DEVELOPMENT OF NEURAL RESPONSE TO NOVEL SOUNDS IN FRAGILE X SYNDROME: POTENTIAL BIOMARKERS

Keywords: fragile x syndrome; eeg; erp; auditory; mismatch negativity; development

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Abstract

Auditory processing abnormalities in fragile X syndrome (FXS) may contribute to difficulties with language development, pattern identification, and contextual updating. METHOD: Participants with FXS (N=41) and controls (N=27) underwent auditory ERP with a 32 lead EEG cap during presentation of an oddball paradigm. Analyses included log age as a covariate. RESULTS: Data was adequate for analysis for 33 participants with FXS and 27 controls (age 4-51y, 13 females (FXS); 4-54y,11 females (control)). Participants with FXS showed larger N1 and P2 amplitudes (p's<0.05), abnormal modulation of ERP amplitudes in response to oddball stimuli including lack of normal increases in P1 (p=0.037) and P2 (p=0.008) amplitudes and normal slowing of P2 latency (p<0.001) relative to controls: Females with FXS were more similar to controls. Participants with FXS showed a marginal speeding of the P2 latency during the task, suggesting potentiation to oddball stimuli rather than habituation, F(1,55)=3.7, p=0.05. Participants with FXS showed a heightened N1 habituation effect to standards compared to controls, F(1,55)=4.5, p=0.03. Gamma power was significantly higher for participants with FXS F(1,55)=10.1, p=0.002. Participants with FXS and controls did not differ on mismatch negativity. Both controls and participants with FXS showed significant decreases in P1 amplitude, and increases in N1 amplitude, P2 latency, and gamma power with age. However, controls but not participants with FXS show a decrease in P2 amplitude with age. Retest analyses performed in 14 participants with one month retest suggest strong test-retest reliability (ICC range 0.65 to 0.96, p's <0.05) for most measures, and borderline reliability for mismatch negativity (ICC =0.57, p=0.06), and P2 amplitude and latency to oddball (p's>0.05). CONCLUSION: Individuals with FXS show previously demonstrated increased in response amplitude and high frequency neural activity. Additionally, despite an overall normal developmental trajectory for most measures, individuals with FXS show age-independent but gender-dependent decreases in complex processing of novel stimuli. Many markers show strong retest reliability even in children and thus are potential biomarkers for clinical trials in FXS.

Introduction.

Sensory processing abnormalities are a common clinical characteristic of individuals with fragile X syndrome (FXS) with plausible links to pathophysiology (Sinclair et al., 2016). Sensory sensitivities and central sensory processing abnormalities are often present at a young age (Baranek et al., 2008) and may contribute to difficulties in language development and other executive function abilities such as sound and pattern identification and understanding context (Ludlow et al., 2014). Behavioral tasks can identify many of these executive function and language abnormalities but may be difficult to conduct in individuals with intellectual disability, as in FXS, can be difficult to link back to sensory processing abnormalities and may take extended treatment periods to see change with intervention.

Electroencephalography (EEG) and event-related potentials (ERP) can be used to not only evaluate higher order cognition but also identify linked sensory processing ERP components, potentially serving as a marker for target engagement and even subject selection in clinical trials of interventions targeting underlying brain function in FXS. One common task used to examine sensory processing and higher-order contextual updating is the auditory oddball task, which uses repeated identical tones (standards) to set up a sequence in which a relatively rare tone of a different pitch (oddball) becomes novel in context. Typically developing individuals show a stronger, more prolonged sensory response to the oddball tone, including a large negative component approximately 100-200 ms post-stimulus-onset called the mismatch negativity (Naatanen et al 1993). The amplitude of the mismatch negativity, arising from bilateral superior temporal and right inferior frontal cortices (Garrido et al., 2008; 2009; Cooray et al., 2016; Ranlund et al., 2016) tracks with language development, including specific language impairments (Paquette et al., 2013; Rocha-Muniz et al., 2015; Kujala & Leminen, 2017).

Individuals with FXS commonly show delays with deficits in both expressive and receptive language skills (Thurman et al., 2017), particularly in auditory sequential memory (Oakes et al., 2013). While a growing body of EEG/ERP studies support basic cortical sensory processing abnormalities in FXS (Castren et al., 2003; Ethridge et al., 2016; 2017; Knoth et al., 2018) and fmr1 knockout (KO) mouse models (Sinclair et al., 2017a; 2017b; Lovelace et al., 2016; 2018; Wen et al. 2019), the extent to which these abnormalities extend to higher order cognitive processing is unclear. Animal models suggest that auditory and frontal cortical deficits are present in early development and increase with age (Wen et al., 2019). If similar developmental patterns occur in humans with FXS, early sensory abnormalities could compound, leading to the progressive impairment of language skills observed in FXS (Lee et al., 2016). Most ERP studies support increased amplitude sensory ERPs, reduced habituation of sensory ERPs, and reduced signal to noise ratio in the gamma frequency band in auditory cortex in adolescents and adults with FXS (Ethridge et al., 2016; 2017; 2019; Knoth et al., 2018; Schneider et al., 2013) with limited evidence for increased N1 ERP amplitudes in children with FXS (Castren et al., 2003). It is unknown whether very early auditory processing ERP components such the P1 ERP are also increased in FXS. Auditory processing phenotypes, in particular the N1 and P2 ERPs (Schneider et al., 2013) and gamma power (Sinclair et al., 2017a), may be amenable to pharmacological intervention but developmental effects on these phenotypes are unknown, limiting their usefulness for studying language development or use in clinical trials with children. In addition, most ERP studies in FXS include few or no females. Because the disorder is X-linked, females with FXS, while still impaired relative to typically developing children, show a milder phenotype in general and more typical developmental trajectories for language acquisition than do males with FXS (Komesidou et al., 2017; Sterling &

Abbeduto, 2012), based on expression of the normal allele in a fraction of neurons. How the corresponding neural trajectories differ between genders in FXS is unknown. The current study examined age and gender-related effects on sensory and cognitive processing of auditory stimuli using EEG/ERP in a wide age-range of individuals with FXS. We focused on identifying stimulus-invariant auditory processing deficits and appropriate biomarkers for clinical trials based on age and gender variance as well as test-retest reliability. We hypothesized that developmental trajectories would be flatter for the FXS group than for controls, leading to wider divergence in auditory phenotypes with increased age. We also predicted that females with FXS would show reduced auditory processing deficits relative to males with FXS, potentially due to the known milder phenotype and differences in developmental trajectory.

