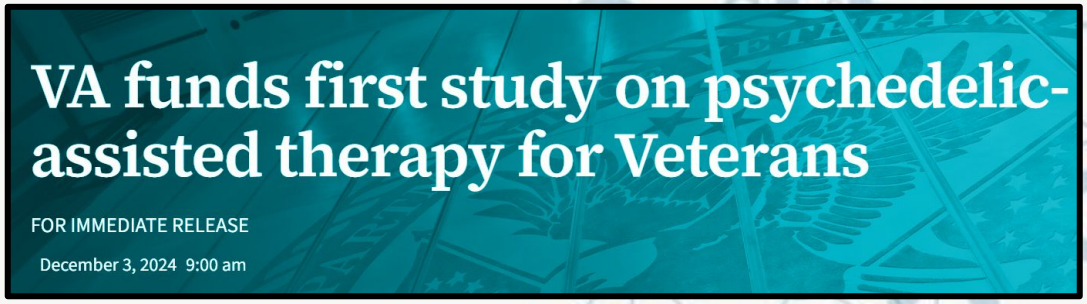
Exploring Psychedelics, Stress, and the Brain

An analog of psychedelics restores functional neural circuits disrupted by unpredictable stress - Ju Lu, Yi Zuo, et. al

Some Social Commentary

‘Current federal drug laws make psychedelics difficult to study and illegal to prescribe. Ocasio-Cortez spoke to that issue at the press conference with Crenshaw. “Right now our hands are tied in terms of getting the science we need,” ’-Texas Public Radio



“Veterans Affairs ... announced a $1.5 million grant to study MDMA-assisted therapy for post-traumatic stress disorder and alcohol use disorder among veterans, the first department-funded research on psychedelic benefits in more than five decades.” -Military Times

Potential in Alzheimer's Disease?

"A significant treatment gap exists for late-stage AD, characterized by extensive neuronal damage and severe cognitive decline" (make font bigger)

"Terminal lucidity suggests that cognitive decline might be reversible, at least momentarily"

"It seems more plausible that these lucidity episodes arise from the spontaneous formation of neural bypasses."

"(LSD) and psilocybin have [the] ability to induce changes in neuroplasticity-the brain's capacity to form and reorganize synaptic connections ... by promoting the growth of dendritic spines and synapses"

- A perspective on Alzheimer's disease: exploring the potential of terminal/paradoxical lucidity and psychedelics

Psychedelics: untapped potential

- Psychedelics, serotonergic hallucinogens, are substances which induce an altered perception and altered cognitive processes
- Long stigmatized due to their hallucinogenic and potentially addictive traits are being studied in modern research for its possible therapeutic applications.
- Multiple different compounds including LSD, DMT, and Psilocybin are showing promising contributions to treating mental health conditions such as depression, anxiety, PTSD, and addiction.

- These hallucinogens show the ability to influence brain connections and promote neuroplasticity. These effects can be used to improve mental health conditions
- Clinical use is limited as the concern for altered state of mind and addictive properties still linger
- The solution to this involves non hallucinogenic analogs of these common psychedelics which exhibit similar neuroplasticity effects without the psychoactive component

Stress and how it impacts the brain

- Chronic subduction to stressors over time lead to an allostatic overload by the body when prolonged. Stress is associated with multiple mental health disorders and impaired cognitive ability.
- The neural effects of stress reach physical levels. Alterations include dendritic atrophy and overall loss of spines in the hippocampus. The hippocampus is associated with memory formation, spatial navigation,regulation of emotions, learning, and overall cognition
- Dendritic atrophy leads so impairment of processing of sensory information and cognitive flexibility

Experimental Setups

Experimental Mice & Unpredictable Mild Stress

Supplementary Table 1: Protocol of 7-day UMS.

Day	Light Cycle		Dark Cycle
	First half	Second half	
1	Restraint stress 30 min	Restraint stress 30 min	Home cage space reduction
2	Exposure to a new room 30 min + orbital shaker 30 min	Exposure to loud sudden noise 5 times + tail suspension 6 min	Wet bedding
3	Exposure to new mice		Light exposure
4	Social isolation		Tilted cage
5	Tilted cage	Island isolation	No bedding
6	No bedding	No bedding + random air puff 5-10 times	Foreign objects
7	Foreign objects	Food deprivation	Food deprivation + continuous exposure to loud music

- C57BL/6J- Main mice used unless noted- used in
  - Standard studied mouse breed
- *Thyl*-GFP-M- used in dendritic spine imaging (figure 2)
  - Thy1 = promoter (wide expression in brain)
- Housed together with littermates, with 12 h light/day cycle
- Aged 1-2 months before experiments, randomly assigned to different treatments

Drug Preparation & Administration

- Fluoxetine Hydrochloride (TBG)
- Administered intraperitoneally
- Dosage
  - 10 mg/kg
  - USP-grade saline (0.9%) = vehicle
  - Based on previous pharmacokinetic studies indicating it as the necessary concentration that activate 5-HT2 receptors

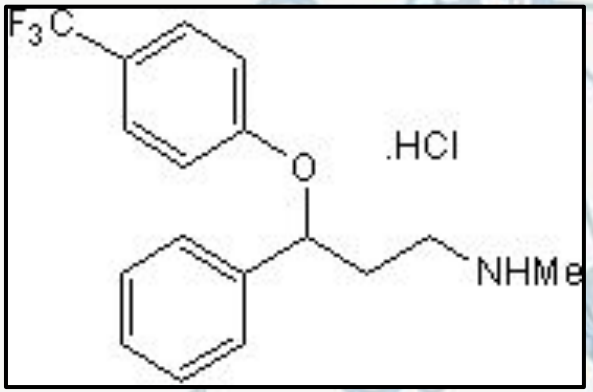
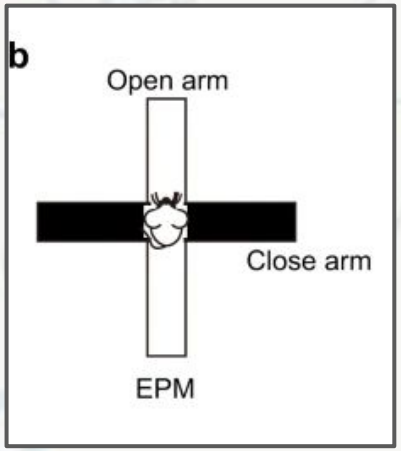


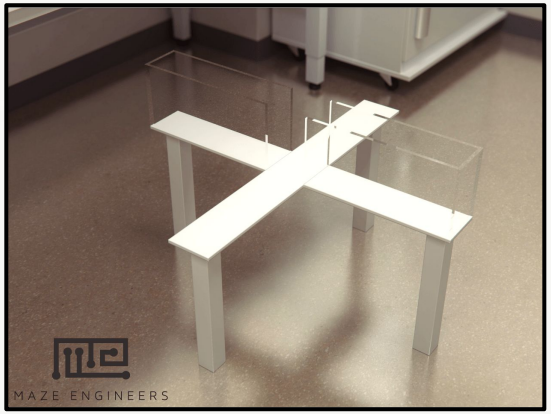
Fig. 1a
Timeline between groups before behavioral tests

Behavioral Assays

Elevated Plus Maze



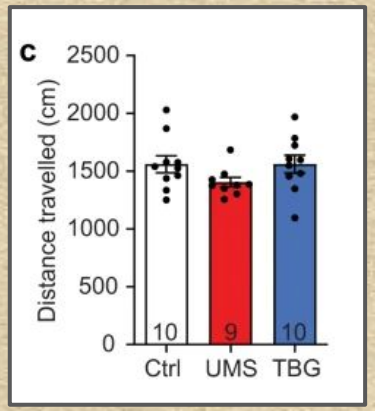
From Fig 1



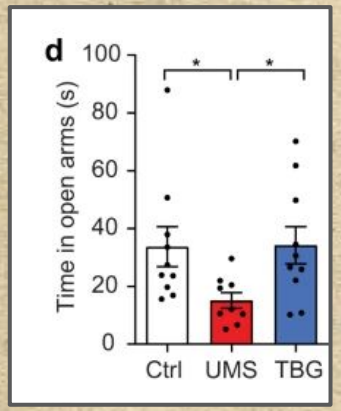
- Tests:
  - Natural aversion to open heights vs their spontaneous curiosity driven exploration
- **General Protocol:**
- 4 paths
  - 2 open
  - 2 closed
- Mice allowed to explore for 5 minutes
- Video recording of mice to track behavior
- DeepLab Cut software tracked points of interest on mice
  - Nose, head, neck, body, tail base
- Custom written programs quantified
  - Distance traveled,
  - Time in open, closed arms

Behavioral Assays Results

Elevated Plus Maze

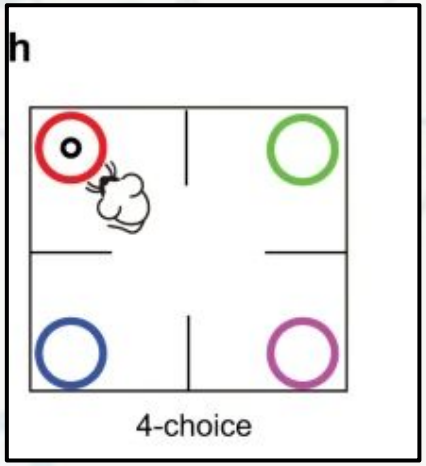


No difference in distance traveled
(fig. 1c)



