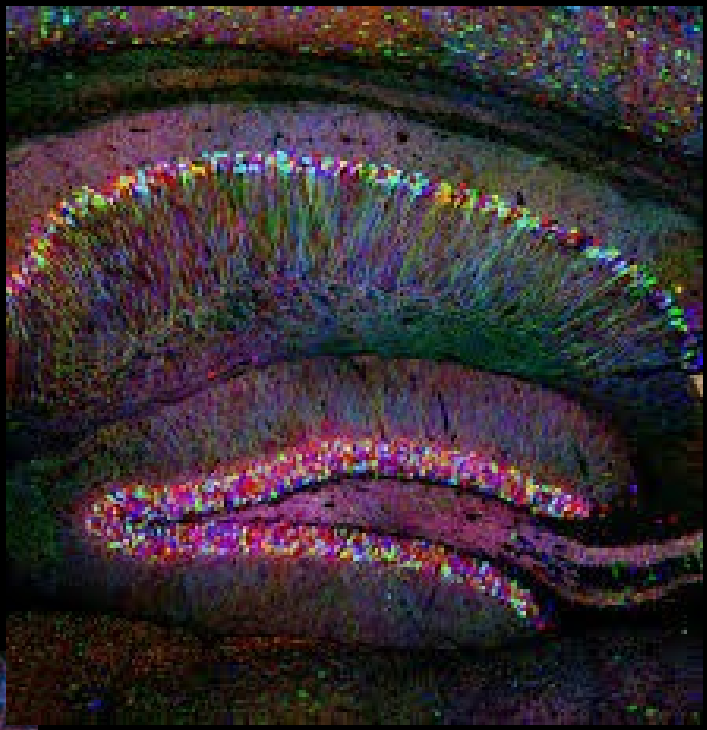
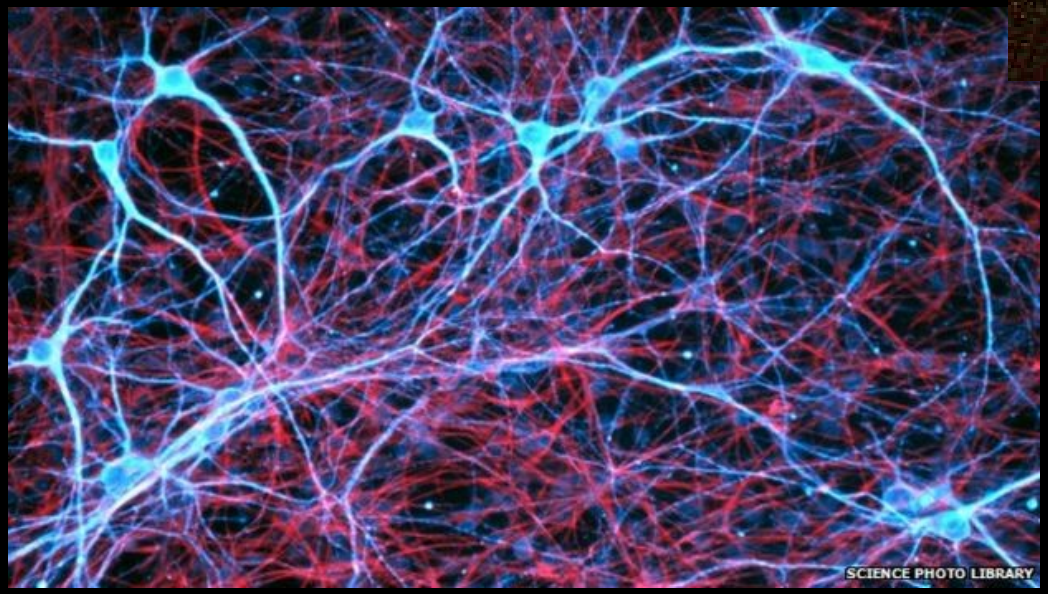
Imaging Voltage in Neurons

Overview and recent advances

Technical Journal Club
January 26, 2016
Angie Wulf

motivation

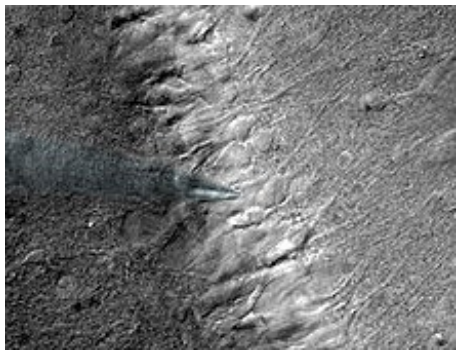




Methods to measure neuronal activity

- electrodes
  - extracellular: invasive

  - intracellular: limited to cell soma and large dendrites, few cells



- calcium imaging

Methods to measure neuronal activity

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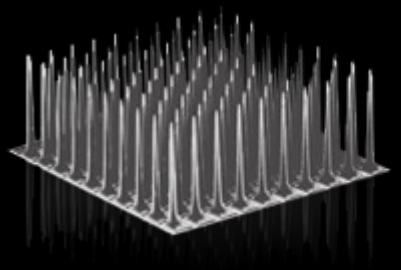
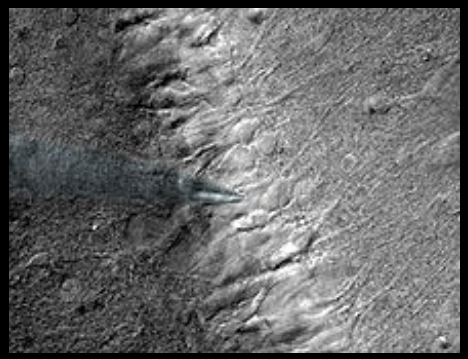


  - intracellular: limited to cell soma and large dendrites, few cells



- calcium imaging
  - Biased to suprathreshold events
  - slow

Methods to measure neuronal activity

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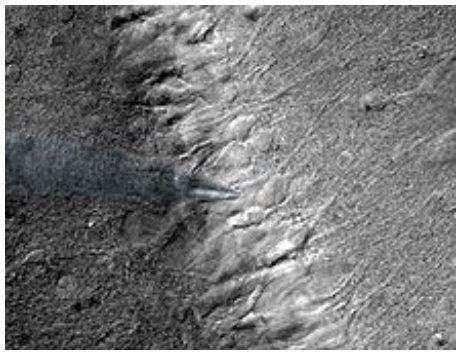


- calcium imaging
  - Biased to suprathreshold events
  - slow

- voltage imaging
  - all events
  - all intracellular compartments
  - many cells

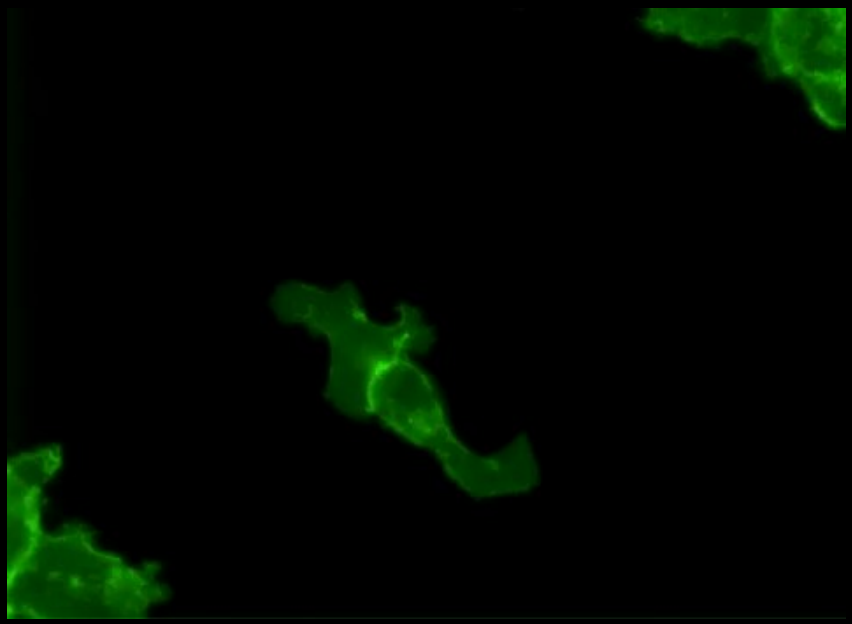
Definition: voltage indicator

- indicator that changes its fluorescence upon changes of membrane voltage

https://www.youtube.com/watch?v=AhufY9wwJzM

Definition: voltage indicator

- indicator that changes its fluorescence upon changes of membrane voltage

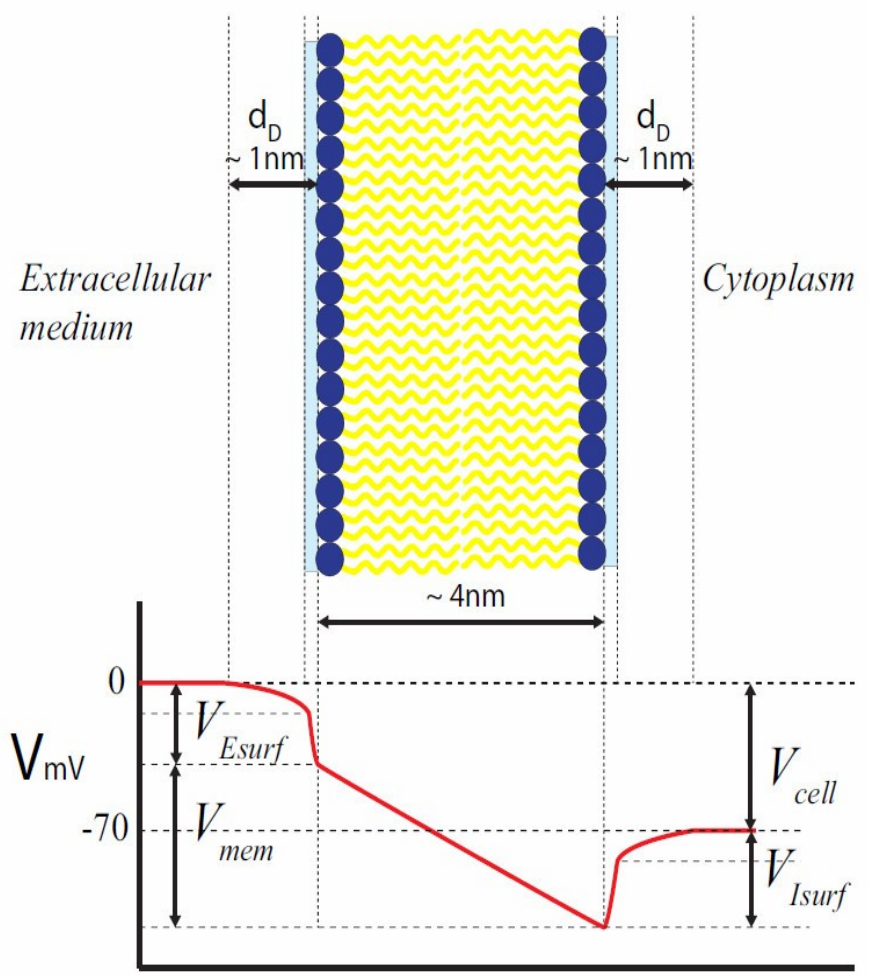


https://www.youtube.com/watch?v=AhufY9wwJzM

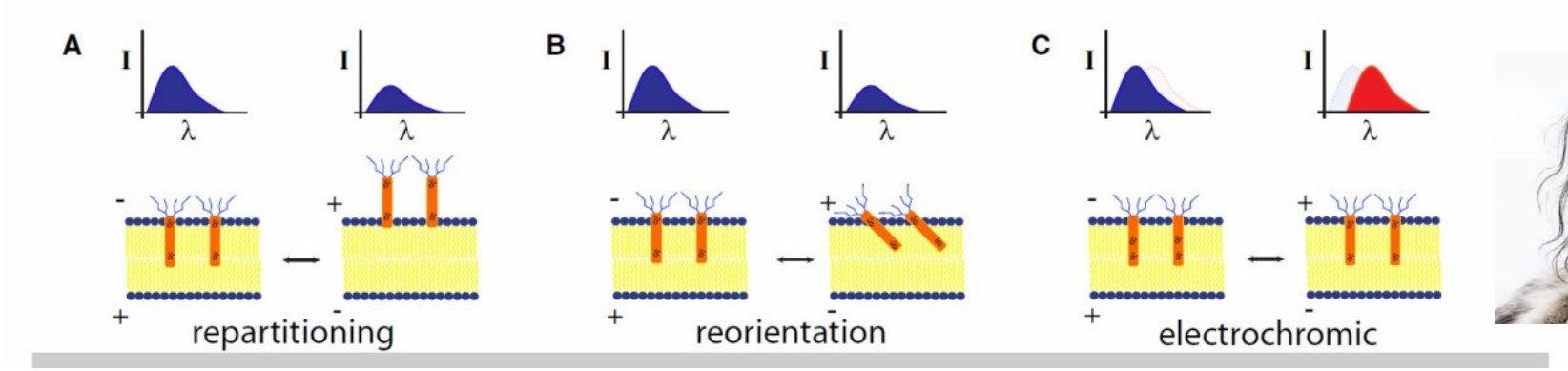
Requirements

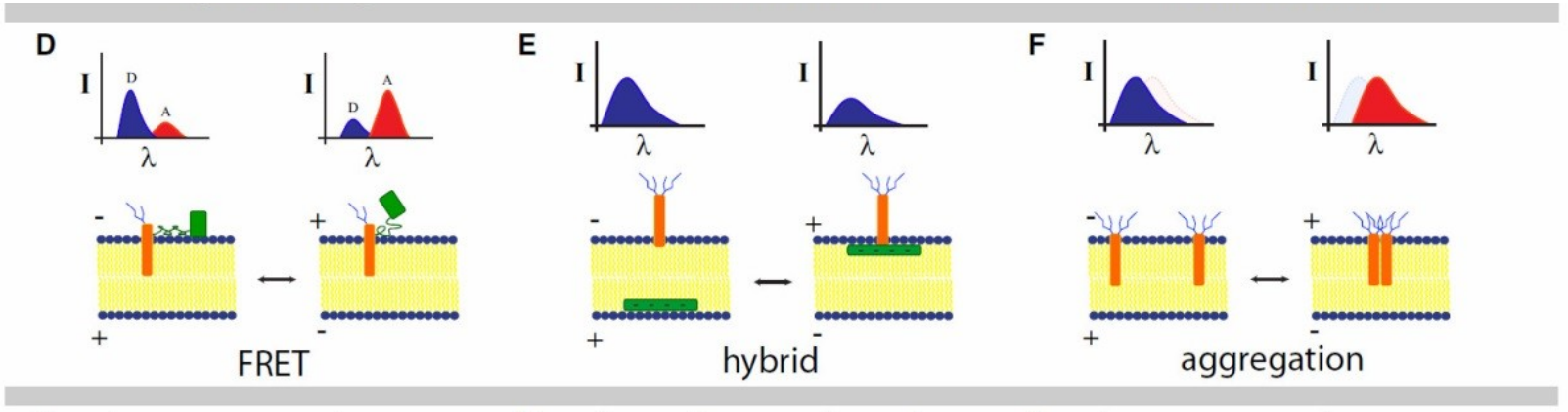
- Challenges
  - Membrane properties
  - (photo-) toxicity
  - Biological activity

