Children watched an extended version of the video described above, lasting for approximately 5 minutes. Methods were otherwise identical to the SPA and *SYNGAPI* cohorts.

SYNGAPI: EEG data were collected in dim lighting, and methods were otherwise identical to those described for the ABC-CT cohort.

Data Analysis

EEG Pre-Processing

EEG data from all cohorts were exported to MATLAB and pre-processed using the Batch EEG Automated Processing Platform.¹⁸⁸ Data were filtered using a bandpass filter from 0.1-100 Hz and resampled to 250 Hz. Artifact detection and correction was completed using the Harvard Automated Preprocessing Pipeline for EEG (HAPPE) pipeline, which is optimized for short recording EEG data collected from children with neurodevelopmental conditions.¹⁸⁹ HAPPE applies line noise removal at 60 Hz, then detects and removes bad channels and artifacts in the data, such as eyeblinks and eye or muscle movements, using wavelet-enhanced independent component analysis (w-ICA) and ICA with the multiple artifact rejection algorithm (MARA).^{190,191} The following channels, in addition to the 10–20 electrodes, were used for ICA with MARA: FC3, CP3, C1, C5, FC4, CP4, C2, C6, AF3, F1, AFz, AF4, F2. Electrodes were spread evenly across the

scalp, and the number of electrodes was chosen relative to our recording length to maximize ICA performance and prevent overfitting of the algorithm.^{188,228} Following artifact removal, bad channels were interpolated, and data were re-referenced to an average reference. Data were then segmented into 5 s windows. Segments were inspected again for artifact and segments rejected using HAPPE's recommended amplitude cutoff (40 μ V cutoff) and joint probability criteria. The 40 μ V cutoff reflects the smaller amplitude that results from the wavelet thresholding and ICA steps during artifact detection.¹⁸⁹

EEG Rejection Criteria

EEGs were rejected if they had fewer than 25 segments (125 sec) or fell outside of 3 standard deviations from the mean across participants on the following HAPPE quality control parameters: percent good channels (3 SD: < 76%), mean retained artifact probability (3 SD: > 0.27), median retained artifact probability (3 SD: > 0.25), percent of independent components rejected as artifact (3 SD: > 75%), and percent of EEG signal variance retained after artifact removal (3 SD: < 15%). Based on the above criteria 20 of 594 (3.4%) EEGs collected were rejected.

For all participants who had more than 25 segments available, Spearman's correlation revealed no significant association between segment number and TBE-rates ($p = .864$). See Supplementary Table 3.7 for a summary of EEG exclusion by cohort. "Usable" EEGs for each cohort are defined as EEGs that were collected and not rejected based on the above criteria.

Transient Beta Event Detection and Event Rate Calculation

Transient beta events were detected in single trial segmented data from using the SpectralEvents Toolbox.²²⁹ Each 5-second segment was convolved with 5-cycle Morlet wavelets to calculate the time-frequency response (TFR) every 4 Hz between 13 and 30 Hz.²³⁰ Each TFR was then normalized by the median power in each frequency; thus, units of the normalized TFR are in factors of median. Transient events were then detected using the “minimal overlap” option of the toolbox; local maxima were found in the normalized TFR using MATLAB’s `imregionalmax` function (R2021a). The TFR was thresholded to a cutoff of 6 factors of median, and the center of each beta event was then defined as the single local maximum in each suprathreshold region of the TFR.

The rate of event occurrence was found in each segment by counting the number of detected events and dividing by 5, in order to yield an event rate in Hertz for each 5-second segment. For each participant, the TBE-rates was defined as the average of this value across all segments of a channel’s data. The averaged TBE-rates in channels C3 and C4 for each individual were used for all analyses because prior studies of TBE have primarily focused on somatosensory cortex.^{216,219} Topoplots (Figure 3.2) show TBE-rates in other channels.

Statistical Analyses

Prior to analyses, we confirmed that TBE-rates did not vary for TD participants across cohorts using a Kruskal-Wallis test (Figure S3.1) and therefore collapsed all TD participants into one group. Because our EEG data were non-normally distributed, we used non-parametric tests for all EEG analyses. All analyses were performed by IBM SPSS Statistics version 27.²³¹

Comparing Transient Beta Event Rate

Provided (1) we only had sensory phenotyping data for ASD participants in the SPA cohort and (2) the study description in SPA may have self-selected for participants with elevated sensory processing difficulties, the ASD participants from the SPA and ABC-CT cohorts are treated as separate groups (hereafter referred to as ASD_SPA and ASD_ABC-CT). To compare transient beta event rate, we tested whether TBE-rates at C3/C4 in ASD participants (ASD_SPA and ASD_ABC-CT) differed significantly from our collapsed TD group via two independent samples Mann-Whitney U tests.

We next evaluated TBE-rates in a variety of neurogenetic conditions, all of which increase the likelihood of ASD. Specifically, we used five Mann-Whitney U tests, Bonferroni corrected for five comparisons, to evaluate whether TBE-rates in TD participants significantly differed from TBE-rates in each neurogenetic group (PMS, TSC, PHTS, RTT, *SYNGAPI*).

Evaluating the test-retest reliability of TBE-rates across two time points

Since evaluation of TBE-rates in EEG and in neurodevelopmental conditions more generally is a novel analysis, and with an eye towards potential utility of TBE-rates as a clinically relevant biomarker of atypical sensory processing in these conditions, we also wished to assess the test-retest reliability of TBE-rates. For participants in the ABC-CT cohort with usable EEG data at both visits, we ran intraclass correlations (ICC) to assess the short-term test-retest reliability of TBE-rates. First, we ran ICCs with TD and ASD collapsed into one group and subsequently ran ICCs by diagnostic group. ICC values were interpreted as poor (< 0.4), fair (0.4 to .59), good (0.6 to 0.74), or excellent (0.75 to 1.0).²³²

Evaluating the relationship between TBE-rates and Tactile Sensory Processing

For participants who provided sensory data (from the SPA study), we evaluated the relationship between TBE-rates and tactile processing using Spearman's rank correlations for the ASD and TD groups separately. Our reason for examining groups separately is that ASD is known to be associated with atypical sensory responsiveness and we wanted to ensure our findings reflected sensory responsiveness rather than differences in diagnostic groups. First, we correlated TBE-rates with the SP2 touch domain score. Next, to evaluate how TBE-rates related to subdomains of touch, we carried out four separate Spearman's rank correlations to test the relationship between TBE-rates and Tactile Hyper-responsivity and Tactile Hypo-responsivity, within the ASD and TD groups separately. For these subdomain analyses, we thus use p-values Bonferroni corrected for 4 comparisons.

Evaluating the relationship between TBE-rates and other demographic and clinical characteristics

We evaluated whether other demographic and clinical characteristics (age, NVIQ, epilepsy, medications, ASD) could account for a positive association between TBE-rates and tactile responsivity. We used Spearman's rank correlations to evaluate the relationship between TBE-rates and age for all participants. We used Spearman's rank correlations to evaluate the relationship between TBE-rates and NVIQ for all participants in whom NVIQ data were available (ABC-CT, SPA, DSC). Across all neurogenetic conditions that measured ASD (DSC, *SYNGAPI*), we used one Mann-Whitney U-test to evaluate whether TBE-rates differed in participants with or without co-occurring ASD. Since neurogenetic conditions had higher rates of seizures than idiopathic ASD, across all neurogenetic conditions we compared TBE-rates in participants with versus without history of seizures using a Mann-Whitney U-test. To determine whether medications could contribute to particularly elevated TBE-rates, the small numbers of participants taking a wide

variety of medications precluded direct statistical comparisons. Instead, we compiled medication lists for participants with “extreme” TBE-rates. TBE-rates was defined as “extreme” if it was an outlier based on the TD group’s threshold (i.e., if it fell above the top TD “whisker”, exceeding the 75th percentile by more than 1.5 x the IQR value) (Supplementary Table 3.4).

Results

Demographics and clinical characteristics of transdiagnostic, multi-site participants in our sample are reported in Tables S3.1 and S3.2. We first tested whether TBE-rates differ in ASD relative to typically developing (TD) participants, using EEG at rest from two studies of idiopathic ASD with different recruitment goals. “Sensory Processing and Adaptation” (SPA) studied sensory processing in preschoolers (ASD_SPA; n=26). The “Autism Biomarkers Consortium for Clinical Trials” studied biomarkers of social communication in school-aged children (ASD_ABC-CT; n=262). TD participants were recruited from these studies and studies of 5 neurogenetic conditions described below (n=180; Supplementary Table 3.1).

We calculated TBE-rates (Hz) from electrodes over somatosensory cortex (C3/C4) (Supplementary Table 3.3). Since TBE-rates did not differ for TD participants by study (Fig. S3.1), we collapsed them into one group. Next, we tested whether TBE-rates in ASD participants from ASD_SPA and/or ASD_ABC-CT differed from TD. TBE-rates were higher in ASD_SPA ($p < .001$) but not ASD_ABC-CT relative to TD.

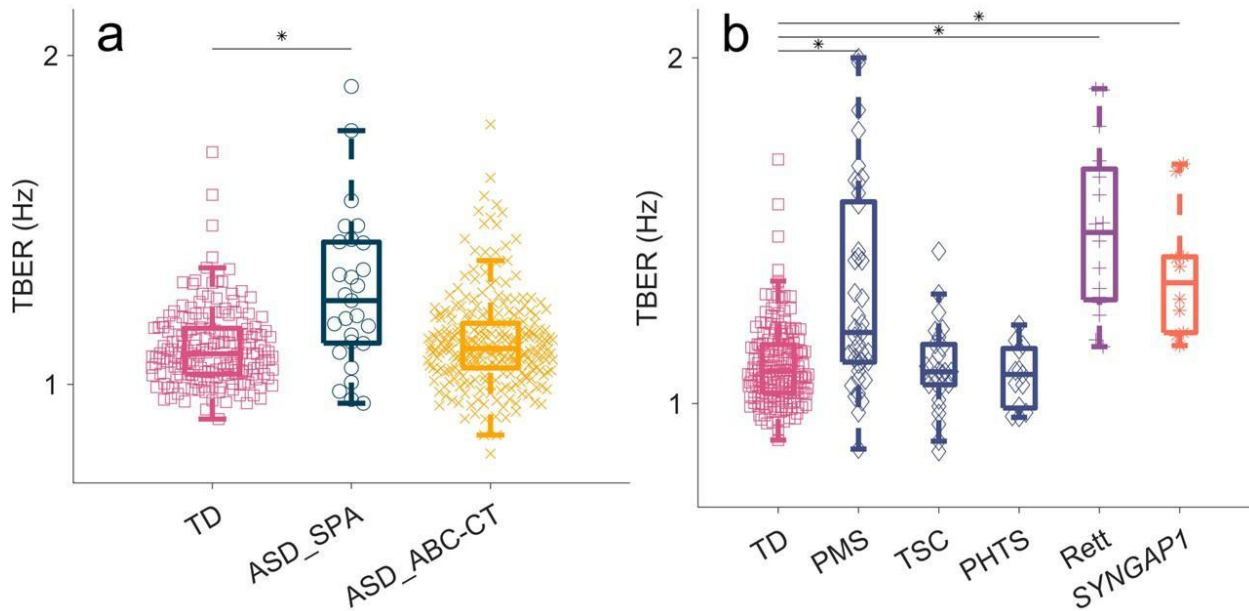


Figure 3.1. Transient Beta Event Rate measured over electrodes C3/C4 across diagnostic groups. Each shape reflects one participant. Within each box, horizontal middle line denotes group median; boxes extend over each group's interquartile range (IQR); vertical dashed whiskers denote values 1.5 times the IQR value. **(a)** * indicates significant group differences assessed using a Mann-Whitney U test ($p < .001$). TBE-rates did not differ between TD and ASD_ABC-CT ($p = .127$). **(b)** * indicates significant group differences assessed using repeated Mann-Whitney U tests ($p < .001$, Bonferroni corrected for five comparisons). TBE-rates did not significantly differ between TD and TSC or PHTS (both adjusted $p = 1.0$).

Considering the heterogeneity of ASD and the findings above, we next aimed to understand whether TBE-rates could stratify biologically meaningful sub-groups. To this end, we evaluated TBE-rates in five neurogenetic conditions associated with ASD (Phelan McDermid Syndrome (PMS; $n=41$); Tuberous Sclerosis Complex (TSC; $n=25$); *PTEN* Hamartoma Tumor Syndrome (PHTS; $n=12$); Rett syndrome (RTT; $n=15$); and *SYNGAP1*-Related Disorder (*SYNGAP1*; $n=11$)). TBE-rate was elevated in PMS, RTT, and *SYNGAP1* participants relative to TD (all adjusted p 's $< .001$). TBE-rates did not significantly differ between TD and TSC or PHTS. Across neurogenetic groups, there was no significant difference in TBE-rates based on co-occurring ASD diagnostic

status (Supplementary Table 3.4; $p = .379$). These results show that TBEs occur more frequently in some ASD and a subset of ASD-related neurogenetic conditions (Fig. 3.1, Fig. 3.2, Supplementary Table 3.3), but ASD itself does not seem to be the primary driver of elevated TBE-rates.

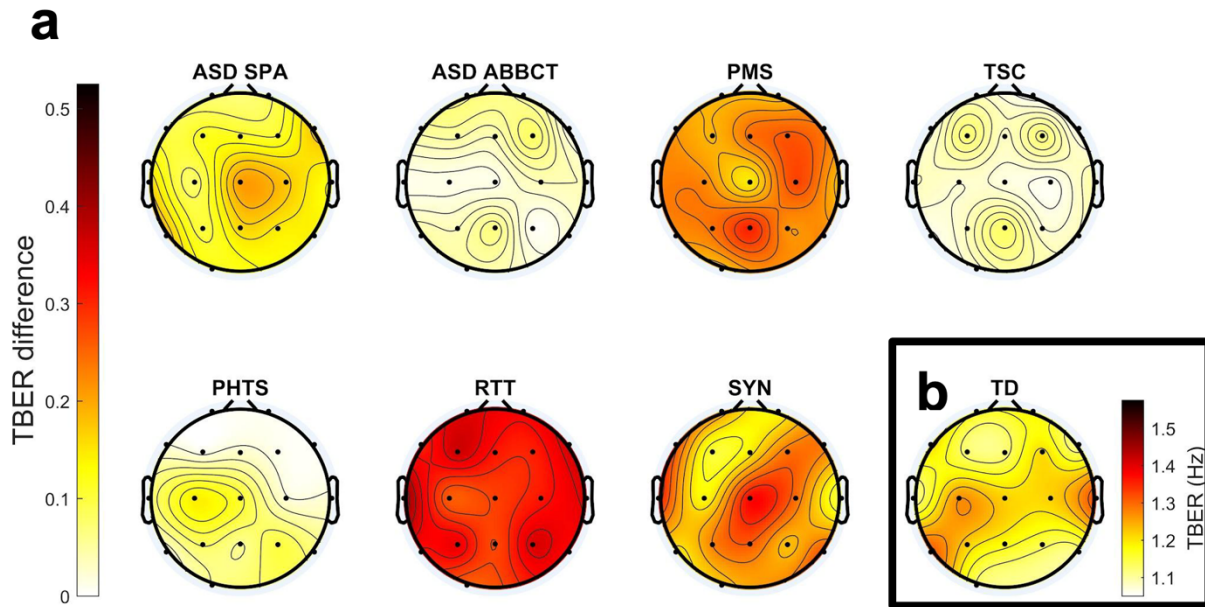


Figure 3.2. Difference in transient beta event rate across the scalp and across diagnostic groups. Topoplots showing (a) Difference in TBE-rates (Hz) across the scalp (Cohort minus TD) and (b) TD TBE-rates values.

To begin considering potential clinical utility of TBE-rates, we next evaluated its short-term reliability. We utilized longitudinal data from the ABC-CT cohort collected six weeks post initial visit. TBE-rates showed fair test-retest reliability when TD ($n=108$) and ASD ($n=247$) groups were collapsed (intraclass correlation (ICC) .547, 95% CI [.470, .616]) (Fig. S3.2). When separated by diagnostic group, TBE-rates test-retest reliability was poor for the TD group, (ICC .365, 95% CI [.189, .517]) and good for the ASD group (ICC .606, 95% CI [.520, .679]) (Fig. S3.2). These results support the reliability of TBE-rates as a functional biomarker for ASD.

Finally, we used the Sensory Profile 2 (SP2),¹⁷⁶ a caregiver-reported questionnaire collected from a subset of our SPA sample to test whether thalamocortically-driven TBE-rates may act as a biomarker for atypical tactile responsiveness. We evaluated the overall Touch subscale and two subdomains: Tactile Hyper-responsivity and Tactile Hypo-responsivity (Supplementary Table 3.5). We evaluated the association between tactile processing and TBE-rates for the ASD (n=22) and TD (n=20) groups separately to ensure our findings reflected sensory responsiveness rather than group differences, since ASD is known to be associated with atypical sensory responsiveness (Figure 3.3a).

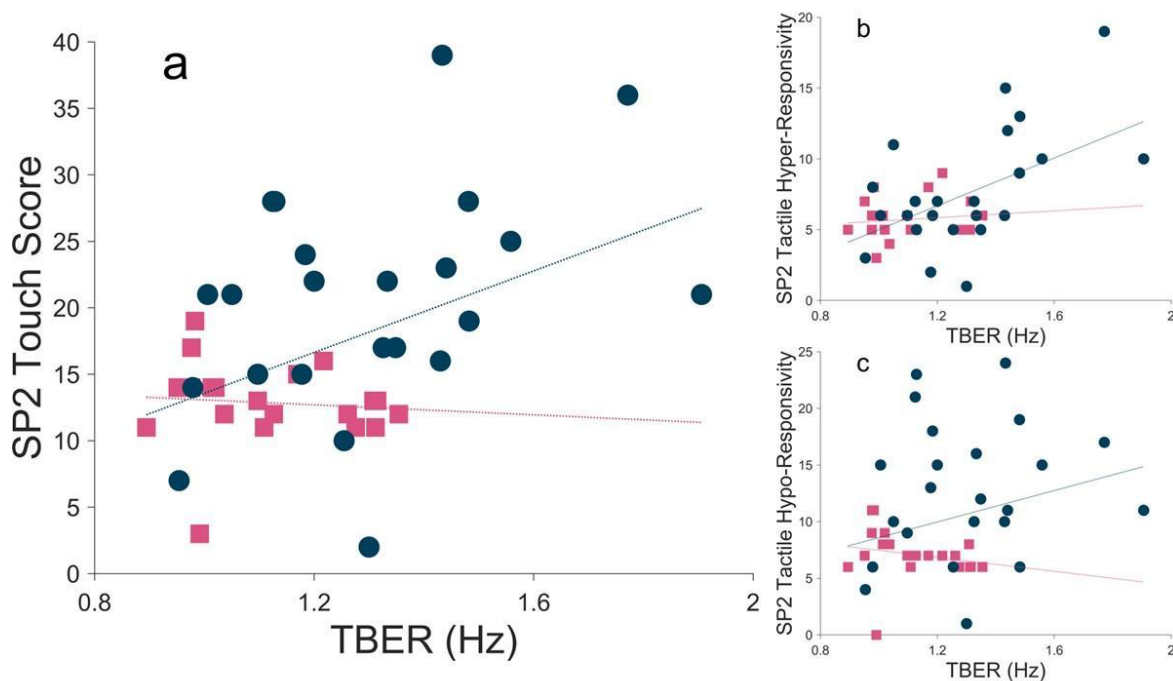


Figure 3.3. Transient beta event rate is associated with tactile responsiveness. Each dot represents one participant. ASD blue circles; TD pink squares. Dashed lines reflect lines of best fit. r = Spearman's Rank correlation coefficients. **(a)** TBE-rates and SP2 Touch subscale were positively correlated in ASD ($r = .475$, $p = .026$), but not TD ($r = -.360$, $p = 0.119$). **(b)** TBE-rates and Tactile Hyper-responsivity were positively correlated in ASD ($r = .559$, adjusted $p = .028$) but not TD ($r = -.014$, adjusted $p = 1.000$). **(c)** TBE-rates and Tactile Hypo-responsivity were not significantly correlated for either group (ASD: $r = .275$, adjusted $p = .859$; TD: $r = -.465$, adjusted $p = .156$). (b,c) p -values Bonferroni corrected adjusting for 4 comparisons.

The overall Touch subscale and TBE-rates were positively correlated in ASD ($\rho=.475$, $p=.026$), but not TD. Furthermore, Tactile Hyper-responsivity and TBE-rates were positively correlated in the ASD group ($\rho=.559$, adjusted $p = .028$; Figure 3.3b). Tactile Hypo-responsivity and TBE-rates were not significantly correlated for either group (Figure 3.3c). These data demonstrate TBEs behavioral relevance to a tactile Hyper-responsivity phenotype.

Neither age ($\rho = -.038$, $p = .360$) nor NVIQ ($\rho = -.021$, $p=.611$) were significantly associated with TBE-rates. Medication likely does not account for high TBE-rates as there was no consistent pattern of medications for participants with extreme TBE-rates values (Supplementary Table 3.6). Among neurogenetic conditions, TBE-rates were not significantly higher based on seizure history ($p=.150$).

Discussion

Taken together, TBE-rates do not appear to be a diagnostic biomarker for autism itself, but rather a transdiagnostic biomarker associated with tactile hyper-responsivity. Biomarker development is a crucial unmet need necessary to support clinical trials in neurodevelopmental conditions.²³³ Across a wide range of individuals with varying types and severities of neurodiversity, addressing sensory processing challenges is repeatedly noted as a common goal that would improve quality of life^{1,162,206,234}; TBE-rates reflect a step towards this shared goal. We find that TBE-rates are elevated in multiple rare neurogenetic conditions associated with atypical neurodevelopment (i.e., PMS, RTT, and *SYNGAP1*), and for a subset of children with idiopathic

ASD, suggesting shared atypicalities of neural circuit function and thus potential shared opportunities for treatment in these conditions.

The thalamocortical mechanisms underlying TBE and the relationship between TBE and tactile processing have been previously well described in wild type animal, computational, and neurotypical human models.^{215–217} This knowledge, in combination with the present findings describing atypical TBE-rates in neurodiverse populations, suggests multiple future directions spanning the bench-to-bedside spectrum. Since bursting activity from thalamus is sufficient to drive TBE,²¹⁷ it will be of benefit to determine whether TBEs originate primarily in thalamus or whether these bursts are driven by more central (“top-down”) or peripheral (“bottom-up”) activity. This work will inform targets for potential interventions addressing sensory hyper-responsivity and offer clues as to whether TBE-rates may be best used as a biomarker for stratification and/or outcome monitoring in clinical trials. Notably, shared treatments for tactile hyper-responsivity in mouse models of PMS and RTT are already being developed,¹⁰ and future studies can measure TBE-rates in mouse models of various ASD-related neurogenetic conditions (e.g. *Shank3b*, *Mecp2*), including before and after genetic or pharmacological treatment in the periphery, thalamus, or cortex.

In the present study, we focused on tactile processing and evaluated TBE-rates over somatosensory cortex (C3/C4) due to previous literature relating transient beta events and tactile perception. However, TBEs were found across the scalp and TBE-rate was elevated at many electrode locations (particularly in individuals with RTT, who exhibited the highest TBE-rate of all diagnostic groups) as shown in Figure 3.2. Further work is needed to characterize TBEs in other electrodes (Oz, Fz) and investigate their relationship to sensory responsivity in other modalities such as vision and audition.

Clinically, our findings suggest strong scalability for TBE-rates in future clinical trials. We leveraged resting EEG data from 9 collection sites, across ages 1-25 years, and 2 EEG acquisition setups. However, our findings also highlight an unmet need for further characterization of sensory processing challenges in neurodevelopmental and neurogenetic conditions. To date, while detailed sensory phenotyping has begun in several studies of the conditions we include here,^{235,236} many of the large consortia developing and testing biomarkers for these conditions did not initially include targeted measures of sensory processing; this should be rectified in future iterations of these studies. Inadvertent recruitment biases should also be considered. For example, we suspect that the study titled, “Sensory Processing and Adaptation” likely over-enrolled children with sensory processing challenges (and thus children with elevated TBE-rates, leading to group differences in TBE-rates for SPA_ASD vs. TD), which did not occur in ABC-CT. (Ages were also different in these samples; however, we found no association between age and TBE-rates). Determining expected population distributions of TBE-rates will also be of benefit; we suspect that higher test-retest reliability of TBE-rates in our ASD group may be the result of low inter-individual variability, rather than high intra-individual variability, in the TD group, since ICC depends on both values.

In summary, TBE-rates reflect an opportunity to develop a meaningful transdiagnostic biomarker relevant to neural circuit functions relevant to several genotypes and conditions. Whether they reflect an aspect of daily function (e.g. tactile hyper-responsivity) requires further investigation by the scientific community given its clear contribution to quality of life in neurodivergent individuals. TBE-rates may thus serve as a springboard and model for further understanding, measurement, and eventual treatment for clinically and biologically meaningful subgroups of individuals with neurodevelopmental and neurogenetic conditions.

Data Availability

The data that support the findings of this study are available, in collaboration with the principal investigator for each respective study, upon reasonable request. Data from the Autism Biomarkers Consortium for Clinical Trials are also available via the NIMH Data Archive (identifier: 2288; https://nda.nih.gov/edit_collection.html?id=2288).

Code Availability

EEG preprocessing was conducted using the Batch EEG Preprocessing Platform (BEAPP), available at <https://github.com/lcnbeapp/beapp>. Within BEAPP we used the Harvard Automated Preprocessing Pipeline for EEG (HAPPE), which is embedded in BEAPP and available separately at <https://github.com/PINE-Lab/HAPPE>. To calculate TBE-rates we used the SpectralEvents Toolbox, available at <https://github.com/jonescompneurolab/SpectralEvents>.

Author Contributions

GP, KS, YB, DK, and AL conceptualized the study. GP, KS, YB, DK, and AL wrote and prepared the initial manuscript draft. GP, KS, and YB performed the analysis. AL supervised the project. All authors contributed to interpretation of the results and critical revision of the manuscript.

Acknowledgements

The Developmental Synaptopathies Consortium acknowledges the support of Simon K. Warfield, Benoit Scherrer, Rajna Filip-Dhima, Kira Dies, Paige Siper, Ellen Hanson, and Jennifer M. Phillips.

The Autism Biomarkers Consortium for Clinical Trials acknowledges the support of Madeline Aubertine, Jessica Benton, Cynthia Brandt, Carter Carlos, Shou-An A. Chang, Kelsey Dommer, Alyssa Gateman, Simone Hasselmo, Julie Holub, Toni Howell, Ann Harris, Alexander Hoslet, Kathryn Hutchins, Kelsey Jackson, Scott Johnson, Lily Katsovitch, Minah Kim, Beibin Li, Kelsey MacDonald, Samantha Major, Samuel Marsan, Adriana S. Méndez Leal, Takumi McAllister, Lisa Nanamaker, Leon Rozenblit, Megha Santosh, Helen Seow, Laura Simone, Dylan Stahl, Cindy Voghell, Andrew Yuan, and Taylor Hoffman. Consultation for the ABC-CT EEG was provided by the EU Aims LEAP team, including Declan Murphy, Eva Loth, Emily J.H. Jones and Luke Mason.

Funding

This work was supported by grants from the Simons Foundation/SFARI (Award number 648277, ARL), the Hock E. Tan and K. Lisa Yang Center for Autism Research at Harvard University (ARL), the Clark Family (ARL/MF), the Eagles Autism Foundation (ARL), the National Institute of Mental Health (U19 MH108206, JCM; R01 MH122428, D\$), Intramural Research Program ZICMH002961, National Institute of Neurological Disorders and Stroke (U54 NS092090, MS), National Institute of Child Health and Human Development (P50HD105351,

MS; P50HD105351 Project 001, MF), and the Syngap Research Fund (ARL/MS/AP/KW). We are grateful to all the children and families who participated in these studies.

Competing interests

AD, LC, CE, SF, JAMA, CAN, DMR, CAS have no relevant conflicts of interest.

ARL previously received consulting fees from Lab 1636.

FS consults for and has received research funding from Roche and Janssen.

CS receives royalties from Pearson Assessments for the Vineland-3.

JCM consults with Customer Value Partners, Bridgebio, Determined Health, and BlackThorn Therapeutics, has received research funding from Janssen Research and Development, serves on the Scientific Advisory Boards of Pastorius and Modern Clinics, and receives royalties from Guilford Press, Lambert, Oxford, and Springer.

MS reports grant support from Biogen, Astellas, Bridgebio, and Aucta. He has served on Scientific Advisory Boards for Roche, SpringWorks Therapeutics, Jaguar Therapeutics and Alkermes.

Chapter 4. Altered oscillatory coupling reflects possible inhibitory interneuron dysfunction in Rett syndrome

Devorah Kranz,^{1,2} Yael Braverman,¹ Michelle McCarthy,³ Claire Mackay, Karen Sabol,¹ Tim A. Benke, David Lieberman,^{1,2} Eric D. Marsh,⁴ Jeffrey L Neul,⁵ Fleming Peck,⁶ Alan K Percy,⁷ Joni Saby,⁴ Nancy Kopell,³ Charles A. Nelson,^{1,2} April R Levin,^{*1,2} Michela Fagiolini^{*1,2}

*Co-last authors

Abstract

Rett syndrome is a rare neurodevelopmental disorder caused primarily by pathogenic variants in the *MECP2* gene, leading to lifelong cognitive impairments. To understand the broad neural disruptions in Rett syndrome, it is essential to examine large-scale brain dynamics at the level of neural oscillations. Phase-amplitude coupling—a form of cross-frequency interaction that supports information integration across temporal and spatial scales—is a promising candidate measure for capturing such widespread neural dysfunction. Phase-amplitude coupling depends on the coordinated activity of specific neuronal subtypes, and while multiple subtypes are implicated in different aspects of the Rett syndrome phenotype, their role in shaping large-scale oscillatory dynamics in Rett syndrome is not well understood. To investigate this, we utilized a multi-level approach, combining EEG recordings with computational modeling to identify alterations in phase-amplitude coupling in Rett syndrome and probe their underlying cellular and circuit-level mechanisms.

