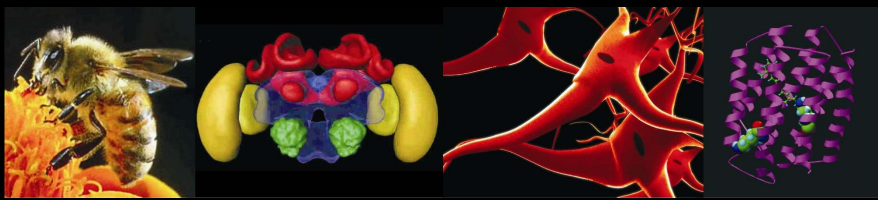
Cognition and miniature brain: What we can learn from a honeybee brain



Martin Giurfa

Research Centre on Animal Cognition
Toulouse, France

http://cognition.ups-tlse.fr

UNIVERSITE TOULOUSE III PAUL SABATIER
Faculté des Sciences et d'Ingénierie

cnrs

The honeybee *Apis mellifera*: a model for the study of learning and memory

- ***Developed learning and memory capabilities in a natural context***
  The worker honeybee learns and memorizes different sensory stimuli in a foraging context → *flower constancy*

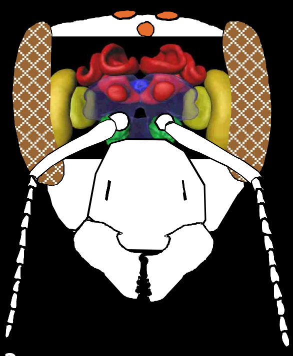
- ***Experimental accessibility***
  Possibility of training and testing in controlled laboratory conditions

- A relatively accessible and 'simple' *nervous system; a genome* fully sequenced

- *Higher-order forms of learning so far not demonstrated in Drosophila*

Cognition in a mini brain

In natural conditions, bees learn and memorize different kinds of information.



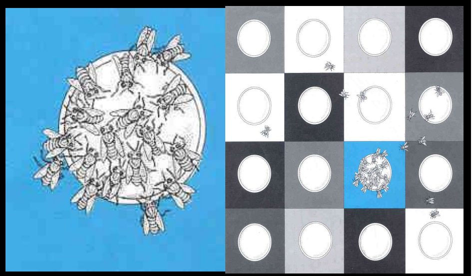
- ❑ Do they exhibit just simple forms of learning? Or can they achieve even complex, non-elemental forms of learning, akin to cognitive processing?
- ❑ How does such a learning occur in the brain?
- ❑ Does the bee brain allow identifying neuronal architectures underlying cognitive processing?

Studying cognitive components of visual learning in free flying-bees under controlled experimental conditions

First experimental demonstration of bee visual learning

Karl von Frisch

Marked Bee



Training with a color reinforced with sucrose solution

Learning and discrimination

=> Association 'Color – Reward'

Concept learning in honey bees

What is conceptual learning?

Thomas Zentall

Relational concepts : relationships encoded independently of the physical nature of the objects linked by the relation.

- Concepts can be formulated in terms of **rules/relations:** *> or < ; heavier than; different from , better than; above of, etc*

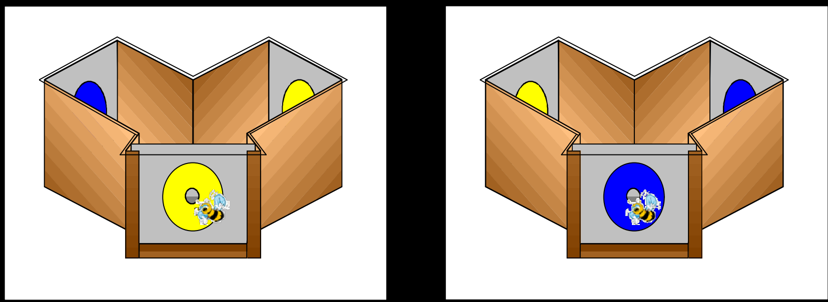
Learning of a relational concept

- ❑ Can honeybees learn a rule based on a **principle of sameness?**

- ❑ Can they solve a **delayed matching-to-sample problem**?

