# Hypothalamic histaminergic modulation of brain regions involved in fear memory


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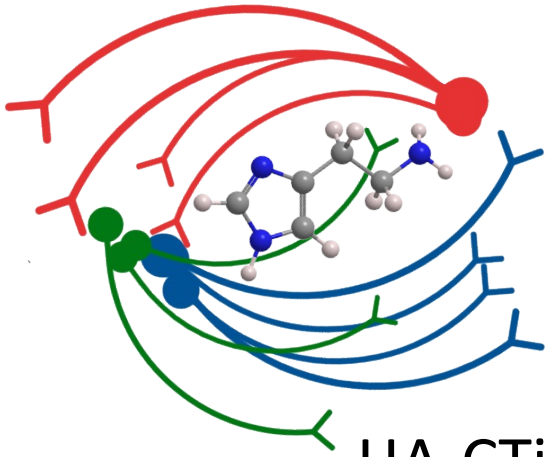

HA-CTion

BORDEAUX neurocampus NutriNeuro

Lens European Laboratory for Non-Linear Spectroscopy

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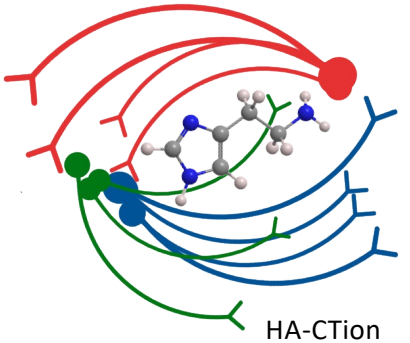

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*Specific questions*:

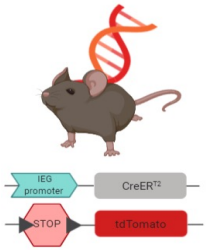
1.What are the spatio-temporal frames allowing hypothalamic HA neurons to organize behavioural responses associated with adverse events?
*Inhibitory avoidance test + functional anatomical maps, + electrophysiological characterisation*
*UNIFI LENS*

2. Are selective HA pathways recruited at different time points during the various steps of emotional memory formation?
*Chemogenetic approach - Inhibitory avoidance test + DREADD*
*NutriNeuro*

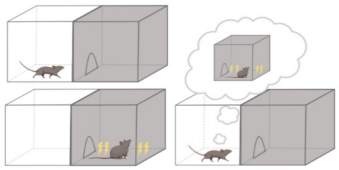
3. What is the temporal precision of HA neurotransmission in the control of the behavioural responses under study?
*Photo-pharmacology with selective ligands for HA receptors + Inhibitory Avoidance*
*VUA*

RESULTS 2021

Development of a pipeline able to represent and analyze different neuronal patterns involved in aversive memory

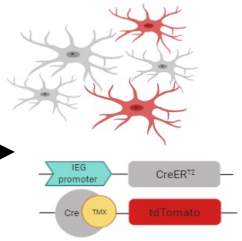



FosTrap mice

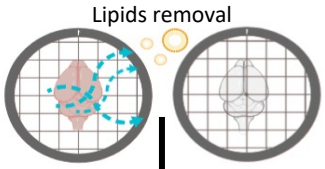


Behavioural task

+ 4-TMX injection

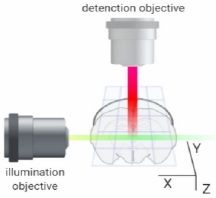



Neuron activation



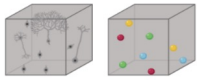

CLARITY/TDE

Cleared brain

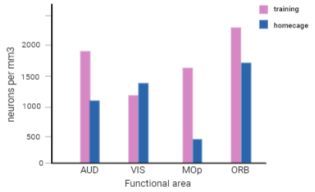



Whole-brain imaging

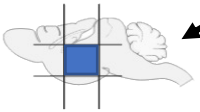
Atlas



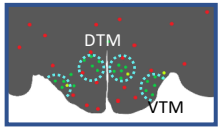
Cell detection



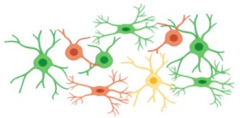

Quantification



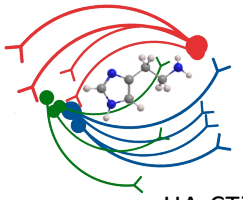
Hypothalamus isolation and immunohistochemistry




TMN dual-colour imaging



Colocalization



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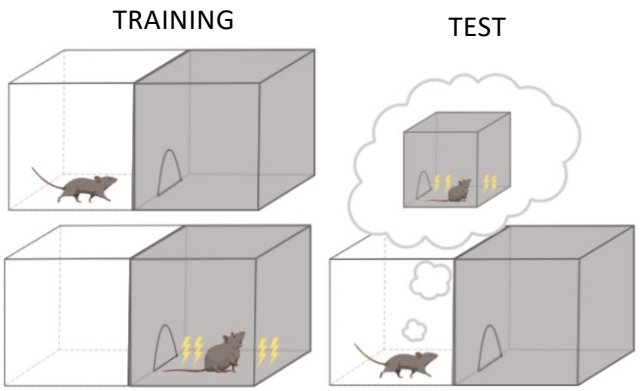
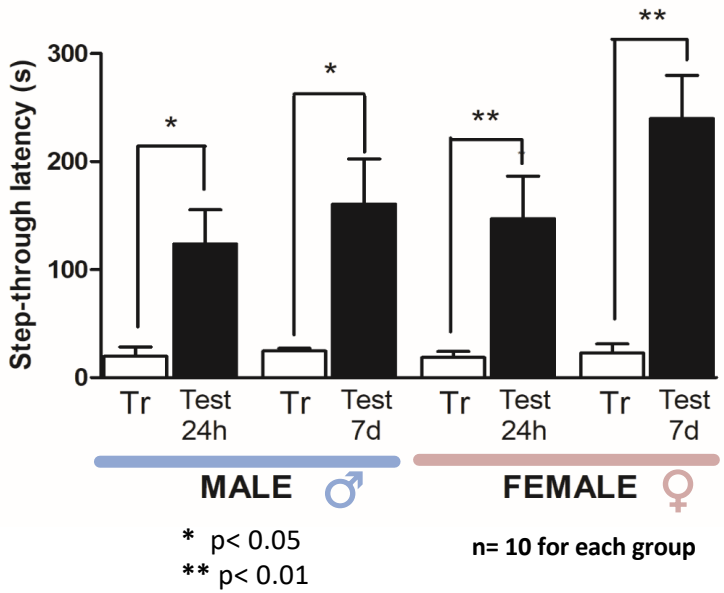
# STEP-THROUGH PASSIVE AVOIDANCE

It is used to assess aversive memory on small laboratory animals, where they learn to associate a particular context with an aversive event (mild foot-shock)




EXPERIMENTAL CLASSES ♂ ♀

- HOMECAGE
- TRAINING
- TEST 24 H (FOR SHORT- LONG TERM MEMORY SLTM)
- TEST 7D (FOR LONG TERM MEMORY LTM)