UMS Mice Spend less time in open arms than controls
TBG restored to controls
(fig. 1d)

Four Odor Discrimination & Reversal



From Fig 1

Physical Layout:

- Four chamber arena and stimuli in ramekins
- digging medium on with odor stimuli(5)
- Food reward stimuli
  - Honey Nut Cheerios

Test day:

- Initial Discrimination Phase
  - Became familiar with 4 odors
  - Association between odor & reward
- In between trials
  - Ramekins reset and replaced pseudorandomly
- Reversal Session
  - New odor/reward combination presented

Mouse handling:

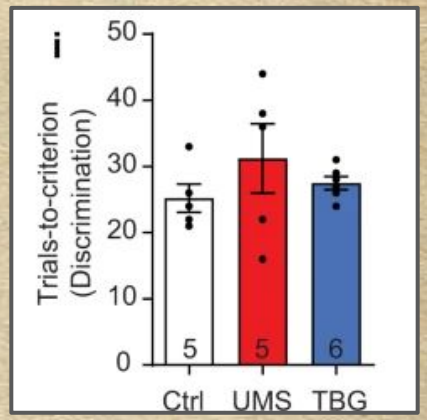
- Diet prior to trials
  - Reduced weight to 80-85% of normal
  - 2 day pre-training
    - Day 1(acclimation): familiarized w/setup
    - Day 2(shaping): presented digging medium w/stimuli

Tests:

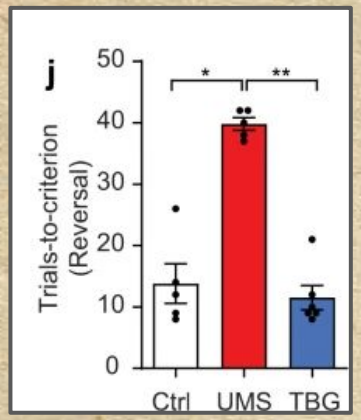
- Odor distinguishment
- Reward driven association learning
- Plasticity of previous associations(reversal)

Behavioral Assays Results(cont.)

Four Choice Odor Discrimination & Reversal

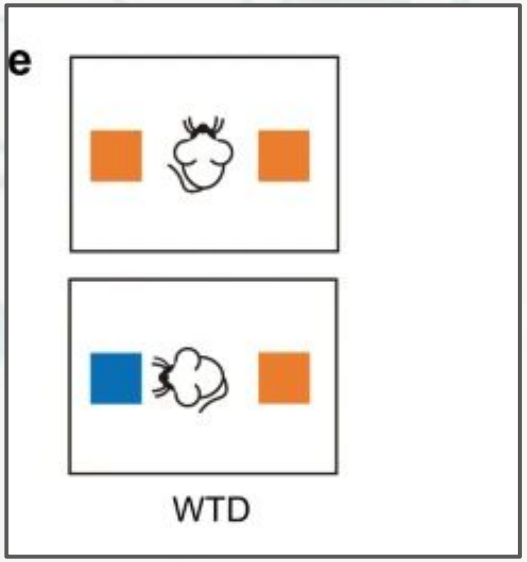


How many trails each took to meet criterion (8/10 correct choices) (fig. 1i)



Normalized cognitive flexibility (fig. 1j)

Whisker dependent texture discrimination (WTD)



From Fig 1

Tests:

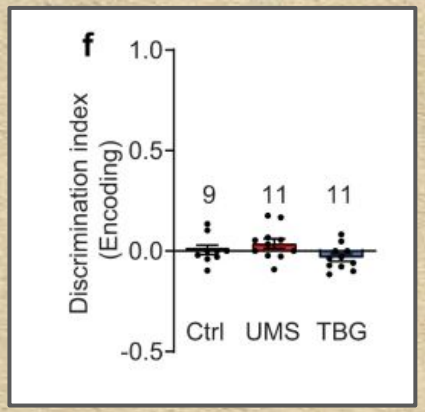
- Natural preference for novelty (neophilia)

Protocol:

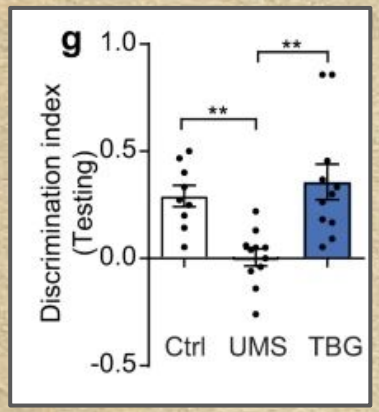
- Habituation Period
  - 10 min. per day for 2 days prior to testing
- Testing:
  - Habituation(3 min)
  - Encoding(5 min)
    - Presented 2 columns with same texture (top of 1e)
  - Rest(2 min)
  - Test (3 min)
    - 1 texture as before & 1 novel one (bottom of 1e)

Behavioral Assays Results(cont.)

Whisker-dependent Texture Discrimination



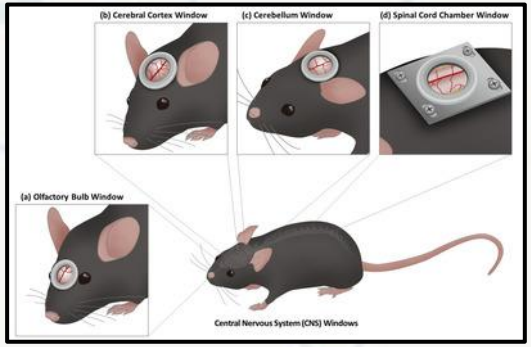
How well mice groups discriminated textures (fig. 1f)



TBG restored novel texture preference (fig. 1g)

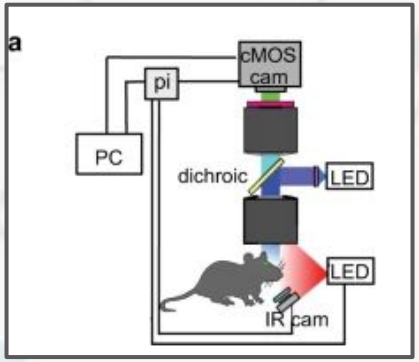
Imaging Assays

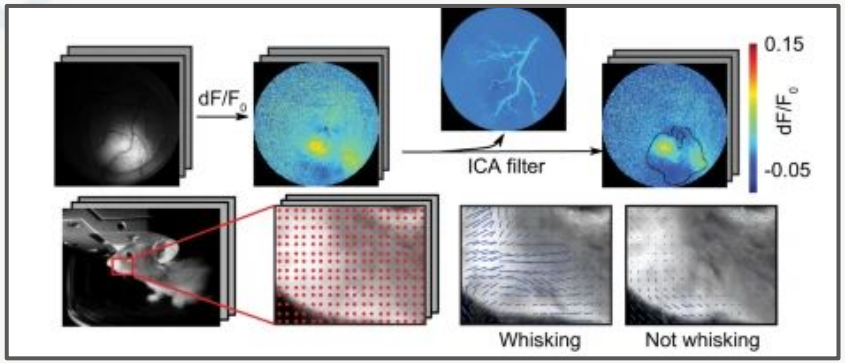
Experimental Setup: Cranial Window



- Post-natal day 30
  - Cranial window
- Trephine micro drill removed circular pieces of skull
- Covered with round glass coverslip
  - Secured with stainless-steel head plate
- Treatment/Care
  - anesthetization before and maintenance
  - Anti-inflammatories
  - Pain killers
  - Antibiotics
- Allowed for further imaging techniques
  - 2 photon imaging (2P)
  - Mesoscope

In Vivo Wide Field Ca Imaging (Mesoscope)

