Multisite imaging of neural activity using a genetically encoded calcium sensor in the honeybee

Jana Seiler

5th International Workshop on Regulatory Approaches for Agricultural Applications of Animal Biotechnologies

Virtual Workshop, 28th August 2024

The caste system – the basis of the social bee state

Heinrich Heine Universität Düsseldorf

Reproduction

➢ Queen and drones

- **Mating flight**
- **Production of offspring**
- **Live inside the hive**

Sterile assistants

➢ Worker

• **Cell cleaning**	Young bees	0 - 3 days
• **Brood care** • **Queen retinue** • **Trophallaxis**	Nurse bees	4 - 12 days
• **Food processing** • **Wax production/ proccesing**	Middle-aged bees	13 - 21 days
• **Foraging**	Foraging bees	From day 21

1. http://www.waldeneffect.org/blog/Natural_vs._artificial_bee_reproduction/
2. https://www.britannica.com/animal/honeybee#/media/1/270903/110883

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What are the possibilities for measuring neuronal activity?

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Genetic toolbox

Genome editing

RNAi
Specific suppression of gene expression

Transposons
Insertions of DNA fragments
Generation of mutations

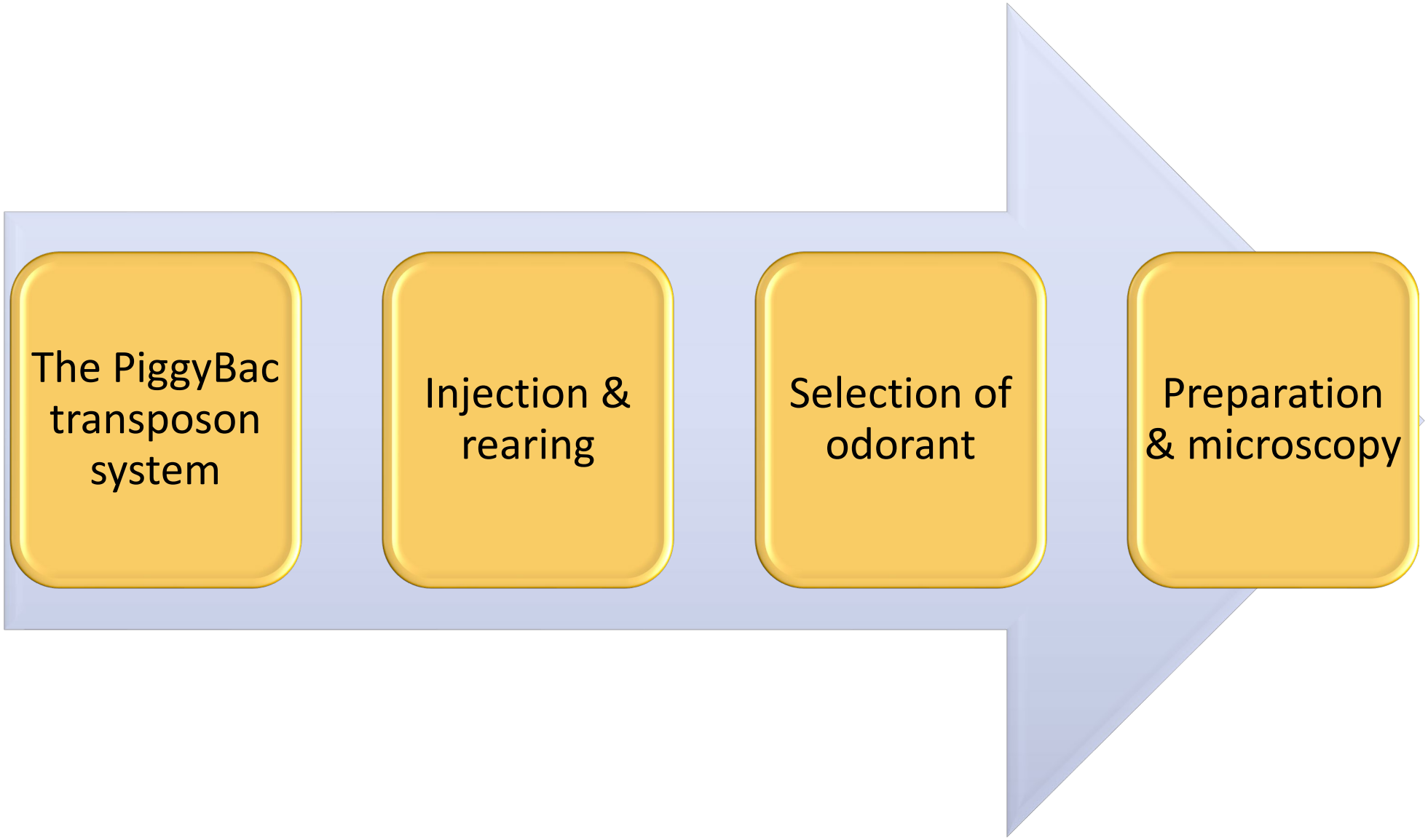
CRISPR/Cas9
Precise modification of the genome
Generation of knock-outs and knock-ins

Visualization and analysis

Flourescent protein
Labeling with fluorescent protein for visualization in tissue

Calcium sensor
Labeling of the presynapse by binding calcium

Calcium imaging – Procedure

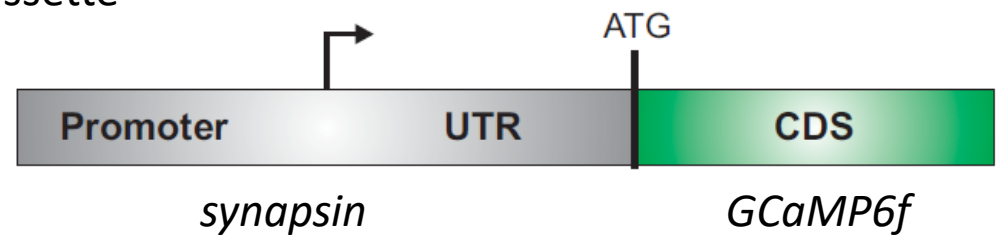


Calcium imaging – Procedure

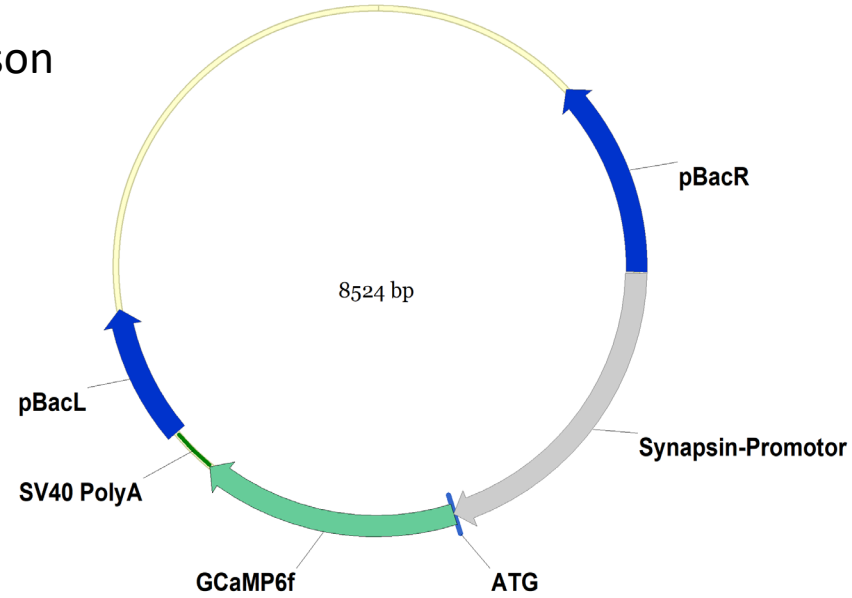
Heinrich Heine Universität Düsseldorf

PiggyBac transposon | Injection & rearing | Selection of odorant | Preparation & microscopy

