# Expanding the toolbox of fluorescence protein-based sensors

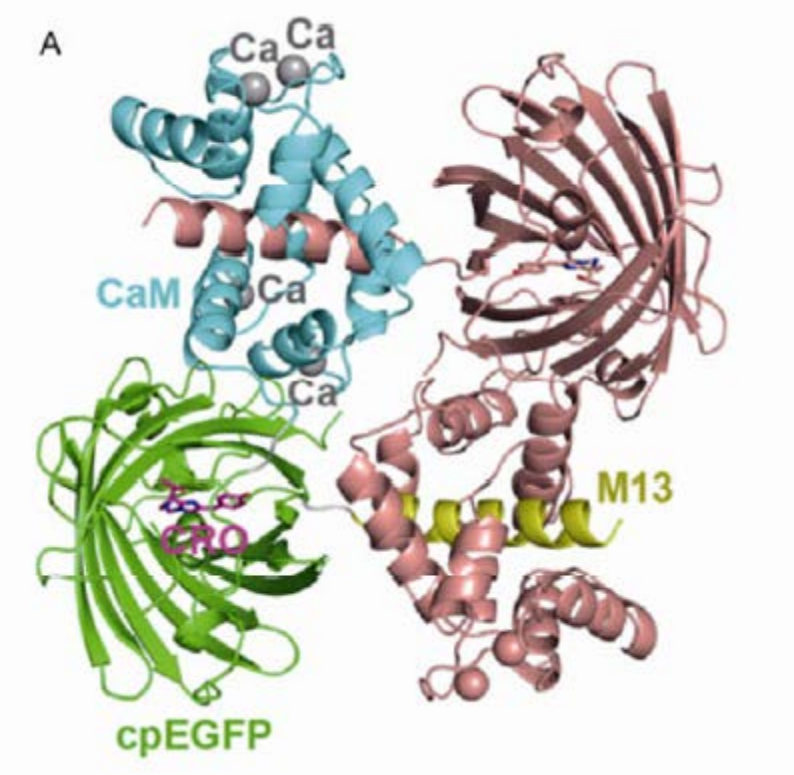
Measure what is measurable, and make measurable what is not so.

Galileo Galilei

# Outline

1) Introduction
2) dLight – a new dopamine sensor (Patriarchi, Science, 2018)
3) An application (Corre, eLife, 2018)
4) Conclusion and comparison

# Fluorescence protein-based sensors




**Structure of GCaMP6 (dimer)**
*Ding, Sci China Life Sci, 2014*
*Chen, Nature, 2013*

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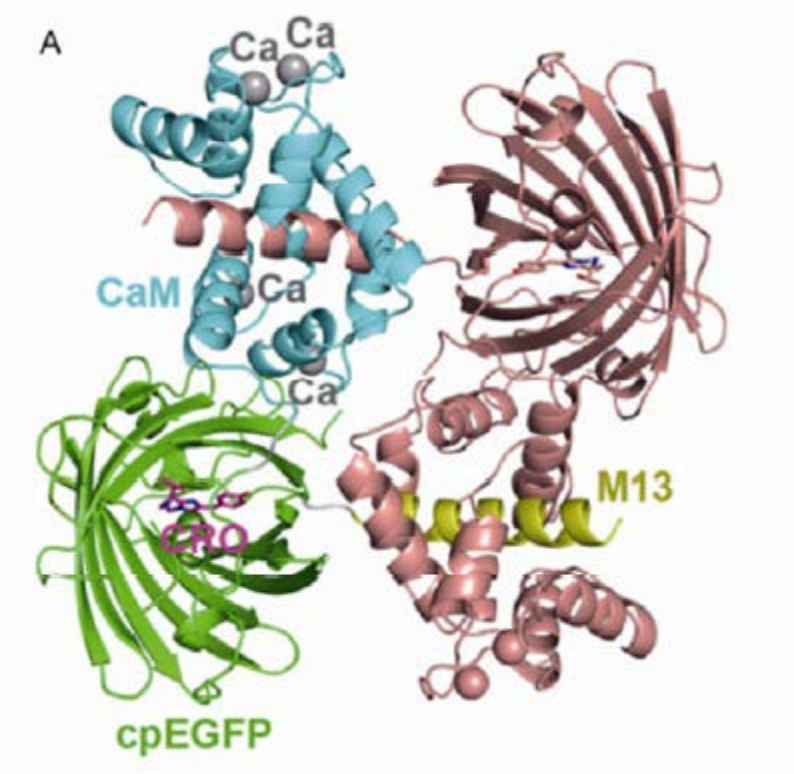


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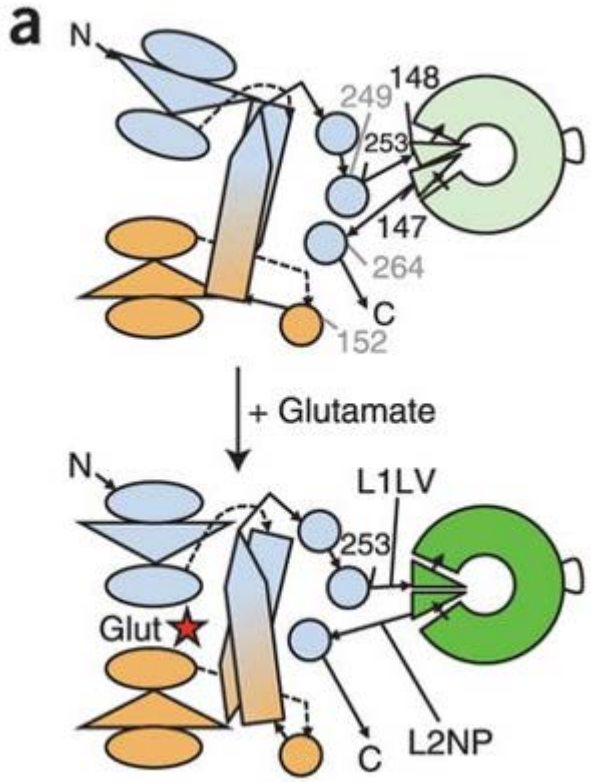


**iGluSnFR**
*Marvin, Nature Methods, 2013*

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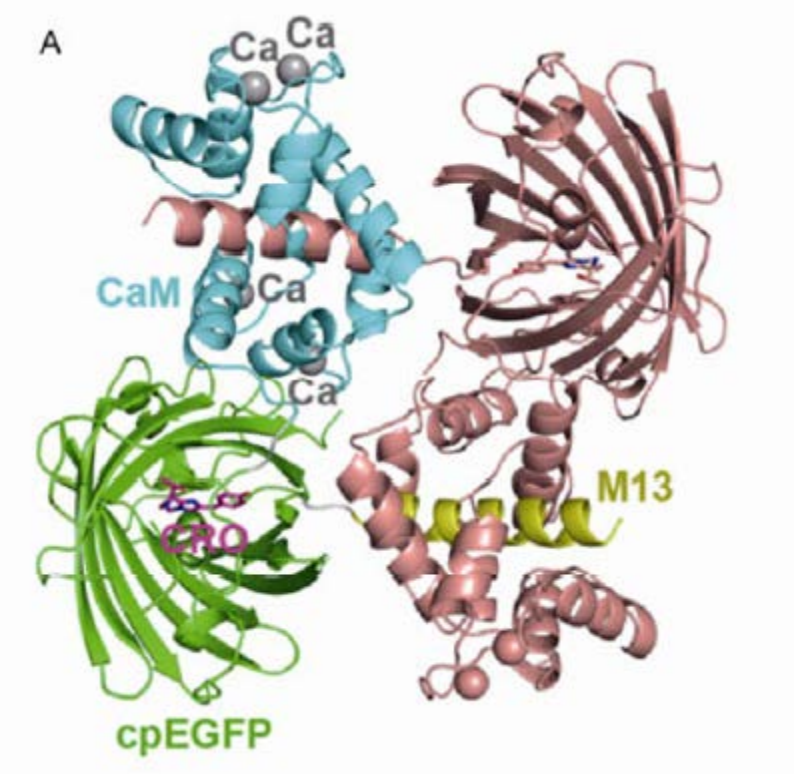


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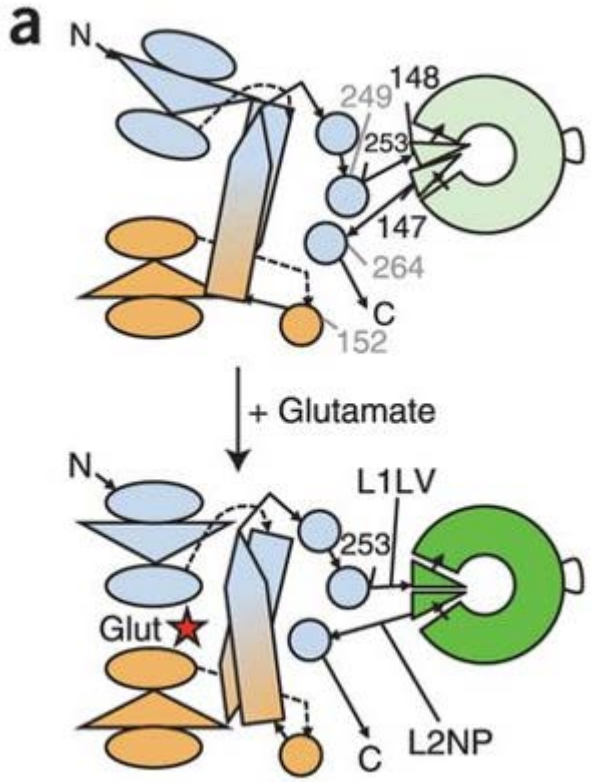


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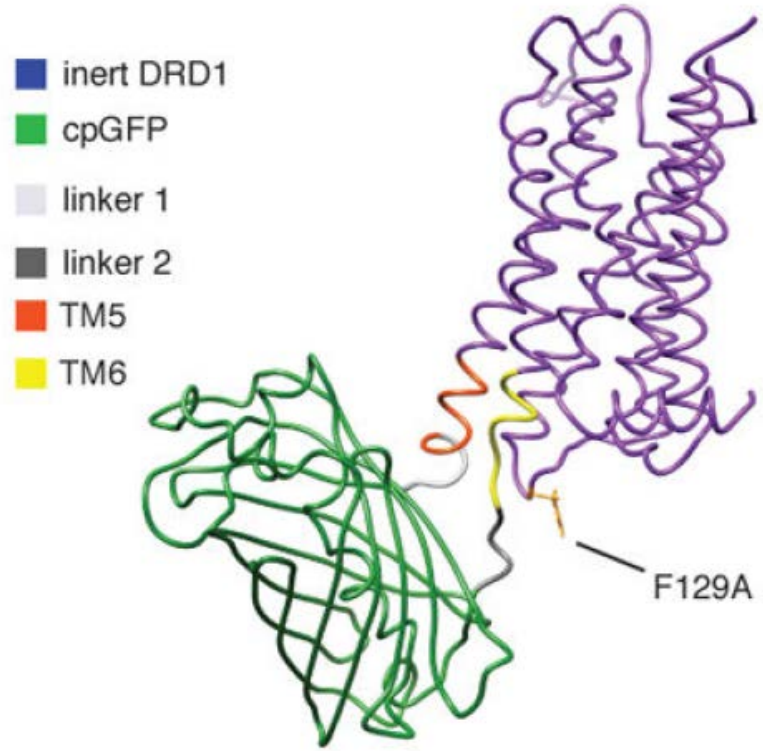



**dLight1**
*Patriarchi, Science, 2018*

# Neuromodulators

- Neuromodulators > neuronal circuit dynamics > neural function and behavior
- Neuromodulator targeting drugs

- Little is known about how the neuromodulators alter the function of their target circuits

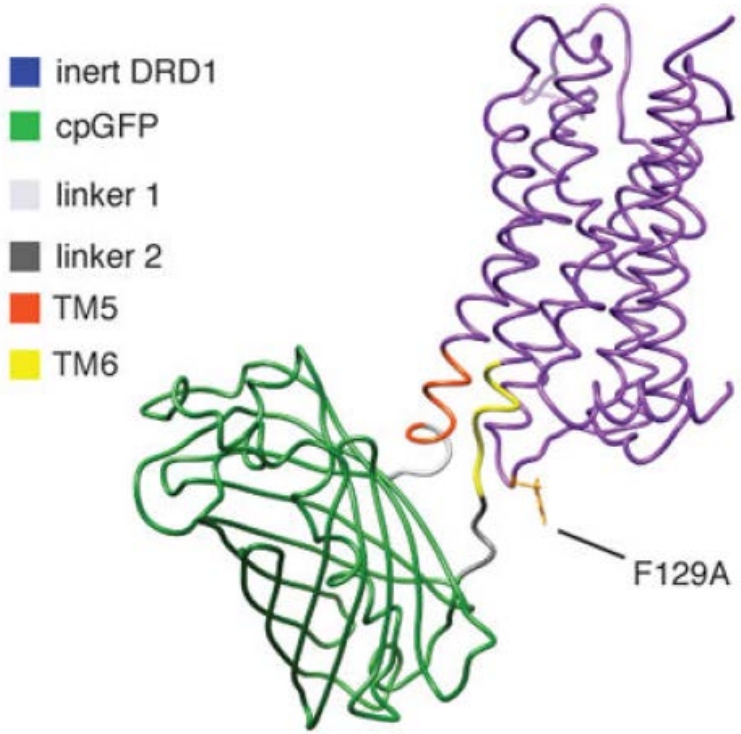
- Combine genetically encoded indicators based on fluorescent proteins with modern microscopy

# Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors

Tommaso Patriarchi[†,1], Jounhong Ryan Cho[†,2], Katharina Merten[3], Mark W. Howe[4], Aaron Marley[5], Wei-Hong Xiong[6], Robert W. Folk[3], Gerard Joey Broussard[1], Ruqiang Liang[1], Min Jee Jang[2], Haining Zhong[6], Daniel Dombeck[4], Mark von Zastrow[5], Axel Nimmerjahn[3], Viviana Gradinaru[2], John T. Williams[6], and Lin Tian[1,*]

Science, June 2018

Couple the dopamine (DA) binding-induced conformational changes in human DA receptor to changes in fluorescence intensity of cpGFP.