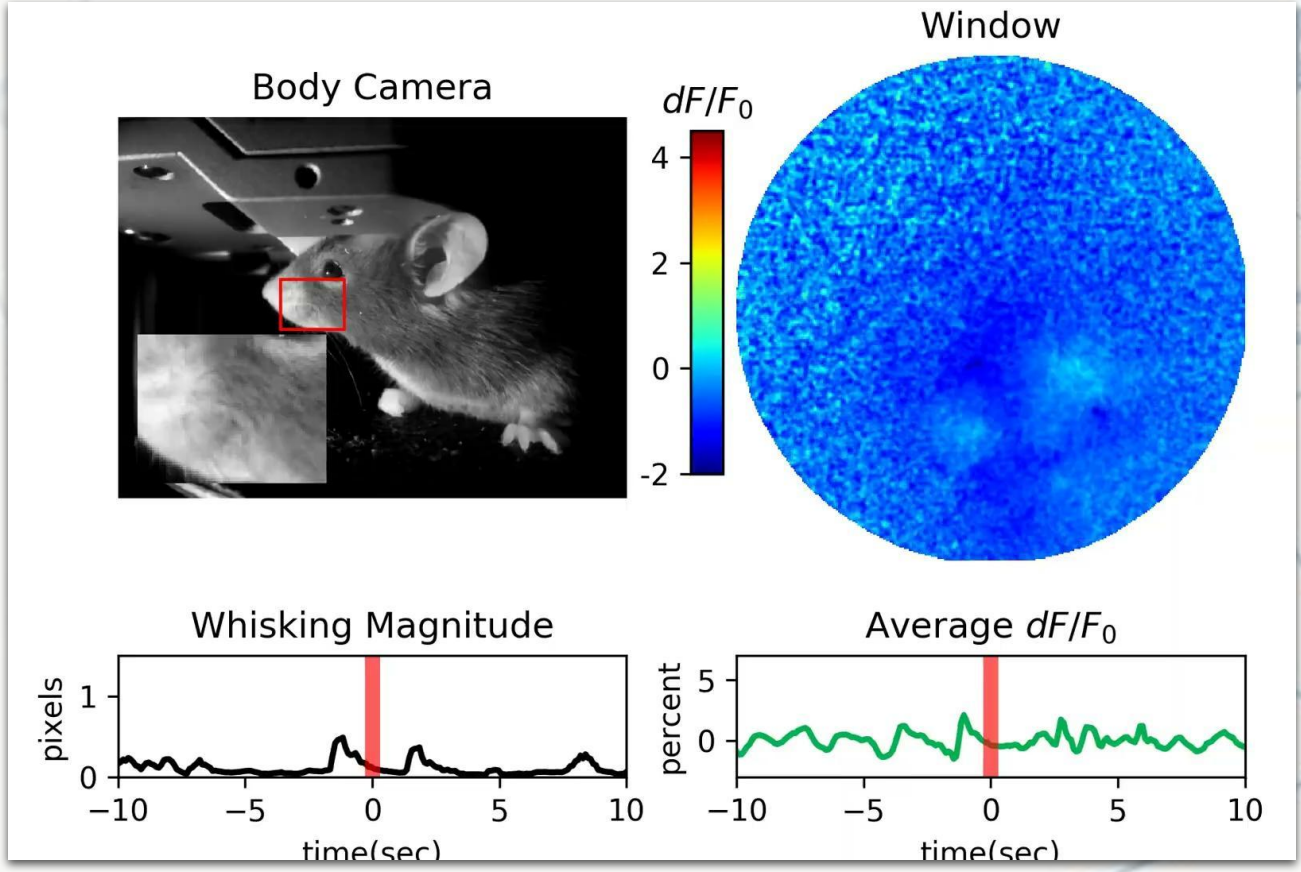




Awake mouse head-fixed on a rotating disk

- Injected with AAV
  - Carry GCaMP6f genes- Ca indicator
  - Expression driven by Synapsin promoter
    - Specifies neuronal expression
- Measured neuronal activities among brain regions during different behaviors
  - Eg. whisking
- Looked at Barrel cortex(S1BF)
  - Neurons associated w/whisker coordination
- Established correlation magnitudes of behavior (whisking) and neuronal excitation/activity through Ca imaging (fig 3b,3c)

Video of Wide Field Imaging



Imaging Assay Results

Wide-field Ca Imaging with Simultaneous Behavioral Monitoring

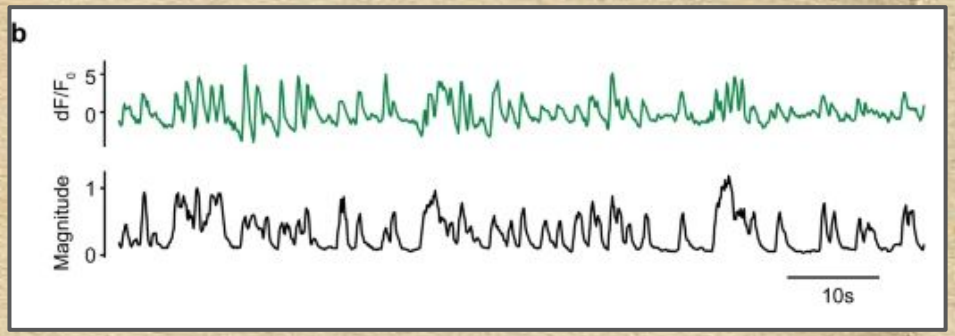


Fig. 3b correlates whole-field Ca imaging magnitudes in control mice to whisking behavior

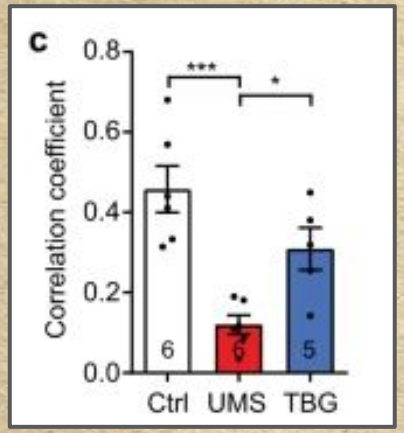
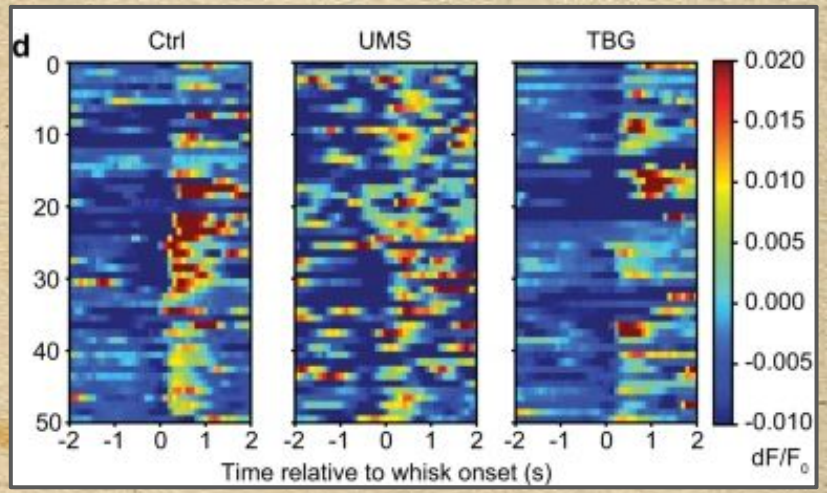


Fig. 3c correlates magnitudes of neuronal activity to whisking and includes other treatments

Imaging Assay Results

Wide-field Ca Imaging with Simultaneous Behavioral Monitoring cont.

Ca_{WF}= whole field Ca activity



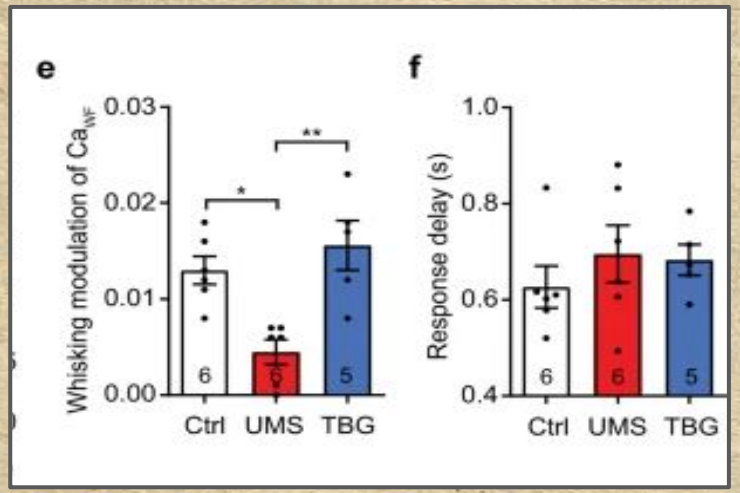


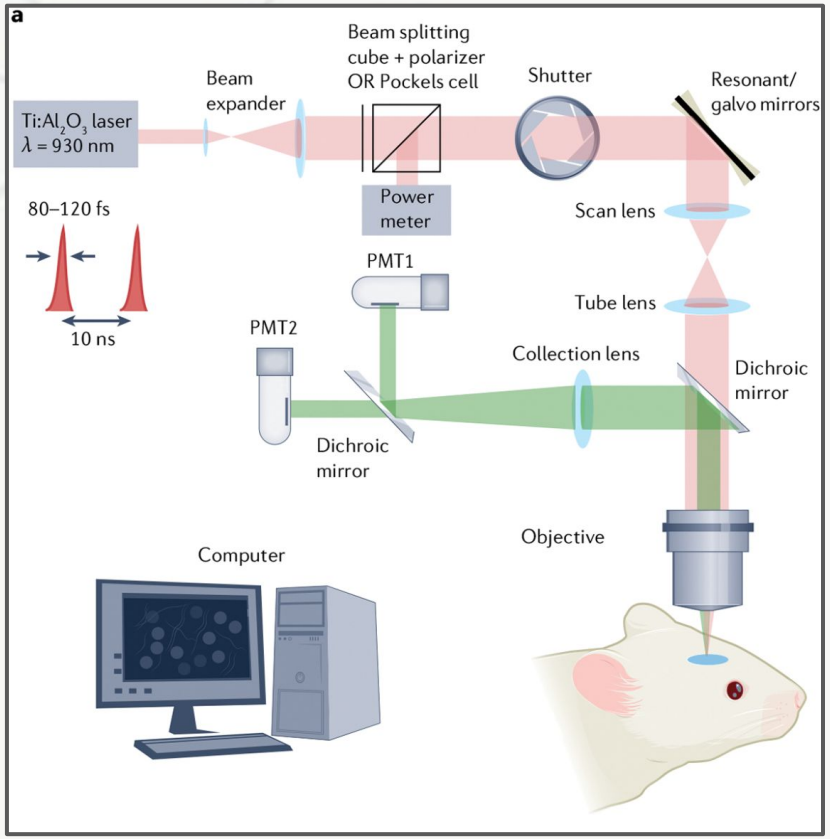
Fig. 3d , more activity in UMS before 0,

