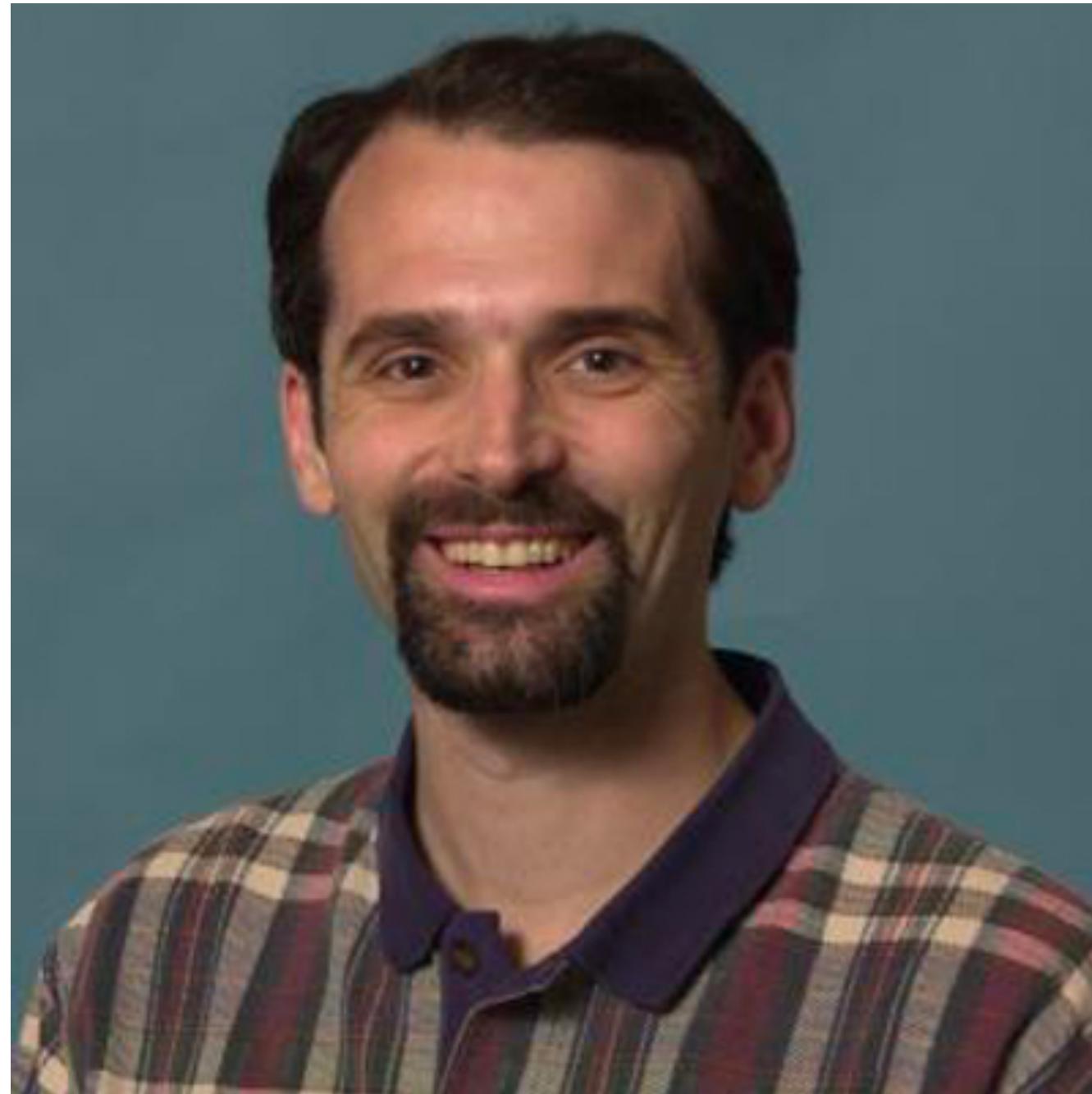


**NEUROSCIENCE**

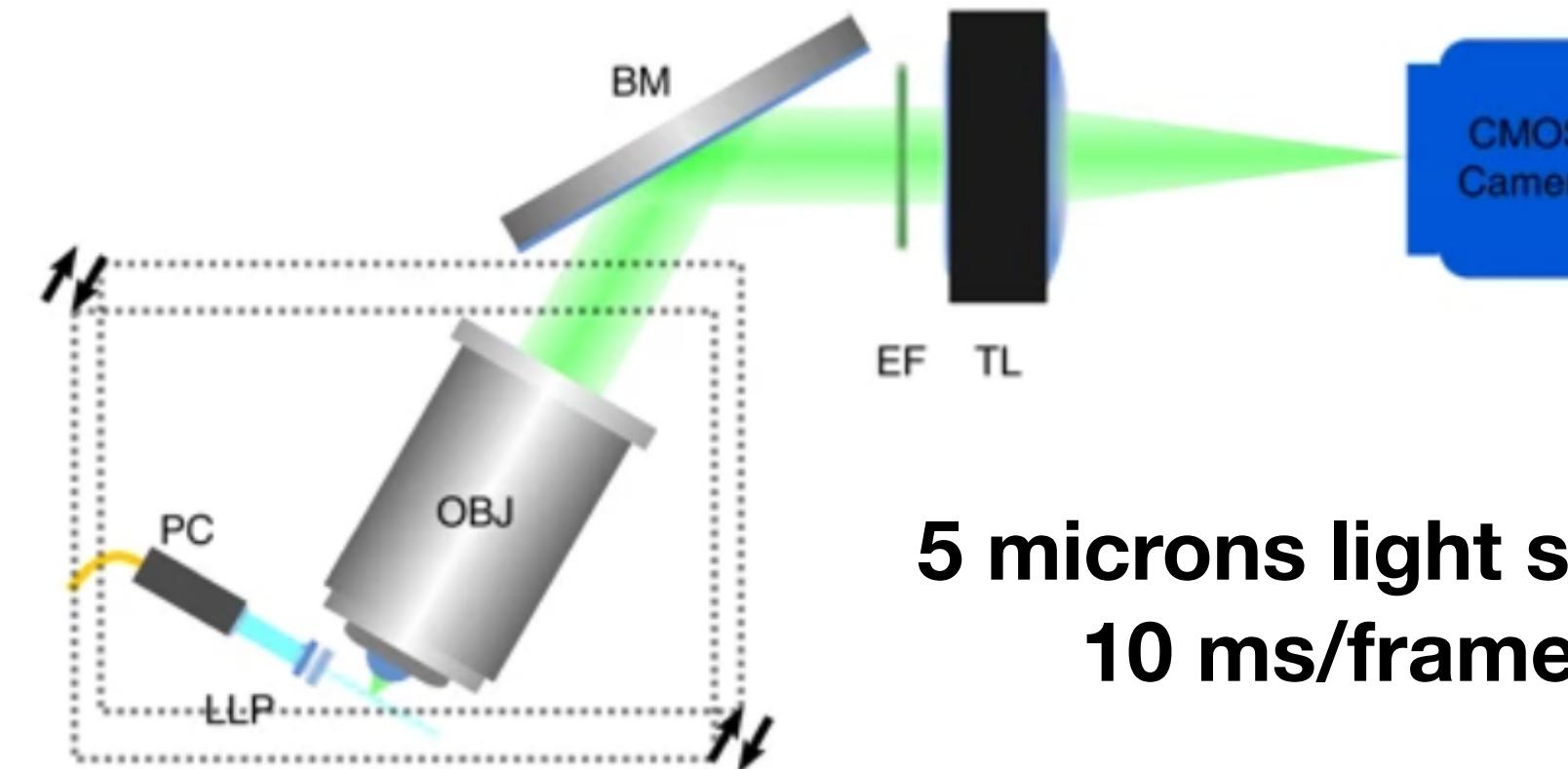
# **Sensory coding mechanisms revealed by optical tagging of physiologically defined neuronal types**

**Donghoon Lee, Maiko Kume, Timothy E. Holy\***



# Tim Holy

- Technology Driven Lab @ Washington University in St. Louis
- Simultaneous neural population activity
- Objective Coupled Planar Illumination (OCPI) microscopy

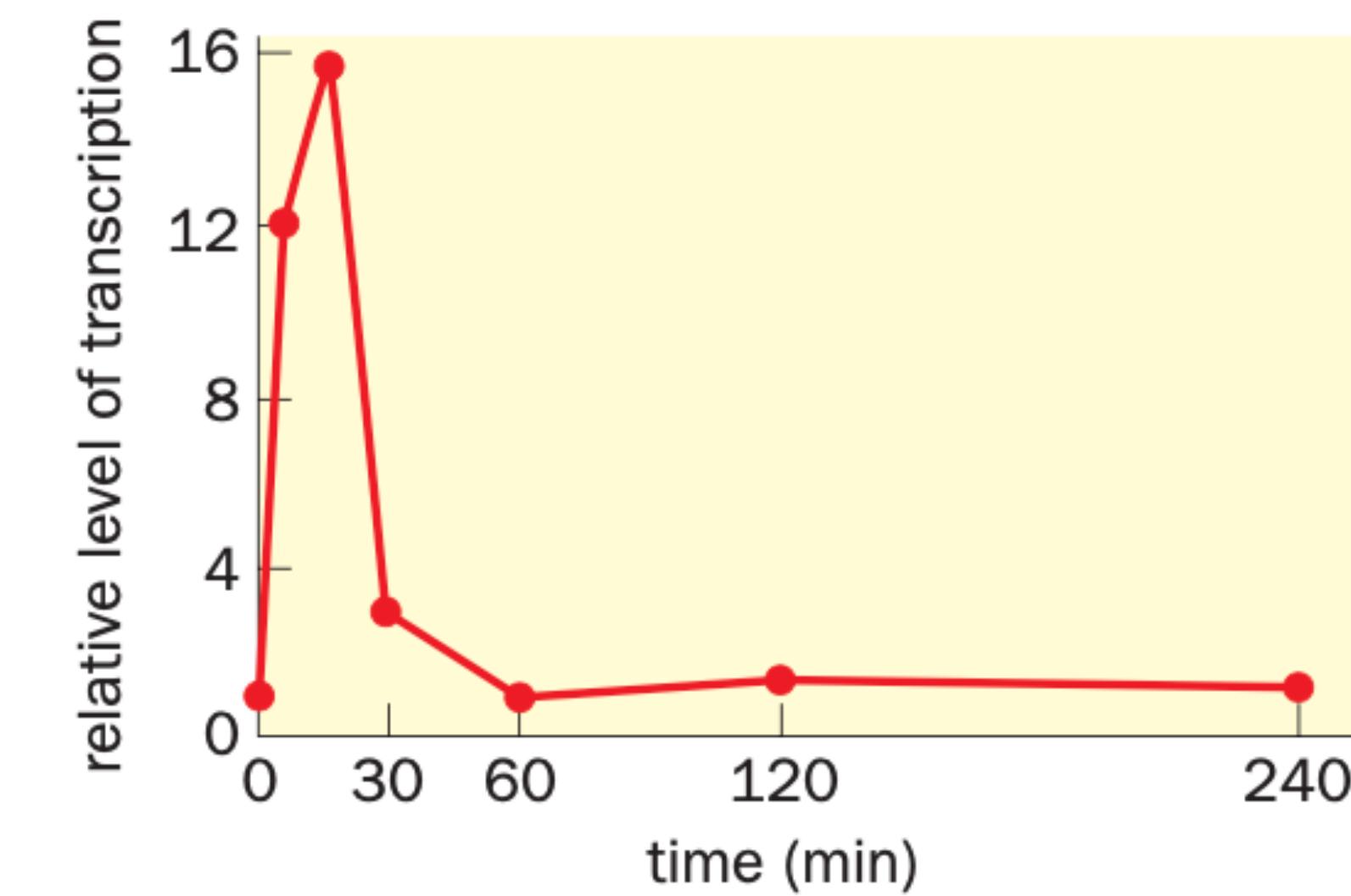


5 microns light sheet  
10 ms/frame

Greer, Cody J., and Timothy E. Holy. "Fast objective coupled planar illumination microscopy." *Nature communications* 10.1 (2019): 1-14.

# Part I Indicators

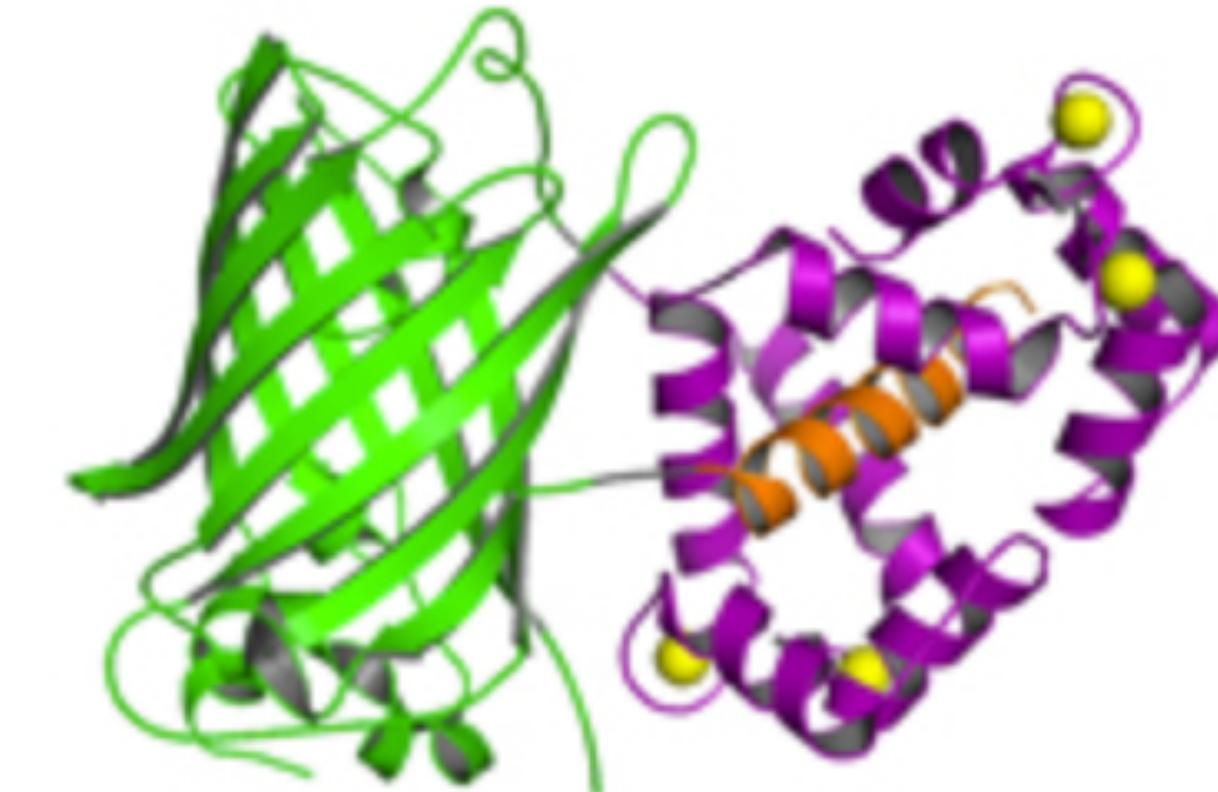
- Internal indicators: immediate early genes (IEGs)  
e.g. Fos, Egr1, etc.