Method

Participants. Forty-one individuals with FXS and an *FMR1* full-mutation (>200 repeats) and 27 age and gender matched typically developing controls participated in the ERP study. Data from 8 individuals with FXS was removed due to excessive movement-related artifact, leaving 33 FXS (mean age = 17.3, SD= 8.9 years, range 4-51 years, 13 females) and 27 controls (mean age = 21.0, SD = 10.4 years, range 4-54 years, 11 females) included in the final analyses (Table 1). In addition to the ERP task, FXS participants completed the Stanford-Binet 5 (SB5, Roid, 2003) to measure IQ, the interview version of the Vineland Adaptive Behavior Scales Version 3 (Sparrow et al., 2016) was administered to caregivers to assess adaptive behavior and caregivers completed the Aberrant Behavior Checklist – Community Edition with subdomain scores factored for FXS populations (ABC_{FX}, Sansone et al. 2012) to evaluate maladaptive behavior and the Sensory Profile-2 (SP2, Dunn, 2014) to evaluate abnormal sensory behaviors. Controls completed a screening questionnaire, had no sign of cognitive compromise, neurological or psychiatric diagnoses and had a normal *FMR1* allele. All participants provided written consent or verbal assent with parental consent as appropriate for age and intellectual ability.

Stimuli. Participants completed a passive auditory oddball task presented using Presentation software (Neurobehavioral Systems, Albany, CA). Stimuli consisted of 432 "standard" tones (1000 Hz; 90% of stimuli) and 48 "oddball" tones (2000 Hz; 10% of stimuli) presented at 70 dB SPL via headphones. Tones were 70 ms in duration including a 10ms rise/fall with 1000 ms inter-stimulus interval. Order of stimuli was pseudorandomized with the caveat that at least 6 standard stimuli must be presented sequentially before an oddball stimulus would occur once at either the 7^{th}, 8^{th}, 9^{th}, or 10^{th} position in a 10 stimulus train (Schneider et al., 2013). Participants watched a silent video of their choice during stimulus presentation to improve comfort and reduce movement.

EEG Recording. EEG was continuously recorded and digitized at 512 Hz, with a 5^{th}-order Bessel anti-aliasing filter at 200 Hz, using a 32 channel BioSemi ActiveTwo system (BioSemi, Inc; Amsterdam, Netherlands) with sensors placed according to the International 10/10 system (Chatrian, 1985). All sensors were referenced to a monopolar reference feedback loop connecting a driven passive sensor and a common-mode-sense active sensor, both located on posterior scalp. Participant behavior was recorded on notes sheets with EEG time stamps for major events.

EEG Analyses. Raw data were visually inspected offline and bad sensors (maximum 1 sensor per file) interpolated using spherical spline interpolation implemented in BESA 6.1 (MEGIS Software, Grafelfing, Germany), digitally filtered from 0.5-100 Hz (12 and 24 db/octave rolloff, respectively; zero-phase, 60 Hz notch) and re-referenced to average reference.

Eye movement, cardiac, and muscle movement artifacts were removed blind to participant group using independent components analysis (ICA) implemented in EEGLAB 13 (Delorme & Makeig, 2004) in Matlab (The Mathworks, Natick, MA). ERP data were epoched into 1000 ms trials (-500 ms to 500 ms), averaged across trials and baseline-corrected using the 500 ms pre-stimulus period. Trials with post-ICA amplitude exceeding 120 μV were rejected. Number of valid trials did not differ between groups for the oddball stimulus (FXS M=44.9, SD=4.2; Control M=46.0, SD=1.9, t(58)=1.2, p=0.21). While number of trials was significantly different between groups for the standard stimulus, t(58)=2.1, p=0.03, all participants retained at least 75% of trials, creating large trial counts (FXS M=404.9, SD=31.7, Control M=419.5, SD=16.5) that equate the group difference to 3%, which is unlikely to produce systematic effects on ERP signal quality.

Epoched waveforms were averaged over 8 sensors distributed across the fronto-central scalp (Figure 1 inset), selected a priori based on the spatial distribution most consistent with previous literature capturing auditory cortex activity (Luck, 2014). ERP amplitudes and latencies for each participant were calculated at the waveform peak within 80 ms time windows centered on the peak of each ERP component in the grand average waveform (Table 2). Mismatch negativity was calculated as mean standard waveform amplitude minus mean oddball waveform amplitude between 70-160ms post-stimulus. To equate signal-to-noise ratio based on trial count between stimulus types, only standard stimuli occurring immediately prior to oddball stimuli were included in the standard averages for mismatch negativity calculations.

Previous studies suggest a deficit in auditory habituation in FXS (Schneider et al., 2013; Ethridge et al., 2016; Knoth et al., 2018). Because oddball stimuli effectively "reset" neural processing of repeated stimuli, habituation of the N1ERP was calculated as the difference in N1 amplitude and latency between standard stimuli immediately following an oddball stimulus and the following repeated standard stimulus. To assess habituation across the entire task, similar to Schneider and colleagues (2013), habituation was also calculated as the difference in N1 and P2 amplitude and latency between an average of the first 15% and the last 15% of standard stimulus trials presented in the EEG session.

To obtain estimates of gamma single-trial power, single-trials were concatenated and analyzed in the time-frequency domain from 30-100 Hz using Morlet wavelets with 1 Hz frequency step using a linearly increasing cycle length from 6 cycles at 30 Hz to 20 cycles at 100 Hz. (Delorme and Makeig, 2004). To be consistent with previous literature (Ethridge et al., 2016; 2017), gamma power was averaged across the entire frequency range and epoch.

Statistical analyses. Separate mixed-effects ANOVAs were calculated for amplitude and latency of each ERP peak with between-subjects factors group (FXS, Control) and gender (M,F) and within-subjects factor trial type (standard, oddball). N1 habituation was calculated in a mixed-effects ANOVA with the between-subjects factors group (FXS, Control) and gender (M,F) and within-subjects factor stimulus repetition (initial stimulus, first repeated stimulus). Habituation across the entire task was calculated in a mixed-effects ANOVA with the between-subjects factors group (FXS, Control) and gender (M,F) and within-subjects factor stimulus position (first 15%, last 15% of trials). Differences in N1 and P2 habituation were quantified using the group by stimulus repetition interaction, indicating a difference between groups in amplitude or latency change across repetitions/stimulus position. Age was log transformed to approximate a normal distribution and used as a covariate in all analyses. Clinical parameters including SB5 z-deviation IQ to eliminate floor effects for ID populations (Sansone et al.2015), Vineland 3 Composite and Language Subdomain Scores, SP2 subscale scores, and ABC_{FX}

domain scores were compared to EEG outputs using partial Pearson correlations accounting for age and corrected for multiple comparisons using a 25% False Discovery Rate. To account for gender differences correlations were conducted separately for males and females.

One month test-retest reliability was calculated on a subset of 14 younger individuals (FXS N=8, Control N=6, Mean age = 10.2, SD=3.7, age range= 4-15 years, 7 females) using intraclass correlation mixed model with absolute agreement 95% confidence intervals on a difference from null. Group size on this subset was too small to calculate separate ICCs for FXS and controls, so calculations were collapsed across group membership.

Results.

Demographics. There were no significant differences between participants with FXS and controls on age $t(55)=1.4$ or gender distribution $\chi^2(1,N=60)=0.01$.