- Before 0 = baseline activity
- 0 = whisking/behavior
- After 0 = activity
- Y = cells

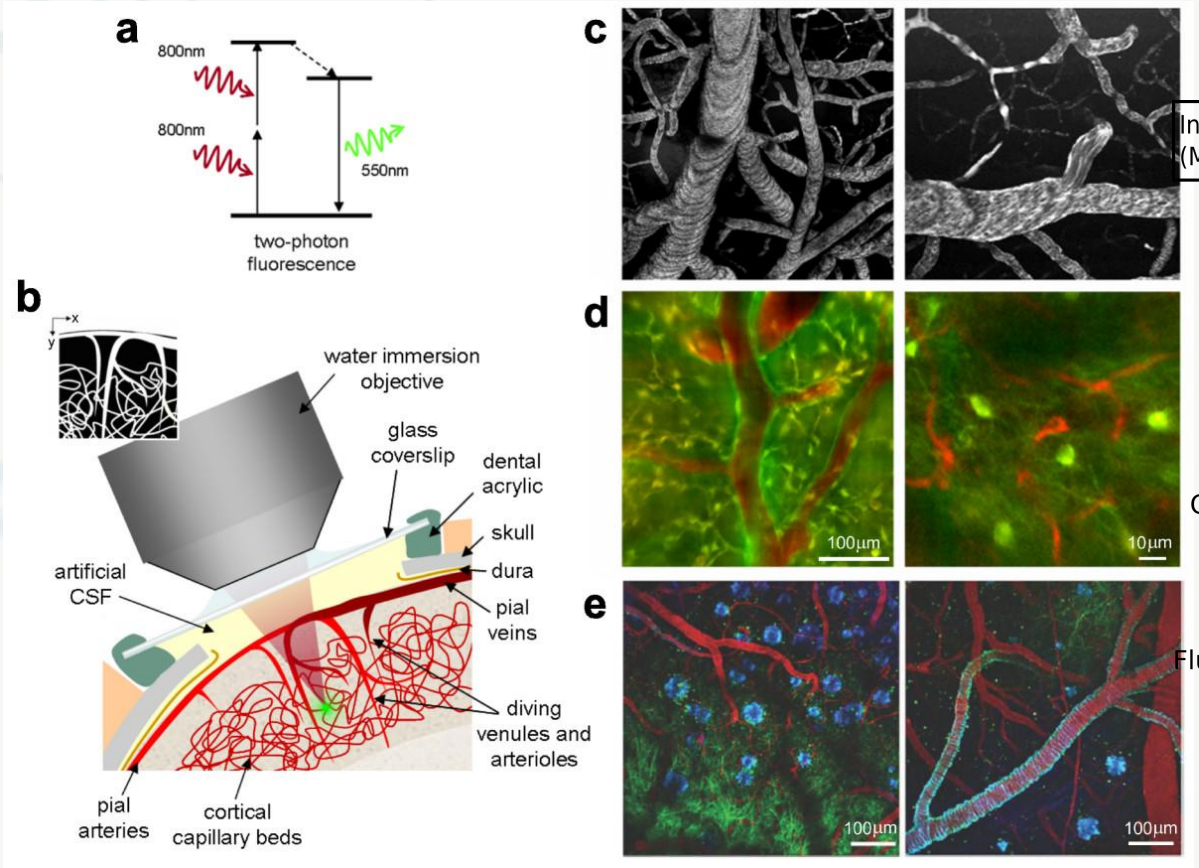
Fig. 3e
How well Ca_{WF} translated to whisking behavior

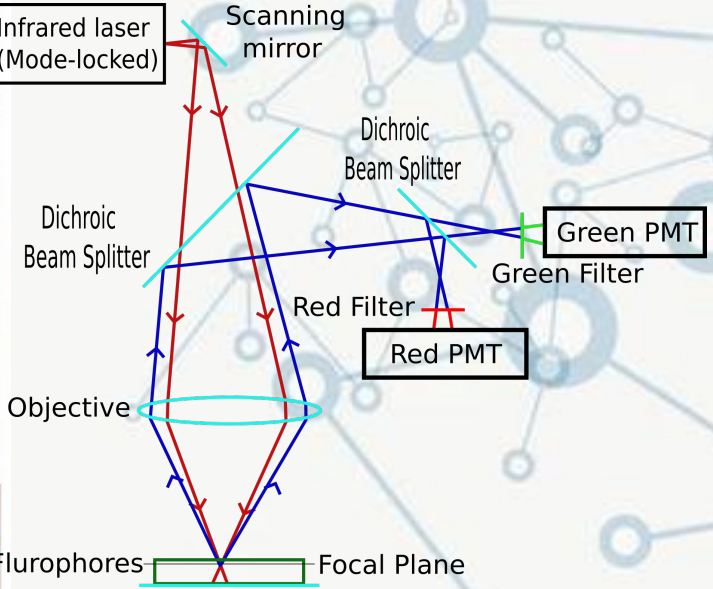
Fig. 3f
Delay between whisking & peak Ca_{WF}

2P (Photon) Microscopy

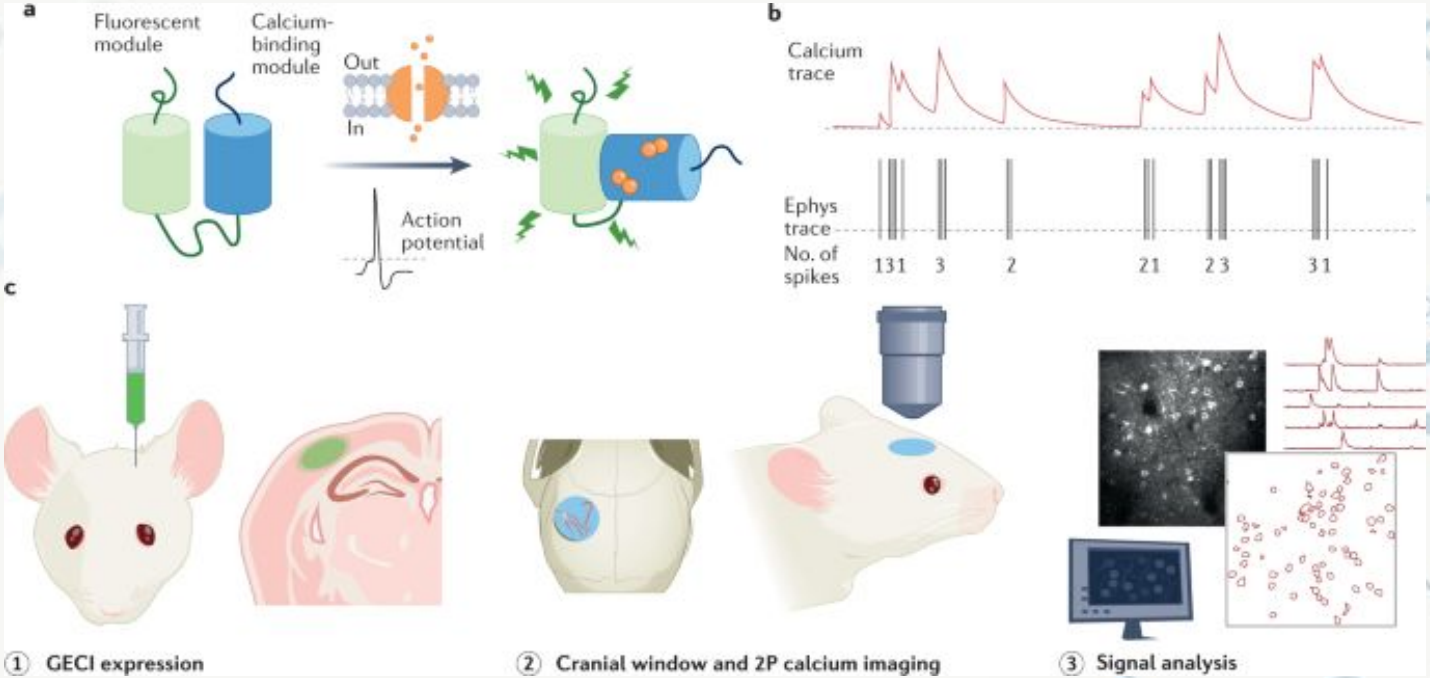


- How it works:
  - 2 infrared (low energy) photons work to excite fluorescent molecule
  - Short pulses (~100 femtoseconds or 10^-15) while scanning over tissue
  - Get real time resolved images of dendrites deep in the brain
- Why use it?
  - Less disruptive(don't have to dig around the brain)
  - Great for *in vivo*
    - Exciting fluorophore takes high energy
    - Especially to deep tissue (2 mm below surface)
      - 2P is less destructive


a
800nm
800nm
550nm
two-photon
fluorescence
b
water immersion
objective
glass
coverslip
dental
acrylic
skull
dura
pial
veins
artificial
CSF
diving
venules and
arterioles
pial
arteries
cortical
capillary beds
c
d
e
100μm
10μm
100μm
100μm