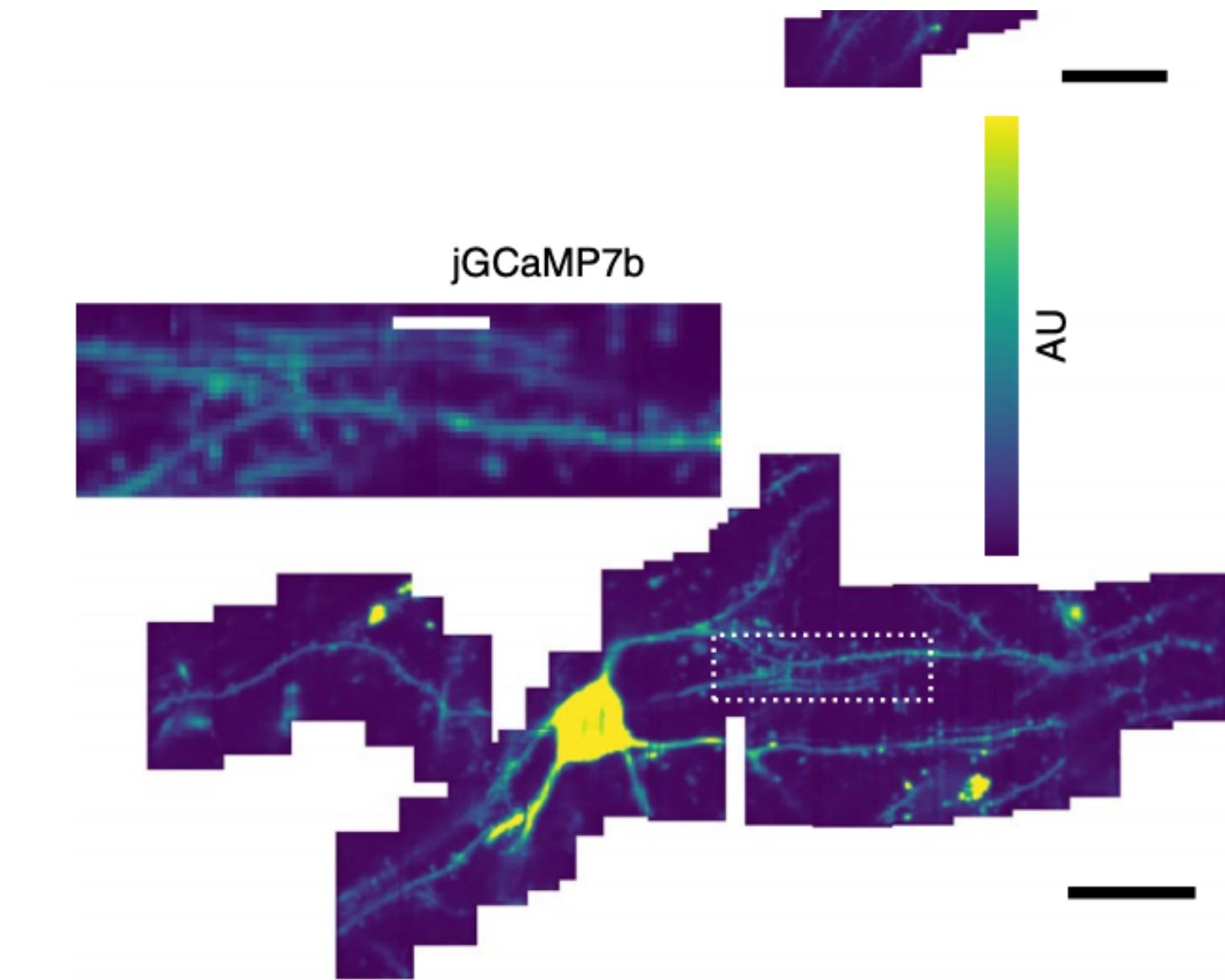
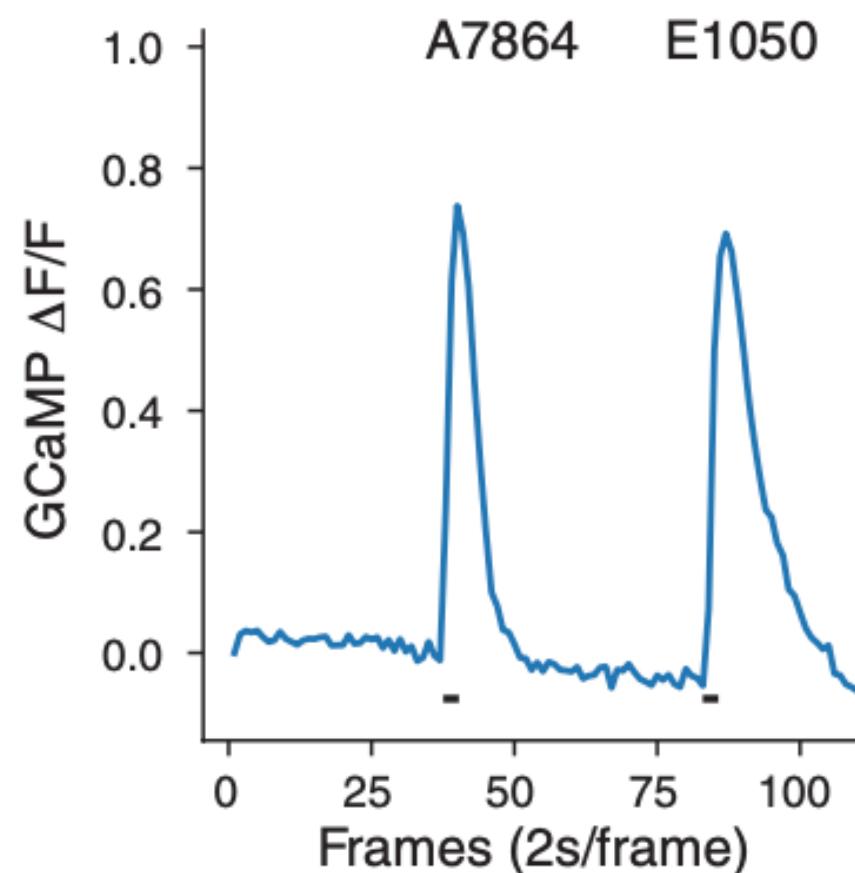
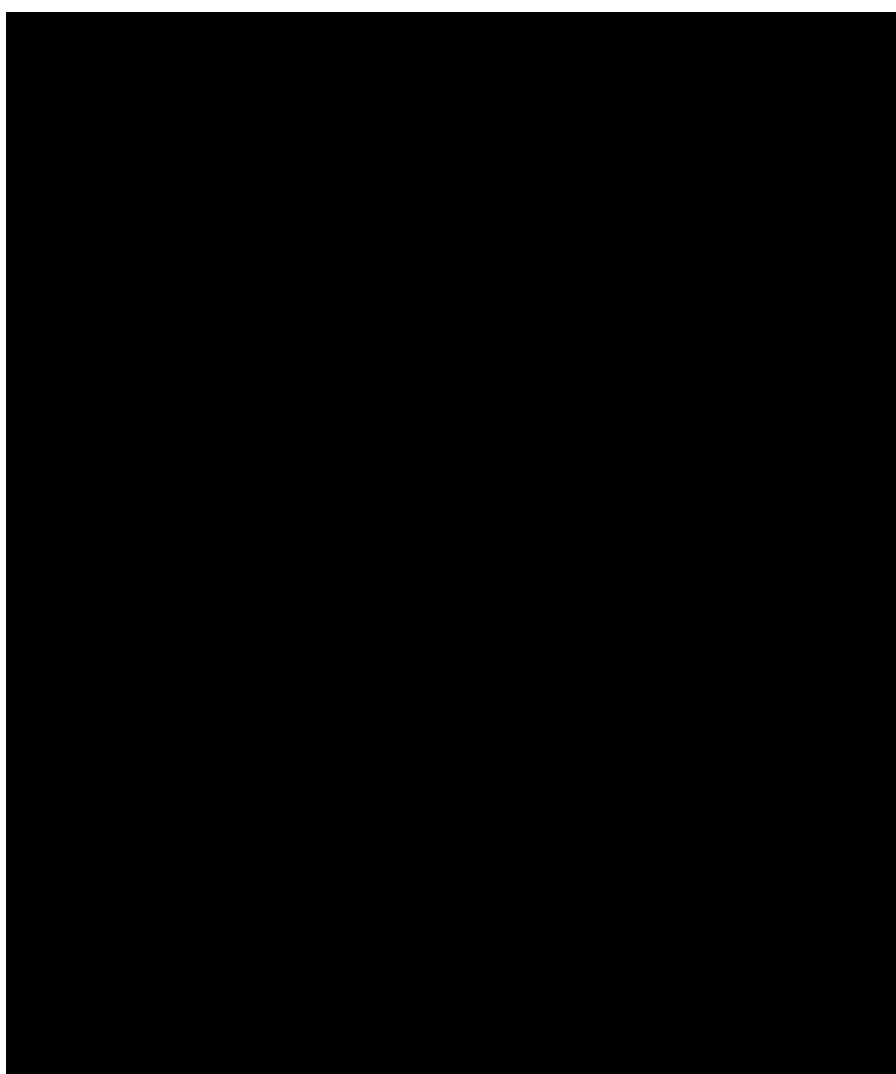
Liqun Luo. *Principle of Neuroscience*. 2015, Page 109

# Part I Indicators

- Internal indicators: immediate early genes (IEGs)  
e.g. Fos, Egr1, etc.
- Calcium indicators  
e.g. dye & GECI (GCaMP, CaMPARI)



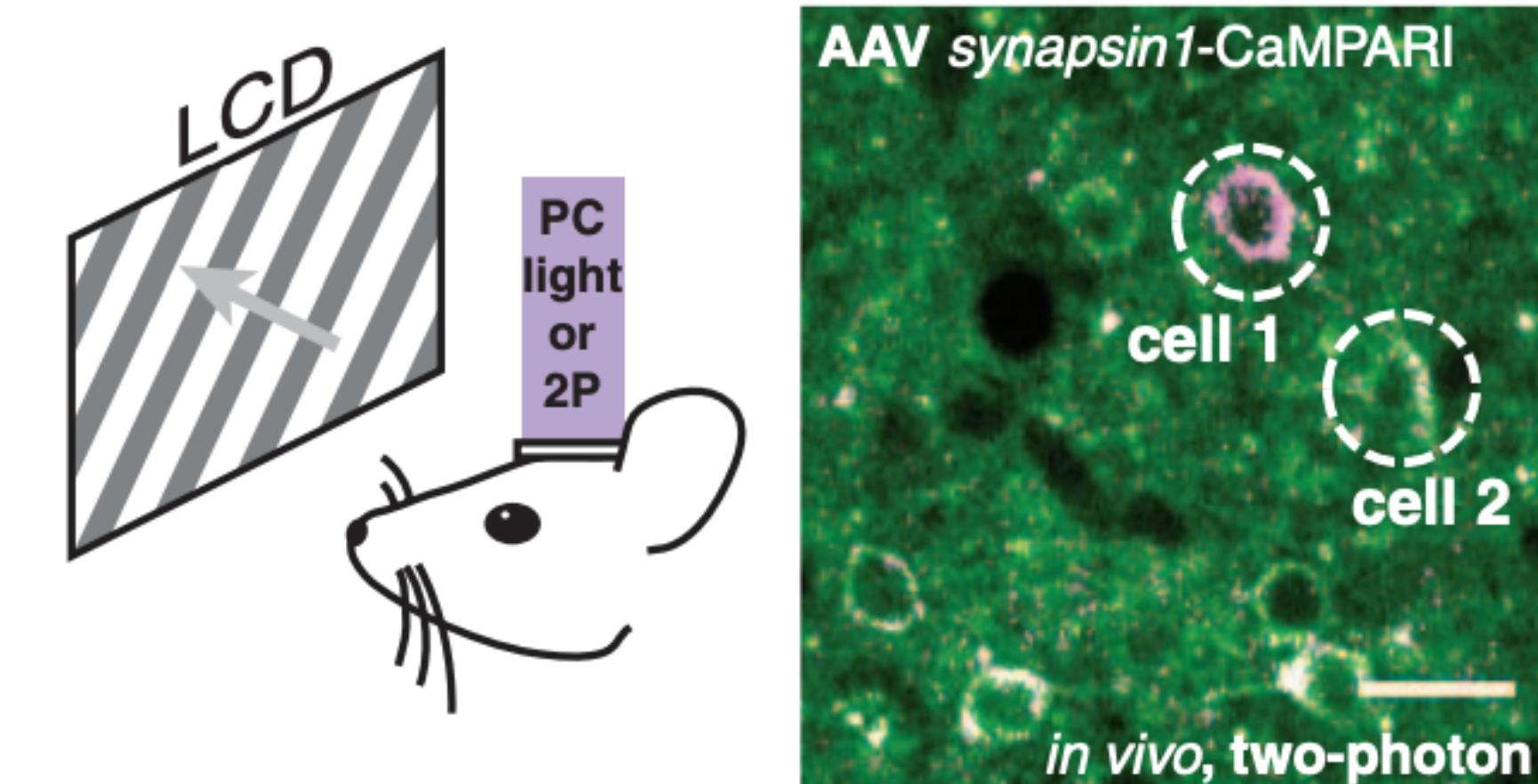
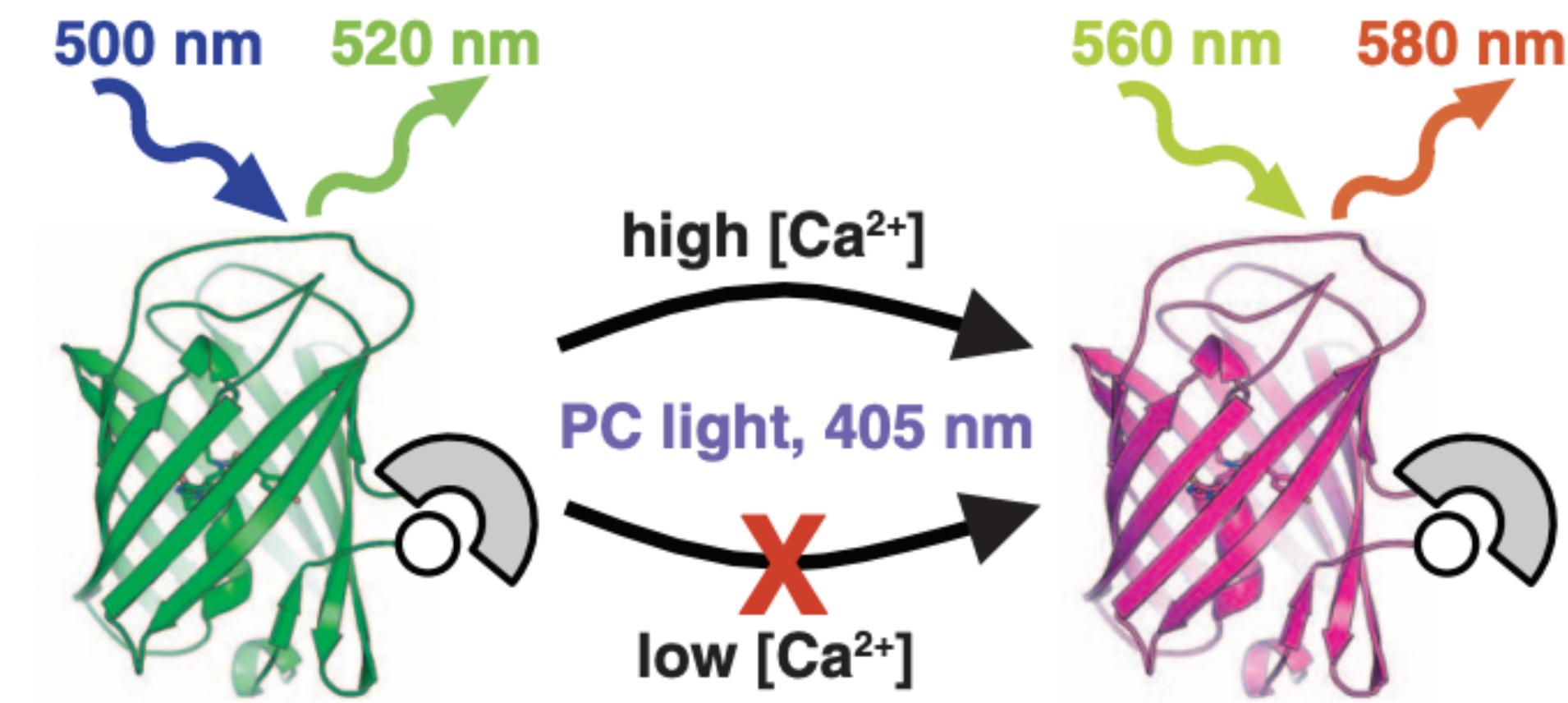
GCaMP7, from Janelia Farm



Dana, Hod, et al. "High-performance calcium sensors for imaging activity in neuronal populations and microcompartments." *Nature methods* 16.7 (2019): 649-657.

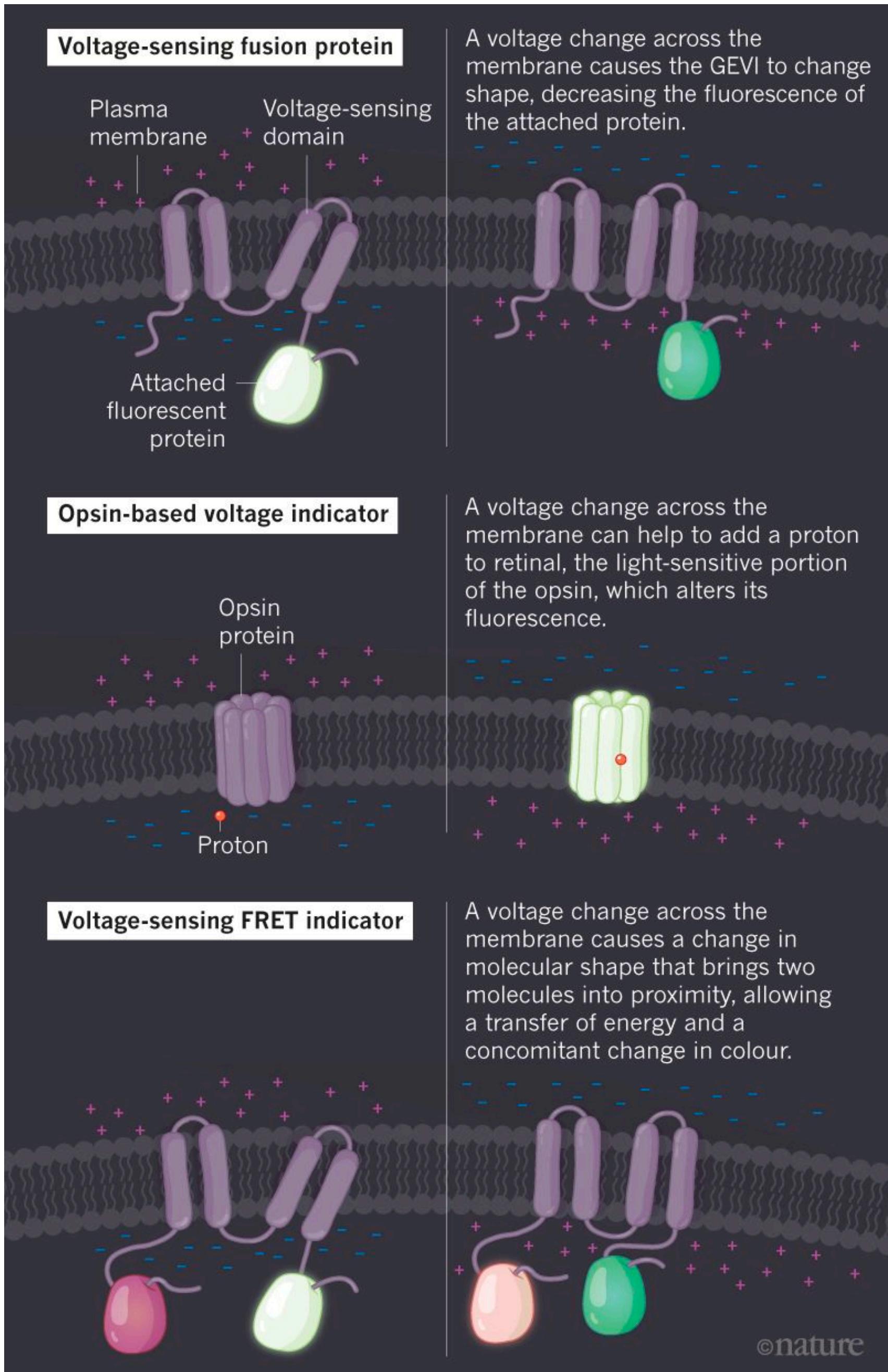
# Part I Indicators

- Internal indicators: immediate early genes (IEGs)  
e.g. Fos, Egr1, etc.
- Calcium indicators  
e.g. dye & GECI (GCaMP, CaMPARI)