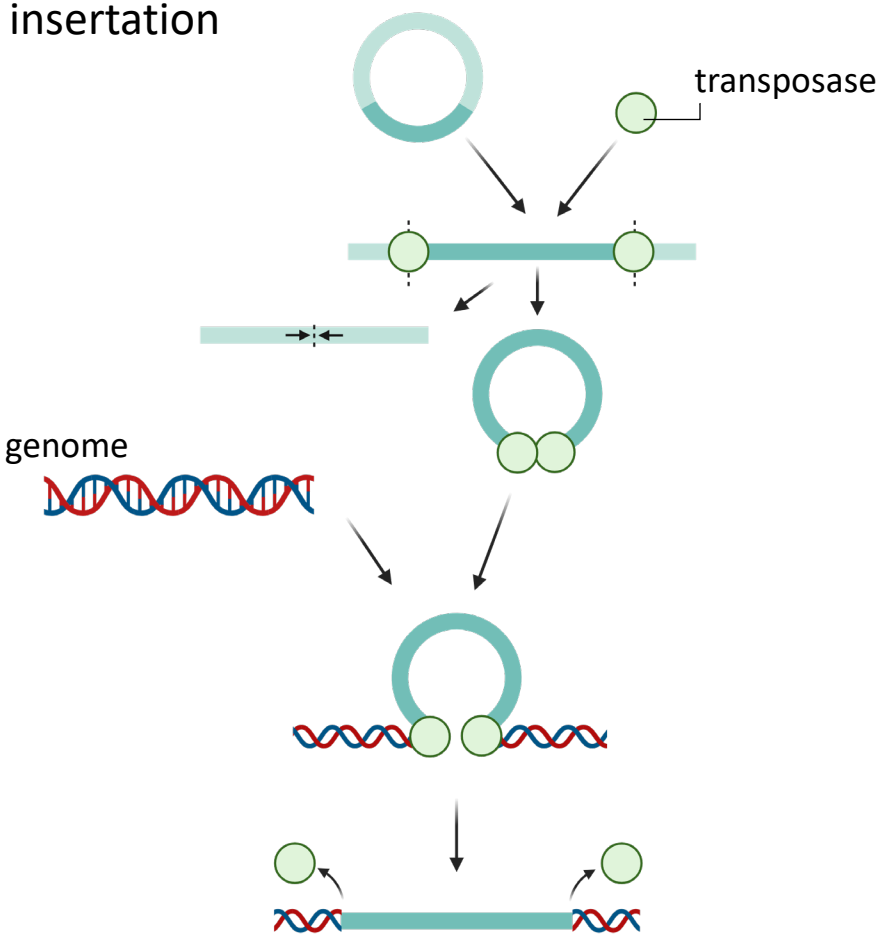
cassette



transposon



insertation



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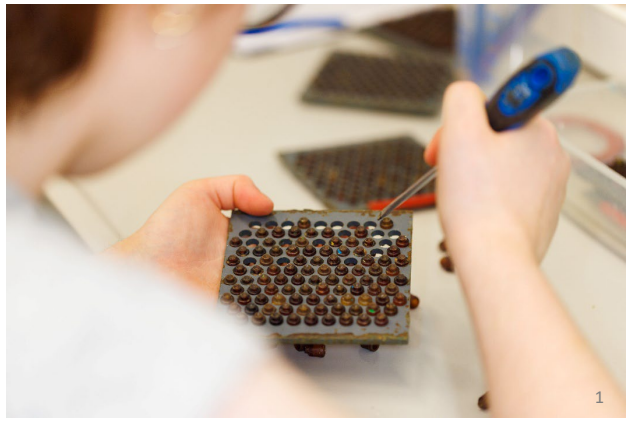
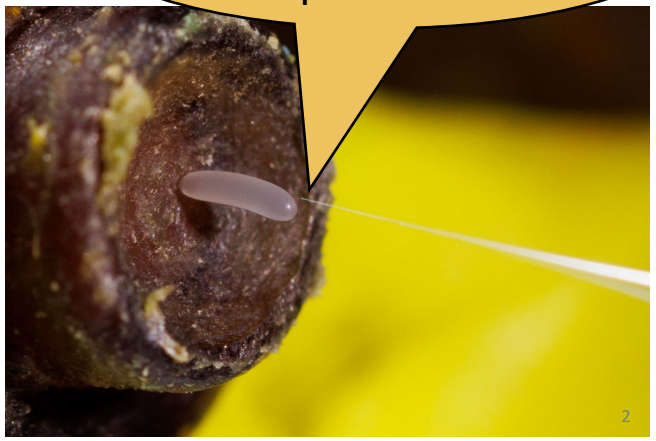
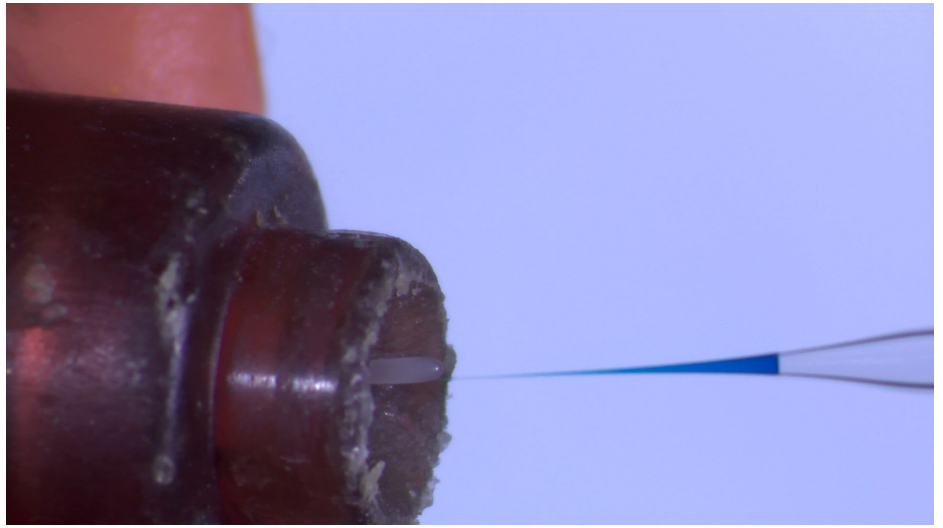
PiggyBac transposon → Injection & rearing → Selection of odorant → Preparation & microscopy