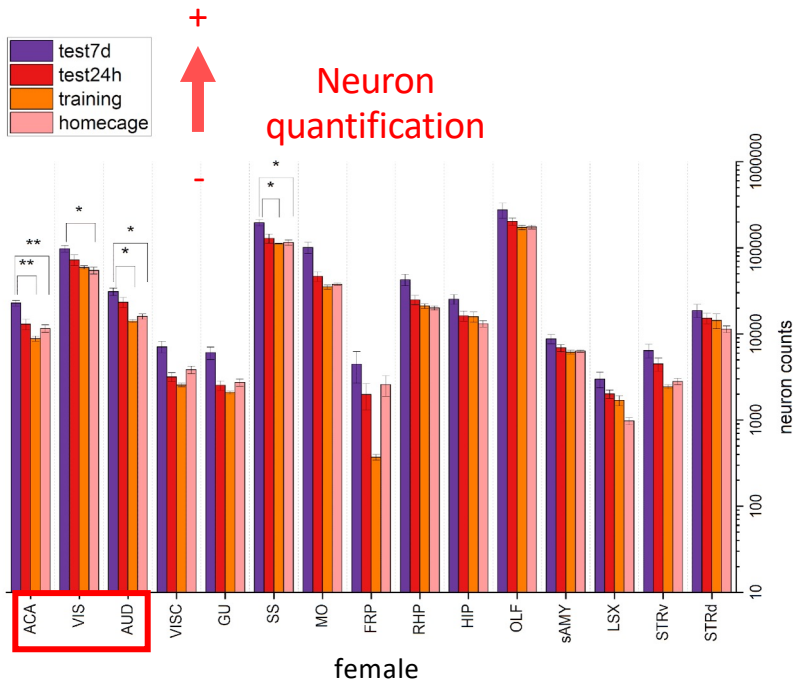

* p< 0.05
** p< 0.01

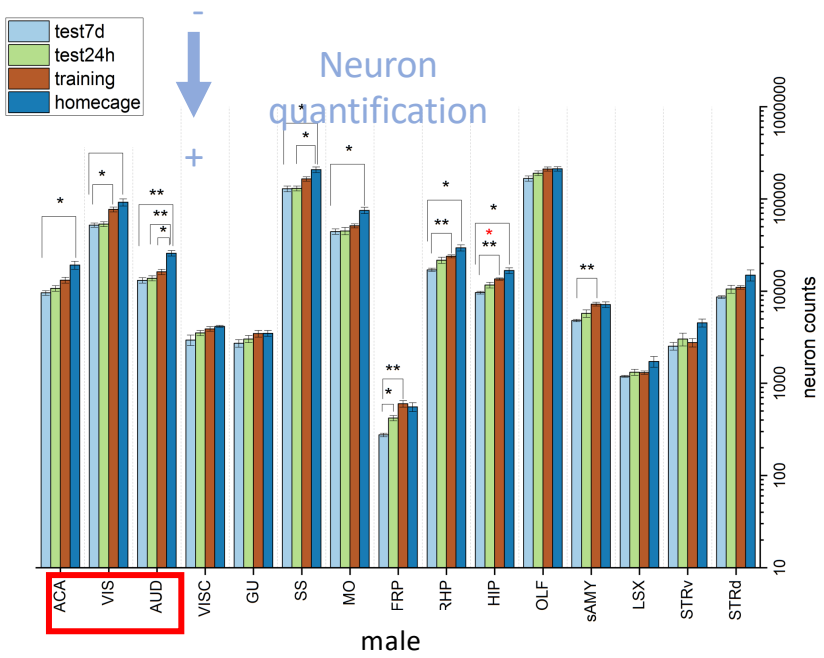
**n= 10 for each group**

Comparisons of acquisition and retention times are analyzed by 2-way ANOVA, followed by Bonferroni's post-hoc comparisons tests

HA-CTion

# Activation of neurons during aversive memory formation: OPPOSITE TREND BETWEEN GENDERS




***p < 0.01
** p < 0.05
* p < 0.1
NO CORRECTION
* p < 0.1
** p < 0.05
FDR CORRECTION

**Cortex**
**ACA** anterior cingulate area
**VIS** visual area
**AUD** auditory areas
**VISC** visceral areas
**GU** gustatory areas
**SS** somatosensory areas
**MO** somatomotor areas
**FRP** frontal pole

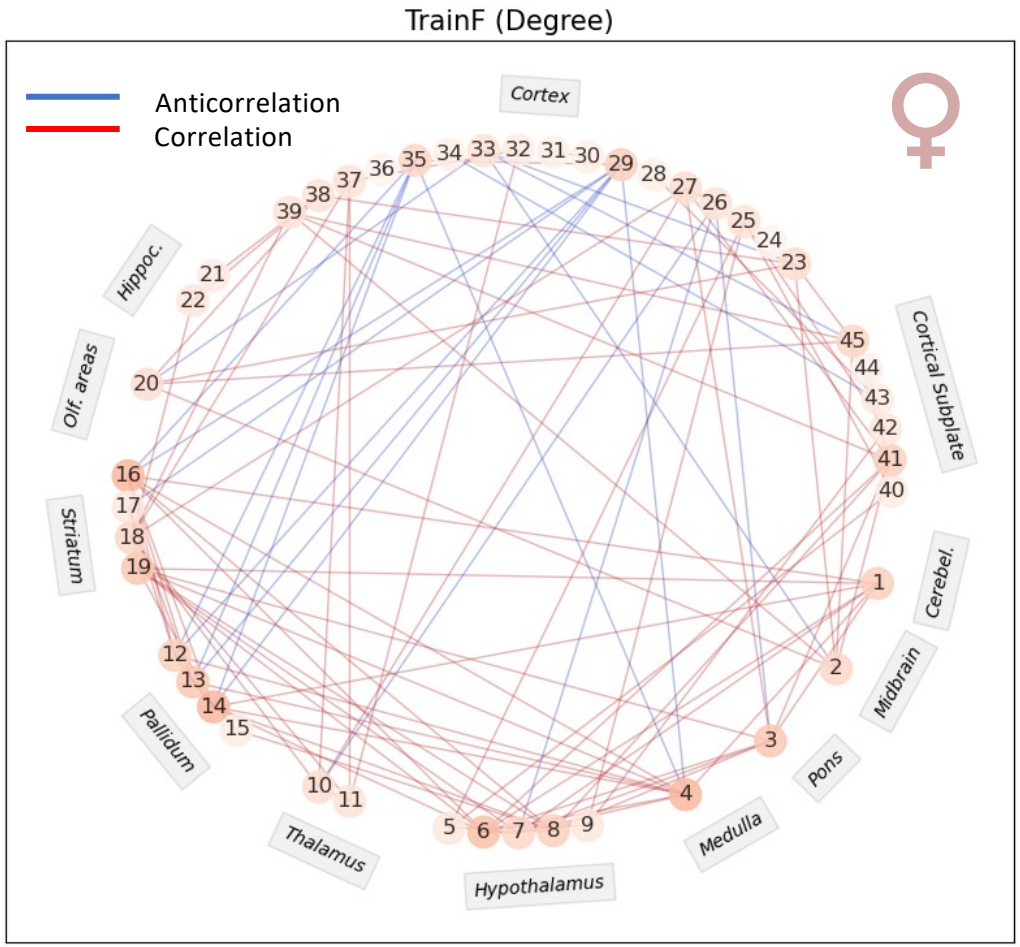
**Hippocampus**
**HIP** hippocampal region
**RHP** retrohippocampal region