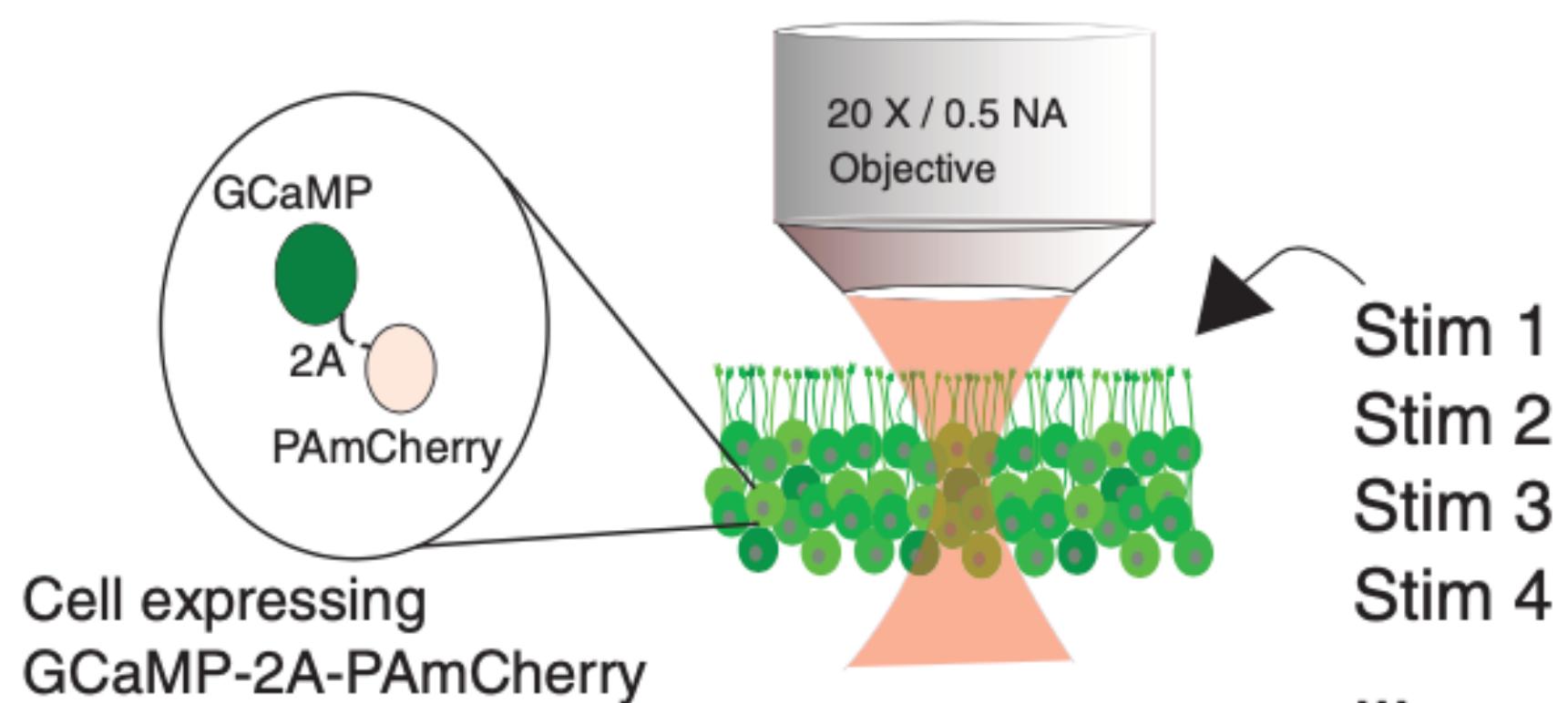
# Part I Indicators

- Internal indicators: immediate early genes (IEGs)  
e.g. Fos, Egr1, etc.
- Calcium indicators  
e.g. dye & GECI (GCamP, **CaMPARI**)
- Voltage indicators  
e.g. Dye, GEVI

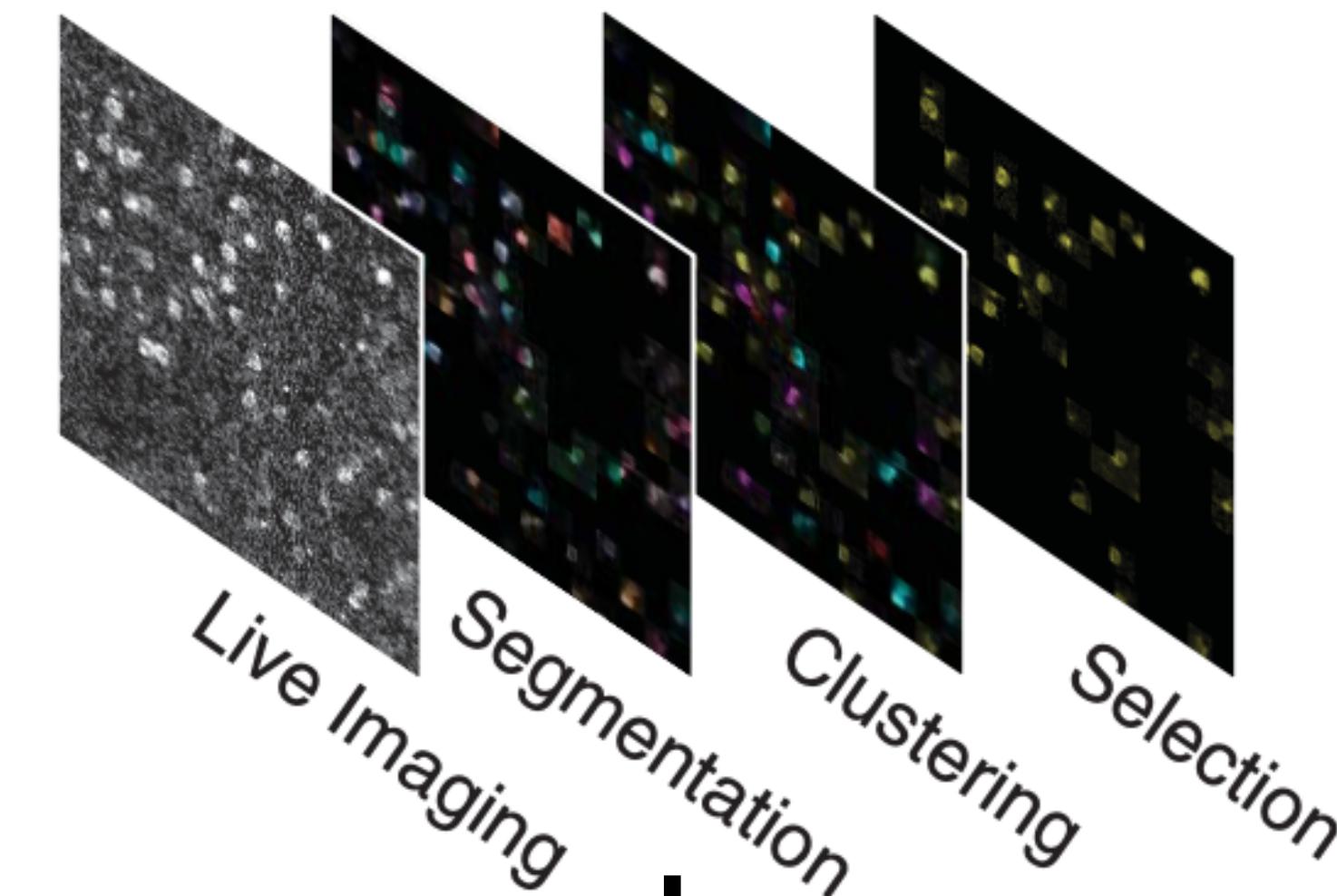


# Part I PhOT-seq

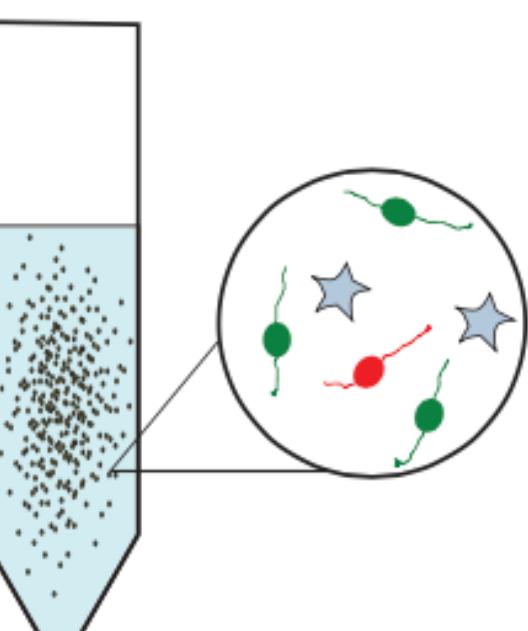
## Two-photon calcium imaging



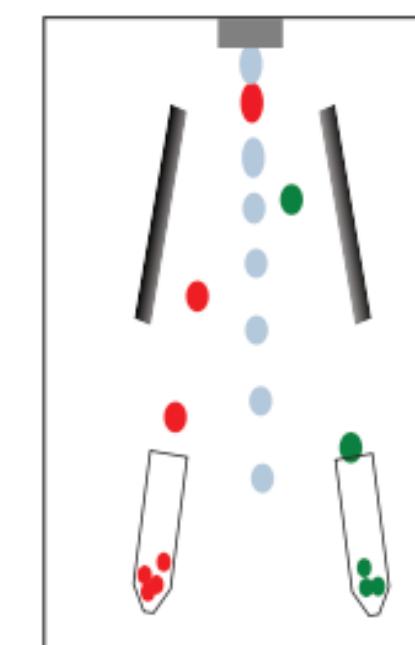
## Online image analysis



## Tissue dissociation

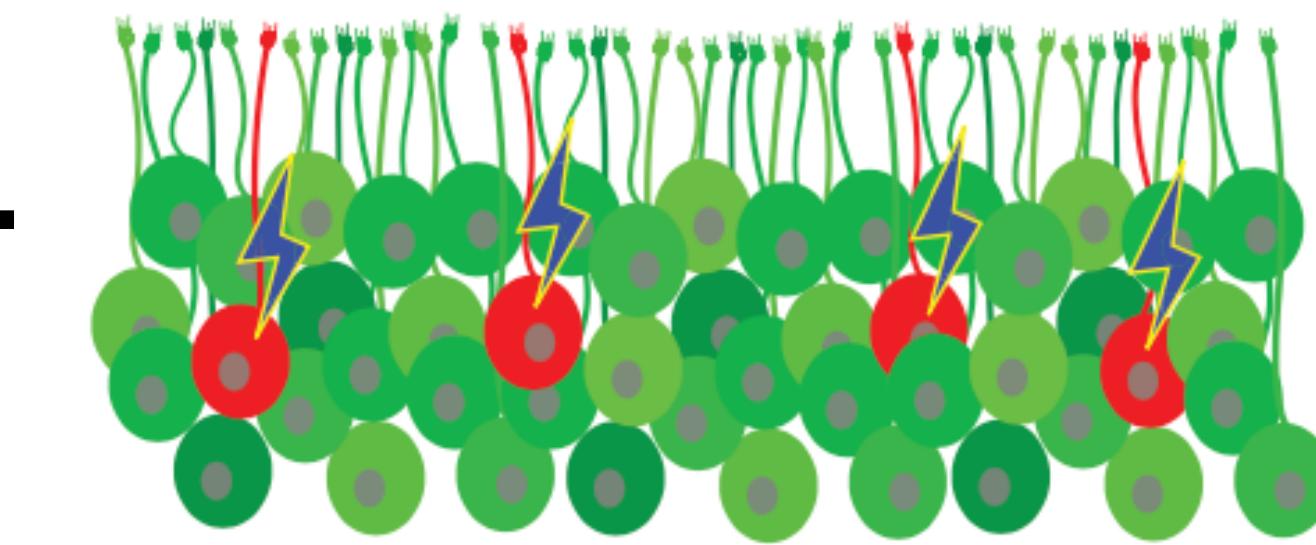


## FACS



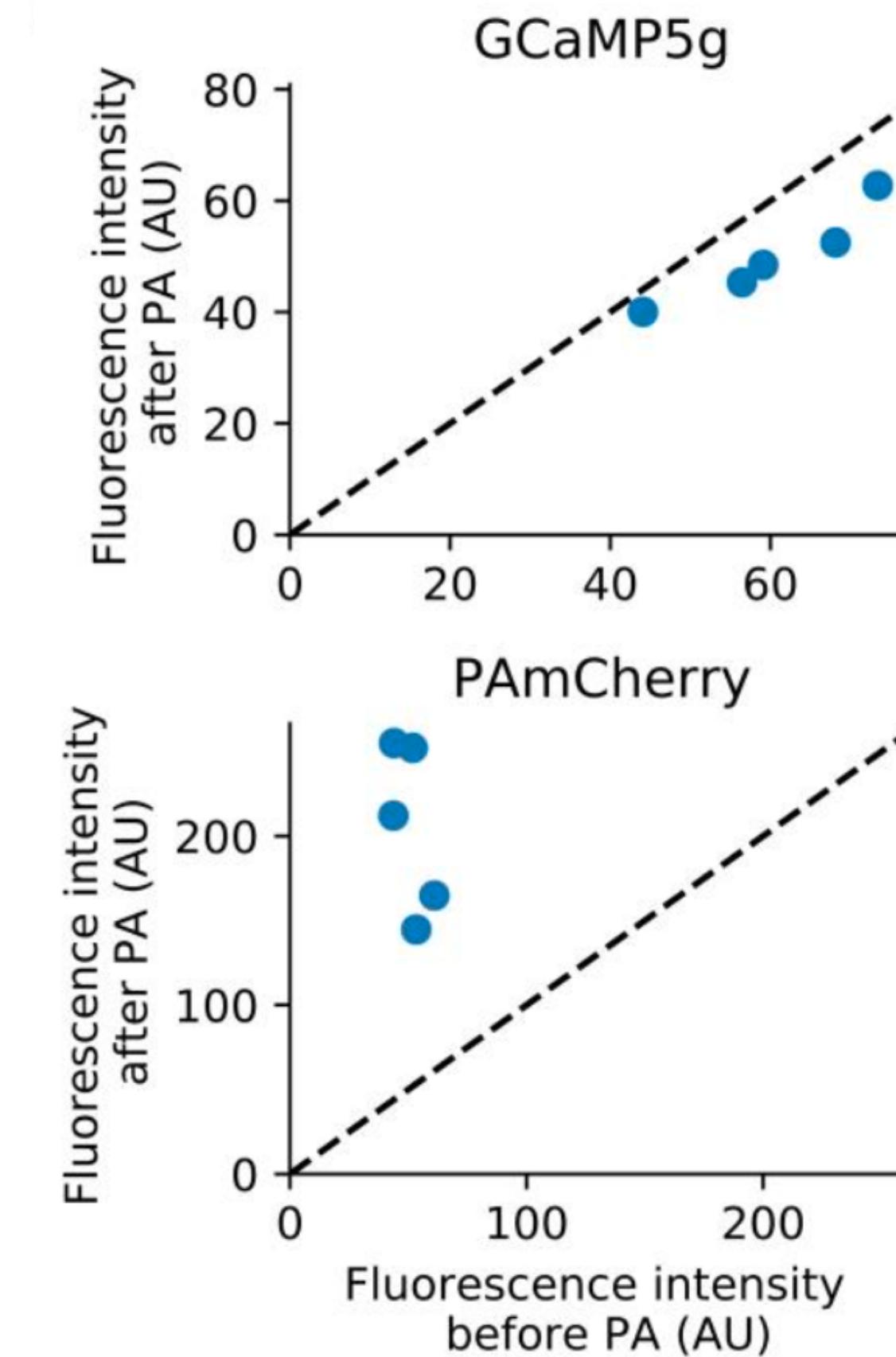
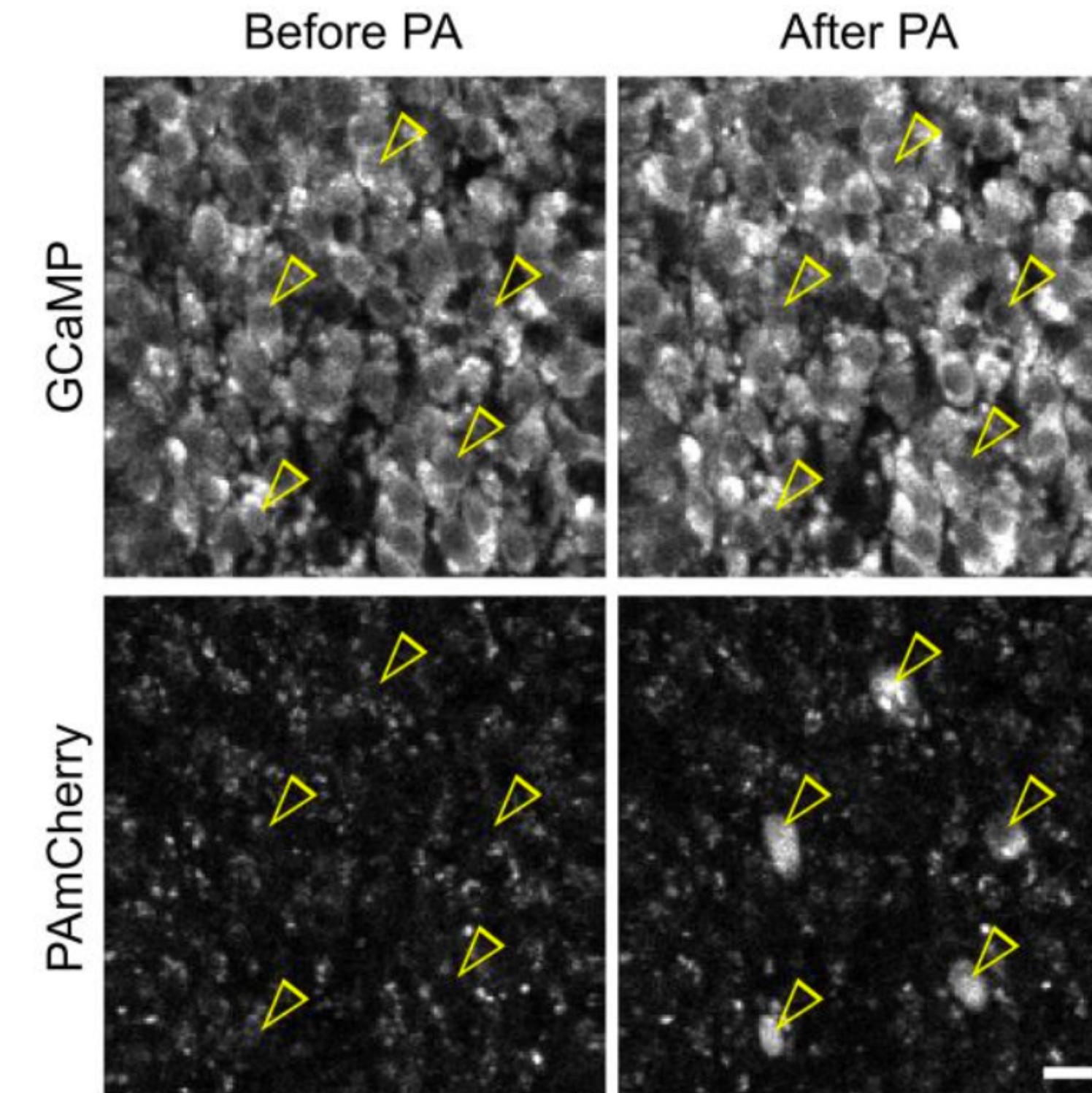
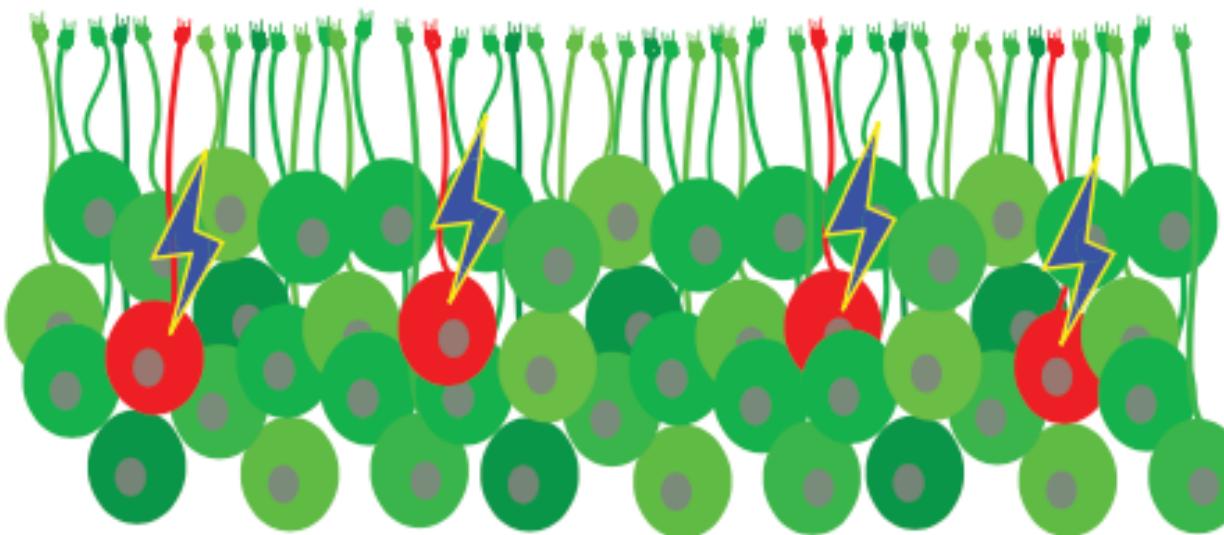
**Seq**

