Visual Evoked Potential Abnormalities in Phelan-McDermid Syndrome

RH = VEPs in Phelan-McDermid Syndrome

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Supplemental Materials

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Abstract

Objective: The current study utilized visual evoked potentials (VEPs) to examine excitatory and inhibitory postsynaptic activity in children with Phelan-McDermid syndrome (PMS) and the association with genetic factors. PMS is caused by haploinsufficiency of *SHANK3* on chromosome 22 and represents a common single-gene cause of autism spectrum disorder (ASD) and intellectual disability.

Method: Transient VEPs were obtained from 175 children, including 31 with PMS, 79 with idiopathic ASD, 45 typically developing controls, and 20 unaffected siblings of children with PMS. Stimuli included standard and short-duration contrast-reversing checkerboard conditions and the reliability between these two conditions was assessed. Test-retest reliability and correlations with deletion size were explored in the group with PMS.

Results: Children with PMS and, to a lesser extent, those with idiopathic ASD, displayed significantly smaller amplitudes and decreased beta and gamma band activity relative to TD controls and PMS siblings. Across groups, high intraclass correlation coefficients were obtained between standard and short-duration conditions. In children with PMS, test-retest reliability was strong. Deletion size was significantly correlated with P_{60}-N_{75} amplitude for both conditions.

Conclusion: Children with PMS displayed distinct transient VEP waveform abnormalities in both time and frequency domains that might reflect underlying glutamatergic deficits which were associated with deletion size. A similar response pattern was observed in a subset of children with idiopathic ASD. VEPs offer a noninvasive measure of excitatory and inhibitory neurotransmission that holds promise for stratification and surrogate endpoints in ongoing clinical trials in PMS and ASD.

Key words: Phelan McDermid syndrome, autism spectrum disorder, visual evoked potential, transient VEP

Introduction

Excitatory and inhibitory (E/I) changes in the brain represent one mechanistic hypothesis for autism spectrum disorder (ASD).[1-3] From a clinical perspective, this hypothesis is exciting, as impairments in excitatory (glutamatergic) and inhibitory (*γ*-aminobutyric acid [GABAergic]) neurotransmission offer a potential biomarker of disease that can be measured through electroencephalography (EEG) and targeted through pharmacological interventions. However, to date, empirical support for E/I changes in ASD remains inconsistent, likely due to biological heterogeneity.[4] The current study examined EEG markers of excitatory and inhibitory activity in individuals with Phelan-McDermid syndrome (PMS), a syndrome in which glutamatergic activity is disrupted.[5-7]

PMS is one of the most common single-gene causes of ASD and intellectual disability (ID), accounting for 1-2% of cases of ID and more than 0.5% of ASD cases.[8] Over 80% of individuals with PMS also meet criteria for ASD[5,6,8-10] and present with global developmental delays, ID, language, sensory and motor impairment, as well as a host of medical conditions.[10-12] PMS involves the loss of one functional copy (haploinsufficiency) of *SHANK3* on chromosome 22 through deletion or mutation (OMIM 606232). Point mutations in *SHANK3* are sufficient to cause clinical features, although better developed speech and motor abilities were identified in certain individuals with point mutations compared to those with multi-gene deletions.[10] *SHANK3* is a scaffolding protein in glutamatergic synapses and findings from animal studies indicate glutamatergic dysregulation in *Shank3*-deficient rodents.[5,7,13] Thus, PMS offers a unique opportunity to develop tools for measuring neuronal regulation as it relates to individuals with ASD and related conditions.

Visual evoked potentials (VEPs) reflect the sum of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs)[14-17] and offer a promising electrophysiological measure for ASD and related disorders. The visual system is known to involve approximately 50% of neocortex and all known brain mechanisms[18,19], which makes VEPs a good candidate to assess brain function. The major positive and negative deflections in VEP waveforms represent specific cortical cellular events that follow afferent activity from the dorsal lateral geniculate nucleus of the thalamus (dLGN) to the primary visual cortex (V1). Transient VEPs (tVEPs) to a contrast-reversing checkerboard produce a characteristic waveform with well-understood mechanisms.[14,20,21] The earliest activity, an initial positive deflection, reflects solely the depolarization of cortical neurons receiving afferent input from the dLGN. The following negative deflection reflects the spread of excitation through the cortical layers of V1, and the later positive deflection reflects intracortical GABAergic inhibition. The depolarization (excitatory postsynaptic potentials) occurring on recipient cortical neurons in V1, presumably involves activation of glutamatergic receptors and the following major positive deflection has been shown to reflect the sum of inhibitory postsynaptic potentials resulting from activation of GABA-A receptors.[20,21] The times to the negative trough and positive peak depend upon the strength of the overlapping EPSP and IPSP activity.

It was recently shown that short-duration stimuli are effective in capturing EEG alterations in idiopathic autism spectrum disorder (iASD).[20] While tVEP abnormalities have been identified in other psychiatric conditions [22,23] and in several single-gene causes of ASD, including Rett syndrome,[24] Fragile X syndrome (FXS),[25] and 16p11.2 deletion syndrome[26] where glutamatergic dysregulation has also been described, this is the first study to examine tVEPs in PMS.

The current study (1) tested the utility of tVEPs in PMS and its overlap with iASD and (2) examined PMS-specific variables including correlations with deletion size and test-retest reliability.