EEG. Overall, participants with FXS showed larger N1 and P2 amplitudes compared to controls (Figure 1, Table 2). Participants with FXS also showed abnormal modulation of ERP amplitudes in response to stimulus frequencies: while controls showed heightened P1 and P2 response to novel (oddball) stimuli, participants with FXS did not modulate P1 amplitude based on stimulus type and decreased P2 amplitude to novel stimuli. Controls also slowed P2 latency to novel stimuli, while participants with FXS showed no difference between stimulus types. However a gender x group x trial type interaction suggests that females with FXS modulate P2 latency more similarly to controls. Age was a significant covariate for all comparisons except P2 amplitude.

P2 latency to standards also differed marginally between groups across the duration of the task: when comparing the first 15% of trials to the last 15% of trials in a session, controls showed no difference in P2 latency from the beginning of the task to the end, whereas participants with FXS showed a speeding of the P2 latency, suggesting an ERP response potentiation rather than habituation. This effect did not differ by gender. Contrary to previous literature, participants with FXS also showed a heightened N1 habituation effect to standards compared to controls.

Similar to previous findings, gamma power to both stimulus types was significantly higher for participants with FXS. This did not differ by gender. Although the group average waveforms (Figure 2) and the P1/P2 group differences suggest more modulation in controls between trial types, participants with FXS and controls did not differ significantly on mismatch negativity specifically.

Developmental correlations. Both controls and participants with FXS showed significant or marginal decreases in P1 amplitude to standards and oddballs (Table 2), increases in N1 amplitude for both standards and oddballs, increases in P2 latency to standards, and increases in gamma power to standards and oddballs with age, suggesting normal developmental trajectory for FXS despite abnormal amplitudes and task-based modulation of these responses. However, controls show a decrease in P2 amplitude to standards with age, while participants with FXS do not.

Clinical correlations. The only correlations surviving FDR correction were in males with FXS, for whom gamma power in both conditions was correlated with sensory avoidance (Standards $r=-.77$, $p=.002$; oddball $r=-.76$, $p=.002$) and sensitivity (Standards $r=-.76$, $p=.002$; oddball $r=-.72$, $p=.005$).

Retest reliability. Exploratory retest analyses performed in a subset of 14 younger participants with one month follow-ups suggest strong test-retest reliability (ICC range .65 to .96, p's <.05) for most of the ERP (Figure 3) and power measures, the exceptions being mismatch negativity with marginally significant reliability (ICC =.57, p=.06), and P2 amplitude and latency in response to the oddball stimulus only (p's>.05) (Table 2).

Discussion.

A growing body of translational literature supports particular neural correlates of sensory processing abnormalities in FXS (Ethridge et al., 2016; 2017; Lovelace et al., 2018; Sinclair et al, 2017). New findings from the current study also support those neural correlates, namely increased N1 and P2 ERP amplitude and increased gamma power in individuals with FXS, and expand those findings to include abnormalities in neural modulation in response to changing stimulus properties in context of stimulus expectancies.

Typically developing controls showed a heightened response to the novel (oddball) stimulus compared to the common standard stimulus while participants with FXS showed either no modulation or a decreased response, consistent with the only other ERP study of change detection in FXS (Van der Molen et al., 2012). These findings could be due to a neurobiological ceiling effect in FXS ERP amplitudes, which were elevated compared to controls. This is unlikely, however, given the lack of modulation in participants with FXS for the P1, which was not significantly larger overall in the FXS group. Participants with FXS also did not slow their P2 latency to novel stimuli like controls, suggesting that lack of earlier sensory differentiation may lead to a lack of later cognitive or sensory integrative processing. Importantly, females with FXS appeared similar to males in early sensory processing modulation deficits but showed P2 latency slowing similar to controls, perhaps due to compensation from relatively more intact neural networks and cognitive ability. For both genders, participants with FXS showed a potentiation rather than habituation of P2 latency over the course of the session. This finding is interesting, as the task was short enough (~5.5 minutes) to avoid fatigue effects that can dampen neural response, but the oddball stimulus was salient enough to continually "reset" neural processing in controls to avoid long-term attentional habituation over the course of the session. Therefore, controls responded similarly throughout. Continual stimulation actually speeded sensory integration/context processing for participants with FXS, which may indicate increased efficiency or decreased response complexity. Since participants with FXS do not modulate P2 latency with stimulus complexity, the latter interpretation may be more appropriate. However, gender effects differed between these two comparisons. Further work specifically targeted at P2 modulation will be necessary to disentangle gender effects on early cognitive processing in FXS.

Unlike previous literature, participants with FXS showed a heightened N1 short-term habituation refractory response (comparing the first standard after the oddball "reset" and the next standard repetition). This finding may be due to a number of factors. First, participants with FXS showed an overall heightened, unmodulated N1 amplitude, and thus a higher starting point for the habituation calculation. The inter-stimulus interval for a typical oddball task is twice as long (1000 ms) as that normally employed in habituation tasks (500 ms)(Ethridge et al., 2016; Potter et al., 2006). Sensory gating of the P1 and N1 ERP is strongest at 500ms (Pereira et al., 2014), tapering off with longer inter-stimulus intervals. It is possible that sensory processing deficits in FXS change the ideal latency at which stimulus properties can be registered, leading to habituation response at longer intervals that is characteristic of shorter interval processing in

controls. Indeed, ERP work with fmr1 KO mice shows that KO mice did not show auditory habituation deficits at longer inter-stimulus intervals (Lovelace at al., 2016). Enhanced registration of some stimulus properties at this latency however does not lead to appropriate modulation related to stimulus expectancies (i.e. contextual novelty).

While the current study's findings are largely consistent with the only other study of change detection in FXS (Van der Molen et al., 2012), there was one contrary finding: participants with FXS did not show deficits in mismatch negativity. This may be due to added heterogeneity introduced by the developmental age range and both genders in our sample. We also found that retest reliability was only moderate for MMN, suggesting that state variability may also contribute to differential findings. Mismatch negativity is considered a pre-attentive perceptual prediction error indicator (Friston, 2005) based on statistical regularities in the repeated stimuli creating an expectancy of continued repetition; the P2 ERP is more associated with complex stimulus discrimination and sensory memory related to these expectancies. P2 amplitude and latency modulation abnormalities in FXS suggest decreases in complex processing of novel stimuli that may lead to abnormal response to common and unusual environmental stimuli.

Participants with FXS and controls show similar developmental trajectories on all measures except the P2 response. This finding is particularly interesting in comparison to findings of differential developmental trajectories in autism for gamma power (De Stefano et al. 2019; Gabard-Durnam et al., 2019). The developmental similarities between participants with FXS and controls strengthens clinical trial design specific to FXS using these variables: accurate prediction of appropriate neural measures depending on the trial sample age-range can be done based on the large body of developmental data available regarding the auditory oddball paradigm in typically developing populations. Group differences seen here coupled with similar developmental trajectories also suggest that these neural differences are already present in young children with FXS and may be appropriate for use in clinical trials involving children.