Infrared laser
(Mode-locked)
Scanning
mirror
Dichroic
Beam Splitter
Dichroic
Beam Splitter
Green PMT
Green Filter
Red Filter
Red PMT
Objective
Flurophores
Focal Plane

In Vivo 2P (photon) Calcium Imaging

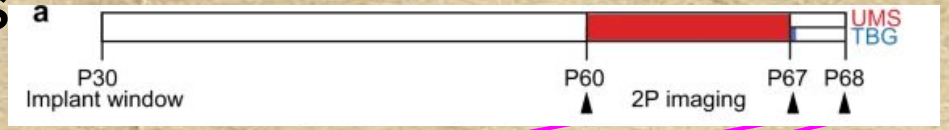


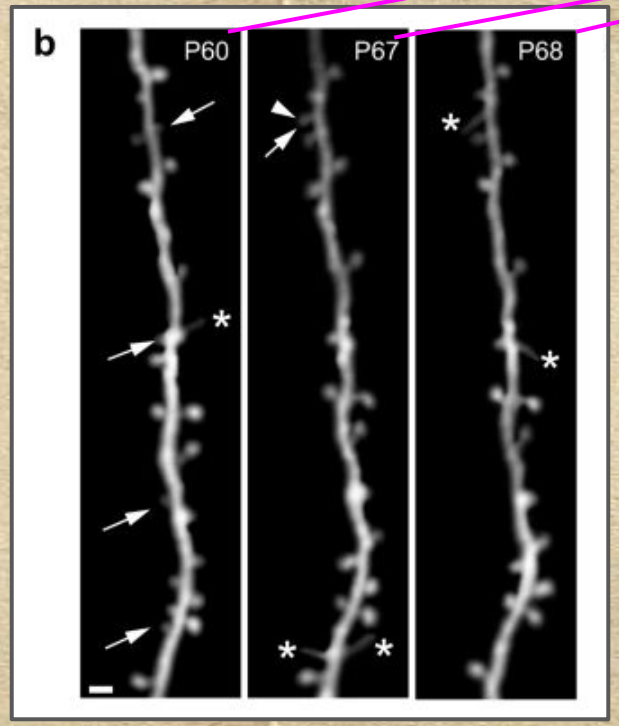
How it works:

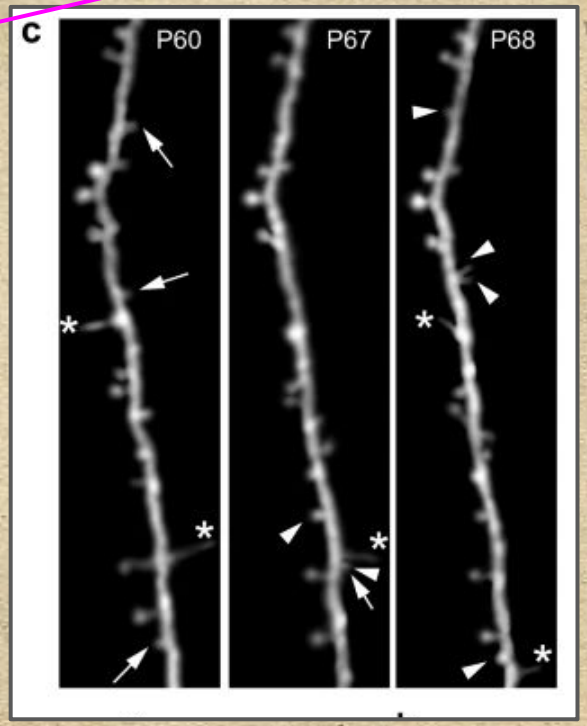
- Genetically Encoded Calcium indicators are expressed in the mouse brain which readily binds to Calcium molecules
- A laser shines 2 photons of low energy light into the injected tissue, when they meet the injected dye/protein it excites.
- When neurons fire action potentials, Calcium rushes into the neuron via Voltage Gated Calcium channels.
- Administrators measure the changes in calcium levels with fluorescence detectors.
- A difference in Calcium fluorescence indicates changes in neuronal activity. Calcium is used as a measure for activation.

Imaging Assay Results

Thy1-GFP-M *in vivo* 2 Photon (2P) Microscopy (fig 2a-c)







Both show different sets of S1BF spines

Fig. 2b

- P60 = before UMS
- P67 = after UMS
- P68 = 1 day recovery from UMS

Fig. 2c

- P60 = before UMS
- P67 = after UMS but w/TBG treatment
- P68 = 1 day rest

Imaging Assay Results

Thy1-GFP-M *in vivo* 2 Photon (2P) Microscopy cont.

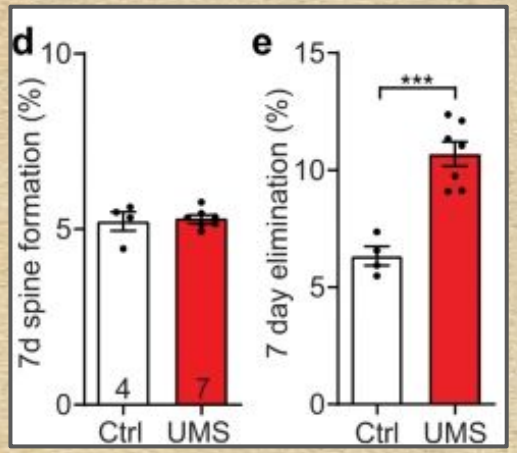


Fig 2d. UMS vs ctrl spine formation over 7 day UMS period

Fig 2e. UMS vs ctrl spine elimination over 7 day UMS period

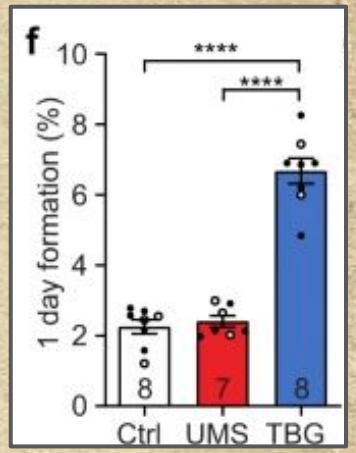


Fig 2f. Spine formation post 7d UMS period

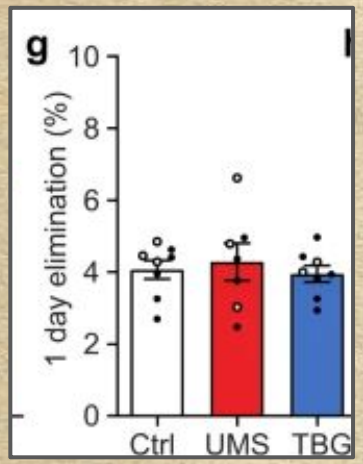


Fig 2g. Spine elimination post 7d UMS period

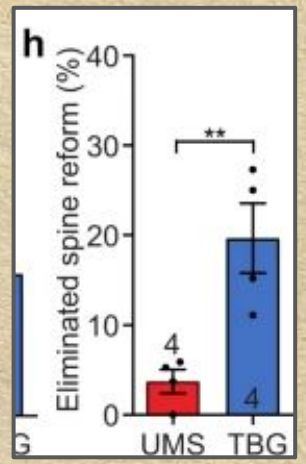
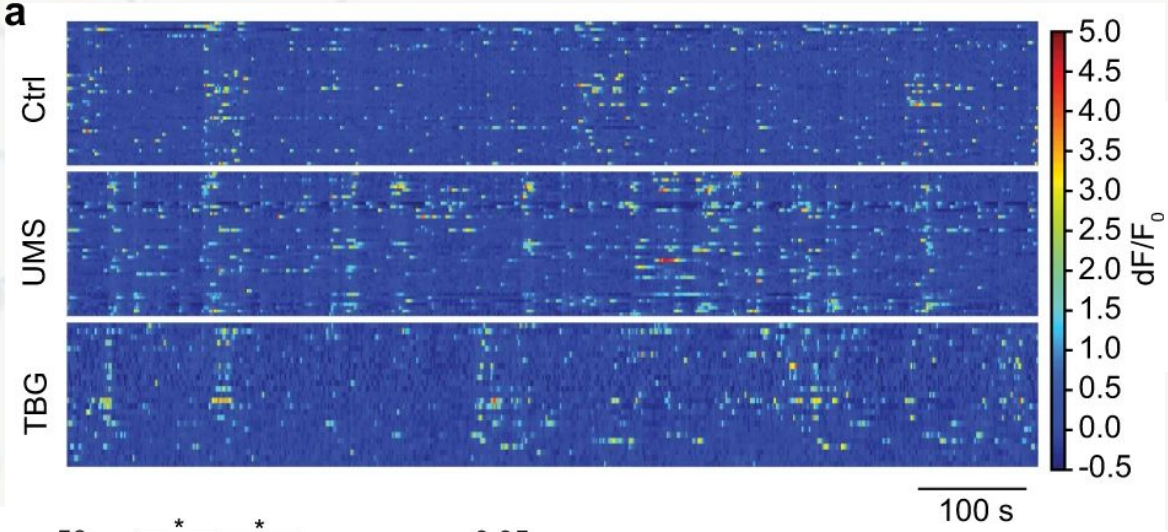
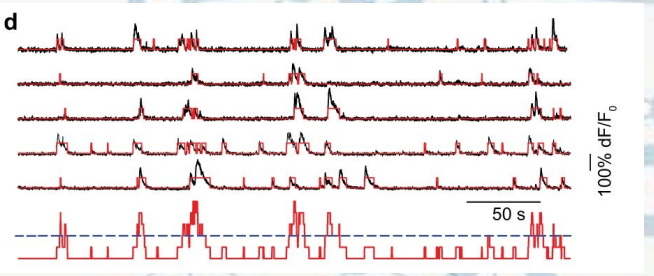
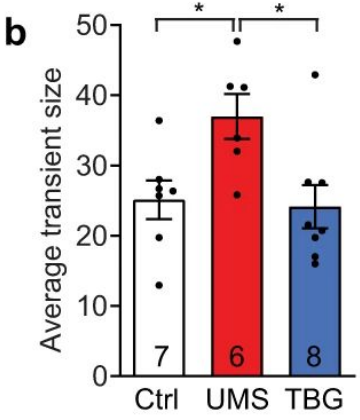