## Two-photon photoactivation

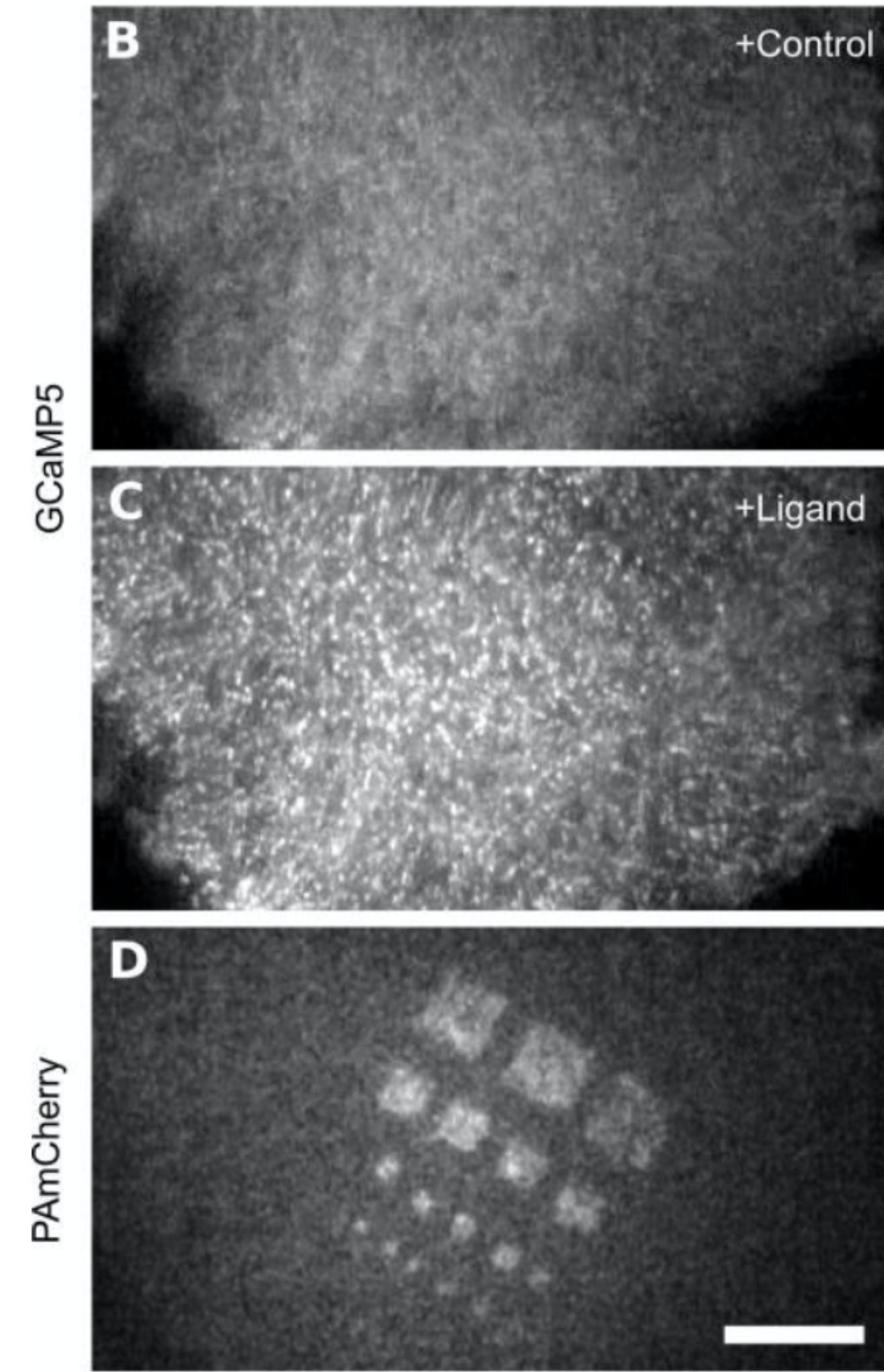
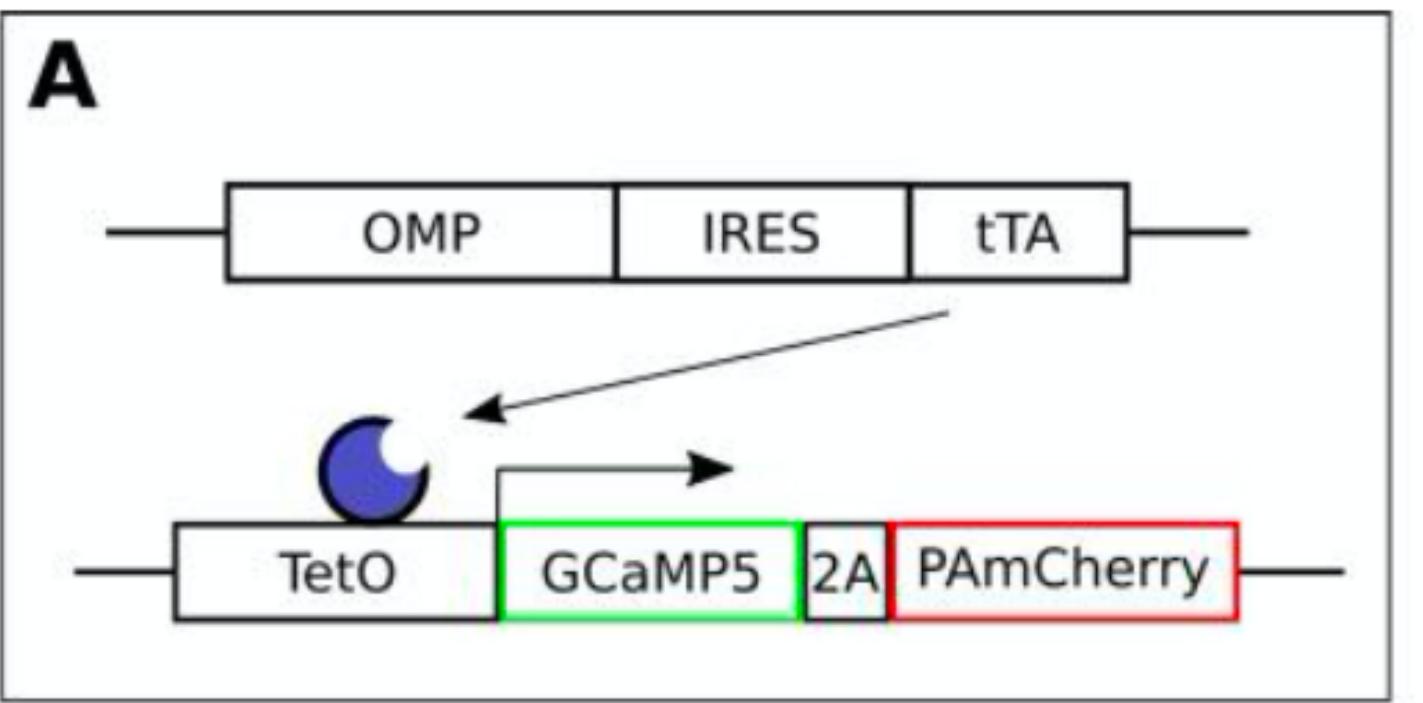