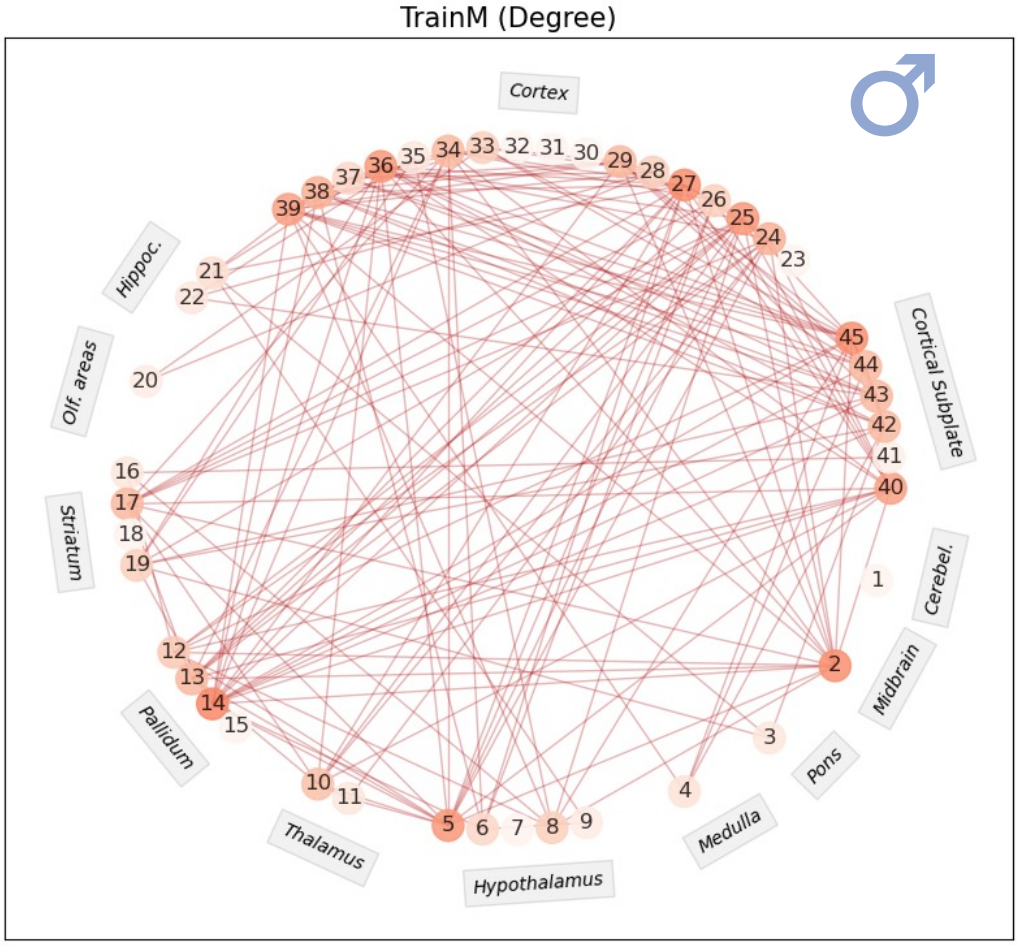
**Striatum**
**sAMY** Striatum-like amygdalar nuclei
**LSX** lateral septal complex
**STRv** striatum ventral region
**STRd** striatum dorsal region

HA-CTion

Brain connectivity during aversive memory formation: OPPOSITE TREND BETWEEN THE GENDERS