PiggyBac plasmid
+
transposase mRNA





1+2. HHU / Christoph Kawan

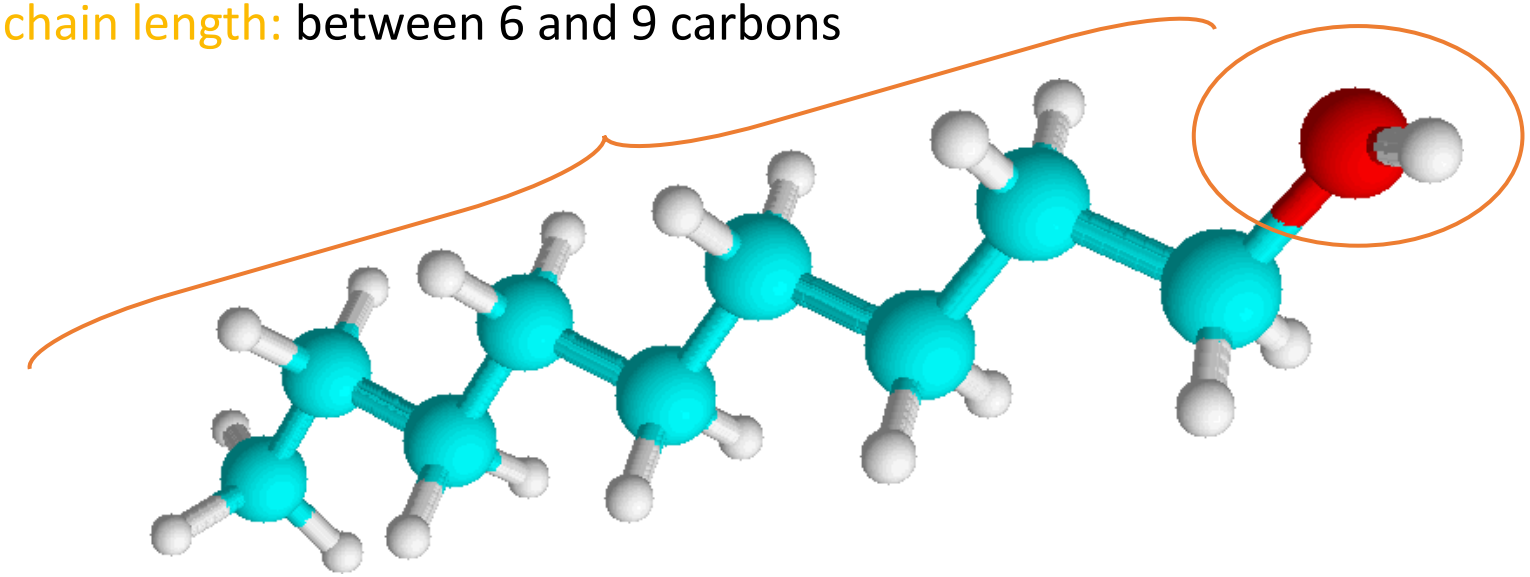
Calcium imaging – Procedure

Heinrich Heine Universität Düsseldorf

PiggyBac transposon | Injection & rearing | Selection of odorant | Preparation & microscopy

use of 16 aliphatic odorants:

chain length: between 6 and 9 carbons

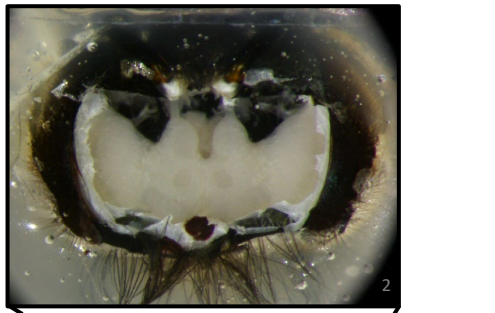


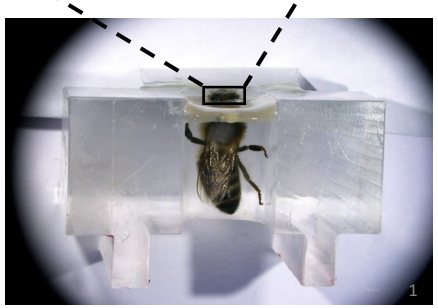
functional group :

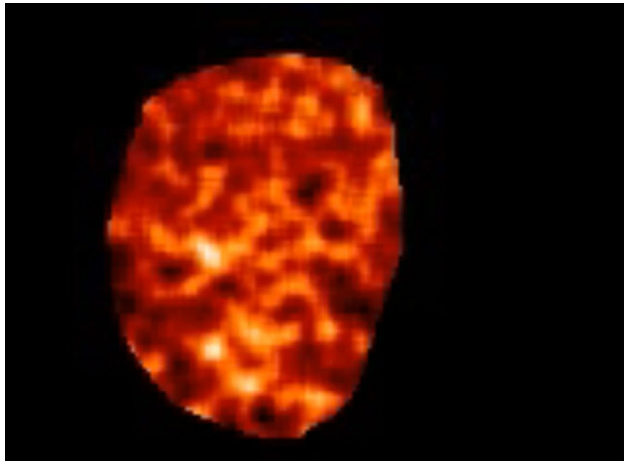
- primary alcohols
- secondary alcohols
- aldehydes
- ketones

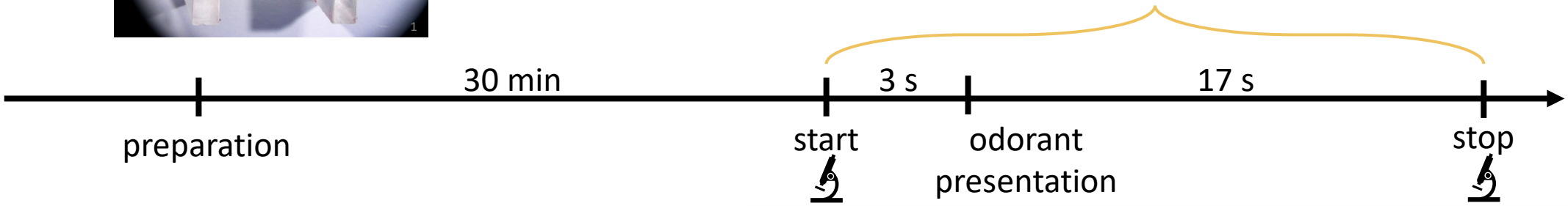
Calcium imaging – Procedure

PiggyBac transposon → Injection & rearing → Selection of odorant → Preparation & microscopy



