ESPCI PARIS | PSL

BIG DATA ANALYSIS APPLIED TO BEHAVIOURAL NEUROSCIENCE

Gisella Vetere
ESPCI Paris
Dec 2023

C4TEAM

CEREBRAL CODES AND CIRCUITS CONNECTIVITY

veterelab.weebly.com

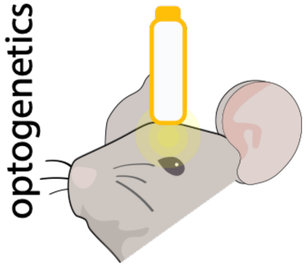
BIG DATA ANALYSIS APPLIED TO BEHAVIOURAL NEUROSCIENCE

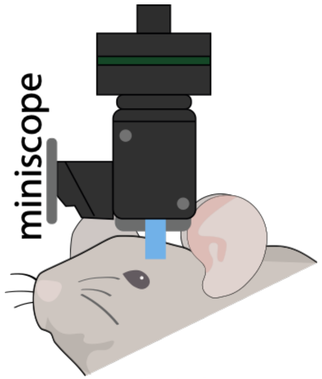
FEAR MEMORY



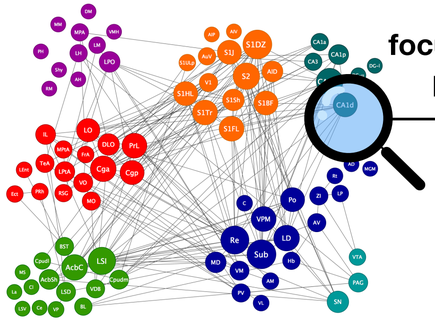
focus on the brain





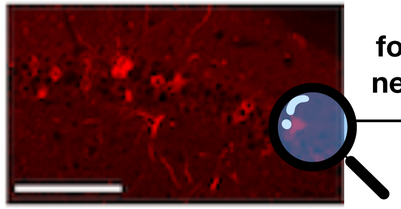


freezing activated neurons

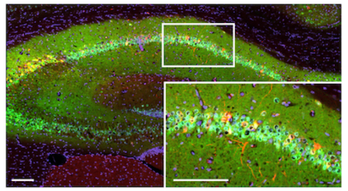


focus on neuronal populations

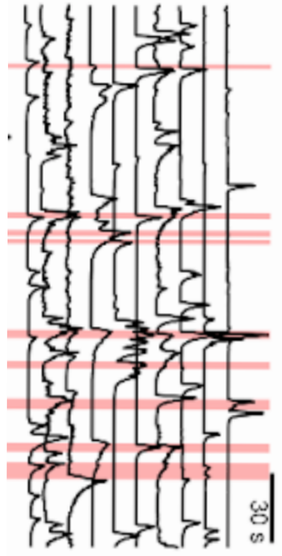
NETWORK of brain regions



focus on single neurons activity



ENGRAM neurons



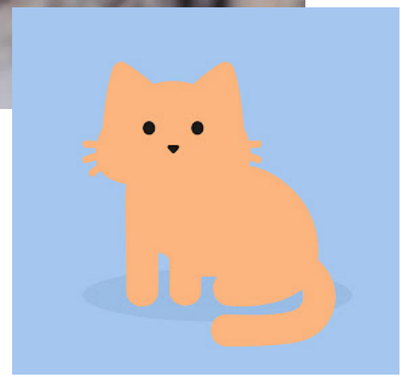
single cells

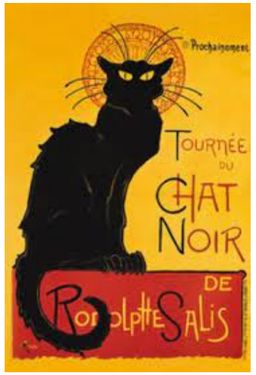
Vetere Gisella



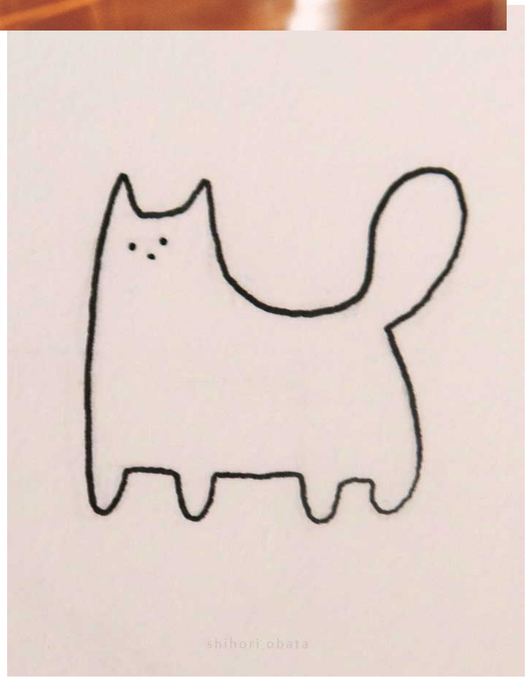






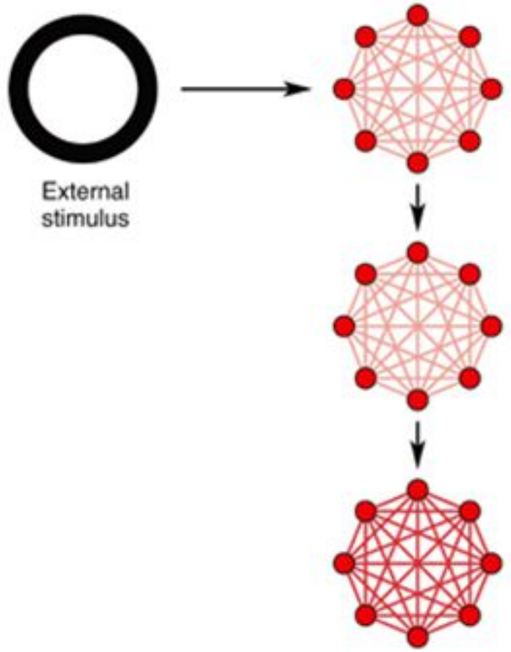

Prochainement
TOURNÉE
DU
CHAT
NOIR
DE
Rodolphe SALIS




shihori obata

Memory location - the Engram

Hebb, in 1949, proposed that the internal representation of an object consists of all cortical cells that are activated by the external stimulus.



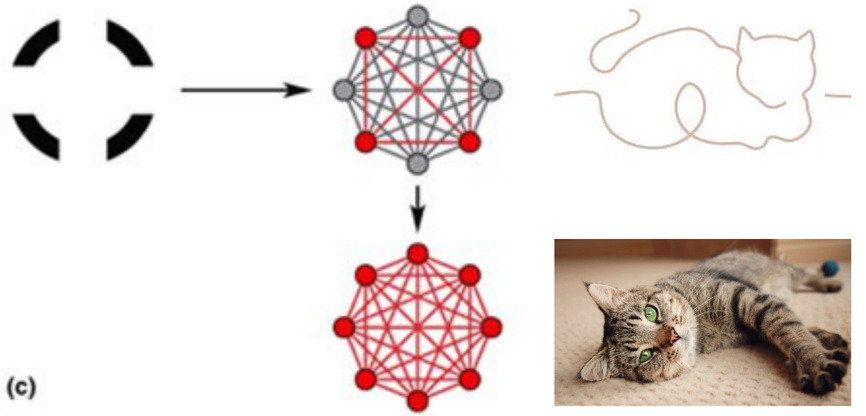
All these cells should be <u>reciprocally interconnected.</u>

If the activation of the cell assembly persist over time, consolidation would occur by a “growth process” that made these reciprocal connections more effective.

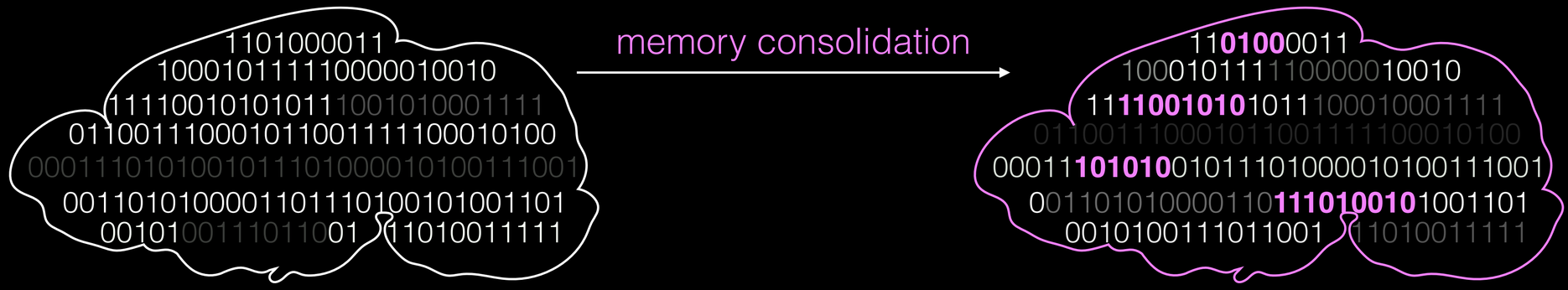
<u>Neurons that FIRE together would WIRE together.</u>

Subsequently, if only a fraction of the cells are activated by a later stimulus (a segment of the circle), this would cause the whole assembly to become active again

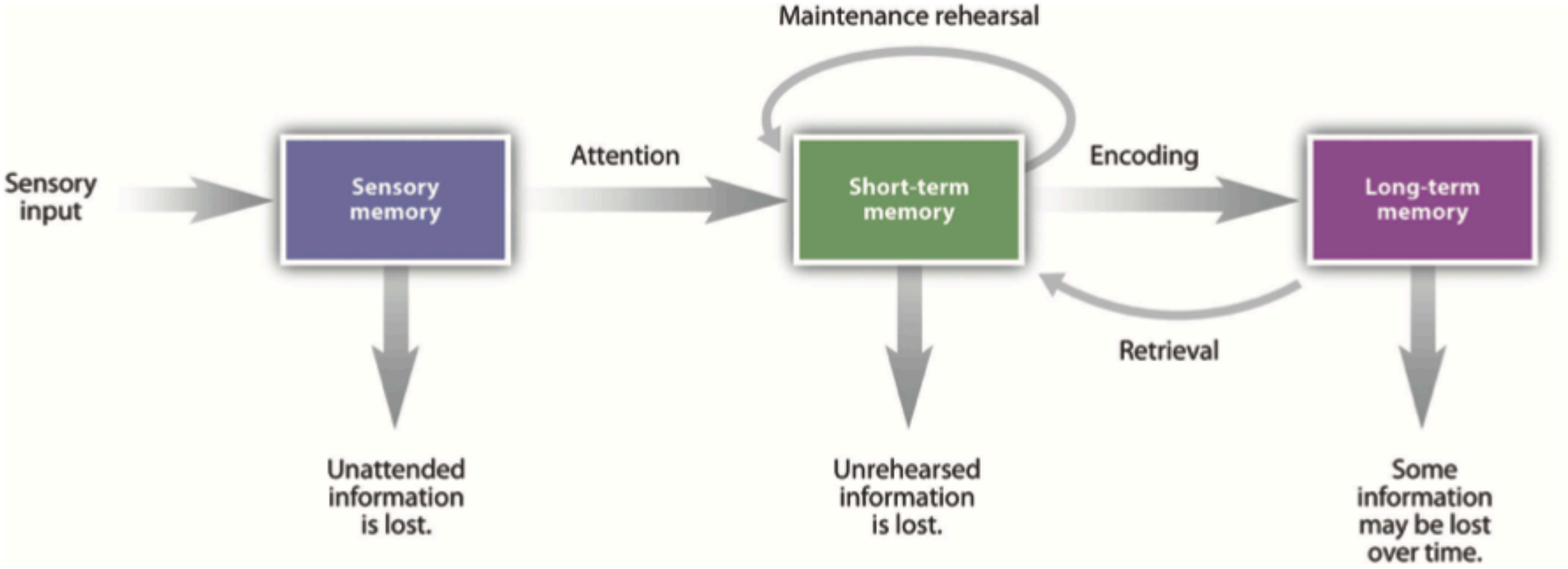
RECALLING THE ENTIRE INTERNAL REPRESENTATION OF THE EXTERNAL STIMULUS



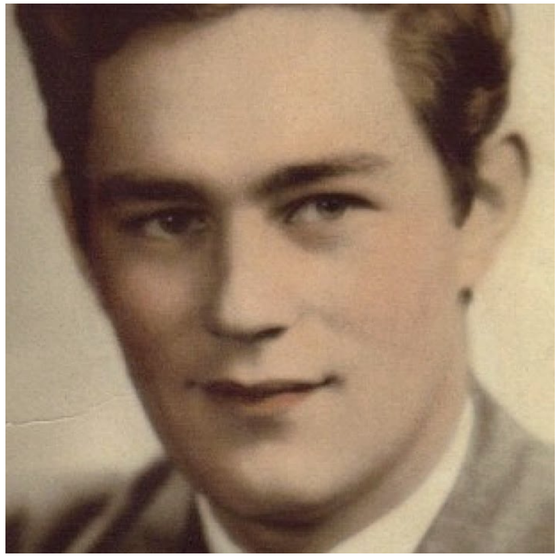
How memory consolidation modulates the reorganization of specific circuits in the brain

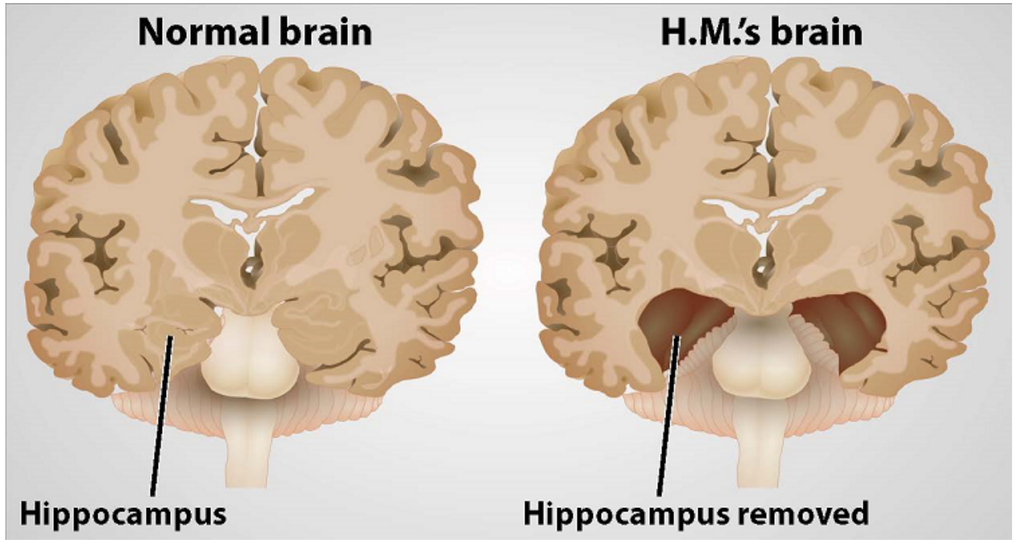


How memory consolidation modulates the reorganization of specific circuits in the brain

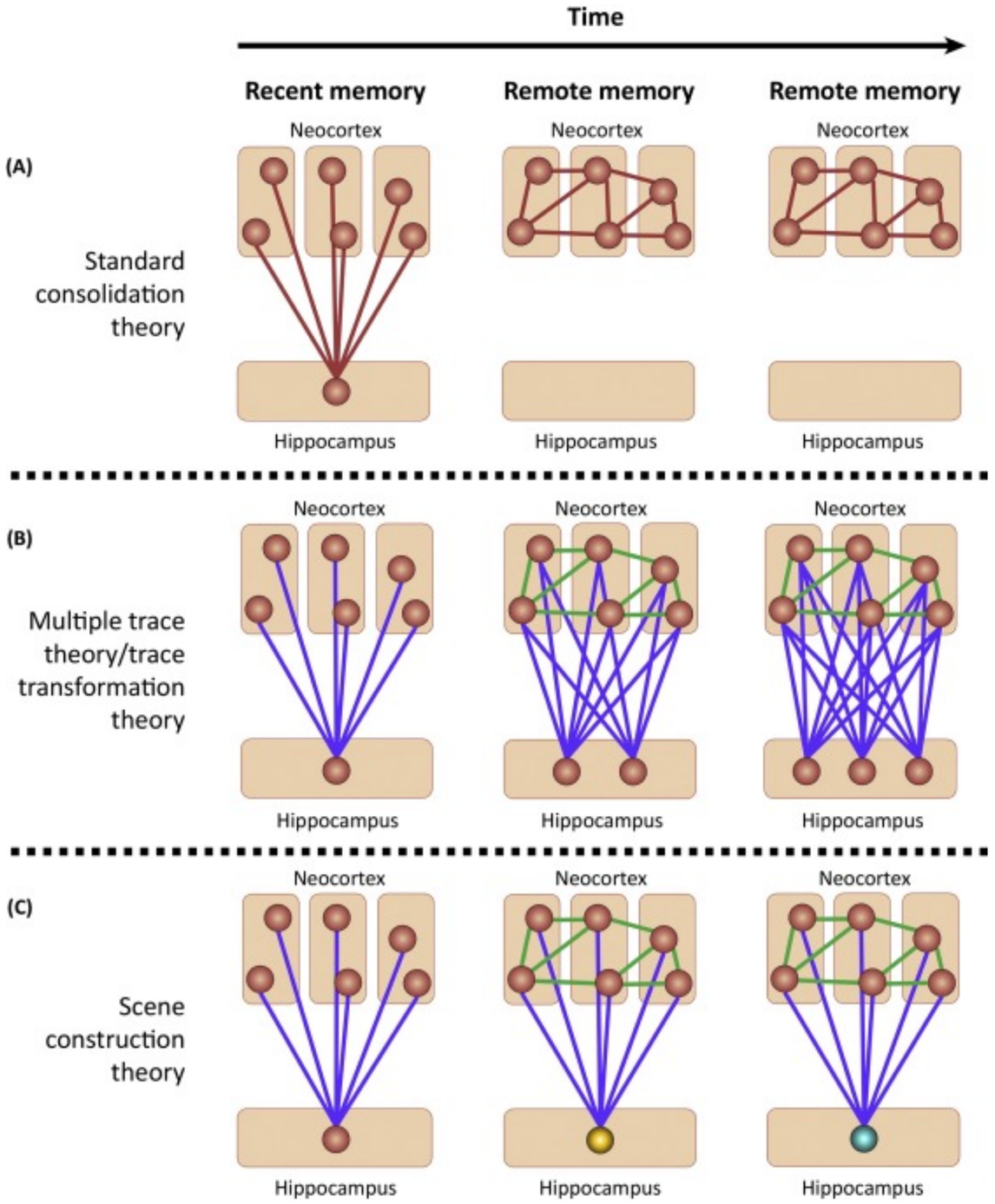


patient H.M.

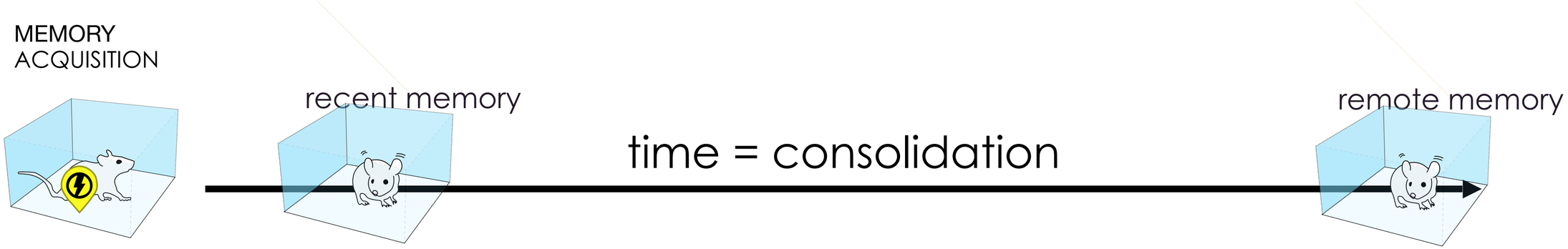




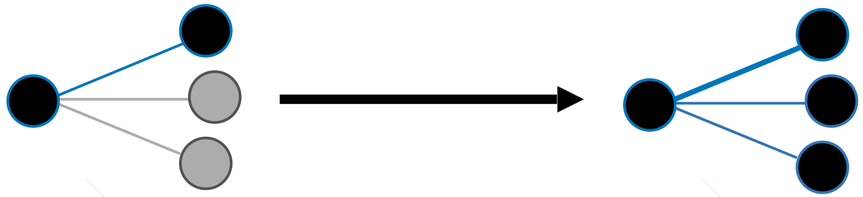



Time
Recent memory
Remote memory
Remote memory
(A)
Standard consolidation theory
Neocortex
Hippocampus
(B)
Multiple trace theory/trace transformation theory
(C)
Scene construction theory

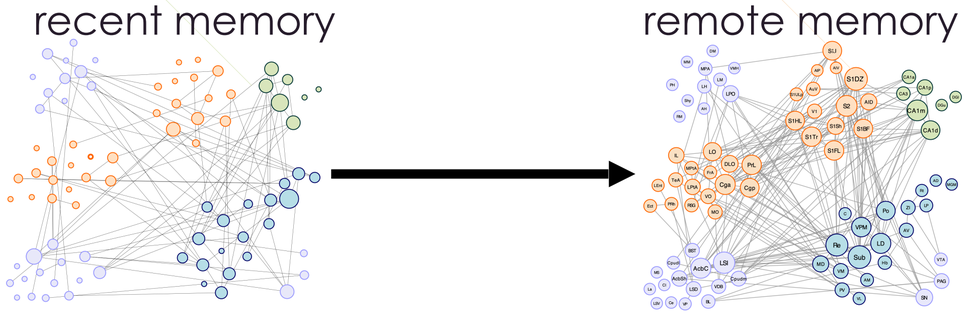
memory consolidation



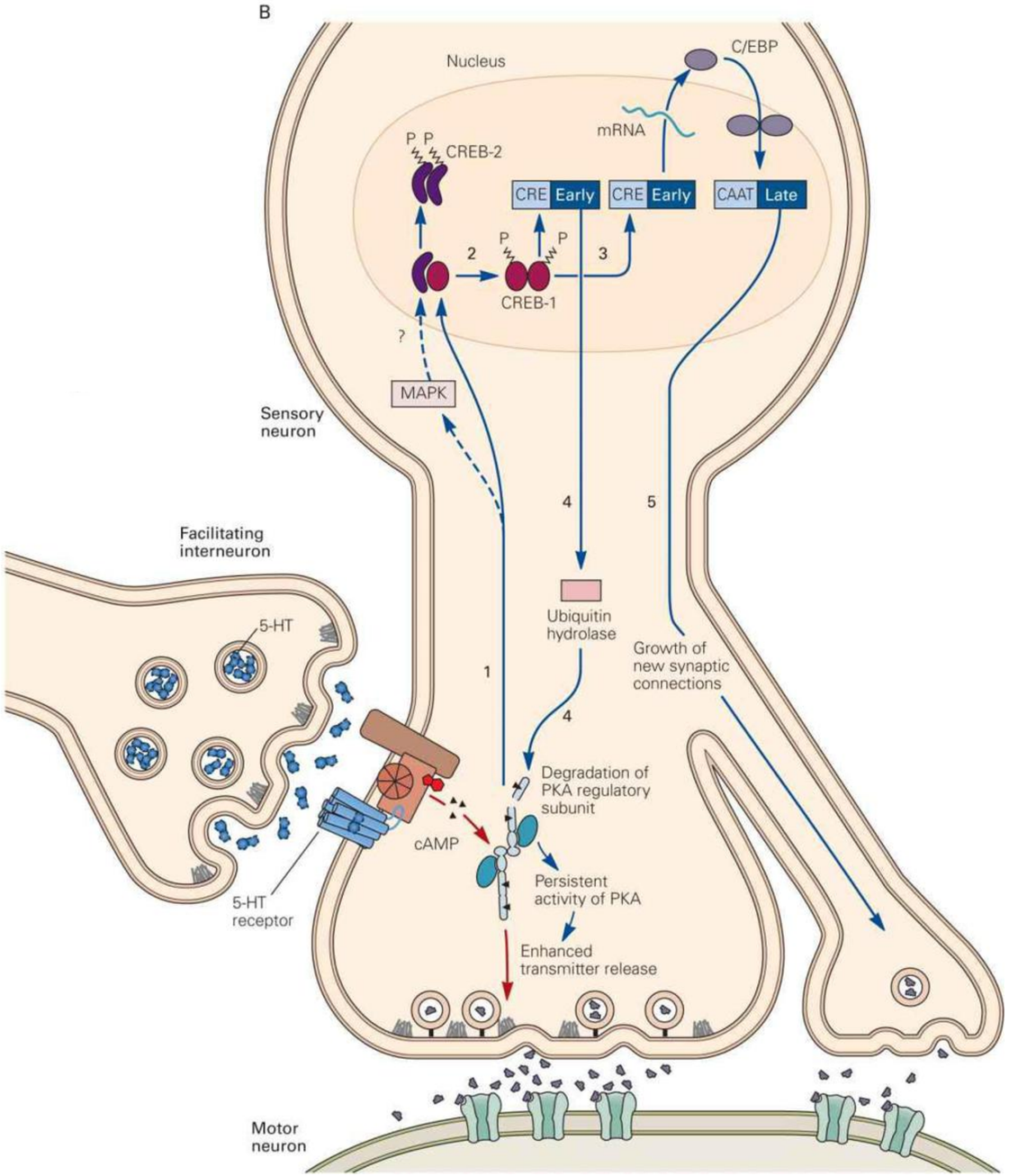
synaptic consolidation

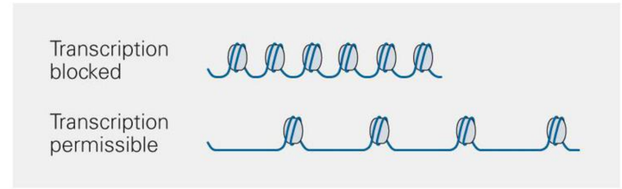


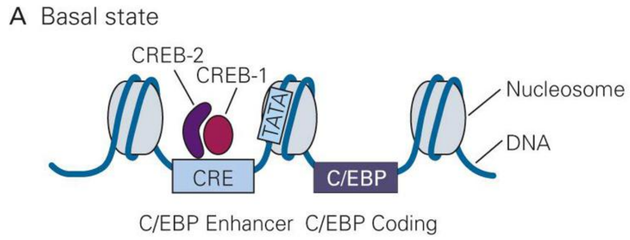
system consolidation

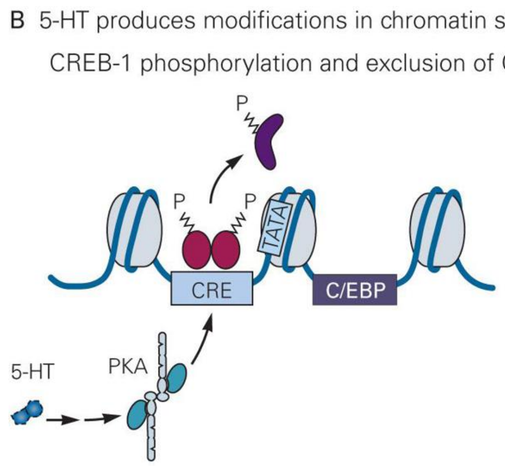


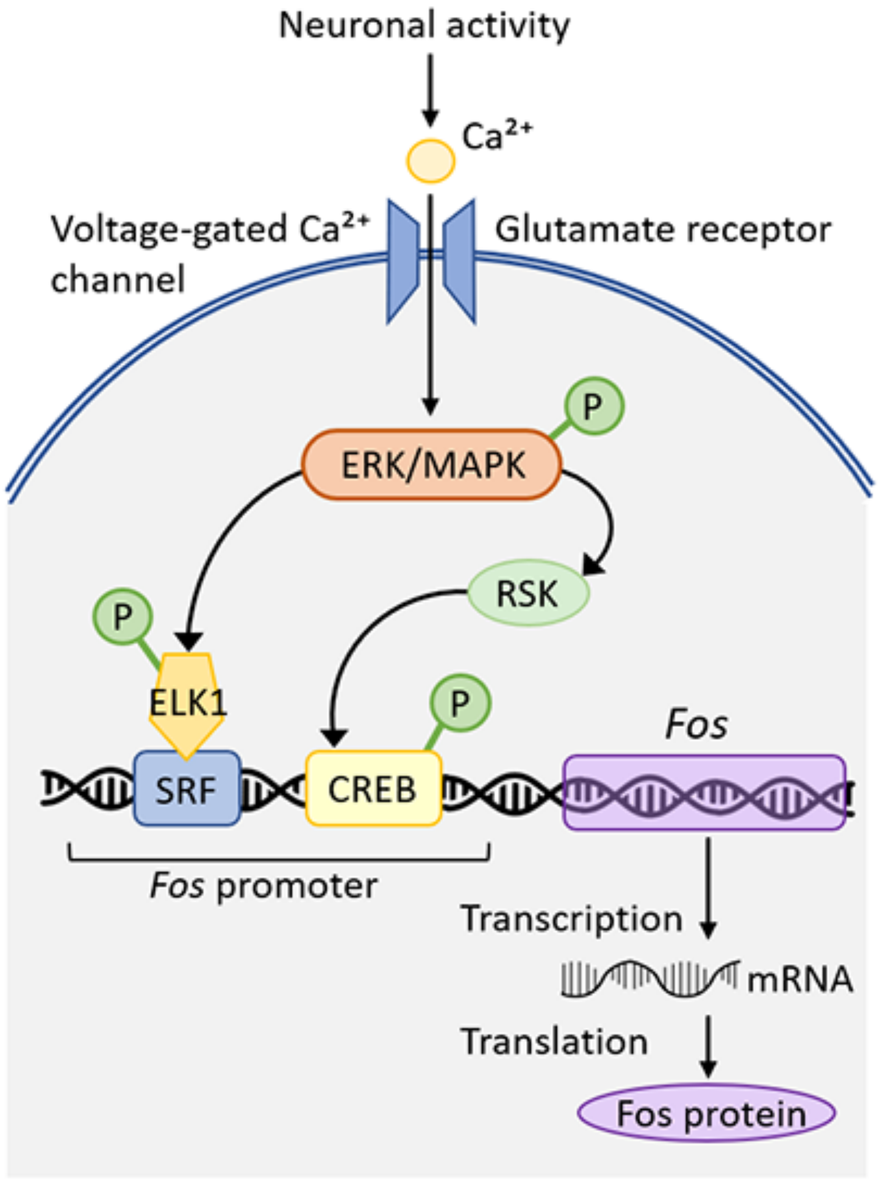
changes in memory quality


B
Nucleus
C/EBP
mRNA
P P CREB-2
CRE Early
CRE Early
CAAT Late
P P
2
3
CREB-1
?
MAPK
Sensory neuron
4
5
Facilitating interneuron
Ubiquitin hydrolase
Growth of new synaptic connections
5-HT
1
4
Degradation of PKA regulatory subunit
cAMP
Persistent activity of PKA
5-HT receptor
Enhanced transmitter release
Motor neuron


Transcription blocked
Transcription permissible


A Basal state
CREB-2
CREB-1
TATA
Nucleosome
DNA
CRE
C/EBP
C/EBP Enhancer C/EBP Coding

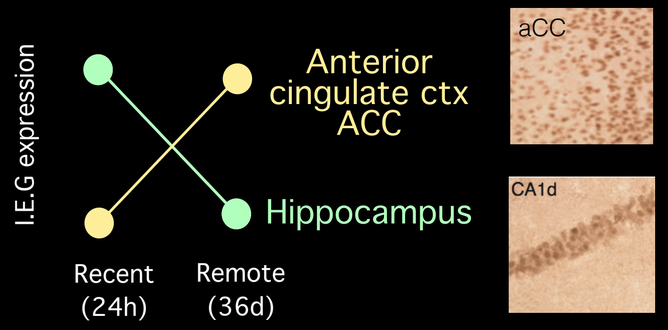

B 5-HT produces modifications in chromatin structure
CREB-1 phosphorylation and exclusion of CREB-2
P
P
P
TATA
CRE
C/EBP
5-HT
PKA


Neuronal activity
Ca2+
Voltage-gated Ca2+ channel
Glutamate receptor
P
ERK/MAPK
RSK
P
ELK1
P
Fos
SRF
CREB
Fos promoter
Transcription
mRNA
Translation
Fos protein

Specific regions have different roles in recent or remote memory recall

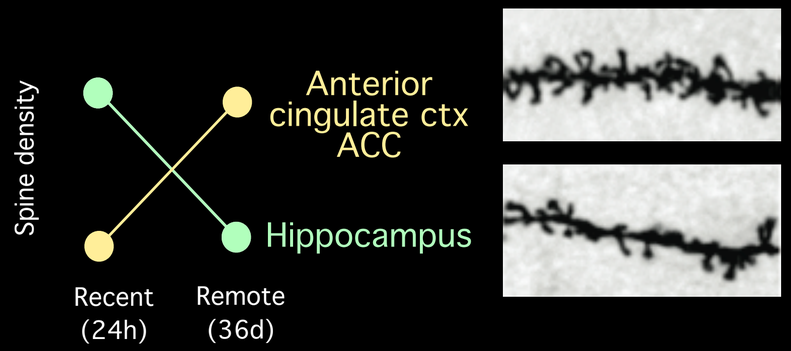
OBSERVATIONAL

neuronal activation



Frankland et al, Science, 2004

morphology of neurons



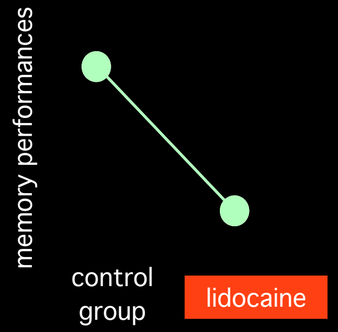
Restivo*, **Vetere*** et al, **JN**, 2009

CAUSALITY

neuronal activation

lidocaine

pharmacological inactivation **of ACC**

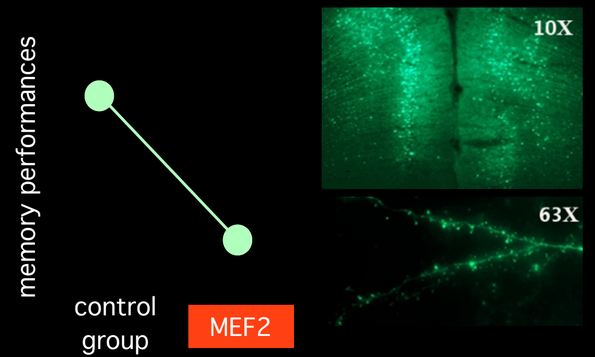


Frankland et al, Science, 2004

morphology of neurons

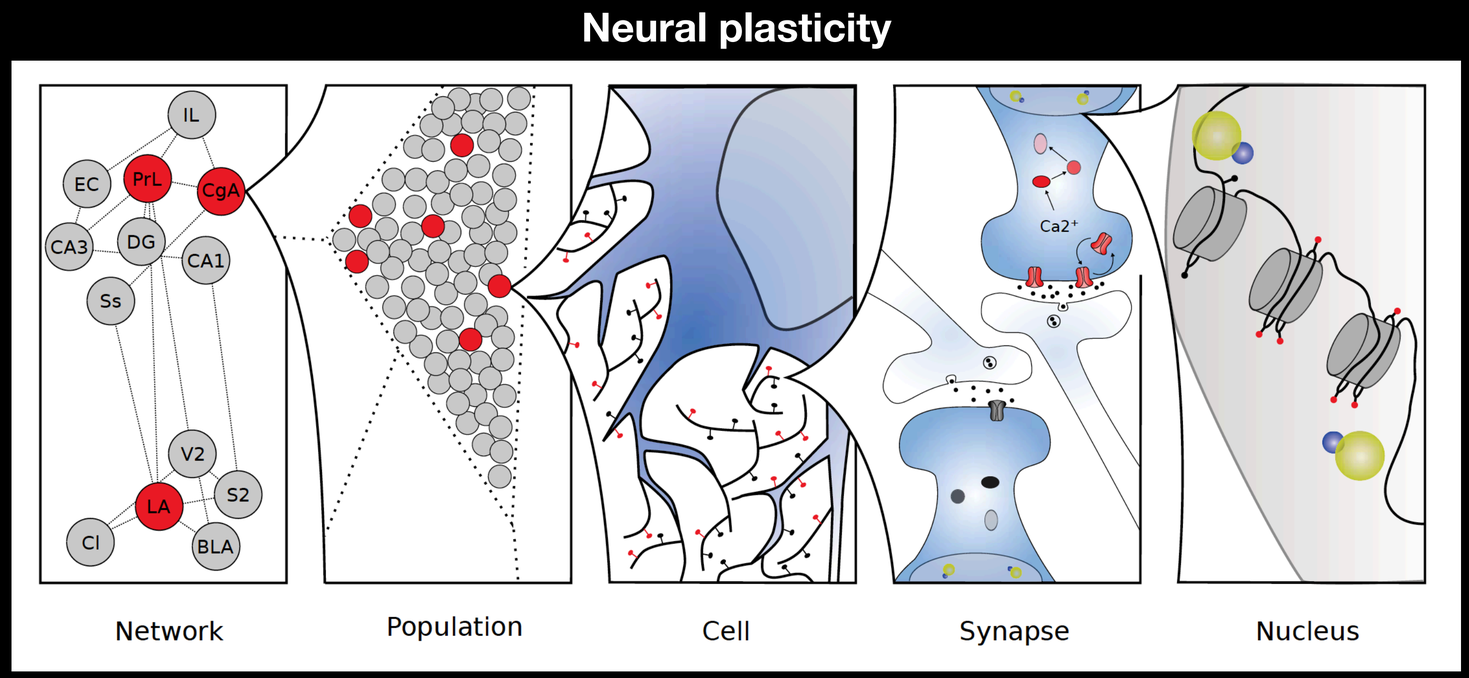
MEF2

prevent the formation of new spines **in ACC**



Vetere et al, **PNAS**, 2011

Enduring morphological changes induced by learning



Vetere, Neuron, 2017

Vetere, NN, 2021

Vetere, PNAS, 2011
Restivo*, Vetere*, JN, 2009
Vetere, Hippocampus, 2014
Vetere, LM, 2011

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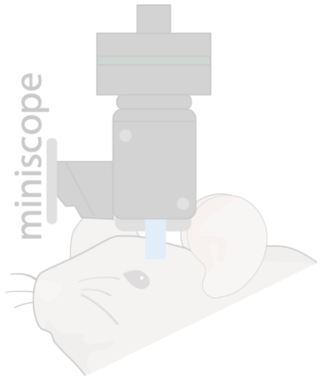
FEAR MEMORY

focus on the brain



optogenetics

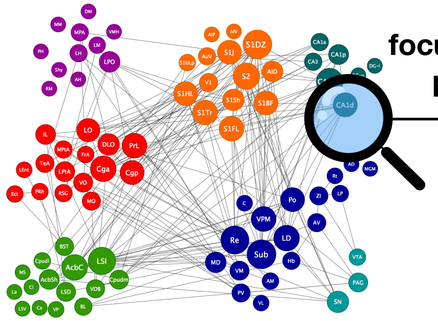
miniscope



freezing activated neurons



focus on single neurons activity



focus on neuronal populations

NETWORK of brain regions

ENGRAM neurons

single cells

Studying memory consolidation: a network approach



84 different regions

Sensory ctx: FrA, DLO, LO, VO, MO, IL, PrL, Cg-a, Cg-p, RSG, Ect, LEnt, LPtA, MPtA, PRh, TeA, AuV, V1, AIP, AID, AIV, S1BF

Association ctx: S1DZ, S1FL, S1HL, S1J, S1Sh, S1Tr, S1ULp, S2

Thalamus: AD, AM, AV, C, Hb, LD, LP, MD, Po, PV, Re, Sub, VL, VM, VPM, Rt, ZI, MGM, AH, PH, DM, VMH

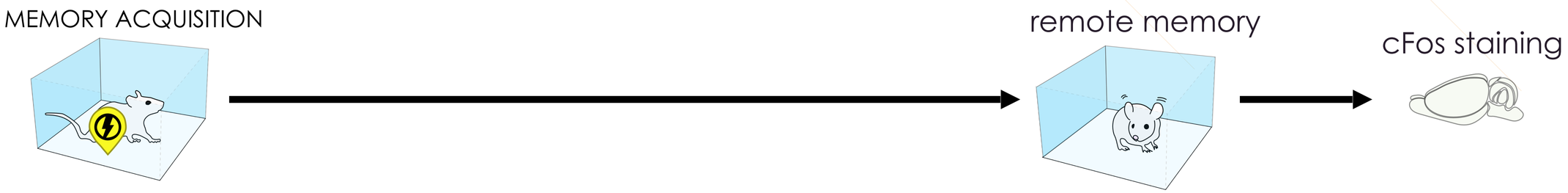
Hypothalamus: LH, LPO, MPA, SHy, LM, RM, MM

Cerebral nuclei: BST, Cpu-dl, Cpu-dm, AcbC, AcbSh, VP, VDB, CI, La, Ce, BL, MS, LSD, LSI, LSV

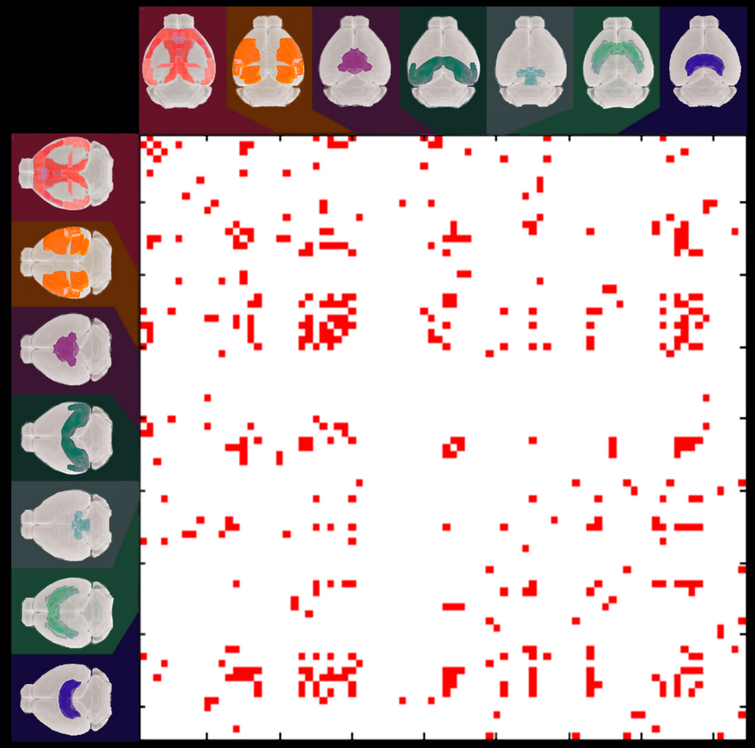
Hippocampus: CA1 -anterior, CA1 -medial, CA1 -posterior, CA3, DG-up, DG-low

Midbrain: VTA, PAG, SNC

Studying memory consolidation: a network approach

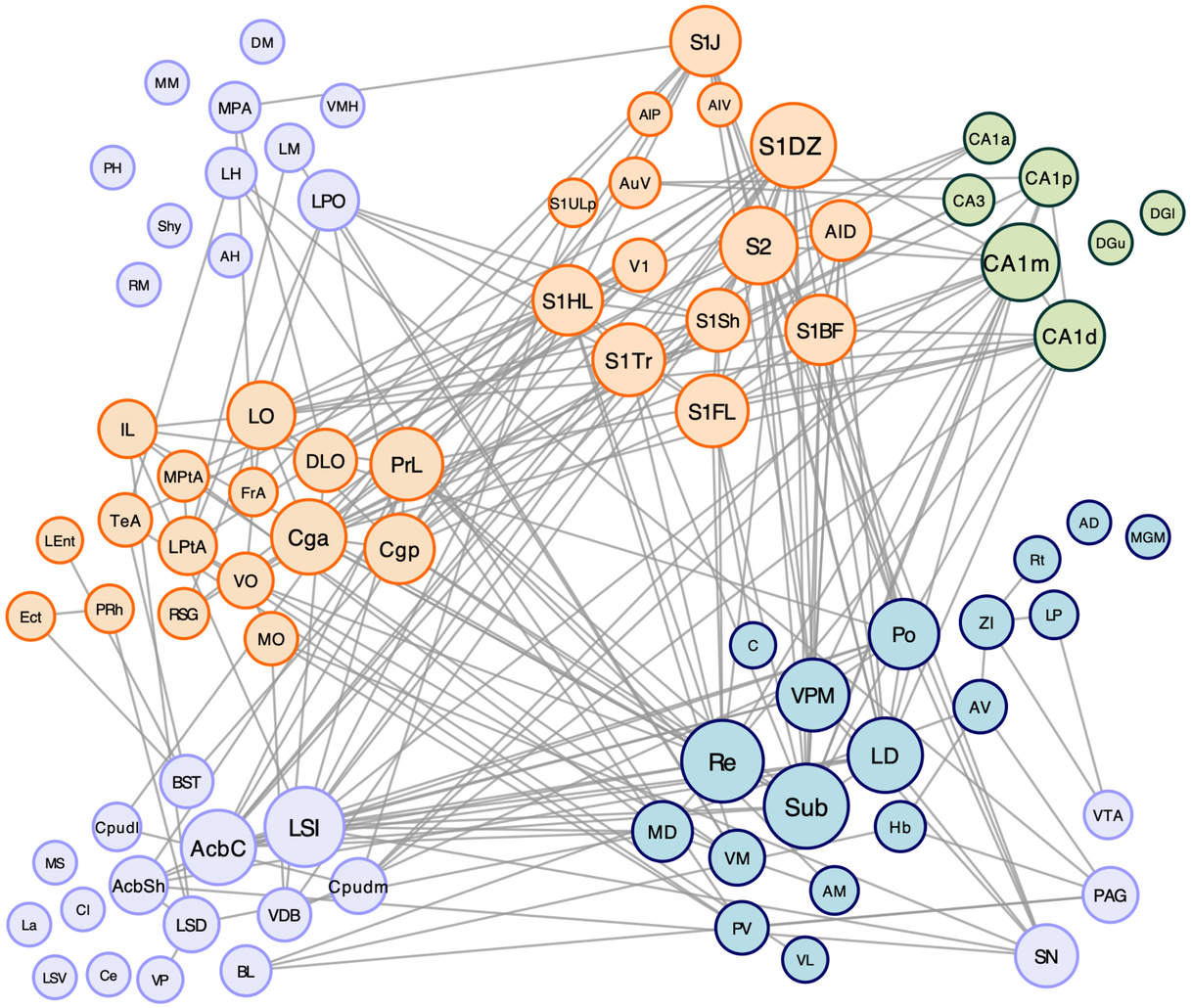


Wheeler et al., 2013, PLOS Computational Biology

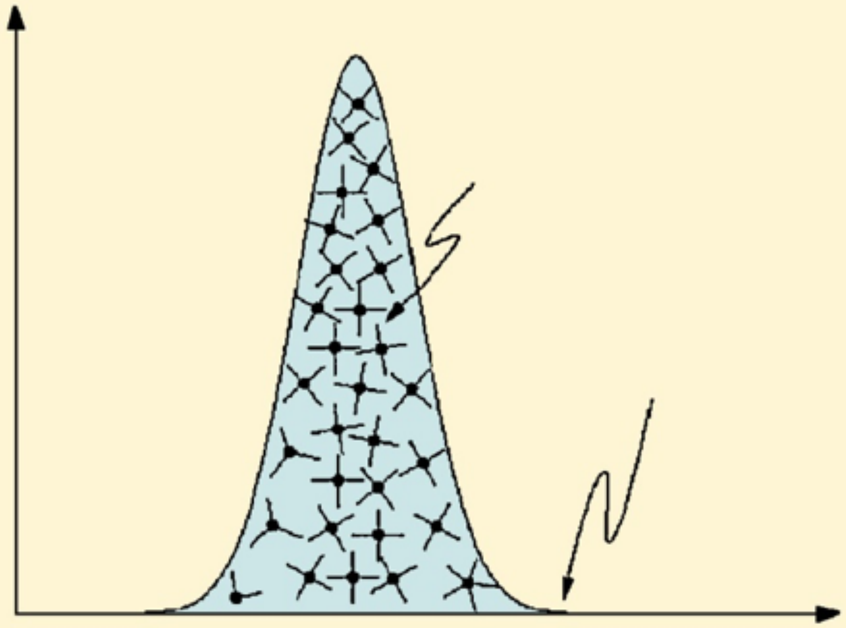


A MEMORY NETWORK

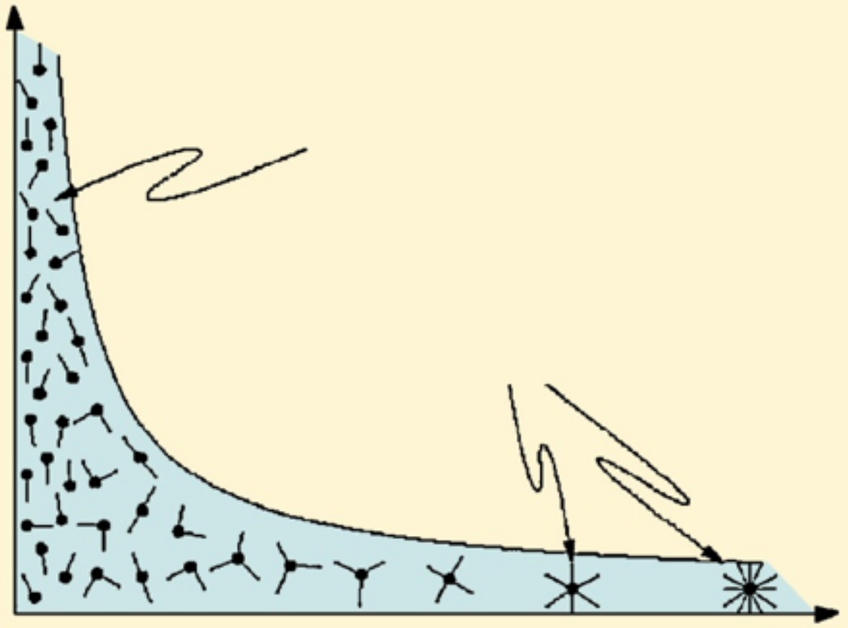
<u>functionally connected regions</u>=
c-Fos expression
co-varies across mice



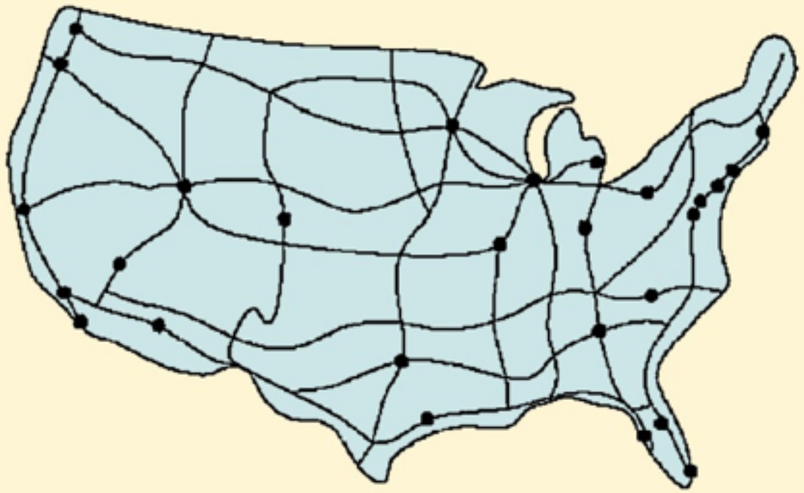
Wheeler et al., 2013, PLOS Computational Biology

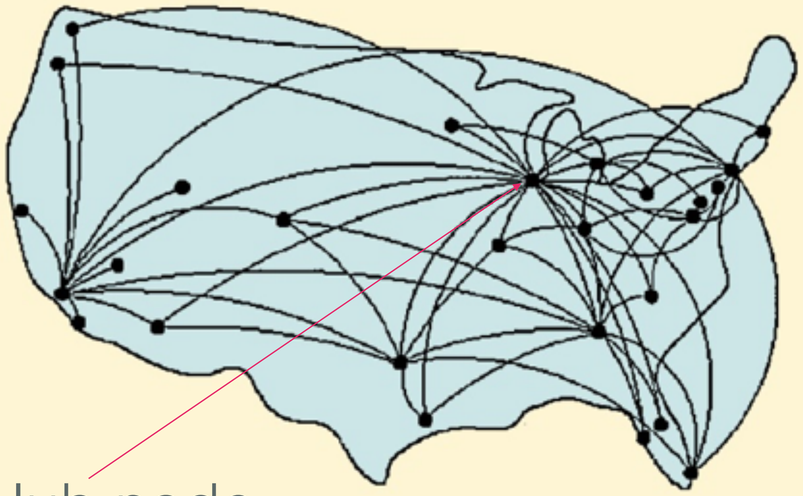


Highway network



air traffic network





Hub node

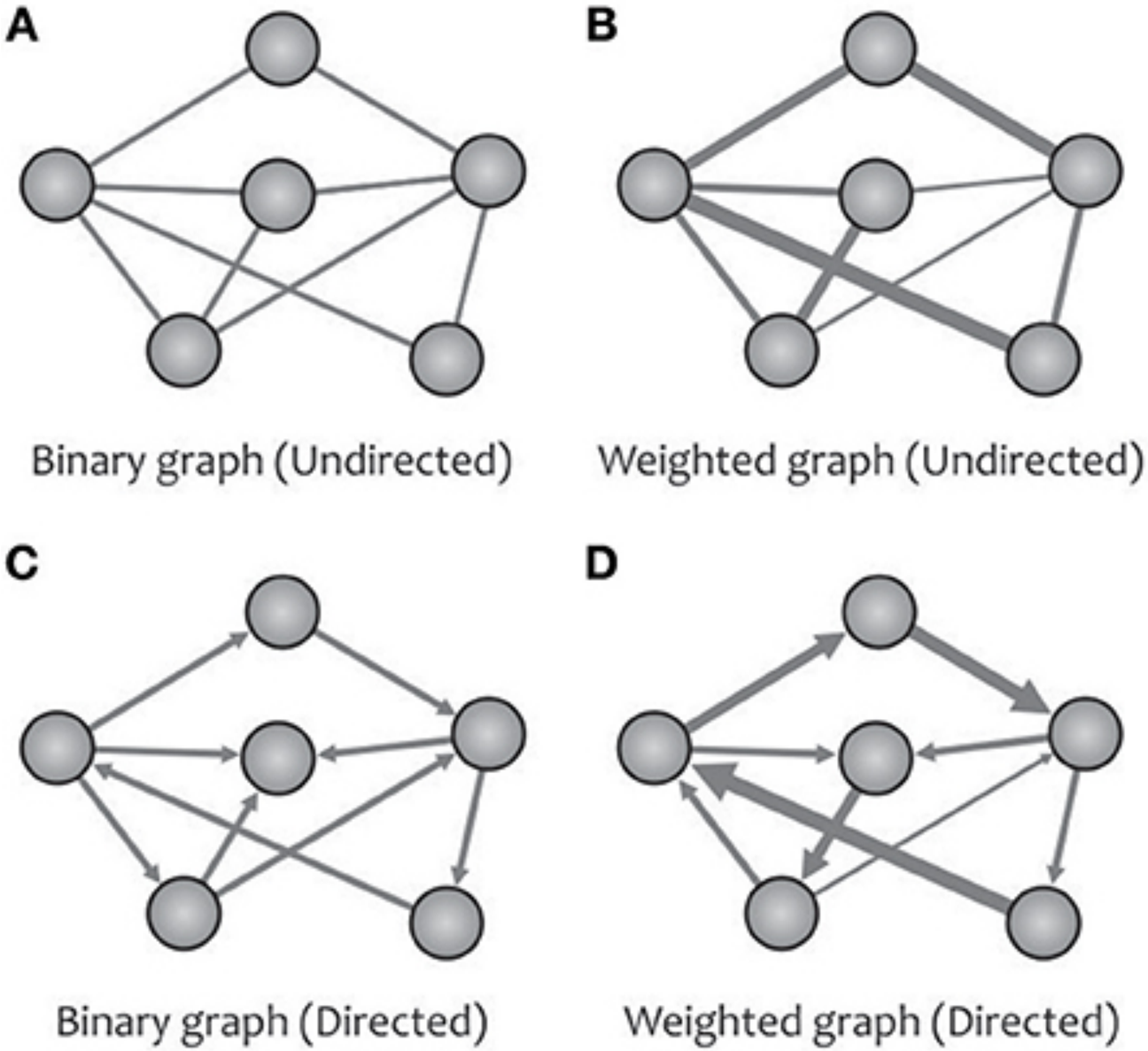
the network

In mathematics, **graph theory** is the study of *graphs*, which are mathematical structures used to model pairwise relations between objects.

A graph in this context is made up of *vertices* (also called *nodes* or *points*) which are connected by *edges* (also called *links* or *lines*). A distinction is made between **undirected graphs**, where edges link two vertices symmetrically, and **directed graphs**, where edges link two vertices asymmetrically.



Visualization of social network analysis


A
B
Binary graph (Undirected)
Weighted graph (Undirected)
C
D
Binary graph (Directed)
Weighted graph (Directed)

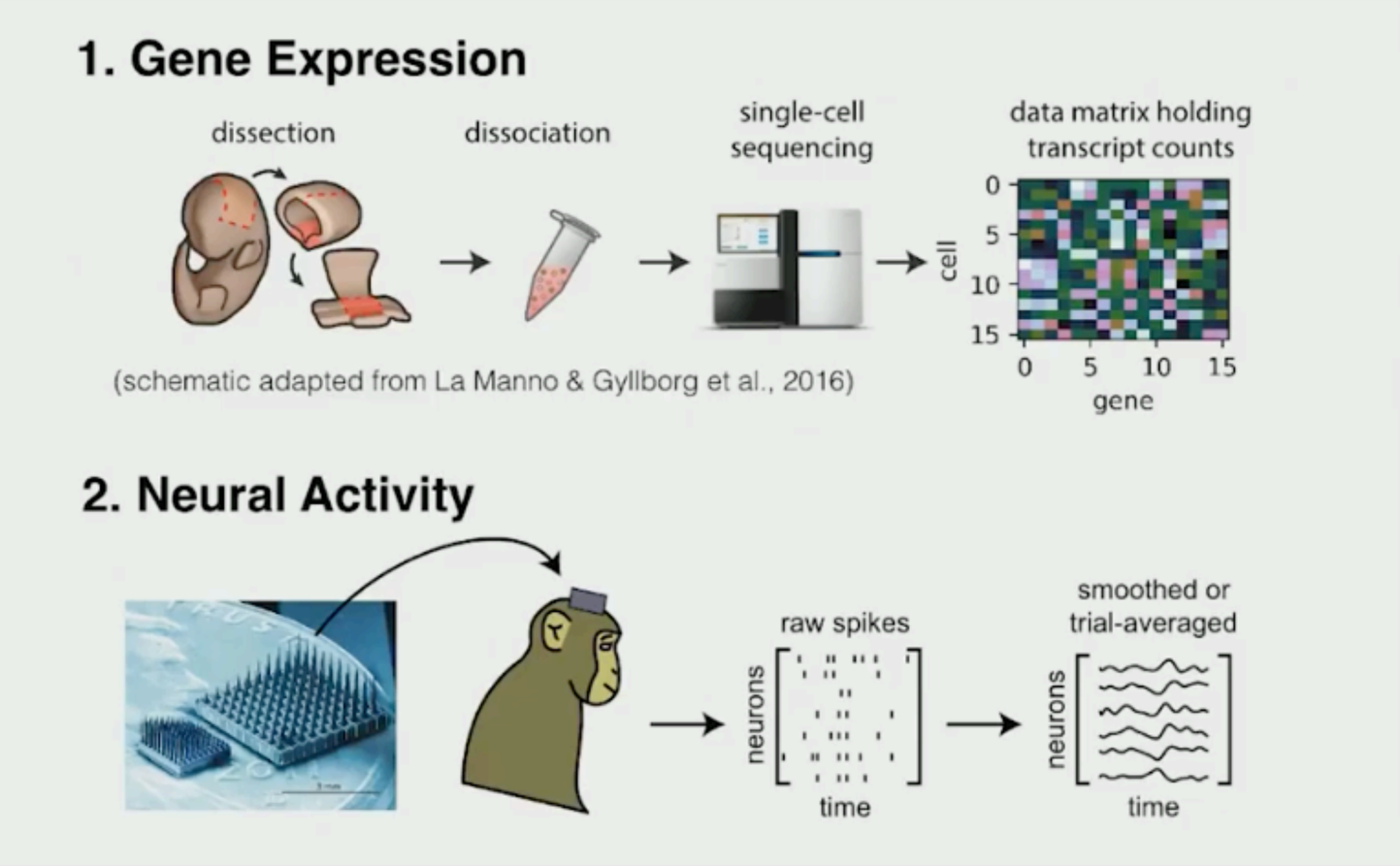
Applications

Graphs can be used to model many types of relations and processes in physical, biological, social and information systems.

Many practical problems can be represented by graphs.

In the field of **microbiology**, graph can express the molecular structure, where cell, gene or protein can be denoted as a nodes, and the connect element can be regarded as an edge. In this way, the biological activity characteristic can be measured via topological index computing in the corresponding graphs

Large-scale data analysis via matrix



Biology

Graph theory is useful in biology and conservation efforts where a node can represent regions where certain species exist (or inhabit) and the edges represent migration paths or movement between the regions. This information is important when looking at breeding patterns or tracking the spread of disease, parasites or how changes to the movement can affect other species.

Graphs are also commonly used in molecular biology and genomics to model and analyze datasets with complex relationships. For example, graph-based methods are often used to ‘cluster' cells together into cell-types in single-cell transcriptome analysis. Another use is to model genes or proteins in a pathway and study the relationships between them, such as metabolic pathways and gene regulatory networks. Evolutionary trees, ecological networks, and hierarchical clustering of gene expression patterns are also represented as graph structures.

Graph theory is also used in connectomics; nervous systems can be seen as a graph, where the nodes are neurons or regions and the edges are the connections between them.

Network Neuroscience

High-throughput molecular screening

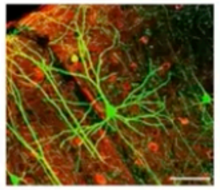


statistical associations

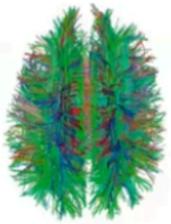


molecular binding

High-resolution anatomical mapping

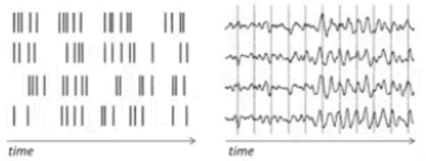


synaptic connections



projections

High-density functional recording

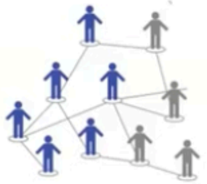


statistical dependence
causal efficacy
information flow

High-volume behavioral tracking



individual: sensorimotor coupling



collective: social interactions

Network construction

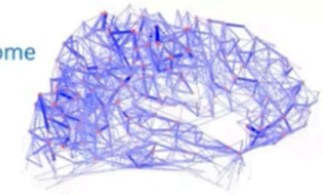
Statistical inference, normalization, quality control, reduction of measurement bias and noise, artifact detection/elimination

transcriptome

interactome



connectome



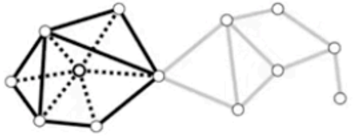


functional/effective connectivity



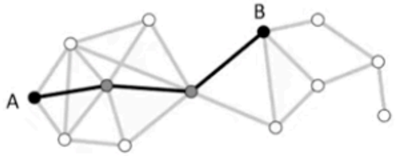
social network

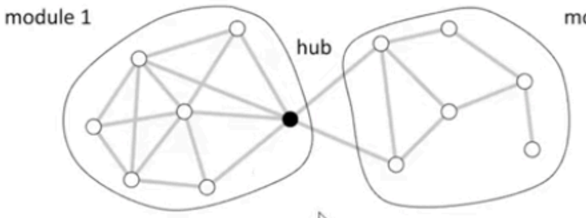
Network analysis



local topology (degree, clustering)

paths, distances, communication





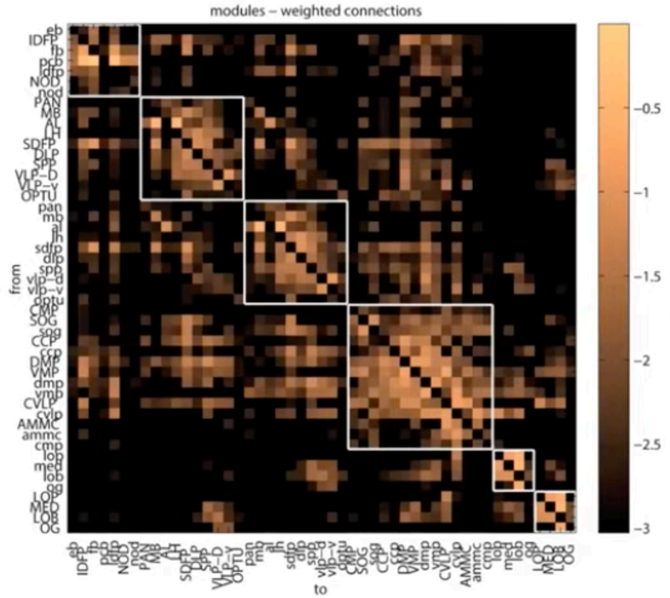
module detection
centrality

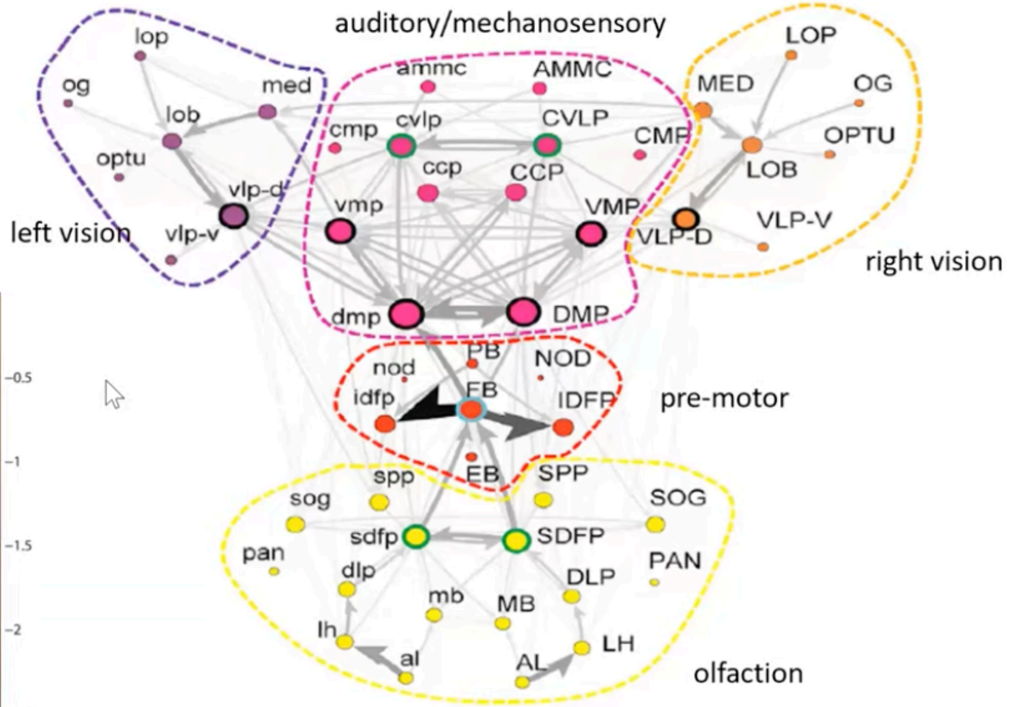
Data sharing and deposition in bio/neuroinformatics data banks, visualization, computer simulation and integrative modeling, experimental validation, refinement of measurement and analysis techniques

Mesoscale Connectome of *Drosophila*

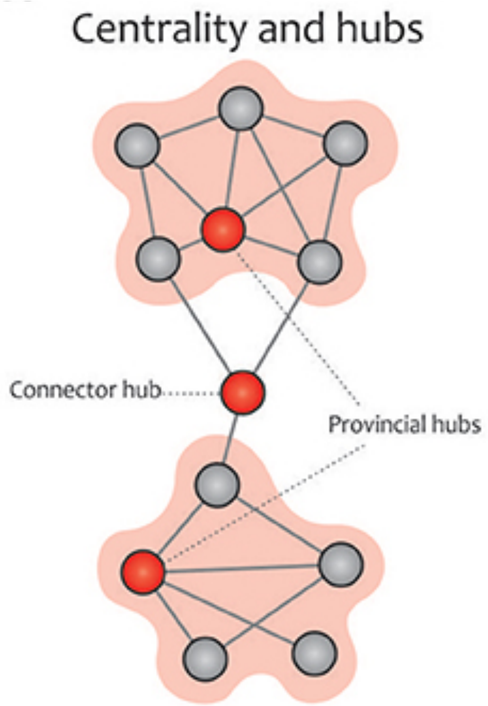
Modular organization of the Drosophila connectome – structural modules relate to different domains of behavioral function



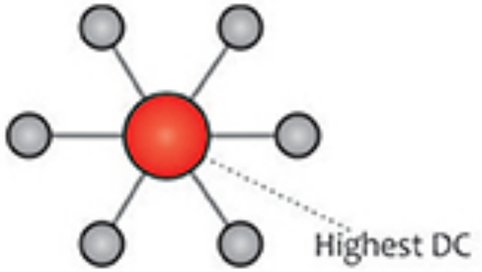




Shi, Sporns et al. (2015) *Current Biol* 25, 1249

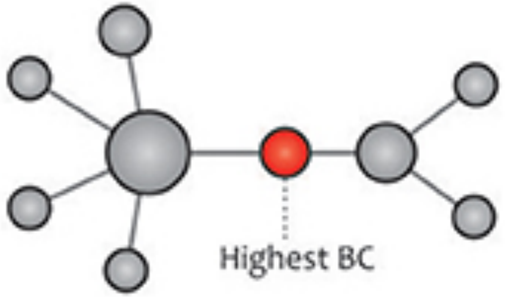


Degree centrality



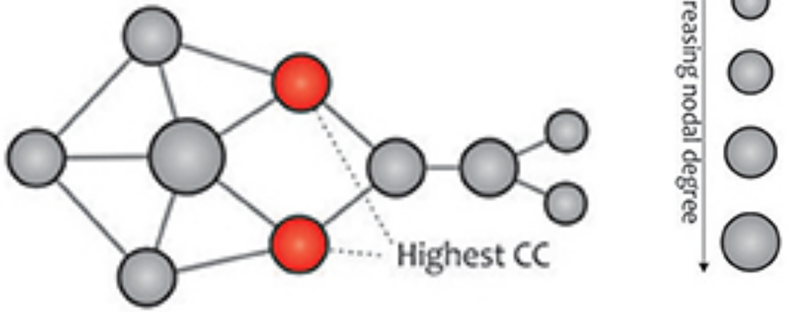
the number of links incident upon a node

Betweenness centrality



quantifies the number of times a node acts as a bridge along the shortest path between two other nodes.