- An ideal voltage indicator is
  - Sensitive & bright with good S/N ratio
  - Fast
  - Non-toxic
  - Biologically „inert“



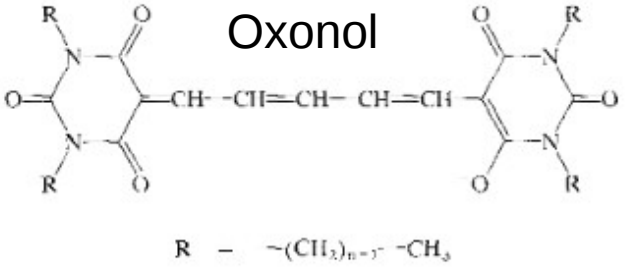
Mechanisms of voltage sensing



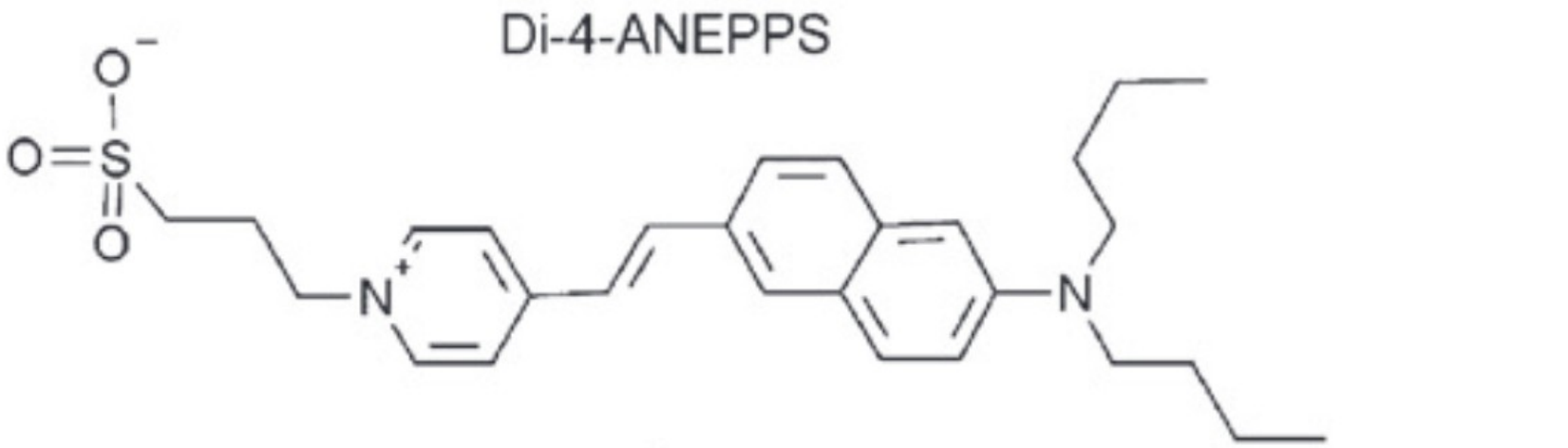


Peterka & Yuste: Imaging Voltage in Neurons, Neuron 69 (2011)

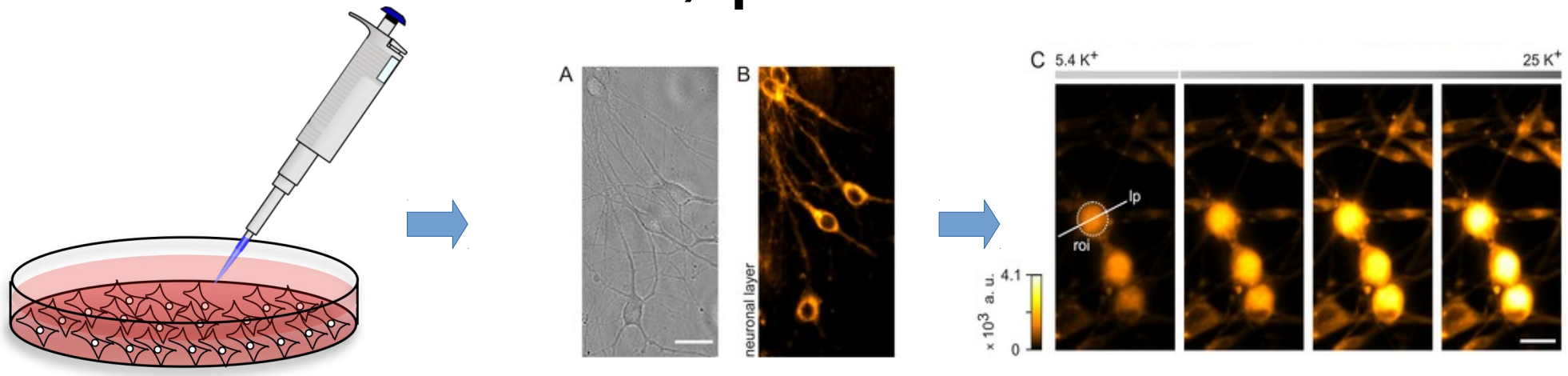
Voltage Sensitive Dyes (VSDs)



- Organic molecules
- Enrich in the membrane
- Change absorption or emission spectra upon changes in the electric field around them



VSDs: use, pro's and con's



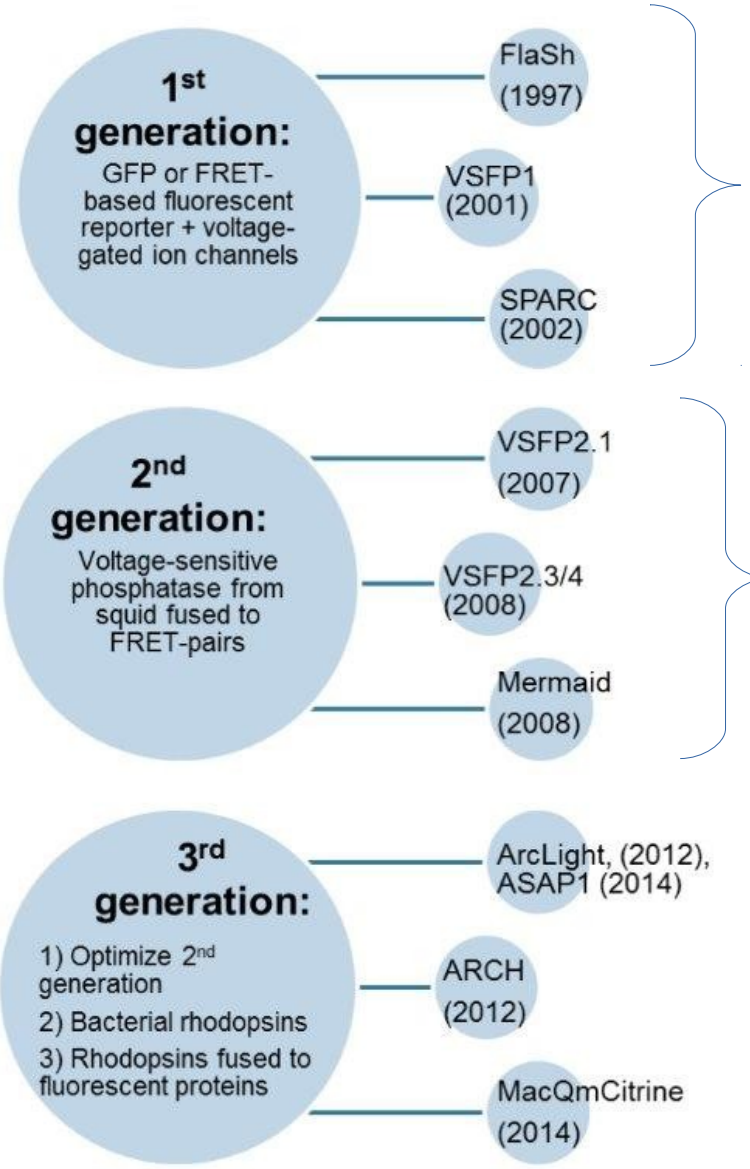
- simple to use
- fast kinetics

- some cells hard to label
- only transient
- no targeting, unspecific labeling

=> excellent for single use where cell labelling is not so important

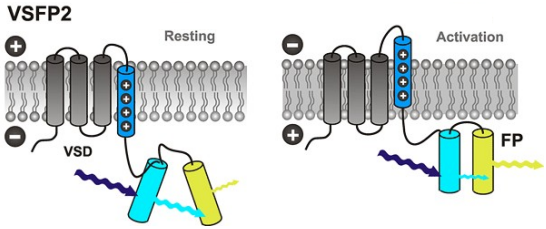
Fairless R et.al. PLoS ONE 8(3): e58260.

Genetically Encoded Voltage Indicators



No expression on the plasma membrane in mammalian cells

Work well in mammalian cells but
- small signals
- slow



Canepari (Ed) et.al. 2015, Chapter 20

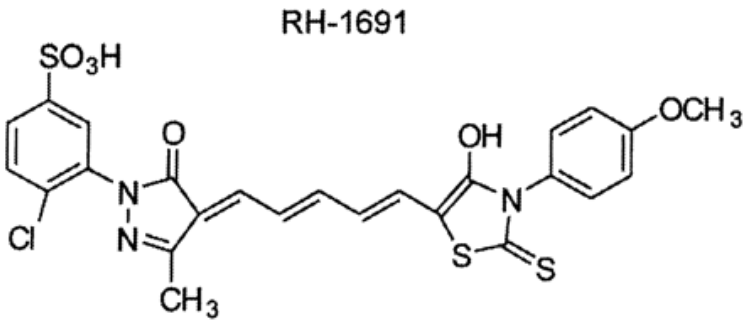
Paper 1

Front Cell Neurosci. 2015 Apr 24;9:147. doi: 10.3389/fncel.2015.00147. eCollection 2015.

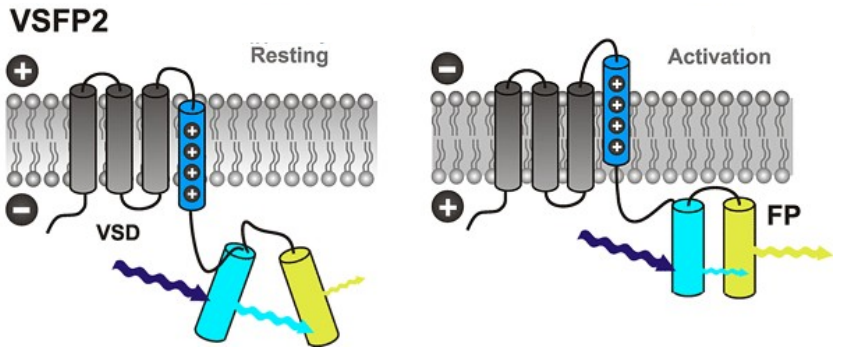
Comparative performance of a genetically-encoded voltage indicator and a blue voltage sensitive dye for large scale cortical voltage imaging.

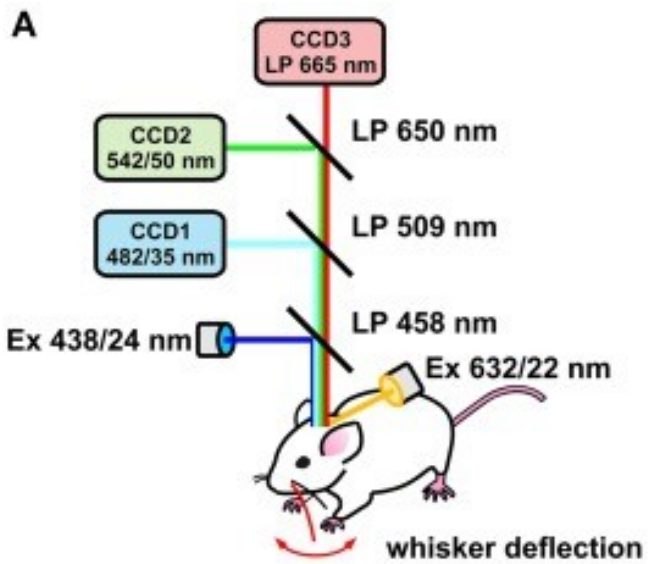
Mutoh H[1], Mishina Y[2], Gallero-Salas Y[3], Knöpfel T[4].