Many measures showed weak correlations with ERP parameters when males and females were analyzed together. Correlations were not significant for individual genders likely because the N for the individual genders is too small to see significant correlations. It could also be that the full cognitive range is needed to show correlation, and only females will be in the cognition range >60, while males will contribute the lower end of the spectrum of cognition on FXS, suggesting analysis of males and females together is most appropriate for this purpose. Nonetheless, the only clinical correlations surviving FDR correction for multiple comparisons were between gamma power and sensory avoidance and sensitivity from the SP2. Individuals with FXS who had more sensory avoidance and sensitivity showed lower gamma power. This finding contradicts previous findings showing increased gamma power in FXS participants with higher sensory sensitivity scores (Ethridge et al., 2016; 2017). Together these findings suggest that gamma power abnormalities are related to sensory processing abnormalities but the nature of this relationship requires further evaluation in future research. Interestingly, in this sample all SP2 measures were highly correlated, including seemingly contradictory measures such as sensory avoidance and sensory seeking ($r=.83$, $p<.001$), suggesting that sensory hypersensitivity is not a binary measure. Individuals with more extreme sensory experiences in general showed reduced gamma, whereas previous work (Ethridge et al., 2016; 2017) examined correlations with auditory processing specifically.

Any measure evaluated as a candidate biomarker for clinical trials must show strong retest reliability. The majority of the measures in this study showed very strong retest reliability,

with some practical considerations: increased trial count for standard stimuli increases signal-to-noise ratio of the waveform and provides a more reliable test-retest measure than oddball stimuli. The sensory components P1, N1 and gamma power show strong retest reliability regardless of trial count, and may be more useful targets for biomarker evaluation in clinical trials. Test-retest reliability on duration and omission mismatch is high using magnetoencephalography (MEG) (Recasens & Uhlhaas, 2017). The marginally significant retest reliability in our sample may then be due to the difference in stimulus (pitch vs duration/omission), measurement (EEG vs MEG), or increased variability of this response in FXS samples vs the typically developing samples in previous literature. It should be noted that a limitation of this study is that the portion of our sample with retest data were all in the younger age group. While retest reliability for oddball task ERPs is generally high in typically developing adults (Williams et al., 2005), it is unknown whether retest reliability for these measures changes systematically with age or whether reliability differs for adults with FXS.

It may be possible to utilize the more stable gamma power measure as a proxy for some processes underlying mismatch negativity. Gamma-band connectivity between temporal and frontal cortices has been associated with mismatch negativity and response to novel stimuli, suggesting a role for gamma in processing auditory context outside of sensory cortices alone (Zhang et al., 2018). Gamma power has also been linked to language processing (Brederoo et al. 2015; Bastiaansen & Hagoort, 2006), language development (Benasich et al., 2008), and item prediction in language comprehension (Wang et al., 2012; Monsalve et a., 2014). Gamma power is 1) increased in FXS, 2) likely stimulus invariant as it has been found in other studies using different (or no) stimulation (Ethridge et al., 2016;2017; Wang et al., 2017) and shows no significant modulation by stimulus type in the current study, 3) follows a predictable developmental trajectory, 4) has translational stability to mouse models (Lovelace et al., 2018; Sinclair et al., 2017) including responsiveness to pharmaceutical manipulation (Sinclair et al, 2017), 5) probable links to FXS pathophysiology (Goswami et al., 2019) and 6) strong retest reliability even at reduced trial counts. Thus, increased gamma power may provide the most promising target for biomarker development in FXS, particularly in relation to clinical features of sensory processing or behavior. In addition, putative mechanisms for altered gamma power in FXS, namely reduced excitatory drive onto inhibitory interneurons (Gibson et al., 2008) and increased rigidity of layer-specific oscillatory behavior (Goswami et al., 2019) suggest that gamma power measures may be useful not just for evaluation of sensory phenotypes but also as an index of more general cortical function in FXS.

The current study reinforces support for previous findings of enhanced ERP amplitude in FXS, provides additional evidence for the strength of gamma power as a candidate biomarker, and extends findings to a new developmental sample with broader age range and increased representation of females with FXS. New findings suggest that despite an overall normal developmental trajectory for most of these measures, individuals with FXS show decreases in complex processing of novel stimuli even in young children. With the replication of gamma power increases and additional evidence for their complex connection to extremes sensory behaviors, this study provides increased support for early sensory neural components (P1, N1, gamma power) as potential biomarkers for target engagement, response to intervention or subject selection in clinical trials of targeted treatments, further supported by strong retest reliability of these measures in children.

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Conflicts of Interest
EBK has been a consultant for Seaside Therapeutics, Novartis, Roche, Alcobra, Neuren, Cydan, Fulcrum, GW, Neurotrope, Marinus, Zynerba, BioMarin and Ovid Pharmaceuticals; a consultant for Novartis, Roche and Neurotrope regarding the conduct of clinical trials in fragile X syndrome (FXS) and other rare neurological diseases; on the scientific advisory board for Vteese/Mallinckrodt to provide guidance on clinical trials in Niemann–Pick type C (NP-C) disease; and has received research funding to conduct clinical trials in FXS and/or other neurodevelopmental disorders from Novartis, Roche, Seaside Therapeutics, Alcobra and Neuren, from Ovid and Zynerba to conduct a clinical trial in NP-C, from Vtesse/Mallinckrodt and from Asuragen to develop fragile X mental retardation 1 testing standards.
LE has consulted for Novartis, Fulcrum, and Ovid Pharmaceuticals.
The remaining authors report no potential conflicts.

Table 1. Clinical Measures For FXS Participants

Measure	Males	Females	Percent Participants with Completed Reports
Nonverbal Deviation IQ	47.71 (14.73)) [22.85 - 79.52]	70.82 (12.50) [46.26 – 92.69]	91%
Verbal Deviation IQ	53.65 (16.96) [24.80 - 91.96]	70.69 (13.49) [42.72 – 90.59]	91%
Full scale Deviation IQ	50.68 (15.37) [23.83 – 85.74]	70.75 (12.15) [44.44 – 91.64]	91%
SP2 Sensory Seeking	21.50 (15.83) [0 - 48]	22.88 (15.73) [0 – 43]	76%
SP2 Sensory Avoidance	31.42 (19.82) [0 - 60]	40.77 (26.49) [0 – 72]	76%
SP2 Sensory Sensitivity	29.50 (21.12) [2 – 69]	40.66 (26.08) [3 – 77]	76%
SP2 Sensory Registration	28.78 (21.23) [0 – 68]	36.11 (25.66) [2 – 83]	76%
Vineland Adaptive Behavior	50.47 (20.62) [22 – 98]	71.41 (21.84) [38 – 110]	85%
Vineland Communication	43.41 (24.04) [20 - 98]	67.08 (24.40) [26 – 106]	85%
Vineland Daily Living	53.00 (19.77) [26 – 104]	76.58 (26.91) [20 – 118]	85%
Vineland Social	54.35 (22.19) [20 – 94]	70.58 (21.20) [40 – 102]	85%
ABC_{FX} Irritability	9.45 (10.72) [0 – 35]	6.76 (7.54) [0 - 26]	100%
ABC_{FX} Lethargy	3.90 (4.25) [0 – 16]	5.61 (7.22) [0 – 26]	100%
ABC_{FX} Stereotypy	3.05 (3.17) [0 – 9]	2.46 (4.35) [0 - 16]	100%
ABC_{FX} Hyperactivity	5.84 (6.03) [0 – 17]	4.84 (6.25) [0 – 23]	97%
ABC_{FX} Inappropriate Speech	4.25 (3.54) [0 – 11]	1.92 (1.70) [0 – 5]	100%
ABC_{FX} Social Avoidance	2.85 (2.97) [0 - 8]	3.53 (4.33) [0 -12]	100%

Means are presented for males and females with standard deviation in parentheses and range in brackets.