Training

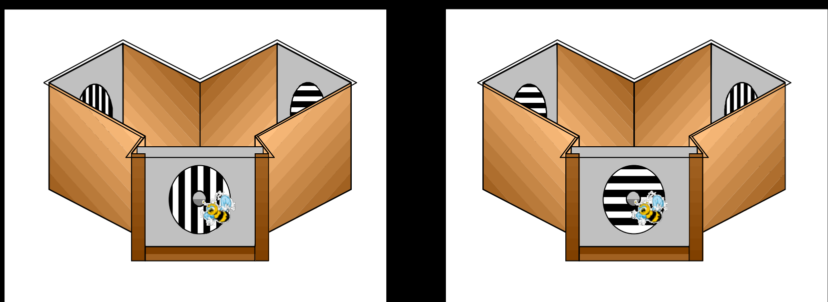
Bees are trained in a color discrimination problem. In a delayed-matching-to-sample problem they have to choose the stimulus identical to the sample shown at the entrance of the maze.



Giurfa et al (2001) Nature

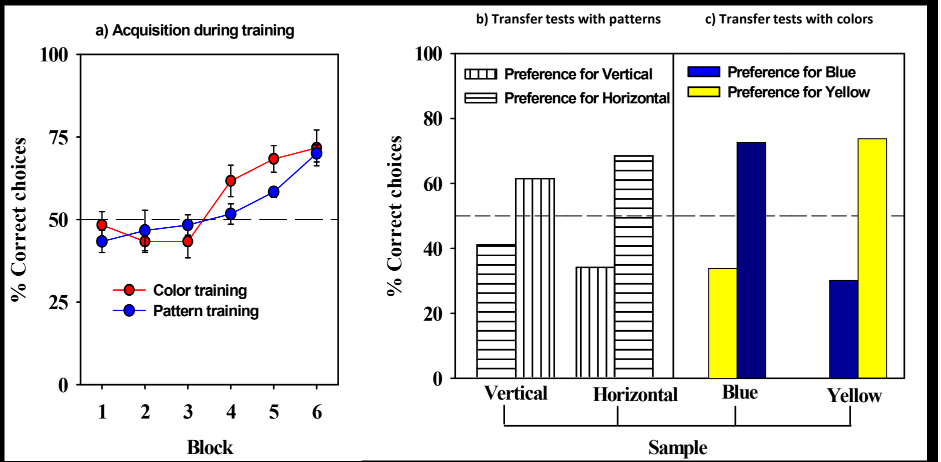
Test

After learning the previous rule, bees are confronted with novel stimuli that they never saw before. They have to show whether they learned the abstract concept of sameness and thus choose accordingly



Giurfa et al (2001) Nature

Bees transferred the relational rule from color to pattern and from pattern to color



Giurfa et al (2001) Nature

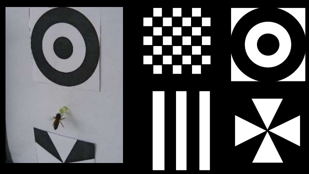
Conclusion: Conceptual learning in bees

- Bees learn a rule based on a principle of sameness independently of the physical nature of the stimuli trained
- Transfer between colors and achromatic patterns, but also between colors and odors
- They also learn a rule of difference (DNMS)

Conceptual learning in bees: further relational concepts, concept combination

Aurore Avarguès-Weber
International L'Oréal Prize 2014

- Meanwhile, our team showed that bees learn other relational concepts: for instance…'above of', 'below of', 'to the right of', 'to the left of', etc.



