Novel 5-HT2A receptor agonists exhibit translational antidepressant and psychedelic drug-like profiles in a model of treatment-resistant depression

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Neuroscience 2023
PSTR426.15 / NN1
Session Title: Treatment and Drugs for Mood Disorders

Background/Objective

Mood disorders, particularly treatment-resistant depression, remain a significant unmet medical need [1]. Clinical and preclinical research suggest the potential for serotonergic psychedelic compounds, such as psilocybin, to produce rapid and lasting antidepressant activity after a single dose [2-3].

Wistar Kyoto (WKY) rats exhibit behavioral, neurobiological and endocrine phenotypes that are consistent with symptoms observed in clinical depression [4]. In particular, this strain shows increased rapid eye movement (REM) sleep [5], a hallmark of depression in humans that is suppressed by acute and chronic antidepressant treatment [6-8]. As REM sleep in WKY rats is resistant to suppression by traditional antidepressant drugs (e.g., SSRIs) [5], it is a potentially useful genetic model of treatment-resistant depression (Table 1).

Novel 5-HT2A receptor agonists, EGX-A and EGX-B, discovered using an artificial intelligence/ machine learning approach were investigated for antidepressant drug-like effects using translational electroencephalography (EEG)-based measures in the WKY rat model.

Table 1. WKY Rat Phenotypes

Measure	WKY rat effect*
Behavioral measures of stress	↑ (anhedonia, neophobia, LH)
Other measures of stress	↑ (ACTH, CORT, TSH, stomach ulcers)
Measures of neurogenesis	↓ (BDNF, HPC volume, BrdU+ cells)
REM sleep	↑ (amount); ↓ (latency)
Response to antidepressants	↓ (SSRIs); √ (Ketamine)

Based on Millard et al., 2020, Ivarsson et al., 2005; *compared to other rat strains (e.g., outbred Wistar or Sprague-Dawley); ↑: Increased; ↓: Decreased; √: Responsive; LH: Learned helplessness; ACTH: Adrenocorticotropic hormone; CORT: Corticosterone; TSH: Thyroid stimulating hormone; BDNF: Brain derived neurotrophic factor; HPC: Hippocampus; BrdU+: 5-Bromo-2'-deoxyuridine positive; SSRI: Selective serotonin reuptake inhibitor

Methods

Adult male WKY rats (n=7) were implanted with an EEG dipole electrode (frontal positive, occipital negative) and EMG (nuchal muscle) electrodes. An intraperitoneal radio transmitter was used to transmit signals.

One hour after lights-on, animals were placed individually in recording boxes similar to their home cages (with food, water and limited environmental enrichment). After 1 hour of habituation, each animal was briefly removed from its box to be dosed and then recordings continued uninterrupted for 23 hours.

All animals received all 8 treatments in a pseudo randomized cross-over design with a minimum of 7 days washout between doses. Each treatment condition was represented during each weekly test session. Treatment conditions included: Vehicle negative control , Psilocybin positive control (10 mg/kg IP), EGX-A (3, 10, 30 mg/kg IP) or EGX-B (10, 17, 30 mg/kg IP). Novel compound doses were based on the free base weights and prepared in 40% (w/v) hydroxypropyl-β-cyclodextrin in water.

Automatic scoring of wake and REM sleep was performed on 10-second epochs of EEG/ EMG recordings using proprietary software. Compound effects on the percentage of time spent in REM sleep were analyzed over the initial 6 hours of EEG recording post-dosing.

REM sleep latencies were defined as the time (minutes) after dosing at which the first 3 consecutive REM sleep epochs occurred.

Quantitative EEG (qEEG) spectra (Delta (1-4Hz), Theta (4-7Hz), Alpha (8-12Hz), Beta (14-30Hz), Low Gamma (L. Gam, 30-50Hz), High Gamma (H. Gam, 50-100Hz) were computed using fast Fourier Transformation on 1-second non-overlapping consecutive epochs (unpadded Hann windows, 1 Hz resolution) and bandpass filtered at 0.5-100 Hz. Compound effects on waking qEEG power were analyzed as the average of the initial 4 hours post-dosing.

