HHS Public Access

Author manuscript

J Psychopharmacol. Author manuscript; available in PMC 2016 July 27.

Published in final edited form as:

J Psychopharmacol. 2013 October ; 27(10): 956–963. doi:10.1177/0269881113494105.

Electrocortical changes associated with minocycline treatment in fragile X syndrome

Schneider Andrea[1,2], **Leigh Mary Jacena**[2], **Adams Patrick**[1,2], **Nanakul Rawi**[3], **Chechi Tasleem**[1,2], **Olichney John**[4], **Hagerman Randi**[1,2], and **Hessl David**[1,5]

[1]MIND Institute, University of California at Davis Medical Center, Sacramento, California, USA

[2]Department of Pediatrics, University of California at Davis Medical Center, Sacramento, California, USA

[3]University of Washington, Seattle, USA

[4]Department of Neurology, University of California at Davis Medical Center, Sacramento, California, USA

[5]Department of Psychiatry and Behavioral Sciences, University of California at Davis Medical Center, Sacramento, California, USA

Abstract

Minocycline normalizes synaptic connections and behavior in the knockout mouse model of fragile X syndrome (FXS). Human-targeted treatment trials with minocycline have shown benefits in behavioral measures and parent reports. Event-related potentials (ERPs) may provide a sensitive method of monitoring treatment response and changes in coordinated brain activity. Measurement of electrocortical changes due to minocycline was done in a double-blind, placebo-controlled crossover treatment trial in children with FXS. Children with FXS ($Mean_{age}$ 10.5 years) were randomized to minocycline or placebo treatment for 3 months then changed to the other treatment for 3 months. The minocycline dosage ranged from 25–100 mg daily, based on weight. Twelve individuals with FXS (eight male, four female) completed ERP studies using a passive auditory oddball paradigm. Current source density (CSD) and ERP analysis at baseline showed high-amplitude, long-latency components over temporal regions. After 3 months of treatment with minocycline, the temporal N1 and P2 amplitudes were significantly reduced compared with placebo. There was a significant amplitude increase of the central P2 component on minocycline. Electrocortical habituation to auditory stimuli improved with minocycline treatment. Our study demonstrated improvements of the ERP in children with FXS treated with minocycline, and the potential feasibility and sensitivity of ERPs as a cognitive biomarker in FXS treatment trials.

Corresponding author: Andrea Schneider, UC Davis MIND Institute, 2825 50th Street, Sacramento, CA 95817, USA., andrea.schneider@ucdmc.ucdavis.edu.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The study was approved by the Institutional Review Board at University of California, Davis. All participants and parents/caretakers of participants gave their written consent to participate in the study.

Keywords

Fragile X syndrome; EEG; ERP; cortical parameters; minocycline

Introduction

Fragile X syndrome (FXS) is the most common known inherited cause of intellectual disability and autism. It is a single-gene disorder (*FMR1*) with an expanded CGG trinucleotide repeat on the long arm of the X chromosome, Xq27.3. Fragile X Mental Retardation Protein (FMRP) is significantly diminished or absent in FXS through a methylation of a CpG island (Sutcliffe et al., 1992), silencing the *FMR1* gene. Individuals with the FXS full mutation (>200 CGG repeats) show symptoms of hyperactivity, short attention span, emotional problems, and hyper-responsiveness to sensory stimuli (Schneider et al., 2009). Lack of *FMR1* protein in FXS is considered to play a role in cortical hyperexcitability and abnormal synaptic transmission (Bear et al., 2004; Musumeci et al., 2000). The neuropathological basis for this cortical excitation is thought to be the result of enhanced dendritic connections and immature pruning (Irwin et al., 2001). In addition, GABA A receptors are down regulated leading to deficits in inhibition and hyperarousal (D'Hulst and Kooy, 2007).

Minocycline normalizes synaptic connections and behavior in the knockout (KO) mouse model of FXS, which is thought to occur through decreasing levels or activity of MMP9 (matrix metalloproteinase 9) (Bilousova et al. 2009). An open-label treatment trial and a retrospective review of minocycline treatment in FXS demonstrated significant benefits in behavior (Paribello et al., 2010; Utari et al., 2010).

However, most outcome measures in human trials are dependent on the feedback of caregivers and research staff assessments. The use of quantitative electroencephalography (EEG) and event-related potentials (ERPs) may provide an objective and sensitive method of monitoring changes in brain activity due to treatment.

EEG research in individuals FXS remains a challenging area, and only few studies have been published. Among the common findings are seizures (Berry-Kravis, 2002; Musumeci et al., 1999), abnormally large somatosensory evoked potentials (Ferri et al., 1995), and interictal paroxysmal EEG activity in prepubertal participants with FXS (Musumeci et al., 1994). A magnet-encephalographic (MEG) study in FXS showed significantly higher amplitude N100m auditory evoked field component with a less lateralized N100m at anterior-posterior dipole locations (Rojas et al., 2001), which was explained by a more widespread activation of neurons in response to acoustic stimuli. Prepulse inhibition and recent ERP findings from Van der Molen et al. also provide neurophysiological evidence of enhanced sensitivity to auditory stimuli in FXS (Hessl et al., 2009; Van der Molen et al., 2011b), which could be used as a biomarker in targeted treatment trials. Van der Molen and colleagues reported abnormal auditory information processing in FXS with enhanced N1, N2, and P2 components in a standard oddball task with auditory tones. A significant finding is the lack of habituation to repeated auditory stimuli, both in short-term and long-term

conditions in FXS, caused by a hypersensitive auditory feature detection system (Castren et al., 2003; Van der Molen et al., 2012).