Method

Participants

Informed consent was obtained from legal guardians and assent was obtained from participants when appropriate. This study was approved by the Program for the Protection of Human Subjects. One hundred and eighty-four children enrolled in this study. Usable VEP data were collected from a total of 175 participants between the ages of 20 months and 12 years, including 31 children with PMS, 79 children with iASD, 45 typically-developing controls (TD), and 20 unaffected siblings of children with PMS (PMS sibs). The age range was chosen based on existing clinical trials in PMS. PMS sibs were included as an additional group to control for genetic and environmental factors, therefore reducing the possibility of confounding familial risks. Participants with PMS were recruited through ongoing PMS studies at the Center. Siblings of PMS participants, individuals with iASD, and TD controls who met study inclusion criteria were eligible to enroll during the same period.

Standard tVEPs (long condition) were successfully obtained from 20 children with PMS, 66 with iASD, 39 TD controls, and 20 PMS sibs. Short-duration tVEPs (short condition) were obtained from 28 children with PMS, 74 with iASD, 40 TD controls, and 18 PMS sibs. Three children with PMS completed the long condition, but not the short condition. The TD and PMS sibs samples were screened for the presence of psychiatric and medical conditions in themselves and immediate family members. Past diagnoses of vision problems (e.g., strabismus, amblyopia,

cerebral visual impairment (CVI)) were exclusionary for all groups. Two children with PMS were excluded due to vision problems. Four additional PMS participants and five iASD participants were excluded due to noncompliance or repetitive behaviors (e.g., motor mannerisms, excessive teeth grinding, overactivity). One iASD participant was excluded due to a variant of unknown significance identified in the *SHANK3* gene. The presence of psychiatric and medical (e.g., seizures) comorbidity was carefully assessed in all PMS and iASD participants, as was medication use. The stimuli used in this study were appropriate for use in individuals with seizure history due to the low photopic range of luminance, stability in space-average luminance, imperceptible frame rate, and pattern modulation below the frequency range that typically can evoke seizure activity.[27]

PMS diagnosis was confirmed using chromosomal microarray or sequencing. Eleven children in the PMS group had pathogenic frameshift mutations in *SHANK3* and 20 children had deletions ranging from 0.04 to 8.3 megabases in size and included between 2 and 136 genes based on deletion breakpoints provided on clinical microarray reports. Individuals were considered to have iASD if chromosomal microarray analysis confirmed the absence of known pathogenic copy number variants and FXS testing was negative.

Behavioral Phenotyping

Psychiatric and medical diagnoses were assessed through a comprehensive clinical evaluation by a board-certified psychiatrist or a licensed clinical psychologist. In the iASD and PMS groups, ASD diagnosis was determined based on gold standard diagnostic testing, including the Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2) [28] and the Autism Diagnostic Interview-Revised (ADI-R).[29] A consensus diagnosis was then determined for each

participant based on ADOS-2, ADI-R and clinical evaluation (DSM-5). The TD and PMS sibs samples were screened with the Social Responsiveness Scale, 2nd edition (SRS-2)[30] and through a detailed demographic history form and brief clinical exam.

Cognitive and adaptive functioning was measured using an appropriate IQ test, including the Mullen Scales of Early Learning (MSEL),[31] the Stanford-Binet, 5th edition (SB-5),[32] or the Differential Ability Scales, 2nd edition.[33] The MSEL is validated in children from birth to 68 months, but also is commonly used in children of older ages who are intellectually-disabled and minimally-verbal.[34] Developmental quotients (DQ) were computed as previously described in the literature.[35] To compare standard scores and DQs, a floor of 40 was set across cognitive measures to be consistent with the lowest possible scores on the SB-5. IQ was evaluated in TD and PMS sibs groups using the SB-5 abbreviated IQ or the Wechsler Abbreviated Intelligence Scale, 2nd Edition (WASI-II).[36] Adaptive behavior was measured in the PMS and iASD groups using the Vineland Adaptive Behavior Scales, 2nd edition, Survey Interview Form.[37]

VEP

The tVEP battery included two test conditions presented in a random order within one testing session. A contrast-reversing checkerboard (~100% contrast) was used to elicit a tVEP, which enabled the examination of multiple frequency mechanisms.[38] Participants viewed a traditional 60-s condition ("standard condition") and a short-duration condition (10 2-s EEG epochs with 1-s of adaptation per run) developed by our group and published previously.[20,38] Stimulus conditions were presented in accordance with the International Society for Clinical Electrophysiology of Vision (ISCEV) VEP standards.[39] Stimulus field size subtended 10° x 10° of visual angle. Background luminance was ~50 cd/m^2. To ensure visual angle was maintained,

participants were seated in a developmentally appropriate chair with a seatbelt, on a parents' lap, or in their stroller in a fully upright position.

A Neucodia system (VeriSci Corp., Raritan, NJ) was used for stimulus presentation, gaze monitoring and data collection. Three gold-cup electrodes were placed on the midline of the scalp based on the 10-20 International System,[40] which includes an active electrode at *Oz* (occipital), a reference electrode at *Cz* (vertex), and a ground electrode at *Pz* (midway between *Oz* and *Cz*). VEPs were recorded and digitized synchronized to the display's frame rate of 150 Hz (4 samples per frame, or 600 samples per second). The EEG was amplified with a gain of 20,000 and a bandpass filter of 0.5-100 Hz. A noise detection feature determined whether the EEG recording was affected by artifacts, 60 Hz noise (i.e., electrical line noise) or drift/saturation. If an artifact was detected, the epoch was deleted and the examiner was prompted to repeat the run. An infrared gaze fixation monitor allowed the examiner to determine whether participants were attending to the screen, and individual runs were only initiated once visual fixation was determined. At the end of each run, the examiner was prompted to save or delete the data depending on whether proper fixation was maintained. Thus, each epoch is collected without interruption and the same number of epochs are included in each VEP run.