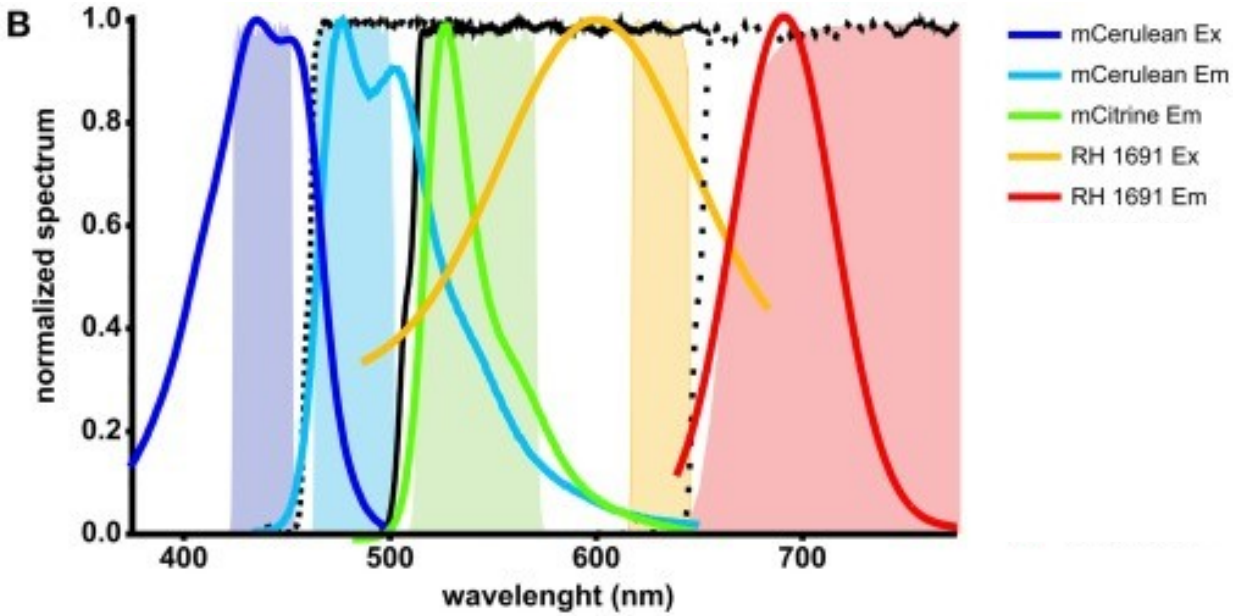
Measurement configuration



vs



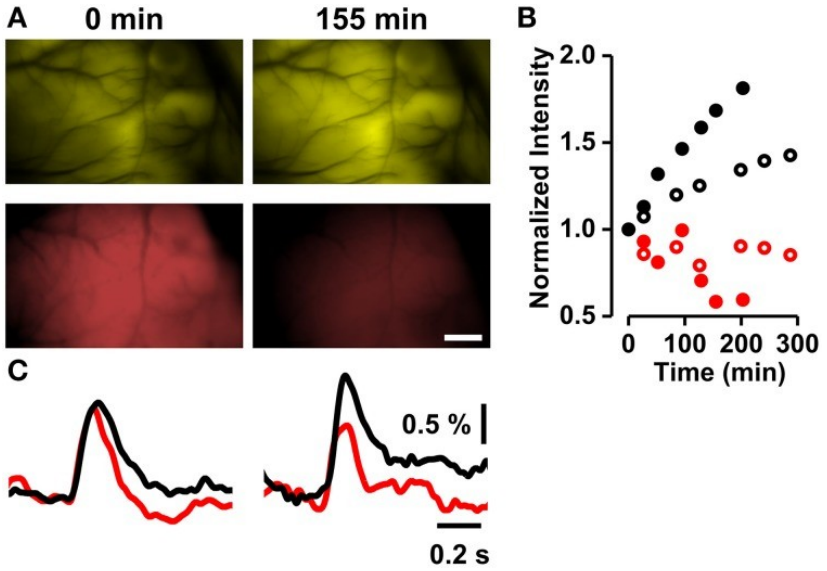


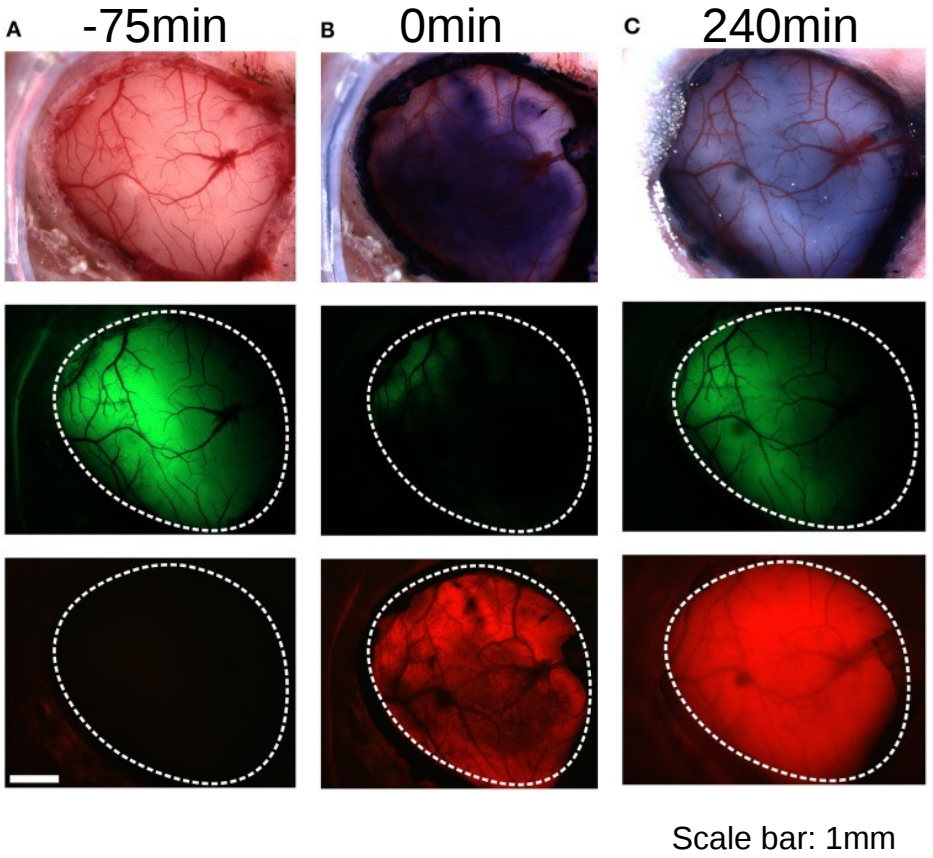


Mesoscopic cortical population voltage imaging

staining and imaging procedure

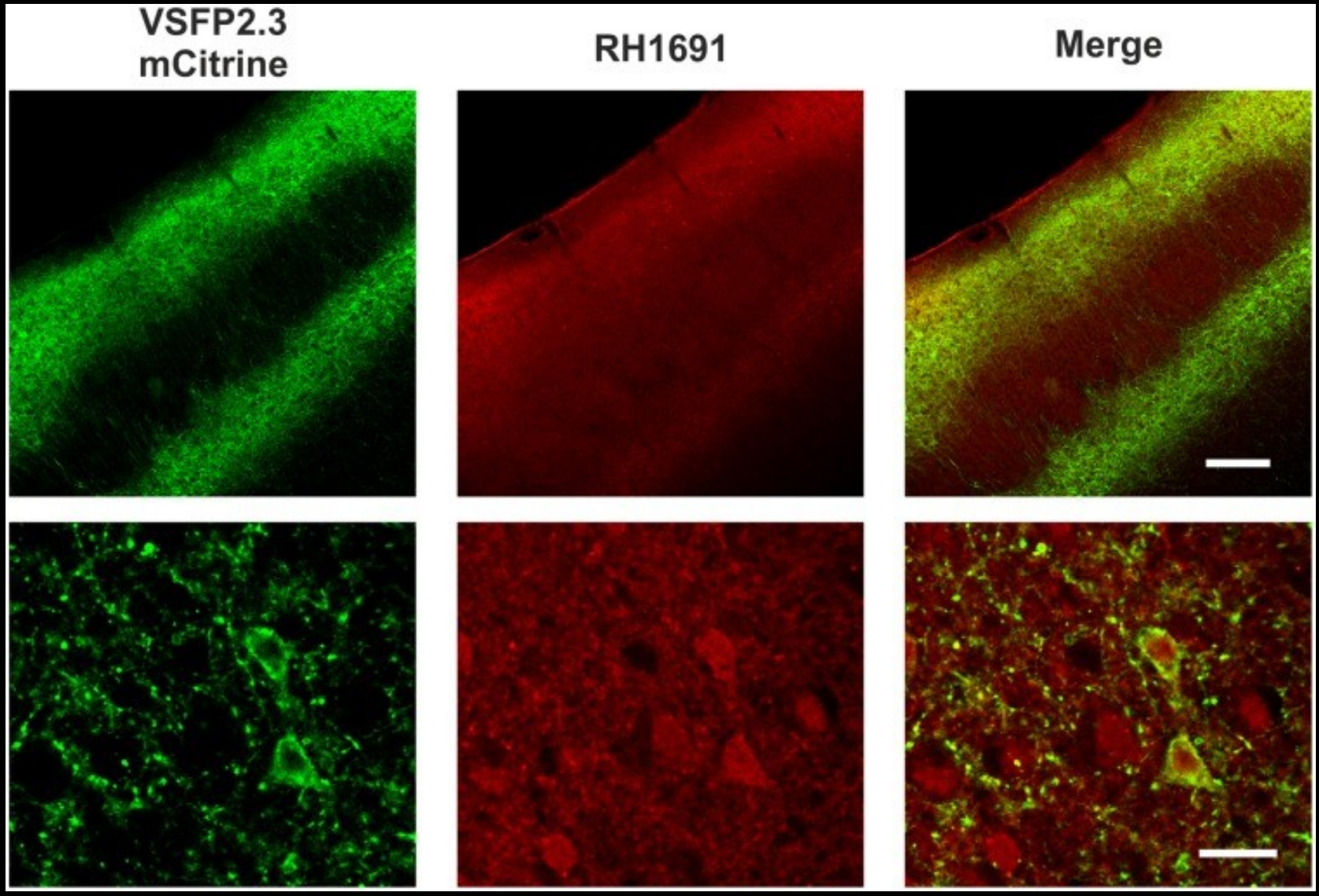
- In utero electroporation of VSFP2
- VSFP2 imaging through intact dura
- dye staining for 1h, 15min wash