# Part I PhOT-seq

Two-photon photoactivation



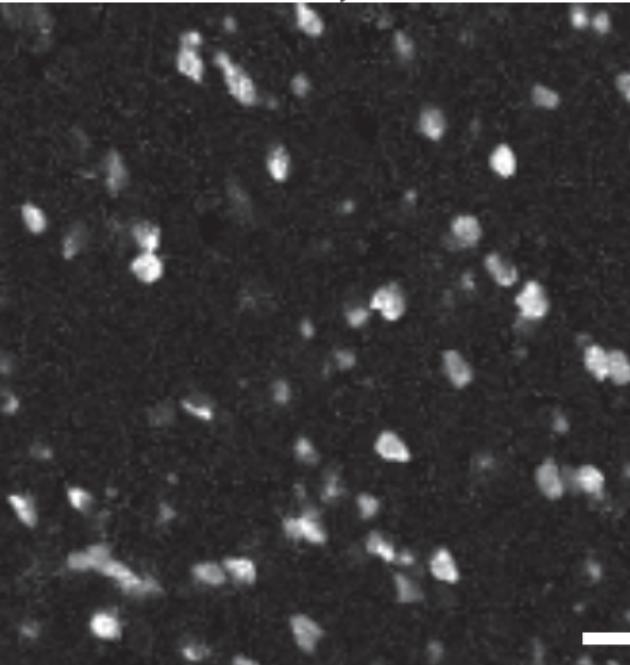
# Part I PhOT-seq



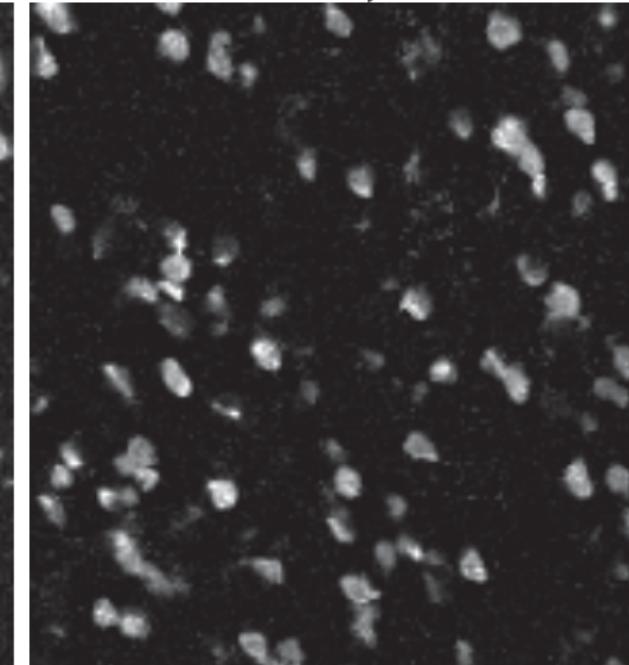
# Part I PhOT-seq

**B**

GCaMP; A7864



GCaMP; E1050

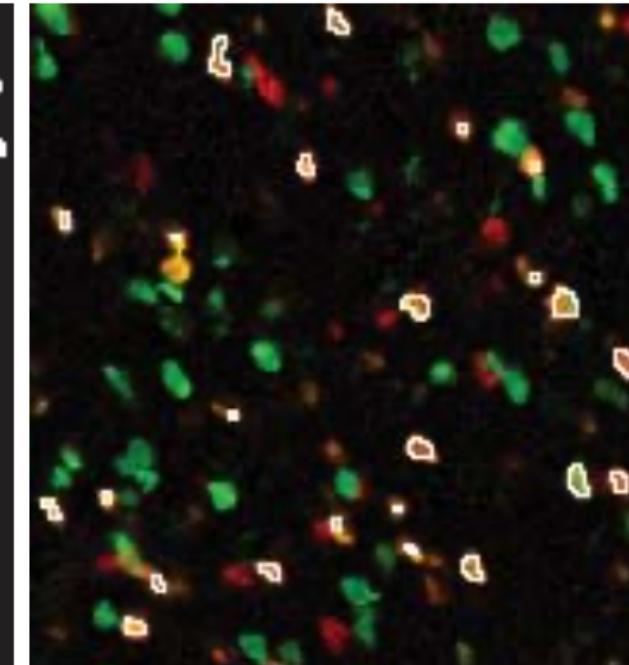


Two Photon imaging

PA mask

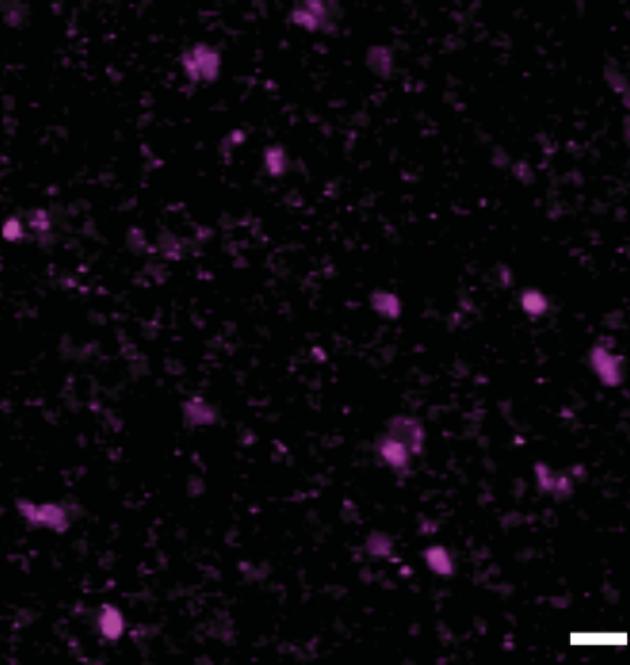


A7864/E1050/PA mask

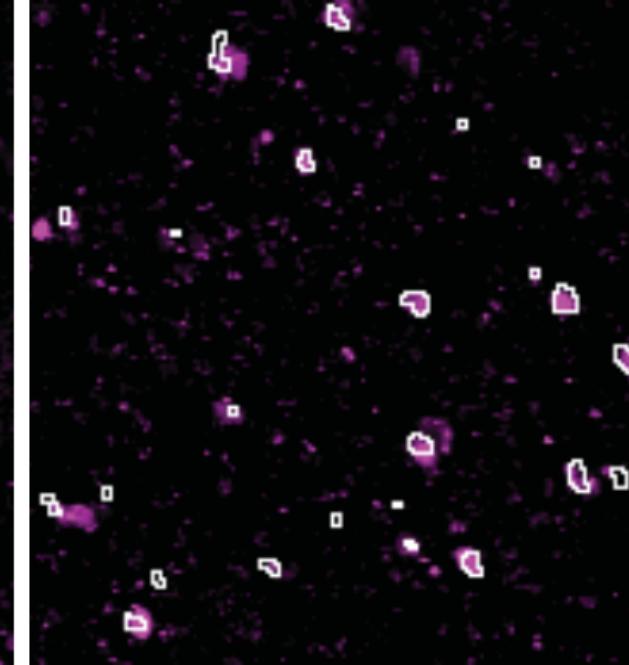


**E**

PAmCherry

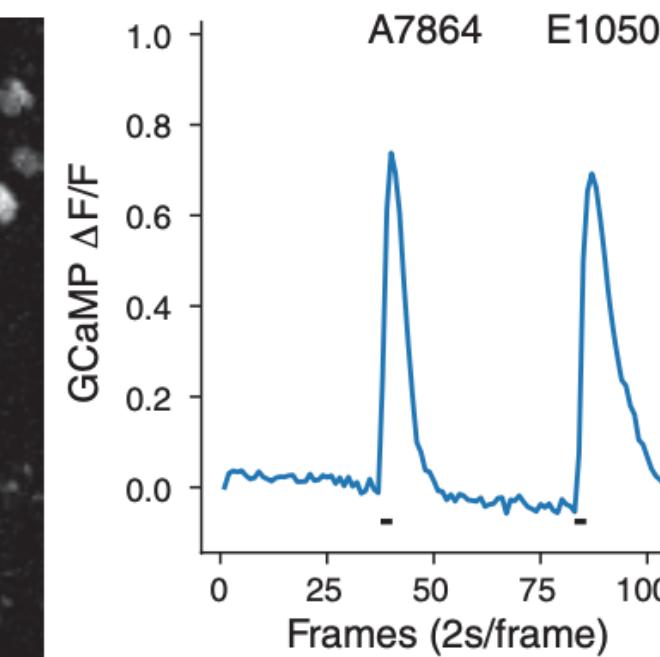


PAmCherry/ PA mask

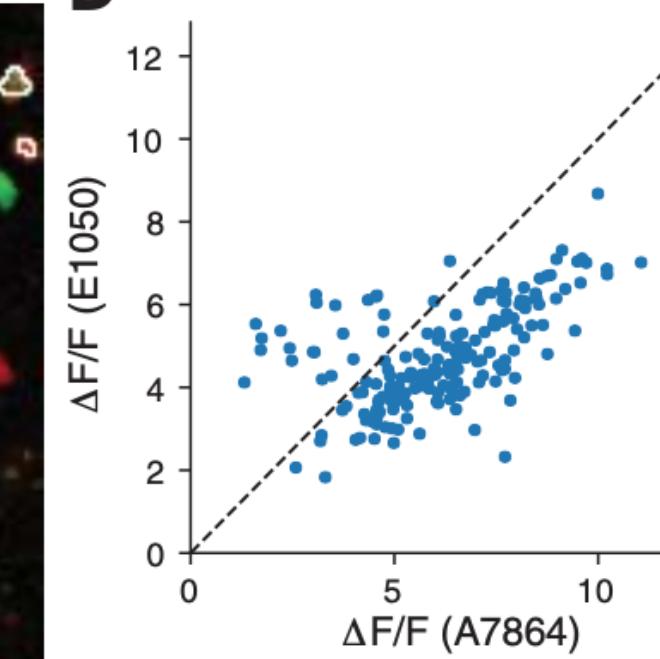


Confocal imaging

**C**



**D**



**Cell Clusters**

Stim 1

Stim 2

I

Yes

No

II

No

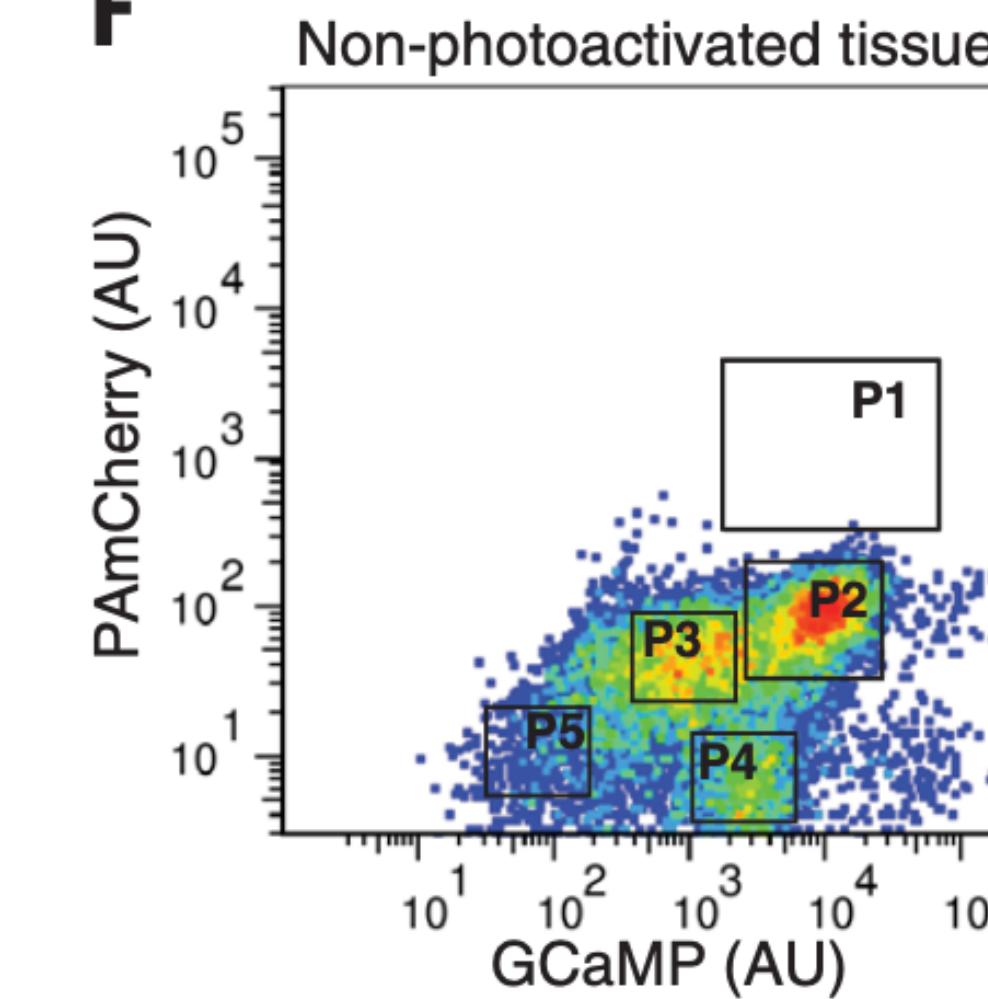
Yes

III\*

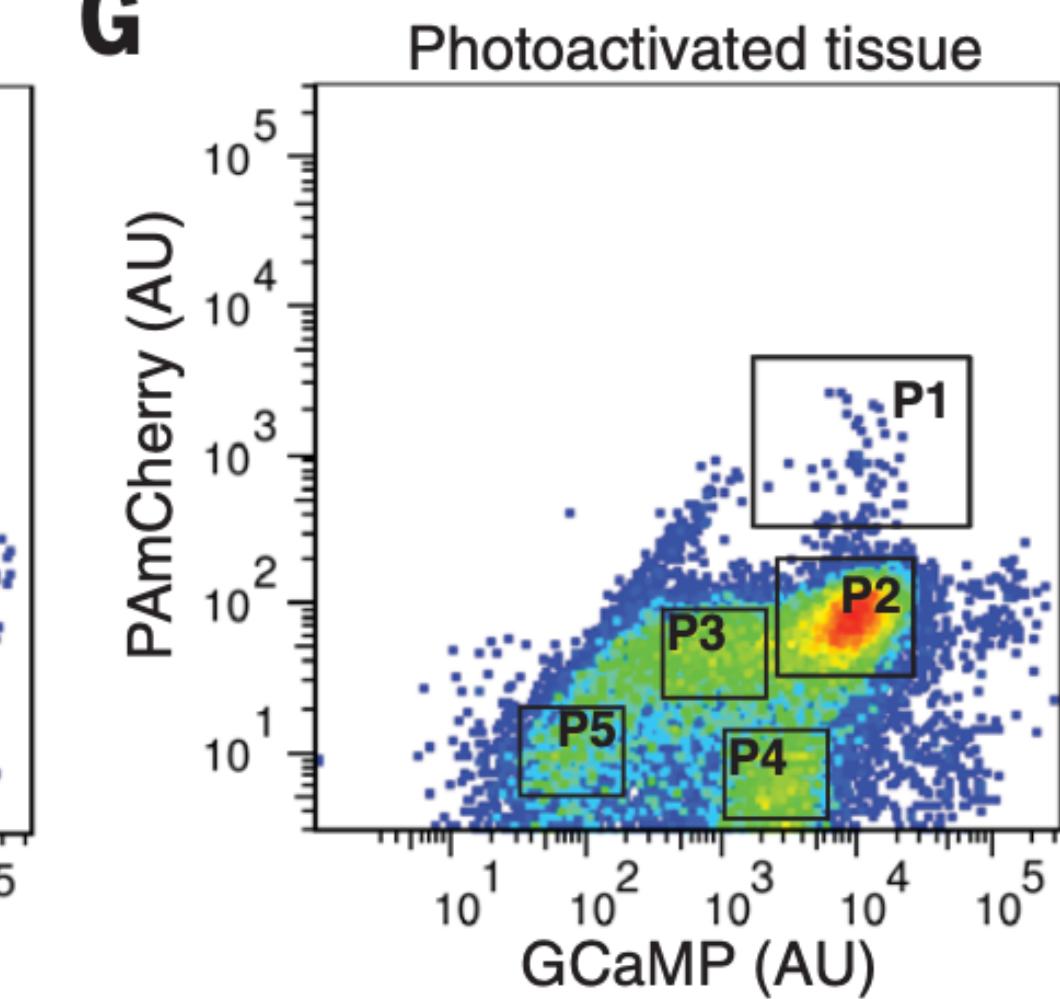
Yes

Yes

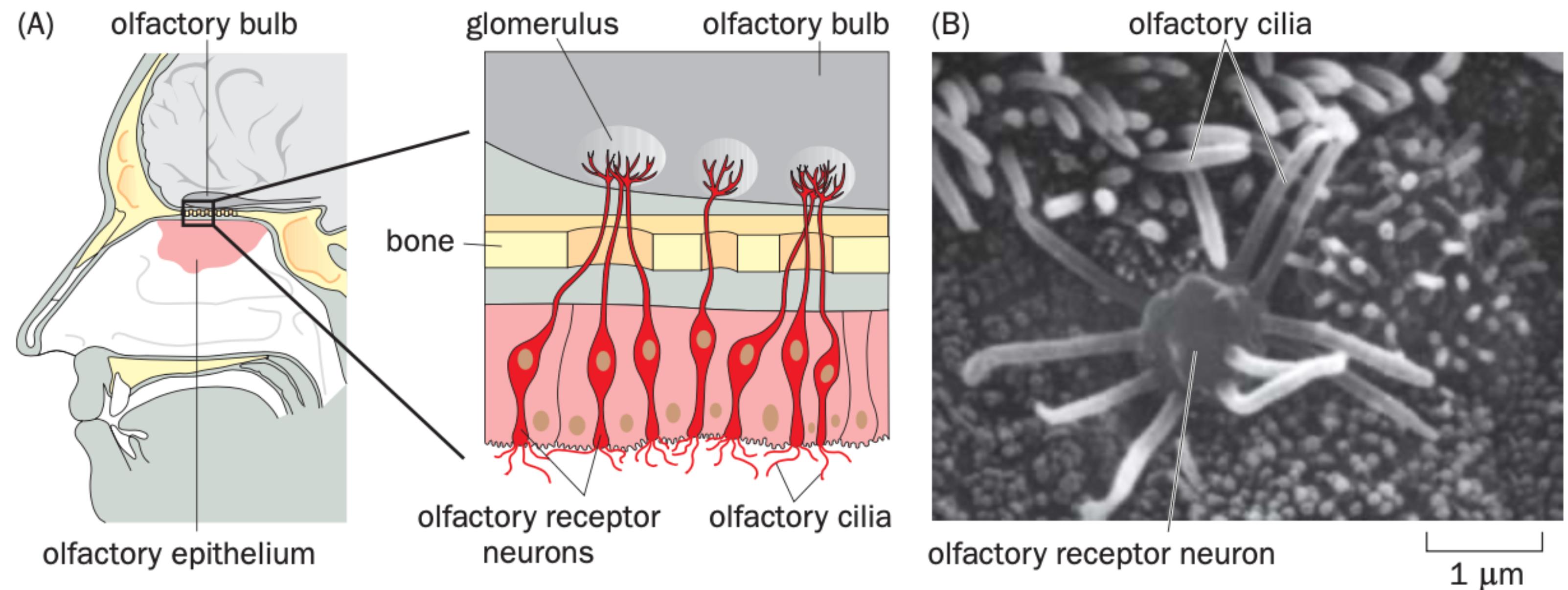
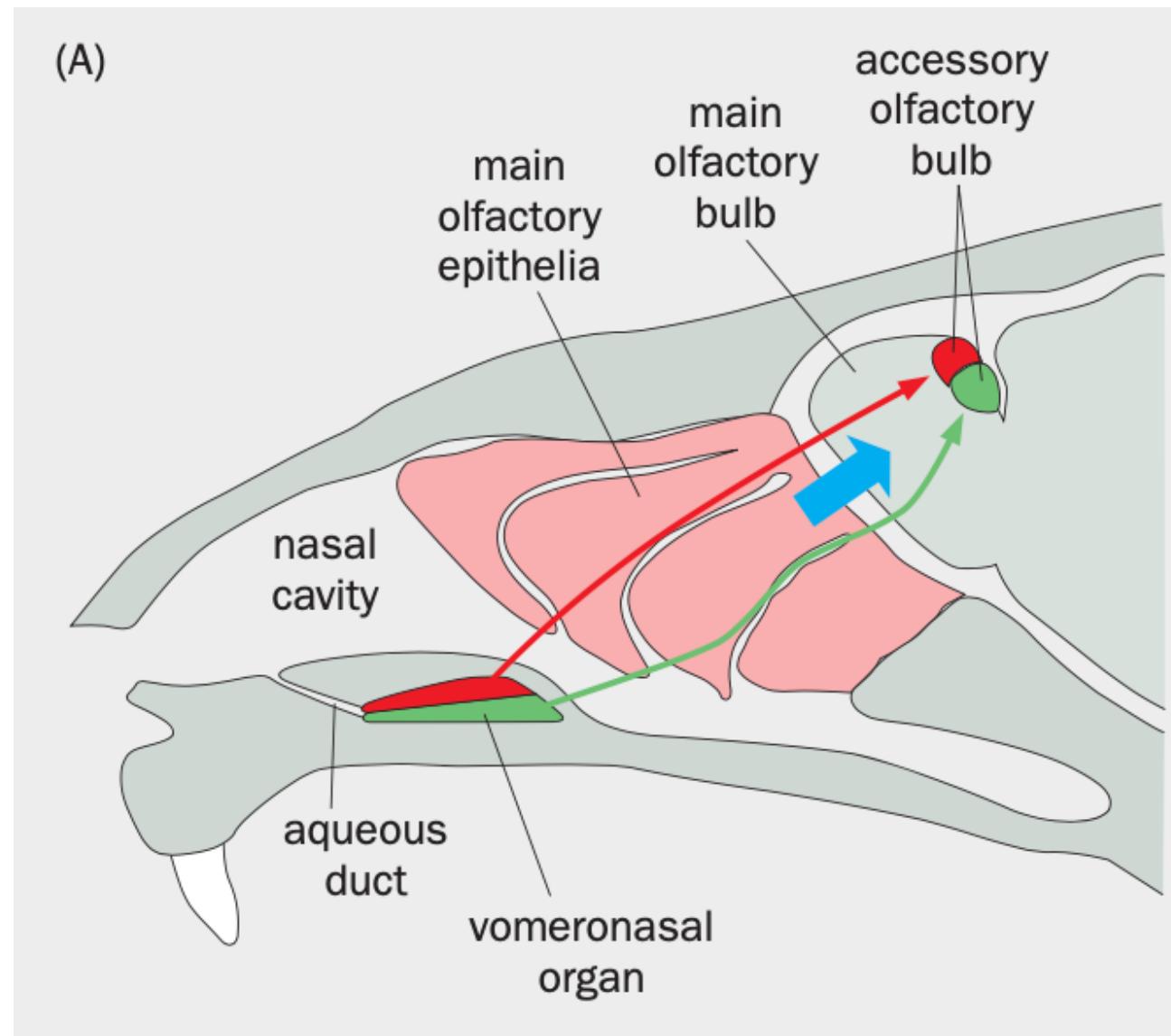
**F**



