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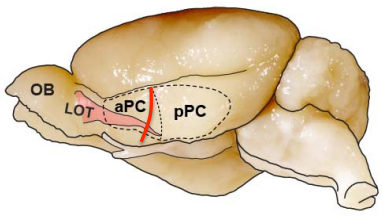
Olfaction 2015 ◆ KITP, UC Santa Barbara

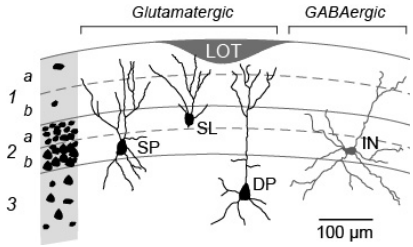
Spontaneous and odor-evoked activity in the mouse piriform cortex *in vivo*

John Bekkers
Eccles Institute of Neuroscience
John Curtin School of Medical Research
The Australian National University, Canberra

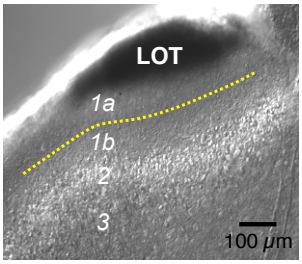
Australian National University

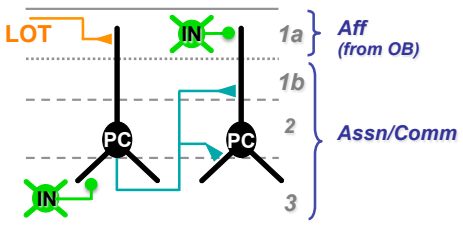
The piriform cortex: A trilaminar paleocortex





SP = superficial pyramidal DP = deep pyramidal
SL = semilunar IN = GABA interneuron





From: Neville & Haberly *(2004) Olfactory cortex. In: The Synaptic Organization of the Brain*

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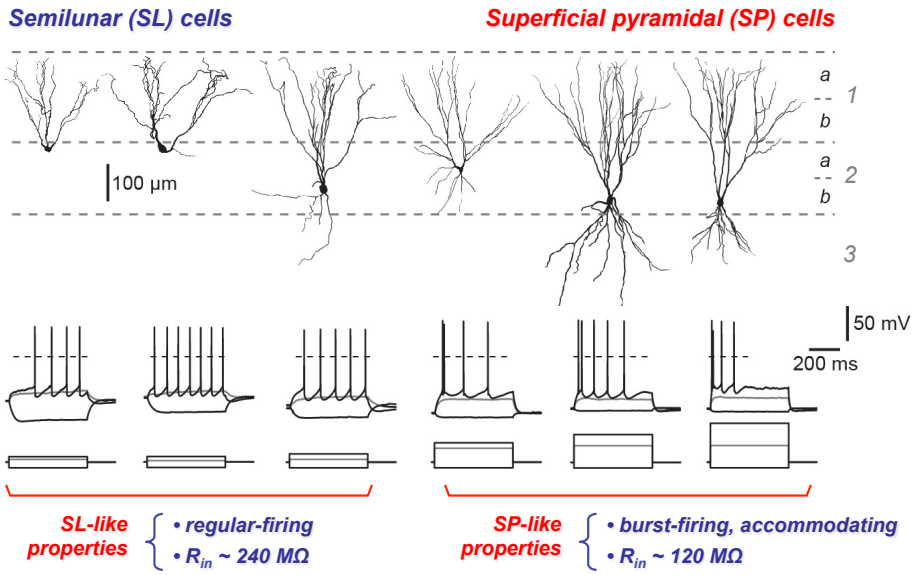
Two (or graded?) types of layer 2 neurons



Suzuki & Bekkers *(2011) J Neurosci 31:2156*

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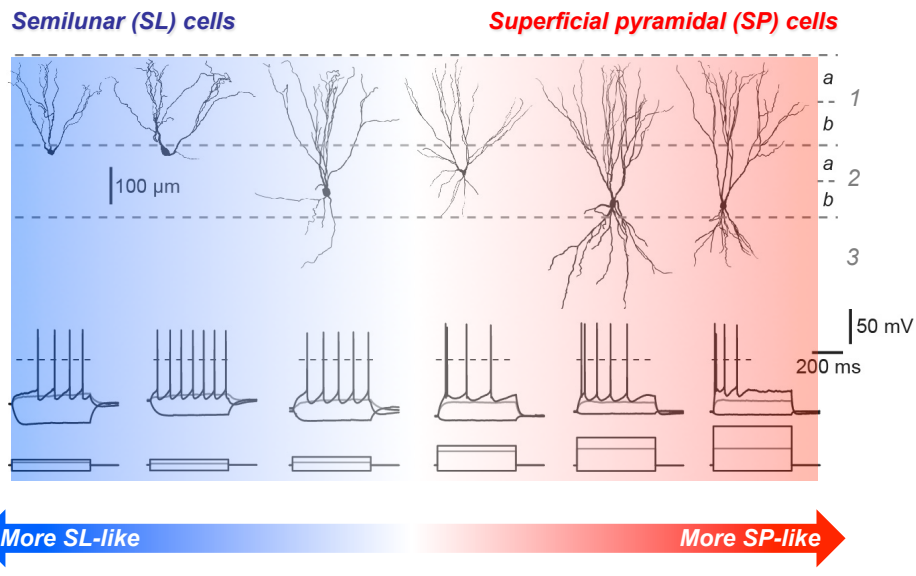
Two (or graded?) types of layer 2 neurons



