

In-vivo optical measurement of activity-dependent fluorescence change in mice striatum

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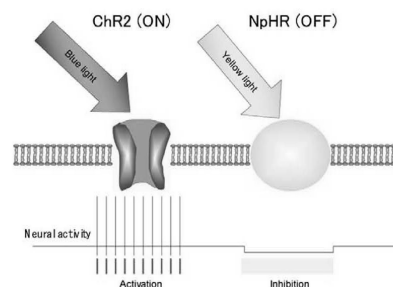


Optogenetics for neuromodulation

Optogenetics?

- Optical methods for controlling and probing genetically targeted neurons in neural circuits
- The principle was discovered by Dr. Miesenboeck in 2002
- “Optogenetics” was coined in 2006 by Dr. Karl Deisseroth.

- Channelrhodopsin (ChR2)
 - light-activated cation channel
- Halorhodopsin (NpHR)
 - light-driven ion pump, specific for Cl^- ions



Optogenetics for neuromodulation

Millisecond-timescale, genetically targeted optical control of neural activity.

Boyden ES, et al. Nat Neurosci. 2005

Circuit-breakers: optical technologies for probing neural signals and systems.

Zhang F, et al. Nat Rev Neurosci. 2007

An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology.

Aravanis AM, et al. J Neural Eng. 2007

Optical deconstruction of parkinsonian neural circuitry.

Gradinaru V, et al. Science. 2009

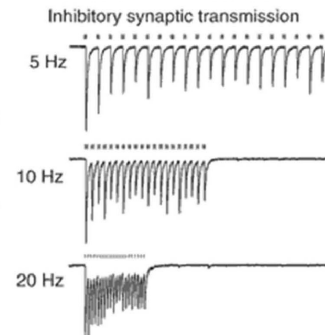
Global and local fMRI signals driven by neurons defined optogenetically by type and wiring.

Lee JH, et al. Nature. 2010

Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry.

Kravitz AV, et al. Nature. 2010

→ Optogenetics has become a very popular and useful tools to study neural pathways.



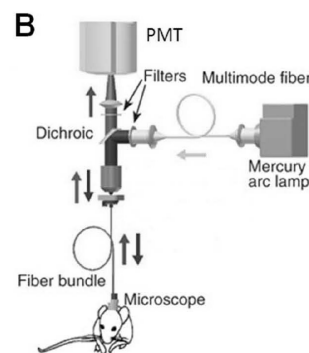
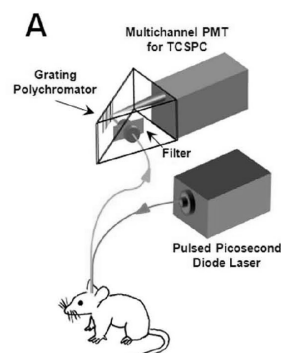
Mouse implanted with an optic fiber
- Karl Deisseroth at Stanford Univ.

Optogenetics for measurement of neural activity

Optogenetics for neural recording?

1) Activity-dependent fluorescence change

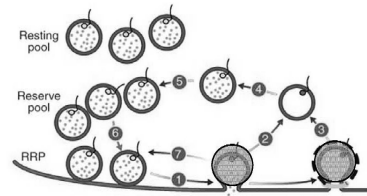
2) Sensitive fluorescence measurement methods for in vivo detection



Activity-dependent fluorescence

1. Synaptophluorin (spH21)

- A fusion protein of the synaptic vesicle protein VAMP2 and the **ecliptic pHluorin (pH-sensitive GFP)** targeted inside the vesicle lumen.
- Upon neurotransmitter releases, fluorescence increases.