Table 2. EEG/ERP Result Summary Table

Variable	Time window	Main effects	Interactions	Correlation with age	Retest reliability
P1 amp.	40-120ms	NS	Trial type x group F(1,55)=5.3* η^2=.09	FXS STD r=-.45** Control STD r=-.76*** FXS OB r=-.31^ Control OB r=-.61***	STD ICC=.72** OB ICC=.88***
N1 amp.	70-150ms	Trial type F(1,55)=3.3^ OB>STD η^2=.06 Group F(1,56)=3.2^ FXS>Control η^2=.06	NS	FXS STD r=-.59*** Control STD r=-.70*** FXS OB r=-.42** Control OB r=-.63***	STD ICC=.93*** OB ICC=.92***
P2 amp.	150-250ms	Trial type F(1,55)=11.9*** STD>OB η^2=.18	Trial type x age F(1,55)=11.5*** η^2=.17 Trial type x group F(1,55)=8.5** η^2=.13	FXS STD r=.03 Control STD r=-.44* FXS OB r=.30^ Control OB r=.27	STD ICC=.89*** OB ICC=.35
P2 latency	150-250ms	Trial type F(1,55)=5.4* OB>STD η^2=.09 Gender F(1,55)=3.0^ M>F η^2=.05	Trial type x group F(1,55)=21.2*** η^2=.28 Trial type x gender x group F(1,55)=3.1^ η^2=.05	FXS STD r=.61*** Control STD r=.57** FXS OB r=.07 Control OB r=.34^	STD ICC=.93*** OB ICC=.02
Gamma power	-500-1000ms	Group F(1,55)=10.1** FXS>Control η^2=.16	NS	FXS STD r=.54*** Control STD r=.66*** FXS OB r=.52** Control OB r=.67***	STD ICC=.66* OB ICC=.65*
N1 habituation	70-150ms	Group F(1,55)=4.5* FXS N1 > Control N1 η^2=.08	Repetition x group F(1,55)=7.3** η^2=.12	-	-
Mismatch negativity	70-160ms	NS	NS	FXS r=-.03 Control r=.20	ICC=.57^
N1 amp. habituation first/last 15%	70-150ms	Group F(1,55)=2.9^ FXS N1 > Control N1 η^2=.05	NS	-	-
P2 amp. habituation first to last	150-250ms	Group F(1,55)=8.6** FXS>Control	NS	-	-

15%		η^2=.14			
N1 latency habituation first to last 15%	70-150ms	Group F(1,55)-5.4* FXS>Control η^2=.09 Gender F(1,55)=3.7^ M>F η^2=.06	NS	-	-
P2 latency habituation first to last 15%	150-250ms	Gender F(1,55)=2.9^ M>F η^2=.05	First/last x group F(1,55)=3.7^ η^2=.06	-	-

^p<.10 (marginal)
*p<.05
**p=<.01
***p=<.001
Effect sizes reported as partial eta squared.
Note: Measures without entries for age correlations and re-test reliability were calculated via statistical interaction and thus did not provide appropriate single variables for ICC calculation

Figure Captions.

Figure 1. Grand average waveforms for standard and oddball stimuli by group. Inset figure indicates sensors selected a priori (red sensors) and averaged for waveform creation and subsequent analyses. Stimulus onset occurs at 0 ms on the x axis.

Figure 2. Mismatch negativity waveforms by group. Mismatch negativity is calculated from 70-160 ms post-stimulus onset (at 0 ms), centered on the large negative waveform peak created by subtracting the negative-going ERP to standard stimuli from the larger negative-going ERP to oddball stimuli. Larger negative amplitude indicates larger negative response to oddball stimuli.

Figure 3. Retest-reliability of the waveforms from a subset of the sample with one month re-test data.

Figure 4. Correlations for EEG variables with age by group and condition. R values and significance levels for each correlation are presented in Table 2. Age is plotted on a log scale.

