Blarcamesine corrects EEG biomarkers of cortical dysfunction in a model of fragile X syndrome

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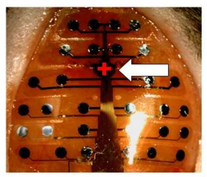
Background

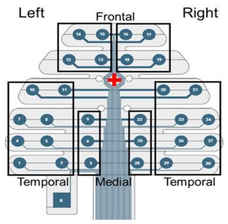
Fragile X syndrome (FXS) is characterized by cognitive impairment, behavioral abnormalities, and aberrant cortical processing including imbalanced excitation-inhibition. Translational electroencephalographic (EEG) biomarkers, which can be assessed in both humans and animal models of FXS, have emerged as promising tools for investigating the neural underpinnings of FXS and evaluating the efficacy of novel therapeutics. Sensory-evoked paradigms, such as the auditory steady-state response (ASSR), can be used to evaluate neural processes (e.g., auditory processing) that have implications for higher-level cortical functions.

Notably, auditory processing deficits are observed in both individuals with FXS and the *Fmr1* KO mouse model. Individuals with FXS have enhanced gamma power and reduced inter-trial phase coherence, particularly at gamma frequencies, compared to healthy controls.

In individuals with FXS intensified background gamma oscillations ("gamma noise“) may contribute to hypersensitivities and interfere with stimulus-evoked synchronization. These EEG abnormalities correlate with FXS phenotypes of social communication deficits and hypersensitivities. Previous studies using multi-electrode arrays (MEAs) in *Fmr1* KO mice and EEG biomarkers in affected individuals with FXS have identified similar EEG abnormalities, which constitute biomarkers of cortical dysfunction and could serve as outcome measures for drug treatments in clinical stages of development.

Materials and Methods

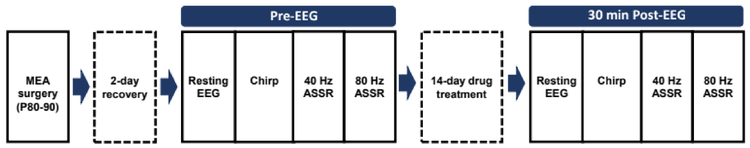




Brain Regions

- Left and Right (L/R)
  - Frontal (F)
  - Medial (M)
  - Temporal (T)

Oral administration of Blarcamesine



Study Groups

1) *Fmr1* KO Blarcamesine 1 mg/kg (n=10)
2) *Fmr1* KO Blarcamesine 10 mg/kg (n=15)
3) *Fmr1* KO Blarcamesine 30 mg/kg (n=15)

EEG Parameters and Analyses

- Resting Power
  - Frequency Bands: Delta to Low/High Gamma
- Inter-Trial Phase Coherence (ITPC)
  - Chirp and ASSR (40 and 80 Hz)

Results

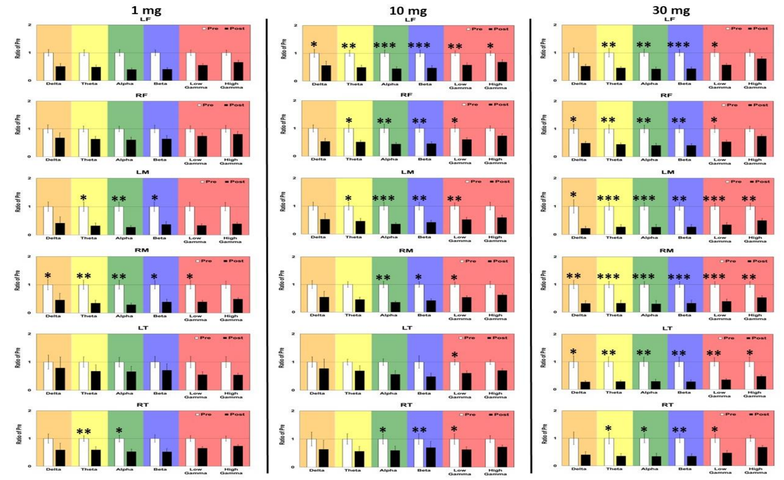


Figure 1. Resting state power in *Fmr1* KO mice before treatment (pre) *vs. Fmr1* KO mice after 14-day treatment (post) of Blarcamesine 1, 10 and 30 mg/kg. Ratio of *Fmr1* KO post-treatment (*black bars*) to *Fmr1* KO pre-treatment (*white bars*) represent EEG resting state power across frequency bands for distinct brain regions. Bars represent group means and error bars represent standard error of the mean (SEM). Statistical significance (*p < 0.05; ** p < 0.01; *** p < 0.001).