Design based on structure of B2AR (Rasmussen, Nature, 2011)

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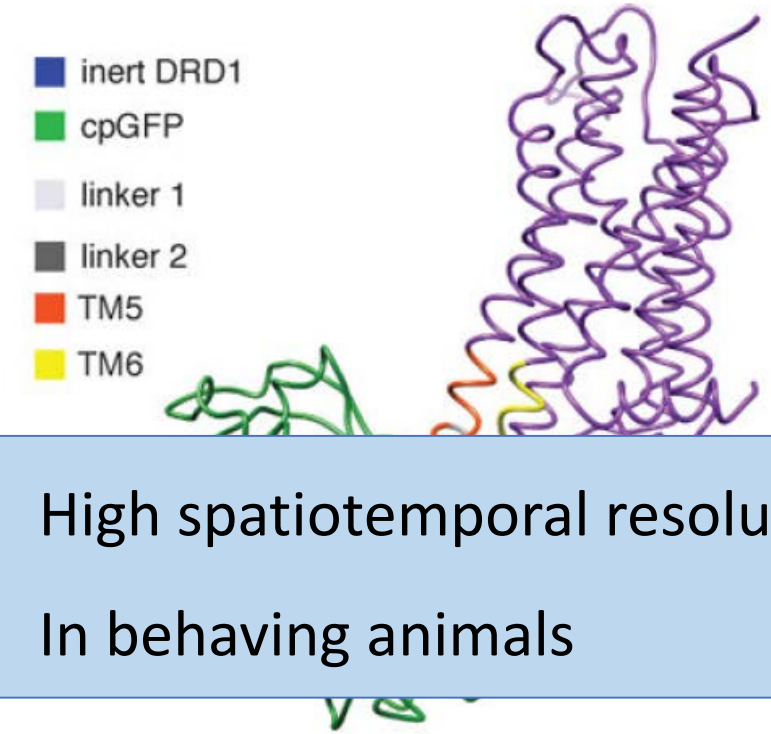
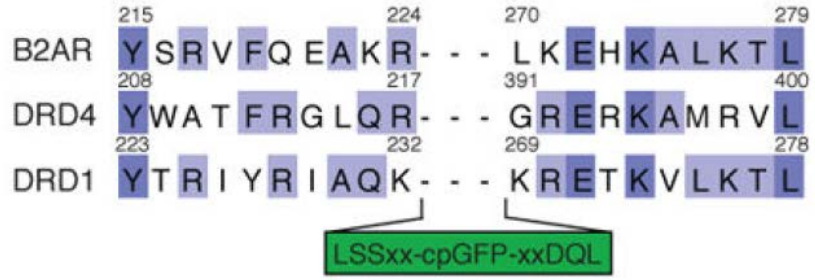



- High spatiotemporal resolution
- In behaving animals

Rasmussen, Nature, 2011

# Sensor engineering

- Sequence alignment



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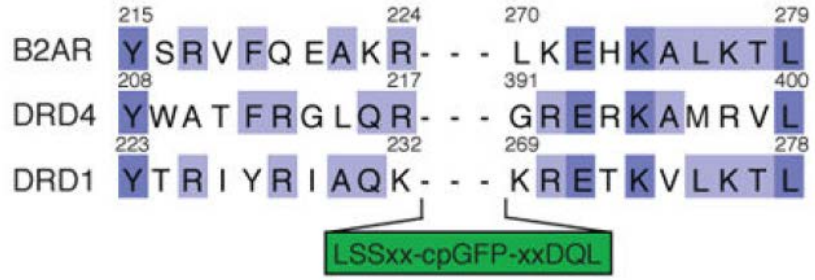
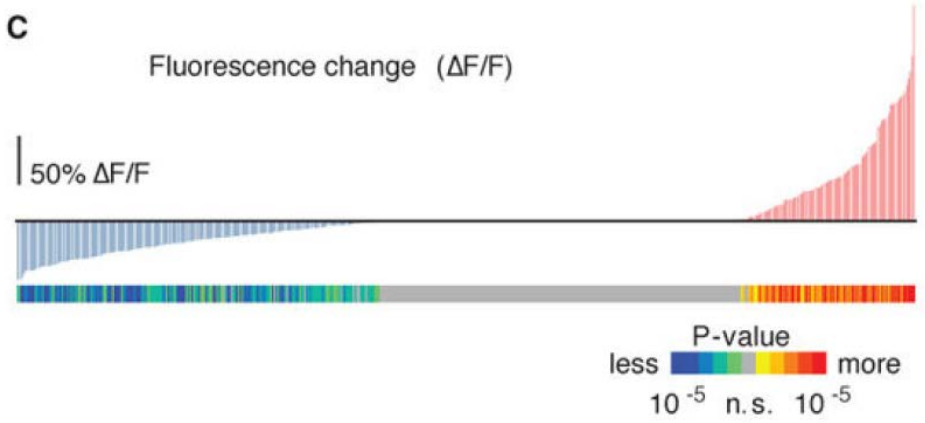



- Screening of linker variants in HEK293 cells



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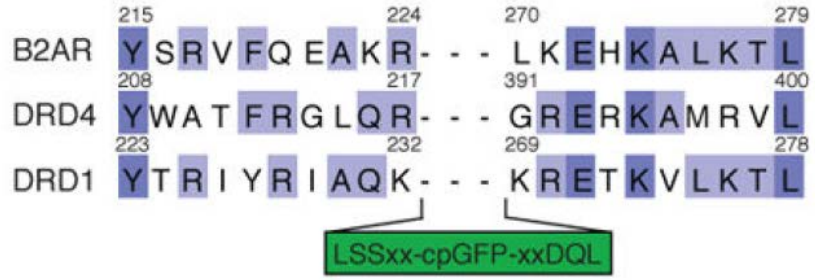


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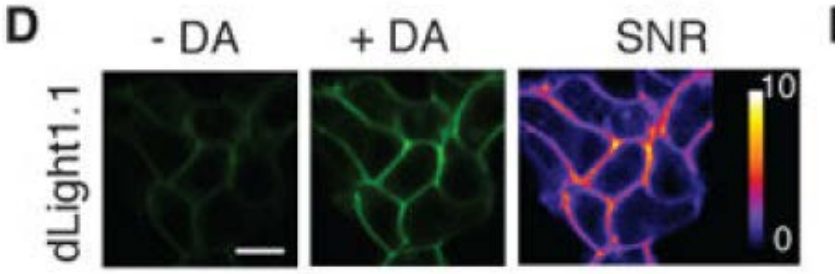
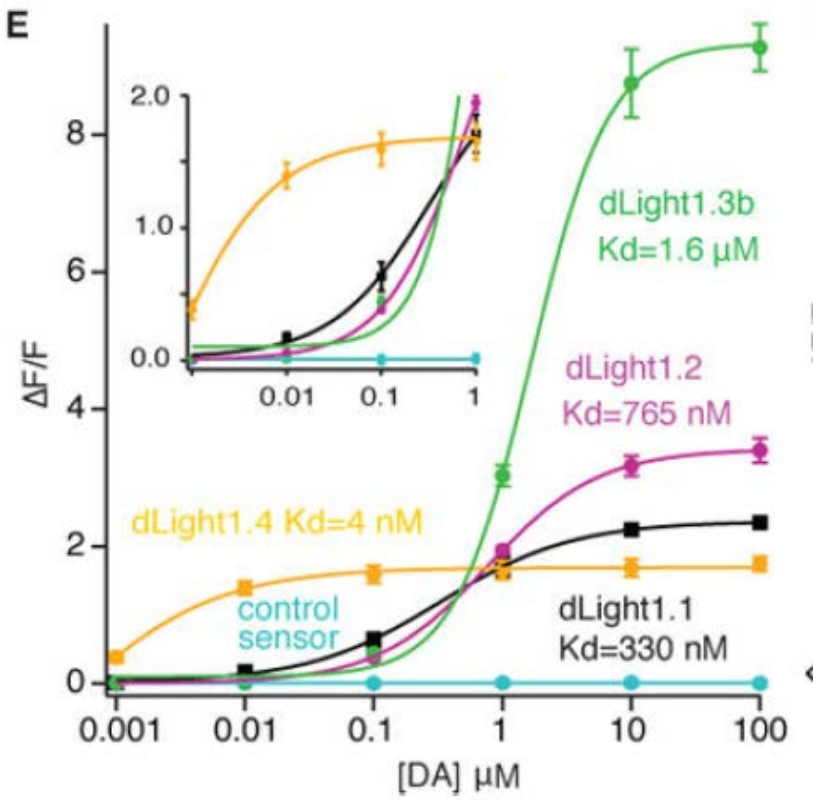


# Sensor engineering

- DA titration: affinity




dLight1.1

dLight1.2: mutation of  Phe129

# Sensor engineering

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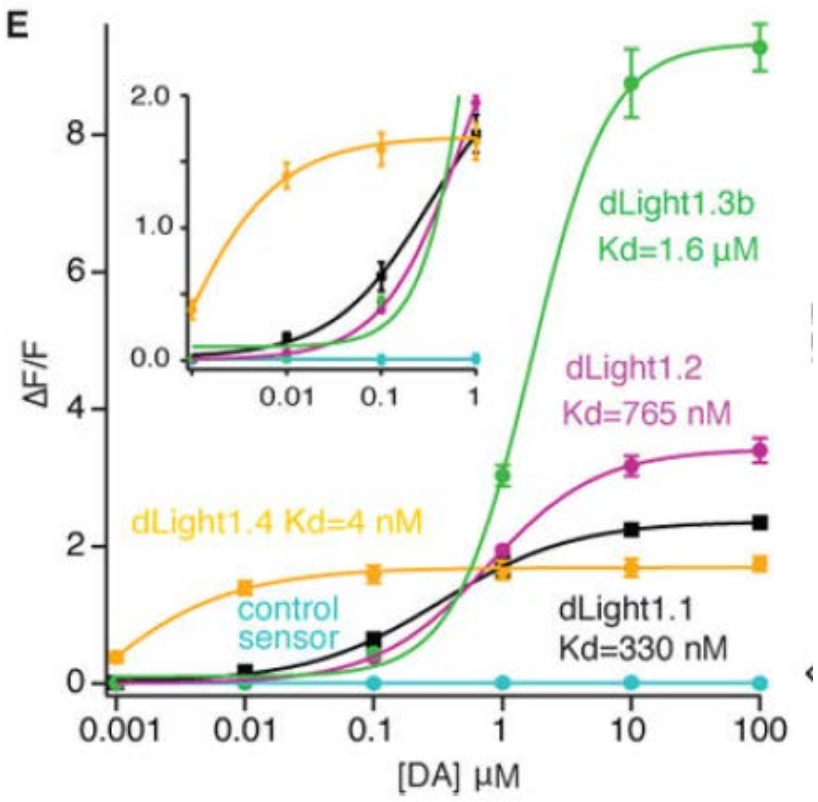
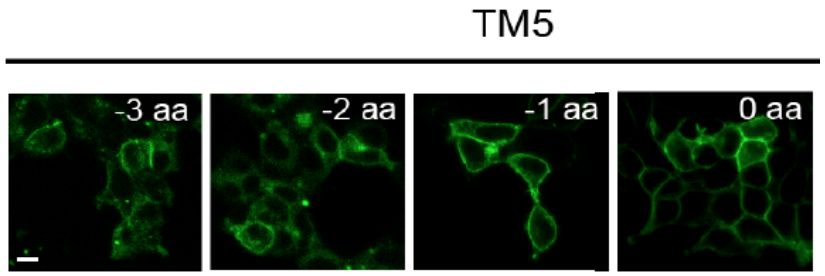



dLight1.1

dLight1.2: mutation of Phe129

- Optimization of cpGFP insertion site



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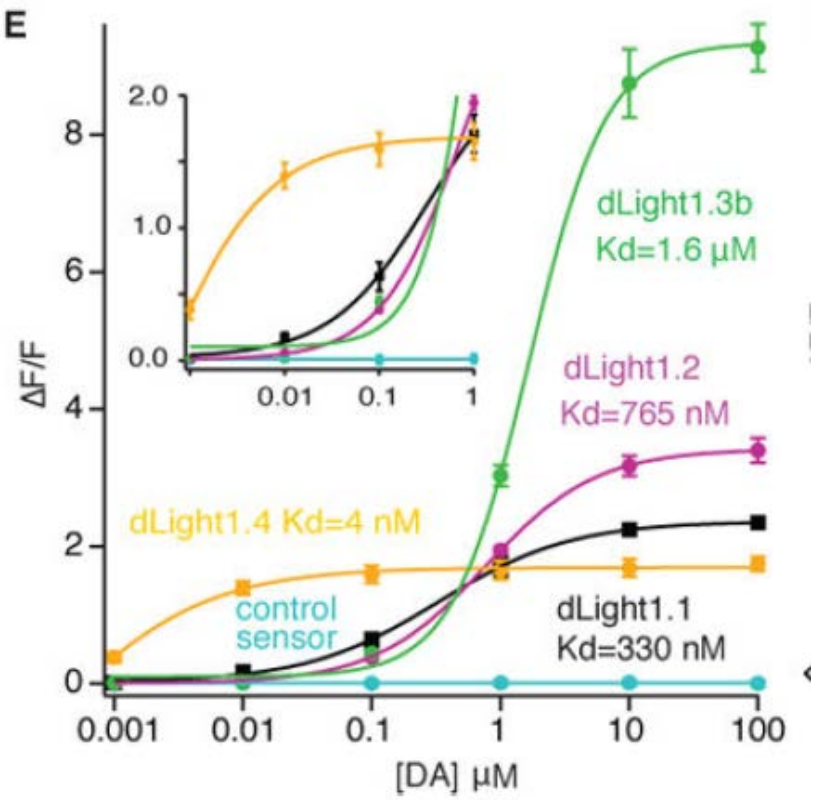


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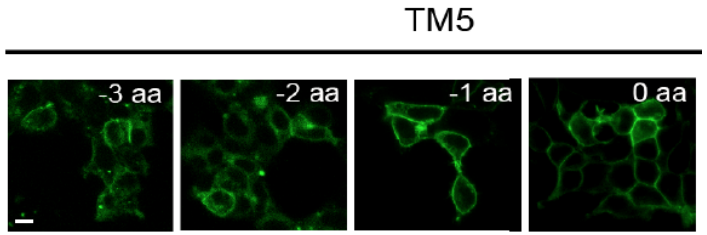
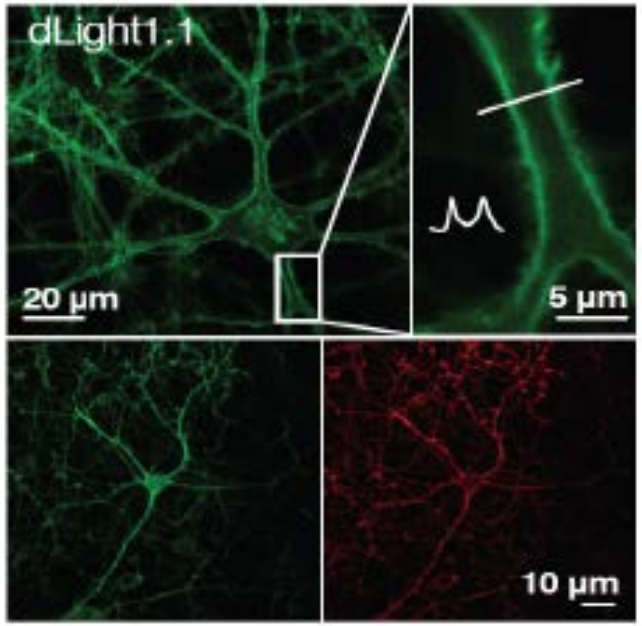