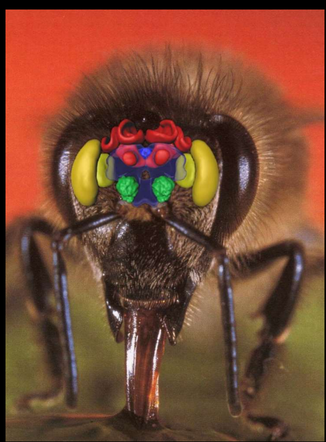
- Bees also learn to use at least two concepts simultaneously

Avargues-Weber et al (2012) PNAS

But these are free flying bees…

Performance is appealing but it does not reveal the underlying mechanisms.

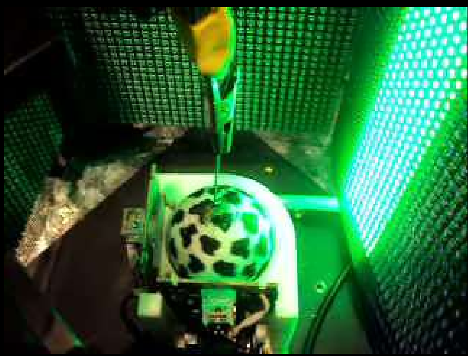
For accessing the neural mechanisms underlying associative learning in the laboratory other protocols are necessary…

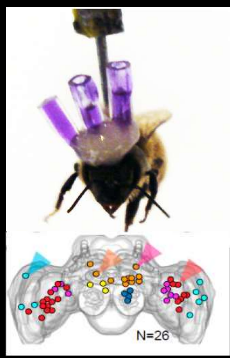


Recording the neural signature of complex visual learning in a virtual environment

Jeff Riffell

- Moving away from the drawback of studying free-flying bees while keeping behavioral richness
- Recording from cell in the central brain while the bee behaves





Studying associative learning in bees in the laboratory

Controlled conditioning protocols with harnessed bees

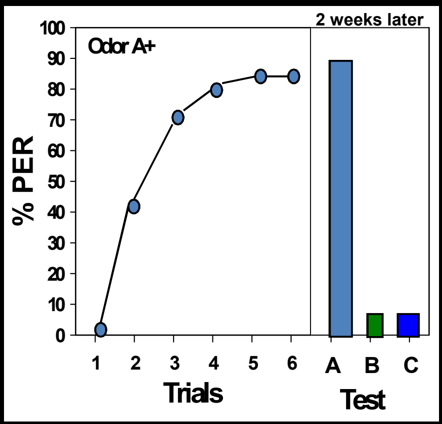
Honeybee Learning in the Laboratory

Olfactory conditioning of the proboscis extension reflex: a Pavlovian bee

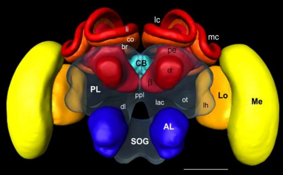
A case of Pavlovian Conditioning 'Odor (CS) – Reward (US)'

Odor (CS) has to precede Sucrose (US) for learning to occur

Fast learning in few trials
Robust memory lasting the whole life



+ access to the brain….



Giurfa & Sandoz Learn Mem 2012

The olfactory circuit in the bee brain: the antennal lobes the odor-encoding stage

Mushroom bodies (higher-order centers)