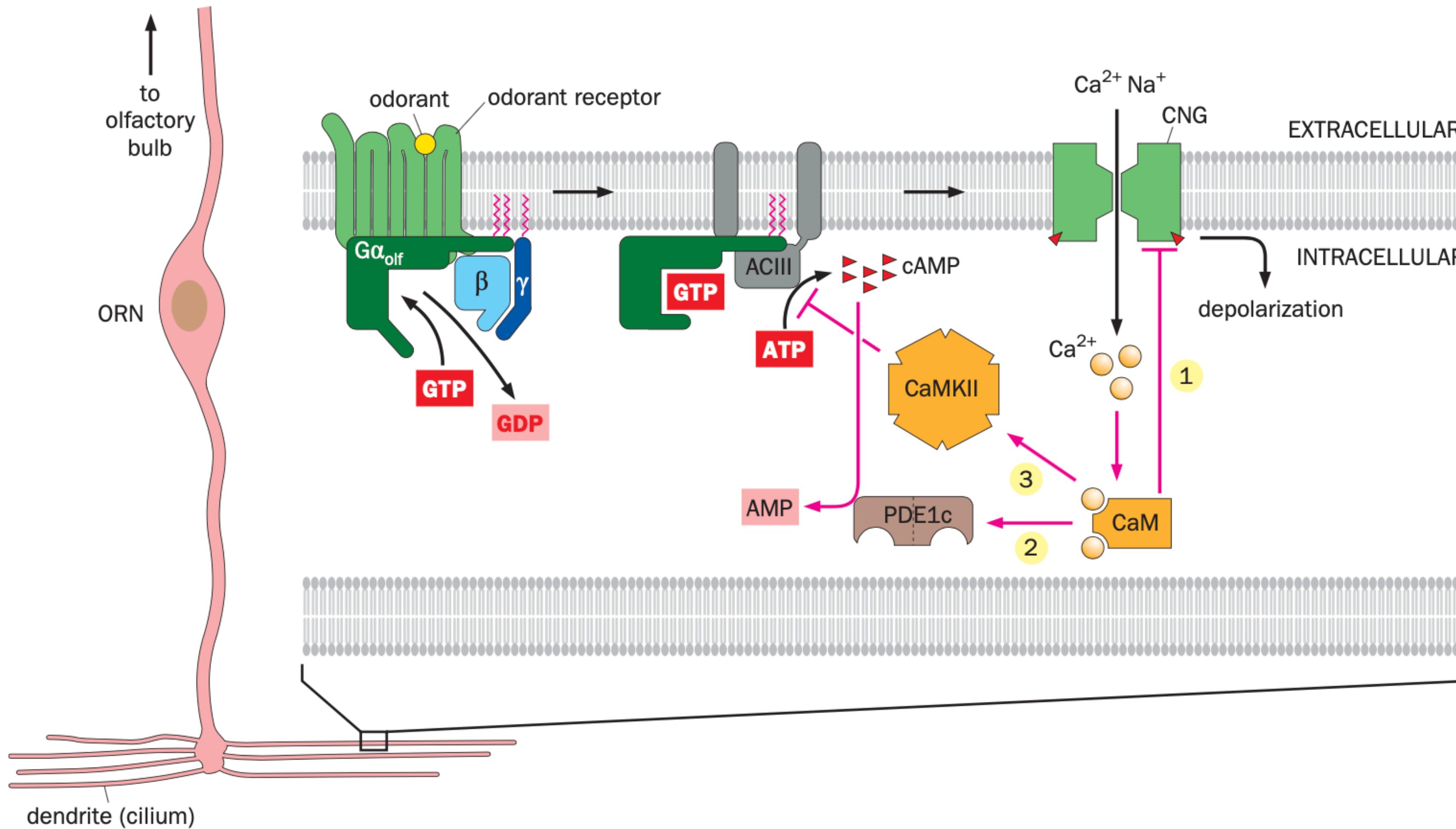
**G**



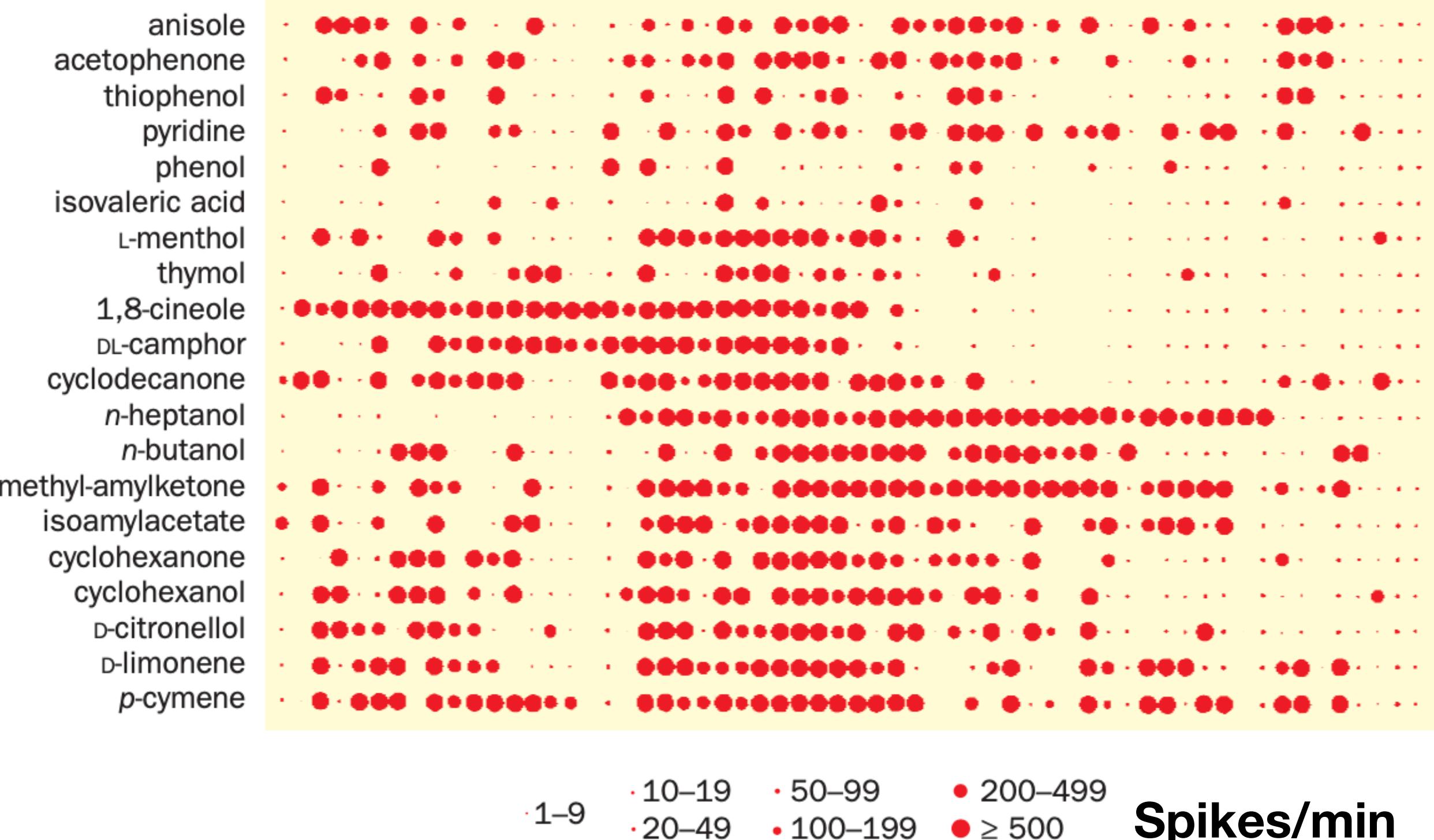
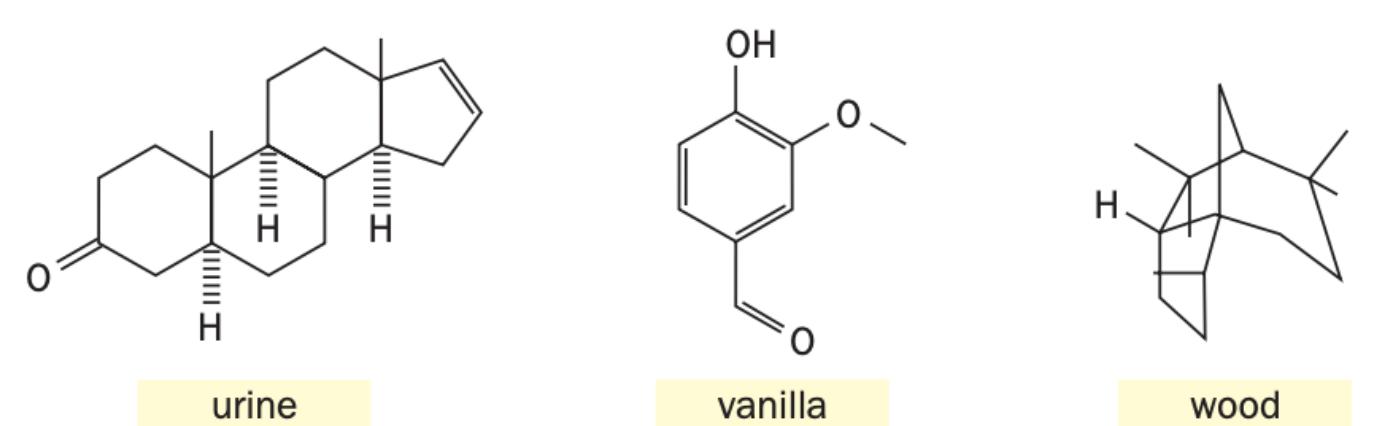
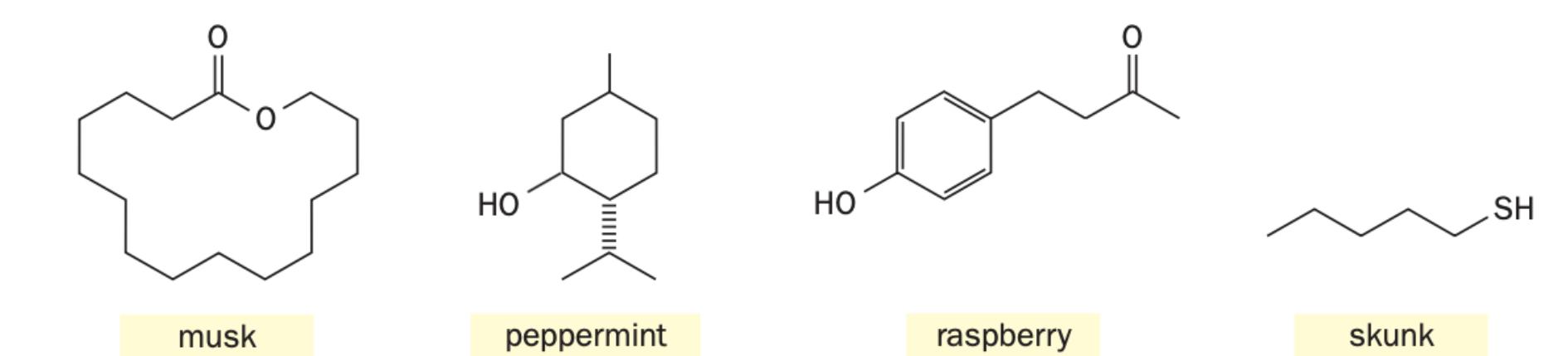
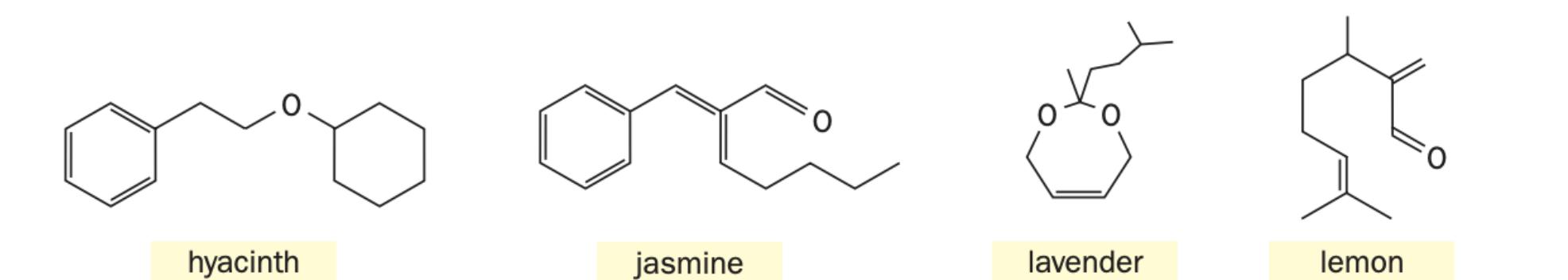
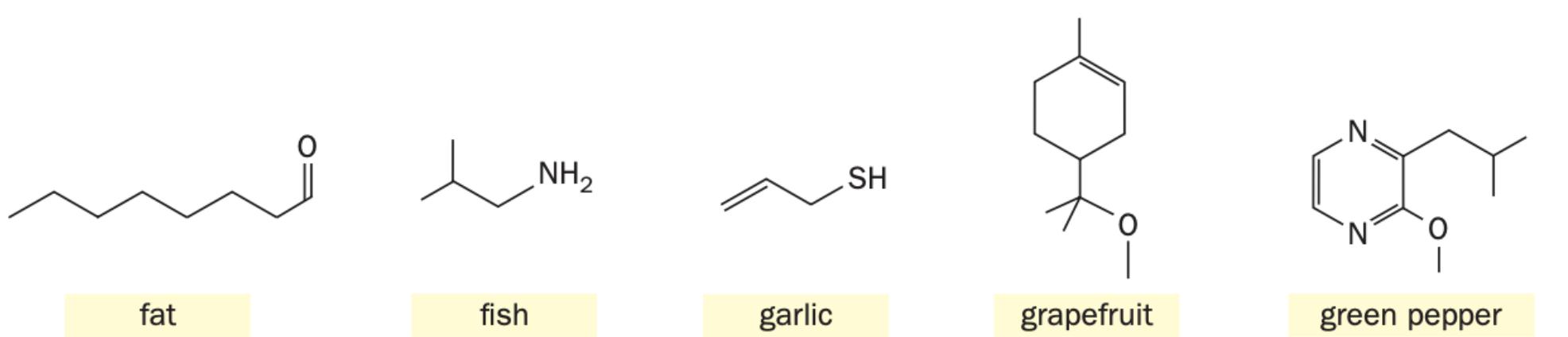
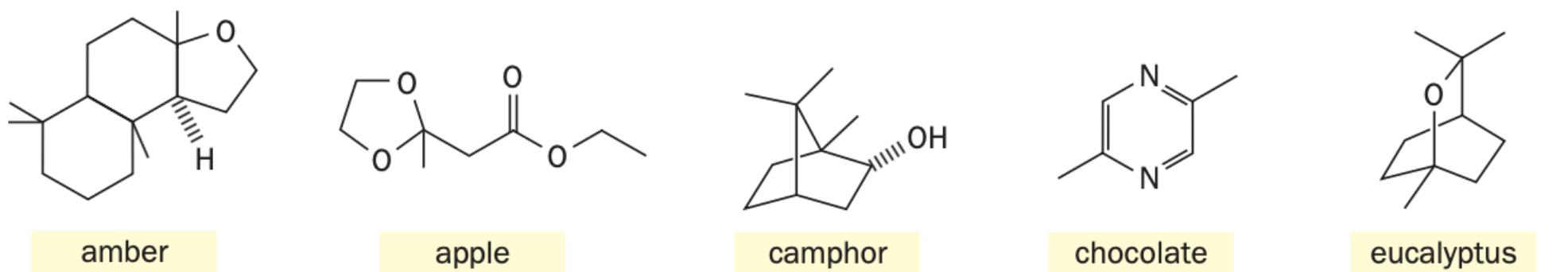
# Part II Olfactory Sensation



## Part II Olfactory Sensation

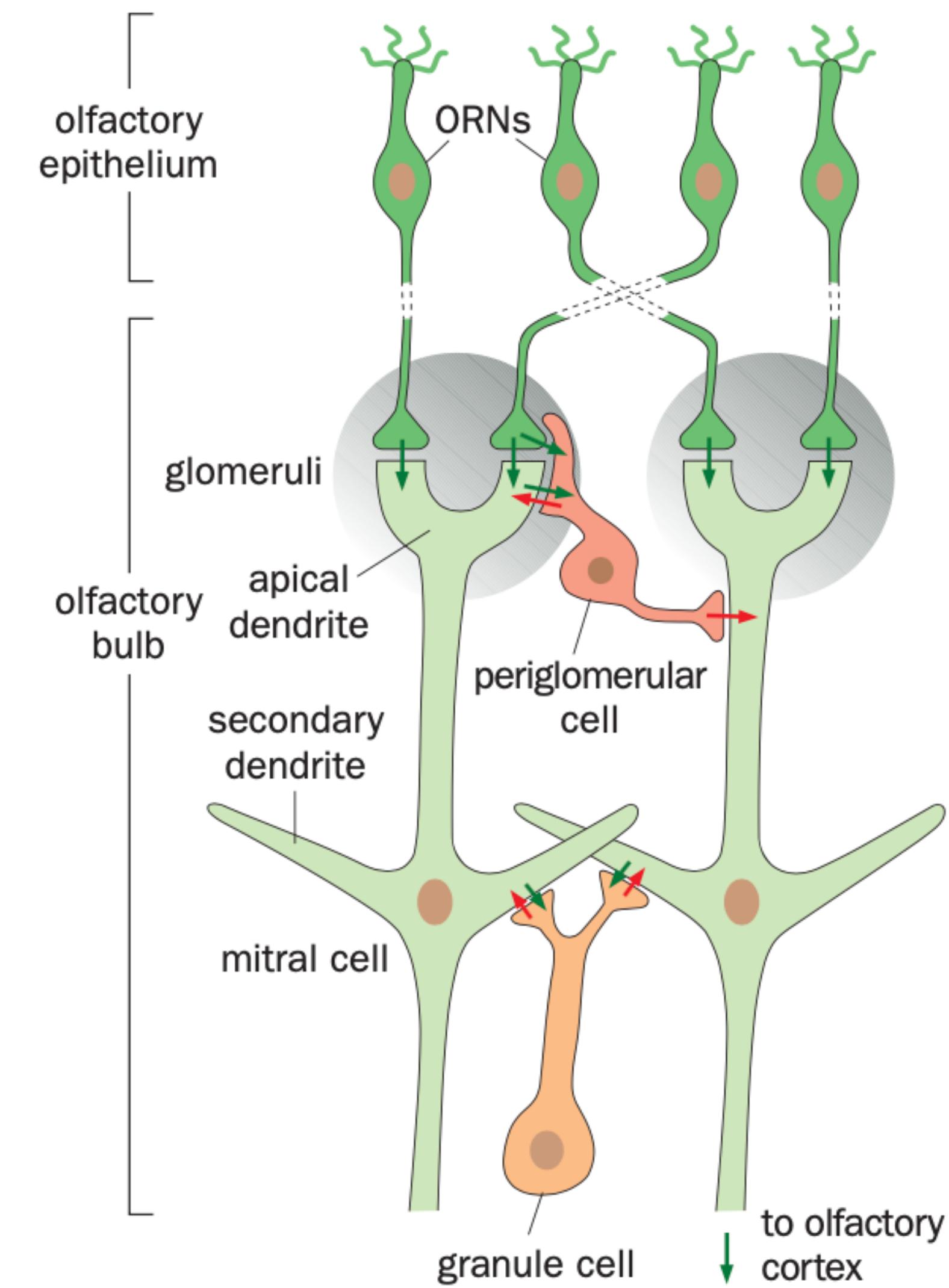
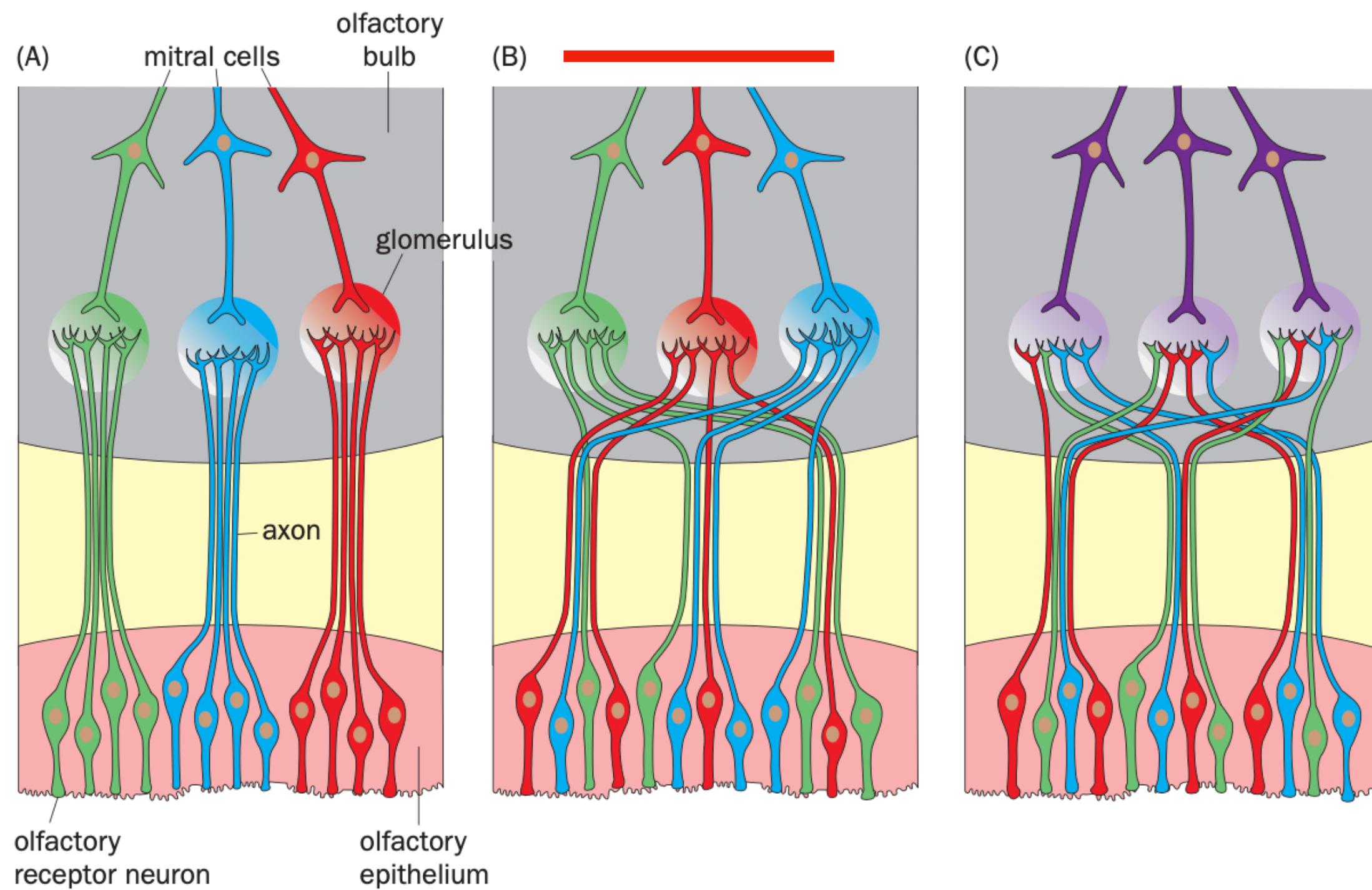