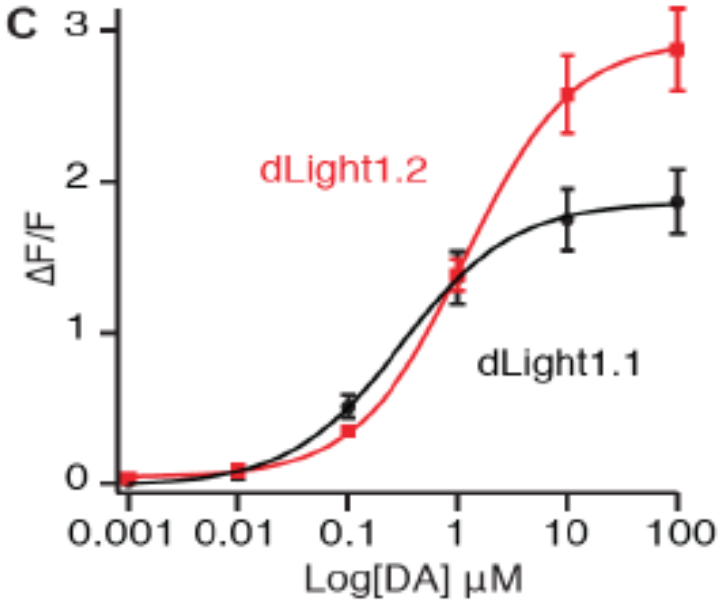

dLight1.4: DRD4 (small dynamic range)

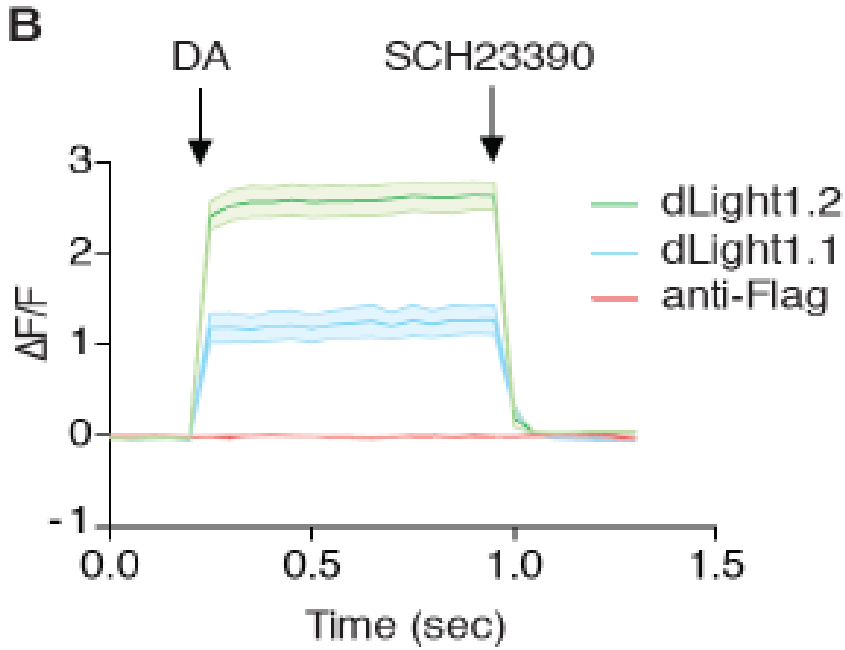
Control: dLight1.1 with D103A mutation > no DA binding

# Sensor characterization

- dLight1.1, 1.2
- Hippocampal neurons (culture)

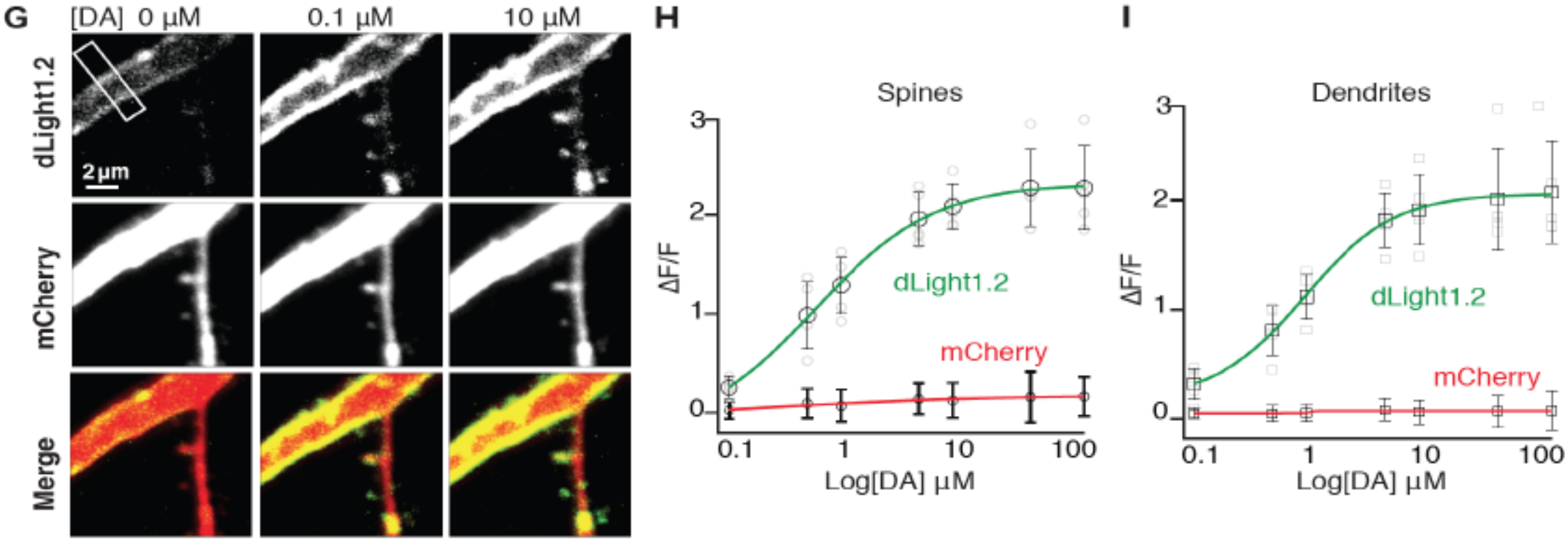


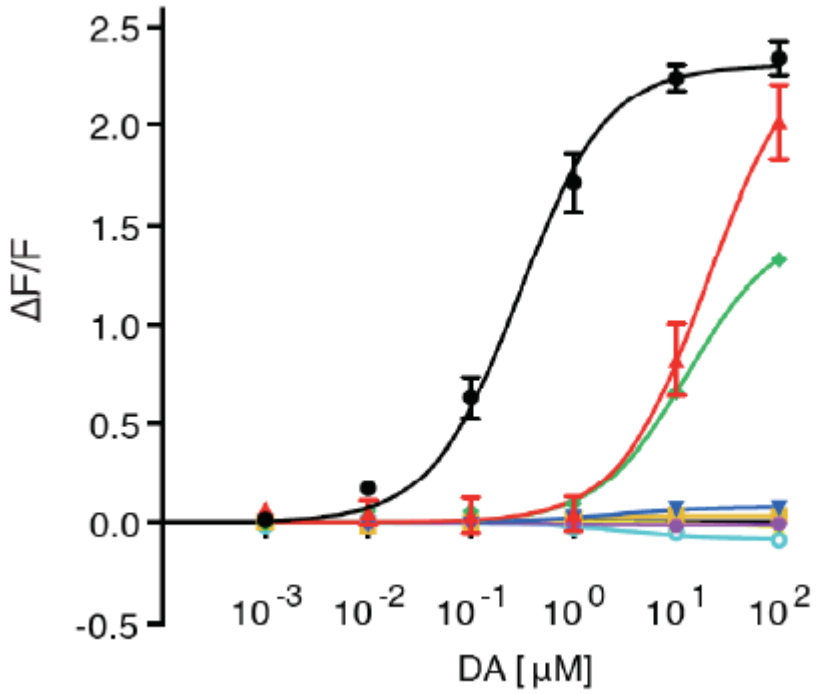
# Sensor characterization

- Hippocampal slices

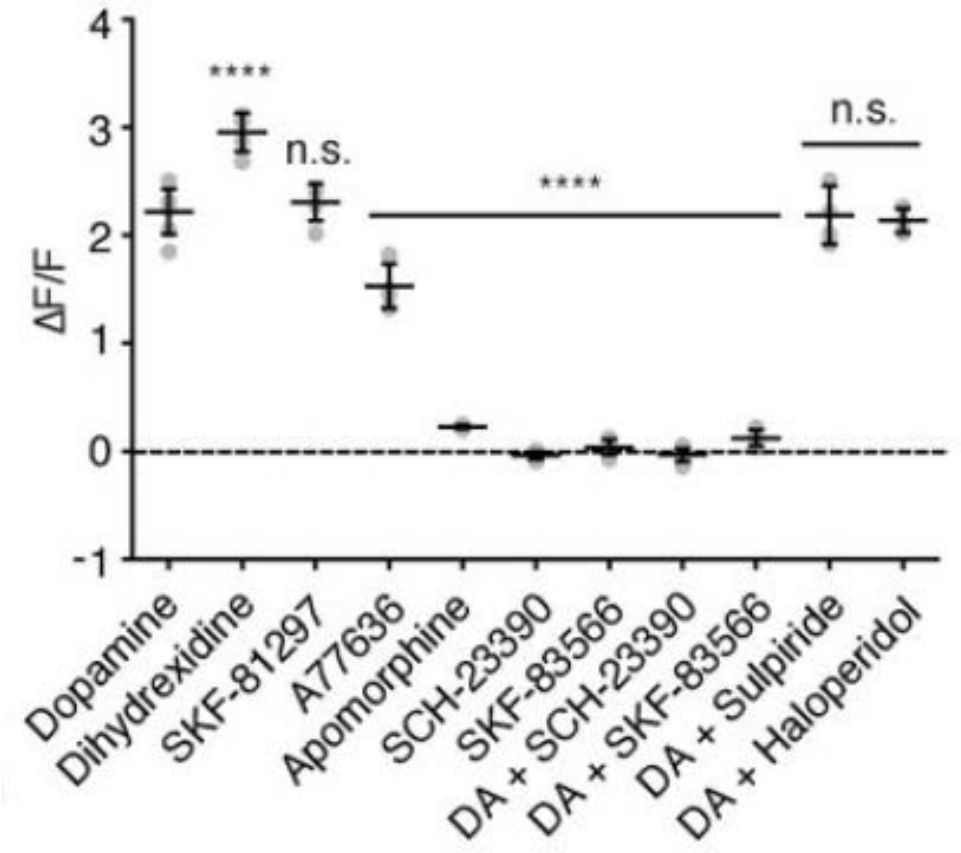


# Sensor characterization

- Endogenous and pharmacological molecular specificity







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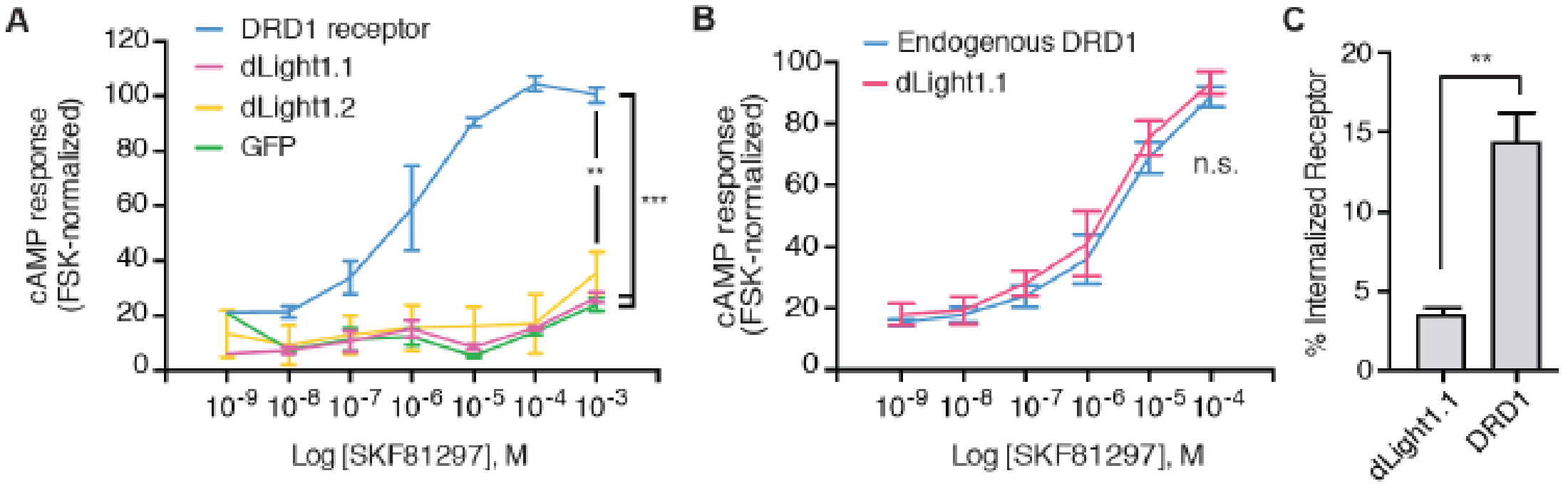
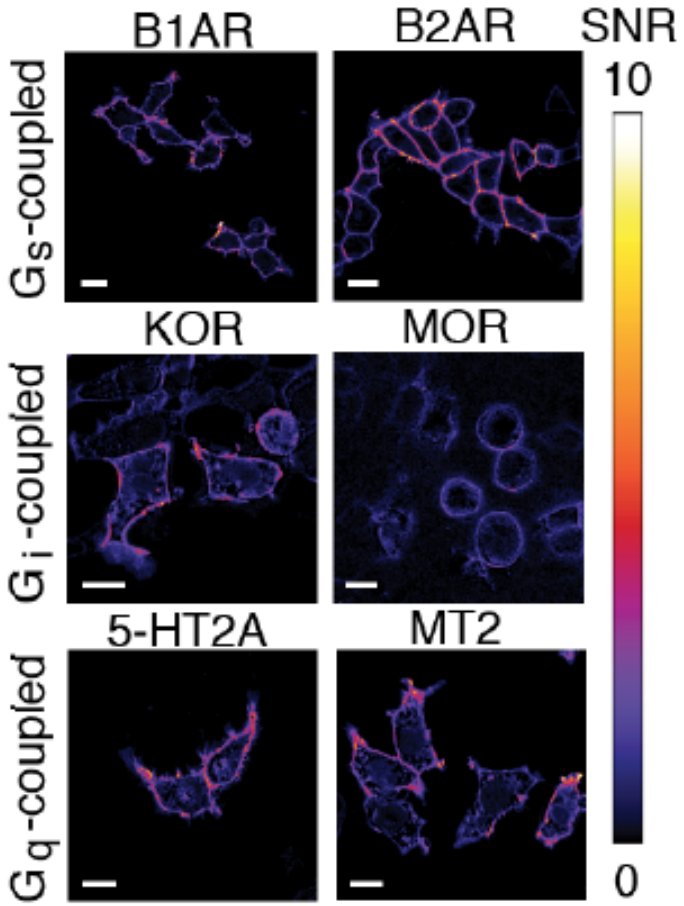