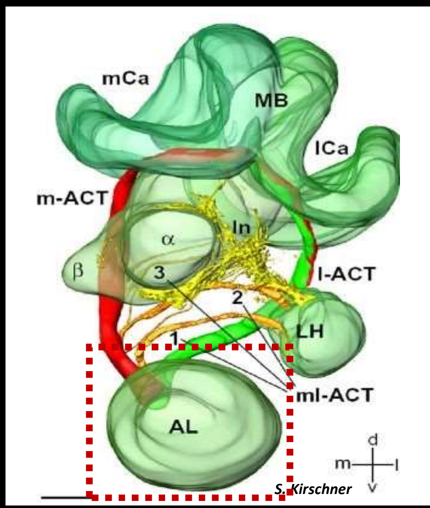
Lateral horn

Antennal lobe: 160 glomeruli

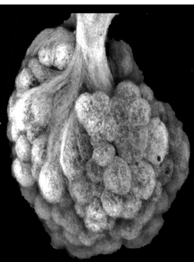
800 projection neurons

4 000 local interneurons

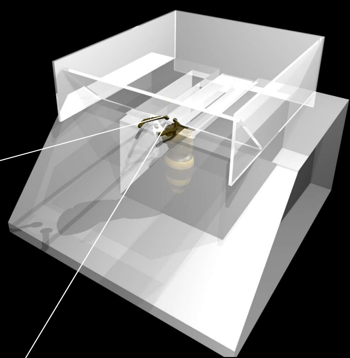
60 000 olfactory receptor neurons (at the level of the antennae)

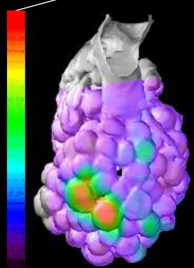


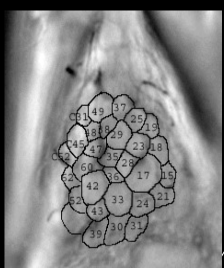
Imaging antennal lobe activity upon olfactory stimulation

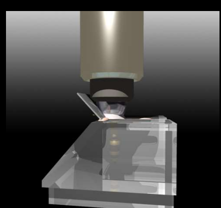


- Calcium probes of different selectivity (e.g; calcium green; fura dextran)
- Standardized atlas available