Suzuki & Bekkers *(2011) J Neurosci 31:2156*

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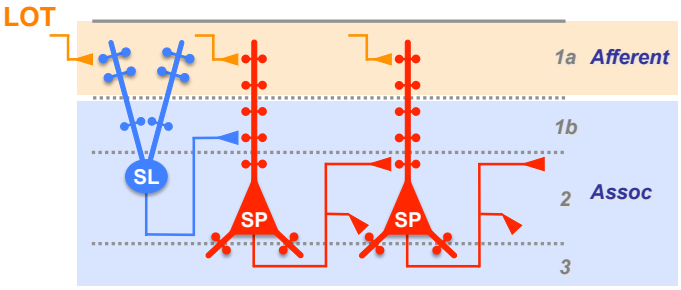
An elaborated layer 2 excitatory circuit



- *SL cells are more specialized for receiving afferent (LOT) input*
- *SP cells receive much greater associational input than SL cells and so experience more recurrent excitation*

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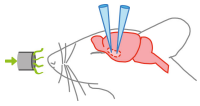
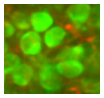
Talk outline

1. Anatomy:
Layers of excitation & inhibition



2. 'In vivo' patching:
Odor coding by SL and SP cells



3. Ca imaging 'in vivo':
Network activity

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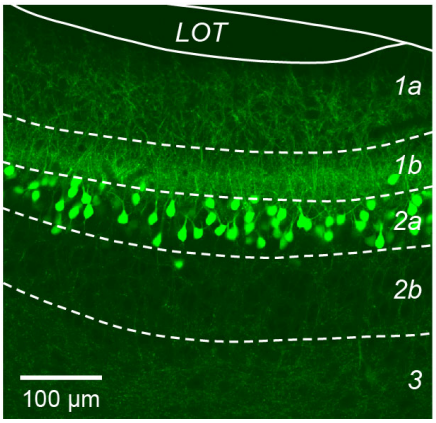
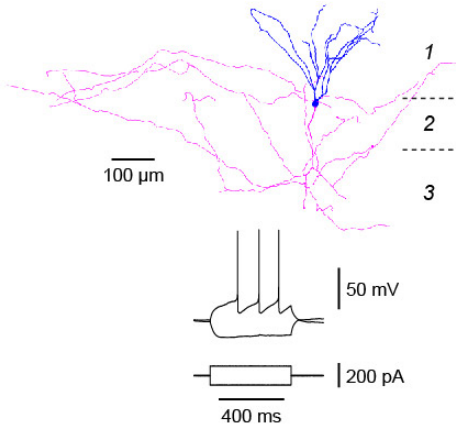
A transgenic mouse with labeled neurons in layer *2a*

48L mouse: *(mCitrine-tTA)*



Sacha Nelson (Brandeis Univ, Waltham MA)



Lentiviral enhancer trap screen: Kelsch *et al.* (2012) *PLoS One* 7:e38593

Choy, Suzuki, Nelson & Bekkers *(unpublished)*

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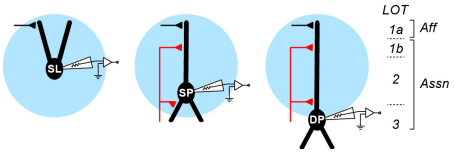
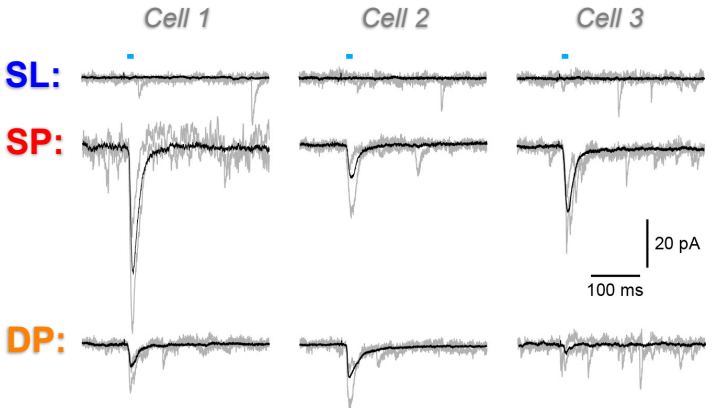
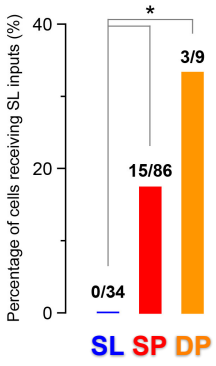
SL cells excite deeper cells but not each other



- ***Record from ChR2-neg SL, SP, DP cells***
- ***0.5 µM TTX, 100 µM 4-AP, 100 µM picro***