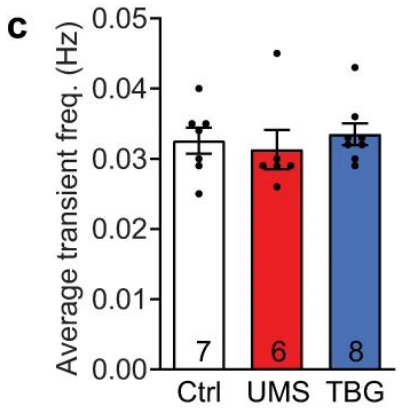
Fig 2h. Spine Recovery of previously eliminated post TBG treatment

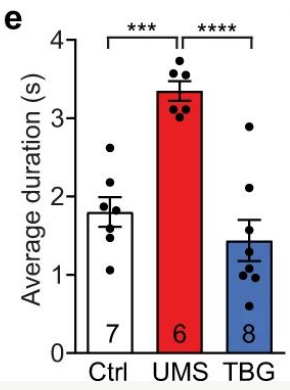
Figure 4

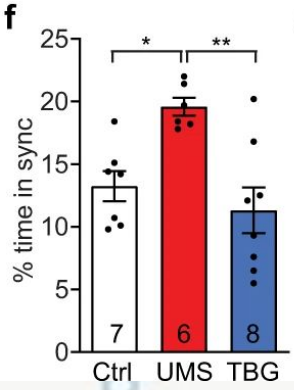

a
Ctrl
UMS
TBG
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
dF/F0
100 s


d
50 s
100% dF/F0


b
Average transient size
50
40
30
20
10
0
*
*
7
6
8
Ctrl
UMS
TBG


c
Average transient freq. (Hz)
0.05
0.04
0.03
0.02
0.01
0.00
7
6
8
Ctrl
UMS
TBG


e
Average duration (s)
4
3
2
1
0
***
****
7
6
8
Ctrl
UMS
TBG


f
% time in sync
25
20
15
10
5
0
*
**
7
6
8
Ctrl
UMS
TBG

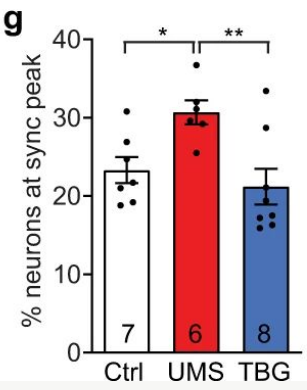
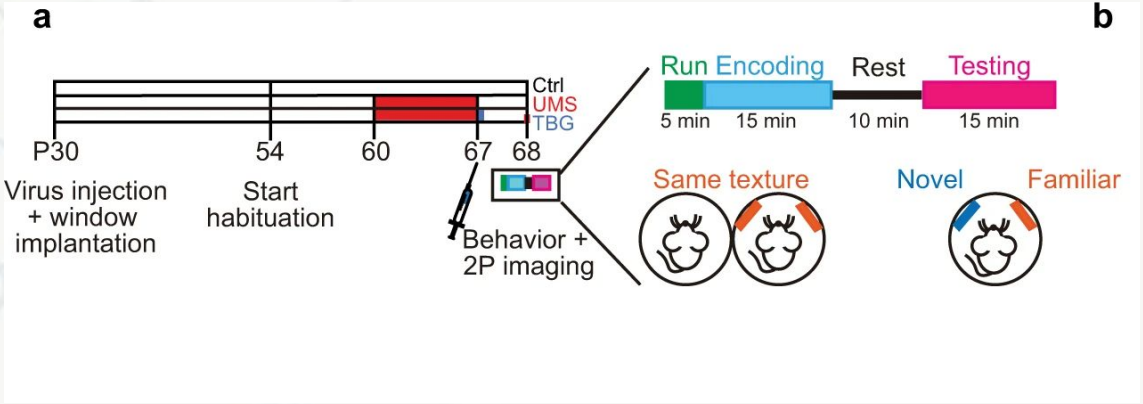
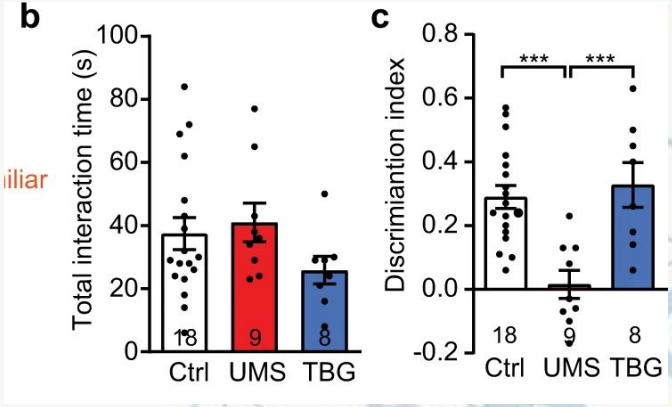

g
% neurons at sync peak
40
30
20
10
0
*
**
7
6
8
Ctrl
UMS
TBG

Figure 5


a
b
Ctrl
UMS
TBG
P30
54
60
67
68
Virus injection
+ window
implantation
Start
habituation
Behavior +
2P imaging
Run Encoding
Rest
Testing
5 min
15 min
10 min
15 min
Same texture
Novel
Familiar


b
Total interaction time (s)
100
80
60
40
20
0
18
9
8
Ctrl
UMS
TBG
c
Discrimiantion index
0.8
0.6
0.4
0.2
0.0
-0.2
***
***
18
9
8
Ctrl
UMS
TBG