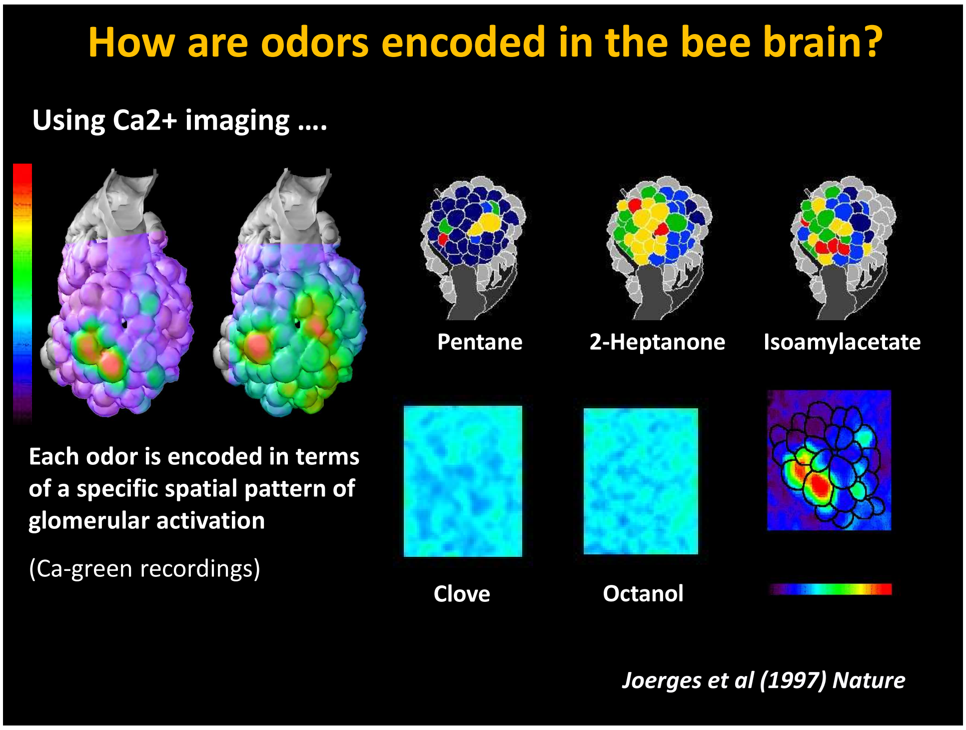


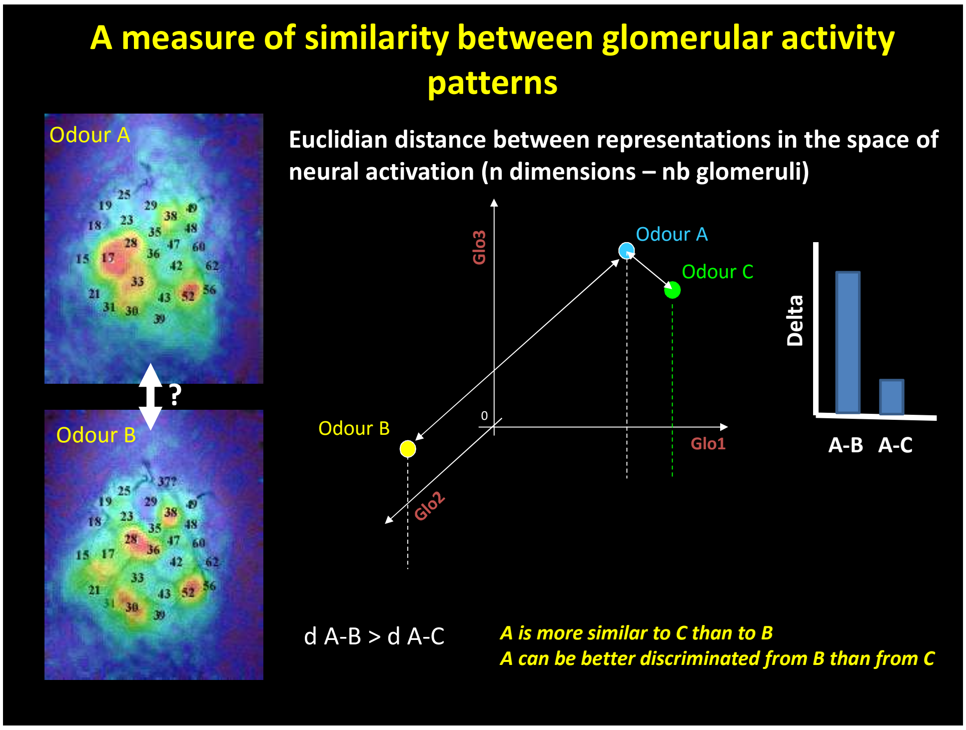






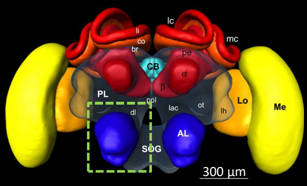
CCD camera


How are odors encoded in the bee brain?
Using Ca2+ imaging ….
Pentane
2-Heptanone
Isoamylacetate
Each odor is encoded in terms of a specific spatial pattern of glomerular activation
(Ca-green recordings)
Clove
Octanol
Joerges et al (1997) Nature

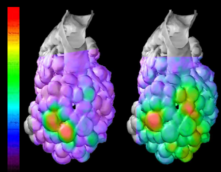

A measure of similarity between glomerular activity patterns
Odour A
Odour B
?
Euclidian distance between representations in the space of neural activation (n dimensions – nb glomeruli)
Glo3
Glo1
Glo2
0
Odour A
Odour C
Odour B
Delta
A-B
A-C
d A-B > d A-C
A is more similar to C than to B
A can be better discriminated from B than from C

Encoding of odor mixtures in the bee brain preserves to a large extent odor-component information

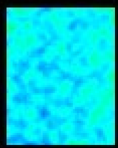

Julie Carcaud JC Sandoz



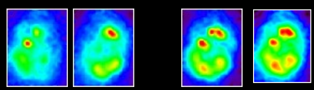
Using Ca2+ imaging ….