## CONNECTIVITY during training

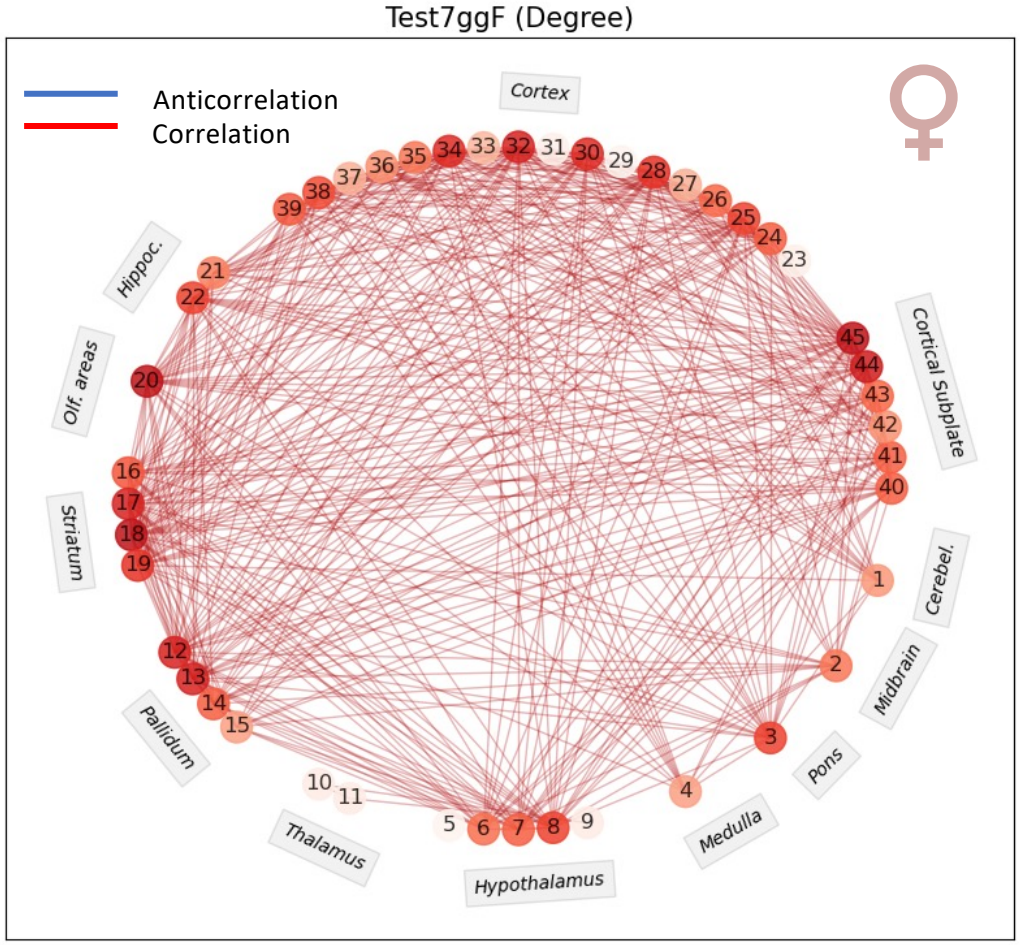


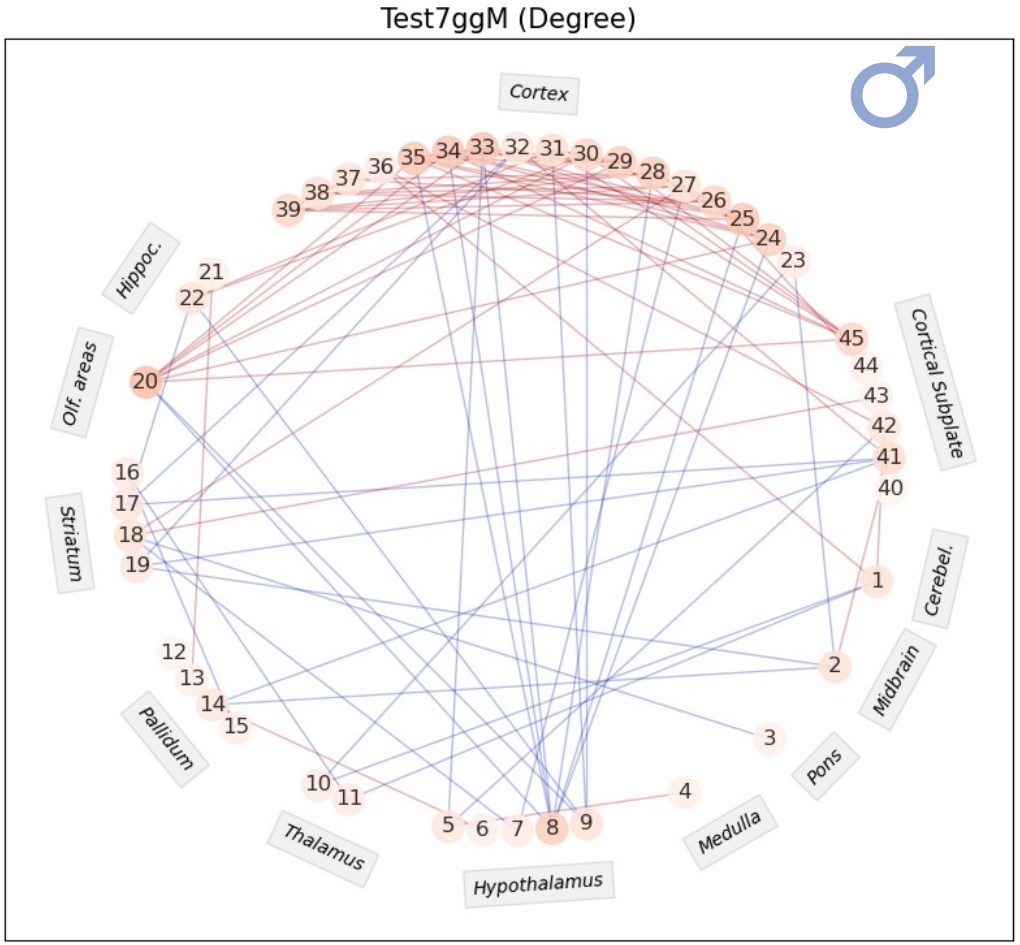




DEGREE= is the number of connections of a brain area to other areas

Brain connectivity during aversive memory formation: OPPOSITE TREND BETWEEN THE GENDERS

## CONNECTIVITY during Retrieval



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HA-CTion

# Unravelling the role of histamine neurons in memory processes through chemogenetics

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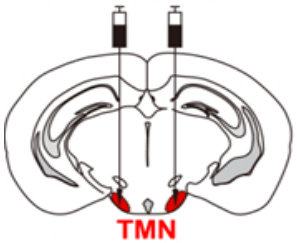
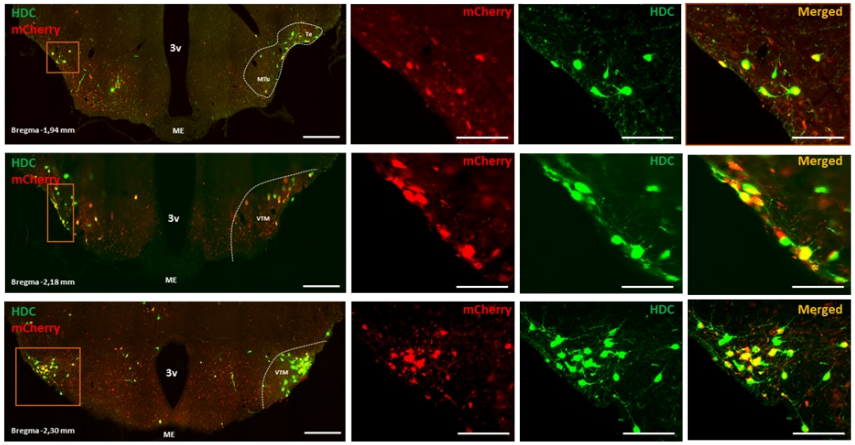
# Viral Injections in the TMN of HDC-Cre mice




