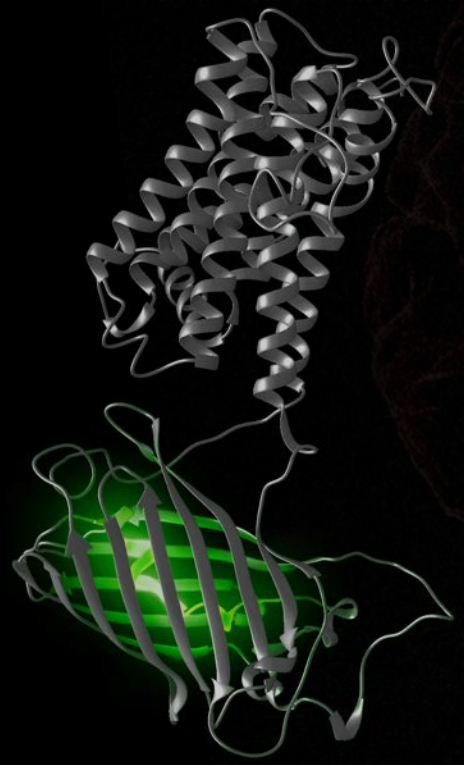
Monitoring real-time pharmacology and serotonin dynamics with serotonin biosensor



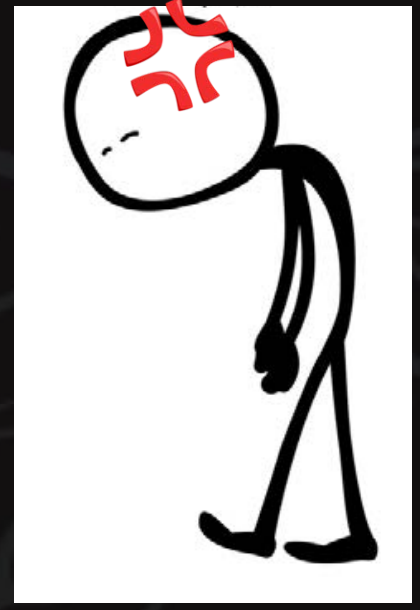
Chunyang (Jason) Dong

Biochemistry and Molecular Medicine
School of Medicine
University of California, Davis

WCBR 2020
Big Sky Montana

Depression

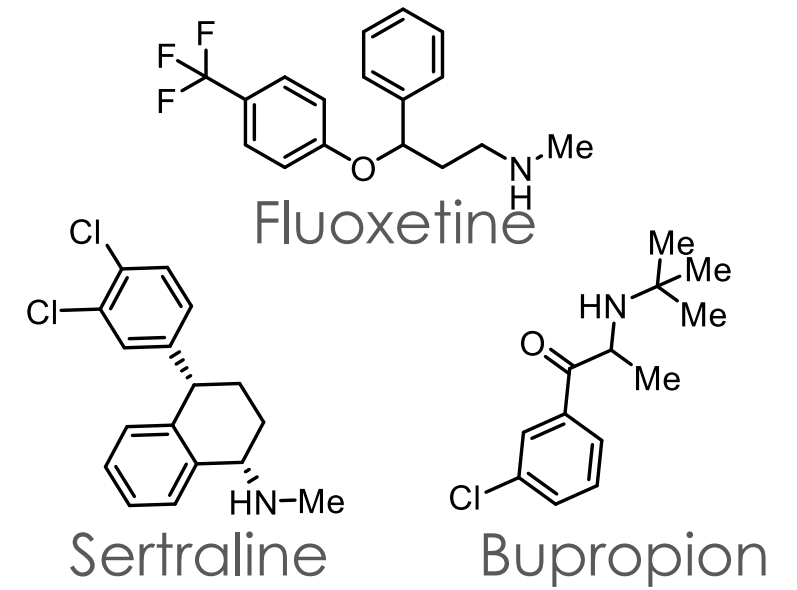
- *Affects over 16.2 million adults*
- *45,000 lives lost to suicide in 2016*
- *Chronic stress is a factor leading to depression*



Treatment?

From the National Institute of Mental Health, 2016
Centers for Disease Control, 2018

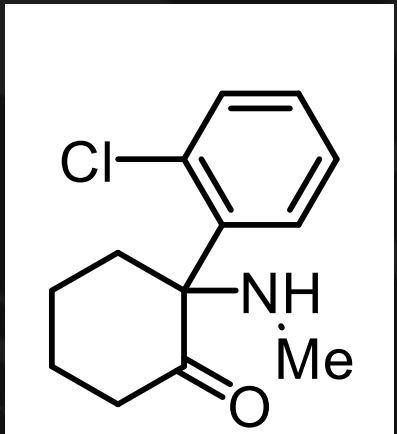
Slow-acting antidepressant



Drug Type	Drug Name	Trade Name	Year Discovered
SSRI	Fluoxetine	Prozac	1987
SSRI	Sertraline	Zoloft	1991
NDRI	Bupropion	Wellbutrin	1996

- Takes 4-6 weeks to have effects
- No matter how to combine different types of SSRIs, still 1/3 of the population do not achieve remission.

Fast-acting antidepressant



Ketamine

FDA approved for treatment-resistant depression in 2019

- Affect with in 1 hour
- Last for a week

- **However..**
  - Strictly controlled due to **drug abuse** and **hallucination** side effect.

Major challenges

- Develop a handy platform to screen novel compounds that could be effective but without hallucination side effect.
  - What's the mechanistic actions of antidepressant drugs on receptors?

- Understand neuromodulation signaling pathways by precisely measure serotonin dynamics in behaving animals.

Current method of classifying hallucinogen

Head twitch experiment

Pro

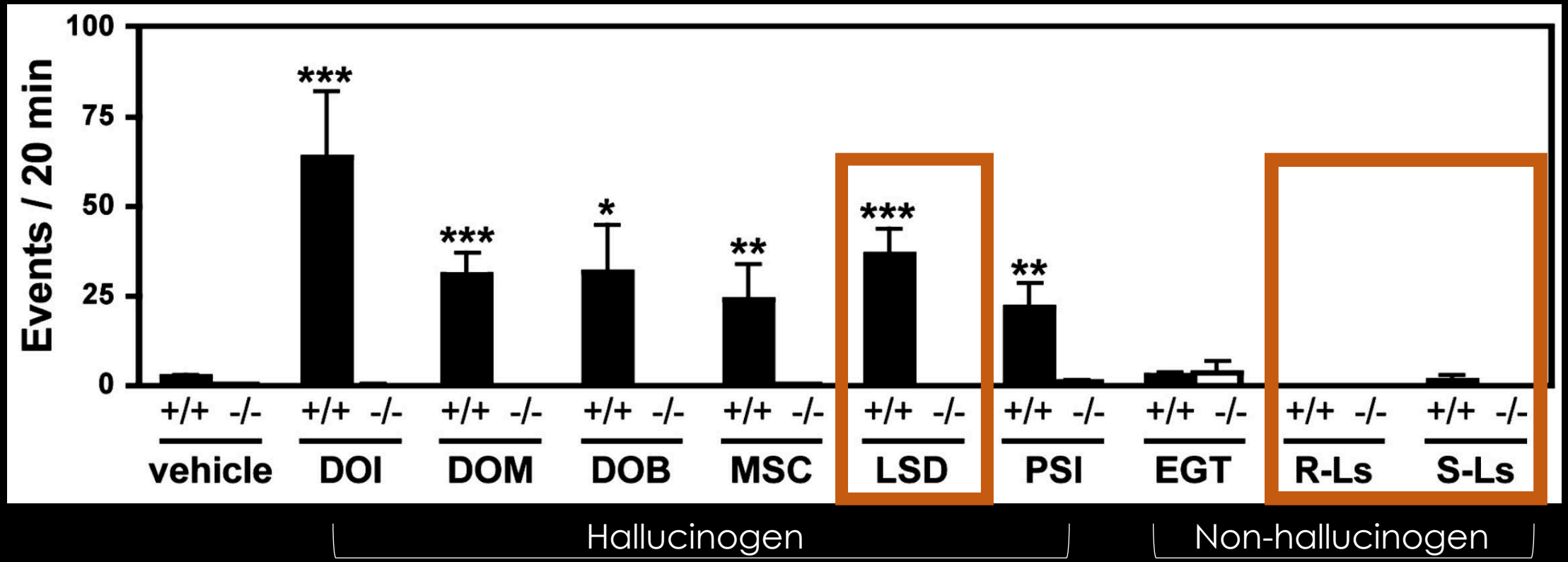
- suitable animal model for predicting hallucinogenic potency in humans

Con

- Time consuming
- Costly
- Low throughput

Head twitch response is directly related to 5-HT 2A receptor

HTR comparison between WT and 5-HT2A receptor KO mice



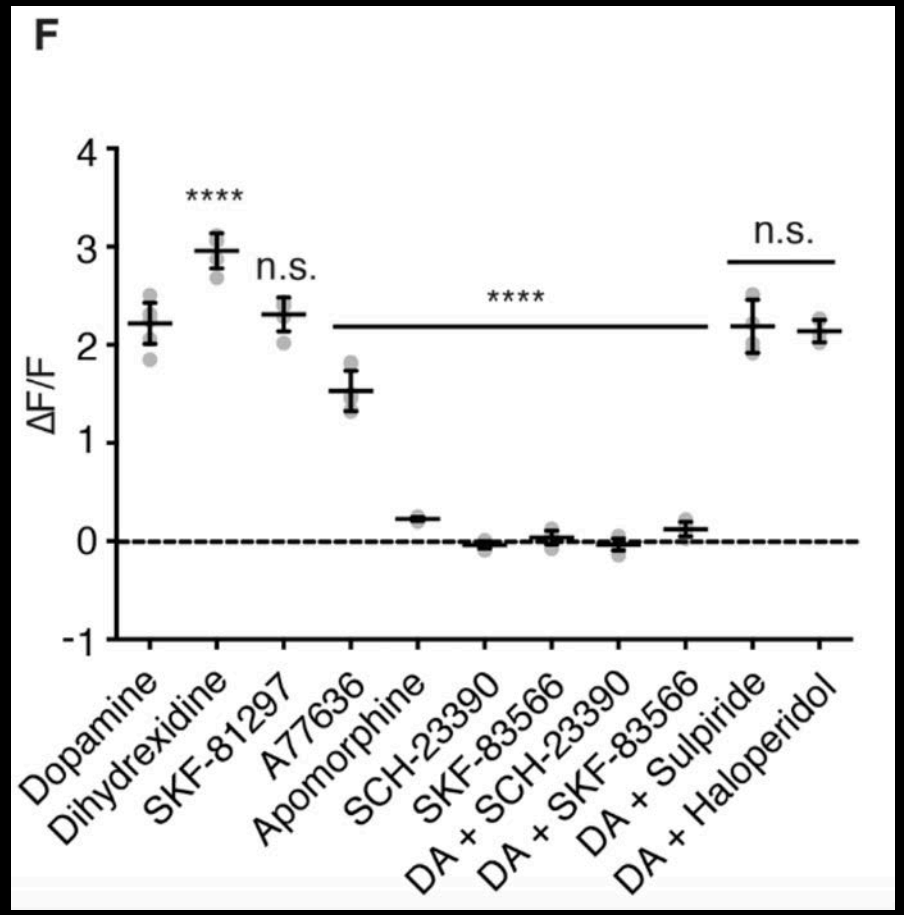
Gonzaʹlez-Maeso et al., 2017, Neuron53, 439–452