Choy, Suzuki, Nelson & Bekkers *(unpublished)*

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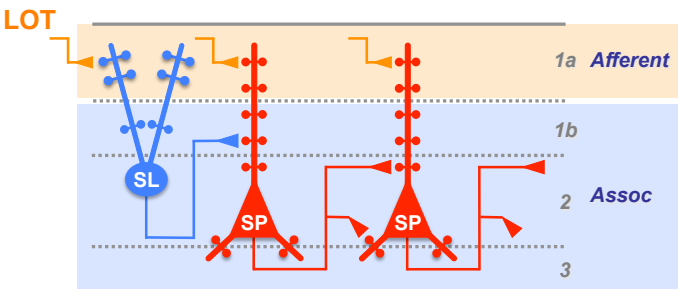

SL = semilunar

SP = superficial pyramidal

- *SL cells are more specialized for receiving afferent (LOT) input*
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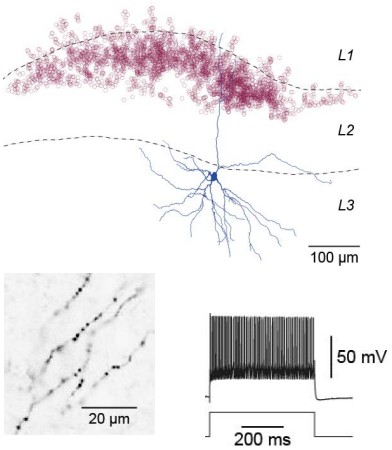
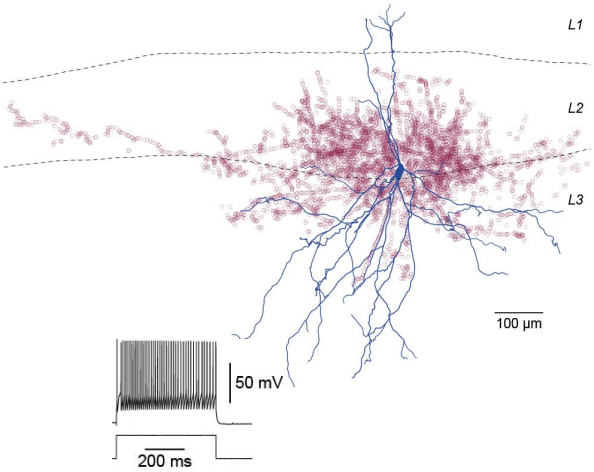
Fast-spiking cells provide layered feedback inhibition

Neuron 1:



Neuron 2:



Suzuki & Bekkers *(unpublished)*

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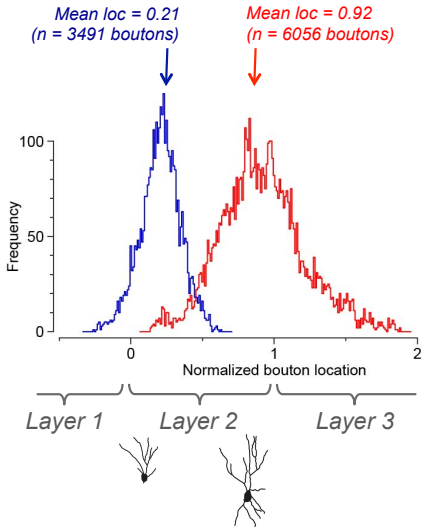

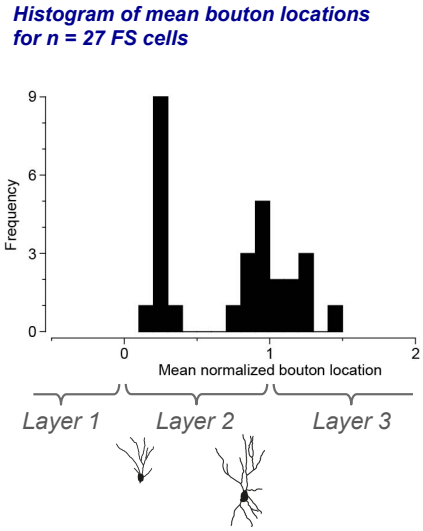

Suzuki & Bekkers *(unpublished)*

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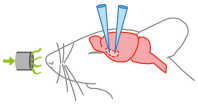

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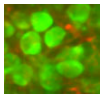

3. Ca imaging 'in vivo':
Network activity

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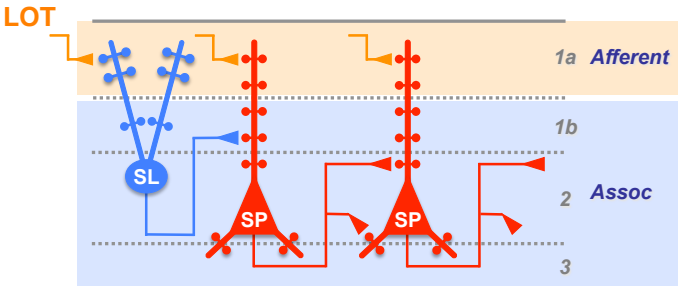
Are SL and SP cells functionally distinctive *in vivo*?