We recorded resting-state EEG from 38 individuals with Rett syndrome and 30 age- and sex-matched typically developing individuals. Phase-amplitude coupling was quantified: modulation index was obtained to determine coupling strength, and phase bias was assessed to examine the preferred phase of coupling. We characterized phase-amplitude coupling across all

low and high frequency combinations and electrodes, as well as within canonical theta-gamma and alpha-gamma frequency pairs across four predefined cortical regions. Finally, we modeled a biophysically-constrained Layer 4 cortical network to propose a possible mechanism underlying changes to oscillatory dynamics.

We found significantly stronger phase-amplitude coupling in Rett syndrome across widespread cortical regions and frequency pairs, with a pronounced increase in theta-gamma and alpha-gamma coupling in anterior, posterior, and whole-brain regions ($P < 0.05$). Individuals with Rett syndrome also exhibited a more positive alpha-gamma phase bias in anterior and whole-brain regions ($P < 0.05$). Biophysically constrained modeling demonstrated that reduced VIP-expressing interneuron activity alone could recapitulate the pattern of increased theta-gamma and alpha-gamma phase-amplitude coupling observed in Rett syndrome ($P < 0.001$).

These findings identify alterations in awake-state phase-amplitude coupling in Rett syndrome and propose a mechanistic link to VIP+ interneuron dysfunction. Elevated phase-amplitude coupling may serve as a promising biomarker of cortical dysfunction and a translational bridge from neural circuitry to clinically observable EEG signatures. By implicating VIP+ interneurons, our results open new avenues for testing interventions in preclinical models to identify potential novel therapeutic targets for individuals with Rett syndrome.

Author affiliations

1. Boston Children's Hospital, Boston, MA 02115
2. Harvard Medical School, Boston, MA 02115
3. Boston University, Boston MA 02215

4. Departments of Neurology and Pediatrics, University of Pennsylvania Perelman School of Medicine and Children's Hospital of Philadelphia, Philadelphia PA 19096
5. Department of Pediatrics, Vanderbilt University Medical Center, Nashville TN 37203
6. Department of Psychology, University of California Los Angeles
7. University of Alabama, Heersink School of Medicine, Department of Pediatrics (Neurology)

Correspondence to:

Dr. Michela Fagiolini, michela.fagiolini@childrens.harvard.edu, Center for Life Sciences, 3 Blackfan St, Boston, MA 02115

Dr. April Levin, april.levin@childrens.harvard.edu, 2 Brookline Place, BCH 3433, Brookline, MA 02445

Keywords

Rett syndrome; neurogenetic disorders; EEG; phase-amplitude coupling; VIP+ interneurons

Introduction

Rett syndrome (RTT) is a rare X-linked neurodevelopmental disorder that presents with an initial period of typical development followed by rapid regression resulting in profound cognitive, motor, and autonomic impairments including loss of purposeful hand use and spoken language.^{13,16} The majority of RTT cases are caused by de novo pathogenic variants in the X-linked *MECP2*

gene, which encodes methyl-CpG-binding protein 2 (MeCP2).²² Although MeCP2 is expressed widely throughout the body, its levels are 5 to 10-fold higher in neurons compared to other cell types, and neuronal dysfunction is central to RTT pathology.⁵⁰ MeCP2 influences the brain's cellular and network development across the lifespan: its function affects prenatal neurogenesis, development of neuronal circuitry, and maintenance of adult neural function.^{55–57,237} Given these widespread alterations across diverse brain regions and functions, understanding RTT pathophysiology requires investigating neural dynamics at the level of large-scale brain networks.^{13,117}

Brain-wide dynamics rely heavily on coordination of and by neural oscillations—rhythmic patterns of neuronal activity characterized by frequency, amplitude, and phase—that facilitate communication between distant cortical regions and support cognitive processing.¹¹³ Electroencephalography (EEG) provides a valuable non-invasive and clinically relevant tool to assess these oscillations and is particularly suitable for use in RTT populations as it can be recorded without requiring active task engagement. Clinical EEG in RTT has long revealed visually observed abnormalities such as epileptiform discharges and diffuse slowing of activity that persists into adulthood.^{118,119,121} Recent quantitative studies have expanded upon these findings, revealing evidence of region-specific disturbances to spectral power (which examines the power of each individual frequency present in the signal), including increases in delta (1-3 Hz) and theta power (4-7 Hz), reductions in alpha power (8-12 Hz), and alterations in the 1/f spectral slope.^{122,123} While spectral power analyses offer insight into the overall magnitude of neural oscillations, they provide limited information about how these oscillations dynamically interact to support cognitive processes, or how circuit-level dysfunction in RTT can disrupt communication between brain regions or alter precise timing mechanisms essential for cognition.

Phase-amplitude coupling (PAC), which evaluates how the phase of low-frequency oscillations modulates the amplitude of high-frequency activity, has been proposed as a fundamental mechanism of neural coordination across spatial and temporal scales.^{238–240} PAC has been observed in diverse electrophysiological signals, from intracellular recordings to scalp EEG, highlighting its relevance across different levels of neural activity. It is thought that PAC serves as a clocking mechanism, generating temporal windows that allow for the integration of relevant information.²⁴¹ Notably, PAC strength and phase bias—the phase of the slow wave at which coupling occurs—can vary by cortical layer and are influenced by interneuron function.^{242–244}

PAC between canonical frequency pairs is particularly relevant to specific domains of brain function and has been shown to be functionally important to a range of core cognitive processes. Theta-gamma coupling is implicated in working memory, with theta phase modulating gamma amplitude to organize memory encoding and retrieval in the hippocampus and prefrontal cortex.^{245–248} Alpha-gamma coupling, meanwhile, has been linked to computations in visual cortical areas, where alpha phase modulates local gamma activity, facilitating selective attention, visual information integration, and prioritization of sensory input based on stimulus saliency.²⁴⁹ Altered PAC has been reported in autism spectrum disorder (ASD) and other neurogenetic disorders, both in resting-state EEG²⁶ and during specific cognitive tasks.^{251–255} In RTT, abnormal PAC has recently been identified during slow-wave sleep,²⁵⁶ but its presence during the awake state—particularly at faster frequency pairs associated with memory and attention—has not yet been studied. Moreover, precisely which neuronal subpopulations and circuit mechanisms drive RTT-related oscillatory abnormalities remains an area of active investigation.

In *Mecp2* mutant mice, which replicate key aspects of the RTT phenotype, cortical circuit dysfunction is characterized by an imbalance in excitatory-inhibitory signaling,^{78,83,86} and studies

employing techniques such as selective deletion of *Mecp2* from specific neuronal subtypes have clarified how distinct cell populations individually contribute to these network disruptions.^{94–96} Pyramidal (PYR) neuron activity is significantly reduced, particularly in sensory cortical regions, leading to progressive silencing of visual circuits and decreased visual acuity.^{78,83,257} Alterations in GABAergic interneurons have also been strongly implicated⁹⁰: parvalbumin-positive interneurons (PV+INs), which normally provide fast-spiking inhibition to PYR neurons, exhibit increased connectivity and activity in *Mecp2* mutant mice.⁸⁶ Vasoactive intestinal peptide-expressing interneurons (VIP+INs) in particular have emerged as especially relevant to circuit and oscillatory dysfunction. VIP+INs synapse onto both PYR cells and other interneurons,^{92,93} modulating sensory gain^{258–262} and driving oscillatory activity.^{263,264} Early disruptions in VIP+IN activity broadly compromise cortical circuits, resulting in weakened synaptic inhibition, impaired experience-dependent plasticity, and abnormal cortical maturation.²⁵⁸ Deficits in VIP+IN excitability and modulation have been reported in mouse models of other neurodevelopmental disorders^{265,266} that share some clinical features with RTT.^{267,268} Notably, recent studies demonstrate that selective deletion of *Mecp2* from VIP+INs alters their intrinsic activity and recapitulates core phenotypic features of RTT⁹⁴, positioning VIP+IN dysfunction as a compelling avenue for understanding altered cortical dynamics in RTT.

In the present study, we adopted a multi-level approach, characterizing neural dynamics from both top-down and bottom-up perspectives. We first investigated phase-amplitude coupling patterns during awake resting state EEG in individuals with RTT. Since the specific frequencies and cortical regions where PAC may be disrupted in RTT are currently unknown, we initially conducted a data-driven analysis examining coupling comprehensively across all possible frequencies and brain regions. Subsequently, we focused our analyses on two canonical frequency

pairs—theta-gamma and alpha-gamma—given their established roles in cognitive processing, and to allow for more detailed assessments of the distribution of PAC across each group. We identified abnormal PAC in individuals with RTT compared to typically developing controls, revealing significantly elevated coupling across multiple frequency bands and cortical regions, particularly in the alpha-gamma and theta-gamma ranges.

To identify potential neuronal and network mechanisms underlying the increased theta-gamma and alpha-gamma coupling seen in RTT, we then took a bottom-up approach by constructing a biophysically-constrained network model of cortical Layer 4 (L4) comprising pyramidal neurons and three key inhibitory interneuron classes: somatostatin (SOM+), PV+, and VIP+ interneurons. We focused our investigation on L4 for three reasons: (1) a major source of cortical alpha is thought to come via thalamocortical interactions which include L4^{242,269}; (2) increased theta-gamma coupling has been associated with thalamocortical dysfunction²⁷⁰; (3) interneurons are key mediators of oscillatory brain activity^{271–273} and L4 consists of a relatively large 7/1 ratio of inhibitory to excitatory neuronal subtypes.²⁷⁴ Of note, biophysically-constrained models have been instrumental in revealing the connection of interneuron activity to rhythmic network dynamics.^{115,275,276} Here, our computational model suggests that decreased excitability of VIP+ interneurons alone could account for the observed PAC alterations in L4 dynamics.

By integrating clinical EEG findings with mechanistic insights from animal models and computational modeling, our approach provides a unique translational framework for linking electrophysiological biomarkers with underlying cellular and circuit-level disruptions in RTT. Establishing PAC as a quantitative marker of cortical dysfunction could enable more precise tracking of disease progression and facilitate the evaluation of therapeutic interventions, and

importantly, our findings open new potential pathways to test in preclinical models in order to identify potential novel therapeutic targets in individuals with RTT.

Materials and Methods

Participants

Participants were enrolled in one of two studies whose data were combined for the analyses presented here:

1. Natural History Study of Rett and Related Disorders (NHS) (U54 HD061222; NCT02738281).²⁷⁷ Data were acquired across three study locations (Boston Children's Hospital (BCH), Vanderbilt University Medical Center (VUMC), or Children's Hospital Colorado (CHCO). The experimental protocol was approved and overseen by the Institutional Review Boards at each site.
2. IDDRC Rett Syndrome Biomarkers Study (RSBS). Data were acquired only at BCH. The experimental protocol was approved and overseen by the Institutional Review Board at Boston Children's Hospital.

Participants were 1-46-year-old individuals with Rett Syndrome (RTT; $n = 51$) and typical development (TD; $n = 30$). While many prior studies in RTT have focused on a narrower age range, we chose this wider range to capture the full spectrum of the disorder, and examined the effects of age on our analyses as described below. Participants were recruited through hospital-based Rett clinics, a research registry, clinical referrals, community sources, and word of mouth. Informed consent was obtained from all guardians or participants above 18 years old, and assent from all participants developmentally able.

Table 4.1 Participant Information

	Diagnostic Group	
	RTT (<i>n</i> = 38)	TD (<i>n</i> = 30)
Clinical Severity Score		
<10	5	
10->19	15	
20->29	13	
30->39	3	
Rett Diagnosis		
Typical	29	
Atypical	9	
Seizures		
None (with or without meds)	28	
Monthly	1	
Weekly	5	
Daily	1	
Sex		
Female	35	28
Male	3	2
Age	Range: 1-45	Range: 1-15
	Median: 8.5	Median: 6.0
	IQR: 11.75	IQR: 6.0
Usable EEG		
Yes	38	30
No	13	0
Collection Site		
VUMC	11	1
BCH	26	25
CHCO	1	4
Study		
RSBS	23	19
NHS	15	11

In the RTT group, inclusion criteria included being 12 months to 50 years old at time of enrollment and a diagnosis of RTT with a previously identified pathogenic or likely pathogenic variant in the *MECP2* gene. TD participants were recruited to be approximately age and sex-matched to the RTT participants and were included if they had no underlying genetic diagnoses or prior medical conditions associated with increased likelihood of ASD or intellectual disability. In both groups, the primary language of participants and caregivers was English. Exclusion criteria for the RTT group included taking an investigational drug as part of another research study within 30 days prior to study enrollment.

Demographic and other characteristics are reported in Table 4.1.

EEG data collection

Participants in the RSBS study watched an approximately five-minute video consisting of repeating silent videos akin to screensavers which directed their attention to the monitor. Each video played forward for 15 seconds and then in reverse for 15 seconds and video display was limited to 7 x 9.3 cm to minimize eye movement during task completion. The procedure for the NHS study differed slightly, in that participants watched a silent video of their choice. A behavioral assistant sat in the room with the participant to keep them as calm as possible.

Continuous EEG data were recorded at "rest" (i.e., with no time-locked stimuli), during the awake quiet state in dim lighting via a Net Amps 400 amplifier using the 128-channel Hydrocel Geodesic Net (Electrical Geodesic, Inc., Eugene, OR) with a sampling rate of 1000 Hz.

EEG data pre-processing

EEG data preprocessing was conducted using the Batch EEG Automated Processing Platform (BEAPP).¹⁸⁸ Within BEAPP, data were artifact detected and corrected using the Harvard Automated Processing Pipeline for Electroencephalography (HAPPE) 1.0, a pipeline optimized for EEG data collected from young children with neurodevelopmental disorders.¹⁸⁹ Data were first bandpass filtered using a high pass (1 Hz) and low pass (100 Hz) filter and resampled to 250 Hz. Only the 18 electrodes in the international 10–20 system were chosen for further processing and analysis. HAPPE applied line noise removal at 60 Hz via CleanLine's multi-taper method and then automatically detected and rejected bad channels, movement, muscle, and eye blink artifact with wavelet-enhanced independent component analysis (ICA) and ICA with Multiple Artifact Rejection Algorithm (MARA).^{190,191} Following artifact removal, bad channels were interpolated using spherical interpolation and data were re-referenced to an average reference. Data were then segmented into overlapping 6 second segments (20% overlap), and a 2-second buffer was included at the beginning and end of each EEG segment to avoid edge effects from filters. (In other words, the buffer resulted in 10-second segments, but only the middle 6 seconds were analyzed). Segments were rejected based on HAPPE's recommended amplitude cut off (40 μ V) and joint-probability rejection criteria. 55 randomly selected segments were chosen for each participant for subsequent analysis.

EEG rejection criteria

Participants were rejected if they had fewer than 55 EEG segments (330 seconds) of usable data or fell outside of 3 standard deviations from the mean on the following HAPPE quality control parameters: percent good channels, mean retained artifact probability, median retained artifact probability, percent of independent components rejected as artifact, and percent of EEG signal

variance retained after artifact removal. Based on the above criteria 13 of the 81 EEGs collected were rejected (these were all from RTT participants, likely due to movement artifact) (Table 4.1).

Phase-amplitude coupling

As previously described in Mariscal²⁷⁸ and Peck²⁵⁰, the following PAC computations were computed using the *pactools* Python toolbox. For each segment, first narrowband neural oscillations at low (2-20 Hz in 2 Hz steps) and high (20-100 Hz in 4 Hz steps) frequencies were extracted. Low frequencies were filtered using a constant bandwidth of 2 Hz, while high frequencies were filtered using an upper sideband variable bandwidth, equal to the low frequency of interest.²⁷⁹ Filtering at this step consisted of a zero-phase cosine-based filter to extract the real component and then a sine-based filter to extract the imaginary component, resulting in a complex-valued output signal.²⁸⁰

The low frequency phase and high frequency amplitude time series were obtained from the filtered signals. The phases of the low frequency signals were binned into 18 20-degree intervals (-180° to 180°), and the mean of the amplitude of the high frequency signals within each phase bin was calculated. The amplitude values were then normalized by dividing each bin average by the sum of all bin averages. The resulting phase-amplitude distributions were averaged across all 55 segments within participant to minimize noise.

After averaging the phase-amplitude distribution across segments, coupling strength, also known as the Modulation Index (*MI_{raw}*), was computed as the Kulback-Leibler divergence of the phase-amplitude distribution from a uniform distribution (this is embedded in *pactools*).²⁸¹ To isolate the effect of PAC strength from related factors that may generate spurious phase-amplitude coupling (e.g. spectral power), phase amplitude distributions and coupling strength were also

computed from a surrogate dataset that did not represent real effects of PAC. This surrogate dataset (*MI_{surr}*) was generated by offsetting the high frequency and low frequency signals randomly from .1 to 1.9 seconds. The modulation index was computed for 200 different iterations of this surrogate, null phase-amplitude coupling data. Next, a normalized z-scored modulation index was computed using the surrogate distribution; this method was proposed in Dupré La Tour.²⁸⁰ In carrying out these steps three measures were obtained from the original PAC strength: *MI_{raw}*, *MI_{surr}*, and *MI_{norm}*, where *MI_{norm}* is computed as the z-score of the *MI_{raw}* compared to the distribution of *MI_{surr}* values (previously termed z-MI in Mariscal²⁷⁸).

Phase-amplitude coupling strength: all frequencies and regions

To characterize diagnostic differences in patterns of PAC agnostic to canonical frequency bands, a data-driven approach was first employed, where analysis was performed across all frequency pairs and all channels.

To identify which frequency pairs and channels had statistically significant PAC within each group, a cluster-based approach was utilized, similar to Mariscal.²⁷⁸ The following steps were repeated for the RTT and TD groups separately.

1. For each low and high frequency pair within every channel, a t-test was conducted to compare the *MI_{raw}* values across all files within the group to the mean *MI_{surr}* values across all files within the group.
2. “Frequency grouping” size was identified as the number of adjacent significant frequency pairs within a channel, excluding diagonals.

To determine the significance of these frequency grouping sizes, *MI_{raw}* and *MI_{surr}* were flipped in a randomly selected subset of half the participants ranging from 1 to half the sample,

and the same test statistics and frequency grouping sizes were computed (steps 1 and 2 above). This process was repeated 1000 times to generate a null distribution of frequency grouping size. Finally, the original frequency grouping sizes that were larger than the 95th percentile of this null frequency grouping size distribution were determined to reflect significant PAC.

To determine differences in PAC strength (MI_{norm}) between groups, a cluster-based approach was utilized again. The same analysis steps were conducted, now comparing MI_{norm} from each group (rather than MI_{raw} and MI_{surr} from all files). In this analysis, t-tests were similarly conducted to compare MI_{norm} between groups at each frequency pair, and frequency grouping sizes that exceeded the permutation-generated null frequency grouping size distribution were determined to reflect statistically significant differences in PAC strength between groups.

Phase-amplitude coupling strength: a-priori defined regions of interest and canonical wavebands

Next, a-priori defined regions of interest and canonical wavebands were examined. Group differences in PAC strength were evaluated using the modulation index averaged over all segments for each individual normalized by the surrogate distribution (MI_{norm}). PAC strength was calculated across four brain regions of interest: anterior (FP1, FP2, F3, F4, F7, F8, Fz), posterior (O1, O2, T5, T6, Pz), midline (C3, C4), and whole-brain (all 10-20 channels), and in the following frequency pairs: theta (4-7 Hz) and gamma (28-56 Hz) and alpha (8-12 Hz) and gamma (28-56 Hz). A series of independent samples t-tests were used to test whether any differences between groups were significant.

Phase bias analysis

While the MI value represents the overall strength of phase-amplitude coupling, phase bias (or phase preference) reflects whether the high-frequency amplitude is preferentially modulated during specific phases of the low-frequency oscillatory cycle. To compute phase bias, the amplitude of the high-frequency oscillation was averaged across 18 phase bins spanning one full low frequency cycle for each participant. For each frequency pair, phase bias was calculated as the proportion of the high frequency amplitude occurring in the positive phase range (0° to $+180^\circ$), divided by the total amplitude across all bins, and then centered by subtracting 0.5. This yielded a scalar value per participant per frequency pair, where:

- Positive values indicate greater high frequency amplitude during the *falling phase* of the low frequency oscillation,
- Negative values indicate greater amplitude during the *rising phase*,
- Values near zero indicate no consistent phase preference.

The low frequency phase was derived using a cosine-based filter, with 0° corresponding to the waveform peak, $\pm 90^\circ$ to the zero crossings (rising at -90° , falling at $+90^\circ$), and $\pm 180^\circ$ to the trough. Group-level comparisons of phase bias were performed using Mann–Whitney U tests.

Phenotypic Assessments

Associations between PAC metrics in all canonical brain regions and frequency pairs and age as well as clinical severity were examined. Clinical severity was assessed by clinicians using the Clinical Severity Scale (CSS)³⁶ and Clinical Global Impressions of Severity (CGI- S).^{178,179} For RSBS participants only, associations with several other phenotypic measurements were also evaluated using parent-reported questionnaires: Rett Syndrome Behavior Questionnaire (RSBQ),^{180–182} Sensory Profile 2 (SP2),^{1,176} Vineland Adaptive Behavior Scales,^{282,283} and Anxiety

Depression and Mood Scale (ADAMS).^{284,285} Scores and questionnaires were obtained close to or on the same day as the EEG recording. Linear regression analyses were computed separately for the RTT and TD groups, and False Discovery Rate was used to correct for multiple comparisons and applied to p-values. MATLAB was used to run all statistical analyses.

Modeling PAC

We constructed a biophysical model of Layer 4 cortical networks using single-compartment neurons with Hodgkin-Huxley-type dynamics (Fig. 4.5). The network model included excitatory pyramidal neurons or stellate neurons (PYR) and inhibitory interneurons. The interneuron populations consisted of the three most common types of interneurons found in cortex: PV+INs, SOM+INs and 5HT3aR+INs.²⁸⁶ We used the VIP+IN subtype of 5HT3aR+ INs in our model since we were particularly interested in how VIP+IN activity affects network dynamics—VIP+INs have been implicated in mediating Rett symptoms by specific deletion of MeCP2 in VIP+ cells.⁹⁴ The model VIP+INs had D-, M- and T-currents which allowed for bursts of spikes at a delta time scale. L4-targeting SOM+INs (also known as X94 SOM+ interneurons) have a unique electrophysiological profile among cortical SOM+ cells, which includes stuttering behavior at alpha frequency^{94,287}. Our model L4 SOM+INs had D-, A- and h-type membrane currents that produced the alpha frequency stuttering. We note that rodent L4 differs in cell types and circuits depending on cortical area. Specifically, rodent S1—the cortical areas mediating the rodent’s more specialized sense of somatosensation—includes X94 SOM+ cells, whereas rodent V1 does not. We chose the more specialized cortical area of rodent S1 to model human L4. The PV+INs and PYRs were modeled using previous formulations of these cell types.²⁸⁸ Each cell type was connected to other types of neurons and to other cells of its own type with varying types, strengths

and degrees of connectivity constrained by existing literature that we were familiar with (see Appendix for more details). The network consisted of 110 neurons, with the number of each cell type roughly proportional to their prevalence in cortex.²⁸⁶ All neuronal subtypes were connected to each other through excitatory (AMPA) or inhibitory (GABA_A) synapses, with weights assigned according to available information. All neurons within the same class were connected to each by electrical and/or chemical synapses.

We studied the network activity under typical (baseline) and low VIP+IN activity levels, running 20 simulations for each condition. To decrease the activity of VIP+INs we lowered the maximal sodium current conductance of the VIP+INs by 22.5 mS/cm², which lowered the spiking rate of VIP+INs, but did not silence them. In simulations where specific types of interneurons were removed from the network, we sufficiently lowered either the maximal sodium conductance or the background excitation to those neurons such that spiking was eliminated in that neuronal subtype. We ran 7 simulations under each condition of removing one type of interneuron.

The local field potential (LFP), which is similar to the EEG in that it records extracellular voltage fluctuations but is different in that it is more spatially restricted than EEG, was modeled as the sum of all excitatory currents among PYR neurons in the network (as in Adam et al., 2024).²⁸⁹ To compute phase-amplitude coupling we followed the methods in Kramer et al., 2016.²⁹⁰ Phase amplitude coupling was determined by first bandpass filtering the model LFP at gamma, theta and alpha frequencies (where the ranges of all frequencies were the same as those used for the clinical EEG data) followed by extraction of phase and amplitude information of the low (theta and alpha) and high (gamma) frequencies, respectively using the Hilbert transform; this procedure generates phase and amplitude time-series vectors. We then computed the average amplitude of gamma for each phase of the low frequency oscillations and found the difference (h) between the

maximum and minimum values of the average amplitude. To determine the statistical significance of h , we compared h to the difference between the maximum and minimum values of h produced from 1000 surrogate phase-amplitude vectors generated by randomly permuting the amplitude time series to assign each time point of the phase vector to a random amplitude. Significant phase-amplitude coupling was determined by calculating the p-value, which was computed as the proportion of h -values from the surrogate phase-amplitude data greater than the h -value from the non-randomized phase-amplitude data. The raw modulation index (MI) for each high and low frequency pair was calculated using the method described above (Kulback-Leibler divergence of the phase-amplitude distribution from a uniform distribution).²⁸¹ A two-sample t-test was used to evaluate for a significant difference between the mean of the MI values for simulations run under different conditions.

The code for the model was written in C++. Both the analysis of data and the graphics used MATLAB. All code will be made available on GitHub. See Appendix for more details.

Results

To investigate brain-wide oscillatory dynamics in RTT, we analyzed phase-amplitude coupling during resting awake-state EEG recorded from 38 individuals with a confirmed diagnosis of RTT and 30 typically developing participants. We obtained low frequency phase and high frequency amplitude time series from the EEG data and extracted phase-amplitude distributions, used throughout the following analyses (see Methods).

PAC in Rett syndrome and typical development across all possible frequencies and regions

We used a data-driven approach agnostic to pre-defined canonical frequency ranges to characterize any patterns in PAC that may not be captured otherwise, evaluating coupling between all possible frequency pairs and across all 18 electrodes.

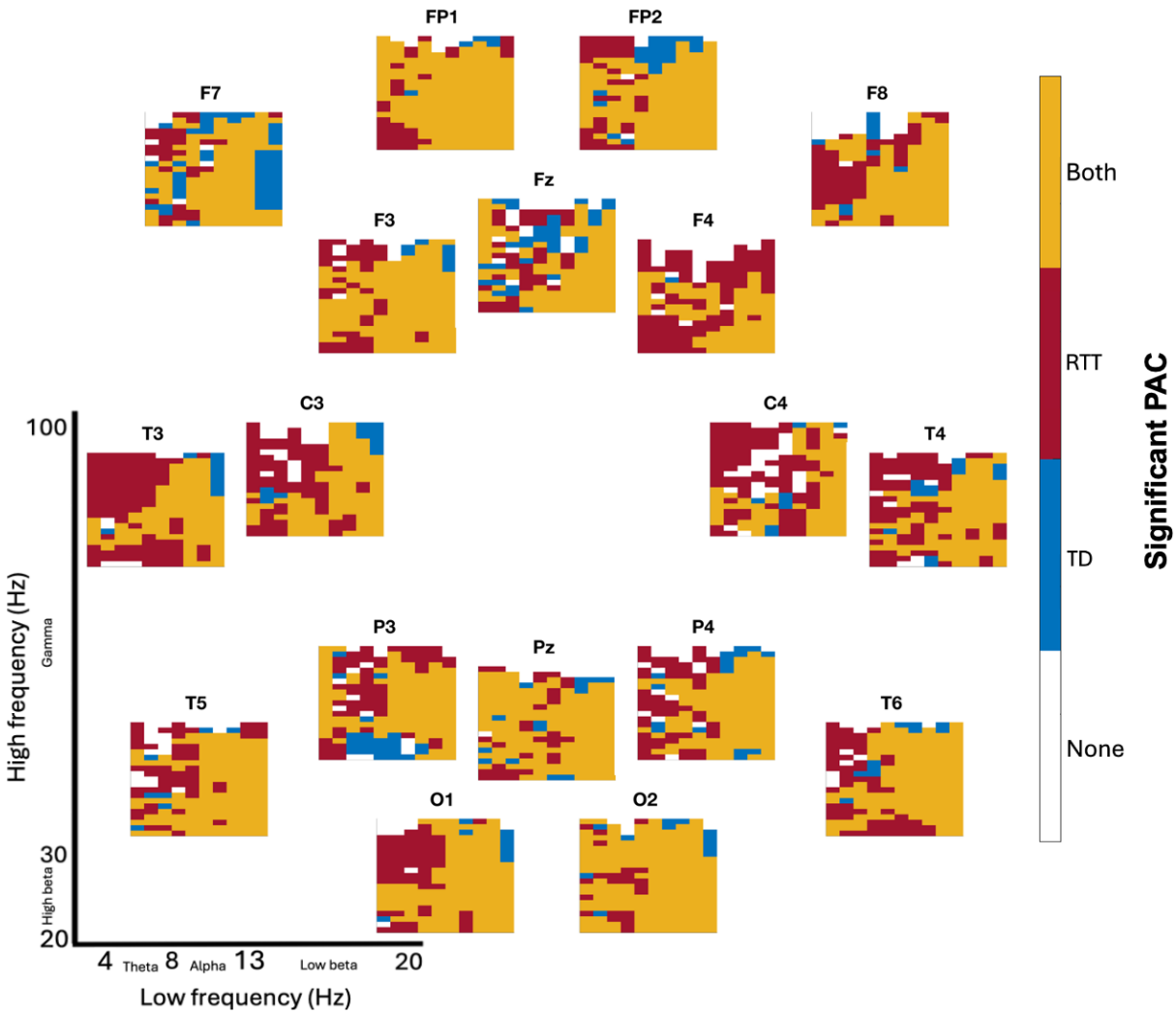


Figure 4.1. Instances of significant phase-amplitude coupling for RTT and TD independently across all possible frequencies and regions. Topoplot of comodulagrams visualizing significant PAC frequency pairs in RTT ($n = 38$) and TD ($n = 30$) for all 18 electrodes of the 10-20 channel array. Each subplot represents one electrode, and all subplots share the same axes of low and high frequency filtered signal, as shown in the lower left corner. Significant PAC frequency pairs are color-coded for RTT (red) and TD groups (blue), with any overlapping significant coupling shown in orange. On every subplot, each (x, y) coordinate with colored shading represents an instance of

significant coupling between the corresponding low frequency (x) and high frequency (y) signals. Significance was calculated using a cluster-based permutation test.

