**A. SIGNIFICANCE**

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by social impairments and restricted, repetitive behaviors [1] that arises from altered brain development [2, 3]. Despite sharing a single diagnosis, the heterogeneity of behavior, sensory processing [4], genetics [5, 6], and underlying neural mechanisms [7] in autism contributes to a wide range of poorly understood sub-clinical phenotypes [8, 9]. Advancing our understanding of this heterogeneity requires integration of neurophysiological and behavioral measures to quantify the diverse patterns of brain activity underlying ASD.

A promising avenue for capturing this neural variability lies in the study of neuro-oscillatory activity. Neuro-oscillatory activity—which reflects synchronized neural activity within and between cortical regions [10]—is ubiquitously reported to be atypical in ASD [11-13], offering a potential assay of disrupted information processing and network connectivity [14]. However, findings are often difficult to compare across studies due to variability in methodology, participant characteristics, and task demands. To address these challenges, we have collected a comprehensive battery probing diverse cognitive, sensory, and motor domains within a single cohort—an approach well-suited to capturing the full spectrum of neuro-oscillatory impairments in autism.

The standardized collection of quantitative neuro-oscillatory markers spanning multiple cognitive and sensory modalities offers an invaluable opportunity to examine how neural activity relates to clinical phenotype at the individual-level—thus addressing a longstanding challenge in characterizing heterogeneity in autism. Previous works are typically limited to between-group comparisons, which risk oversimplification of subtle neurophysiological differences that may underly phenotypic variation within autism. Critically, such variability likely reflects differing neurobiological mechanisms that may respond differently to treatment. To address these limitations, we propose integration of novel machine learning approaches, which enables a) rigorous evaluation of the strength of neural biomarkers associated with ASD and b) identification of meaningful subgroups within ASD to begin disentangling clinical and behavioral heterogeneity. While cluster-based identification of ASD subgroups has been attempted, prior efforts have largely relied on functional Magnetic Resonance Imaging (fMRI), which lacks temporal resolution and typically focuses on resting-state activity or a single sensory domain—failing to capture the full extent of neural dysfunction likely present in autism.

This project, therefore, is designed to significantly improve our understanding of the atypical neural measures commonly reported in ASD through electroencephalography (EEG)—a robust measure of cortical activity. We assume there is variability in the clinical and behavioral profiles of individuals with autism and that a single deficit model will be inadequate. Therefore, deployment of cluster-based machine learning algorithms will allow us to elucidate how impaired brain mechanisms in ASD fundamentally relate to continuous clinical measures of autism severity, both at the individual- and subgroup-level. Moreover, neurophysiological markers of autism (and subgroups) identified in this work may serve as quantitative indicators of treatment efficacy, and support the development of more personalized diagnostic and therapeutic interventions [15, 16].

**B. APPROACH**

**B.1 METHODS**

B.1.1. Project Overview.We have curated a rich clinical dataset compromising high-density EEG data from 136 individuals and eight paradigms, alongside rigorous clinical assessments. This data provides a rare opportunity to systematically evaluate neuro-oscillatory activity across diverse sensory modalities within a single cohort.

B.1.2. Research Design. Selected paradigms were chosen to probe neural activity that i) span multiple cognitive, motor and sensory domains and ii) are potentially relevant to autism. Each paradigm targets distinct neural processes likely implicated in ASD pathology, which we evaluate here in a standardized manner within a single cohort. This offers a comprehensive battery of neural markers reflecting the integrity of diverse cognitive and sensory processes that may be impaired in autism, both at the group- and individual-level.

**B.1.2.1 Auditory Steady State Response (ASSR).** ASSR—which elicits a sinusoidal frequency-following response in the primary auditory cortex—provides a non-invasive measure of neural oscillatory function. Here, we evoke ASSR by periodic auditory stimulation in the gamma-frequency range (40-Hz), which is thought to probe intact synchronization of GABAergic interneurons [17] and offers insight into underlying excitatory-inhibitory (E/I) imbalance in ASD [18, 19]—a popular idea supported by findings from both animal models [20, 21] and human studies [22, 23]. Previous studies evaluating ASSR in ASD demonstrate an ASD-specific reduction in evoked gamma power and inter-trial coherence (ITC) [24-28], although findings are conflicting. Analyses will evaluate gamma-band activity and the broad-band evoked response.