SL = semilunar

SP = superficial pyramidal

- *Does the greater intracortical connectivity of SP cells mean that these cells are more broadly responsive to odors?*

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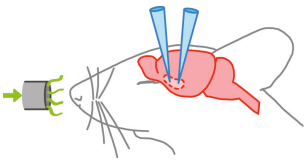
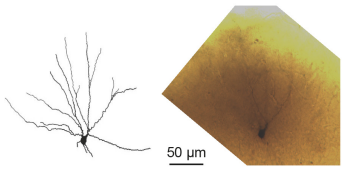
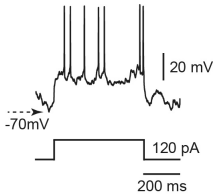
In vivo blind patch clamp in the piriform cortex

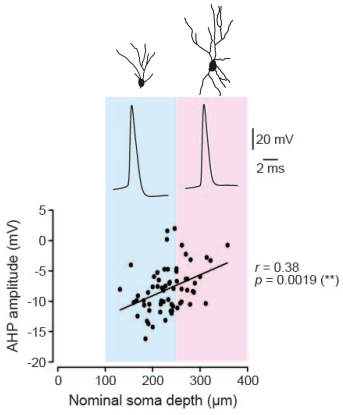


- *Urethane anesthesia (0.75 g/kg)*
- *Olfactometer delivering 15 odorants @ 10% dilution*
- *Whole-cell patch and field recording electrodes*

- *Cells identified by:*
  - *Depth*
  - *Morphology*
  - *Electrophysiology*







Huang & Bekkers *(unpublished)*

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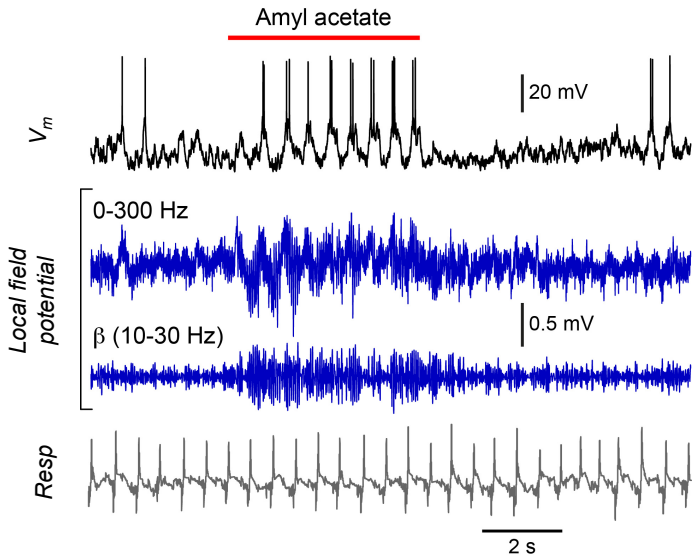
Single-cell and local field responses to odors



Huang & Bekkers *(unpublished)*

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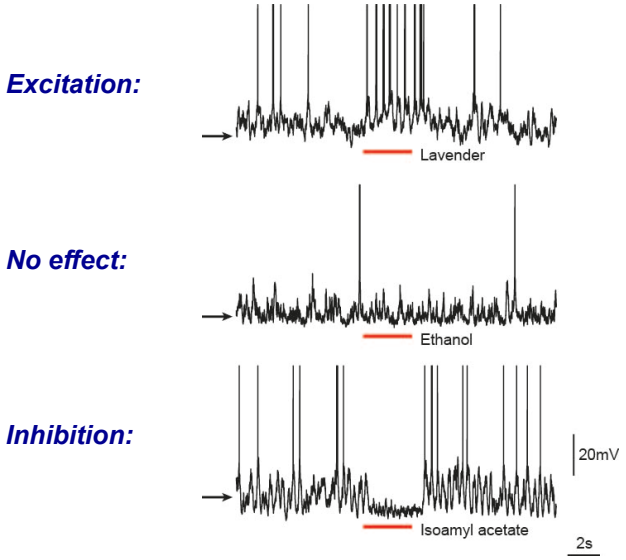
Diverse responses of Layer 2 principal cells to odors



Huang & Bekkers *(unpublished)*

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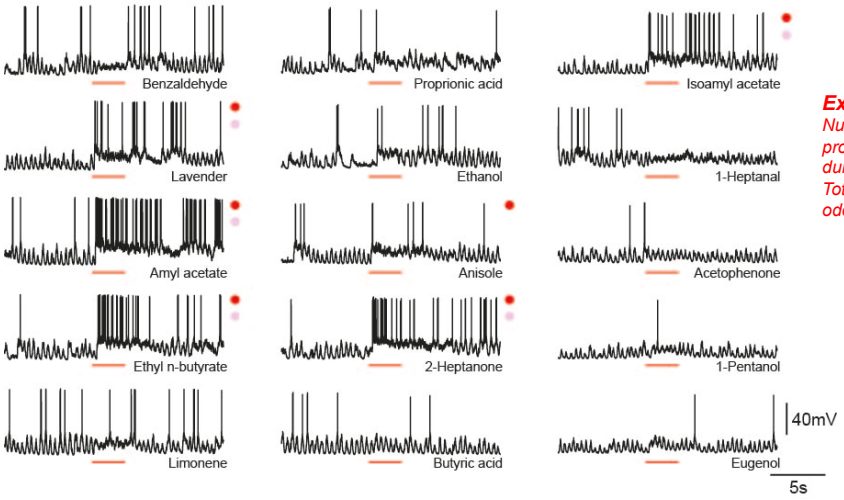
Layer 2 principal cells respond variably to odors

