Gamma Band EEG Sample Entropy, a Patient Selection Biomarker for ALTO-300, is Increased by Dopamine Depletion in Humans and Mice

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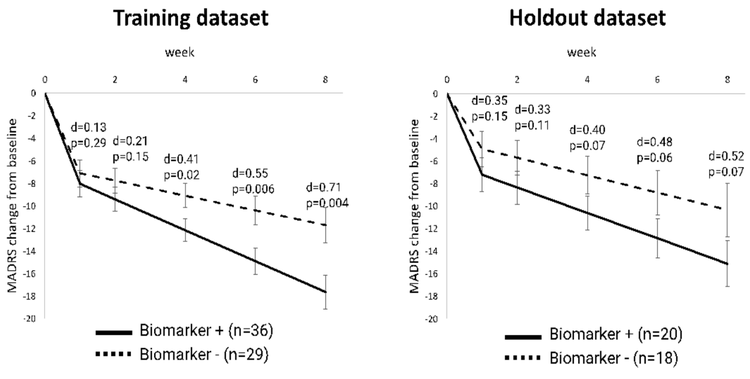
1. Introduction

- Major depressive disorder (MDD) is a complex and heterogeneous condition. Targeting independent, biomarker-defined patient populations has the potential to improve treatment outcomes.
- Our completed ALTO-300 (agomelatine) Phase 2a trial in MDD identified a reproducible, readily scalable and easily administered machine learning-derived electroencephalography (EEG) biomarker, which was further validated through prospective replication.



- The ALTO-300 biomarker, measured by gamma band sample entropy, is indicative of reduced neural signaling stability. Higher values indicate decreased neural connectivity and greater EEG irregularity.
- Dopaminergic circuits are critical for stabilizing neural representations and network dynamics; disruptions in these pathways are hypothesized to manifest as aberrantly reduced neural signal regularity, detectable via scalp EEG.
- We hypothesized that reducing dopamine levels would increase gamma band sample entropy, consistent with the notion that elevated sample entropy indicates reduced dopaminergic tone.
- We analyzed data from two studies designed to manipulate dopamine levels, with the specific goal of assessing resultant changes in sample entropy:
  - (1) using acute dopamine precursor depletion (APTD) in humans.
  - (2) employing alpha-methyl-p-tyrosine (AMPT) in mice.

2. Study Design – APTD Human Study

- Data from a previously conducted study of twelve healthy male participants aged 18–30 years were enrolled in a double-blind, placebo-controlled, crossover dietary dopamine depletion study.
- In a counterbalanced design, participants consumed both a nutritionally balanced amino acid mixture (placebo) and a mixture deficient in tyrosine and phenylalanine (APTD) separated by 7 days.
- Approximately four hours after ingestion, EEG data were recorded during a cognitive task, and gamma band sample entropy was calculated from the continuous recordings.

3. Study Design – AMPT Mice Study

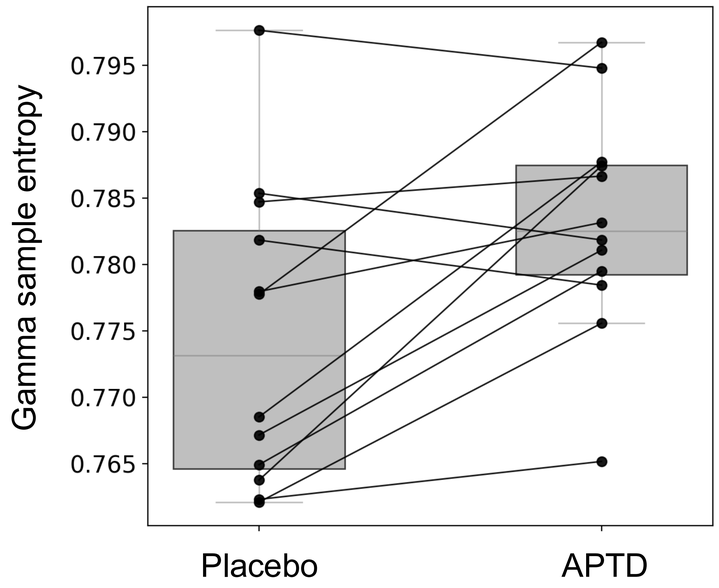
- EEG was recorded from nine male mice from frontal and parietal transcranial screws.
- Each animal received either a dose of AMPT (50, 100, or 150 mg/kg) or a vehicle control, in a randomized sequence.
- Drugs were administered at 10AM each day (7AM – 7PM light cycle).