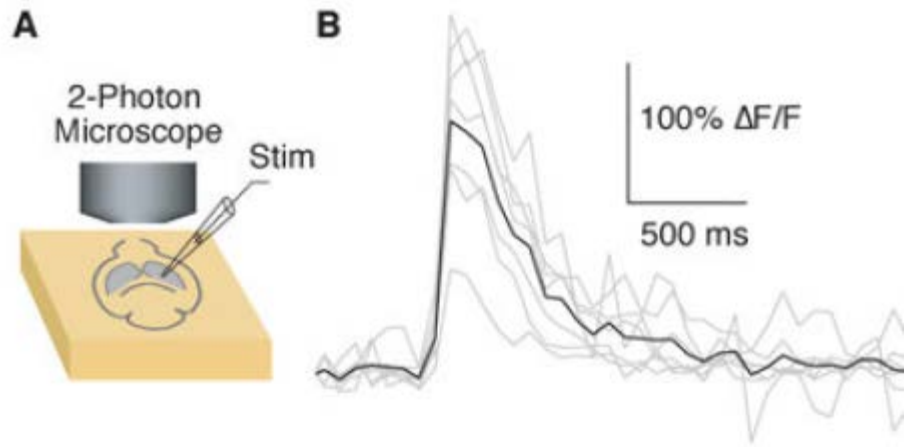

dLight does not affect endogenous signaling

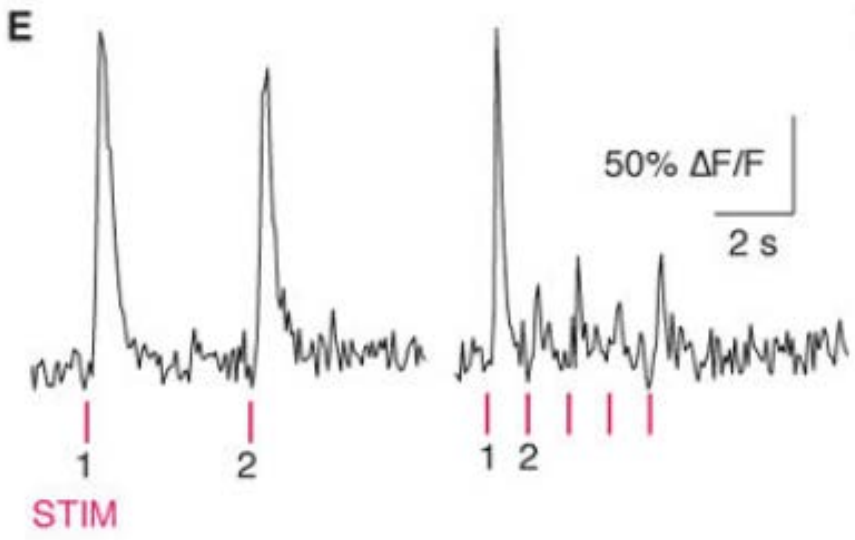
# Versatile application to other neuromodulators



# Endogenous DA release: ex vivo

- 2-photon imaging
- Acute striatal slices
- Electrical stimulation and drug modification
- AAV9.*hSynapsin1*.dLight1.2



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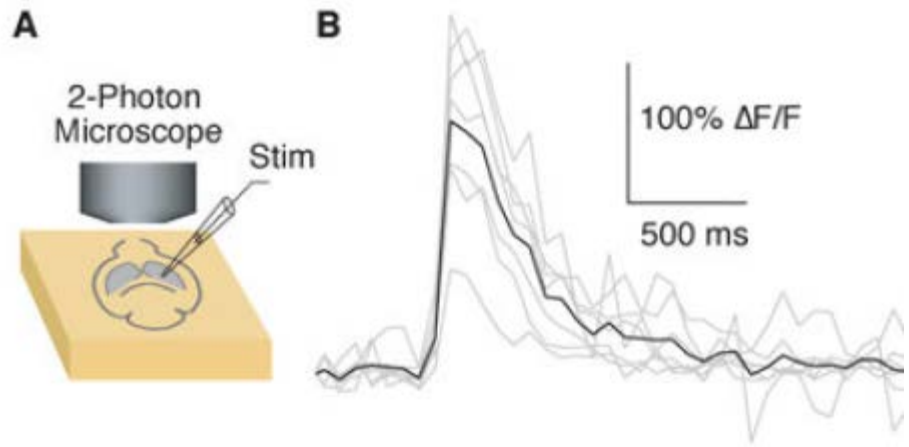
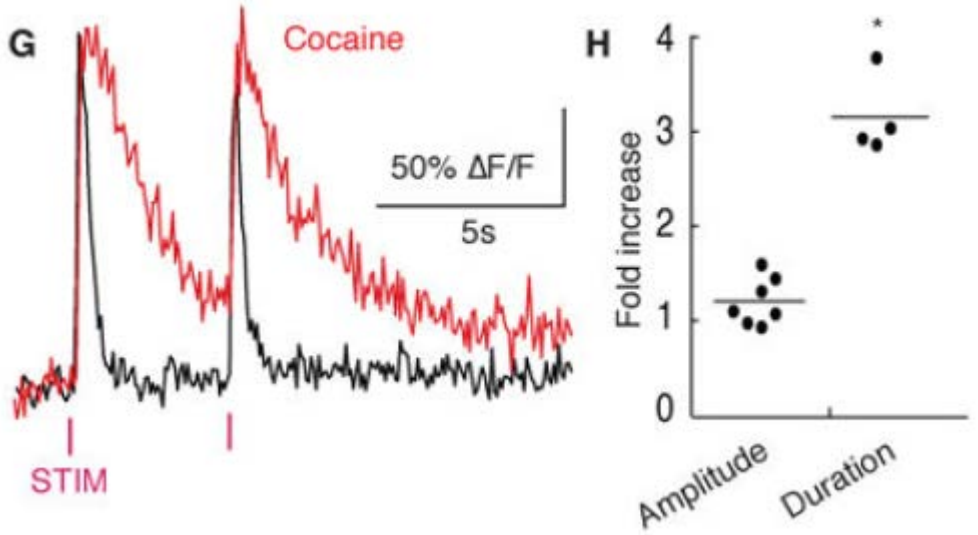
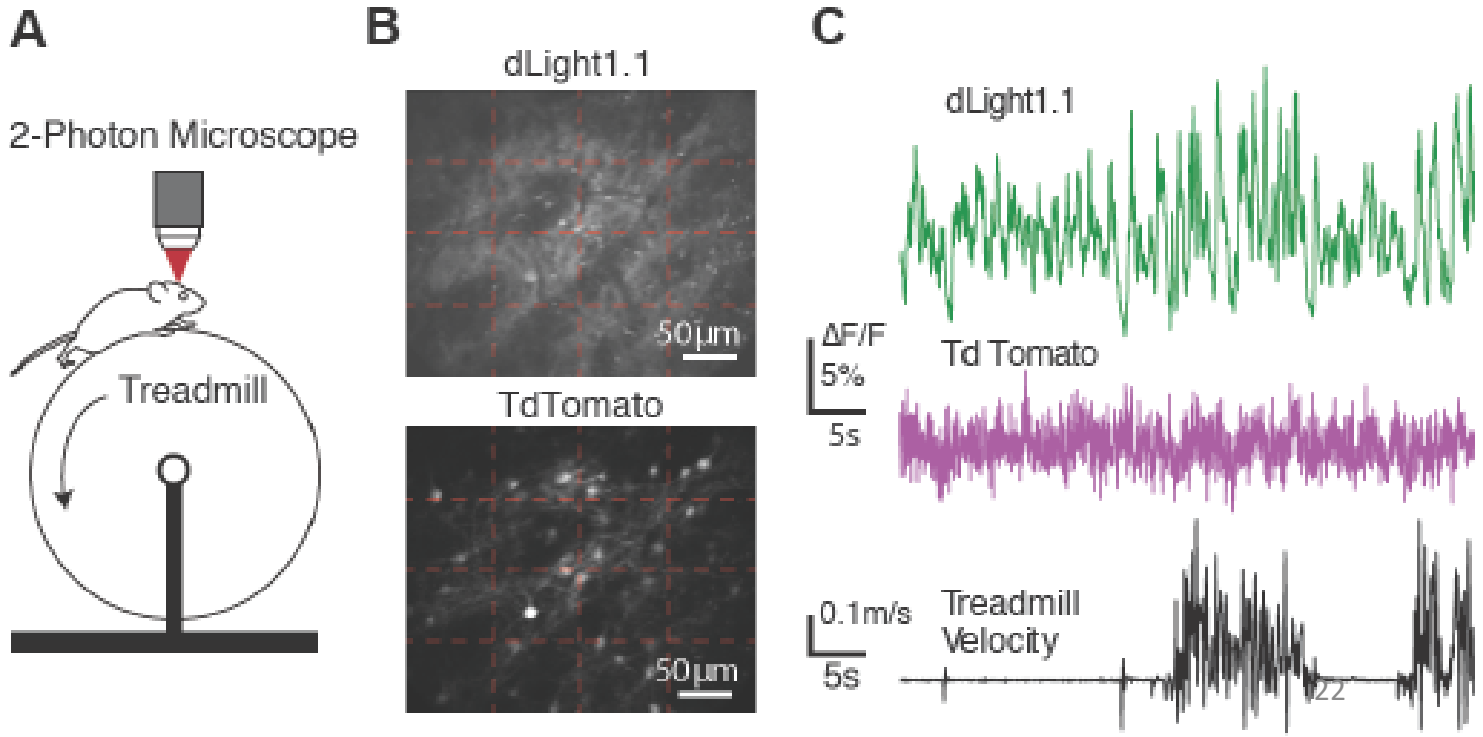


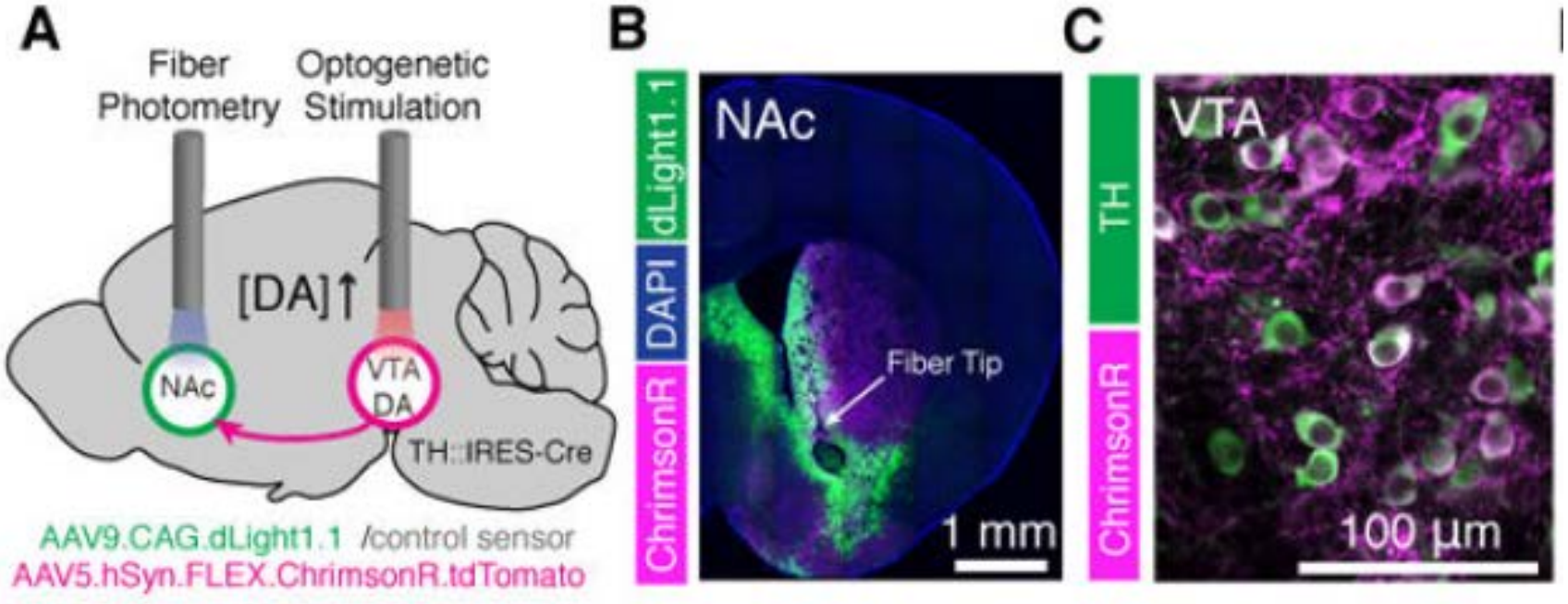
# Endogenous DA release: in vivo

- 2-photon imaging
- Mouse dorsal striatum
- Rest and self-initiated locomotion
- AAV9.*hSynapsin1*.