Each odor mixture is represented in terms of a rather linear sum of individual odor representations



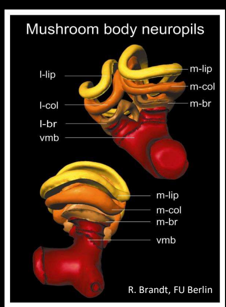
Octanol
(S. Sachse)



1 hexanol linalool Mixture Arithme-tic sum

Deisig et al J Neurophysiol 2010
Roussel et al Current Biology 2014
Carcaud et al J Neuroscience 2015

Accessing the neural bases of non-elemental learning

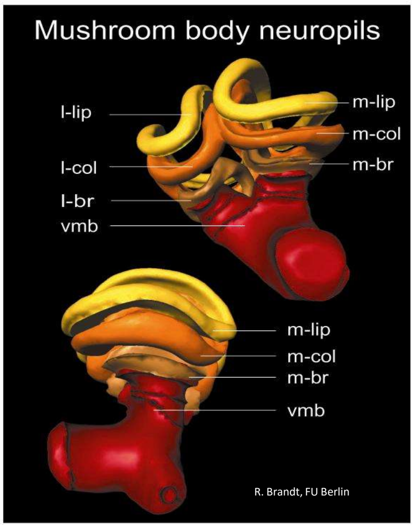


Besides the storage and retrieval of elemental memories, are there other additional functions of mushroom bodies?

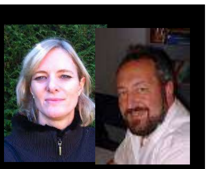
Focusing on the mushroom bodies

Mushroom bodies (higher-order centers)

- Central, prominent structures in the insect brain (ca. 30% of the brain) : calyx, neck, pedunculus, vertical + medial lobe
- Multimodal, segregated input (visual, olfactory, mechanosensory, etc) - Multimodal, integrated output → Higher-order multimodal, integration centres
- Historically associated with long-term memory storage and retrieval



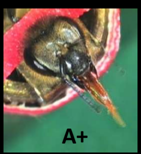
Studying non-linear forms of olfactory conditioning amenable to a neural analysis:
The case of negative patterning



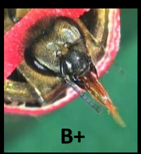
Nina Deisig Harald Lachnit

Non-linear, ambiguous discrimination:
Negative Patterning: A+, B+, AB-

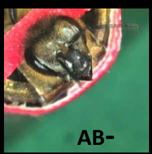
Blank trials



Odor A rewarded



Odor B rewarded



Mixture AB non-rewarded

Learning that AB ≑ A + B

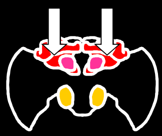
Odorants chosen represented linearly (additive sum) when delivered simultaneously

Studying the role of mushroom bodies in non-linear discrimination problems

Jean-Marc Devaud

Local injections of anesthetics **produce** reversible blocking of the mushroom bodies

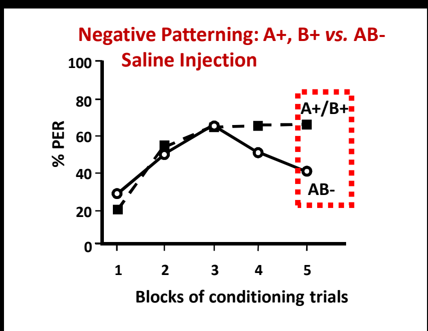
Localized injection of procaine in the mushroom bodies

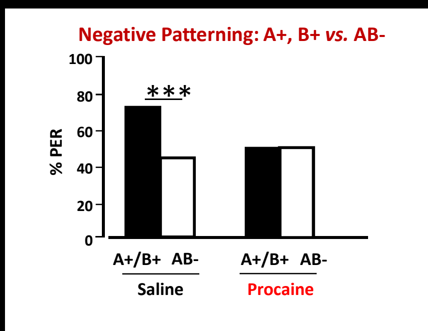


Procaine blocks voltage-gated Na+ and K+ currents

Devaud et al Eur J Neurosci (2007)

Bees require mushroom body integrity to solve the negative-patterning discrimination