Scale bar: 1mm

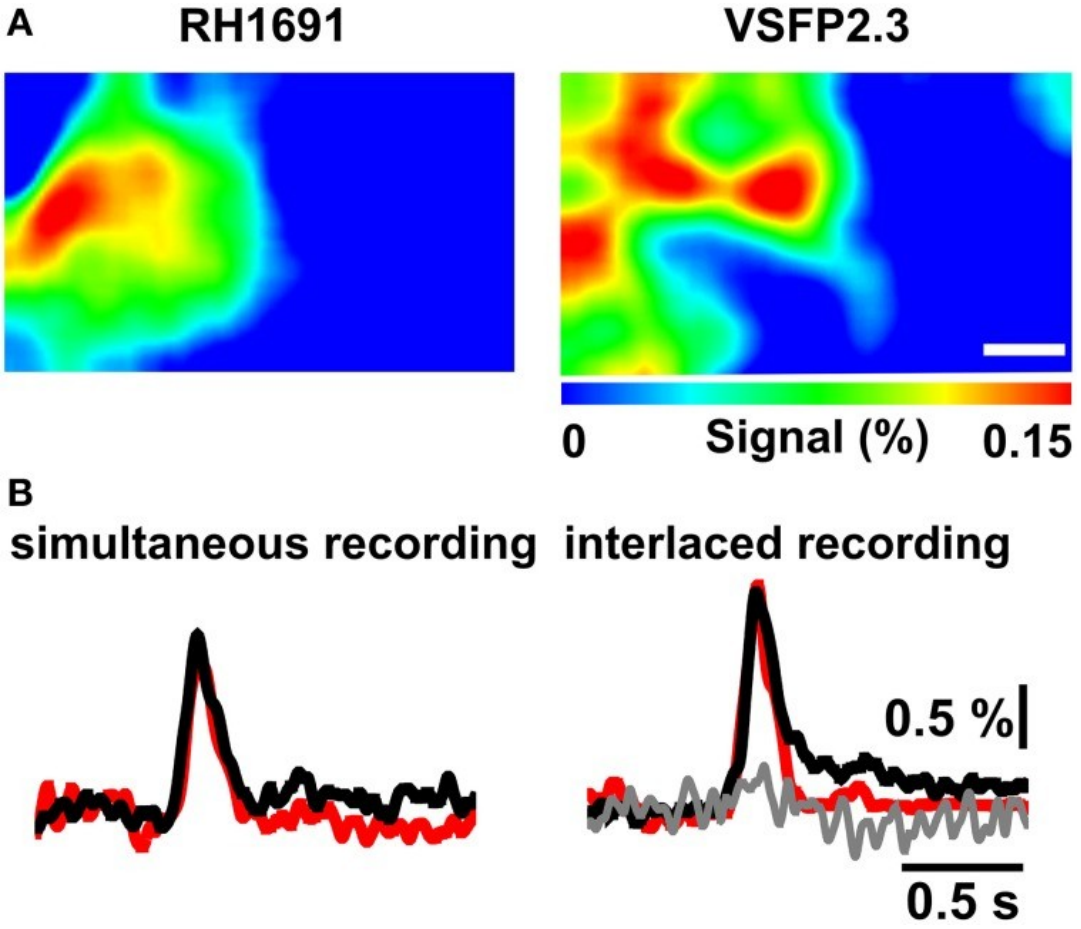
Post-fixation imaging



=> VSD labels all cells while GEVI is specific

Scale bar
Top: 200um
Bottom: 20um

direct comparison I

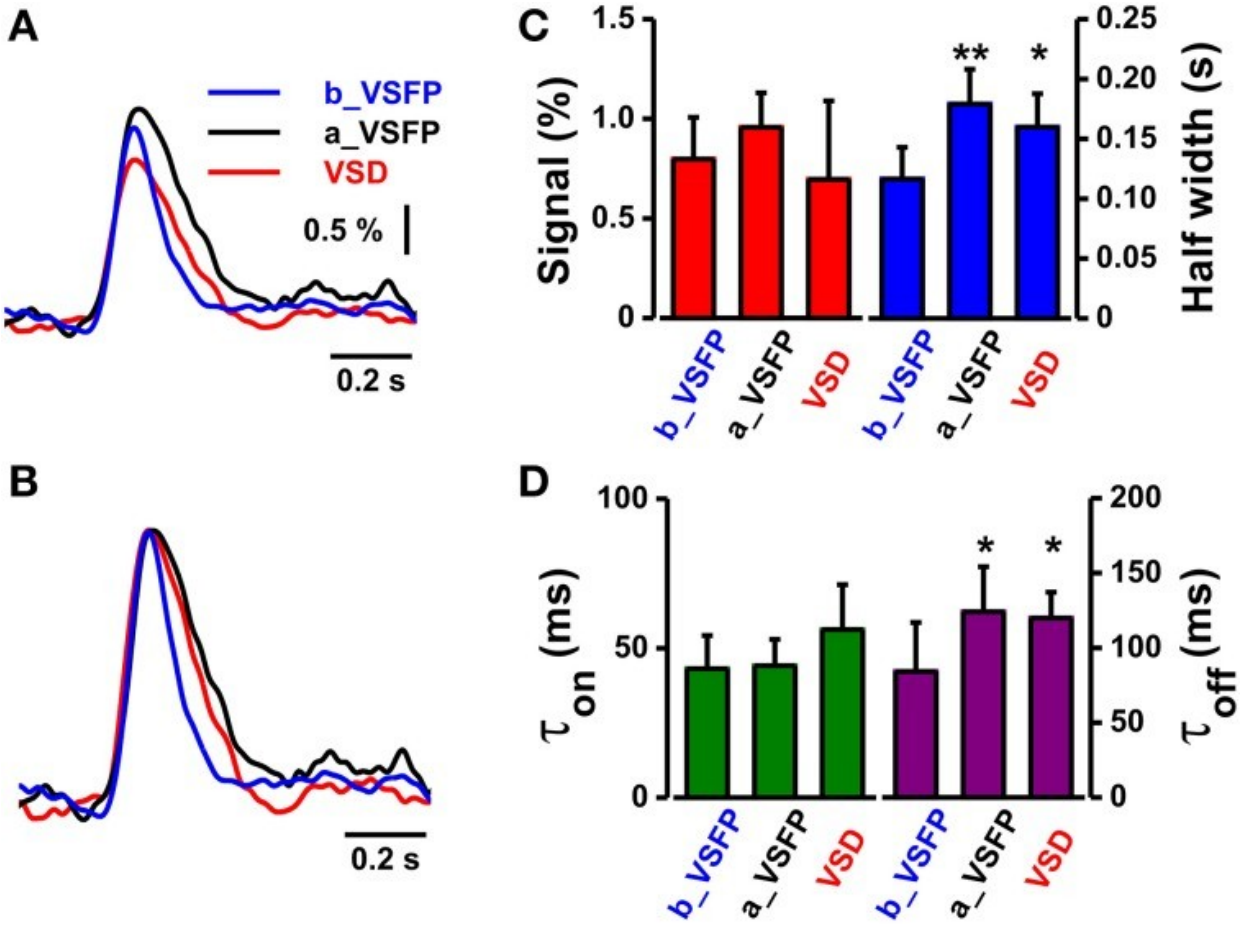


50-trial average!

=> no difference

Scale bar: 1mm

direct comparison II



=> VSFP is better before opening the dura, after, both are same

Paper 1: discussion and conclusion

- VSFP2 and RH1691 show similar responses to whisker deflection
- VSFP2 is faster if dura/skull is left intact

- GEVIs have many advantages
  - more stable signal
  - targeting
  - non-invasive imaging conditions
  - chronic imaging

Paper 2

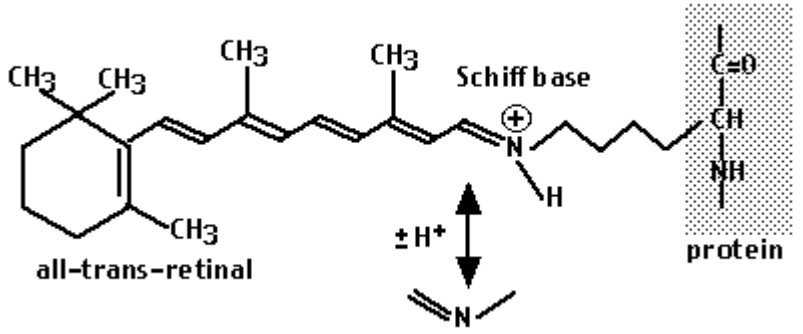
Science. 2015 Dec 11;350(6266):1361-6. doi: 10.1126/science.aab0810. Epub 2015 Nov 19.

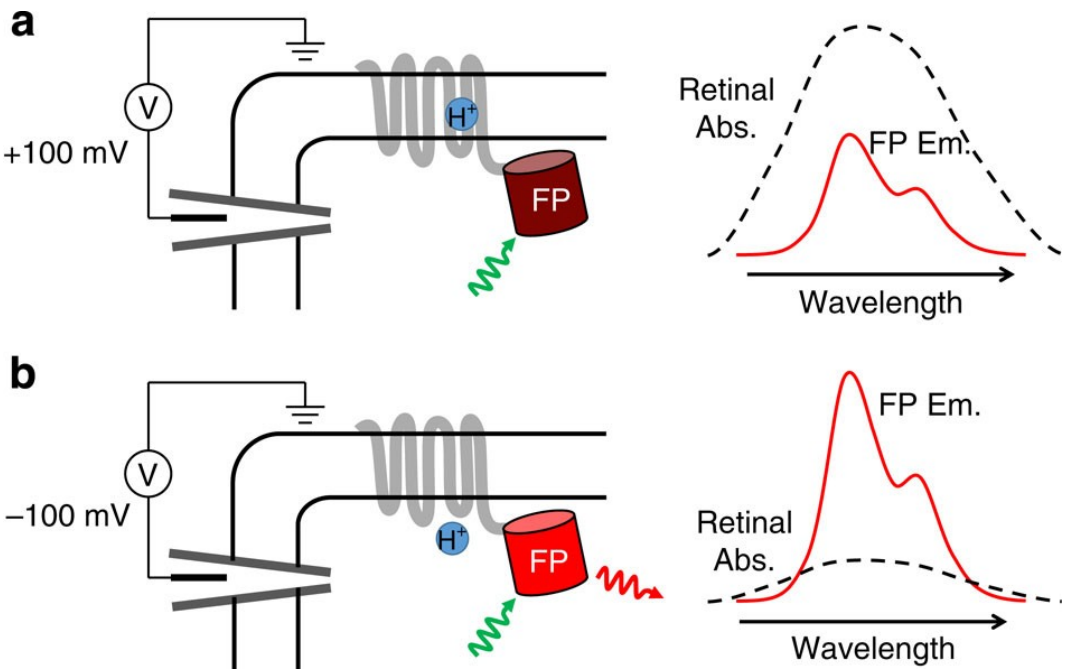
High-speed recording of neural spikes in awake mice and flies with a fluorescent voltage sensor.

Gong Y[1], Huang C[2], Li JZ[3], Grewe BF[3], Zhang Y[4], Eismann S[3], Schnitzer MJ[5].