dLight1.2



# Combination with optogenetics



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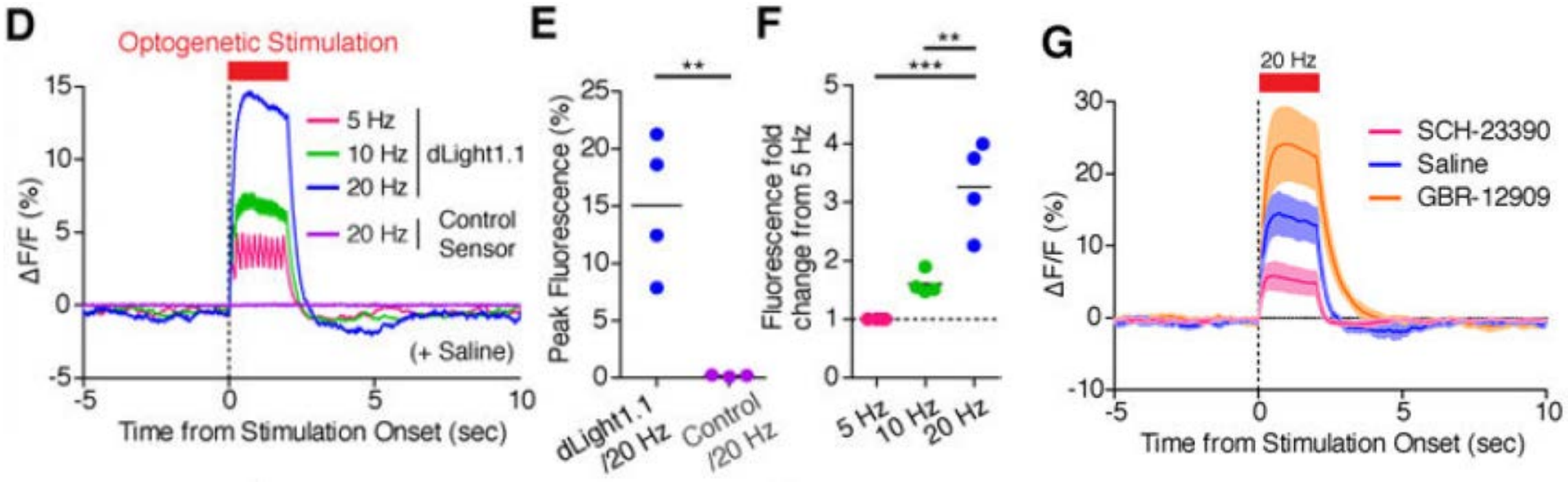
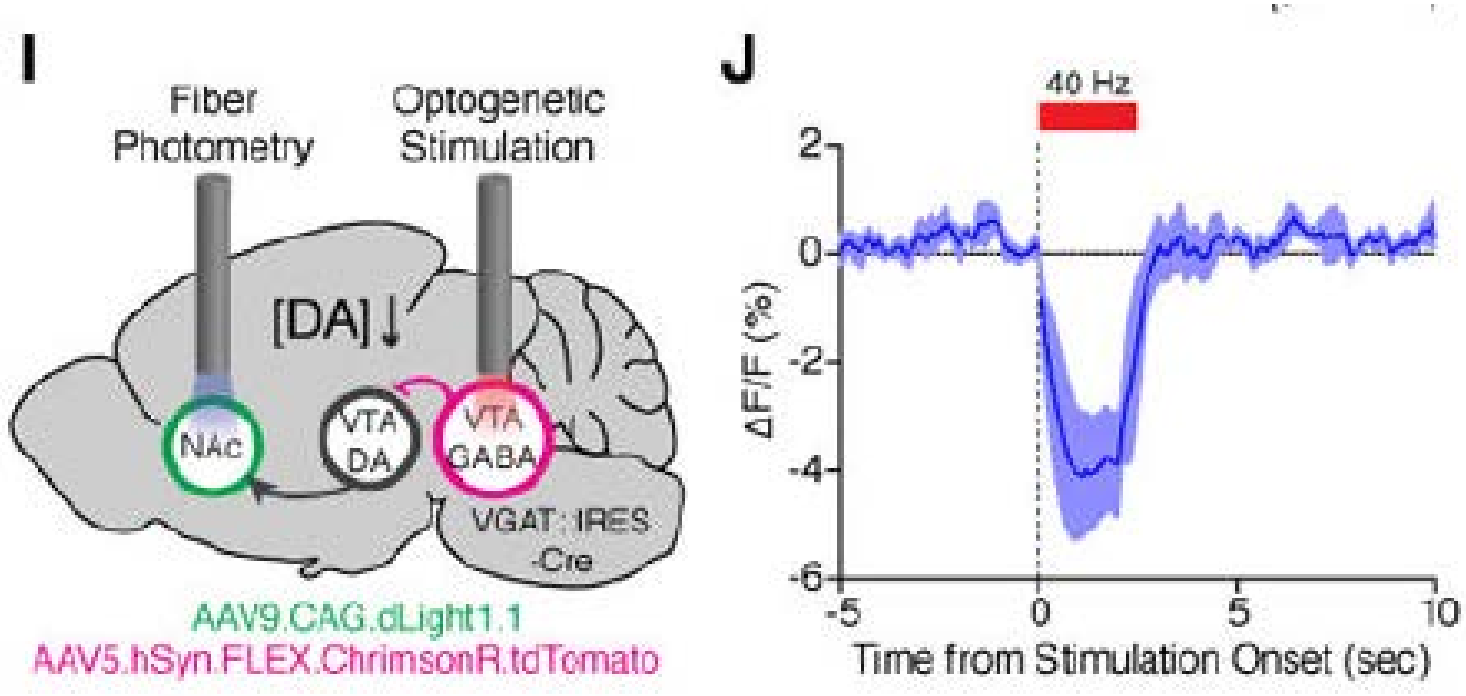


# Combination with optogenetics

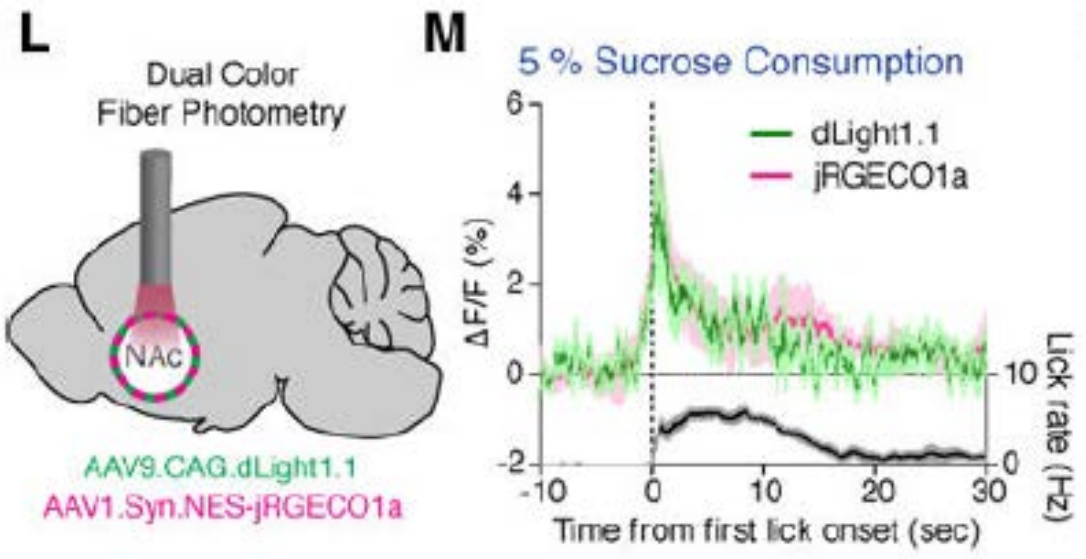
inhibition of DA transients



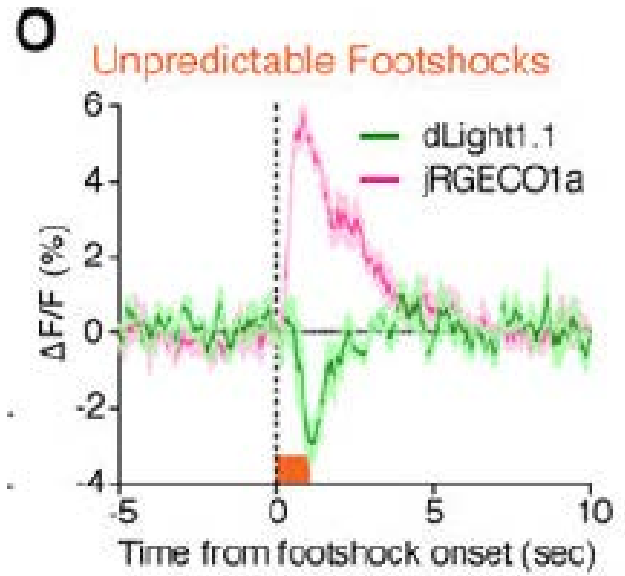

dLight can report bidirectional changes in local DA release.

# Combination with calcium imaging

- dLight1.1 > local DA release
- Red-shifted calcium indicator jRGECO1a > neuronal activity
- Dual color fiber photometry



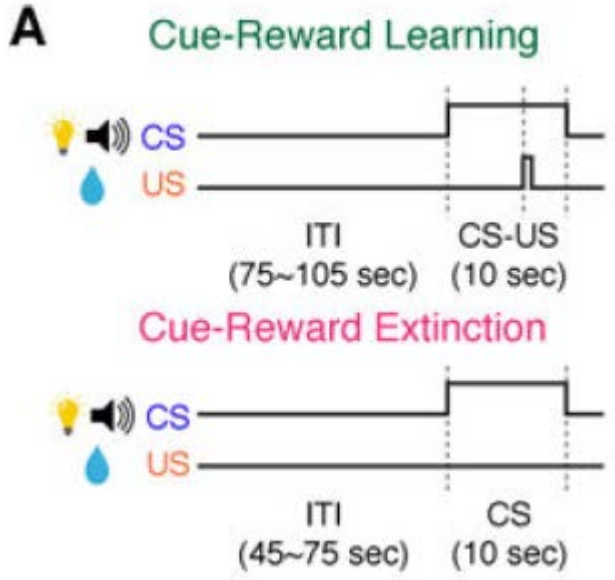

concordant

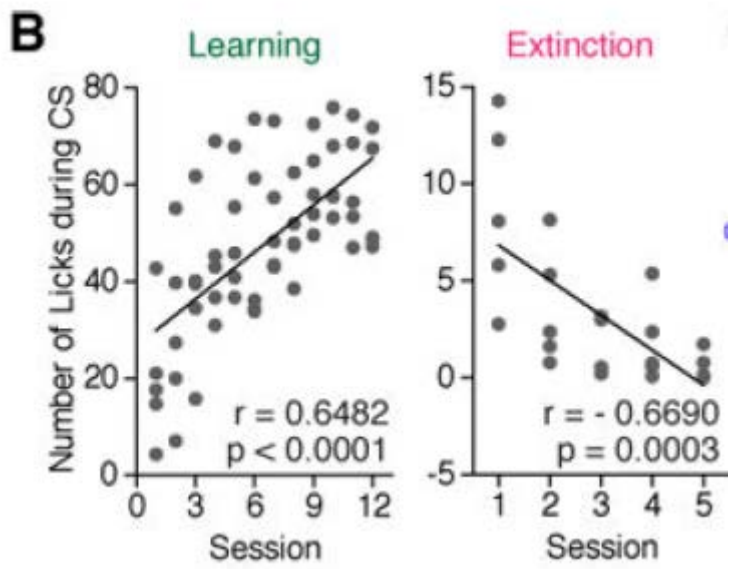


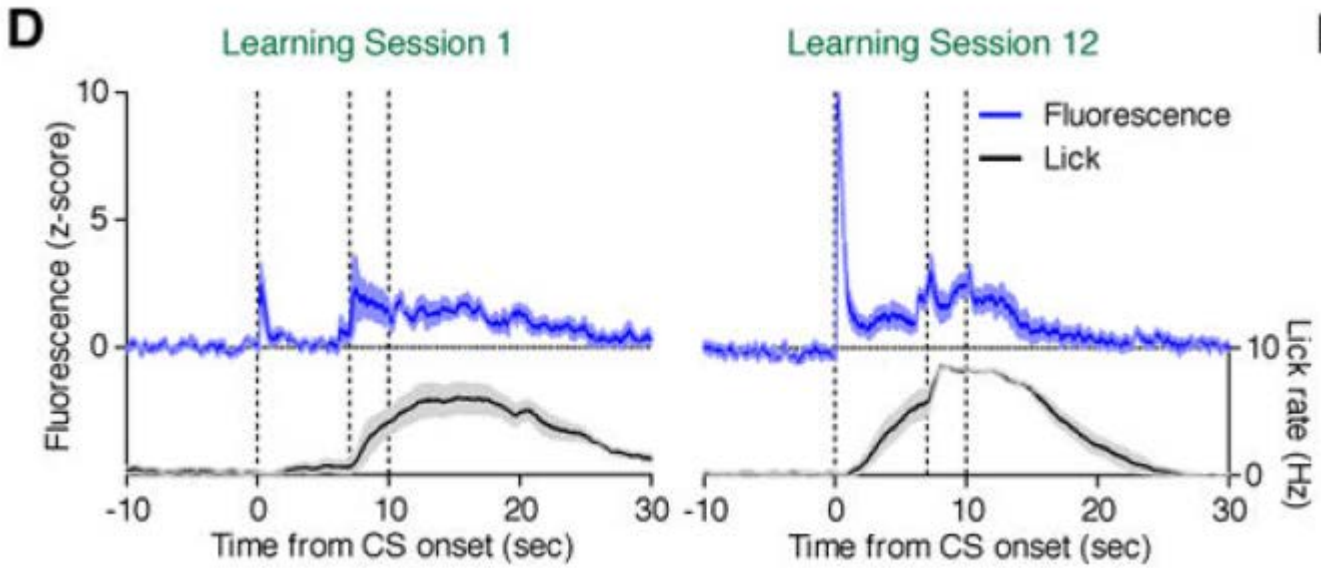

Dissociation DA vs. local activity

# Chronic imaging

- Cue-reward learning
- Repeated fiber photometry recordings in NAc



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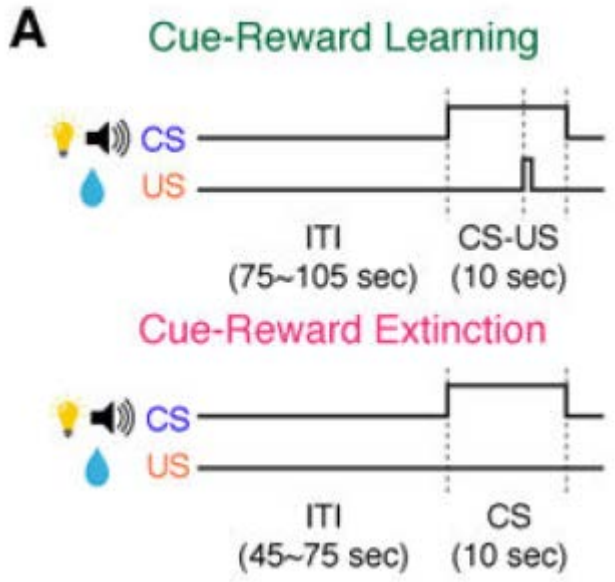