Because PAC has not been previously described in the awake state in RTT, we first asked at what channels and at what frequency pairs significant PAC occurs. Using a cluster-based permutation analysis, we compared observed PAC to randomized data to identify regions and frequency pairs where coupling was significant. Figure 4.1 visualizes instances of significant coupling for RTT and TD independently, in a binary manner, agnostic to the strength of the coupling. We identified significant coupling in all electrodes for both groups with a high level of overlap (Fig. 4.1). Interestingly, in many electrodes (most strikingly F4, F8, T3, T4, C3, C4, and O1), the RTT group, but not the TD group, exhibited significant PAC in slower frequencies paired with gamma (Fig. 4.1).

To investigate the differences in PAC between RTT and TD in this data-driven approach, we next quantified the *strength* of coupling by calculating normalized modulation index, or MI, and averaging within each group. In Figure 4.2, RTT MI was subtracted from TD and plotted to visualize the difference in coupling strength between groups; regions and frequency pairs where differences were significant were identified using independent samples t-tests and indicated. We found that PAC was stronger in RTT compared to TD in a large portion of frequency pairs and electrodes, and that a portion of these differences in MI were statistically significant in every electrode ($P < .05$) (Fig. 4.2). Notably, slower frequencies paired with gamma appeared particularly stronger in RTT, and lower gamma was predominantly affected (Fig. 4.2).

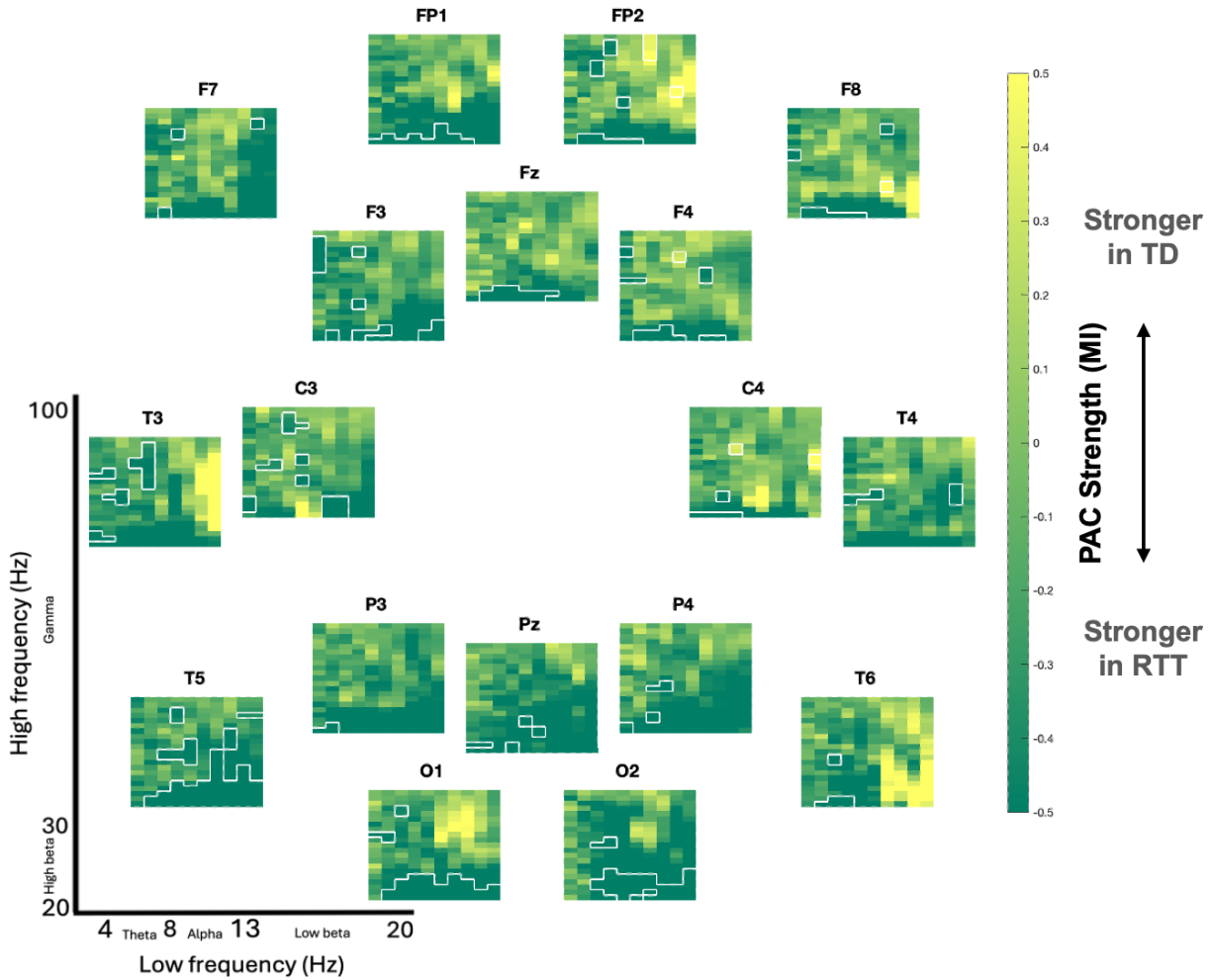


Figure 4.2. Difference between RTT and TD in phase-amplitude coupling strength across all possible frequencies and regions. Topoplot of comodulagrams visualizing PAC strength difference between RTT ($n = 38$) and TD ($n = 30$) for all 18 electrodes of the 10-20 channel array. Each subplot represents one electrode, and all subplots share the same axes of low and high frequency filtered signal, as shown in the lower left corner. On every subplot, each (x, y) coordinate represents RTT subtracted from TD strength of coupling between the corresponding low frequency x and high frequency y signal. Dark green shading corresponds to higher PAC strength in RTT than TD, whereas bright yellow shading corresponds to higher PAC strength in TD than RTT. Lighter green indicates areas of low PAC strength difference. White boundaries outline areas where coupling strength is significantly different between groups. Significance was calculated using independent sample t-tests with a threshold of .05.

PAC strength is increased in Rett syndrome in canonical theta-gamma and alpha-gamma frequency pairs

We next quantified coupling strength for each participant within the groups, focusing on four regions of interest (anterior, posterior, midline, and whole-brain) and the two canonical frequency pairs that have been most affected in other neurodevelopmental disorders (alpha-gamma and theta-gamma).^{250,255,278,279} We found that RTT participants had significantly higher MI compared to TD in the alpha-gamma frequency pair in anterior, posterior, and whole-brain regions, and in the theta-gamma frequency pair in posterior, midline, and whole-brain regions (independent samples t-test, $P < .05$) (Fig. 4.3). While a subset of participants in the RTT group showed MI values comparable to those in the TD group in each region, overall variability was greater across individuals, and PAC strength was markedly elevated in the RTT group in most regions we evaluated (Fig. 4.3).

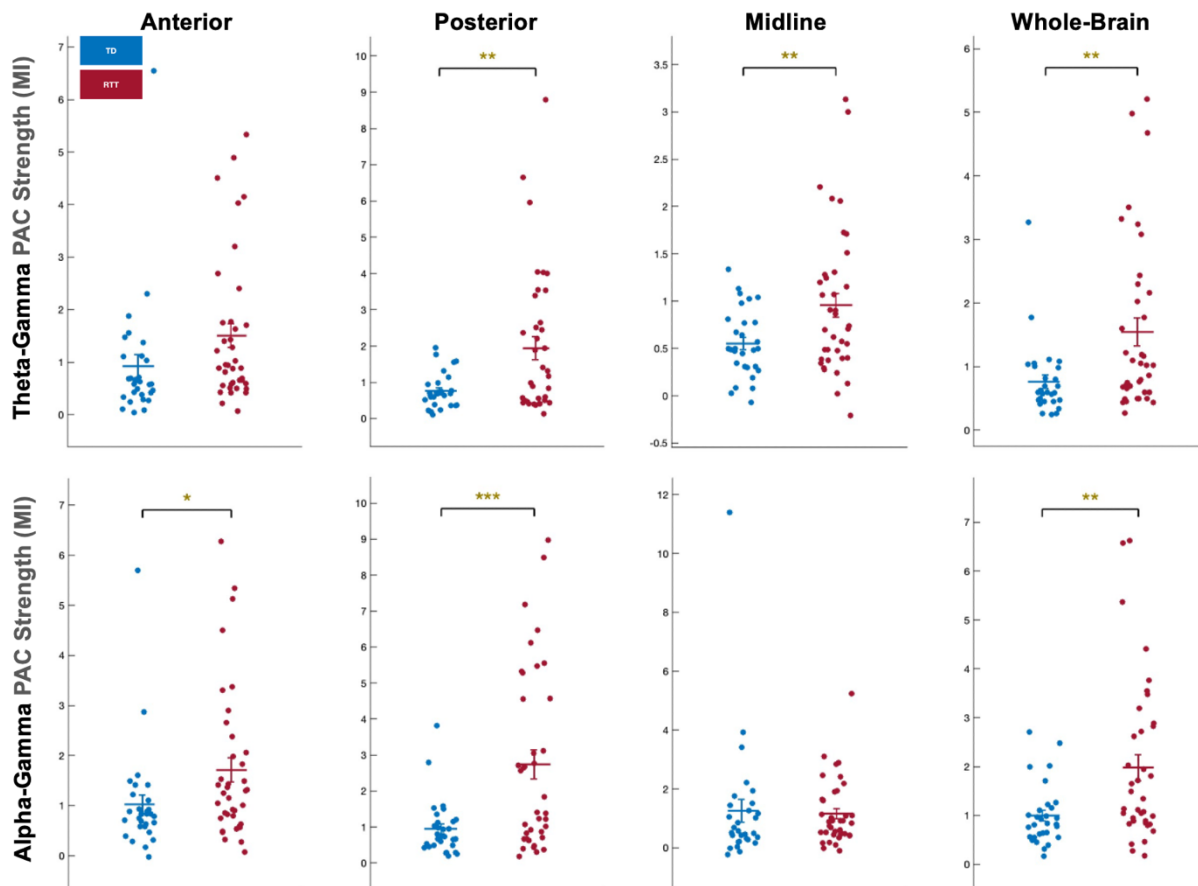


Figure 4.3. Theta-gamma and alpha-gamma PAC strength elevated in individuals with Rett syndrome. Markers in each subplot signify a single individual's normalized PAC strength for a given frequency pair and region (indicated on the top and left). TD participants ($n = 30$) are shaded in blue and RTT participants ($n = 38$) are shaded in red. Difference between groups was calculated using an independent samples t-test, where *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$. Group mean (horizontal lines) and standard error (vertical lines) are plotted.

PAC phase preference is more positive in the alpha-gamma frequency range in Rett syndrome

To further probe group differences in PAC, we examined at what point within the low-frequency cycle this coupling occurred, known as phase bias. Specifically, we computed phase bias using a metric that captures whether the high-frequency amplitude tended to occur preferentially during the rising or falling phases of the low frequency oscillation, where positive

values reflect greater coupling during the falling phase (from peak to trough) and negative values indicate greater coupling during the rising phase (from trough to peak).

We focused on the same canonical frequency pairs and plotted individual alpha-gamma and theta-gamma phase bias across anterior, posterior, midline, and whole-brain regions. RTT participants exhibited significantly more positive alpha-gamma phase bias compared to TD in anterior and whole-brain regions (Mann-Whitney U test, $P < .05$) (Fig. 4.4A). Correspondingly, in RTT, gamma amplitude peaked slightly earlier in the alpha cycle compared to TD (Fig. 4.4B). We did not observe a significant group difference in alpha-gamma phase bias in the posterior region or in theta-gamma phase bias in any region.

Phenotypic Assessments

Despite the robust group differences in PAC between RTT and TD participants, no associations between PAC metrics and clinical or phenotypic measures reached significance.

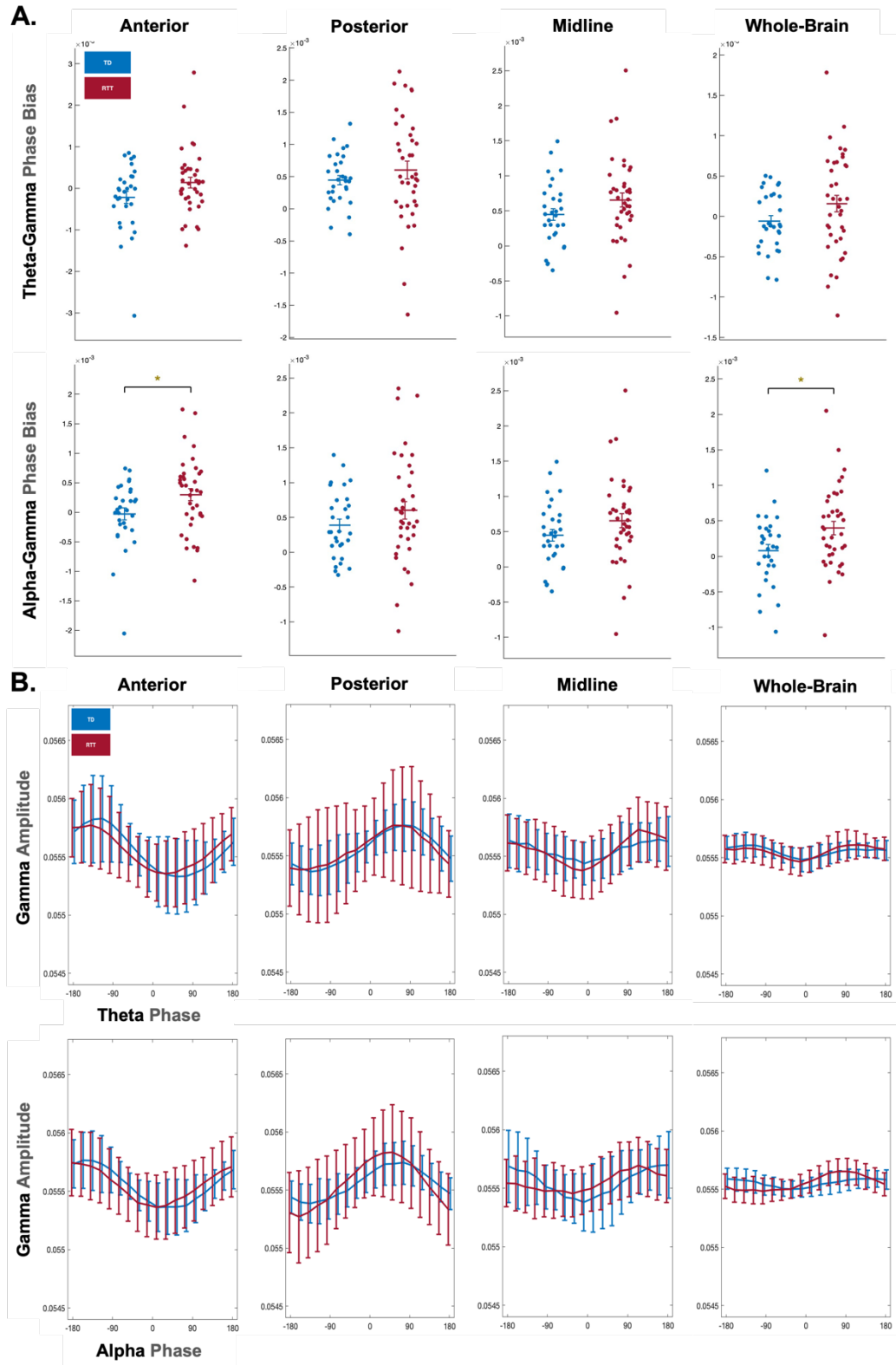


Figure 4.4. Alpha-gamma phase preference altered in Rett syndrome. TD participants ($n = 30$) are shaded in blue and RTT participants ($n = 38$) are shaded in red. **(A)** Markers in each subplot

signify a single individual's theta-gamma or alpha-gamma phase bias values in four given regions. Difference between groups was calculated using a Mann-Whitney U test, where *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$. Group mean (horizontal lines) and standard error (vertical lines) are plotted. **(B)** Gamma amplitude plotted as a function of alpha phase in four given regions. Traces represent group averages, where amplitude means are plotted. Error bars represent standard deviation values. 0° corresponds to the peak of the low frequency, $+90^\circ$ corresponds to the falling phase of the low frequency, 180° or -180° corresponds to the trough of the low frequency, and -90° corresponds to the rising phase of the low frequency.

Modeling of Layer 4 network implicates VIP+IN dysfunction in increased theta-gamma and alpha-gamma PAC

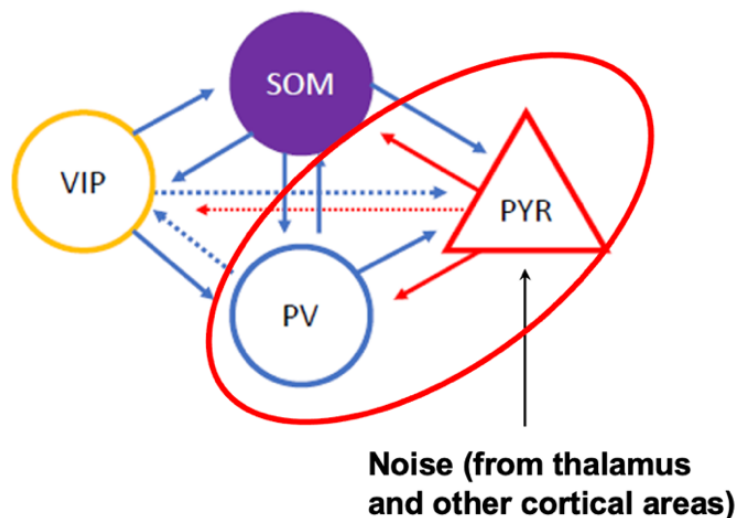


Figure 4.5. Schematic of L4 network. PYR represents excitatory stellate and/or pyramidal cells, PV represents fast spiking parvalbumin+ cells, SOM represents somatostatin+ cells projecting to L4, and VIP represents VIP+ cells. Each represents a population of cells of a given cell type. In isolation (without network connections), VIP+ cells produce delta/theta (2 – 4 Hz), SOM+ cells produce alpha, and the reciprocal interaction between PYR and PV+ cells produces gamma via PING (pyramidal cell – interneuron gamma). Noisy input is given to pyramidal cells to represent thalamic input and/or input from other cortical areas. Excitatory synaptic connections are represented by red arrows and inhibitory connections are represented by blue arrows.

To explore the underlying cellular and network mechanisms of changes to PAC in RTT, we constructed a biophysically constrained model of cortical Layer 4 containing excitatory neurons (PYR) as well as PV+, SOM+ and VIP+ interneurons (Fig. 4.5). Since VIP+INs have been implicated in mediating RTT symptoms,⁹⁴ we were particularly interested in whether deficiency in VIP+IN activity could account for the elevated PAC we observed in RTT. We therefore studied the model network activity under typical (baseline) and low VIP+IN activity levels.

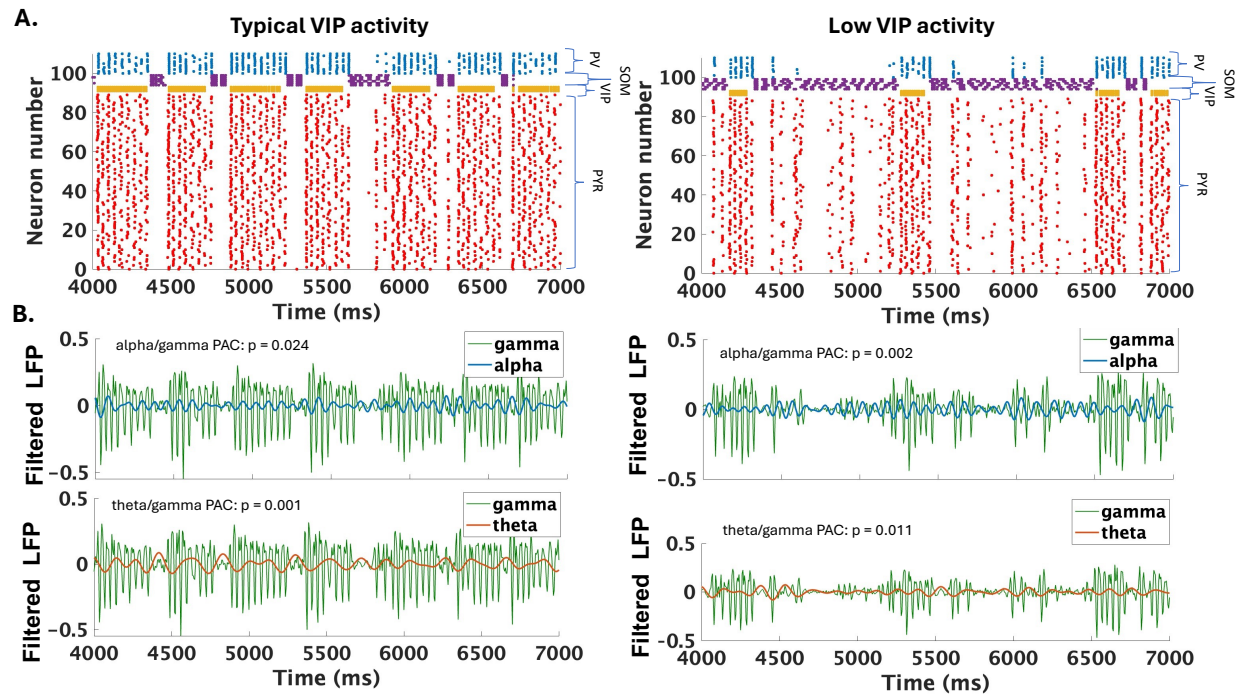


Figure 4.6. VIP+ cell hypoactivity alters local network dynamics but PAC remains significant in L4 model. (A) Raster plots of simulations of L4 network under conditions of typical VIP+IN activity and when VIP+IN activity is lowered. (B) Filtered LFP signals show high (gamma in green) and low (theta in blue and alpha in red) frequency bands and their coordination under typical and low VIP+IN activity conditions. Significant PAC was calculated using a permutation test.

At typical (baseline) levels of VIP+IN activity, networks showed significant participation of all neuronal subtypes (Fig. 4.6A, left). VIP+INs and SOM+INs were mutually inhibitory, and the network toggled between periods of SOM+IN-dominated activity and periods of VIP+IN-dominated activity. The brief periods of SOM+IN activity were accompanied by pauses in network activity, whereas the relatively longer VIP+IN activation periods allowed PYR and PV+INs spiking. Gamma oscillations emerged in the network spiking during periods of VIP+IN activity (Fig. 4.6A left, 6B left) when PYR and PV+INs interacted to form a type of gamma rhythm known as PING (pyramidal-interneuron network gamma)²⁹¹ as observed in the filtered model LFP (Fig. 4.6B, left). Although theta and alpha oscillations were also apparent, they were not clearly attributable to the spiking activity of any one neuronal subtype.

Using a permutation test similar to the one used to identify significant PAC in the clinical EEG data (Fig. 4.1), significant LFP PAC was found in the baseline condition between both theta and gamma ($P < 0.01$) as well as between alpha and gamma ($P < 0.05$) (Fig. 4.6B, left).

We next considered simulations in which VIP+IN excitability was decreased. The overall spiking rate of PYR was greatly reduced (Fig. 4.6A, right), consistent with findings in mouse models of RTT.⁷⁸ The reduction of activity was a consequence of overactivity of SOM+INs, which were released from baseline levels of VIP+IN inhibition, thereby strongly inhibiting all other network components. Although the population spiking of VIP+INs was decreased compared to baseline, gamma still emerged during PING oscillations, which occurred robustly during the brief VIP+IN active periods and sparsely during the periods of SOM+IN overactivity.

Remarkably, despite obvious changes in network dynamics (compared to baseline) when VIP+IN activity was low, the PAC between low frequency (theta and alpha) and high frequency (gamma) oscillations in the model LFP remained statistically significant ($P < 0.05$ in both cases).

These results are consistent with our finding of significant theta-gamma and alpha-gamma coupling in the EEG of *both* TD and RTT participants as shown in Fig. 4.1.

Importantly, if reduction in VIP+IN activity is related to the changes in PAC strength as found in our EEG data, simulations in which VIP+IN activity is lowered should have increased PAC coupling strength compared to the PAC strength under baseline conditions. Therefore, we next investigated if the strength of the gamma modulation by theta and alpha (coupling strength, or MI) changed when VIP+IN activity was lowered. When we compared baseline and low VIP+IN activity conditions, both theta-gamma and alpha-gamma MI were significantly increased in the low VIP+IN condition versus the baseline condition ($P < 0.001$ in both cases) (Fig. 4.7A), matching the observations in the EEG of RTT participants as shown Figures 4.2 and 4.3.

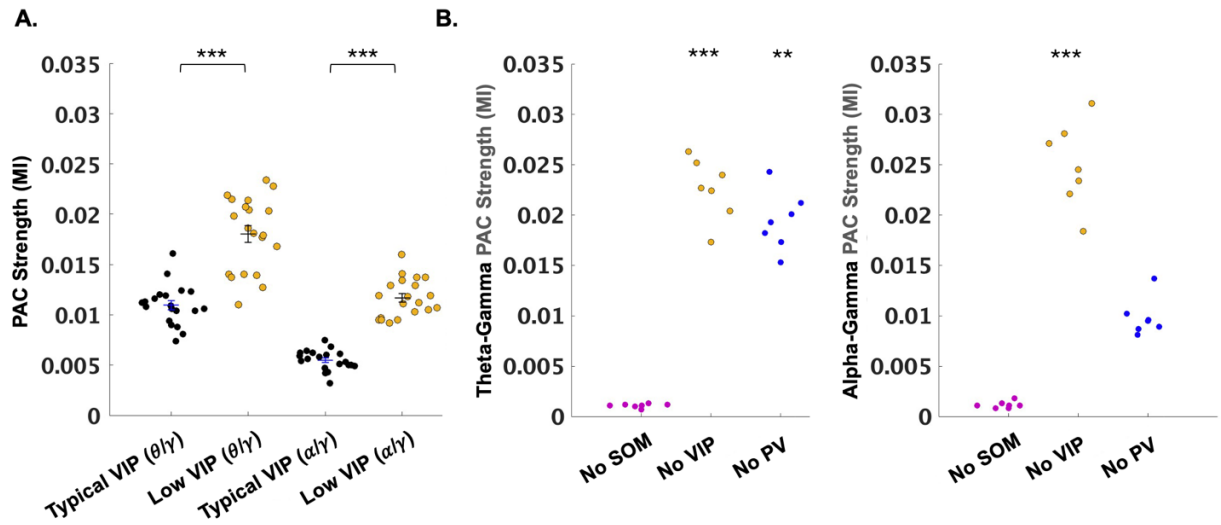


Figure 4.7. Increased modulation of gamma by slower theta and alpha frequencies with loss of VIP+ cell excitability. (A) Markers represent the modulation index of the theta-gamma or alpha-gamma PAC in the model LFP after 9s of simulation time. Blue markers represent typical VIP+IN activity conditions and red markers represent low VIP+IN activity conditions. $n = 20$ for each group. Difference between groups was calculated using an independent samples t-test, where *** indicates $P < 0.001$. Group mean (horizontal lines) and standard error (vertical lines) are plotted. (B) Modulation index of the theta-gamma and alpha-gamma PAC in the model LFP after 9s of simulation time after one interneuron type (SOM+, VIP+ or PV+) was removed from the simulation. An independent samples t-test was used to determine if there was an increase in the

mean MI for a particular condition (loss of one type of interneuron) compared to the mean MI in simulations with typical interneuron activity levels (i.e., the “typical VIP+IN activity” level condition), where ** indicates $P < 0.01$ and *** indicates $P < 0.001$.

Finally, we probed whether other interneuron subtypes could account for some of the changes to PAC coupling strength seen in the RTT group by repeating the simulation and alternately removing one interneuron subtype at a time. When compared to baseline conditions, removal of SOM+IN activity did not account for increased PAC strength in either theta-gamma or alpha-gamma PAC ($P > 0.05$ in both cases) (Fig. 4.7B). Removal of PV+IN activity could account for increases in theta-gamma PAC strength ($P < 0.01$) but not alpha-gamma PAC strength ($P > 0.05$). Crucially, only removal of VIP+INs could account for changes to both alpha-gamma and theta-gamma PAC strength. Notably, compared to lowered VIP+IN activity (Fig. 4.7A), PAC strength increased even more when VIP+IN activity was completely removed (Fig. 4.7B), indicating that PAC strength may be modulated by the level of VIP+IN deficiency.

Overall, our results suggest that RTT-induced VIP+IN deficiency could contribute to the elevated PAC strength observed in RTT.

Discussion

Rett syndrome is a disorder of widespread brain dysfunction, yet its impact on large-scale network activity remains incompletely understood. Oscillatory dynamics—specifically phase–amplitude coupling—offer a powerful, non-invasive window into these disruptions in RTT. With few disease-altering therapeutics currently available, such measures are especially valuable as potential biomarkers that can guide the development and evaluation of targeted interventions.