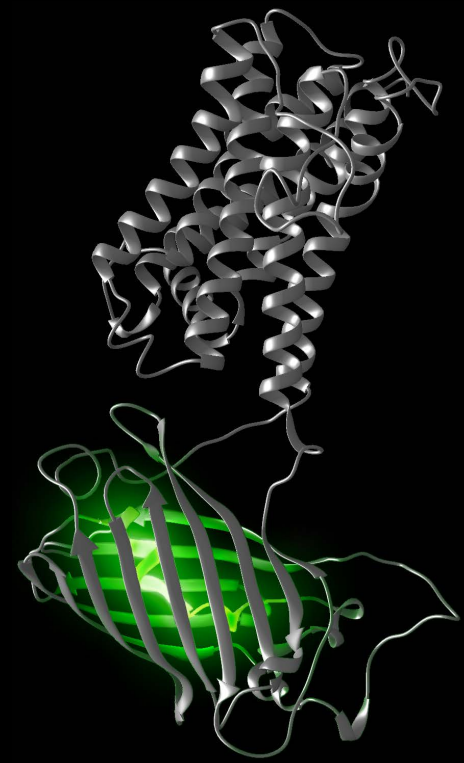
Hypothesis: Functional selectivity

- the hallucinogenic effect is linked to functional selectivity to 5HT2A receptor.

- We can develop a sensor to specifically detect the conformational states of 5HT2A receptor by coupling a specific conformation of the receptor induced by ligand binding to the fluorescence.

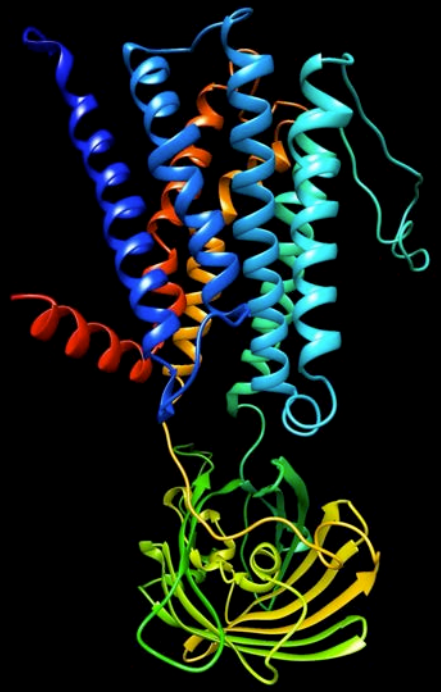
From dLight to sLight

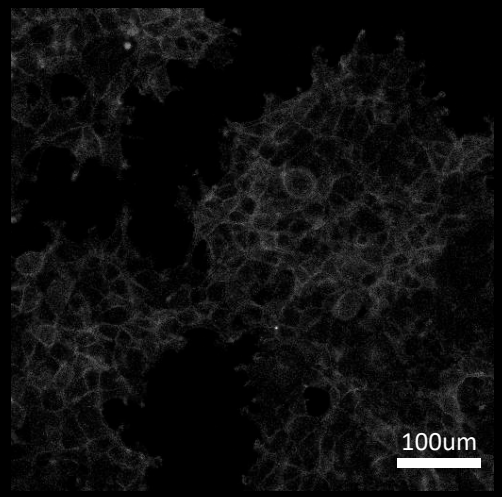




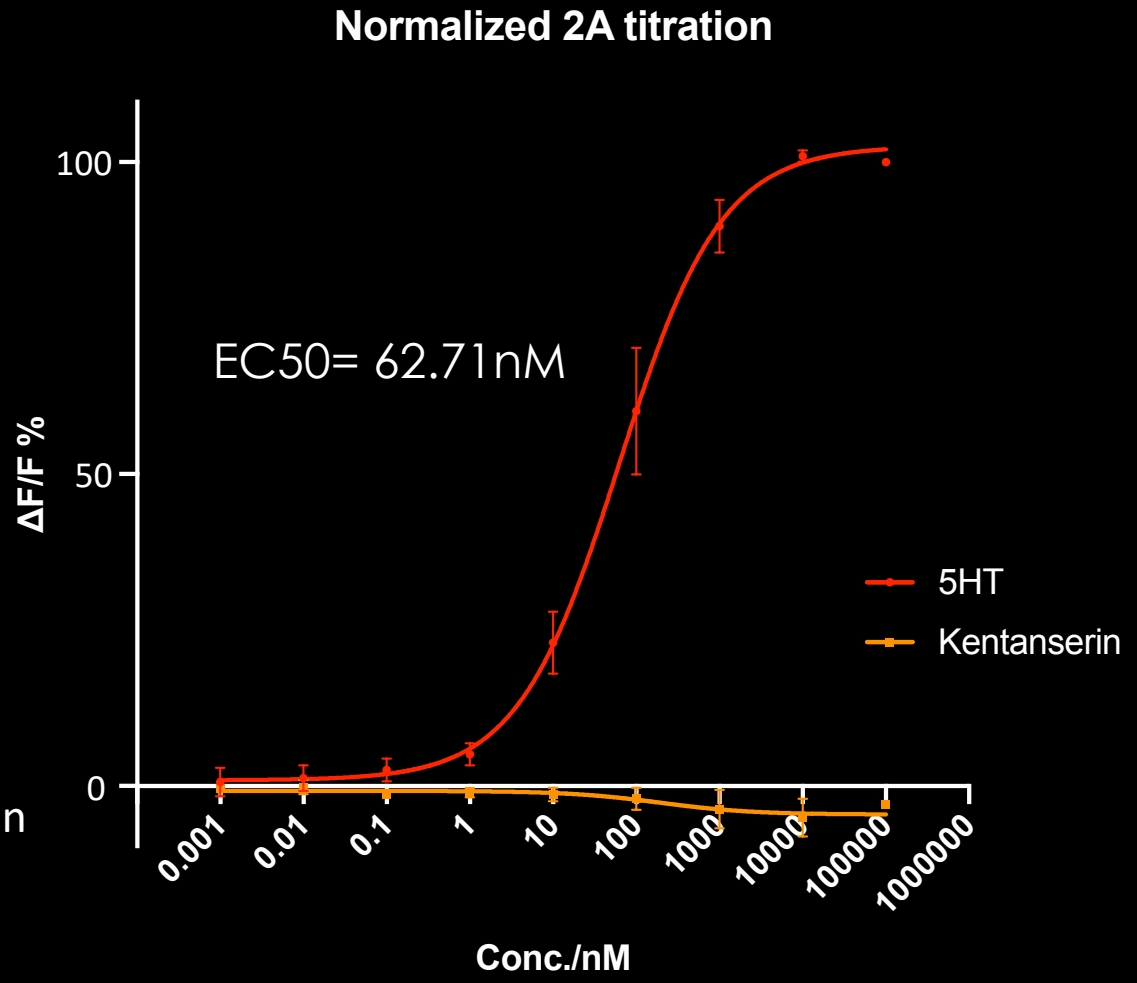
T. Patriarchi et al., Science 10.1126/science.aat4422 (2018)

sLight: a 5-HT2A based sensor



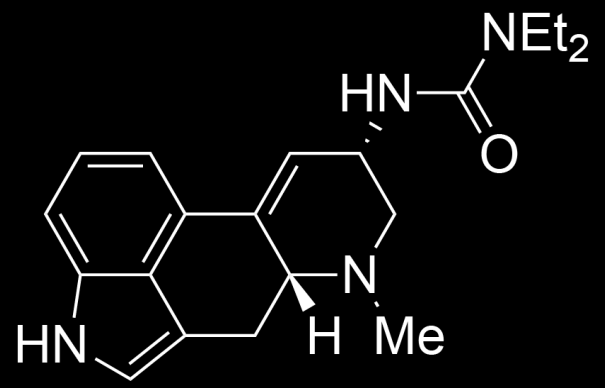


sLight + 10uM 5-HT + 10uM kentanserin

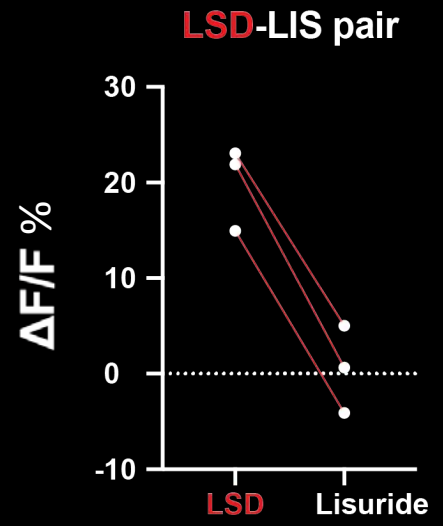


sLight can differentiate hallu vs non-hallu

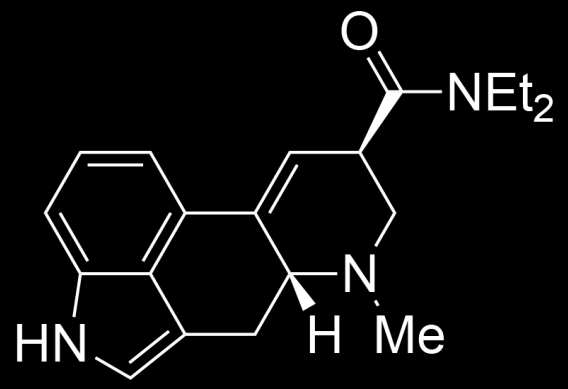
LSD



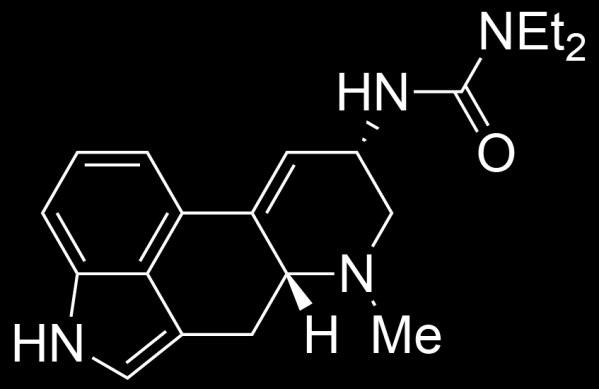
Lisuride



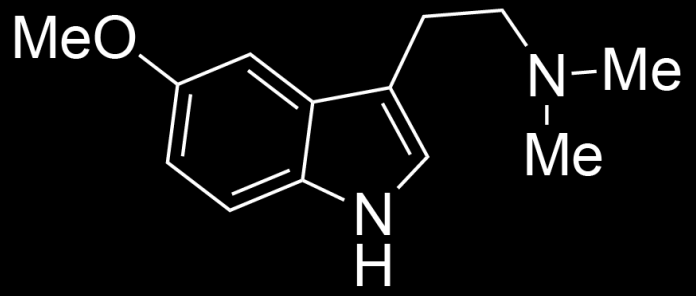
sLight can differentiate hallu vs non-hallu