**B.1.2.2 Illusory Contours (IC).** Kanizsa IC stimuli invoke increased gamma-band activity compared to control stimuli, reflecting synchronized activity that underlies automatic binding of visual contours.Kanizsa IC stimuli offer a powerful means of probing local versus global visual processing, which is ubiquitously shown to be atypical in individuals with ASD. Here, we passively present both IC and control stimuli to test the integrity of gamma-band activity in ASD.

**B.1.2.3 Face Processing.** Social impairment—a core diagnostic feature of ASD—is likely linked to atypical face processing, which has been reported by multiple behavioral, eye-tracking, and neuroimaging studies. We present a mix of social (faces) and non-social (objects) stimuli to assay alpha and theta function—neural oscillatory measures previously associated with altered face processing in autism.

**B.1.2.4 Inter-sensory Attention.** Alpha-band activity is thought to modulate suppression of task-irrelevant visual information, a process shown to be atypical in individuals with ASD. We deploy a cued-target S1-S2 cross-sensory attention task in which participants are required to attend to a target in either the visual or auditory modality during S2 presentation. This assay is designed to evaluate alpha oscillatory activity as a measure of attentional suppression in the cue-target window—particularly relevant given the widespread attentional difficulties observed in autism.

**B.1.2.5 Audio-visual Speeded Reaction Time (AVSRT).** AVSRT—a simple task in which participants respond to visual, auditory, and audio-visual targets—probes both early unisensory processing and multisensory integration. This allows for evaluation of multisensory integration at the behavioral level (reaction time) and its association with cortical activity, as indexed by alpha desynchronization. This approach is motivated by consistent evidence that individuals with autism exhibit alterations in early visual and auditory processing, as well as impaired multisensory performance and electrophysiological responses.

**B.1.2.6 Motor Processing (Treadmill Walking).** Atypical gait and other motor abnormalities are commonly reported in ASD. Here, we use treadmill walking to assess oscillatory activity during motor behavior, particularly focusing on alpha and beta synchronization, which has been shown to increase in the motor and posterior parietal cortices with rising gait demands.

**B.1.2.7 Go-NoGo Cognitive Control (Single & Dual-Task).** We employ a classic Go-NoGo task in which participants respond when a centrally presented cross changes into an X, thereby requiring cognitive control to inhibit a prepotent response. This enables interrogation of theta function—a neural marker of cognitive control shown to be reduced in ASD—across single-task (Go-NoGo while sitting), motor dual-task (Go-NoGo while walking), and cognitive dual-task (Go-NoGo with a visual optic flow distractor) conditions. Here, we plan to evaluate group differences in theta across conditions, which systematically vary cognitive control demands.

**B.1.2.8 Rest.** Resting state (RS) data—which is collected in the absence of a task—offers a powerful assay of neural oscillatory function across all frequency bands that is both i) clinically relevant and ii) easily translatable to animal work. Spectral analysis of alpha- and gamma-activity during RS has revealed significant correlation with measures of autistic traits and language function in ASD, respectively. Moreover, RS alpha- and theta-activity offer measures of cognitive control, as measured in TD adults. RS data analysis will include evaluation of mean power (absolute and relative) and peak frequency across all major oscillatory bands.

B.1.3. Participants. This large, heterogeneous sample includes autistic children (n=66), age-, sex- and IQ-matched typically developing peers (n=41), and unaffected siblings of individuals with ASD (n=29), drawn from the Bronx—a highly diverse and historically underrepresented population in biomedical research. Participants are age-restricted (8-12 years) to minimize developmental confounds. Inclusion of unaffected siblings of individuals with ASD in our clinical cohort allows us to investigate whether observed neural markers reflect heritable mechanisms contributing to ASD risk, or whether they instead represent consequences of overt disease expression [14]—thereby addressing a critical challenge in the search for neural biomarkers in autism.

