Neural and molecular mechanistic correlates of an EEG biomarker predictive of the antidepressant response to ALTO-300 in patients with major depression

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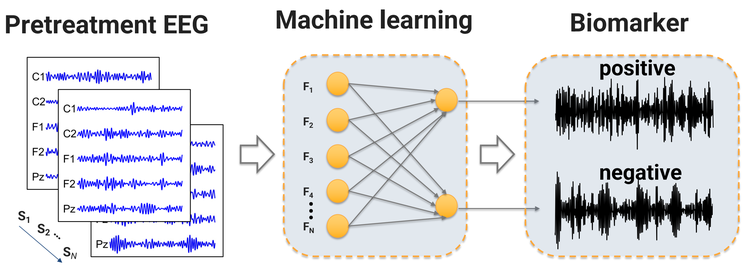
1. Introduction

- Major Depressive Disorder (MDD) is a complex and heterogeneous condition. Identifying specific sub-populations for targeted treatment through objective biomarkers can enhance treatment outcomes.
- Previously, we demonstrated the benefit of this approach by identifying, and prospectively replicating, a machine learning-derived biomarker based on resting-state electroencephalography (rsEEG), which can predict response to ALTO-300 (agomelatine) treatment in MDD.
- This biomarker yielded an MMRM effect size of 0.61 in the training set and an effect size of 0.51 in the holdout set at week 6 in treated patients positive versus negative on the biomarker for change in MADRS.
- The biomarker in the model was sample entropy (gamma band), which is a measure of complexity or irregularity of rsEEG. Higher values indicate more irregular rhythms.



Schematic diagram to illustrate the biomarker pipeline

- We hypothesized that irregularity of rsEEG may reflect changes in underlying neural network dynamics, as measured through EEG functional connectivity. Specifically, we proposed that more connected networks would exhibit higher synchronous activity and thereby higher regularity.
- ALTO-300, a melatonergic agonist and 5-HT2C antagonist, increases dopamine and noradrenaline release via 5-HT2C antagonism.
- 5-HT2C agonists (i.e. opposite action of drug effect) have been shown to induce depressive and anhedonia-like behaviors, such as reduced sucrose preference, while agomelatine has been found to rescue anhedonia in various animal models of depression.
- To further understand the molecular mechanistic relevance of this biomarker, we evaluated the association between sample entropy and 5-HT2C agonism in mice.

2. Methods

2.1. Human rsEEG connectivity analysis:

- Functional connectivity: orthogonalized power envelope connectivity (PEC) in gamma band; PEC measures the correlation of power fluctuations in oscillations over time.
- Sensor level analysis: age and sex corrected partial correlation of biomarker with gamma band PEC.
  - 19 channel eyes closed rsEEG from 5 independent MDD datasets.
- Source analysis: minimum norm estimation in BEAM (64 ch); correlation of biomarker to nodal gamma PEC.

2.2. Animal rsEEG 5-HT2C agonist analysis:

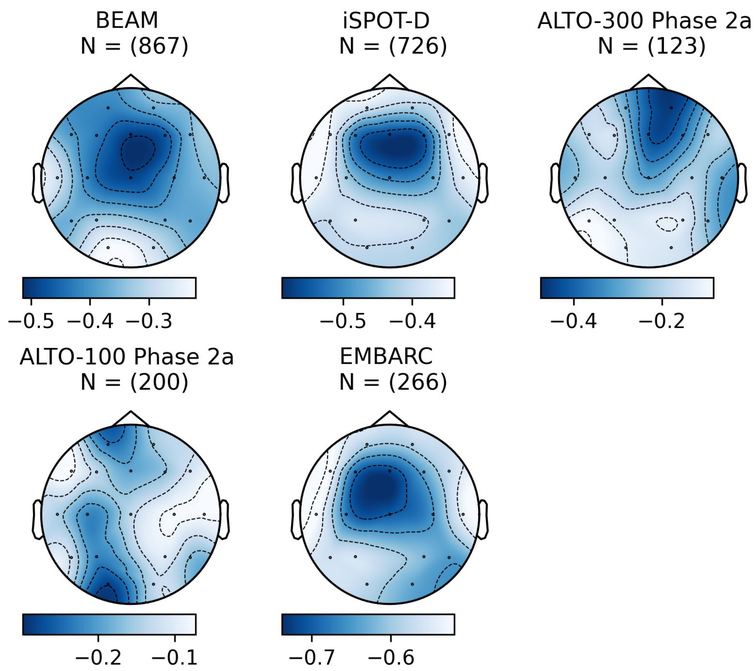
- Evaluate the association between gamma sample entropy and 5-HT2C agonism (R0 60-0175).
- Male mice (N=13); Cross-over within-animal dosing
- Subcutaneous administration.
- 72 hours washout between doses.
- Four doses RO-0175 were tested (0.75, 1.25, 2.5, 4 mg/kg) plus vehicle.
- Two-channel (frontal and parietal) EEG recording.
- Evaluate gamma sample entropy over the period of 0.5-to-1.5-hour post dosing and compare w.r.t. vehicle.