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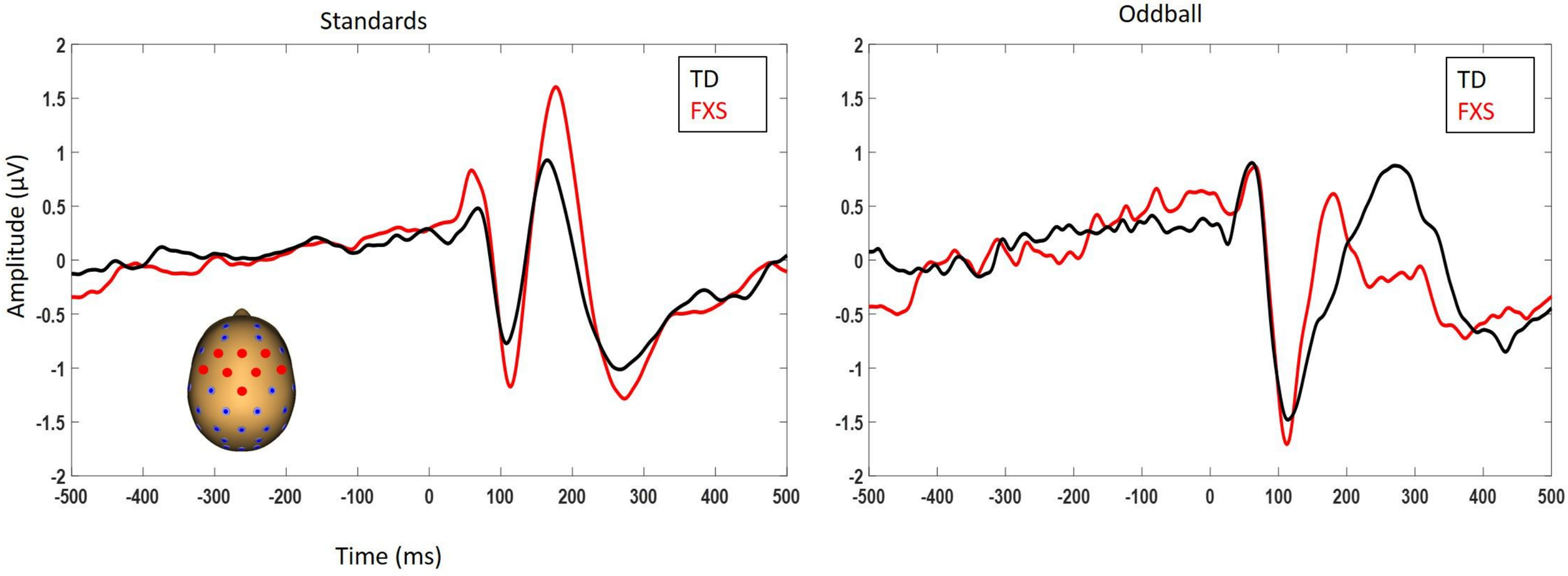
Standards
Oddball
TD
FXS
TD
FXS
Amplitude (μV)
Time (ms)
2
1.5
1
0.5
0
-0.5
-1
-1.5
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-500
-400
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-200
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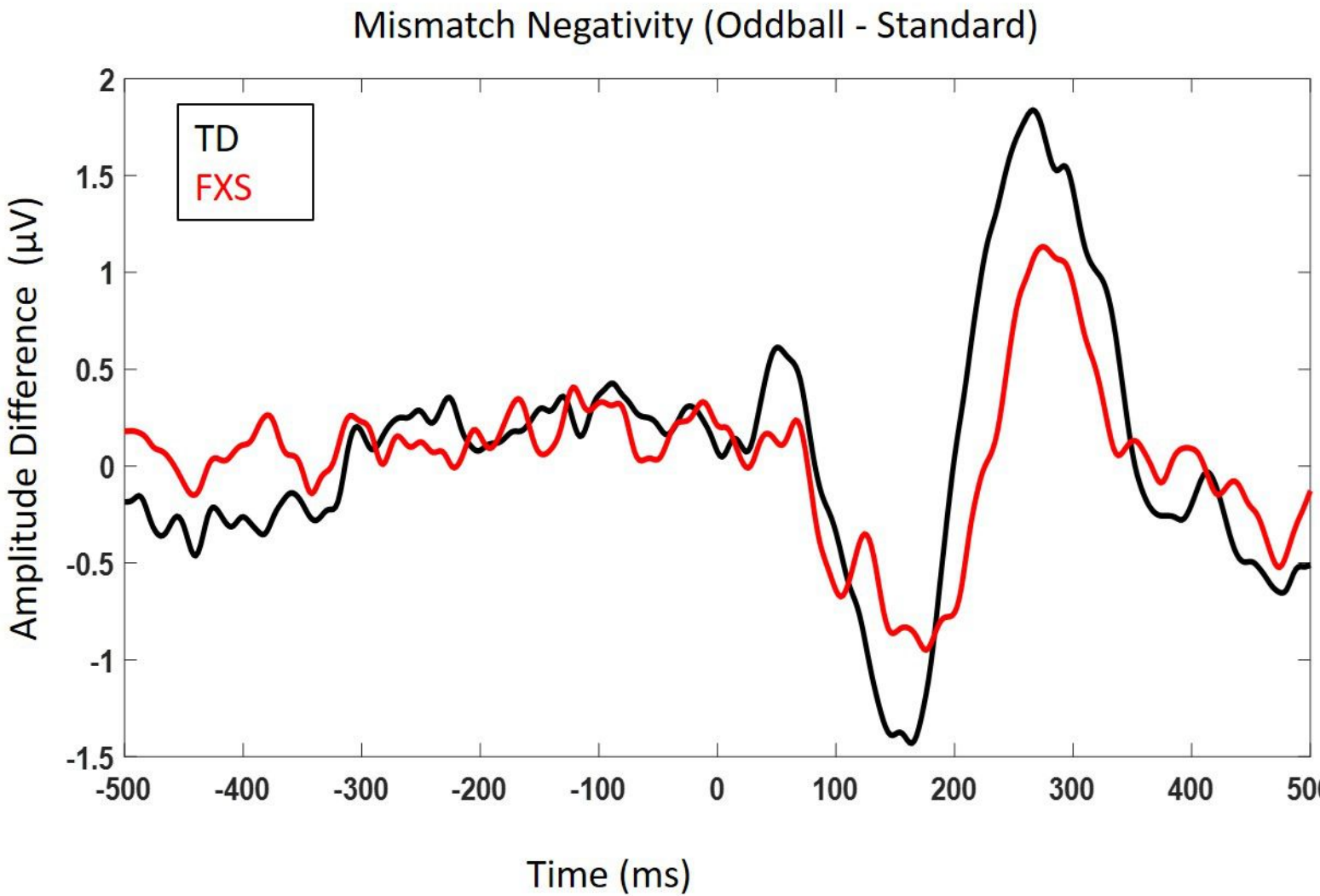
Mismatch Negativity (Oddball - Standard)
TD
FXS
Amplitude Difference (μV)
2
1.5
1
0.5
0
-0.5
-1
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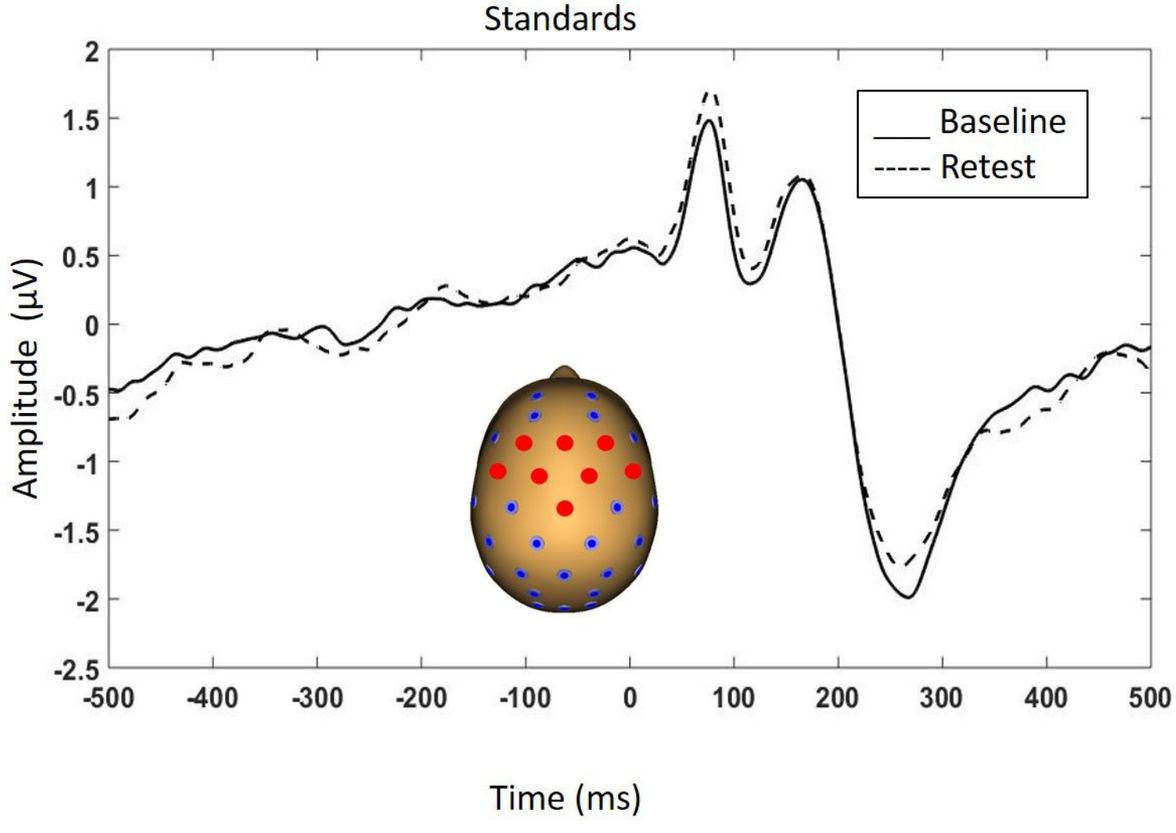
-500
-400
-300
-200
-100
0
100
200
300
400
500
Time (ms)