Statistical Analysis

A discrete Fourier transform was applied to the EEG data to extract harmonic frequency components of the response; tVEP waveforms were reconstructed using even harmonics 2-84 Hz (minus the 60 Hz component). Amplitudes (in μV) were measured peak-to-trough (P_{60}-N_{75}, N_{75}-P_{100}) and latencies (in ms) (P_{60}, N_{75}, P_{100}) were measured by time-to-peak (subscripted numbers indicate typical time-to-peak values). Frequency-domain analyses were conducted using

magnitude-squared coherence (MSC) statistics[20,38] to quantitatively assess the integrity of entire responses in each distinct frequency band. MSC measures coherence from one trial to the next for a given frequency component in terms of relative power. A pure signal (response without noise) produces a value of 1, and no signal (only noise) produces a value of approximately 0.1 (bias level given random activity). Higher MSC values reflect stronger and more consistent oscillatory activity in a given frequency band synchronized to the stimulus. Four distinct frequency bands were assessed: Band 1, 6-10 Hz, Band 2, 12-28 Hz, Band 3, 30-36 Hz, and Band 4, 38-48 Hz [20,38,41]. Band 1 reflects activity in the theta- and alpha-wave range, Band 2 reflects activity in the beta range, and Bands 3 and 4 reflect activity in the gamma range.

First, to test for differences among groups, multivariate analysis of covariance (MANCOVA) with Pillai's trace (PT) was used with an alpha level of 0.05 for all multivariate statistical tests. Evidence suggest that PT is robust to violations of the statistical model[42]. Age significantly affected the MANCOVA and was included as a covariate. There was no significant effect of IQ on any variable. Pairwise comparisons were run as post hoc analyses with Bonferroni correction. Standard and short-duration conditions were analyzed separately. Second, to examine agreement between standard and short-duration conditions, intraclass correlation coefficients (ICCs) were used. Third, Spearman's rho (r_s) was used to examine the relationship between VEP responses and variables specific to the PMS group (deletion size, number of genes deleted) to assess whether larger deletions resulted in a more severe phenotype. Finally, test-retest reliability of the short-duration condition was assessed with ICCs in a subset of the PMS sample who were able to return for a follow-up visit between 4-12 weeks post baseline VEP (n = 10).

Results

Group demographics

There was no significant difference in age between groups ($F_{3,171} = 0.26$, $p = 0.85$). The iASD group had more male participants compared to the other three groups ($X_3^2 = 42.18$, $p < 0.001$; post-hoc $p's < 0.05$), which is expected given the higher ratio of male:female participants in iASD.[43] Intelligence and developmental quotients were obtained from the majority of the sample ($n = 156$) and differed significantly among groups (nonverbal IQ (NVIQ)/DQ: $F_{3,152} = 53.59$, $p < 0.001$, $\eta_p^2 = 0.51$). The PMS group had significantly lower NVIQs than all other groups ($p's < 0.001$). The iASD group had significantly lower scores compared with the TD ($p < 0.001$) and PMS sibs groups ($p = 0.013$). There was no difference in IQ between the PMS sibs and TD groups ($p = 1.00$). Analyses revealed no differences in any result when individuals on anticonvulsant medications ($n = 4$ participants with PMS) were removed from the sample, and accordingly, all participants were included in the analyses. All TD and PMS sibs were below clinical cutoffs on SRS-2 total score and individual domain scores, with the exception of one PMS sibling whose scores fell in the mild range on two SRS-2 domains. Group demographics are summarized in Table 1.

Amplitude

Amplitudes were measured peak-to-trough at P_{60}-N_{75} and N_{75}-P_{100}. There was a significant multivariate difference among groups for both the standard condition ($F_{6,280} = 6.73$, $p < 0.001$, $\eta_p^2 = 0.13$, PT = 0.25) and the short condition ($F_{6,310} = 10.06$ $p < 0.001$, $\eta_p^2 = 0.16$, PT = 0.33). A main effect of group was found for both the P_{60}-N_{75} amplitude (standard: $F_{3,140} = 13.11$, $p < 0.001$, $\eta_p^2 = 0.22$; short: $F_{3,155} = 19.42$, $p < 0.001$, $\eta_p^2 = 0.27$) and N_{75}-P_{100} amplitude

(standard: $F_{3,140} = 7.64$, $p < 0.001$, $\eta_p^2 = 0.14$; short: $F_{3,155} = 14.66$, $p < 0.001$, $\eta_p^2 = 0.22$). While the multivariate analyses found a significant effect of age in both the standard ($F_{2,139} = 4.88$, $p = 0.009$, $\eta_p^2 = 0.07$, PT = 0.07) and short ($F_{2,154} = 9.82$, $p < 0.001$, $\eta_p^2 = 0.11$, PT = 0.11) conditions, only the main effect of age of the P_{60}-N_{75} amplitude in the short condition was found to be significant ($F_{1,155} = 6.02$, $p < 0.015$, $\eta_p^2 = 0.37$) such that older participants generated a larger amplitude. Age did not significantly impact the other conditions (standard P_{60}-N_{75} amplitude: $F_{1,140} = 3.76$, $p = 0.055$, $\eta_p^2 = 0.07$; standard P_{60}-N_{75} amplitude: $F_{1,155} = 0.001$, $p = 0.98$, $\eta_p^2 < 0.001$; short P_{60}-N_{75} amplitude: $F_{1,155} = 0.28$, $p = 0.60$, $\eta_p^2 = 0.002$).