**Diagnostic categorization:** To be included in the ASD group, diagnosis was confirmed on the basis of: (1) *Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2; [29, 30]);* (2) Diagnostic Criteria for Autistic Disorder from the DSM-5; (3) Clinical impression of experienced licensed psychologist.

**Autism severity/autistic traits:** Scores from the ADOS-2 can be used to determine calibrated severity scores (CSS) [31, 32]. The *Social-Responsiveness Scale, 2nd Edition (SRS-2; [33])*—which is collected for all participants, irrespective of group—will be used as continuous measures of autistic traits.

**Cognitive function:** Cognitive function is assessed by *WASI-II Intelligence Quotient (IQ; [34]),* which is an excellent tool for reliably assessing full-scale intelligence in individuals aged 6-89 years old.

**Attention function:** Continuous measures of attention were obtained from *Conners’ Continuous Performance Test, 3rd edition (CPT-3; [35]),* which is a computerized test to evaluate attention in individuals >8 years old.

**Exclusion criteria:** Uncorrected vision; genetic syndromes. TD participants may not have a first degree relative with ASD or language impairment, or a history of special education services.

B.1.4 EEG Acquisition.EEG recordings were made using a Biosemi 70-channel electrode system. Artifact rejection is performed by visual inspection of continuous EEG waveforms, automated rejection of trials with EEG amplitude exceeding a preset threshold, and ICA capture of artifactual activity including eye movements. We have collected sufficient data to yield a minimum of 100 accepted trials per condition, for a high signal-to-noise ratio. Standard approaches to selection of electrode sites and latencies for extraction of component latency and amplitude values, based on the literature and confirmatory inspection of data, will be applied.

B.1.5. Analysis and Potential Outcomes. Preliminary analyses to identify group-level neural differences in each paradigm will be done using traditional statistical methods. Linear mixed-effects modeling (LMM) will be deployed to investigate the effects of our independent measures [36, 37]. Regression analyses enable identification of group differences, while also examining individual variability across dependent measures. In all LMMs, participant is treated as a random effect, with covariates of age, sex, and IQ, with a fixed effect of group (ASD, TD, siblings). Multiple linear regression will examine the relationship between EEG measures, behavioral performance, and measures of ASD symptom severity (e.g. SRS-2), with covariates of age, sex, and IQ.

In addition to traditional methods, we propose a two-pronged analytic approach that combines supervised and unsupervised learning. First, we will apply support vector machine (SVM) and regression algorithms [38] to predict both discrete (diagnostic group) and continuous (clinical or behavioral) outcomes from neural markers of interest, allowing us to quantify the strength of the relationship between neural activity and phenotype.

A collage of graphs and diagrams

AI-generated content may be incorrect.We will also implement a complementary unsupervised approach (k-means clustering with elbow method; [39]) to identify data-driven sub-groups based on neural features. Clusters will be compared on continuous measures of behavior and ASD symptom severity to assess how neural profiles relate to variations in clinical presentation. Importantly, inclusion of multiple tasks spanning diverse sensory and cognitive domains allows us to examine how neural signatures map onto distinct clinical features (i.e. neural markers of heightened sensory processing may be associated with greater sensory arousal symptoms).

**B.2 PRELIMINARY DATA**

**Figure 1** highlights preliminary group-level differences (p<0.05) in neural markers between ASD and TD individuals across selected paradigms, using traditional between-group analyses. Key findings include significantly reduced amplitude in ASD during evoked response to gamma-band click trains **(B.1.2.1)**, increased theta power to inverted faces **(B.1.2.3)**, reduced parieto-occipital alpha desynchronization during multisensory integration **(B.1.2.5)**, and fewer rhythmic alpha events during resting state **(B.1.2.8)**—each suggesting atypical sensory and cognitive processing in ASD. These case examples represent just a subset of the neural measures that will be included in machine learning models—alongside those detailed in the methodology above—to test association with the clinical phenotype at the individual- and subgroup-level.