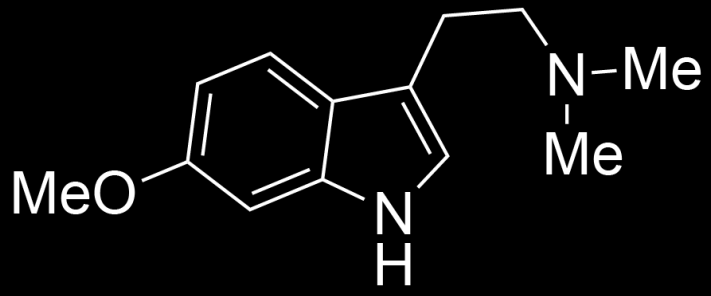
LSD



Lisuride



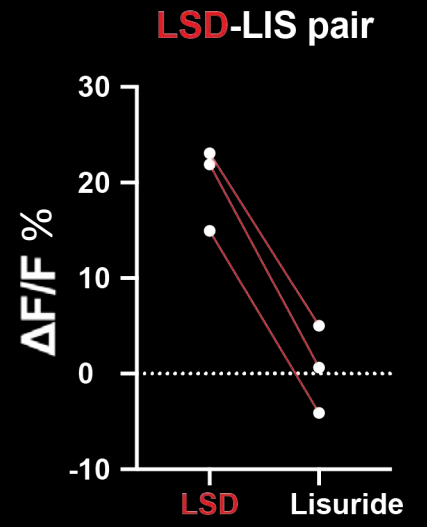
5-MeO-DMT

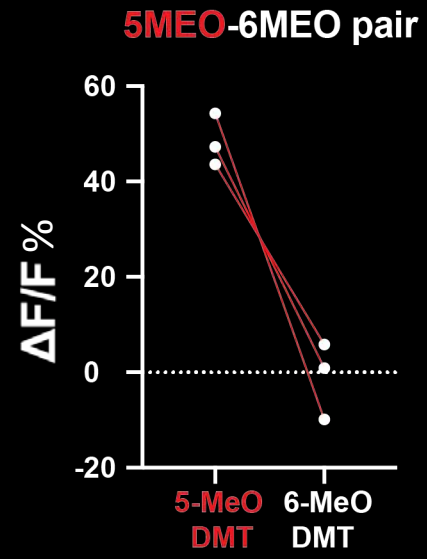


6-MeO-DMT

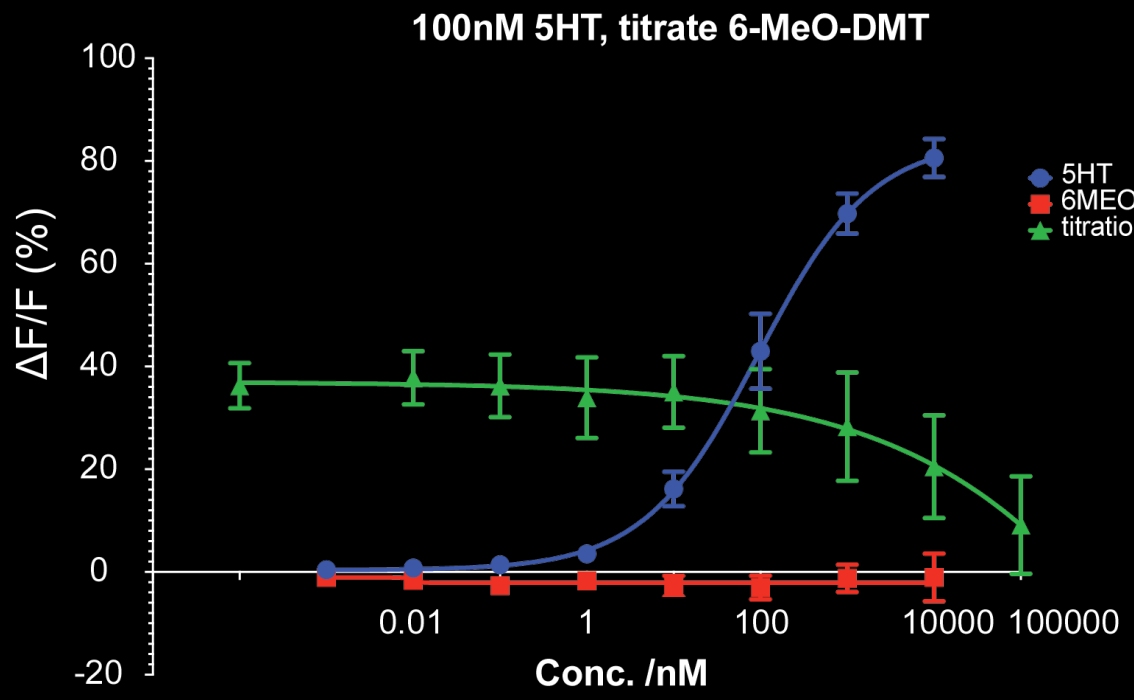
Known hallucinogen

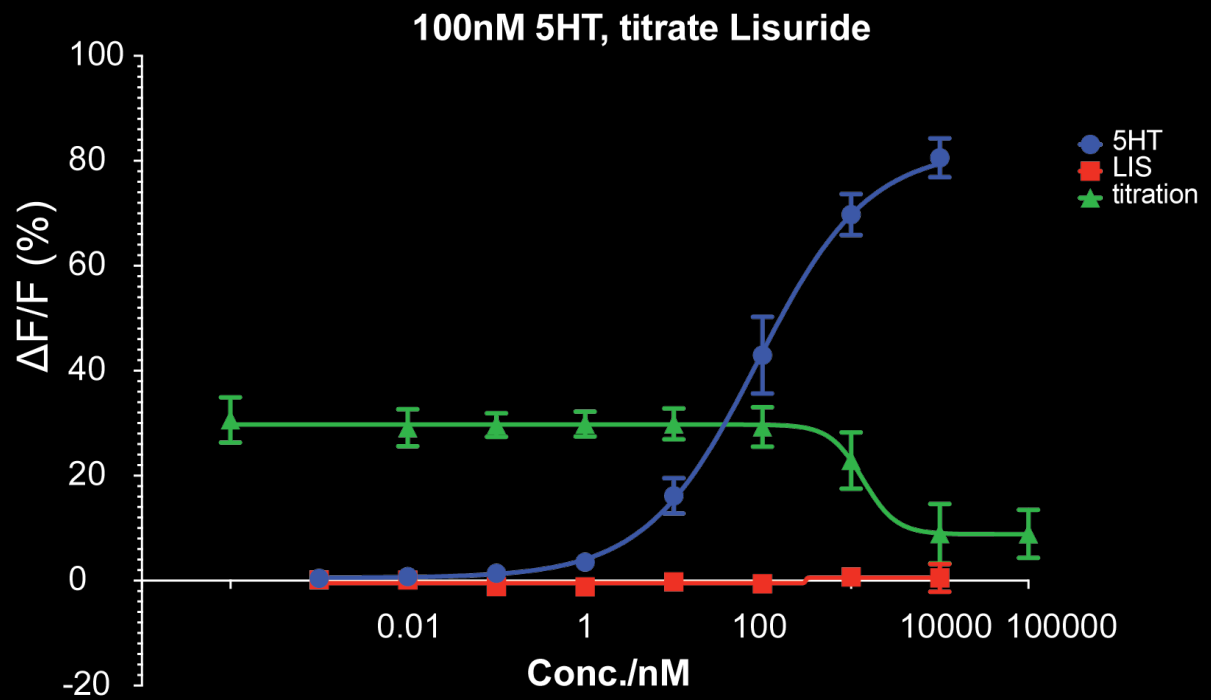
Known non-hallucinogen



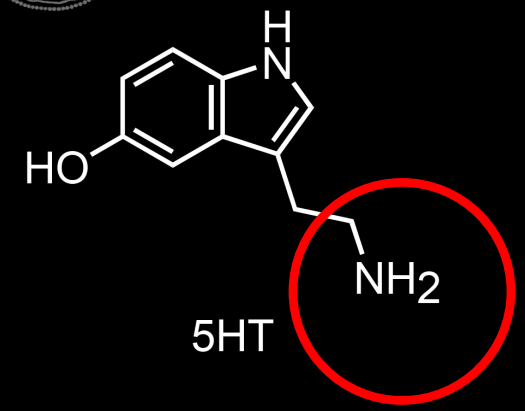


No-hull compounds bind the same pocket as 5-HT

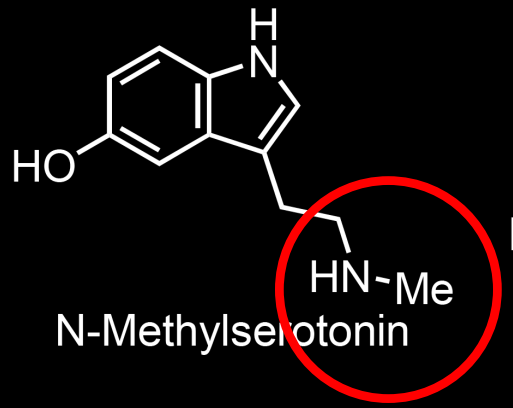




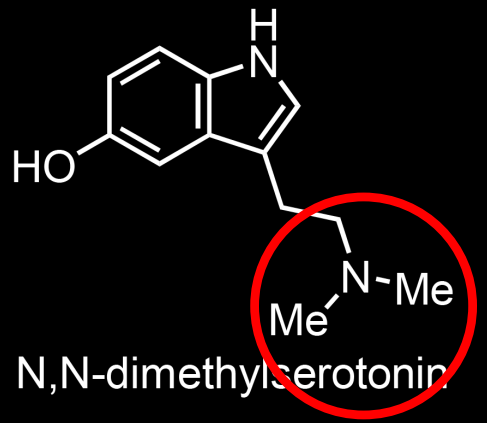
Larger functional group leads to lower fluorescent intensity