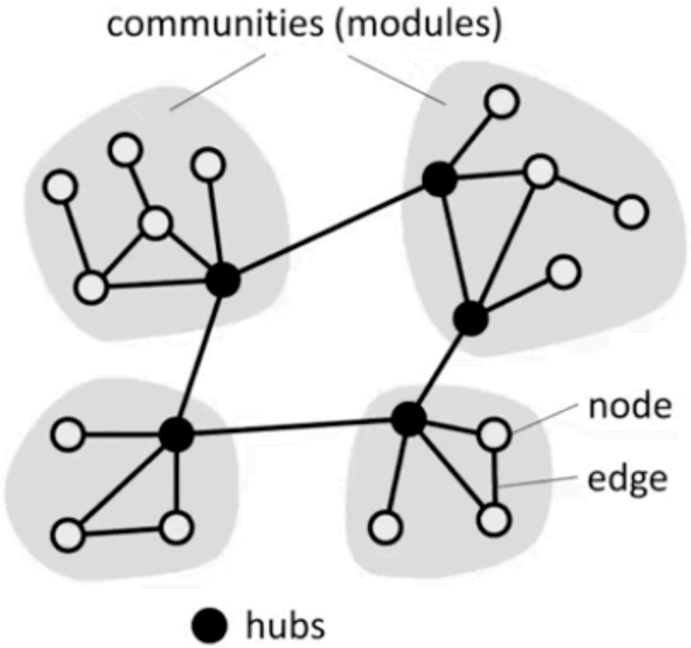
Closeness centrality

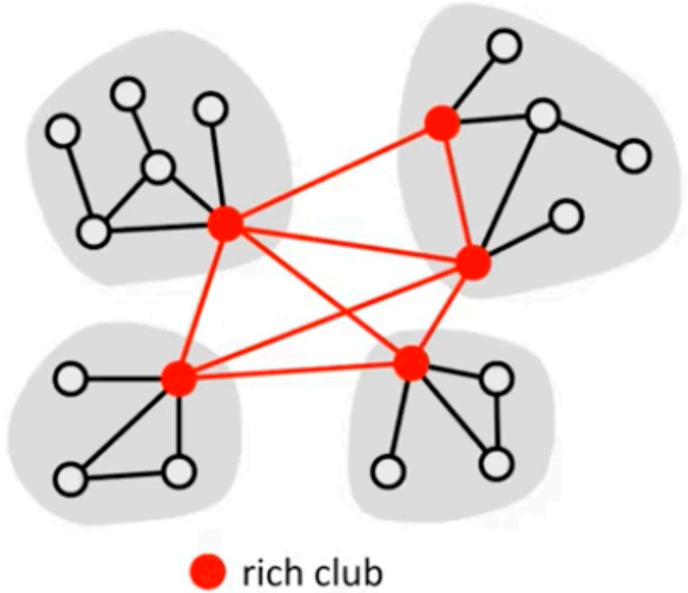


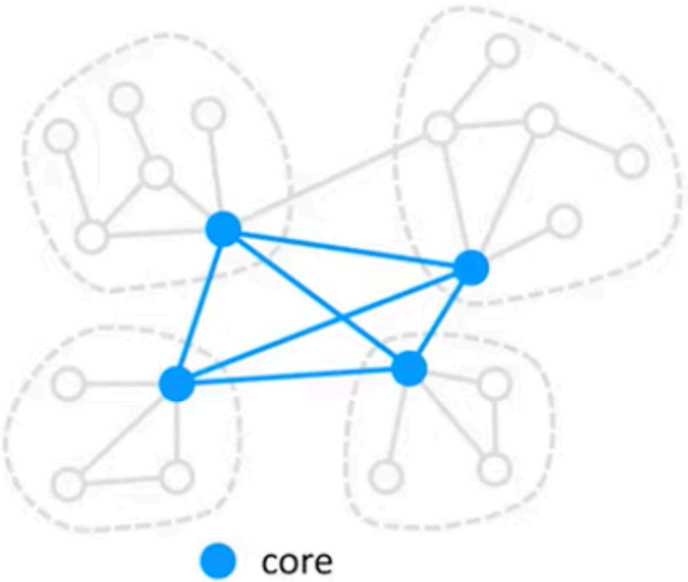
of a node is the average length of the shortest path between the node and all other nodes in the graph.

Modules, Cores and Rich Clubs

In some networks, highly connected/central hub nodes have a tendency to be highly connected to each other ("rich-club" organization).







Colizza et al. (2006) *Nature Physics* 2, 110.

Bullmore & Sporns (2012) *Nature Rev. Neurosci.* 13, 336.

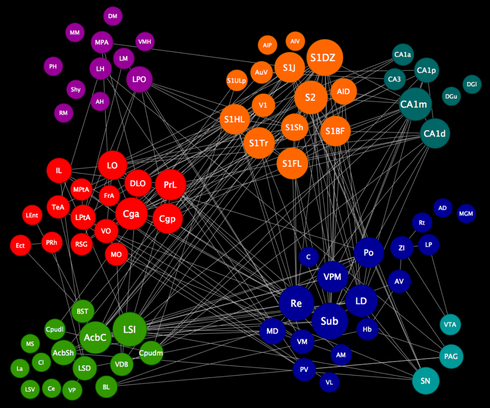
The most important goals of brain network analyses are to:

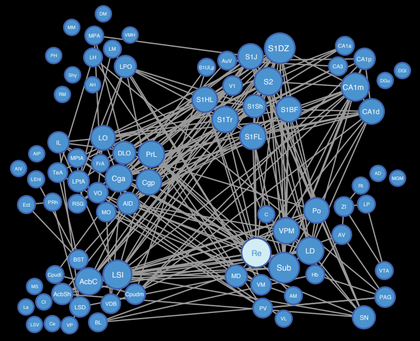
(a) **detect** pivotal regions and connections that contribute to disproportionate communication flow,

(b) **integrate** global information, and

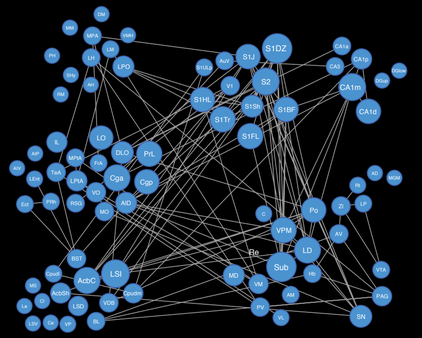
(c) **increase** the brain network efficiency.

deletion of network nodes: *in silico* and *in vivo*





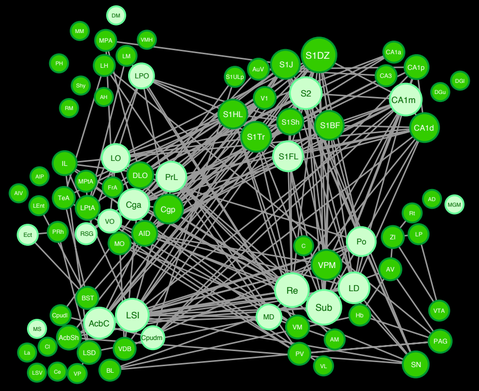
in silico

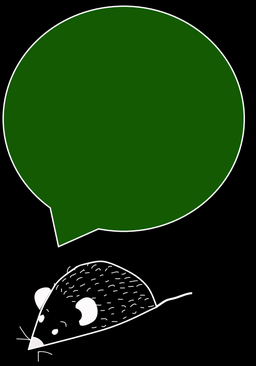


disruption propagation model

effect on Network Global efficacy

in vivo



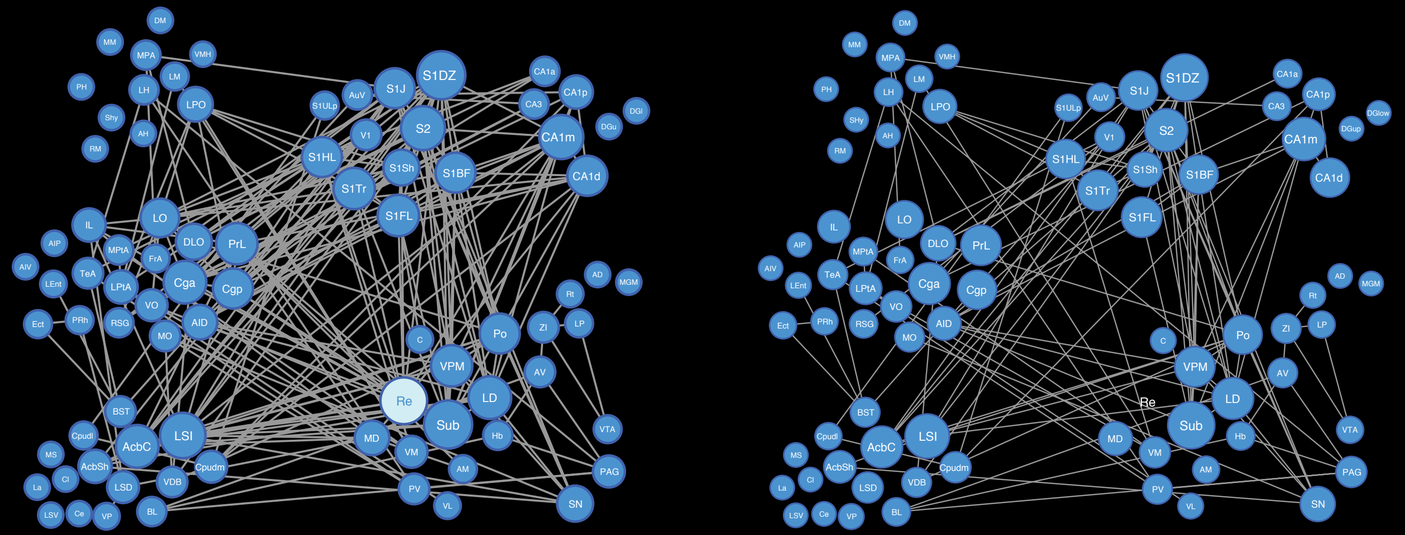


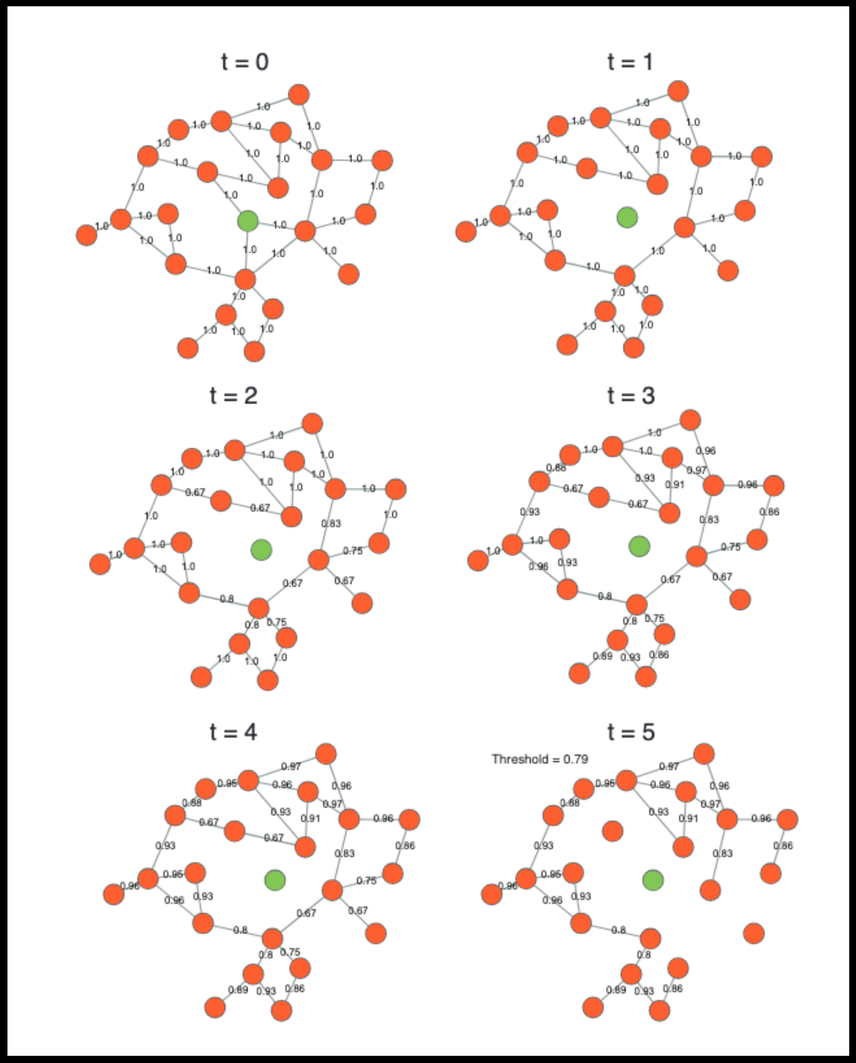
chemogenetic inhibition of nodes

effect on memory: freezing levels

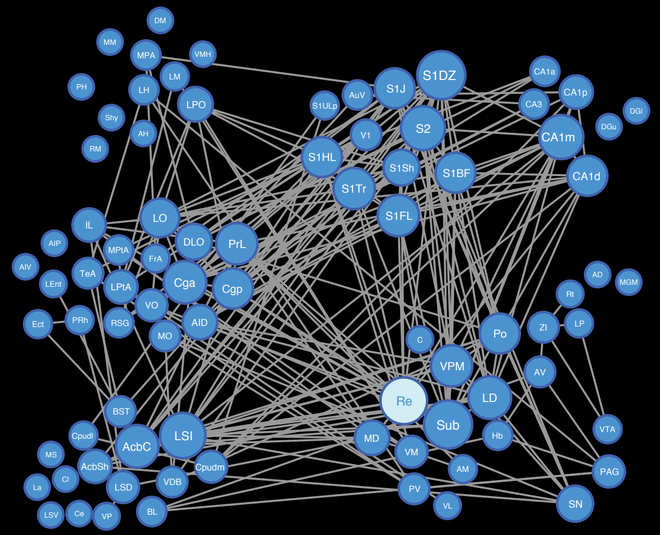
Vetere, 2017 *Neuron*

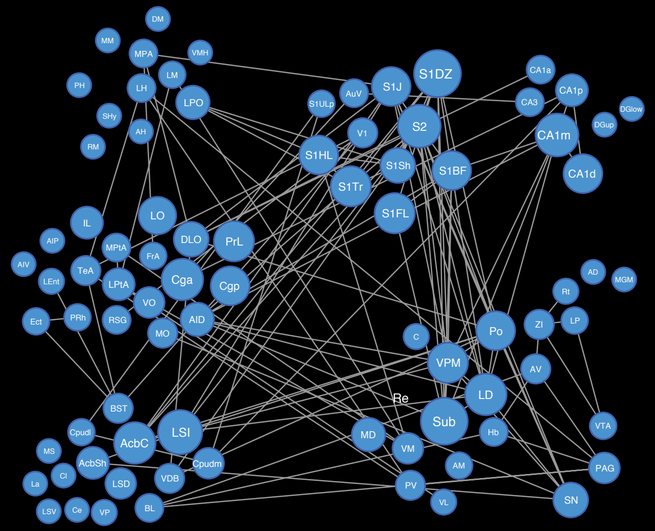
In silico: silencing of network nodes

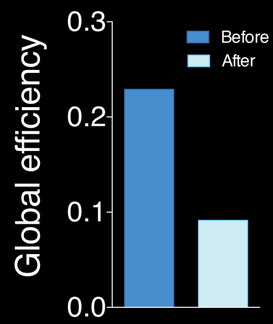




In silico: silencing of network nodes



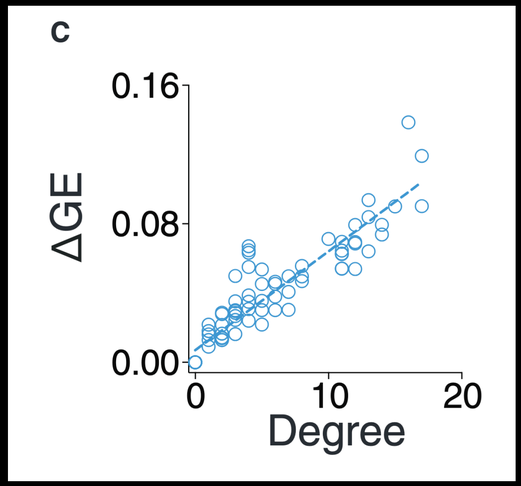




Global efficiency:

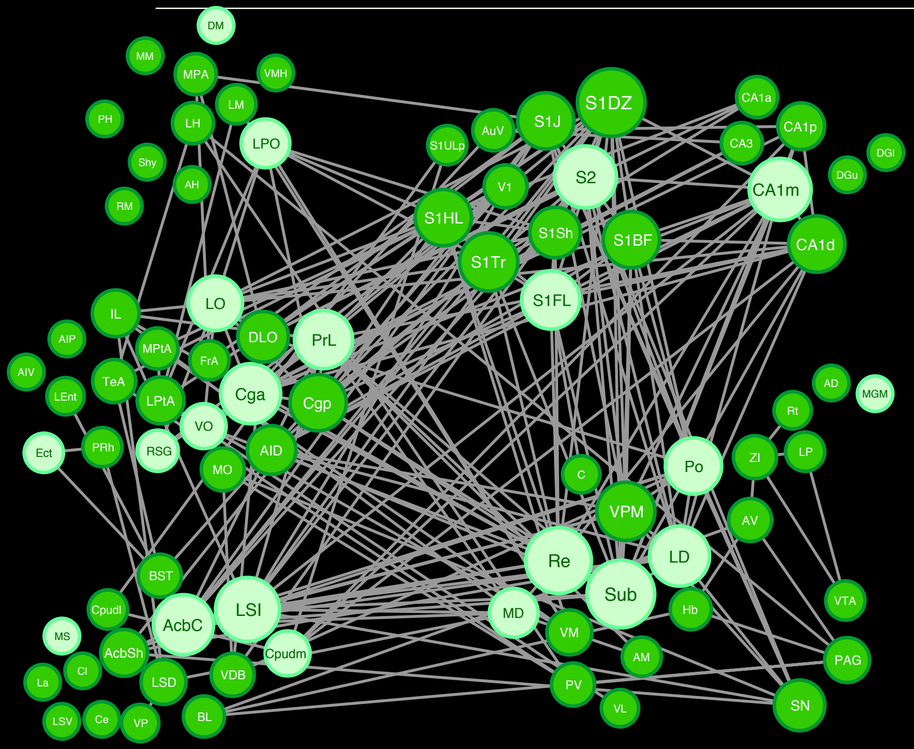
quantifies the exchange of information across the whole network where information is concurrently exchanged

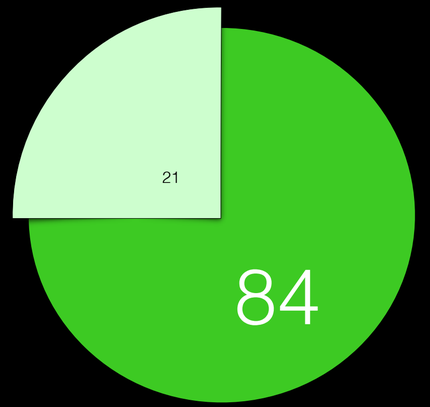
Calculated as the average of the efficiencies over all pairs of vertices

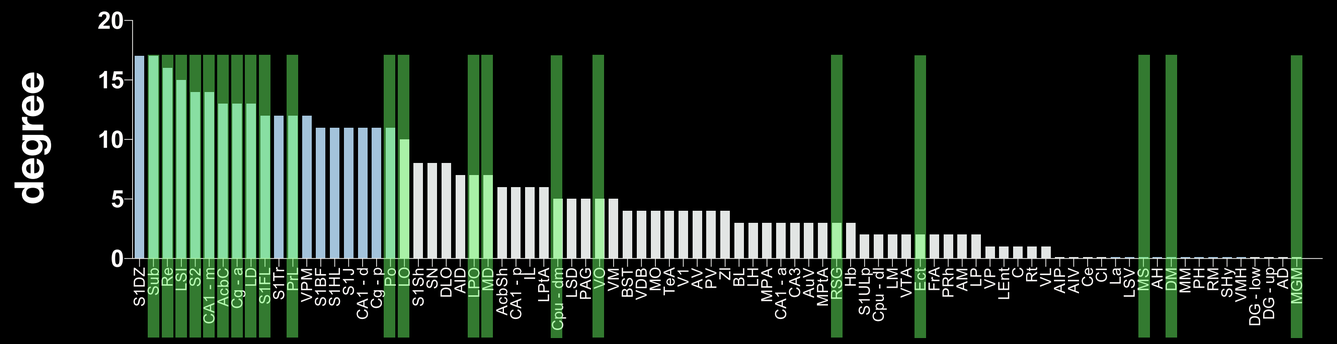


the efficiency of a network is a measure of how efficiently it exchanges information

In vivo: chemogenetic inhibition of nodes

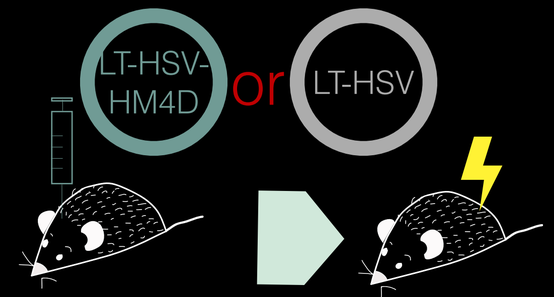






verify the reliability of brain networks to predict regions involved in memory consolidation

Inactivate selected regions during consolidation
CHEMOGENETIC APPROACH



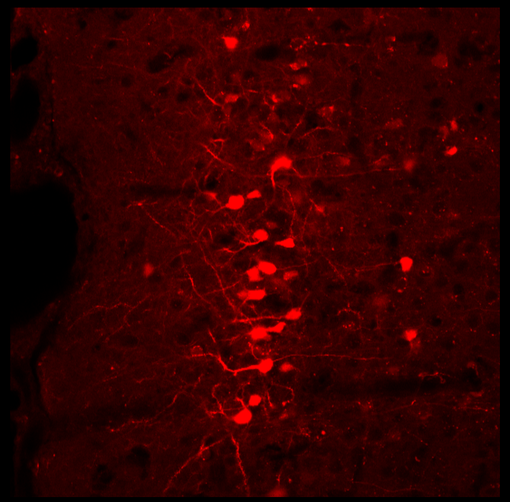
VIRUS INFECTION

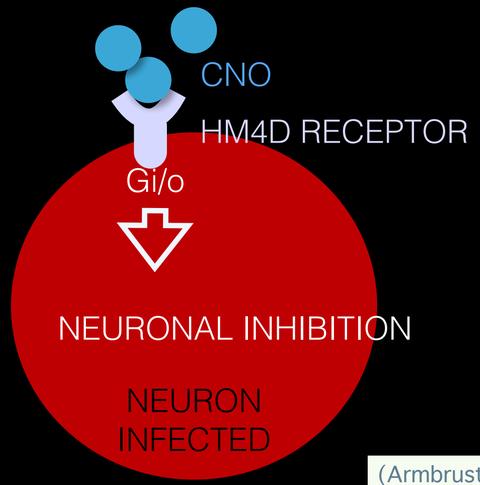
CFC

CNO (5mg/Kg)/vehicle

CONSOLIDATION

TEST

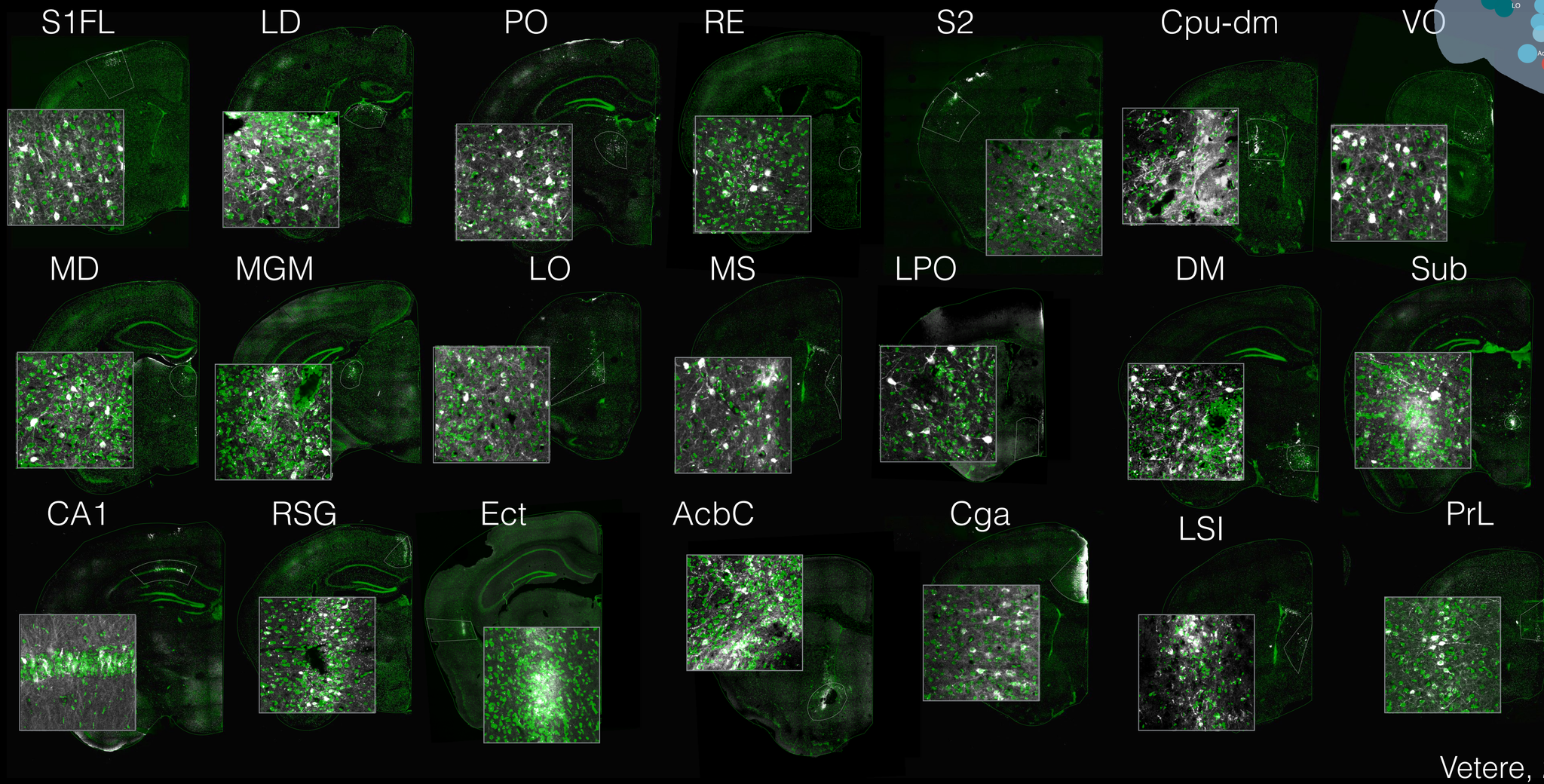




(Armbruster et al, 2007)

Inhibition of selected region of the network using CHEMOGENETICS



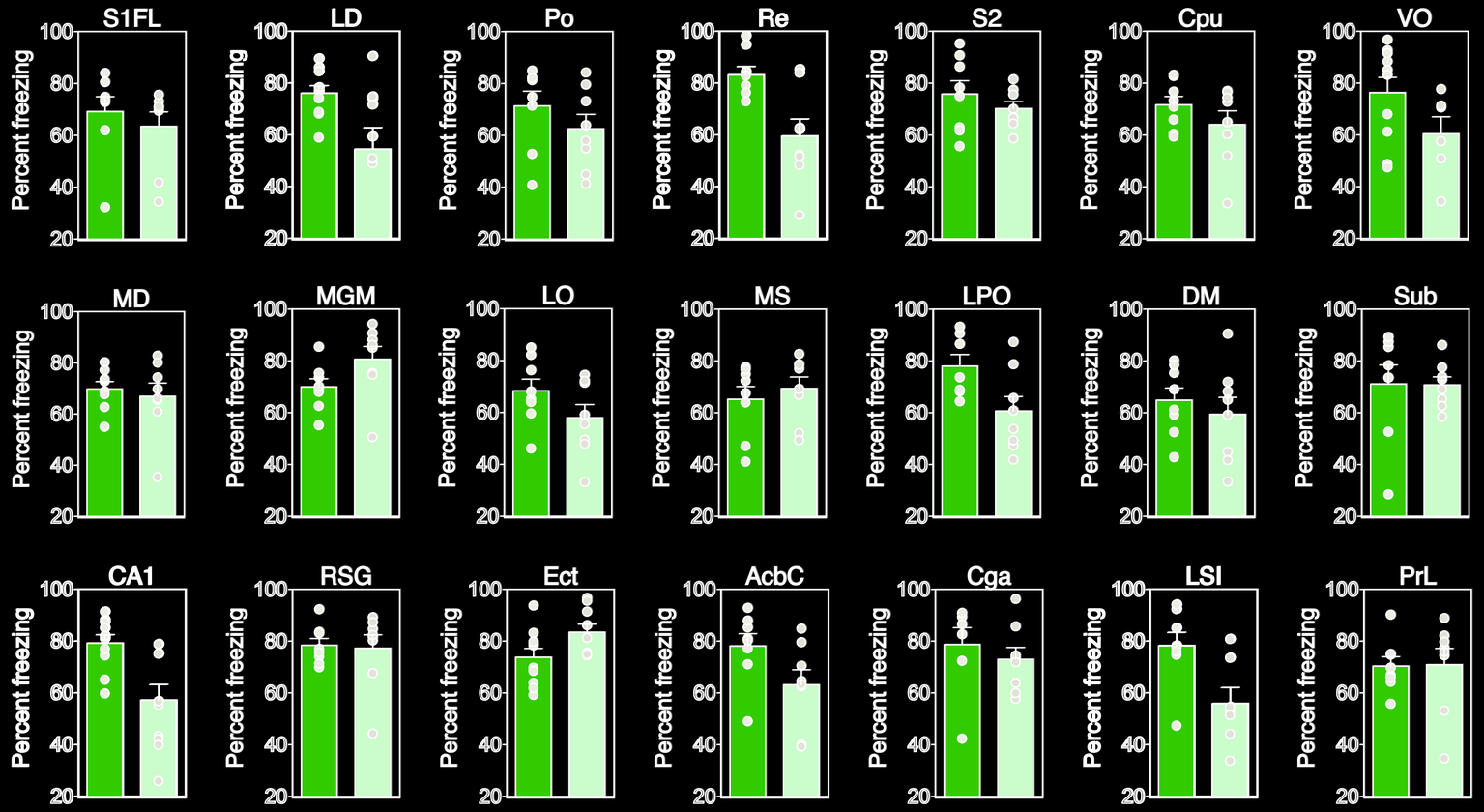


Vetere, 2017 *Neuron*

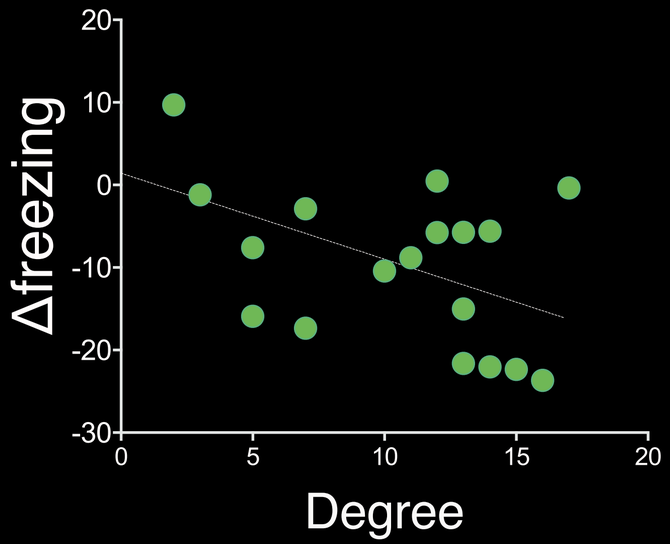
validating the network approach

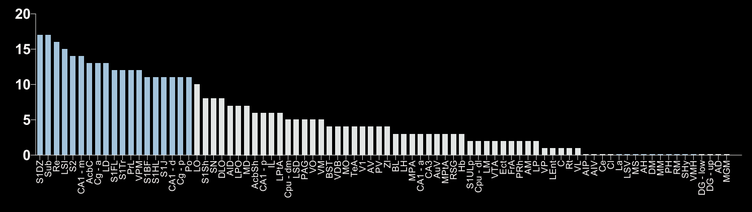
vehicle

CNO





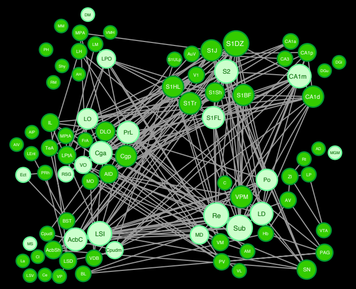


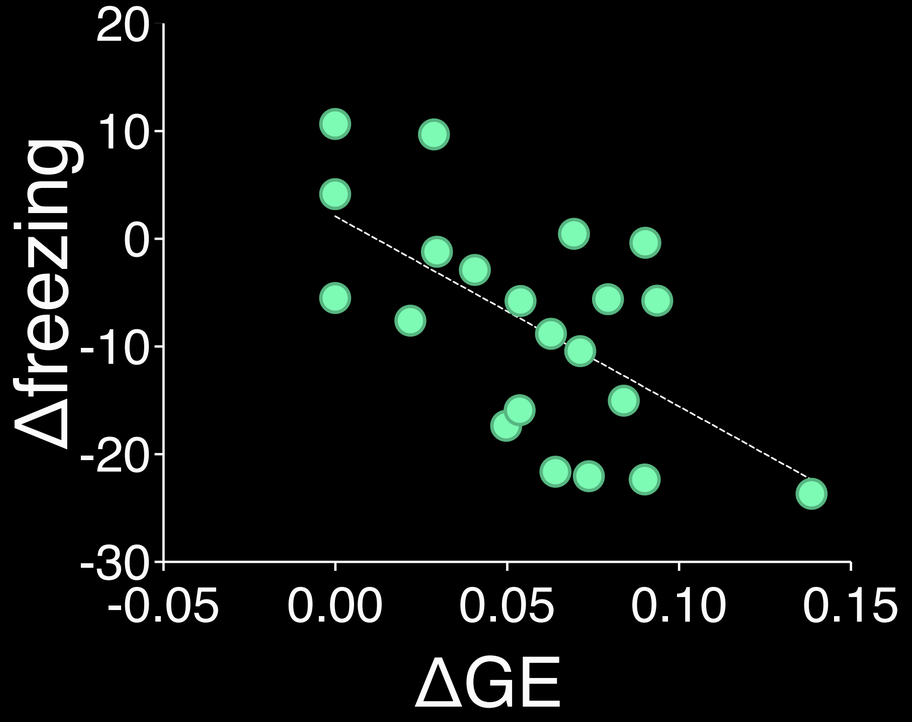


Vetere G, Kenney JW, Tran LM, Xia F, Steadman PE, Parkinson J, Josselyn SA, Frankland PW. **Neuron 2017**
Chemogenetic interrogation of a brainwide fear memory network in mice.

The fear memory network predicts the effect of *in vivo* silencing of memory network nodes

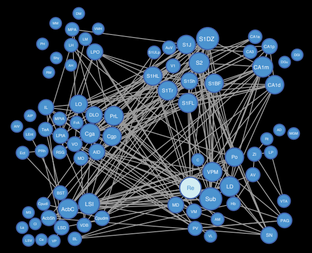
in vivo

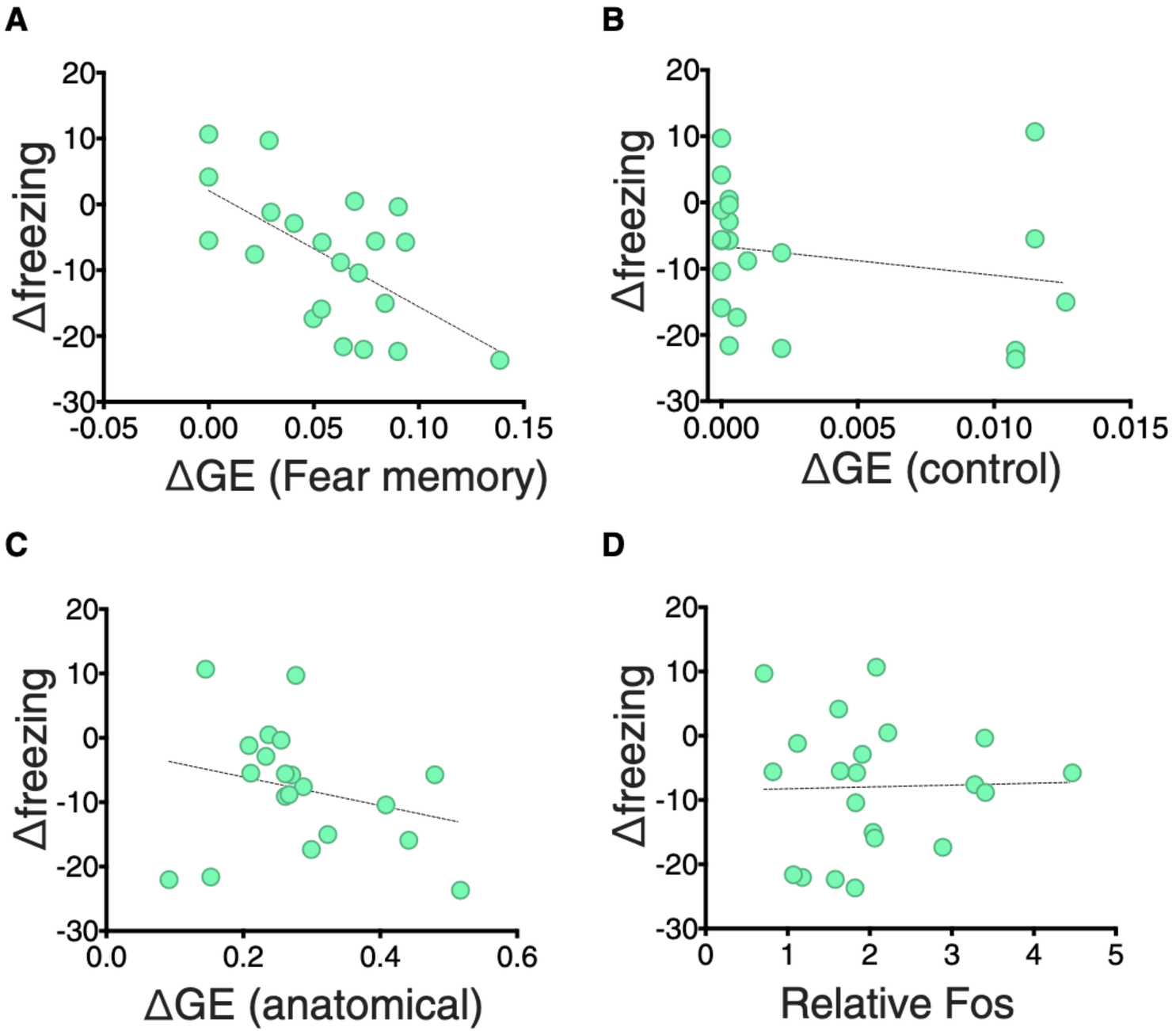




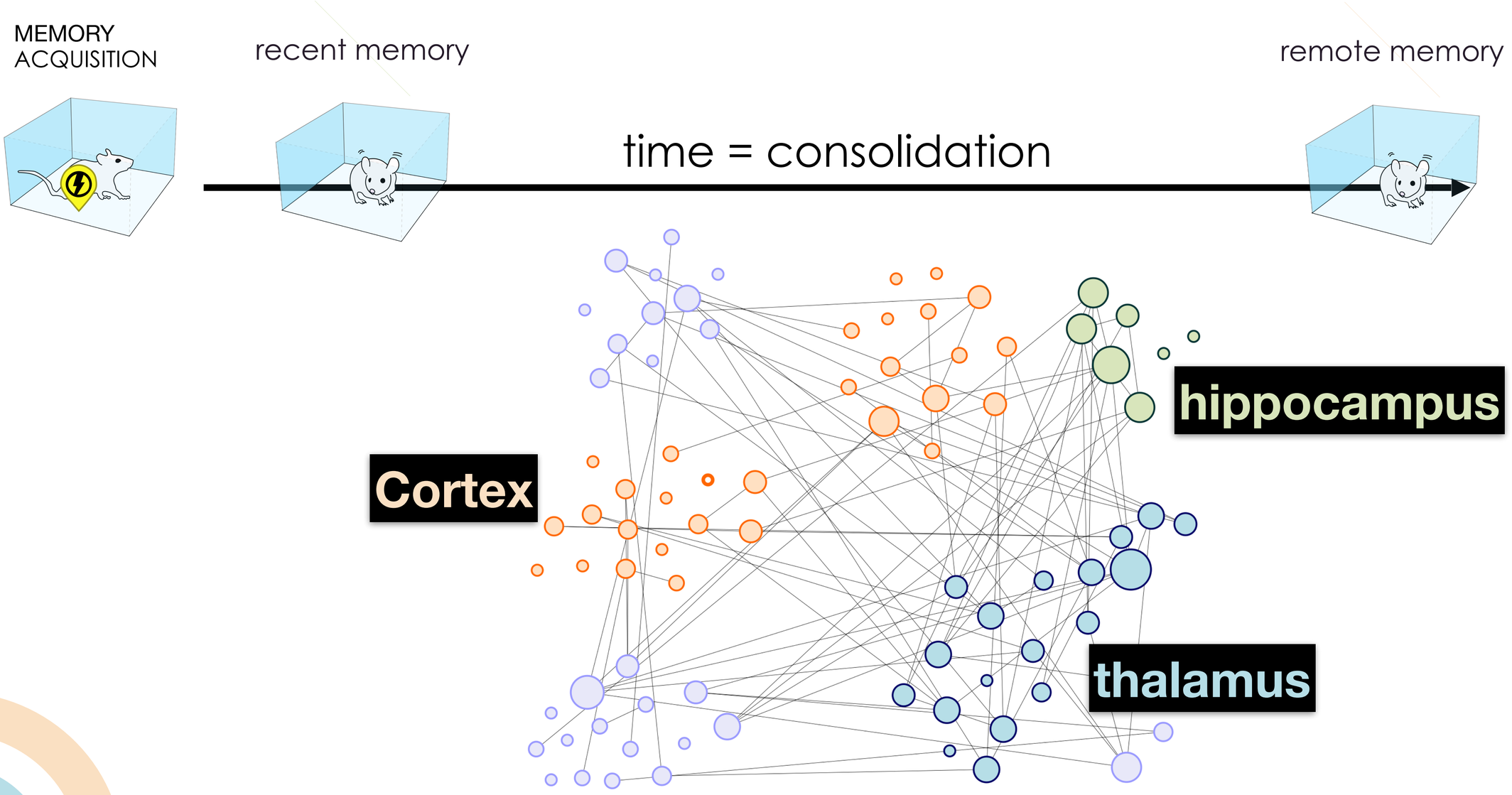
r=-0.61
p<0.01

in silico




A
B
C
D
Δfreezing
ΔGE (Fear memory)
ΔGE (control)
ΔGE (anatomical)
Relative Fos
20
10
0
-10
-20
-30
-0.05
0.00
0.05
0.10
0.15
0.000
0.005
0.010
0.015
0.0
0.2
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0
1
2
3
4
5

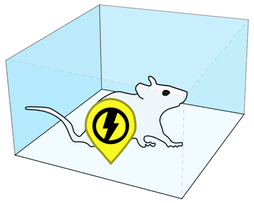
How regions change their functional connectivity during memory consolidation



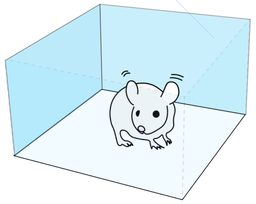
Vetere G, Kenney JW, Tran LM, Xia F, Steadman PE, Parkinson J, Josselyn SA, Frankland PW. **Neuron 2017**
Chemogenetic interrogation of a brainwide fear memory network in mice.

LONG TERM MEMORIES

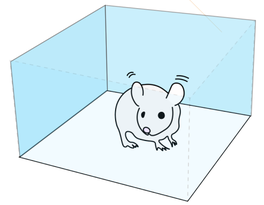
MEMORY ACQUISITION



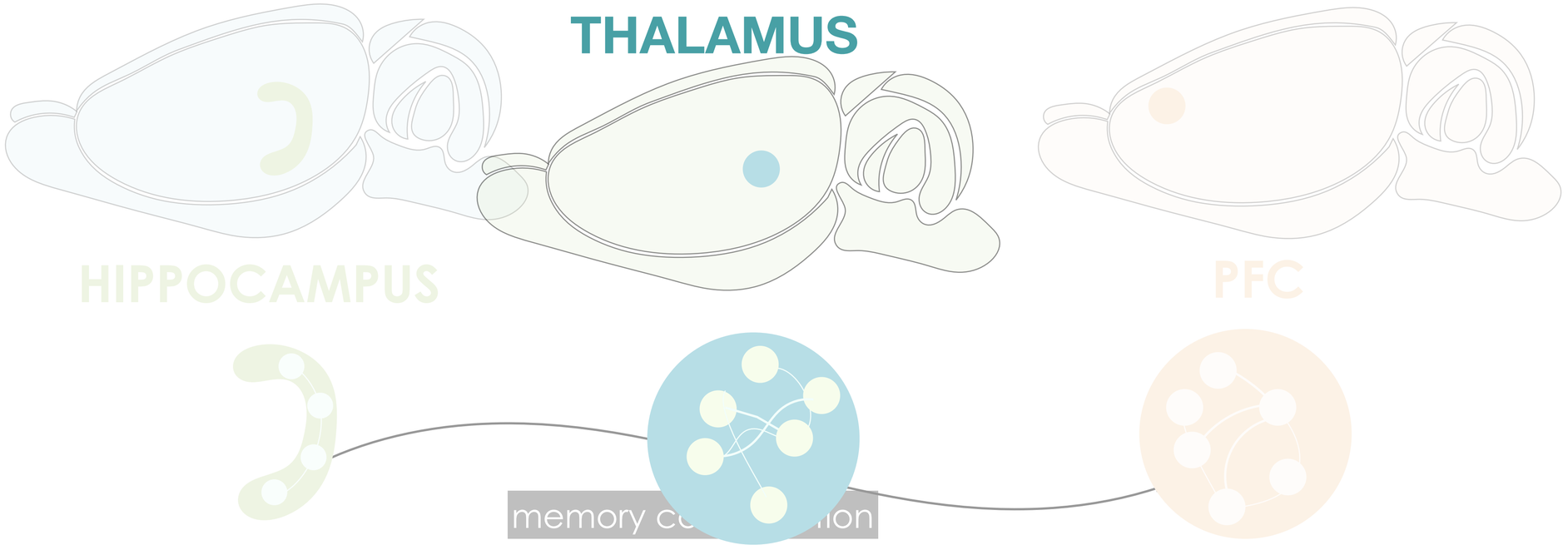
recent memory



remote memory

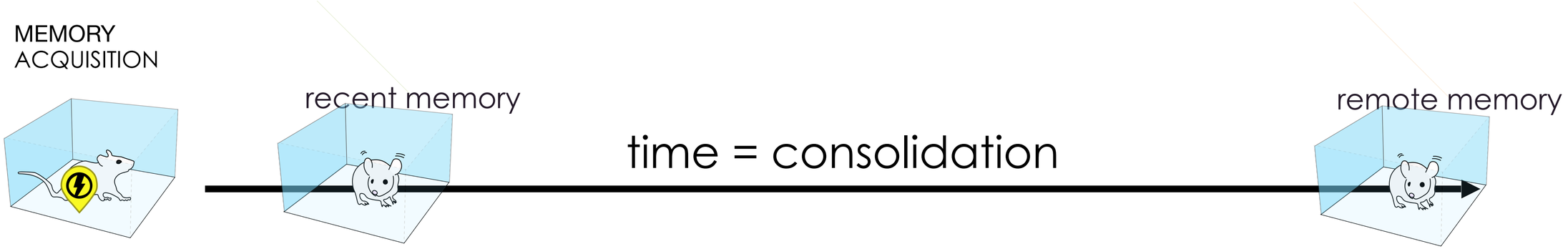


time = consolidation

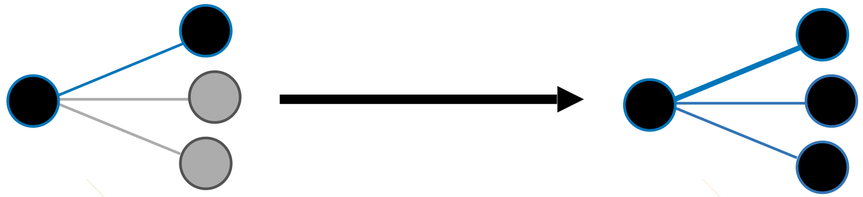


Vetere G, Restivo L, Ross PJ, Ammassari-Teule M, Josselyn SA & Frankland PW. **PNAS** 2011
Spine growth in the anterior cingulate cortex is necessary for the consolidation of contextual fear memory

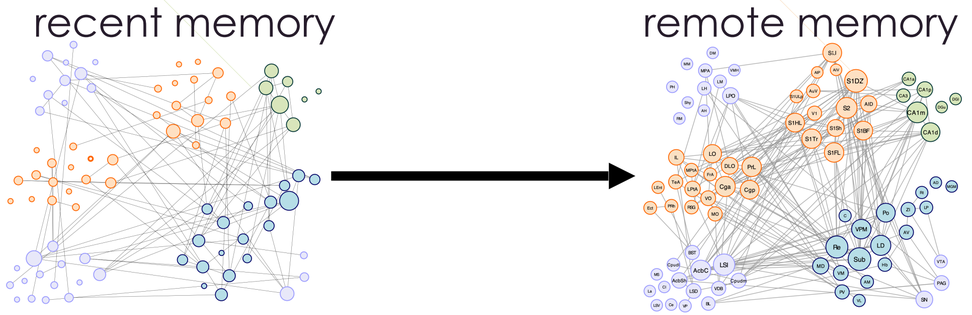
memory consolidation



synaptic consolidation

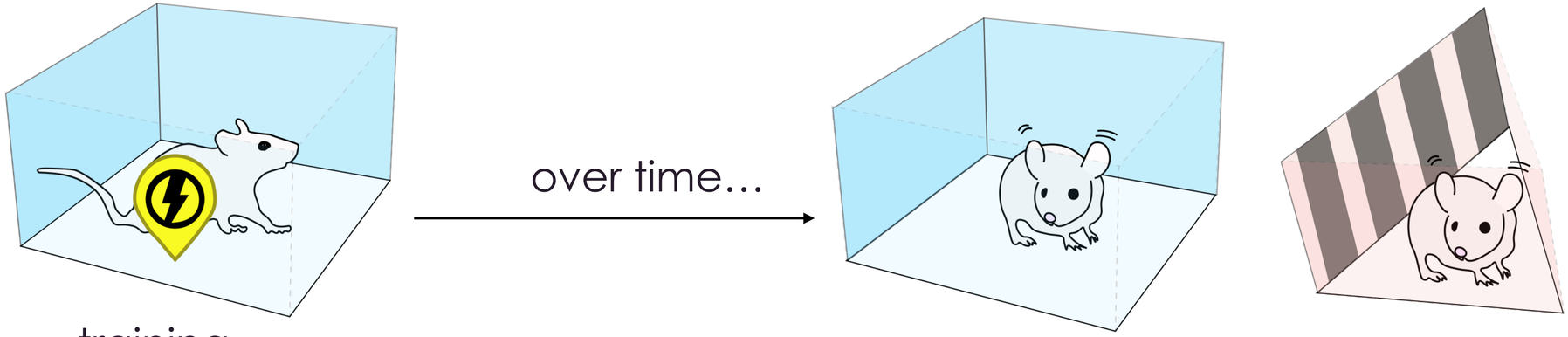


system consolidation



changes in memory quality

MEMORY OVER TIME LOSE SPATIAL DETAILS



training

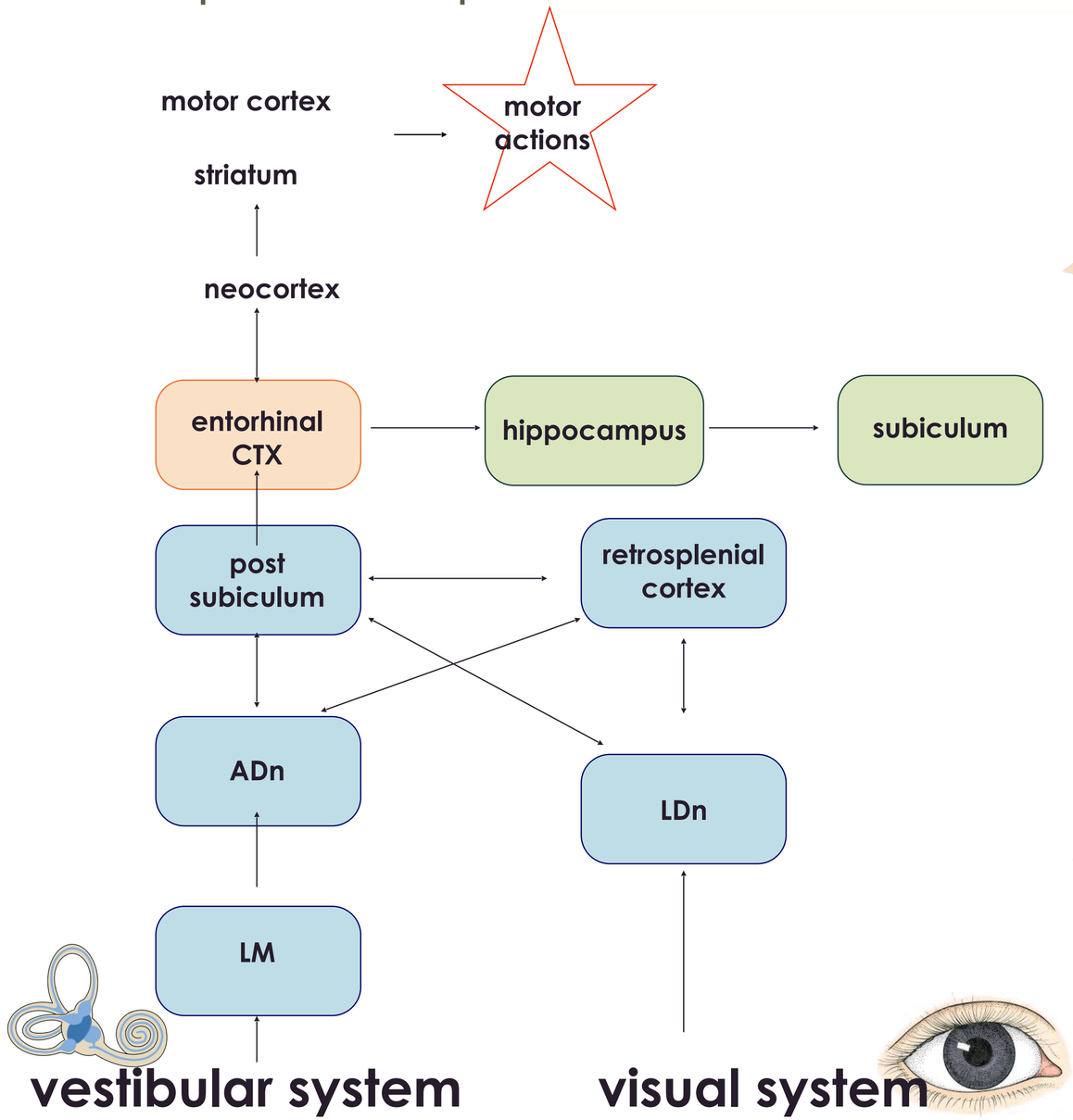
GENERALIZATION=
freezing in both contexts

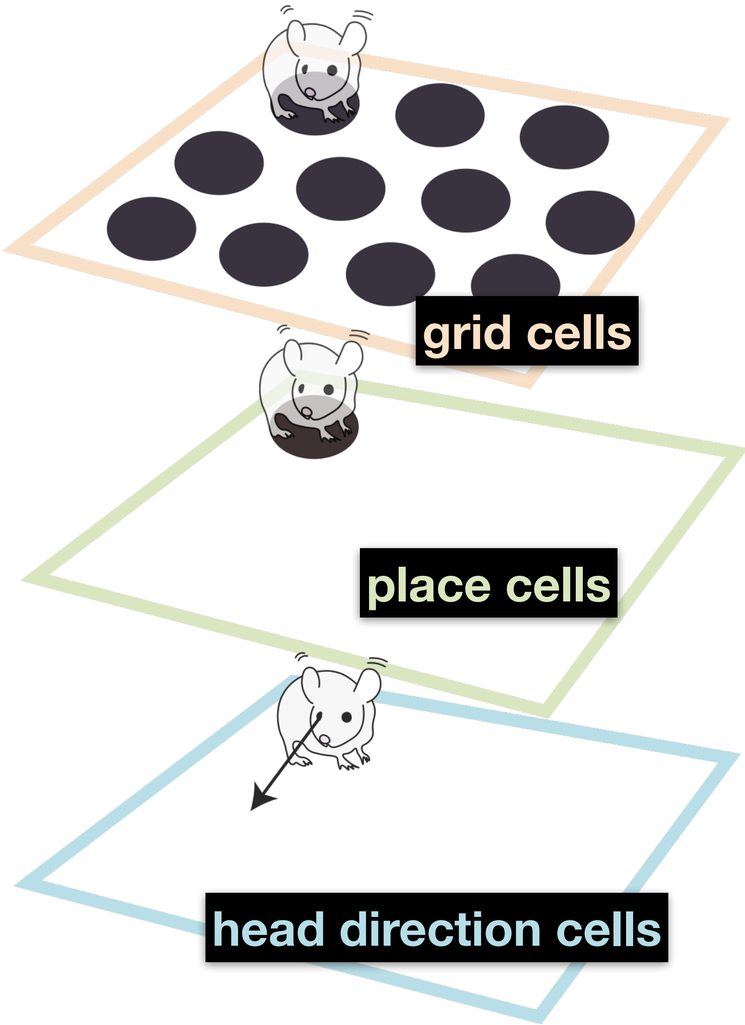
PTSD



WHERE THE SPATIAL INFORMATION GOES??