*HDC-Cre mice*

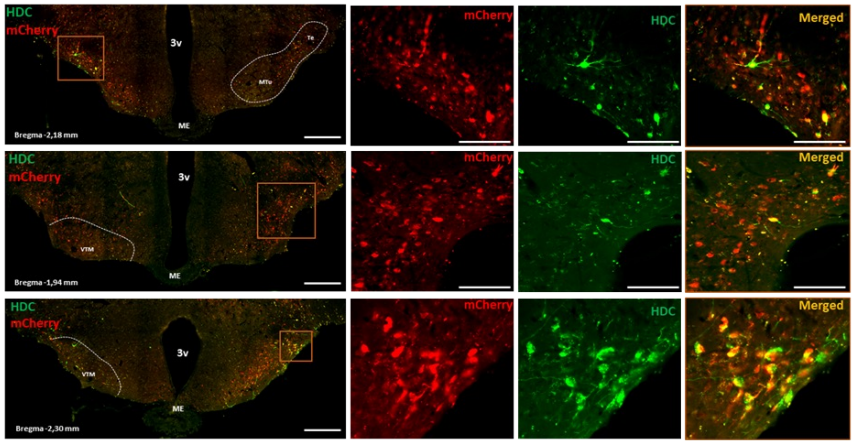
**AAV8-hSyn-DIO-mCherry**


*Cre recombination*

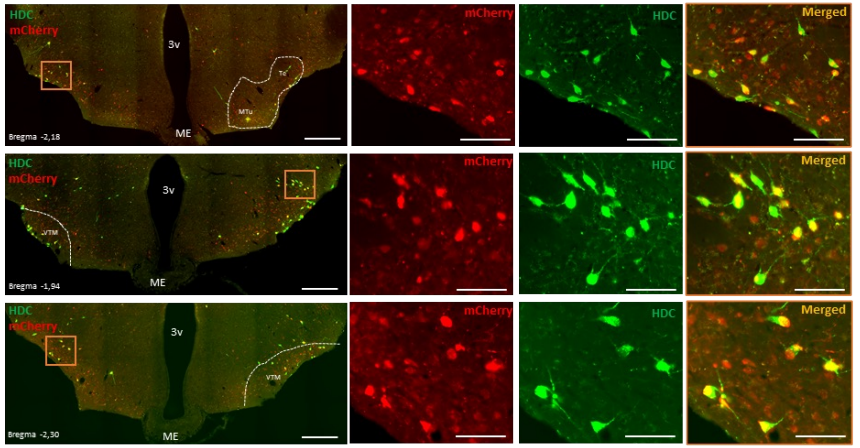

**AAV8-hSyn-DIO-hM3D(Gq)-mCherry**


*Cre recombination*

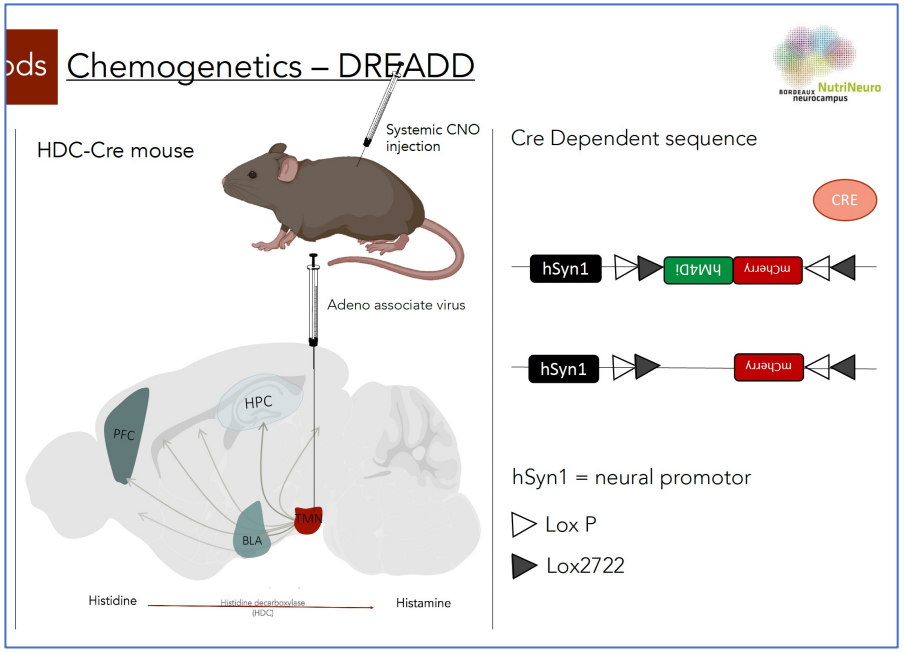



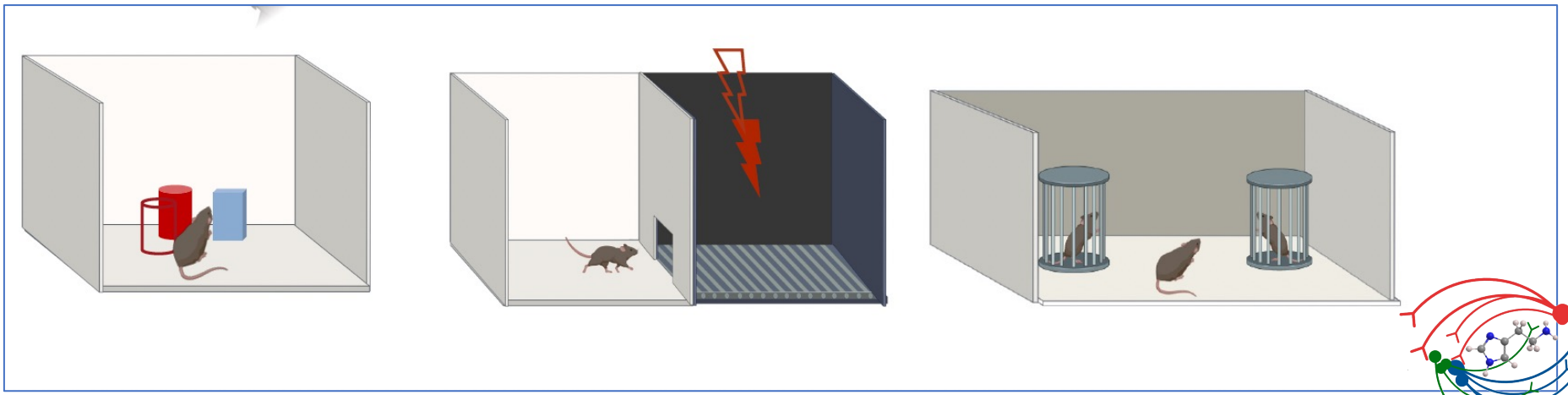

**AAV8-hSyn-DIO-hM4D(Gi)-mCherry**


*Cre recombination*

# Chemogenetic inhibition of HA neurons impairs memory formation



HA-CTion

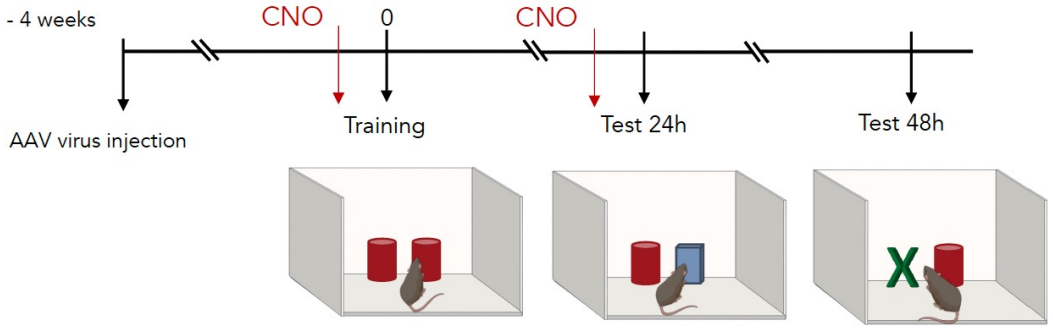
# Chemogenetic inhibition of HA neurons impairs memory consolidation

**AAV8-hSyn-DIO-hM4D(Gi)-mCherry**


*Cre recombination*


## Results

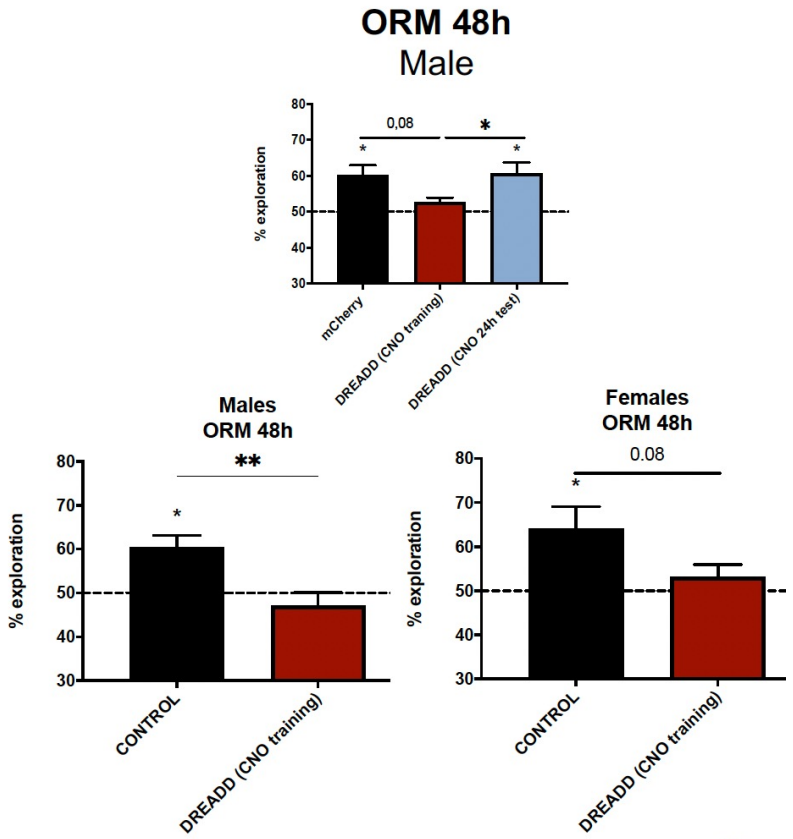
## Object recognition




Implication of histamine neurons in:

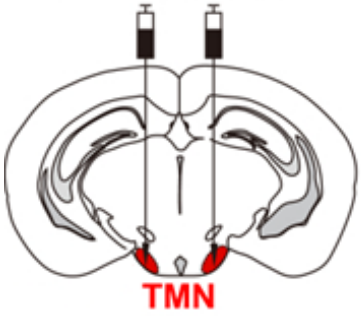
Memory consolidation

Not retrieval



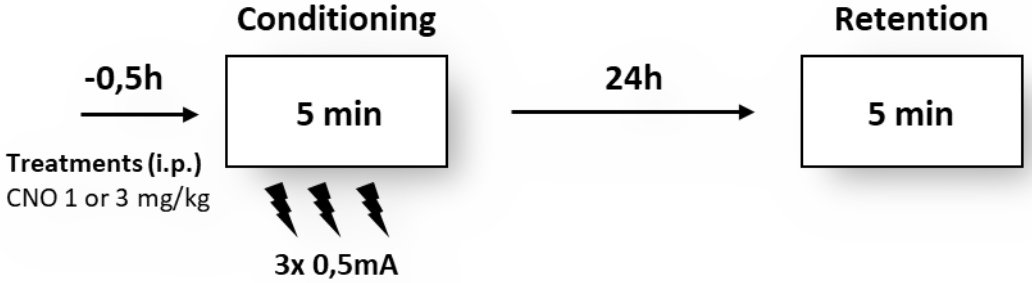
# Chemogenetic modification of HA neurons affect aversive memory formation