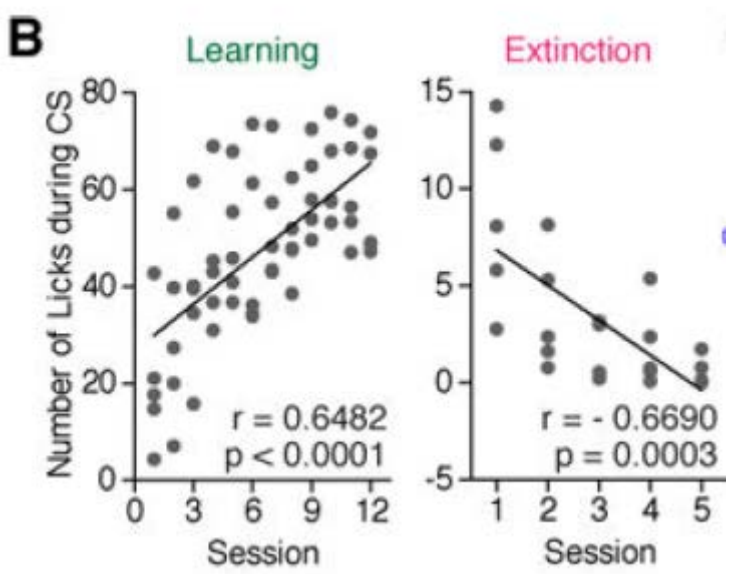


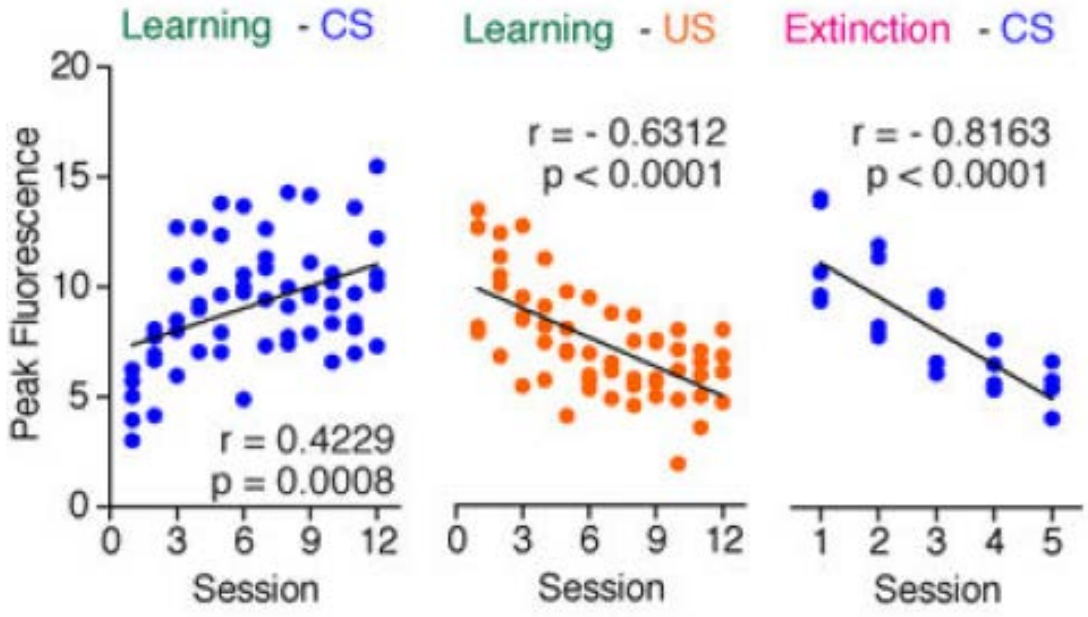

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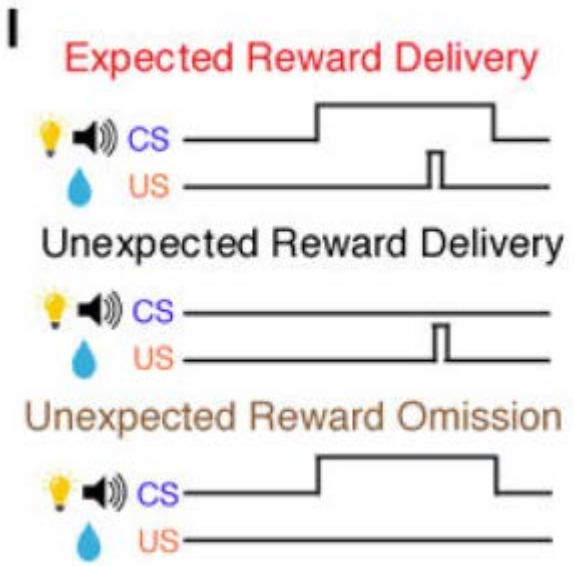

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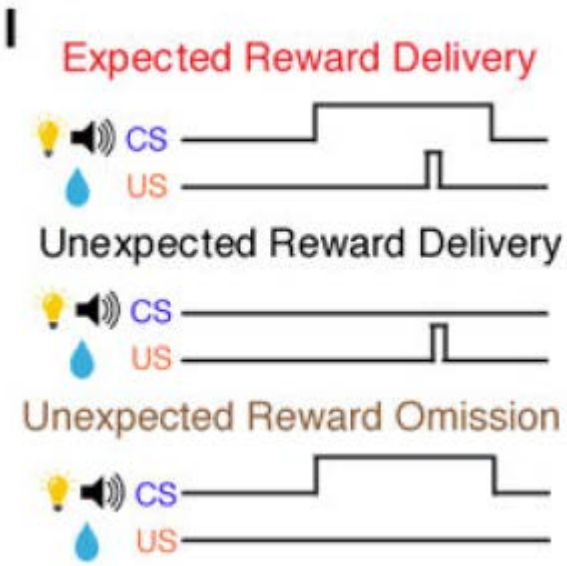



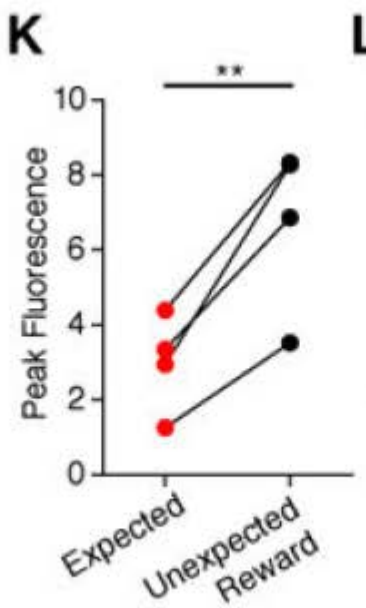


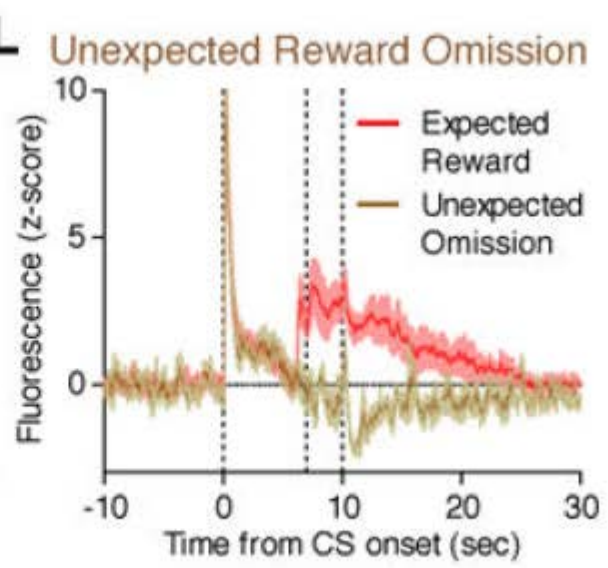


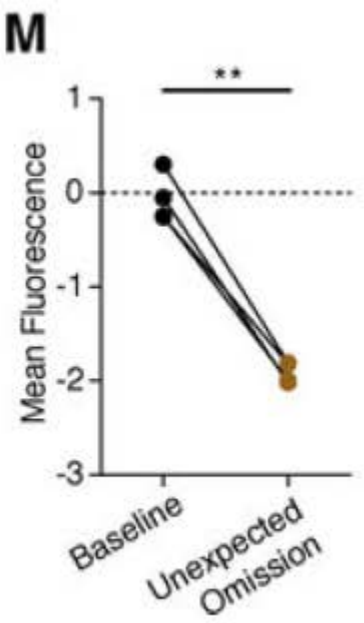

# Conclusions

- New class of genetically encoded indicators
- High resolution imaging of DA dynamics in brain slices and behaving mice
- Submicromolar affinity
- Fast kinetics (10ms on, 100ms off)
- Detect physiologically relevant DA levels
- Higher molecular specificity than existing electrochemical or cell based probes
- Combination with Calcium imaging and optogenetics

# An application…

## Dopamine neurons projecting to medial shell of the nucleus accumbens drive heroin reinforcement

**Julie Corre[1], Ruud van Zessen[1], Michaël Loureiro[1], Tommaso Patriarchi[2], Lin Tian[2], Vincent Pascoli[1], Christian Lüscher[1,3]***

[1]Department of Basic Neurosciences, Medical Faculty, University of Geneva, Geneva, Switzerland; [2]School of Medicine, Department of Biochemistry and Molecular Medicine, University of California Davis, California, United States; [3]Service of Neurology, University of Geneva Hospital, Geneva, Switzerland

eLife, 2018

# Dopamine in heroin reinforcement

- DA hypothesis

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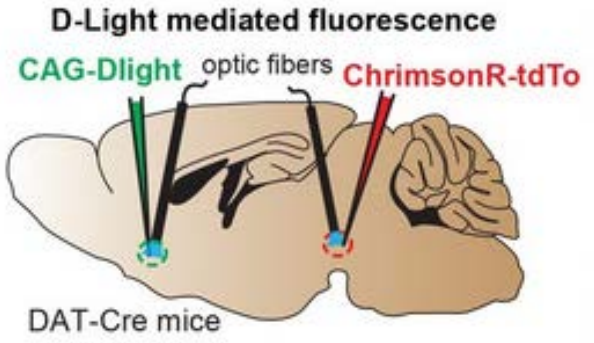
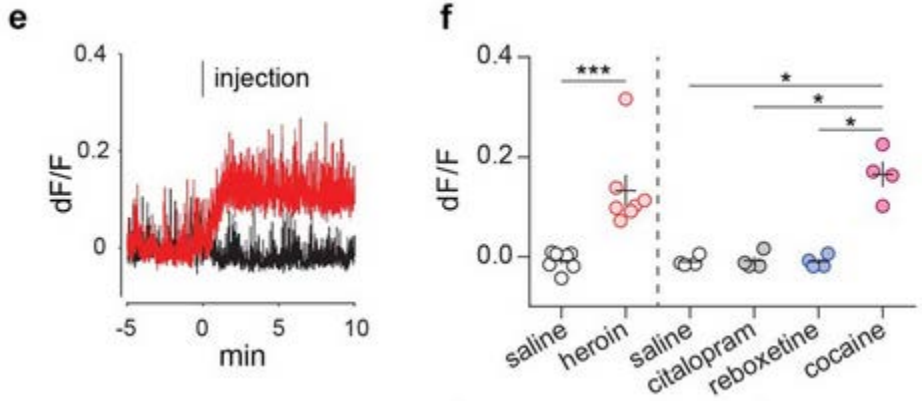
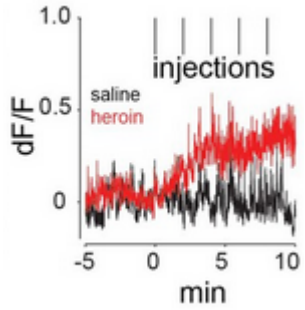



NAc dopamine

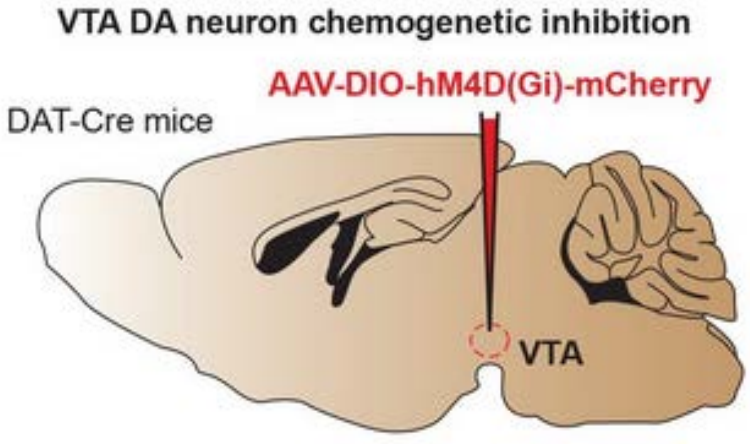



VTA activity (calcium)



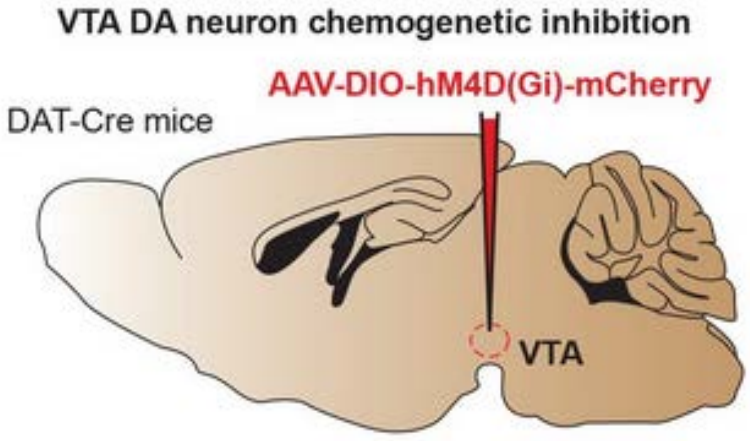
# Dopamine in heroin reinforcement

- DA hypothesis
- Causal relationship between enhanced mesolimbic dopamine and heroin reinforcement

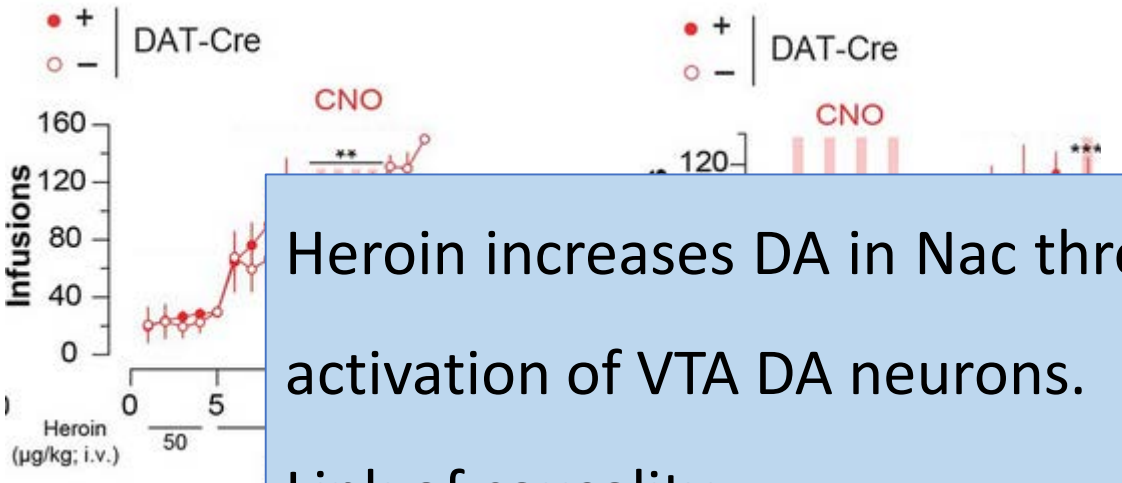


# Dopamine in heroin reinforcement

- DA hypothesis
- Causal relationship between enhanced mesolimbic dopamine and heroin reinforcement




Late early




Heroin increases DA in Nac through activation of VTA DA neurons.