Responses of an SP cell to 15 odorants:



Excitation index = *Number of odorants producing excitation during application / Total number of odorants tested*

● Spikes
● EPSPs

Huang & Bekkers *(unpublished)*

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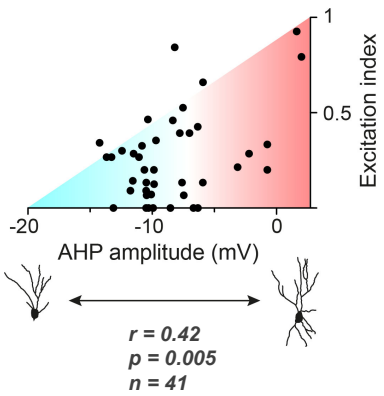
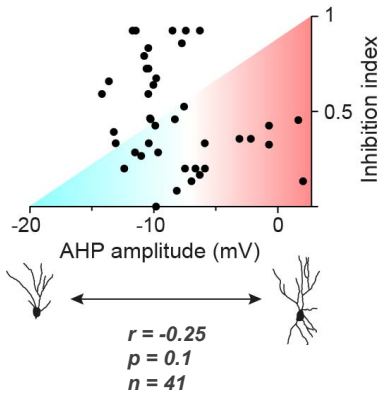
Excitatory odor tuning appears to be graded across layer 2

***Spikes** (excitation):*



r = 0.42
p = 0.005
n = 41

***EPSPs** (inhibition):*



r = -0.25
p = 0.1
n = 41

Huang & Bekkers *(unpublished)*

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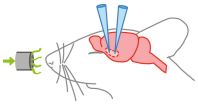

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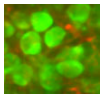

3. Ca imaging 'in vivo':
Network activity

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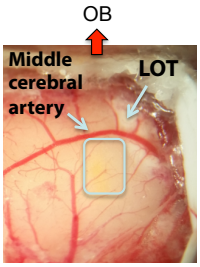
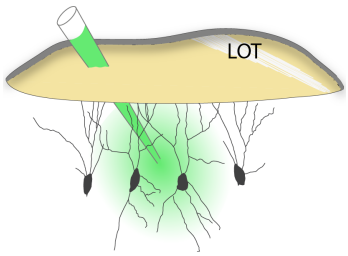
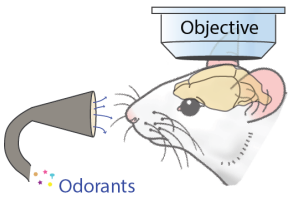
In vivo Ca imaging in the piriform cortex







- ***Craniotomy in freely-breathing anesthetized P50-70 mice***
- ***Two Ca indicators used:***
  - ***Bulk-loaded membrane permeable dye Cal-520 AM (1 mM)***
  - ***Virally-infected GCaMP6s applied 3-4 weeks before imaging (AAV1-Syn-GCaMP6s)***
- ***In vivo 2-photon microscopy:***
  - ***Timed presentation of up to 15 structurally diverse odorants***
  - ***Respiration monitored***

Tantirigama & Bekkers *(unpublished)*

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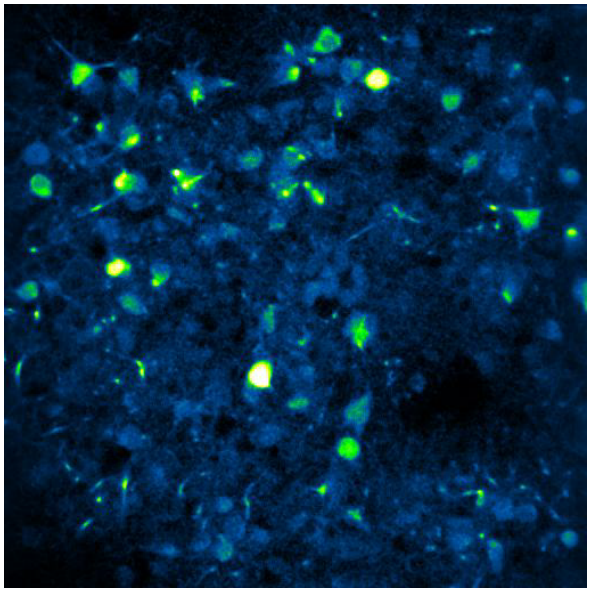
Odor-evoked responses (Cal-520)



(playing at 3.3x real time)