indicator mechanism: eFRET

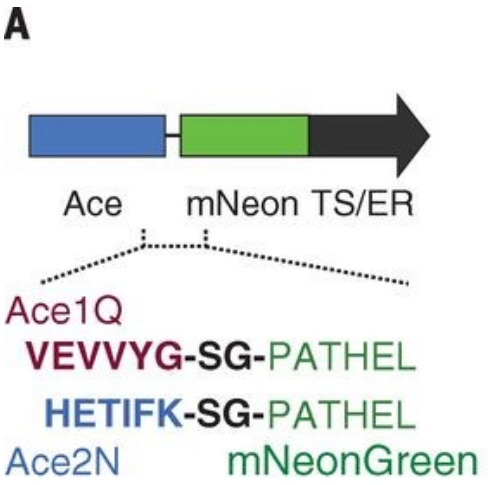
rhodopsin

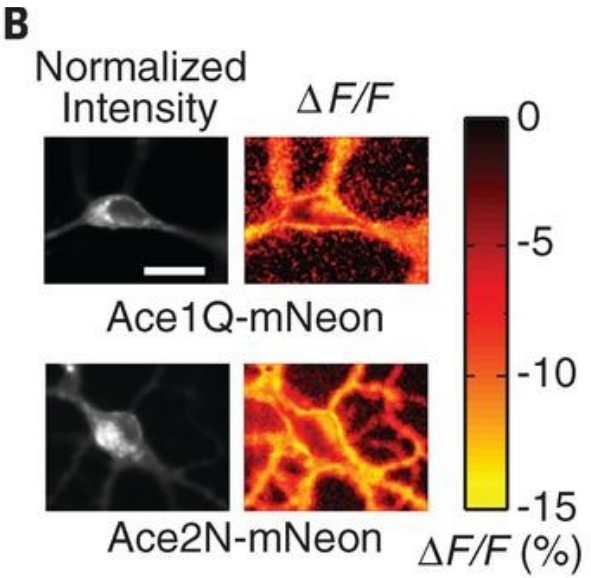




indicator generation & characterization I

Acetabularia acetabulum

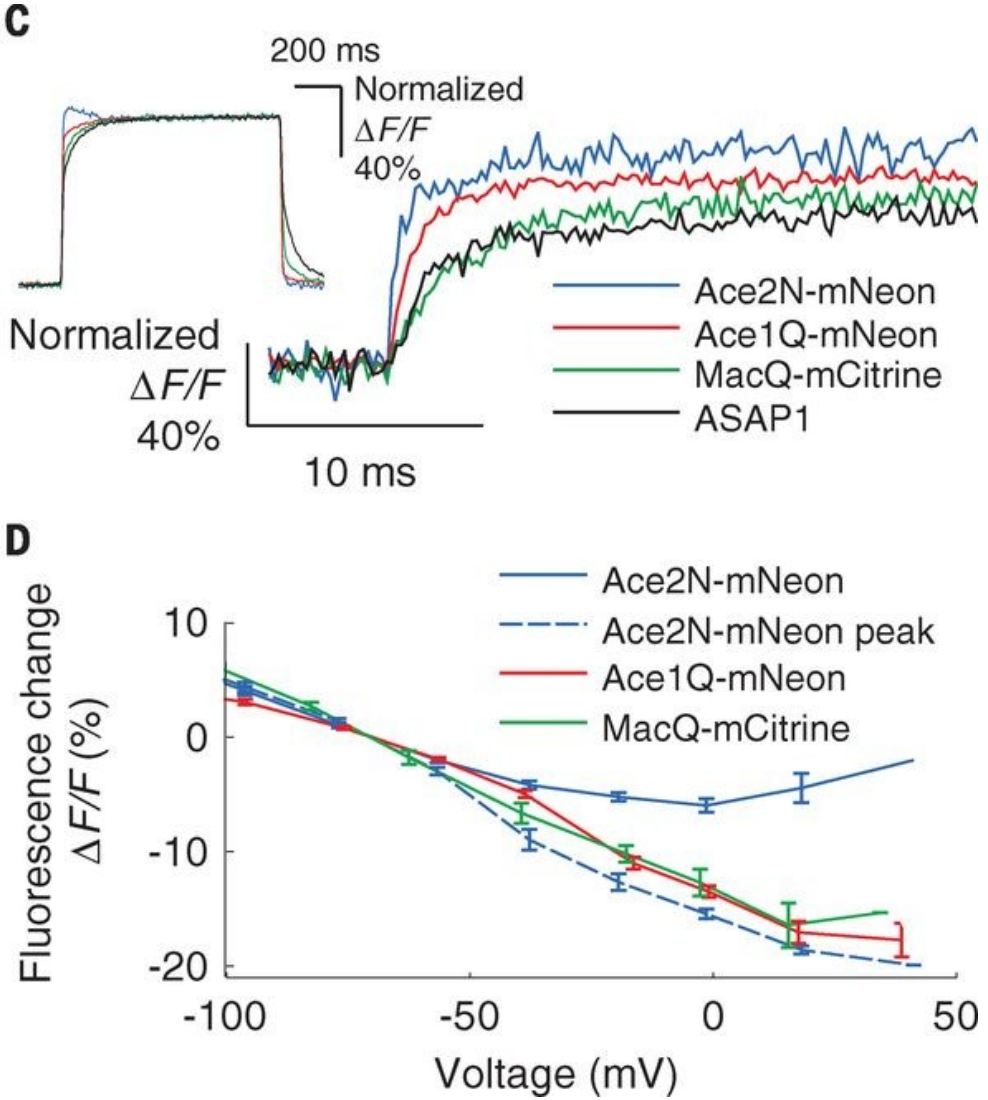




=> high FRET-efficiency
=> little intracellular aggregation

HEK293 cells
100mV depolarization step

indicator generation & characterization I

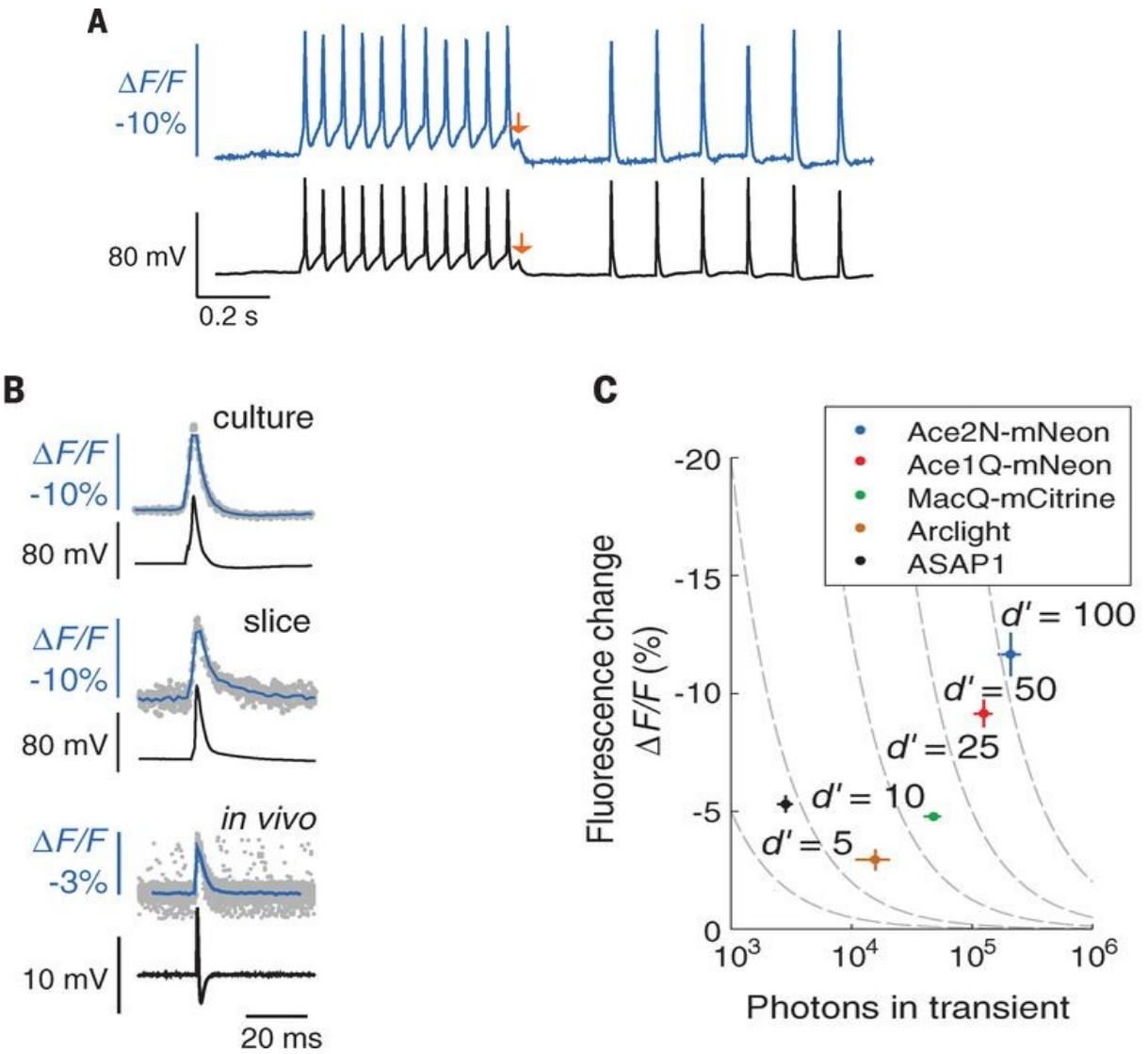


=> <1ms response time

=> linear relationship between membrane voltage and signal

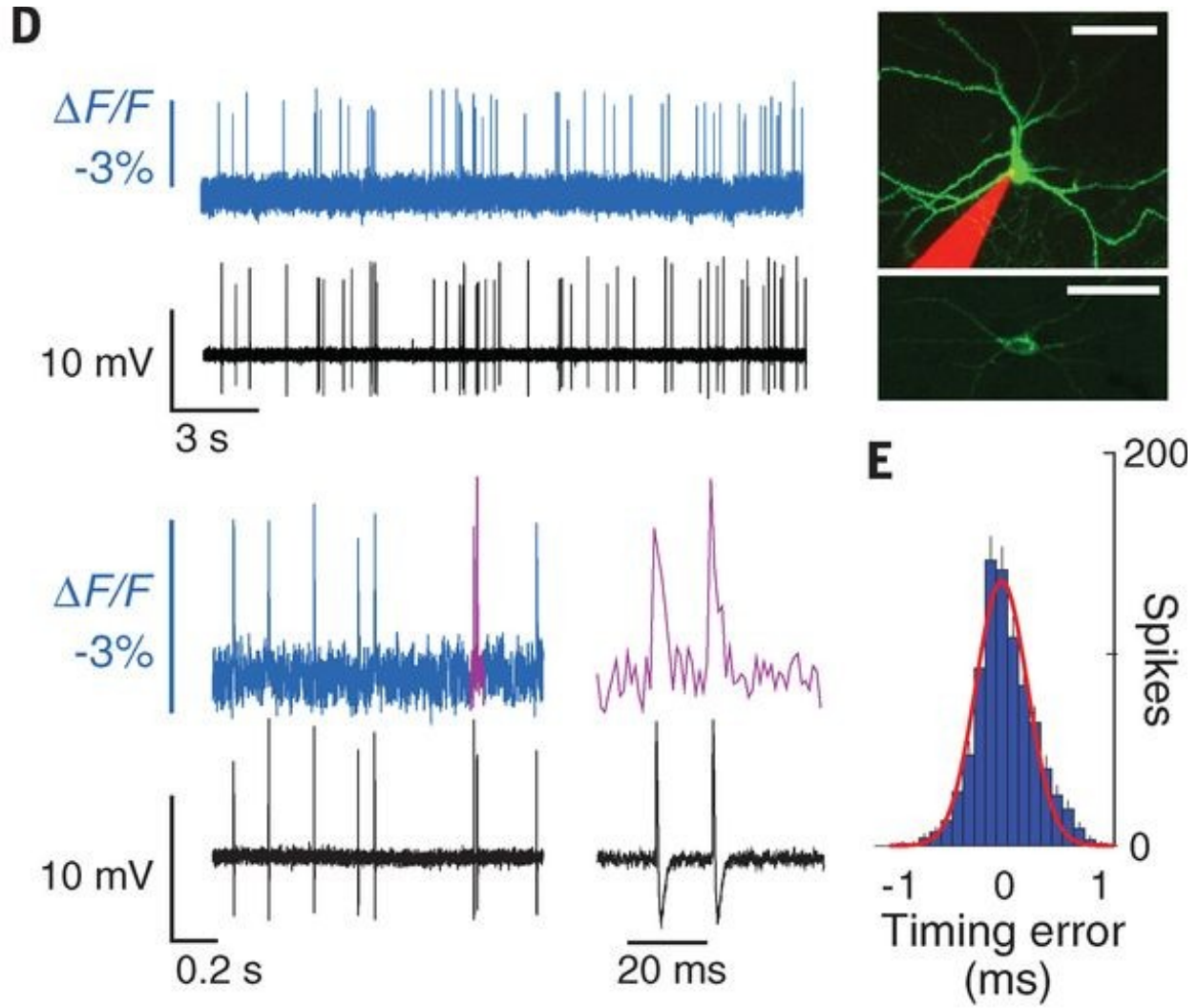
HEK293 cells
100mV depolarization step

performance assessment I



=> 3-10fold better spike detection fidelity

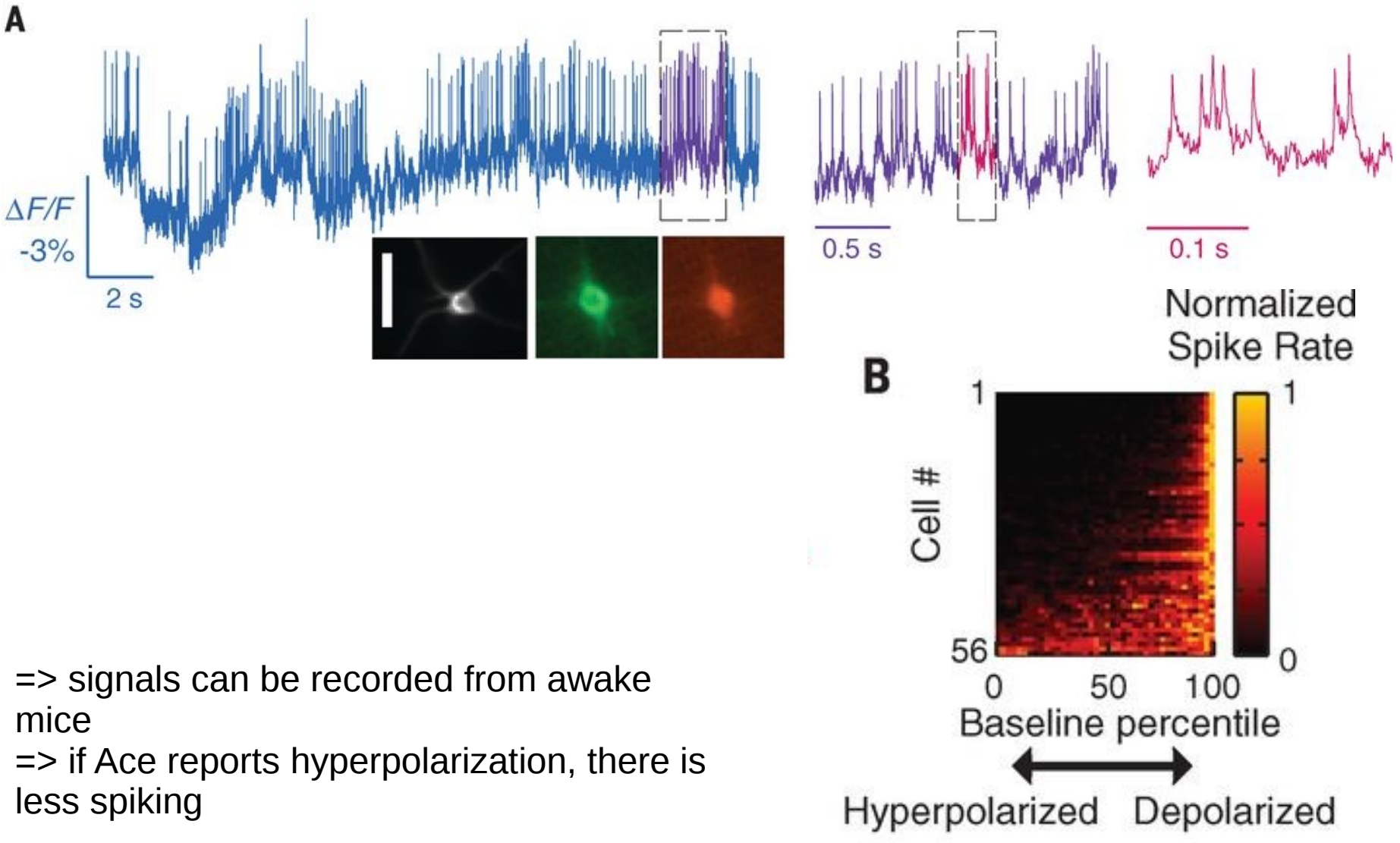
performance assessment II



Anesthesized mouse, cortical neuron

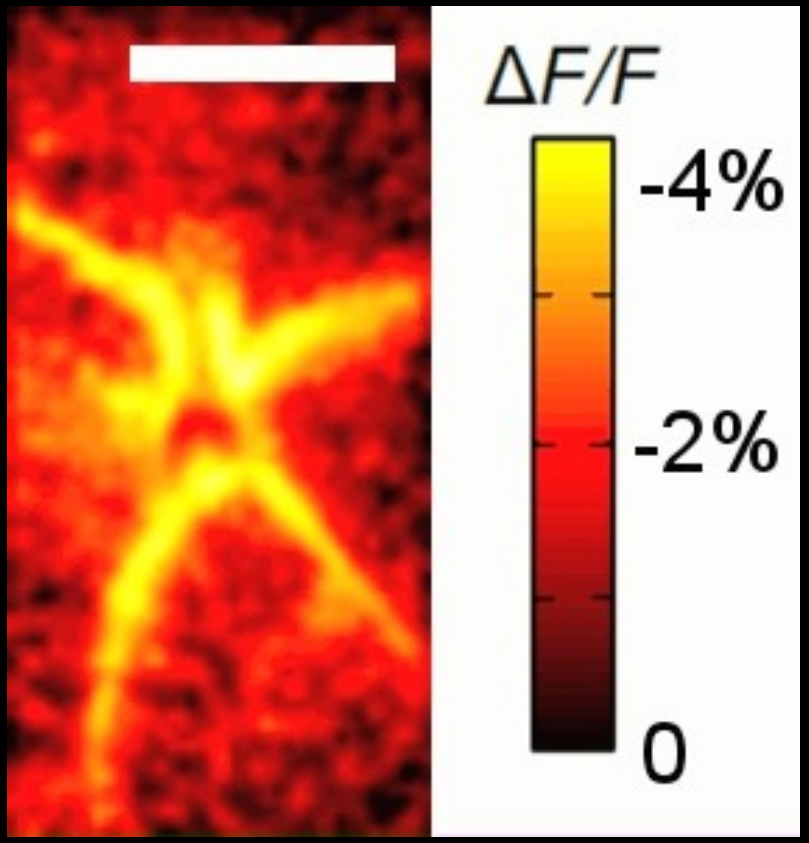
=> detection of fast spiking with small timing error