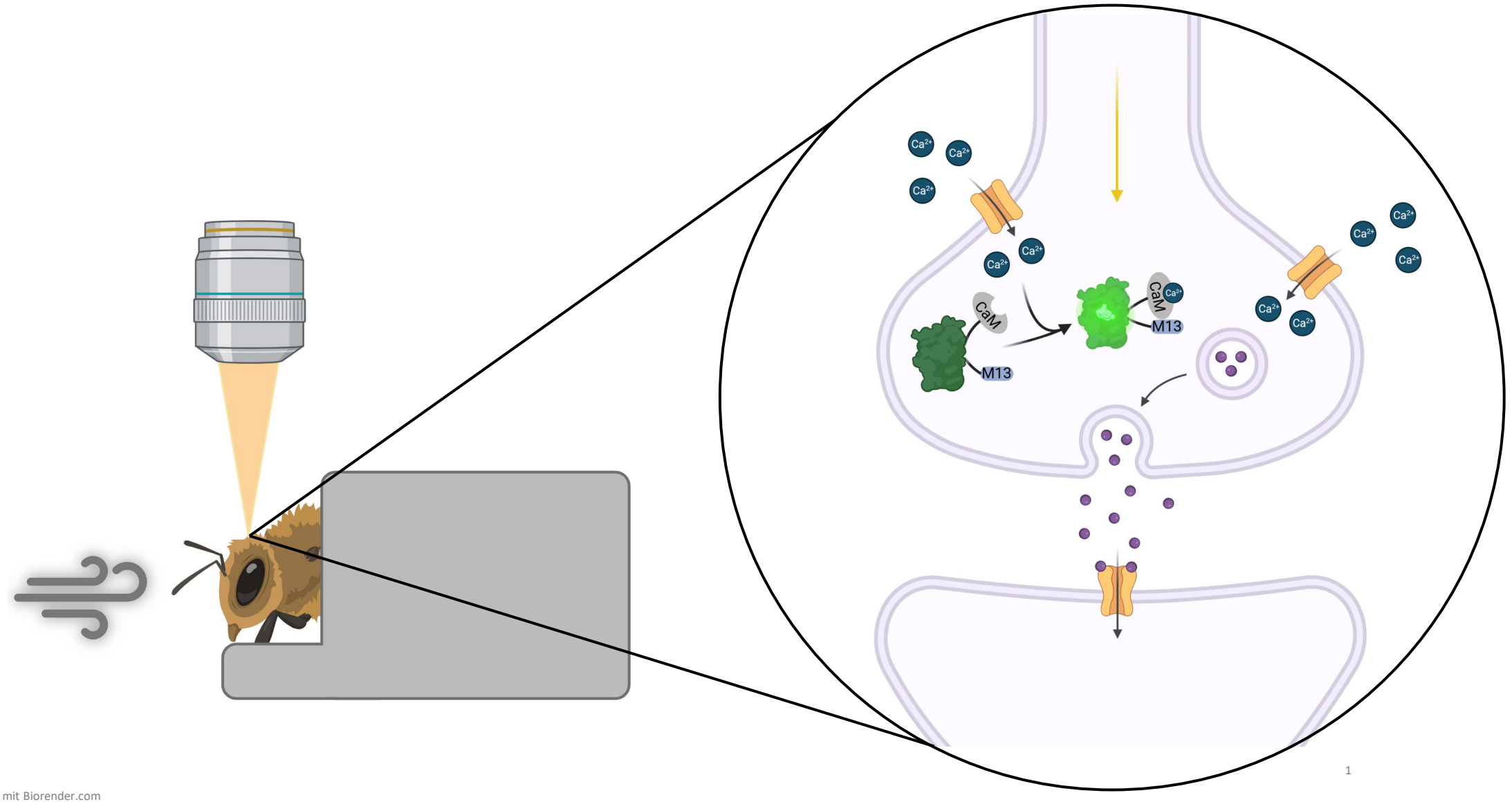






1.+ 2. Julie Carcaud

Calcium imaging – Meachnism



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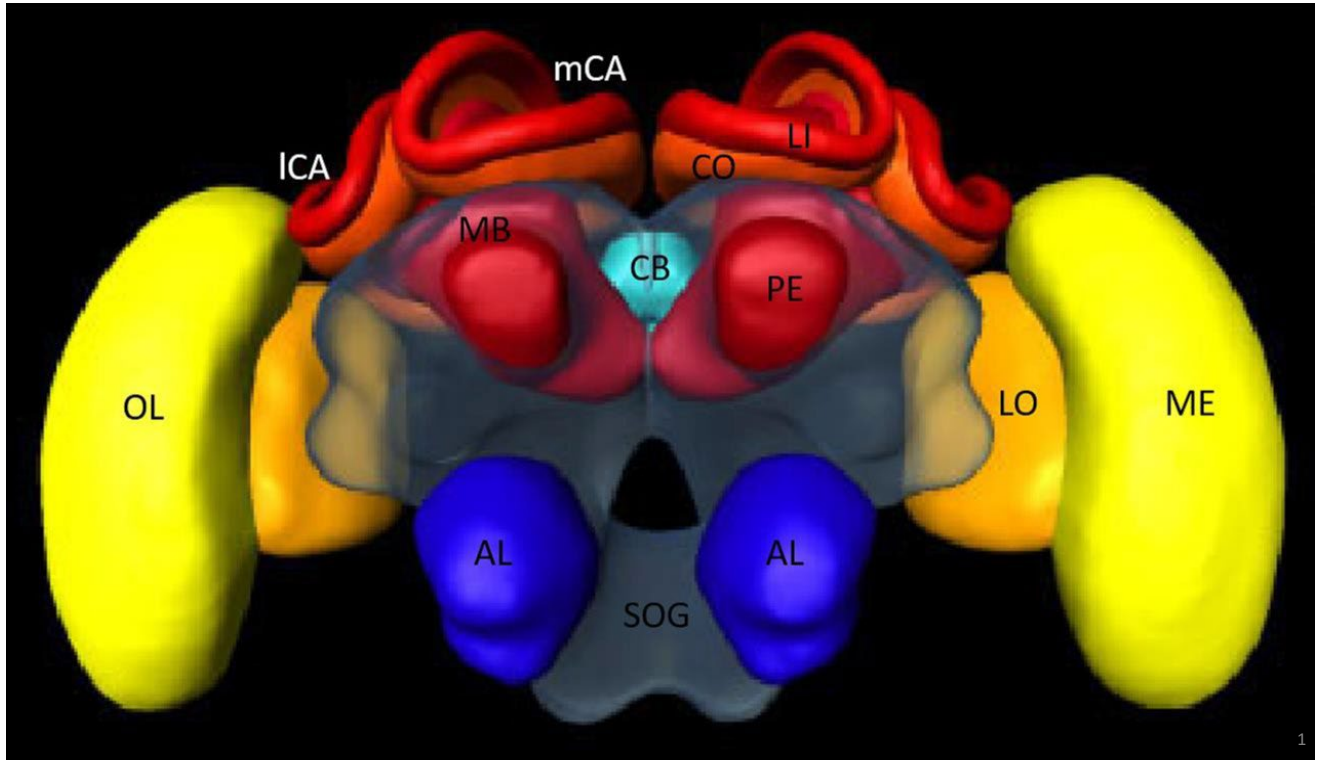
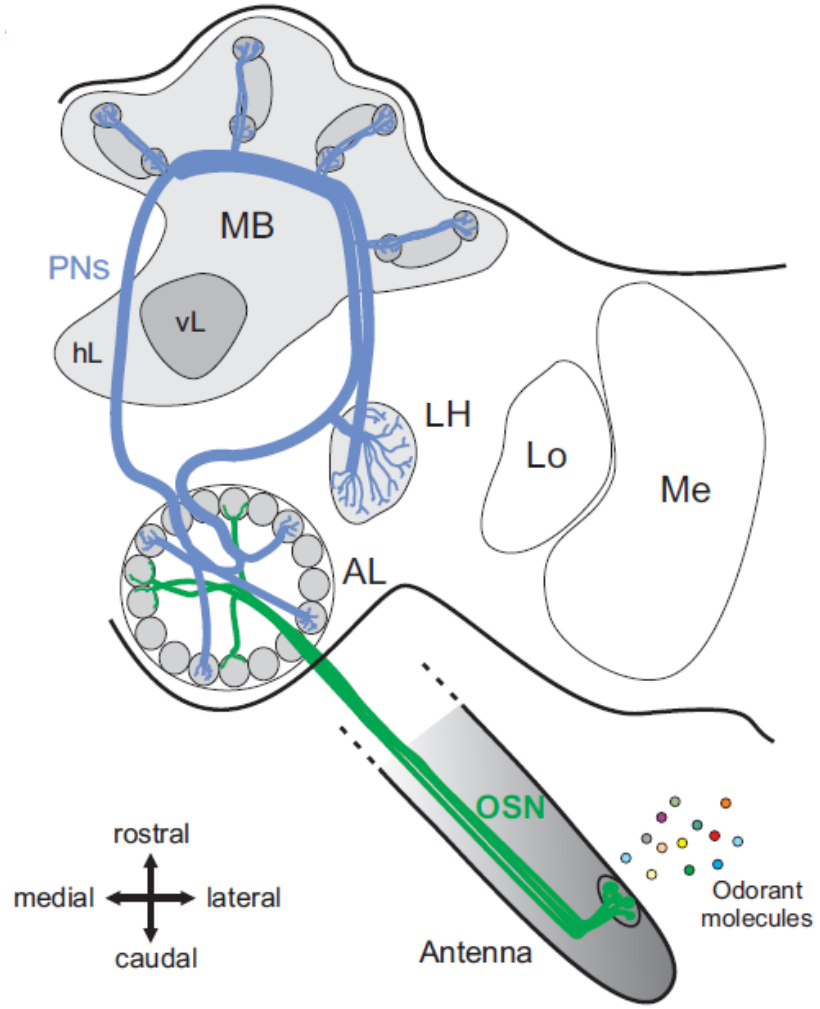
1. erstellt mit Biorender.com

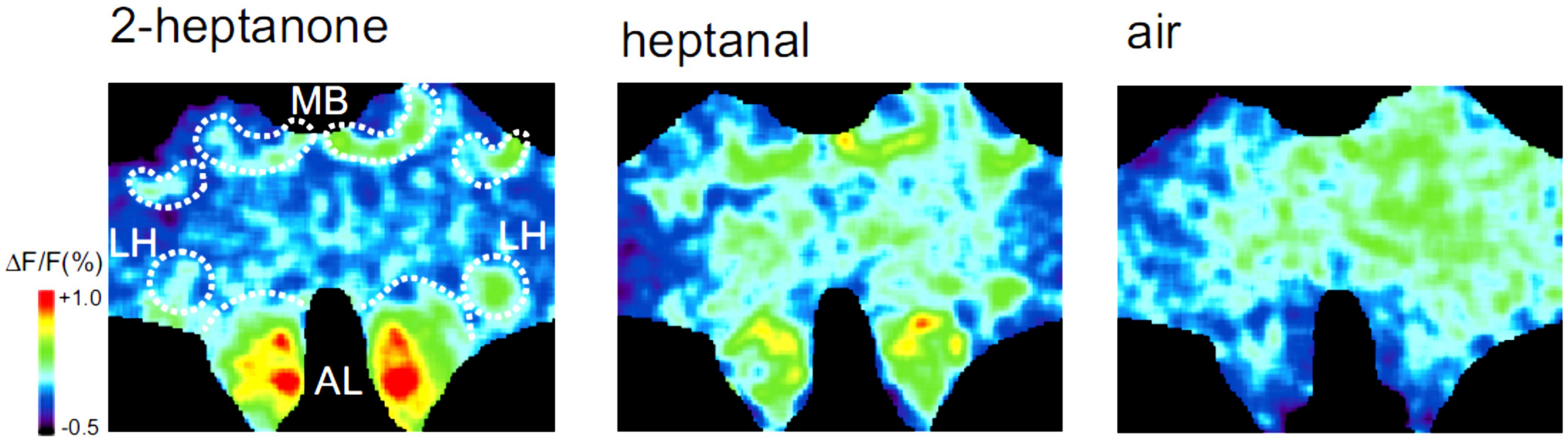
Focus on the processing of odor information