Nat Protoc. 2006;1(6):2970-8

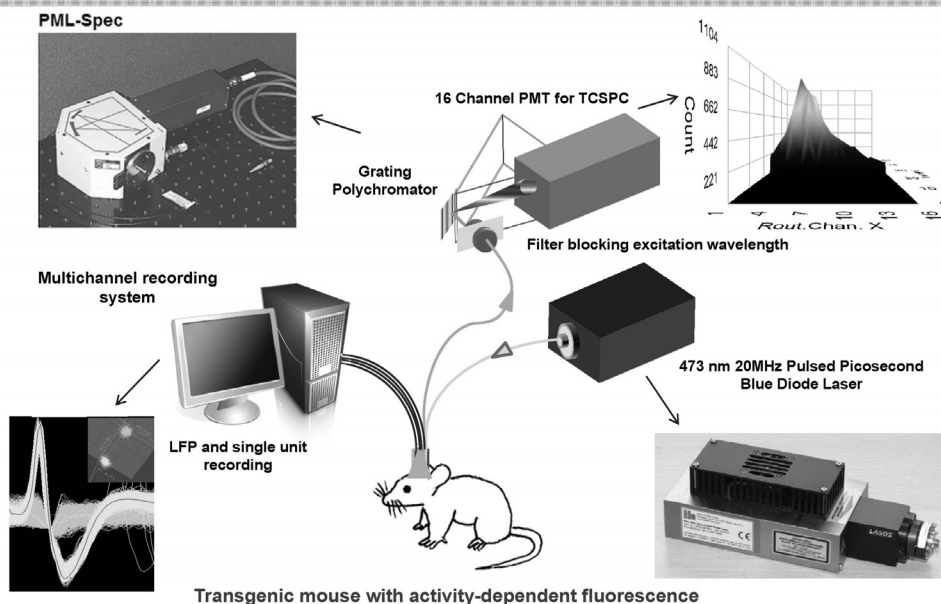
2. Calcium-sensitive indicator (GCaMP3)

- Genetically-encoded Ca^{2+} indicator .
- AP-induced calcium entry transiently increases fluorescence.

3. Immediate early gene (Arc-GFP)

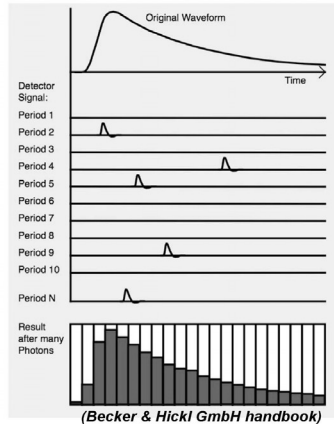
- Activated at the transcription in response to external stimuli, before new protein synthesis.
- Arc-GFP (activity-regulated cytoskeleton-associated GFP).

In-vivo optical measurement of neural activity using TCSPC



Principles of TCSPC

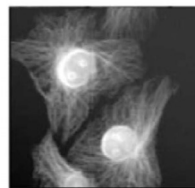
The principle of TCSPC



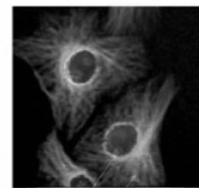
Typical applications

- Ultra-fast recording of optical waveforms
- FLIM (Fluorescence Lifetime Imaging Microscopy)
- FRET (Fluorescence Resonance Energy Transfer)
- DNA sequencing