in vivo recordings awake mouse

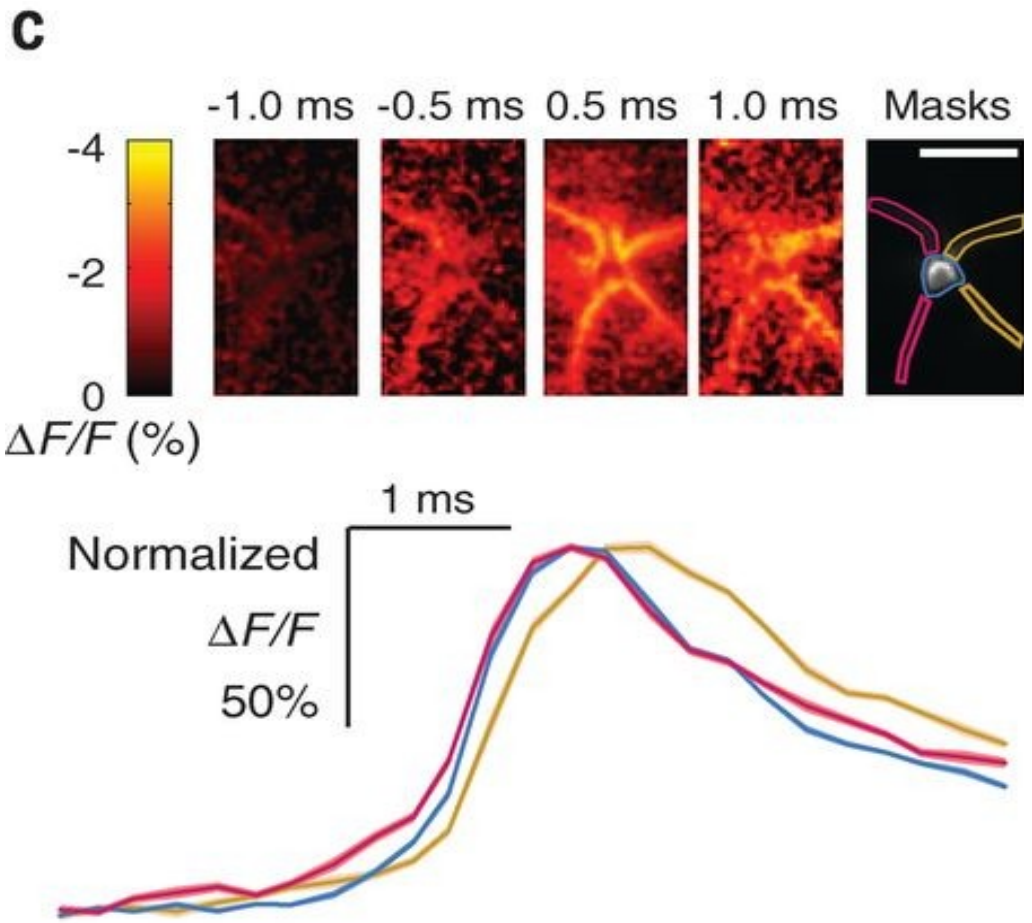


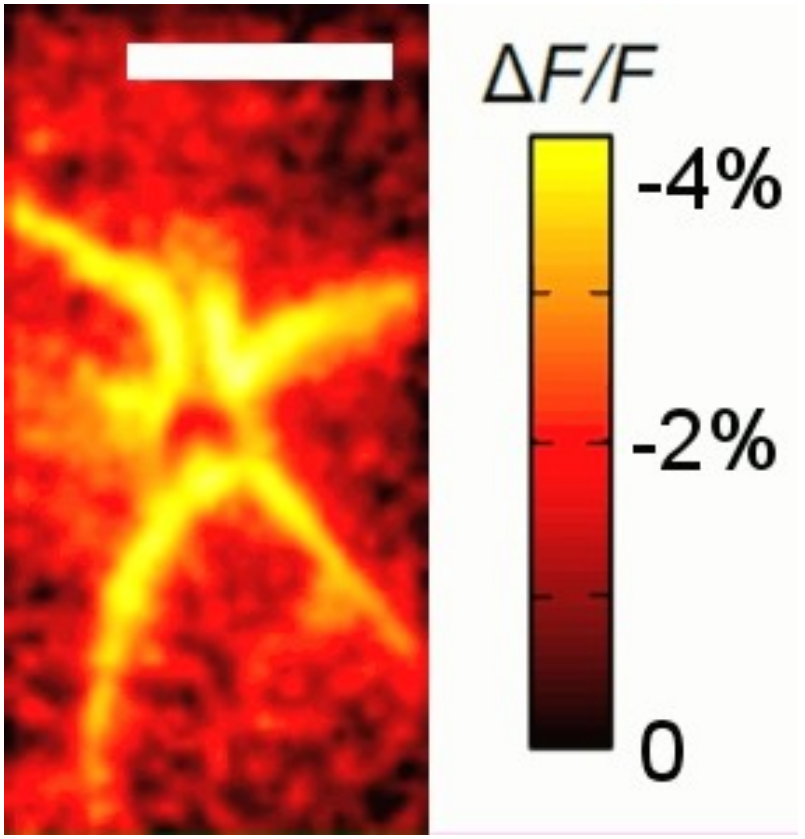
=> signals can be recorded from awake mice
=> if Ace reports hyperpolarization, there is less spiking

in vivo recordings awake mouse



in vivo recordings awake mouse

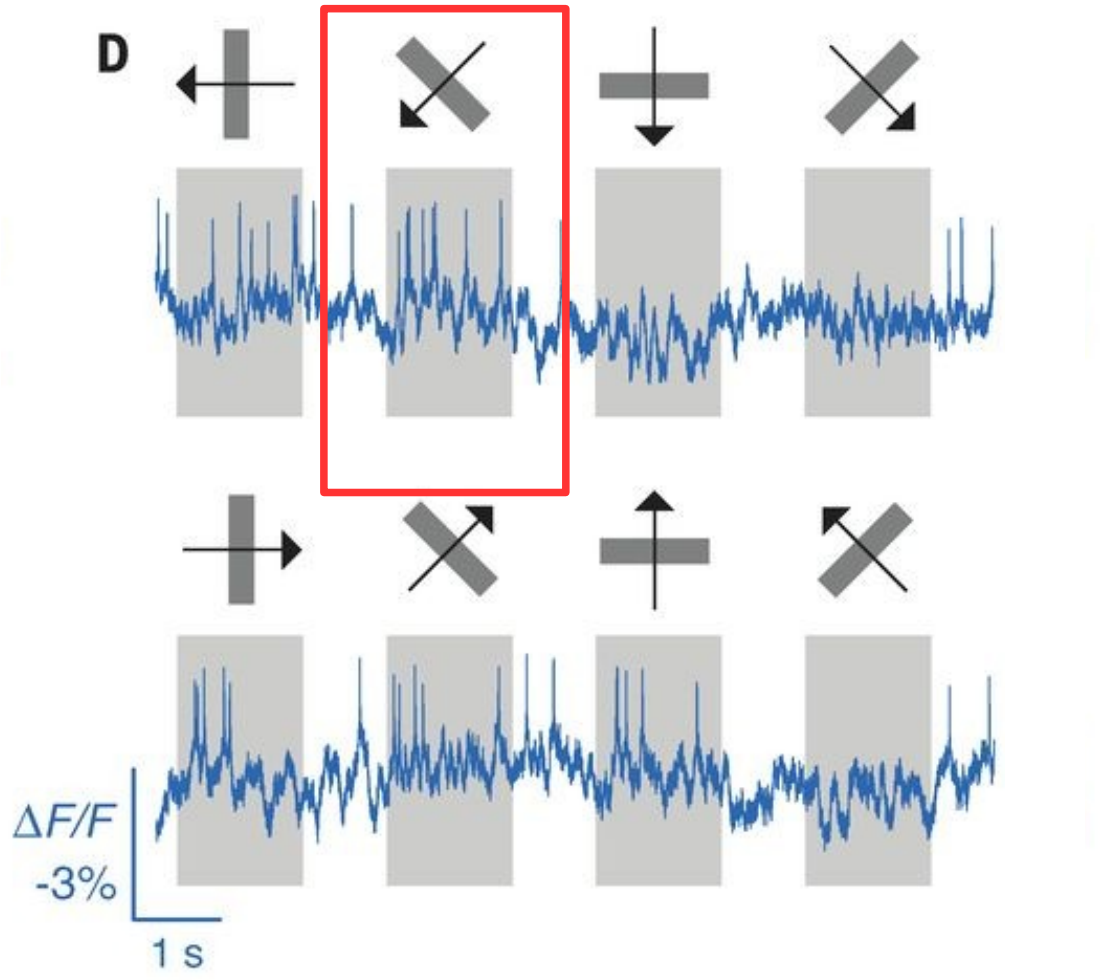


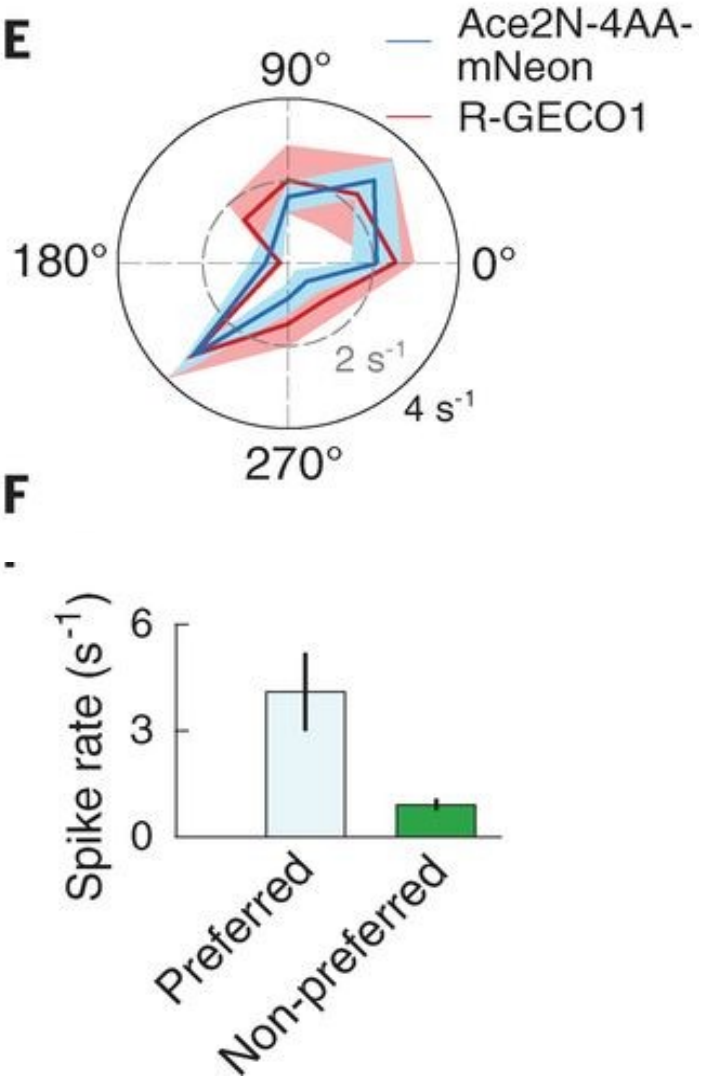


1900 spikes averaged

=> subcellular resolution possible

in vivo recordings awake mouse





Visually evoked responses of a V1 cortical neuron to moving gratings (10 trials per stimulus)

Conclusion paper 2

- New indicator outperforms
  - other published GEVIs
  - Calcium indicators

- But
  - only in sparse labelling

    => development of new imaging techniques is required

Paper 3

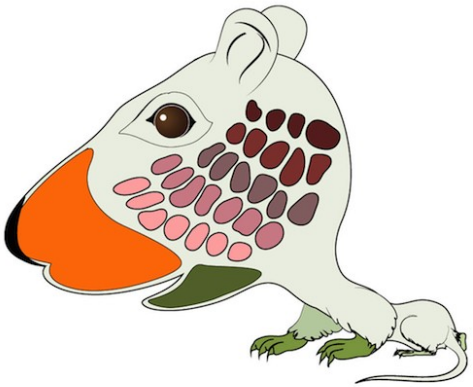
Sci Rep. 2015 Nov 27;5:17325. doi: 10.1038/srep17325.

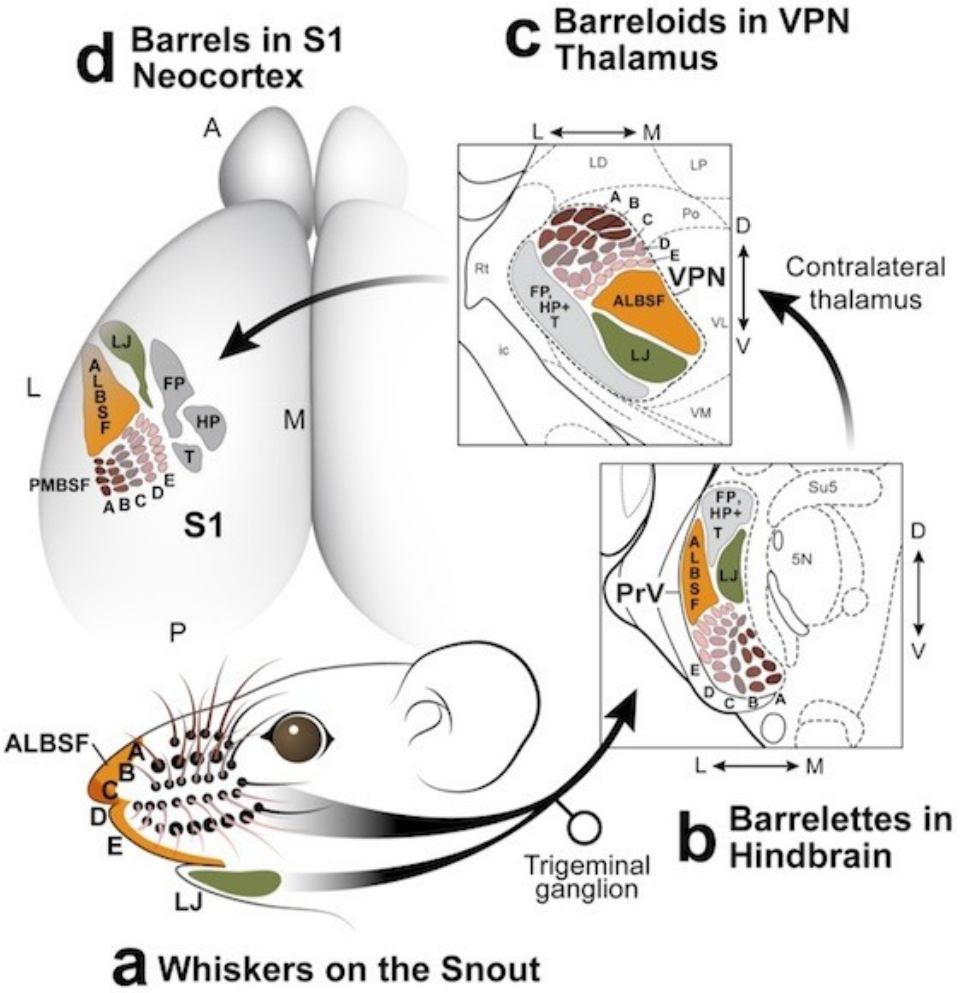
In Vivo Voltage-Sensitive Dye Imaging of Subcortical Brain Function.

Tang Q[1], Tsytsarev V[1,2], Liang CP[1], Akkentli F[2], Erzurumlu RS[2], Chen Y[1].