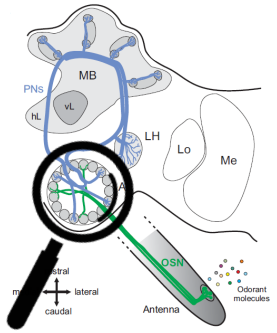
1. Brandt et al., (2005)

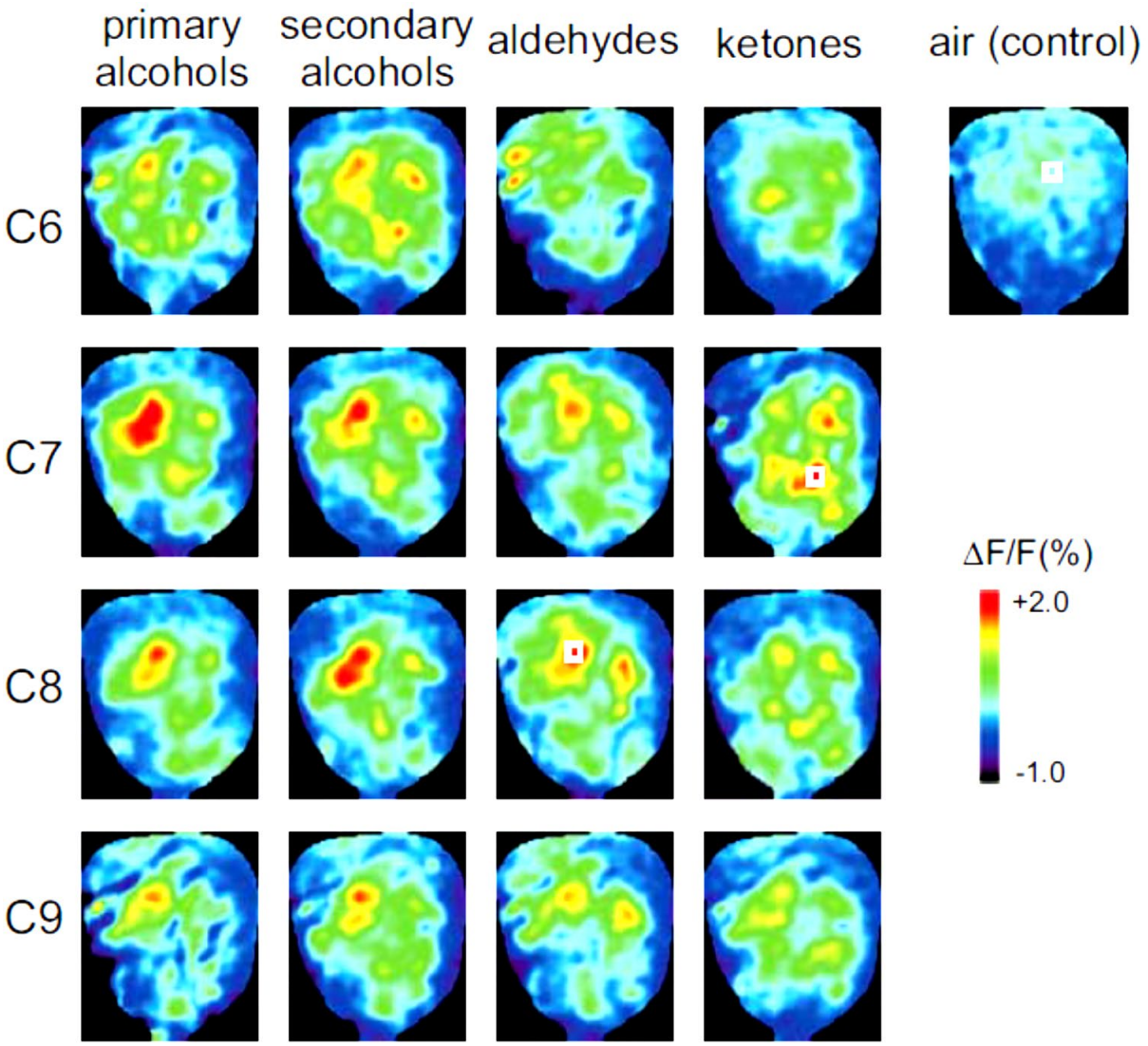
Proof of concept :
focus on processing odor information