Tantirigama & Bekkers *(unpublished)*

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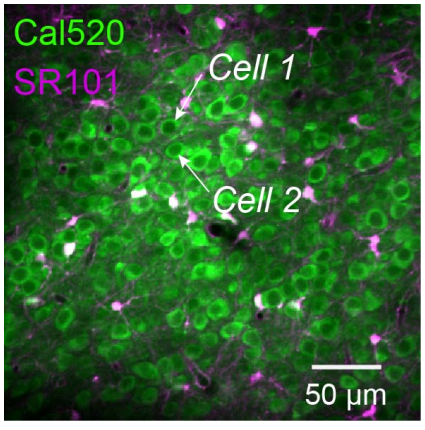
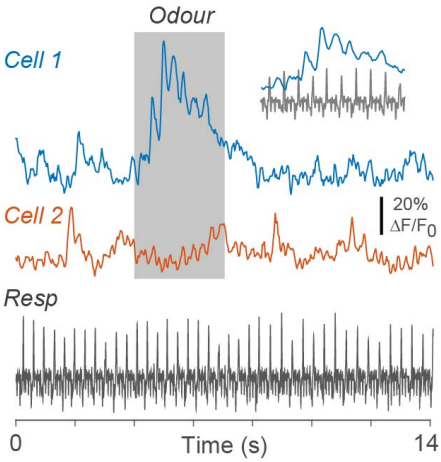
Oscillatory Ca responses to odor





Tantirigama & Bekkers *(unpublished)*

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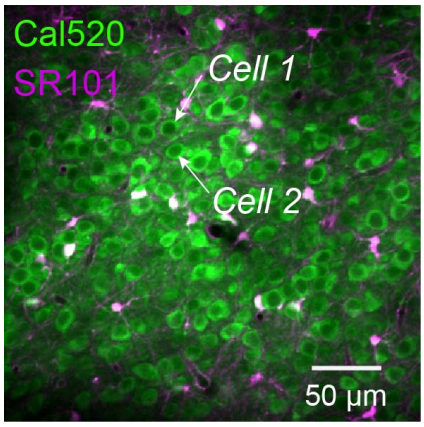
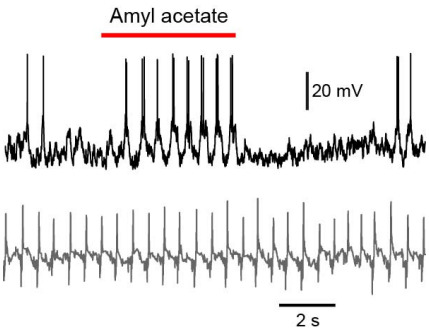
Oscillatory Ca responses to odor





Tantirigama & Bekkers *(unpublished)*

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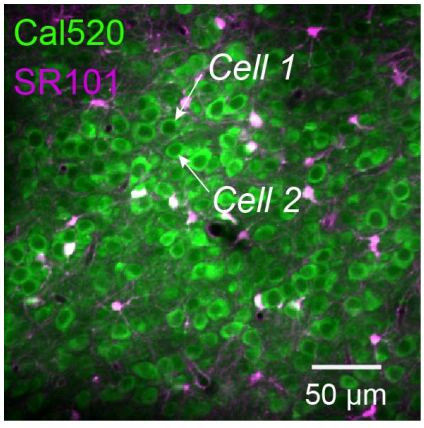
Analysis of imaging data



1. Spontaneous activity

2. Odor coding

Tantirigama & Bekkers *(unpublished)*

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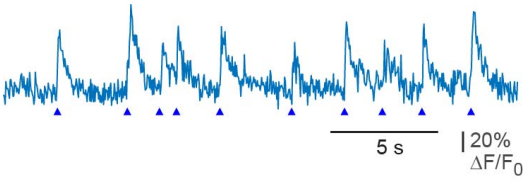
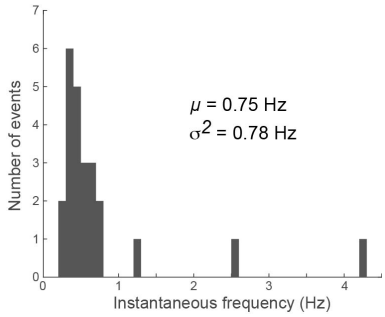
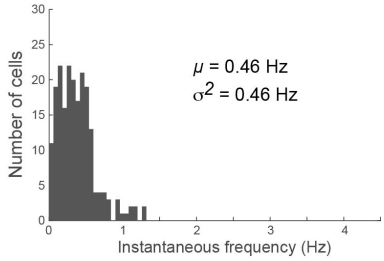
Measuring spontaneous Ca transients



Inst freq for above neuron:



Distribution of mean _Inst freq_ for _n_ = 227 neurons:



Tantirigama & Bekkers *(unpublished)*

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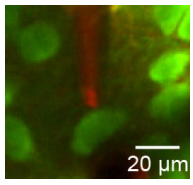
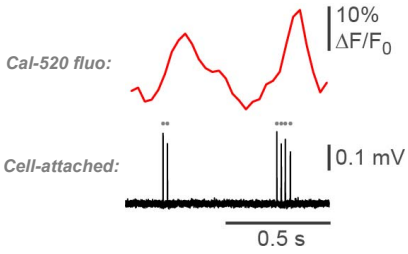
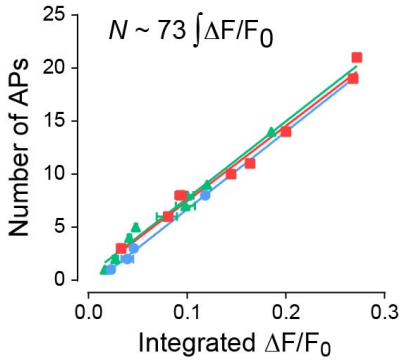
Dye calibration