Post hoc analyses revealed significantly smaller amplitudes in the PMS group for both VEP components when compared to the TD group (standard: P_{60}-N_{75}: $p < 0.001$, N_{75}-P_{100}: $p = 0.003$; short: P_{60}-N_{75}: $p < 0.001$; N_{75}-P_{100}: $p < 0.001$) and the PMS sibs group (standard: P_{60}-N_{75}: $p = 0.001$; N_{75}-P_{100}: $p = 0.001$; short: P_{60}-N_{75}: $p < 0.001$, N_{75}-P_{100}: $p < 0.001$). The iASD group also showed a significant attenuation in amplitude relative to the TD group at P_{60}-N_{75} (p's < 0.001 for both conditions) and at N_{75}-P_{100} (standard: $p = 0.040$; short: $p = 0.001$). Similarly, the iASD group showed significantly reduced amplitudes as compared to the PMS sibs group at P_{60}-N_{75} (standard: $p = 0.015$; short: $p < 0.001$) and N_{75}-P_{100} amplitude (standard: $p = 0.012$; short: $p < 0.001$). The TD group did not differ from the PMS sibs group for any condition. There were no significant differences between the PMS and iASD group for either condition (standard: P_{60}-N_{75}: $p = 0.33$, N_{75}-P_{100}: $p = 0.55$; short: P_{60}-N_{75}: $p = 0.25$, N_{75}-P_{100}: $p = 1.00$). In the standard condition, 30% (20/66) of participants in the iASD group fell within one SD (+/-) of the PMS group mean for P_{60}-N_{75} amplitude and 61% (40/66) for N_{75}-P_{100} amplitude. In the short condition, 55% (41/74) of the iASD group fell within one SD of the PMS group mean for P_{60}-N_{75} amplitude

and 54% (40/74) for N_{75}-P_{100} amplitude. Amplitude values are summarized in Table 2 and displayed in Figure 1.

Latency

There were no statistically significant differences among groups for latency in the standard condition ($F_{9,420}$ = 1.43, p = 0.17, η_p^2 = 0.03, PT = 0.09). There was a significant multivariate difference in latency among groups in the short condition ($F_{9,465}$ = 2.37, p = 0.012, η_p^2 = 0.04, PT = 0.13). Follow-up analyses revealed a significant difference in latency at N_{75} ($F_{3,155}$ = 3.00, p = 0.032, η_p^2 = 0.06), but no significant differences between individual pairs of groups at the other latencies (P_{60}: $F_{3,155}$ = 2.07, p = 0.11, η_p^2 = 0.04; P_{100}: $F_{3,155}$ = 0.82, p = 0.48, η_p^2 = 0.02). Post hoc pairwise comparisons indicate N_{75} occurred earlier in the PMS group (68.02 ± 1.23 ms [Mean, SE]) compared with the iASD group (71.94 ± 0.76 ms), p = 0.044. No other pairwise comparisons were significant. Age was not statistically significant in either the standard ($F_{3,138}$ = 1.48, p = 0.22, η_p^2 = 0.03, PT = 0.03) and short condition ($F_{3,135}$ = 0.78, p = 0.51, η_p^2 = 0.02, PT = 0.02).

Frequency domain

MSC multivariate group differences were statistically significant for both standard ($F_{12,417}$ = 6.10, p < 0.001, η_p^2 = 0.15, PT = 0.45) and short ($F_{12,462}$ = 5.26, p < 0.001, η_p^2 = 0.12, PT = 0.36) conditions. In particular, MSC group differences were identified within Bands 2-4 in both the standard (Band 2: $F_{3,140}$ = 13.74, p < 0.001, η_p^2 = 0.22; Band 3: $F_{3,140}$ = 15.82, p < 0.001, η_p^2 = 0.25; Band 4: $F_{3,140}$ = 13.60, p < 0.001, η_p^2 = 0.23) and short conditions (Band 1: $F_{3,155}$ = 2.94, p = 0.035, η_p^2 = 0.05; Band 2: $F_{3,155}$ = 17.16, p < 0.001, η_p^2 = 0.25; Band 3: $F_{3,155}$ = 18.85, p

< 0.001, η_p^2 = 0.27; Band 4: $F_{3,155}$ = 9.84, p < 0.001, η_p^2 = 0.16). Overall, responses were significantly weaker in the iASD and PMS groups relative to the TD and PMS sibs groups in bands encompassing activity in the beta- and gamma-wave ranges (Figure 2). Post hoc tests are summarized in Table 3. Multivariate age differences were found in both standard ($F_{4,137}$ = 6.45, p < 0.001, η_p^2 = 0.16, PT = 0.16) and short conditions ($F_{4,152}$ = 6.59, p < 0.001, η_p^2 = 0.15, PT = 0.15). All bands, except Band 1 in the short condition ($F_{1,155}$ = 0.16, p = 0.69, η_p^2 = 0.001), had a main effect of age, in which older participants produced larger MSC values (standard Band 1: $F_{1,140}$ = 4.99, p = 0.027, η_p^2 = 0.03; standard Band 2: $F_{1,140}$ = 6.72, p = 0.011, η_p^2 = 0.05; standard Band 3: $F_{1,140}$ = 24.04, p < 0.001, η_p^2 = 0.15; standard Band 4: $F_{1,140}$ = 7.46, p = 0.007, η_p^2 = 0.05; short Band 2: $F_{1,155}$ = 4.03, p = 0.047, η_p^2 = 0.03; short Band 3: $F_{1,155}$ = 21.15, p < 0.001, η_p^2 = 0.12; short Band 4: $F_{1,155}$ = 11.37, p = 0.001, η_p^2 = 0.07).