Simultaneous recording of olfactory responses in different brain structures

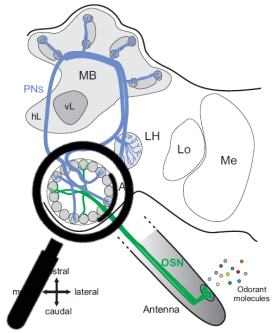
Activity maps in the AL

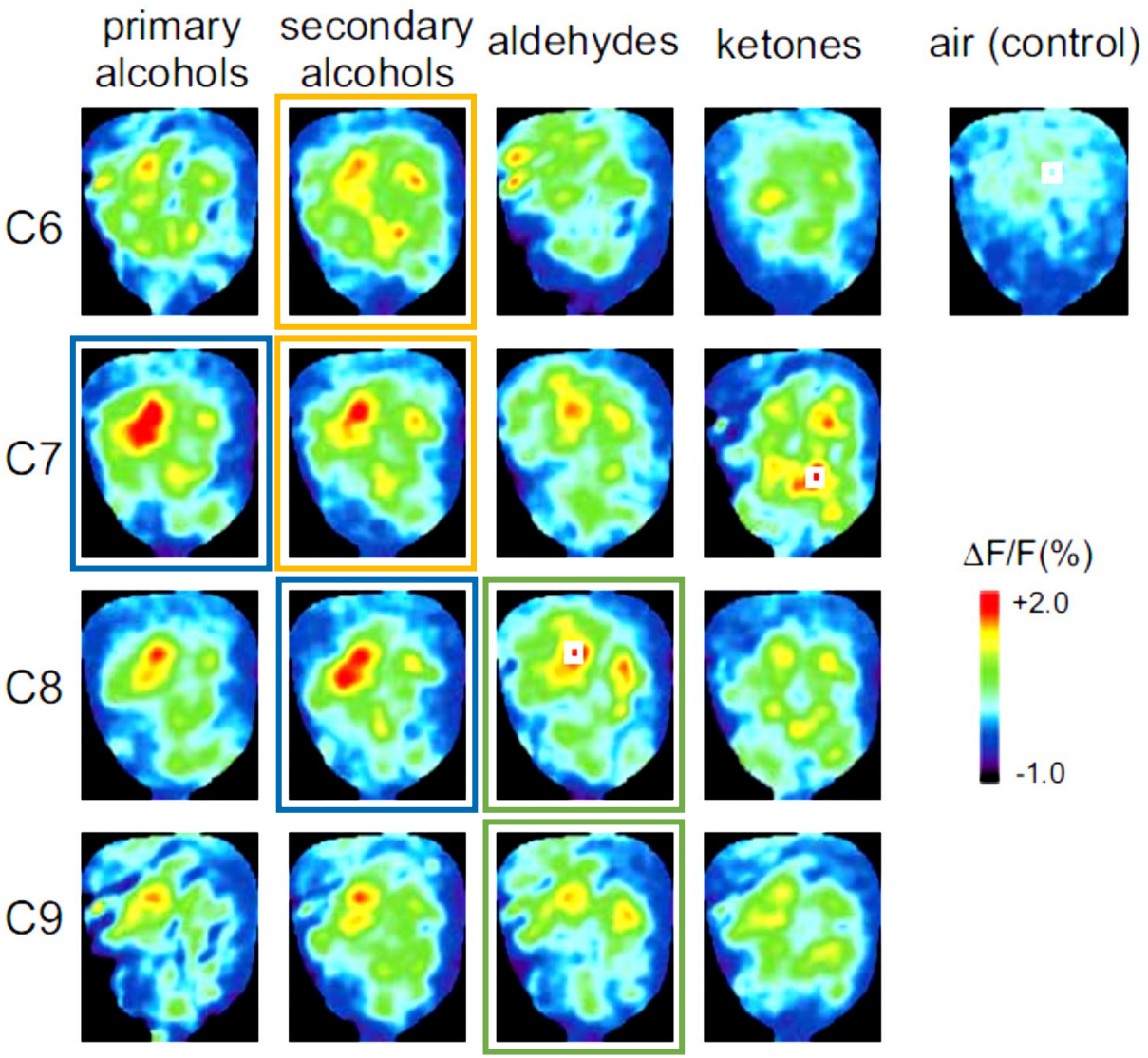


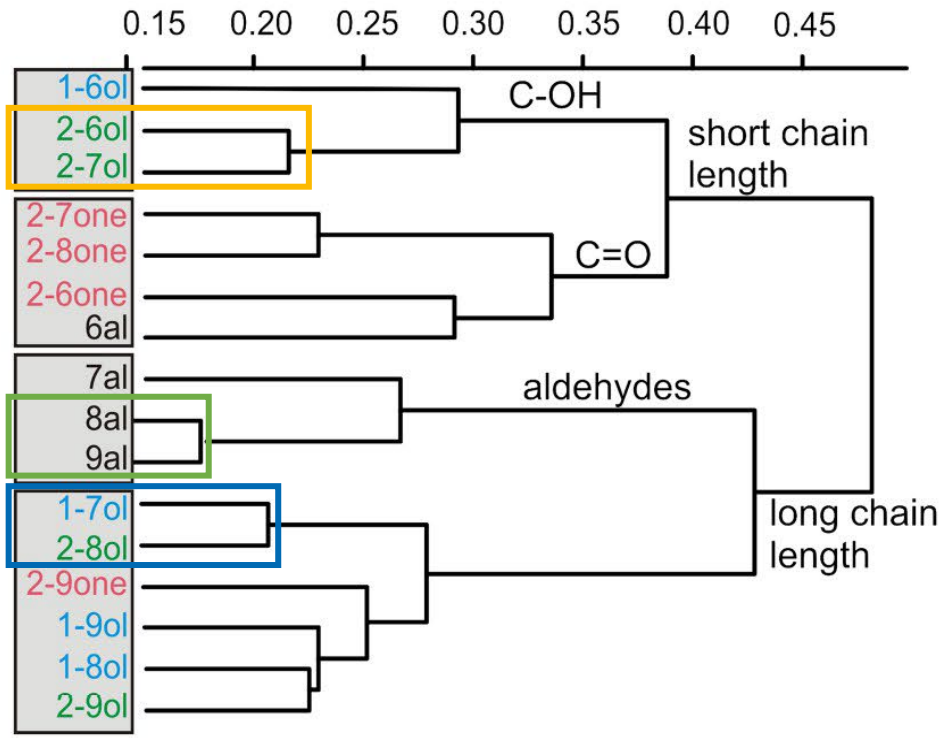


Presentation of each odorant induced a signal in a different set of AL glomeruli

Activity maps in the AL







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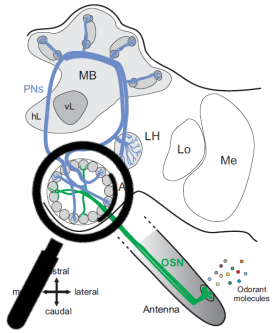
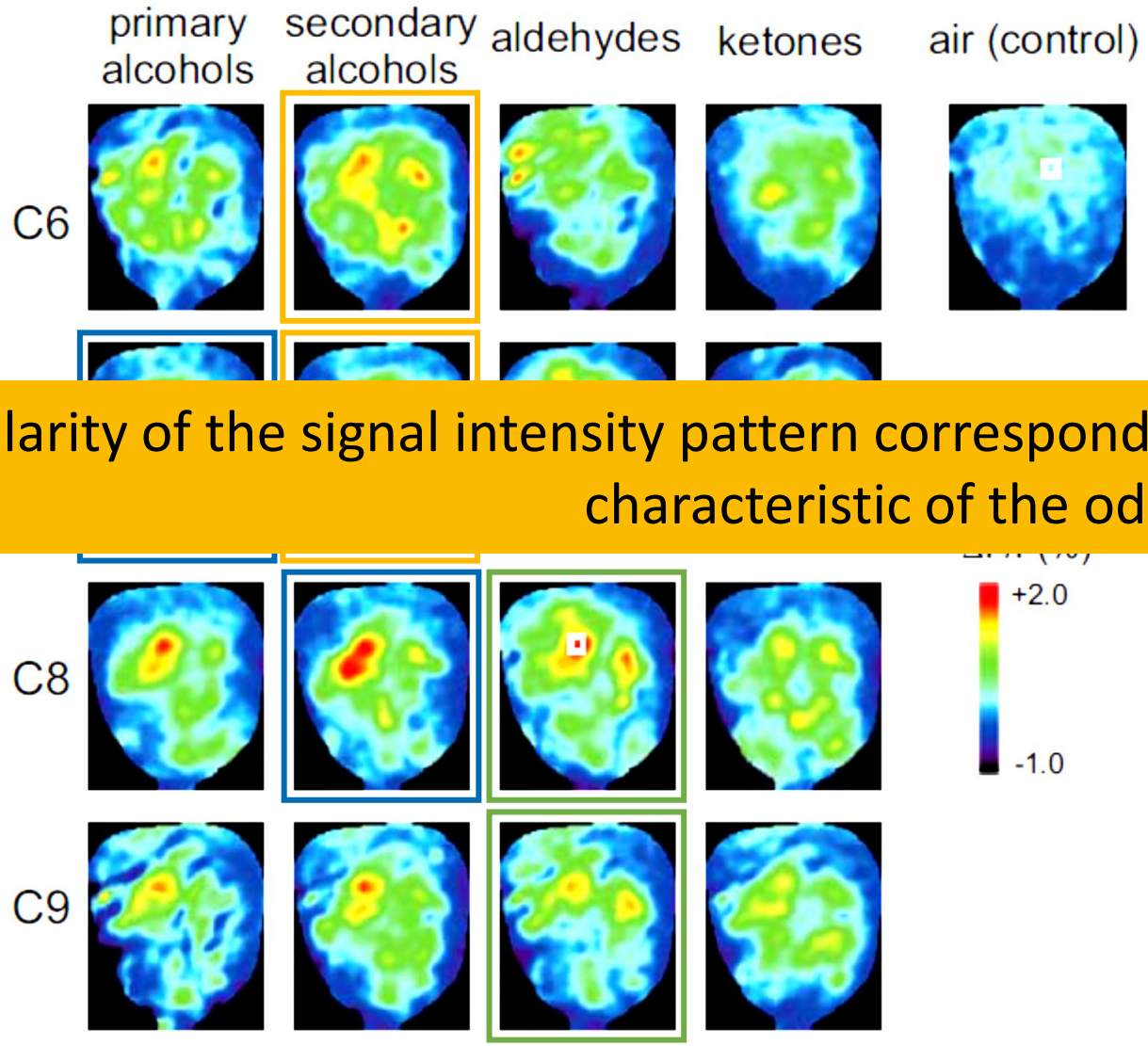
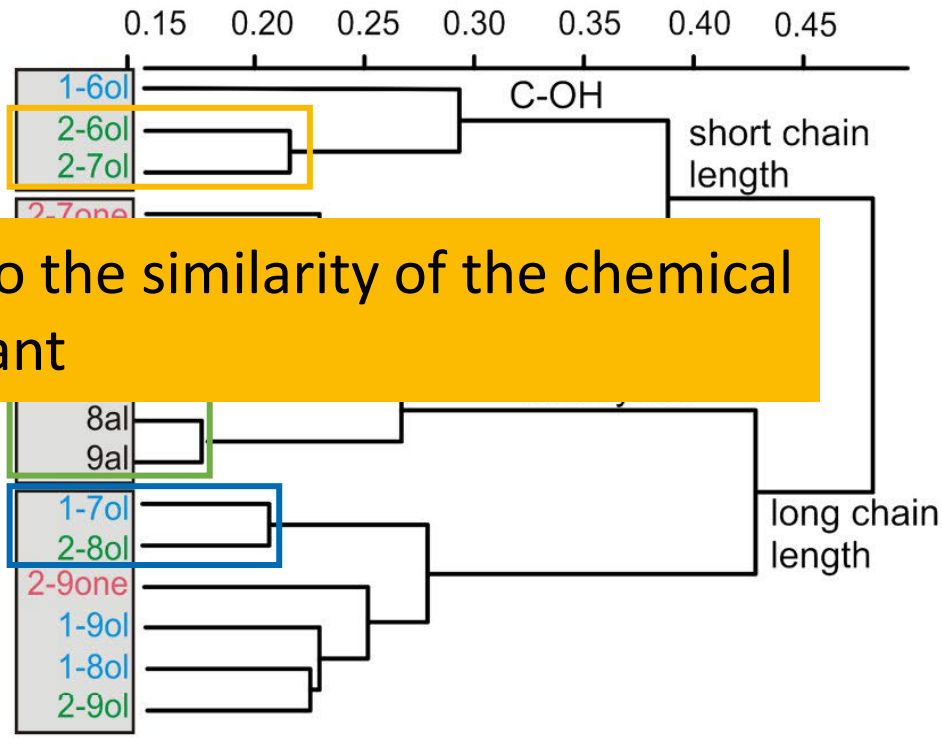
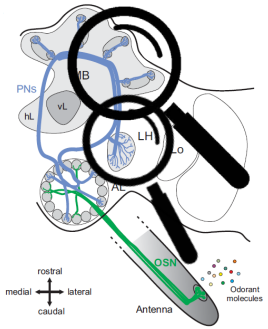