Body temperature and locomotor activity (EMG data) were computed using 10-second epoch values. Compound effects were analyzed over the initial 6 hours of recording post-dosing.

Data were analyzed by 1- or 2-way repeated measures Analysis of Variance (ANOVA, R version 3.6.3 or GraphPad Prism version 10.0.3) followed by Dunnett's test to compare test articles to the vehicle group. The level of significance was set at $p<0.05$.

Compound Profiles

EGX-A and EGX-B are selective agonists for 5-HT2A relative to 5-HT2B receptors, and induce the head twitch response in mice, supporting 5-HT2A receptor activation in vivo.

Table 2. Compound In Vitro & In Vivo Pharmacological Profiles

In Vitro IPOne EC50 & Emax%	EGX-A	EGX-B	Psilocin
Human 5-HT2A receptor agonism[1]	2.8 nM (99%)	9.2 nM (98%)	18 nM (83%)
Human 5-HT2B receptor agonism[1]	35 nM (78%)	81 nM (49%)	22 nM (73%)
5-HT2A/5-HT2B selectivity[2]	185	204	16
In Vivo Mouse Head Twitch Response (ED50, mg/kg IP)	1.7	3.2	0.17 [16]

[1]In vitro agonist activity normalized to the assay reference agonist, α-Me-5-HT;
[2]Selectivity calculated based on ΔΔlog(Emax/EC50) relative to α-Me-5-HT 5-HT2A/5-HT2B selectivity = 1

Figure 1. EGX-A and EGX-B showed similarly high brain relative to plasma concentrations for ~6-8h following dosing of 10 mg/kg IP to male rats. Thus, analyses of compound effects were focused on the initial 4-6 hours post dosing.

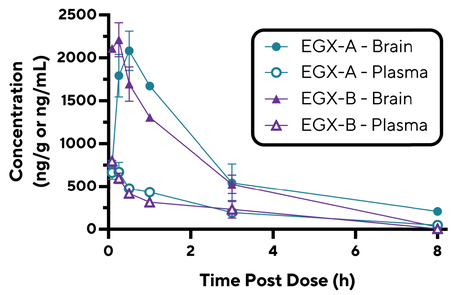

Figure 1. Brain (ng/g) and plasma (ng/mL) concentrations (mean ± sem) over time following administration of 10 mg/kg EGX-A or EGX-B IP to male Sprague-Dawley rats.

Compound Effects on REM Sleep Latency

Figure 2. EGX-A and EGX-B significantly increased REM sleep latencies in male WKY rats, consistent with the effect of Psilocybin (Psi).

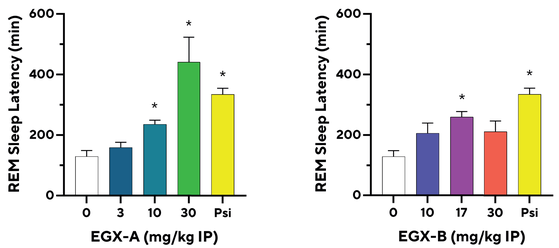

Figure 2. Latencies (mean ± sem) to REM sleep following administration of EGX-A (3, 10, 30 mg/kg IP, left), EGX-B (10, 17, 30 mg/kg IP, right) or Psilocybin (10 mg/kg IP) to male WKY rats. *p<0.05 vs. vehicle.

Compound Effects on REM Sleep Duration

Figure 3. EGX-A and EGX-B significantly reduced REM sleep duration in male WKY rats, consistent with the effect of Psilocybin (Psi).