Intensity image

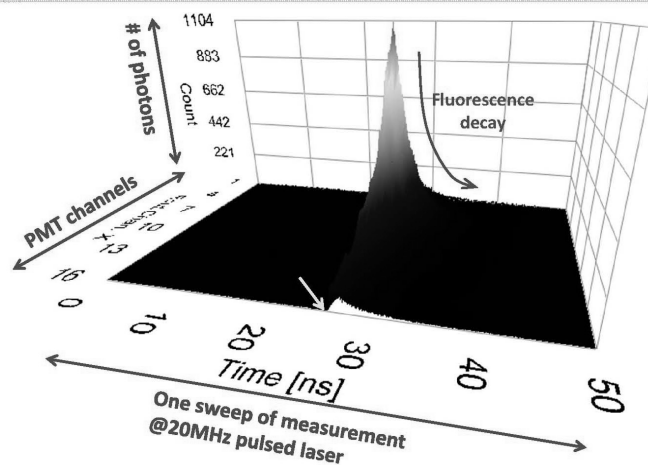


Intensity-Life time image



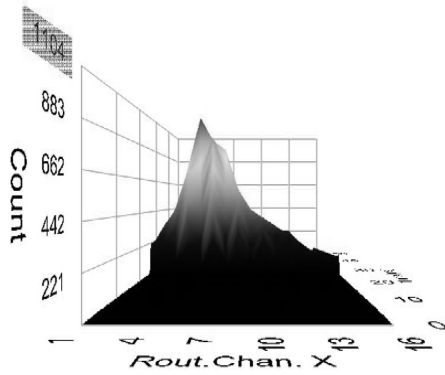
- Ultra-high time resolution - 25 ps
- Ultra-high sensitivity – single photon detection
- Excellent signal-to-noise ratio
- High gain stability

TCSPC measurement

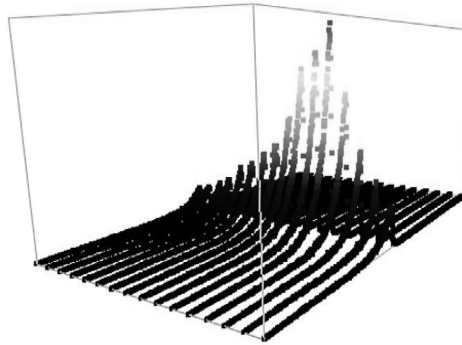


- This spectrum was obtained by 1 s collection (=accumulation of 20×10^6 sweeps)
- Time resolution in 50 ns : 1024 (selectable from 1 to 4096)
- Width of wavelength from ch1 to ch16 : 200 nm \rightarrow single channel width: 12.5nm
- Wavelength region is adjustable in 340-820nm

TCSPC measurement in spH mouse



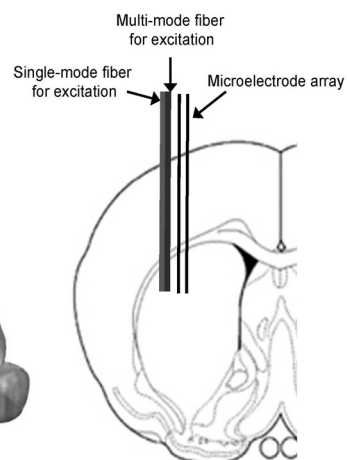
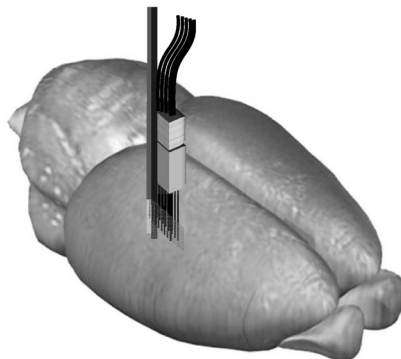
- Width of wavelength from ch1 to ch16 : 200nm
→ Width of single channel: 12.5nm
- Wavelength region is adjustable in 340-820nm