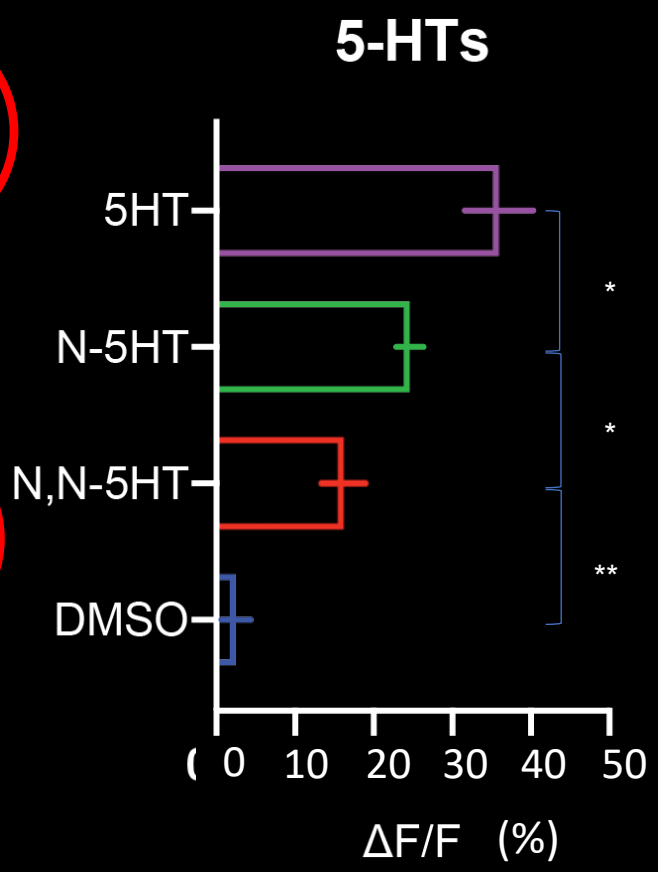
5HT

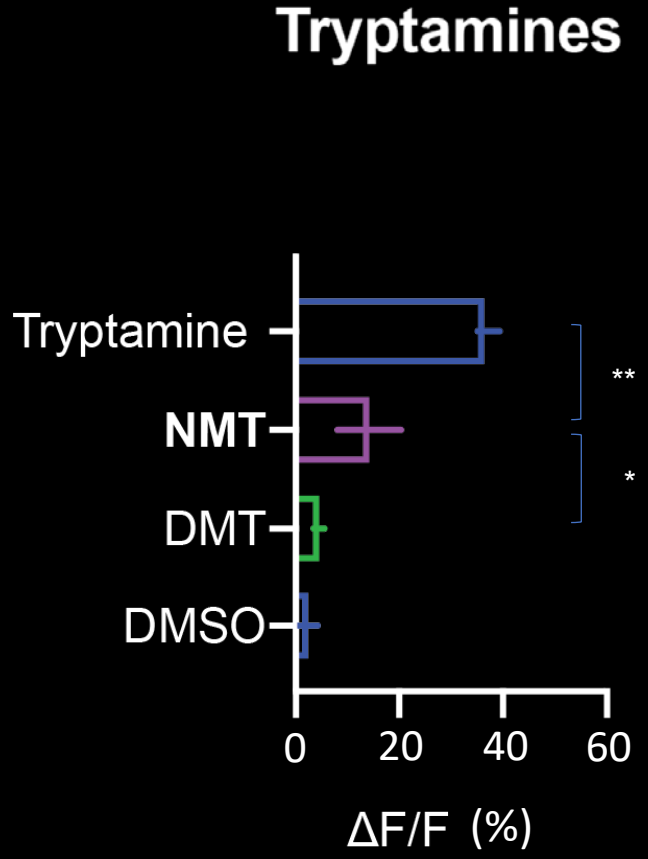


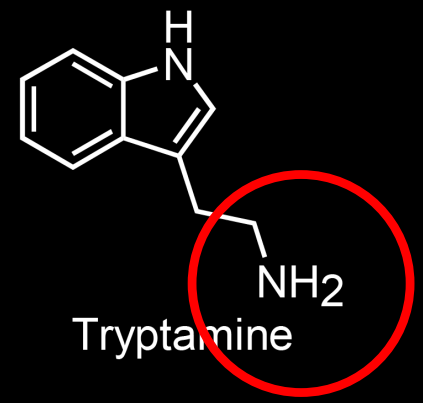
N-Methylserotonin



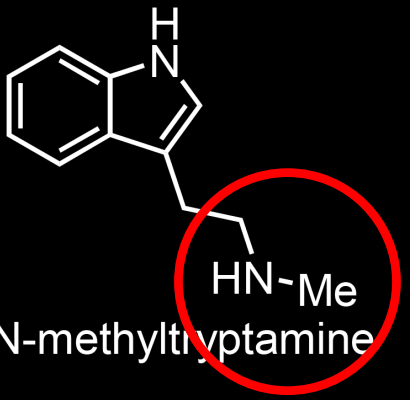
N,N-dimethylserotonin



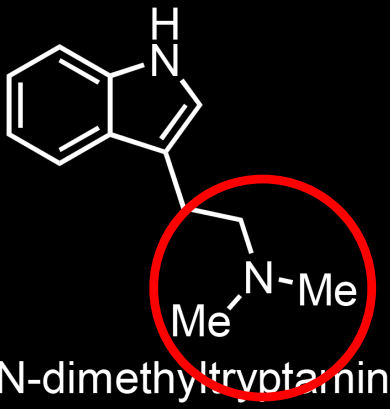




Tryptamine

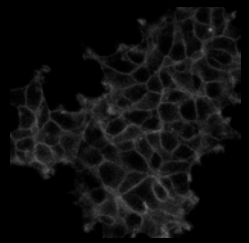


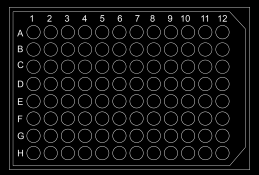
N-methyltryptamine

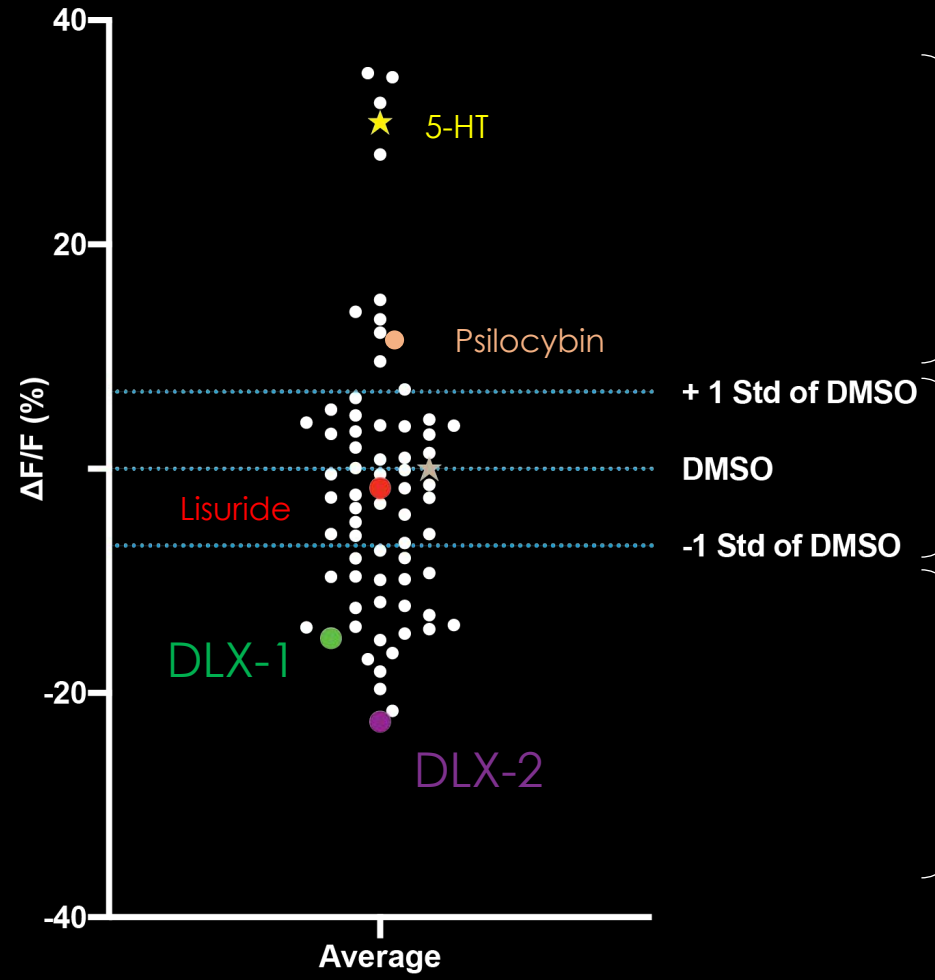


N,N-dimethyltryptamine

A counter-screening platform