AAV8-hSyn-DIO-mCherry or
AAV8-hSyn-DIO-hM4D(Gi)-mCherry or
AAV8-hSyn-DIO-hM3D(Gq)-mCherry

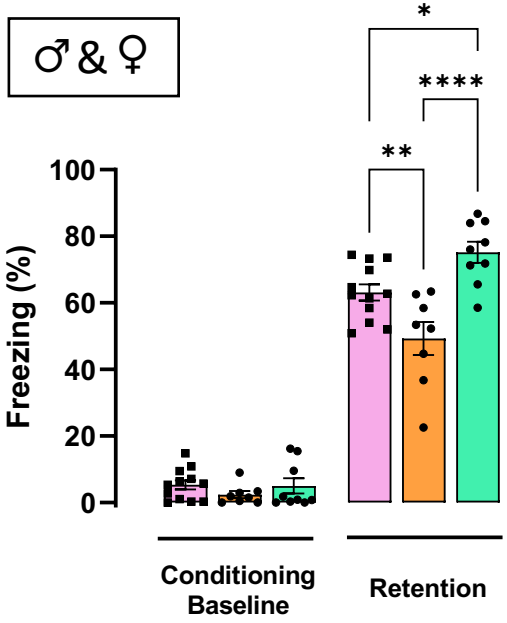


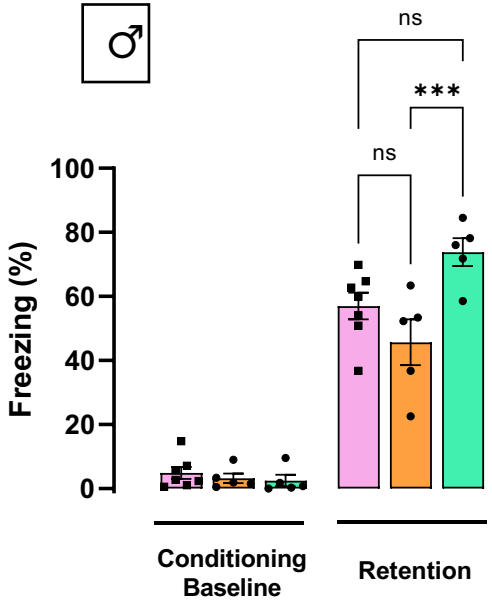

*HDC-Cre mice*

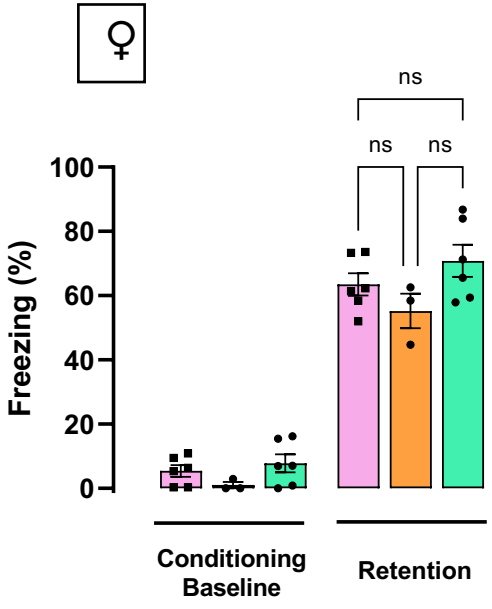
**Protocol:**



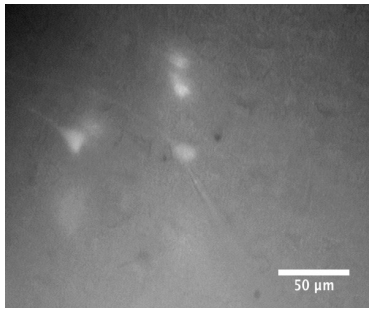

## Contextual Fear Conditioning Test



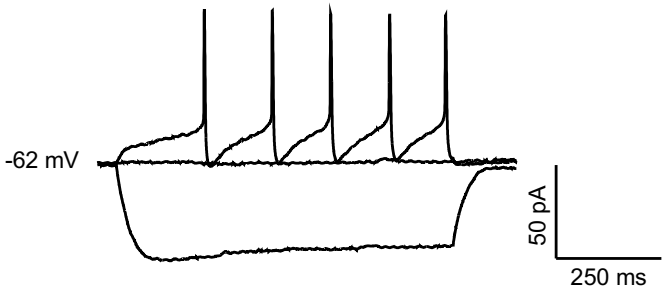



# Electrophysiological Recording of HA Neurons during memory formation

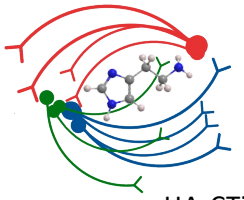


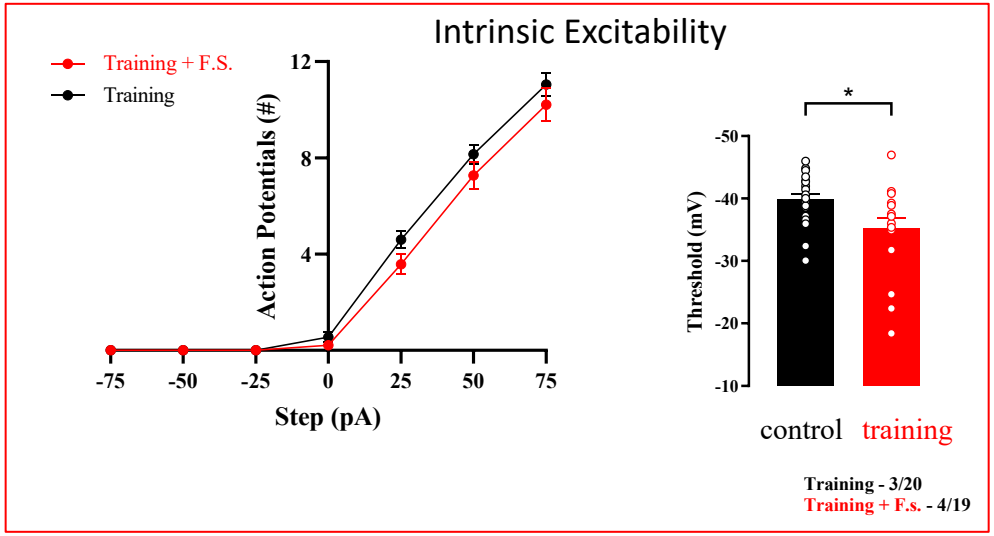

Patch clamp recordings from
HDC-Cre/TdTomato neurons

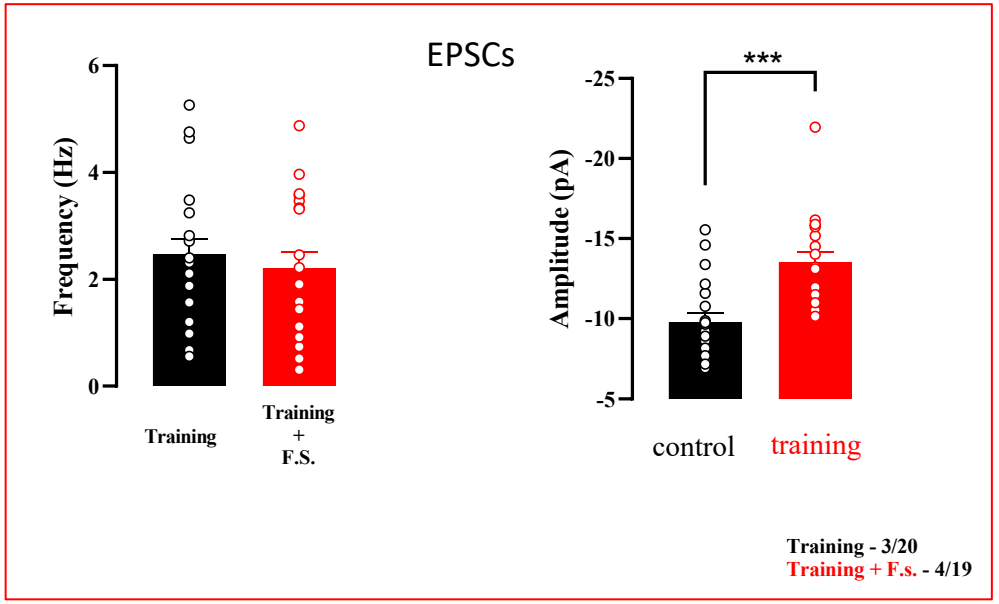



**Experimentals groups**:

- Control
- Training
- Test 24h
- Test 7d



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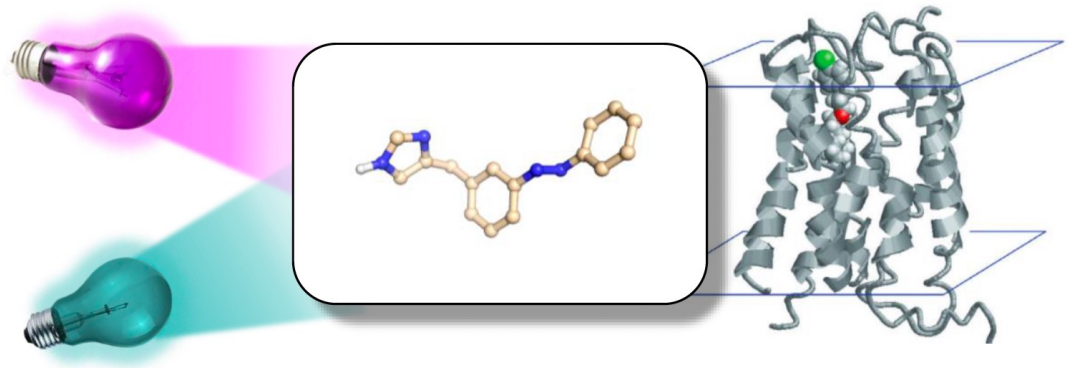
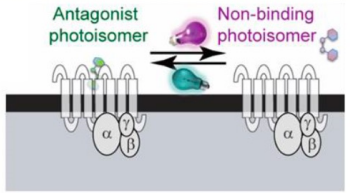
VU University Medicinal Chemistry

# Concept

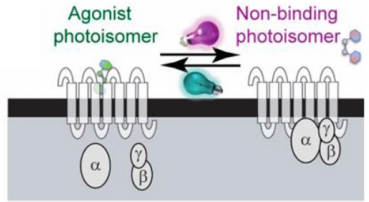
- **Photoswitchpharmacology = modulation of drug targets with light-sensitive molecules**
  - Dynamic, reversible manner