4. Data Analysis

- Mixed-effects models were used to assess the treatment effects on EEG sample entropy, comparing treatment and placebo (or vehicle) while accounting for individual variability.

5. APTD Increases Gamma Sample Entropy in humans

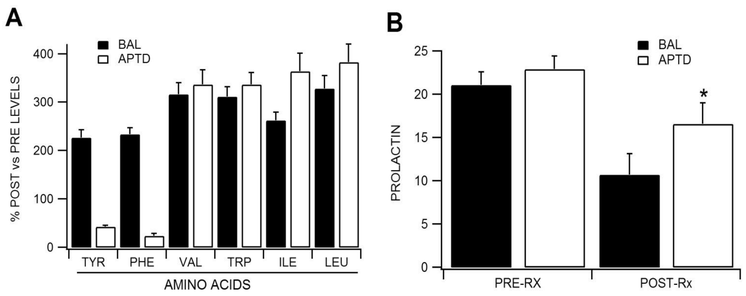
APTD significantly increased gamma-band sample entropy compared to placebo (Cohen's d=0.94, p=0.006).



6. APTD treatment in humans

A) APTD treatment depletes the essential amino acid precursors required for dopamine synthesis.

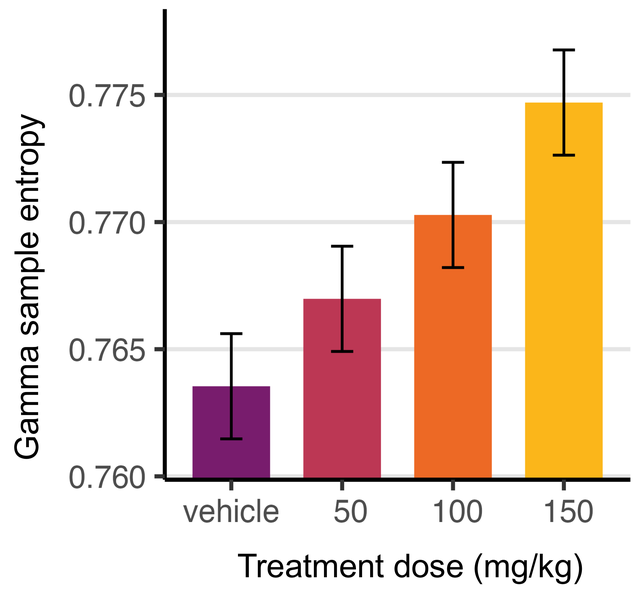
B) Since dopamine exerts an inhibitory action on prolactin release in the hypothalamus, increased prolactin levels are indicative of a decrease in dopamine transmission (reprinted from the original publication reporting these data).



Larson, M. J., Clayson, P. E., Primosch, M., Leyton, M., & Steffensen, S. C. (2015). The Effects of Acute Dopamine Precursor Depletion on the Cognitive Control Functions of Performance Monitoring and Conflict Processing: An Event-Related Potential (ERP) Study

7. AMPT Increases Gamma Sample Entropy in mice

Sample entropy values were computed in 10-seconds windows and averaged from 30 – 90 minutes post-dose.



8. AMPT treatment in rats

- AMPT directly inhibits tyrosine hydroxylase, the enzyme that converts tyrosine to L-DOPA, thereby reducing dopamine synthesis pharmacologically.

9. Conclusions

- Both the APTD study in humans and the AMPT study in mice demonstrate that reducing dopamine availability increases gamma band sample entropy, an index of EEG signal irregularity.
- These results provide mechanistic evidence that elevated gamma sample entropy may reflect reduced dopaminergic tone.
- ALTO-300 has been shown to increase dopamine release and stabilize neural signaling. Dopamine depletion drives the biomarker change, as measured by gamma sample entropy.
- These findings demonstrate the link between the mechanism of ALTO-300 and the EEG biomarker used to identify patients who are more likely to be responders in Alto's ongoing Phase 2b trial of ALTO-300 in MDD.

10. Acknowledgments

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