Here we investigated phase-amplitude coupling in the awake resting state in individuals with RTT compared to typically developing controls. We found that both groups exhibited significant PAC across all electrodes and frequency combinations, confirming widespread cross-frequency coordination at rest, independent of any presence of disorder. However, the RTT group showed more extensive and spatially distributed coupling, particularly in frontal, temporal, and occipital electrodes. PAC strength was significantly elevated in RTT, especially for slower frequencies coupled with low gamma. Analyses of canonical frequency bands further revealed increased theta–gamma PAC strength in posterior, midline, and whole-brain regions, and increased alpha–gamma PAC strength in anterior, posterior, and whole-brain regions. Additionally, alpha–gamma phase bias was shifted toward the falling phase of the low-frequency oscillation in anterior and whole-brain regions. Finally, simulations from a biophysically constrained model showed that reduced excitability in VIP+ interneurons (but not other interneuron subtypes) produced similar elevations in theta–gamma and alpha–gamma PAC strength, supporting a mechanistic link between VIP+IN dysfunction and the empirical data. Together, these findings suggest that PAC captures systems-level abnormalities in neural coordination that may be driven by a particular cell type in RTT.

Elevated theta–gamma coupling in Rett syndrome is notable given the established role of theta–gamma coupling in working memory, episodic encoding, and cognitive control.^{245–248} In both humans and animal models, theta phase organizes gamma bursts into discrete representational windows, supporting flexible information integration.^{245–248} In the context of RTT, increased theta–gamma coupling may reflect compensatory recruitment of neural resources or inefficient top-down modulation. Alternatively, it could indicate disrupted thalamocortical coordination, a feature implicated in both RTT and other neurodevelopmental conditions.^{241,257,269,292} Altered alpha–

gamma coupling and phase bias in RTT further implicate disruptions in sensory integration and attentional gating. Alpha rhythms modulate cortical excitability and control sensory gain, while gamma activity reflects local circuit computation^{242,293–295}; increased alpha–gamma coupling may therefore reflect a compensatory mechanism aimed at stabilizing sensory processing through enhanced top-down control. The shift in phase bias toward the falling phase of the alpha cycle suggests a disruption in the temporal precision of this gating process. Evaluating PAC metrics in event or task-related paradigms will further clarify the functional role of coupling during active cognitive or sensory engagement.

Disruptions in theta–gamma coupling—characterized by reduced specificity or abnormal timing—have been reported in Fragile X syndrome and ASD,^{253,255} while altered alpha–gamma phase bias has been observed in both Phelan-McDermid syndrome²⁷⁸ and ASD. These findings suggest that aberrant phase-amplitude coupling may reflect shared network-level vulnerabilities across neurodevelopmental disorders, despite divergent genetic etiologies. PAC metrics may therefore help stratify mechanistically relevant subgroups within heterogeneous conditions like ASD and serve as a systems-level framework for comparing circuit dysfunction across syndromic forms.^{253,255}

Despite the robust group differences in PAC between RTT and TD participants, we did not find significant associations between PAC metrics and clinical or phenotypic measures. This may be due to the complexity of the RTT phenotype, which encompasses a broad range of cognitive, motor, sensory, and autonomic dysfunctions. The clinical scales utilized in this study, while valuable for assessing overall disease severity, may not be sufficiently sensitive to detect more subtle cognitive deficits, memory retention, or sensory processing abnormalities that are reflected

in PAC dynamics. Developing more specific cognitive and behavioral assessments could help clarify the functional relevance of these neurophysiological findings.

Our computational model of L4 demonstrates that lowered VIP+IN activity may lead to a general reduction of local neural activity along with an increase in the amount of coupling between lower (theta and alpha) and higher (gamma) frequencies. Dysfunction in VIP+INs alone could recapitulate the elevated theta–gamma and alpha–gamma coupling observed in the EEG data, suggesting that lowered VIP+IN activity could contribute to PAC abnormalities in RTT. Our computational results also suggest that VIP+IN deficiency alters network function by increasing SOM+IN modulated inhibitory tone, which restricts signal transmission that would typically occur with higher VIP+IN activity levels. To establish causality between deficiency of VIP and increased theta/gamma and alpha/gamma coupling in RTT, future studies could directly manipulate VIP+IN function in RTT preclinical models. Selective deletion of *Mecp2* from VIP+INs followed by *in vivo* EEG recording would test whether VIP+IN activity is necessary and sufficient to produce elevated PAC. If causality is established between deficiency of VIP+INs and increased theta/gamma and alpha/gamma coupling, future studies can combine experimental event or task-related paradigms with further modeling to identify functional consequences of the altered PAC. Complementary experiments restoring VIP+IN function—either genetically or through targeted neuromodulation—could provide critical insight into reversibility. Notably, non-invasive stimulation approaches that entrain gamma rhythms have been shown to improve pathology via VIP peptide (a peptide co-released with GABA from VIP+INs) in a mouse model of Alzheimer's disease, and may offer a translatable strategy to modulate VIP+IN activity in RTT.²⁹⁶

Interestingly, we observed inter-individual variability in PAC strength in the RTT group, with a subset exhibiting MI values similar to TD and a subset exhibiting higher MI. Our model

suggests that the elevation in PAC strength may scale with the level of VIP+IN deficiency, and it is possible that some of the heterogeneity observed in the PAC strength of individuals with RTT could be attributable to differing levels VIP+IN dysfunction across individuals or as disease progresses (with greater deficiency of VIP+INs in more severe cases of RTT compared to less severe ones). Most participants in the present study were in the post-regression phase, which may reflect distinct stages of circuit pathology. Longitudinal studies or studies spanning pre- and post-regression ages could help determine whether PAC changes arise early in disease progression or emerge as a consequence of ongoing network changes. Additionally, the type or location of *MECP2* mutations, as well as differential patterns of X chromosome inactivation skewing (on a cell-by-cell and regional basis), influence disease severity^{32,33,35–37} and could thus impact the nature of PAC alterations and impairment to VIP+INs. Incorporating different disorder stages along with genetic subtype analyses in both preclinical and clinical studies will help clarify inter-individual variability in electrophysiological phenotypes and inform personalized treatment strategies.

Overall, our study identifies altered phase–amplitude coupling as a robust signature of cortical dysfunction in Rett syndrome. By bridging molecular and cellular mechanisms with non-invasive neurophysiological readouts, PAC offers a scalable and clinically accessible tool for tracking circuit-level pathology. Given the widespread use of EEG, PAC could serve not only as a functional biomarker in RTT but also as a generalizable framework for monitoring dysfunction across neurodevelopmental and neuropsychiatric disorders. As our understanding of cell-type-specific contributions to oscillatory dynamics grows, PAC may ultimately help link genetic and cellular pathology to systems-level outcomes and therapeutic response in a broad range of brain disorders.

Data Availability

Data is available upon reasonable request from both NHS and RSBS studies. Code used for modeling will be made available on GitHub.

Author Contributions

DK, AL, and MF conceptualized the study. DK wrote and prepared the initial manuscript draft; YB contributed to the EEG/PAC Methods section. DK, CM, and KS collected clinical data for the RSBS study. DK performed the analysis. YB developed custom analysis scripts. MM provided the main computational contribution. AL, MF, and CN supervised the project. All authors contributed to interpretation of the results and critical revision of the manuscript.

Acknowledgements

Thank you very much to all the participants in these studies and their families, especially those who travelled and extended themselves to contribute to research in Rett syndrome. Thank you to the Rett Syndrome Angels (originally Rett Syndrome Association of Massachusetts) for their incredible support. Thank you to the Rett syndrome clinics and to all members of the labs of Dr.'s April Levin, Michela Fagiolini, Charles Nelson, and Takao Hensch for your invaluable feedback and contributions. This work could not have been completed without all of you.

Funding

This work was supported partially by:

P50HD105351 - Project 1 - Michela Fagiolini

3U54HD061222 - For Rett syndrome, MECP2 Duplications, and Rett-related Disorders Natural History - Alan Percy, Eric Marsh, Tim A. Benke, Jeffrey Neul

P50HD103537 – Jeffrey Neul

Competing interests

Dr. Percy has received research support from the NIH and has been a site PI for Acadia Pharmaceuticals. He is a consultant for Acadia Pharmaceuticals, Taysha Gene Therapies, and Neurogene. He has prepared educational materials for WebMD, Medscape, Pharmacy Times Continuing Education, Prime Inc., and the CME Institute.

Chapter 5. General Discussion

Understanding how disruptions to neuronal function gives rise to atypical sensory processing—and how these disturbances cascade into broader impairments in cognition, social behavior, and daily functioning—remains an area of active investigation in the study of neurodevelopmental disorders, including both idiopathic and genetically defined forms of ASD. Here, we focus mainly on Rett syndrome, a monogenic disorder with well-characterized genetic origins, which provides a unique opportunity to examine how molecular and cellular pathology manifests in large-scale neural dynamics and behavioral outcomes. Across three studies, we leverage EEG-based methods to interrogate the neural mechanisms by which the brain processes, gates, and coordinates information. Together, these studies characterize disruptions to both stimulus-evoked and spontaneous brain activity and propose candidate cellular and circuit-level mechanisms underlying these patterns.

Summary of findings

Atypical responsivity to sensory stimuli is a hallmark of many neurodevelopmental conditions including ASDs, with individuals often exhibiting both hypo- and hyper-reactivity to sensory stimuli. In the first study, we quantified this Sensory Paradox in RTT and investigated whether variability in neural responses to sensory stimuli might contribute to these phenotypes. We measured inter-trial phase coherence, a time-frequency analysis of consistency in the phase response across trials, and found that individuals with RTT showed significantly reduced ITPC in both auditory- and visual-evoked potentials compared to typically developing individuals, indicating greater trial-by-trial variability in neural responses. This variability was strongly associated with attenuated VEP and AEP amplitudes. Notably, reduced ITPC in VEPs also correlated with elevated sensory responsivity and greater behavioral symptom burden and was

impacted by mutation severity. These findings suggest that instability in the temporal precision of neural responses to sensory stimuli may contribute to dampened ERPs previously observed in RTT and atypical sensory responsivity.

The second study focused on resting-state beta-band dynamics in EEG as a window into thalamocortical function, a key pathway for sensory processing. We employed transient beta events—brief, high-power bursts in the beta frequency range that are thought to reflect thalamocortical drive and are implicated in tactile processing²⁹⁷—as a mechanistically informed marker of sensory gating. This study included individuals with idiopathic ASD as well as several neurogenetic conditions with increased rates of ASD, allowing us to assess whether differences in TBEs may be shared across related disorders despite distinct genetic etiologies. We found that compared to typically developing individuals, the rate of TBEs was significantly elevated in individuals with RTT, as well as in Phelan-McDermid syndrome, SYNGAP1-related disorder, and a subset of children with idiopathic ASD (but not in those with Tuberous Sclerosis Complex or PTEN hamartoma tumor syndrome). These results highlight both convergent and condition-specific profiles. Given the role of TBEs in sensory gain and perception, these findings suggest that increased TBE rate may reflect disruptions in thalamocortical signaling and contribute to altered sensory responsivity that are common to many neurodevelopmental disorders including RTT.

The final study once again focuses on resting EEG and explores large-scale oscillatory coordination through the lens of phase-amplitude coupling. PAC measures how the phase of slow rhythms modulates the amplitude of fast activity, enabling integration of information across spatial and temporal scales, and is implicated in key cognitive and sensory functions.^{238,240} Individuals with RTT showed widespread and significantly elevated PAC, especially in theta-gamma and

alpha-gamma frequency ranges. A shift in alpha-gamma phase bias was also observed. Biophysically constrained computational modeling of a Layer 4 cortical network further revealed that reduced excitability of VIP+ interneurons alone could reproduce the PAC elevations observed in RTT, identifying a candidate mechanism linking cellular dysfunction to altered oscillatory dynamics.

Disrupted neural timing in Rett syndrome

Changes in oscillatory dynamics offer a window into how the temporal coordination of neural activity is altered in Rett syndrome (RTT). Across all three studies, this dissertation highlights disruptions to the temporal structure of neural activity in RTT — not just in terms of magnitude or spectral content, but in how the brain organizes when neurons are most excitable, responsive, or coordinated. This framing has been previously described as the concept of the *dynome*—the brain’s dynamic counterpart to the connectome—which captures how neural activity is organized across time to enable perception, cognition, and behavior.¹⁹⁴ While the connectome describes what is connected, the *dynome* describes when and how those connections are active across behavioral states and timescales. The alterations observed in ITPC, TBEs, and PAC can reflect disruptions in this temporal architecture—in how the brain transitions between more and less excitable states, how it gates incoming input, and how it synchronizes rhythmic activity to support computation.

Rapid, moment-to-moment fluctuations in arousal and brain state during wakefulness strongly modulate cortical responsiveness and behavioral performance; cortical networks rapidly shift between high- and low-excitability states even during “rest,” and these fluctuations influence the gain of sensory responses and the timing of spontaneous and evoked activity, shaping how

stimuli are encoded (and whether the brain is more or less likely to respond).²⁹⁸ Traditional EEG measures can miss this layer of dynamic variability, but measures like ITPC and TBEs can be especially sensitive to capturing moment-to-moment variability in circuit readiness. Reduced ITPC may reflect dysregulation in these state transitions, resulting in inconsistency in how the brain responds to repeated stimuli, and an increased rate of TBEs may indicate aberrant or excessive transitions into high-excitability states, disrupting the balance of sensory gating and contributing to atypical sensory responsivity.

Importantly, network oscillations that can be measured using EEG emerge from reciprocal interactions between excitatory and inhibitory pools of neurons that lead to rhythmic population activity;¹¹⁵ gradients of excitatory/inhibitory balance across cortex govern both the stability of cortical states and the generation of network rhythms.^{77,115} Interestingly, although individual neurons can fire stochastically, population-level coherence still emerges through dynamically regulated rhythms, creating temporal windows for information processing. This population-level coordination is fundamental to how the brain segments and integrates information across space and time. In Chapter 4, our modeling work identifies VIP+ interneuron dysfunction—a key regulator of local circuit gain—as sufficient to produce the elevated PAC observed in RTT, reinforcing the idea that cell-type-specific dysfunction can disrupt temporal dynamics in network oscillations observable with EEG.

Together, the findings presented here point to disruption in the temporal architecture of neural computation in RTT.

Translational implications and conclusions

The ability to use EEG to measure circuit-relevant signals from human participants opens powerful avenues for “back-translation” into preclinical models, enabling a mechanistic interrogation of the findings and testing of targeted interventions; each of the EEG-based biomarkers in this dissertation offers a distinct entry point into circuit-level dysfunction that can be further clarified using manipulations in model organisms. For example, future studies can test the causal role of VIP+ interneurons in PAC abnormalities by selectively deleting or restoring *Mecp2* and measuring changes to PAC, while manipulations of interneuron populations in *Mecp2* mutant mice could clarify how disruptions to E/I balance affect ITPC.

Furthermore, even in distinct monogenic disorders, circuit dysfunction may arise through partially overlapping physiological processes. Rett syndrome shares many clinical, behavioral, and neurophysiological features with a broader class of neurodevelopmental disorders that have high rates of ASD, including atypical sensory processing, disrupted E/I balance, and altered network dynamics. Chapter 3 highlights this convergence: elevated TBE rates were not exclusive to RTT, but were also observed in Phelan-McDermid syndrome, SYNGAP1-related disorder, and a subset of idiopathic ASD. Furthermore, similar patterns of aberrant PAC and reduced ITPC have also been reported in Fragile X syndrome,¹³⁵ Phelan-McDermid syndrome,²⁷⁸ and idiopathic ASD,^{173,250} supporting the idea that these biomarkers may represent vulnerabilities across diagnostic groups. In this context, mechanistically grounded EEG-based biomarkers could help stratify biologically meaningful subgroups within heterogeneous clinical populations and serve as a unifying framework for comparing circuit-level dysfunction across neurodevelopmental disorders.

Collectively, these studies may ultimately help bridge the gap between molecular pathology and clinical phenotype in RTT and other neurodevelopmental disorders and offer EEG-

based biomarkers with translational potential for tracking circuit dysfunction and guiding therapeutic development, advancing a bidirectional path between preclinical models and clinical research.

References

1. Tomchek SD, Dunn W. Sensory Processing in Children With and Without Autism: A Comparative Study Using the Short Sensory Profile. *The American Journal of Occupational Therapy*. 2007;61(2):190-200. doi:10.5014/ajot.61.2.190
2. Ben-Sasson A, Gal E, Fluss R, Katz-Zetler N, Cermak SA. Update of a Meta-analysis of Sensory Symptoms in ASD: A New Decade of Research. *J Autism Dev Disord*. 2019;49(12):4974-4996. doi:10.1007/s10803-019-04180-0
3. Hazen EP, Stornelli JL, O'Rourke JA, Koesterer K, McDougale CJ. Sensory Symptoms in Autism Spectrum Disorders. *Harvard Review of Psychiatry*. 2014;22(2):112-124. doi:10.1097/01.hrp.0000445143.08773.58
4. Robertson CE, Baron-Cohen S. Sensory perception in autism. *Nat Rev Neurosci*. 2017;18(11):671-684. doi:10.1038/nrn.2017.112
5. Marco EJ, Hinkley LBN, Hill SS, Nagarajan SS. Sensory Processing in Autism: A Review of Neurophysiologic Findings. *Pediatric Research*. 2011;69(5 Part 2):48R-54R. doi:10.1203/pdr.0b013e3182130c54
6. Espenhahn S, Godfrey KJ, Kaur S, et al. Tactile cortical responses and association with tactile reactivity in young children on the autism spectrum. *Molecular Autism*. 2021;12(1):26. doi:10.1186/s13229-021-00435-9
7. Mayer JL, Hannent I, Heaton PF. Mapping the Developmental Trajectory and Correlates of Enhanced Pitch Perception on Speech Processing in Adults with ASD. *J Autism Dev Disord*. 2016;46(5):1562-1573. doi:10.1007/s10803-014-2207-6
8. Oakley BF, Tillmann J, Ahmad J, et al. How do core autism traits and associated symptoms relate to quality of life? Findings from the Longitudinal European Autism Project. *Autism*. 2021;25(2):389-404. doi:10.1177/1362361320959959
9. Lin LY, Huang PC. Quality of life and its related factors for adults with autism spectrum disorder. *Disability and Rehabilitation*. 2019;41(8):896-903. doi:10.1080/09638288.2017.1414887
10. Thyne MD, Bednarz HM, Herringshaw AJ, Sartin EB, Kana RK. The impact of atypical sensory processing on social impairments in autism spectrum disorder. *Developmental Cognitive Neuroscience*. 2018;29:151-167. doi:10.1016/j.dcn.2017.04.010
11. Horder J, Wilson CE, Mendez MA, Murphy DG. Autistic Traits and Abnormal Sensory Experiences in Adults. *J Autism Dev Disord*. 2014;44(6):1461-1469. doi:10.1007/s10803-013-2012-7
12. An JY, Claudianos C. Genetic heterogeneity in autism: From single gene to a pathway perspective. *Neuroscience & Biobehavioral Reviews*. 2016;68:442-453. doi:10.1016/j.neubiorev.2016.06.013

13. Chahrour M, Zoghbi HY. The Story of Rett Syndrome: From Clinic to Neurobiology. *Neuron*. 2007;56(3):422-437. doi:10.1016/j.neuron.2007.10.001
14. Hagberg BA, Skjeldal OH. Rett variants: A suggested model for inclusion criteria. *Pediatric Neurology*. 1994;11(1):5-11. doi:10.1016/0887-8994(94)90082-5
15. Ivy AS, Standridge S. Rett Syndrome: A Timely Review From Recognition to Current Clinical Approaches and Clinical Study Updates. *Seminars in Pediatric Neurology*. 2021;37:100881. doi:10.1016/j.spen.2021.100881
16. Jeffrey L. Neul, Kaufmann WE, Glaze DG, et al. Rett syndrome: Revised diagnostic criteria and nomenclature. *Ann Neurol*. 2010;68(6):944-950. doi:10.1002/ana.22124
17. Brima T, Molholm S, Molloy CJ, et al. Auditory sensory memory span for duration is severely curtailed in females with Rett syndrome. *Transl Psychiatry*. 2019;9(1):130. doi:10.1038/s41398-019-0463-0
18. Neklyudova A, Smirnov K, Rebreikina A, Martynova O, Sysoeva O. Electrophysiological and Behavioral Evidence for Hyper- and Hyposensitivity in Rare Genetic Syndromes Associated with Autism. *Genes*. 2022;13(4):671. doi:10.3390/genes13040671
19. Devarakonda KM, Lowthian D, Raghavendra T. A case of Rett syndrome with reduced pain sensitivity. *Pediatric Anesthesia*. 2009;19(6):625-627. doi:10.1111/j.1460-9592.2009.03018.x
20. Downs J, Géranton SM, Bebbington A, et al. Linking *MECP2* and pain sensitivity: The example of Rett syndrome. *American J of Med Genetics Pt A*. 2010;152A(5):1197-1205. doi:10.1002/ajmg.a.33314
21. Suter B, Pehlivan D, Ak M, Harris HK, Lyons-Warren AM. Sensory experiences questionnaire unravels differences in sensory profiles between *MECP2* -related disorders. *Autism Research*. Published online March 3, 2024;aur.3112. doi:10.1002/aur.3112
22. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2. *Nat Genet*. 1999;23(2):185-188. doi:10.1038/13810
23. Amir RE, Van den Veyver IB, Schultz R, et al. Influence of mutation type and X chromosome inactivation on Rett syndrome phenotypes. *Ann Neurol*. 2000;47(5):670-679.
24. Samaco RC. Multiple pathways regulate *MeCP2* expression in normal brain development and exhibit defects in autism-spectrum disorders. *Human Molecular Genetics*. 2004;13(6):629-639. doi:10.1093/hmg/ddh063
25. Samaco RC, Hogart A, LaSalle JM. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: *MECP2* deficiency causes reduced expression of *UBE3A* and *GABRB3*. *Human Molecular Genetics*. 2005;14(4):483-492. doi:10.1093/hmg/ddi045

26. Shibayama A, Cook EH, Feng J, et al. MECP2 structural and 3'-UTR variants in schizophrenia, autism and other psychiatric diseases: A possible association with autism. *American J of Med Genetics Pt B*. 2004;128B(1):50-53. doi:10.1002/ajmg.b.30016
27. Kaufmann WE, Capone GT, Clarke M, Budimirovic DB. Autism in Genetic Intellectual Disability. In: *Autism*. Humana Press; 2008:81-108. doi:10.1007/978-1-60327-489-0_4
28. Percy AK. Rett syndrome: exploring the autism link. *Arch Neurol*. 2011;68(8):985-989. doi:10.1001/archneurol.2011.149
29. Nagarajan R, Hogart A, Gwyne Y, Martin MR, LaSalle JM. Reduced MeCP2 Expression is Frequent in Autism Frontal Cortex and Correlates with Aberrant MECP2 Promoter Methylation. *Epigenetics*. 2006;1(4):172-182. doi:10.4161/epi.1.4.3514
30. Neul JL. The relationship of Rett syndrome and *MECP2* disorders to autism. *Dialogues in Clinical Neuroscience*. 2012;14(3):253-262. doi:10.31887/DCNS.2012.14.3/jneul
31. Bienvenu T. MECP2 mutations account for most cases of typical forms of Rett syndrome. *Human Molecular Genetics*. 2000;9(9):1377-1384. doi:10.1093/hmg/9.9.1377
32. Bebbington A, Anderson A, Ravine D, et al. Investigating genotype-phenotype relationships in Rett syndrome using an international data set. *Neurology*. 2008;70(11):868-875. doi:10.1212/01.wnl.0000304752.50773.ec
33. Cuddapah VA, Pillai RB, Shekar KV, et al. *Methyl-CpG-binding protein 2 (MECP2)* mutation type is associated with disease severity in Rett syndrome. *J Med Genet*. 2014;51(3):152-158. doi:10.1136/jmedgenet-2013-102113
34. Cheadle JP. Long-read sequence analysis of the MECP2 gene in Rett syndrome patients: correlation of disease severity with mutation type and location. *Human Molecular Genetics*. 2000;9(7):1119-1129. doi:10.1093/hmg/9.7.1119
35. Frullanti E, Papa FT, Grillo E, et al. Analysis of the Phenotypes in the Rett Networked Database. *International Journal of Genomics*. 2019;2019:1-9. doi:10.1155/2019/6956934
36. Neul JL, Fang P, Barrish J, et al. Specific mutations in Methyl-CpG-Binding Protein 2 confer different severity in Rett syndrome. *Neurology*. 2008;70(16):1313-1321. doi:10.1212/01.wnl.0000291011.54508.aa
37. Merritt JK, Fang X, Caylor RC, et al. Normalized Clinical Severity Scores Reveal a Correlation between X Chromosome Inactivation and Disease Severity in Rett Syndrome. *Genes*. 2024;15(5):594. doi:10.3390/genes15050594
38. Gulmez Karaca K, Brito DVC, Oliveira AMM. MeCP2: A Critical Regulator of Chromatin in Neurodevelopment and Adult Brain Function. *IJMS*. 2019;20(18):4577. doi:10.3390/ijms20184577

39. Young JI, Hong EP, Castle JC, et al. Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *Proc Natl Acad Sci USA*. 2005;102(49):17551-17558. doi:10.1073/pnas.0507856102
40. Tillotson R, Bird A. The Molecular Basis of MeCP2 Function in the Brain. *Journal of Molecular Biology*. 2020;432(6):1602-1623. doi:10.1016/j.jmb.2019.10.004
41. Liu Y, Whitfield TW, Bell GW, et al. Exploring the complexity of MECP2 function in Rett syndrome. *Nat Rev Neurosci*. 2025;26(7):379-398. doi:10.1038/s41583-025-00926-1
42. Kinde B, Wu DY, Greenberg ME, Gabel HW. DNA methylation in the gene body influences MeCP2-mediated gene repression. *Proc Natl Acad Sci USA*. 2016;113(52):15114-15119. doi:10.1073/pnas.1618737114
43. Sharifi O, Yasui DH. The Molecular Functions of MeCP2 in Rett Syndrome Pathology. *Front Genet*. 2021;12. doi:10.3389/fgene.2021.624290
44. Leonard H, Cobb S, Downs J. Clinical and biological progress over 50 years in Rett syndrome. *Nat Rev Neurol*. 2017;13(1):37-51. doi:10.1038/nrneurol.2016.186
45. Urbanowicz A, Downs J, Girdler S, Ciccone N, Leonard H. Aspects of speech-language abilities are influenced by *MECP2* mutation type in girls with Rett syndrome. *American J of Med Genetics Pt A*. 2015;167(2):354-362. doi:10.1002/ajmg.a.36871
46. Zhou Z, Goffin D. Modeling Rett Syndrome with MeCP2 T158A Knockin Mice. In: Patel VB, Preedy VR, Martin CR, eds. *Comprehensive Guide to Autism*. Springer New York; 2014:2723-2739. doi:10.1007/978-1-4614-4788-7_181
47. Goffin D, Allen M, Zhang L, et al. Rett syndrome mutation MeCP2 T158A disrupts DNA binding, protein stability and ERP responses. *Nat Neurosci*. 2012;15(2):274-283. doi:10.1038/nn.2997
48. Lamonica JM, Kwon DY, Goffin D, et al. Elevating expression of MeCP2 T158M rescues DNA binding and Rett syndrome-like phenotypes. *Journal of Clinical Investigation*. 2017;127(5):1889-1904. doi:10.1172/JCI90967
49. Johnson BS, Zhao YT, Fasolino M, et al. Biotin tagging of MeCP2 in mice reveals contextual insights into the Rett syndrome transcriptome. *Nat Med*. 2017;23(10):1203-1214. doi:10.1038/nm.4406
50. Chen RZ, Akbarian S, Tudor M, Jaenisch R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat Genet*. 2001;27(3):327-331. doi:10.1038/85906
51. Fukuda T, Itoh M, Ichikawa T, Washiyama K, Goto Y ichi. Delayed Maturation of Neuronal Architecture and Synaptogenesis in Cerebral Cortex of *Mecp2* -Deficient Mice. *J Neuropathol Exp Neurol*. 2005;64(6):537-544. doi:10.1093/jnen/64.6.537

52. Shahbazian MD. Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Human Molecular Genetics*. 2002;11(2):115-124. doi:10.1093/hmg/11.2.115
53. Jung BP, Jugloff DGM, Zhang G, Logan R, Brown S, Eubanks JH. The expression of methyl CpG binding factor MeCP2 correlates with cellular differentiation in the developing rat brain and in cultured cells. *J Neurobiol*. 2003;55(1):86-96. doi:10.1002/neu.10201
54. Cheval H, Guy J, Merusi C, De Sousa D, Selfridge J, Bird A. Postnatal inactivation reveals enhanced requirement for MeCP2 at distinct age windows. *Human Molecular Genetics*. 2012;21(17):3806-3814. doi:10.1093/hmg/dds208
55. Feldman D, Banerjee A, Sur M. Developmental Dynamics of Rett Syndrome. *Neural Plasticity*. 2016;2016:1-9. doi:10.1155/2016/6154080
56. Lyst MJ, Bird A. Rett syndrome: a complex disorder with simple roots. *Nat Rev Genet*. 2015;16(5):261-275. doi:10.1038/nrg3897
57. McGraw CM, Samaco RC, Zoghbi HY. Adult Neural Function Requires MeCP2. *Science*. 2011;333(6039):186-186. doi:10.1126/science.1206593
58. Mellios N, Feldman DA, Sheridan SD, et al. MeCP2-regulated miRNAs control early human neurogenesis through differential effects on ERK and AKT signaling. *Mol Psychiatry*. 2018;23(4):1051-1065. doi:10.1038/mp.2017.86
59. Bedogni F, Cobolli Gigli C, Pozzi D, et al. Defects During *Mecp2* Null Embryonic Cortex Development Precede the Onset of Overt Neurological Symptoms. *Cereb Cortex*. 2016;26(6):2517-2529. doi:10.1093/cercor/bhv078
60. Gao Y, Su J, Guo W, et al. Inhibition of miR-15a Promotes BDNF Expression and Rescues Dendritic Maturation Deficits in MeCP2-Deficient Neurons. *Stem Cells*. 2015;33(5):1618-1629. doi:10.1002/stem.1950
61. Guy J, Cheval H, Selfridge J, Bird A. The Role of MeCP2 in the Brain. *Annu Rev Cell Dev Biol*. 2011;27(1):631-652. doi:10.1146/annurev-cellbio-092910-154121
62. Zhou Z, Hong EJ, Cohen S, et al. Brain-Specific Phosphorylation of MeCP2 Regulates Activity-Dependent Bdnf Transcription, Dendritic Growth, and Spine Maturation. *Neuron*. 2006;52(2):255-269. doi:10.1016/j.neuron.2006.09.037
63. Chapleau CA, Calfa GD, Lane MC, et al. Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. *Neurobiology of Disease*. 2009;35(2):219-233. doi:10.1016/j.nbd.2009.05.001
64. Na ES, Monteggia LM. The role of MeCP2 in CNS development and function. *Hormones and Behavior*. 2011;59(3):364-368. doi:10.1016/j.yhbeh.2010.05.014

65. Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat Genet.* 2001;27(3):322-326. doi:10.1038/85899
66. Vashi N, Justice MJ. Treating Rett syndrome: from mouse models to human therapies. *Mamm Genome.* 2019;30(5-6):90-110. doi:10.1007/s00335-019-09793-5
67. Goffin D, Brodtkin ES, Blendy JA, Siegel SJ, Zhou Z. Cellular origins of auditory event-related potential deficits in Rett syndrome. *Nat Neurosci.* 2014;17(6):804-806. doi:10.1038/nn.3710
68. Meng X, Wang W, Lu H, et al. Manipulations of MeCP2 in glutamatergic neurons highlight their contributions to Rett and other neurological disorders. *eLife.* 2016;5:e14199. doi:10.7554/eLife.14199
69. Zhang Y, Cao SX, Sun P, et al. Loss of MeCP2 in cholinergic neurons causes part of RTT-like phenotypes via $\alpha 7$ receptor in hippocampus. *Cell Res.* 2016;26(6):728-742. doi:10.1038/cr.2016.48
70. Shahbazian MD, Young JI, Yuva-Paylor LA, et al. Mice with Truncated MeCP2 Recapitulate Many Rett Syndrome Features and Display Hyperacetylation of Histone H3. *Neuron.* 2002;35(2):243-254. doi:10.1016/S0896-6273(02)00768-7
71. Schaevitz LR, Gómez NB, Zhen DP, Berger-Sweeney JE. MeCP2 R168X male and female mutant mice exhibit Rett-like behavioral deficits. *Genes Brain and Behavior.* 2013;12(7):732-740. doi:10.1111/gbb.12070
72. Pitcher MR, Herrera JA, Buffington SA, et al. Rett syndrome like phenotypes in the R255X *Mecp2* mutant mouse are rescued by MECP2 transgene. *Human Molecular Genetics.* 2015;24(9):2662-2672. doi:10.1093/hmg/ddv030
73. Rangasamy S, Olfers S, Gerald B, Hilbert A, Svejda S, Narayanan V. Reduced neuronal size and mTOR pathway activity in the *Mecp2* A140V Rett syndrome mouse model. *F1000Res.* 2016;5:2269. doi:10.12688/f1000research.8156.1
74. Stearns NA, Schaevitz LR, Bowling H, Nag N, Berger UV, Berger-Sweeney J. Behavioral and anatomical abnormalities in *Mecp2* mutant mice: A model for Rett syndrome. *Neuroscience.* 2007;146(3):907-921. doi:10.1016/j.neuroscience.2007.02.009
75. Samaco RC, McGraw CM, Ward CS, Sun Y, Neul JL, Zoghbi HY. Female *Mecp2*^{+/-} mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies. *Human Molecular Genetics.* 2013;22(1):96-109. doi:10.1093/hmg/ddt406
76. Van Vreeswijk C, Sompolinsky H. Chaos in Neuronal Networks with Balanced Excitatory and Inhibitory Activity. *Science.* 1996;274(5293):1724-1726. doi:10.1126/science.274.5293.1724

77. Wang XJ. Macroscopic gradients of synaptic excitation and inhibition in the neocortex. *Nat Rev Neurosci.* 2020;21(3):169-178. doi:10.1038/s41583-020-0262-x
78. Durand S, Patrizi A, Quast KB, et al. NMDA Receptor Regulation Prevents Regression of Visual Cortical Function in the Absence of *Mecp2*. *Neuron.* 2012;76(6):1078-1090. doi:10.1016/j.neuron.2012.12.004
79. Gogolla N, Takesian AE, Feng G, Fagiolini M, Hensch TK. Sensory Integration in Mouse Insular Cortex Reflects GABA Circuit Maturation. *Neuron.* 2014;83(4):894-905. doi:10.1016/j.neuron.2014.06.033
80. Sceniak MP, Lang M, Enomoto AC, James Howell C, Hermes DJ, Katz DM. Mechanisms of Functional Hypoconnectivity in the Medial Prefrontal Cortex of *Mecp2* Null Mice. *Cereb Cortex.* 2016;26(5):1938-1956. doi:10.1093/cercor/bhv002
81. Krishnan K, Lau BYB, Ewall G, Huang ZJ, Shea SD. MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. *Nat Commun.* 2017;8(1):14077. doi:10.1038/ncomms14077
82. Nelson ED, Kavalali ET, Monteggia LM. MeCP2-Dependent Transcriptional Repression Regulates Excitatory Neurotransmission. *Current Biology.* 2006;16(7):710-716. doi:10.1016/j.cub.2006.02.062
83. Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett Syndrome. *Proc Natl Acad Sci USA.* 2005;102(35):12560-12565. doi:10.1073/pnas.0506071102
84. Wood L, Shepherd GMG. Synaptic circuit abnormalities of motor-frontal layer 2/3 pyramidal neurons in a mutant mouse model of Rett syndrome. *Neurobiology of Disease.* 2010;38(2):281-287. doi:10.1016/j.nbd.2010.01.018
85. Krishnan K, Wang BS, Lu J, et al. MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. *Proc Natl Acad Sci USA.* 2015;112(34). doi:10.1073/pnas.1506499112
86. Patrizi A, Awad PN, Chattopadhyaya B, Li C, Di Cristo G, Fagiolini M. Accelerated Hyper-Maturation of Parvalbumin Circuits in the Absence of MeCP2. *Cerebral Cortex.* 2020;30(1):256-268. doi:10.1093/cercor/bhz085
87. Sigal YM, Bae H, Bogart LJ, Hensch TK, Zhuang X. Structural maturation of cortical perineuronal nets and their perforating synapses revealed by superresolution imaging. *Proc Natl Acad Sci USA.* 2019;116(14):7071-7076. doi:10.1073/pnas.1817222116
88. Morello N, Schina R, Pilotto F, et al. Loss of *Mecp2* Causes Atypical Synaptic and Molecular Plasticity of Parvalbumin-Expressing Interneurons Reflecting Rett Syndrome-Like Sensorimotor Defects. *eNeuro.* 2018;5(5):ENEURO.0086-18.2018. doi:10.1523/ENEURO.0086-18.2018

89. Mierau SB, Patrizi A, Hensch TK, Fagiolini M. Cell-Specific Regulation of N-Methyl-D-Aspartate Receptor Maturation by Mecp2 in Cortical Circuits. *Biological Psychiatry*. 2016;79(9):746-754. doi:10.1016/j.biopsych.2015.05.018
90. Chao HT, Chen H, Samaco RC, et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*. 2010;468(7321):263-269. doi:10.1038/nature09582
91. Tomassy GS, Morello N, Calcagno E, Giustetto M. Developmental abnormalities of cortical interneurons precede symptoms onset in a mouse model of Rett syndrome. *Journal of Neurochemistry*. 2014;131(1):115-127. doi:10.1111/jnc.12803
92. Kullander K, Topolnik L. Cortical disinhibitory circuits: cell types, connectivity and function. *Trends in Neurosciences*. 2021;44(8):643-657. doi:10.1016/j.tins.2021.04.009
93. Wall NR, De La Parra M, Sorokin JM, Taniguchi H, Huang ZJ, Callaway EM. Brain-Wide Maps of Synaptic Input to Cortical Interneurons. *J Neurosci*. 2016;36(14):4000-4009. doi:10.1523/JNEUROSCI.3967-15.2016
94. Mossner JM, Batista-Brito R, Pant R, Cardin JA. Developmental loss of MeCP2 from VIP interneurons impairs cortical function and behavior. *eLife*. 2020;9:e55639. doi:10.7554/eLife.55639
95. He L jie, Liu N, Cheng T lin, et al. Conditional deletion of Mecp2 in parvalbumin-expressing GABAergic cells results in the absence of critical period plasticity. *Nat Commun*. 2014;5(1):5036. doi:10.1038/ncomms6036
96. Ito-Ishida A, Ure K, Chen H, Swann JW, Zoghbi HY. Loss of MeCP2 in Parvalbumin-and Somatostatin-Expressing Neurons in Mice Leads to Distinct Rett Syndrome-like Phenotypes. *Neuron*. 2015;88(4):651-658. doi:10.1016/j.neuron.2015.10.029
97. Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, Monteggia LM. Postnatal Loss of Methyl-CpG Binding Protein 2 in the Forebrain is Sufficient to Mediate Behavioral Aspects of Rett Syndrome in Mice. *Biological Psychiatry*. 2006;59(5):468-476. doi:10.1016/j.biopsych.2005.07.025
98. Xu P, Yue Y, Su J, et al. Pattern decorrelation in the mouse medial prefrontal cortex enables social preference and requires MeCP2. *Nat Commun*. 2022;13(1). doi:10.1038/s41467-022-31578-9
99. Boyle N, Li Y, Sun X, et al. MeCP2 deficiency alters the response selectivity of prefrontal cortical neurons to different social stimuli. *eNeuro*. Published online September 12, 2024:ENEURO.0003-24.2024. doi:10.1523/eneuro.0003-24.2024
100. Lau BYB, Krishnan K, Huang ZJ, Shea SD. Maternal Experience-Dependent Cortical Plasticity in Mice Is Circuit- and Stimulus-Specific and Requires MECP2. *J Neurosci*. 2020;40(7):1514-1526. doi:10.1523/JNEUROSCI.1964-19.2019

101. Lau BYB, Layo DE, Emery B, et al. Lateralized Expression of Cortical Perineuronal Nets during Maternal Experience is Dependent on MECP2. *eNeuro*. 2020;7(3):ENEURO.0500-19.2020. doi:10.1523/ENEURO.0500-19.2020
102. Bhattacharjee A, Mu Y, Winter MK, et al. Neuronal cytoskeletal gene dysregulation and mechanical hypersensitivity in a rat model of Rett syndrome. *Proc Natl Acad Sci USA*. 2017;114(33). doi:10.1073/pnas.1618210114
103. Chao HT, Zoghbi HY, Rosenmund C. MeCP2 Controls Excitatory Synaptic Strength by Regulating Glutamatergic Synapse Number. *Neuron*. 2007;56(1):58-65. doi:10.1016/j.neuron.2007.08.018
104. Calfa G, Li W, Rutherford JM, Pozzo-Miller L. Excitation/inhibition imbalance and impaired synaptic inhibition in hippocampal area CA3 of *Mecp2* knockout mice: E/I Imbalance In *Mecp2* Knockout Hippocampus. *Hippocampus*. 2015;25(2):159-168. doi:10.1002/hipo.22360
105. Lu H, Ash RT, He L, et al. Loss and Gain of MeCP2 Cause Similar Hippocampal Circuit Dysfunction that Is Rescued by Deep Brain Stimulation in a Rett Syndrome Mouse Model. *Neuron*. 2016;91(4):739-747. doi:10.1016/j.neuron.2016.07.018
106. Moretti P, Levenson JM, Battaglia F, et al. Learning and Memory and Synaptic Plasticity Are Impaired in a Mouse Model of Rett Syndrome. *J Neurosci*. 2006;26(1):319-327. doi:10.1523/JNEUROSCI.2623-05.2006
107. Kron M, Howell CJ, Adams IT, et al. Brain Activity Mapping in *Mecp2* Mutant Mice Reveals Functional Deficits in Forebrain Circuits, Including Key Nodes in the Default Mode Network, that are Reversed with Ketamine Treatment. *Journal of Neuroscience*. 2012;32(40):13860-13872. doi:10.1523/JNEUROSCI.2159-12.2012
108. Huang TW, Kochukov MY, Ward CS, et al. Progressive Changes in a Distributed Neural Circuit Underlie Breathing Abnormalities in Mice Lacking MeCP2. *J Neurosci*. 2016;36(20):5572-5586. doi:10.1523/jneurosci.2330-15.2016
109. Noutel J, Hong YK, Leu B, Kang E, Chen C. Experience-Dependent Retinogeniculate Synapse Remodeling Is Abnormal in MeCP2-Deficient Mice. *Neuron*. 2011;70(1):35-42. doi:10.1016/j.neuron.2011.03.001
110. Ballinger EC, Schaaf CP, Patel AJ, et al. *Mecp2* Deletion from Cholinergic Neurons Selectively Impairs Recognition Memory and Disrupts Cholinergic Modulation of the Perirhinal Cortex. *eNeuro*. 2019;6(6):ENEURO.0134-19.2019. doi:10.1523/eneuro.0134-19.2019
111. Samaco RC, Mandel-Brehm C, Chao HT, et al. Loss of MeCP2 in aminergic neurons causes cell-autonomous defects in neurotransmitter synthesis and specific behavioral abnormalities. *Proc Natl Acad Sci USA*. 2009;106(51):21966-21971. doi:10.1073/pnas.0912257106

112. Orefice LL, Zimmerman AL, Chirila AM, Sleboda SJ, Head JP, Ginty DD. Peripheral Mechanosensory Neuron Dysfunction Underlies Tactile and Behavioral Deficits in Mouse Models of ASDs. *Cell*. 2016;166(2):299-313. doi:10.1016/j.cell.2016.05.033
113. Buzsáki G, Draguhn A. Neuronal Oscillations in Cortical Networks. *Science*. 2004;304(5679):1926-1929. doi:10.1126/science.1099745
114. Uhlhaas PJ, Singer W. Neuronal Dynamics and Neuropsychiatric Disorders: Toward a Translational Paradigm for Dysfunctional Large-Scale Networks. *Neuron*. 2012;75(6):963-980. doi:10.1016/j.neuron.2012.09.004
115. Wang XJ. Neurophysiological and Computational Principles of Cortical Rhythms in Cognition. *Physiological Reviews*. 2010;90(3):1195-1268. doi:10.1152/physrev.00035.2008
116. Schnitzler A, Gross J. Normal and pathological oscillatory communication in the brain. *Nat Rev Neurosci*. 2005;6(4):285-296. doi:10.1038/nrn1650
117. Moser SJ, Weber P, Lütschg J. Rett Syndrome: Clinical and Electrophysiologic Aspects. *Pediatric Neurology*. 2007;36(2):95-100. doi:10.1016/j.pediatrneurol.2006.10.003
118. Glaze DG. Neurophysiology of Rett syndrome. *Ment Retard Dev Disabil Res Rev*. 2002;8(2):66-71. doi:10.1002/mrdd.10024
119. Niedermeyer E, Rett A, Renner H, et al. Rett syndrome and the electroencephalogram. *American Journal of Medical Genetics*. 1986;25(S1):195-199. doi:https://doi.org/10.1002/ajmg.1320250522
120. Smirnov K, Stroganova T, Molholm S, Sysoeva O. Reviewing Evidence for the Relationship of EEG Abnormalities and RTT Phenotype Paralleled by Insights from Animal Studies. *IJMS*. 2021;22(10):5308. doi:10.3390/ijms22105308
121. Tarquinio DC, Hou W, Berg A, et al. Longitudinal course of epilepsy in Rett syndrome and related disorders. *Brain*. 2017;140(2):306-318. doi:10.1093/brain/aww302
122. Roche KJ, LeBlanc JJ, Levin AR, O'Leary HM, Baczewski LM, Nelson CA. Electroencephalographic spectral power as a marker of cortical function and disease severity in girls with Rett syndrome. *J Neurodevel Disord*. 2019;11(1):15. doi:10.1186/s11689-019-9275-z
123. Saby JN, Mulcahey PJ, Benke TA, et al. ELECTROENCEPHALOGRAPHIC Correlates of Clinical Severity in the Natural history study of RTT and Related Disorders. *Annals of Neurology*. Published online May 9, 2024;ana.26948. doi:10.1002/ana.26948
124. Fabio RA, Billeci L, Crifaci G, Troise E, Tortorella G, Pioggia G. Cognitive training modifies frequency EEG bands and neuropsychological measures in Rett syndrome. *Research in Developmental Disabilities*. 2016;53-54:73-85. doi:10.1016/j.ridd.2016.01.009

125. Thibodeau R, Jorgensen RS, Kim S. Depression, anxiety, and resting frontal EEG asymmetry: A meta-analytic review. *Journal of Abnormal Psychology*. 115(4):715-729. doi:https://doi.org/10.1037/0021-843X.115.4.715
126. Khwaja OS, Ho E, Barnes KV, et al. Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome. *Proc Natl Acad Sci USA*. 2014;111(12):4596-4601. doi:10.1073/pnas.1311141111
127. Jensen SKG, Kumar S, Xie W, et al. Neural correlates of early adversity among Bangladeshi infants. *Sci Rep*. 2019;9(1):3507. doi:10.1038/s41598-019-39242-x
128. Saby JN, Benke TA, Peters SU, et al. Multisite Study of Evoked Potentials in Rett Syndrome. *Ann Neurol*. 2021;89(4):790-802. doi:10.1002/ana.26029
129. Boggio EM, Pancrazi L, Gennaro M, et al. Visual impairment in FOXP1-mutated individuals and mice. *Neuroscience*. 2016;324:496-508. doi:10.1016/j.neuroscience.2016.03.027
130. Courchesne E. Chronology of postnatal human brain development: Event-related potential, positron emission tomography, myelinogenesis, and synaptogenesis studies. *Event-related brain potentials: Basic issues and applications*. Published online 1990:210-241.
131. Webb SJ, Emerman I, Sugar C, et al. Identifying Age Based Maturation in the ERP Response to Faces in Children With Autism: Implications for Developing Biomarkers for Use in Clinical Trials. *Front Psychiatry*. 2022;13:841236. doi:10.3389/fpsy.2022.841236
132. Karrer JH, Karrer R, Bloom D, Chaney L, Davis R. Event-related brain potentials during an extended visual recognition memory task depict delayed development of cerebral inhibitory processes among 6-month-old infants with Down syndrome. *International Journal of Psychophysiology*. 1998;29(2):167-200. doi:10.1016/S0167-8760(98)00015-4
133. Stauder JEA, Brinkman MJR, Curfs LMG. Multi-modal P3 deflation of event-related brain activity in Prader-Willi syndrome. *Neuroscience Letters*. 2002;327(2):99-102. doi:10.1016/S0304-3940(02)00377-4
134. Ethridge LE, White SP, Mosconi MW, Wang J, Byerly MJ, Sweeney JA. Reduced habituation of auditory evoked potentials indicate cortical hyper-excitability in Fragile X Syndrome. *Transl Psychiatry*. 2016;6(4):e787-e787. doi:10.1038/tp.2016.48
135. An WW, Nelson CA, Wilkinson CL. Neural response to repeated auditory stimuli and its association with early language ability in male children with Fragile X syndrome. *Front Integr Neurosci*. 2022;16:987184. doi:10.3389/fnint.2022.987184
136. Key AP, Jones D, Peters S, Dold C. Feasibility of using auditory event-related potentials to investigate learning and memory in nonverbal individuals with Angelman syndrome. *Brain and Cognition*. 2018;128:73-79. doi:10.1016/j.bandc.2018.11.001

137. Brittenham C. Objective Measures of Electrophysiological Responses of Children with Idiopathic Autism Spectrum Disorder and Phelan-McDermid Syndrome to a Contrast-Reversing Checkerboard. Published online 2017. doi:10.13140/RG.2.2.35857.38241
138. Demarest ST, Olson HE, Moss A, et al. CDKL5 deficiency disorder: Relationship between genotype, epilepsy, cortical visual impairment, and development. *Epilepsia*. 2019;60(8):1733-1742. doi:https://doi.org/10.1111/epi.16285
139. Kálmánchey R. Evoked potentials in the rett syndrome. *Brain and Development*. 1990;12(1):73-76. doi:10.1016/S0387-7604(12)80181-1
140. Verma NP, Nigro MA, Hart ZH. Rett syndrome — a gray matter disease? Electrophysiologic evidence. *Electroencephalography and Clinical Neurophysiology*. 1987;67(4):327-329. doi:10.1016/0013-4694(87)90118-0
141. Bader GG, Witt-Engerström I, Hagberg B. Neurophysiological findings in the Rett syndrome, I: EMG, conduction velocity, EEG and somatosensory-evoked potential studies. *Brain and Development*. 1989;11(2):102-109. doi:10.1016/S0387-7604(89)80077-4
142. Stach BA, Stoner WR, Smith SL, Jerger JF. Auditory evoked potentials in Rett syndrome. *J Am Acad Audiol*. 1994;5(3):226-230.
143. Stauder JEA, Smeets EEJ, van Mil SGM, Curfs LGM. The development of visual- and auditory processing in Rett syndrome: An ERP study. *Brain and Development*. 2006;28(8):487-494. doi:10.1016/j.braindev.2006.02.011
144. Brima T, Beker S, Prinsloo KD, et al. Probing a neural unreliability account of auditory sensory processing atypicalities in Rett Syndrome. *J Neurodevelop Disord*. 2024;16(1):28. doi:10.1186/s11689-024-09544-x
145. Sysoeva OV, Molholm S, Djukic A, Frey HP, Foxe JJ. Atypical processing of tones and phonemes in Rett Syndrome as biomarkers of disease progression. *Transl Psychiatry*. 2020;10(1):188. doi:10.1038/s41398-020-00877-4
146. Foxe JJ, Burke KM, Andrade GN, Djukic A, Frey HP, Molholm S. Automatic cortical representation of auditory pitch changes in Rett syndrome. *J Neurodevelop Disord*. 2016;8(1):34. doi:10.1186/s11689-016-9166-5
147. Engineer CT, Rahebi KC, Borland MS, et al. Degraded neural and behavioral processing of speech sounds in a rat model of Rett syndrome. *Neurobiology of Disease*. 2015;83:26-34. doi:10.1016/j.nbd.2015.08.019
148. Peters SU, Katzenstein A, Jones D, Key AP. Distinguishing response to names in Rett and MECP2 Duplication syndrome: An ERP study of auditory social information processing. *Brain Research*. 2017;1675:71-77. doi:10.1016/j.brainres.2017.08.028
149. Key AP, Jones D, Peters S. Spoken word processing in Rett syndrome: Evidence from event-related potentials. *Int j dev neurosci*. 2019;73(1):26-31. doi:10.1016/j.ijdevneu.2019.01.001

150. Lenassi E, Likar K, Stirn-Kranjc B, Breclj J. VEP maturation and visual acuity in infants and preschool children. *Doc Ophthalmol*. 2008;117(2):111-120. doi:10.1007/s10633-007-9111-8
151. Ponton CW, Eggermont JJ, Kwong B, Don M. Maturation of human central auditory system activity: evidence from multi-channel evoked potentials. *Clinical Neurophysiology*. 2000;111(2):220-236. doi:10.1016/S1388-2457(99)00236-9
152. Saunders KJ, McCulloch DL, Kerr AM. Visual function in Rett syndrome. *Dev Med Child Neurol*. 1995;37(6):496-504. doi:10.1111/j.1469-8749.1995.tb12037.x.
153. LeBlanc JJ, DeGregorio G, Centofante E, et al. Visual evoked potentials detect cortical processing deficits in Rett syndrome: VEP in Rett Syndrome. *Ann Neurol*. 2015;78(5):775-786. doi:10.1002/ana.24513
154. Badr GG, Witt-Engerström I, Hagberg B. Brain stem and spinal cord impairment in rett syndrome: somatosensory and auditory evoked responses investigations. *Brain and Development*. 1987;9(5):517-522. doi:10.1016/S0387-7604(87)80076-1
155. Guerrini R, Bonanni P, Parmeggiani L, Santucci M, Parmeggiani A, Sartucci F. Cortical reflex myoclonus in Rett syndrome. *Ann Neurol*. 1998;43(4):472-479. doi:10.1002/ana.410430410
156. Kimura K, Nomura Y, Segawa M. Middle and short latency somatosensory evoked potentials (SEPM, SEPs) in the Rett syndrome: chronological changes of cortical and subcortical involvements. *Brain Dev*. 1992;14 Suppl:S37-42.
157. Yamanouchi H, Kaga M, Arima M. Abnormal cortical excitability in Rett syndrome. *Pediatric Neurology*. 1993;9(3):202-206. doi:10.1016/0887-8994(93)90085-Q
158. Yoshikawa H, Kaga M, Suzuki H, Sakuragawa N, Arima M. Giant somatosensory evoked potentials in the Rett syndrome. *Brain and Development*. 1991;13(1):36-39. doi:10.1016/S0387-7604(12)80295-6
159. Webb SJ, Shic F, Murias M, et al. Biomarker Acquisition and Quality Control for Multi-Site Studies: The Autism Biomarkers Consortium for Clinical Trials. *Front Integr Neurosci*. 2020;13:71. doi:10.3389/fnint.2019.00071
160. Ewen JB, Levin AR. Neurobehavioral Biomarkers: An EEG Family Reunion. *Journal of Clinical Neurophysiology*. 2022;39(2):129-134. doi:10.1097/WNP.0000000000000836
161. Sandweiss AJ, Brandt VL, Zoghbi HY. Advances in understanding of Rett syndrome and MECP2 duplication syndrome: prospects for future therapies. *The Lancet Neurology*. 2020;19(8):689-698. doi:10.1016/S1474-4422(20)30217-9
162. MacLennan K, O'Brien S, Tavassoli T. In Our Own Words: The Complex Sensory Experiences of Autistic Adults. *J Autism Dev Disord*. 2022;52(7):3061-3075. doi:10.1007/s10803-021-05186-3

163. Sapey-Triomphe LA, Moulin A, Sonié S, Schmitz C. The Glasgow Sensory Questionnaire: Validation of a French Language Version and Refinement of Sensory Profiles of People with High Autism-Spectrum Quotient. *J Autism Dev Disord.* 2018;48(5):1549-1565. doi:10.1007/s10803-017-3422-8
164. Sysoeva OV, Smirnov K, Stroganova TA. Sensory evoked potentials in patients with Rett syndrome through the lens of animal studies: Systematic review. *Clinical Neurophysiology.* 2020;131(1):213-224. doi:10.1016/j.clinph.2019.11.003
165. Helfrich RF, Knight RT. Cognitive neurophysiology: Event-related potentials. In: *Handbook of Clinical Neurology.* Vol 160. Elsevier; 2019:543-558. doi:10.1016/B978-0-444-64032-1.00036-9
166. Luck SJ. *An Introduction to the Event-Related Potential Technique.* MIT Press; 2005.
167. Makeig S, Westerfield M, Jung TP, et al. Dynamic Brain Sources of Visual Evoked Responses. *Science.* 2002;295(5555):690-694. doi:10.1126/science.1066168
168. Makeig S, Debener S, Onton J, Delorme A. Mining event-related brain dynamics. *Trends in Cognitive Sciences.* 2004;8(5):204-210. doi:10.1016/j.tics.2004.03.008
169. Ding N, Simon JZ. Power and phase properties of oscillatory neural responses in the presence of background activity. *J Comput Neurosci.* 2013;34(2):337-343. doi:10.1007/s10827-012-0424-6
170. Klimesch W, Sauseng P, Hanslmayr S. EEG alpha oscillations: The inhibition–timing hypothesis. *Brain Research Reviews.* 2007;53(1):63-88. doi:10.1016/j.brainresrev.2006.06.003
171. Milne E. Increased Intra-Participant Variability in Children with Autistic Spectrum Disorders: Evidence from Single-Trial Analysis of Evoked EEG. *Front Psychology.* 2011;2. doi:10.3389/fpsyg.2011.00051
172. Kovarski K, Malvy J, Khanna RK, Arsène S, Batty M, Latinus M. Reduced visual evoked potential amplitude in autism spectrum disorder, a variability effect? *Transl Psychiatry.* 2019;9(1):341. doi:10.1038/s41398-019-0672-6
173. Yu L, Wang S, Huang D, Wu X, Zhang Y. Role of inter-trial phase coherence in atypical auditory evoked potentials to speech and nonspeech stimuli in children with autism. *Clinical Neurophysiology.* 2018;129(7):1374-1382. doi:10.1016/j.clinph.2018.04.599
174. Edgar JC, Fisk IV CL, Berman JJ, et al. Auditory encoding abnormalities in children with autism spectrum disorder suggest delayed development of auditory cortex. *Molecular Autism.* 2015;6(1):69. doi:10.1186/s13229-015-0065-5
175. Van Noordt S, Wu J, Venkataraman A, Larson MJ, South M, Crowley MJ. Inter-trial coherence of medial frontal theta oscillations linked to differential feedback processing in