**Fig. 1.** Preliminary case examples of neural markers that significantly differ between ASD and TD (p<0.05) at the group-level (highlighted in yellow). **B.1.2.1. ASSR.** Fronto-central evoked response to 40-Hz auditory click trains (gamma ASSR), highlighting a significantly reduced amplitude in ASD (vs. TD; p-val<0.05) at 180-250 ms, which potentially reflects a broad delay in early auditory processing. Responses are separated by group (TD: grey, ASD: red, siblings: green). **B.1.2.3 Face Processing.** Fronto-central theta power (%-compared to fixation baseline) in response to faces (red) and inverted faces (orange) during face processing task, highlighting a significant ASD-only increase in theta power to inverted faces (p<0.05). **B.1.2.5** **AVSRT.** Parieto-occipital alpha desynchronization (dB) in response to multi-sensory audiovisual stimuli, separated by group (TD: grey, ASD: red, siblings: green). Significantly reduced alpha desynchronization in ASD (vs. TD; p-val <0.05) at ~150 ms post-stimulus onset highlighted in yellow. **B.1.2.8 Rest.** Topographical maps of the number of rhythmic alpha (7-13 Hz) events during eyes-closed resting-state, as calculated by eBOSC. Electrode clusters demonstrating significant reduction in the number of rhythmic events in the ASD group (vs. TD, p-val<0.05) are highlighted in yellow.

1. Association, A.P., *Diagnostic and statistical manual of mental disorders*. 5 ed. 2013.

2. Courchesne, E., *Brain development in autism: early overgrowth followed by premature arrest of growth.* Ment Retard Dev Disabil Res Rev, 2004. **10**(2): p. 106-11.

3. Lord, C., et al., *Autism spectrum disorder.* Lancet, 2018. **392**(10146): p. 508-520.

4. Lane, A.E., et al., *Sensory processing subtypes in autism: association with adaptive behavior.* J Autism Dev Disord, 2010. **40**(1): p. 112-22.

5. Jeste, S.S. and D.H. Geschwind, *Disentangling the heterogeneity of autism spectrum disorder through genetic findings.* Nat Rev Neurol, 2014. **10**(2): p. 74-81.

6. Willsey, A.J. and M.W. State, *Autism spectrum disorders: from genes to neurobiology.* Curr Opin Neurobiol, 2015. **30**: p. 92-9.

7. Salmond, C.H., et al., *Heterogeneity in the patterns of neural abnormality in autistic spectrum disorders: evidence from ERP and MRI.* Cortex, 2007. **43**(6): p. 686-99.

8. Masi, A., et al., *An Overview of Autism Spectrum Disorder, Heterogeneity and Treatment Options.* Neurosci Bull, 2017. **33**(2): p. 183-193.

9. Mottron, L. and D. Bzdok, *Autism spectrum heterogeneity: fact or artifact?* Mol Psychiatry, 2020. **25**(12): p. 3178-3185.

10. Lakatos, P., et al., *An oscillatory hierarchy controlling neuronal excitability and stimulus processing in the auditory cortex.* J Neurophysiol, 2005. **94**(3): p. 1904-11.

11. Beker, S., J.J. Foxe, and S. Molholm, *Oscillatory entrainment mechanisms and anticipatory predictive processes in children with autism spectrum disorder.* J Neurophysiol, 2021. **126**(5): p. 1783-1798.

12. Dickinson, A., et al., *Peak alpha frequency is a neural marker of cognitive function across the autism spectrum.* Eur J Neurosci, 2018. **47**(6): p. 643-651.

13. Murphy, J.W., et al., *Susceptibility to distraction in autism spectrum disorder: probing the integrity of oscillatory alpha-band suppression mechanisms.* Autism Res, 2014. **7**(4): p. 442-58.

14. Uhlhaas, P.J. and W. Singer, *Neuronal dynamics and neuropsychiatric disorders: toward a translational paradigm for dysfunctional large-scale networks.* Neuron, 2012. **75**(6): p. 963-80.

15. Kim, S.H., et al., *Examining the phenotypic heterogeneity of early autism spectrum disorder: subtypes and short-term outcomes.* J Child Psychol Psychiatry, 2016. **57**(1): p. 93-102.

16. Ousley, O. and T. Cermak, *Autism Spectrum Disorder: Defining Dimensions and Subgroups.* Curr Dev Disord Rep, 2014. **1**(1): p. 20-28.