Arm	Doses (mg/kg, s.c.)					Effect time	Wash out
Ro 60-0175	Vehicle	0.75	1.25	2.5	4	40m-3h	3d

3. Results

3.1. Human rsEEG connectivity analysis:

- Greater sample entropy is associated with decreased medial prefrontal connectivity across all the datasets tested, reflecting high consistency in the findings, and generalization across EEG hardware.



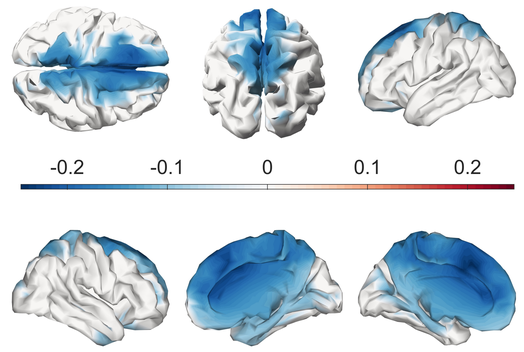
Pearson correlation of biomarker to lower gamma PEC (r's plotted)

- Partial correlation of the biomarker with lower gamma Fz PEC, corrected for age and sex, are reported below.

	Beam	iSPOTD	ALTO-300 Phase 2a	ALTO-100 Phase 2a	EMBARC
Partial correlation	-0.493*	-0.568*	-0.417*	-0.19	-0.717*
N	867	726	123	200	266

Correlation of the biomarker to Fz PEC in the lower gamma band;
**significant after Bonferroni correction*

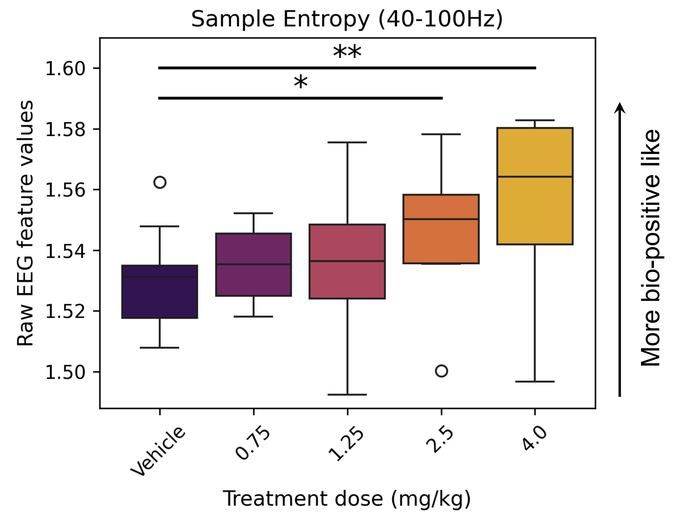
- Greater sample entropy is associated with decreased global connectivity, as measured by gamma PEC in the medial prefrontal cortex at the source level, aligning with its location at the scalp level.



Pearsons partial correlation of biomarker to source level lower gamma PEC localized to the mPFC; FDR corrected using Benjamini-Hochberg method (N =784)

3.2. Animal rsEEG PD analysis:

- Dose-dependent increase in gamma sample entropy compared to the vehicle group.
- One way ANOVA for dose effect: **pre-dose**: p = 0.526; **0.5hr - 1.5hr post dose**: p = 1e-4.
- Post-hoc pairwise t-test vs. vehicle: p = 0.01 for 2.5 mg/kg, p = 0.003 for 4.0 mg/kg.



Lower gamma sample entropy for 0.5 -1-hour post dosing

4. Conclusions

- Here we describe the network functional relevance of the ALTO-300 biomarker in defining patients with reduced medial prefrontal neural connectivity across multiple independent datasets.
- In mice, we showed that 5-HT2C agonism leads to higher (i.e. more biomarker positive-like) gamma sample entropy values, tying the human-discovered biomarker to ALTO-300's molecular mechanism of action.

5. Acknowledgements

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