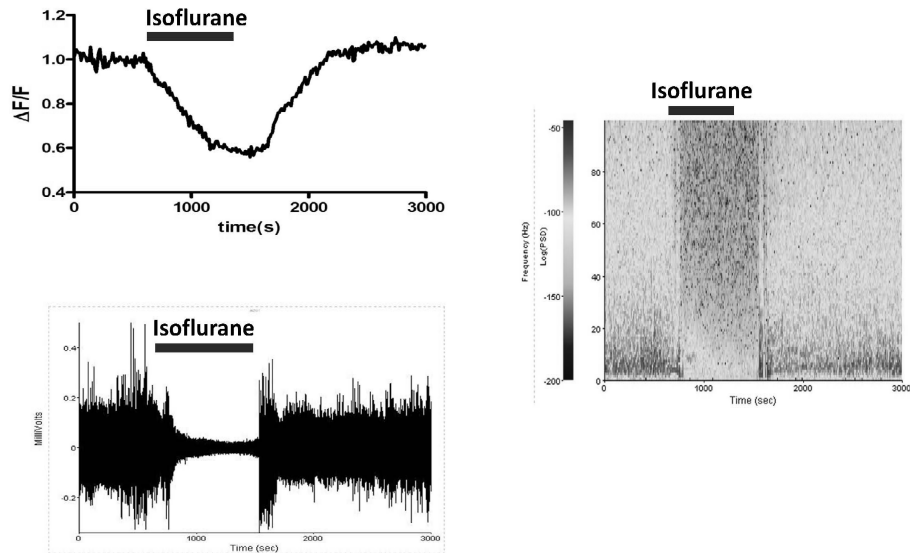
- 4 Channels (#4~7) were used for analysis

Simultaneous e-phys and fluorescence recording

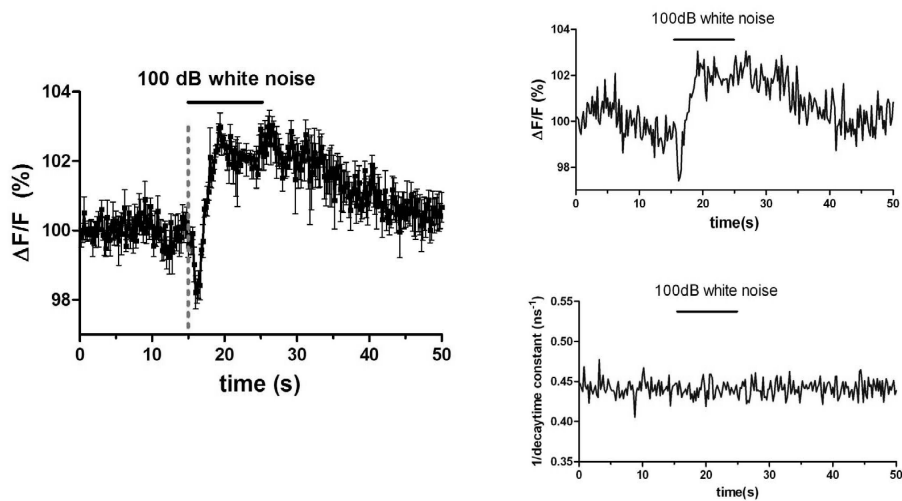
- Dorsolateral striatum
- A-P: + 0.5 mm from Bregma
 - M-L: + 2.5 mm
 - Insertion Depth: 1.8 mm