  - Spatially restricted, complementary to **optogenetics**



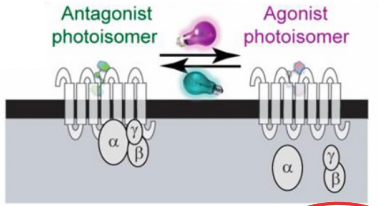

Antagonist affinity switch




Agonist affinity switch




Agonist efficacy switch



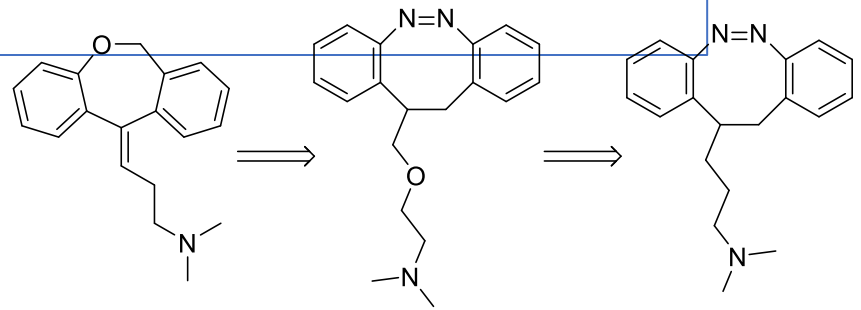

Hauwert *et al., J. Am. Chem. Soc.* **2018,** *140,* 4232
Hauwert *et al., Angew. Chem. Intl. Ed.* **2019**, *58,* 4531
Gómez-Santacana *et al.*, *Angew. Chem. Intl. Ed.* **2018**, *57*, 11608



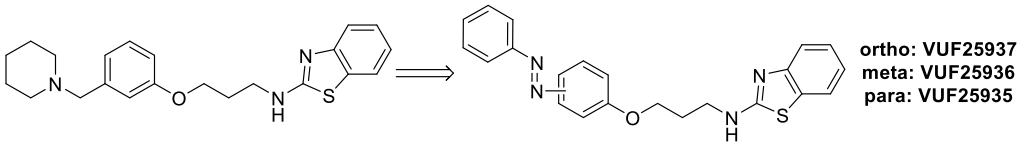
HA-CTion

# Photoswitch projects

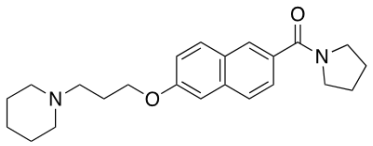
- $H_1R$
  - switch antagonist
  - doxepin

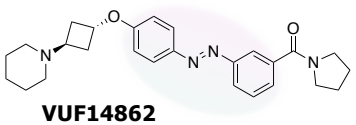


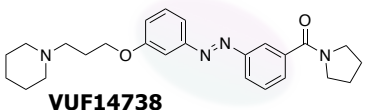

- $H_2R$
  - Switch antagonist
  - Zolantidine

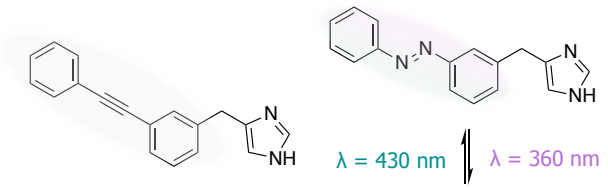


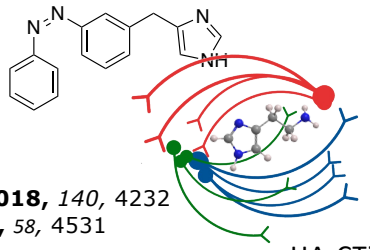

- $H_3R$
  - Switch agonist VUF15000
  - Switch antagonist VUF





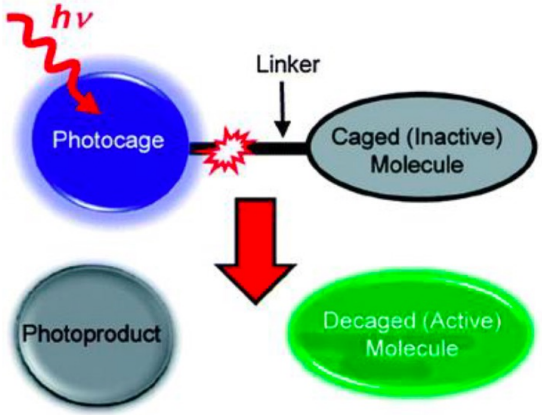



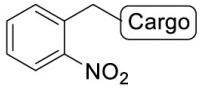



Hauwert *et al., J. Am. Chem. Soc.* **2018,** *140,* 4232
Hauwert *et al., Angew. Chem.* **2019,** *58,* 4531