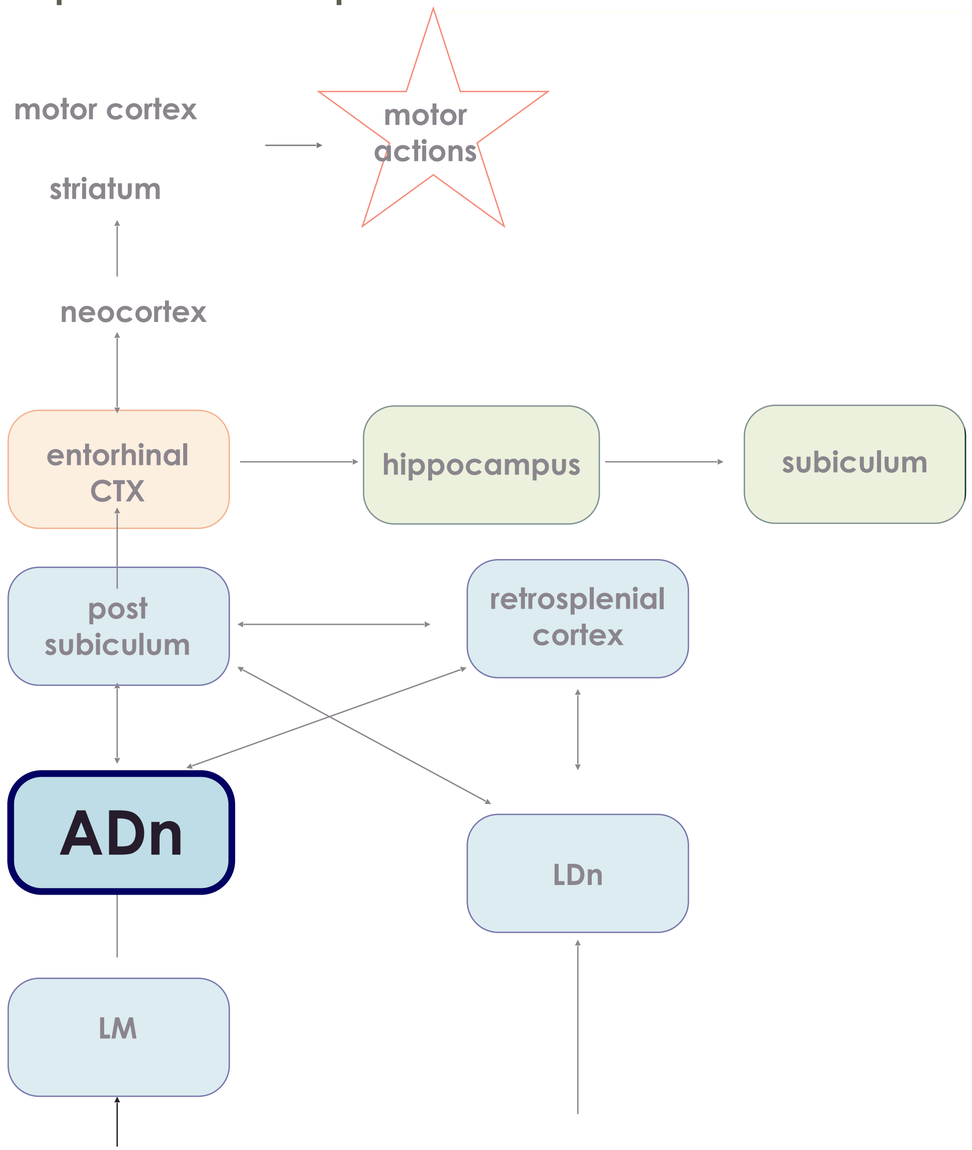
Spatial representation network

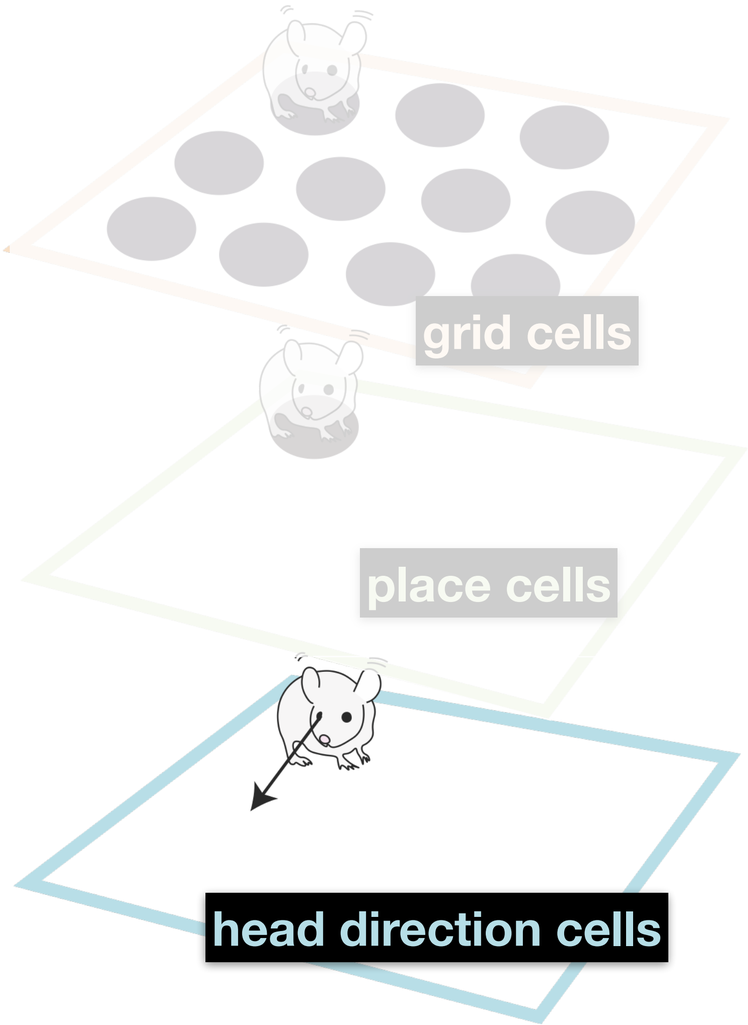




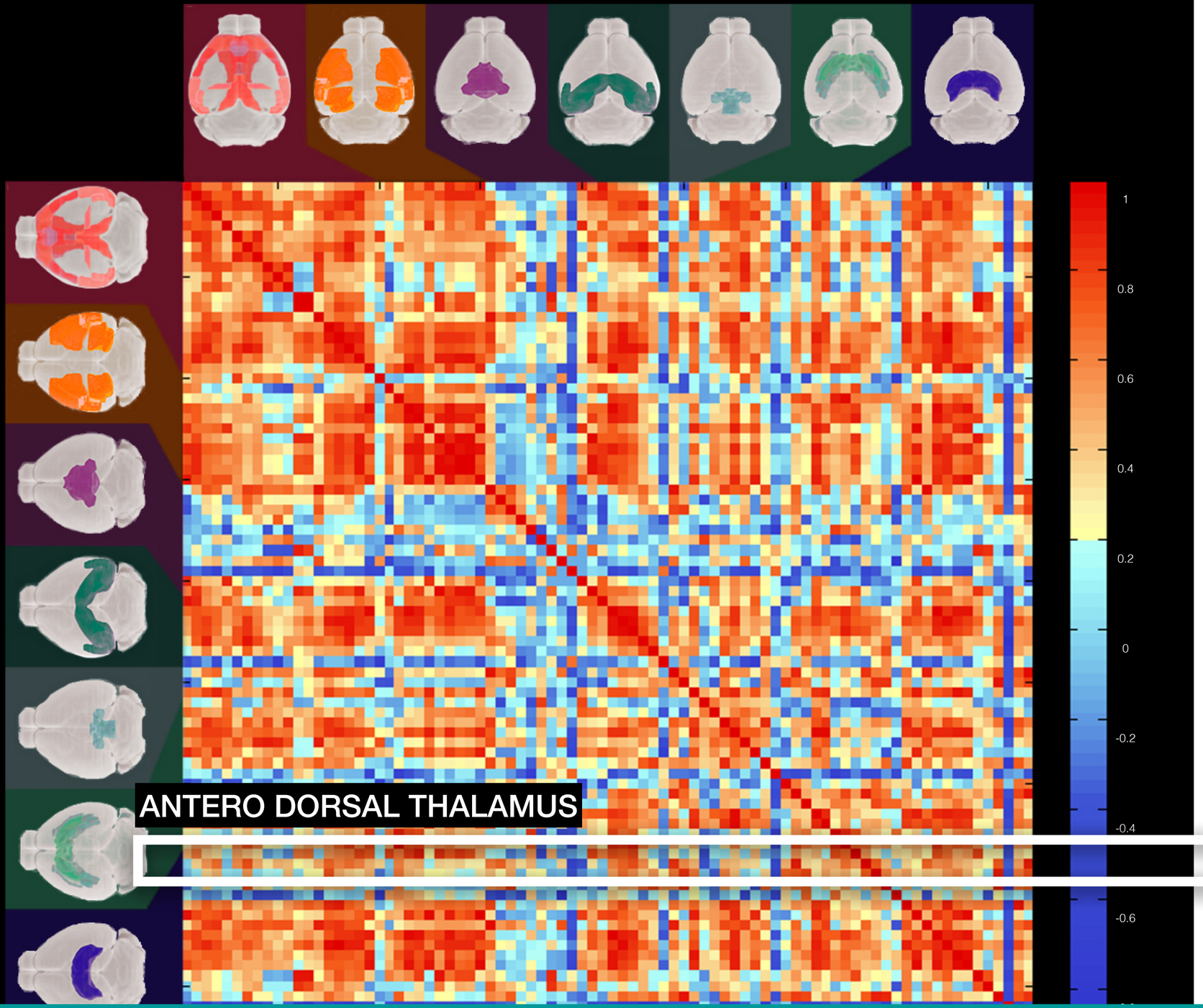
Burger et al., 2010

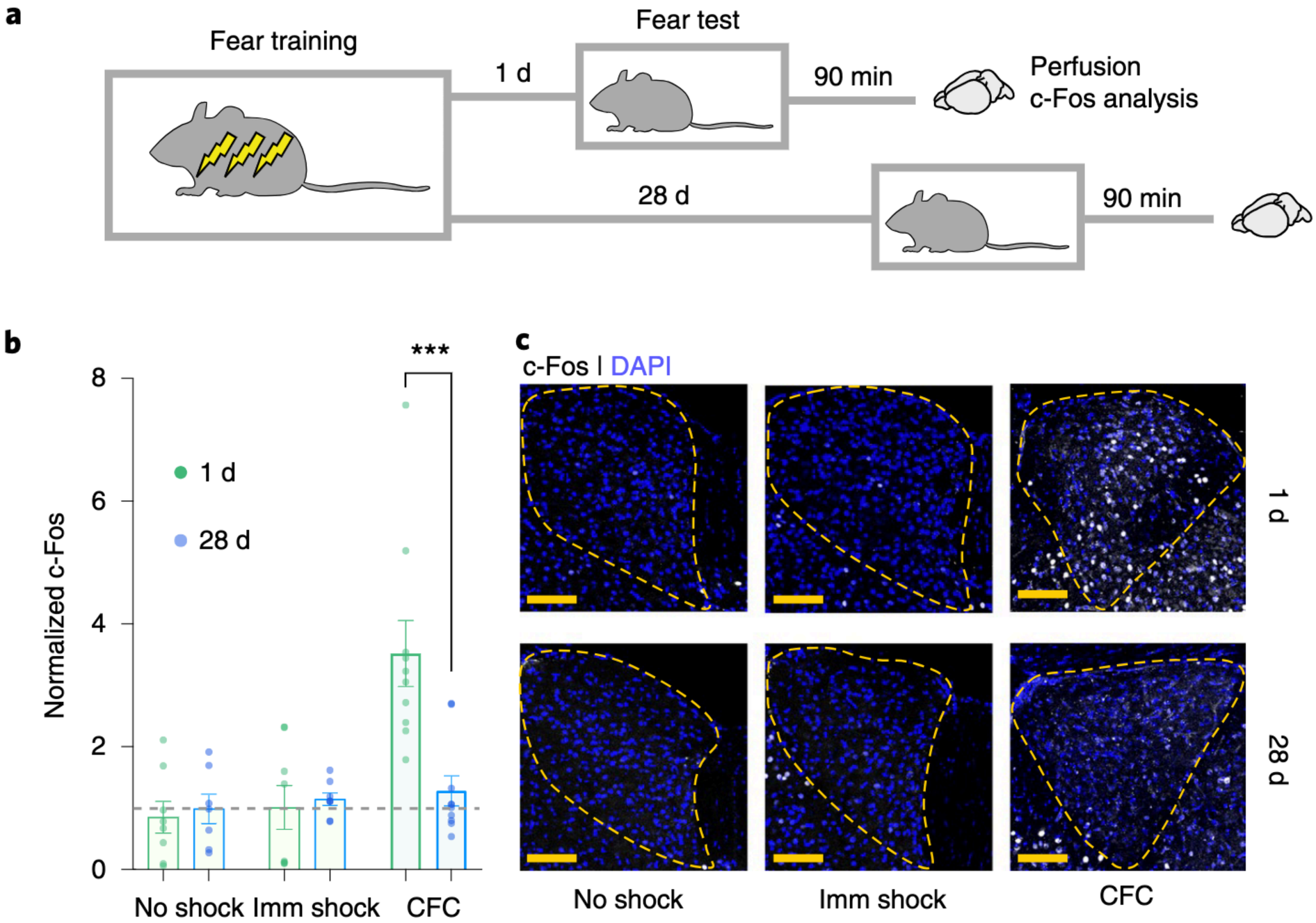
Spatial representation network



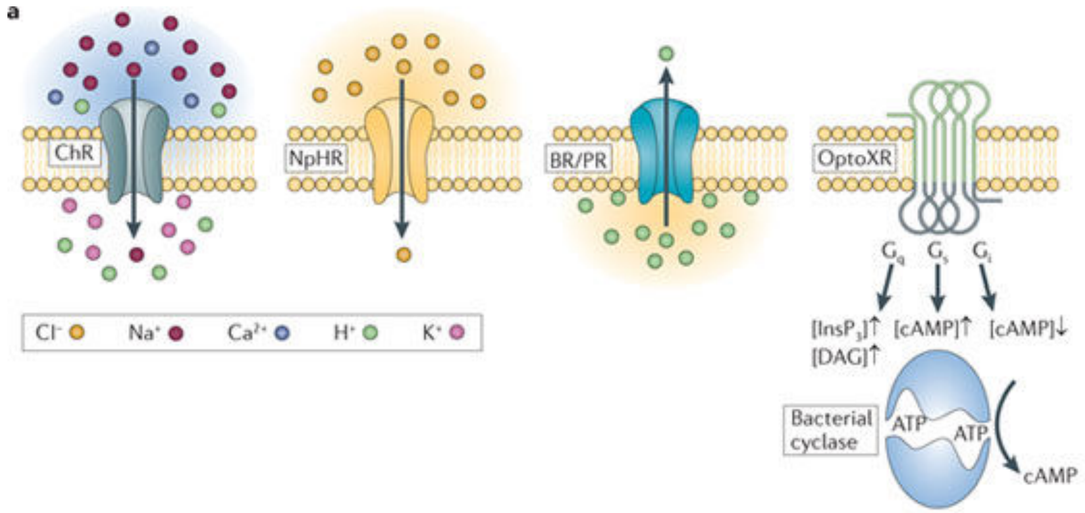


Burger et al., 2010

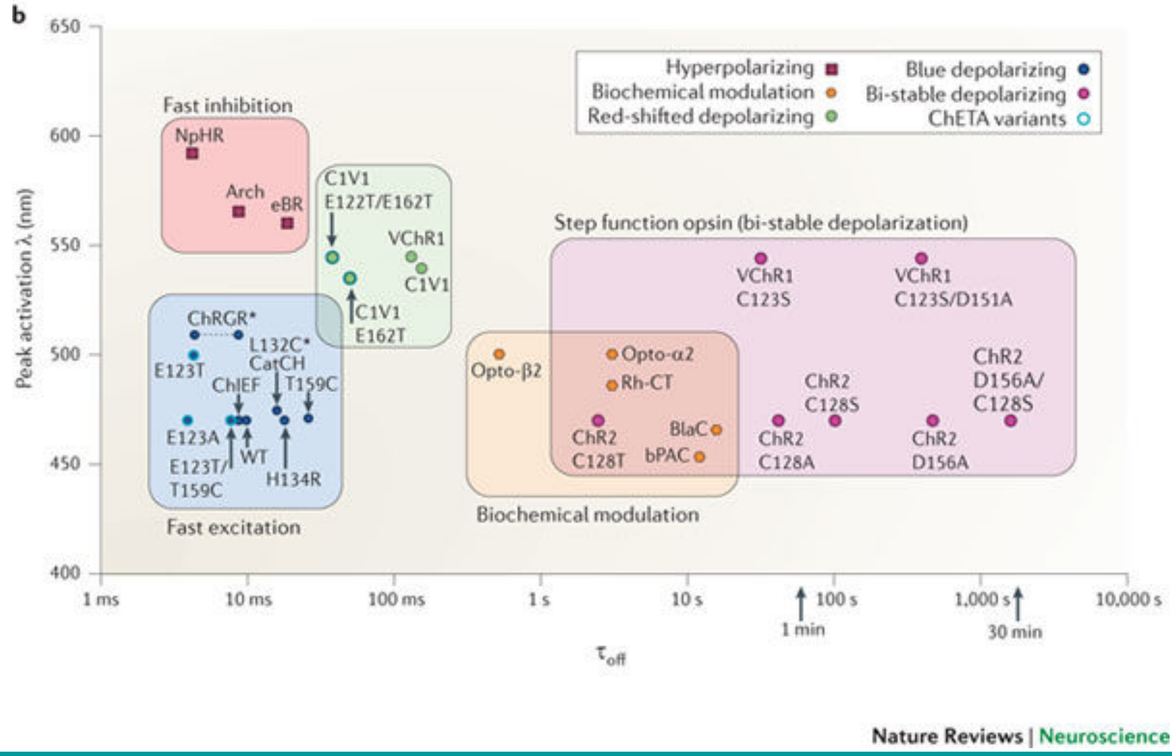

1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
ANTERO DORSAL THALAMUS


a
Fear training
1 d
Fear test
90 min
Perfusion
c-Fos analysis
28 d
90 min
b
***
Normalized c-Fos
8
6
4
2
0
1 d
28 d
No shock
Imm shock
CFC
c
c-Fos | DAPI
1 d
28 d
No shock
Imm shock
CFC

optogenetics to mimic physiological activation/inhibition of targeted neuronal populations



specifically control **<u>genetically defined neuronal populations</u>** in **<u>real time</u>** in behaving animals performing cognitive tasks.

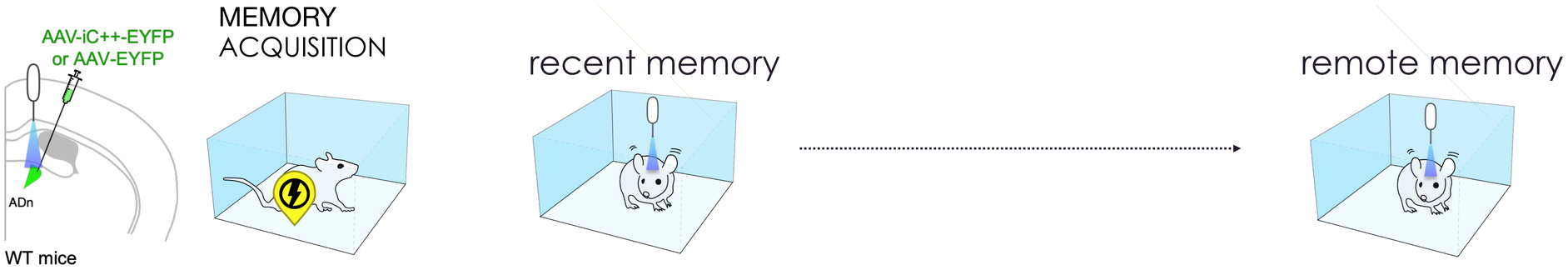


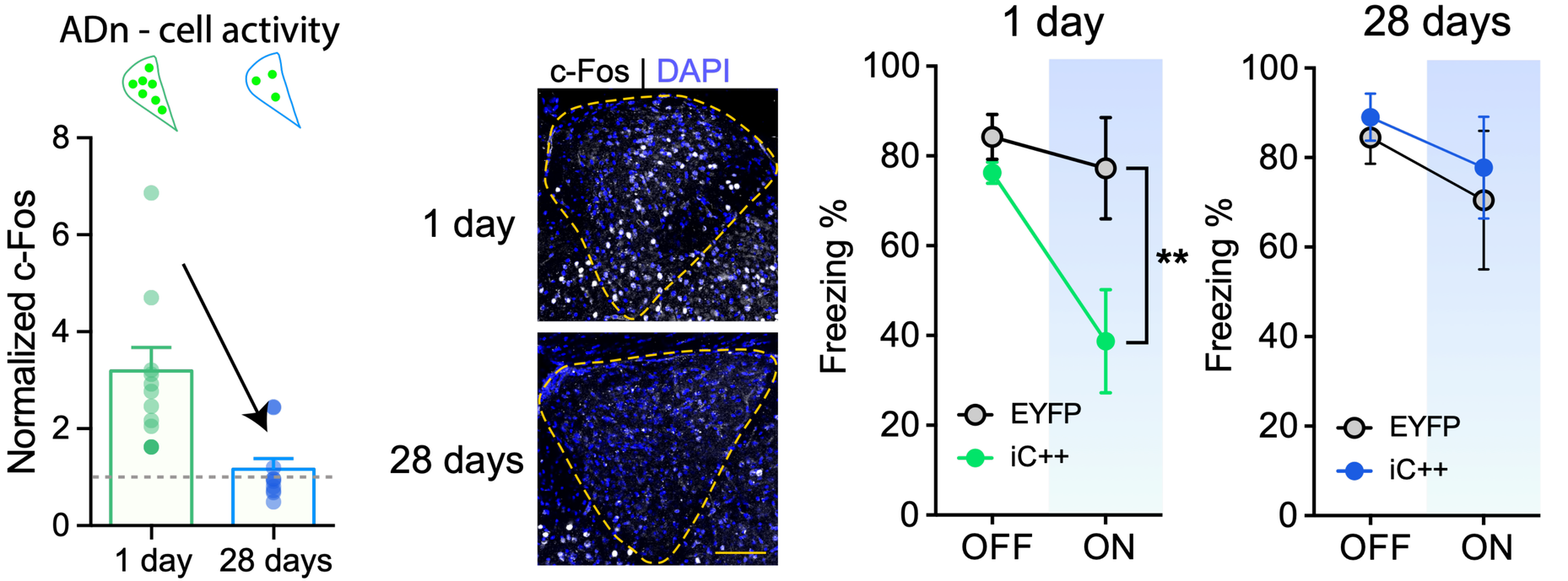
Nature Reviews | Neuroscience



ADn

essential for **recent** memory recall

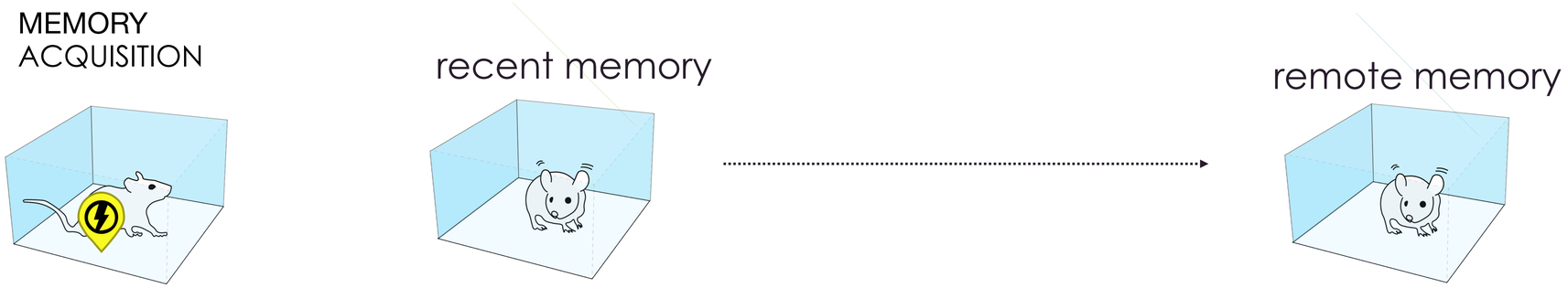


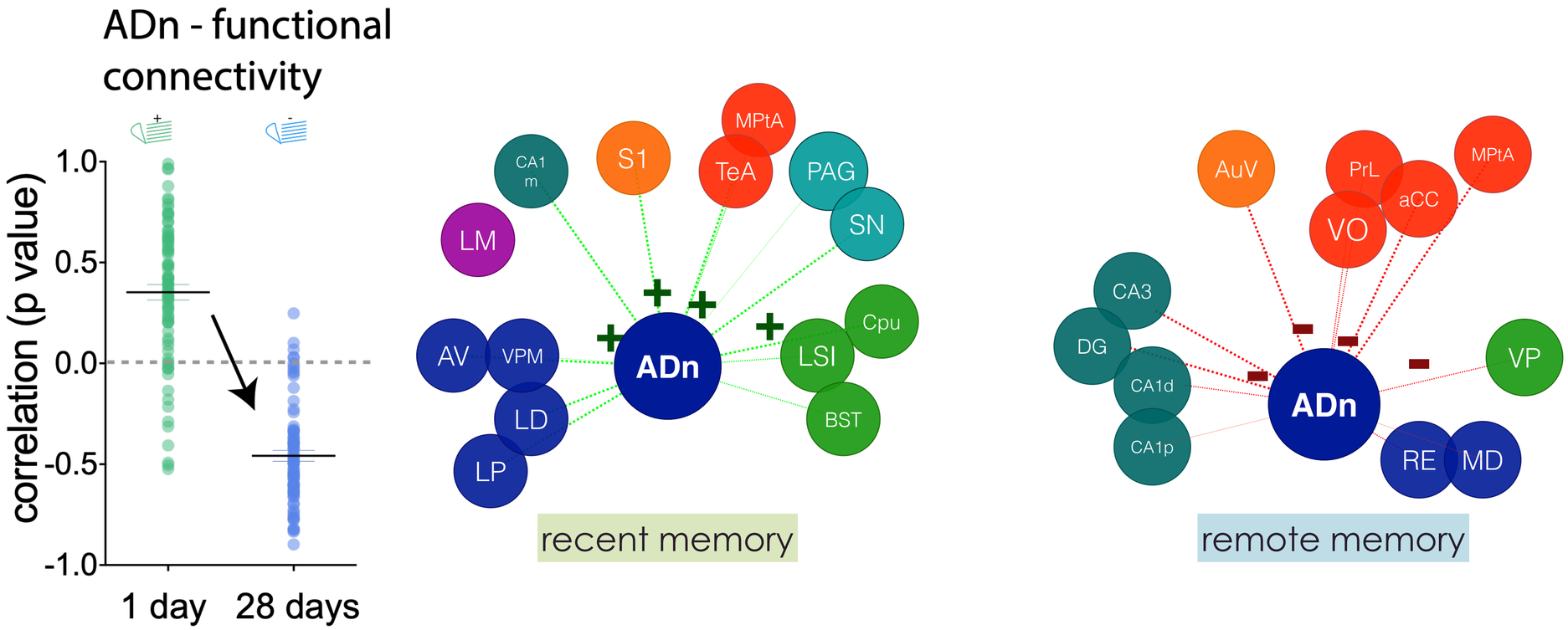


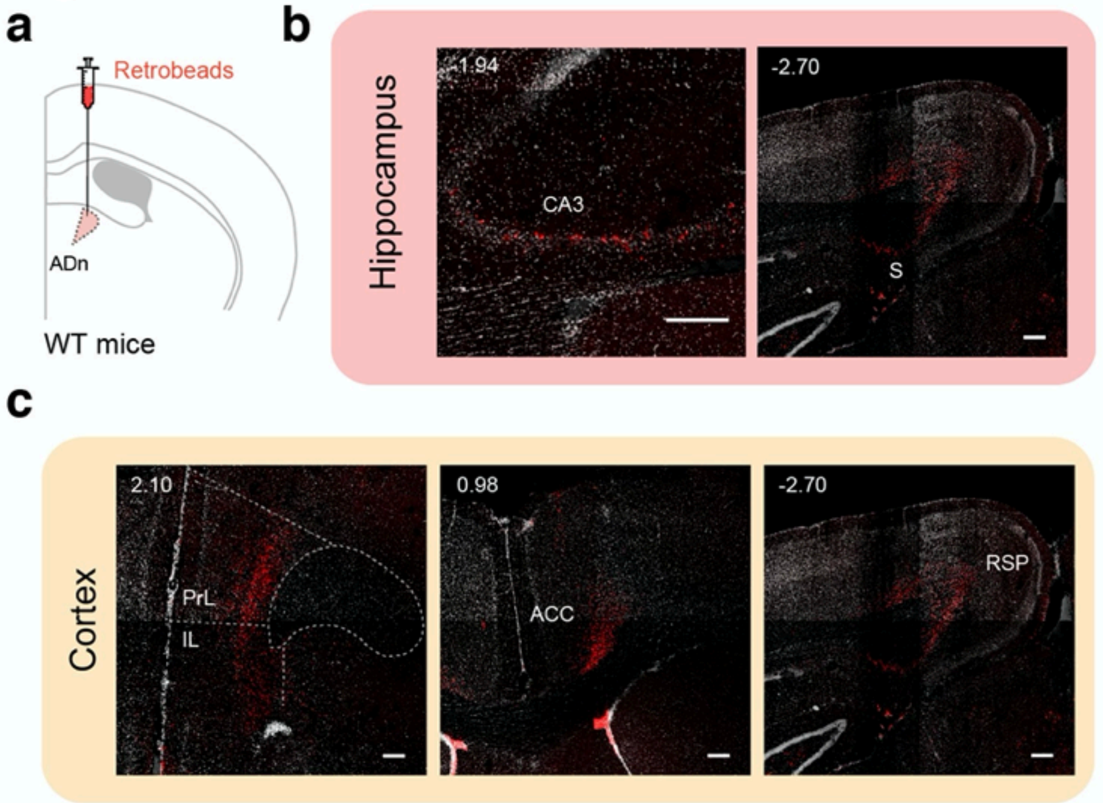
Vetere G, Xia F, Ramsaran A,Tran L, Josselyn S, Frankland P. **Nature Neuroscience, 2021**
Identification of an inhibitory hippocampal-thalamic pathway that is necessary for remote memory retrieval

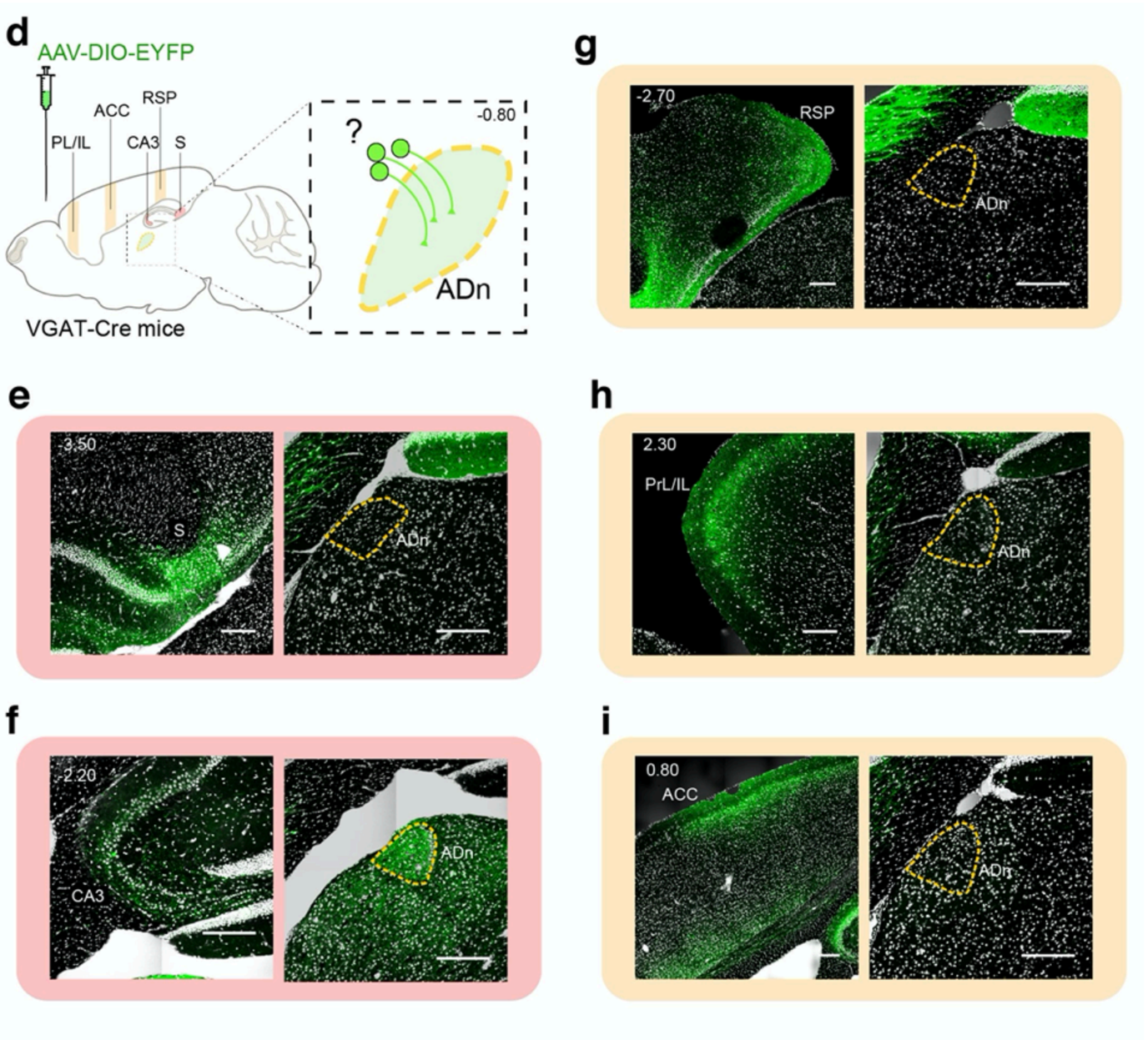
ADn

functional connectivity at recent vs remote



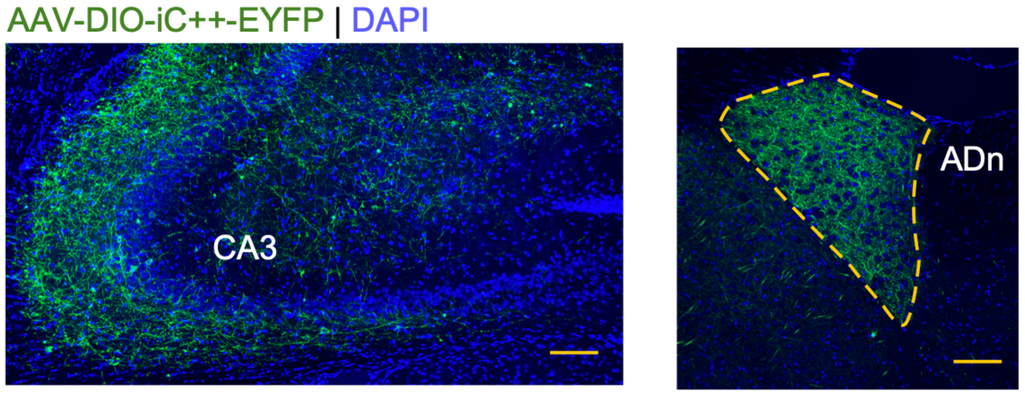


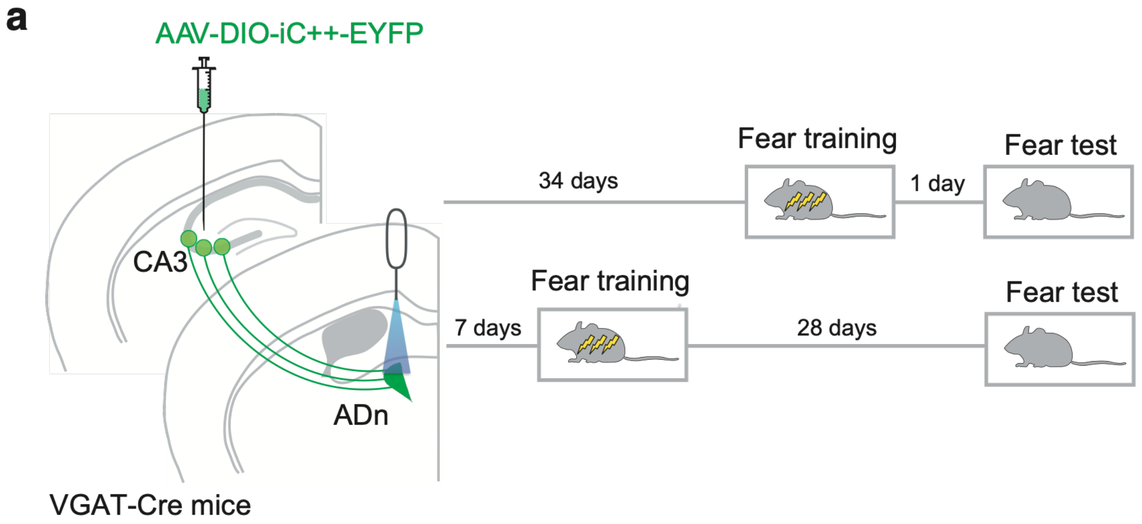

a
Retrobeads
ADn
WT mice
b
Hippocampus
-1.94
CA3
-2.70
S
c
Cortex
2.10
PrL
IL
0.98
ACC
-2.70
RSP

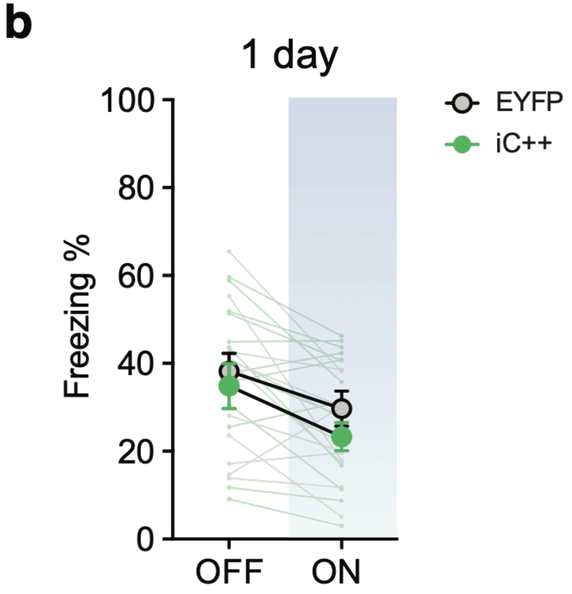

d
AAV-DIO-EYFP
ACC
RSP
PL/IL
CA3
S
?
-0.80
ADn
VGAT-Cre mice
g
-2.70
RSP
ADn
e
3.50
S
ADn
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2.30
PrL/IL
ADn
f
-2.20
CA3
ADn
i
0.80
ACC
ADn

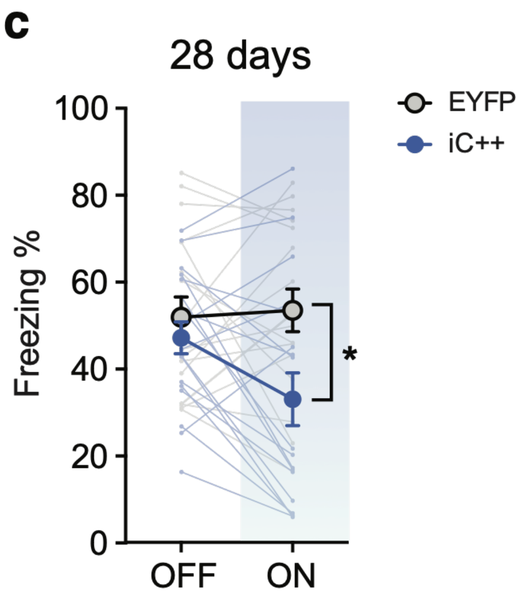
ADn

active inhibition of **ADn** during remote memory recall





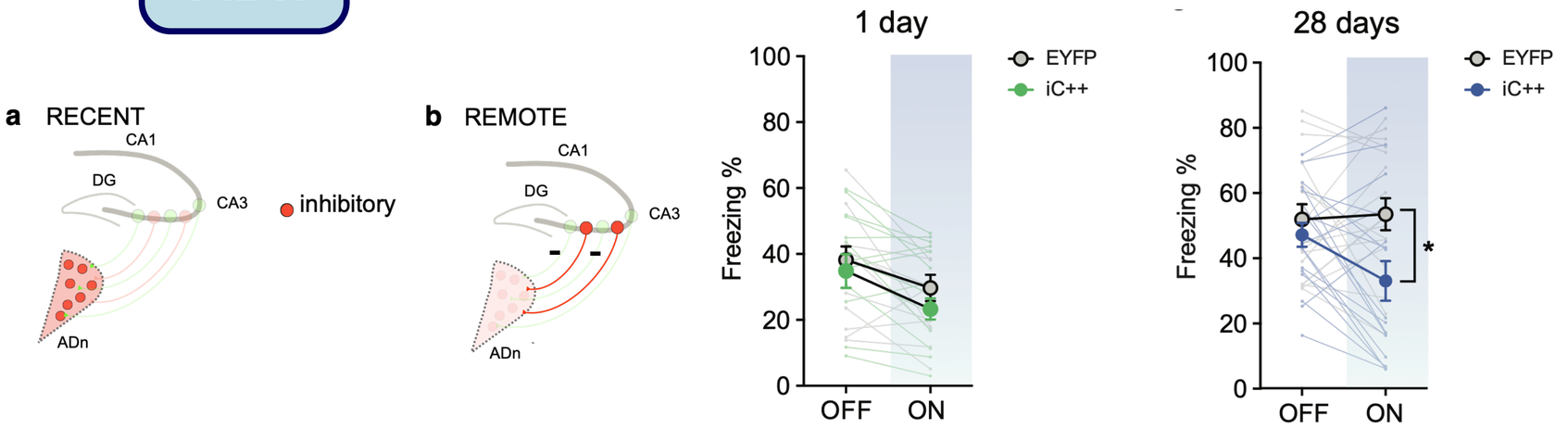




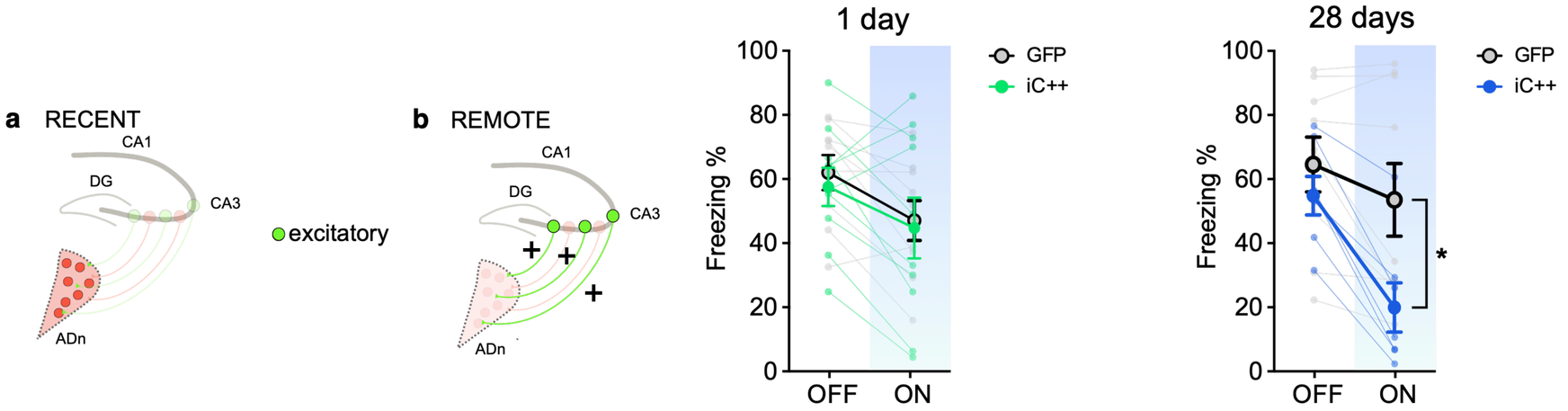
Vetere G, Xia F, Ramsaran A,Tran L, Josselyn S, Frankland P. **Nature Neuroscience, 2021**
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ADn

activation of **ADn** during remote memory recall



Vetere G, Xia F, Ramsaran A,Tran L, Josselyn S, Frankland P. **Nature Neuroscience, 2021**
Identification of an inhibitory hippocampal-thalamic pathway that is necessary for remote memory retrieval

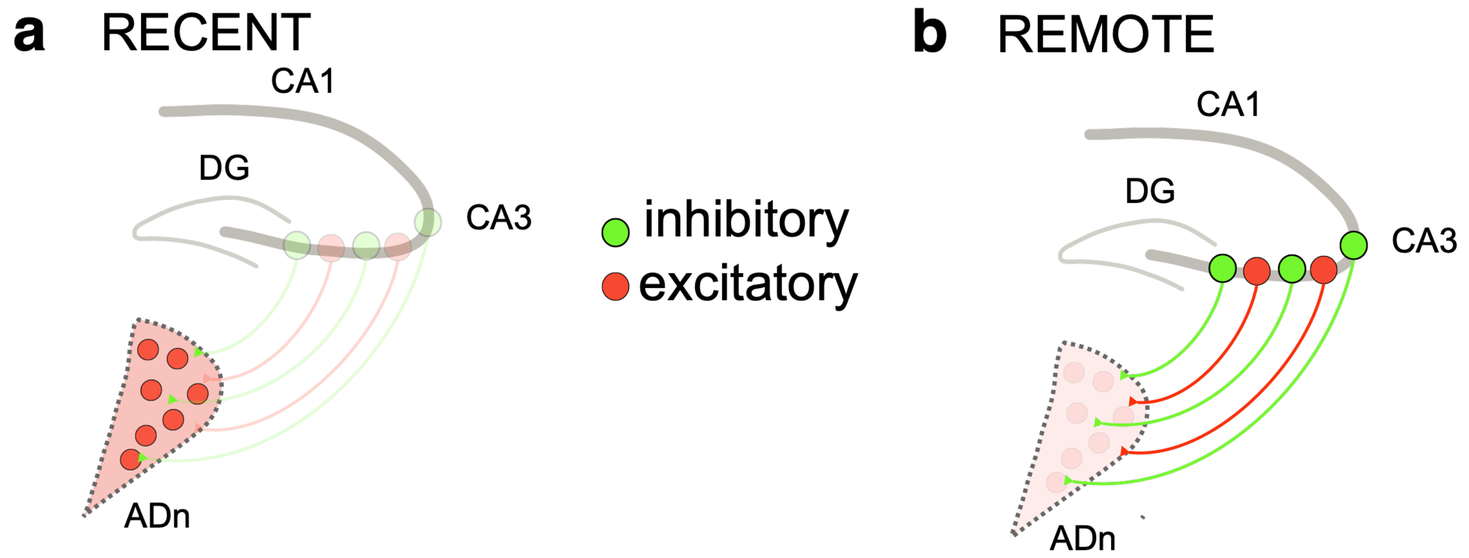


Andrews C, Christiaens M, Avida F, Balcells Picaza N, Vetere G. In preparation
Excitatory hippocampal-thalamic projections play a role in remote memory recall

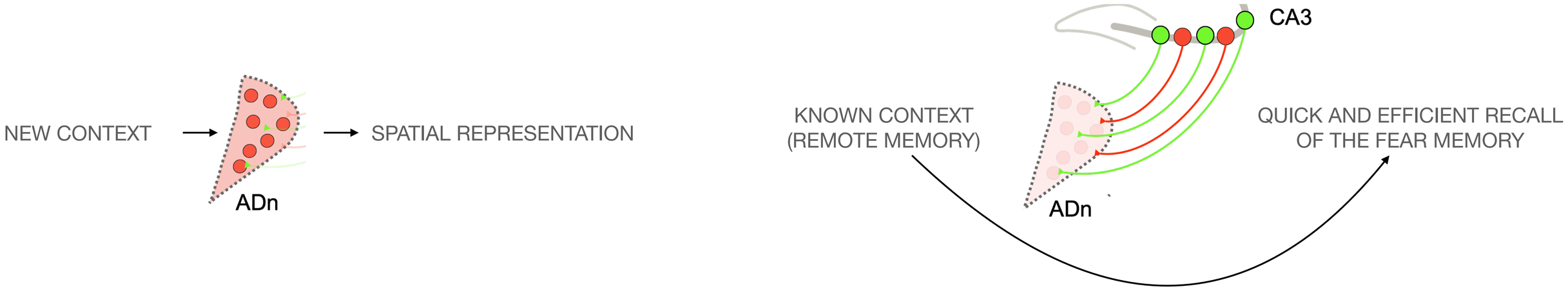


ADn

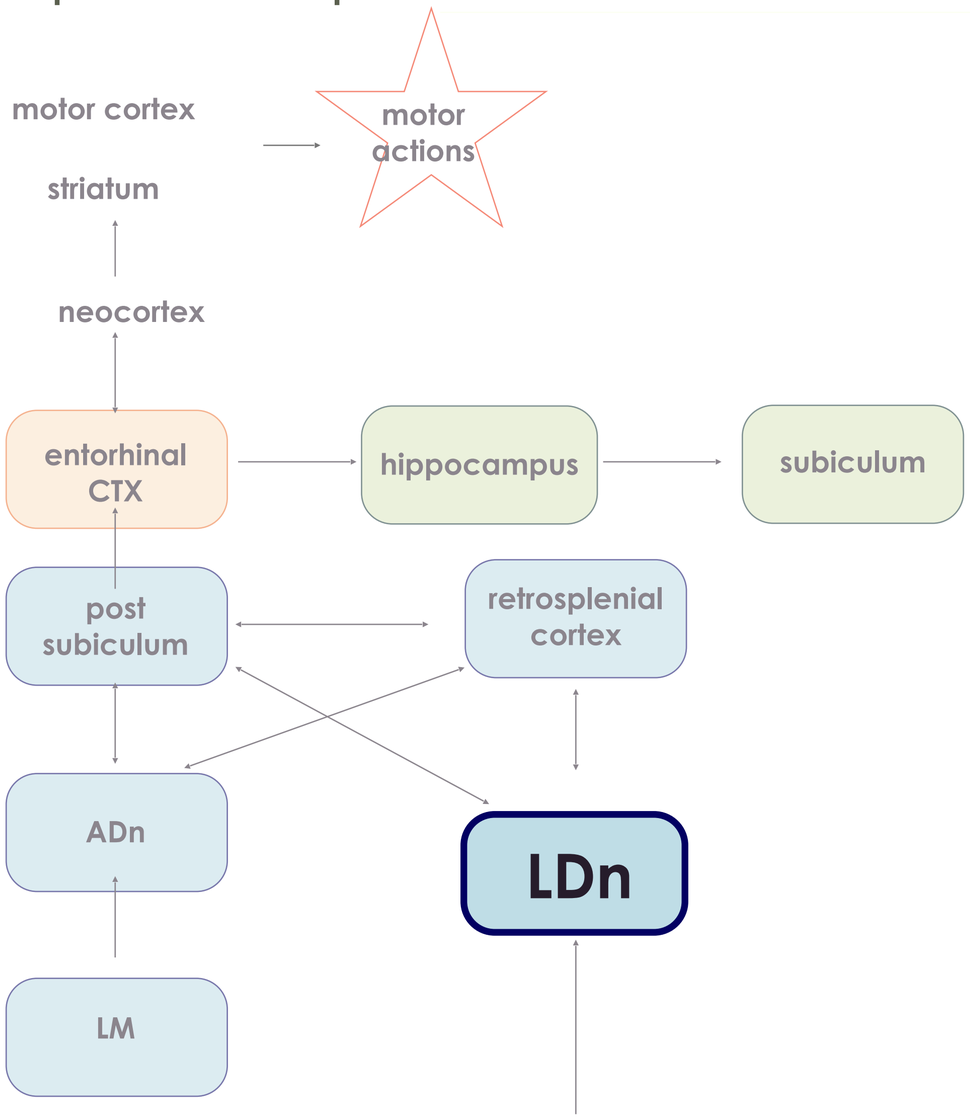
Hypothesis of change in CA3-ADn **connectivity**

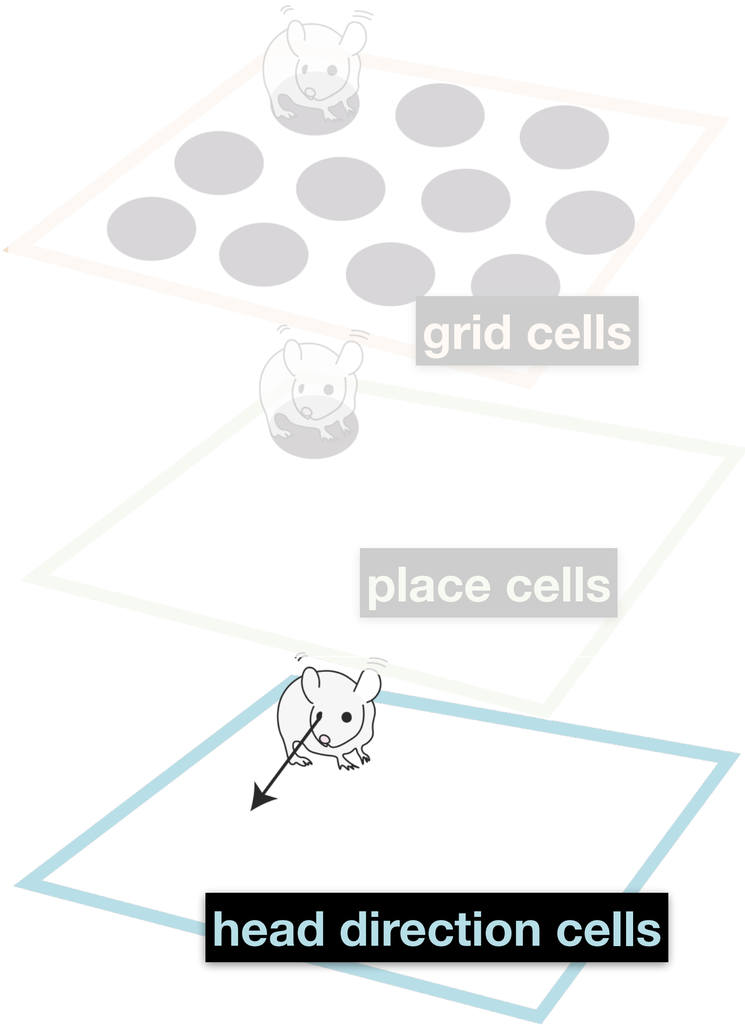


Why the ADn is inactivated during consolidation



Spatial representation network



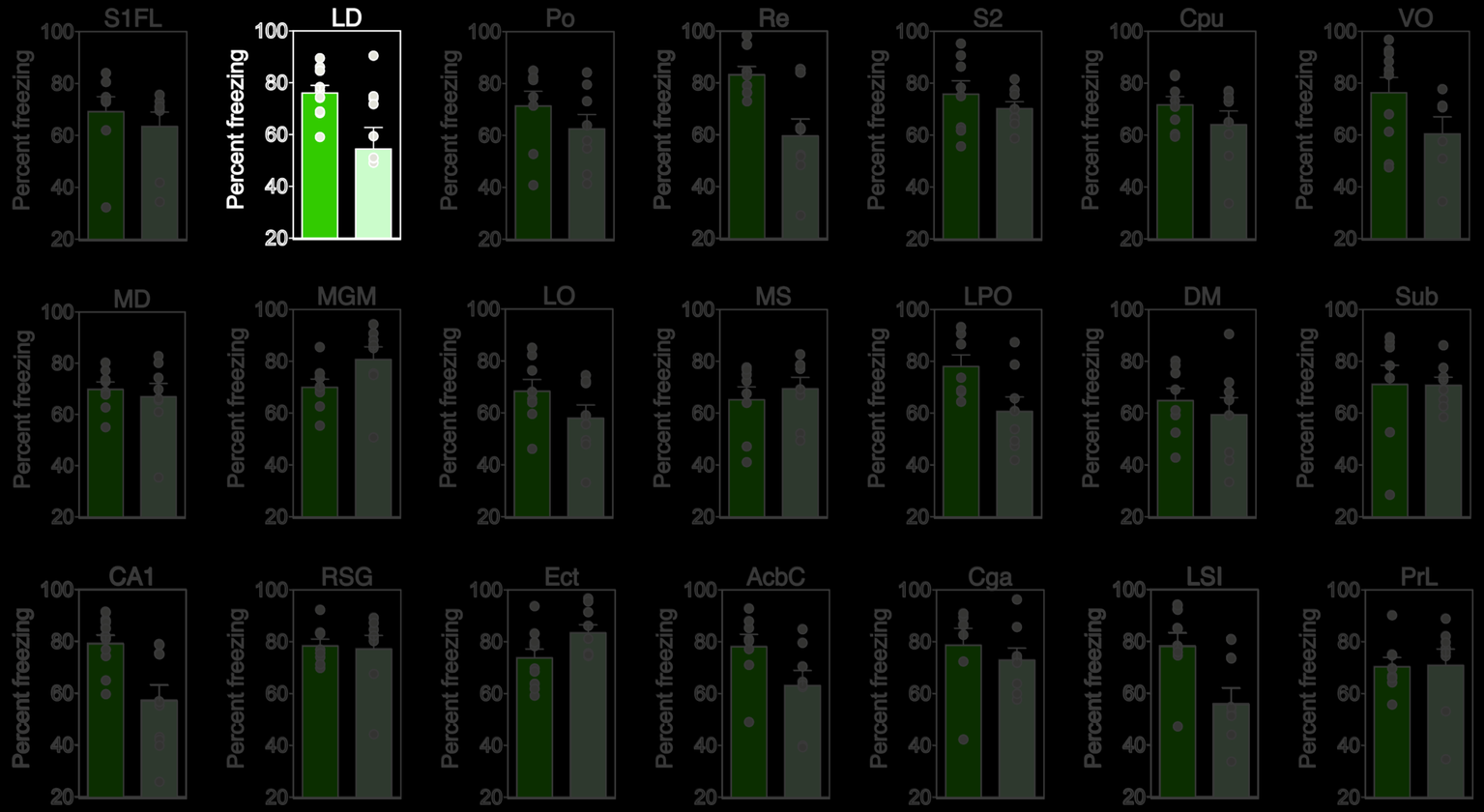


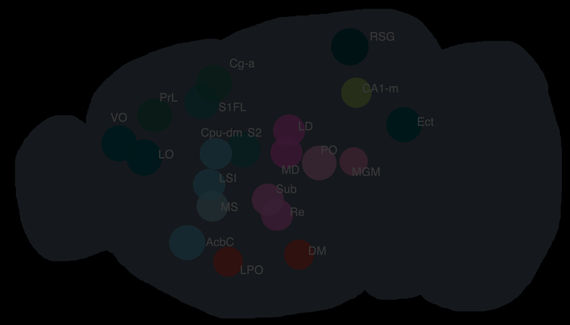
Burger et al., 2010

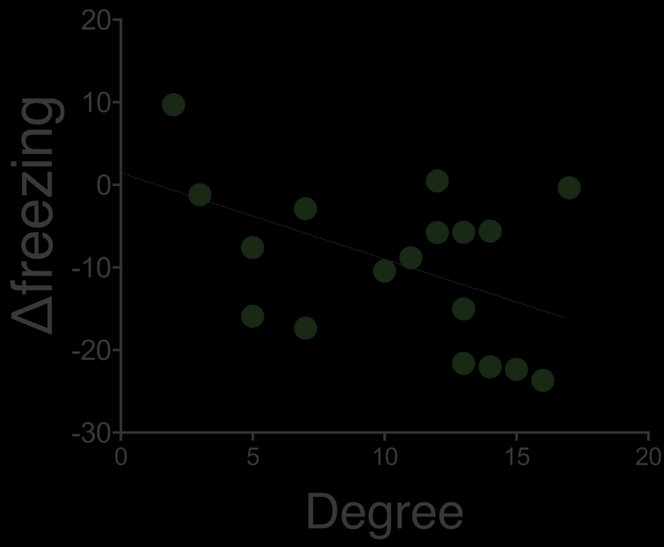
validating the network approach

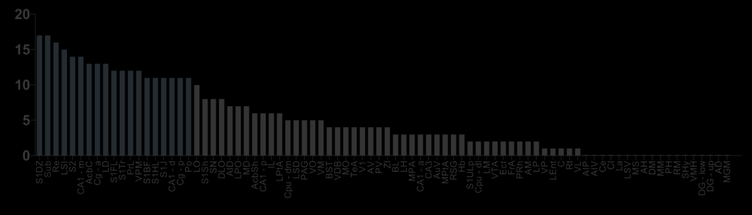
vehicle

CNO







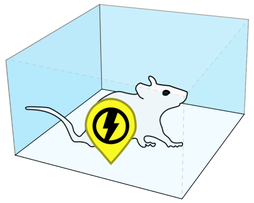


Vetere G et al, **Neuron 2017**

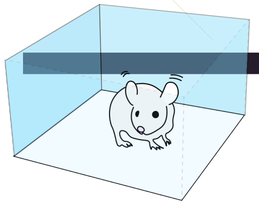
essential for **remote** memory recall

LDn

MEMORY ACQUISITION

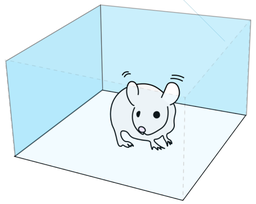


recent memory

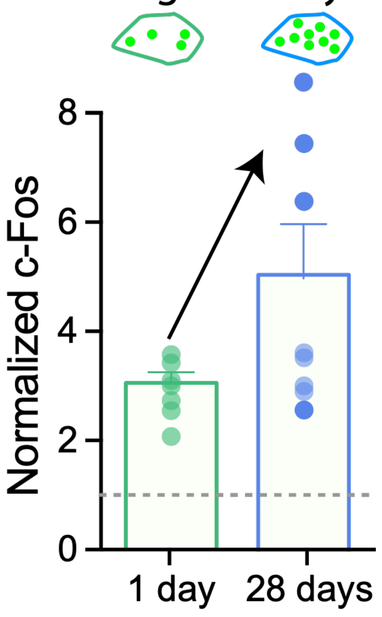


CNO (in drinking water)

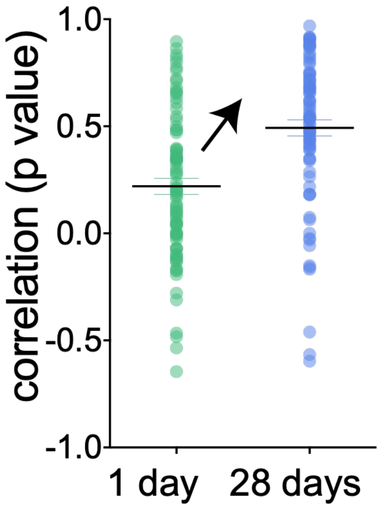
remote memory



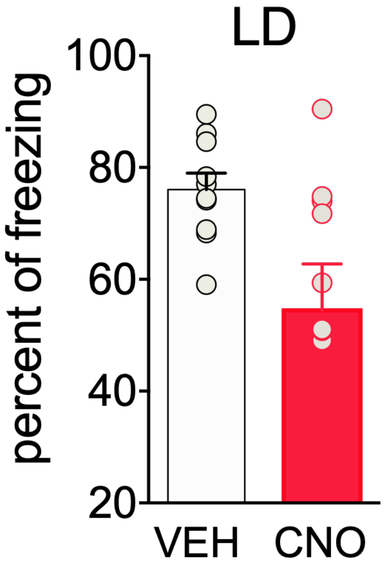
LDn - cell activity during memory

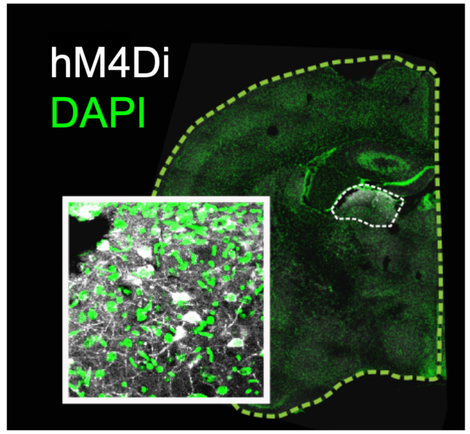


LDn - functional connectivity



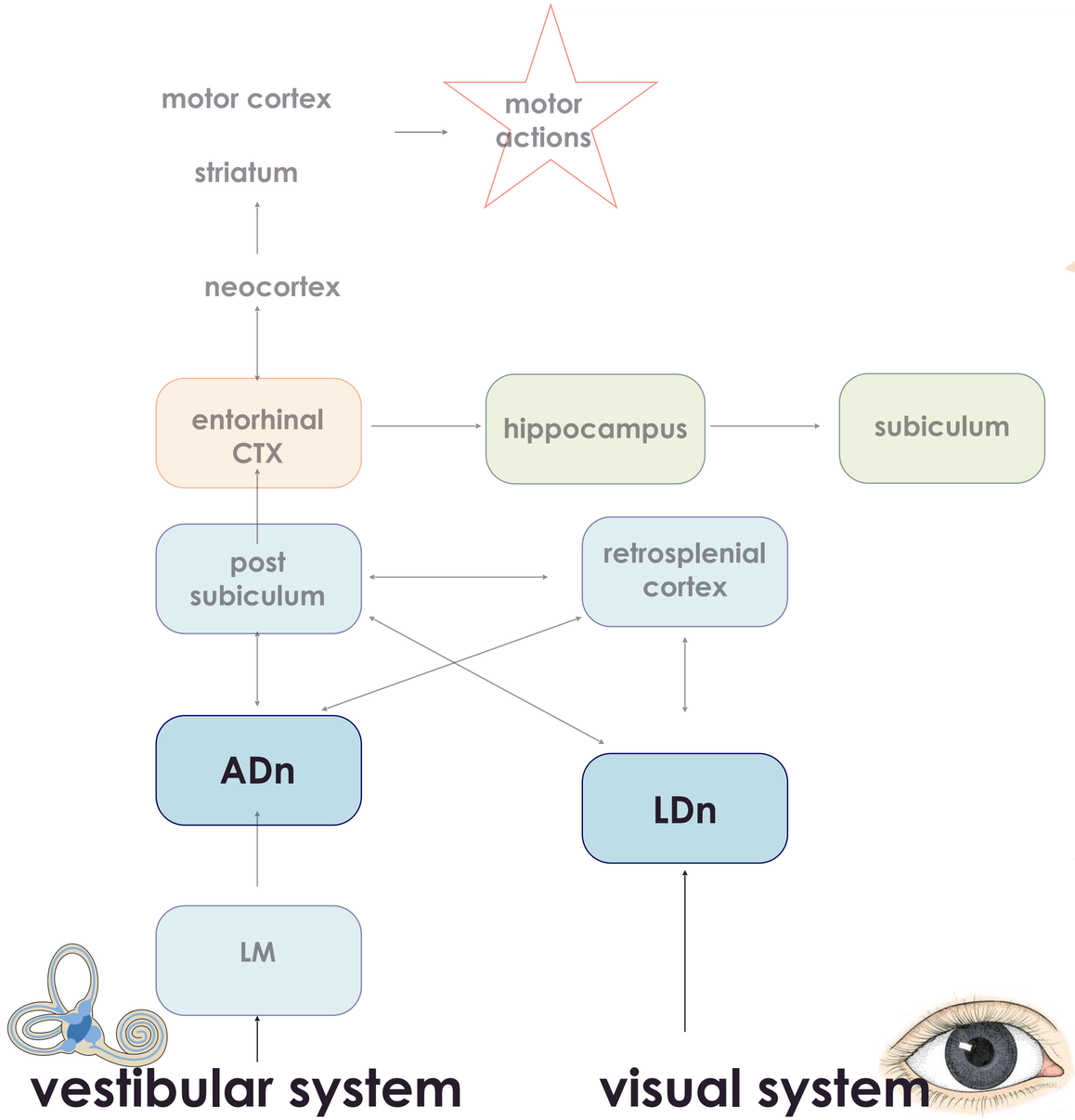
LDn chemogenetic inactivation - remote memory recall

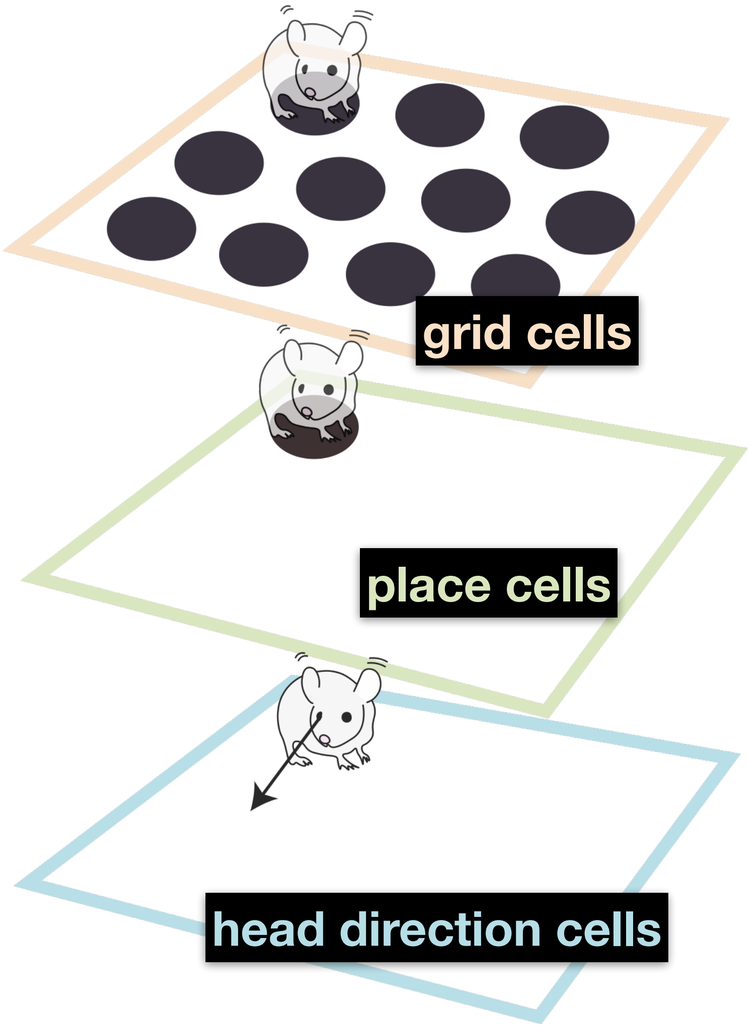




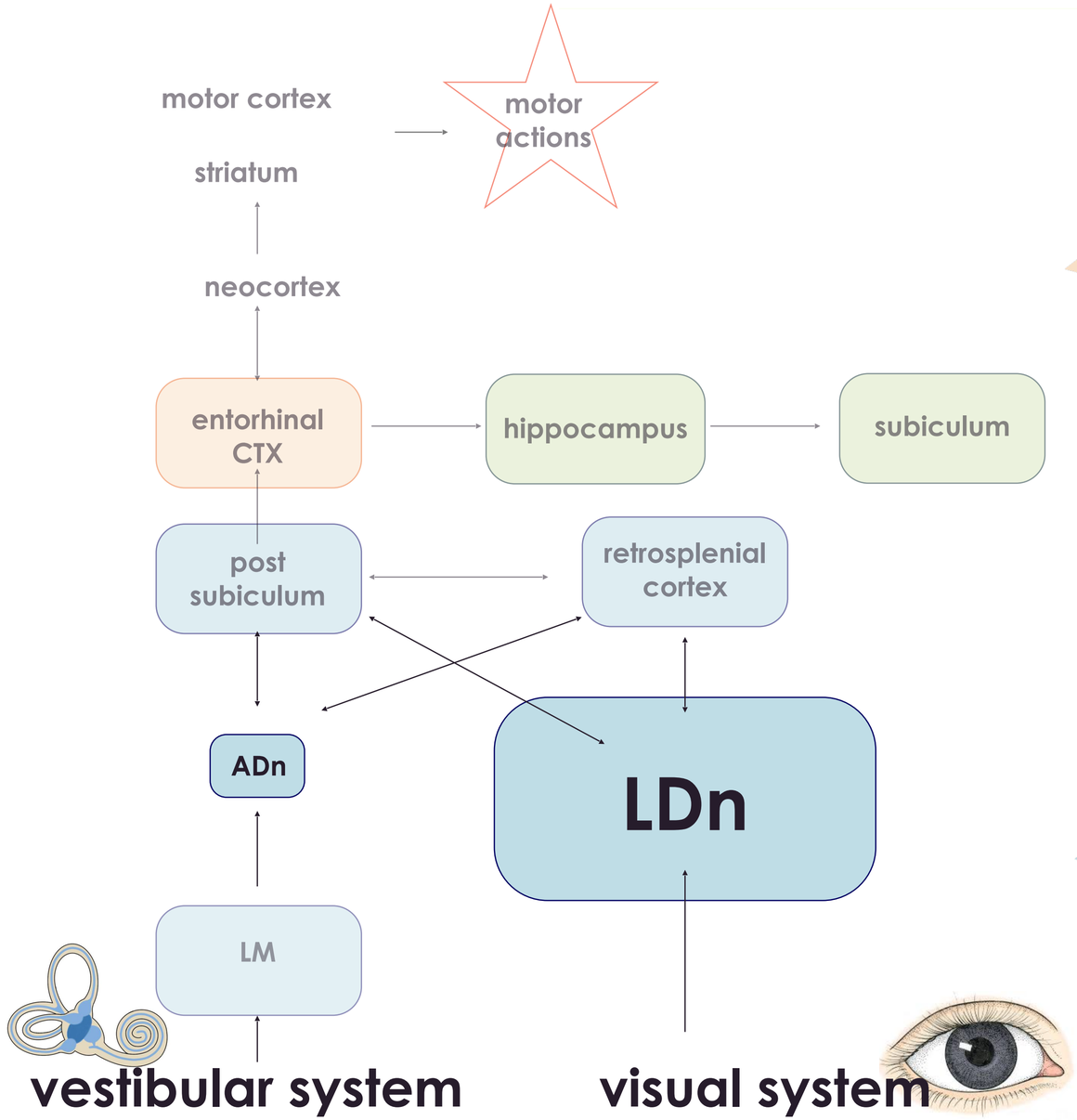
Vetere G, Kenney JW, Tran LM, Xia F, Steadman PE, Parkinson J, Josselyn SA, Frankland PW. **Neuron 2017**
Chemogenetic interrogation of a brainwide fear memory network in mice.