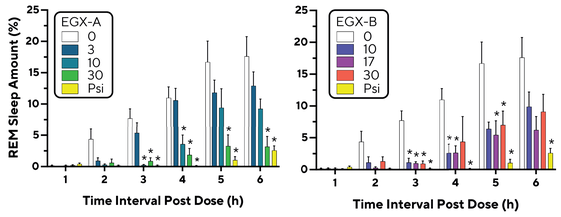

Figure 3. Percentage of time in REM sleep (mean ± sem) over 6h following administration of EGX-A (3, 10, 30 mg/kg IP, left), EGX-B (10, 17, 30 mg/kg IP, right) or Psilocybin (10 mg/kg IP) to male WKY rats. *p<0.05 vs. vehicle.

Compound Effects on Waking qEEG Power Spectra

Figure 4. EGX-A and EGX-B significantly increased waking theta & beta power, and decreased high gamma power, over the initial 4 hours following dosing to male WKY rats, consistent with the effects of Psilocybin (Psi).

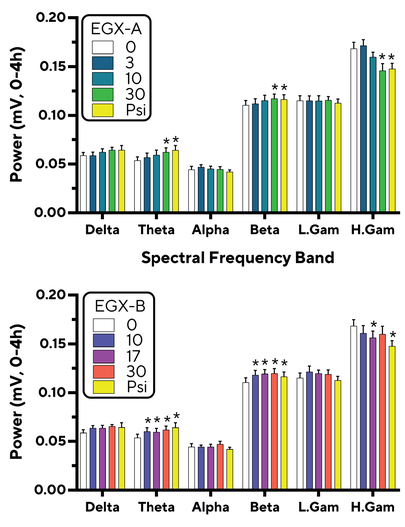

Figure 4. Mean (± sem) spectral frequency band power (mV) during wake over the initial 4 hours following administration of EGX-A (3, 10, 30 mg/kg IP, top), EGX-B (10, 17, 30 mg/kg IP, bottom) or Psilocybin (10 mg/kg IP) to male WKY rats. *p<0.05 vs. vehicle

Compound Effects on Temperature & Activity

EGX-B exhibited minimal effects on body temperature and locomotor activity in male WKY rats

Table 3. Compound Effects over 6 Hours Post Dose

Measure	EGX-A	EGX-B	Psilocybin
Body temperature	↓ (for 6h)	↓ (2h)	↓ (for 6h)
Locomotor activity	↓ (1h) / ↑ (2h)	No Δ	↓ (1h)

↓: Significant decrease; ↑: Significant increase; No Δ: No significant change vs. vehicle

Summary and Conclusions

REM SLEEP:

- The current work demonstrated that acute administration of novel 5-HT2A agonists increased REM sleep latency and suppressed REM sleep duration in male WKY rats, a model of treatment-resistant depression, similar to the effects of the serotonergic psychedelic psilocybin.
- The REM sleep effects of the novel compounds are highly consistent with reported effects of psychedelic 5-HT2A agonists (e.g., psilocybin/ psilocin, DOI) in healthy subjects and rodents [9-12] and indicate potential antidepressant drug-like activity [7-8].

qEEG

- Under the current study conditions, novel 5-HT2A agonists acutely increased theta and beta power and decreased high gamma power during wake in male WKY rats, similar to psilocybin.
- Acute administration of serotonergic psychedelics (e.g., psilocybin/ psilocin, LSD, mescaline) has been shown to generate broadband EEG power decreases during wake in healthy subjects and rodents [11-15].
- The present findings may differ from other rodent psychedelic EEG studies due to a) qEEG setup (dipole electrode vs. multi-electrode array, showing different spatial topological changes), b) EEG quantification (without vs. with differentiation of active vs. resting wake), and c) EEG analysis techniques (extended time analysis aligned with novel compound brain exposure vs. time-limited analysis).

Together, these results demonstrate that

- Novel 5-HT2A agonists exhibited effects on translational brain functional activity measures that were consistent with serotonergic psychedelic- and antidepressant drug-like activity
- EGX-B demonstrated agonist selectivity for 5-HT2A over 5-HT2B receptors and limited effects on body temperature and activity
- EEG measures may be useful to confirm compound-induced modulation of brain functional activity in future clinical trials

Acknowledgement: atai Life Sciences would like to thank all colleagues involved in the Entheogenix Biosciences program.

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