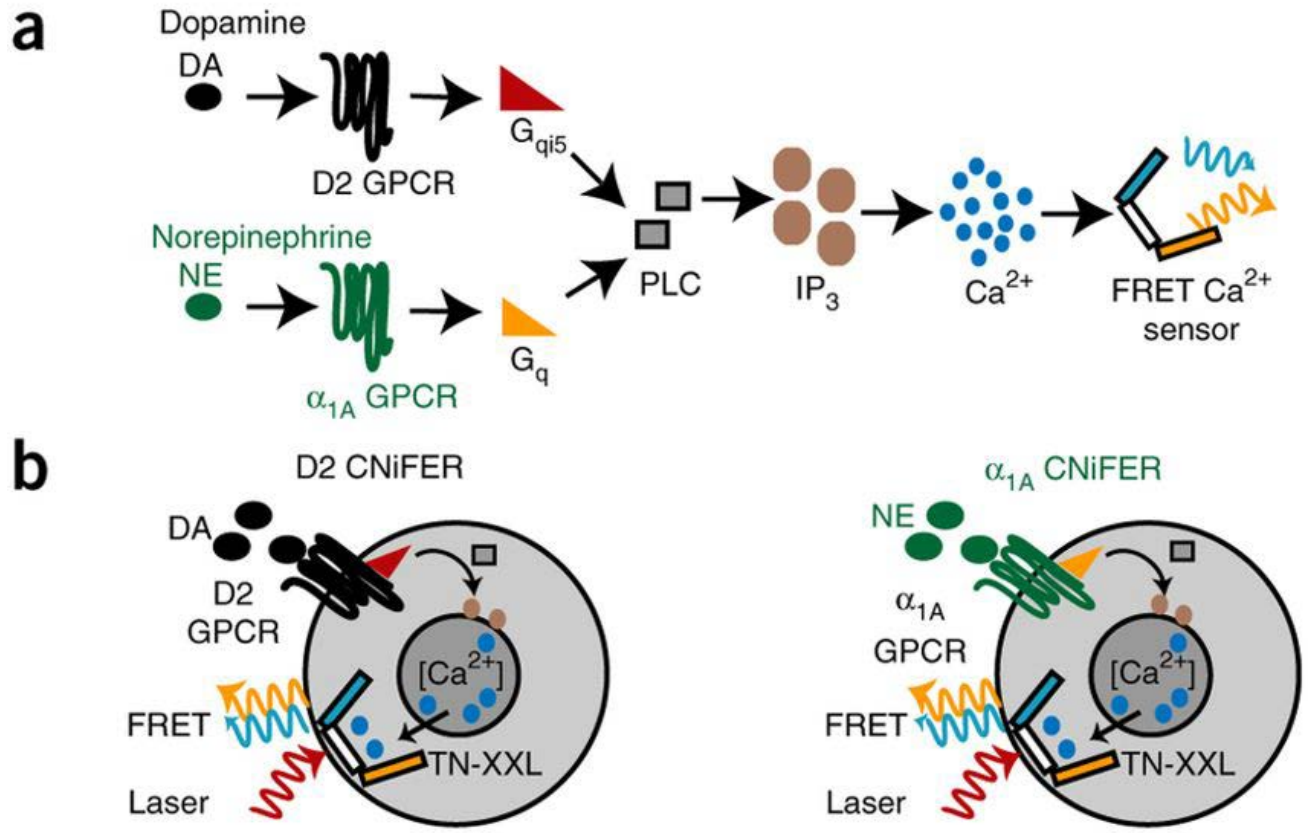
Link of causality.

# Comparison

- Microdialysis
- Fast-scan cyclic voltammetry
- CNiFER (**c**ell-based **n**euro-transmitter **f**luorescent **e**ngineered **r**eporter)

# Comparison

- Microdialysis
- Fast-scan cyclic voltammetry
- CNiFER




- injected cell based system
- high molecular specificity (in vitro sensitivity for respecitve monoamine: nanomolar! 2.5nm for DA and 19nm for NE – vs. dLight1.1: 330nm)
- poor temporal resolution

(2s on, 20s off vs dLight: 10ms on, 100ms off)



Muller, Nat Methods, 2014

# Were they the first/only ones?

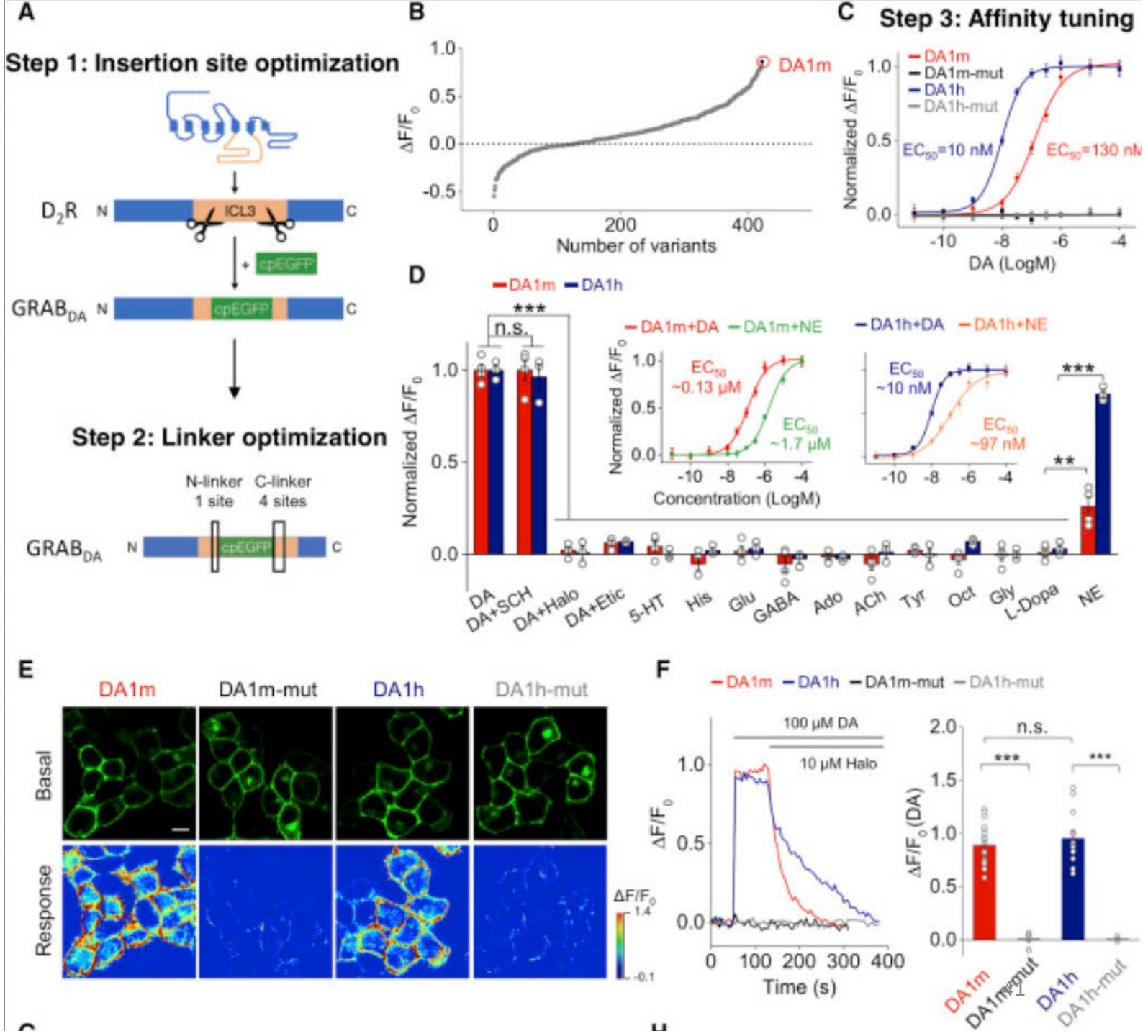
# A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in Flies, Fish, and Mice

Fangmiao Sun [13] • Jianzhi Zeng [13] • Miao Jing [13] • ... Anatol C. Kreitzer • Guohong Cui • Yulong Li [14] • Show all authors • Show footnotes

Cell, July 2018

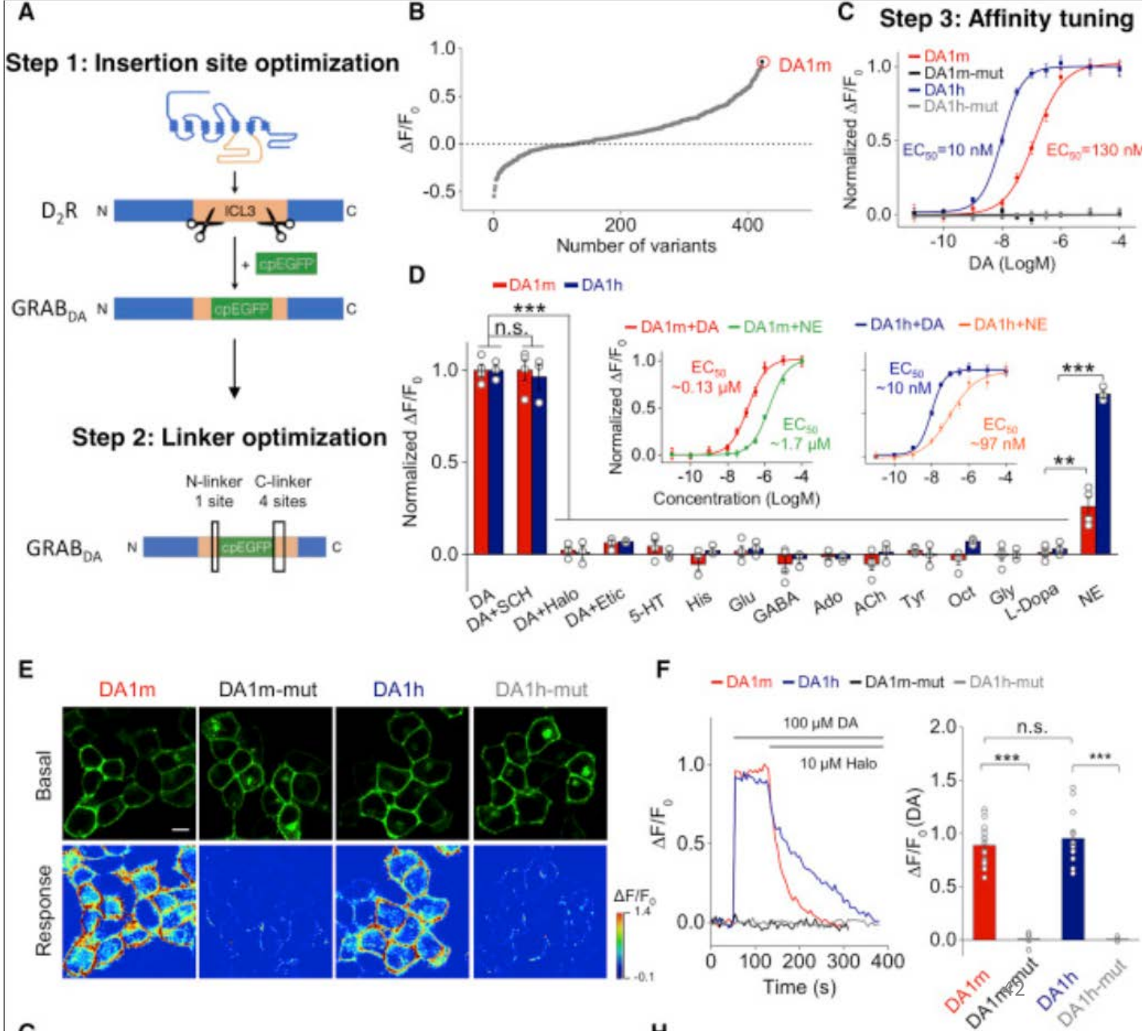
$GRAB_{DA}$ (genetically encoded, GPCR activation based DA sensor)

# $GRAB_{DA}$


A
Step 1: Insertion site optimization
D2R
ICL3
cpEGFP
GRABDA
Step 2: Linker optimization
N-linker 1 site
C-linker 4 sites
B
DA1m
ΔF/F0
Number of variants
C
Step 3: Affinity tuning
DA1m
DA1m-mut
DA1h
DA1h-mut
EC50=10 nM
EC50=130 nM
Normalized ΔF/F0
DA (LogM)
D
DA1m DA1h
n.s.
DA1m+DA DA1m+NE
DA1h+DA DA1h+NE
EC50 ~0.13 μM
EC50 ~1.7 μM
EC50 ~10 nM
EC50 ~97 nM
Concentration (LogM)
DA
DA+SCH
DA+Halo
DA+Etic
5-HT
His
Glu
GABA
Ado
ACh
Tyr
Oct
Gly
L-Dopa
NE
E
DA1m
DA1m-mut
DA1h
DA1h-mut
Basal
Response
ΔF/F0
F
DA1m DA1h DA1m-mut DA1h-mut
100 μM DA
10 μM Halo
Time (s)
ΔF/F0 (DA)
n.s.

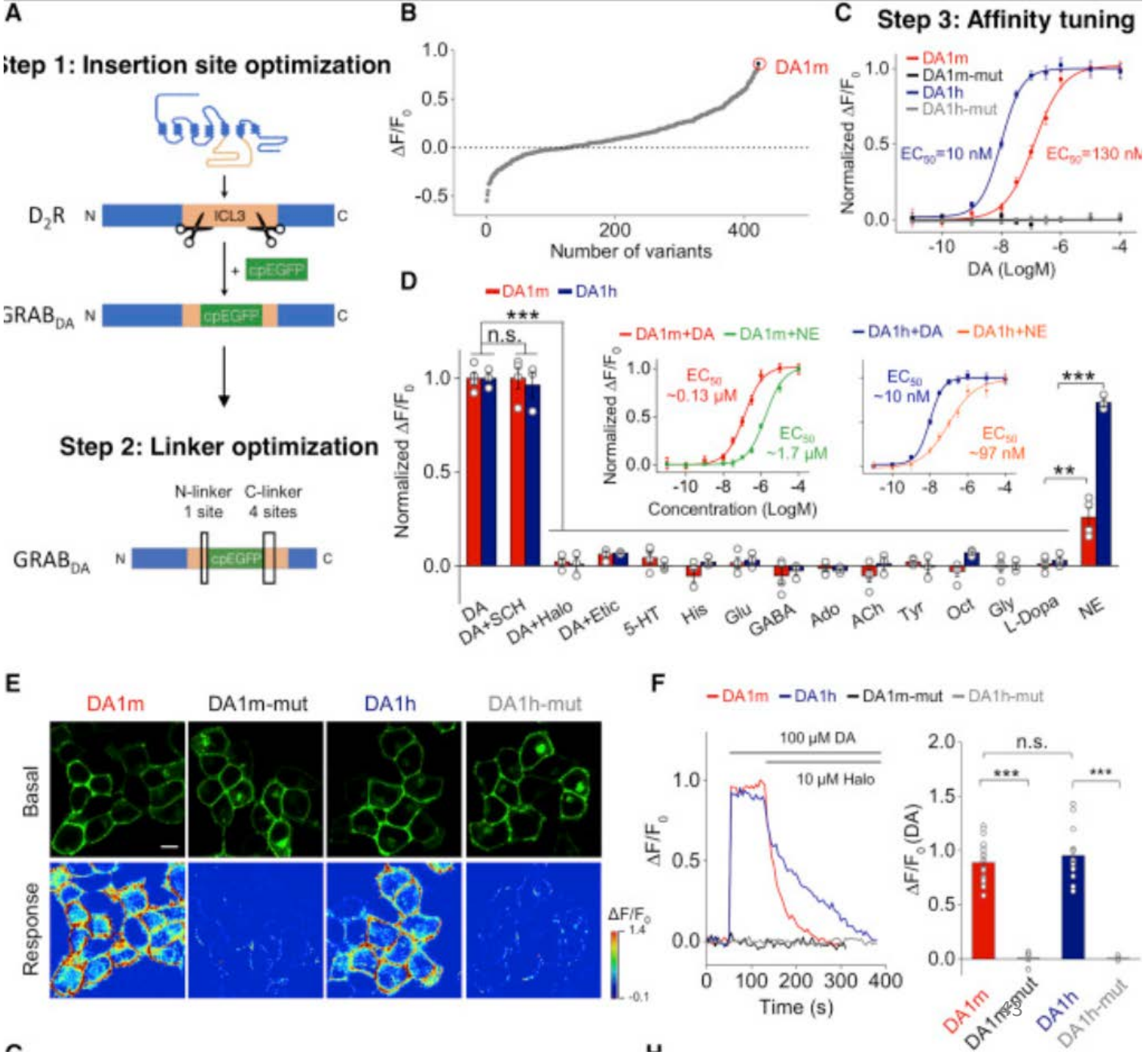
# $GRAB_{DA}$

- Cultured HEK293 cells and neurons
- Acute brain slices
- Drosophila, zebrafish, freely moving mice
- Combination with optogenetics in Pavlovian conditioning and male mating behavior