Agreement between standard and short conditions

The standard condition was successfully obtained from 65% of the PMS group and 84% of the iASD group compared to 90% of the PMS group and 94% of the iASD under the short condition. ICCs for amplitude and MSC measures were computed to assess the absolute agreement between the standard and short conditions. The estimated agreement between conditions for the P_{60}-N_{75} amplitude was 0.87 with a 95% CI [0.61, 0.94], and for N_{75}-P_{100} amplitude, it was 0.81 with a 95% CI [0.60, 0.90]. ICC for latency at N_{75} was .55 (95% CI [.36, .68] and at P_{100} was .66 (95% CI [.52, .76]). The agreement of frequency bands was moderate in Band 1 and very strong for Bands 2-4: Band 1 (ICC = 0.57, 95% CI [0.33, 0.72]), Band 2 (ICC = 0.81, 95% CI [0.54, 0.90]), Band 3 (ICC = 0.84, 95% CI [0.66, 0.92]) and Band 4 (ICC = 0.82, 95% CI [0.69, 0.88]).

PMS group analyses

Visual inspection of waveforms in the PMS group indicates an absent or significantly diminished P_{60}-N_{75} in all children presenting with deletions in *SHANK3* (Figure 1A; Figure S1, available online) and greater variability in children with point mutations. Deletion size was significantly correlated with P_{60}-N_{75} amplitude for both the standard (r_s = -.445, p = .049) and the short (r_s = -.401, p < .035) conditions. Number of genes deleted was significantly correlated with activity in MSC Band 3 (r_s =-.449, p = .035) and Band 4 (r_s = -.473, p = .035) for the standard condition.

Test-retest reliability

Test-retest reliability in 10 children with PMS was strong for both P_{60}-N_{75} amplitude (ICC = .871), N_{75}-P_{100} amplitude (ICC = .889), N_{75} latency (ICC = .803) and P_{100} latency (ICC = .847).

Discussion

This is the first known study to examine visual electrophysiological markers of excitatory and inhibitory neurotransmission in PMS. Our results indicate early-stage visual processing abnormalities characterized by significant reductions in VEP amplitudes and decreased beta- and early gamma-band activity in children with PMS. Similar to our previously reported findings in iASD[20], the smaller amplitudes at P_{60}-N_{75} found in both the PMS and iASD groups appear to indicate weaker excitatory input to the cortex and that loss likely results in the subsequent diminishment of the N_{75}-P_{100} amplitudes. It is notable that all participants with deletions in

SHANK3 displayed remarkably abnormal waveforms characterized by the absence or significant diminishment of the P_{60}-N_{75} deflection (Figure S1, available online). Individuals with point mutations showed weak P_{60}-N_{75} responses, although the N_{75} peak was consistently distinguishable. Correlations indicated that the larger the deletion, the smaller the P_{60}-N_{75} amplitude. These results further suggest a discernable link between the magnitude of loss of function in *SHANK3* and subsequent glutamatergic dysregulation. There were no overall differences in the latencies of responses at P_{60} or P_{100}. Differences observed at N_{75} latency in the PMS group indicating shorter time to peak are likely a result of weaker excitatory input to the primary visual cortex. In the frequency domain, deficits in beta- and gamma-band activity also suggest diminished excitatory activity. Responses in these high frequencies are likely dependent on fast-acting ionotropic glutamate receptors, which support preclinical work in PMS demonstrating the deleterious effects of *SHANK3* deficiency on glutamatergic system function[5,6]. The lack of significant group differences in MSC values in theta and alpha bands support preserved later cortical (inhibitory) activity.

Our results replicate previous findings in iASD[20] and demonstrate an overlap between children with PMS and children with iASD. Results suggest approximately 30-50% of the iASD group fell within one standard deviation of the PMS group mean for P_{60}-N_{75} amplitude and 54-60% for N_{75}-P_{100} amplitude. Thus, while the literature on excitatory and inhibitory disturbances in ASD samples has been mixed, our findings suggest that a subset of individuals with iASD have pronounced excitatory deficits within the visual system, despite the presence of more normative excitatory tone in others. VEPs may be useful for identifying individuals with iASD who have marked visual system excitatory deficits, representing a possible stratification technique for treatments targeting the glutamate system. Interestingly, there were no significant

differences in clinical variables between participants with iASD who fell within the "PMS-like" VEP range.

When considering time versus frequency domain measures in the context of clinical trials, the MSC statistic adjusts for the typical inter-individual variability in amplitude[44] seen for participants within a diagnostic group by normalizing the responses obtained from individuals, and therefore may emerge as a superior measure to examine differences among groups, although amplitude exhibits better agreement over time within an individual as compared to MSC. Therefore, amplitude may be a useful and sensitive tool to examine how a particular individual change in response to a given treatment or intervention.