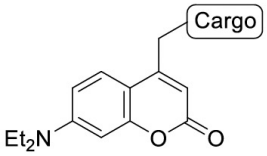
HA-CTion

# Photocage

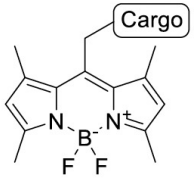



**Nitro aryl**

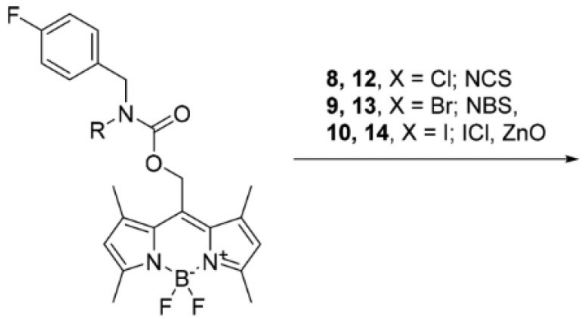



**Coumarin**



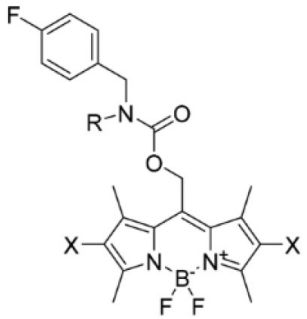

**BODIPY**

$\lambda_{max}$ up to 560 nm
Deprotection time in minutes
Deprotected primary and secondary amines
Preparation from easily accessible substrates




**7**, R = H
**11**, R = $CH_3$

**8, 12**, X = Cl; NCS
**9, 13**, X = Br; NBS,
**10, 14**, X = I; ICl, ZnO



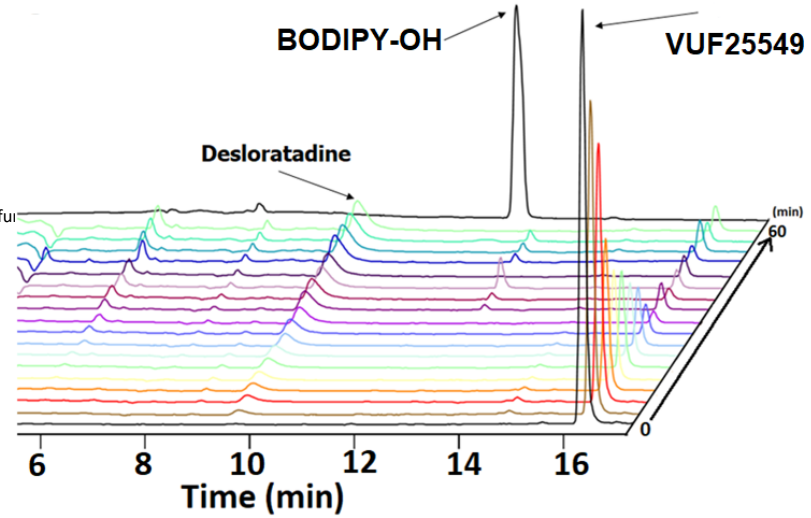

**8**, X = Cl, R = H, y = 73%
**9**, X = Br, R = H, y = 80%
**10**, X = I, R = H, y = 76%
**12**, X = Cl, R = $CH_3$, y = 69%
**13**, X = Br, R = $CH_3$, y = 81%
**14**, X = I, R = $CH_3$, y = 73%

| compound no. | X | R | half-life [min] |
|---|---|---|---|
| 7 | H | H | 0.73 |
| 8 | Cl | H | 0.94 |
| 9 | Br | H | 1.62 |
| 10 | I | H | 1.99 |
| 11 | H | $CH_3$ | 2.12 |
| 12 | Cl | $CH_3$ | 2.06 |
| 13 | Br | $CH_3$ | 0.96 |
| 14 | I | $CH_3$ | 1.87 |

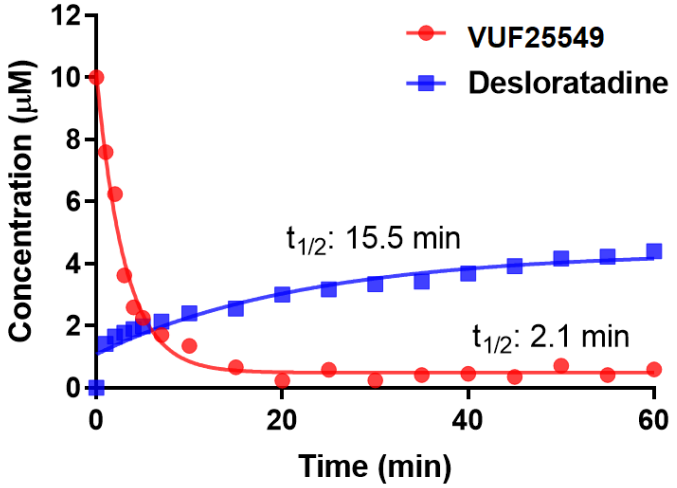
Yang DM et al. *Chem Soc Rev* **2015**, 1416-1448.

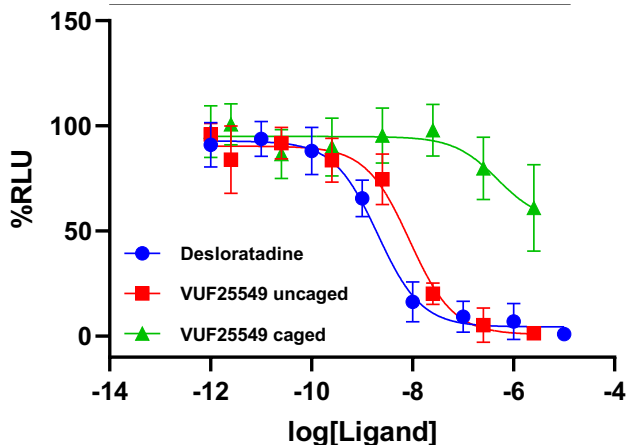
# BODIPY caged Desloratadine

- Photo characterization

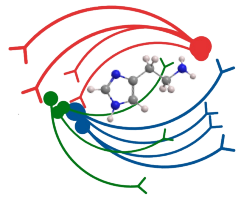



- $H_1R$ fu




| Compound | Condition | p$K_i$ ± SD |
|---|---|---|
| **VUF25549** | Dark | 5.2 ± 0.8 |
| **VUF25549** | Pre-illumination | 8.1 ± 0.1 |
| **Desloratadine** | Dark | 8.4 ± 0.3 |



HA-CTion

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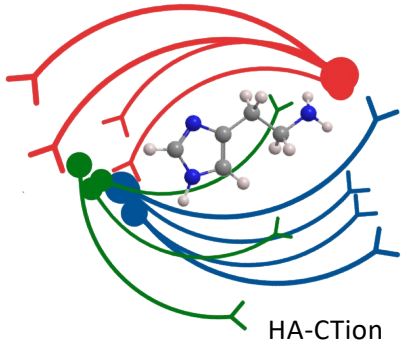
UNIVERSITÀ DEGLI STUDI FIRENZE



HA-CTion

# Achievements

- Whole map connectivity of brain nuclei during aversive memory formation. Gender differences
  - In progress: role of the histaminergic system
- Chemogenetic modulation of HA neurotransmission during memory formation
  - In progress: activation/inhibition of selected histaminergic pathways during memory formation
- Analysis of electrophysiological signature of HA neurons during memory formation
  - Work in progress
- Synthesis of new photoswitchable and photocaged HA ligands
  - In progress: in vitro test of compounds