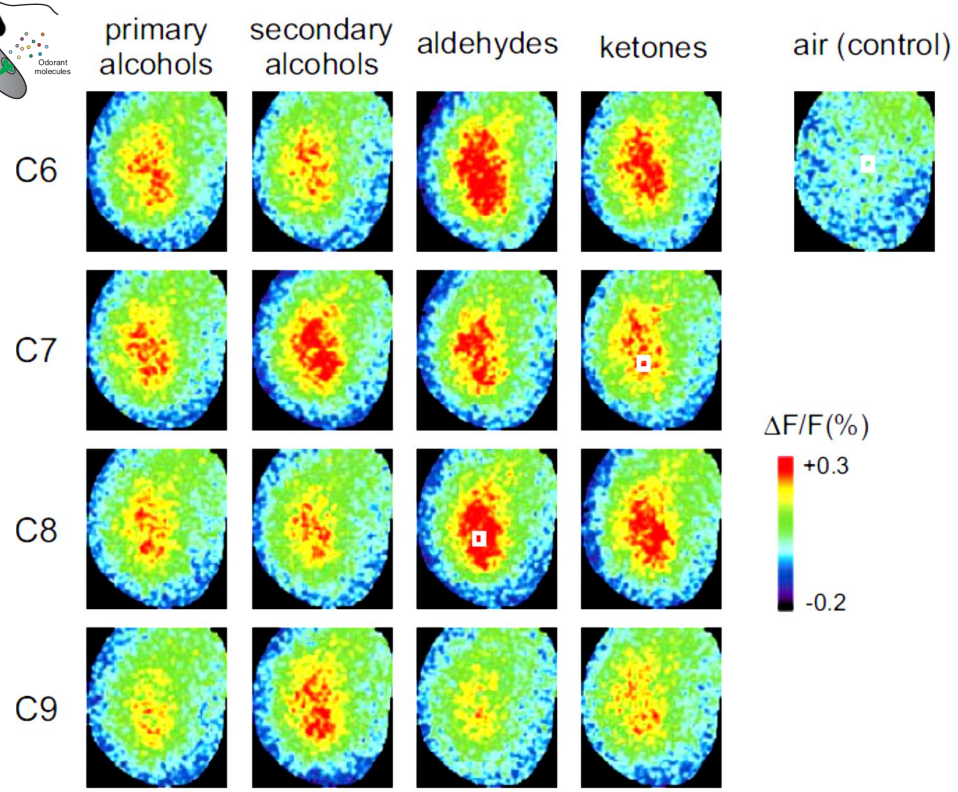


Similarity of the signal intensity pattern corresponds to the similarity of the chemical characteristic of the odorant

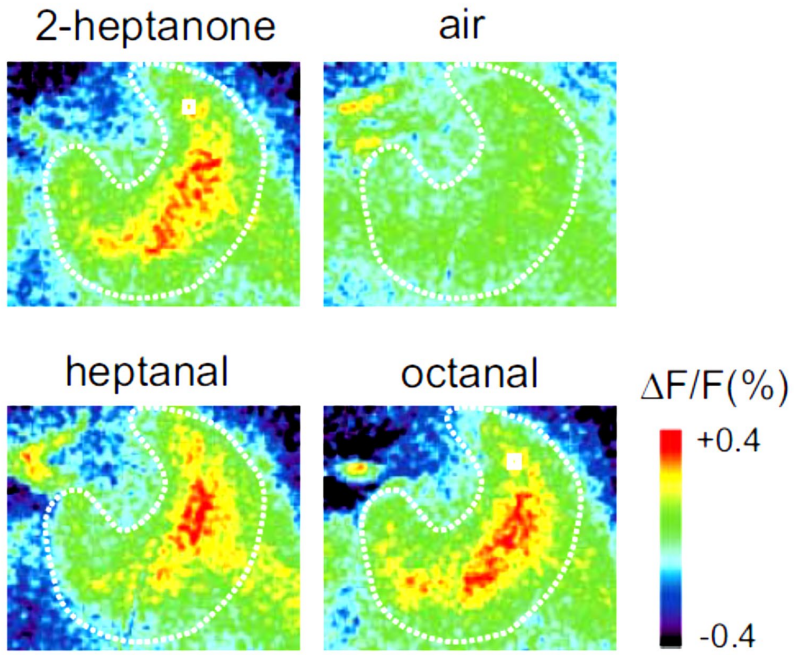
Olfactory coding in higher-order centers



The lateral horn:



The mushroom bodies:



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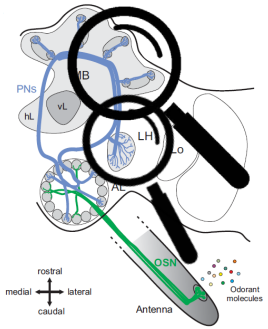