Standards
Baseline
Retest
Amplitude (μV)
Time (ms)
2
1.5
1
0.5
0
-0.5
-1
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-2
-2.5
-500
-400
-300
-200
-100
0
100
200
300
400
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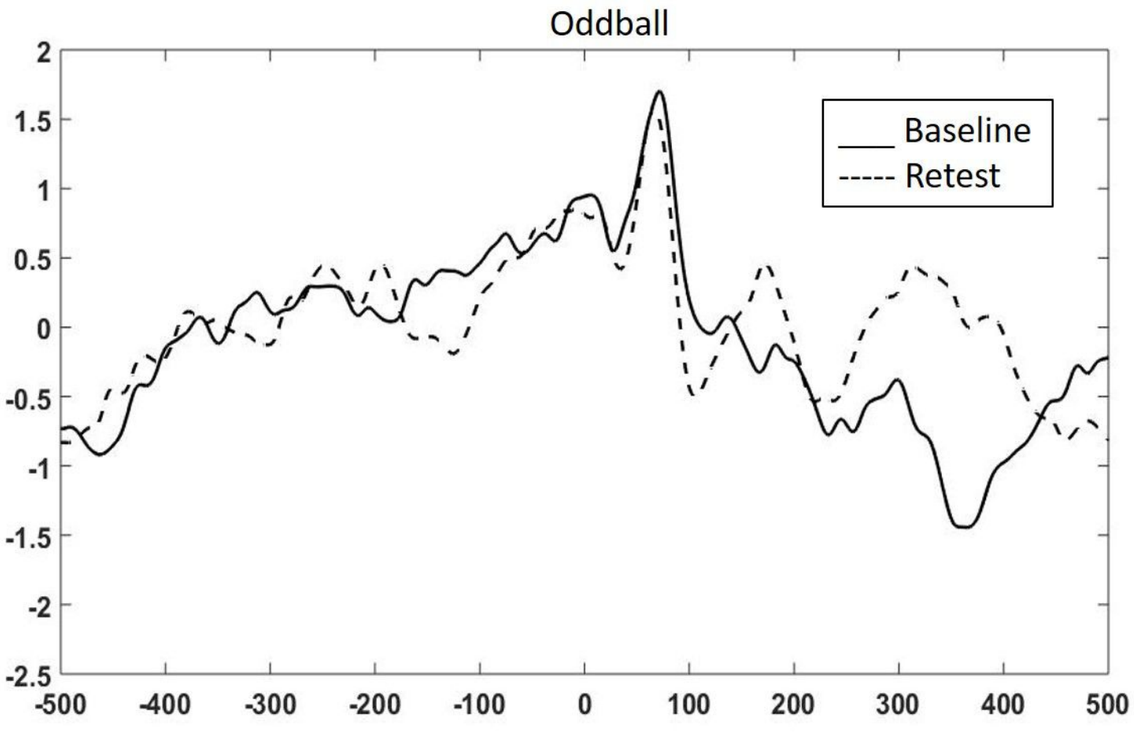
Oddball
Baseline
Retest
2
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-2
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-500
-400
-300
-200
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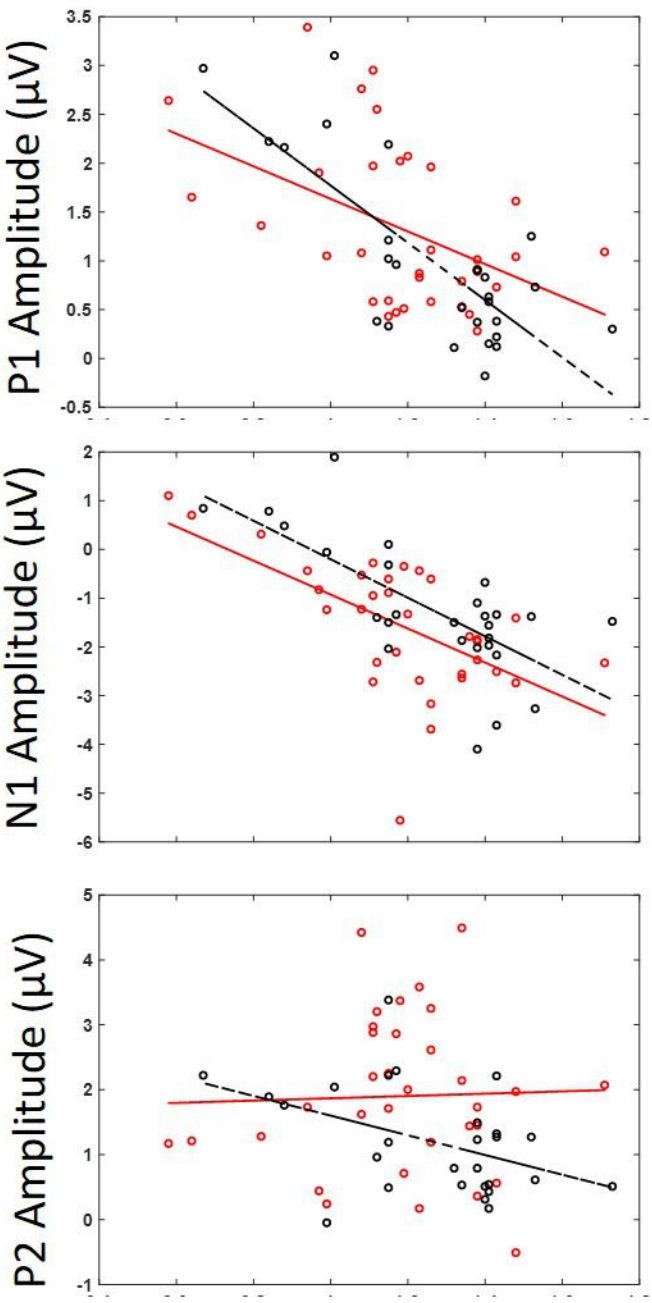
Standard

