3D RECONSTRUCTION OF BRAIN WIDE NEURONAL CIRCUITS INVOLVED IN AVERSIVE MEMORY FOLLOWING A-FLUOROMETHYLHISTIDINE

A.Franceschini*[1,2], C.Checcucci[3], Giacomo Mazzamuto[2], F.S.Pavone[2,4], P.Blandina[5], M.B.Passani[1], L.Silvestri[2,4]

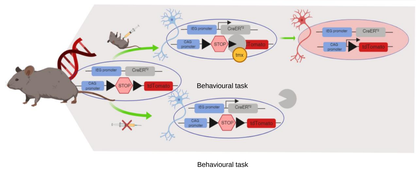
1 DSS Dipartimento di Scienze della Salute Umana, University of Florence, 50139, Florence, Italy, 2 LENS European Laboratory for Non-Linear Spectroscopy, 50019, Sesto Fiorentino, Italy, 3 DINFO Department of Information Engineering, University of Florence, 50139, Florence, Italy , 4 Department of Physics and Astronomy, University of Florence, 50019, Sesto Fiorentino, Italy, 5 Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, University of Florence, 50139, Florence, Italy

Abstract

The central histaminergic system is an important modulator of memory related to adverse events. Researchers have proved that the histamine neurotransmitter is necessary for long-term memory (LTM) but not short-term memory of step-through inhibitory avoidance (IA).
Our aim is to understand how neuronal patterns, involved in formation and storage of fear memory, change with a histamine depletion using whole brain mapping of c-fos expression.

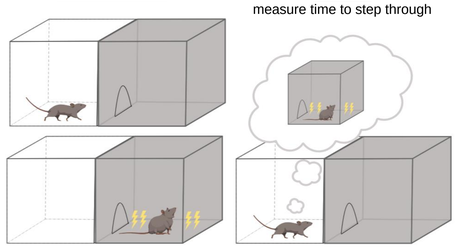
Animal model

We use TRAP mice [Gunther at al. Neuron, 2013] to permanently label neurons which are activated during different behavioral conditions. In Fos-TRAP mice, injection of tamoxifen induces cre-driven recombination in cells, providing a permanent access to a transiently active population, expressing the fluorescent protein tdTomato



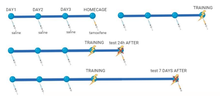
Behavioural learning

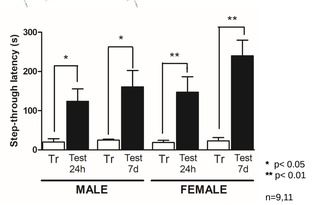
We use the step-through passive avoidance test that is a fear-motivated test classically used to assess short-term or long-term memory on mice [Eriksson et al. Neuropharmacology, 2001].
During test, the latency time is considered a direct measurement of memory



Experimental classes
- ✓ Homecage
- ✓ Training
- ✓ Test 24 h (for short term memory STM)
- ✓ Test 7d (for long term memory LTM)

Experimental timeline

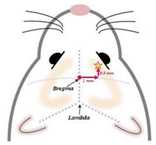


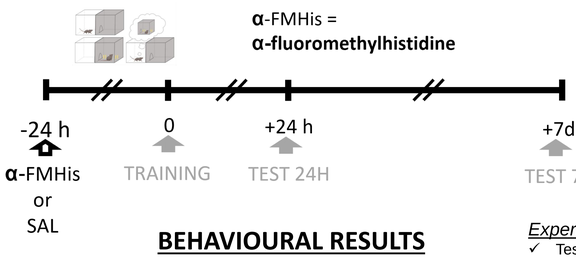


0,3 mA shock intensities have a comparable impact on both male and female STM and LTM. The comparisons of acquisition and retention times are analyzed by 2-way ANOVA, followed by Bonferroni's post-hoc comparisons tests