The mouse whisker system

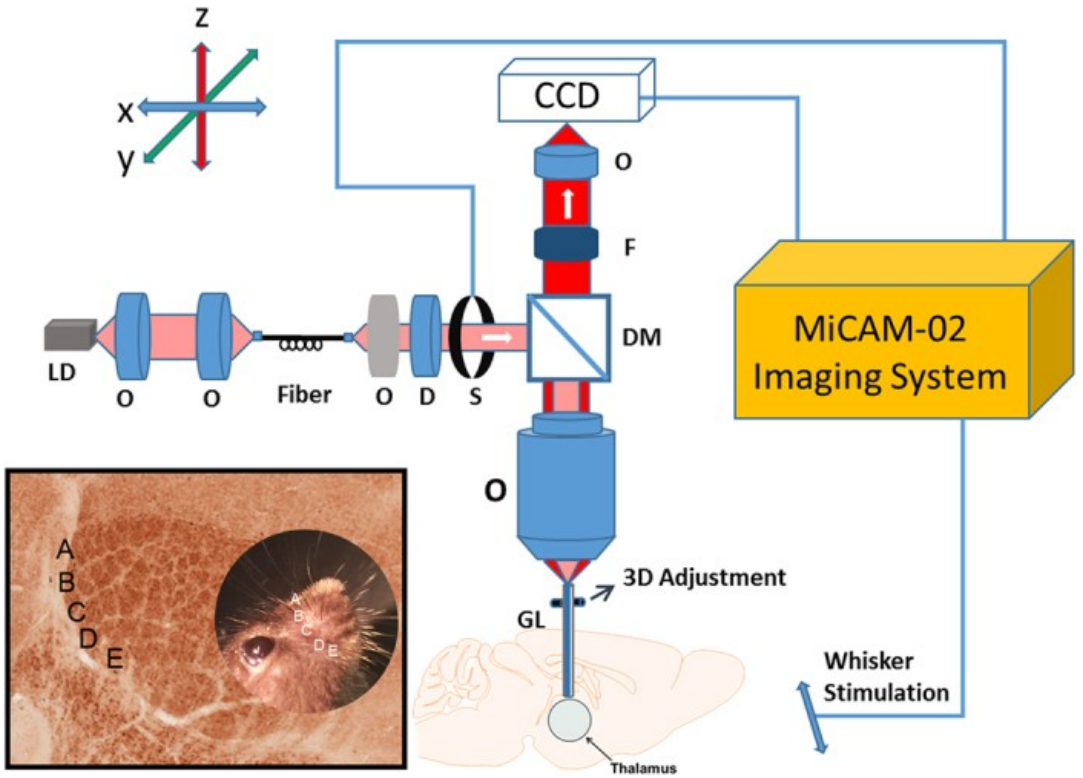
the mouseunculus

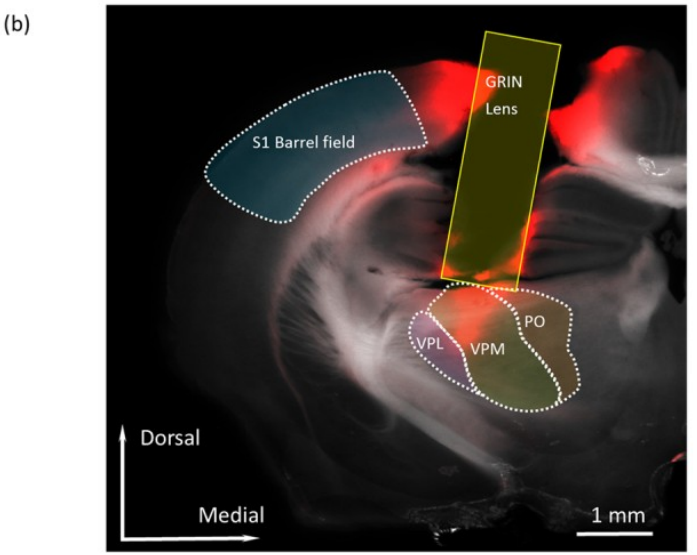




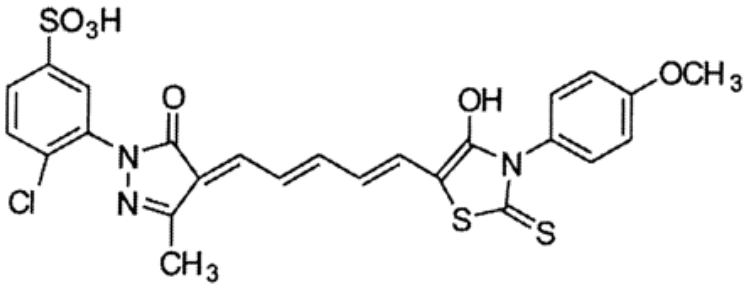
Zembrzycki et.al Nat.Neurosci. 2013

Recording Configuration





RH-1691



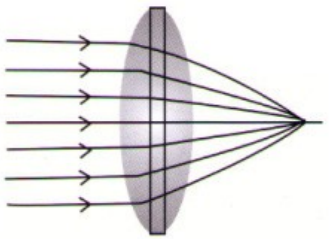


Figure1: Conventional Lens

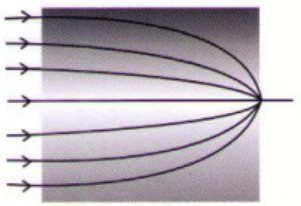
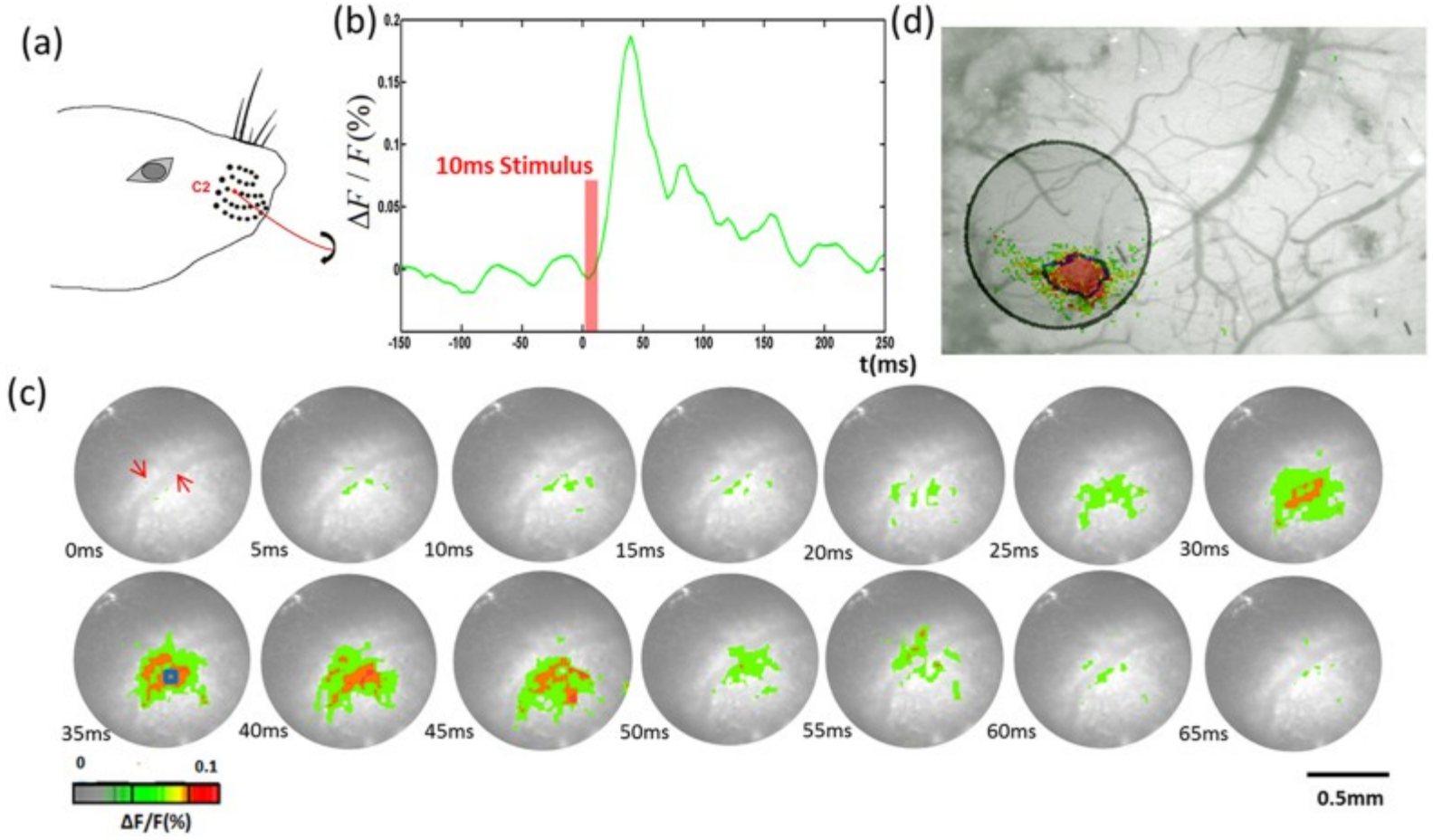


Figure 2:Grin Lens

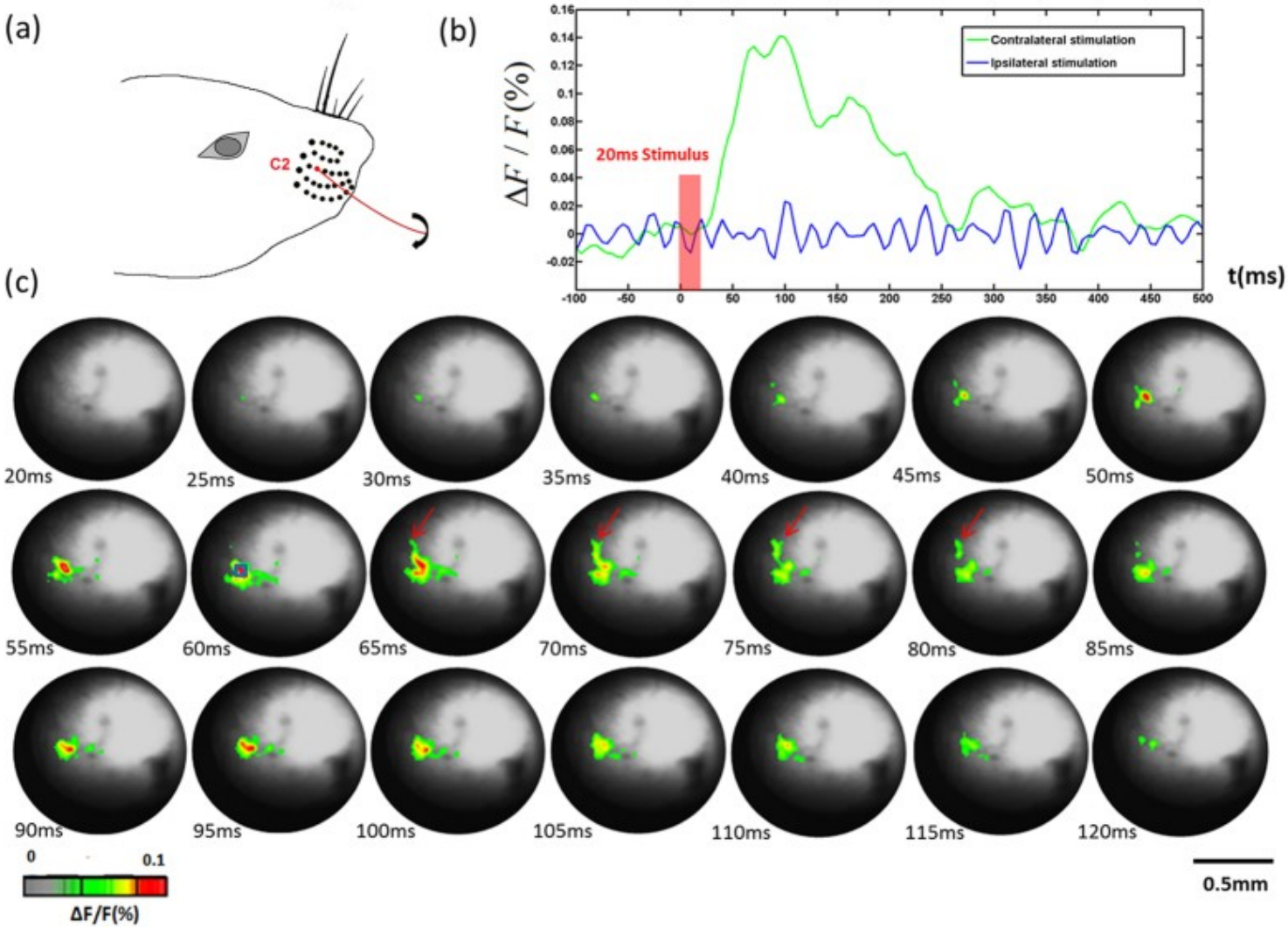
http://www.micro-lens.com/content/8-introduction-of-grin-lens