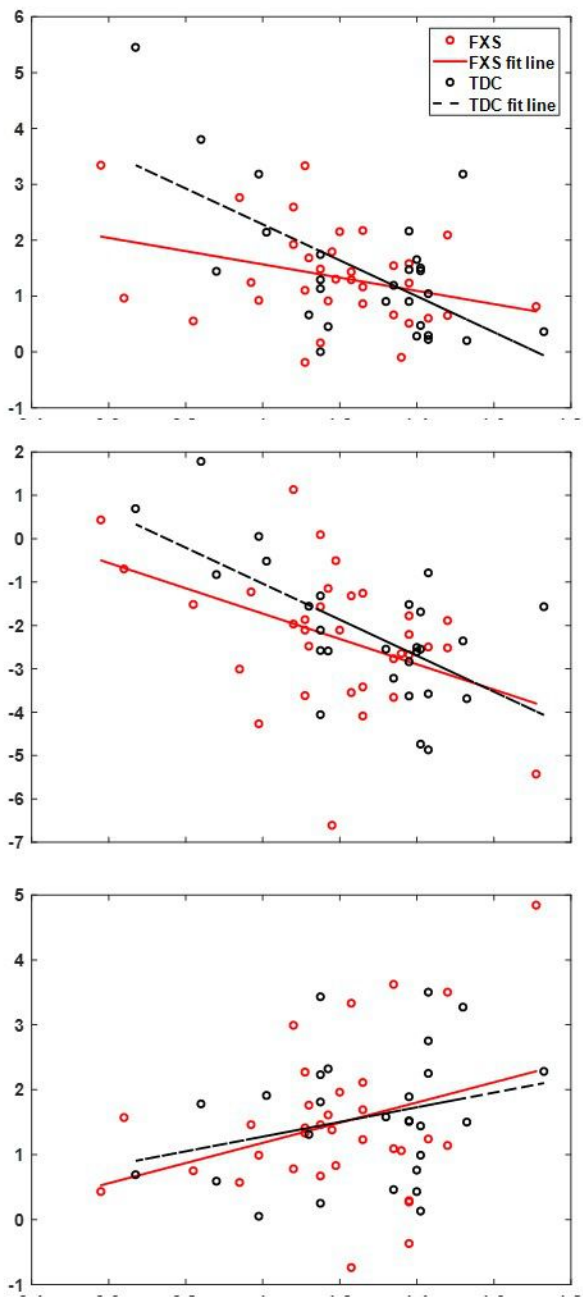
Oddball

P1 Amplitude (μV)

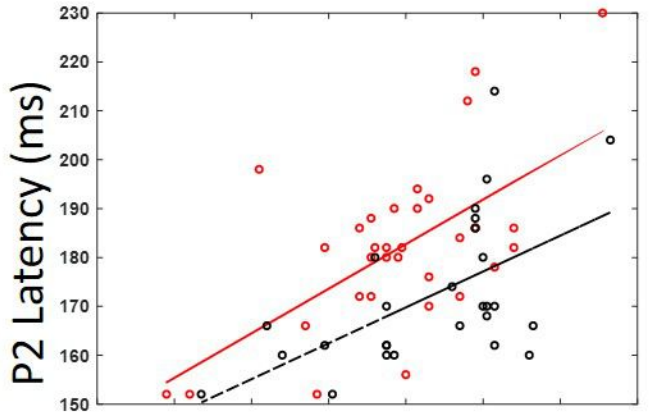
N1 Amplitude (μV)

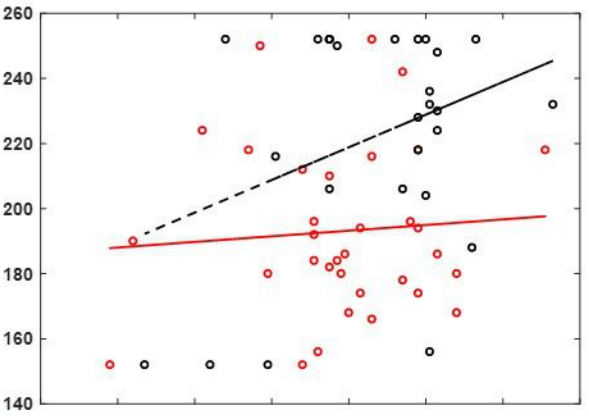
P2 Amplitude (μV)



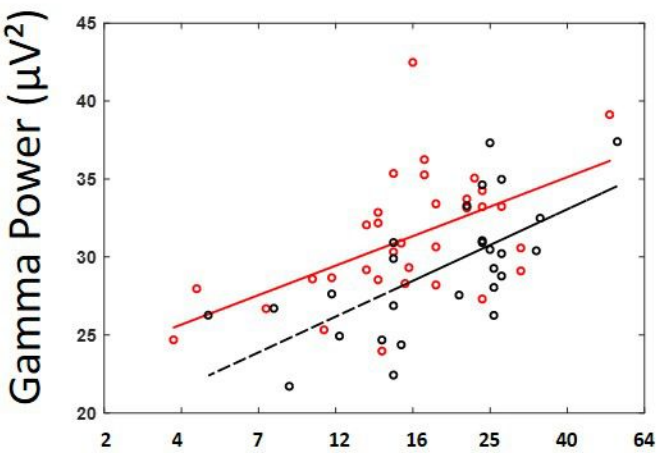


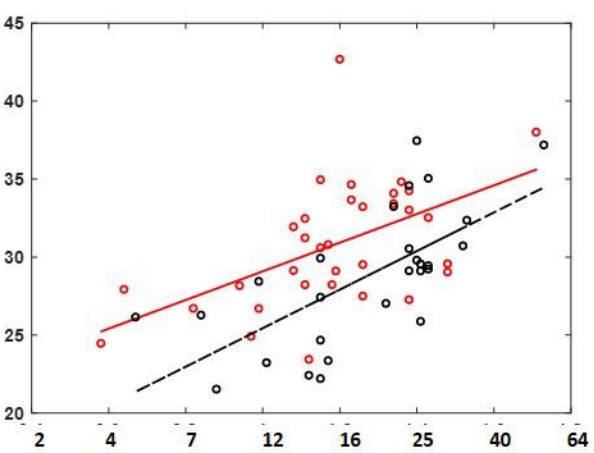
P2 Latency (ms)





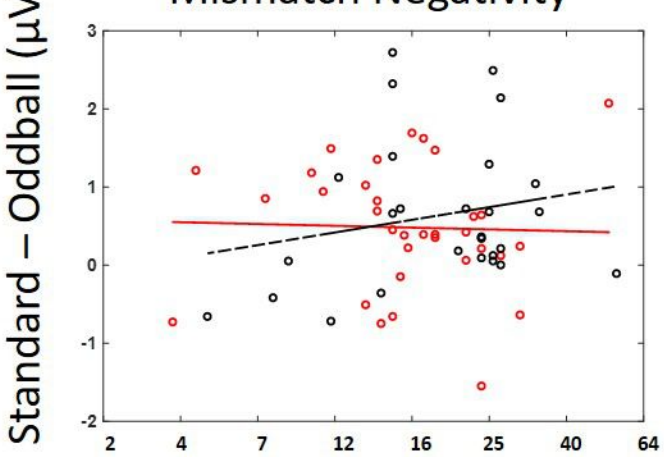
Gamma Power (μV²)





Mismatch Negativity

Standard – Oddball (μV)



Age (years)