Our EEG study is a pilot project on electrocortical changes in a subsample of children with FXS during a crossover trial with minocycline (Leigh et al., 2012). This larger clinical trial (Clinicaltrials.gov, http://www.clinicaltrials.gov/ct2/show/NCT01053156) was a 6-month, single center, placebo-controlled, double-blind crossover trial of minocycline treatment. In total, 55 participants received at least 3 months of either minocycline or placebo, and 48 received 3 months of minocycline treatment and 3 months of placebo treatment. Medication dosage was assigned based on weight, with patients weighing up to 25 kg receiving 25 mg once daily, those weighing between 25 kg and 45 kg receiving 50 mg once daily, and those weighing >45 kg receiving 100 mg once daily. In this study, minocycline treatment was associated with improvements in global functioning by 0.5 points (CGI, Clinical Global Impression Scale) compared with placebo. On the Visual Analog Scale, the minocycline treatment was linked to a significant improvement in various behaviors, predominantly those related to anxiety and mood. No significant carry-over effects were observed from the first treatment period to the next.

Methods and materials

The study was approved by the Institutional Review Board at University of California, Davis. All participants and parents/caretakers of participants gave their written consent to participate in the study.

Participants

Out of the 55 individuals with FXS that participated in the controlled trial of minocycline, 22 individuals participated in EEG recording first sessions. Twelve individuals successfully completed the EEG recordings (four females, eight males, Mean age 10.5 years, SD 3.7) at baseline, after 3 months of minocycline/placebo treatment, and again at 6 months, following the second arm of placebo or minocycline treatment (crossover trial). The reasons for drop-outs were incomplete data for all three visits (N=5; two individuals discontinued the trial), data loss because of behavioral problems that interfered with the data quality (e.g. taking off the cap during the recording, hyperactivity, repetitive speech, N=4), and technical problems (N=1). The mean IQ in the sample was 64 (SD 23.7). There was a non-significant difference in IQ scores between the group that received the minocycline treatment first (see Table 1) that may have been clinically meaningful. Four individuals had mosaicism with partially methylated alleles in the premutation range in addition to a fully methylated full mutation. To compare the EEGs of the individuals with FXS in the minocycline trial, we included the results of a typically developing control group (N=40, Mean age 13.93 years, SD 10.58, 20 males, 20 females, Mean IQ 106.6, SD 12.01) that was presented previously (Schneider et al., 2012, paper in preparation).

Stimuli and procedure

Participants were presented with a passive auditory oddball paradigm using Presentation software (Neurobehavioral Systems, Albany, CA). The auditory stimuli were 350 sinusoidal

tones with frequencies of 1000 Hz (*N*=315, standard tone), and 2000 Hz (*N*=35, target/oddball), generated with the Tone Generator software of NCH (http://nch.com.au). The tones had a 10 ms rise/fall, 50 ms plateau, and a sound pressure intensity of 70 dB. The randomized order consisted of first six 1000 Hz standard tones, then one target tone (2000 Hz) either at 7th, 8th, 9th, or 10th position, with standard tones presented in the remaining positions. The tones were presented with a consistent inter-stimulus interval (ISI) of 1000 ms over stereo-speakers. At the beginning of the experiment, the sound intensity at the participant's head location was confirmed with a digital sound level meter.

Before the experiment, the participants chose a favorite movie, which was shown without sound during the preparation and the oddball task. The movie was required in order to provide a comforting environment for the patients and provide a fixation point for their eyes to reduce eye and head movements. Before the experiment, 2 min of resting EEG was recorded and, in compliant participants, an Alpha-block paradigm was completed with four 30-s blocks of alternating eyes-open and eyes-closed continuous EEG recording. Also, positive reinforcement through stickers and a reward sheet was utilized to encourage compliance.

EEG acquisition

EEG data were acquired using a Brain Products Quickamp system with an Acticap 32-channel $Ag^{+}/Ag^{+}Cl^{-}$ active EEG electrode array (International 10–20 system, positions (Fp1, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, TP9, CP5, CP1, CP2, CP6, TP10, P7, P3, Pz, P4, P8, PO9, O1, Oz, O2, PO10)) using a common average reference and a ground electrode positioned between Fz and Pz sites. Electrode impedances were maintained below 10 kΩ and electrical activities amplified and recorded with Brain Vision Recorder and Quickamp amplifier (Brain Products, Germany). During the recording, bandpass filters set at 0.3–100 Hz, and data were digitized continuously at 250 Hz. Raw data were then imported into Brainvision Analyzer software (Version 2.0.1.558, Brainproducts) for analysis.

Data processing

The continuous data were segmented according to the event type (standard or target tone with a 1000 ms time window, −100 ms before the event until 900 ms after the event) and filtered (Butterworth Zero Phase Filters with low cutoff 0.5 Hz, time constant 0.3, 12 dB/oct, high cutoff: 40 Hz, 12 dB/oct, a notch filter was not applied because of the active shield technology).

For artifact rejection, we defined the maximal allowed voltage step in a segment to 50 μV/ms, with a maximal allowed difference of values in intervals of 1000 μV, minimal allowed amplitude −500 μV, maximal allowed amplitude 500 μV, minimum activity in intervals 0.5 μV. For the detection and correction of blinks we used the electrode sites Fp1 and Fp2 as source for an Independent Component Analysis (ICA) Infomax restricted slope algorithm. The components relevant for vertical activity were selected by computing the global power field power. The number of ICA steps and convergence bound were selected individually according to the quality of the data; in general, the ocular correction ICA

converged between 90–120 steps, with the last step's matrix modification usually smaller than 9.575E-08. In general, there was a loss of ~10% of all trials. We excluded participants without a sufficient number of artifact-free trials (>30 required for oddball tones, >200 for standard tones).