# Part II Olfactory Sensation



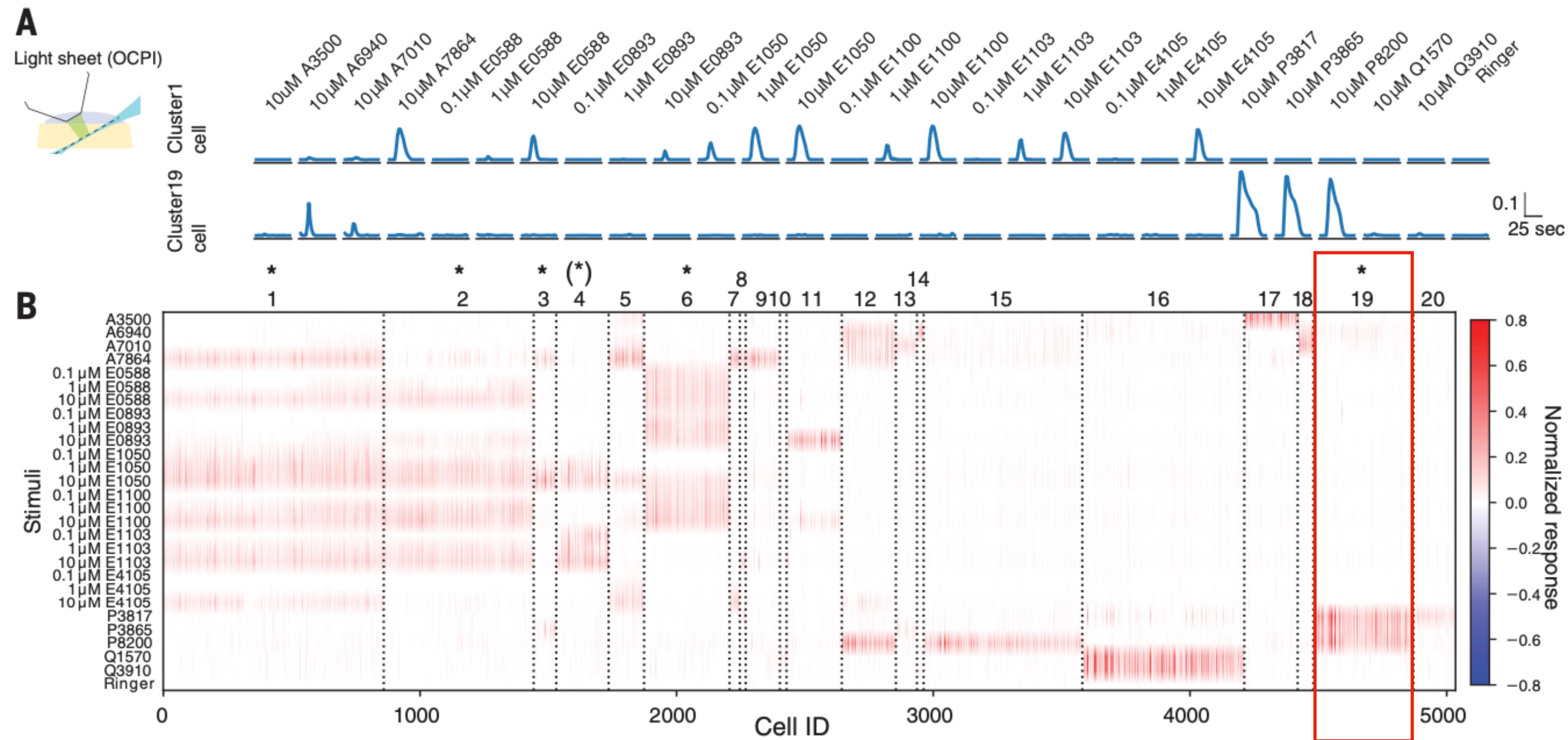
? Single receptor or multi-receptors

# Part II Olfactory Sensation

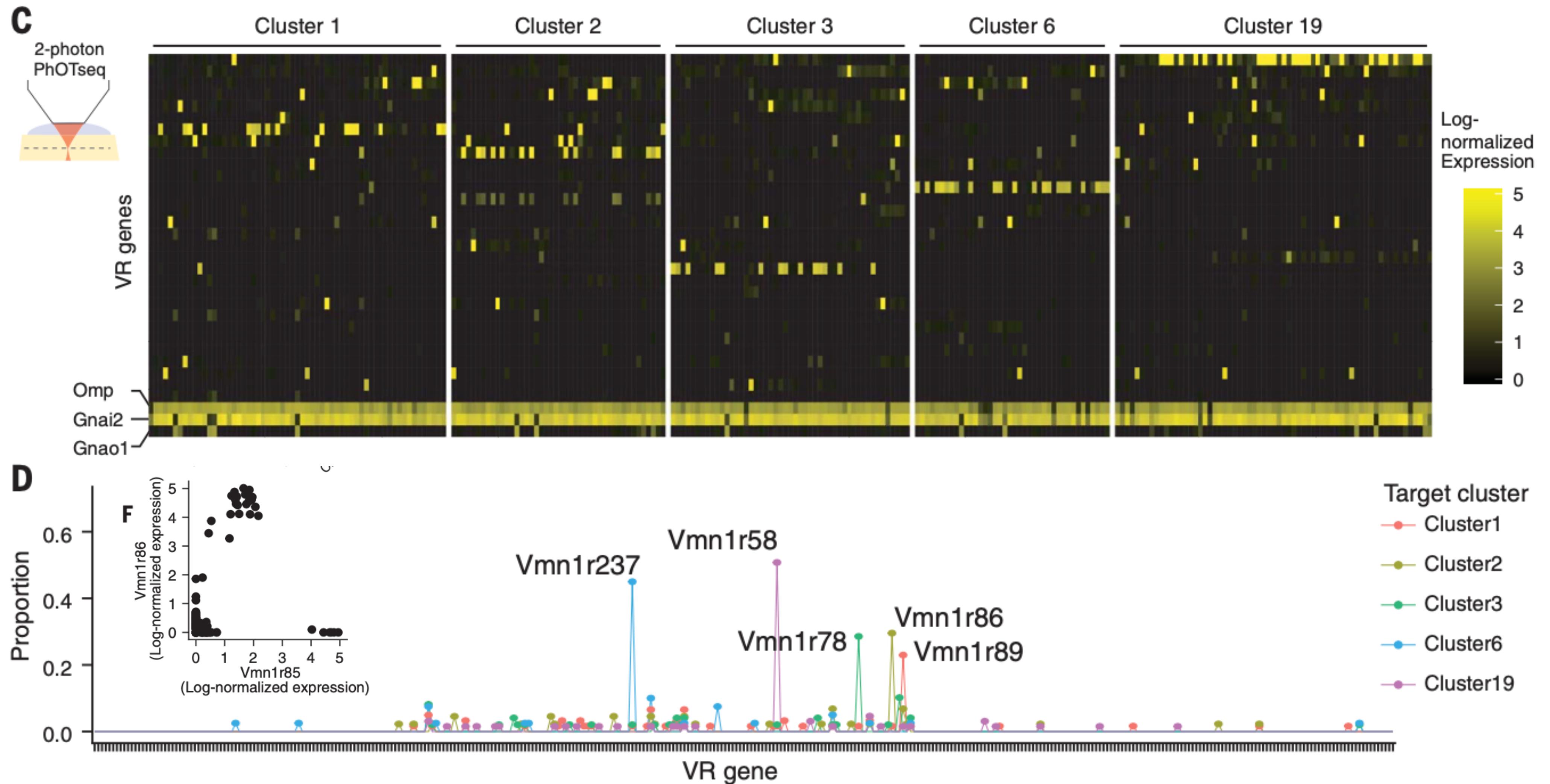


# Part II Olfactory Sensation

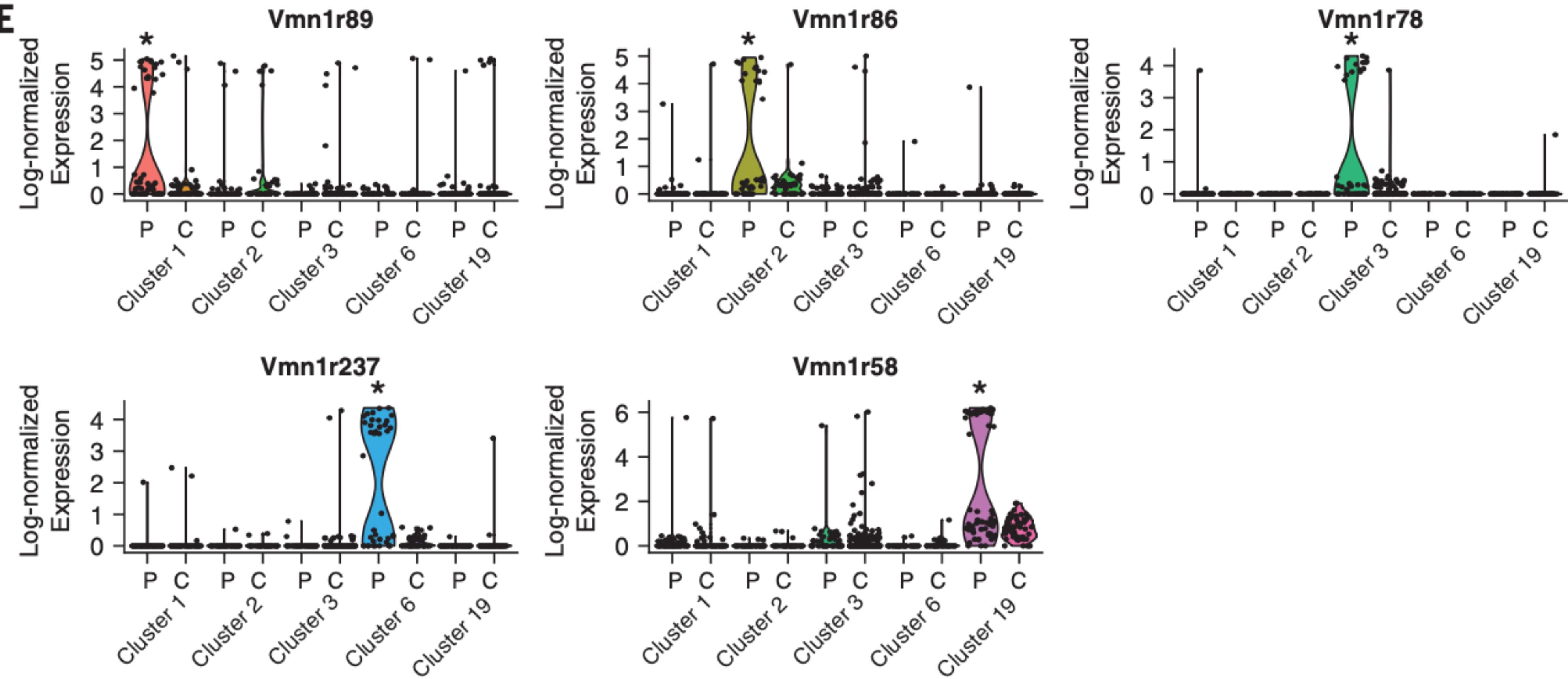
Question: Correlation between sequence and Chemosensation



## Part II Olfactory Sensation

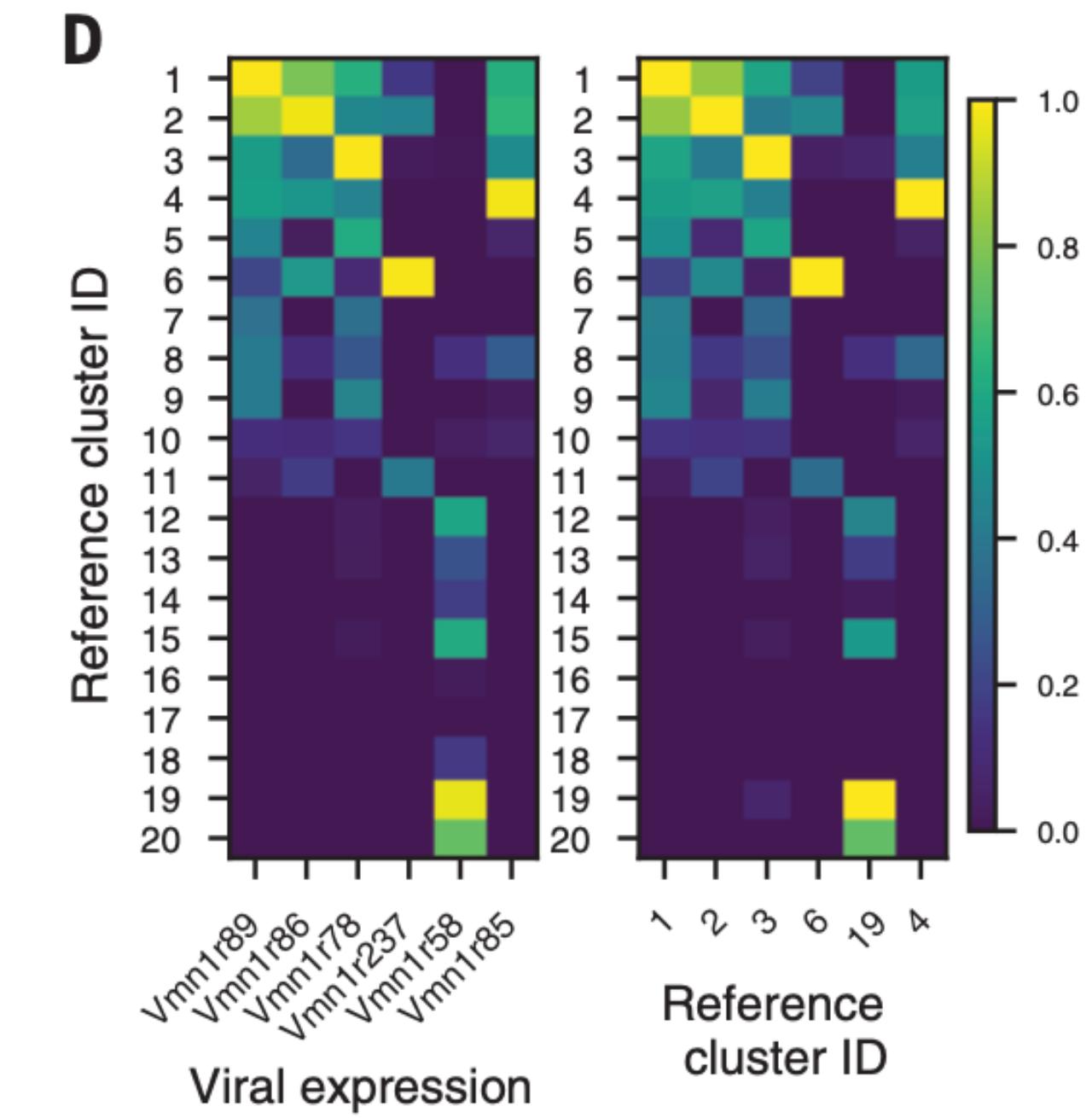
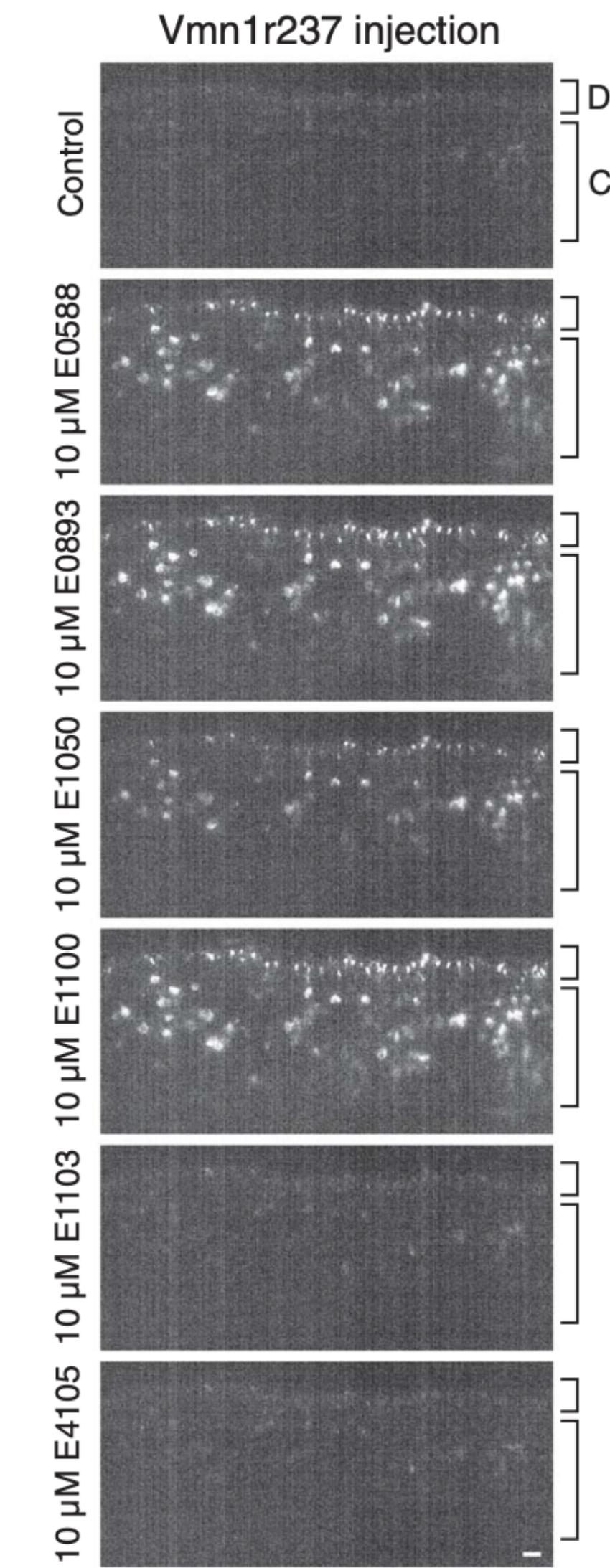
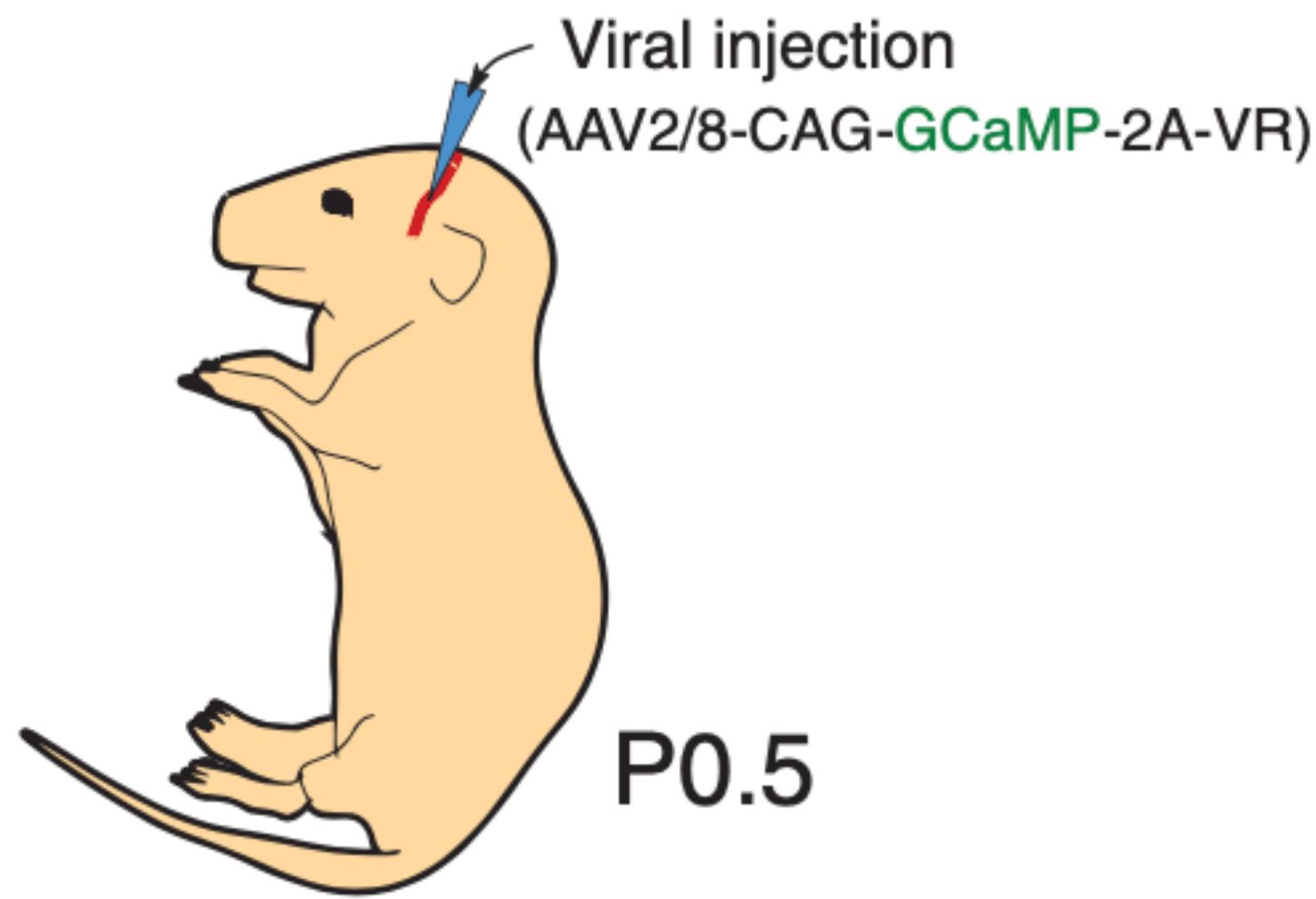


