Acute and durable miniscope-based neurobehavioral profiling of 5-HT2A receptor agonists with hallucinogenic and non-hallucinogenic potential in mouse medial prefrontal cortex

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Introduction & Objectives

Antidepressant potential of 5-HT2AR agonists

- Classical **psychedelics** such as psilocybin, LSD or DMT produce rapid and lasting antidepressant effects in humans[1,2]

Non-hallucinogenic 5-HT2AR agonists

- Includes compounds such as lisuride and 2-Br-LSD
- Preclinical neuroplasticity and behavioral studies suggest antidepressant-like potential[3-5]

Role of medial prefrontal cortex (mPFC)

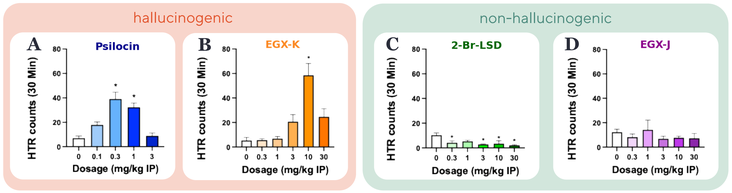
- Associated with stress-related disorders and antidepressant activity[6,7]
- 5-HT2AR highly expressed[8]
- Does it mediate acute and durable 5-HT2AR agonist effects?

Question: How do hallucinogenic and non-hallucinogenic 5-HT2AR agonists affect mPFC neuronal activity and behavior?

1. Acute neuronal metrics
2. Behavior
3. Durable effects?
4. Hall. vs. non-hall. fingerprints?

Methods

Panel of reference and novel hallucinogenic (hall.) and non-hallucinogenic (non-hall.) compounds



To study neurobehavioral differences of hall. and non-hall. compounds, we tested a panel of reference (**psilocin**, **2-Br-LSD**) and novel (**EGX-K**, **EGX-J**) compounds. Hallucinogenic potential was assessed by the head twitch response (HTR) in male C57BL/6 mice. **Psilocin** and **EGX-K** significantly induced HTR (A, B). Neither **2-Br-LSD** (C) nor **EGX-J** (D) up to 30 mg/kg induced HTR, suggesting a lack of hallucinogenic potential.

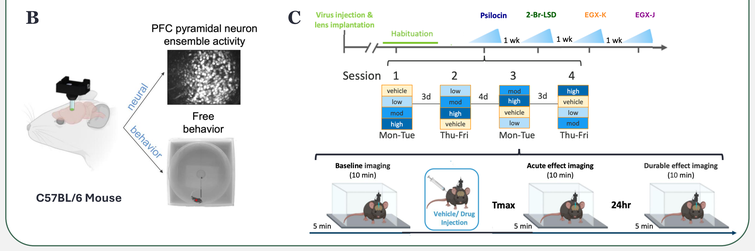
Novel behavior + neurophysiology-based platform for comparative compound evaluation

Acute and durable effects on locomotion and pyramidal neurons' activity in mouse mPFC were measured via *in vivo* cellular calcium imaging during free behavior (B). Fourteen mice were implanted with *nVista miniscopes* and injected with AAV1.CaMK2.GCaMP6f (B, C), followed by 4 weeks of recovery.

For each compound, 1 vehicle control and 3 doses ("low", "mod", "high"; A) were tested within a 2-week period (4 sessions, randomized and counter-balanced, each separated by >72h; C). Each compound's testing block was separated from the next one by 1 week (C).