Event-related potentials

ERPs were baseline corrected using the 100 ms pre-stimulus interval and averaged for standards and target tones separately. Peak amplitude and latency of the N1, P2, and N2 components were determined at the Fz, F3, F4, Cz, C3, C4, Pz, P3, P4, T7, and T8 electrode positions by the largest voltage deflection within the 1000 ms time window relative to stimulus onset, depending on the specific latency range for each component (N1=80–140 ms, P2=120–200 ms, N2=200–350 ms) according to established ERP guidelines (Duncan et al., 2009; Picton et al., 2000). The peak detection was performed semi-automatically, and a large voltage deflection also determined as a peak manually after visual inspection if it was outside the pre-defined latency range.

Source localization

Current source density—The current source density (CSD) is a measure of cortical activity that replaces the voltage values at electrodes that have valid head coordinates with the current source density at these points. The averaged ERP waveforms were transformed into CSD estimates ($\mu V/m^2$units) using a spherical spline surface Laplacian method (order of splines: 4, maximal degree of Legendre polynominals: 10, approximation parameter lambda: 1.00e-005), based on the method by Perrin and colleagues (Perrin et al., 1989; Tenke and Kayser, 2012).

Statistical analysis

For the analysis, we only included the standard tone stimulus because the number of artifact-free trials of the non-standard tones was too limited (N<30 for most participants). Due to the small sample sizes, and non-normal data distributions, our analyses were restricted to non-parametric comparisons between baseline and minocycline conditions (collapsed across both the minocycline and placebo treatment arms) and between baseline and placebo conditions (also collapsed across arms).

Statistical analysis of the ERP/CSD data was performed with a non-parametric Friedman Rank Test, Bonferroni correction, and post-hoc Wilcoxon procedure for condition comparison: baseline to minocycline condition, baseline to placebo condition, and placebo to minocycline condition. For the analysis of habituation to the tone stimuli we analyzed the N1/P2 waveforms to the first 45 tones, compared with the last 45 tones (Van der Molen et al., 2012). We also performed an exploratory analysis to test the hypothesis that larger P2 amplitude at Cz (where the P2 is normally maximal) in association with minocycline treatment would correlate (using Spearman's rho) with global clinical improvement, i.e. higher CGI (Guy, 1976) scores. As this was a directional hypothesis, a one-tailed p-value of ≤0.05 was considered significant.

Results

Figure 1 shows the grand average surface potentials (ERP) for the standard tones at baseline, placebo, and minocycline conditions for electrode positions Cz, T7, and T8. We selected these electrode positions in the figure because they most reliably showed the ERP components of interest across all participants. The P2 component at the Cz electrode shows a significant difference between baseline, placebo, and minocycline, with the highest amplitude for the minocycline treatment (z=−2.66, p .008). The N1 amplitude shows a similar trend at the Cz electrode, but it is not statistically significant (z=.549, p .583). The temporal waveforms at T7 and T8 show significantly reduced amplitudes for N1 on minocycline treatment compared with baseline (T7 and T8) or placebo (T8). The P2 amplitude was also increased at T8 on minocycline treatment compared with baseline/placebo ($P2_{T8}$, z=−2., p .041). There were no significant differences in peak latencies. Compared with typically developing controls, the N1 component at T7 and T8 of the minocycline FXS group shows comparable amplitudes, the N1 and P2 amplitudes at the Cz electrode location are significantly higher than controls.

Table 2 gives an overview of the mean amplitudes, standard deviations (SD), and statistical comparisons of N1, P2, and N2 at electrode positions Cz, T7, and T8. Our exploratory correlational analysis found that an increase in the P200_amplitude at Cz (from baseline to minocycline treatment) was correlated with CGI improvement in the expected direction (rho = .54, one-tailed p = 0.045).

Table 3 gives an overview of the mean amplitudes, SDs, and statistical analyses of N1, P2, and N2 at electrode positions Cz, T7, and T8 of a typical developing control group in comparison to the minocycline treatment FXS group.

Figure 2 shows the surface Laplacian topography (CSD) maps in 50 ms steps from 50–200 ms after stimulus presentation. There is a similar bi-temporal negative activation pattern in both hemispheres at baseline and placebo condition (between 150–250 ms). With minocycline treatment, the temporal negative activation pattern is counterbalanced with a strong central positive activation pattern (increased P2 amplitude at Cz). These CSD maps following minocycline treatment resemble the cortical activation patterns of typical developing individuals (Schneider et al., 2012).

For the analysis of habituation to repeated stimuli (Van der Molen et al., 2012), we compared the ERP waveforms for the first 45 stimuli to the last 45 stimuli, dependent on baseline, placebo, or minocycline conditions. Figure 3 shows the grand average waveforms, Figure 3(a) the N1 amplitude attenuation, and Figure 3(b) the P2 attenuation.