In the search for ASD biomarkers, VEPs represent an objective measure that can be obtained from individuals at all levels of functioning. This study demonstrated successful modification of well-established VEP methods for use in severely affected and difficult to test populations, as well as strong test-retest reliability in a subset of participants with PMS. While amplitudes were larger in the short condition as compared to the standard condition—due to adaptation effects resulting from repeated stimulation in the standard condition [44]—the short-duration stimulus condition correlated strongly with the standard condition, which is consistent with findings in typically developing and iASD populations[20,38] and may be useful in the context of clinical trials where the ability to reliably collect data across time points is critical. The application of VEPs in clinical trials is appealing, given the challenges of other objective tools such as functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG)[45,46] that may be challenging to obtain without sedation in severely affected populations.

This study has several limitations. Determining an appropriate control group is a challenge. Due to the level of intellectual disability common in PMS,[10,11] individuals with PMS

are not fully matched on IQ. However, IQ did not significantly affect the MANCOVA for any variable. Our inclusion of a PMS sibling group as an additional control group is novel and further validates the VEP abnormalities identified. Given the *de novo* nature of most PMS cases, PMS siblings typically do not have ASD, and present with similar environmental exposures to their affected siblings. PMS siblings did not differ from other controls, whereas VEP deficits in the PMS group were pronounced. It is possible that some children classified as iASD may have genetic variants not captured through microarray and FXS testing. This study targeted a wide age range to obtain a sufficient number of PMS cases. VEPs are known to mature early in infancy[47] and were present even in our youngest TD controls. Age was included as a covariate given a significant effect on the analyses, which is consistent with evidence of developmental changes, particularly in amplitude, throughout childhood.[48] As genetic testing becomes increasingly accessible, we anticipate the numbers of individuals diagnosed with PMS will grow substantially, which will allow for VEP testing in more refined age cohorts. Additionally, this is largely a cross-sectional study. While we did complete test-retest reliability on a subset of the PMS group, ongoing natural history studies are critical to understand the progression of the syndrome. Future studies examining VEPs in the context of regression, which has been described in a number of PMS cases, will be particularly interesting[10,49] as well as studies examining clinical associations with electrophysiological responses in this population. Finally, this study was restricted to transient VEPs. The examination of additional sensory systems is important to determine whether there are differences in E/I changes based on brain region. Preclinical studies are also necessary to examine analogous paradigms in model systems and to answer questions regarding cell type and circuit specific aspects of E/I that may impact neuronal dysregulation.

In summary, our findings provide information about the underlying neurophysiology of PMS where pathway disturbances have been identified and where clinical trials are underway. A shift from the reliance on subjective caregiver and clinician ratings to objective biomarkers is critical for successful clinical trials in severely affected and minimally verbal populations. VEPs can be collected rapidly, repeated frequently, and are a cost-effective method with strong translational potential across human and animal studies. With this approach, the availability of treatments targeting core mechanistic disturbances in PMS and ASD may progress more rapidly, thereby making a significant clinical contribution.

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Table 1. Group Demographics

	TD	iASD	PMS	PMS sibs	Test Statistic	*p*
Age (*M(SD)*)	6.69 (2.67)	6.72 (2.58)	6.57 (2.68)	7.20 (2.31)	F(3,171)=0.26	0.85
Sex (% female)	44.44	11.39	58.06	75.00	$X^2(3)=42.18$	< 0.001
Race and Ethnicity (%)						
Asian/Pacific Islander	11.11	8.86	12.90	5		
Black/African American	6.67	18.99	0	0		
White (Non-Hispanic)	68.89	46.84	83.87	95		
Hispanic/Latino	6.67	20.25	0	0		
More than one race	6.67	5.06	3.23	0		
NVIQ/NVDQ *(M(SD))*	113.21 (18.37)	89.99 (25.69)	48.79 (13.88)	107.94 (16.00)	F(3,152)=53.59	<0.001
Vineland Adaptive Behavior Composite *(M(SD))*	--	77.19 (11.00)	59.04 (12.99)	--	F(1,86)=43.89	<0.001
ADOS-2 Comparison Score *(M(SD))*	--	7.59 (1.80)	6.64 (2.04)	--	F(1,100)=5.28	0.024
SRS-2 Total Score *(M(SD))*	43.00 (4.69)	72.30 (10.43)	71.03 (10.36)	42.92 (6.01)	F(3,147)	<0.001

Note: ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition; iASD = idiopathic autism spectrum disorder; NVIQ/NVDQ = nonverbal intelligence quotient/nonverbal developmental quotient; PMS = Phelan-McDermid syndrome; SRS-2 = Social Responsiveness Scale, Second Edition; TD = typically developing.

Table 2. Standard and Short Condition Amplitude Measures

	Standard Condition		Short Condition	
	P_{60}-N_{75}	N_{75}-P_{100}	P_{60}-N_{75}	N_{75}-P_{100}
TD	16.43 [13.99 18.88]	27.59 [23.63 31.55]	21.43 [18.64 24.22]	34.01 [30.03 37.99]
iASD	9.16 [7.28 11.04]	20.63 [17.58 23.67]	12.01 [9.96 14.06]	23.95 [21.02 26.87]
PMS	5.35 [1.94 8.77]	15.22 [9.68 20.75]	7.94 [4.61 11.28]	21.06 [16.3 25.82]
PMS sib	15.24 [11.83 18.66]	30.7 [25.16 36.23]	22.31 [18.15 26.48]	41.28 [35.34 47.23]

Note: Values (in μV) reported for amplitude (estimated mean), 95% CI [LL UL]. Abbreviations: iASD = idiopathic autism spectrum disorder; PMS = Phelan-McDermid syndrome; TD = typically developing.