- youth and young adults with autism. *Research in Autism Spectrum Disorders*. 2017;37:1-10. doi:10.1016/j.rasd.2017.01.011
176. Dunn, W. Sensory Profile 2. NCS Pearson, Inc., 2014.
 177. He JL, Williams ZJ, Harris A, et al. A working taxonomy for describing the sensory differences of autism. *Molecular Autism*. 2023;14(1). doi:10.1186/s13229-022-00534-1
 178. Guy W. Clinical global impressions. In: Guy W, editor. ECDEU assessment manual for psychopharmacology (Revised) National Institute of Mental Health; Rockville, Maryland: 1976. pp. 217–221.
 179. Neul JL, Glaze DG, Percy AK, et al. Improving Treatment Trial Outcomes for Rett Syndrome: The Development of Rett-specific Anchors for the Clinical Global Impression Scale. *J Child Neurol*. 2015;30(13):1743-1748. doi:10.1177/0883073815579707
 180. Mount RH, Charman T, Hastings RP, Reilly S, Cass H. The Rett Syndrome Behaviour Questionnaire (RSBQ): refining the behavioural phenotype of Rett syndrome. *Child Psychology Psychiatry*. 2002;43(8):1099-1110. doi:10.1111/1469-7610.00236
 181. Percy AK, Neul JL, Benke TA, Marsh ED, Glaze DG. A review of the Rett Syndrome Behaviour Questionnaire and its utilization in the assessment of symptoms associated with Rett syndrome. *Front Pediatr*. 2023;11:1229553. doi:10.3389/fped.2023.1229553
 182. Oberman LM, Leonard H, Downs J, et al. Rett Syndrome Behaviour Questionnaire in Children and Adults With Rett Syndrome: Psychometric Characterization and Revised Factor Structure. *American Journal on Intellectual and Developmental Disabilities*. 2023;128(3):237-253. doi:10.1352/1944-7558-128.3.237
 183. Glaze DG, Neul JL, Kaufmann WE, et al. Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome. *Neurology*. 2019;92(16). doi:10.1212/WNL.00000000000007316
 184. Neul JL, Percy AK, Benke TA, et al. Design and outcome measures of LAVENDER, a phase 3 study of trofinetide for Rett syndrome. *Contemporary Clinical Trials*. 2022;114:106704. doi:10.1016/j.cct.2022.106704
 185. O’Leary HM, Kaufmann WE, Barnes KV, et al. Placebo-controlled crossover assessment of mecasermin for the treatment of Rett syndrome. *Ann Clin Transl Neurol*. 2018;5(3):323-332. doi:10.1002/acn3.533
 186. Robertson L, Hall SE, Jacoby P, Ellaway C, Klerk ND, Leonard H. The association between behavior and genotype in Rett syndrome using the Australian Rett Syndrome Database. *American J of Med Genetics Pt B*. 2006;141B(2):177-183. doi:10.1002/ajmg.b.30270
 187. Espenhahn S, Yan T, Beltrano W, et al. The effect of movie-watching on electroencephalographic responses to tactile stimulation. *NeuroImage*. 2020;220:117130. doi:10.1016/j.neuroimage.2020.117130

188. Levin AR, Méndez Leal AS, Gabard-Durnam LJ, O’Leary HM. BEAPP: The Batch Electroencephalography Automated Processing Platform. *Front Neurosci.* 2018;12:513. doi:10.3389/fnins.2018.00513
189. Gabard-Durnam LJ, Mendez Leal AS, Wilkinson CL, Levin AR. The Harvard Automated Processing Pipeline for Electroencephalography (HAPPE): Standardized Processing Software for Developmental and High-Artifact Data. *Front Neurosci.* 2018;12:97. doi:10.3389/fnins.2018.00097
190. Winkler I, Haufe S, Tangermann M. Automatic Classification of Artifactual ICA-Components for Artifact Removal in EEG Signals. *Behav Brain Funct.* 2011;7(1):30. doi:10.1186/1744-9081-7-30
191. Winkler I, Brandl S, Horn F, Waldburger E, Allefeld C, Tangermann M. Robust artifactual independent component classification for BCI practitioners. *J Neural Eng.* 2014;11(3):035013. doi:10.1088/1741-2560/11/3/035013
192. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods.* 2004;134(1):9-21. doi:10.1016/j.jneumeth.2003.10.009
193. Brandwein AB, Foxe JJ, Russo NN, Altschuler TS, Gomes H, Molholm S. The Development of Audiovisual Multisensory Integration Across Childhood and Early Adolescence: A High-Density Electrical Mapping Study. *Cerebral Cortex.* 2011;21(5):1042-1055. doi:10.1093/cercor/bhq170
194. Kopell NJ, Gritton HJ, Whittington MA, Kramer MA. Beyond the Connectome: The Dynome. *Neuron.* 2014;83(6):1319-1328. doi:10.1016/j.neuron.2014.08.016
195. Senkowski D, Schneider TR, Foxe JJ, Engel AK. Crossmodal binding through neural coherence: implications for multisensory processing. *Trends in Neurosciences.* 2008;31(8):401-409. doi:10.1016/j.tins.2008.05.002
196. Wang X, Wang S, Fan Y, Huang D, Zhang Y. Speech-specific categorical perception deficit in autism: An Event-Related Potential study of lexical tone processing in Mandarin-speaking children. *Sci Rep.* 2017;7(1):43254. doi:10.1038/srep43254
197. Čeponienė R, Shestakova A, Balan P, Alku P, Yläguchi K, Naatanen R. Children’s Auditory Event-Related Potentials Index Sound Complexity and “Speechness.” *International Journal of Neuroscience.* 2001;109(3-4):245-260. doi:10.3109/00207450108986536
198. Xie W, McCormick SA, Westerlund A, Bowman LC, Nelson CA. Neural correlates of facial emotion processing in infancy. *Dev Sci.* 2019;22(3). doi:10.1111/desc.12758
199. Maenner MJ, Warren Z, Williams AR, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. *MMWR Surveill Summ.* 2023;72(2):1-14. doi:10.15585/mmwr.ss7202a1

200. Rogers SJ, Ozonoff S. Annotation: What do we know about sensory dysfunction in autism? A critical review of the empirical evidence. *Child Psychology Psychiatry*. 2005;46(12):1255-1268. doi:10.1111/j.1469-7610.2005.01431.x
201. Diagnostic and Statistical Manual of Mental Disorders: DSM-5. American Psychiatric Association, 2013.
202. Baranek GT, David FJ, Poe MD, Stone WL, Watson LR. Sensory Experiences Questionnaire: discriminating sensory features in young children with autism, developmental delays, and typical development: SEQ. *Journal of Child Psychology and Psychiatry*. 2006;47(6):591-601. doi:10.1111/j.1469-7610.2005.01546.x
203. Espenhahn S, Godfrey KJ, Kaur S, et al. Tactile cortical responses and association with tactile reactivity in young children on the autism spectrum. *Molecular Autism*. 2021;12(1):26. doi:10.1186/s13229-021-00435-9
204. Mikkelsen M, Wodka EL, Mostofsky SH, Puts NAJ. Autism spectrum disorder in the scope of tactile processing. *Developmental Cognitive Neuroscience*. 2018;29:140-150. doi:10.1016/j.dcn.2016.12.005
205. Puts NAJ, Wodka EL, Tommerdahl M, Mostofsky SH, Edden RAE. Impaired tactile processing in children with autism spectrum disorder. *Journal of Neurophysiology*. 2014;111(9):1803-1811. doi:10.1152/jn.00890.2013
206. Robertson CE, Baron-Cohen S. Sensory perception in autism. *Nat Rev Neurosci*. 2017;18(11):671-684. doi:10.1038/nrn.2017.112
207. Zhang Z, Sun QQ. The Balance Between Excitation And Inhibition And Functional Sensory Processing In The Somatosensory Cortex. In: *International Review of Neurobiology*. Vol 97. Elsevier; 2011:305-333. doi:10.1016/B978-0-12-385198-7.00012-6
208. Orefice LL, Mosko JR, Morency DT, et al. Targeting Peripheral Somatosensory Neurons to Improve Tactile-Related Phenotypes in ASD Models. *Cell*. 2019;178(4):867-886.e24. doi:10.1016/j.cell.2019.07.024
209. Tavassoli T, Bellesheim K, Tommerdahl M, Holden JM, Kolevzon A, Buxbaum JD. Altered tactile processing in children with autism spectrum disorder: Tactile processing, inhibition, ASD. *Autism Research*. 2016;9(6):616-620. doi:10.1002/aur.1563
210. Ben-Sasson A, Carter AS, Briggs-Gowan MJ. Sensory Over-Responsivity in Elementary School: Prevalence and Social-Emotional Correlates. *J Abnorm Child Psychol*. 2009;37(5):705-716. doi:10.1007/s10802-008-9295-8
211. Green D, Chandler S, Charman T, Simonoff E, Baird G. Brief Report: DSM-5 Sensory Behaviours in Children With and Without an Autism Spectrum Disorder. *J Autism Dev Disord*. 2016;46(11):3597-3606. doi:10.1007/s10803-016-2881-7

212. Lane SJ, Reynolds S, Dumenci L. Sensory Overresponsivity and Anxiety in Typically Developing Children and Children With Autism and Attention Deficit Hyperactivity Disorder: Cause or Coexistence? *The American Journal of Occupational Therapy*. 2012;66(5):595-603. doi:10.5014/ajot.2012.004523
213. Mangeot SD, Miller LJ, McIntosh DN, et al. Sensory modulation dysfunction in children with attention-deficit-hyperactivity disorder. *Dev Med Child Neurol*. 2001;43(06):399. doi:10.1017/S0012162201000743
214. Green SA, Ben-Sasson A, Soto TW, Carter AS. Anxiety and Sensory Over-Responsivity in Toddlers with Autism Spectrum Disorders: Bidirectional Effects Across Time. *J Autism Dev Disord*. 2012;42(6):1112-1119. doi:10.1007/s10803-011-1361-3
215. Sherman MA, Lee S, Law R, et al. Neural mechanisms of transient neocortical beta rhythms: Converging evidence from humans, computational modeling, monkeys, and mice. *Proc Natl Acad Sci USA*. 2016;113(33). doi:10.1073/pnas.1604135113
216. Shin H, Law R, Tsutsui S, Moore CI, Jones SR. The rate of transient beta frequency events predicts behavior across tasks and species. *eLife*. 2017;6:e29086. doi:10.7554/eLife.29086
217. Law RG, Pugliese S, Shin H, et al. Thalamocortical Mechanisms Regulating the Relationship between Transient Beta Events and Human Tactile Perception. *Cerebral Cortex*. 2022;32(4):668-688. doi:10.1093/cercor/bhab221
218. Jones SR. When brain rhythms aren't 'rhythmic': implication for their mechanisms and meaning. *Current Opinion in Neurobiology*. 2016;40:72-80. doi:10.1016/j.conb.2016.06.010
219. Bonaiuto JJ, Little S, Neymotin SA, Jones SR, Barnes GR, Bestmann S. Laminar dynamics of high amplitude beta bursts in human motor cortex. *NeuroImage*. 2021;242:118479. doi:10.1016/j.neuroimage.2021.118479
220. Szul M, Papadopoulos S, Alavizadeh S, et al. Diverse beta burst waveform motifs characterize movement-related cortical dynamics. *Progress in Neurobiology*. 2023;228. doi:10.1016/j.pneurobio.2023.102490
221. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop SL. Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). Western Psychological Services, 2012.
222. McPartland JC, Bernier RA, Jeste SS, et al. The Autism Biomarkers Consortium for Clinical Trials (ABC-CT): Scientific Context, Study Design, and Progress Toward Biomarker Qualification. *Front Integr Neurosci*. 2020;14. doi:10.3389/fnint.2020.00016
223. Elliott, C. D. Differential ability scales, Second edition. Pearson Assessments, 2007.
224. Mullen, E. M. Mullen Scales of Early Learning Manual. American Guidance Service, 1995.
225. Rutter, M. & Le Couteur, A, & Lord, C..... Autism Diagnostic Interview-Revised. Western Psychological Services, 2003.

226. Schopler, E., Van Bourgondien, M.E., Wellman, G.J., & Love, S.R. Childhood Autism Rating Scale, Second Edition. Western Psychological Services, 2010.
227. Roid, G.H. Stanford-Binet Intelligence Scales, Fifth Edition. Riverside Pub, 2003.
228. Sarela, J. & Vigario, R. The problem of overlearning in high-order ICA approaches: analysis and solutions. *Lect. Notes Comput. Sci.* 2001.
229. SpectralEvents: Toolbox for detecting and analyzing rhythms as transient spectral events. Github.
230. Neuhaus E, Lowry SJ, Santhosh M, et al. Resting state EEG in youth with ASD: age, sex, and relation to phenotype. *J Neurodevelop Disord.* 2021;13(1). doi:10.1186/s11689-021-09390-1
231. IBM Corp. IBM SPSS Statistics for Windows, 2020.
232. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychological Assessment.* 1994;6(4):284-290. doi:10.1037/1040-3590.6.4.284
233. Sahin M, Jones SR, Sweeney JA, et al. Discovering translational biomarkers in neurodevelopmental disorders. *Nat Rev Drug Discov.* 2019;18(4):235-236. doi:10.1038/d41573-018-00010-7
234. Lord C, Charman T, Havdahl A, et al. The Lancet Commission on the future of care and clinical research in autism. *The Lancet.* 2022;399(10321):271-334. doi:10.1016/s0140-6736(21)01541-5
235. Tavassoli T, Layton C, Levy T, et al. Sensory Reactivity Phenotype in Phelan–McDermid Syndrome Is Distinct from Idiopathic ASD. *Genes.* 2021;12(7):977. doi:10.3390/genes12070977
236. Lyons-Warren AM, McCormack MC, Holder JL. Sensory Processing Phenotypes in Phelan-McDermid Syndrome and SYNGAP1-Related Intellectual Disability. *Brain Sciences.* 2022;12(2):137. doi:10.3390/brainsci12020137
237. Fagiolini M, Patrizi A, LeBlanc J, et al. Intellectual and Developmental Disabilities Research Centers: A Multidisciplinary Approach to Understand the Pathogenesis of Methyl-CpG Binding Protein 2-related Disorders. *Neuroscience.* 2020;445:190-206. doi:10.1016/j.neuroscience.2020.04.037
238. Canolty RT, Knight RT. The functional role of cross-frequency coupling. *Trends in Cognitive Sciences.* 2010;14(11):506-515. doi:10.1016/j.tics.2010.09.001
239. Gips B, Eerden JPJM, Jensen O. A biologically plausible mechanism for neuronal coding organized by the phase of alpha oscillations. Foxe J, ed. *Eur J Neurosci.* 2016;44(4):2147-2161. doi:10.1111/ejn.13318

240. Hyafil A, Giraud AL, Fontolan L, Gutkin B. Neural Cross-Frequency Coupling: Connecting Architectures, Mechanisms, and Functions. *Trends in Neurosciences*. 2015;38(11):725-740. doi:10.1016/j.tins.2015.09.001
241. Womelsdorf T, Valiante TA, Sahin NT, Miller KJ, Tiesinga P. Dynamic circuit motifs underlying rhythmic gain control, gating and integration. *Nat Neurosci*. 2014;17(8):1031-1039. doi:10.1038/nn.3764
242. Bollimunta A, Mo J, Schroeder CE, Ding M. Neuronal Mechanisms and Attentional Modulation of Corticothalamic Alpha Oscillations. *Journal of Neuroscience*. 2011;31(13):4935-4943. doi:10.1523/JNEUROSCI.5580-10.2011
243. Márton CD, Fukushima M, Camalier CR, Schultz SR, Averbeck BB. Signature Patterns for Top-Down and Bottom-Up Information Processing via Cross-Frequency Coupling in Macaque Auditory Cortex. *eNeuro*. 2019;6(2):ENEURO.0467-18.2019. doi:10.1523/ENEURO.0467-18.2019
244. Sotero RC, Bortel A, Naaman S, et al. Laminar Distribution of Phase-Amplitude Coupling of Spontaneous Current Sources and Sinks. *Front Neurosci*. 2015;9. doi:10.3389/fnins.2015.00454
245. Holz EM, Glennon M, Prendergast K, Sauseng P. Theta–gamma phase synchronization during memory matching in visual working memory. *NeuroImage*. 2010;52(1):326-335. doi:10.1016/j.neuroimage.2010.04.003
246. Mormann F, Fell J, Axmacher N, et al. Phase/amplitude reset and theta–gamma interaction in the human medial temporal lobe during a continuous word recognition memory task. *Hippocampus*. 2005;15(7):890-900. doi:10.1002/hipo.20117
247. Shirvalkar PR, Rapp PR, Shapiro ML. Bidirectional changes to hippocampal theta–gamma comodulation predict memory for recent spatial episodes. *Proc Natl Acad Sci USA*. 2010;107(15):7054-7059. doi:10.1073/pnas.0911184107
248. Tort ABL, Komorowski RW, Manns JR, Kopell NJ, Eichenbaum H. Theta–gamma coupling increases during the learning of item–context associations. *Proc Natl Acad Sci USA*. 2009;106(49):20942-20947. doi:10.1073/pnas.0911331106
249. Seymour RA, Rippon G, Gooding-Williams G, Schoffelen JM, Kessler K. Dysregulated oscillatory connectivity in the visual system in autism spectrum disorder. *Brain*. 2019;142(10):3294-3305. doi:10.1093/brain/awz214
250. Peck F, Naples AJ, Webb SJ, et al. *Phase-Amplitude Coupling in Autism Spectrum Disorder: Results from the Autism Biomarkers Consortium for Clinical Trials*. *Neurology*; 2022. doi:10.1101/2022.09.25.22279830
251. Gregoriou GG, Gotts SJ, Zhou H, Desimone R. High-Frequency, Long-Range Coupling Between Prefrontal and Visual Cortex During Attention. *Science*. 2009;324(5931):1207-1210. doi:10.1126/science.1171402

252. Khan S, Gramfort A, Shetty NR, et al. Local and long-range functional connectivity is reduced in concert in autism spectrum disorders. *Proc Natl Acad Sci USA*. 2013;110(8):3107-3112. doi:10.1073/pnas.1214533110
253. Mamashli F, Khan S, Bharadwaj H, et al. Maturational trajectories of local and long-range functional connectivity in autism during face processing. *Human Brain Mapping*. 2018;39(10):4094-4104. doi:10.1002/hbm.24234
254. Mamashli F, Kozhemiako N, Khan S, et al. Children with autism spectrum disorder show altered functional connectivity and abnormal maturation trajectories in response to inverted faces. *Autism Research*. 2021;14(6):1101-1114. doi:10.1002/aur.2497
255. Radwan B, Dvorak D, Fenton AA. Impaired cognitive discrimination and discoordination of coupled theta–gamma oscillations in Fmr1 knockout mice. *Neurobiology of Disease*. 2016;88:125-138. doi:10.1016/j.nbd.2016.01.003
256. Davis P, Takach K, Maski K, Levin A. A circuit-level biomarker of Rett syndrome based on ectopic phase-amplitude coupling during slow-wave-sleep. *Cerebral Cortex*. Published online May 31, 2022:bhac226. doi:10.1093/cercor/bhac226
257. Banerjee A, Rikhye RV, Breton-Provencher V, et al. Jointly reduced inhibition and excitation underlies circuit-wide changes in cortical processing in Rett syndrome. *Proc Natl Acad Sci USA*. 2016;113(46). doi:10.1073/pnas.1615330113
258. Batista-Brito R, Vinck M, Ferguson KA, et al. Developmental Dysfunction of VIP Interneurons Impairs Cortical Circuits. *Neuron*. 2017;95(4):884-895.e9. doi:10.1016/j.neuron.2017.07.034
259. Ma G, Liu Y, Wang L, et al. Hierarchy in sensory processing reflected by innervation balance on cortical interneurons. *Sci Adv*. 2021;7(20):eabf5676. doi:10.1126/sciadv.abf5676
260. Mardinly AR, Spiegel I, Patrizi A, et al. Sensory experience regulates cortical inhibition by inducing IGF1 in VIP neurons. *Nature*. 2016;531(7594):371-375. doi:10.1038/nature17187
261. Mesik L, Ma W pei, Li L yun, et al. Functional response properties of VIP-expressing inhibitory neurons in mouse visual and auditory cortex. *Front Neural Circuits*. 2015;9:22. doi:10.3389/fncir.2015.00022
262. Millman DJ, Ocker GK, Caldejon S, et al. VIP interneurons in mouse primary visual cortex selectively enhance responses to weak but specific stimuli. *Elife*. 2020;9:e55130. doi:10.7554/eLife.55130
263. Hakim R, Shamardani K, Adesnik H. A neural circuit for gamma-band coherence across the retinotopic map in mouse visual cortex. *Elife*. 2018;7:e28569. doi:10.7554/eLife.28569
264. Veit J, Handy G, Mossing DP, Doiron B, Adesnik H. Cortical VIP neurons locally control the gain but globally control the coherence of gamma band rhythms. *Neuron*. 2023;111(3):405-417.e5. doi:10.1016/j.neuron.2022.10.036

265. Goff KM, Goldberg EM. Vasoactive intestinal peptide-expressing interneurons are impaired in a mouse model of Dravet syndrome. *eLife*. 2019;8:e46846. doi:10.7554/eLife.46846
266. Rahmatullah N, Schmitt LM, De Stefano L, et al. Hypersensitivity to Distractors in Fragile X Syndrome from Loss of Modulation of Cortical VIP Interneurons. *J Neurosci*. 2023;43(48):8172-8188. doi:10.1523/JNEUROSCI.0571-23.2023
267. Bach S, Shovlin S, Moriarty M, Bardoni B, Tropea D. Rett Syndrome and Fragile X Syndrome: Different Etiology With Common Molecular Dysfunctions. *Front Cell Neurosci*. 2021;15:764761. doi:10.3389/fncel.2021.764761
268. Henriksen MW, Ravn K, Paus B, Von Tetzchner S, Skjeldal OH. De novo mutations in SCN1A are associated with classic Rett syndrome: a case report. *BMC Med Genet*. 2018;19(1):184. doi:10.1186/s12881-018-0700-z
269. Hindriks R, Van Putten MJAM. Thalamo-cortical mechanisms underlying changes in amplitude and frequency of human alpha oscillations. *NeuroImage*. 2013;70:150-163. doi:10.1016/j.neuroimage.2012.12.018
270. Vanneste S, Song JJ, De Ridder D. Thalamocortical dysrhythmia detected by machine learning. *Nat Commun*. 2018;9(1):1103. doi:10.1038/s41467-018-02820-0
271. Bastos G, Holmes JT, Ross JM, et al. Top-down input modulates visual context processing through an interneuron-specific circuit. *Cell Reports*. 2023;42(9):113133. doi:10.1016/j.celrep.2023.113133
272. Chen TW, Li N, Daie K, Svoboda K. A Map of Anticipatory Activity in Mouse Motor Cortex. *Neuron*. 2017;94(4):866-879.e4. doi:10.1016/j.neuron.2017.05.005
273. Whittington MA, Traub RD. Interneuron Diversity series: Inhibitory interneurons and network oscillations in vitro. *Trends in Neurosciences*. 2003;26(12):676-682. doi:10.1016/j.tins.2003.09.016
274. Scala F, Kobak D, Shan S, et al. Layer 4 of mouse neocortex differs in cell types and circuit organization between sensory areas. *Nat Commun*. 2019;10(1):4174. doi:10.1038/s41467-019-12058-z
275. Börgers C, Epstein S, Kopell NJ. Background gamma rhythmicity and attention in cortical local circuits: A computational study. *Proc Natl Acad Sci USA*. 2005;102(19):7002-7007. doi:10.1073/pnas.0502366102
276. Chartove JAK, McCarthy MM, Pittman-Polletta BR, Kopell NJ. A biophysical model of striatal microcircuits suggests gamma and beta oscillations interleaved at delta/theta frequencies mediate periodicity in motor control. Migliore M, ed. *PLoS Comput Biol*. 2020;16(2):e1007300. doi:10.1371/journal.pcbi.1007300
277. Percy AK, Benke TA, Marsh ED, Neul JL. Rett syndrome: The Natural History Study journey. *Annals of the Child Neurology Society*. 2024;2(3):189-205. doi:10.1002/cns3.20086

278. Mariscal MichaelG, Berry-Kravis E, Buxbaum JD, et al. Shifted phase of EEG cross-frequency coupling in individuals with Phelan-McDermid syndrome. *Molecular Autism*. 2021;12(1):29. doi:10.1186/s13229-020-00411-9
279. Berman JI, Liu S, Bloy L, Blaskey L, Roberts TPL, Edgar JC. Alpha-to-Gamma Phase-Amplitude Coupling Methods and Application to Autism Spectrum Disorder. *Brain Connectivity*. 2015;5(2):80-90. doi:10.1089/brain.2014.0242
280. Dupré La Tour T, Tallot L, Grabot L, et al. Non-linear auto-regressive models for cross-frequency coupling in neural time series. Battaglia FP, ed. *PLoS Comput Biol*. 2017;13(12):e1005893. doi:10.1371/journal.pcbi.1005893
281. Tort ABL, Komorowski R, Eichenbaum H, Kopell N. Measuring Phase-Amplitude Coupling Between Neuronal Oscillations of Different Frequencies. *J Neurophysiol*. 2010;104(2):1195-1210. doi:10.1152/jn.00106.2010
282. Wang SY, Lee WT, Shieh JY, et al. Multidimensional Development and Adaptive Behavioral Functioning in Younger and Older Children With Rett Syndrome. *Physical Therapy*. 2022;102(4). doi:10.1093/ptj/pzab297
283. Sparrow SS, Cicchetti DV, Saulnier CA. Vineland Adaptive Behavior Scales, Third Edition (Vineland-3): Manual. Bloomington, MN: Pearson Assessments; 2016.
284. Barnes KV, Coughlin FR, O’Leary HM, et al. Anxiety-like behavior in Rett syndrome: characteristics and assessment by anxiety scales. *J Neurodevelop Disord*. 2015;7(1). doi:10.1186/s11689-015-9127-4
285. Esbensen AJ, Rojahn J, Aman MG, Ruedrich S. Reliability and Validity of an Assessment Instrument for Anxiety, Depression, and Mood Among Individuals with Mental Retardation. *J Autism Dev Disord*. 2003;33(6):617-629. doi:10.1023/b:jadd.00000005999.27178.55
286. Rudy B, Fishell G, Lee S, Hjerling-Leffler J. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Developmental Neurobiology*. 2011;71(1):45-61. doi:10.1002/dneu.20853
287. Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. Distinct Subtypes of Somatostatin-Containing Neocortical Interneurons Revealed in Transgenic Mice. *J Neurosci*. 2006;26(19):5069-5082. doi:10.1523/JNEUROSCI.0661-06.2006
288. Olufsen MS, Whittington MA, Camperi M, Kopell N. New roles for the gamma rhythm: population tuning and preprocessing for the Beta rhythm. *Journal of Computational Neuroscience*. 2003;14(1):33-54. doi:10.1023/A:1021124317706
289. Adam E, Kowalski M, Akeju O, et al. Ketamine can produce oscillatory dynamics by engaging mechanisms dependent on the kinetics of NMDA receptors. *Proc Natl Acad Sci USA*. 2024;121(22). doi:10.1073/pnas.2402732121

290. Kramer MA, Eden UT. *Case Studies in Neural Data Analysis: A Guide for the Practicing Neuroscientist*. The MIT Press; 2016.
291. Whittington MA, Cunningham MO, LeBeau FEN, Racca C, Traub RD. Multiple origins of the cortical gamma rhythm. *Developmental Neurobiology*. 2011;71(1):92-106. doi:10.1002/dneu.20814
292. Tzeng CP, Whitwam T, Boxer LD, et al. Activity-induced MeCP2 phosphorylation regulates retinogeniculate synapse refinement. *Proc Natl Acad Sci USA*. 2023;120(44):e2310344120. doi:10.1073/pnas.2310344120
293. Buzsáki G, Wang XJ. Mechanisms of Gamma Oscillations. *Annu Rev Neurosci*. 2012;35(1):203-225. doi:10.1146/annurev-neuro-062111-150444
294. Jensen O, Mazaheri A. Shaping Functional Architecture by Oscillatory Alpha Activity: Gating by Inhibition. *Front Hum Neurosci*. 2010;4. doi:10.3389/fnhum.2010.00186
295. Cardin JA. Snapshots of the Brain in Action: Local Circuit Operations through the Lens of γ Oscillations. *J Neurosci*. 2016;36(41):10496-10504. doi:10.1523/JNEUROSCI.1021-16.2016
296. Murdock MH, Yang CY, Sun N, et al. Multisensory gamma stimulation promotes glymphatic clearance of amyloid. *Nature*. 2024;627(8002):149-156. doi:10.1038/s41586-024-07132-6
297. Law RG, Pugliese S, Shin H, et al. Thalamocortical Mechanisms Regulating the Relationship between Transient Beta Events and Human Tactile Perception. *Cerebral Cortex*. 2022;32(4):668-688. doi:10.1093/cercor/bhab221
298. McGinley MJ, Vinck M, Reimer J, et al. Waking State: Rapid Variations Modulate Neural and Behavioral Responses. *Neuron*. 2015;87(6):1143-1161. doi:10.1016/j.neuron.2015.09.012

Supplemental Figures and Tables

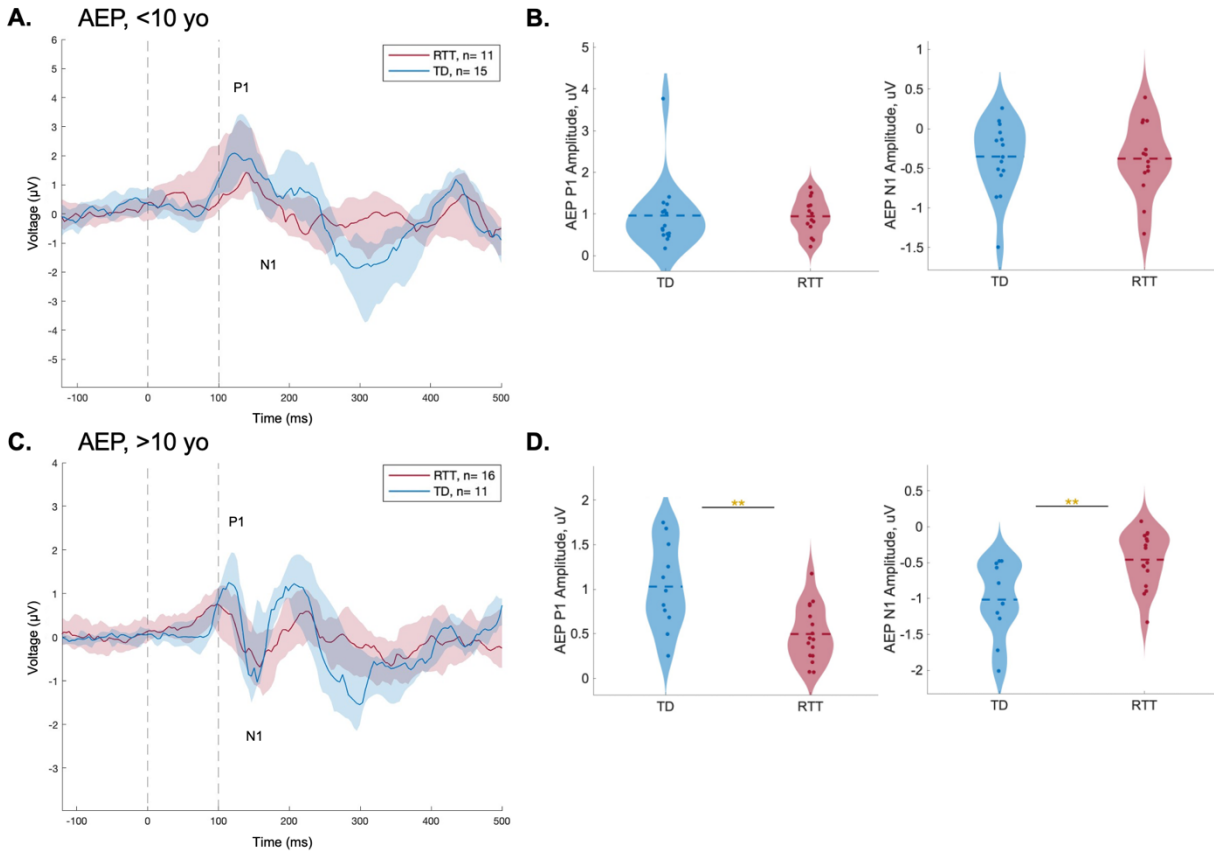


Figure S2.1. Difference in AEP amplitude emerges when stratifying by age. AEPs are shown for RTT (red) and TD (blue) participants in two age groups. **(A)** Grand average AEP waveforms are shown for participants below 10 years old (RTT $n=11$ and TD $n=15$). The P1 and N1 components are indicated. AEP was analyzed at frontal-central electrode Fz. Shaded areas represent the 25th to 75th percentile range across participants. Vertical dashed lines indicate stimulus onset (0ms) and 100ms post-stimulus. **(B)** Violin plots of corresponding AEP P1 and N1 amplitudes, where circles represent individual participants. Statistical significance is indicated, where *** indicates $P<0.001$, ** indicates $P<0.01$, and * indicates $P<0.05$. Violin widths represent kernel density estimates; dashed horizontal lines indicate group means. **(C-D)** Figures and analyses are repeated for participants above 10 years old (RTT $n=16$ and TD $n=11$).