The N1 amplitude at baseline condition for the first vs. last 45 tones does not show a change in amplitude ($Cz_{N1\_first45}$=−1.568, Cz_{N1_last45}=−1.6584, mean difference 0.090 μV), the P2 component shows a tendency for a reduced amplitude, but this is not statistically significant ($Cz_{P2\_first45}$=2.670, Cz_{P2_last45}=2.145, mean difference 0.524 μV). The placebo condition comparisons show a similar pattern ($Cz_{N1\_first45}$=−3.302, Cz_{N1_last45}=−4.061, mean difference 0.75 μV), and the P2 component ($Cz_{P2\_first45}$=5.011, Cz_{P2_last45}=5.697, mean difference −0.686 μV), both not statistically significant. For the minocycline treatment

condition, there were significant amplitude reductions in both the N1 and P2 components ($Cz_{N1\_first45}$=−5.857, Cz_{N1_last45}=−3.336, mean difference −2.520 μV, z=−2.72, *p* .002, and $Cz_{P2\_first45}$=7.370, Cz_{P2_last45}=5.185, mean difference −2.185 μV, z=−2.63, *p* .012).

Discussion

This is the first study to examine electrocortical changes in the context of a controlled targeted treatment trial in FXS. We tested the hypothesis that a simple, auditory ERP paradigm would be sensitive to changes in cortical activation patterns during auditory information processing (0–400 ms) associated with minocycline treatment. In the 2009 Bilosouva study (Bilousova et al., 2009), minocycline reversed the abnormal behaviors in *Fmr1* KO mice, and promoted dendritic spine maturation in vivo and in vitro. One of potentially important EEG findings in our present study is the attenuation of the temporal N1 waveform, which could be an indicator of a reduced auditory hyperexcitability with minocycline. One plausible mechanism might be that dendritic spines become more mature with minocycline treatment, as shown in the KO mouse model (Bilousova et al. 2009). In previous studies, individuals with FXS showed exaggerated N1 and P2 amplitudes to auditory stimuli (Van der Molen et al., 2011a), providing evidence for this auditory hypersensitivity, which may be normalized by lowered MMP9 activity associated with minocycline treatment. Reduction or absence of FMRP is known to play a role in producing cortical hyperexcitability and abnormal synaptic transmission (Chuang et al., 2005; Zhong et al., 2009) The neurobiological basis for the hyperexcitability is thought to be related to GABA and glutamate imbalances and synaptic plasticity deficits, leading to deficits in dendritic connections.

The increase of ERP amplitudes at the central electrode position during the minocycline treatment appears counterintuitive; if minocycline reduces cortical hyperexcitability, the central amplitudes should be decreased. One possibility for the increased P2 at Cz is a summation of dipoles with temporal negative and midline positive peaks. Another possible explanation is the comparison of ERPs elicited by the first 45 standard tones in comparison with the last 45 standard tones, which provides insight into the habituation to stimulus presentation. Participants demonstrated significant ERP amplitude habituation to auditory stimuli only following minocycline treatment, comparable with healthy controls in a prior study (Van der Molen et al., 2012). It is known that enhancements in central processing are associated with improvements in habituation and the enhanced CZ amplitude may relate to this improvement in habituation. A final potential interpretation of the increased P2 is that minocycline exaggerates rather than ameliorates the electrocortical phenotype. However, our preliminary correlational analyses found an association between larger central P2 amplitude and improved global clinical outcome (CGI scores). Clearly, larger sample sizes and independent replication would help to clarify the reliability and nature of this observation.

Study limitations include the small sample size and the behavioral difficulties with lower-functioning, non-verbal participants resulting in EEG data loss due to excessive movement and other artifacts. For example, we only included the response to the standard tone stimulus in the analysis because the number of artifact-free trials of the non-standard tones was too limited (*N*<30 for most participants). However, including the non-standard tones (perhaps by

increasing the proportion of such trials or reducing factors contributing to artifacts) would add further insights into cortical processing, for example into the mismatch negativity (MMN) component, believed to be an indicator of early sensory change detection and sensory memory (Naatanen et al., 2011). A study by Van der Molen (2011a) found a significantly reduced MMN in FXS males relative to controls in a passive auditory odd-ball paradigm. The small sample size and non-normal distribution of data also prevented us from using standard parametric analyses that would allow for robust examination of treatment effects and carry-over. However, we performed an effect size analysis, depending on the treatment order, minocycline on the first arm vs. placebo on the first arm before the crossover (supplemental analysis). The general finding shows a bigger effect size for the group that started with minocycline on the first treatment arm. This modest order effect is most likely a cohort effect, in which the subjects randomized to minocycline on the first arm were in some way different (perhaps related to level of functioning) and more responsive than the second group. Also, our study sample included male and female participants. Generally, the phenotype in female individuals with FXS is milder, with a higher IQ and higher adaptive functioning, and it can be expected that the electrocortical patterns differ significantly. However, to our knowledge there have been no ERP studies on gender differences in FXS, and our study sample is too small to compare the effects.

Also, the different molecular status of four participants with methylation mosaicism adds to the data heterogeneity. The limited sample size prevents clear comparison of these individuals; however, differential treatment response to an mGluR5 negative modulator associated with differences in methylation has been reported (Jacquemont et al., 2011). We are not aware of any other EEG studies in FXS that looked at differences between cortical activation patterns in individuals with partially and fully methylated alleles. The treatment period of only 3 months could be too short for the minocycline to reach full effect, especially in adolescents. There was no formal wash-out period in the design of the study and carry-over effects may be possible. However, the analysis of the original sample in the larger minocycline study did not show a statistically significant carry-over effect on clinical outcome measures collected 14 days after stopping treatment (Leigh et al., 2012).

In the present study, we showed the potential sensitivity of an EEG biomarker as an indicator of cortical changes in FXS in a targeted treatment trial. It provides a measure for the human equivalent of the cortical hyperexcitability demonstrated in the mouse model of FXS. ERP/EEG studies can provide important additional treatment outcome measures and their use is recommended in future targeted treatment trials for FXS.