Isoflurane anesthesia-induced fluorescence change

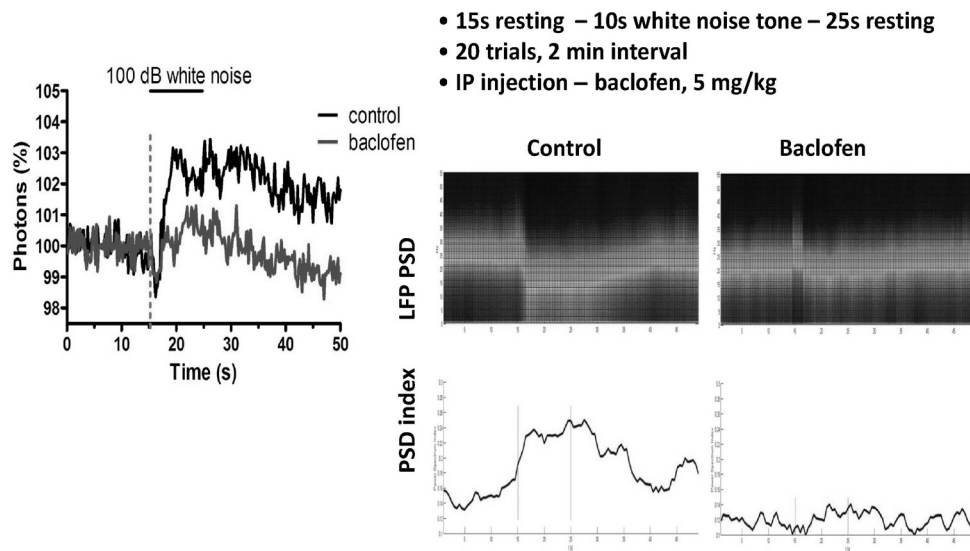


Fluorescence response to auditory stimulation

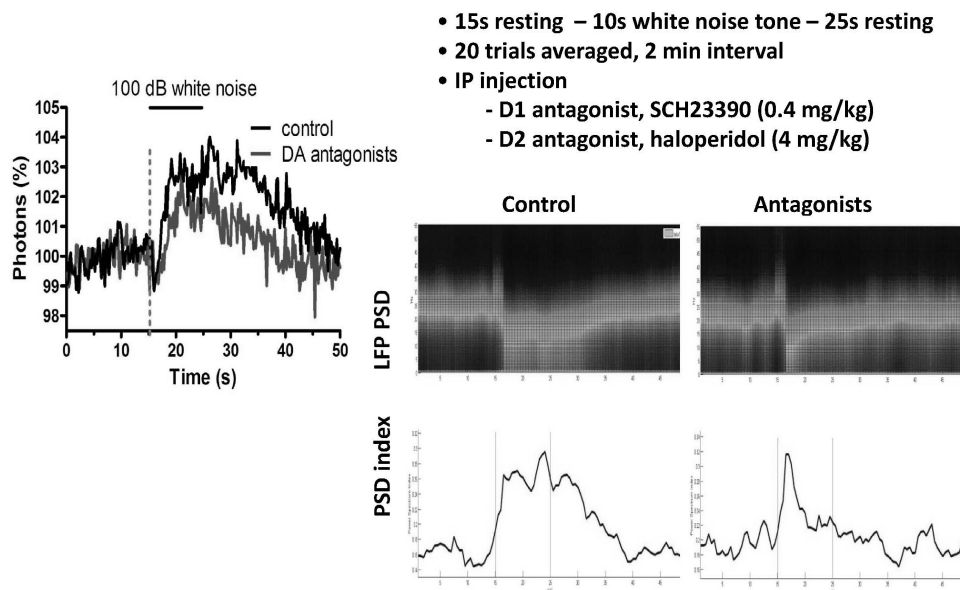


Fluorescence life time did not change with the intensity.

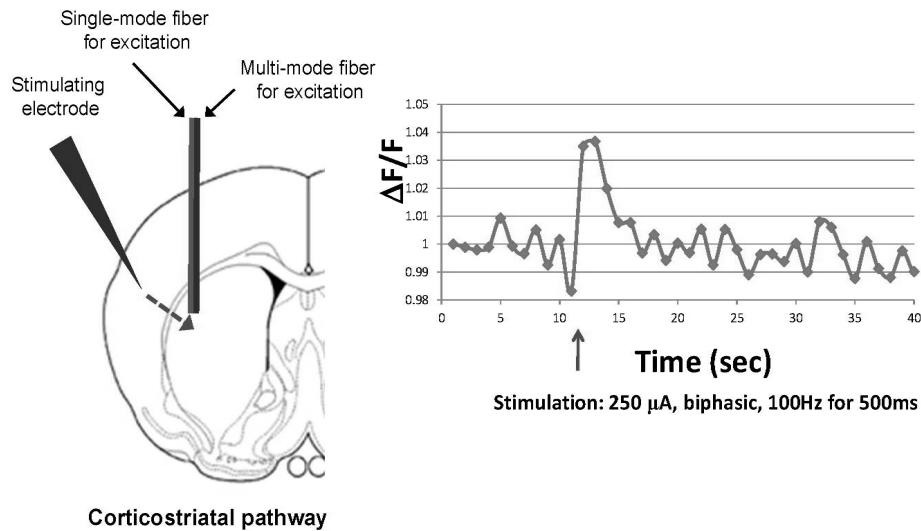
GABA_BR agonist effect on the response to sound