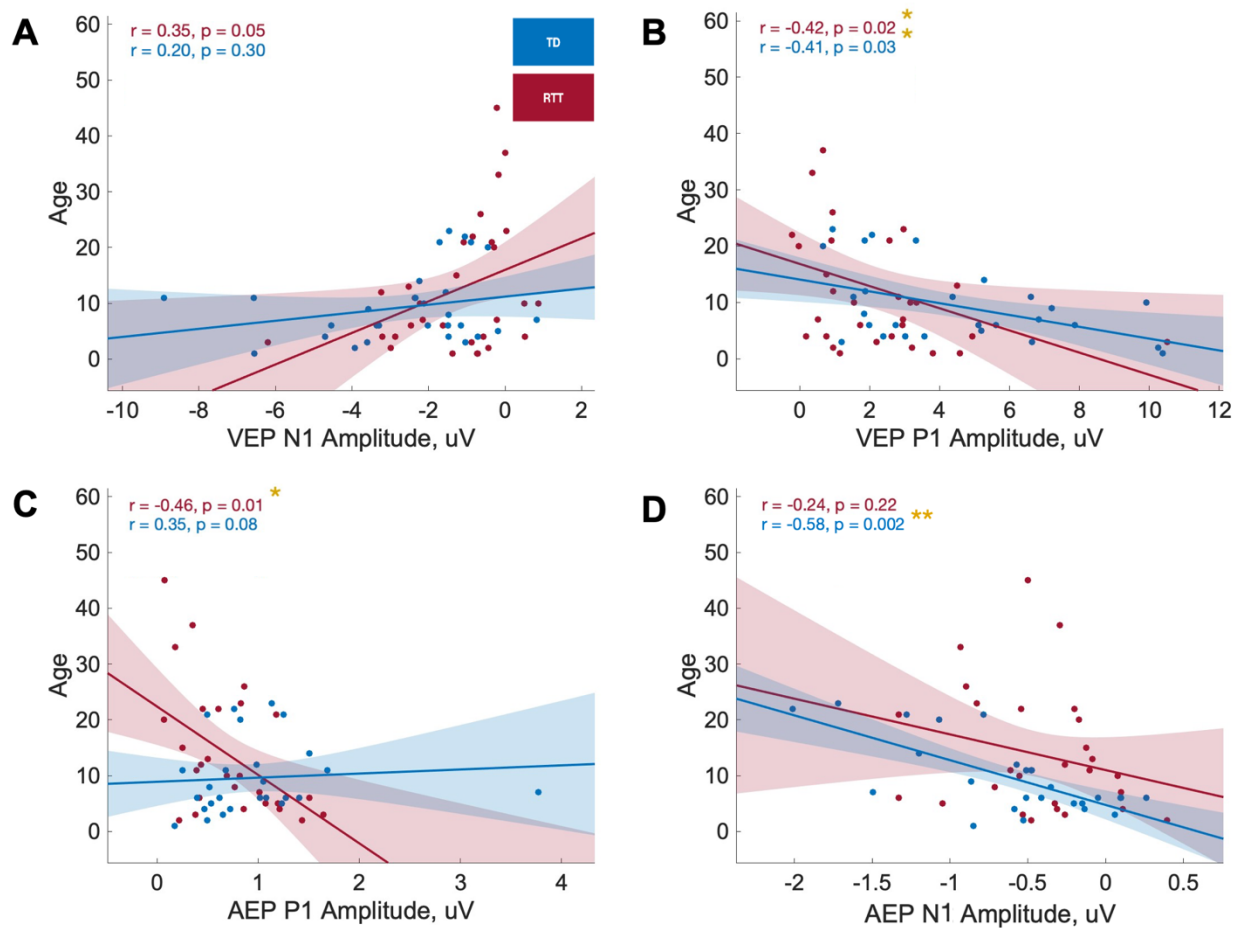


Figure S2.2. Amplitude in some ERP components is associated with age. Association between amplitude in VEP components and age (RTT $n=31$, TD $n=28$) (A-B), and between amplitude in AEP components and age (RTT $n=27$, TD $n=26$) (C-D). Shaded areas indicate 95% confidence intervals around linear regression lines. Statistical significance is indicated with r and P values, where: *** indicates $P<0.001$, ** indicates $P<0.01$, and * indicates $P<0.05$.

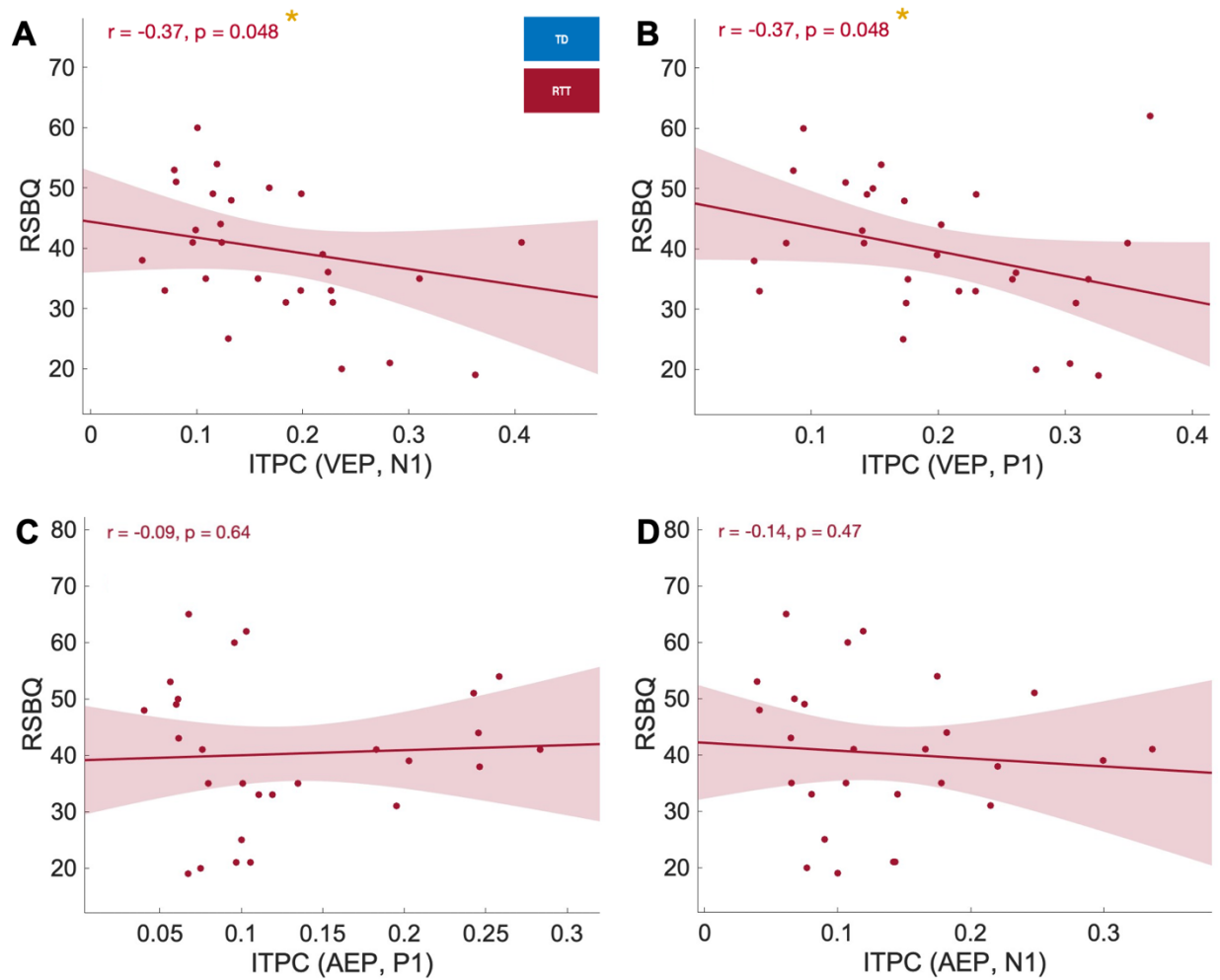


Figure S2.3. VEP phase coherence is associated with behavioral symptom severity in RTT. Association between ITPC in VEP components and RSBQ score ($n=29$) (A-B), and between ITPC in AEP components and RSBQ score ($n=27$) (C-D). Shaded areas indicate 95% confidence intervals around linear regression lines. Statistical significance is indicated with r and P values, where: *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$.

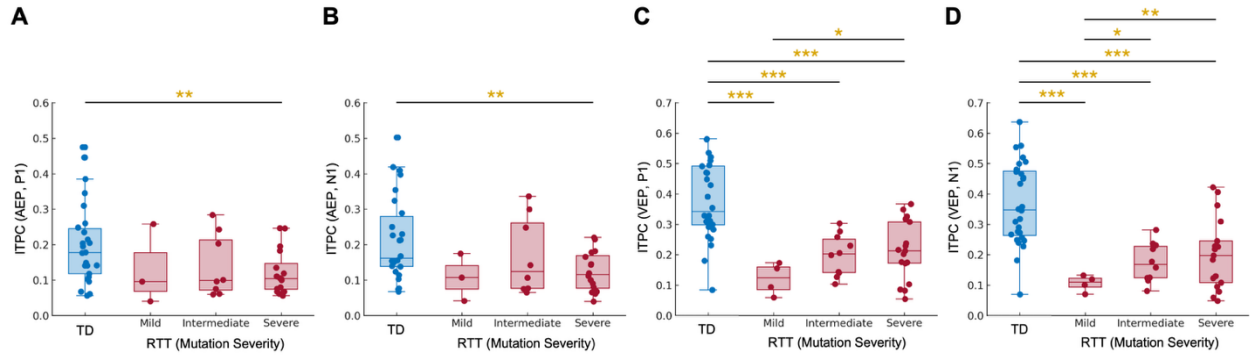


Figure S2.4. ITPC in visual-evoked potentials is impacted by *MECP2* mutation severity. Circles represent individual participant ITPC values. TD participants are shown in blue and RTT participants are shown in red, with RTT subgroups stratified by *MECP2* mutation severity (mild, intermediate, or severe). Statistical significance is indicated with horizontal brackets above the relevant group comparisons, where: *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$. **(A, B)** ITPC in AEP P1 (A) and AEP N1 (B) components. TD $n=26$, RTT: mild $n=3$, intermediate $n=8$, severe $n=16$. **(C, D)** ITPC in VEP P1 (C) and VEP N1 (D) components. TD $n=28$, RTT: mild $n=4$, intermediate $n=10$, severe $n=17$.

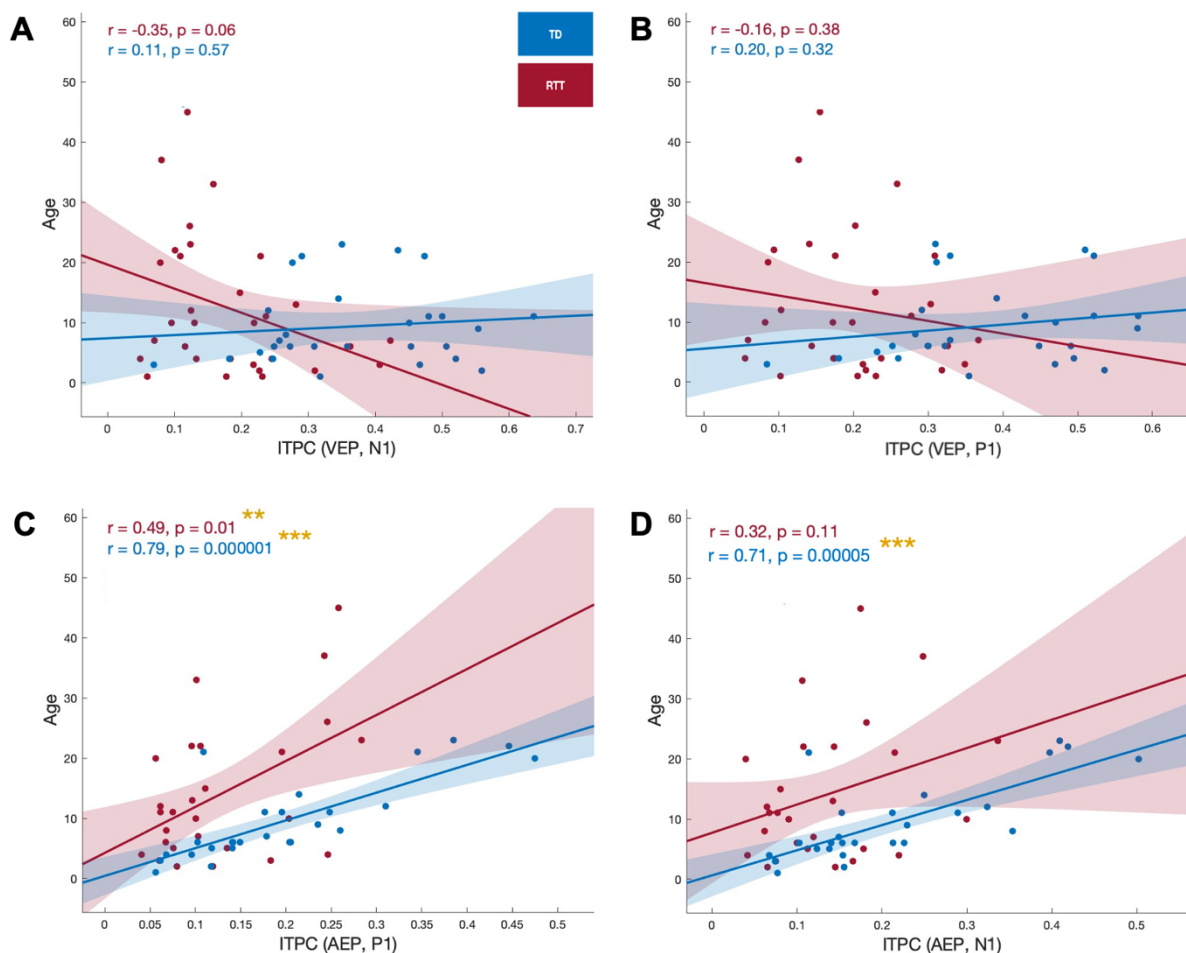


Figure S2.5. ITPC in some ERP components is associated with age. Association between ITPC in VEP components and age (RTT $n=31$, TD $n=28$) (A-B), and between ITPC in AEP components and age (RTT $n=27$, TD $n=26$) (C-D). Shaded areas indicate 95% confidence intervals around linear regression lines. Statistical significance is indicated with r and P values, where: *** indicates $P<0.001$, ** indicates $P<0.01$, and * indicates $P<0.05$.

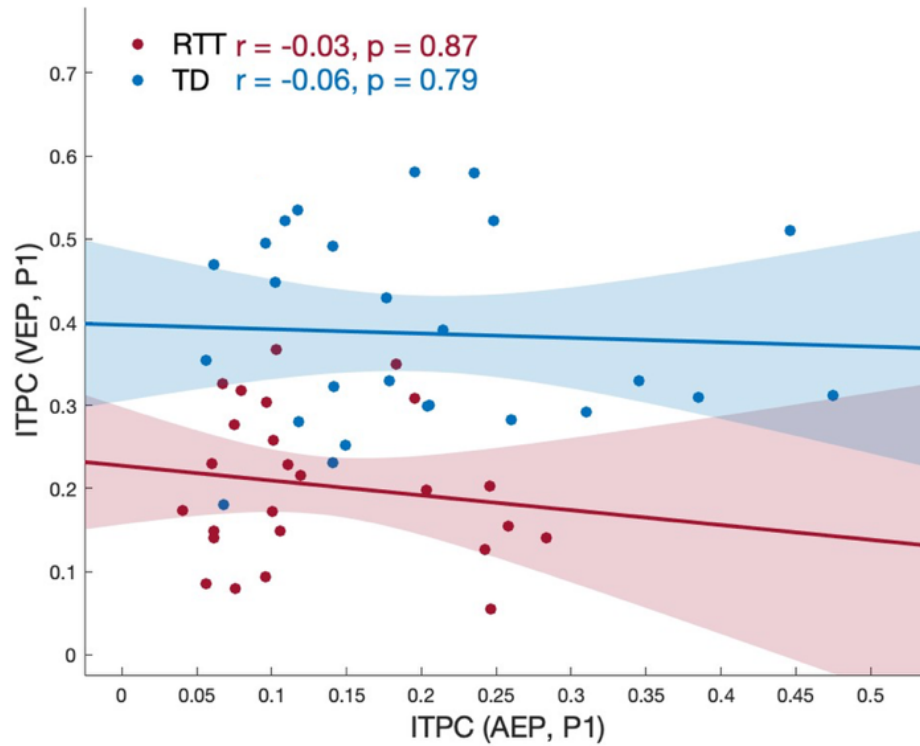


Figure S2.6. ITPC in visual- and auditory-evoked responses are not associated with each other. Association between ITPC in the VEP P1 component and ITPC in the AEP P1 component. RTT $n=25$ and TD $n=26$. Shaded areas indicate 95% confidence intervals around linear regression lines. Statistical significance is indicated with r and P values, where: *** indicates $P<0.001$, ** indicates $P<0.01$, and * indicates $P<0.05$.

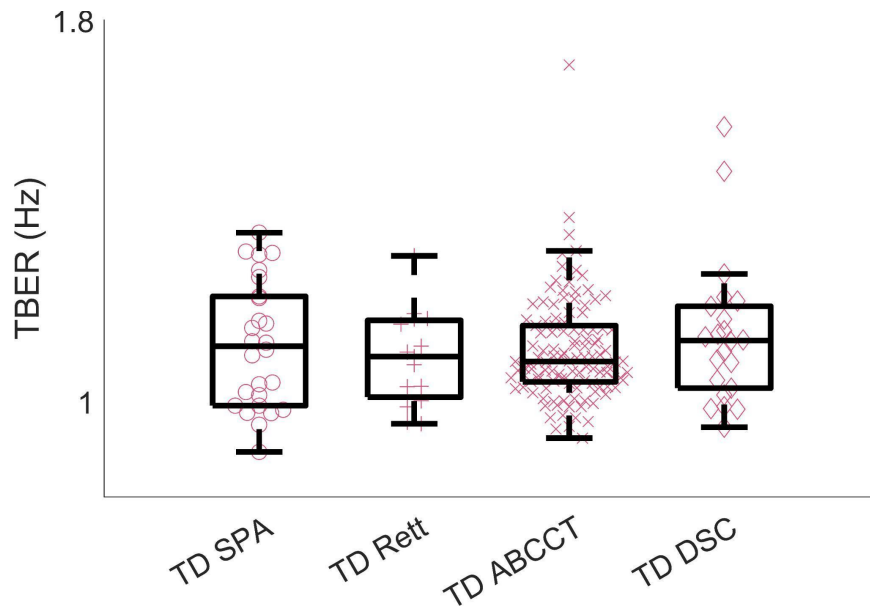


Figure S3.1. TBE-rates do not differ across TD cohorts. Kruskal-Wallis test demonstrated no significant differences in TD groups across studies ($p = .669$).

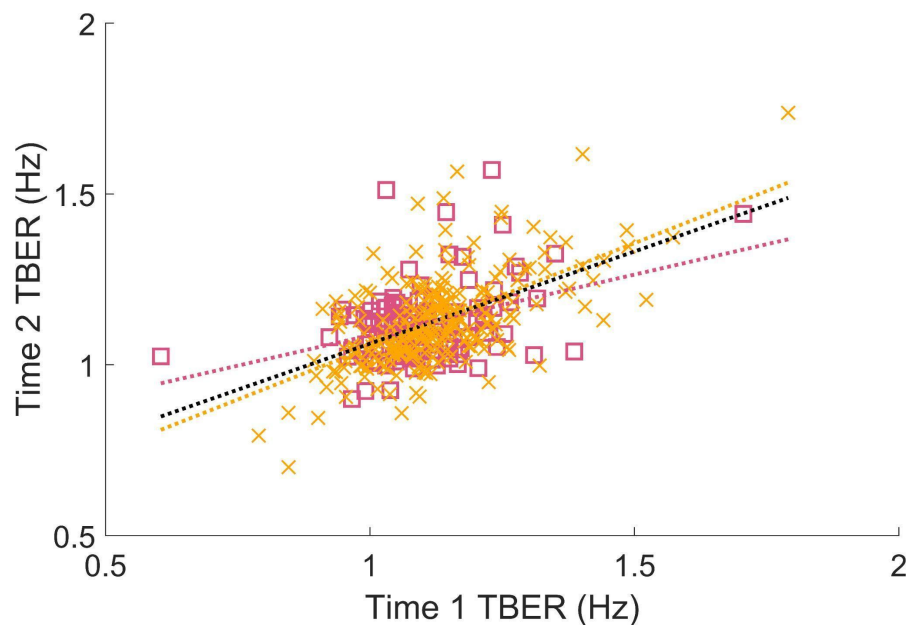


Figure S3.2. Test-retest reliability for ASD and TD groups. Test-tetest with ICCs for TD ABCCT (ICC = .365, 95% CI [.189, .517]) and ASD ABCCT (ICC = .606, 95% CI [.520, .679]) individually and collapsed into one group (ICC = .547, 95% CI [.470, .616]). Time 2 data was collected 6 weeks after Time 1. TD is pink square, $n = 108$; ASD is orange X, $n = 247$

Supplementary Table 3.1 | Demographics and Clinical Characteristics for TD Groups from Different Studies

Demographics	Group			
	TD ABC-CT	TD SPA	TD DSC	TD RTT
Sample Size	117	26	22	15
Age in Years				
Mean (SD)	8.5 (1.6)	3.5 (0.5)	8.2 (4.7)	5.5 (2.1)
Sex n (%)				
Male	81 (69%)	16 (62%)	10 (46%)	2 (13%)
Female	36 (31%)	10 (38%)	12 (54%)	13 (87%)
Race n (%)				
Asian	2 (2%)	2 (8%)	3 (14%)	2 (13%)
Black and/or African American	4 (3%)	1 (4%)	0	2 (13%)
White	97 (83%)	22 (84%)	18 (82%)	11 (74%)
Multiracial	13 (11%)	0	0	0
Other	1 (1%)	0	0	0
No Response	0	1 (4%)	1 (4%)	0
Ethnicity n (%)				
Hispanic	7 (6%)	3 (12%)	7 (32%)	2 (13%)
Not Hispanic	110 (94%)	22 (84%)	15 (68%)	13 (87%)
No Response	0	1 (4%)	0	0
Clinical Characteristics				
NVIQ				
Mean (SD)	112 (12.8)	107 (16.9)	113 (14.0)	

Supplementary Table 3.2 | Demographics and Clinical Characteristics for ASD, Neurogenetic, and Combined TD Groups

Demographics	Diagnostic Group							
	ASD ABC-CT	ASD SPA	PMS	TSC	PHTS	RTT	SYNGAPI	TD
Sample Size	262	26	41	25	12	15	9	180
Age in Years								
Mean (SD)	8.6 (1.6)	3.7 (0.6)	7.2 (3.5)	6.7 (2.3)	6.1 (2.6)	8.5 (6.7)	7.2 (2.3)	7.5 (2.8)
Sex n (%)								
Male	201 (77%)	21 (81%)	18 (44%)	13 (52%)	4 (33%)	2 (13%)	4 (44%)	71 (39%)
Female	61 (23%)	5 (19%)	23 (56%)	12 (48%)	8 (67%)	13 (87%)	5 (55%)	109 (61%)
Race n (%)								
Asian	14 (5%)	6 (23%)	2 (5%)	2 (8%)	0	0	0	9 (5%)
Black and/or African American	22 (8%)	2 (8%)	2 (5%)	0	0	0	0	7 (4%)
White	176 (67%)	13 (50%)	35 (85%)	20 (80%)	11 (92%)	14 (93%)	7 (78%)	148 (82%)
Multiracial	42 (16%)	3 (11%)	0	0	0	0	0	13 (7%)
Other	6 (2%)	0	2 (5%)	0	0	1 (7%)	0	1 (<1%)
No Response	2 (<1%)	2 (8%)	0	3 (12%)	1 (8%)	0	2 (22%)	2 (1%)
Ethnicity n (%)								
Hispanic	47 (18%)	7 (27%)	3 (7%)	5 (20%)	2 (17%)	2 (13%)	1 (11%)	19 (11%)
Not Hispanic	215 (82%)	17 (65%)	38 (93%)	19 (76%)	10 (83%)	13 (87%)	6 (67%)	160 (89%)
No Response	0	2 (8%)	0	1 (4%)	0	0	2 (22%)	1 (<1%)
Clinical Characteristics								
ASD diagnosis ^a	262 (100%)	26 (100%)	25 (60.9%)	7 (30.4%)	5 (62.5%)		7 (77.7%)	0
NVIQ								
Mean (SD)	98 (16.6)	75 (24.8)	42 (21.3)	59 (33.4)	72 (23.6)	NA ^b	NA ^b	111 (13.6)
Seizures n (%)	^c	0	7 (17%)	21 (84%)	1 (8%)	12 (80%)	8 (89%)	^c

^a Percent ASD = ASD/ASD + No ASD. Missing data PMS n=0; TSC n=2; PHTS n=4; SYNGAPI n=0.

^b Not Available: No formal cognitive assessments were done

Supplementary Table 3.3 TBE-rates by Group

Diagnostic Group	TBE-rates Median (IQR)
TD	1.10 (1.03 - 1.17)
ASD SPA	1.26 (1.12 - 1.44)
ASD ABC-CT	1.11 (1.05 - 1.18)
PMS	1.21 (1.12 - 1.59)
TSC	1.09 (0.86 - 1.44)
PHTS	1.07 (0.98 - 1.14)
RTT	1.52 (1.29 - 1.52)
SYNGAPI	1.40 (1.20 - 1.55)

Supplementary Table 3.4 ASD in Neurogenetic Groups

	ASD	No ASD	Missing	%
PMS	25	16	0	60.90%
TSC	7	16	2	30.40%
PHTS	5	3	4	62.50%
SYNGAPI	7	2	0	77.70%

Supplementary Table 3.5. SP2 Tactile Measures

Diagnostic Group	Touch Domain Median (IQR)	Tactile Hyper-responsivity Median (IQR)	Tactile Hypo-responsivity Median (IQR)
TD	13.0 (11-14)	5 (5-6)	7.0 (6-8)
ASD	21.0 (15-24.5)	6.5 (5-10.25)	11.5 (8.25-16.25)

Supplementary Table 3.6. Medication List for Participants with Extreme TBE-Rates.