Simultaneous cell-attached & 2P Ca imaging _in vivo_:





Calibration plots:
(_3 neurons in 2 animals_)



Tantirigama & Bekkers *(unpublished)*

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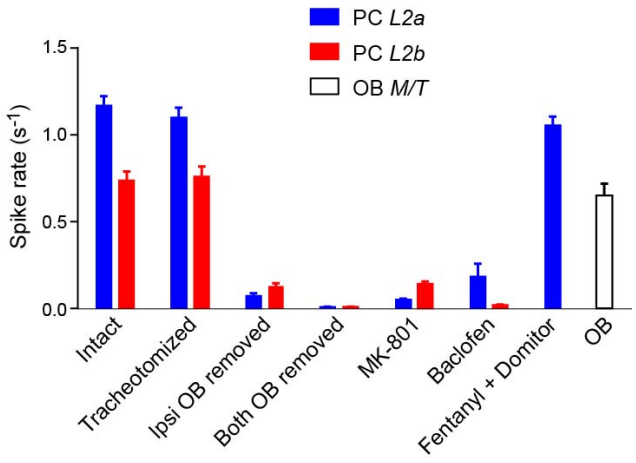
Origins of spontaneous activity



➔ ***Spontaneous activity requires the OB, but also depends on circuits in the PC***

Tantirigama & Bekkers *(unpublished)*

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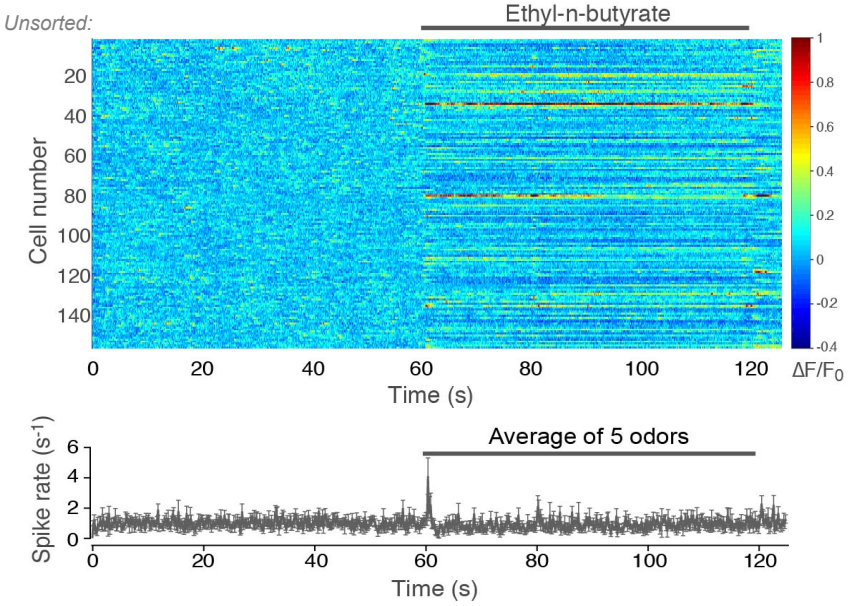
Odor-dependent changes in spontaneous activity



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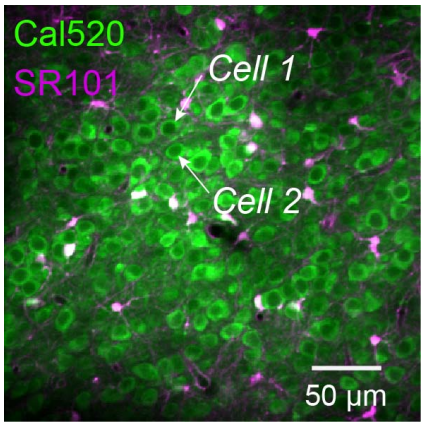
Analysis of imaging data



1. Spontaneous activity

2. Odor coding

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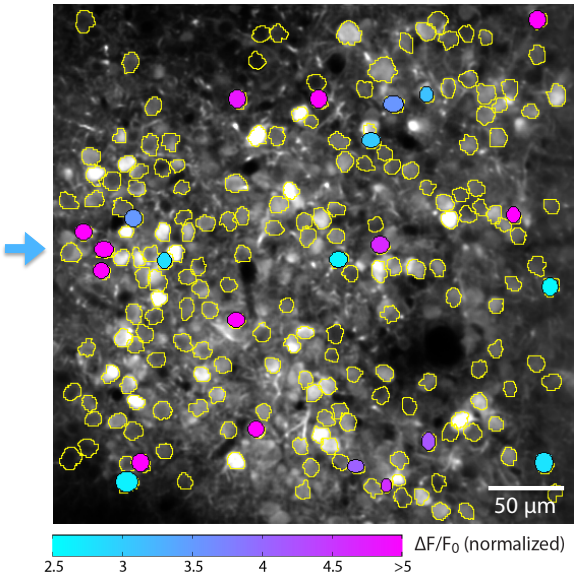
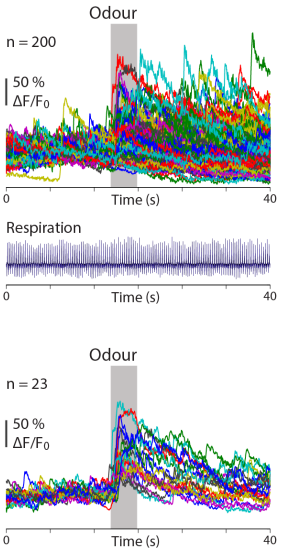
Odor-evoked responses (GCaMP6s)