A

compound	dose [mg/kg IP]	vehicle	Tmax [min]
Psilocin	0.3, 1, 3	saline	30
2-Br-LSD	0.3, 1, 3	saline	10
EGX-K	1, 3, 10	40% (w/v) HPBCD in water	60
EGX-J	1, 3, 10		60



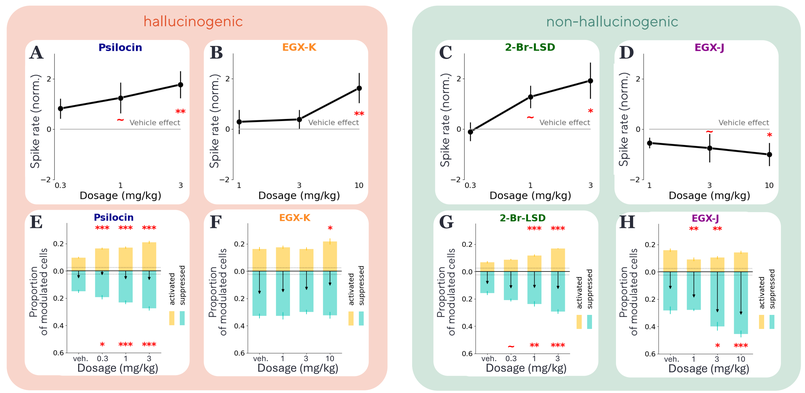
Results

1. Acute neuronal effects

Psilocin, 2-Br-LSD and EGX-K acutely increase neuronal spike rate in mPFC, while EGX-J reduces it

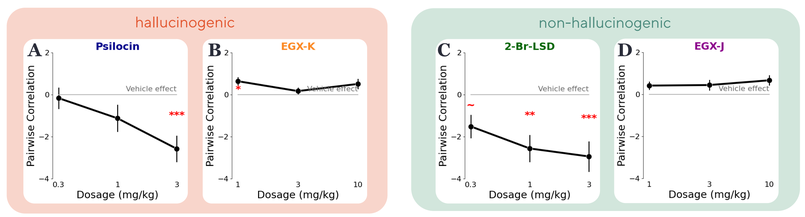
On the neuronal population level, **psilocin** (A), **2-Br-LSD** (C) and **EGX-K** (B) significantly increased average spike rate, while **EGX-J** (D) significantly decreased average spike rate, each in a dose-dependent manner.

On the individual neuron level, we quantified the magnitude of drug-induced spike rate modulation. Then, we calculated the proportion of neurons which were activated (yellow) or suppressed (cyan). **Psilocin** (E) and **2-Br-LSD** (G) increased proportion of both activated and suppressed cells, starting at lower doses. **EGX-K** also increased proportion of activated cells (F). On the other hand, **EGX-J** (H) increased proportion of suppressed cells and decreased proportion of activated neurons.



Psilocin and 2-Br-LSD acutely disrupt coordinated prefrontal neuron activity, while EGX compounds have no effects

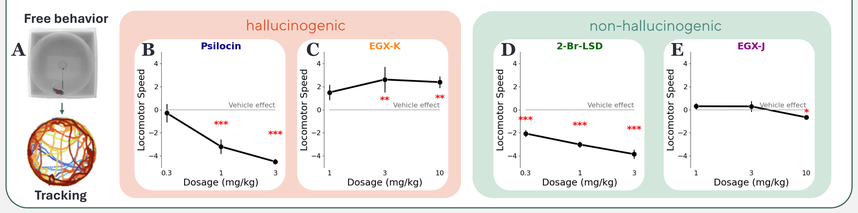
To assess population-level correlation structure, an indicator of neuronal network function, we measured the correlation between fluorescence traces of individual pairs of neurons. **Psilocin** (A) and **2-Br-LSD** (C) reduced magnitude of pairwise correlation, while **EGX-K** and **EGX-J** showed no consistent effects (B, D).



2. Acute behavioral effects

Psilocin, 2-Br-LSD and EGX-J acutely reduce locomotion, while EGX-K increases it

Behavioral changes were assessed by live tracking of mouse movement during free behavior using DeepLabCut (A). Acutely, **psilocin**, **2-Br-LSD** and **EGX-J** significantly reduced average speed of locomotion in a dose-dependent manner (B, D, E), while **EGX-K** increased it (C).