17. Li, J., et al., *The Thalamocortical Mechanism Underlying the Generation and Regulation of the Auditory Steady-State Responses in Awake Mice.* J Neurosci, 2024. **44**(1).

18. Uzunova, G., S. Pallanti, and E. Hollander, *Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics.* World J Biol Psychiatry, 2016. **17**(3): p. 174-86.

19. Cellot, G. and E. Cherubini, *GABAergic signaling as therapeutic target for autism spectrum disorders.* Front Pediatr, 2014. **2**: p. 70.

20. Juarez, P. and V. Martinez Cerdeno, *Parvalbumin and parvalbumin chandelier interneurons in autism and other psychiatric disorders.* Front Psychiatry, 2022. **13**: p. 913550.

21. Yizhar, O., et al., *Neocortical excitation/inhibition balance in information processing and social dysfunction.* Nature, 2011. **477**(7363): p. 171-8.

22. Rojas, D.C., et al., *Decreased left perisylvian GABA concentration in children with autism and unaffected siblings.* Neuroimage, 2014. **86**: p. 28-34.

23. Saleh, M.G., et al., *GABA and glutamate measurements in temporal cortex of autistic children.* Autism Res, 2024. **17**(12): p. 2558-2571.

24. Arutiunian, V., et al., *Neuromagnetic 40 Hz Auditory Steady-State Response in the left auditory cortex is related to language comprehension in children with Autism Spectrum Disorder.* Prog Neuropsychopharmacol Biol Psychiatry, 2023. **122**: p. 110690.

25. Rojas, D.C., et al., *Reduced neural synchronization of gamma-band MEG oscillations in first-degree relatives of children with autism.* BMC Psychiatry, 2008. **8**: p. 66.

26. Seymour, R.A., et al., *Reduced auditory steady state responses in autism spectrum disorder.* Mol Autism, 2020. **11**(1): p. 56.

27. Stroganova, T.A., et al., *Left hemispheric deficit in the sustained neuromagnetic response to periodic click trains in children with ASD.* Mol Autism, 2020. **11**(1): p. 100.

28. Wilson, T.W., et al., *Children and adolescents with autism exhibit reduced MEG steady-state gamma responses.* Biol Psychiatry, 2007. **62**(3): p. 192-7.

29. Lord, C., et al., *The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism.* J Autism Dev Disord, 2000. **30**(3): p. 205-23.

30. Lord, C., et al., *Autism diagnostic observation schedule: a standardized observation of communicative and social behavior.* J Autism Dev Disord, 1989. **19**(2): p. 185-212.

31. Gotham, K., A. Pickles, and C. Lord, *Standardizing ADOS scores for a measure of severity in autism spectrum disorders.* J Autism Dev Disord, 2009. **39**(5): p. 693-705.

32. Shumway, S., et al., *The ADOS calibrated severity score: relationship to phenotypic variables and stability over time.* Autism Res, 2012. **5**(4): p. 267-76.

33. Constantino, J.N., & Gruber, C. P., *Social responsiveness scale: SRS-2, ed.* W.P. Services, Los Angeles, CA, 2012.

34. Wechsler, D., *Wechsler Abbreviated Scale of Intelligence, Second Edition.* Pearson Assessments, 2011.

35. Conners, C.K., *Conners third edition (Conners 3).* Los Angeles, CA: Western Psychological Services, 2008.

36. Payne, B.R., C.L. Lee, and K.D. Federmeier, *Revisiting the incremental effects of context on word processing: Evidence from single-word event-related brain potentials.* Psychophysiology, 2015. **52**(11): p. 1456-69.

37. Tremblay, A. and A.J. Newman, *Modeling nonlinear relationships in ERP data using mixed-effects regression with R examples.* Psychophysiology, 2015. **52**(1): p. 124-39.

38. Cortes C., V.V., *Support-vector networks.* Mach Learn, 1995. **20**: p. 273-297.

39. Steinley, D., *K-means clustering: a half-century synthesis.* Br J Math Stat Psychol, 2006. **59**(Pt 1): p. 1-34.