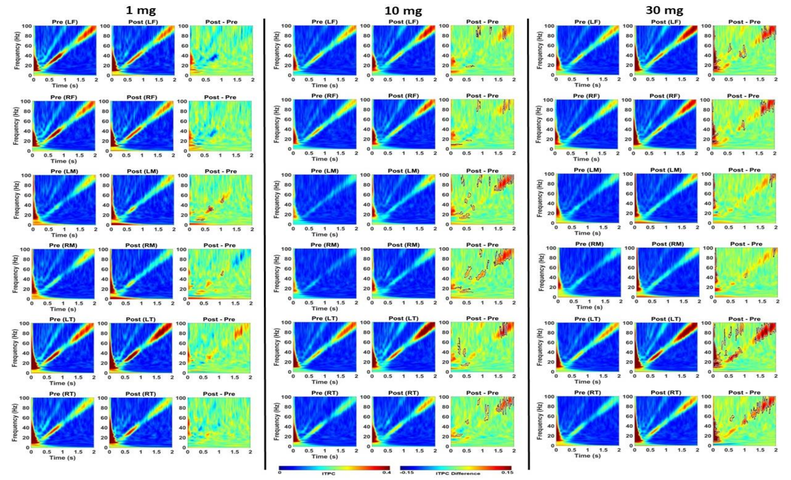


Figure 2. Chirp ITPC in *Fmr1* KO mice before treatment (pre) *vs. Fmr1* KO mice after 14-day treatment (post) of Blarcamesine 1, 10 and 30 mg/kg. For each brain region, the left panel shows the averaged *Fmr1* KO pre-treatment, the middle panel shows the averaged *Fmr1* KO post-treatment, and the right panel shows *Fmr1* KO post-treatment - *Fmr1* KO pre-treatment. Significant increases in ITPC in *Fmr1* KO post compared to *Fmr1* KO pre mice are shown in black outlined areas.

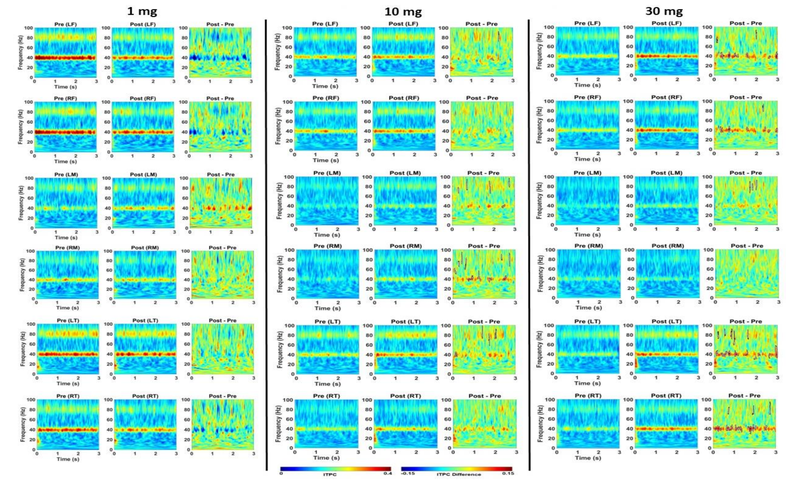


Figure 3. 40 Hz ASSR ITPC in *Fmr1* KO mice before treatment (pre) *vs. Fmr1* KO mice after 14-day treatment (post) of Blarcamesine 1, 10 and 30 mg/kg. For each brain region, the left panel shows the averaged *Fmr1* KO pre-treatment, the middle panel shows the averaged *Fmr1* KO post-treatment, and the right panel shows *Fmr1* KO post-treatment - *Fmr1* KO pre-treatment. Significant increases in ITPC in *Fmr1* KO post compared to *Fmr1* KO pre mice are shown in black outlined areas.

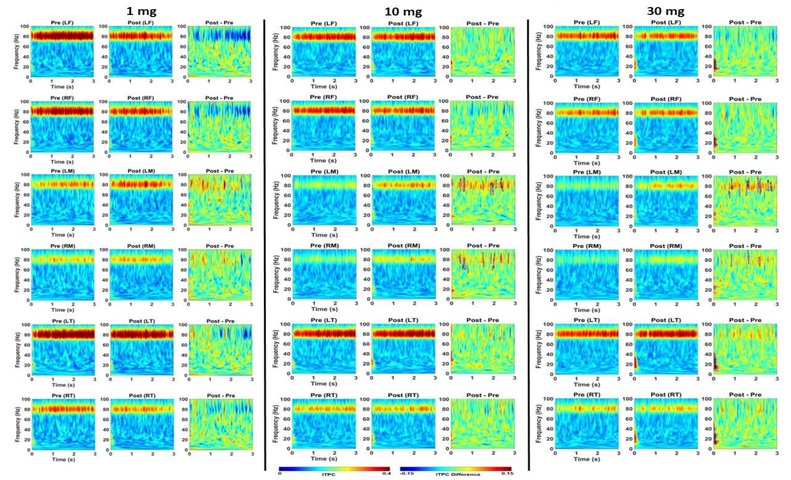


Figure 4. 80 Hz ASSR ITPC in *Fmr1* KO mice before treatment (pre) *vs. Fmr1* KO mice after 14-day treatment (post) of Blarcamesine 1, 10 and 30 mg/kg. For each brain region, the left panel shows the averaged *Fmr1* KO pre-treatment, the middle panel shows the averaged *Fmr1* KO post-treatment, and the right panel shows *Fmr1* KO post-treatment - *Fmr1* KO pre-treatment. Significant increases in ITPC in *Fmr1* KO post compared to *Fmr1* KO pre mice are shown in black outlined areas.

Conclusion

Significant improvements in resting power and ITPC, a measure of cortical circuit synchronization, in a dose-dependent manner demonstrates blarcamesine's target engagement and its capacity to correct multiple EEG biomarkers of cortical dysfunction in the *Fmr1* KO mouse model. Notably blarcamesine's effect on 40 Hz ASSR ITPC, a paradigm reflecting parvalbumin GABAergic deficits, demonstrates a potentially translatable improvement in the well-established feature of GABAergic dysfunction in FXS.

Given that these biomarkers represent fundamental mechanisms underlying cognitive and behavioral abnormalities and that they are shared by FXS mouse models and affected individuals, our findings support the clinical potential of blarcamesine in FXS and other neurodevelopmental disorders.