Hallucinogenic agonist

Non-hallucinogenic agonist

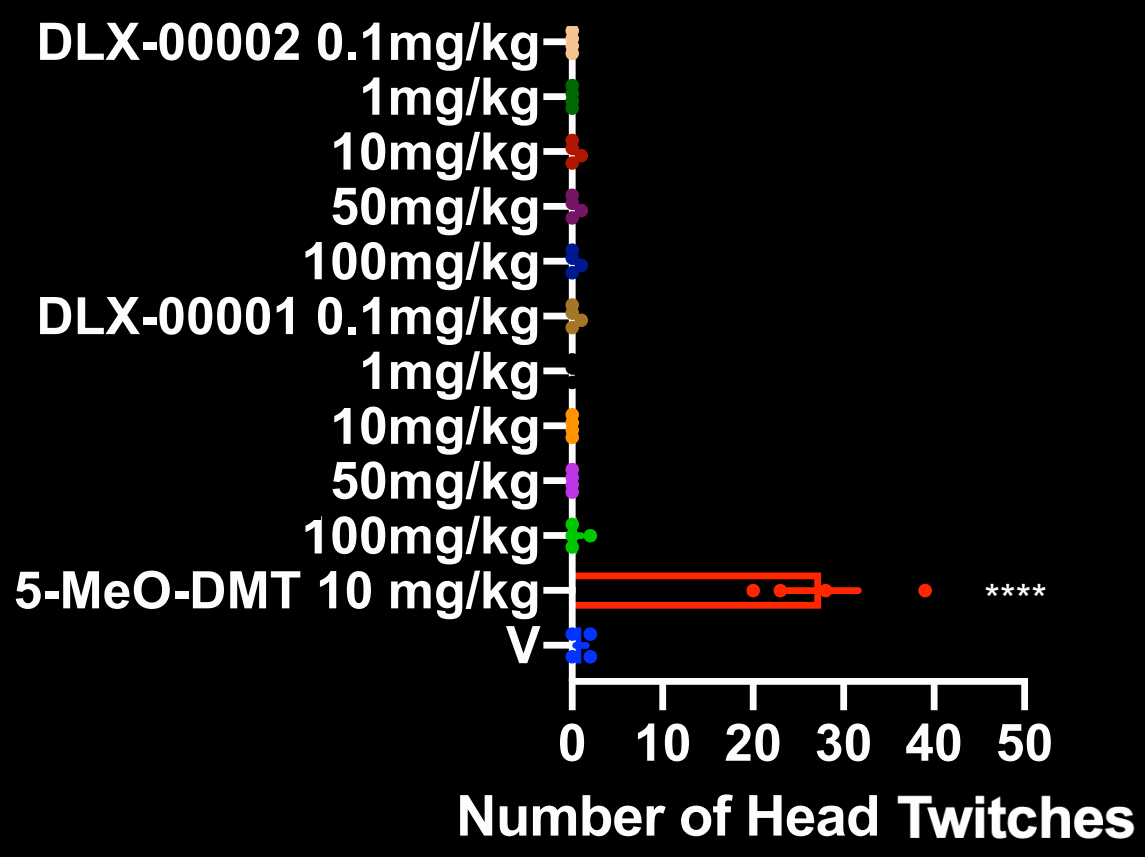
Antagonist

Non-hallucinogens

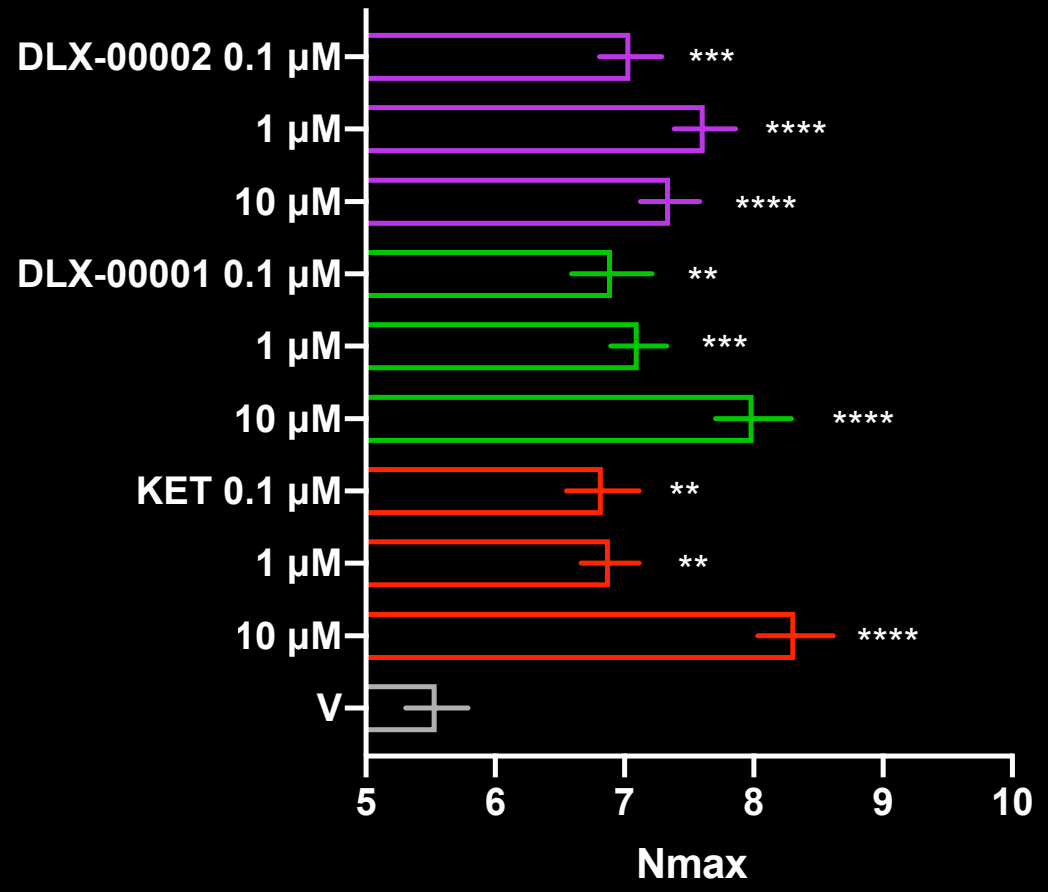
n=3 for each compound
Z-score = 0.6

Characterization of novel compounds: head twitch and structural plasticity

Head twitch response

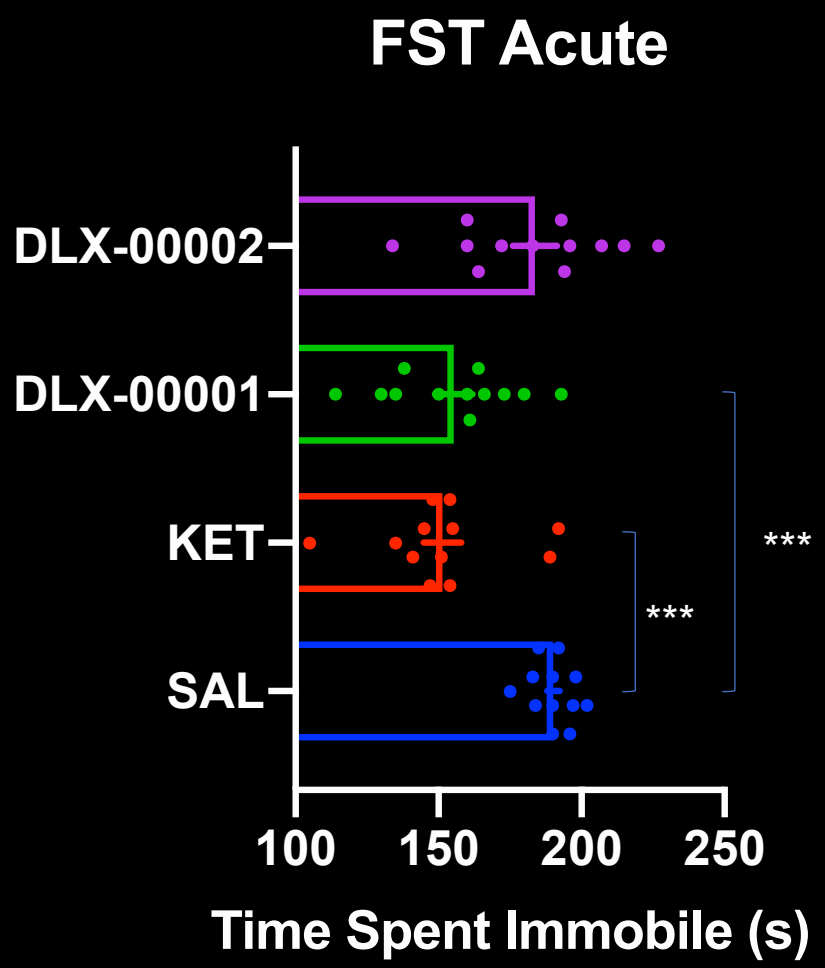


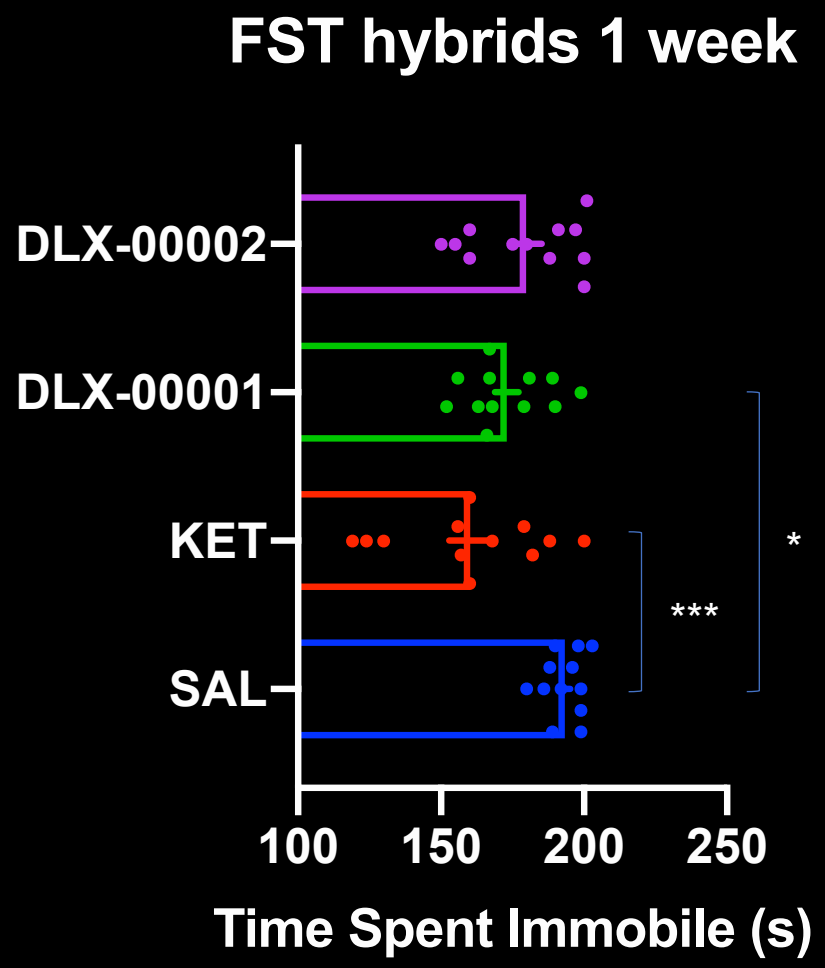
Sholl analysis



Calvin and Lee

DLX-1 showed similar effect as Ketamine in force swim test