Acknowledgments

We are grateful to the research participants and their families; the support of Brainproducts and Brainvision LLC; the generous private financial support of fragile X families; Cecilia Cappeloni for assistance with data collection; Dr. Steve Luck, Dr. Cliff Saron, and Dr. Filipa Viola for support with the EEG analysis; and Louise Gane for assistance with recruitment and genetic counseling to participants.

Funding

Funding for this study was provided by the CTSC 3UL1RR024146-04S4 grant, a National Fragile X Foundation (NFXF) William Rosen/Weingarden Summer Fellowship, and NFXF grant for the minocycline treatment study.

Dr. Hagerman has received funding from Seaside Therapeutics, Roche, Novartis, Forest, and Curemark for treatment studies of fragile X syndrome, autism, and fragile X-associated tremor ataxia syndrome and she has also consulted with Novartis regarding treatment of fragile X syndrome. Funding has also been received from National Institute of Child Health and Human Development Grant HD; Grant NIARL1AG032115; National Center for Research Resources through Clinical and Translational Science Center funding (Grant UL1RR024141); and through the Administration of Developmental Disabilities Grant 90DD0596.

Dr. Hessl reports financial support from Hoffman LaRoche, Novartis, and Seaside Therapeutics for clinical trials of patients with fragile X syndrome and he has also consulted with Novartis and Roche regarding treatment of fragile X syndrome.

Dr. Olichney has served as a paid consultant for Lundbeck.

Dr. Schneider, Dr. Leigh, Rawi Nanakul, Patrick Adams, and Tasleem Chechi report no biomedical financial interests or potential conflicts of interest.

References

Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci. 2004; 27:370–377. [PubMed: 15219735]

Berry-Kravis E. Epilepsy in fragile X syndrome. Dev Med Child Neurol. 2002; 44:724–728. [PubMed: 12418611]

Bilousova TV, Dansie L, Ngo M, et al. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J Med Genet. 2009; 46:94–102. [PubMed: 18835858]

Castren M, Paakkonen A, Tarkka IM, et al. Augmentation of auditory N1 in children with fragile X syndrome. Brain Topogr. 2003; 15:165–171. [PubMed: 12705812]

Chuang SC, Zhao W, Bauchwitz R, et al. Prolonged epileptiform discharges induced by altered group I metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices of a fragile X mouse model. J Neurosci. 2005; 25:8048–8055. [PubMed: 16135762]

D'Hulst C, Kooy RF. The GABAA receptor: A novel target for treatment of fragile X? Trends Neurosci. 2007; 30:425–431. [PubMed: 17590448]

Duncan CC, Barry RJ, Connolly JF, et al. Event-related potentials in clinical research: Guidelines for eliciting, recording, and quantifying mismatch negativity, P300, and N400. Clin Neurophysiol. 2009; 120:1883–1908. [PubMed: 19796989]

Ferri R, Del Gracco S, Elia M, et al. Somatosensory evoked potentials and hyperekplexia outcome. Neurophysiol Clin. 1995; 25:28–30. [PubMed: 7746224]

Guy, W. ECDEU Assessment Manual for Psychopharmacology. Rockville, MD: U.S. Department of Health, Education, and Welfare; 1976. Clinical Global Impression (CGI).

Hessl D, Berry-Kravis E, Cordeiro L, et al. Prepulse inhibition in fragile X syndrome: feasibility, reliability, and implications for treatment. Am J Med Genet B Neuropsychiatr Genet. 2009; 150B: 545–553. [PubMed: 18785205]

Irwin SA, Patel B, Idupulapati M, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: A quantitative examination. Am J Med Genet A. 2001; 98:161–167.

Jacquemont S, Curie A, des Portes V, et al. Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. Sci Transl Med. 2011; 3:64ra61.

Leigh, MJND.; Winarni, TI.; Rivera, SM., et al. A double blind, placebo controlled trial of minocycline in children with fragile X syndrome. Pediatric Academic Societies Annual Meeting; Boston, MA. 2012.

Musumeci SA, Bosco P, Calabrese G, et al. Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. Epilepsia. 2000; 41:19–23. [PubMed: 10643918]

Musumeci SA, Elia M, Ferri R, et al. Evoked spikes and giant somatosensory evoked potentials in a patient with fragile-X syndrome. Ital J Neurol Sci. 1994; 15:365–368. [PubMed: 7698895]

Musumeci SA, Hagerman RJ, Ferri R, et al. Epilepsy and EEG findings in males with fragile X syndrome. Epilepsia. 1999; 40:1092–1099. [PubMed: 10448821]

Naatanen R, Kujala T, Escera C, et al. The mismatch negativity (MMN) – a unique window to disturbed central auditory processing in ageing and different clinical conditions. Clin Neurophysiol. 2011; 123:424–458. [PubMed: 22169062]

Paribello C, Tao L, Folino A, et al. Open-label add-on treatment trial of minocycline in fragile X syndrome. BMC Neurol. 2010; 10:91. [PubMed: 20937127]

Perrin F, Pernier J, Bertrand O, et al. Spherical splines for scalp potential and current density mapping. Electroencephalogr Clin Neurophysiol. 1989; 72:184–187. [PubMed: 2464490]

Picton TW, Bentin S, Berg P, et al. Guidelines for using human event-related potentials to study cognition: Recording standards and publication criteria. Psychophysiology. 2000; 37:127–152. [PubMed: 10731765]

Rojas DC, Benkers TL, Rogers SJ, et al. Auditory evoked magnetic fields in adults with fragile X syndrome. Neuroreport. 2001; 12:2573–2576. [PubMed: 11496151]

Schneider A, Hagerman RJ, Hessl D. Fragile X syndrome – from genes to cognition. Dev Disabil Res Rev. 2009; 15:333–342. [PubMed: 20014363]

Schneider, A.; Leigh, MJ.; Adams, P., et al. Differences in Cortical Hyperexcitability in Fragile X Syndrome. 13th international fragile X conference; Miami, FL, USA. 2012.