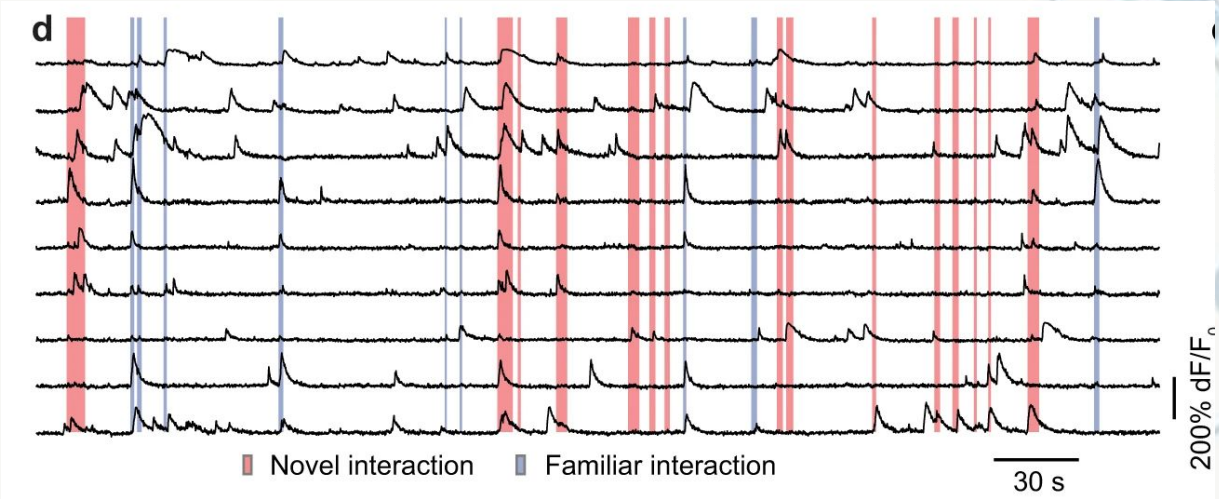
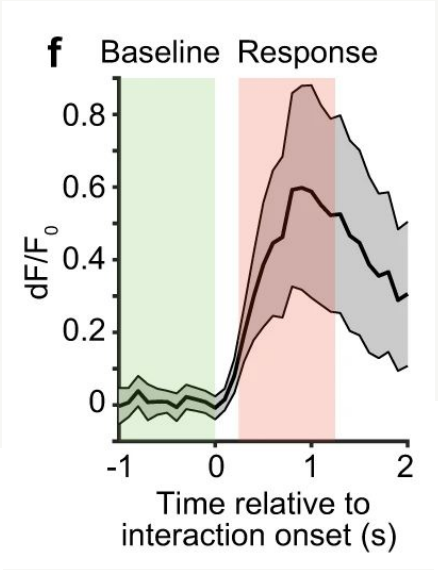
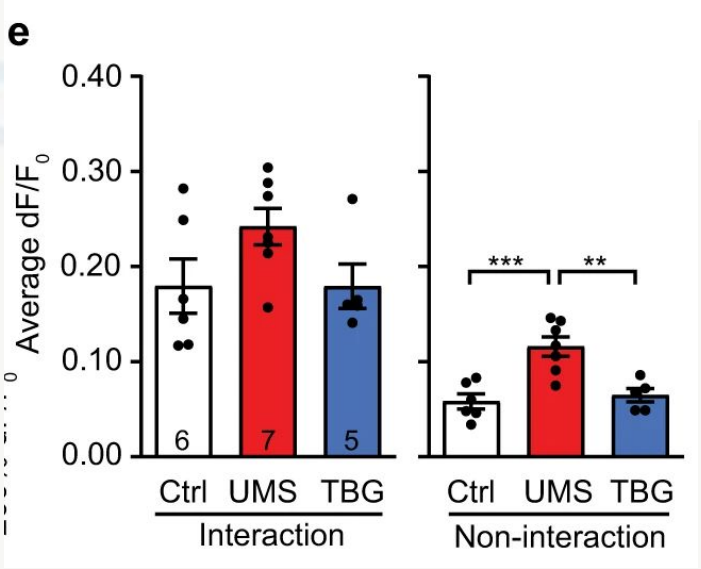

d
Novel interaction
Familiar interaction
30 s
200% dF/F

Figure 5 Contd’





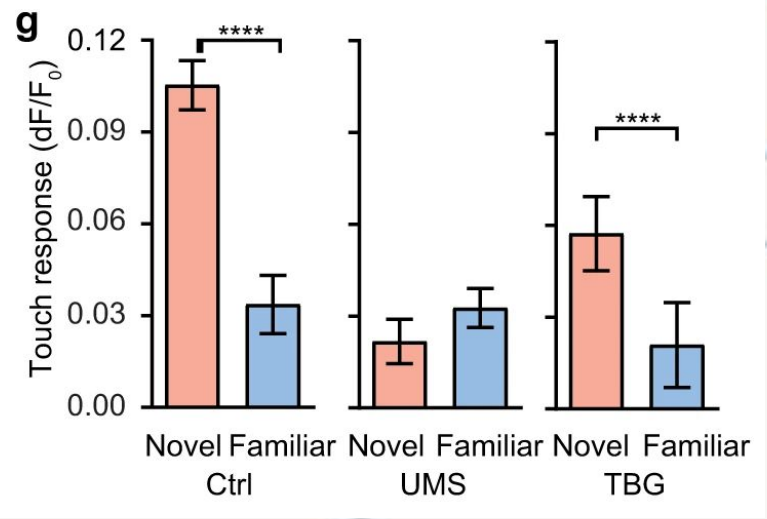
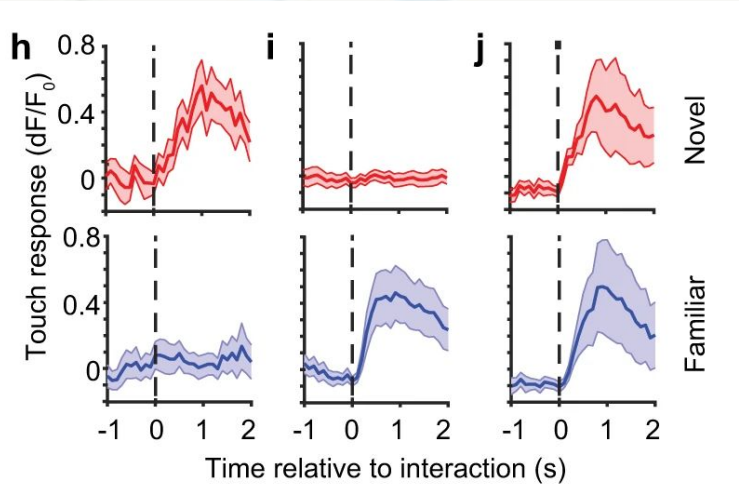
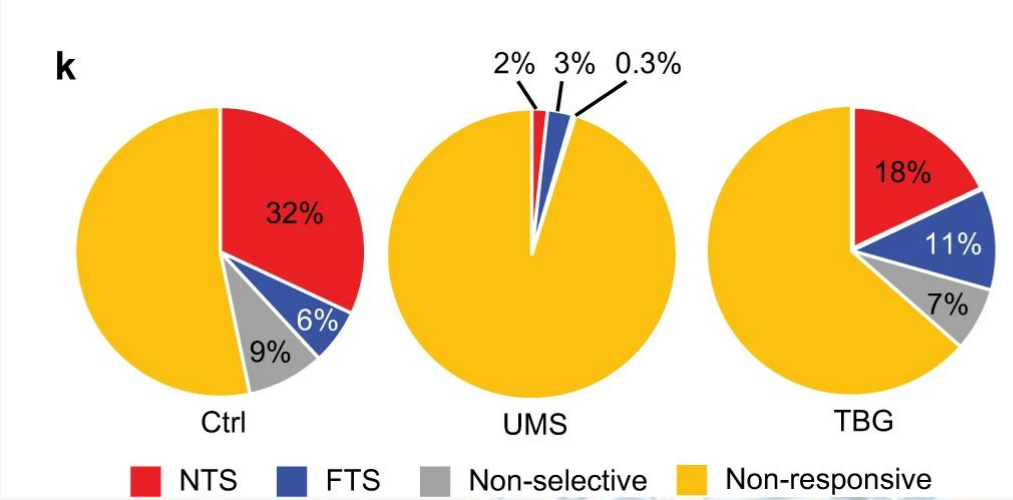
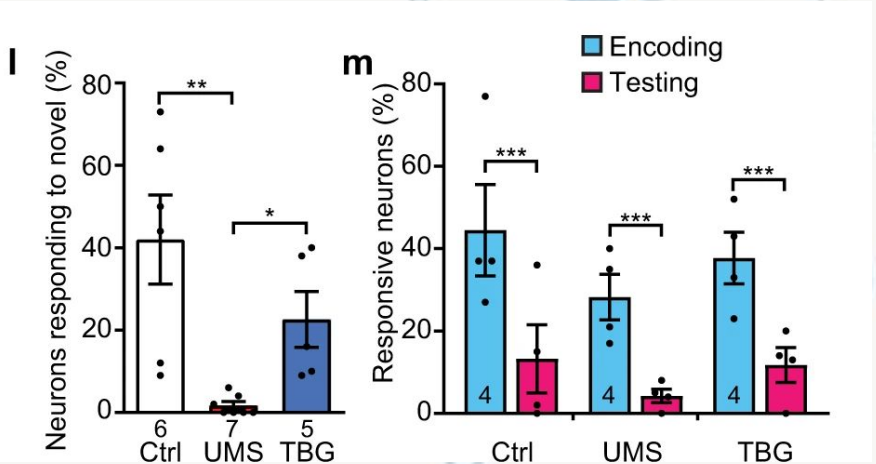