Dopamine antagonists effect



In vivo spH response from DLS evoked by local electrical stimulation in anesthetized mice



In vivo Ca^{2+} sensor imaging: specific to D1 neurons

- GCaMP3 \rightarrow Genetically-encoded Ca^{2+} indicator
- GCaMP3-floxed-AAV injected to D1 CRE mouse
 \rightarrow D1 neuron-specific expression of Ca^{2+} indicator
- For control, Alexa 488 dye was injected instead of the virus.

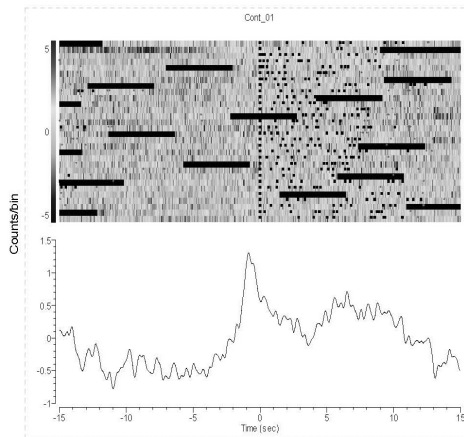
Procedure

1. Virus and control dye injected.
2. Animals were trained to press the lever and receive sucrose solution as a reward. (FR1 \rightarrow FR5 \rightarrow FR10)
3. Fibers implantation
4. Fluorescence measurement during the lever-pressing behavior