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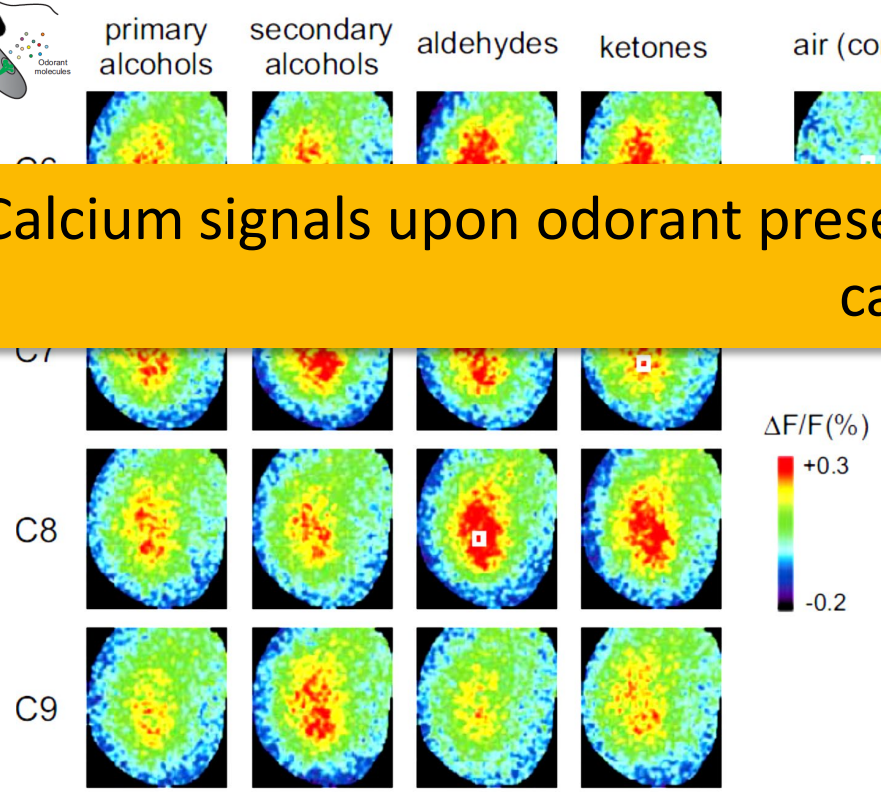

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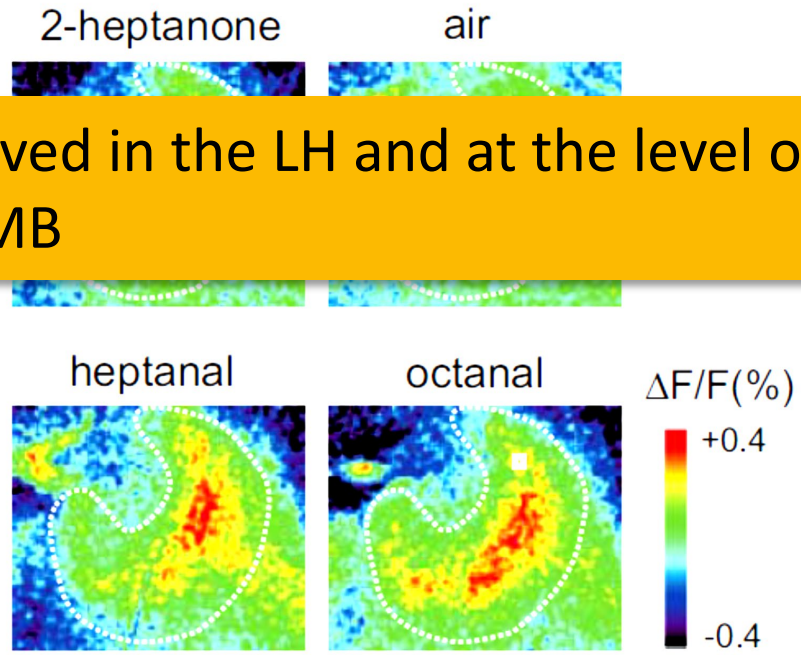
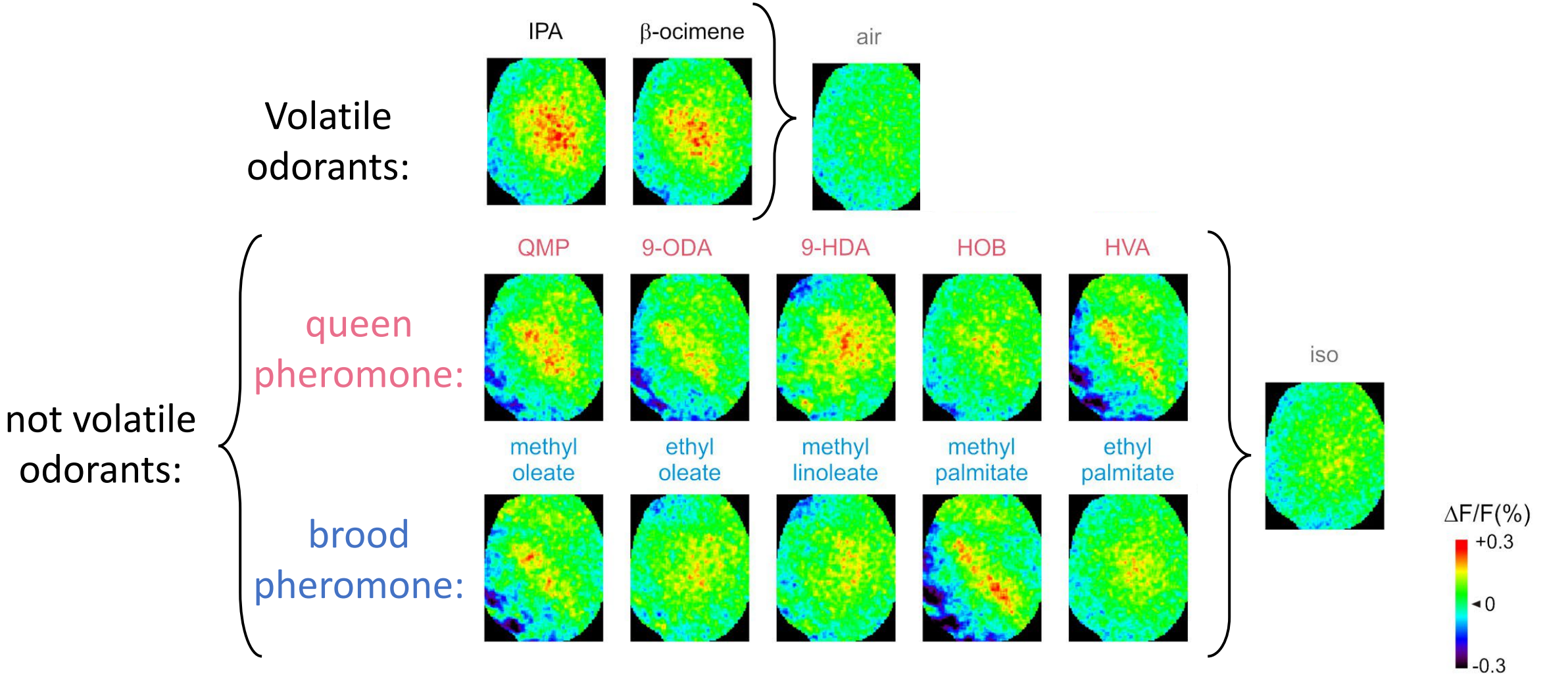


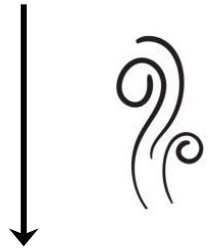
Calcium signals upon odorant presentation observed in the LH and at the level of the calyx lip in the MB

Pheromone reaction in the AL

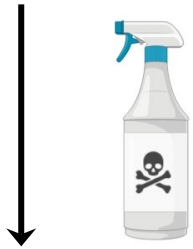


Research use vs. economic use

Neuroethology:



Impact of pesticides:



Development of neurogenetic tools in the honeybee

- Allows simultaneous recordings of different brain structures:
  - useful for dissecting yet undescribed sensory and/or behavior-related pathways
  - recordings of poorly studied structures (i.e. LH or MB)

- Represents a major progress for the neuroethology of social behavior

- Economic use: impact of pesticides

Thank you for your attention!

Thanks also to:

Evolutionary genetics Heinrich-Heine-University Düsseldorf, Germany:

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Marianne Otte
Robin Parlow
Eva Theilenberg
Marion Müller-Borg

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Julie Carcaud
Virginie Larcher

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Bernd Grünewald

Center for Mind/Brain Sciences, University of Trento, Italy:

Albrecht Haase
Sara Bariselli

hhu
Heinrich Heine
Universität Düsseldorf

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Bienenkunde

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