Sutcliffe JS, Nelson DL, Zhang F, et al. DNA methylation represses FMR-1 transcription in fragile X syndrome. Hum Mol Genet. 1992; 1:397–400. [PubMed: 1301913]

Tenke CE, Kayser J. Generator localization by current source density (CSD): Implications of volume conduction and field closure at intracranial and scalp resolutions. Clin Neurophysiol. 2012 in press.

Utari A, Chonchaiya W, Rivera SM, et al. Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. Am J Intellect Dev Disabil. 2010; 115:433–443. [PubMed: 20687826]

Van der Molen MJ, Van der Molen MW, Ridderinkhof KR, et al. Auditory and visual cortical activity during selective attention in fragile X syndrome: a cascade of processing deficiencies. Clin Neurophysiol. 2011a; 123:720–729. [PubMed: 21958658]

Van der Molen MJ, Van der Molen MW, Ridderinkhof KR, et al. Auditory change detection in fragile X syndrome males: A brain potential study. Clin Neurophysiol. 2011b; 123:1309–1318. [PubMed: 22192499]

Van der Molen MJ, Van der Molen MW, Ridderinkhof KR, et al. Attentional set-shifting in fragile X syndrome. Brain Cogn. 2012; 78:206–217. [PubMed: 22261226]

Zhong J, Chuang SC, Bianchi R, et al. BC1 regulation of metabotropic glutamate receptor-mediated neuronal excitability. J Neurosci. 2009; 29:9977–9986. [PubMed: 19675232]

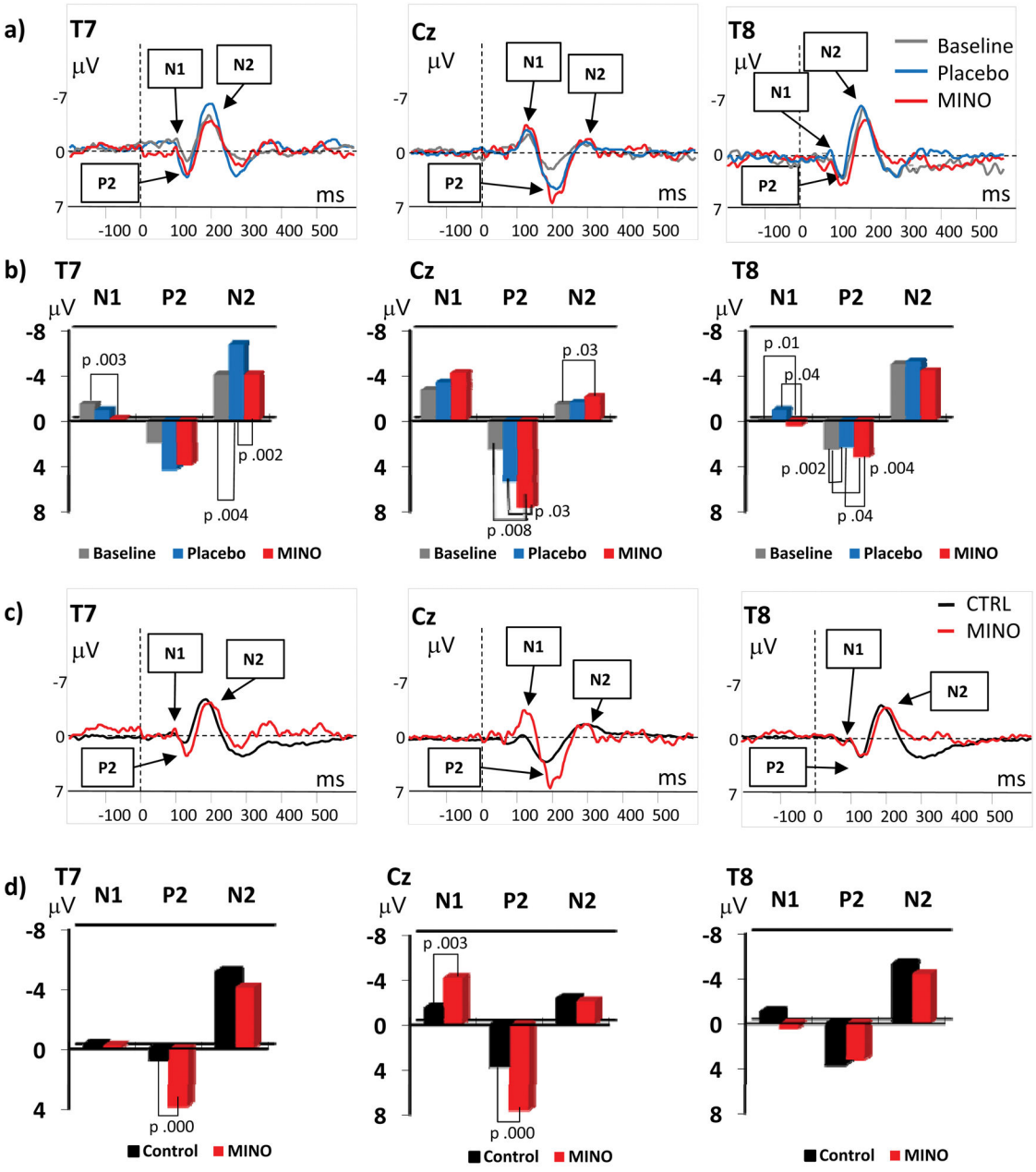

Figure 1.

