Gamma Band EEG Sample Entropy, a Patient Selection Biomarker for ALTO-300 in MDD, is Increased by 5-HT2C Agonists in Mice

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1. Introduction

- ALTO-300, an antidepressant known as agomelatine in the EU and Australia, is a melatonin MT1/MT2 agonist and 5-HT2C antagonist, increases dopamine and noradrenaline release.
- We have previously found that increased parietal gamma band sample entropy (SE) predicts antidepressant response to ALTO-300 and are currently evaluating the ability of this biomarker to enrich for drug responders in an ongoing Phase 2b clinical trial in major depressive disorder (MDD).
- To elucidate the mechanistic basis of this biomarker, we investigated whether SE reflects 5-HT2C receptor activity.
- Dopaminergic circuits are critical for stabilizing neural representations and network dynamics; disruptions in these pathways are hypothesized to manifest as increased gamma SE (a measure of neural signal irregularity), detectable via scalp EEG.
- Given that ALTO-300 blocks the 5-HT2C receptor, we hypothesized that 5-HT2C agonism would increase gamma SE, creating a more biomarker positive-like EEG pattern.
- These findings complement studies of the effect of dopamine depletion on gamma SE (poster S125).

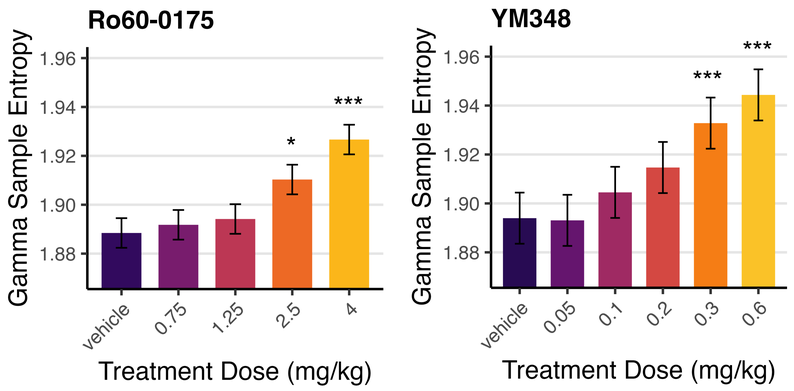
2. Study Design

- Two 5-HT2C agonists were tested in two separate cohorts of mice: RO60-0175 and YM348.
- Each animal received all doses of the relevant drug, including a vehicle control, in a randomized sequence, with a wash-out period at least 72 hours.
- EEG recordings were taken from frontal and parietal transcranial screws.
- Drugs were administered at 10AM each day (7AM – 7PM light cycle) and the recording started around 3PM the previous day and lasted about 24 hours after dosing.
- Sucrose preference tasks (2% sucrose) were conducted in a separate cohort of mice (N = 10 for each drug), with 2 days of habituation, 20 hours pre-treatment baseline measure and post-dose measures at 5 hours and 20 hours after treatment.

	Ro60-0175	YM348
5-HT2C selectivity	Moderate (2C/2A = 7.69)	High (2C/2A = 93)
Doses tested	4 doses: 0.75, 1.25, 2.5, and 4.0 mg/kg	5 doses: 0.05, 0.1, 0.2, 0.3, and 0.6 mg/kg
EEG Sample size	N = 13	N = 10
Key 5-HT2C target engagement properties	Induces behavioral changes (e.g., penile erection) abolished by 5-HT2C antagonists	Minimizes 5-HT2A receptor interference; behavioral effects similar to Ro60-0175

3. Dose-dependent sample entropy increase

- Gamma band frequency range was used to calculate SE.
- SE features were computed for every 10-second epoch and then averaged between 0.5 and 1.5 hours post dosing.
- Estimated values from a mixed model accounting for dosing sequence and individual variances are shown below for both drugs.

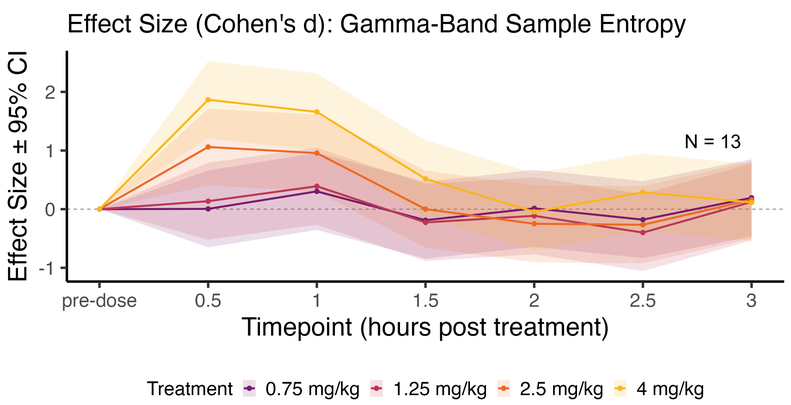


(Treatment effects compared to vehicle: p < 0.05*, p < 0.01**, p < 0.005***)