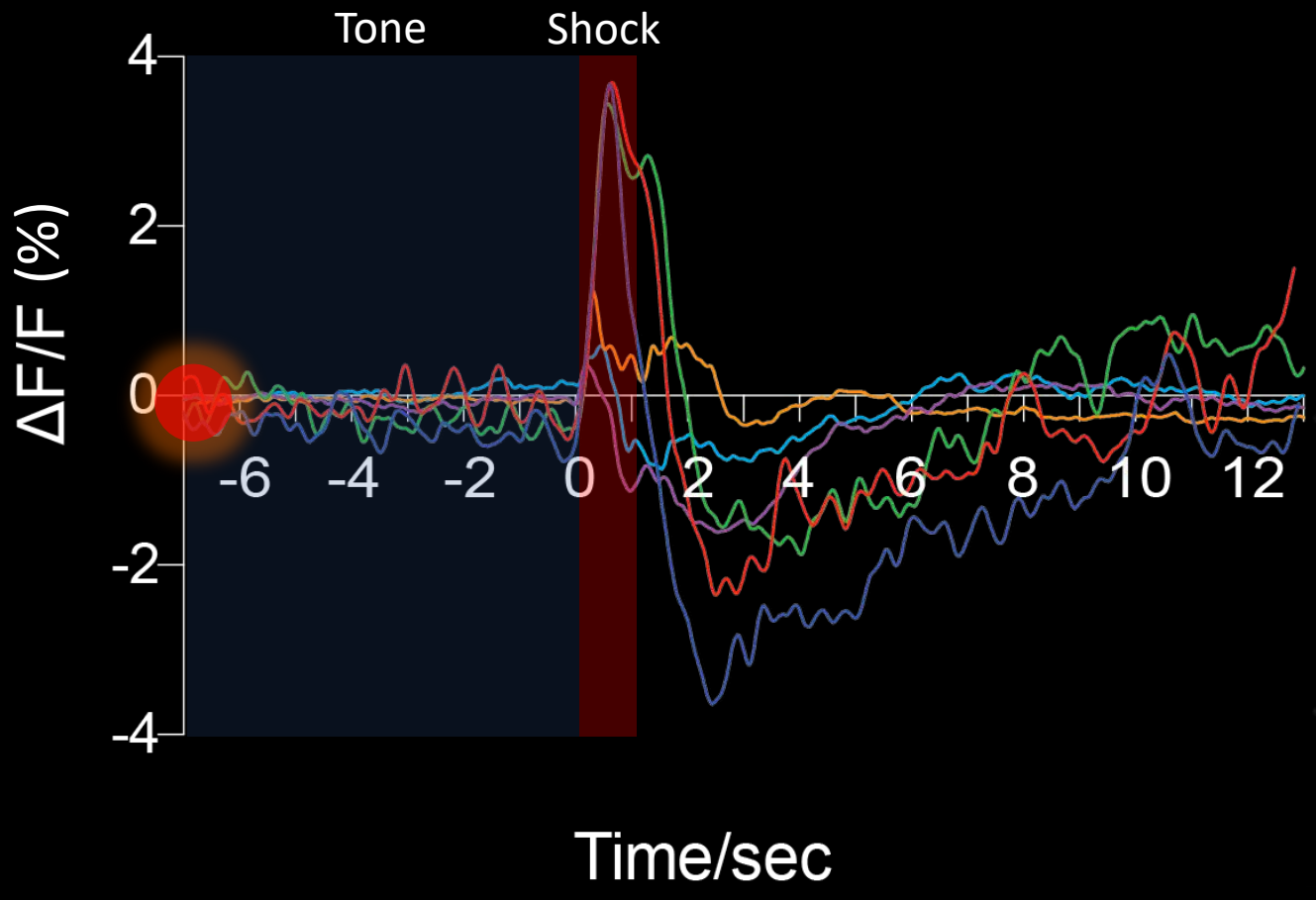
Max Vargas

Conclusions

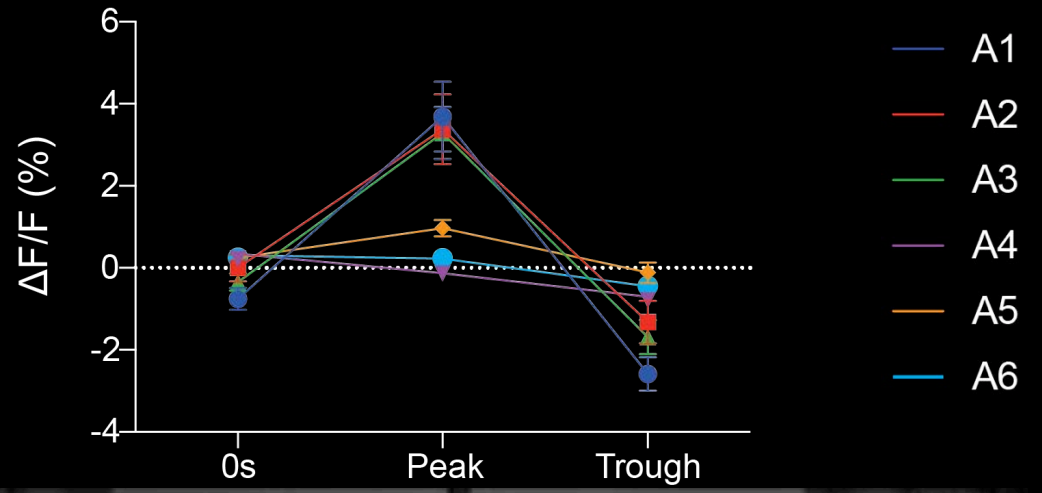
- Developed a 5-HT2A conformer sensor to enable real-time pharmacology to reflect the functional selectivity of HT2AR.

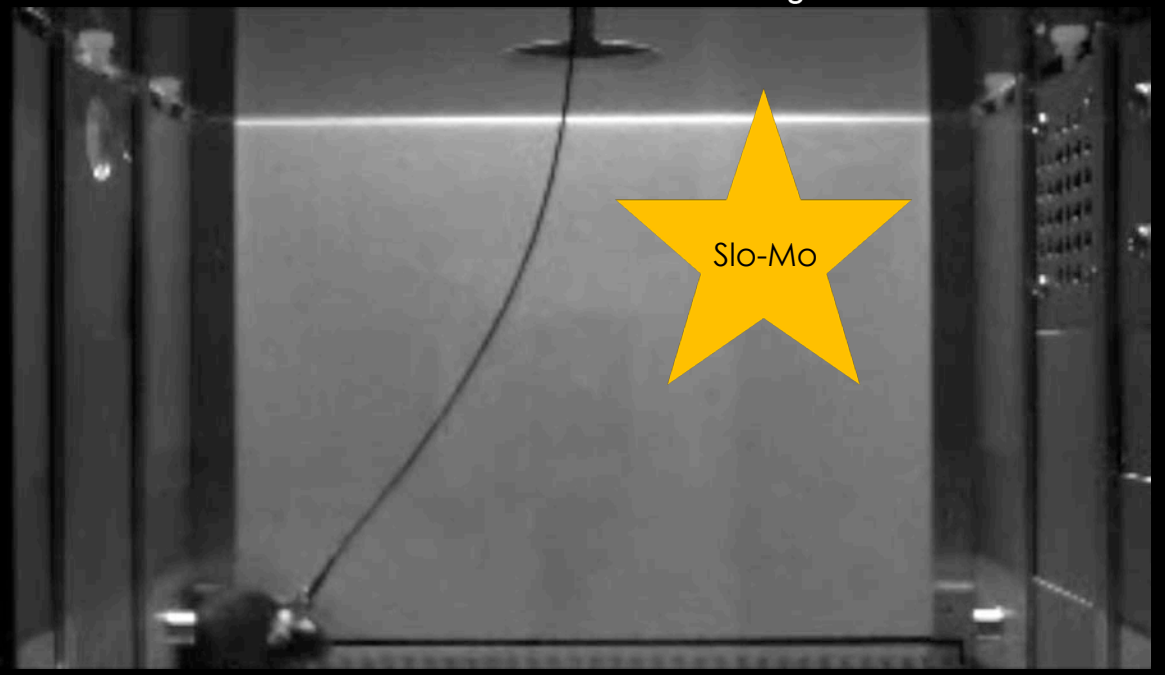
- Developed the counter screening platform to identify compounds that has beneficial effect as fast-acting antidepressant as Ketamine, but without hallucination side effect.