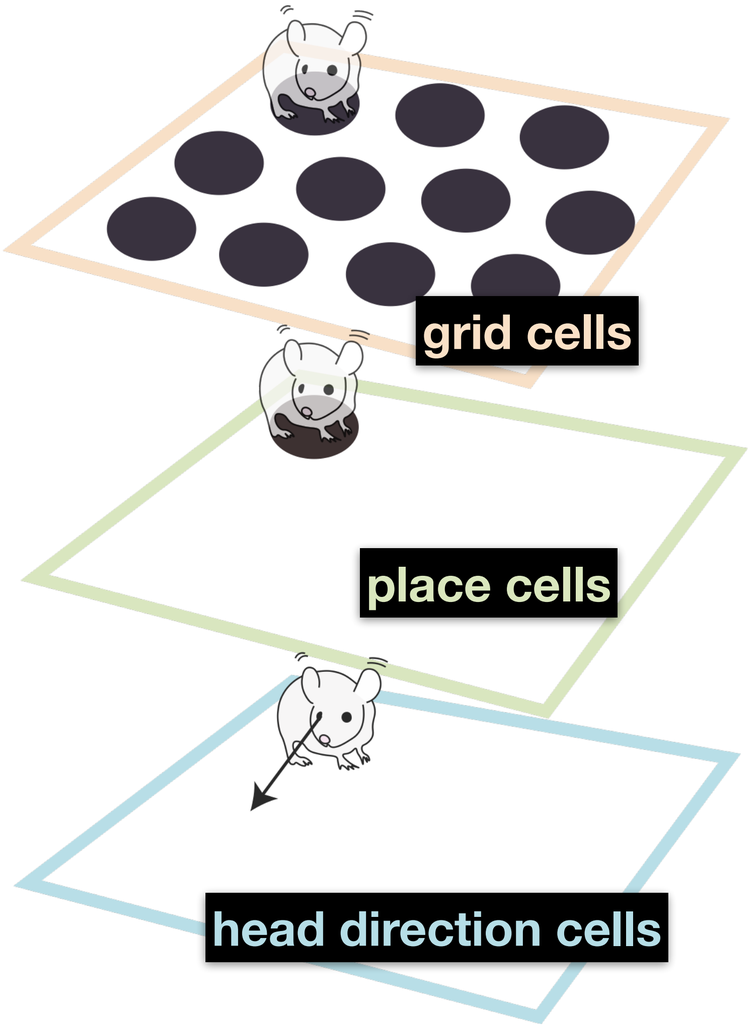
Spatial representation network at **recent** time point





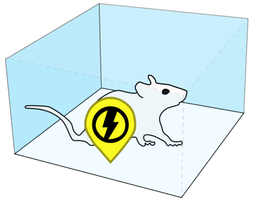
Spatial representation network at **remote** time point



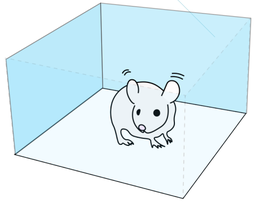


what is the nature of the neuronal ensembles during memory consolidation

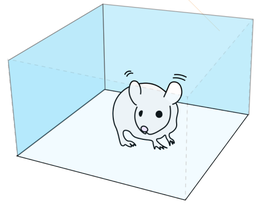
MEMORY ACQUISITION



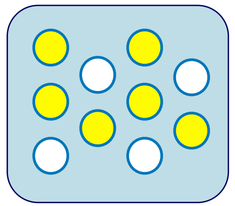
recent memory

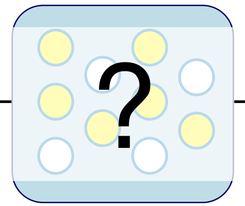


remote memory

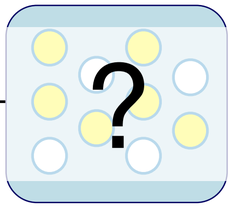


ADn



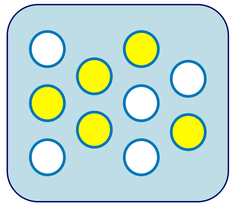


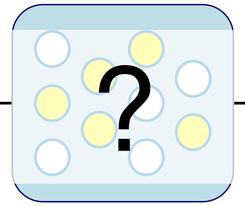
over time…

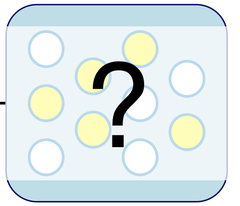




LDn





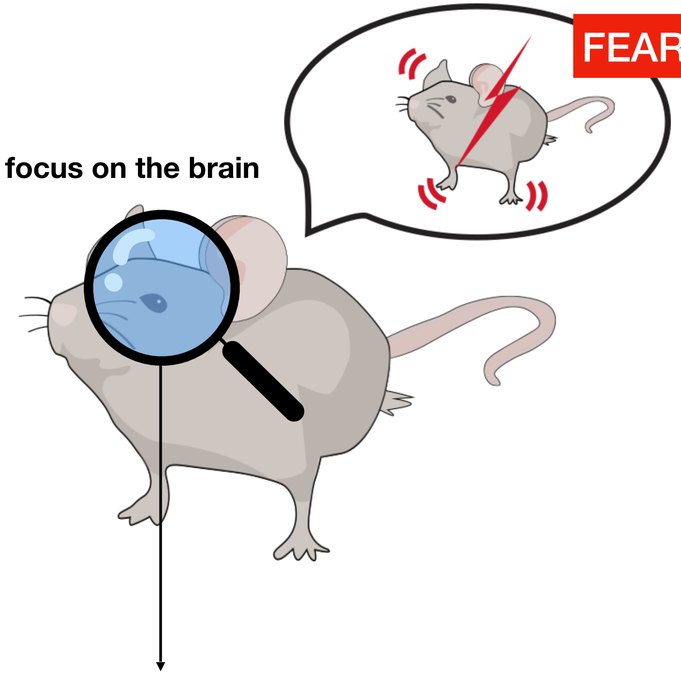




BIG DATA ANALYSIS APPLIED TO BEHAVIOURAL NEUROSCIENCE

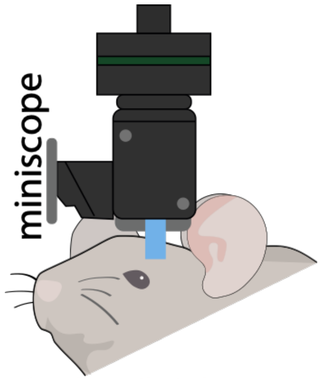
FEAR MEMORY

focus on the brain

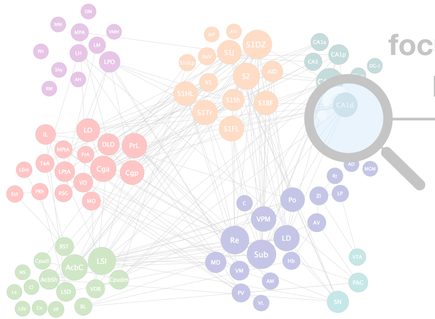


optogenetics

miniscope

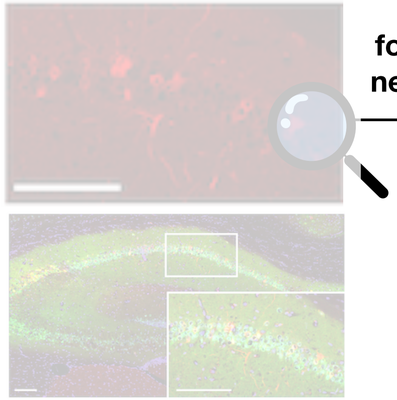


freezing activated neurons



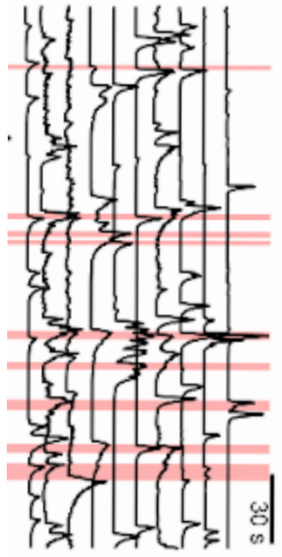
focus on neuronal populations

NETWORK of brain regions

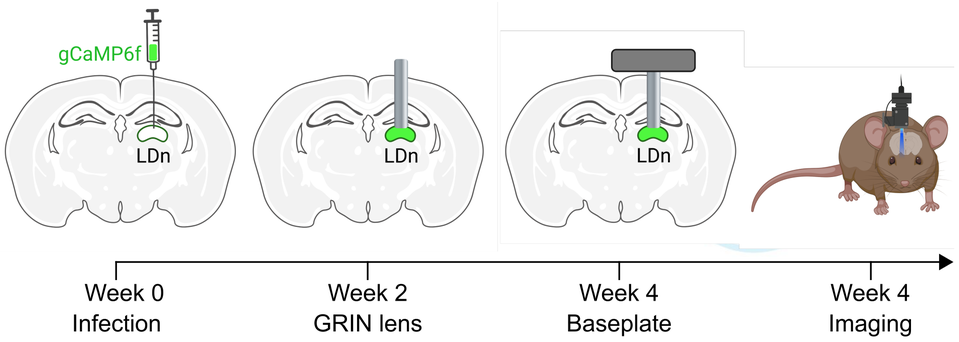


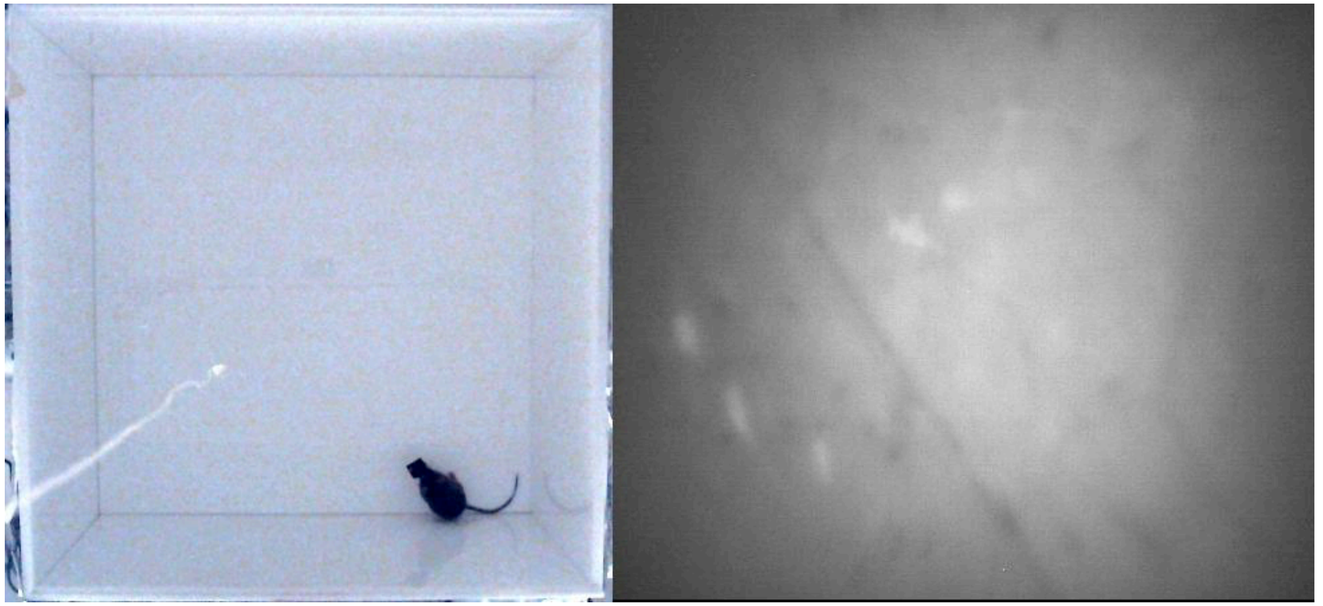
focus on single neurons activity

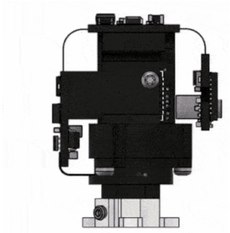
ENGRAM neurons

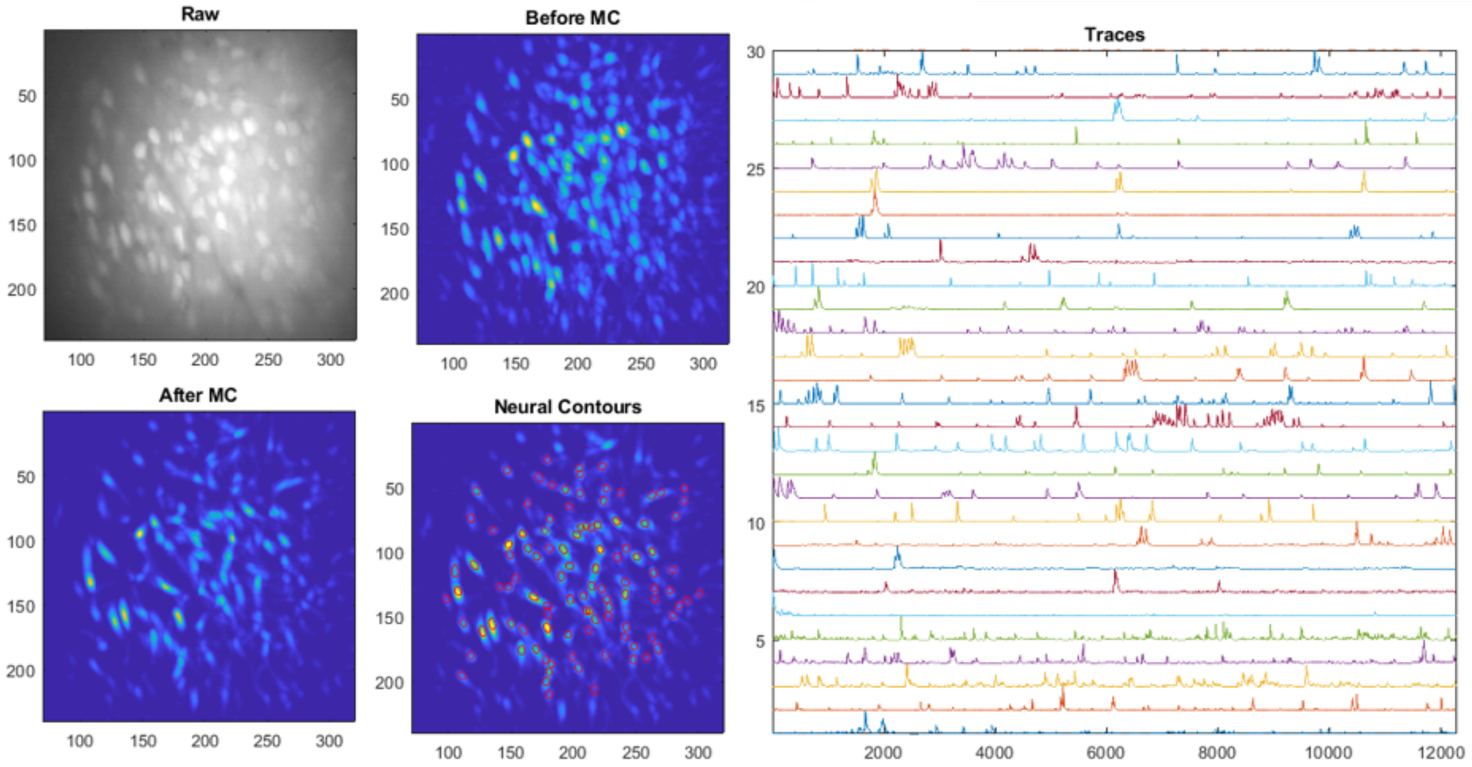


single cells


gCaMP6f
LDn
LDn
LDn
Week 0
Infection
Week 2
GRIN lens
Week 4
Baseplate
Week 4
Imaging



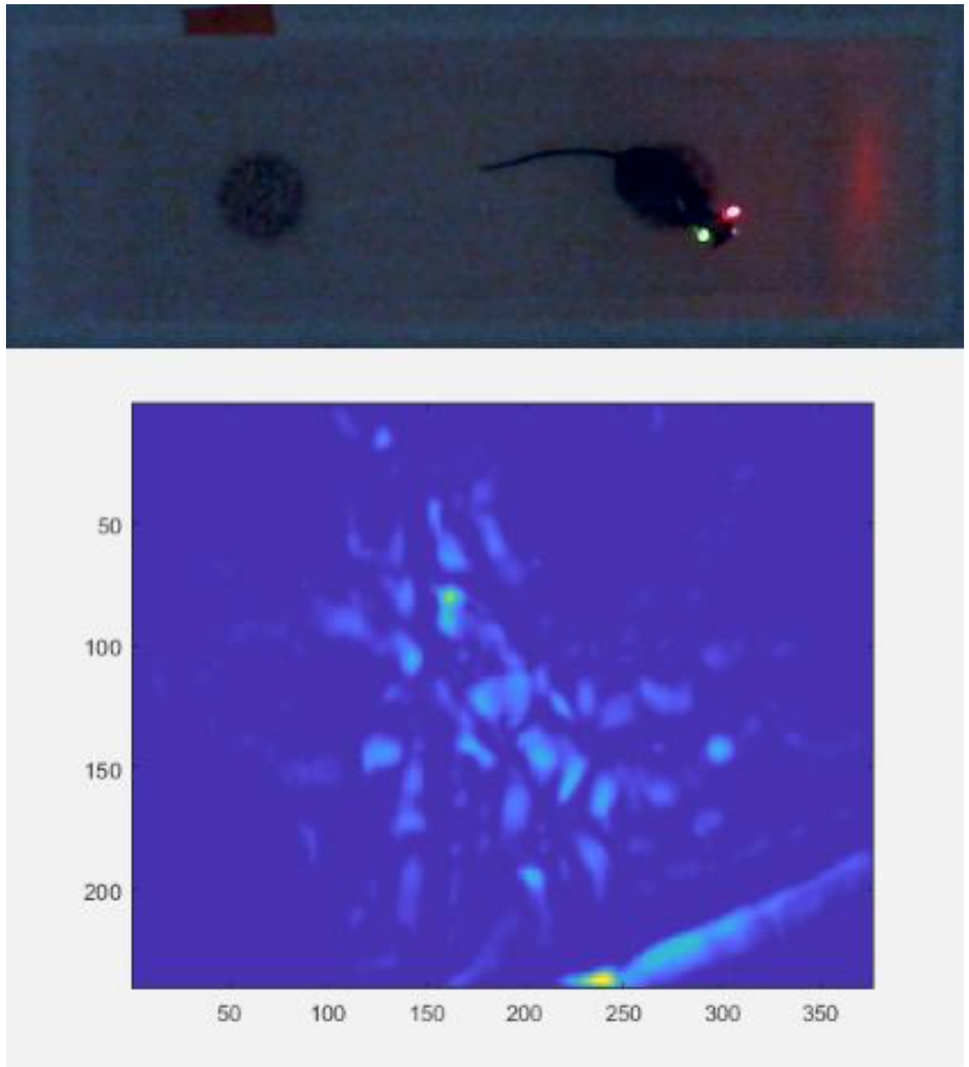


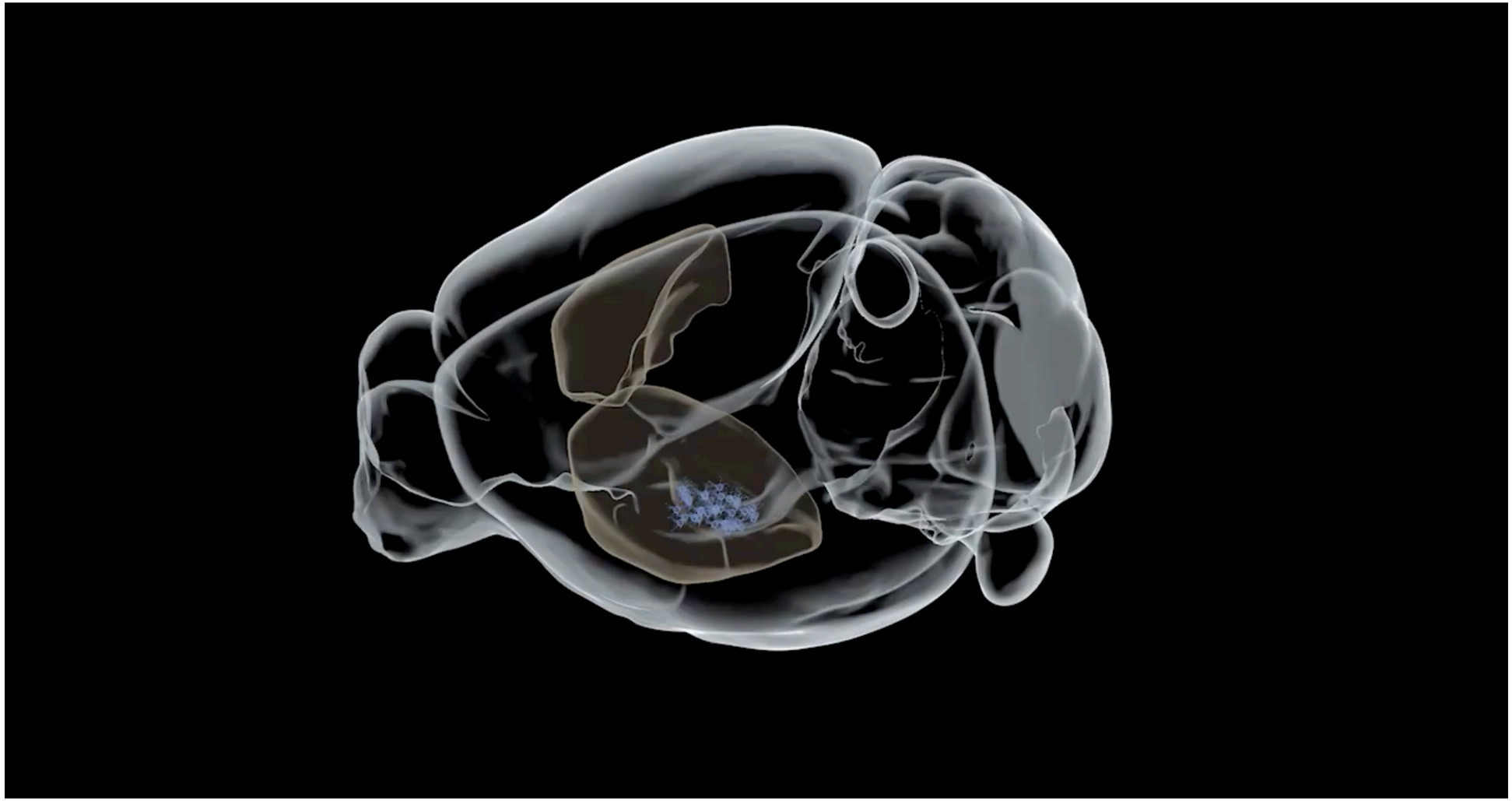

Raw
Before MC
After MC
Neural Contours
Traces
50
100
150
200
100
150
200
250
300
5
10
15
20
25
30
2000
4000
6000
8000
10000
12000

calcium imaging

how the miniscope works

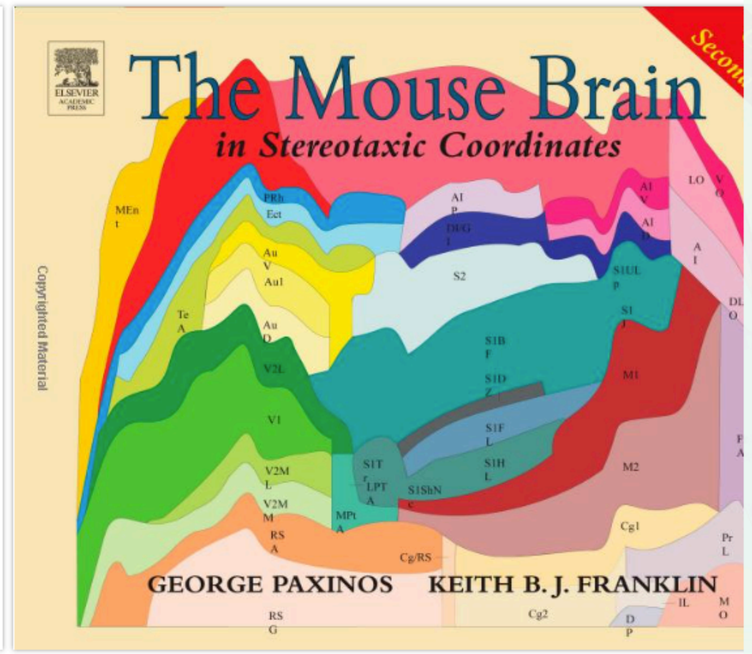
a miniaturized microscope is used for Calcium imaging in freely moving mice

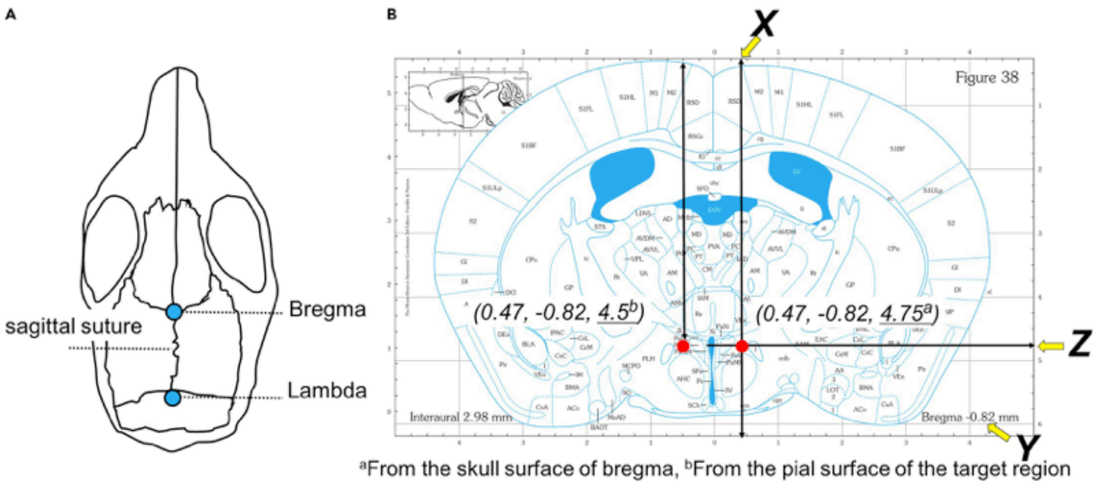


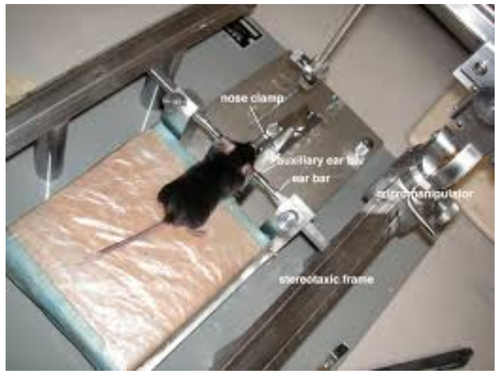


From Inscopix website

Coordinates and Stereotaxic









The miniaturized microscope components

The components of such a miniature microscope overcame cost limitations of table-top lasers and expensive detectors and instead made use of relatively accessible technologies such as :

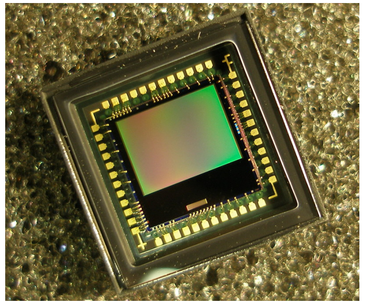
CMOS imaging sensors

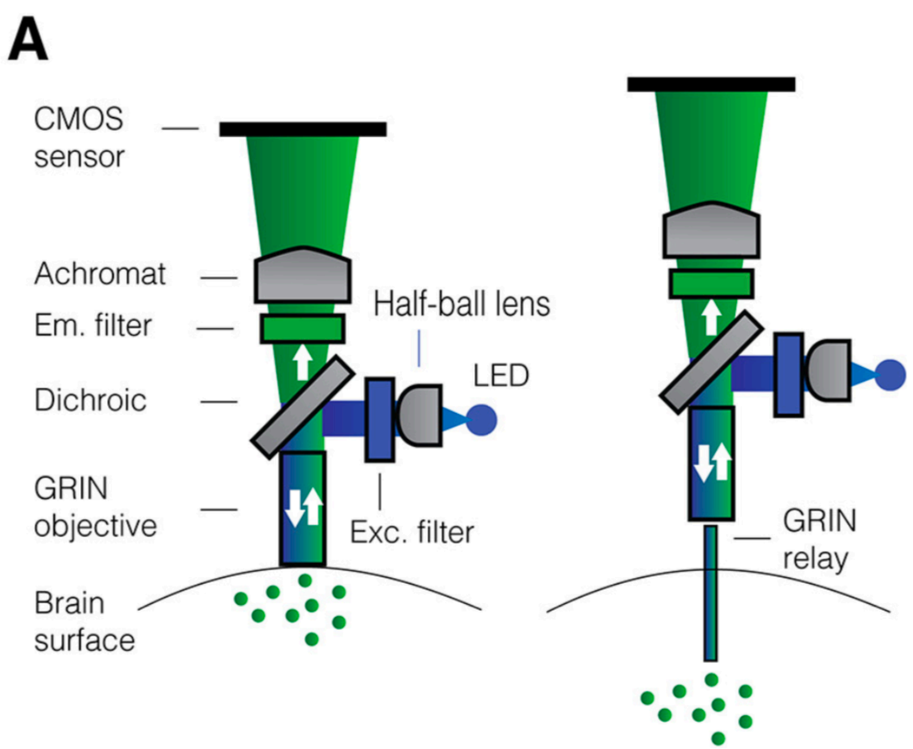
LEDs—as light source—,

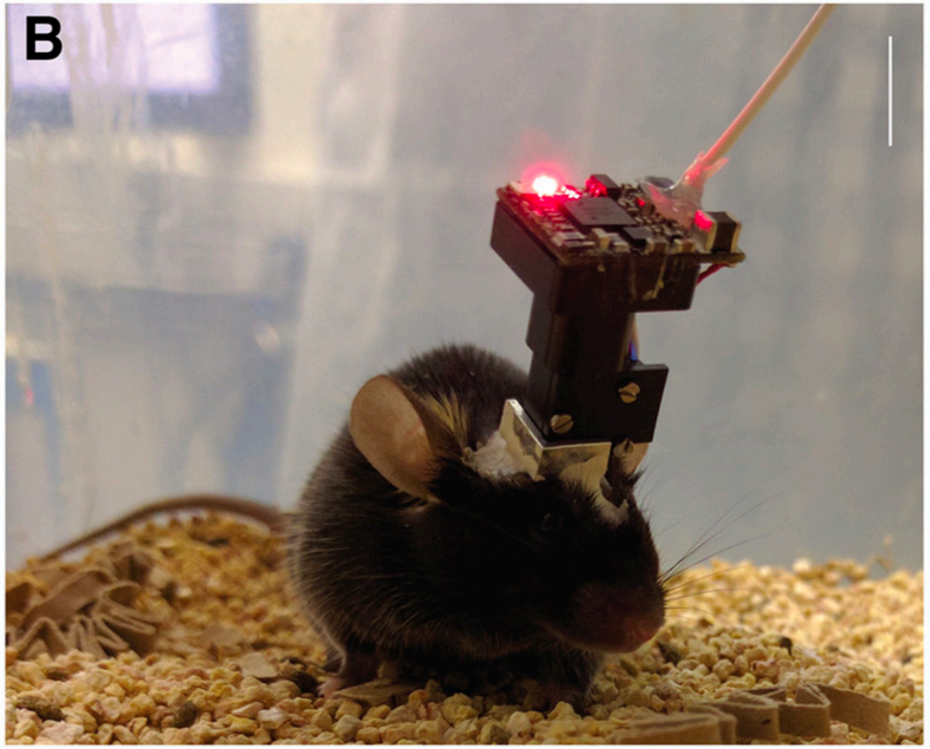
off-the-shelf optical components

Gradient Refractive Index (or GRIN) lenses.

complementary MOS (**CMOS**) APS is An **active-pixel sensor** (**APS**), an image sensor where each pixel sensor unit cell has a photodetector (typically a pinned photodiode) and one or more active transistors.

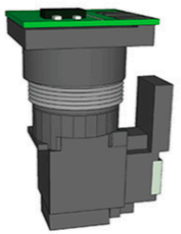






Open source miniscopes released in the public domain

A



FinchScope

Dim: 10 x 6 x 21 mm
Wired: 1.8 gram
Wireless: ~ 4 gram
FOV: 880 x 600 µm
Frame Rate: 30 Hz
Focus: turret
DAQ: Arduino
Software: MacOS

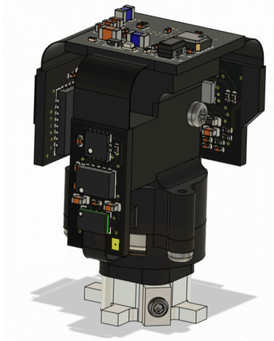
B



miniScope

Dim: 12 x 12 x 20 mm
Wired: 2.4 gram
FOV: 1.1 x 1.1 mm
Frame Rate: 10 Hz
Focus: turret
DAQ: Opal Kelly
Software: Win & Mac

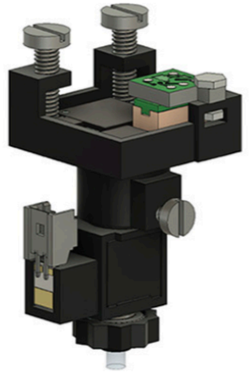
C



UCLA Miniscope

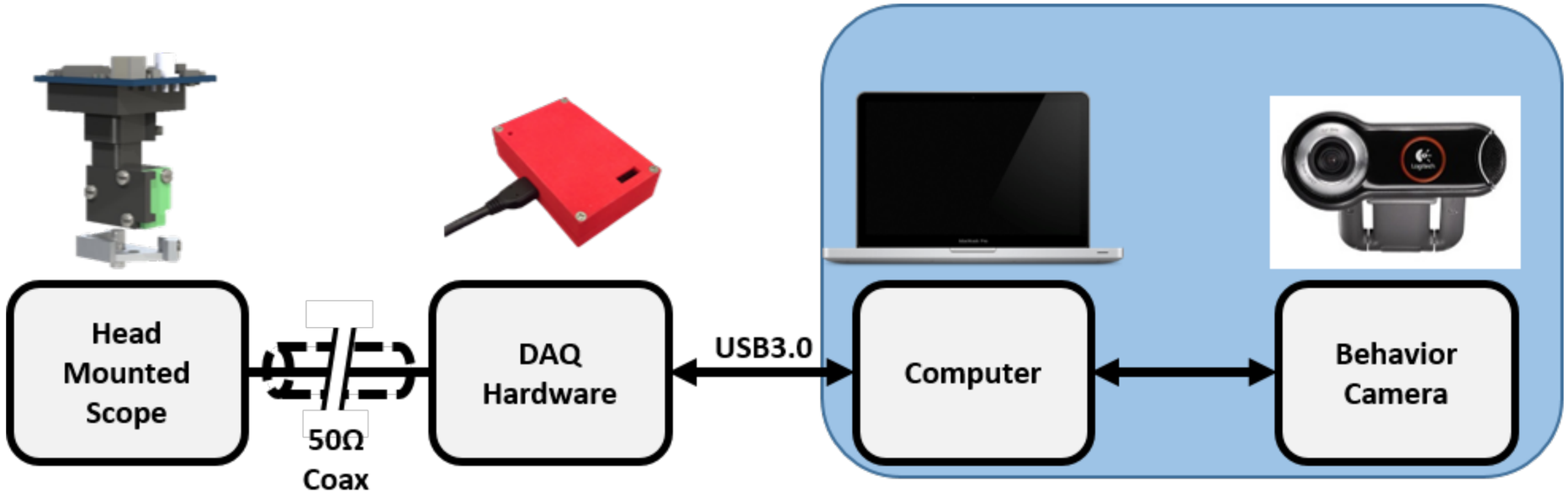
Dim: 16.5 x 13 x 22.5 mm
Wired: ~ 3 gram
Wire-free: 4.5 gram
FOV: 700 x 450 µm
Frame Rate: 60 Hz
Focus: linear slider
DAQ: custom PCB
Software: Win

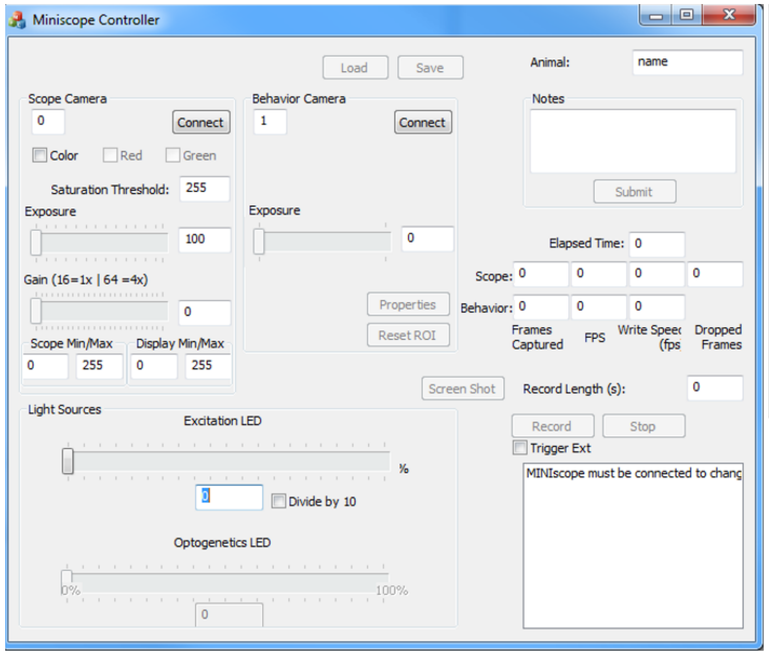
D

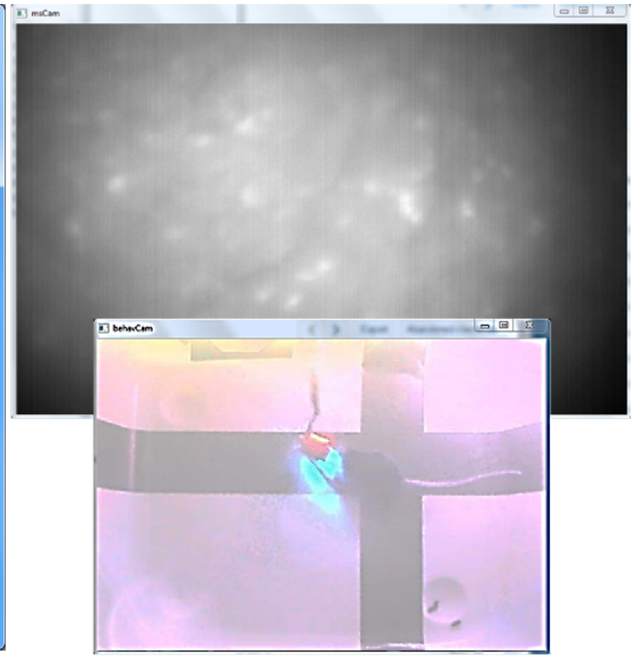


CHEndoscope

Dim: 15.9 x 17 x 32.5 mm
Wired: 4.5 gram
FOV: ~ 500 µm across
Frame Rate: 20 Hz
Focus: turret
DAQ: direct to PC
Software: Win & Linux


Head Mounted Scope
50Ω Coax
DAQ Hardware
USB3.0
Computer
Behavior Camera


Miniscope Controller
Load
Save
Animal:
name
Scope Camera
Connect
Behavior Camera
Connect
Notes
Color
Red
Green
Saturation Threshold:
255
Submit
Exposure
Exposure
Elapsed Time:
Gain (16=1x | 64 =4x)
Properties
Reset ROI
Scope:
Behavior:
Frames Captured
FPS
Write Speed (fps
Dropped Frames
Scope Min/Max
Display Min/Max
Screen Shot
Record Length (s):
Light Sources
Excitation LED
Record
Stop
Trigger Ext
Divide by 10
MINIscope must be connected to chang
Optogenetics LED
0%
100%


msCam
behavCam

Article

Neuron

Anxiety Cells in a Hippocampal-Hypothalamic Circuit

Highlights

- Anxiogenic stimuli are differentially represented along the DV axis of the HPC
- Inhibition of the vHPC in anxiogenic environments reduces avoidance behavior
- vCA1 outputs to LHA but not BA control anxiety-related behavior
- The majority of vCA1-LHA projection neurons represent anxiogenic stimuli

Authors

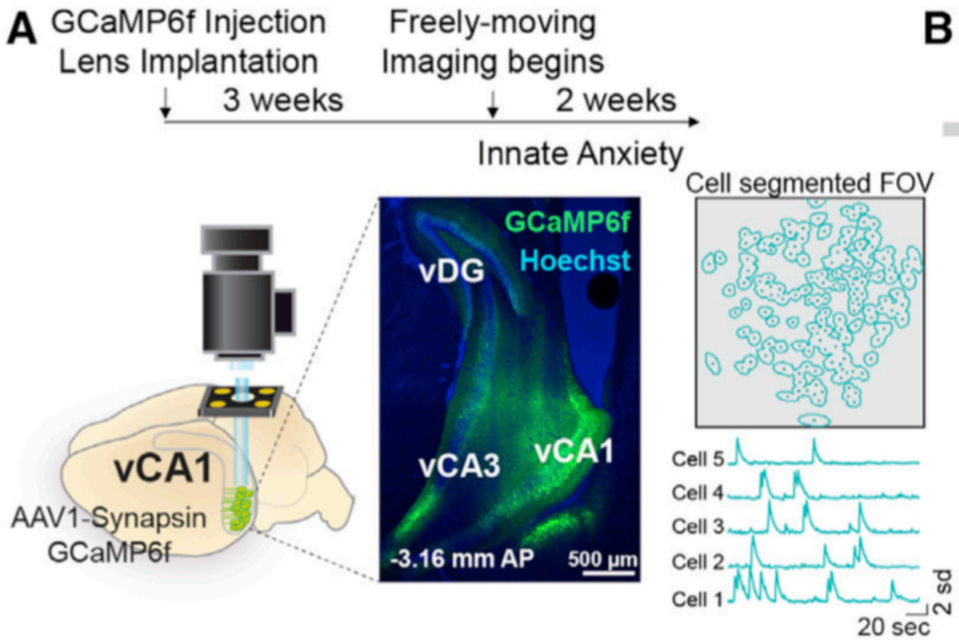
Jessica C. Jimenez, Katy Su, Alexander R. Goldberg, ..., Liam Paninski, René Hen, Mazen A. Kheirbek

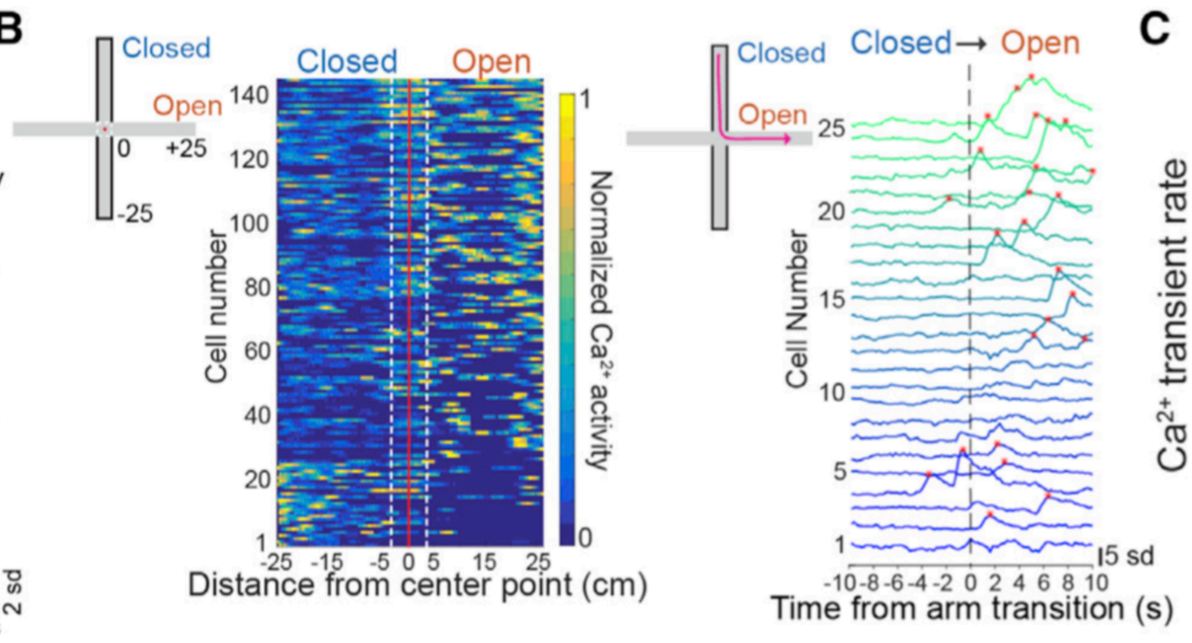
Correspondence

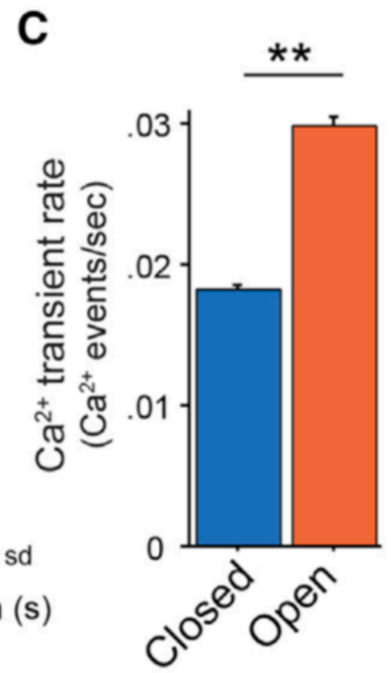
rh95@columbia.edu (R.H.), mazen.kheirbek@ucsf.edu (M.A.K.)

In Brief

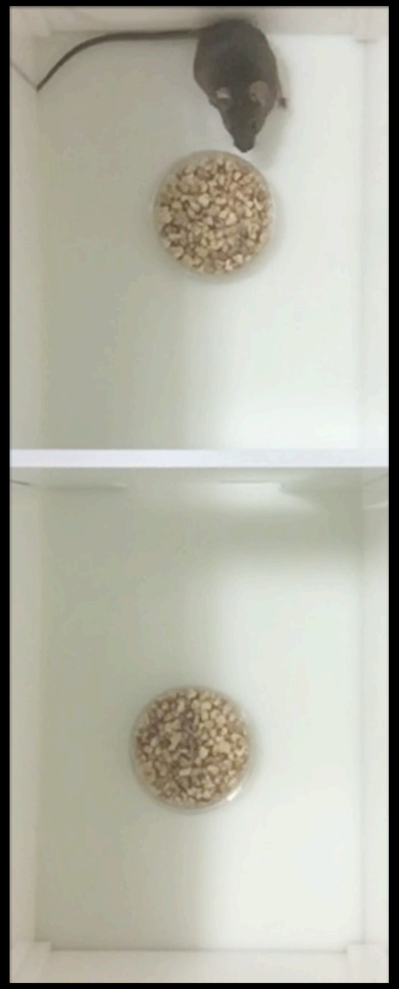
Jimenez et al. show that vCA1 neurons exhibit stable representations of anxiogenic environments that are required for avoidance behavior. The direct projection from vCA1 to the lateral hypothalamus is enriched in anxiety cells and can rapidly control anxiety-related behavior.


A
GCaMP6f Injection
Lens Implantation
3 weeks
Freely-moving
Imaging begins
2 weeks
Innate Anxiety
vCA1
AAV1-Synapsin
GCaMP6f
GCaMP6f
Hoechst
vDG
vCA3
vCA1
-3.16 mm AP
500 µm
Cell segmented FOV
Cell 5
Cell 4
Cell 3
Cell 2
Cell 1
2 sd
20 sec

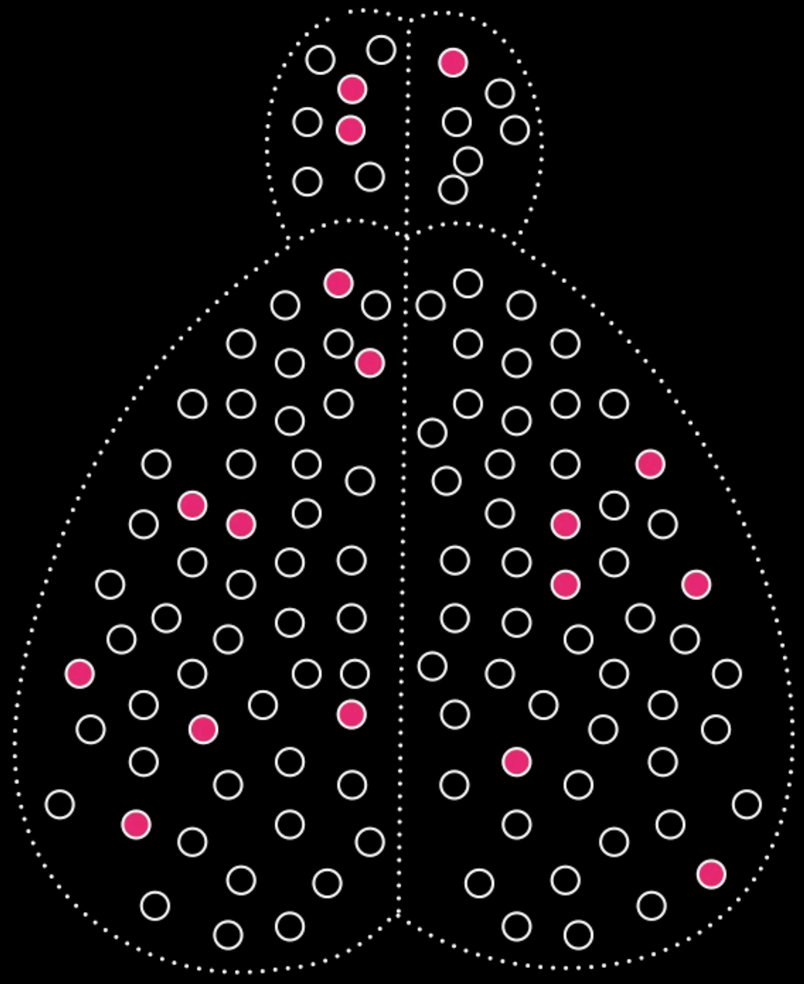

B
Closed
Open
0
+25
-25
Closed
Open
Cell number
140
120
100
80
60
40
20
1
-25 -15 -5 0 5 15 25
Distance from center point (cm)
Normalized Ca2+ activity
1
0
Closed
Open
Closed → Open
Cell Number
25
20
15
10
5
1
-10 -8 -6 -4 -2 0 2 4 6 8 10
Time from arm transition (s)
5 sd


C
**
Ca2+ transient rate
(Ca2+ events/sec)
.03
.02
.01
0
Closed
Open

Correlate…

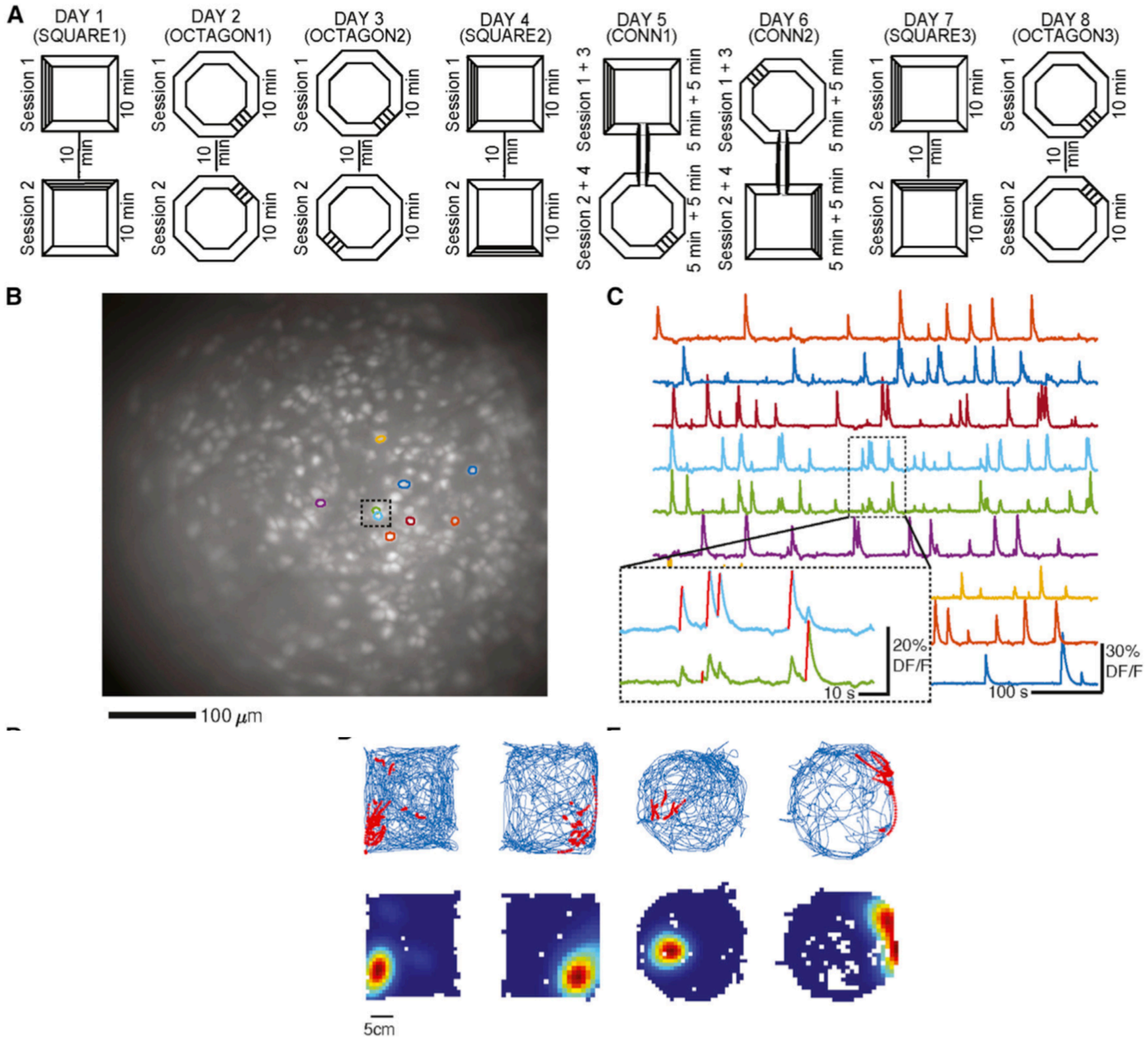


behaviour

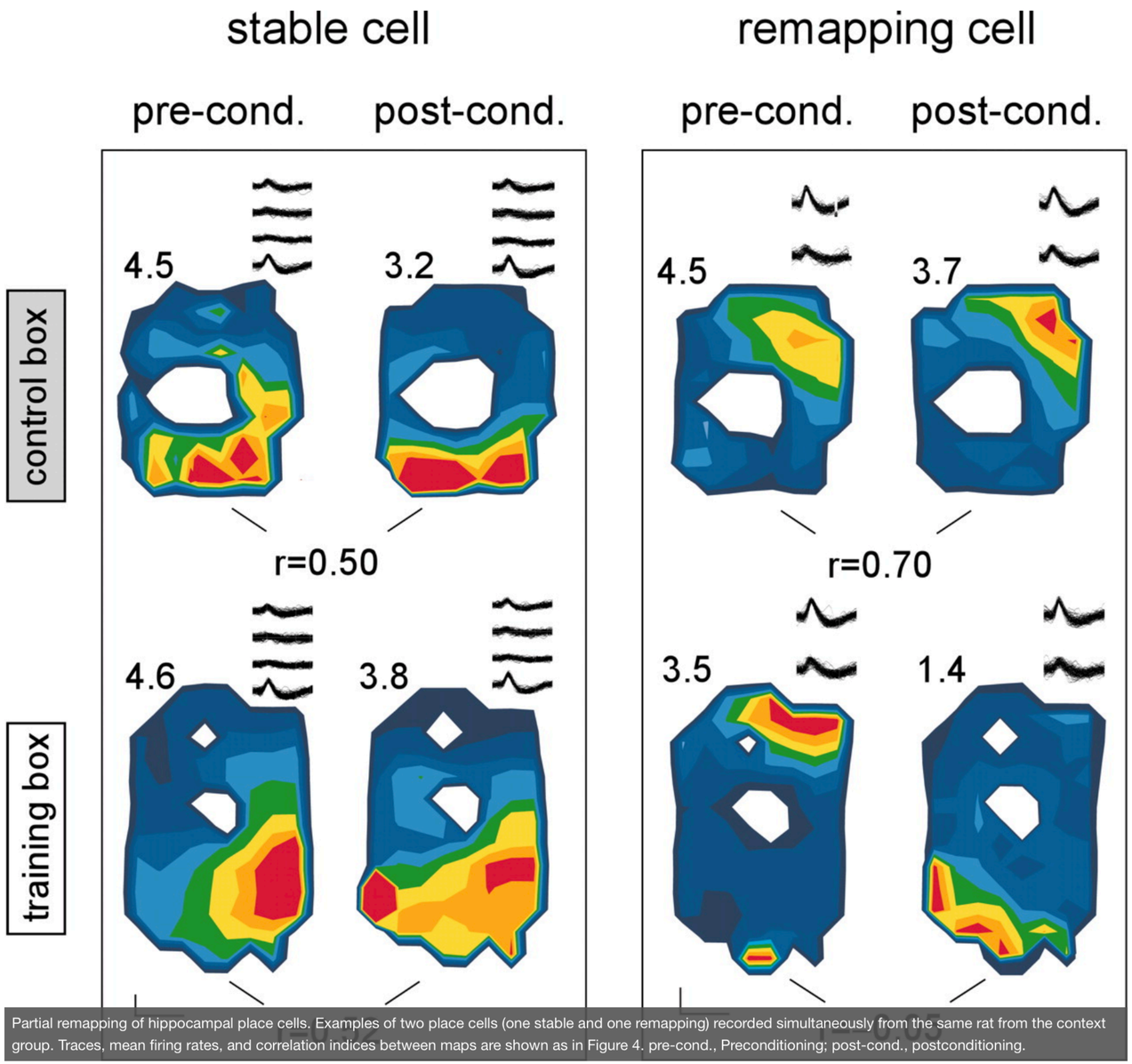


neuronal activity

example of paper showing the place cells in miniscope studies

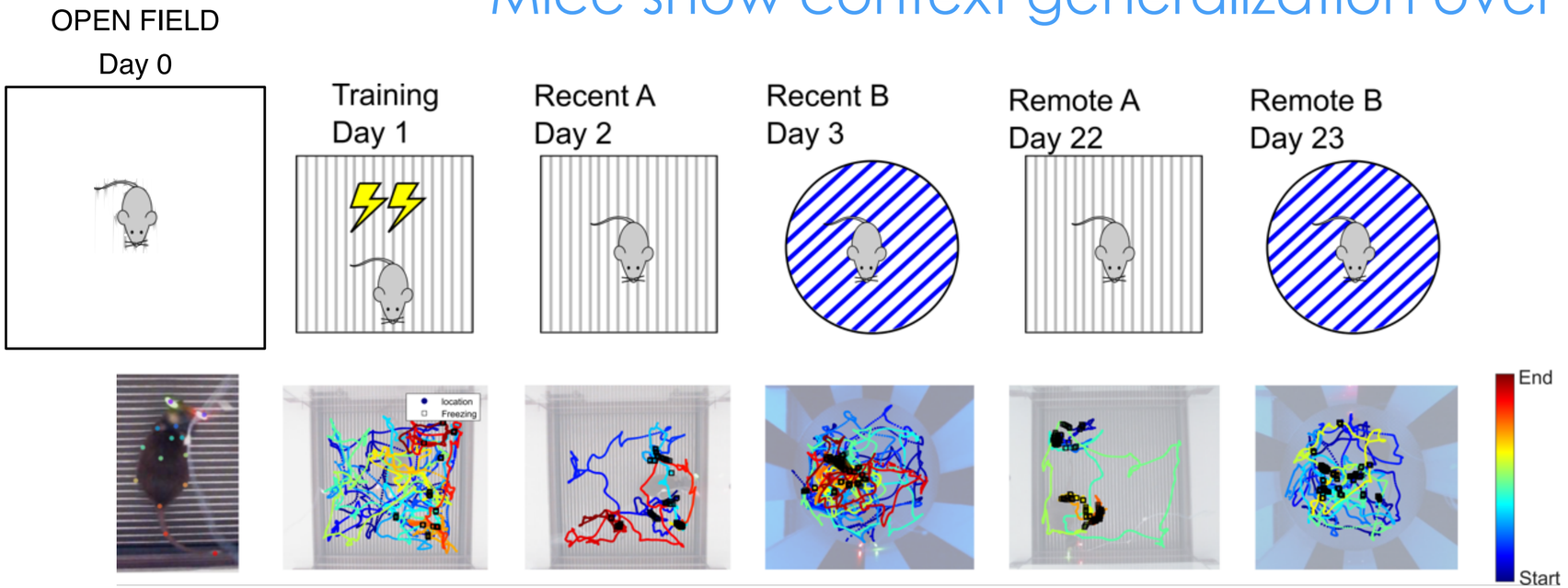


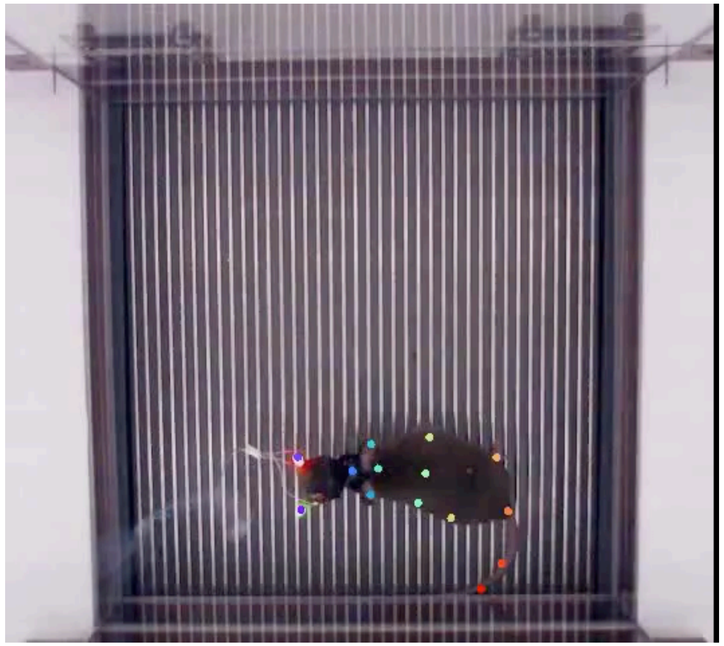
Putting fear in its place: remapping of hippocampal place cells during fear conditioning

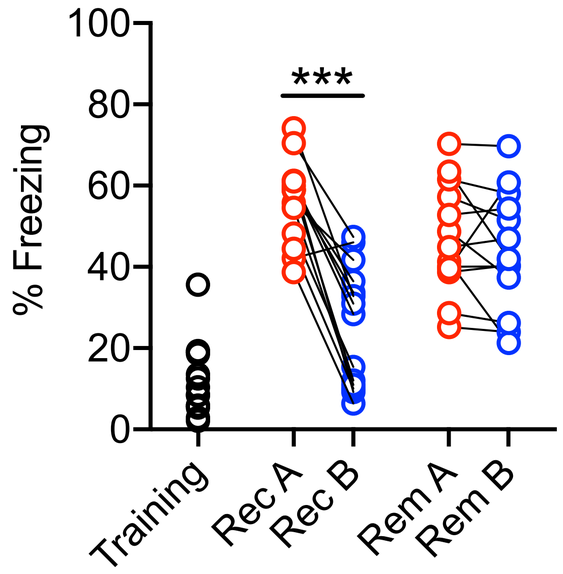


Partial remapping of hippocampal place cells. Examples of two place cells (one stable and one remapping) recorded simultaneously from the same rat from the context group. Traces, mean firing rates, and correlation indices between maps are shown as in Figure 4. pre-cond., Preconditioning; post-cond., postconditioning.