## Part II Olfactory Sensation



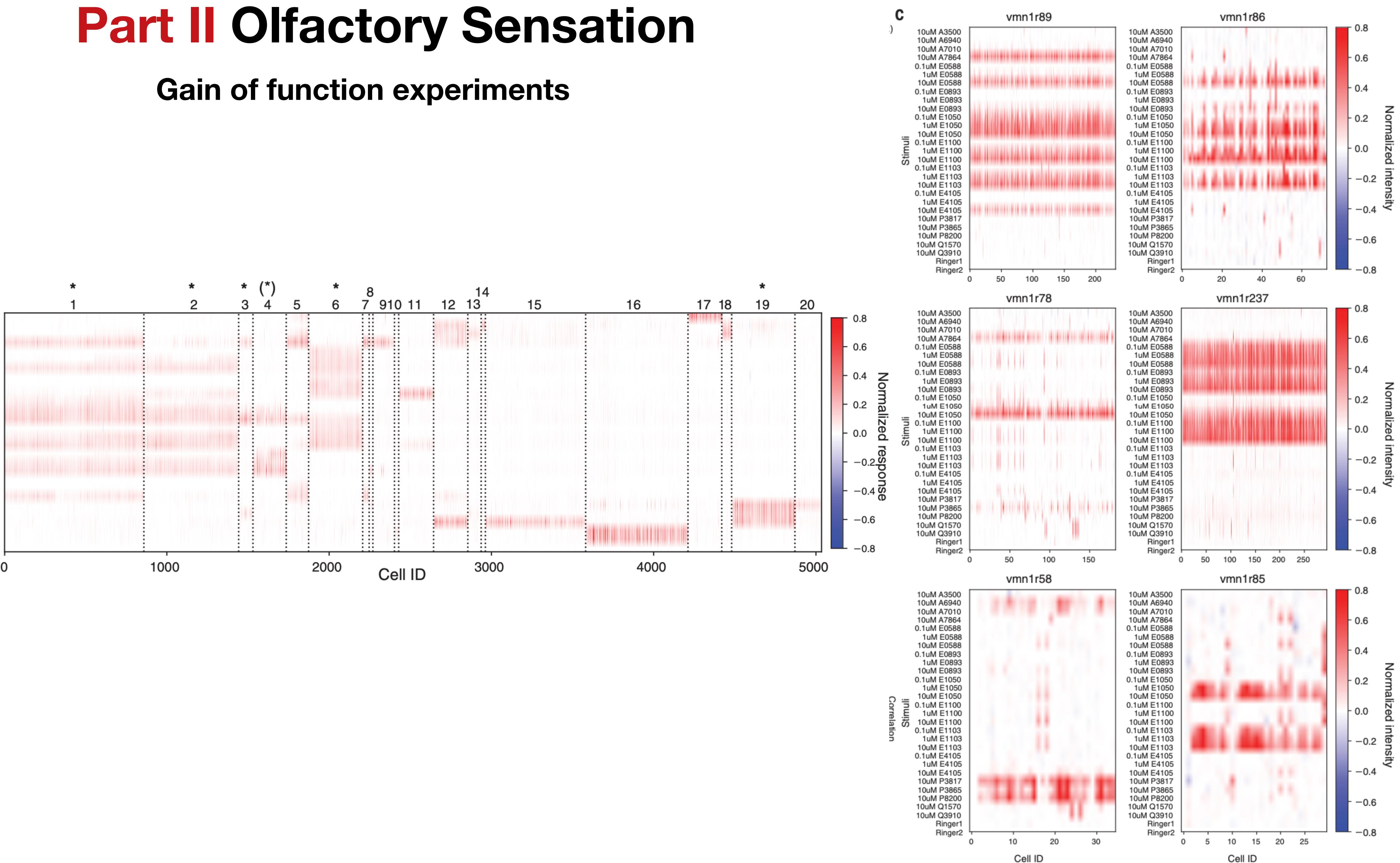
# Part II Olfactory Sensation

## Gain of function experiments

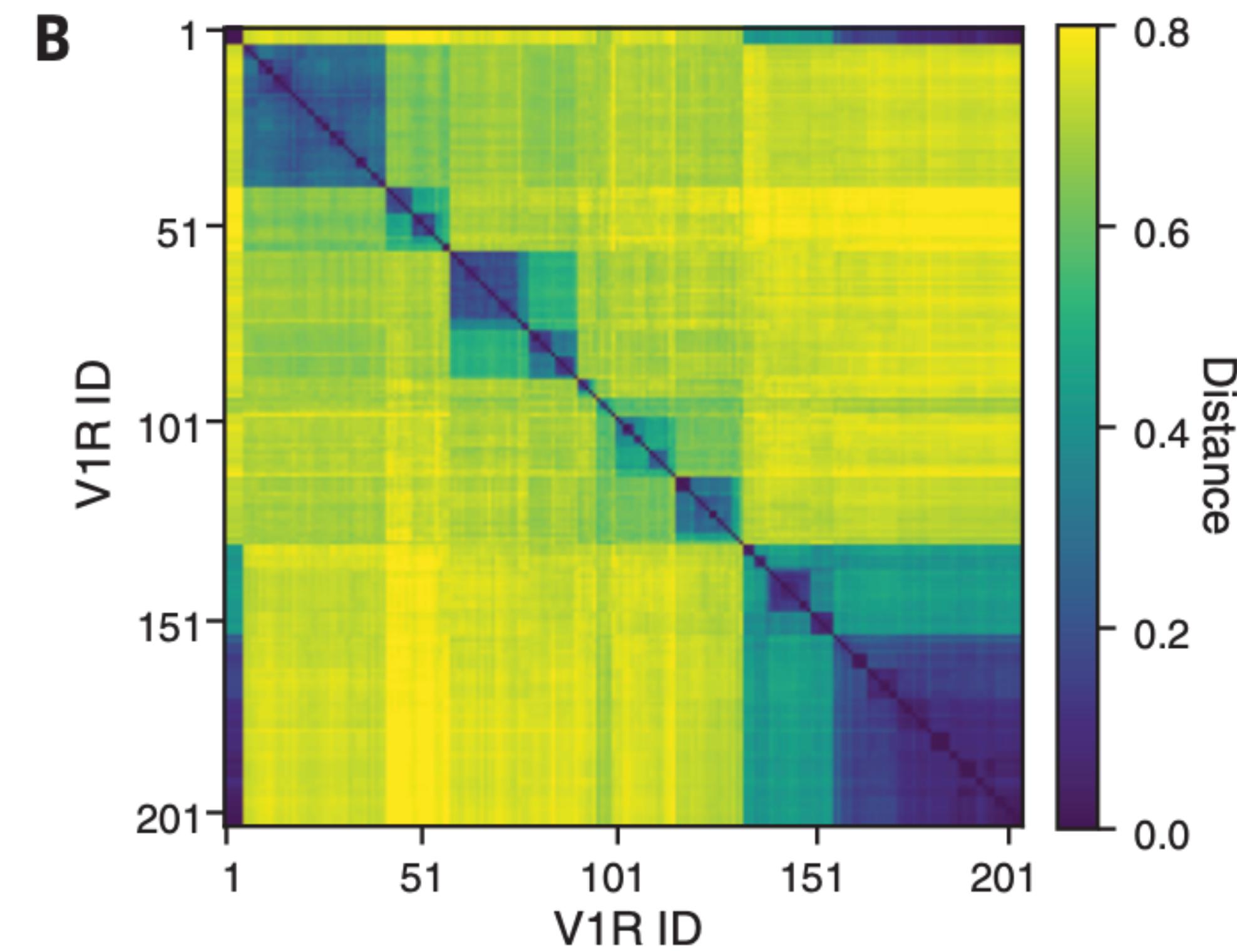
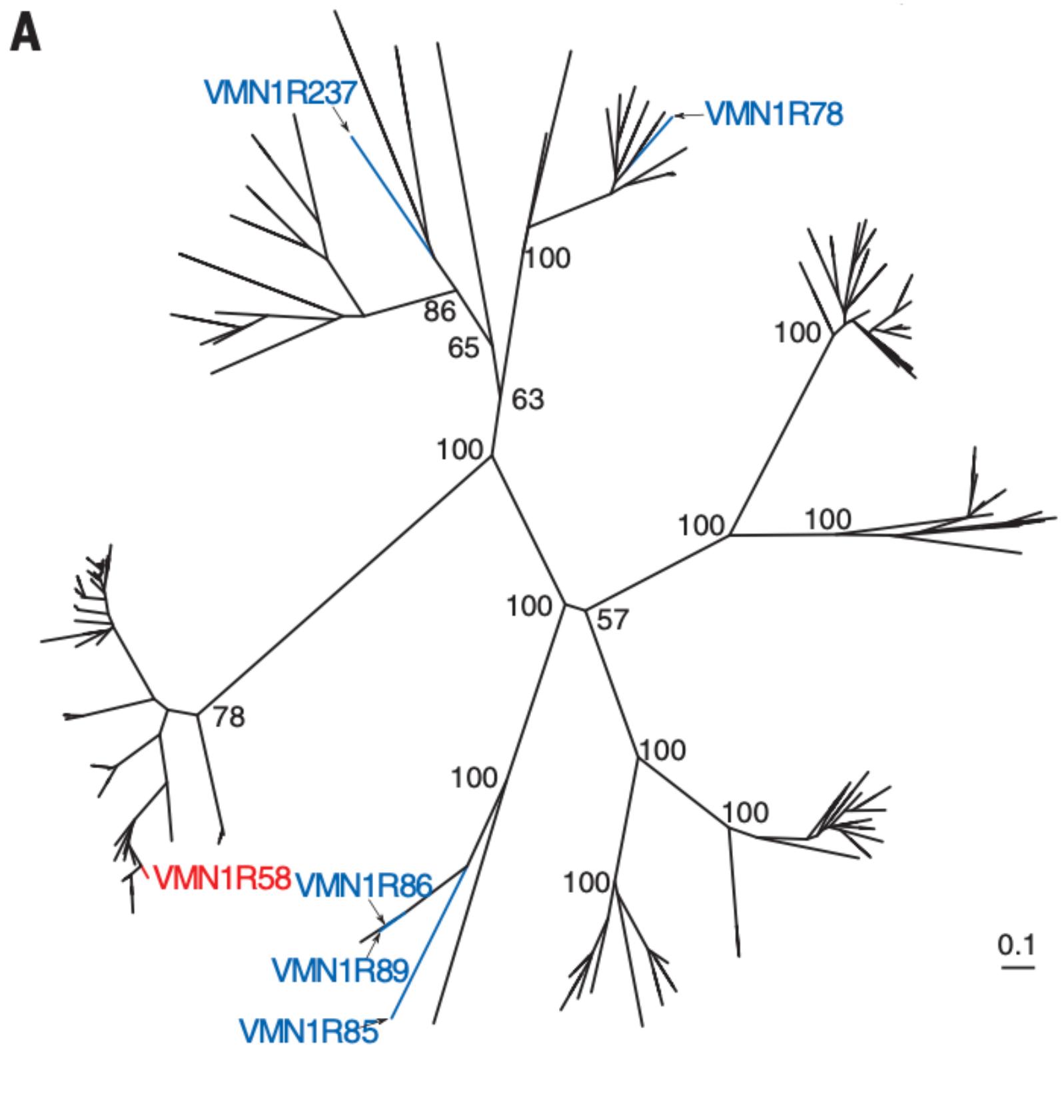


# **Part II Olfactory Sensation**

# Gain of function experiments



## Part II Olfactory Sensation



# Part II Olfactory Sensation

**C**

Rank	VMN1R89	VMN1R86	VMN1R85	VMN1R78	VMN1R237	VMN1R58
1	VMN1R86	VMN1R89	VMN1R86	VMN1R83	VMN1R236	VMN1R62
2	VMN1R88	VMN1R88	VMN1R89	VMN1R75	VMN1R233	VMN1R63
3	VMN1R85	VMN1R85	VMN1R88	VMN1R80	VMN1R234	VMN1R60
4	VMN1R87	VMN1R87	VMN1R87	VMN1R72	VMN1R235	VMN1R57
5	VMN1R81	VMN1R81	VMN1R236	VMN1R76	VMN1R225	VMN1R61
6	VMN1R236	VMN1R236	VMN1R237	VMN1R81	VMN1R226	VMN1R59
7	VMN1R83	VMN1R82	VMN1R233	VMN1R74	VMN1R66	VMN1R56
8	VMN1R82	VMN1R84	VMN1R78	VMN1R79	VMN1R224	VMN1R64
9	VMN1R84	VMN1R215	VMN1R11	VMN1R73	VMN1R78	VMN1R55
10	VMN1R77	VMN1R15	VMN1R31	VMN1R3	VMN1R67	VMN1R65
11	VMN1R237	VMN1R237	VMN1R77	VMN1R238	VMN1R69	VMN1R171
12	VMN1R73	VMN1R77	VMN1R81	VMN1R2	VMN1R184	VMN1R167
13	VMN1R75	VMN1R83	VMN1R28	VMN1R77	VMN1R228	VMN1R169
14	VMN1R78	VMN1R73	VMN1R26	VMN1R82	VMN1R232	VMN1R175
15	VMN1R80	VMN1R70	VMN1R224	VMN1R84	VMN1R76	VMN1R170
16	VMN1R70	VMN1R233	VMN1R75	VMN1R237	VMN1R81	VMN1R176
17	VMN1R233	VMN1R79	VMN1R83	VMN1R236	VMN1R82	VMN1R90
18	VMN1R79	VMN1R201	VMN1R22	VMN1R70	VMN1R68	VMN1R177
19	VMN1R76	VMN1R216	VMN1R234	VMN1R185	VMN1R185	VMN1R125
20	VMN1R74	VMN1R78	VMN1R79	VMN1R184	VMN1R79	VMN1R168