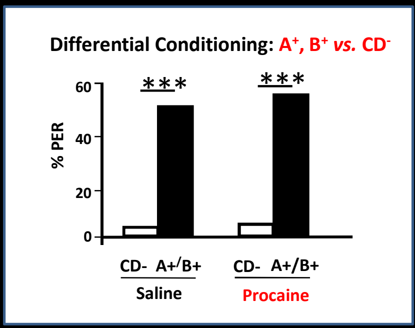
Bees injected with saline were able to solve the negative patterning discrimination

Bees injected with procaine were unable to solve the problem

Devaud et al PNAS (2015)

A control experiment: A+,B+ vs CD-

An elemental discrimination with no ambiguity



Bees were trained to discriminate
A+, B+ vs. CD-
No stimulus ambiguity.

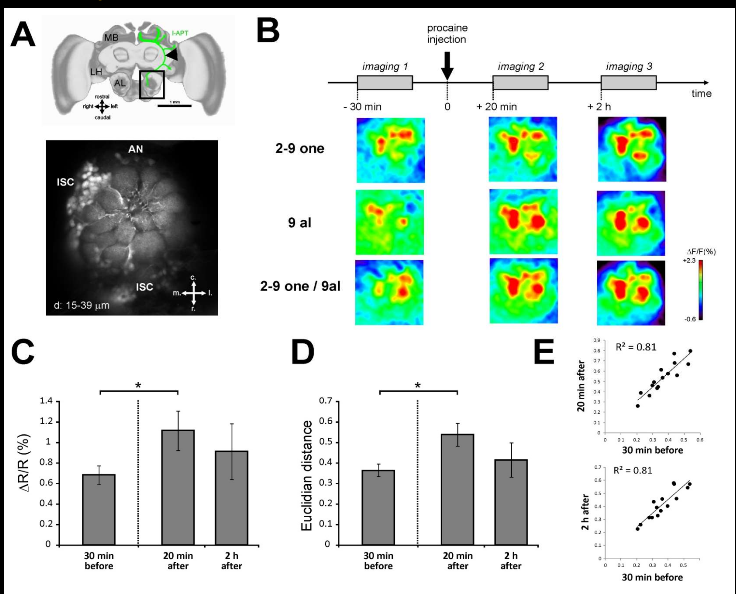
Bees treated with saline were able to solve this elemental discrimination

Bees injected with procaine could also solve equally well the elemental discrimination

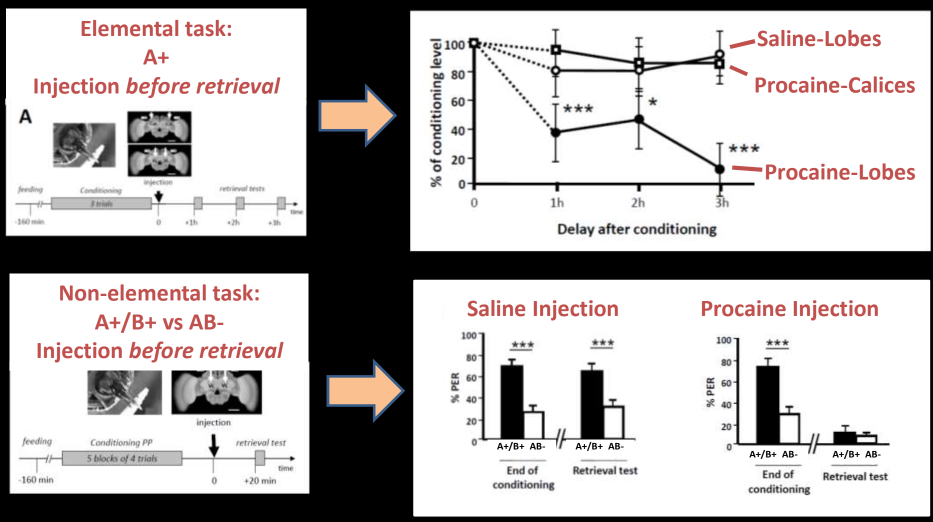
Elemental learning is possible when mushroom bodies are blocked