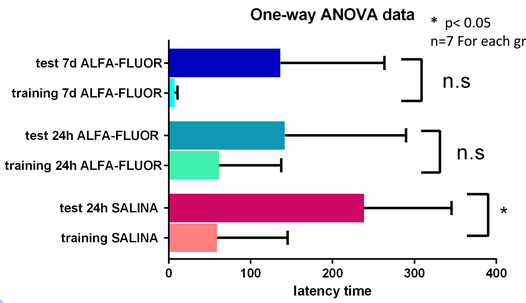
Drug treatment

α -Fluoromethylhistidine (α-FMH) is a potent inhibitor of histidine decarboxylase (HDC) that inhibits the histamine synthesis at brain level in a time-dependent and concentration-dependent manner which is restored to control levels after about 48 h. This drug is somministrated by intracerebroventricular delivery, surgically implanting a cannula





BEHAVIOURAL RESULTS



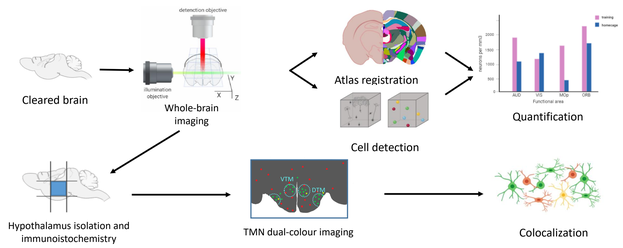
Experimental classes
- ✓ Test 24h saline
- ✓ Test 24h alfa fluor
- ✓ Test 7d alfa fluor

0,3 mA shock intensities have a comparable impact on control group (treated with saline) and drug one, both at 24h and 7d after training. The comparisons of acquisition and retention times are analyzed by 1-way ANOVA

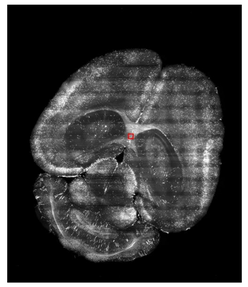
Whole brain activation mapping with light-sheet microscopy and high-throughput analysis

Reconstructions are obtained from the light-sheet microscope (A). Alignment and fusion stacks are performed with the global optimization algorithm ZetaSticher (https://github.com/lens-biophotonics/ZetaStitcher). The mouse brain reconstruction shows the organization of c-fos neurons after activation (B). Each activated fluorescent cell is localized using a neural network. Afterwards, each brain is spatially registered to the Allen Brain Atlas. In this way, we can quantify the number of activated neurons in each specific brain region (C).