In Vivo 5-HT dynamics in response to fear conditioning

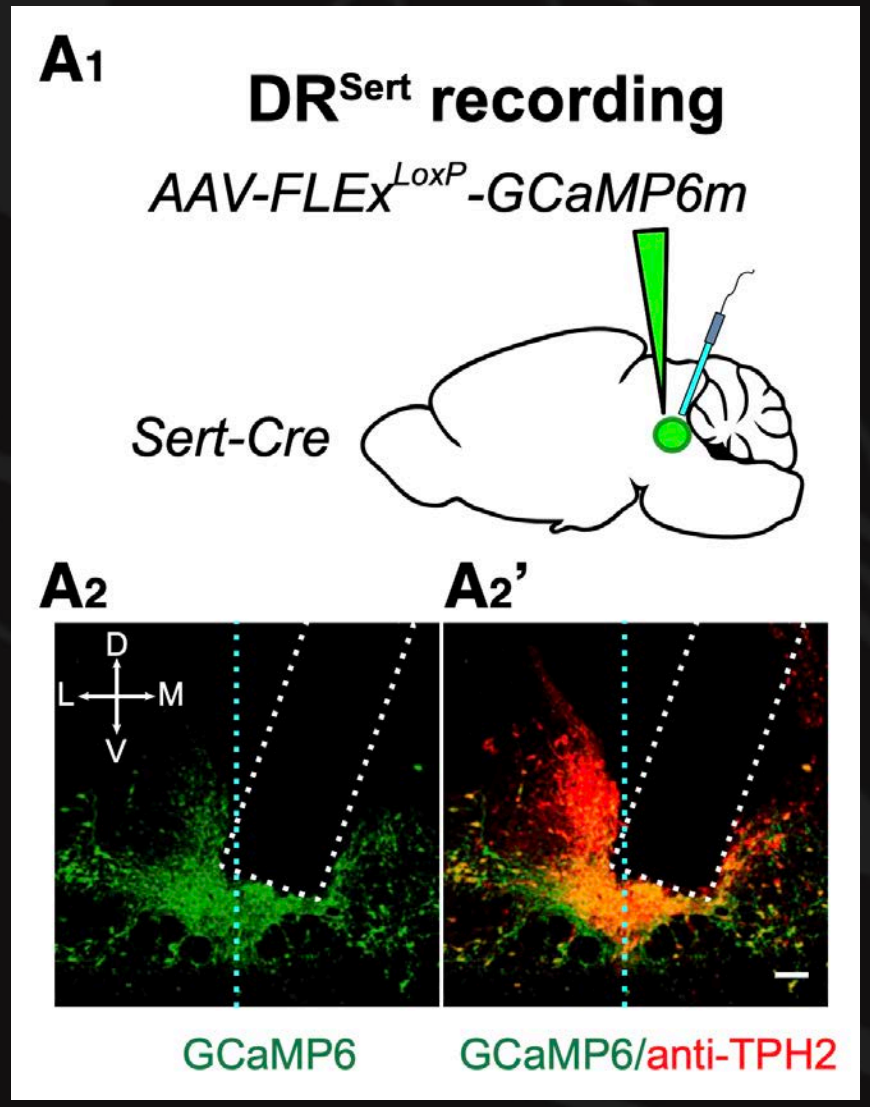


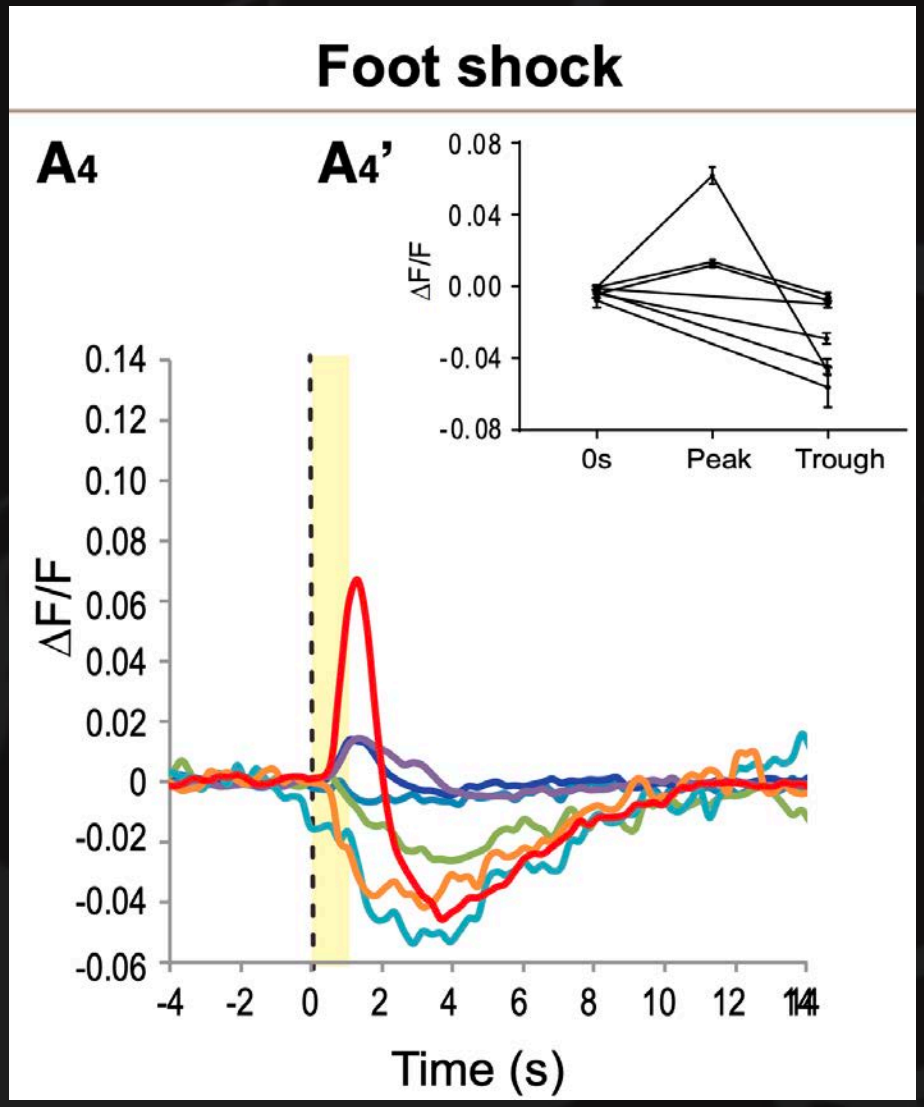
6 Animals, average of 15 trails per trace





Calcium release during AFC

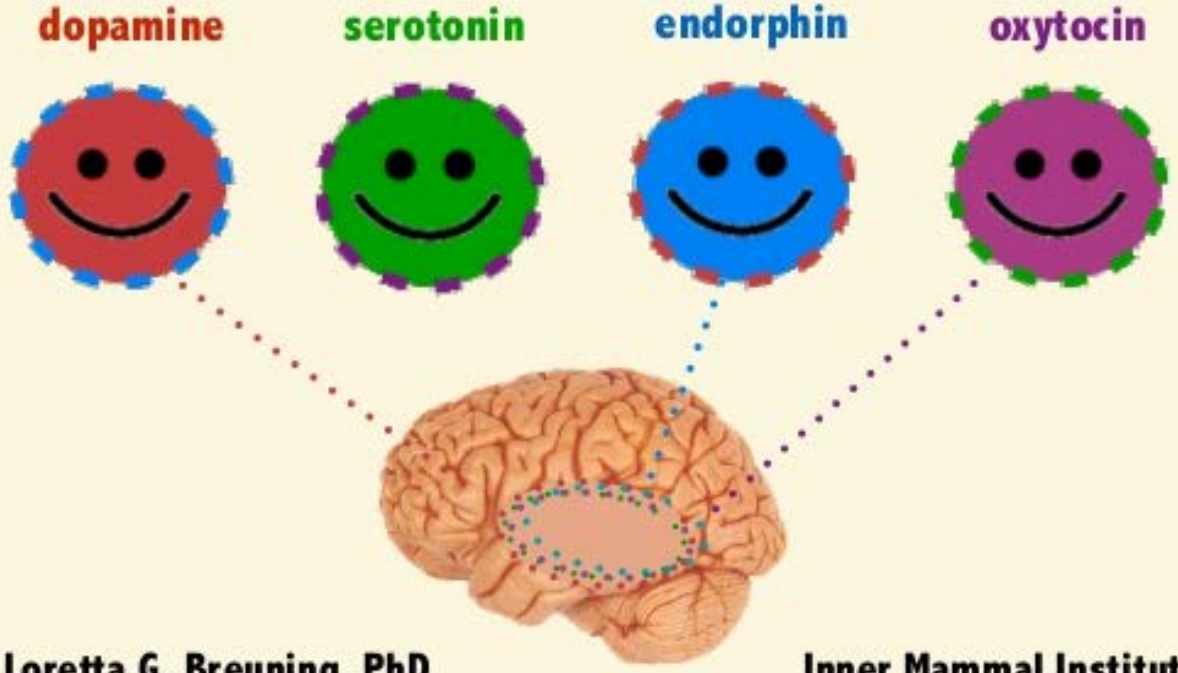




Ren et al., 2018, Cell 175, 472–487

Happy Brain Chemicals

Re-wire Yourself to Turn Them on in New Ways



Loretta G. Breuning, PhD

Inner Mammal Institute

Future directions

- Further validate/optimize sLight screening platform for 2A receptor targeting non-hallucinogenic antidepressant development

- Use sLight to study how spatial and temporal release of 5-HT is related to different types of behavior, e.g fear vs. reward in different brain regions

Acknowledgement

Tian Lab

Lin Tian Ph.D
Tommaso Patriarchi Ph.D.
Elizabeth Unger Ph.D.
Junqing Sun Ph.D.
Ruqiang Liang Ph.D.
Grace Mizuno Ph.D.
Alessio Andreoni Ph.D.
Akash Pal Ph.D
Emily Wright
Carolyn Davis
Karan Mahe

Pharmacology Team

David E. Olson Ph.D. (UC Davis)
Calvin Ly
Lee Dunlap
Max Vargas

Sensor Engineering

Jonathan Marvin Ph.D. (Janelia)
Loren Looger Ph.D. (Janelia)
Henry Lester Ph.D. (CalTech)