Mice show context generalization over time

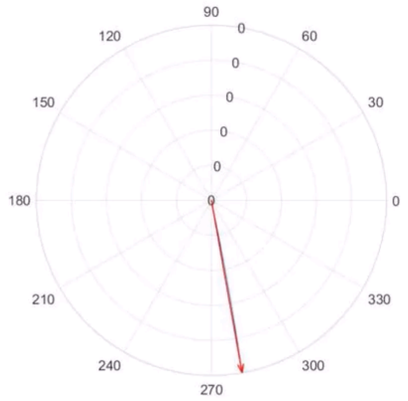




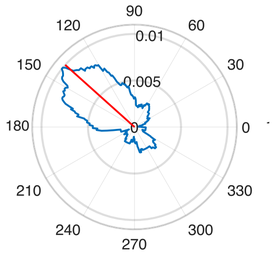


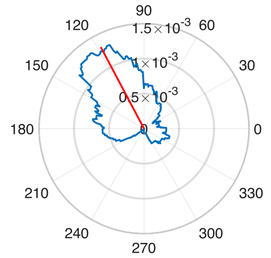
Identify cells encoding spatial information: Head direction cells

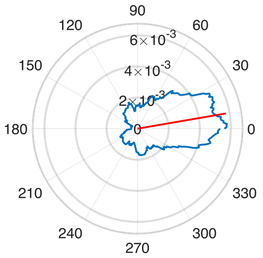




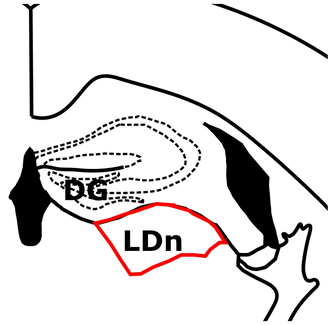
Mouse Head direction

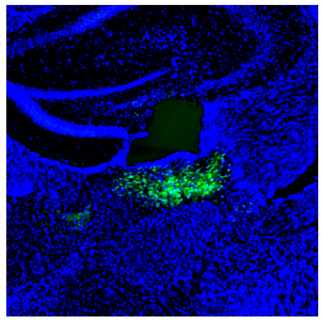


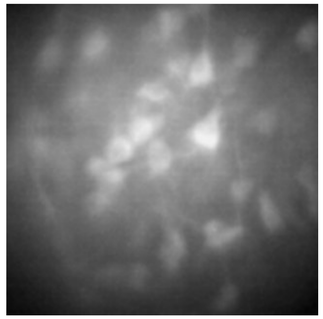




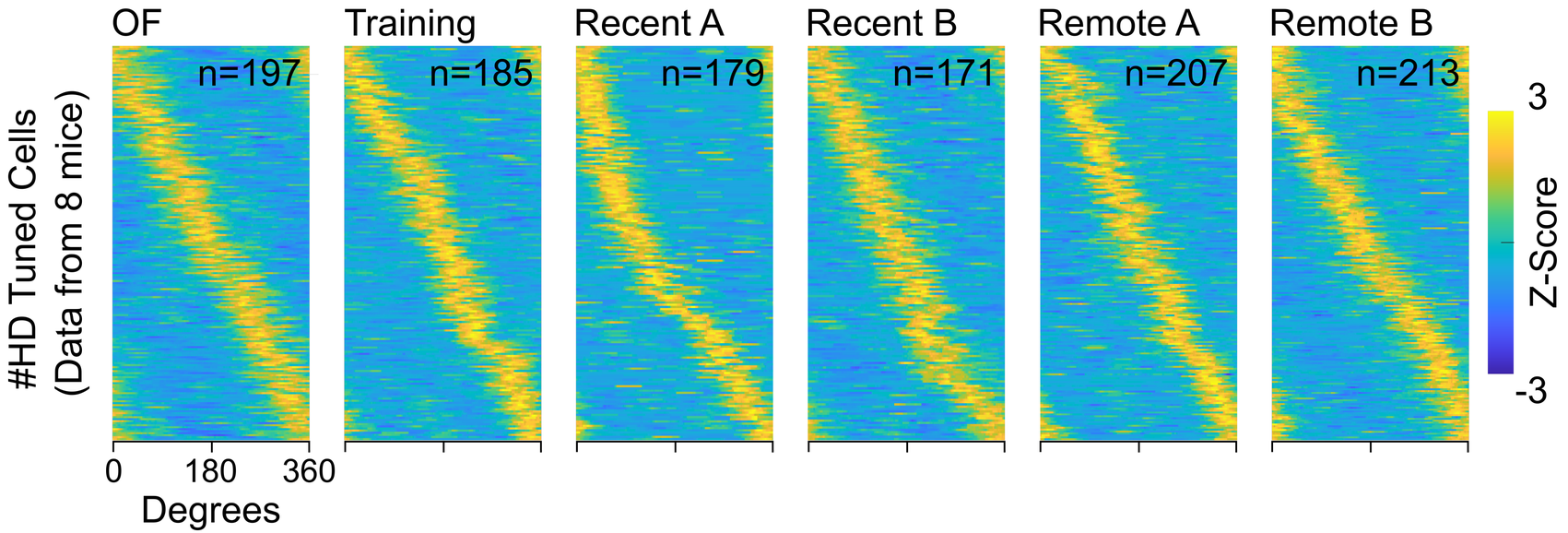
examples of head direction cells







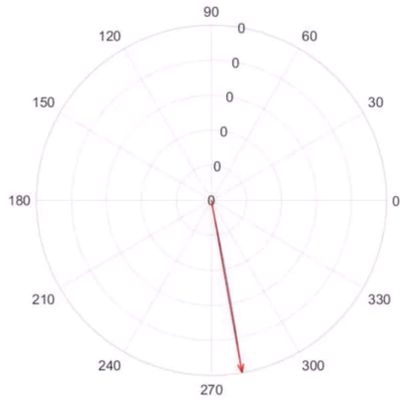
Laterodorsal nucleus of the Thalamus (LDn)



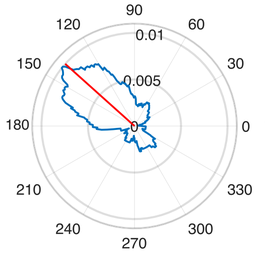


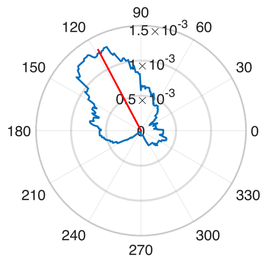
Identify cells encoding spatial information: Head direction cells

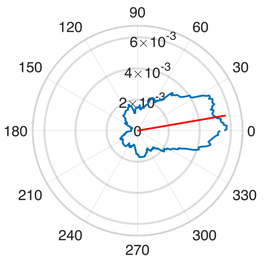




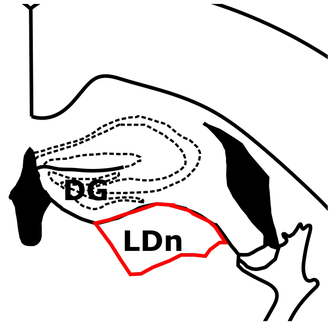
Mouse Head direction

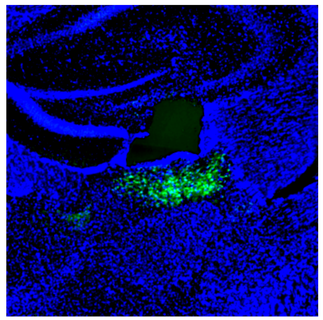


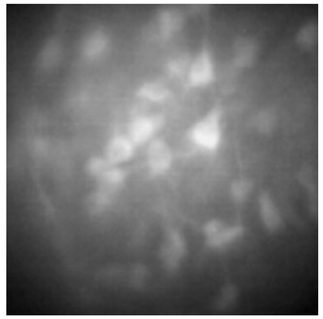




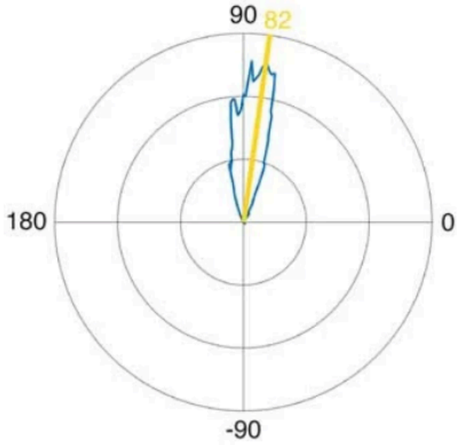
examples of head direction cells

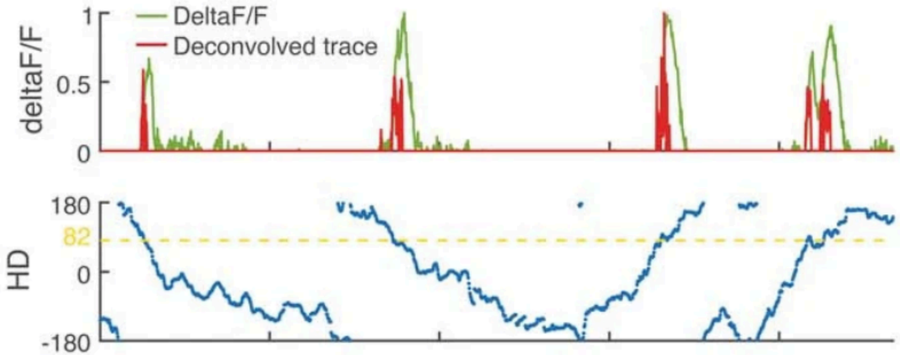






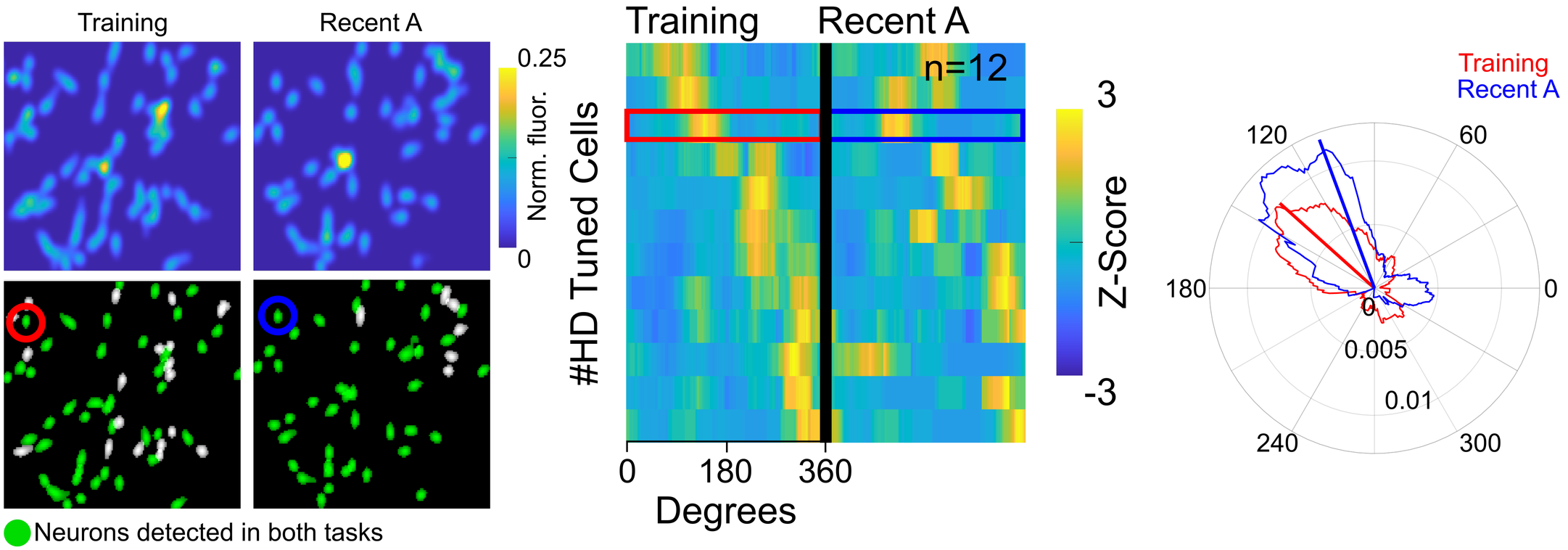
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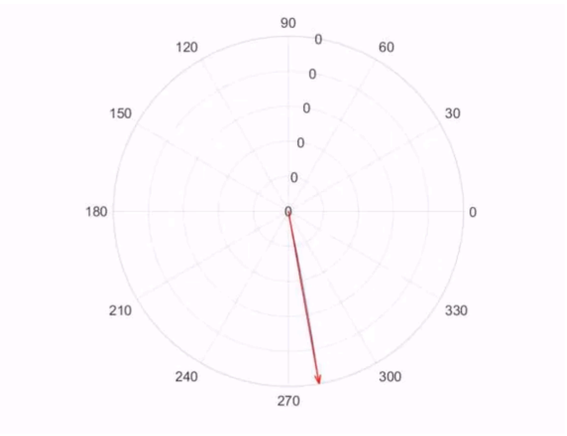
Ajabi et al, Nature 2023

C4TEAM

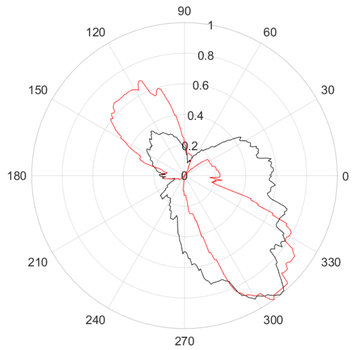

Training
Recent A
0.25
Norm. fluor.
0
Neurons detected in both tasks
Training
Recent A
n=12
#HD Tuned Cells
0
180
360
Degrees
3
Z-Score
-3
Training
Recent A
120
60
180
0
240
300
0
0.005
0.01

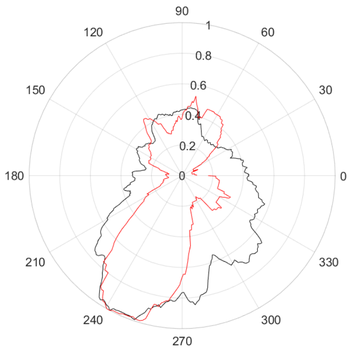
Identify cells encoding spatial information: Head direction cells

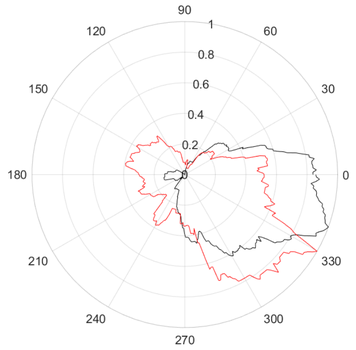




Mouse Head direction





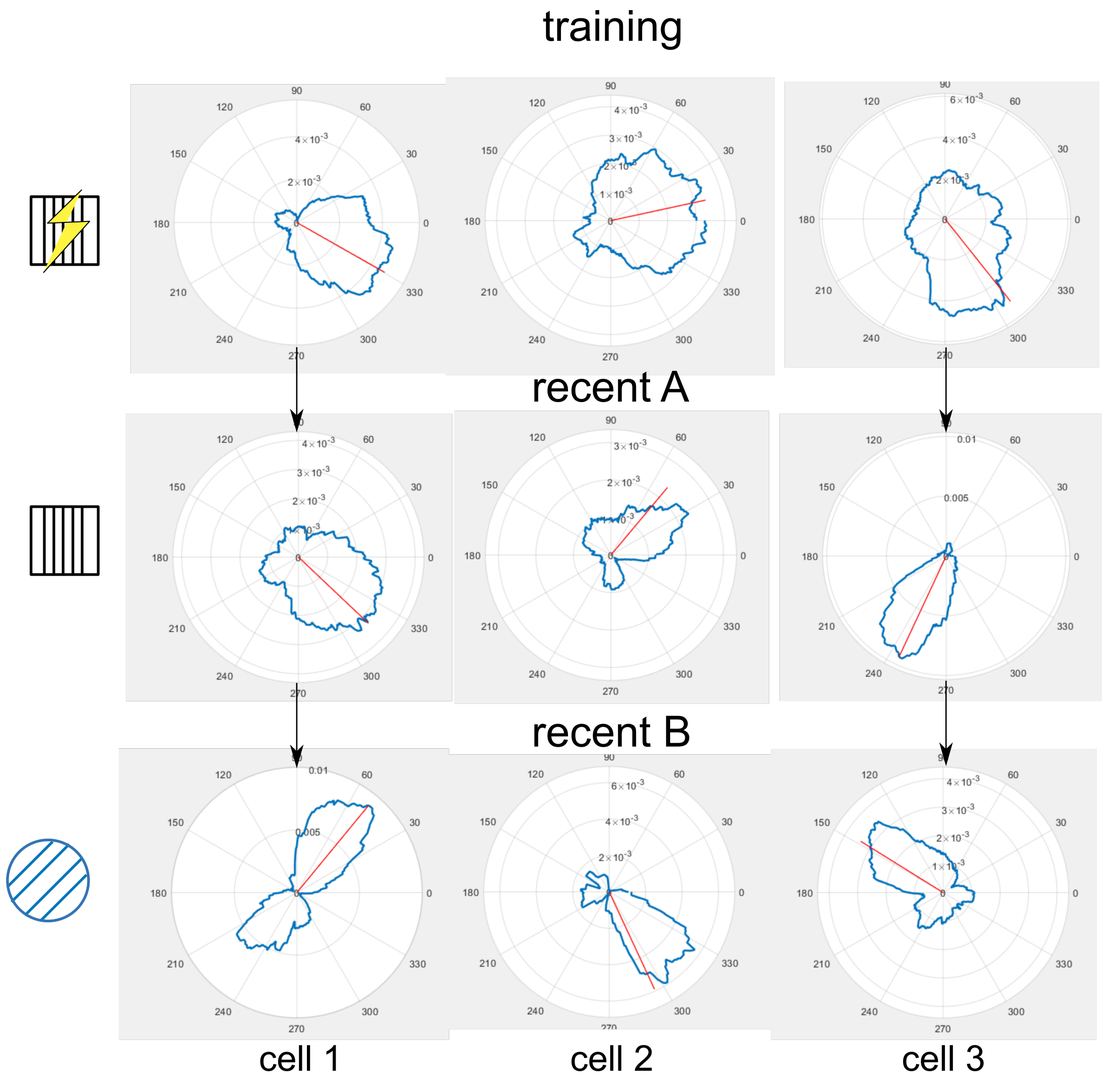


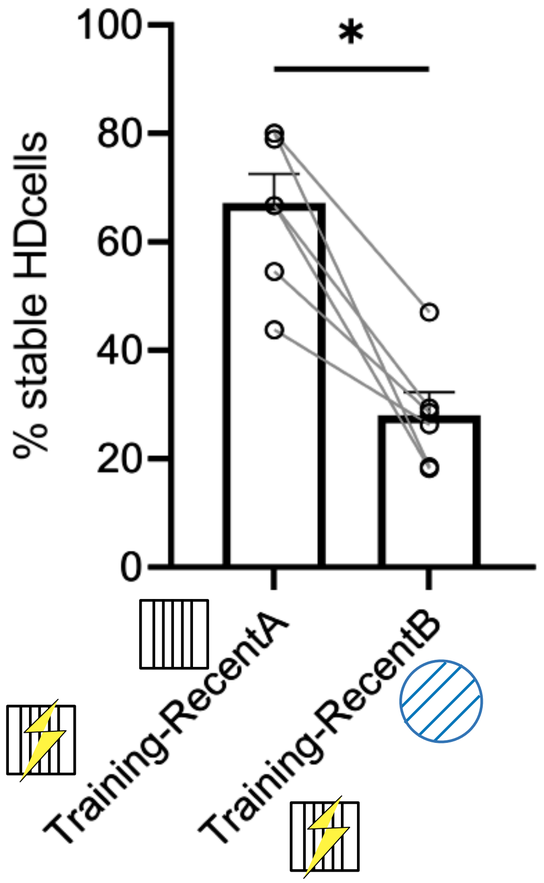
training

recent CtxA

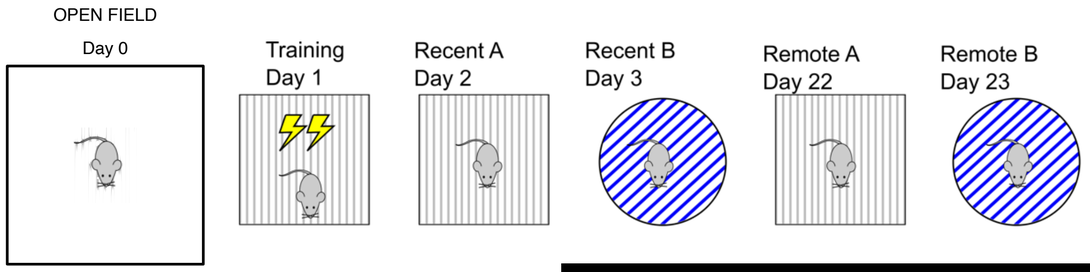
Head direction (HD) cells

majority of identified HDc change their orientation in a different context at recent time point



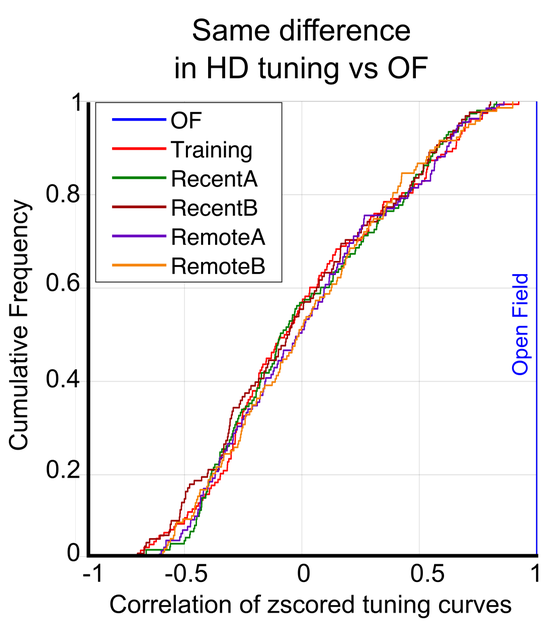


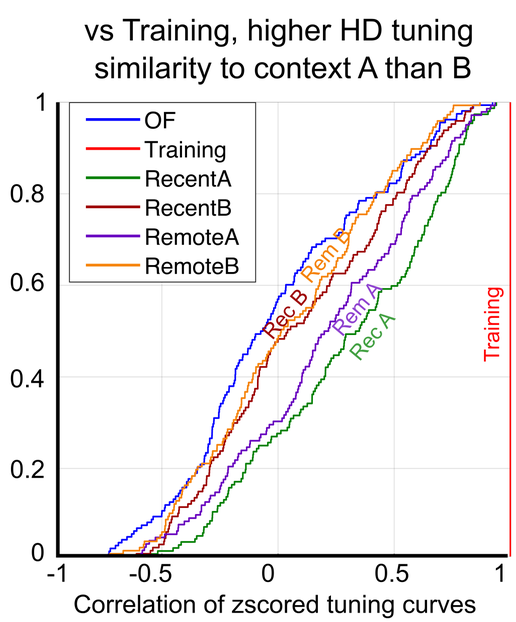
Head direction cells stability over time

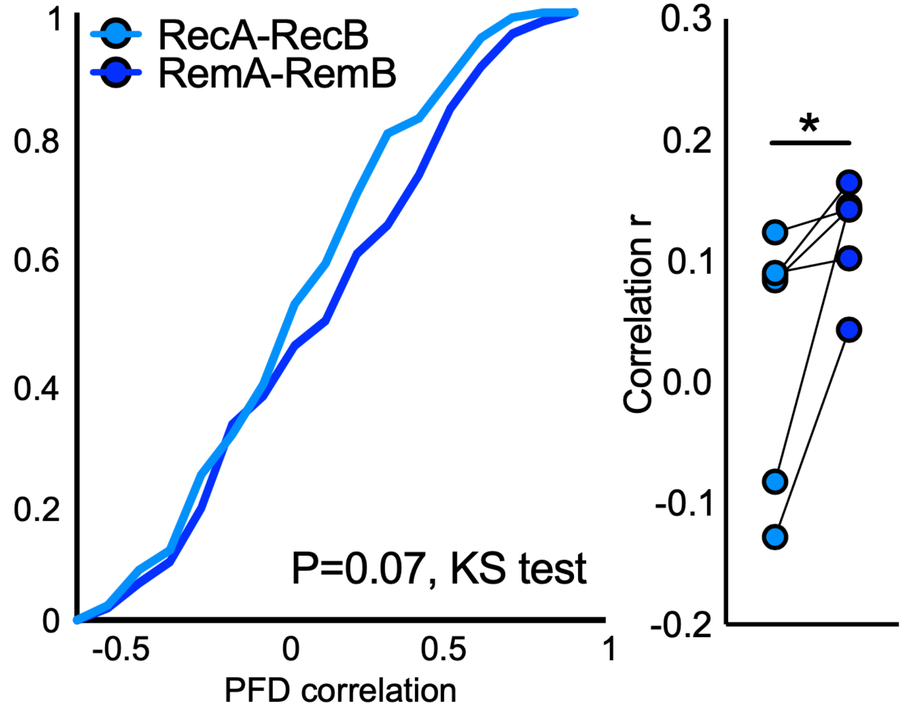


similar contexts have higher correlation HD tuning

OF is the most different context

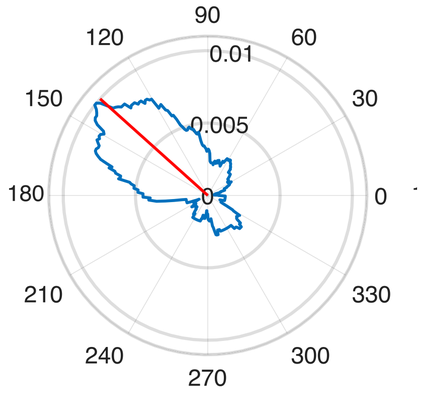


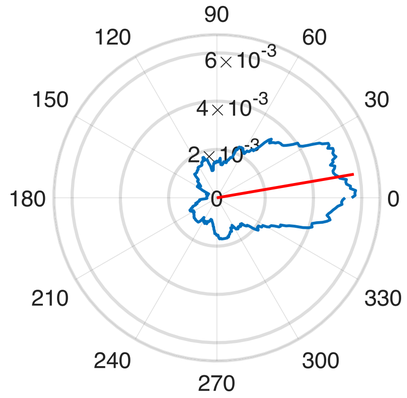


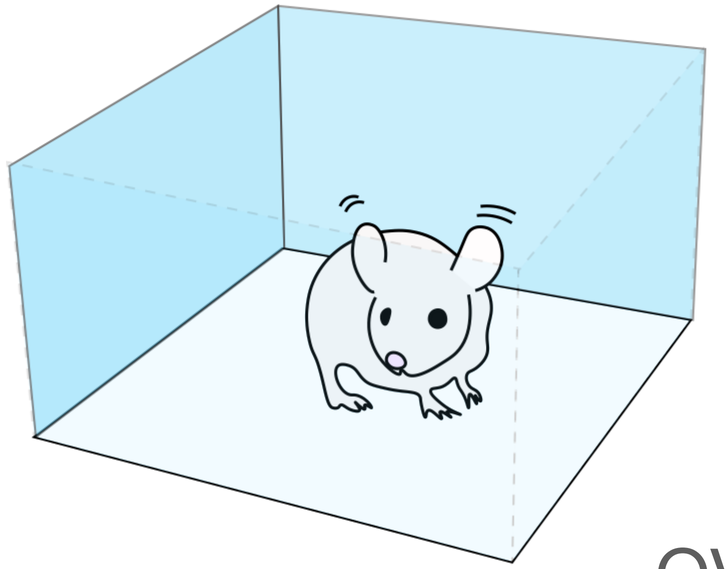


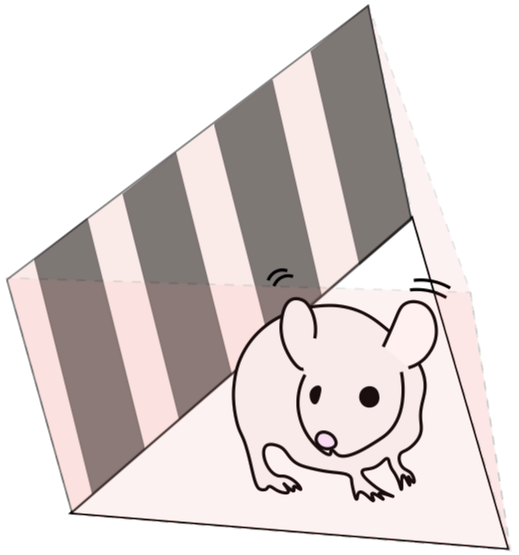
AT A REMOTE TIME POINT, HD tuning of DIFFERENT CONTEXTS CORRELATE MORE

RELATED TO THE GENERALIZATION PROCESS?



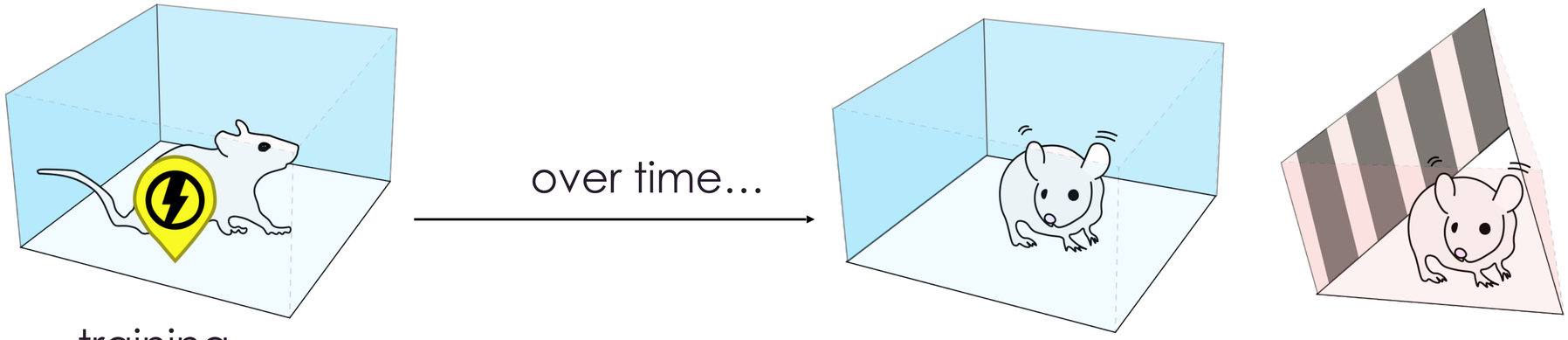






OVER TIME….

MEMORY OVER TIME LOSE SPATIAL DETAILS

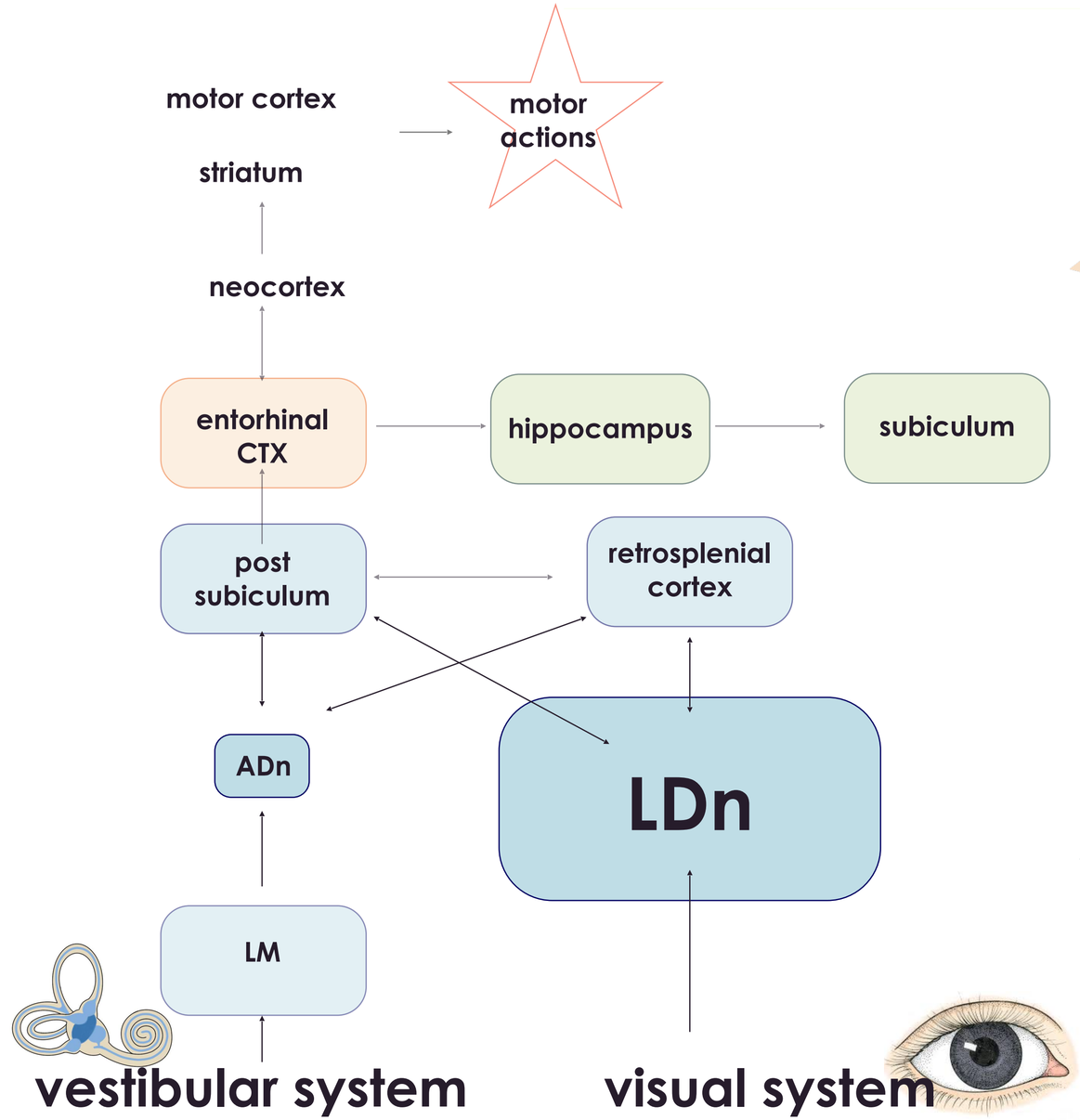


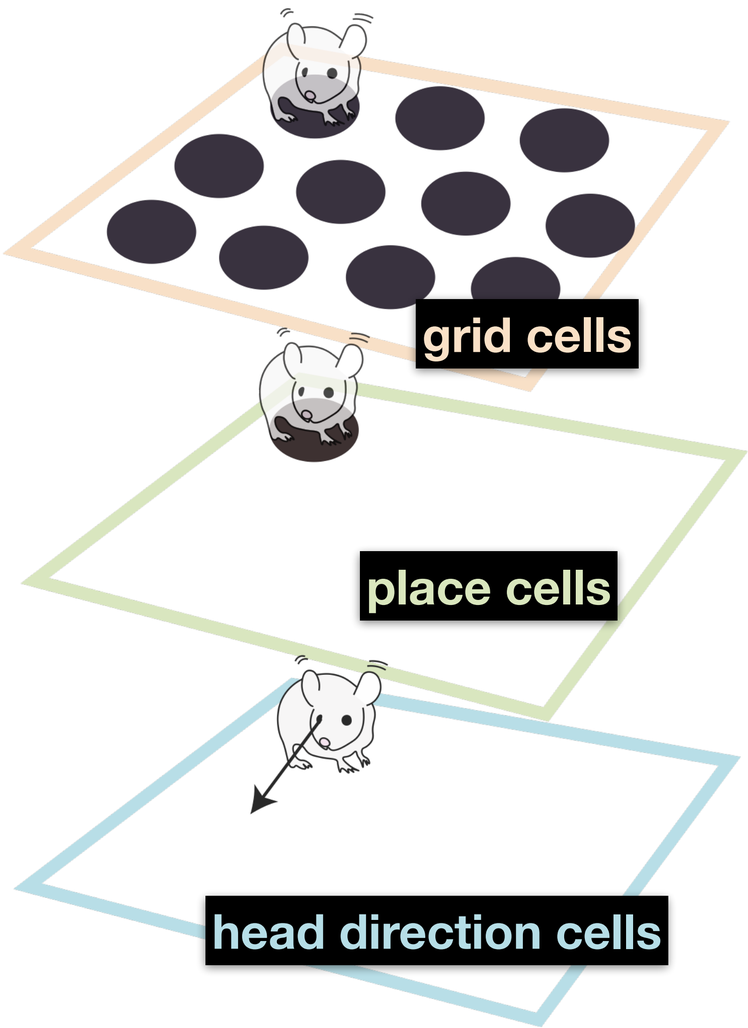
Does memory consolidation change our perception of the space?

Head direction representation of different contexts changes over time

Can it be related to generalization?

Spatial representation network at **remote** time point





Summary

ADn and LDn may play a disproportional role at different time point during memory consolidation

ADn

ADn is actively inactivated at a remote time point





QUICK AND EFFICIENT RECALL
OF THE FEAR MEMORY

remote

LDn

LDn is has a crucial role for remote memory recall





RELATED TO FEAR GENERALIZATION OF MEMORIES
AT A REMOTE TIME POINT?

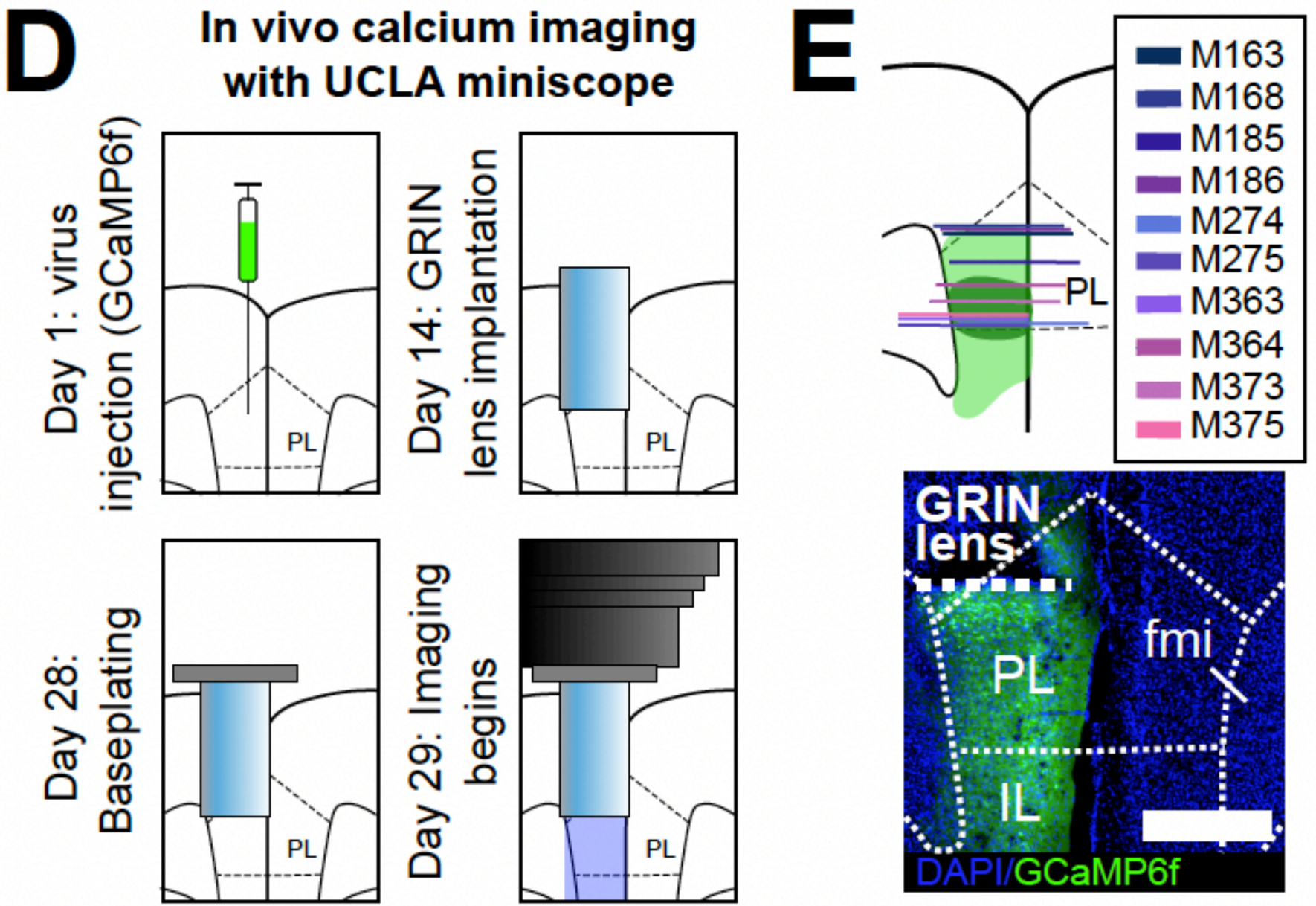
take home message

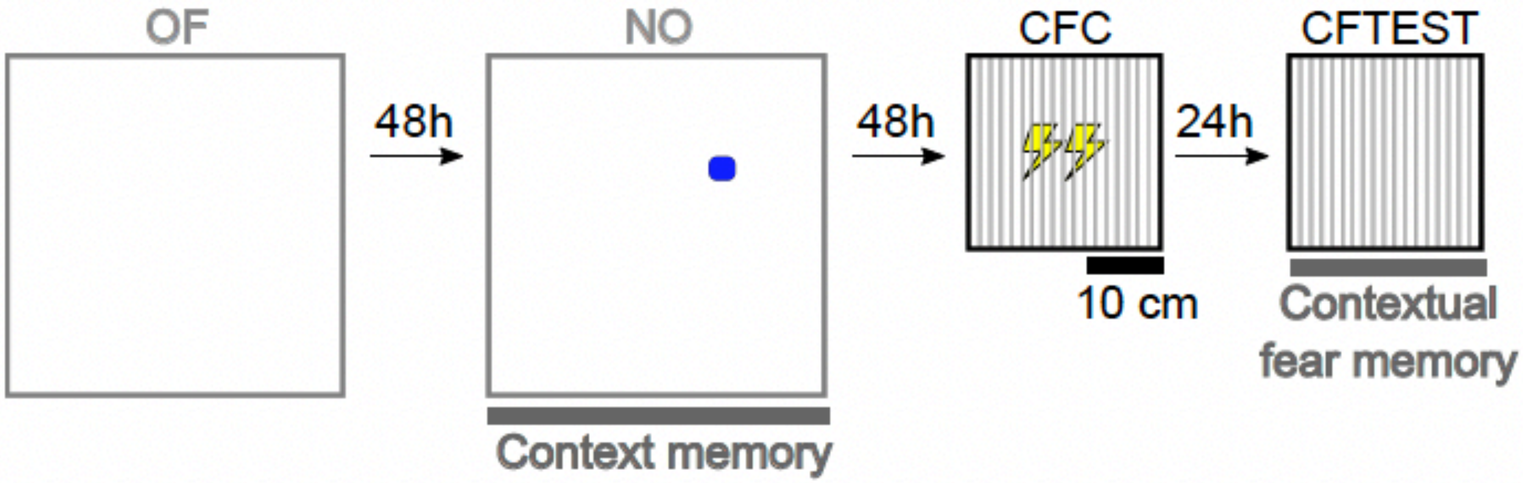
memory consolidation: leads to synaptic and systems changes to allow memories to be remembered later in time

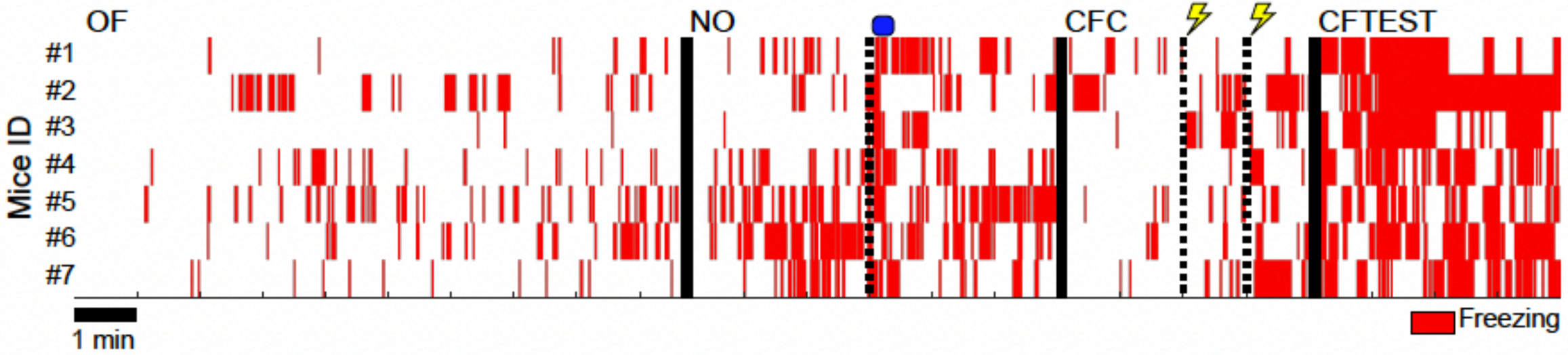
The study of brain networks can help discovering new pathways important for information transfer between regions

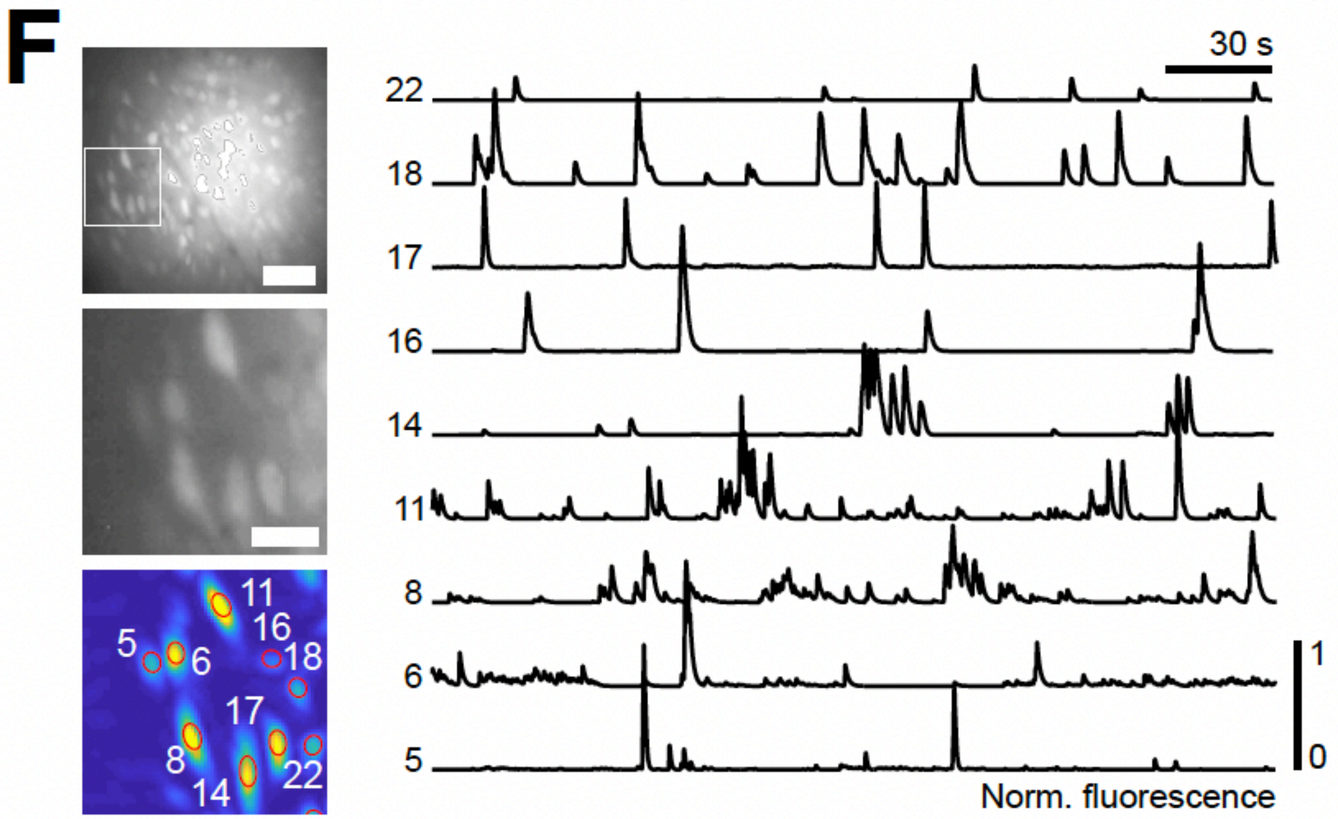
Both activation and inactivation are important!

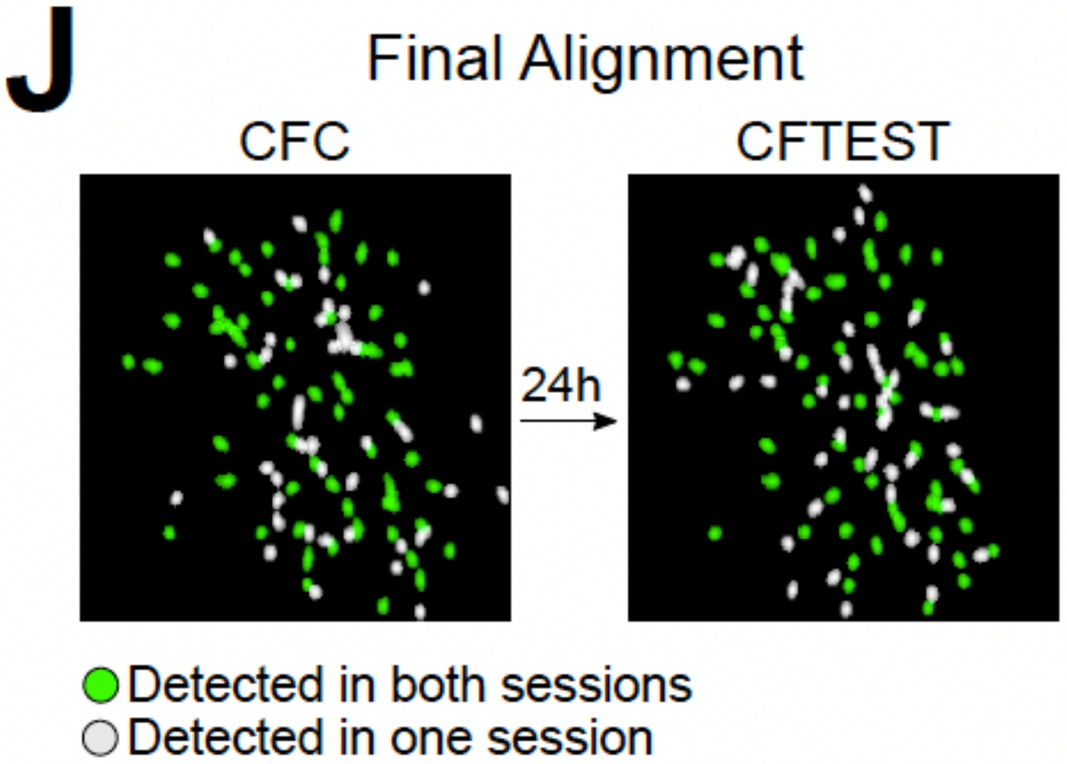
in vivo single cell big data analysis allow us to study specific types of cells correlated with particular behaviours.

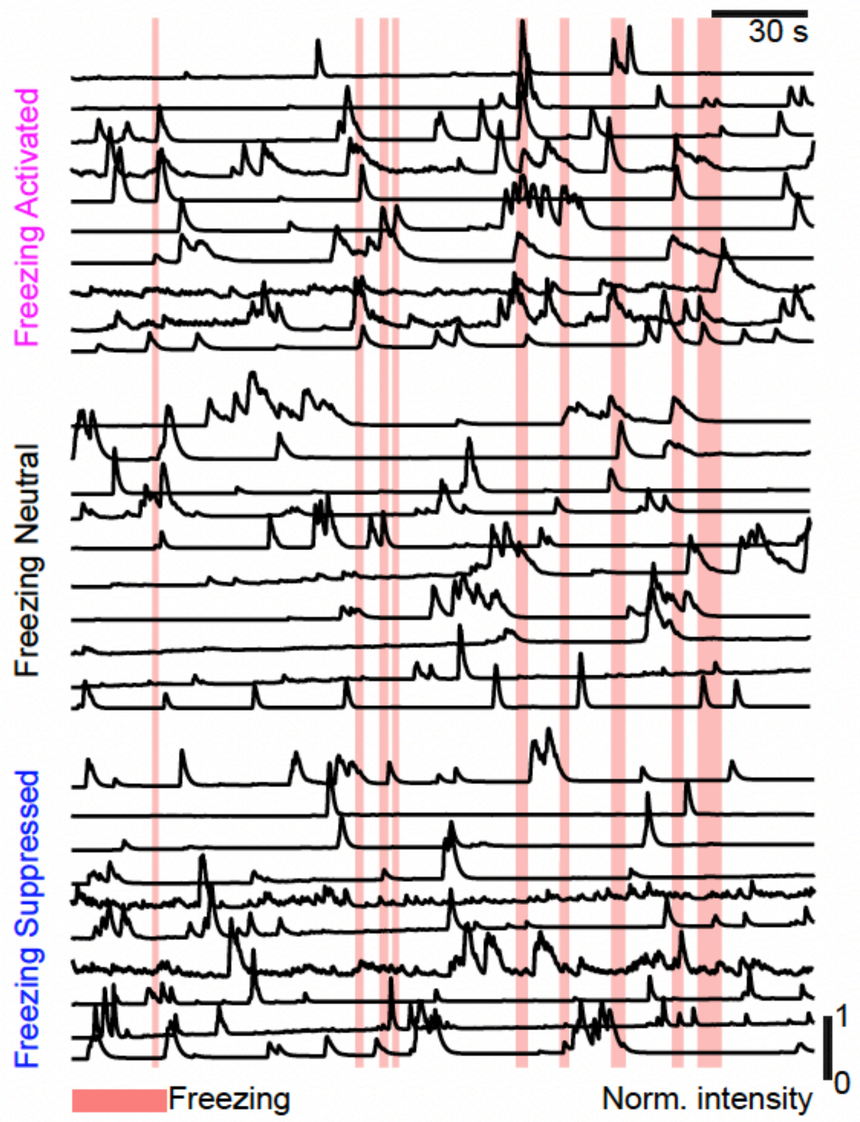

D
In vivo calcium imaging
with UCLA miniscope
Day 1: virus
injection (GCaMP6f)
Day 14: GRIN
lens implantation
Day 28:
Baseplating
Day 29: Imaging
begins
PL
E
M163
M168
M185
M186
M274
M275
M363
M364
M373
M375
GRIN
lens
fmi
PL
IL
DAPI/GCaMP6f

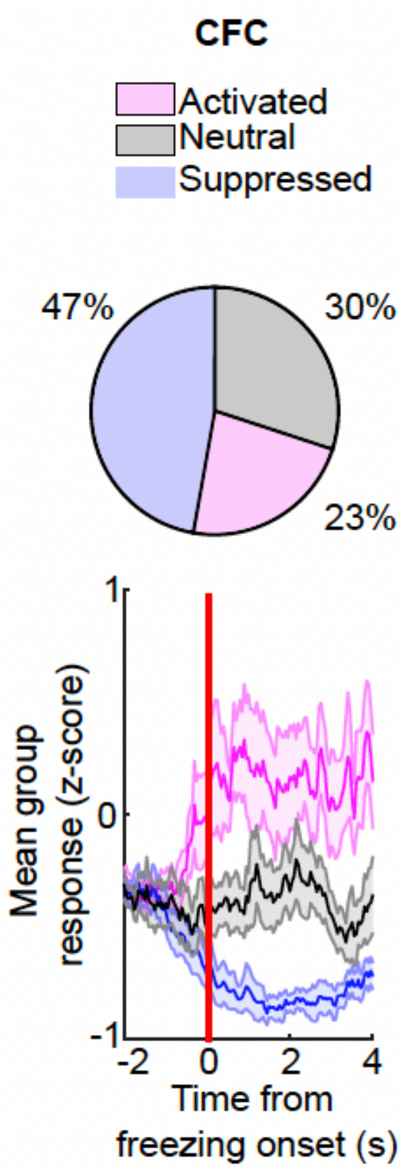

OF
48h
NO
48h
CFC
24h
CFTEST
10 cm
Contextual
fear memory
Context memory

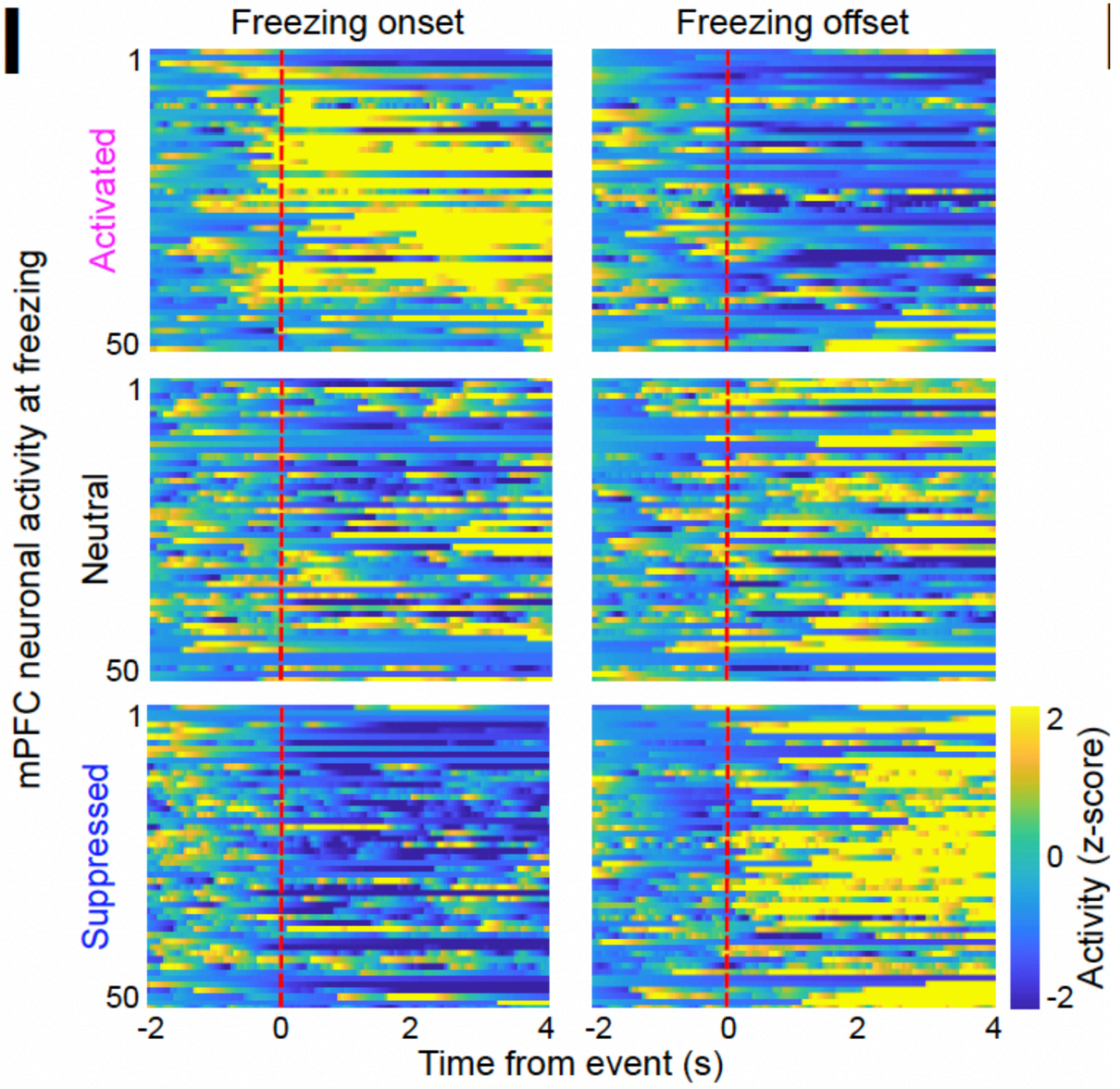

OF
NO
CFC
CFTEST
Mice ID
#1
#2
#3
#4
#5
#6
#7
1 min
Freezing

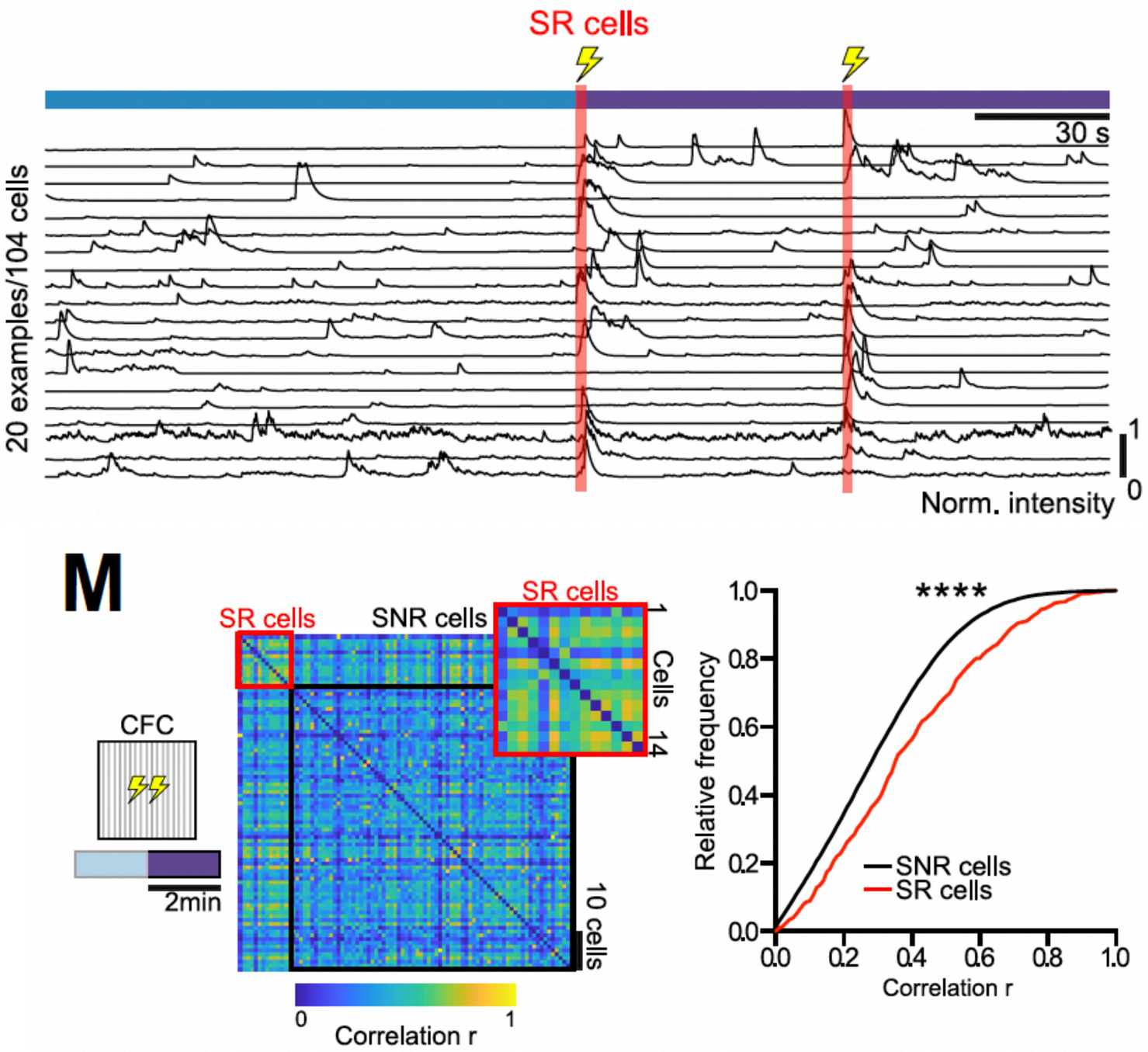

F
30 s
22
18
17
16
14
11
8
6
5
1
0
Norm. fluorescence
11
16
5
6
18
17
8
22
14


J
Final Alignment
CFC
CFTEST
24h
Detected in both sessions
Detected in one session


30 s
Freezing Activated
Freezing Neutral
Freezing Suppressed
1
0
Freezing
Norm. intensity

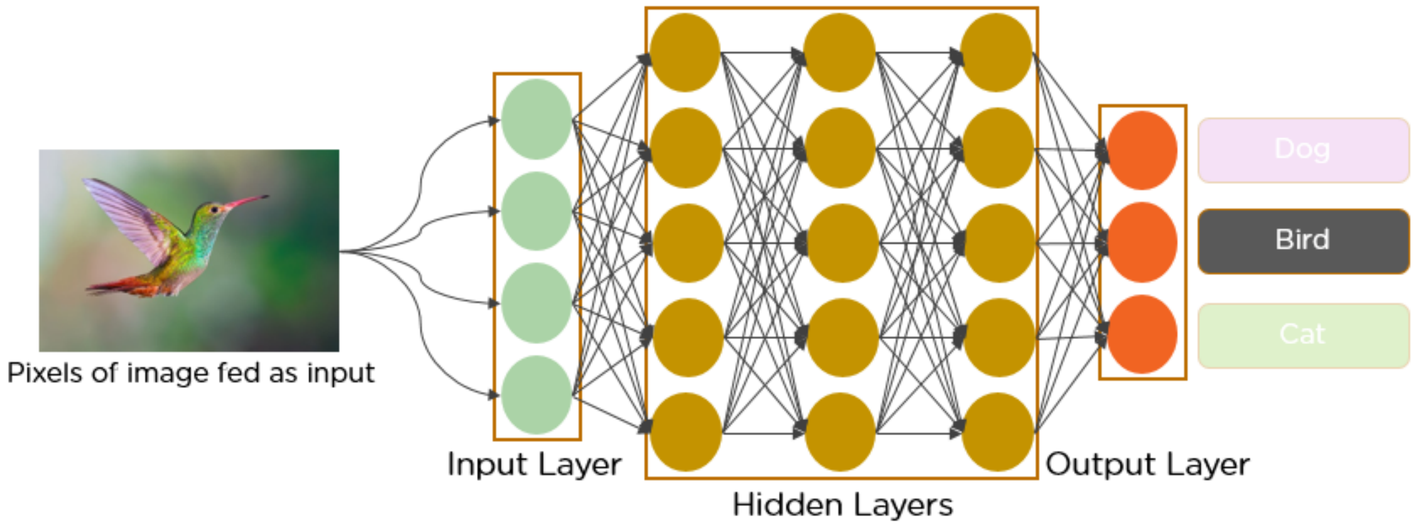

CFC
Activated
Neutral
Suppressed
47%
30%
23%
Mean group response (z-score)
1
0
-1
-2
0
2
4
Time from freezing onset (s)


Freezing onset
Freezing offset
mPFC neuronal activity at freezing
Activated
Neutral
Suppressed
1
50
-2
0
2
4
Time from event (s)
2
0
-2
Activity (z-score)


SR cells
30 s
20 examples/104 cells
1
0
Norm. intensity
M
SR cells
SNR cells
SR cells
1
Cells 14
CFC
2min
10 cells
0
1
Correlation r
****
1.0
0.8
0.6
0.4
0.2
0.0
Relative frequency
SNR cells
SR cells
0.0
0.2
0.4
0.6
0.8
1.0
Correlation r

can we predict when the animals are in freezing just by looking at the neuronal configuration of the Prelimbic cortex?