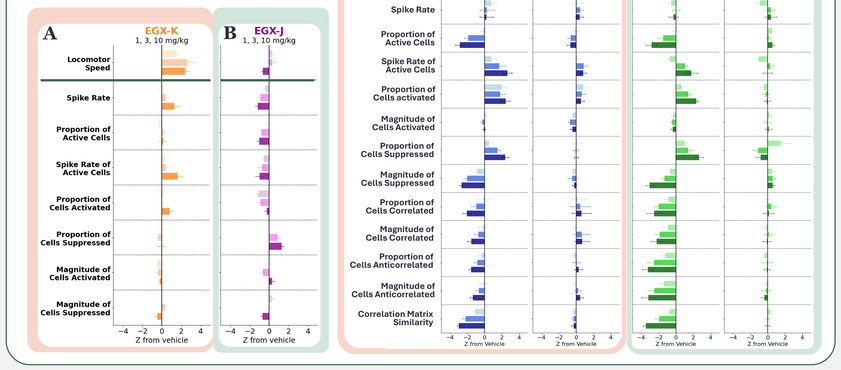
3. Acute vs. durable effects

EGX-K and EGX-J exhibit opposing acute effects

Strikingly, the closely related **EGX-K** and **EGX-J** showed mostly opposite effects on reported measures (A, B), suggesting differential mechanisms for hall. vs. non-hall. compounds in this chemical class. None of the observed changes were present at 24h timepoint (data not shown).

No durable (24h) effects of any tested compound

To verify if any measures, including within-cell effects, persist until 24h, we performed Multi-session Registration Analysis for **psilocin** and **2-Br-LSD**. There were no significant effects at 24h (C, D).

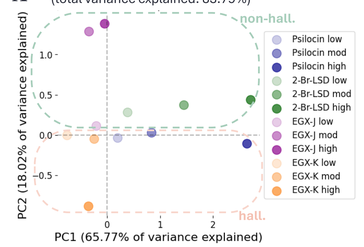


4. Hall. vs. non-hall. fingerprints?

PCA analysis: psilocin and 2-Br-LSD cluster together, PC2 shows a trend toward a hall. vs. non-hall. distinction

The first 2 PCs of a PCA on 13-dimensional neurobehavioral effects clustered **psilocin** and **2-Br-LSD** closely together (A).

PC2, driven primarily by neuronal activity, suggested a potential hall. vs. non-hall. separation (A, B).



B

	PC1	PC2
Locomotor Speed	-0.28	-0.13
Spike Rate	0.08	-0.65
Proportion of Active Cells	-0.04	-0.46
Spike Rate of Active Cells	0.12	-0.48
Proportion of Cells Activated	0.27	-0.06
Proportion of Cells Suppressed	0.27	0.24
Magnitude of Cells Activated	0.20	-0.18
Magnitude of Cells Suppressed	-0.30	0.02
Proportion of Cells Correlated	-0.34	0.01
Proportion of Cells Anticorrelated	0.40	-0.09
Magnitude of Cells Correlated	-0.36	-0.03
Magnitude of Cells Anticorrelated	0.37	-0.07
Correlation Matrix Similarity	-0.30	0.05

Conclusions

- Psilocin and 2-Br-LSD showed nearly identical **acute** neurobehavioral profiles (reduced locomotion, increased spike rate and decreased network correlation), in line with the described increase in cortical spiking activity[9] and simultaneous network disorganization[10–11] in the acute psychedelic state. Hallucinogenic EGX-K elicited similar increases in neuronal spike rate.
- The absence of any effects at 24h, supported by within-cell analysis across timepoints, suggests that durable changes in mPFC pyramidal neurons' activity are unlikely to mediate the lasting antidepressant effects of the tested 5-HT2AR agonists within the experimental parameters used in this study.
- EGX-K and EGX-J, though structurally similar, exhibited opposing neuronal and behavioral effects, suggesting potential distinctive mechanisms underlying hall. vs. non-hall. compounds in this chemical class. Neuronal activity-driven PC2 further emphasized this separation.

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