		Diagnostic Group						
		ASD ABC-CT	ASD SPA	PMS	TSC	Rett	SYNGAPI	TD
Extreme TBER participants (N) ^a		13	8	15	1	10	5	4
Not taking any medications		2	6	1		1	1	3
Centrally acting	Alpha2-Adrenergic Agonist (Clonidine or Guanfacine)			4		5		
	Atypical Antipsychotic (Lurasidone or Quetiapine)	1		1				
	Benzodiazepine (Clobazam, Lorazepam, Clonazepam, Diazepam, or Midazolam)			4		3	2	
	Buspirone					1		
	Cannabidiol					1	2	
	Carbonic Anhydrase Inhibitor (Acetazolamide)				1			
	Dextroamphetamine and amphetamine (Adderall)	1						
	Epinephrine					2		
	Gabapentin			1		2		
	Ketamine					1		
	Glycine-proline-glutamate analog (Trofinetide)					1		
	Histamine H1 Antagonist (Diphenhydramine, Cyproheptadine, Loratadine or Hydroxyzine)	3		2		2		1
	Lacosamide			1		1		
	Lamotrigine			1				
	Levetiracetam			1		1	1	
	Melatonin	5	1	3		3		
	Methylphenidate	2		2				
	Mirtazapine					1		
	Oxcarbazepine			1				
	Phenytoin					1		
	Selective Serotonin Reuptake Inhibitor (Escitalopram, Fluoxetine, Sertraline, Trazodone)	3		1		2	1	
	Topiramate					1	1	
	Valproic Acid					1		
Other medications and supplements	Albuterol Inhaler	1		1		2		
	Antibiotic (Amoxicillin or Azithromycin)	1		1				
	Antifungal (Fluconazole, Nystatin)		1	1				
	Calcium/ magnesium			1				
	Carnitine			1				

Ethinyl estradiol-norethindrone				1	
Fish Oil				1	
Fluoride topical toothpaste				1	
Fluticasone				2	
Folinic Acid		1			
Glycerin Suppository				1	
Glycopyrrolate				1	
Growth hormone		1			
Histamine H2 Antagonist (Famotidine or Ranitidine)		1		1	
Lactulose		1			
Levocarnitine				1	
L-theanine	1				
Milk of magnesia				1	
Multivitamin	4		3		1
Nystatin ointment		1			
Omega 3			1		1
Polyethylene glycol	1		2		
Probiotic			4	1	
Proton Pump inhibitor (Omeprazole or Lansoprazole)			3		
Prucalopride				1	
Ranitidine	1				
Senna			1	1	
Vitamin D	3		3	2	
Zinc			1		

^a Extreme = if individual value was above the top TD “whisker”, exceeding the 75th percentile by more than 1.5 x the IQR value
Note: There were no participants with extreme TBE-rates in the PHTS group.

Supplementary Table 3.7. EEG Exclusion Breakdown by Cohort.

Cohort name	Participants that sat for EEG	EEGs Rejected by Segment Threshold	EEGs Rejected by Quality Control Metrics	Usable EEG
ABC-CT – ASD (T1)	271	0	9	262
ABC-CT – ASD (T2)	253	0	6	247
ABC-CT – TD (T1)	117	0	0	117
ABC-CT – TD (T2)	108	0	0	108
SPA - ASD	29	0	2	26
SPA - TD	29	1	2	26
DSC - PMS	42	0	1	41
DSC - TSC	26	1	0	25
DSC - PHTS	13	0	1	12
DSC - TD	22	0	0	22
RTT	19	0	4	15
RTT - TD	15	0	0	15
SYNGAPI	10	0	1	9

Appendix. Computational Methods

Neurons

Our model of the cortical Layer IV uses single-compartment, biophysical models of four types of neurons: PV+INs, VIP+INs, SOM+INs and excitatory cells (pyramidal cells or stellate cells) called PYR. The voltage (V) of each neuron changes in time according to an equation that equates the capacitive membrane current of each neuron with its membrane ionic currents. This equation is given by:

$$c_m \frac{dV}{dt} = -\sum I_{memb} - \sum I_{syn} + I_{app} \quad (1)$$

In (1), the membrane voltage (V) has units of mV . The membrane ionic currents (I_{memb}), the synaptic current (I_{syn}), and the applied current (I_{app}) all have units of $\mu A/cm^2$. The specific membrane capacitance (c_m) is set to $1\mu F/cm^2$ for all neurons. All ionic membrane and synaptic current have Hodgkin-Huxley-type conductances formulated as:

$$I = \bar{g} m^n h^k (V - E_{ion}) \quad (2)$$

In (2), the maximal ionic conductance ($\bar{g}$) uses units of $\mu S/cm^2$ and the ionic reversal potential (E_{ion}) has units of mV . Each ionic current has n activation gates (m) and k inactivation gates (h). For the membrane currents, the activation and inactivation gating variables evolve according to a two-state kinetic equation formulated (written for the gating variable m) as:

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)} \quad (3)$$

The steady-state function ($m_\infty(V)$) and the time constant of decay ($\tau_m(V)$) are dependent on voltage and may be re-written as rate functions ($\alpha_m(V), \beta_m(V)$) using the transformation:

$$m_{\infty}(V) = \alpha_m(V) / (\alpha_m(V) + \beta_m(V))$$

$$\tau_m(V) = 1 / (\alpha_m(V) + \beta_m(V))$$

The equations for the synaptic currents (I_{syn}) describe the dynamics of the interaction between the presynaptic (*pre*) and postsynaptic (*post*) neurons:

$$I_{post} = \bar{g}s(V_{pre})(V - E_{ion}) \quad (4)$$

Each post synaptic current has a single activation gate (s), which is dependent on the voltage of the pre-synaptic neuron (V_{pre}). The applied current (I_{app}) is either a constant term or a noise term or a sum of both. I_{app} determines the excitability level of an individual cell.

Excitatory Cells

The excitatory neurons have spiking currents (i.e., sodium current (I_{Na}), potassium current (I_K) and leak current (I_L)) as well as an M-current (I_M) and an h-current (I_h), both known to be found in cortical pyramidal cells.^{1,2} Synaptic currents to the excitatory cells come from inhibitory input from SOM+INs (I_{soma}), PV+INs (I_{pve}) and VIP+INs (I_{vip_e}) as well as excitatory input from other excitatory cells (I_{e_e}). The voltage change in an excitatory cell is described by:

$$c_m \frac{dV}{dt} = -I_{Na} - I_K - I_L - I_M - I_h - I_{pve} - I_{vip_e} - I_{soma} - I_{e_e} + I_{app} \quad (5)$$

Models of the spiking currents (I_{Na}, I_K, I_L) are taken from a previous formulation of these currents.³ The maximal sodium conductance is set to $\bar{g}_{Na} = 100 \text{ mS/cm}^2$. The sodium reversal potential is $E_{Na} = 50 \text{ mV}$. This sodium current has three activation gates ($n = 3$) and one inactivation gate ($k = 1$). The rate constants for the activation (m) and inactivation (h) variables are described by:

$$\alpha_m = \frac{0.32(V + 54)}{1 - \exp[-(V + 54)/4]}$$

$$\beta_m = \frac{0.28(V + 27)}{\exp[(V + 27)/5] - 1}$$

$$\alpha_h = 0.128 \exp[-(V + 50)/18]$$

$$\beta_h = \frac{4}{1 + \exp[-(V + 27)/5]}$$

The maximal conductance for the fast potassium channel is $\bar{g}_K = 80 \text{ mS/cm}^2$ and the reversal potential for potassium is $E_K = -100 \text{ mV}$. The fast potassium channel has no inactivation gates and four activation gates described by the rate functions:

$$\alpha_m = \frac{0.032(V + 52)}{1 - \exp[-(V + 52)/5]}$$

$$\beta_m = 0.5 \exp[-(V + 57)/40]$$

The leak current (I_L) has no gating variable. The maximal leak channel conductance is $g_L = 0.1 \text{ mS/cm}^2$. The leak current reversal potential is $E_L = -67 \text{ mV}$.

The formulation of the M-current comes from Mainen et al., 1996.⁴ It has one activation gate and no inactivation gate. The maximal conductance for the M-current, $\bar{g}_M$ is 1.3 mS/cm^2 . The rate functions for the M-current activation variable are:

$$\alpha_m = \frac{Q_s 10^{-4}(V + 30)}{1 - \exp[-(V + 30)/9]}$$

$$\beta_m = -\frac{Q_s 10^{-4}(V + 30)}{1 - \exp[(V + 30)/9]}$$

For the M-current, a Q_{10} factor of 2.3 is used in scaling the rate functions because the kinetics were originally derived from experiments performed at 23°C . To model dynamics of the M-current at a normal body temperature of 37°C , the rate equations for the M-current are scaled by:

$$Q_s = Q_{10}^{(37^\circ\text{C}-23^\circ\text{C})/10} = 3.209$$

The model for the H-current comes from Jones et al., 2000.⁵ It has one activation gate and no inactivation gate. The maximal conductance for the h-current ($\bar{g}_h$) is 1.2 mS/cm². The reversal potential for the h-current is $E_h = -43\text{mV}$. The h-current activation variable (r) steady state function and time constant of decay are given by:

$$r_\infty(V) = \frac{1}{1 + \exp((V + 75)/5.5)}$$

$$\tau_r = \frac{1}{\exp(-14.59 - 0.086 \cdot V) + \exp(-1.87 + 0.0701 \cdot V)}$$

The background excitation to each pyramidal cell was provided by giving independent Gaussian noise to each neuron with an amplitude of $120 * \sqrt{0.01}$ where 0.01 is the time step of integration. Network properties including synaptic currents ($I_{pv_e}, I_{vip_e}, I_{som_e}, I_{e_e}$) are discussed below.

PV Cells

Each PV neuron derives its membrane currents from the I-cell model in Olufsen et al., 2003³:

$$c_m \frac{dV}{dt} = -I_{Na} - I_K - I_L - I_{vip_{pv}} - I_{som_{pv}} - I_{pv_{pv}} - I_{e_{pv}} - I_{elec} \quad (6)$$

The formulations of the I_{Na} , I_K and I_L are the same as for our model excitatory cell including the same values for all maximal conductances and reversal potentials. The PV cells did not have a background excitation term and thus were silent without excitatory input. Electrical (I_{elec}) and ionic synaptic connections ($I_{vip_{pv}}$, $I_{som_{pv}}$, $I_{pv_{pv}}$, $I_{e_{pv}}$) are discussed below.

SOM Cells

Our model SOM cells were constructed to mimic the dynamics of Layer IV projecting SOM cells (also known as X94 cells) as described in Ma et al., 2006, which include delayed spiking, stut-

tering (which could be at alpha frequency) or tonic spiking with increasing levels of depolarizing currents as well as rebound excitation in response to hyperpolarizing currents.⁶ These dynamics were captured by inclusion of the spiking currents (sodium, potassium and leak), an A-type potassium current (for delayed spiking), a D-type potassium current for stuttering dynamics,⁷ and an h-current for rebound to hyperpolarization as suggested by Ma et al., 2006. Our SOM cells is formulated as:

$$c_m \frac{dV}{dt} = -I_{Na} - I_K - I_L - I_A - I_D - I_h - I_{vip_{som}} - I_{pv_{som}} - I_{e_{som}} - I_{elec} + I_{app} \quad (7)$$

The formulations of the I_{Na} , I_K and I_L derive from Golomb et al., 2007.⁷ The maximal sodium conductance is set to $\bar{g}_{Na} = 112.5 \text{ mS/cm}^2$. The sodium reversal potential is $E_{Na} = 50 \text{ mV}$. The sodium current has three activation gates and one inactivation gate. The activation variable (m) is modeled by its the steady state function ($m = m_\infty$) formulated as:

$$m_\infty(V) = \frac{1}{1 + \exp(-(V + 24)/11.5)}$$

The kinetics of the inactivation gate (h) are given by:

$$h_\infty(V) = \frac{1}{1 + \exp((V + 58.3)/6.7)}$$

$$\tau_h = 0.5 + \frac{14}{1 + \exp((V + 60)/12)}$$

The fast potassium current is modeled using two activation gates and no inactivation gates. The maximal conductance of the fast potassium channel is $\bar{g}_K = 225 \text{ mS/cm}^2$. The potassium reversal potential is $E_K = -90 \text{ mV}$. The kinetics of the activation gate (n) are described by:

$$n_\infty(V) = \frac{1}{1 + \exp(-(V + 12.4)/6.8)}$$

$$\tau_n = \left[0.087 + \frac{11.4}{1 + \exp((V + 14.6)/8.6)} \right] \cdot \left[0.087 + \frac{11.4}{1 + \exp(-(V - 1.3)/18.7)} \right]$$

The leak current has maximal conductance $I_L = 0.27 \text{ mS/cm}^2$ and its reversal potential is $E_L = -70 \text{ mV}$.

The D-type potassium current is derived from Golomb et al., 2007, and has a maximal conductance of $\bar{g}_D = 5 \text{ mS/cm}^2$.⁷ The D-current has three activation gates (m) and one inactivation gate (h) with the following kinetics:

$$m_\infty(V) = \frac{1}{1 + \exp(-(V + 50)/20)}$$

$$h_\infty(V) = \frac{1}{1 + \exp((V + 70)/6)}$$

$$\tau_m = 2$$

$$\tau_h = 150$$

The model of the A-type potassium current is derived from Traub et al., 2003.⁸ The maximal conductance of the A-current is $\bar{g}_A = 3 \text{ mS/cm}^2$. The A-current has four activation gates (m) and one inactivation gate (h) with kinetics formulated as:

$$m_\infty = \frac{1}{1 + \exp[-(V + 60)/8.5]}$$

$$\tau_m = 0.185 + \frac{0.5}{\exp[(V + 35.8)/19.7] + \exp[-(V + 79.7)/12.7]}$$

$$h_\infty = \frac{1}{1 + \exp[(V + 78)/6]}$$

$$\tau_h = \begin{cases} \frac{0.5}{\exp[(V + 46)/5] + \exp[-(V + 238)/37.5]} & \text{if } V < -63 \text{ mV} \\ 9.5 & \text{if } V \geq -63 \text{ mV} \end{cases}$$

The model of the SOM cell h-current comes from Kramer et al., 2008.⁹ The maximal h-current conductance is $\bar{g}_h = 2 \text{ mS/cm}^2$, and the h-current reversal potential is $E_h = -35 \text{ mV}$. The h-current has one activation gate (m) and no inactivation gate. The h-current activation variable

steady state function and time constant of decay are given by:

$$r_{\infty}(V) = \frac{1}{1 + \exp((V + 75)/5.5)}$$

$$\tau_r = \frac{1}{\exp(-14.6 - 0.086V) + \exp(-1.87 + 0.07V)}$$

The background excitation to each SOM cell was provided by giving an applied current of $I_{app} = 10\mu A/cm^2$. This value of I_{app} put the SOM cell in its alpha-frequency stuttering mode at baseline (in the absence of any connections with other cells).

VIP Cells

Our VIP cell is modeled as the irregularly spiking (stuttering) type of VIP cell.^{10,11} These cells have a D-type potassium current,¹¹ an M-type potassium current,¹² and a T-type calcium current.¹³ The voltage change of the VIP cells was modeled by the equation:

$$c_m \frac{dV}{dt} = -I_{Na} - I_K - I_L - I_D - I_M - I_T - I_{somvip} - I_{pvvip} - I_{evip} - I_{elec} + I_{app} \quad (8)$$

The sodium current (I_{Na}), the potassium current (I_K) and the D-type potassium current (I_D) are from Golomb et al., 2007.⁷ The formulations of these currents are the same as the ones used for the SOM cell. The sodium current has a maximal conductance of $\bar{g}_{Na} = 112.5 mS/cm^2$ and a reversal potential of $E_{Na} = 50mV$. The potassium current has a maximal conductance of $\bar{g}_K = 225 mS/cm^2$. The potassium reversal potential is $E_K = -90mV$. The maximal conductance of the D-current is $\bar{g}_D = 3 mS/cm^2$. The leak current maximal conductance is $I_L = 0.25 mS/cm^2$ and the leak current reversal potential is $E_L = -70mV$. The background excitation is set to $I_{app} = 10\mu A/cm^2$.

The model of the M-current comes from Mainen et al., 1996.⁴ The formulation of this current is the same as for the excitatory cell. The maximal conductance for the M-current, $\bar{g}_M$ is $1 mS/cm^2$.

The dynamics of the T-type calcium current are governed by a constant field equation as in Huguenard and McCormick, 1992.¹⁴

$$I_T = Pz^2m^2h \cdot \frac{0.001F^2V}{RT} \cdot \frac{[Ca]_i - [Ca]_o \cdot \exp[-z0.001FV/(RT)]}{1.0 - \exp[-z0.001 * F * V/(RT)]} \quad (9)$$

where $P = 0.02 \text{ cm}^3/s$ is the maximal permeability of calcium across the membrane, and $z = 2$ is the valence of calcium. Faraday's constant is $F = 96489 \text{ C/mole}$. The temperature in Kelvin is 310 K , which is equivalent to a body temperature of 37°C . The gas constant is $R = 8.31 \text{ Joules/(mole} \cdot \text{K)}$. The internal calcium concentration ($[Ca]_i$) is 10 nM and the external calcium concentration ($[Ca]_o$) is 3 mM .

The steady state activation curves and time constants of decay for the activation (m) and inactivation (h) gates are:

$$m_\infty(V) = \frac{1}{1 + \exp(-(V + 57)/6.2)}$$

$$h_\infty(V) = \frac{1}{1 + \exp((V + 81)/4)}$$

$$\tau_m = \frac{1}{\exp(\frac{V+132}{-16.7}) + \exp(\frac{V+16.8}{18.2})} + 0.612$$

$$\tau_h = \exp((V + 467)/66.6), \quad \text{if } V < 81 \text{ mV}$$

$$\tau_h = \exp(-(V + 22)/10.5) + 28, \quad \text{if } V \geq 81 \text{ mV}$$

Synaptic currents (I_{somvip} , I_{pvvip} , I_{evip}) and electrical connections between VIP cells (I_{elec}) are discussed below.

Parameter changes to simulate interneuron dysfunction

We model excitatory deficiency in VIP by decreasing its maximal sodium conductance to $I_{Na} = 90 \text{ mS/cm}^2$, which is low enough to decrease VIP excitability but not low enough to eliminate all VIP spiking during network activity. Our reason for choosing to decrease I_{Na} rather than I_{app} was to account for findings that classic Rett syndrome could be associated with mutations in SCN1A, which codes for a subunit in the voltage-gated sodium channel.¹⁵ We note that loss of

function variants in the SCN1A gene are usually associated with Dravet syndrome (also known as severe myoclonic epilepsy in infancy) another severe neurodevelopmental disorder.¹⁶ Mouse models with SCN1A impairment have shown deficits in the voltage-gated sodium channels of irregularly spiking VIP cells,¹² the type of VIP cell modeled in this study. Thus, we choose to model impairment of VIP cell excitability as deficits in the sodium channel conductance.

For investigation regarding the contribution of a particular types of interneurons (SOM, VIP, or PV interneurons) to PAC, we decreased either the maximal conductance of I_{Na} or the value of I_{app} for a particular interneuron type to a level low enough so that no spiking occurred in that cell type, which effectively removed that cell type from the network activity.

Synaptic connections

We modeled two types of ionic synaptic currents (I_{syn}): AMPA currents (I_{AMPA}) from excitatory pyramidal or stellate cells and GABAa currents (I_{GABAa}) from all three interneuron subtypes. Both synaptic currents are derived or modified from Olufsen et al., 2003³ and are formulated as:

$$I_{syn} = \bar{g}s(v_{pre})(V - E_{ion}) \quad (10)$$

Each synaptic current has one activation gate (s), which depends on the voltage of the pre-synaptic neuron (v_{pre}). E_{ion} is the synaptic reversal potential, and the maximal synaptic conductance is denoted by $\bar{g}$. The GABAa current is modeled by:

$$I_{GABAa} = \frac{\bar{g}_i}{N}s(V - E_i)$$

The maximal GABAa conductance ($\bar{g}_i$) of a neuron is normalized by the number (N) of presynaptic neurons of a specific type projecting to that neuron. The value of $\bar{g}_i$ depends on the pre- and post-synaptic neurons. The value of $\bar{g}_i$ in units of mS/cm^2 for each pre- and post-synaptic pair is: $\bar{g}_{VIP-to-PV} = 0.1$, $\bar{g}_{PV-to-VIP} = 0.01$, $\bar{g}_{VIP-to-SOM} = 0.08$, $\bar{g}_{SOM-to-VIP} = 0.5$, $\bar{g}_{SOM-to-PV} = 0.1$, $\bar{g}_{PV-to-SOM} = 0.1$, $\bar{g}_{PV-to-PV} = 0.2$, $\bar{g}_{PV-to-PYR} = 0.4$, $\bar{g}_{SOM-to-PYR} = 0.2$,

and $\bar{g}_{VIP-to-PYR} = 0.01$. The reversal potential for the GABA_A current (E_i) is set to -80 mV for all neurons.

The activation gate (s) of each GABA_A current has kinetics described by:

$$\frac{ds}{dt} = \bar{g}(V_{pre})(1 - s) - s/\tau_i \quad (11)$$

For most GABA_A synaptic connections $\bar{g}(V_{pre})$ is formulated as:

$$\bar{g}(V_{pre}) = 2(1 + \tanh(\frac{V_{pre}}{4}))$$

The exceptions occur for SOM-to-PV connections, where $\bar{g}(V_{pre})$ is

$$\bar{g}(V_{pre}) = 2.5(1 + \tanh(\frac{V_{pre}}{0.1}))$$

and for SOM-to-VIP and SOM-to-PYR connections, where $\bar{g}(V_{pre})$ is:

$$\bar{g}(V_{pre}) = 5(1 + \tanh(\frac{V_{pre}}{0.1}))$$

The time constant of decay (τ_i) of activation gate (s) depends on the type of pre- and post-synaptic neurons. The value of τ_i in units of milliseconds for each pre- and post-synaptic neuron is: $\tau_{PV-to-VIP} = 18$, $\tau_{PV-to-PYR} = 8$, $\tau_{PV-to-PV} = 8$, $\tau_{PV-to-SOM} = 18$, $\tau_{VIP-to-SOM} = 40$, $\tau_{VIP-to-PV} = 6$, $\tau_{VIP-to-PYR} = 40$, $\tau_{SOM-to-PV} = 40$, $\tau_{SOM-to-VIP} = 20$, $\tau_{SOM-to-PYR} = 20$. Values of τ_i were taken from the literature when found.¹⁷⁻¹⁹

The AMPA current is modeled by:

$$I_{AMPA} = \frac{\bar{g}_e}{N} s(V - E_e)$$

The maximal AMPA conductance ($\bar{g}_e$) of a neuron is normalized by the number (N) of presynaptic excitatory neurons projecting to that neuron. The value of $\bar{g}_e$ depends on the type of post-synaptic neuron. The value of $\bar{g}_e$ in units of mS/cm^2 for each pre- and post-synaptic pair is:

$\bar{g}_{PYR-to-PV} = 0.4$, $\bar{g}_{PYR-to-VIP} = 0.3$, $\bar{g}_{PYR-to-SOM} = 0.1$. Additionally, we included extremely weak synaptic currents between PYR cells ($\bar{g}_{PYR-to-PYR} = 0.001$) in order to generate an approximation of the LFP, which was modeled as the sum of all PYR-to-PYR AMPA currents in the network. The reversal potential for the AMPA current (E_e) is set to 0 mV for all neurons.

The activation gate (s) of each AMPA current has kinetics described by:

$$\frac{ds}{dt} = \bar{g}(V_{pre})(1 - s) - s/\tau_e \quad (12)$$

$$\bar{g}(V_{pre}) = 5(1 + \tanh(\frac{V_{pre}}{4}))$$

The value of τ_e was set to 2 ms for all AMPA synapses.

Electrical connections (I_{elec}): Electrical connections (gap junctions) were present between all interneurons of the same type.^{19,20} The gap junction current from interneuron j to interneuron i was modeled as:

$$I_{elec} = \bar{g}_{elec}(V_i - V_j)$$

The maximal gap junction conductance ($\bar{g}_{elec}$) was 0.005 mS/cm^2 between all neurons of the same type.

Networks

Our networks consisted of 110 neurons: 90 PYR, 11 PV+IN, 6 SOM+IN and 3 VIP+IN. These numbers were chosen based on the approximate proportion of each cell type found in cortex.²¹ All-to-all connections were used from SOM, PV and VIP to excitatory cells (PYR). Each PV+IN received AMPA input from 50 randomly chosen PYR. SOM+IN and VIP+INs received input from distinct sets of PYR.¹⁹ Each SOM+IN received input from 6 randomly chosen PYR, and each VIP+IN received input from 45 PYR that did not send projections to SOM+INs. PV+INs were connected all-to-all with each other, excluding autapses. Similarly, PYR were connected

all-to-all with each other (excluding autapses) with very weak connections used to calculate the model LFP. SOM+INs and VIP+INs had no synaptic connections between members of their own subtype. Electrical connections among interneurons of the same type were all-to-all, and synaptic connections between all different subtypes of interneurons were all-to-all.

References

1. Mäki-Marttunen Tuomo, Mäki-Marttunen Verónica. Excitatory and inhibitory effects of HCN channel modulation on excitability of layer V pyramidal cells *PLOS Computational Biology*. 2022;18:e1010506. Publisher: Public Library of Science (PLOS).
2. Hu Wenqin, Bean Bruce P.. Differential Control of Axonal and Somatic Resting Potential by Voltage-Dependent Conductances in Cortical Layer 5 Pyramidal Neurons *Neuron*. 2018;97:1315–1326.e3. Publisher: Elsevier BV.
3. Olufsen Mette S., Whittington Miles A., Camperi Marcelo, Kopell Nancy. New Roles for the Gamma Rhythm: Population Tuning and Preprocessing for the Beta Rhythm *Journal of Computational Neuroscience*. 2003;14:33–54. Publisher: Springer Science and Business Media LLC.
4. Mainen Zachary F., Sejnowski Terrence J.. Influence of dendritic structure on firing pattern in model neocortical neurons *Nature*. 1996;382:363–366. Publisher: Springer Science and Business Media LLC.
5. Jones Stephanie R., Pinto David J., Kaper Tasso J., Kopell Nancy. Alpha-Frequency Rhythms Desynchronize over Long Cortical Distances: A Modeling Study *Journal of Computational Neuroscience*. 2000;9:271–291. Publisher: Springer Science and Business Media LLC.
6. Ma Yunyong, Hu Hang, Berrebi Albert S., Mathers Peter H., Agmon Ariel. Distinct Subtypes of Somatostatin-Containing Neocortical Interneurons Revealed in Transgenic Mice *The Journal of Neuroscience*. 2006;26:5069–5082. Publisher: Society for Neuroscience.
7. Golomb David, Donner Karnit, Shacham Liron, Shlosberg Dan, Amitai Yael, Hansel David. Mechanisms of Firing Patterns in Fast-Spiking Cortical Interneurons *PLoS Computational Biology*. 2007;3:e156. Publisher: Public Library of Science (PLOS).
8. Traub Roger D., Buhl Eberhard H., Gloveli Tengis, Whittington Miles A.. Fast Rhythmic Bursting Can Be Induced in Layer 2/3 Cortical Neurons by Enhancing Persistent Na^+ Conductance or by Blocking BK Channels *Journal of Neurophysiology*. 2003;89:909–921. Publisher: American Physiological Society.
9. Kramer Mark A., Roopun Anita K., Carracedo Lucy M., Traub Roger D., Whittington Miles A., Kopell Nancy J.. Rhythm Generation through Period Concatenation in Rat Somatosensory Cortex *PLoS Computational Biology*. 2008;4:e1000169. Publisher: Public Library of Science (PLOS).

10. Jiang Shao-Na, Cao Jun-Wei, Liu Lin-Yun, et al. *Sncg, Mybp1, and Parm1* Classify subpopulations of VIP-expressing interneurons in layers 2/3 of the somatosensory cortex *Cerebral Cortex*. 2023;33:4293–4304. Publisher: Oxford University Press (OUP).
11. Porter James T., Cauli Bruno, Staiger Jochen F., Lambolez Bertrand, Rossier Jean, Audinat Etienne. Properties of bipolar VIPergic interneurons and their excitation by pyramidal neurons in the rat neocortex *European Journal of Neuroscience*. 1998;10:3617–3628. Publisher: Wiley.
12. Goff Kevin M, Goldberg Ethan M. Vasoactive intestinal peptide-expressing interneurons are impaired in a mouse model of Dravet syndrome *eLife*. 2019;8. Publisher: eLife Sciences Publications, Ltd.
13. Prönneke Alvar, Witte Mirko, Möck Martin, Staiger Jochen F. Neuromodulation Leads to a Burst-Tonic Switch in a Subset of VIP Neurons in Mouse Primary Somatosensory (Barrel) Cortex *Cerebral Cortex*. 2019. Publisher: Oxford University Press (OUP).
14. Huguenard J. R., McCormick D. A.. Simulation of the currents involved in rhythmic oscillations in thalamic relay neurons *Journal of Neurophysiology*. 1992;68:1373–1383. Publisher: American Physiological Society.
15. Henriksen Mari Wold, Ravn Kirstine, Paus Benedicte, Von Tetzchner Stephen, Skjeldal Ola H. De novo mutations in SCN1A are associated with classic Rett syndrome: a case report *BMC Medical Genetics*. 2018;19. Publisher: Springer Science and Business Media LLC.
16. Yu Frank H, Mantegazza Massimo, Westenbroek Ruth E, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy *Nature Neuroscience*. 2006;9:1142–1149. Publisher: Springer Science and Business Media LLC.
17. Donato Cristina, Balduino Victorino Daniella, Cabezas Carolina, et al. Pharmacological Signature and Target Specificity of Inhibitory Circuits Formed by Martinotti Cells in the Mouse Barrel Cortex *The Journal of Neuroscience*. 2023;43:14–27. Publisher: Society for Neuroscience.
18. Pi Hyun-Jae, Hangya Balázs, Kvitsiani Duda, Sanders Joshua I., Huang Z. Josh, Kepecs Adam. Cortical interneurons that specialize in disinhibitory control *Nature*. 2013;503:521–524. Publisher: Springer Science and Business Media LLC.
19. Karnani Mahesh M., Jackson Jesse, Ayzenshtat Inbal, et al. Cooperative Subnetworks of Molecularly Similar Interneurons in Mouse Neocortex *Neuron*. 2016;90:86–100. Publisher: Elsevier BV.
20. Tremblay Robin, Lee Soohyun, Rudy Bernardo. GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits *Neuron*. 2016;91:260–292. Publisher: Elsevier BV.
21. Rudy Bernardo, Fishell Gordon, Lee SooHyun, Hjerling-Leffler Jens. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons *Developmental Neurobiology*. 2011;71:45–61. Publisher: Wiley.