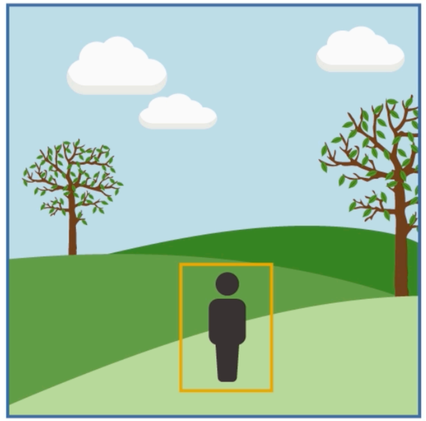
Answer this question using a convoluted neural network



a Convoluted Neural Network is a network architecture for deep learning.
It learns directly from images.
It is made up of several layers that process and transform an input to produce an output.

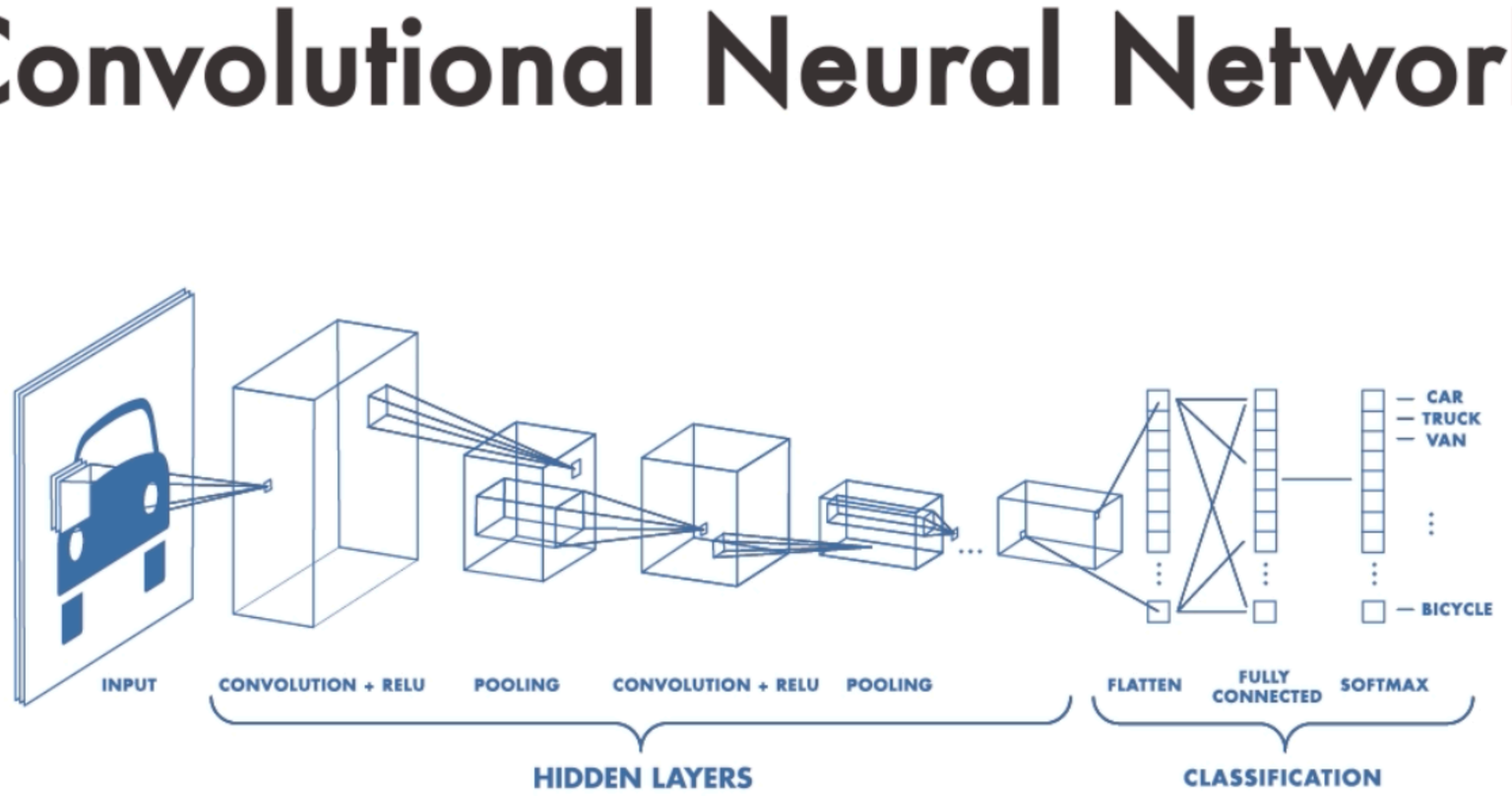
You can train a CNN to do image analysis tasks, including scene classification, object detection and segmentation and image processing.

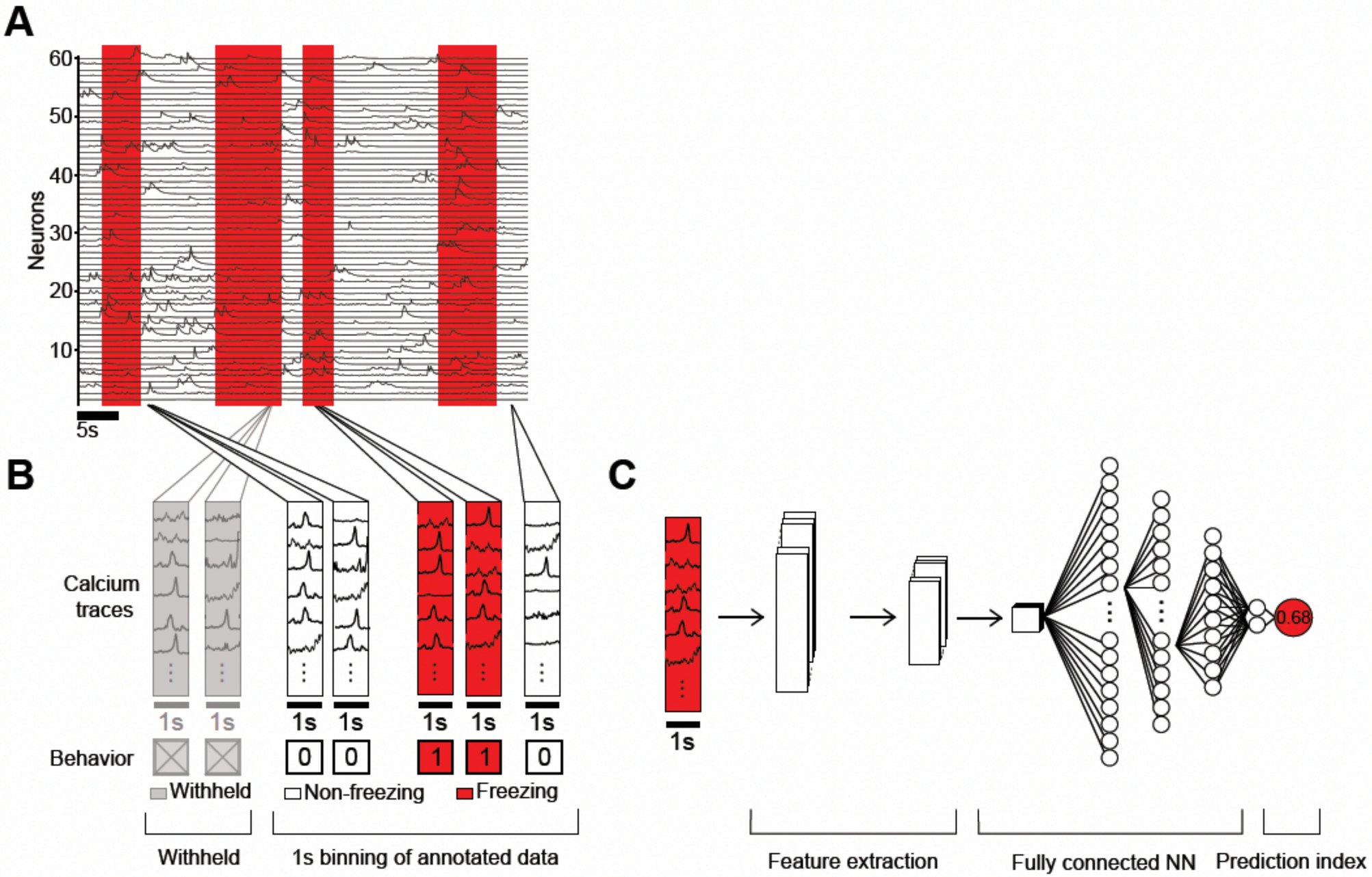




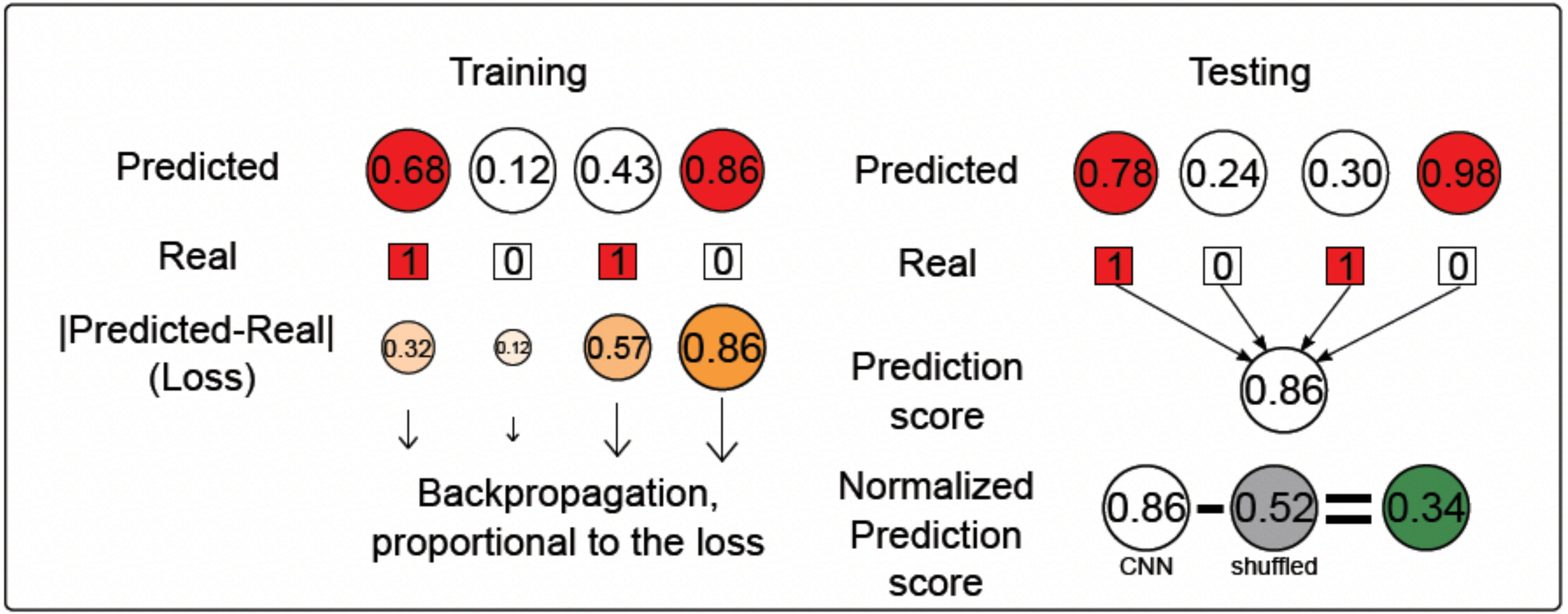
A CNN can have tens or hundreds of hidden layers that each learn to detect different features in an image. In this feature map, we can see that every hidden layer increases the complexity of the learned image features.

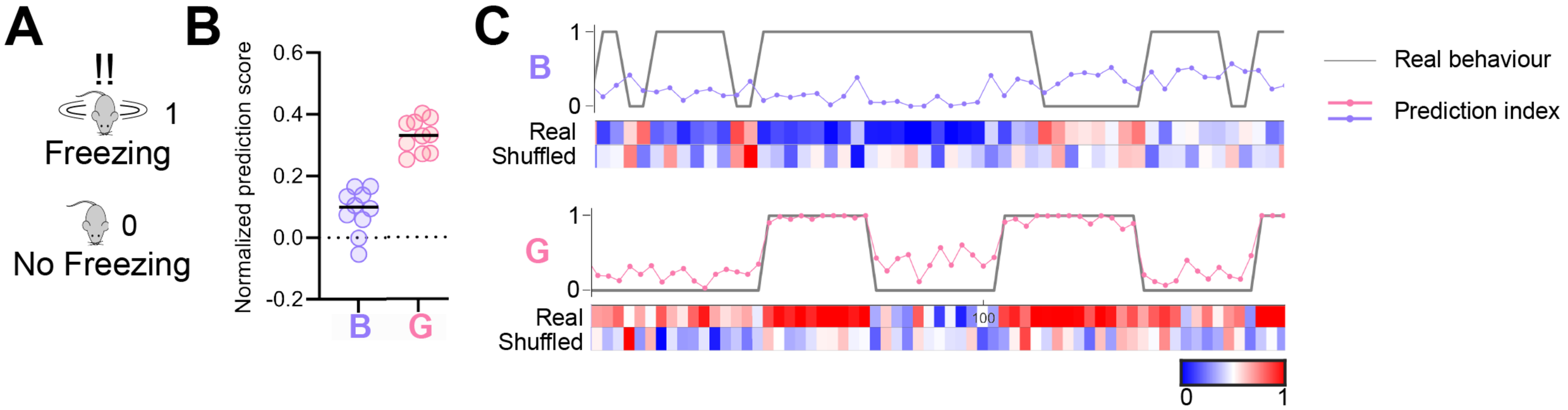
For example, the first hidden layer learns how two detect edges and the last learn to detect more complex shapes. the final layer connects every neruon from the last hidden layer to the output neurons producing the final output

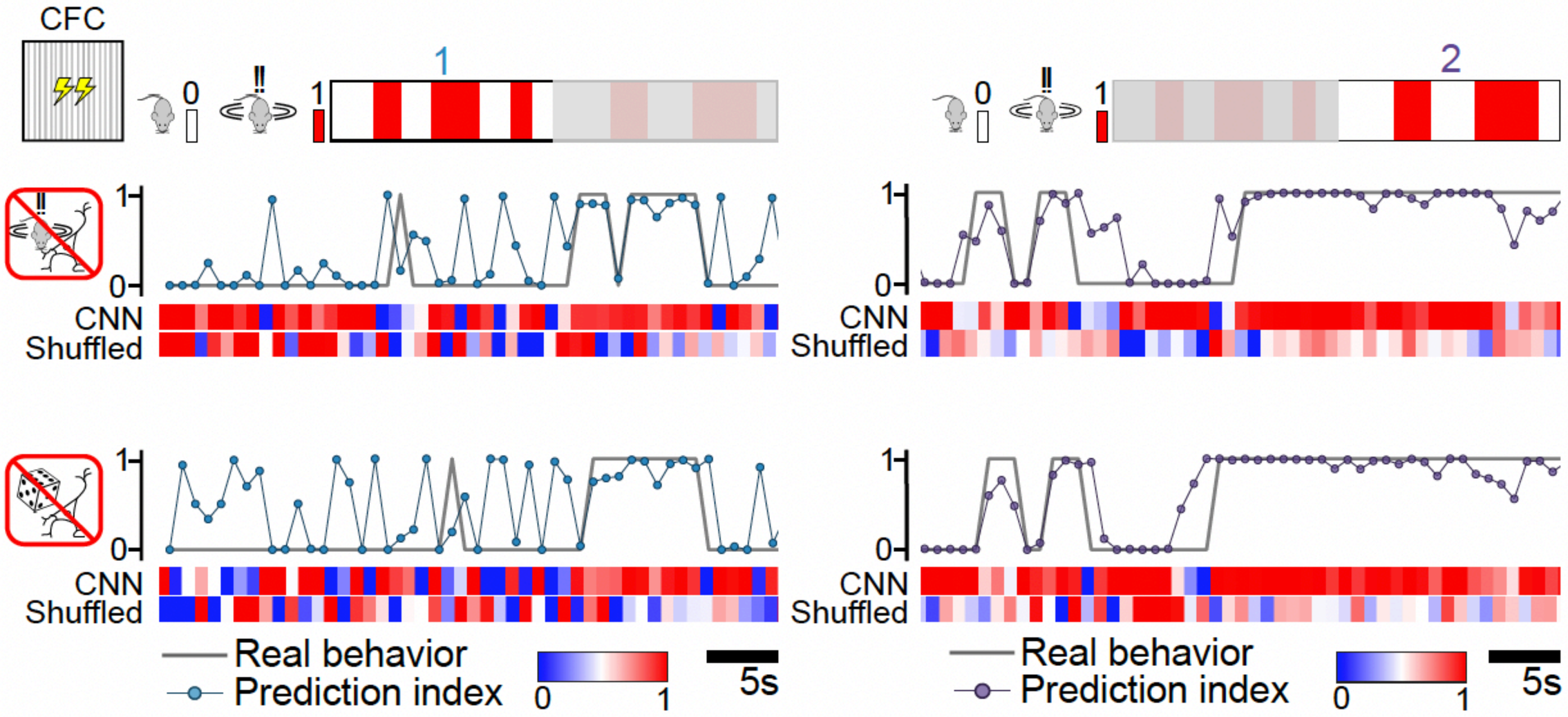



A
60
50
40
30
20
10
Neurons
5s
B
Calcium traces
1s
1s
1s
1s
1s
1s
1s
Behavior
0
0
1
1
0
Withheld
Non-freezing
Freezing
Withheld
1s binning of annotated data
C
1s
0.68
Feature extraction
Fully connected NN
Prediction index

D


Training
Predicted
0.68
0.12
0.43
0.86
Real
1
0
1
0
|Predicted-Real|
(Loss)
0.32
0.12
0.57
0.86
Backpropagation,
proportional to the loss
Testing
Predicted
0.78
0.24
0.30
0.98
Real
1
0
1
0
Prediction
score
0.86
Normalized
Prediction
score
0.86
0.52
0.34
CNN
shuffled


A
!!
1
Freezing
0
No Freezing
B
Normalized prediction score
0.6
0.4
0.2
0.0
-0.2
B
G
C
B
1
0
Real
Shuffled
G
1
0
100
Real
Shuffled
0
1
Real behaviour
Prediction index


CFC
0
1
1
2
1
0
CNN
Shuffled
Real behavior
Prediction index
0
1
5s

BIG DATA ANALYSIS APPLIED TO BEHAVIOURAL NEUROSCIENCE

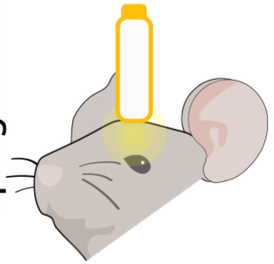
FEAR MEMORY



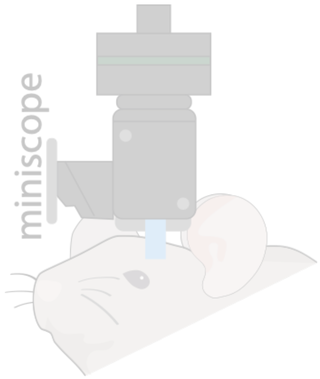
focus on the brain



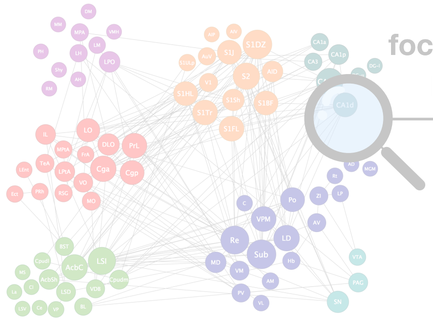
optogenetics



miniscope

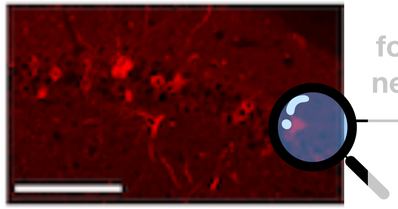


freezing activated neurons

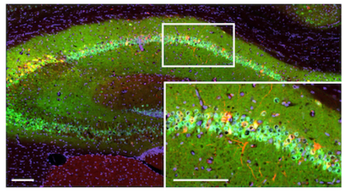


focus on neuronal populations

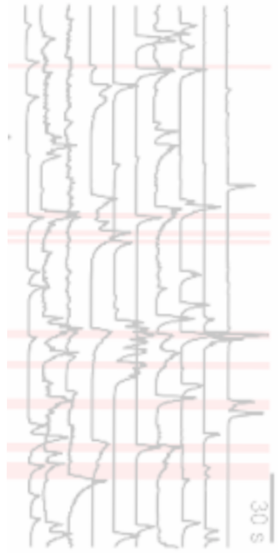
NETWORK of brain regions



focus on single neurons activity



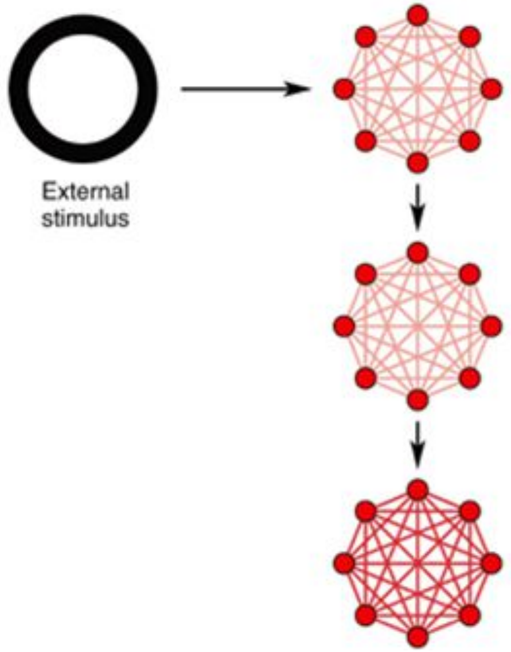
ENGRAM neurons



single cells

Memory location - the Engram

Hebb, in 1949, proposed that the internal representation of an object consists of all cortical cells that are activated by the external stimulus.



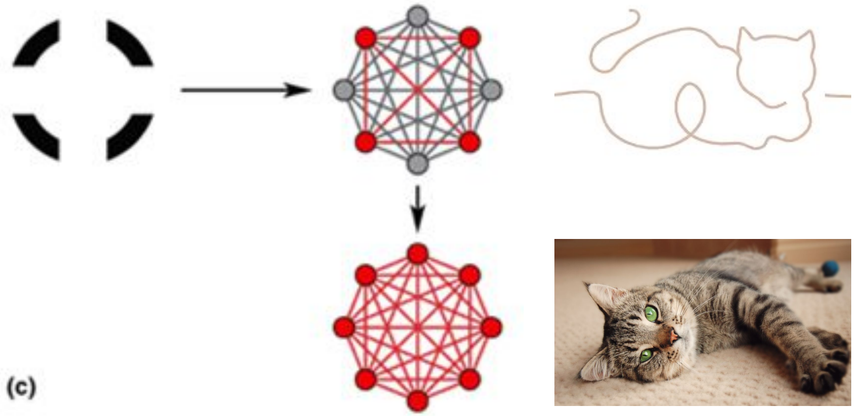
All these cells should be <u>reciprocally interconnected.</u>

If the activation of the cell assembly persist over time, consolidation would occur by a “growth process” that made these reciprocal connections more effective.

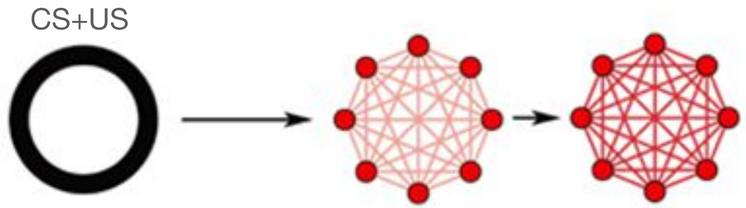
<u>Neurons that FIRE together would WIRE together.</u>

Subsequently, if only a fraction of the cells are activated by a later stimulus (a segment of the circle), this would cause the whole assembly to become active again

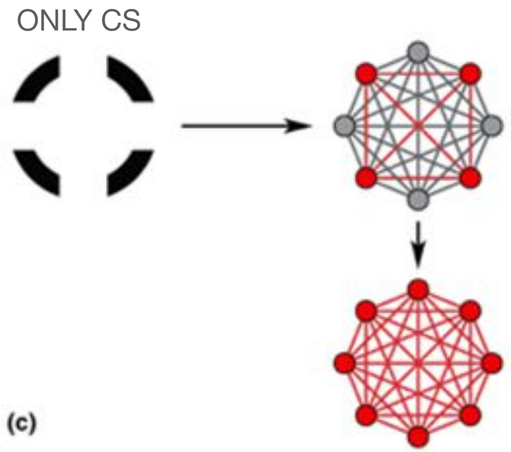
RECALLING THE ENTIRE INTERNAL REPRESENTATION OF THE EXTERNAL STIMULUS

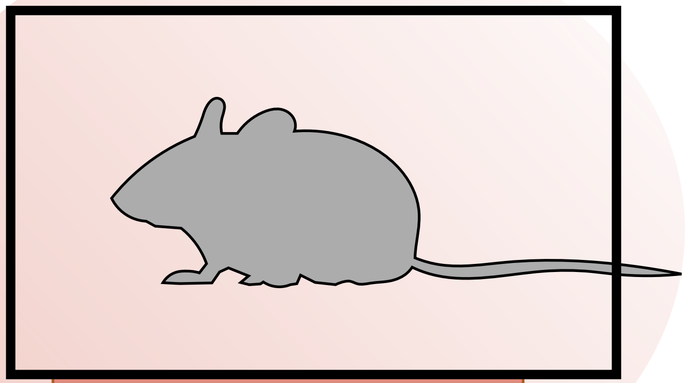


THE FORMATION OF A MEMORY

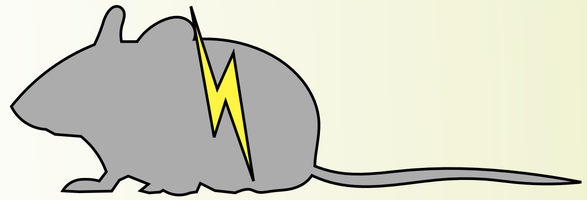


the day after…

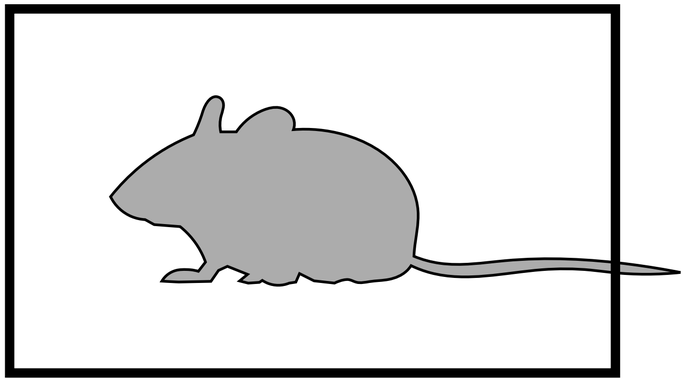




neutral stimulus (CS)



aversive stimulus (US)



conditioned response to CS (FREEZING!)



the nature of a memory engram

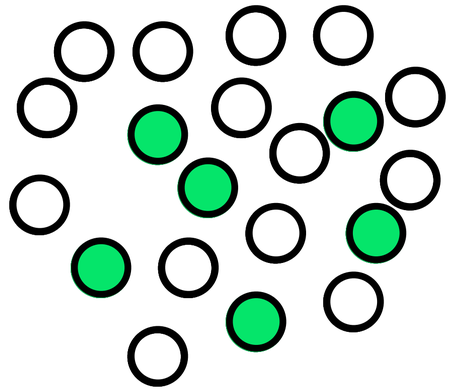
An experience activates a population of neurons that undergo persistent chemical and/or physical changes to become an engram. Subsequent reactivation of the engram by cues available at the time of the experience induces memory retrieval.

From Josselyn and Tonegawa, Science, 2020

1. active during memory acquisition
2. undergo plastic modification
3. reactivated during memory recall

(necessary and sufficient)

1. CS (context A)+US (Shock) presentation **TAG**

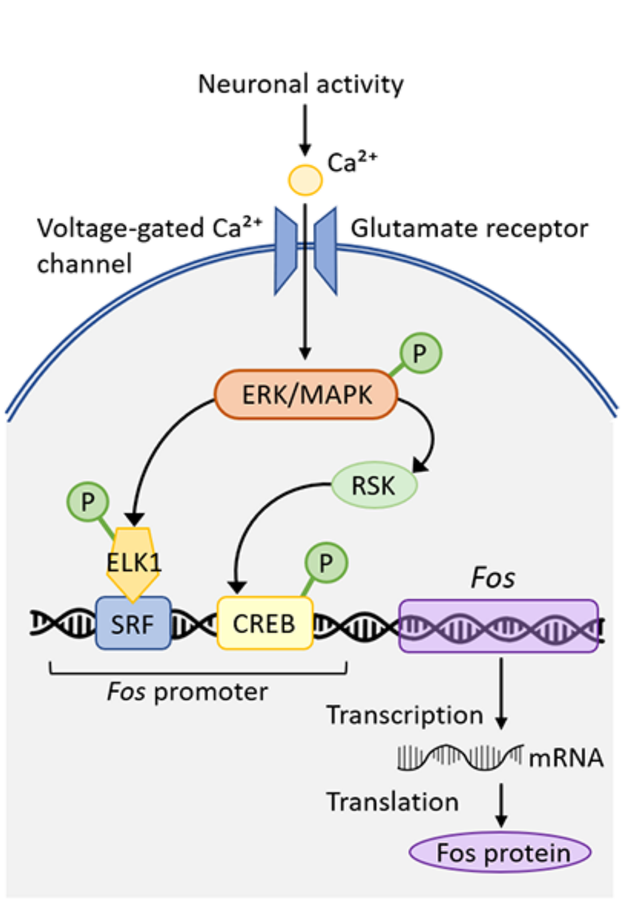


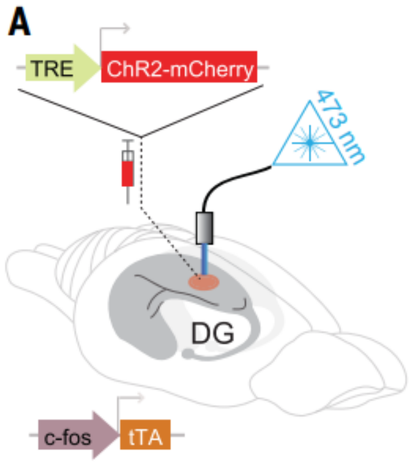
3. context B

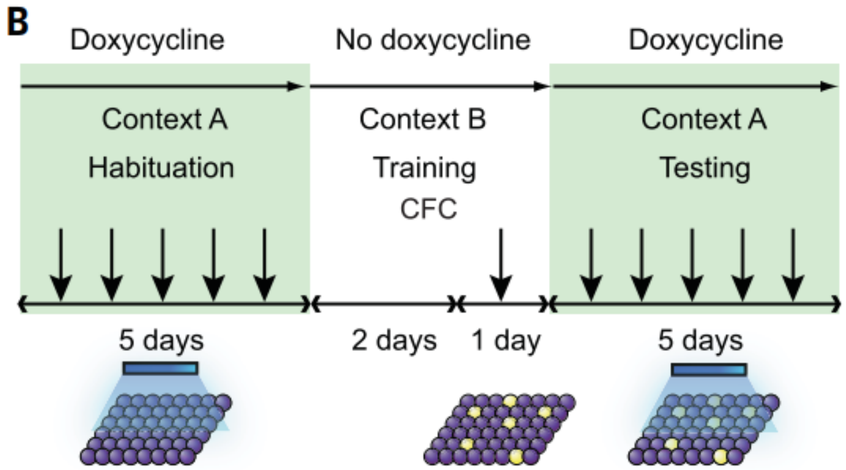
OPTO REACTIVATION

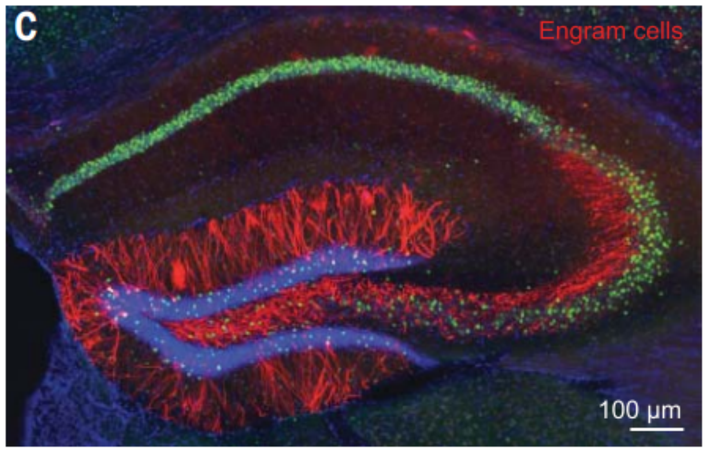
MEMORY RECALL IN NEUTRAL CONTEXT

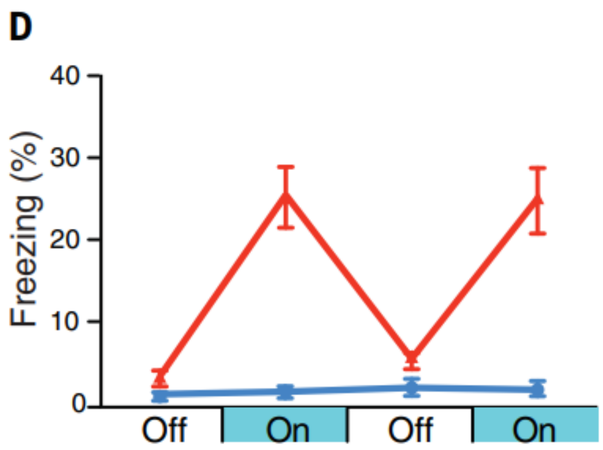
The classic gain-of-function experiment

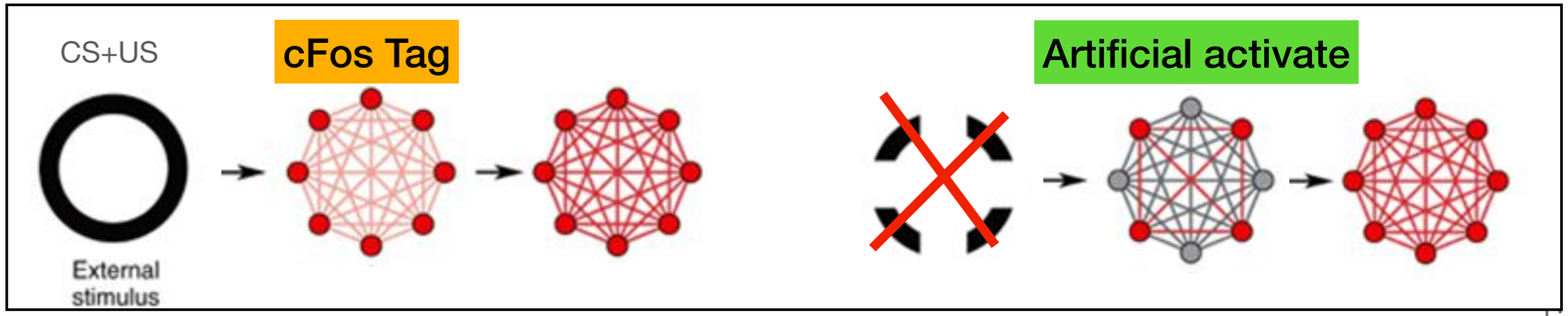








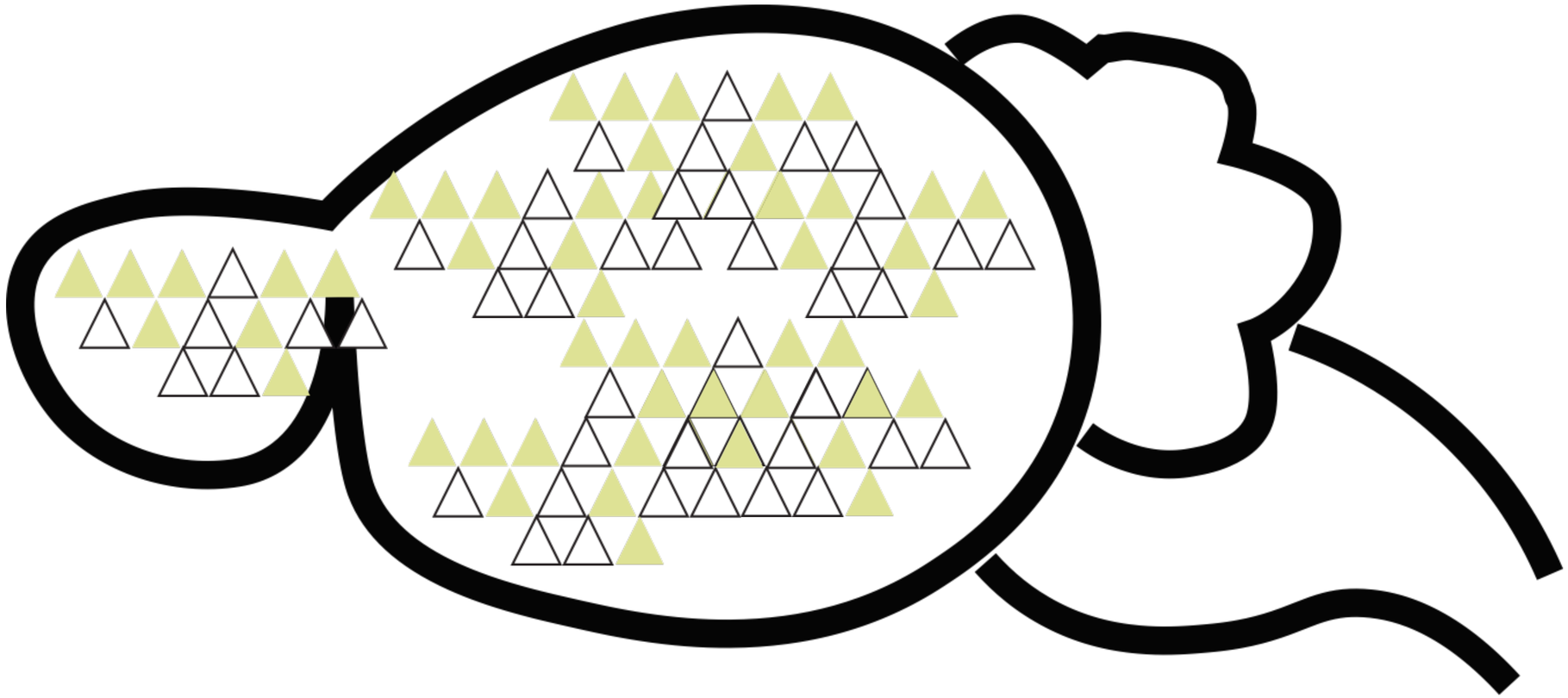




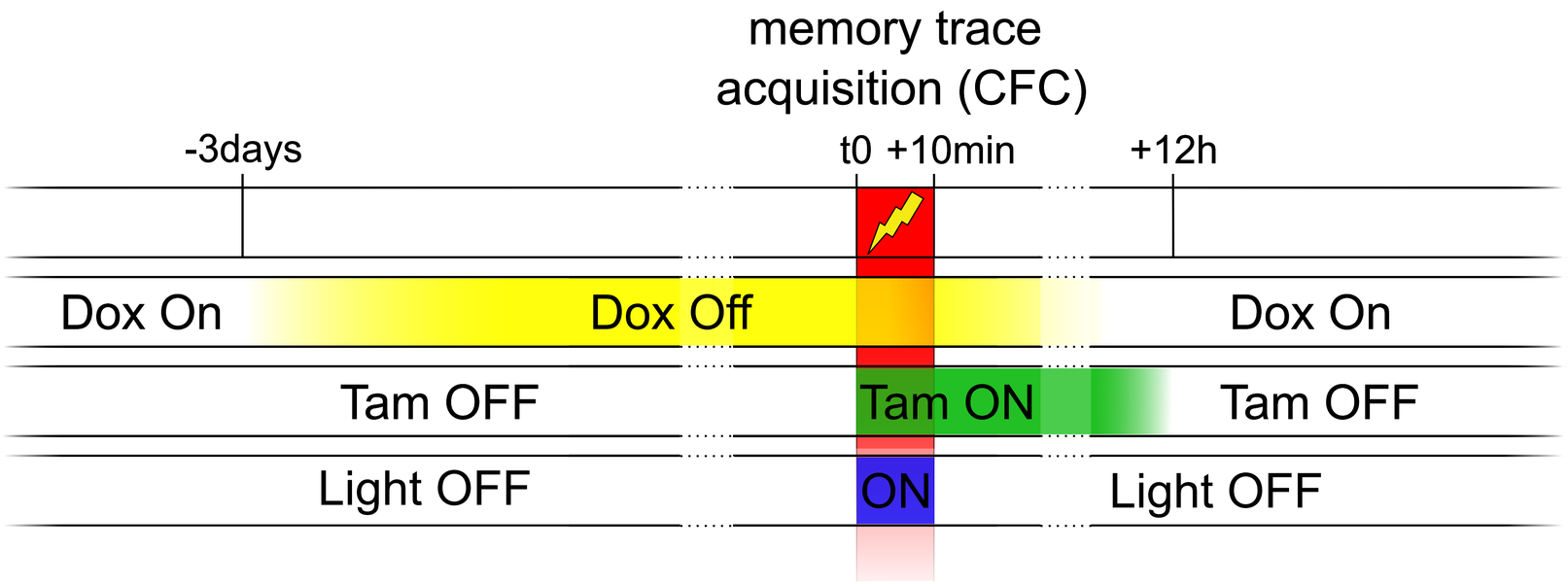
What is the nature of a memory engram?

a novel approach to TAG the ENGRAM

Calcium Influx to tag engram cells



Lack in time specificity of traditional “tagging” techniques

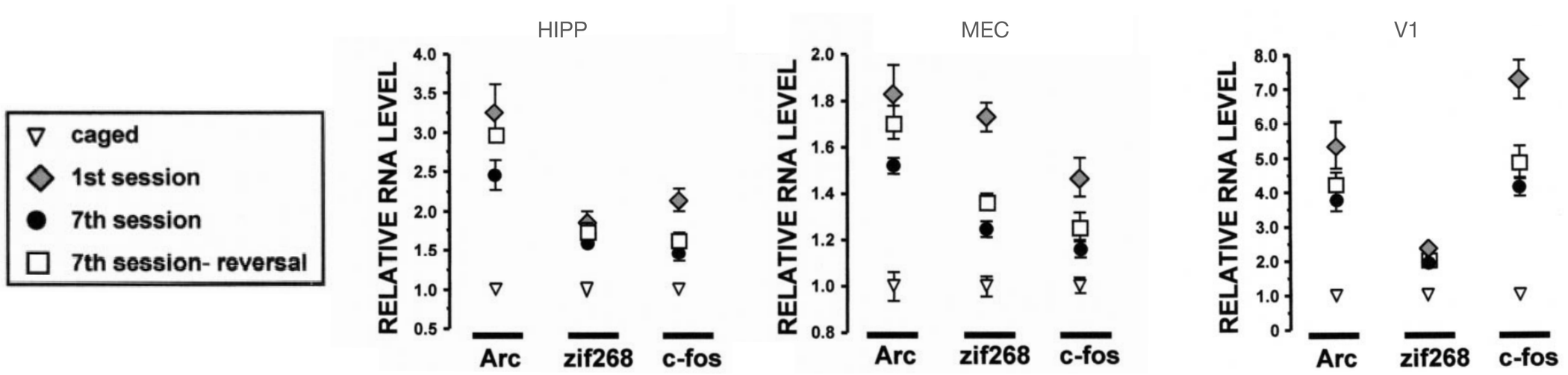


Timeline

IEG/synthetic promoter-tTA

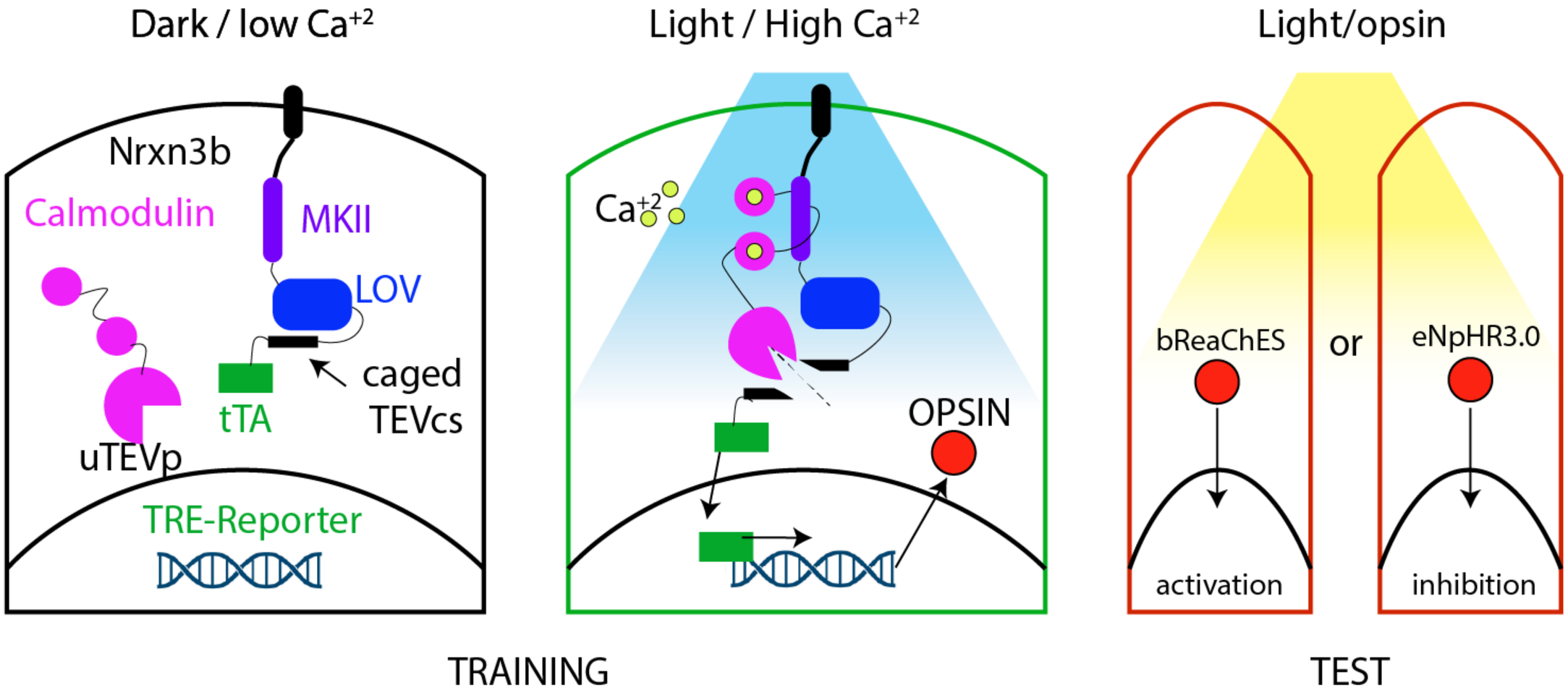
IEG/synthetic promoter-Cre

FLiCRE/Cal-Light



Guzowsky et al., J.Neuroscience, 2001

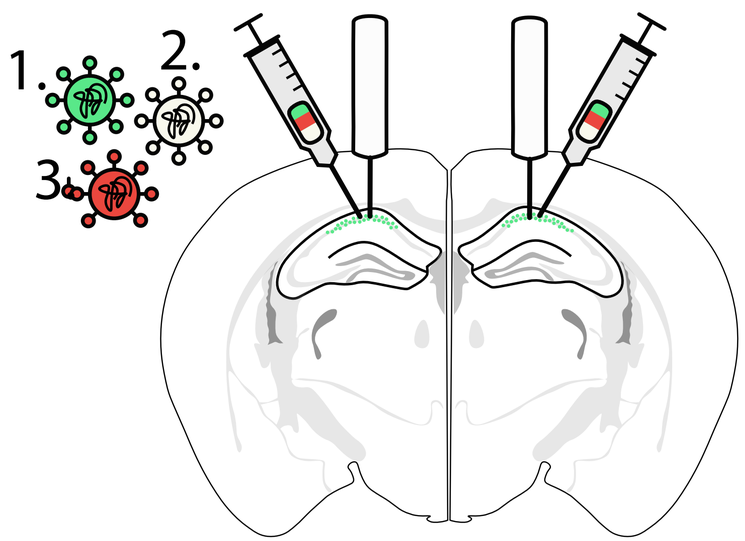
USING FLiCRE TO TAG THE ENGRAM



KIM et al, 2020, Cell

experimental protocol to tag engrams during CFC

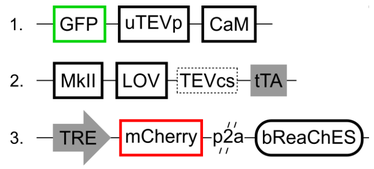
stereotaxic surgeries → 3 weeks → ctxB → 1day → FC ctxA → 1day → ctxC

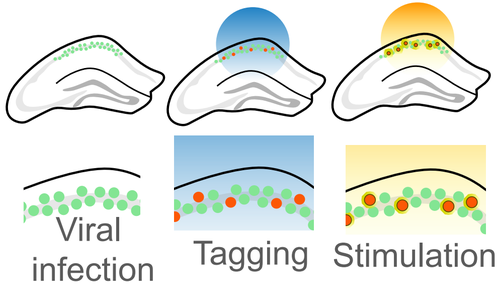


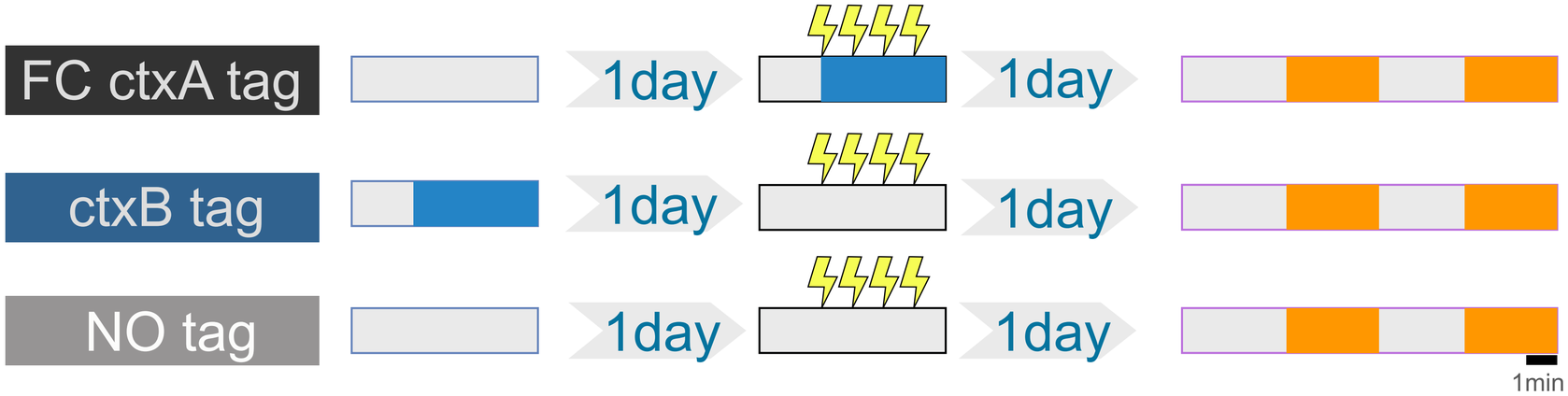


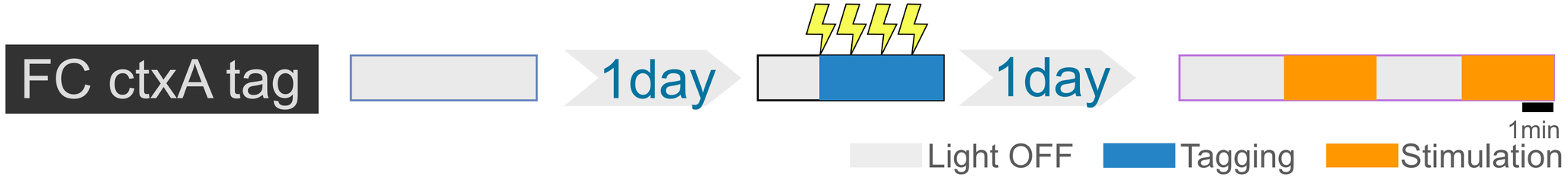


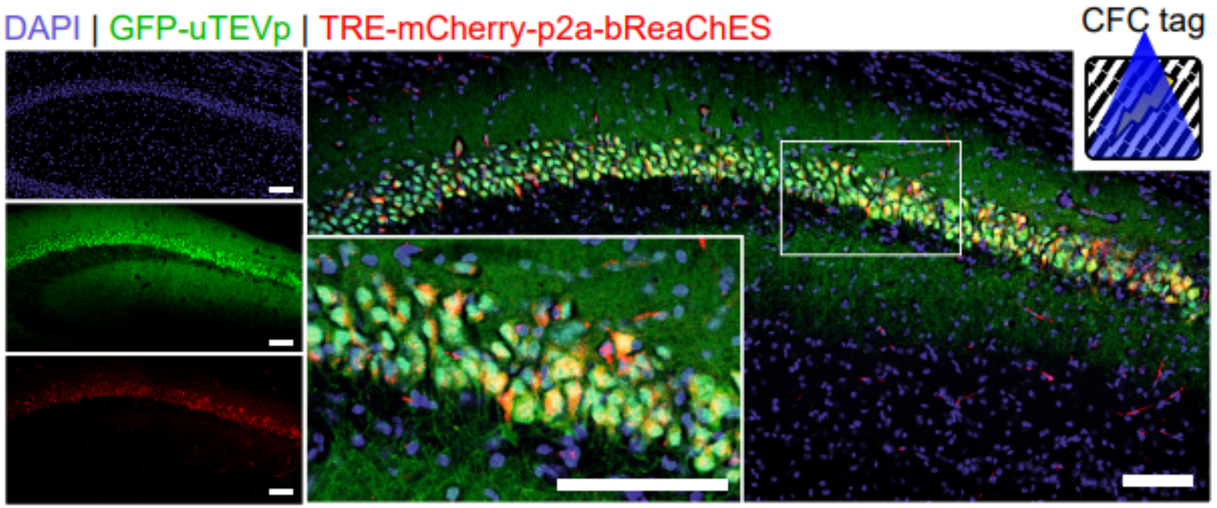




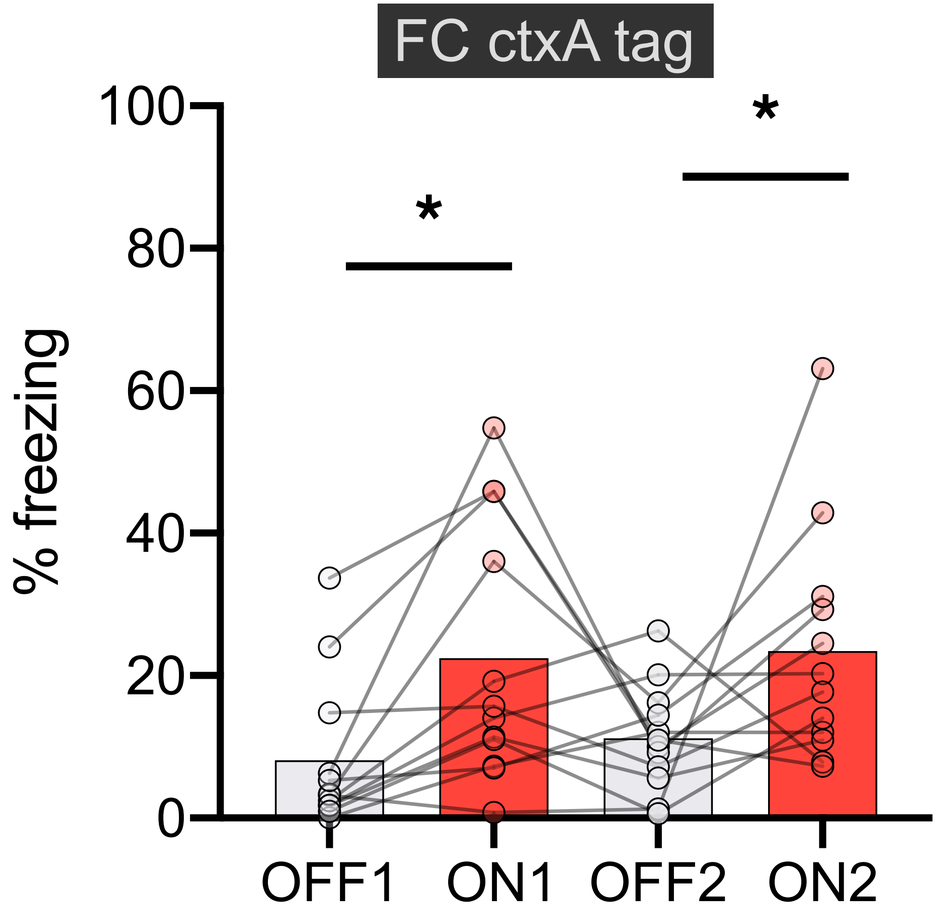








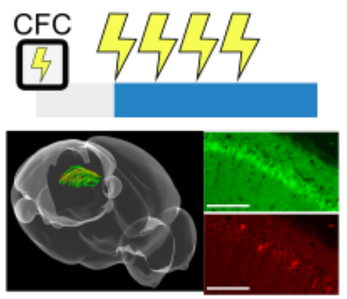


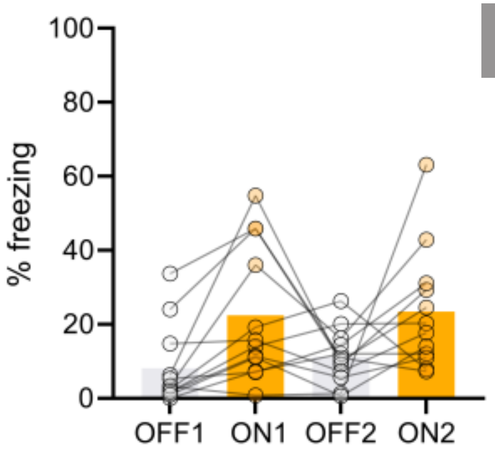


CFC tagging drives freezing during stimulation…

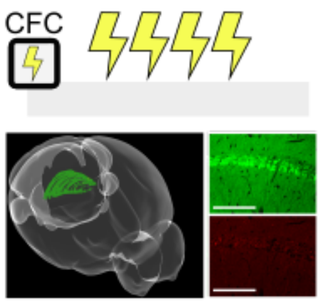
… whilst not tagging or tagging in a different context doesn't

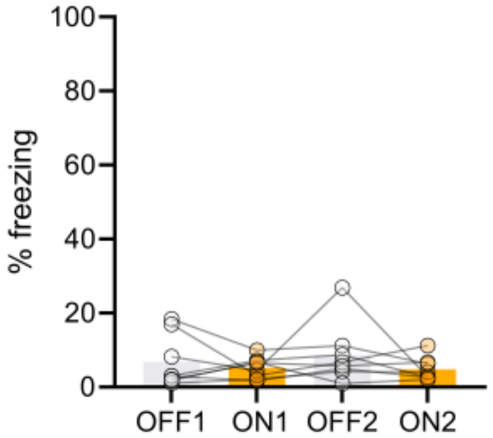
FC ctxA tag



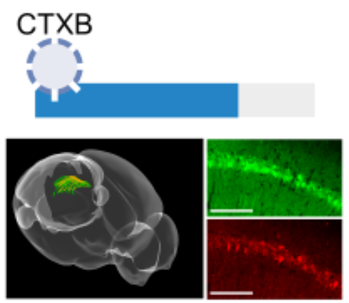


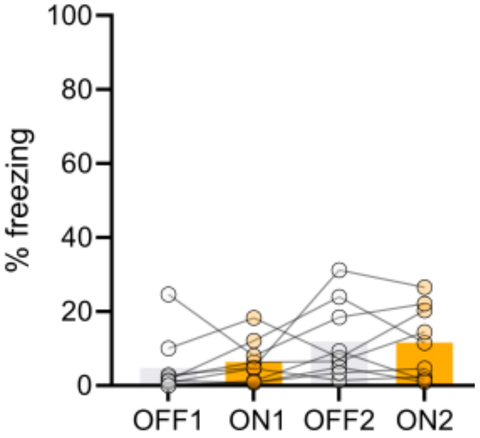
NO tag

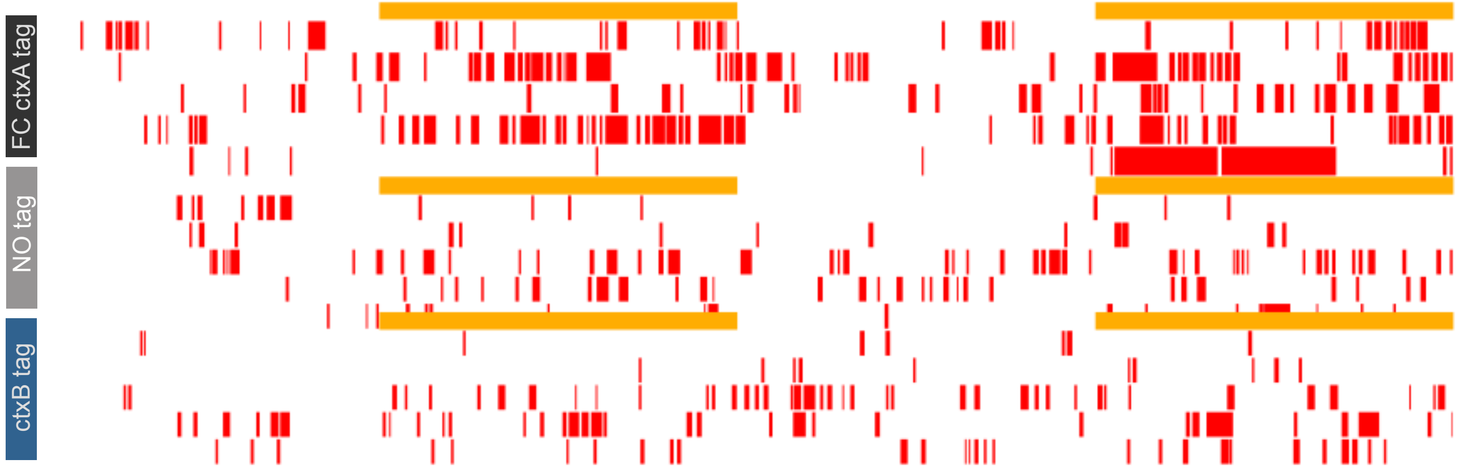


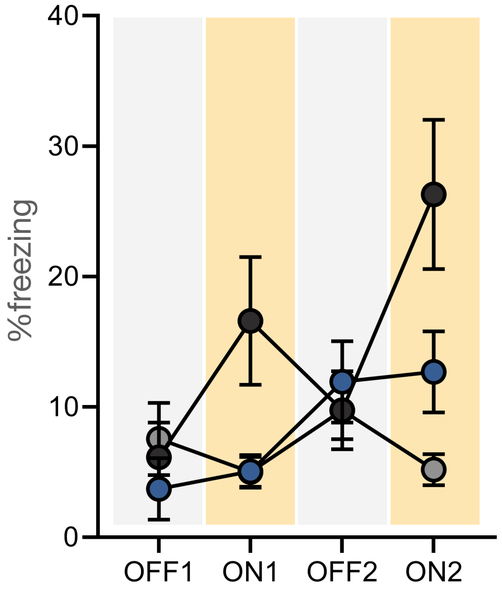


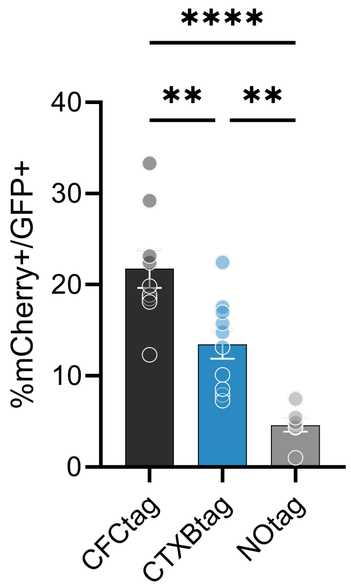
ctxB tag





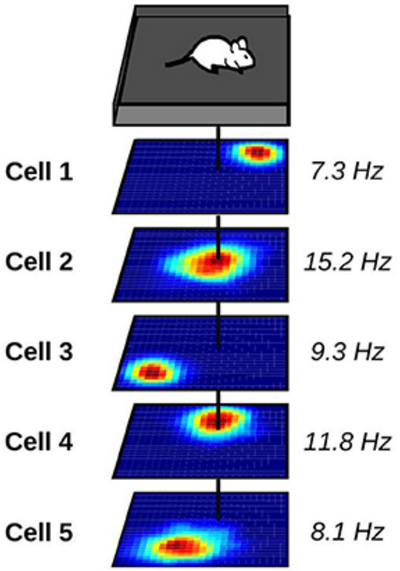




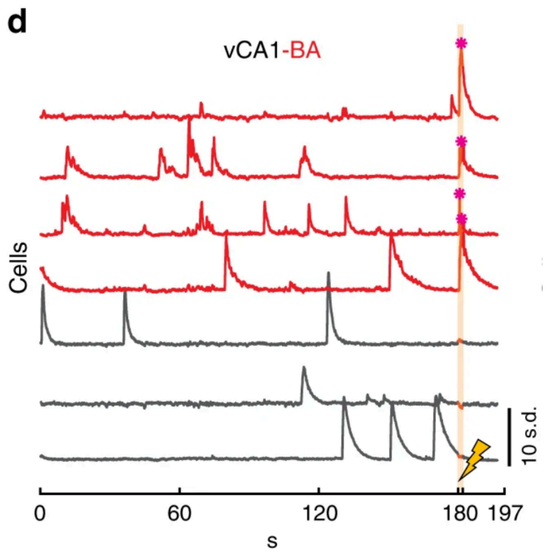


the nature of an hippocampal fear memory engram

PLACE CELLS

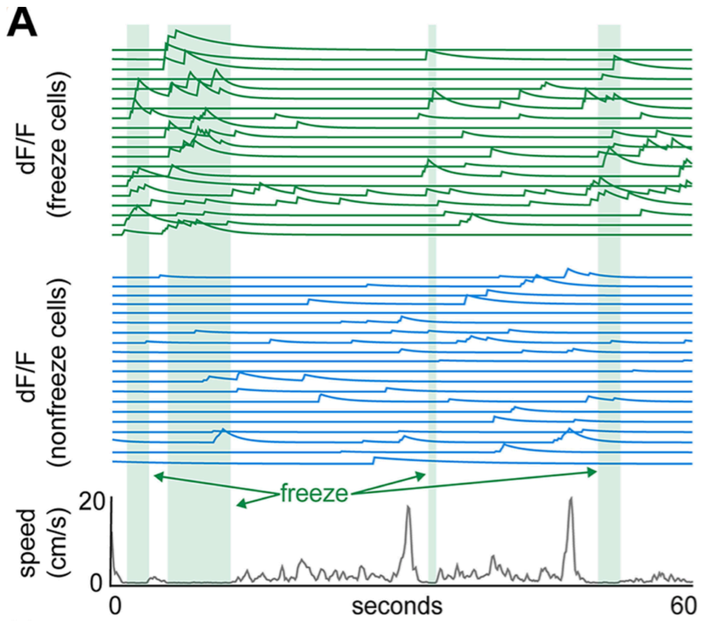


SHOCK CELLS



Jimenez, 2020, Nature comm

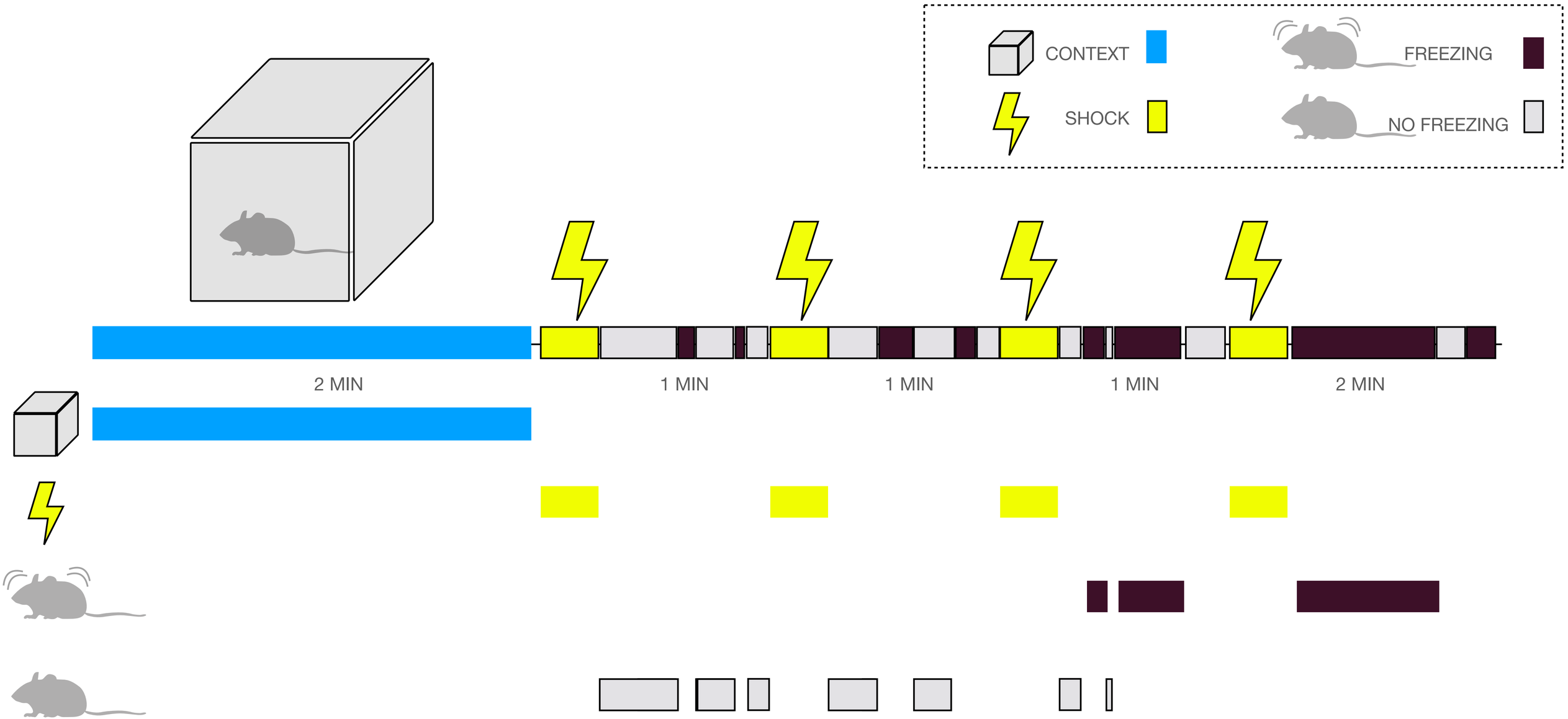
FREEZING CELLS



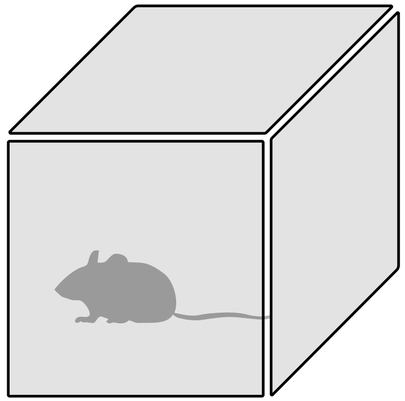
Schuette, 2020, JN

ETC….