Barrel Cortex imaging



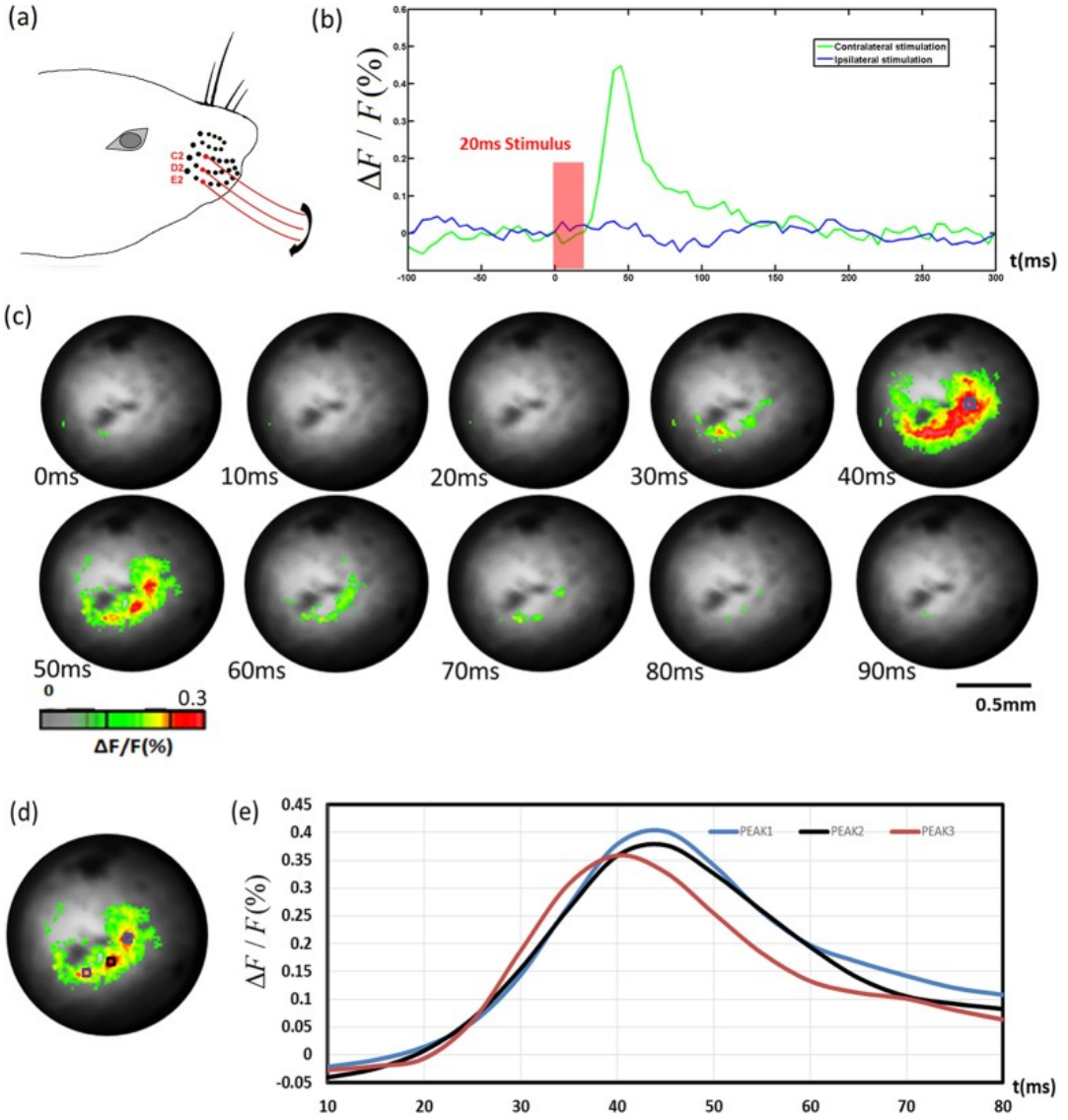
=> GRIN lens gives similar results

Thalamic imaging: one whisker

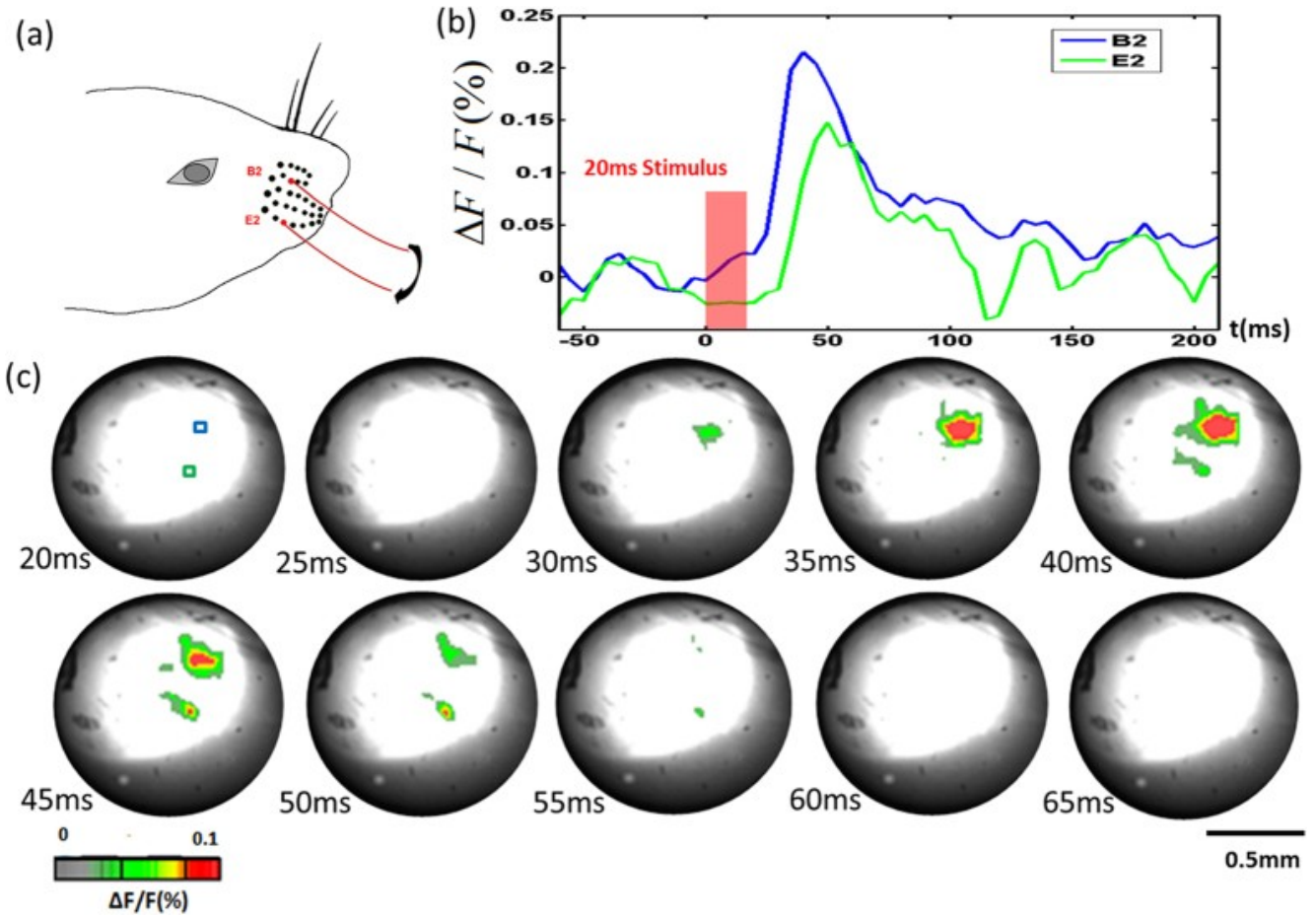


=> barreloid response to stimulation can be detected

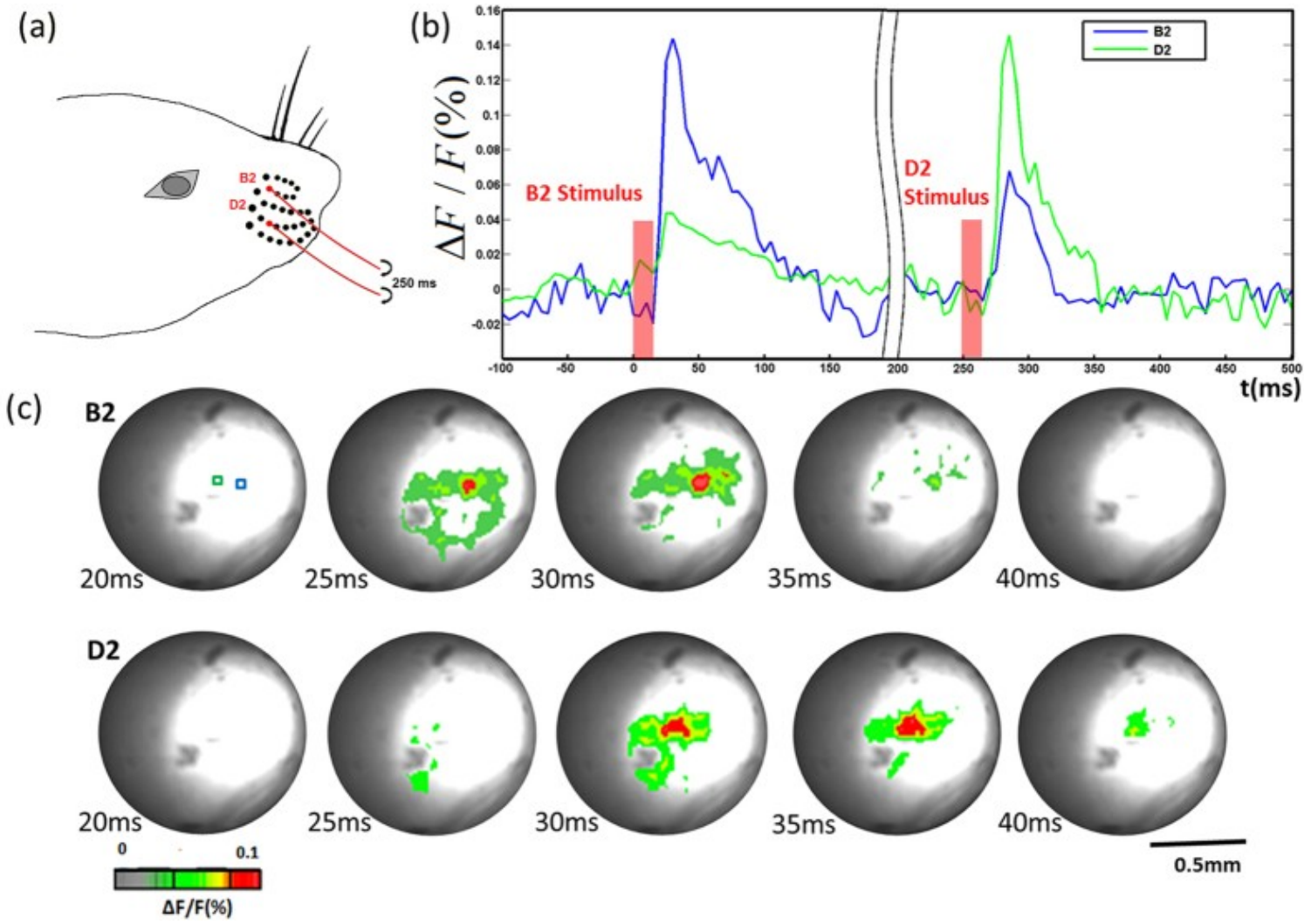
whisker discrimination I



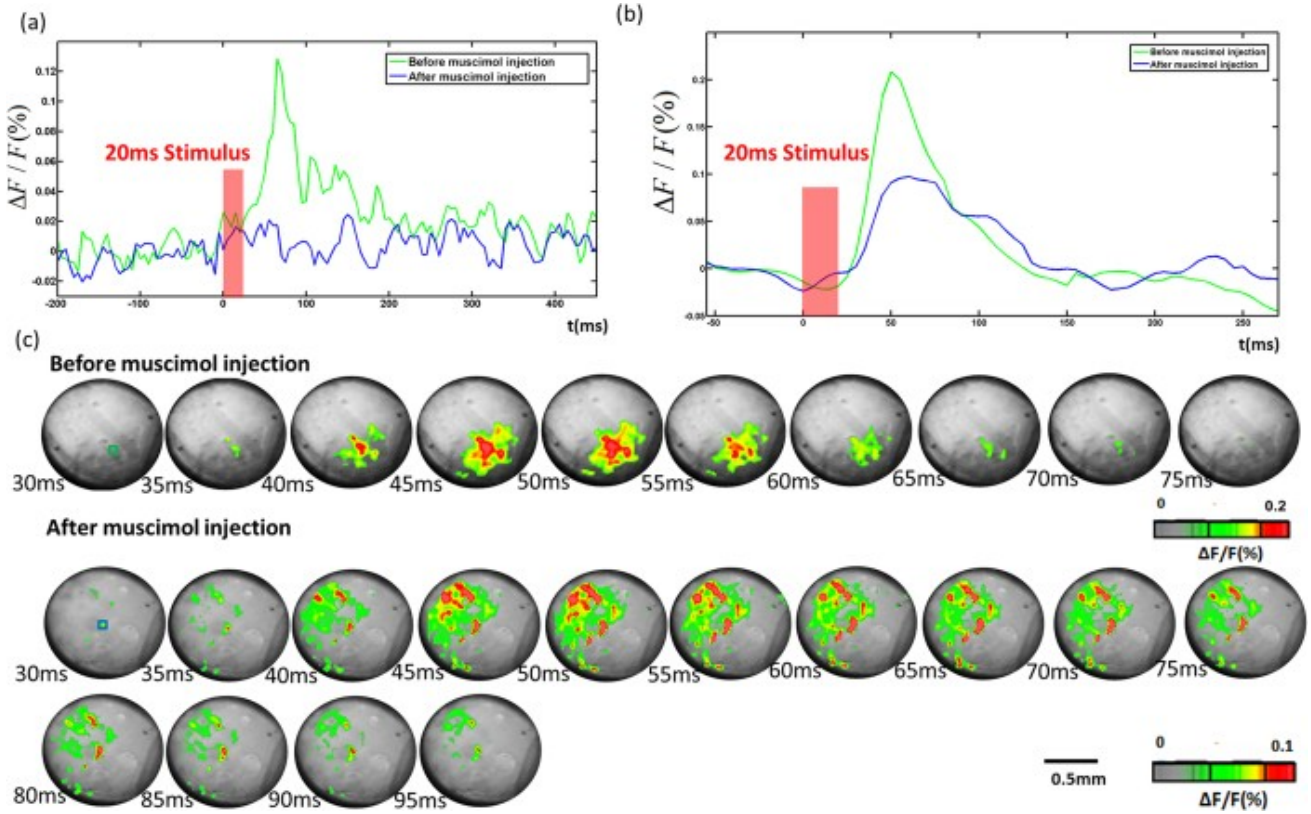
whisker discrimination II



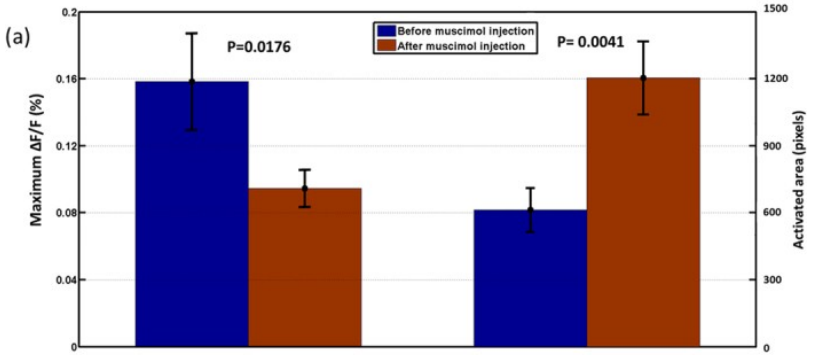
Temporal discrimination of whisker activity



Effect of corticothalamic inputs on barreloid activity



$GABA_A$-activator



Conclusion paper 3

- Provides a method to assess the function of subcortical regions while manipulating the cortex
- Good temporal and spatial resolution

Overall conclusion and outlook

- VSDs are suitable for imaging in dissociated cultures or for „optical field recordings“ *in vivo*

  => maybe several dyes have to be tested to find an appropriate one
- For *in vivo* applications with single cell resolution, GEVIs should be used
- Further advances in voltage-imaging will rely on
  - Development of faster two-photon microscopes
  - Development of dyes with special transition states

Thank you!