Table 3. Frequency Domain Analysis: Magnitude-Squared Coherence (MSC) Pairwise Comparisons' *p* Values

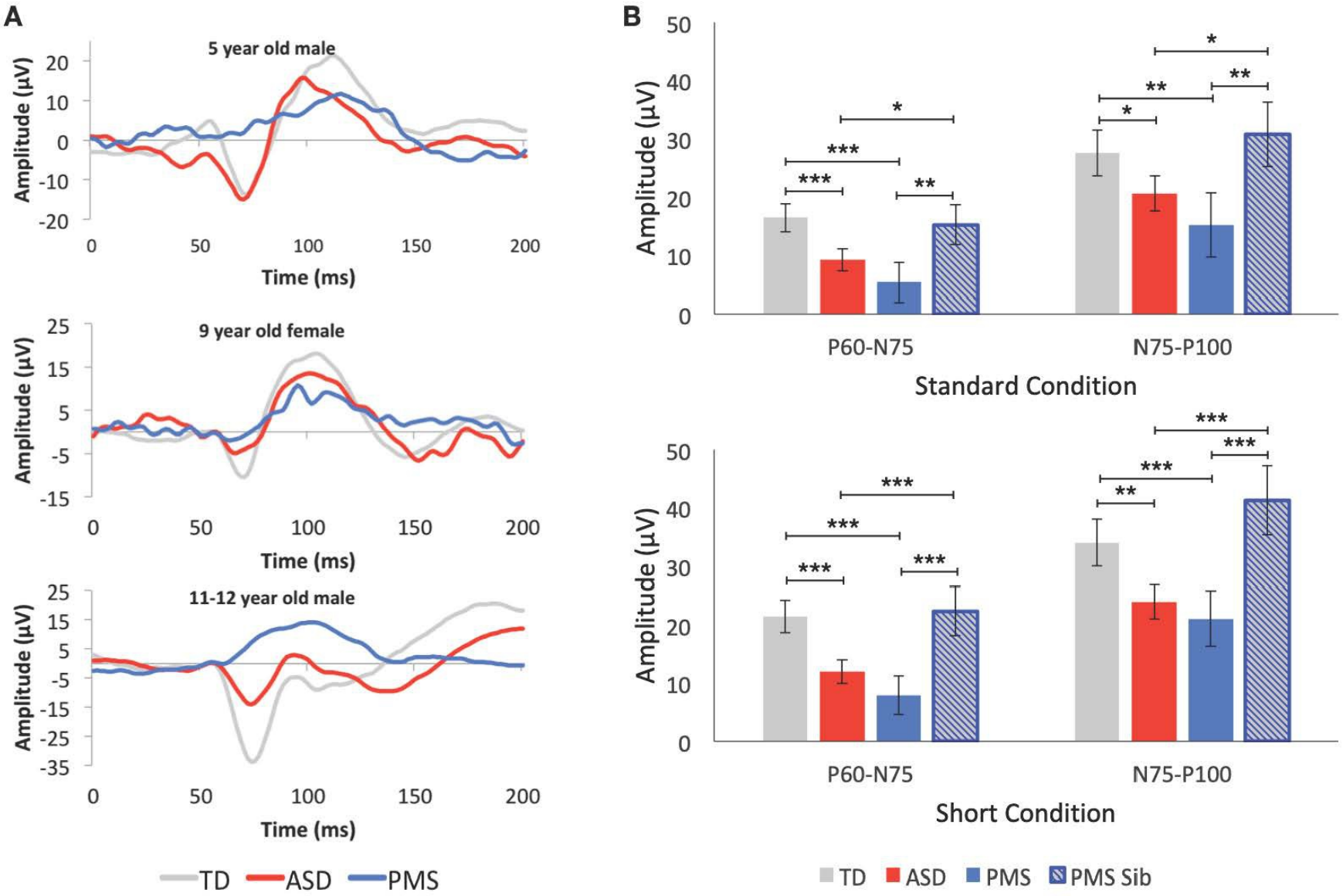
		Standard			Short		
		TD	iASD	PMS	TD	iASD	PMS
Band 1	TD	——			——		
	iASD	1.00	——		1.00	——	
	PMS	0.77	1.00	——	0.45	1.00	——
	PMS sibs	1.00	0.24	0.07	1.00	0.12	0.055
Band 2	TD	——			——		
	iASD	**0.001**	——		**<0.001**	——	
	PMS	**<0.001**	**0.006**	——	**<0.001**	**0.016**	——
	PMS sibs	1.00	0.068	**<0.001**	1.00	**0.002**	**<0.001**
Band 3	TD	——			——		
	iASD	**<0.001**	——		**<0.001**	——	
	PMS	**<0.001**	0.96	——	**<0.001**	0.07	——
	PMS sibs	0.76	**0.024**	**0.004**	1.00	0.043	**<0.001**
Band 4	TD	——			——		
	iASD	**<0.001**	——		**<0.001**	——	
	PMS	**<0.001**	1.00	——	**<0.001**	1.00	——
	PMS sibs	0.77	**0.019**	0.092	1.00	**0.02**	**0.008**