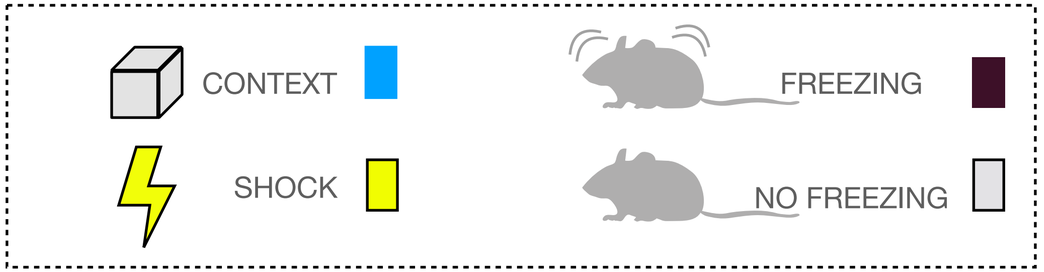
experimental protocol to tag sub-engrams during CFC

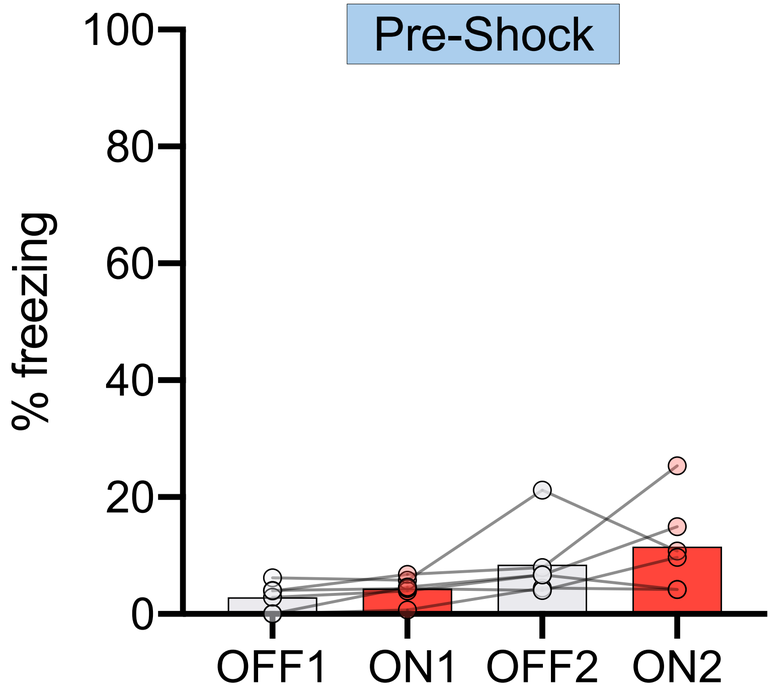


before Shock tagging do not trigger memory recall the day after









Shock cells are driving memory recall?



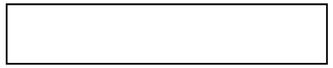
Tag Shocks



1h surgery

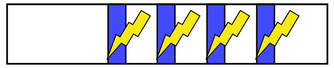


1h surgery



2min OFF

5min ON



3 sec shocks (X4) ON



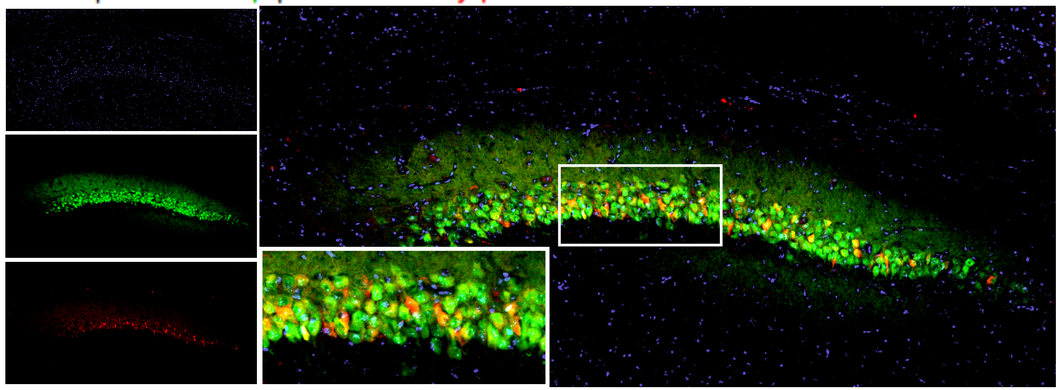
2min OFF

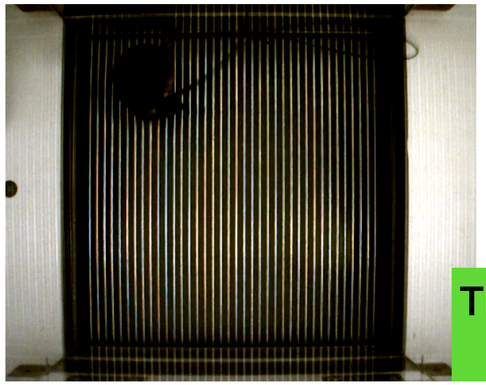
2min ON

2min OFF

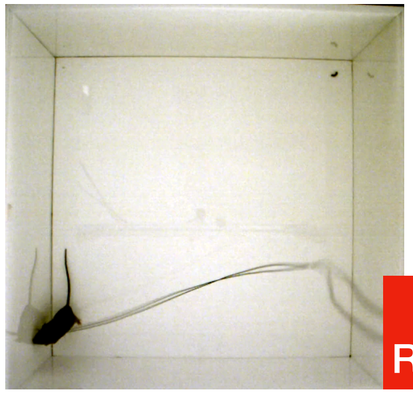
2min ON

DAPI | GFP-uTEVp | TRE-mCherry-p2a-bReaChES

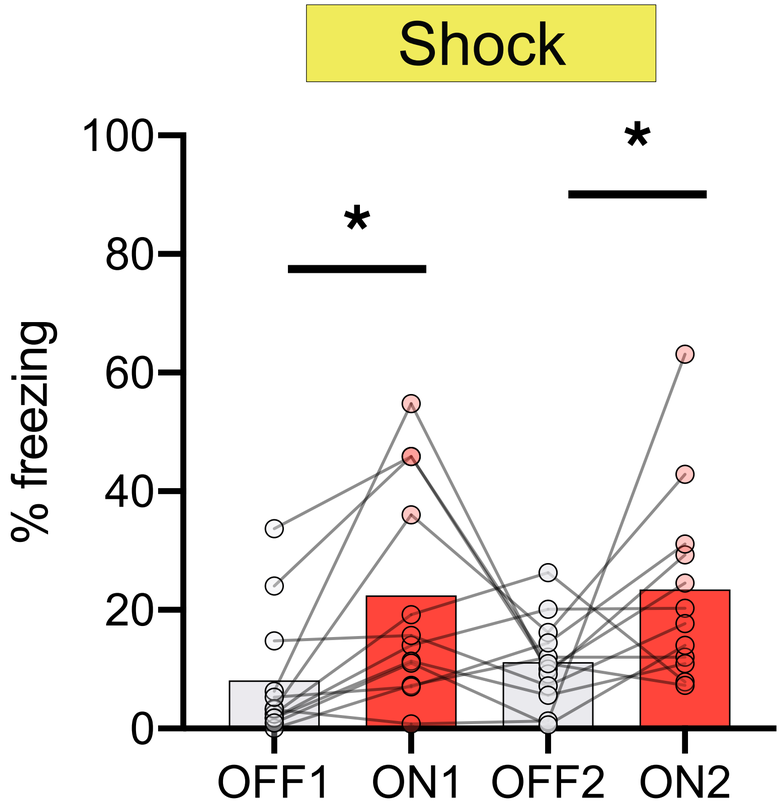




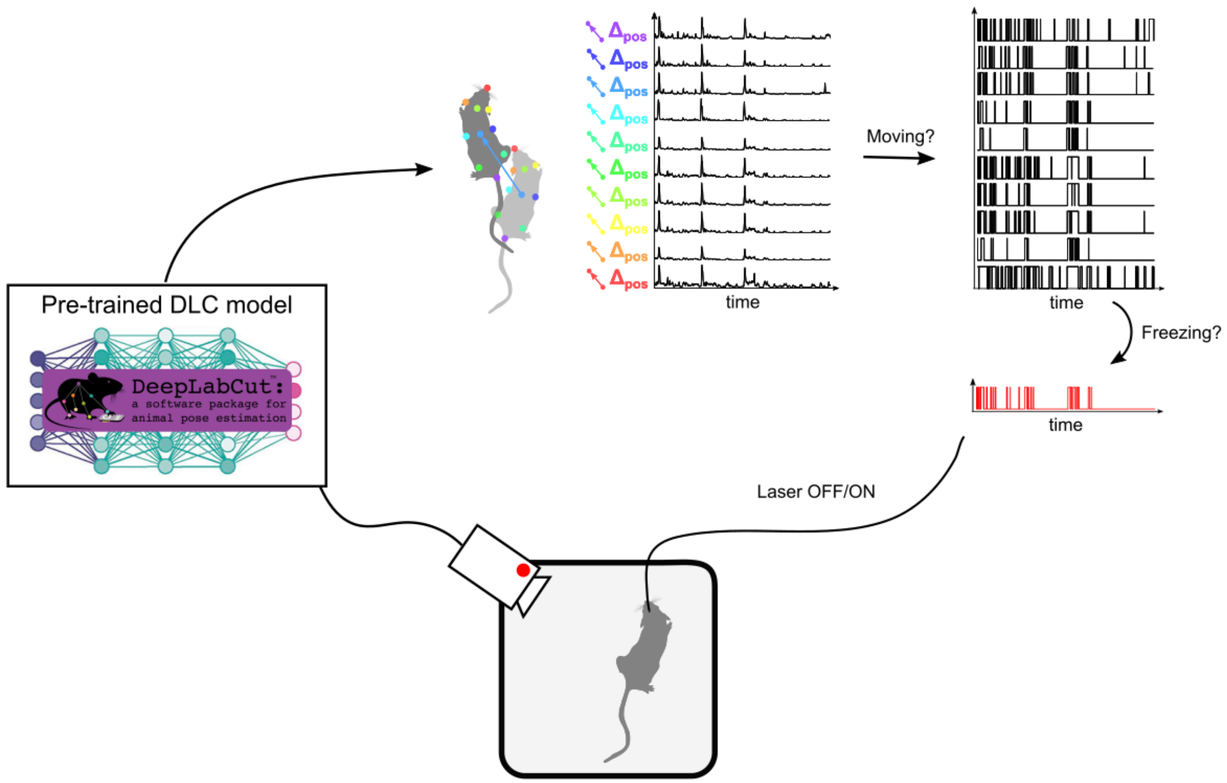
TRAINING TAG

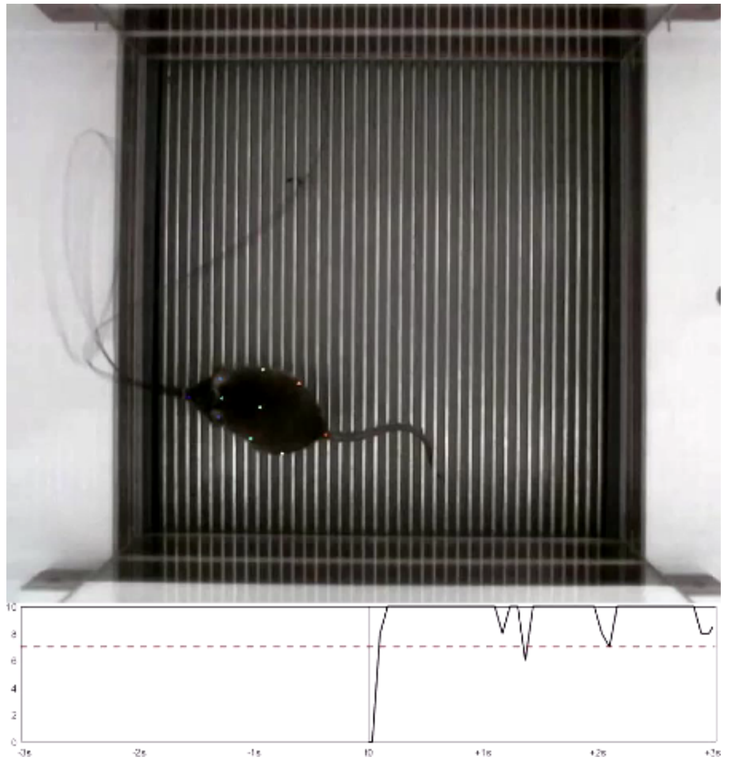


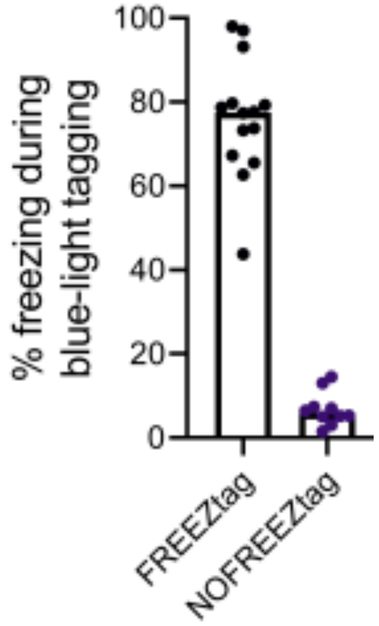
TEST REACTIVATE

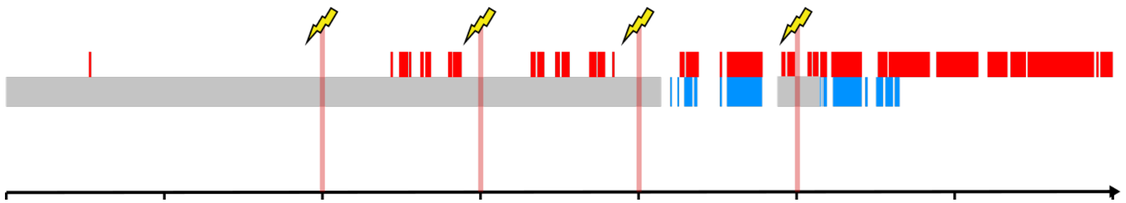


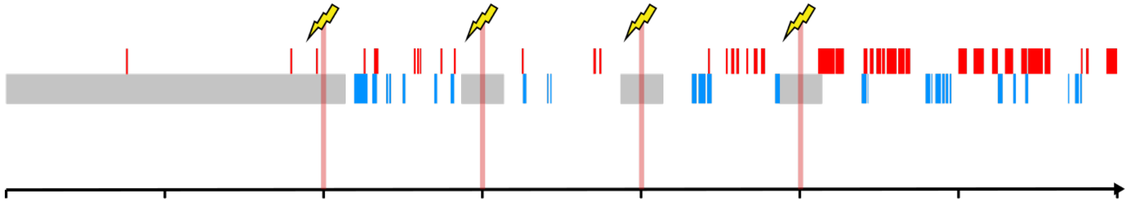
Freezing/NO-freezing tagging



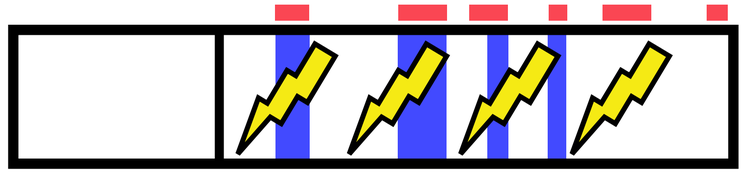


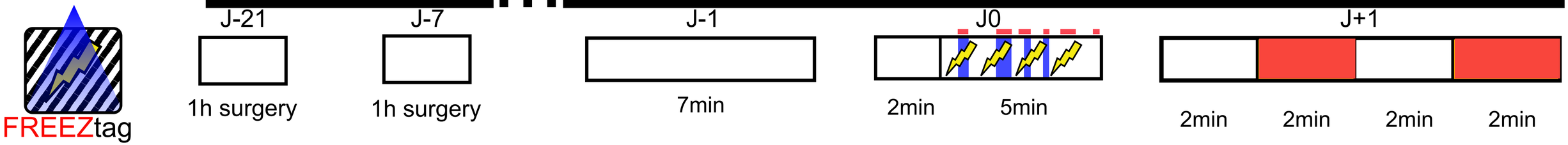


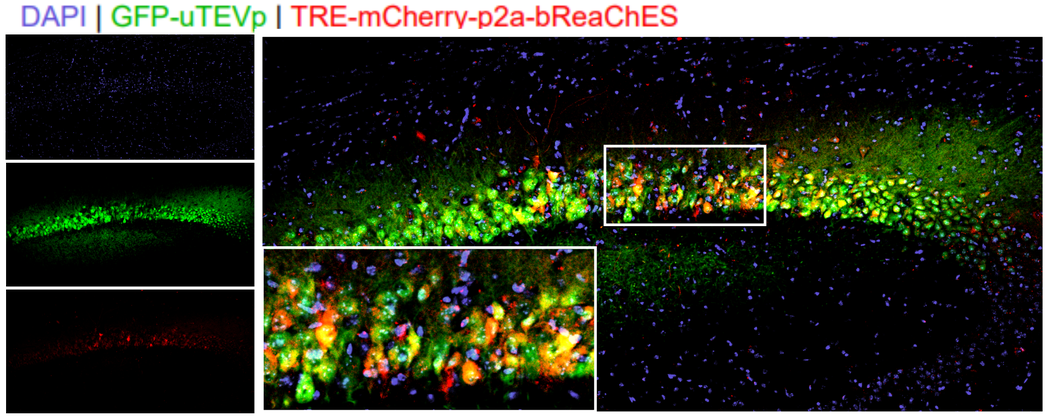


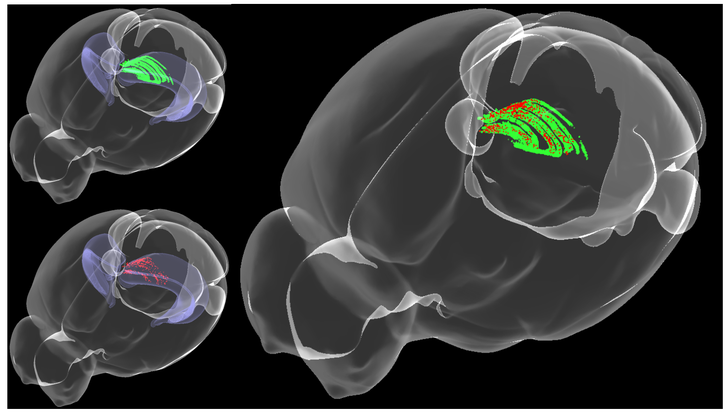


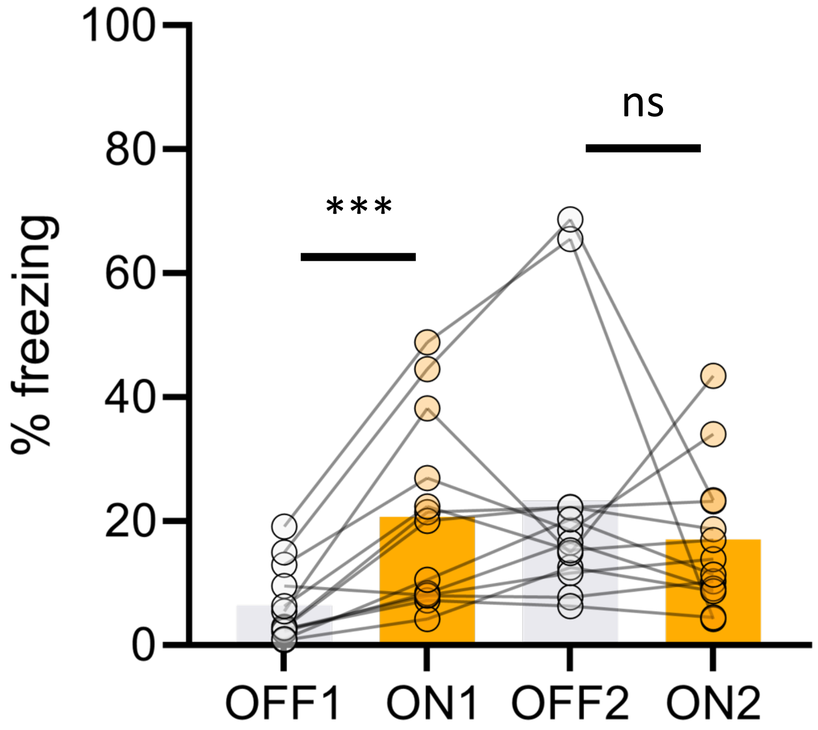
Freezing cells are driving memory recall?



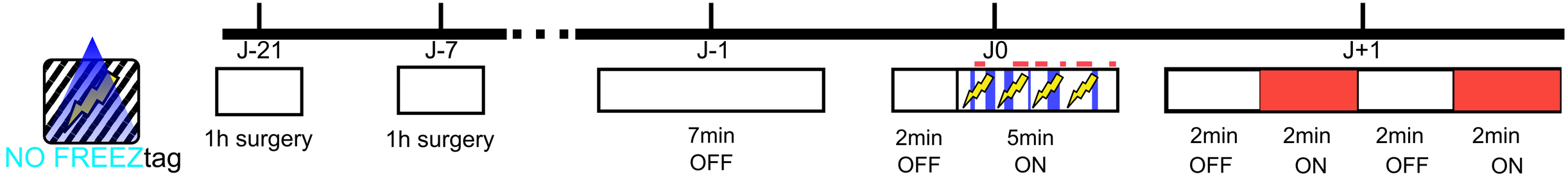


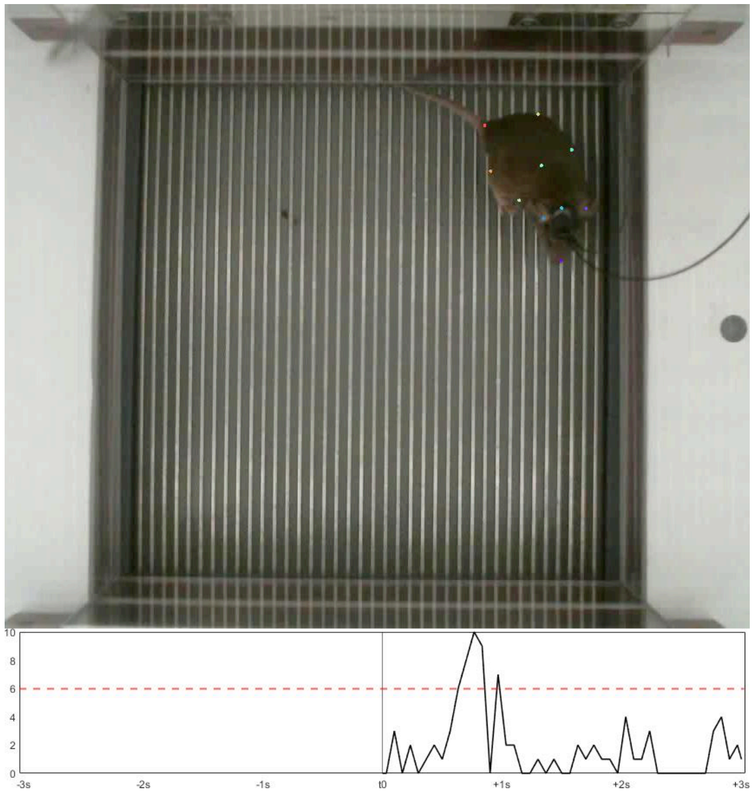


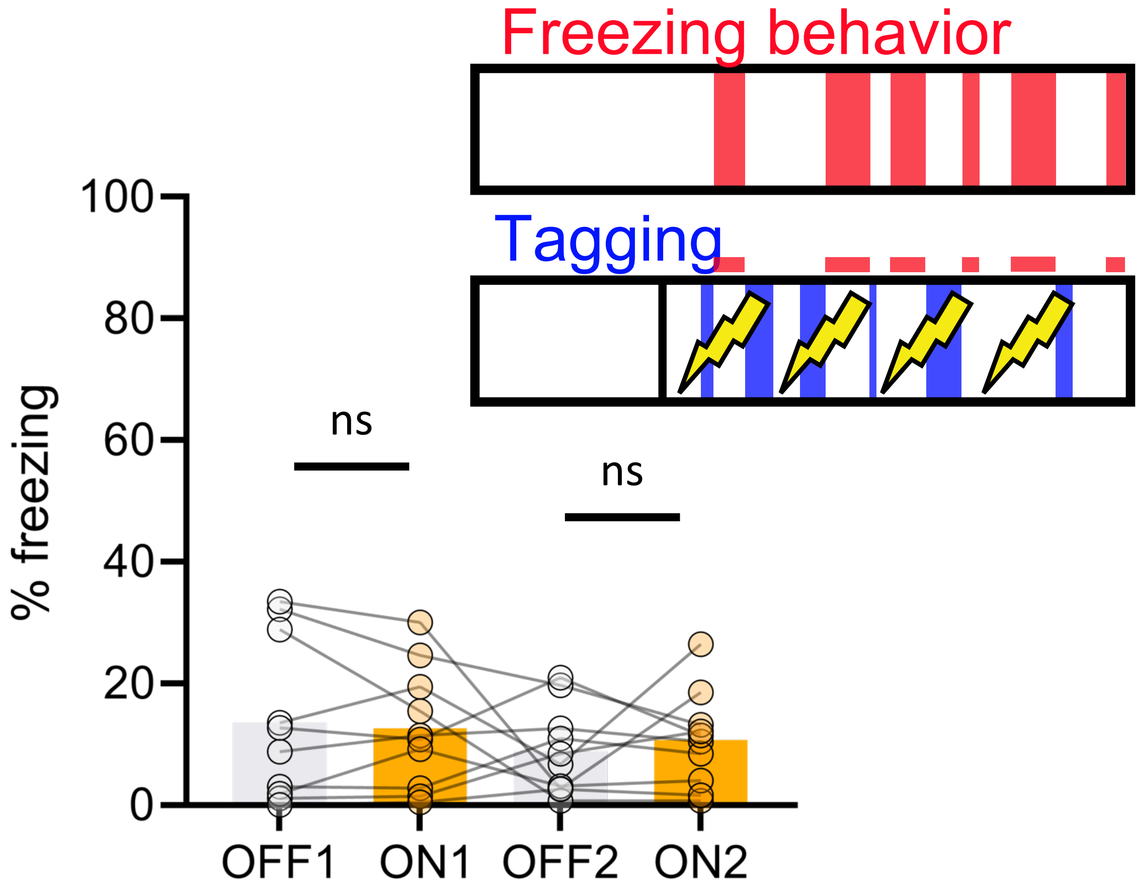




NO-Freezing cells are driving memory recall?

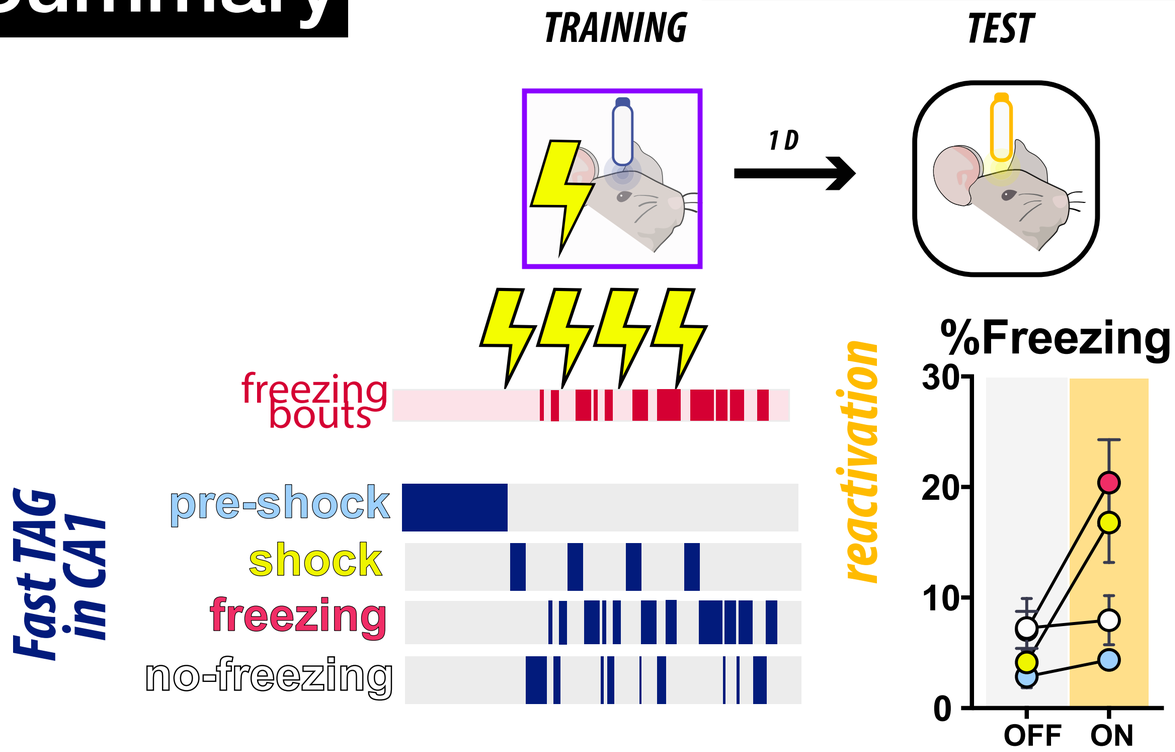


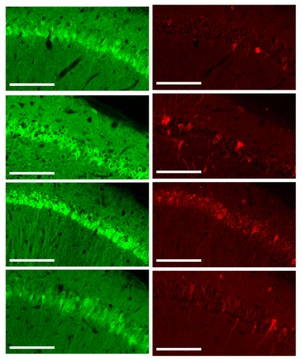


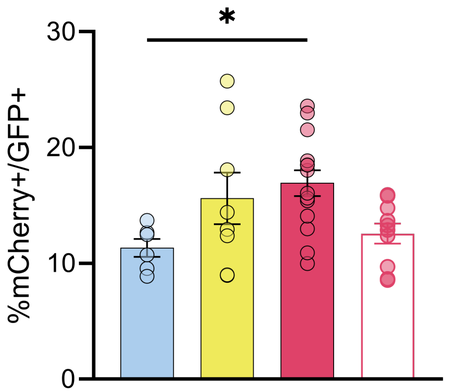


Summary

USING FLiCRE TO DISSECT THE ENGRAM







Clement Pouget

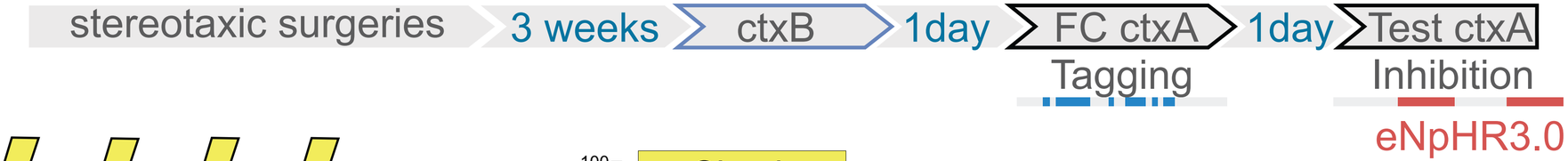


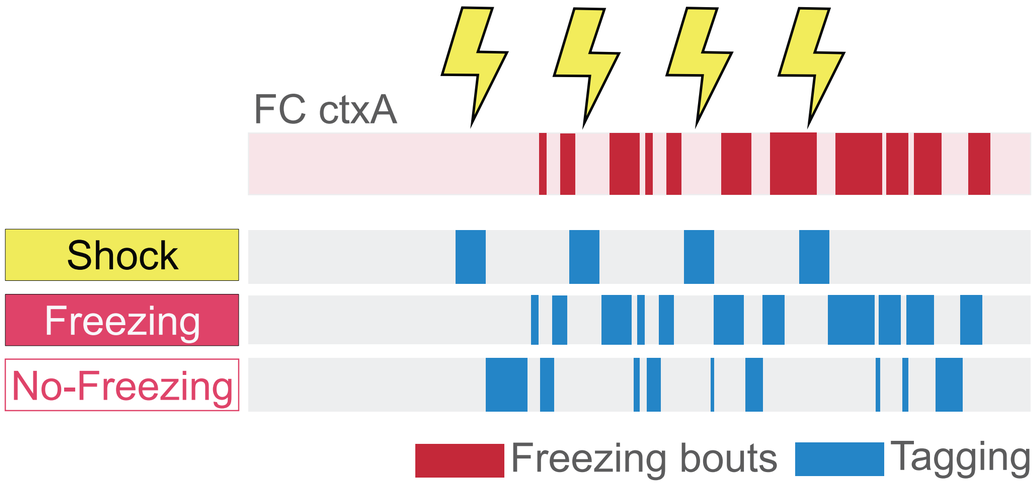
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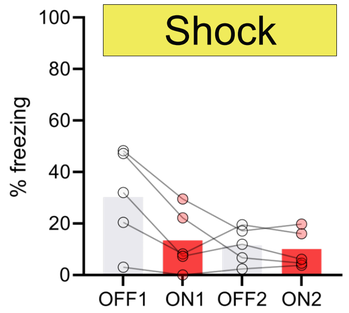
C4TEAM

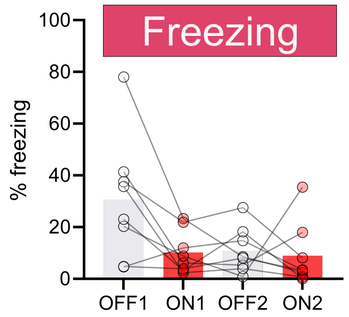
It is sufficient. Is it ALSO NECESSARY?

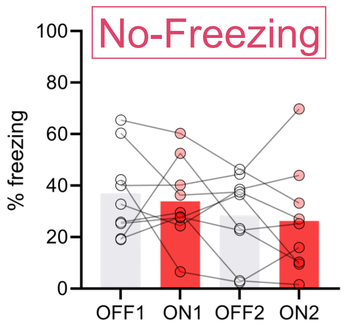
Inhibiting shock cells or freezing cells impairs memory

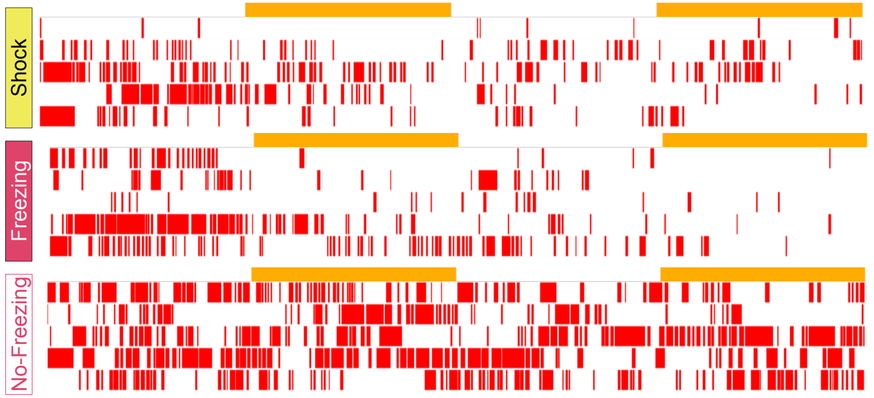


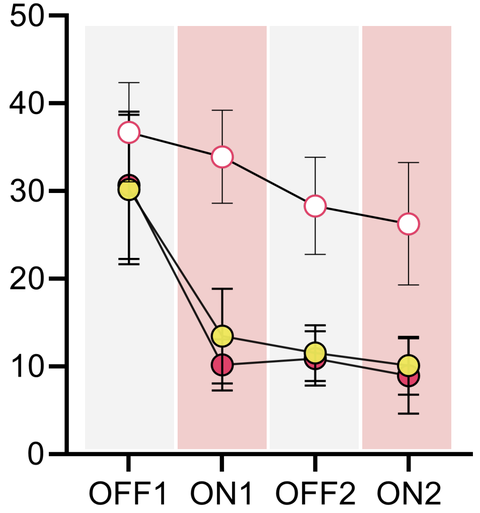


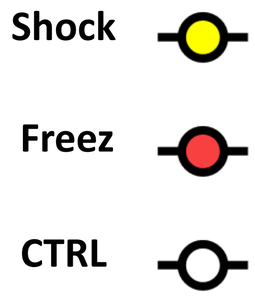




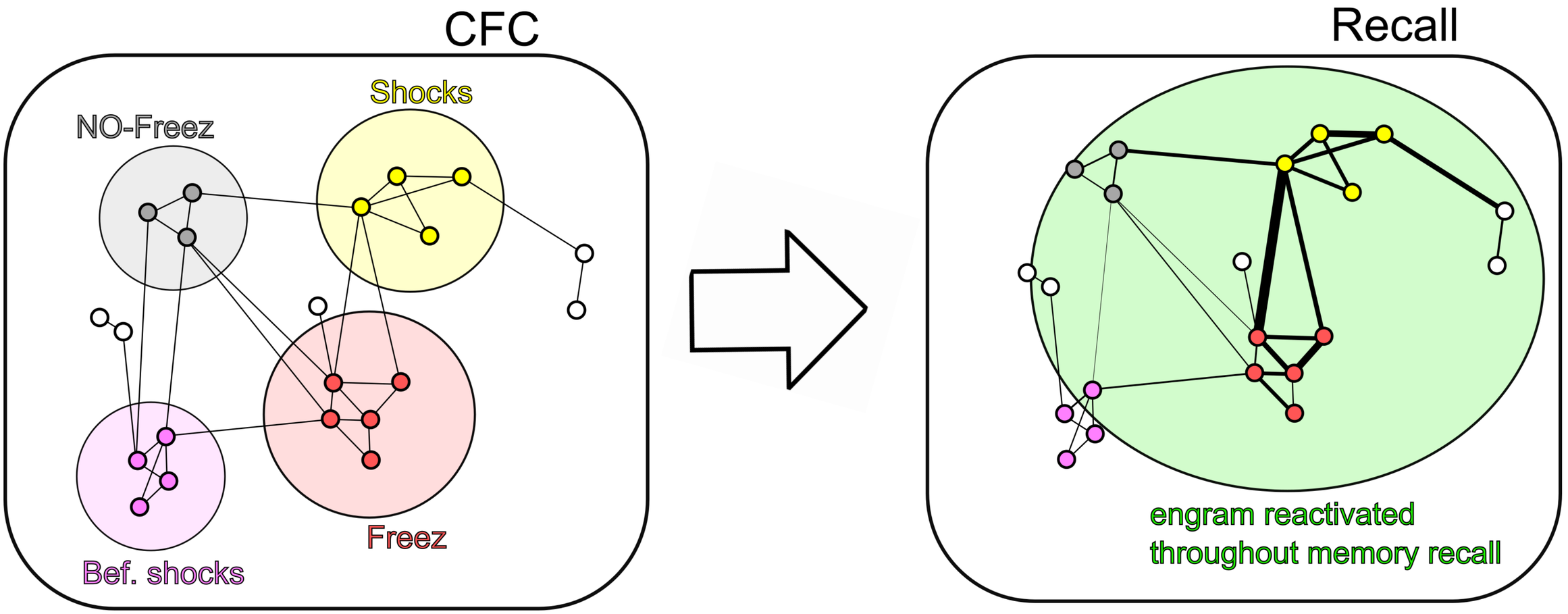


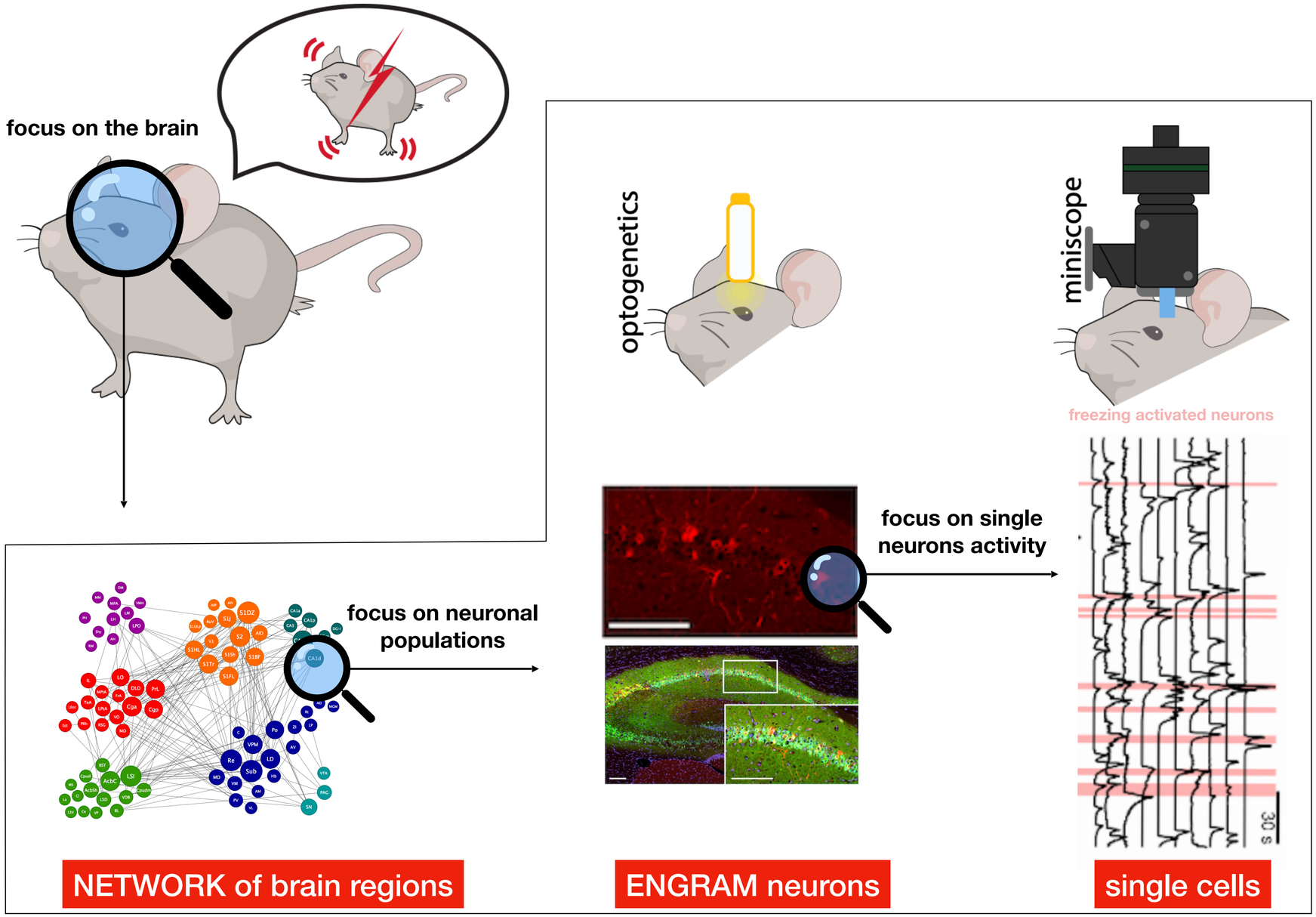






Hypothesis


CFC
Shocks
NO-Freez
Freez
Bef. shocks
Recall
engram reactivated
throughout memory recall


focus on the brain
optogenetics
miniscope
freezing activated neurons
focus on neuronal populations
focus on single neurons activity
30 s
NETWORK of brain regions
ENGRAM neurons
single cells

Is it memory-related Freezing?

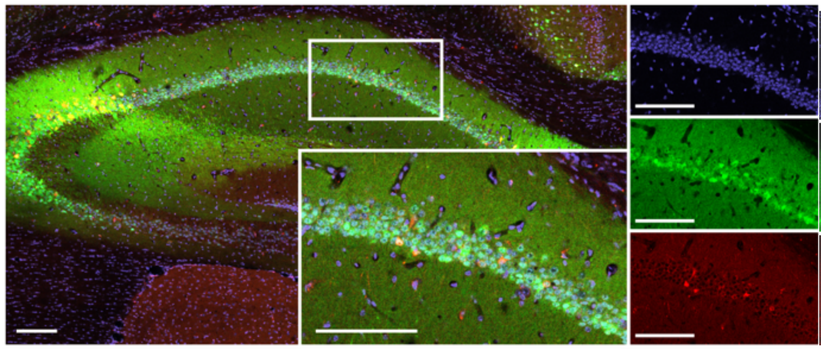




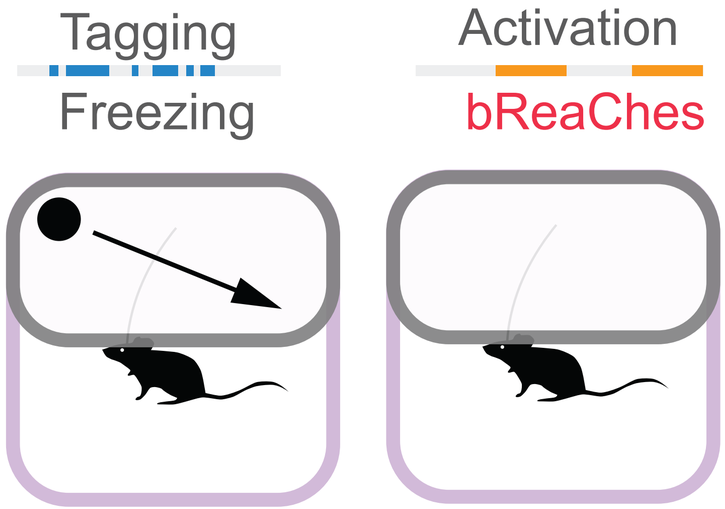
tag freezing behaviour unrelated with an associative memory: the sweeping task

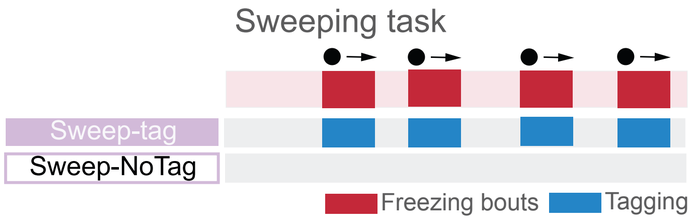
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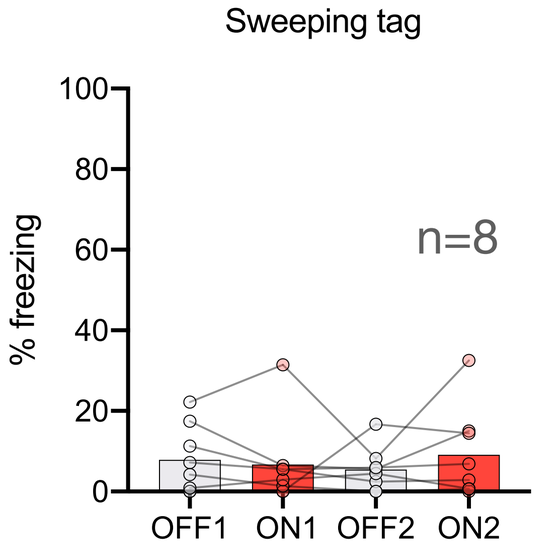
stereotaxic surgeries → 3 weeks → Sweep → 1day → test

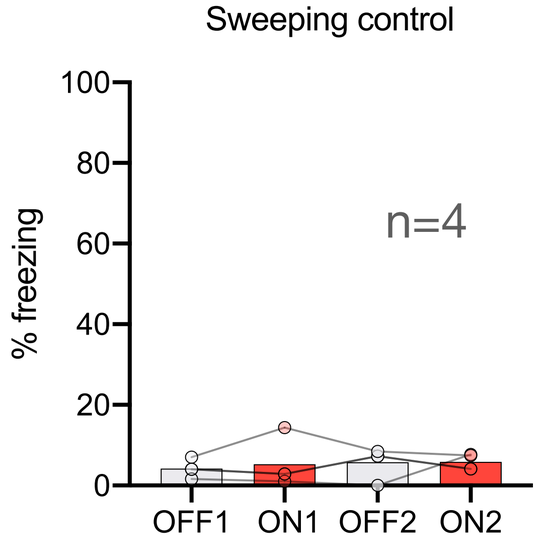


DAPI | GFP-uTEVp | TRE-mCherry-p2a-bReaChES







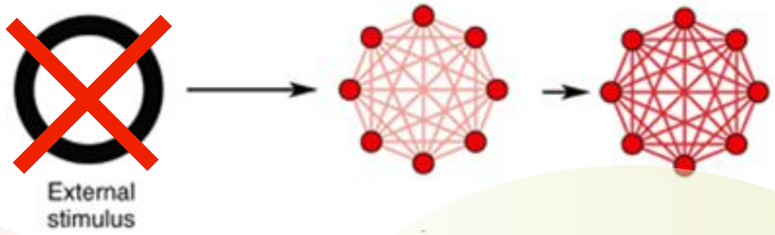


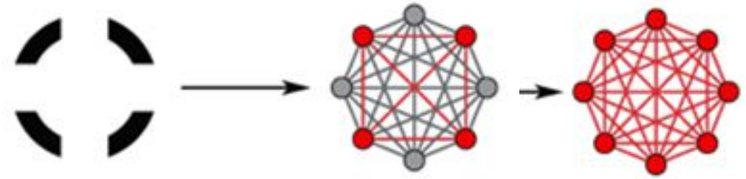
THE FORMATION OF A MEMORY

CS+US

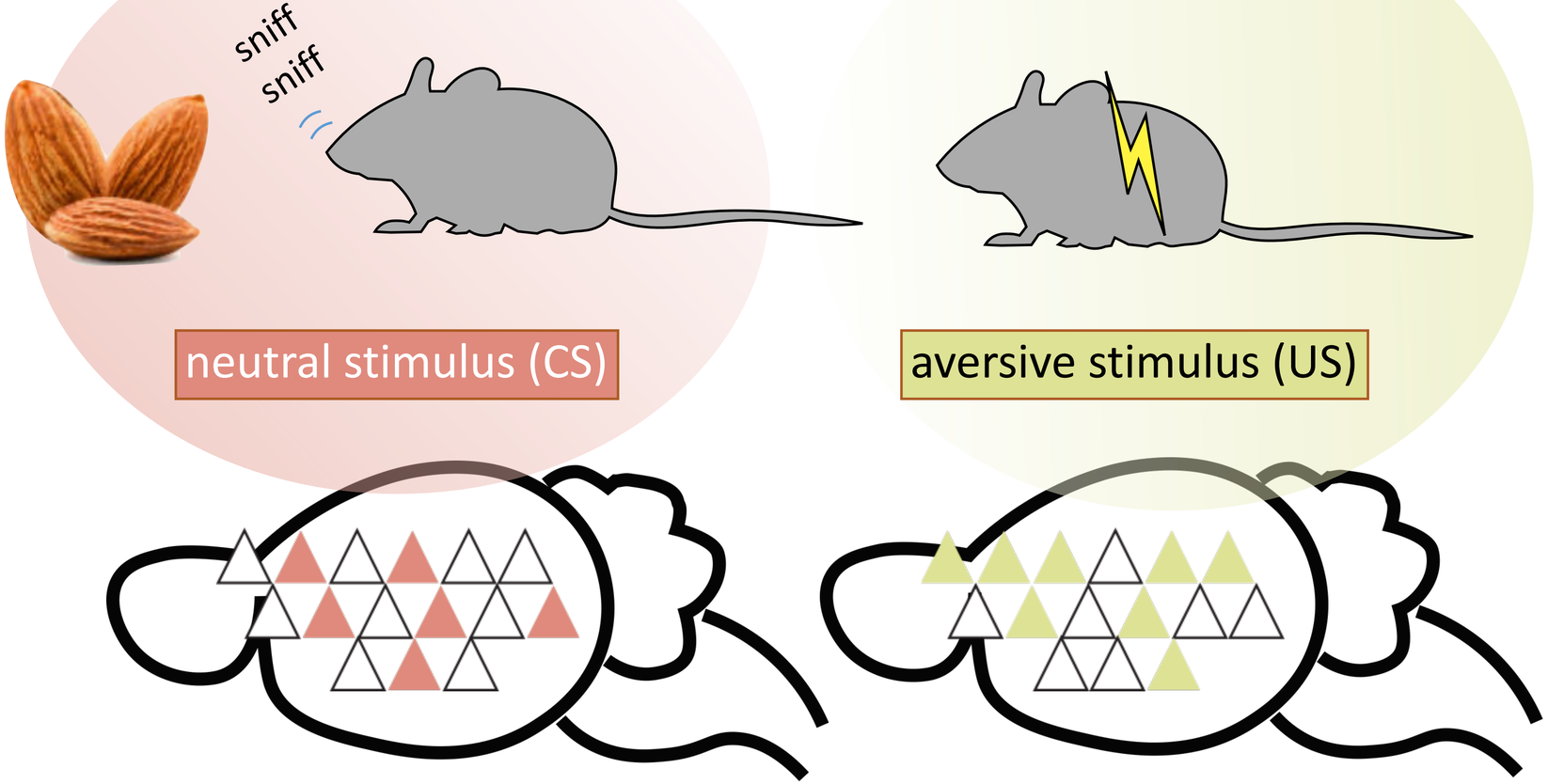
ARTIFICIAL ACTIVATION of the engram

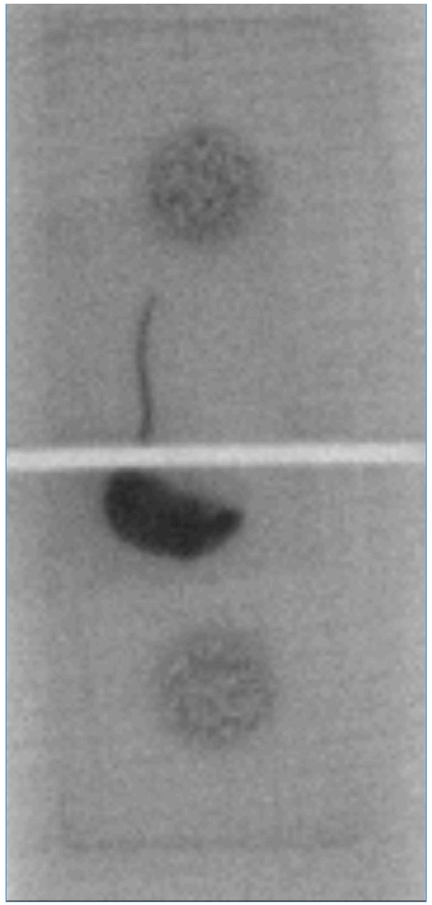
CS ALONE





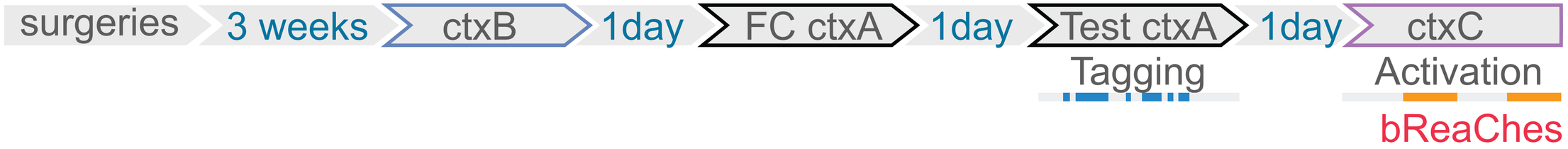
the day after…

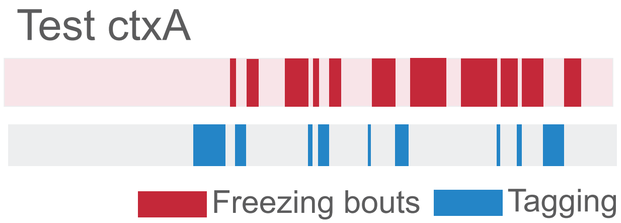




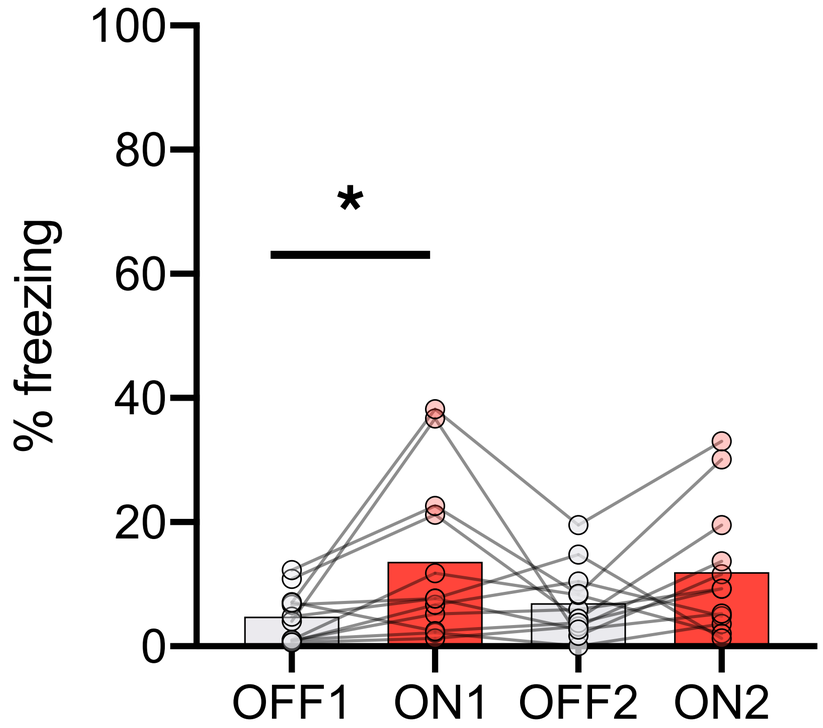
ODOR A avoidance

how is the engram during memory recall?