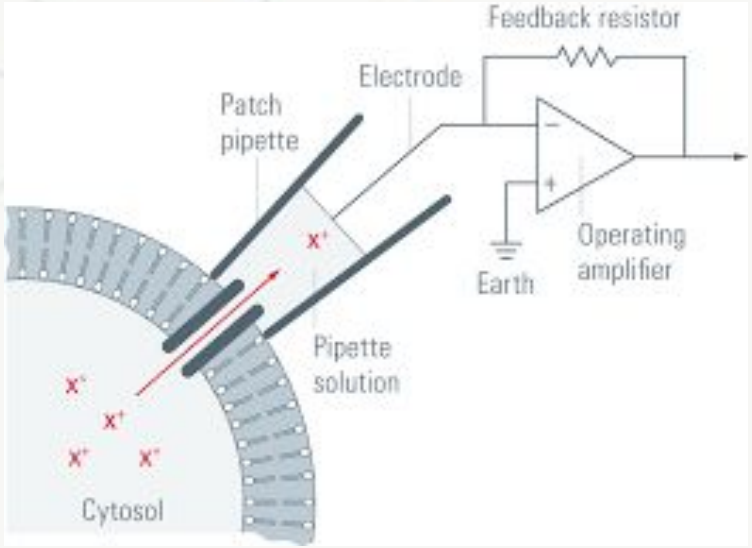
Figure 5 Contd







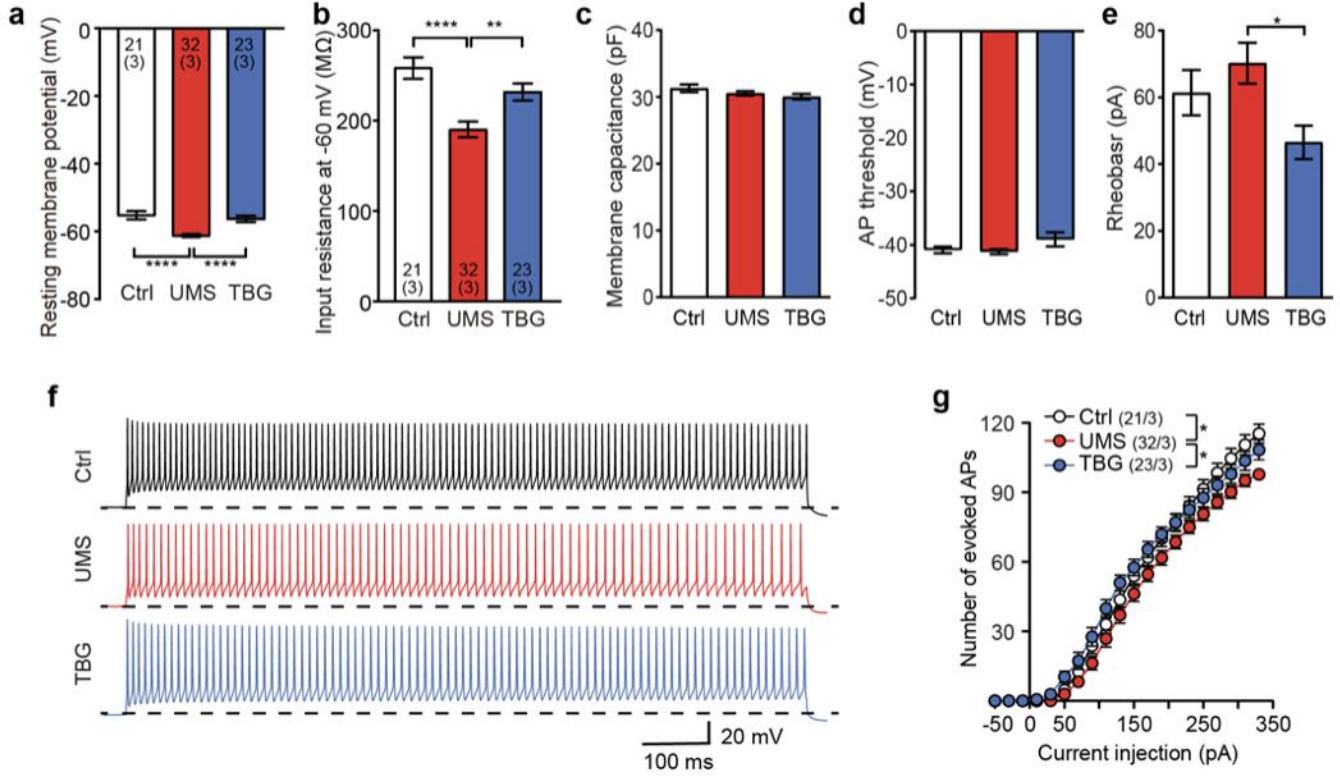
In Vitro Electrophysiology: A Patch Clamp Method



- In essence functions much like a patch clamp experimental setup.
- The brain in thinly sliced into 300 micrometer coronal slices and place in artificial cerebrospinal fluid
- A glass pipette is pressed against the neocortex membrane slice and using suction creates a seal with the membrane
- An electrode is placed along with the pipette and a “command” or chosen voltage is applied
- Once the voltage is applied the membrane will either be a match or be at a difference where the patch clamp method measures the current and if it is positive or negative

“In its human form the neocortex exists at its most complex and evolved state. It is the region of our brain responsible for sensation, action, cognition, and consciousness” - Alejandro L Diaz Pubmed central NIHMSID: NIHMS151063

Supplemental Figure 7



Results : A New Hope

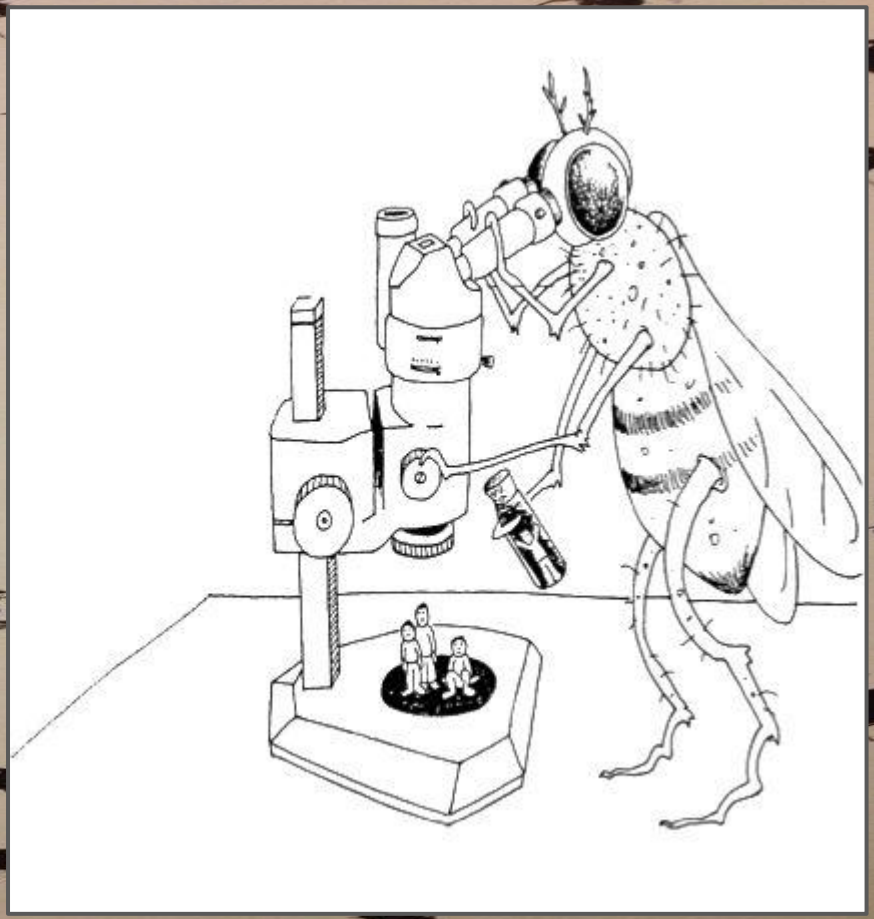
Behavioral Rescue: TBG significantly reduced anxious behavior in mice subjected to UMS - measures through elevated plus maze assay. Mice treated with TBG showed better performance as compared to untreated mice in cognitive behavioral trials.

Neuronal Activity Rescue: Through Calcium imaging it was shown that TBG restored calcium dynamics in mice post UMS implying restored neuronal activity.

Reversal of structure loss - TBG treatment in UMS mice increased dendritic spine density. Untreated mice showed loss of spine density implocating TBG as a method to induce neuronal plasticity

Overall TBG was shown to reverse the effects of Unpredictable mild stress

Cool, so?



Validating Forced Swim Test in *Drosophila* with Drugs- [author]

Tested Wild-type *Canton-Special w/*

- Methamphetamine
- αMT:sedative
- Citalopram:SSRI
- Psilocybin
- WAY:5-HT_{1a} antagonist
- Ketanserin:5-HT_2 antagonist

Forced Swim Test

- Measured response to inescapable adversity
  - Immobility = depressive-state

Potential in Alzheimer's Disease?

"A significant treatment gap exists for late-stage AD, characterized by extensive neuronal damage and severe cognitive decline"

"Terminal lucidity suggests that cognitive decline might be reversible, at least momentarily"

"It seems more plausible that these lucidity episodes arise from the spontaneous formation of neural bypasses."

"(LSD) and psilocybin have [the] ability to induce changes in neuroplasticity-the brain's capacity to form and reorganize synaptic connections ... by promoting the growth of dendritic spines and synapses"

- A perspective on Alzheimer's disease: exploring the potential of terminal/paradoxical lucidity and psychedelics

Jesus’ and Guillermo’s potential project

Our project idea : Can the neuroplasticity caused by psychedelics and their analogs such as TBG be used to reverse the negative effects of the peptide amyloid beta in our AD model?

Currently: Since psychedelics are federally controlled substances the process to get government approval to conduct studies takes over a year. We are currently looking to work with an analog of a psychedelic which deliver similar effects without the hallucinogenic effects.

Our Rationale: Given promising results and well rounded cytotoxicity studies, if psychedelics can promote neuronal plasticity they could potentially be used as a palliative care for patients suffering from Alzheimer’s diseases which may potentially conserve locomotor ability and potentially even extend life.

Gracias !