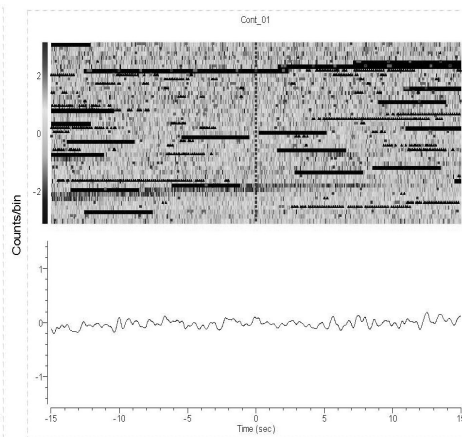


Fluorescence change during lever pressing

GCaMP3

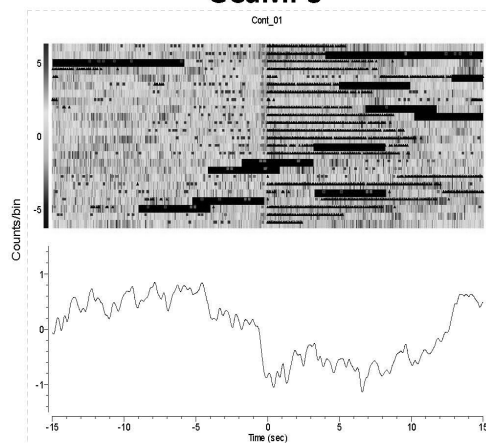


Control

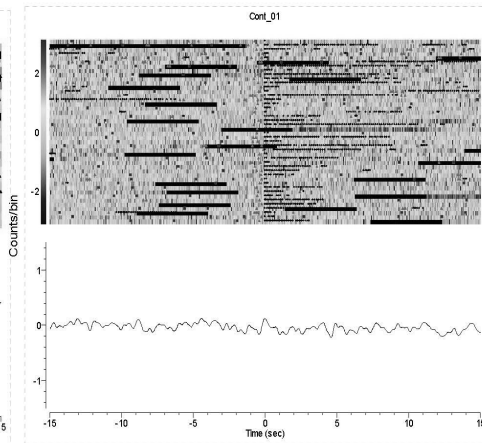


Fluorescence change during licking sucrose

GCaMP3



Control



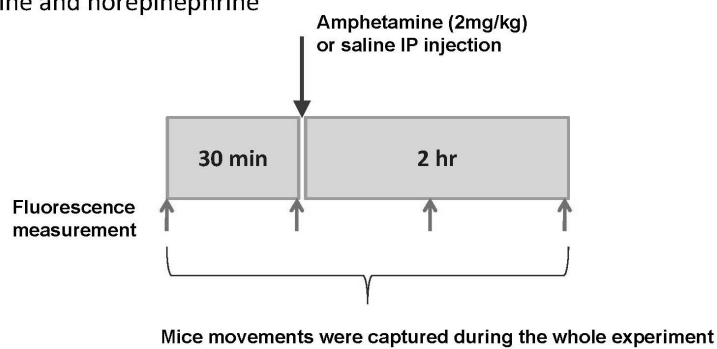
Amphetamine sensitization using Arc-GFP mice

Arc (Activity-regulated cytoskeleton-associated gene)

- An immediate early gene
- The expression is up-regulated by a variety of stimulation
- The function is unclear.

Amphetamine

- Psychostimulant drug
- Increased wakefulness and focus
- Increased dopamine and norepinephrine



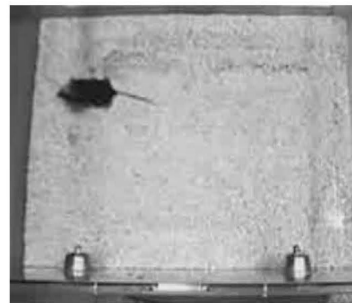
Amphetamine sensitization effect on locomotion