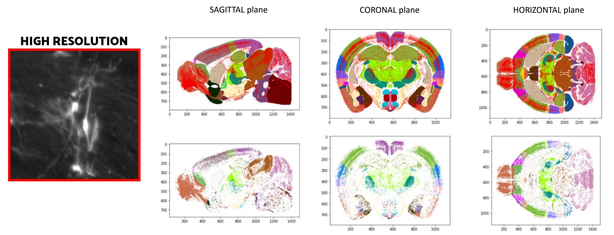
A



B

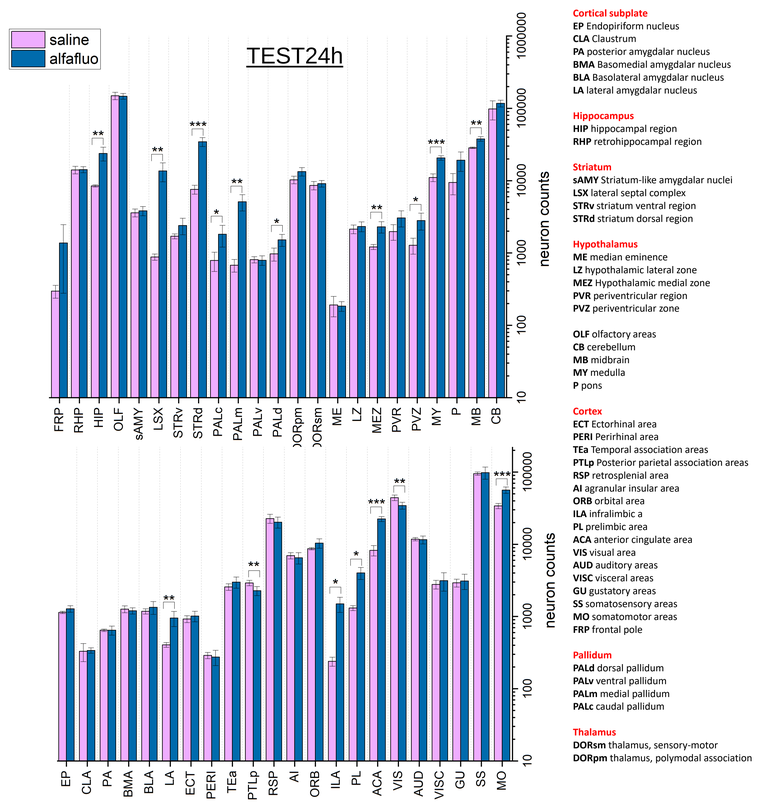


C



Activated neuron quantification

The activated neurons in different memory phases are quantified in all brain areas for these two experimental classes (saline and alfafluor groups). Preliminary results about test 24h are shown in histograms below.



Cortical subplate
EP Endopiriform nucleus
CLA Claustrum
PA posterior amygdalar nucleus
BMA Basomedial amygdalar nucleus
BLA Basolateral amygdalar nucleus
LA lateral amygdalar nucleus

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

Hypothalamus
ME median eminence
LZ hypothalamic lateral zone
MEZ Hypothalamic medial zone
PVR periventricular region
PVZ periventricular zone

OLF olfactory areas
CB cerebellum
MB midbrain
MY medulla
P pons

Cortex
ECT Ectorhinal area
PERI Perirhinal area
TEa Temporal association areas
PTLp Posterior parietal association areas
RSP retrosplenial area
AI agranular insular area
ORB orbital area
ILA infralimbic a
PL prelimbic area
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

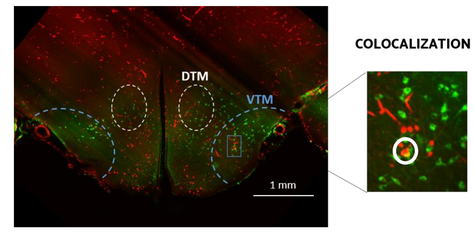
Pallidum
PALd dorsal pallidum
PALv ventral pallidum
PALm medial pallidum
PALc caudal pallidum

Thalamus
DORsm thalamus, sensory-motor
DORpm thalamus, polymodal association

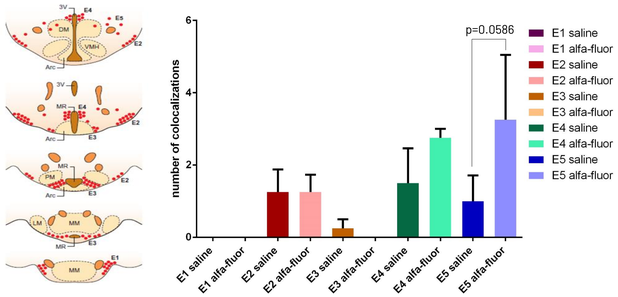
Spatial distribution of histaminergic cells activated at retrieval in mice treated with α-FMH before training

To identify the histaminergic cells among all the activated neurons, we selectively label H-positive neurons of these two experimental classes in the clarified Fos-TRAP brains with whole-mount brain immunohistochemistry (A). This quantification is correlated to the spatial distribution of histaminergic neurons which are activated during the same task (B).

A



B



Future outlook

- Test 7d experimental class treated with **α-fluoromethylhistidine** has yet to be analysed
- To increase the number of mice for α-fluoromethylhistidine experiment in order to study a hypothetical sexual dimorphism (in order to compare this results with previous ones)

Contact

Alessandra Franceschini franceschini@lens.unifi.it
European Laboratory for Non-Linear Spectroscopy, Via Nello Carrara 1-50019, Sesto Fiorentino

This work was supported by the FLAG-ERA grant HA-Ction by the Italian Ministry of Education. The project have also been supported by "Ente Cassa di Risparmio di Firenze" (private foundation). Part of this work was performed in the frame of the Proof of Concept Studies for the ESFRI research infrastructure project Euro-BioImaging at the PCS facility LENS