Devaud et al PNAS (2015)

Procaine does not change odor discriminability upstream the mushroom bodies

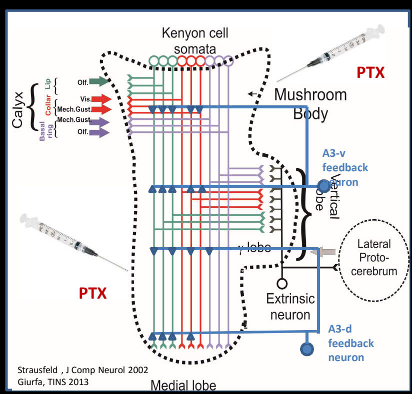


Mushroom bodies are required for elemental and non-elemental retrieval: the song remains the same



Devaud et al PNAS (2015)

Patterning discrimination: potential neural mechanisms

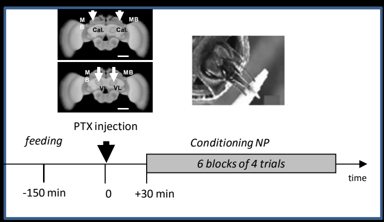


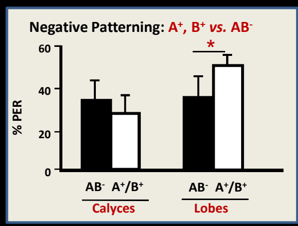
Rybak & Menzel, 1993: Two types of GABA-ergic, inhibitory feedback neurons :

- **A3-v from the vertical lobe to the calyx**
- **A3-d from medial to the vertical lobe**

GABAergic circuits at the level of the mushroom body calyces are required for negative patterning

PTX injections at the level of the MB lobes or the MB calyces





GABAergic feedback signaling from the MB lobes to the calyces is necessary for non-elemental learning: a role for Av3 neurons

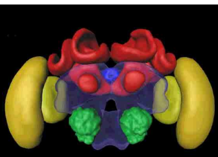
Devaud et al PNAS (2015)

Thus…

- **The incapacity of bees to solve positive & negative patterning in the absence of functional mushroom bodies was not due to side-effects of procaine as elemental differential conditioning was not impaired by mushroom body blockade.**
- **Mushroom bodies are required for solving non-elemental, ambiguous (complex) learning tasks; they disambiguate information and generate adaptive responses to non-linear problems**
- **GABAergic feedback signaling from the MB lobes to the calyces is necessary for non-elemental learning; it may help decreasing responses to non-relevant stimuli**

Devaud et al PNAS (2015)

General conclusions



- The bee brain consists of a network of identifiable neurons and neuropils, which produce stereotyped as well as plastic behavior going beyond elemental learning.
- The bee brain is neither primitive, nor rudimentary. Bees learn and memorize but they are not JUST "associative machines". Their neural circuits may be described as 'simple' in terms of the number of neurons but neither in terms of sophistication nor of performance
- Learning can be modulated by attentional processes, conceptual forms of learning and non-linear problem solving are possible. Specific circuits and brain structures such as the mushroom bodies are necessary for such problem solving.
- The insect brain is not just a perceptual machine; it categorizes, extracts, disentangles and organizes knowledge in such a way that it can be retrieved later to respond adaptively to novel situations

Final quote by a distinguished gentleman: Santiago Ramón y Cajal – Prix Nobel 1906

- *"Insects possess a nervous system that is incredibly complex and differentiated, and that exhibits a level of fineness, which attains ultramicroscopic levels. Comparing the visual and cerebroid ganglia of a bee or a dragonfly with those of a fish or an amphibian yields an extraordinary surprise. [...] It is like pretending to match the rough merit of a wall clock with that of a pocket watch, a marvel of fineness, delicacy and precision. As usually, the genius of life shines more in the construction of smaller than larger pieces"* – Santiago Ramón y Cajal, 1915

(From "Contribución al conocimiento de los centros nerviosos de los insectos ")

The Bee Team…