Follow @LinTianLab

NIH DIRECTOR'S NEW INNOVATOR AWARD

HFSP

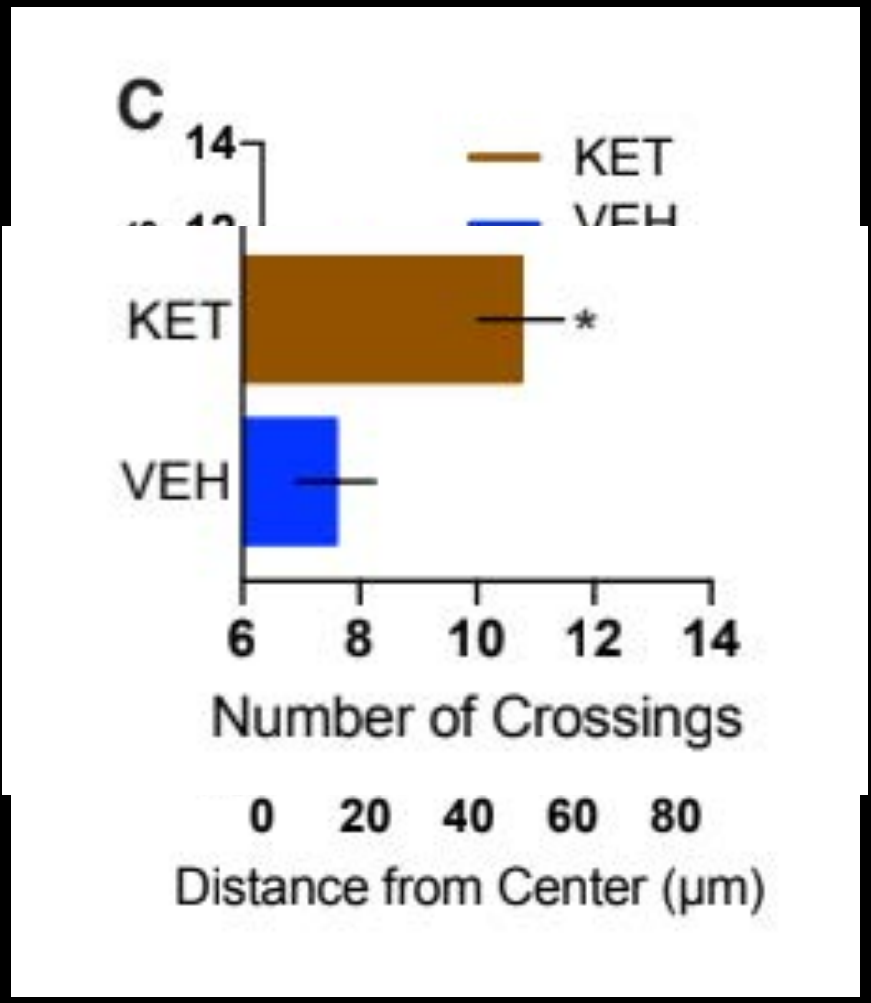
NIGMS

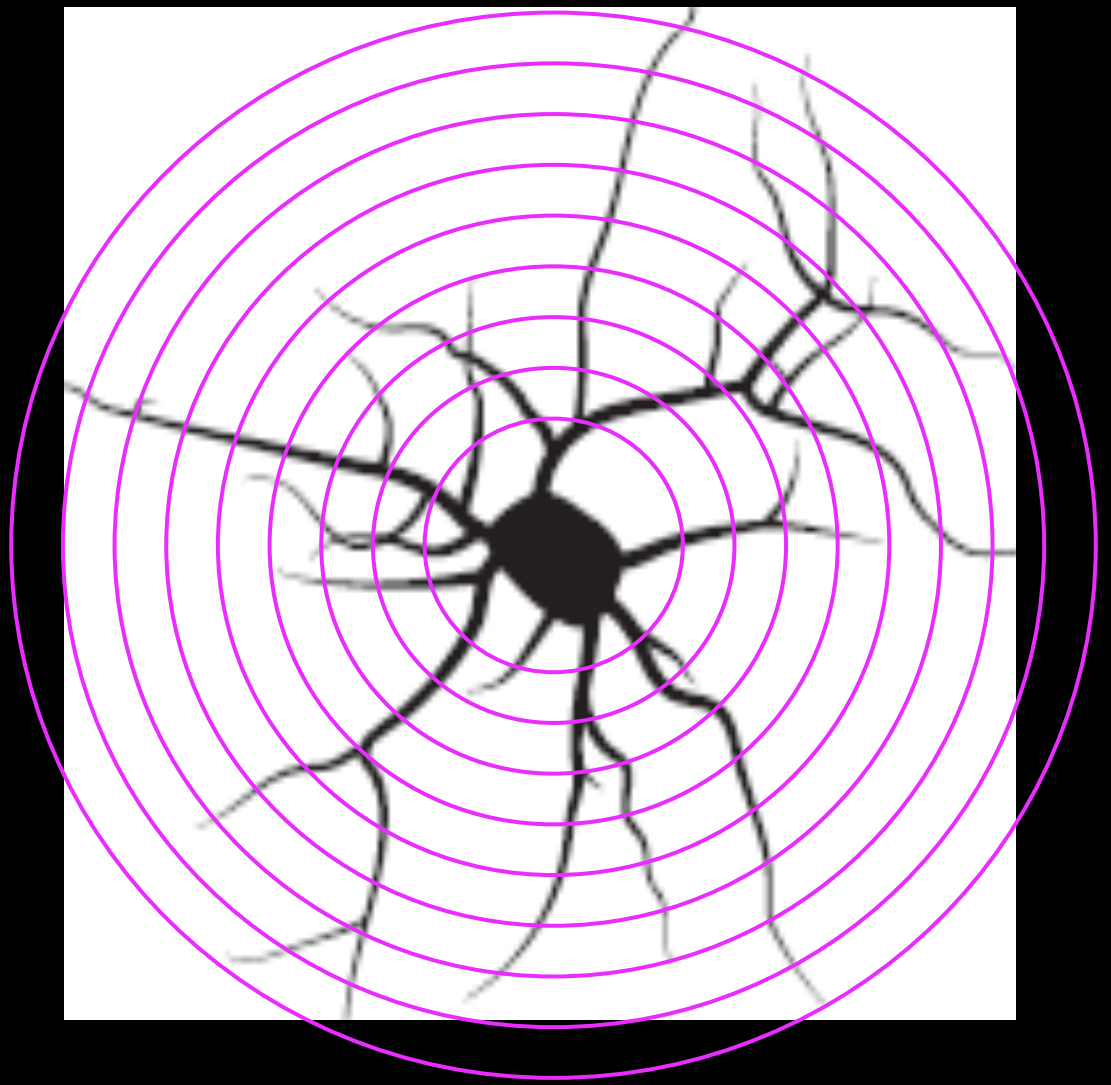
RITA ALLEN FOUNDATION

The BRAIN Initiative

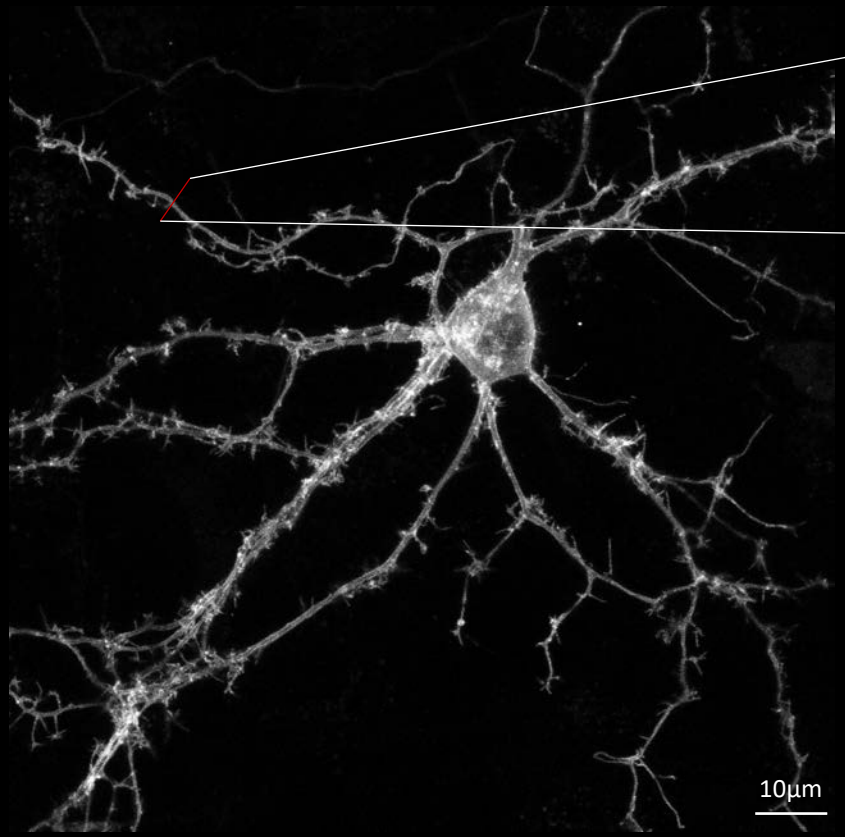
We are recruiting postdocs:
using and engineering new biosensors

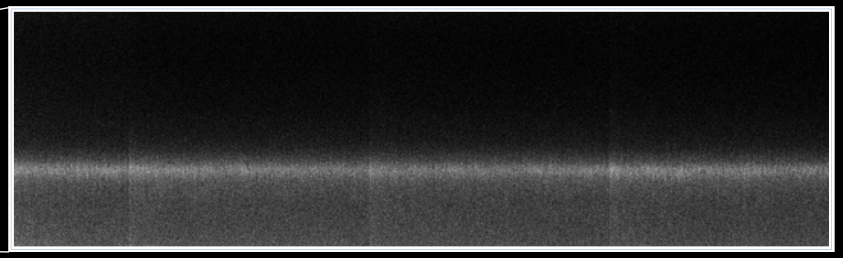
Sholl analysis- promote neuronal growth





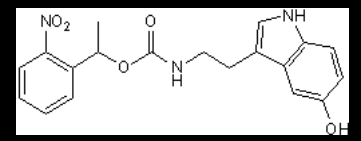
In vitro cultured neurons -kinetics



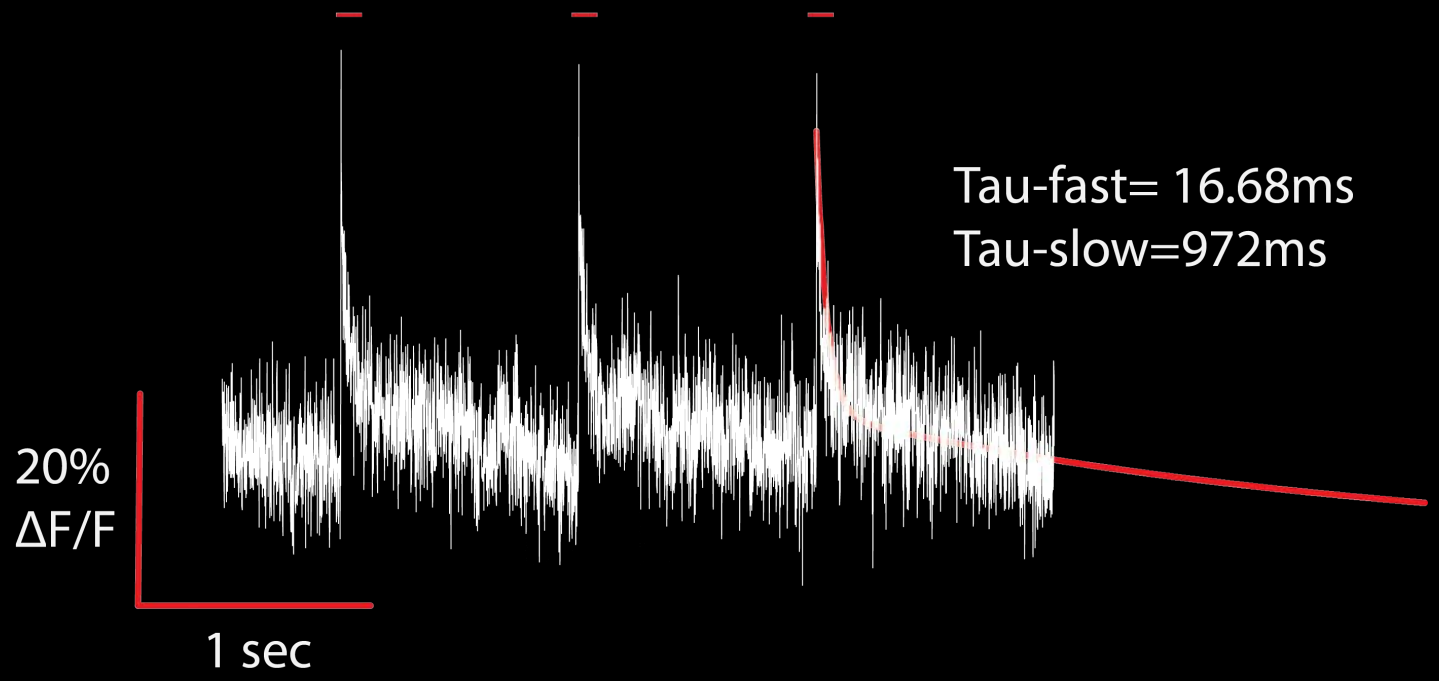


405nm

NPEC-5HT 10uM
bath application



5ms uncaging



Tau-fast= 16.68ms
Tau-slow=972ms