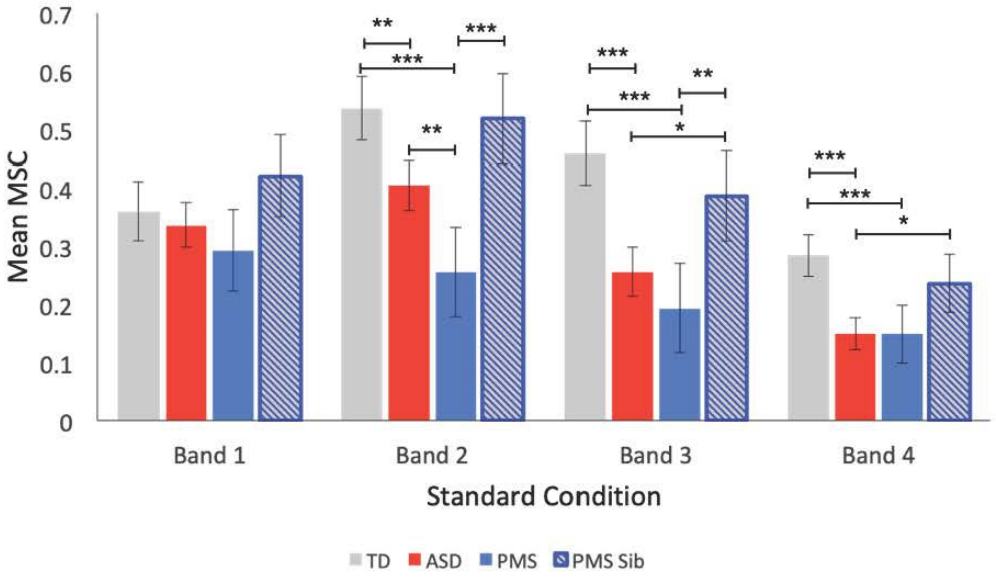
Note: iASD = idiopathic autism spectrum disorder; PMS = Phelan-McDermid syndrome; TD = typically developing.

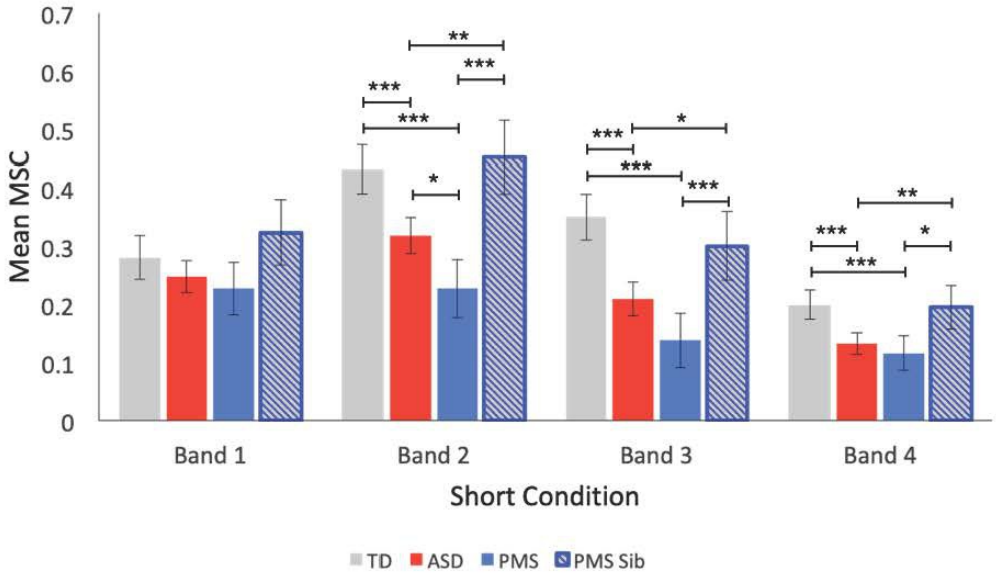
Figure Legends

Figure 1. Representative Transient Visual Evoked Potentials (VEP) Waveforms
Note: (A) Representative transient VEP waveforms from a participant with PMS (blue), iASD (red), and a TD control (grey). (B) Mean amplitudes for the P_{60}-N_{75} and N_{75}-P_{100} tVEP components for the standard condition (top) and short condition (bottom). iASD (red) and PMS (blue) groups show reduced amplitudes compared to TD (grey) and PMS sibs (blue/grey striped). Errors bars represent 95% CI. iASD = idiopathic autism spectrum disorder; PMS = Phelan-McDermid syndrome; TD = typically developing; tVEP = transient visual evoked potential.
*p < 0.05, **p < 0.01, ***p < 0.001.

Figure 2. Frequency Band Activity by Group
Note: PMS (blue) and iASD (red) groups show reduced MSC values across multiple frequency bands relative to TD (grey) and PMS sibs (blue/grey striped) groups in both the standard condition (left) and short condition (right). Errors bars represent 95% CI. iASD = idiopathic autism spectrum disorder; MSC = magnitude-squared coherence; PMS = Phelan-McDermid syndrome; TD = typically developing. *p < 0.05, **p < 0.01, ***p < 0.001.


A
5 year old male
Amplitude (μV)
Time (ms)
9 year old female
11-12 year old male
TD
ASD
PMS
B
Standard Condition
Short Condition
P60-N75
N75-P100
TD
ASD
PMS
PMS Sib


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean MSC
Band 1
Band 2
Band 3
Band 4
Standard Condition
TD
ASD
PMS
PMS Sib


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean MSC
Band 1
Band 2
Band 3
Band 4
Short Condition
TD
ASD
PMS
PMS Sib