(a) Grand averaged waveforms to standard tone at electrode positions T7 (left), Cz (center), and T8 (right) at baseline, placebo and minocycline conditions. Negative is plotted upwards. (b) Amplitudes for N1, P2, and N2 components; patients demonstrated a significant reduction of N1 amplitudes at T7 and T8 on minocycline compared with baseline, increased P2 amplitude at Cz and T8, and an increased N2 amplitude at Cz. (c) Grand averaged waveforms to standard tone at electrode positions T7 (left), Cz (center), and T8 (right), comparison of the minocycline group with a control sample. (d) Amplitudes for N1, P2, and N2 components; individuals with FXS on minocycline compared with controls show similar

N1 amplitudes at T7 and T8, a significant higher N1 component at Cz, and a larger P2 amplitude at T7 and Cz.

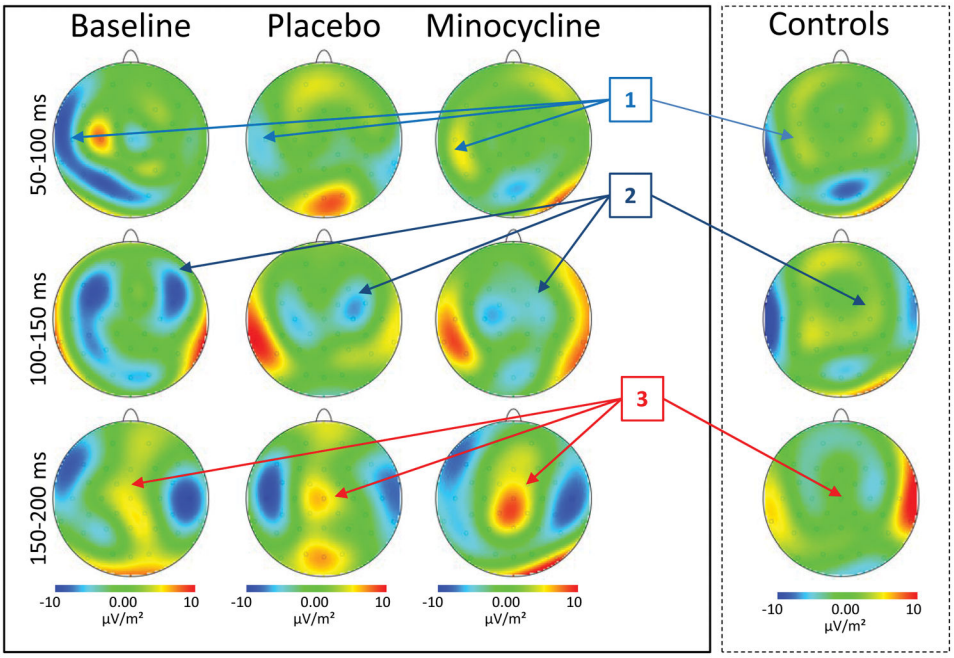

Figure 2.

Current Source Density group grand average maps for 200 ms after stimulus presentation, top view from scalp, nose on top. Arrows indicate differences in activation patterns for the different conditions.

(1) reduced early left-temporal component (N1 equivalent) from baseline to minocycline condition, similar to controls (2) reduced temporal negative components in both hemispheres, a finding that is absent in controls (3) increased central positive component (P2 equivalent at Cz), which is absent in controls.

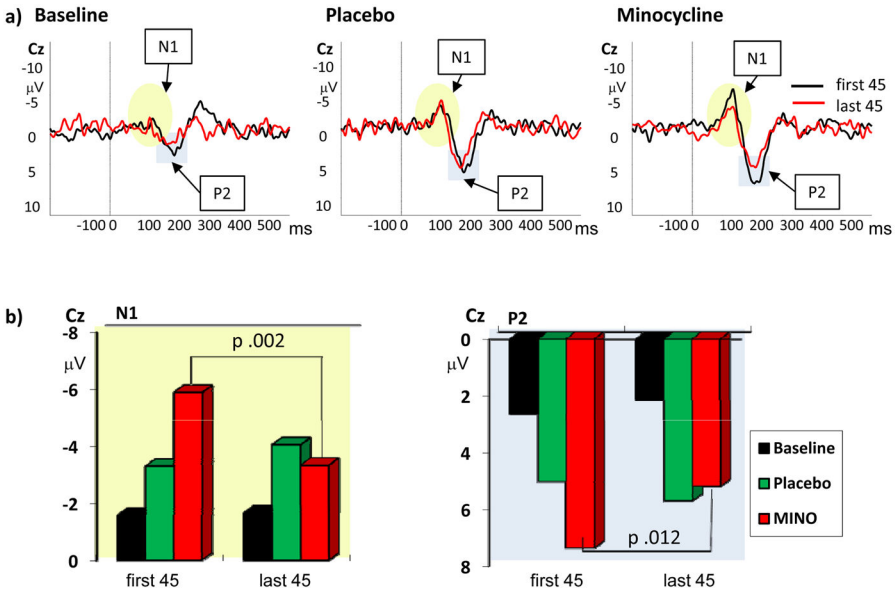

Figure 3.