Tantirigama & Bekkers *(unpublished)*

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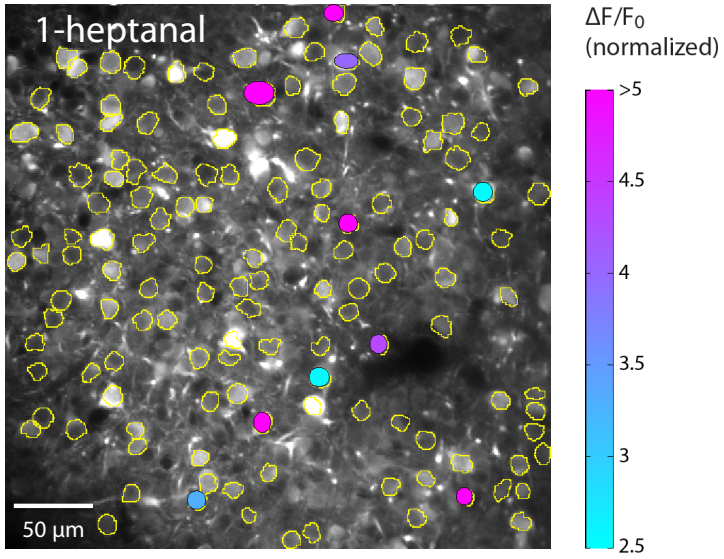
Different odors evoke different sparse responses



Tantirigama & Bekkers *(unpublished)*

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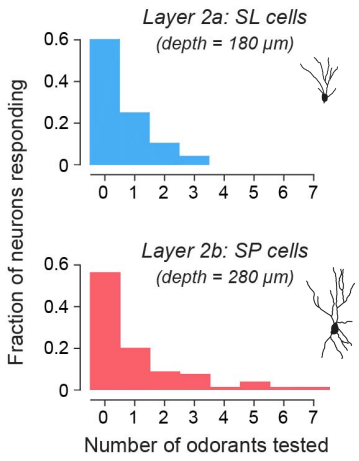
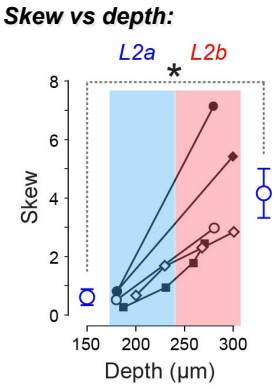
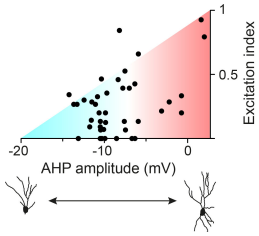
Distribution of odor responses varies with depth







Tantirigama & Bekkers *(unpublished)*

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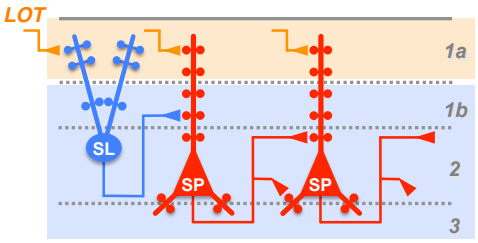
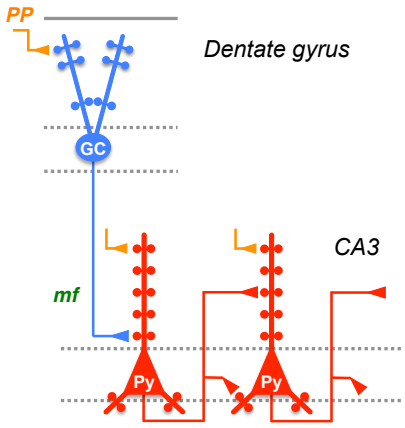
Paleocortex *vs* archicortex

Piriform cortex



Hippocampus



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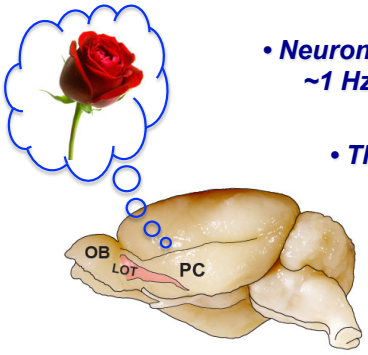
Main findings

- ***Layer 2 of the PC contains sub-networks of SL- and SP-like neurons***
- ***SL neurons are more narrowly tuned to odors than SP neurons***
- ***Neurons in the piriform cortex fire spontaneous spikes at ~1 Hz, and this activity requires input from the bulb***
- ***The existence of sub-networks and spontaneous activity may enrich coding possibilities in the PC***



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