After 4 day injections

saline

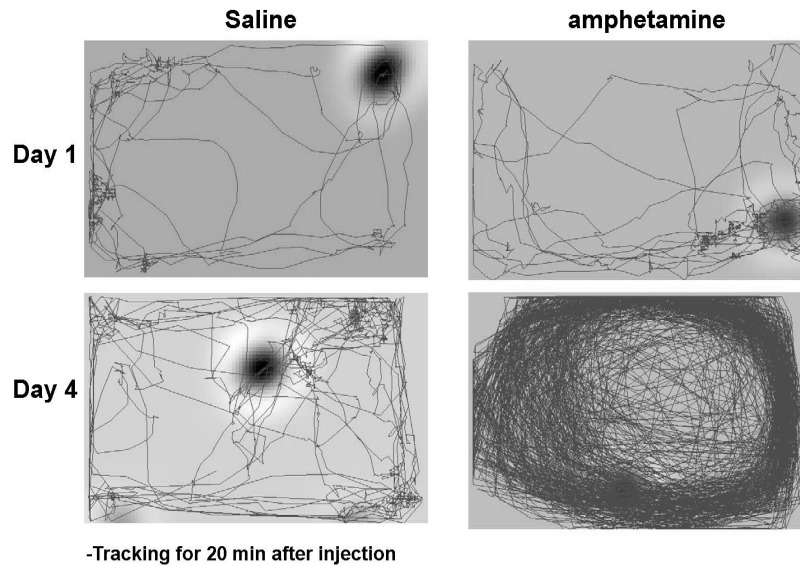


amphetamine

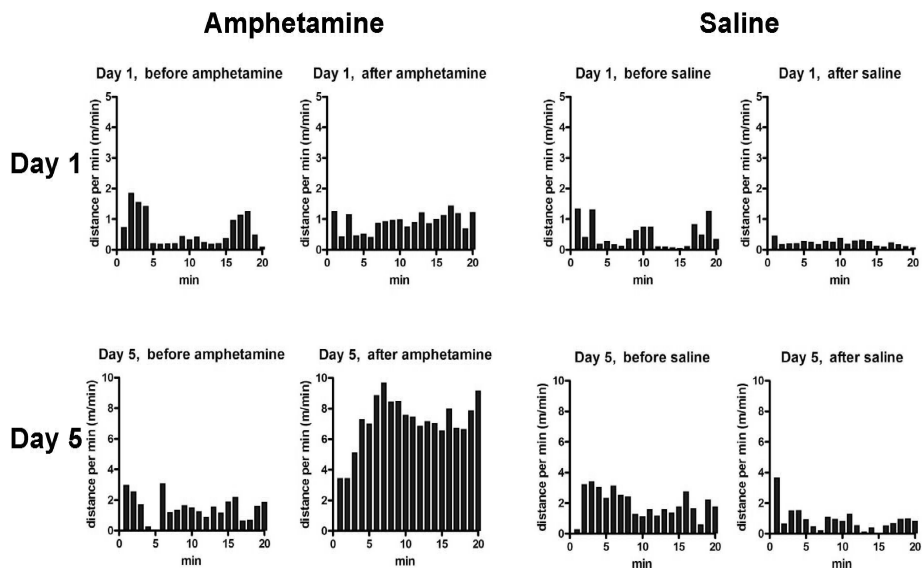


8X speed

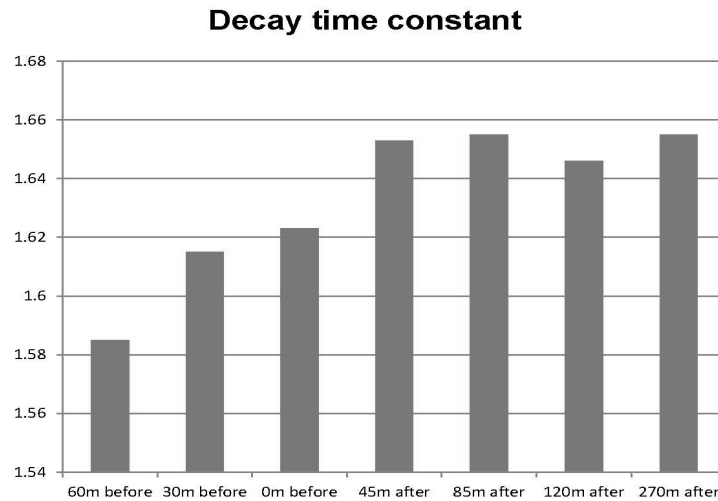
Locomotion trajectory analysis



Amphetamine induced hyperactivity in locomotion



Photon count vs. decay constant

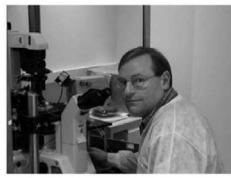


Conclusion and Future work

- Using TCSPC and fiber optics, the activity-dependent fluorescence change was detected in freely moving mice.
 - spH21 transgenic mouse showed fluorescence increase in DLS responding white noise stimulation.
 - GCaMP3-AAV virus injection enabled cell-type specific calcium indicator expression using D1 CRE mice.
- In future, this technique will be applied to several different neural pathways for new findings in neuroscience.
- Stability improvement
 - Ratiometric measurement using additional constant fluorescence signal. → Remove artifact by normalization.
 - Long-term investigation of tissue response to fibers

Acknowledgement

- Dr. Gouhong Cui (Lab. for Integrative Neuroscience)
- Dr. Xin Jin (Lab. for Integrative Neuroscience)
- Dr. Steve Vogel (Lab. Of Molecular Physiology, section on cellular biophotonics)
- Dr. David Lovinger (Lab. for Integrative Neuroscience)
- Dr. Rui Costa (Instituto Gulbenkian de Ciência (Portugal))



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