NO-Freezing cells during memory recall



Summary

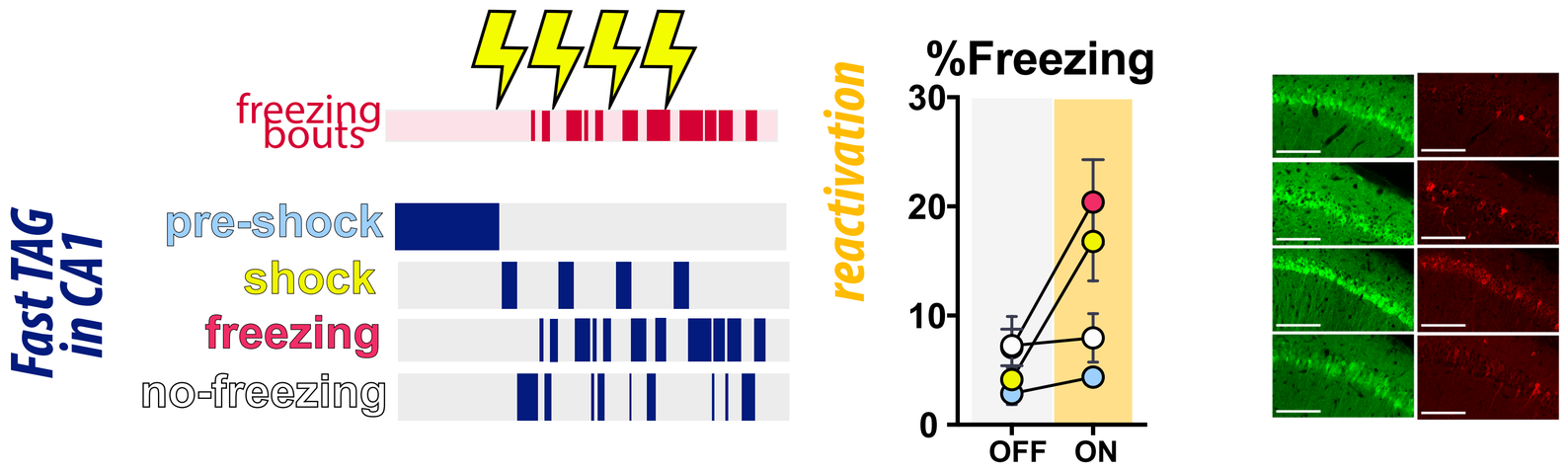
USING FLiCRE TO DISSECT THE ENGRAM

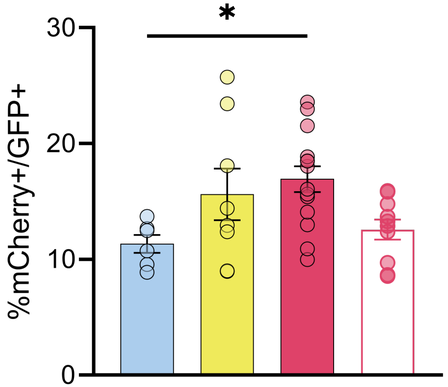


Clement Pouget

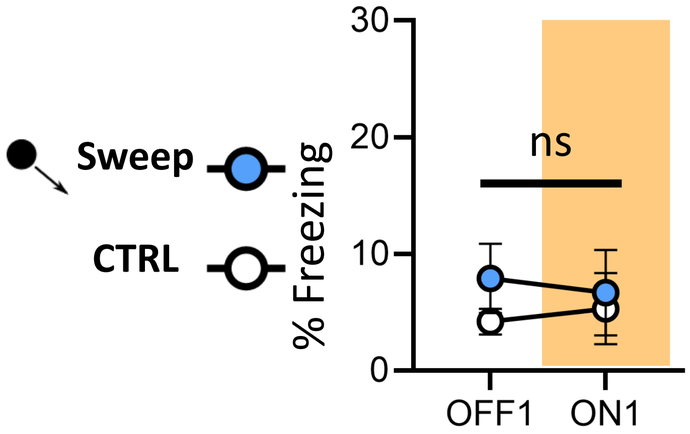
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C4TEAM

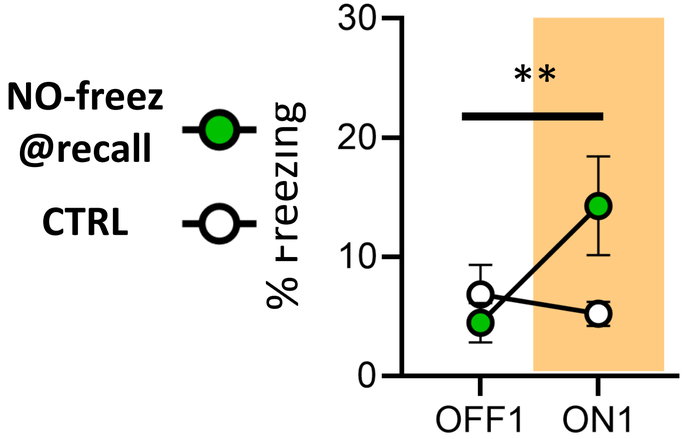




Is it memory-related Freezing?



how is the engram during memory recall?



It is sufficient. Is it ALSO NECESSARY?

Summary

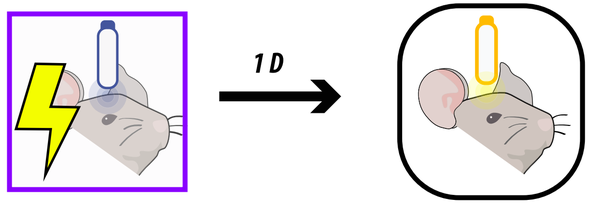
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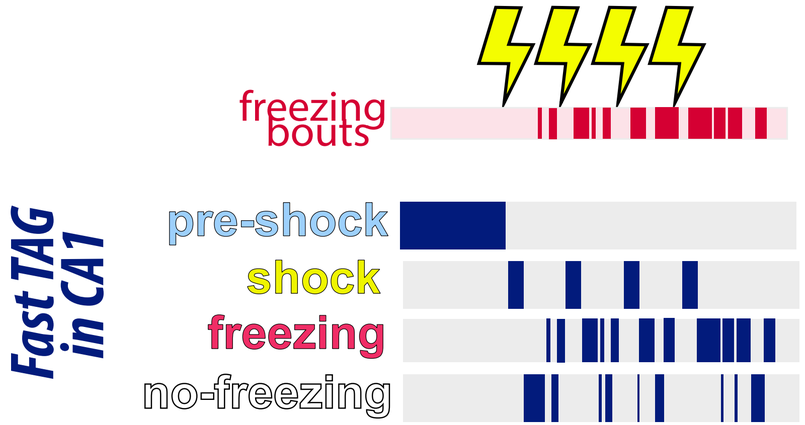


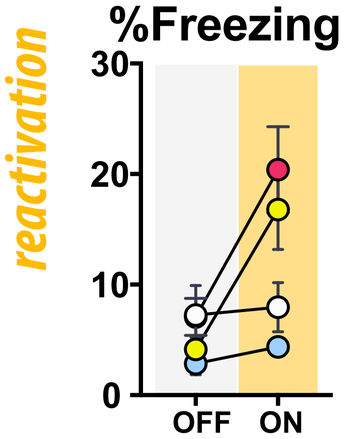
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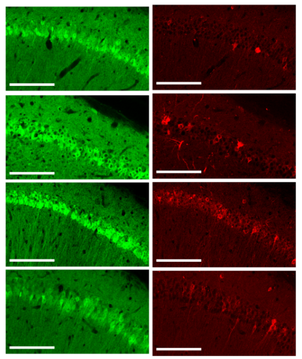
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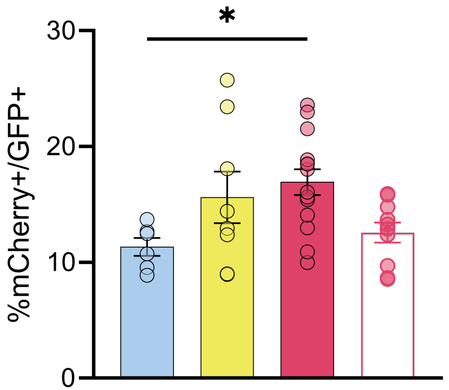
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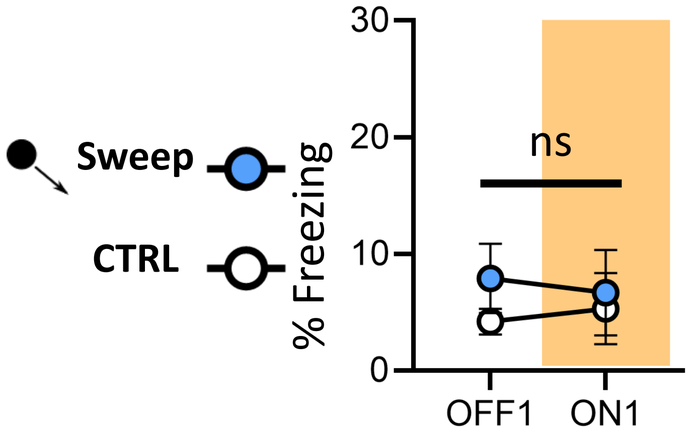




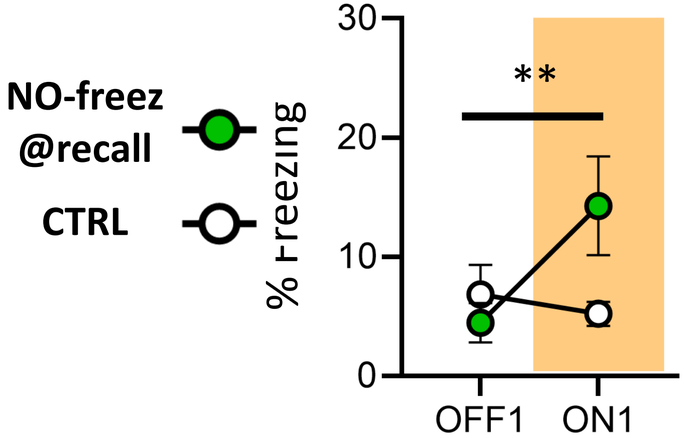




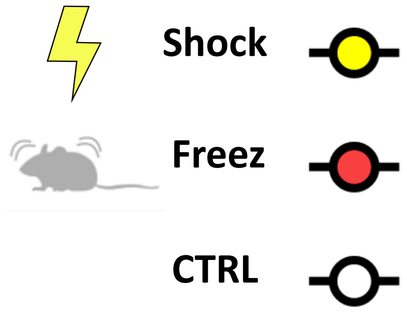
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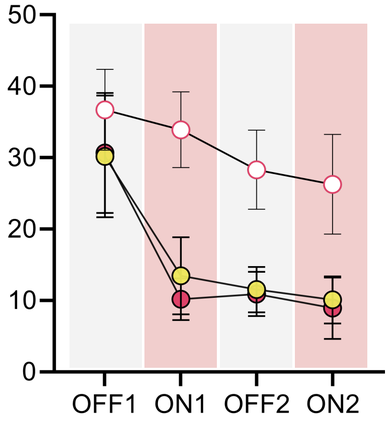


how is the engram during memory recall?



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Summary

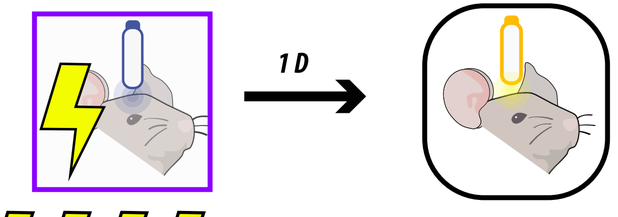
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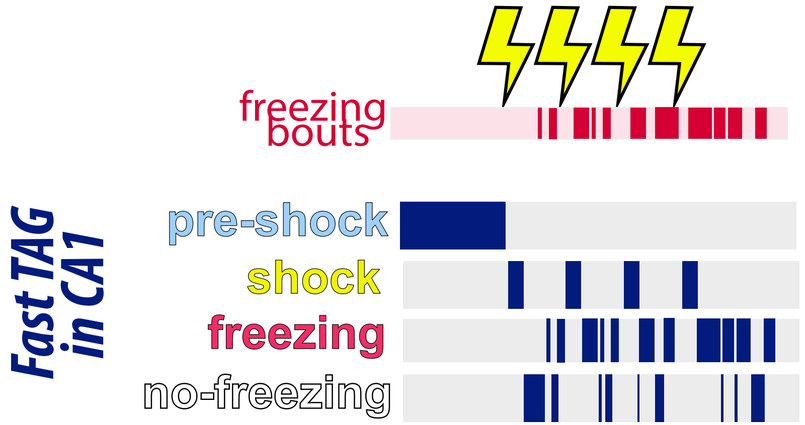


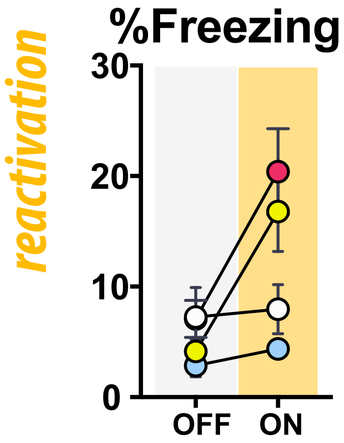
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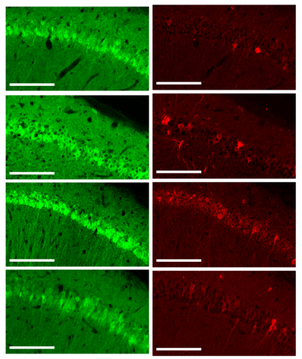
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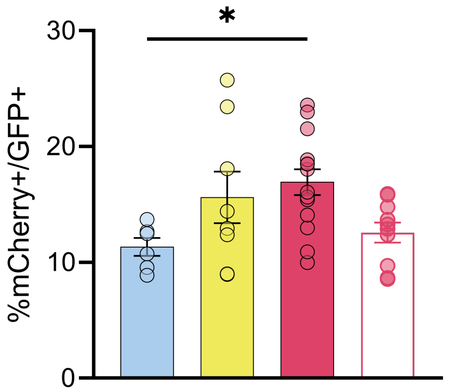
C4TEAM







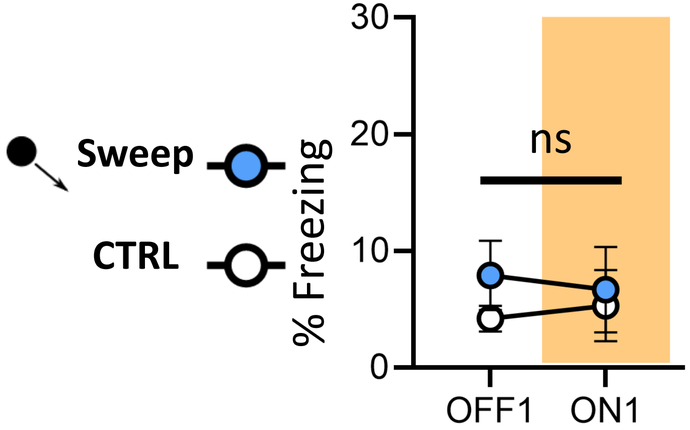




Is it memory-related Freezing?

how is the engram during memory recall?

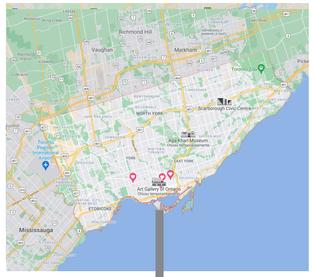
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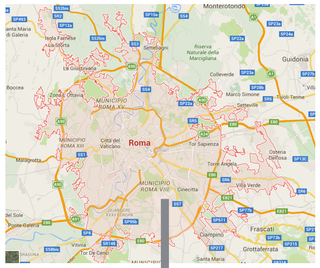
THE HIPPOCAMPAL SYSTEM



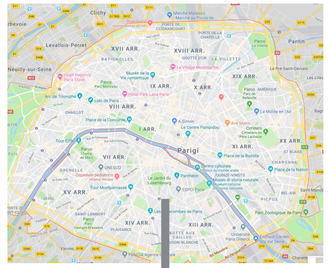
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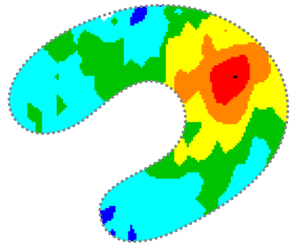


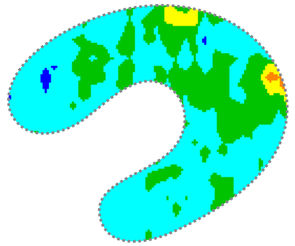
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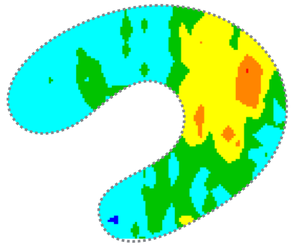


PARIS



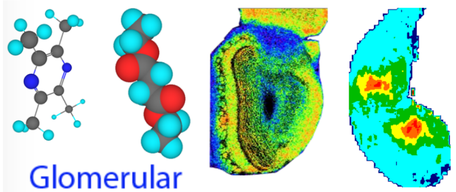




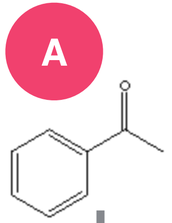


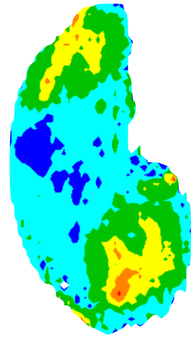
THE OLFACTORY SYSTEM

1 odor=1 map

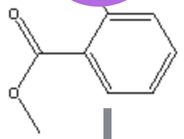


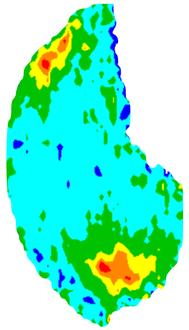
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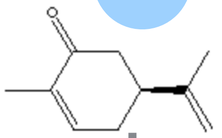


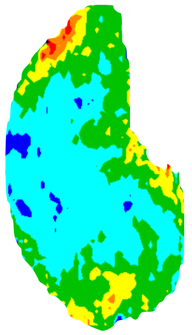
METHYL SALICILATE



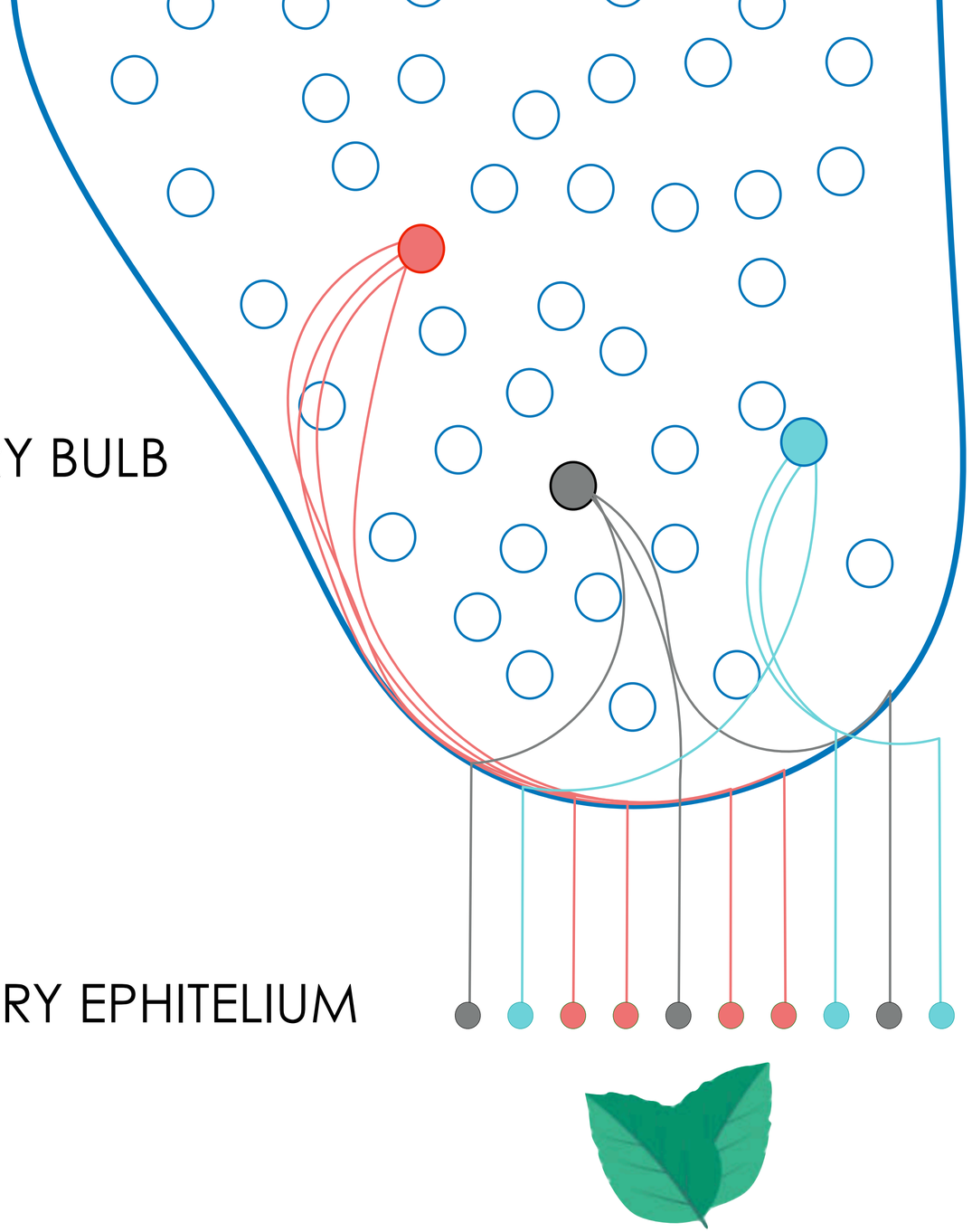


CARVONE





each odor create a unique map of glomeruli activation

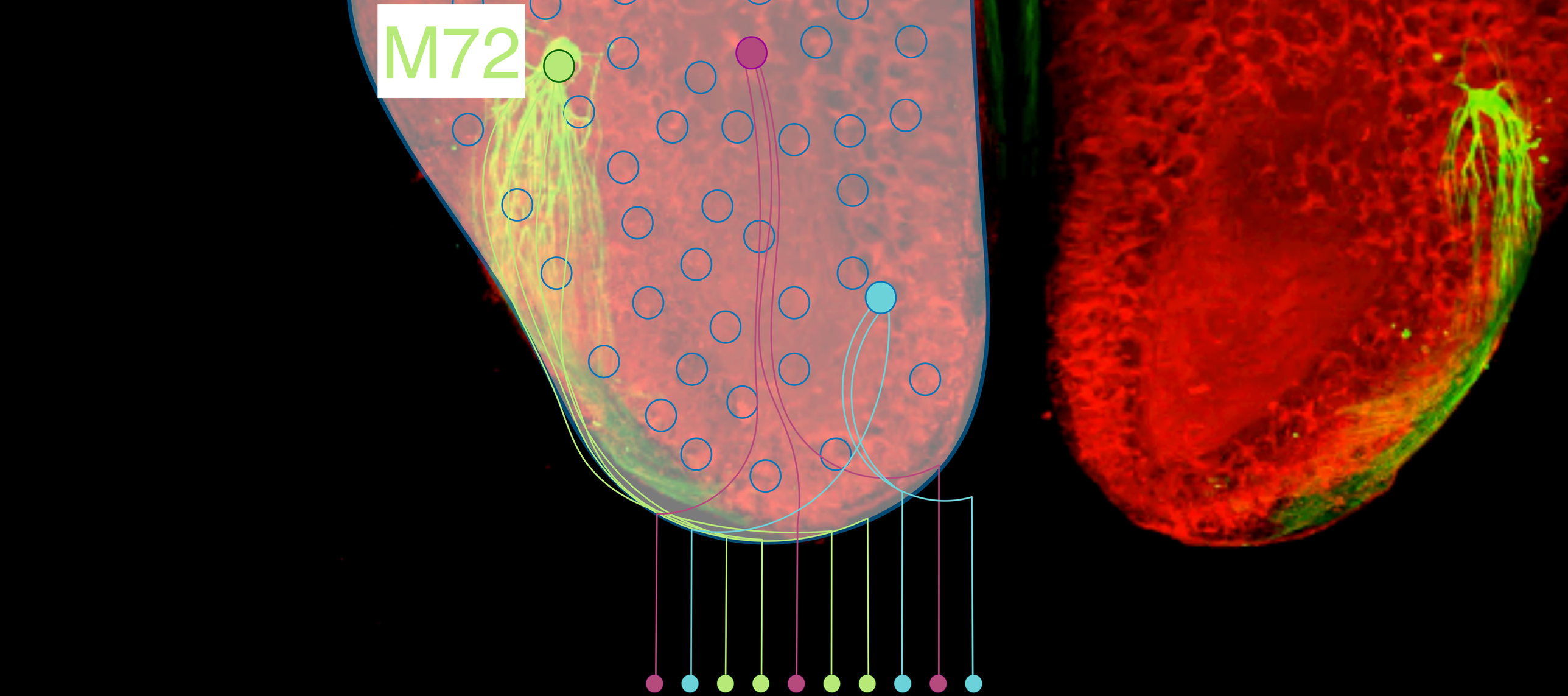

OLFACTORY BULB
OLFACTORY EPHITELIUM

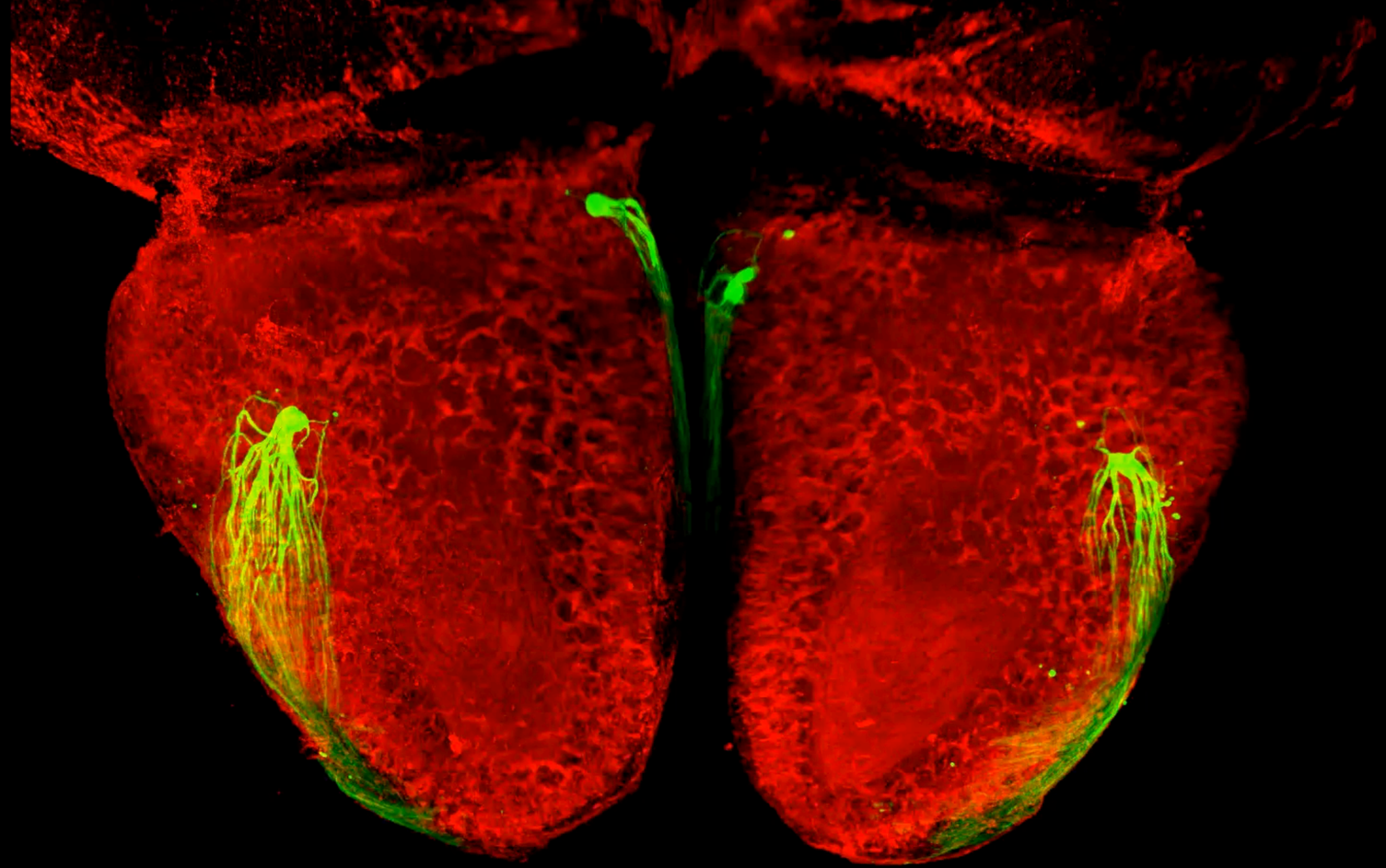
M72

OLFACTORY BULB

OLFACTORY EPHITELIUM

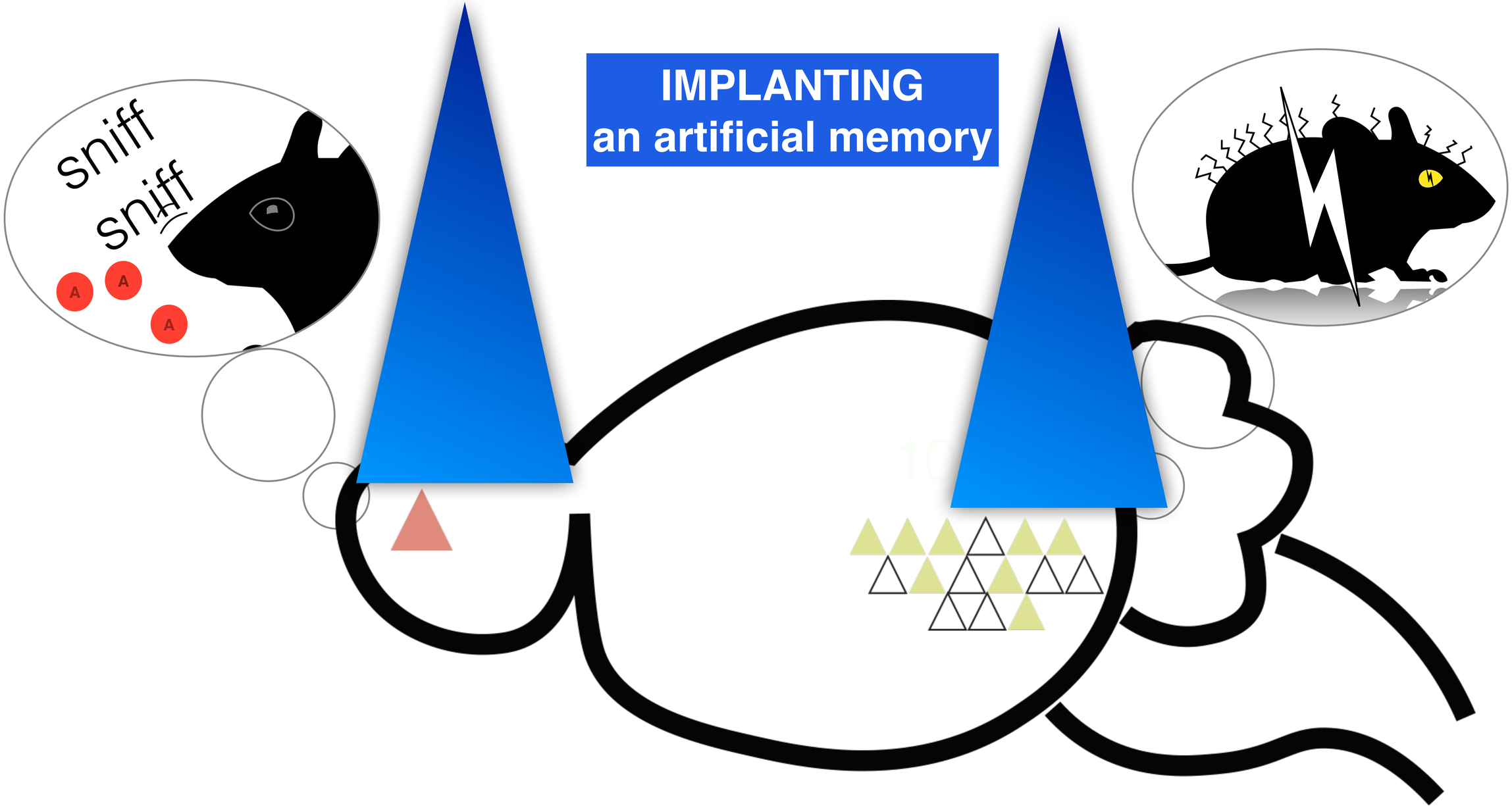



M72

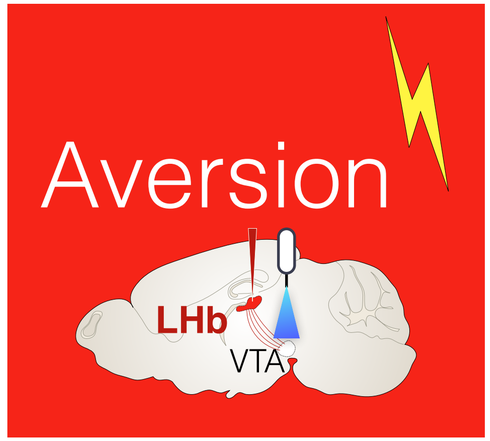


M72-ChR2-YFP mice

optogenetics to mimic the activation of olfactory receptors


IMPLANTING
an artificial memory
sniff
sniff
A
A
A

mimic an emotional experience (aversive or rewarding)





ARTICLE

doi:10.1038/nature11527

Input–specific control of reward and aversion in the ventral tegmental area

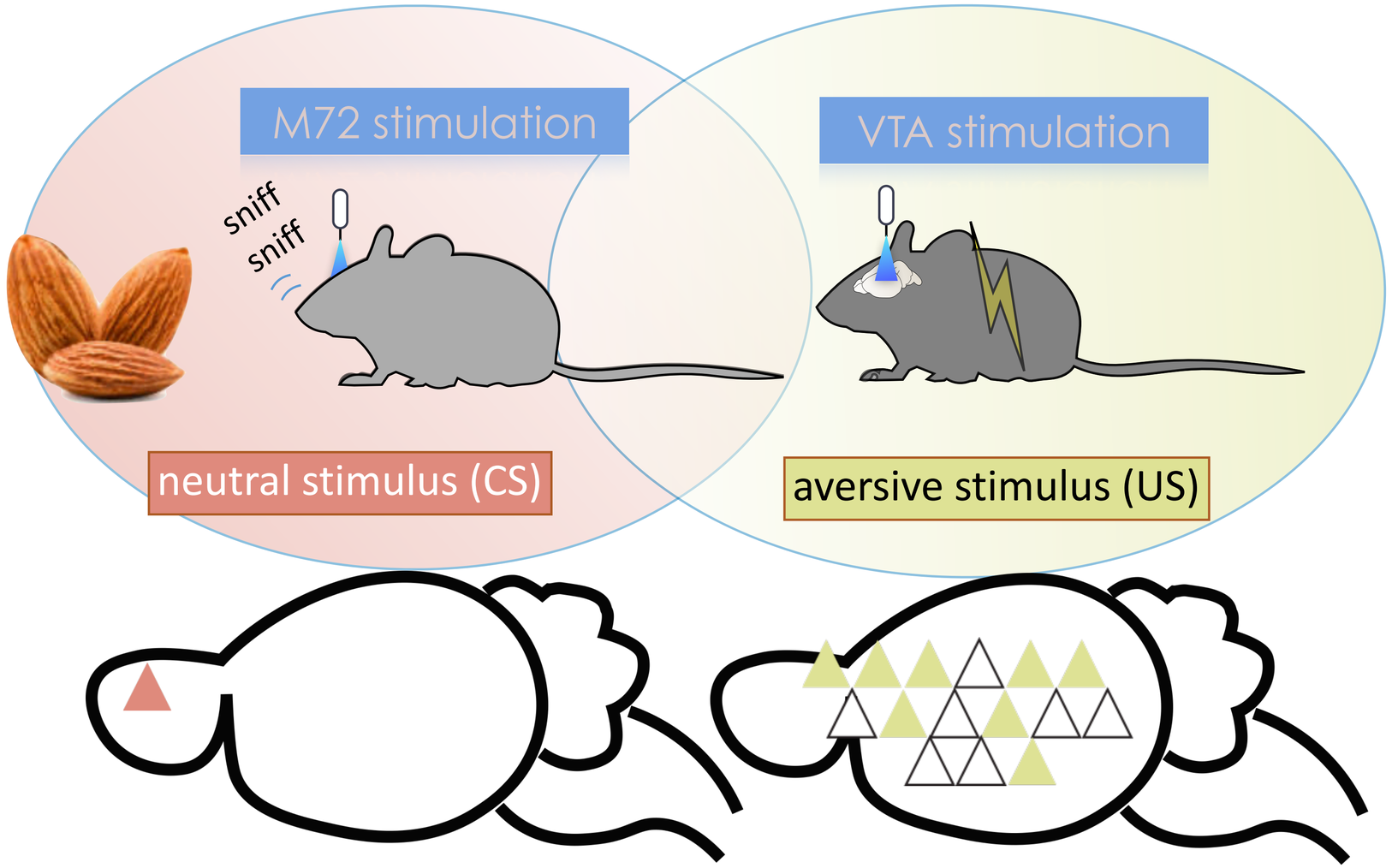
Stephan Lammel[1]*, Byung Kook Lim[1]*, Chen Ran[1], Kee Wui Huang[1], Michael J. Betley[1], Kay M. Tye[2], Karl Deisseroth[3] & Robert C. Malenka[1]

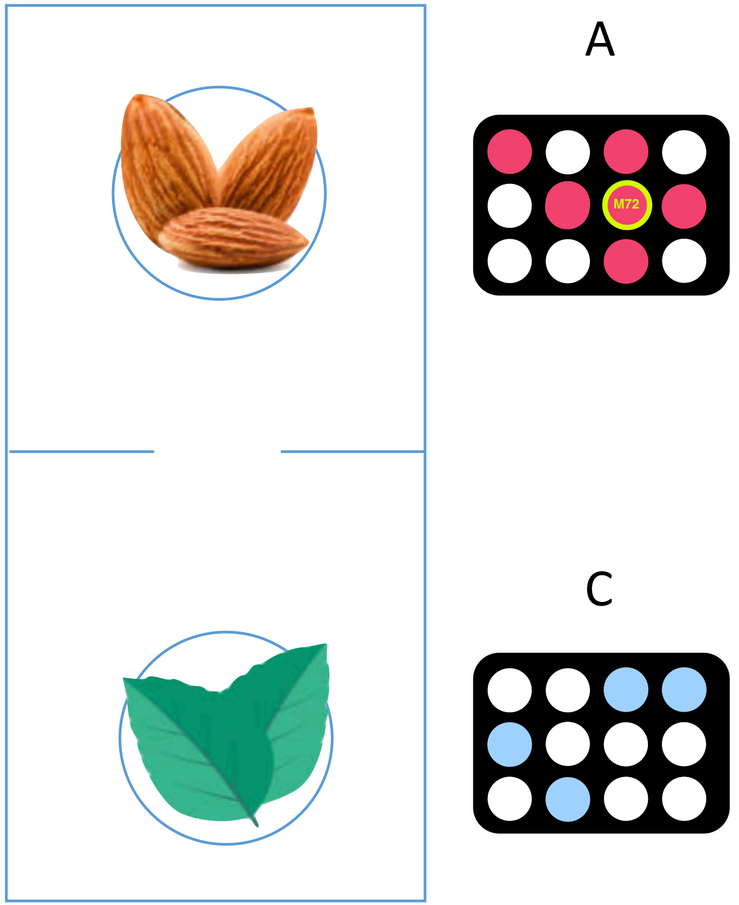
Ventral tegmental area (VTA) dopamine neurons have important roles in adaptive and pathological brain functions related to reward and motivation. However, it is unknown whether subpopulations of VTA dopamine neurons participate in distinct circuits that encode different motivational signatures, and whether inputs to the VTA differentially modulate such circuits. Here we show that, because of differences in synaptic connectivity, activation of inputs to the VTA from the laterodorsal tegmentum and the lateral habenula elicit reward and aversion in mice, respectively. Laterodorsal tegmentum neurons preferentially synapse on dopamine neurons projecting to the nucleus accumbens lateral shell, whereas lateral habenula neurons synapse primarily on dopamine neurons projecting to the medial prefrontal cortex as well as on GABAergic (γ-aminobutyric-acid-containing) neurons in the rostromedial tegmental nucleus. These results establish that distinct VTA circuits generate reward and aversion, and thereby provide a new framework for understanding the circuit basis of adaptive and pathological motivated behaviours.

The functional roles of VTA dopamine neurons have received great attention because they are the primary source of dopamine in target structures such as the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), which have important roles in a broad range of motivated behaviours and neuropsychiatric disorders[1–3]. Although dopamine neuron activity often correlates with a reward prediction error (that is, the difference between expected and actual rewards), these cells can also signal aversion, saliency, uncertainty and novelty[2,3]. They are heterogeneous in their anatomical location, targets to which they project, electrophysiological properties and several molecular

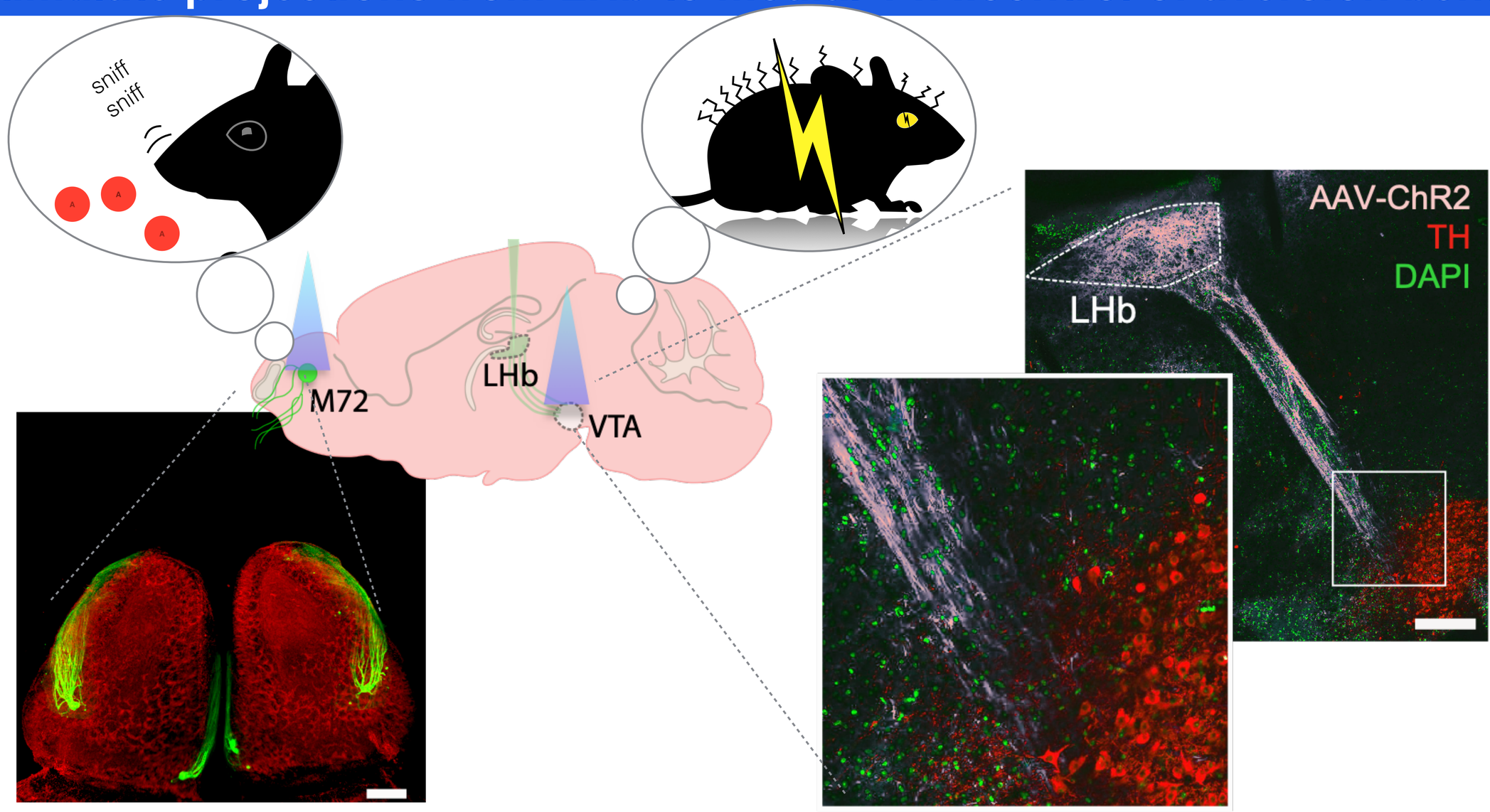
activity and the consequent release of dopamine in target structures[2,7,18]. eGFP-positive LDT neurons expressed markers for both glutamatergic neurons (the glutamate transporter EAAC1 (also known as SLC1A1)) and cholinergic neurons (choline acetyltransferase; ChAT) (Supplementary Fig. 2)[19,20]. However, although 95% of LDT neurons projecting to the VTA expressed EAAC1, only ~7% expressed ChAT. LHb neurons are excited by the absence of an expected reward[18] and probably send direct inputs to GABAergic cells in the tail of the VTA, the rostromedial tegmental nucleus (RMTg)[21,22], that inhibit VTA dopamine neurons[23–26]. eGFP-positive LHb neurons were immunopositive for EAAC1 but

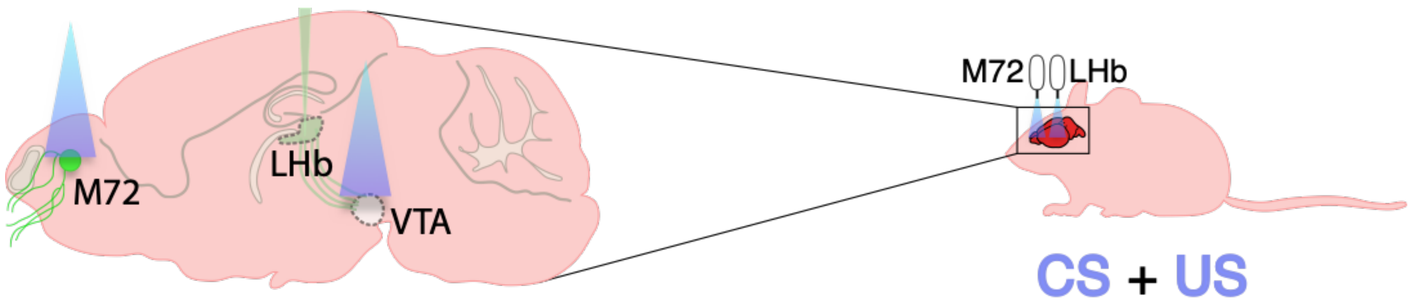
THE IMPLANTATION OF A MEMORY





stimulate projections from LHb to medial VTA:control of aversion behaviour



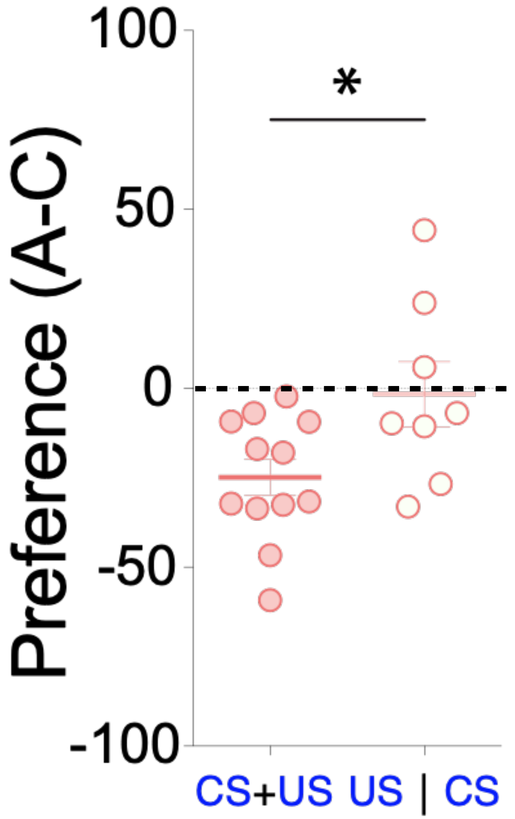




CS + US

US | CS

We created an aversive memory of an event never experienced before

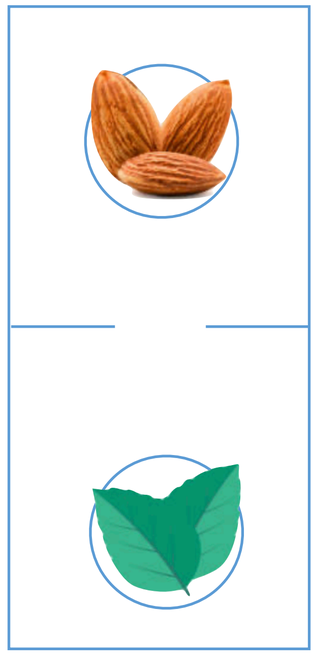


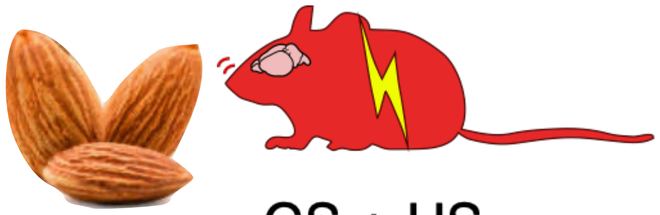


avoidance of room

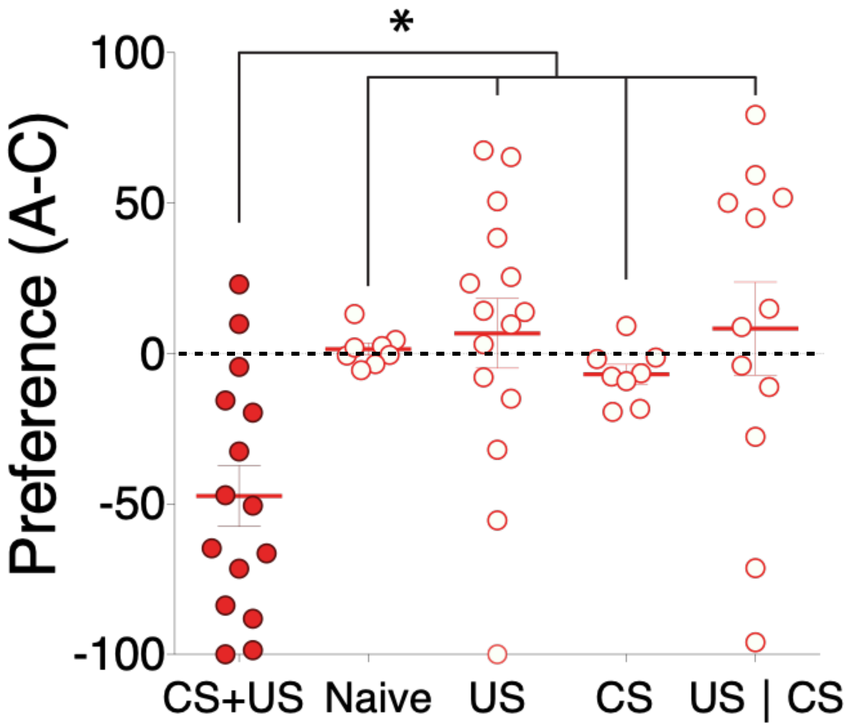
Vetere et al. Nature Neuroscience. 2019

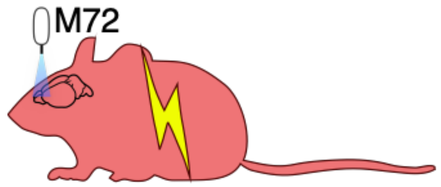
We can use a known code to create a memory of an event never experienced before



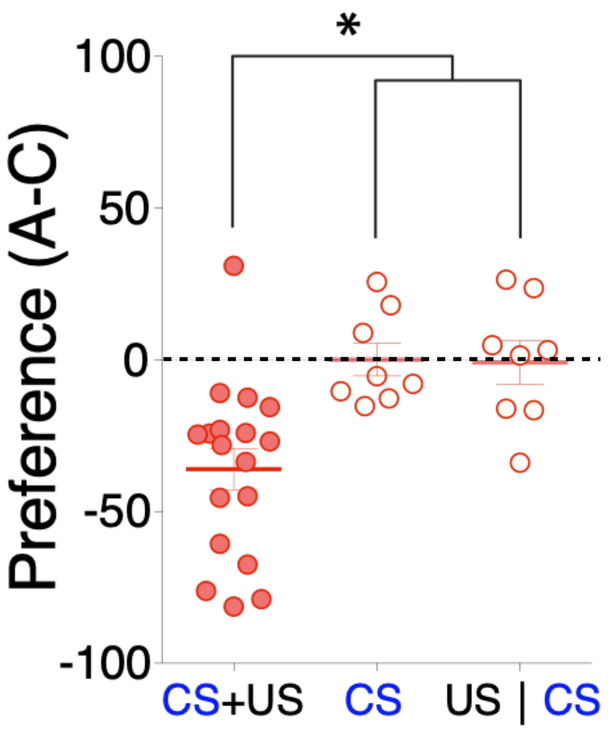


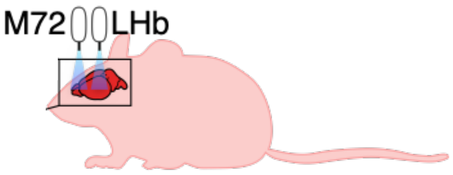
CS + US



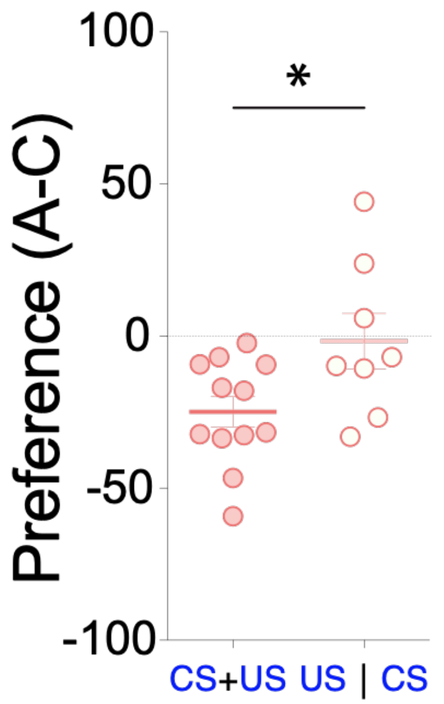


CS + US



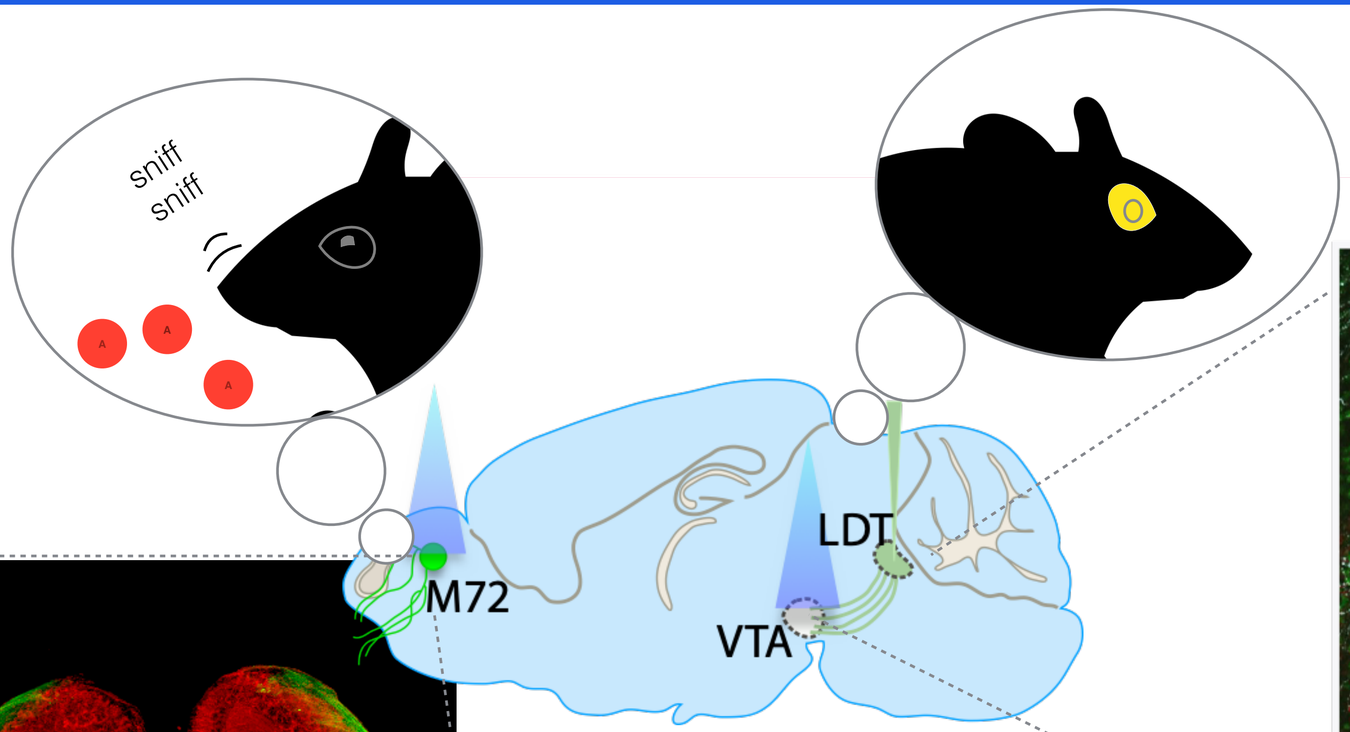


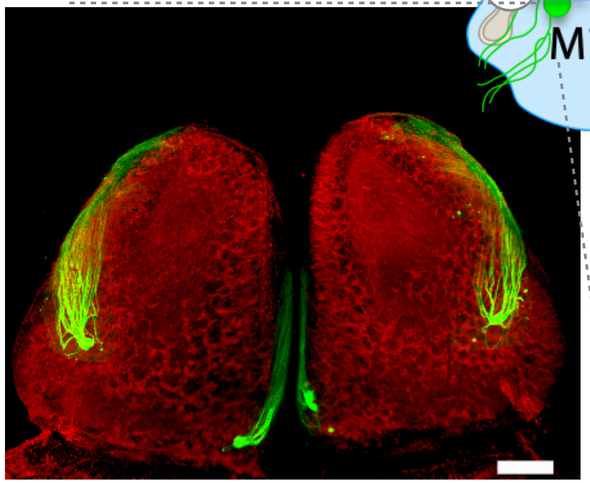
CS + US

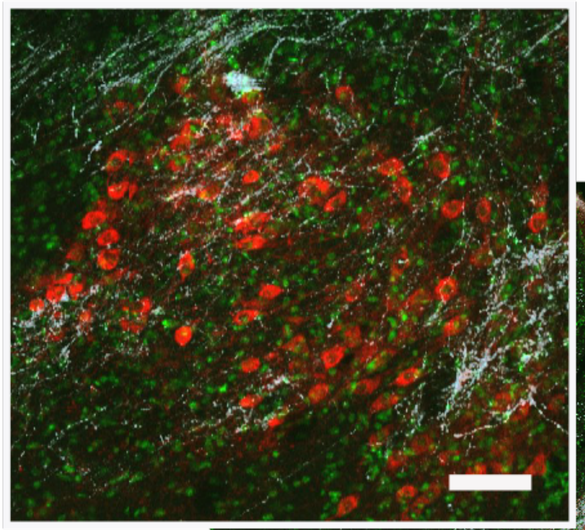


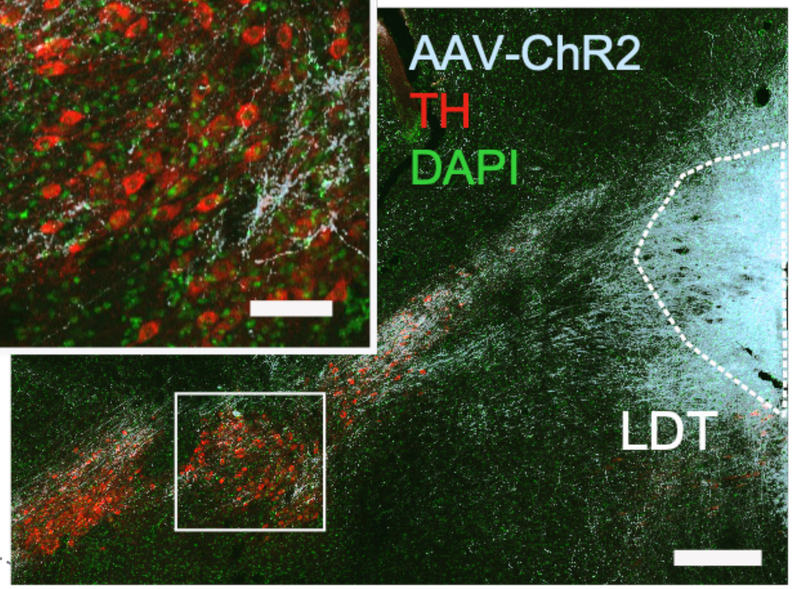
Vetere et al. Nature Neuroscience. 2019

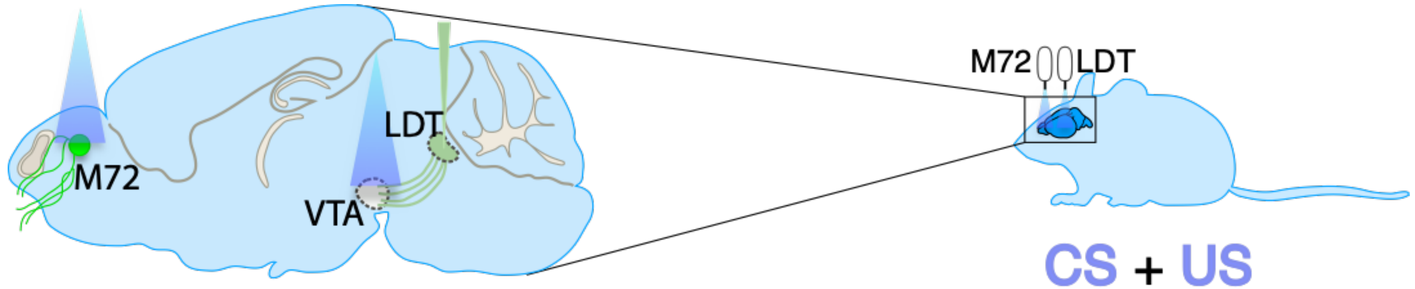
stimulate projections from LDT to medial VTA: control of reward behaviour









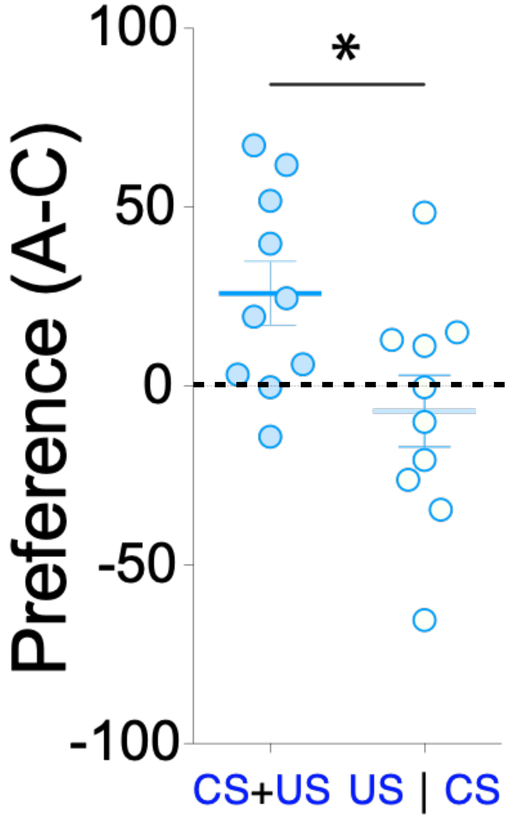




CS + US

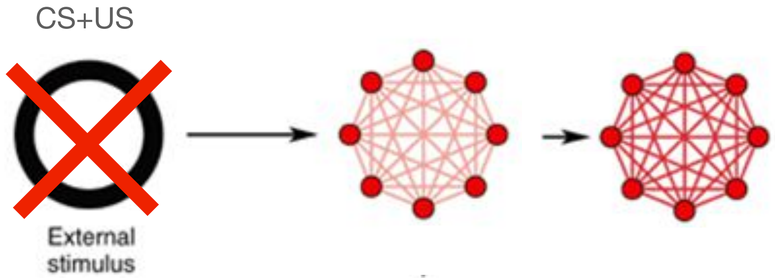
US | CS

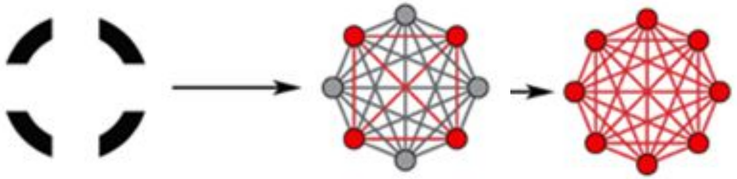
We created an *reward* memory of an event never experienced before



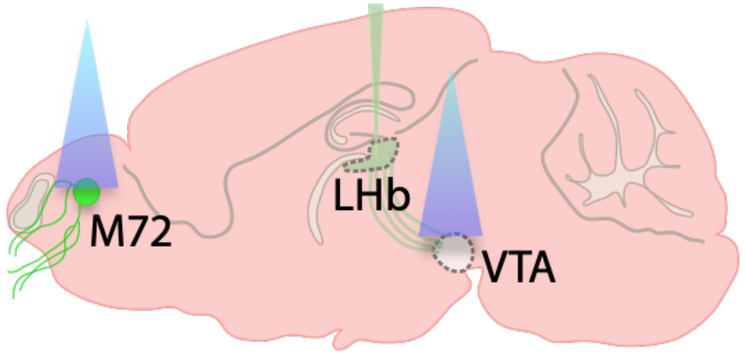


Vetere et al. Nature Neuroscience. 2019

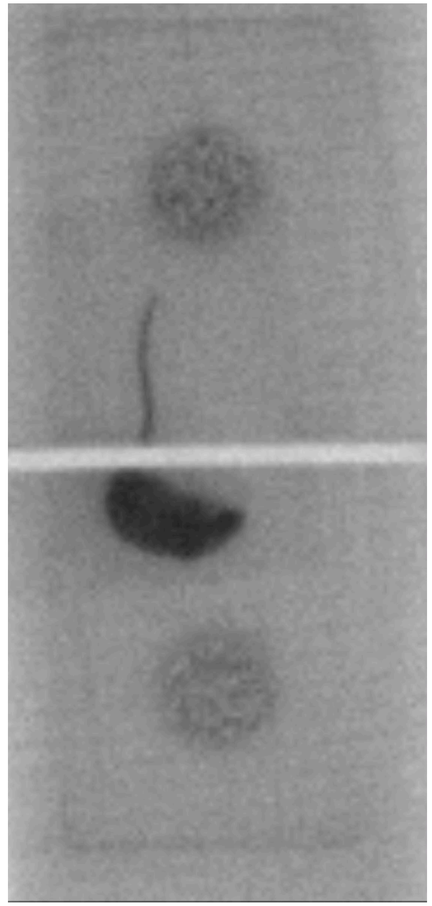




NEGATIVE ARTIFICIAL MEMORY







POSITIVE ARTIFICIAL MEMORY