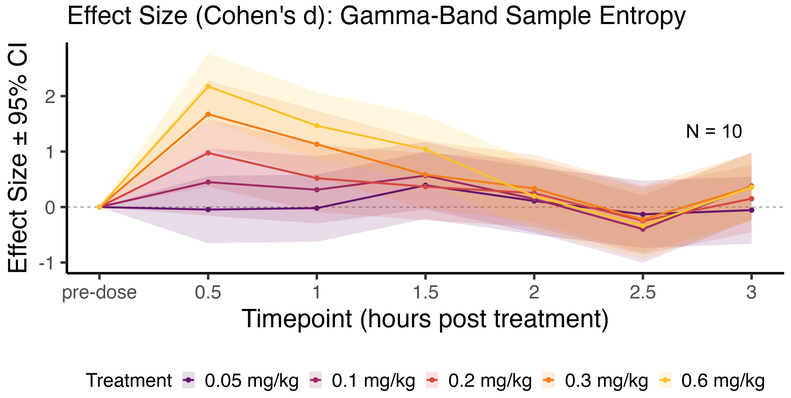
4. Sample entropy change across time

- We performed mixed-effect modeling to quantify the effect sizes of the drugs compared to vehicle across different the course of the recording. The features were averaged within 30-minute bins.
- We modeled EEG features as a function of treatment, time, and dosing sequence, while adjusting for baseline values and accounting for each animal's individual variance.

Ro60-0175



YM348



5. Treatment effect on sample entropy

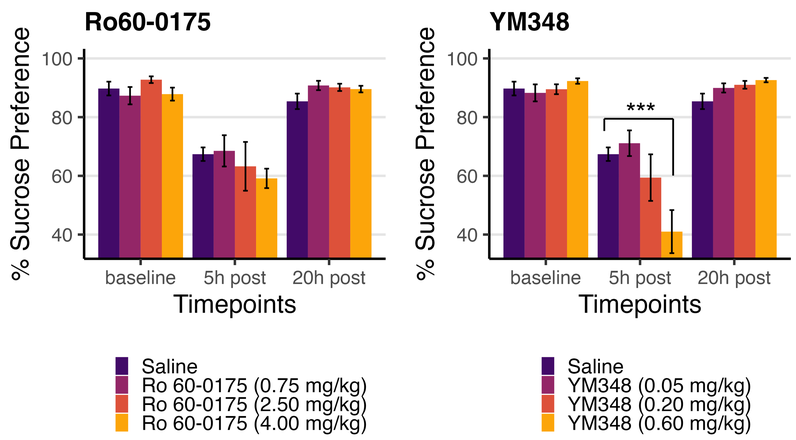
- We calculated the effect sizes (Cohen's d) of the acute treatment effect (EEG features averaged between 0.5 and 1.5 hours post administration) with a mixed model.

Ro60-0175 Dose (mg/kg)		0.75	1.25	2.5	4
Cohen's d	Parietal	0.15	0.26	**1.01***	**1.76*****
	Frontal	-0.68	-0.39	0.38	1.28

YM348 dose		0.05	0.1	0.2	0.3	0.6
Cohen's d	Parietal	-0.03	0.33	0.65	**1.22*****	**1.58*****
	Frontal	-0.55	-0.32	0.05	0.31	0.67

6. Sucrose preference test

- To evaluate the depression-related behavioral effects of 5-HT2C agonism, we performed sucrose preference tests.
- Independent t-tests were performed between each drug arm and the saline vehicle arm.
- At 5 hours post dosing, YM348 induced a significant decrease in sucrose preference (p = 0.004 for 0.6mg/kg), and RO60-0175 showed a trend towards decreased sucrose preference (p = 0.06 for 4mg/kg), suggesting an anhedonia-like behavior.



7. Conclusions

- We observed a dose-dependent increase in gamma-band SE with two 5-HT2C agonists, most strongly in the parietal channel, as hypothesized.
- These findings demonstrate that 5-HT2C agonism, the inverse of one aspect of ALTO-300's mechanism of action, reproducibly induces the same EEG pattern as the ALTO-300 biomarker, and anhedonia-like behavioral change.
- 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 in MDD.

8. Acknowledgments

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