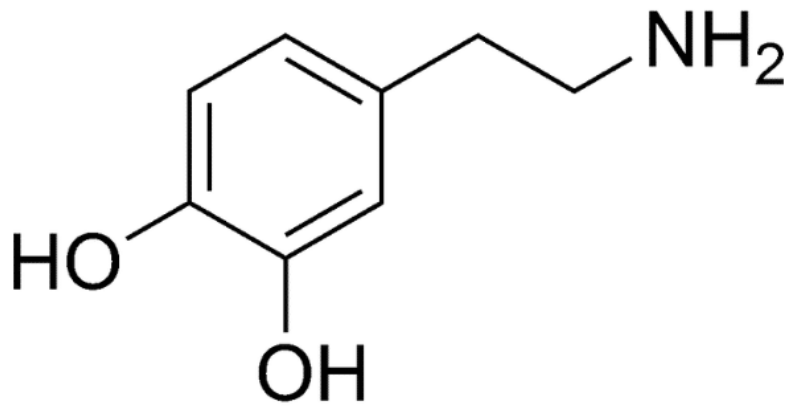


# $GRAB_{DA}$

"A recently described fluorescent DA sensor (named dLight) utilizes a similar detection strategy to report DA signaling *in vivo* (Patriarchi et al., 2018
). The applied dLight variants report *in vivo* DA dynamics in rodent brains with similar kinetics and signal-to-noise ratio as $GRAB_{DA}$ sensors. However, the $GRAB_{DA}$ sensors have been optimized for brightness, have more consistent sensitivity ($EC_{50}$) to DA across different cell types, and have proven efficacy in multiple organisms *in vivo*.»



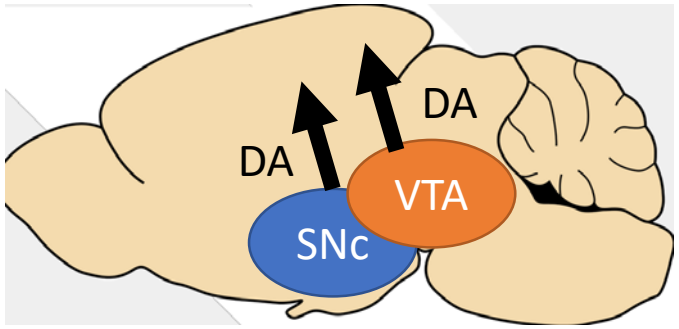
# Thank you for your attention!



# Addendum

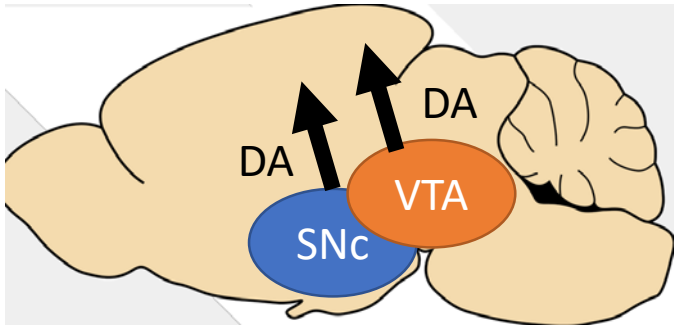
# Cellular level imaging

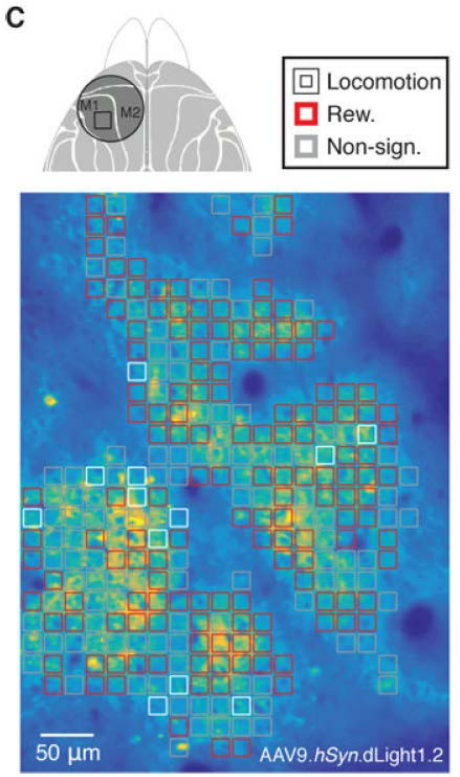
- 2-photon imaging
- In cortex
- Visuomotor association task

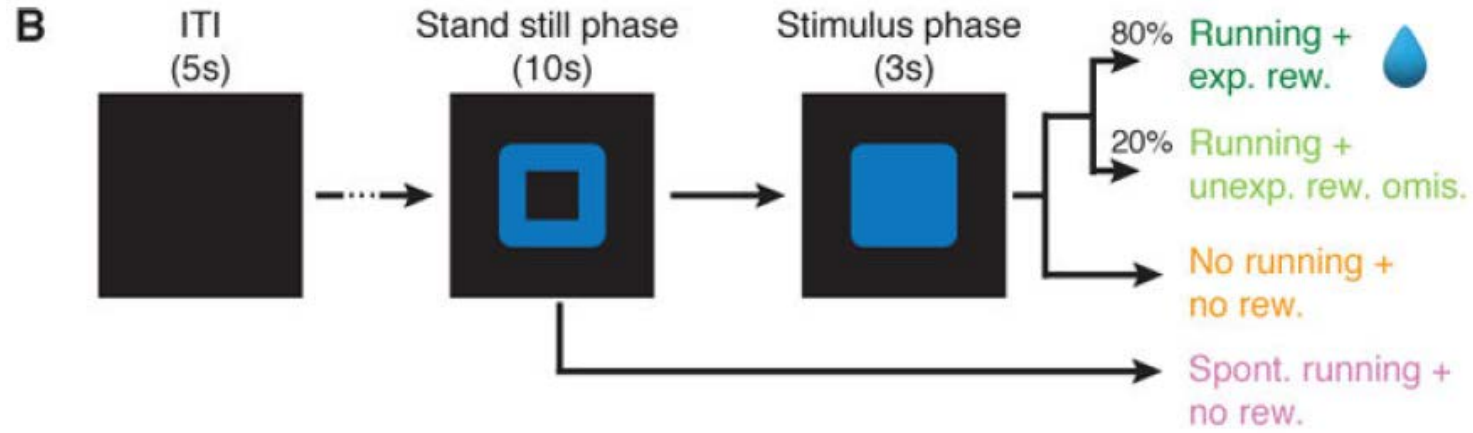


# Cellular level imaging

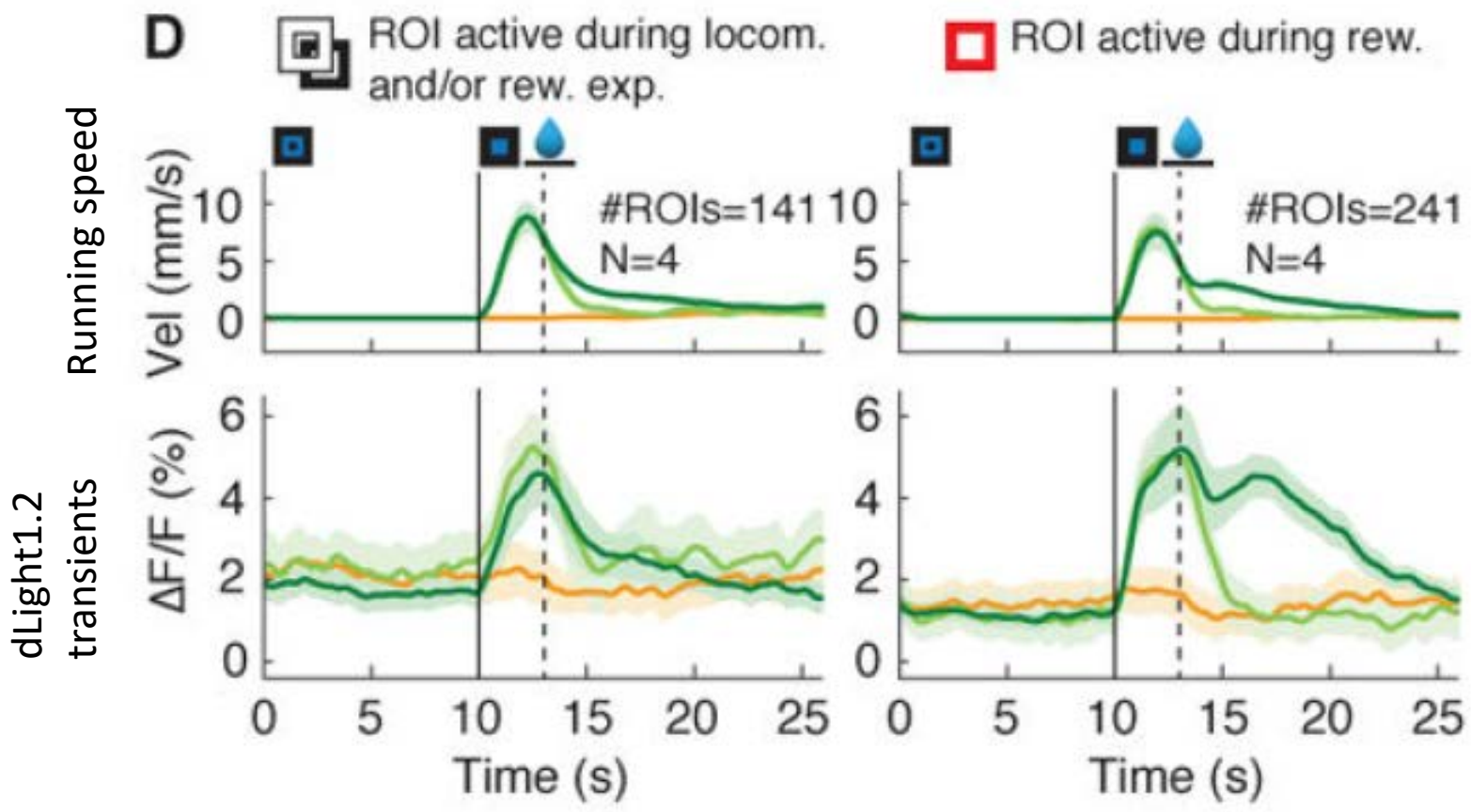
- 2-photon imaging
- In cortex
- Visuomotor association task



# Cellular level imaging

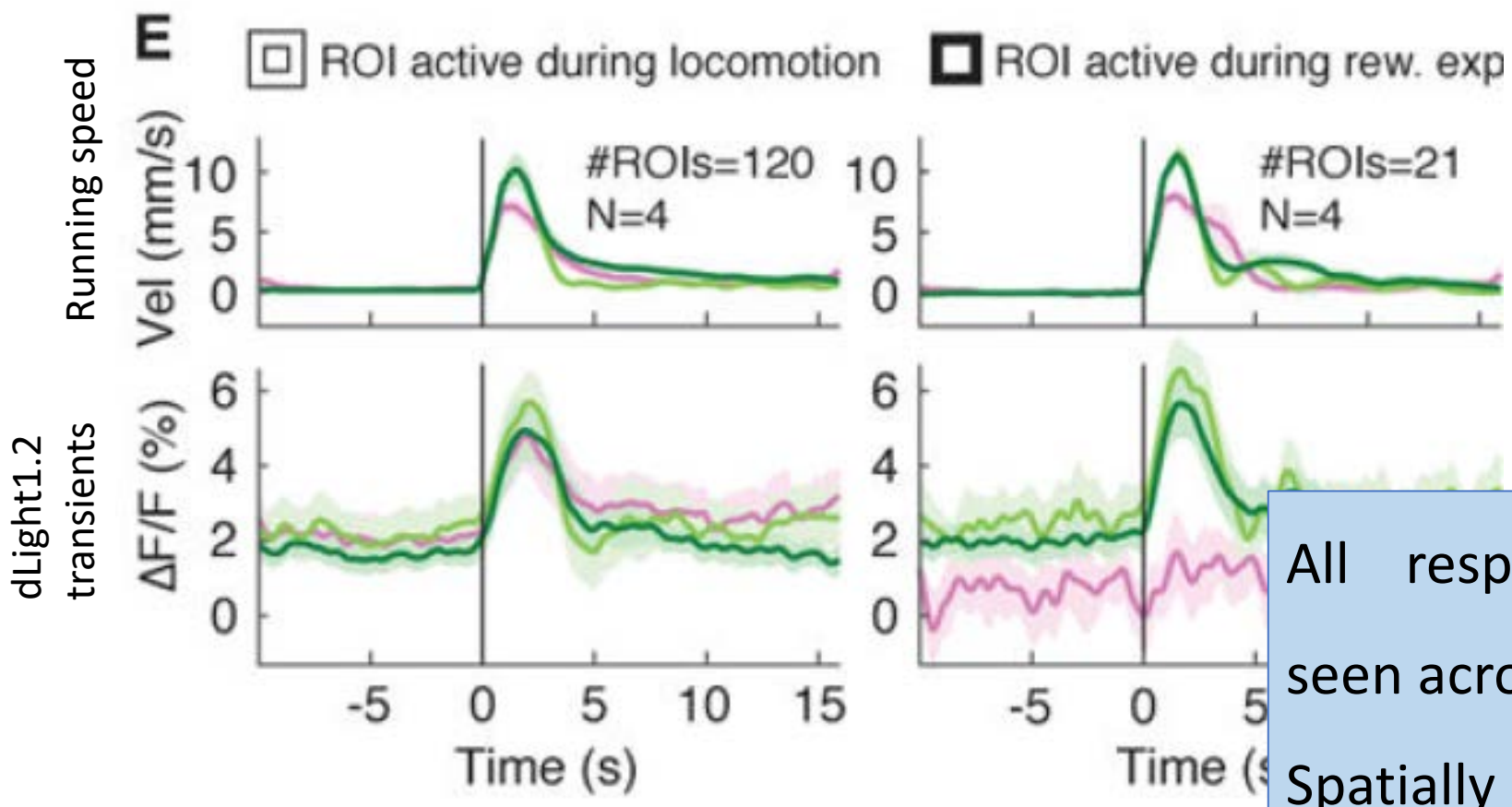



**Reward**

**Unexpected omission**

**Missed trials**

# Cellular level imaging




**Reward**

**Unexpected omission**

**Spontaneous running**

All response types consistently seen across animals.

Spatially intermingled.