(a) Grand average waveforms at Cz, highlighted in yellow the N1 component, the P2 component in blue. Black line signifies first 45 stimuli, the red line last 45 stimuli potentials. Negative plotted upwards. (b) Significantly improved attenuation of N1 and P2 in minocycline condition, not significant for baseline and placebo.

Table 1

Demographics and clinical data of participants randomized first to placebo and those randomized first to minocycline.

	Placebo – Minocycline			Minocycline – Placebo			
	Mean	**N**	**SD**	**Mean**	**N**	**SD**	***p***
Age	11.00	5	3.24	10.14	7	4.33	*0.718*
FSIQ	55.40	5	5.36	70.14	7	30.12	*0.311*
NVIQ	53.25	4	3.30	62.60	5	30.67	*0.568*
VIQ	64.00	4	9.20	66.80	5	33.01	*0.875*
ADOS	8.40	5	3.05	7.83	6	6.08	*0.855*
CGI Baseline	4.00	5	0.00	4.00	6	0.00	*N/A*
CGI 2nd visit	3.80	5	1.10	2.50	6	0.55	0.030[a]
CGI 3rd visit	2.60	5	0.55	3.00	6	1.26	*0.528*

[a] *t*=2.5554, df=9, SE of Difference=0.509

Table 2

ERP amplitudes by treatment condition (Friedman Test, post-hoc Wilcoxon, Bonferroni correction).

ERP (μV)/Electrode position	Baseline (a)	Placebo (b)	Minocycline (c)	a/b (Z, asympt. Sig.)	a/c (Z, asympt. Sig.)	b/c(Z, asympt. Sig.)
N1_Cz	−2.72	−3.40	−4.20	−1.68	−1.84	−.089
	(SD 1.15)	*(SD 1.64)*	*(SD 3.58)*	.09	.06	.92
N1_T7	−1.46	−0.96	−0.19	−1.68	−2.98	−1.88
	(SD 1.19)	*(SD 1.57)*	*(SD 1.49)*	.09	.003*(1)*	.06
N1_T8	−0.01	−0.96	0.46	−1.72	−2.35	−1.96
	(SD 1.43)	*(SD 2.29)*	*(SD 1.80)*	.08	.01*(2)*	.04*(3)*
P2_Cz	2.54	5.33	7.68	−.56	−2.66	−2.11
	(SD 1.27)	*(SD 3.06)*	*(SD 2.60)*	.57	.008*(4)*	.03*(5)*
P2_T7	1.95	4.38	3.87	−.51	−1.33	−1.49
	(SD .88)	*(SD 1.15)*	*(SD 1.00)*	.57	.18	.13
P2_T8	2.50	2.34	3.24	−3.05	−2.90	−2.00
	(SD 2.21)	*(SD .57)*	*(SD 2.23)*	.002*(6)*	.004*(7)*	.04*(8)*
N2_Cz	−1.40	−1.62	−2.09	−.314	−2.15	−.94
	(SD .79)	*(SD 1.06)*	*(SD 1.00)*	.754	.031*(9)*	.347
N2_T7	−4.07	−6.78	−4.08	−2.90	−3.92	−3.05
	(SD 1.72)	*(SD .58)*	*(SD .90)*	.004*(10)*	.695	.002*(11)*
N2_T8	−5.00	−5.25	−4.41	−.800	−1.33	−1.64
	(SD 1.33)	*(SD 1.18)*	*(SD .96)*	.424	.182	.099

Effect sizes:

(1) Cohen's $d = -0.941$, effect size $r = -0.42$,

(2) Cohen's $d = -0.289$, effect size $r = -0.143$,

(3) Cohen's $d = -0.689$, effect size $r = -0.325$,

(4) Cohen's $d = -2.512$, effect size $r = -0.782$,

(5) Cohen's $d = -0.827$, effect size $r = -0.382$,

(6) Cohen's $d = 1.728$, effect size $r = 0.653$,

(7)Cohen's $d = 1.855$, effect size $r = 0.680$,

(8)Cohen's $d = 0.307$, effect size $r = 0.151$,

(9)Cohen's $d = 0.765$, effect size $r = 0.357$,

(10)Cohen's $d = 2.111$, effect size $r = 0.725$.

(11)Cohen's $d = -3.566$, effect size $r = -0.872$.

Table 3

Group differences in ERP amplitudes (*t*-test).

ERP (μV)/Electrode position	Control[a] (N=40)	Minocycline (N=12)	(t, Sig.)
N1_Cz	−1.44	−4.20	3.06
	(SD 2.43)	*(SD 3.58)*	.003
N1_T7	−0.23	−0.19	0.090
	(SD 1.32)	*(SD 1.49)*	.927
N1_T8	−1.02	0.46	1.79
	(SD 2.68)	*(SD 1.80)*	.079
P2_Cz	3.74	7.68	4.58
	(SD 2.60)	*(SD 2.60)*	.000
P2_T7	0.70	3.87	8.11
	(SD 1.23)	*(SD 1.00)*	.000
P2_T8	3.65	3.24	0.32
	(SD 4.22)	*(SD 2.23)*	.745
N2_Cz	−2.34	−2.09	0.52
	(SD 1.57)	*(SD 1.00)*	.604
N2_T7	−5.12	−4.08	0.82
	(SD 4.32)	*(SD .90)*	.413
N2_T8	−5.32	−4.41	0.85
	(SD 3.65)	*(SD .96)*	.397

[a]Control group: *N*=40, Mean age 13.93 (SD 10.58), Mean IQ 106.6 (SD 12.01)