In vivo activity tagging techniques and their applications

Journal Club

06/07/21

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Why neuronal activity tagging?

Obtain a brain-wide scale visualization with cellular resolution of neuronal activation

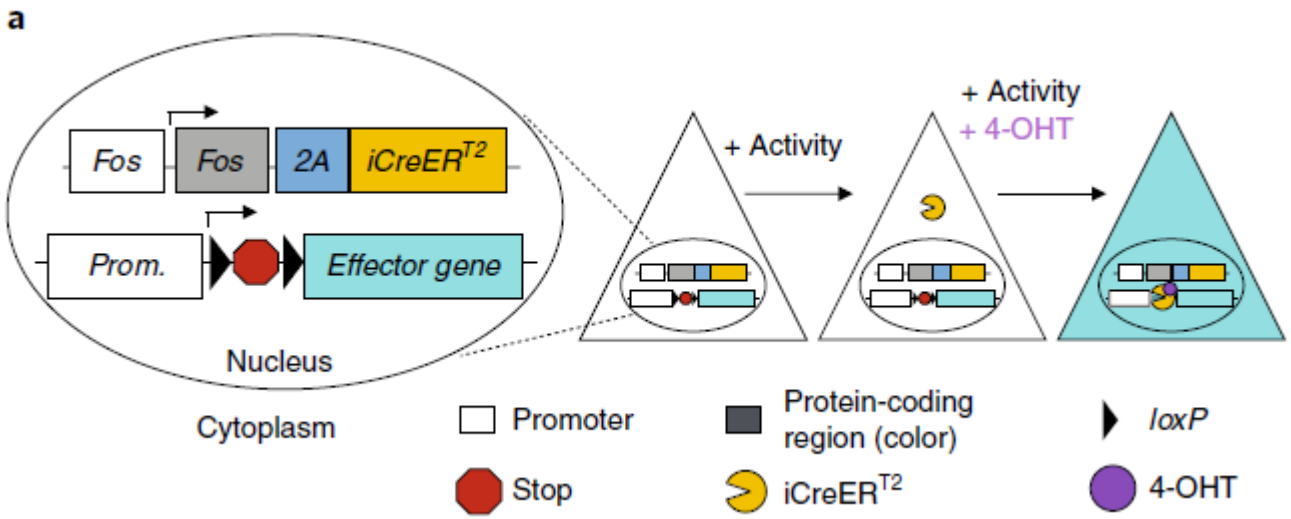
- This allows the analysis of how neuronal circuits are disrupted during pathology or affected by specific behaviors/experiences

- Ideally you would want electrical activity of all neurons in the brain of a living and freely moving animal with single cell resolution **→ so far a bit complicated…**

- Different options with their drawbacks:
  → fMRi or EEG (low resolution but live and whole-brain)
  → Calcium activity/voltage recordings (high resolution but very limited FOV)

- Compromise using *in vivo* tagging and subsequent *ex vivo* imaging

Targeted recombination in active populations



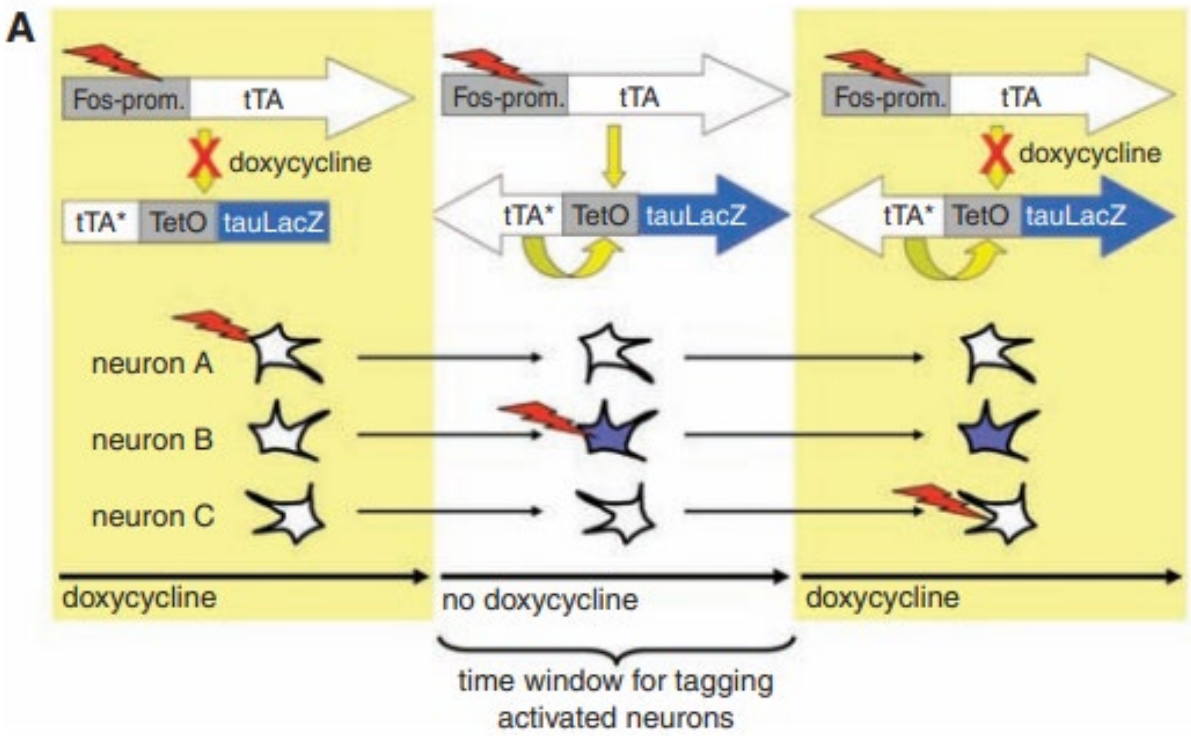
- **TRAP allows <u>permanent</u> genetic access to neurons activated by a specific experience**

- The TRAP system uses an IEG (cFos) locus to drive the expression of tamoxifen-inducible Cre recombinase along with a Cre-dependent effector (e.g. GFP)

- When a neuron is active in the presence of tamoxifen, the Cre can enter the nucleus to catalyze recombination, resulting in permanent expression of the effector

DeNardo, Laura A., et al. *Nature neuroscience* 22.3 (2019): 460-469.

Similar techniques available: TetTag



- Fos promoter to drive the expression of a doxycycline-repressible tetracycline transactivator (tTA), and artificial TF
- During the resting state, tTA is usually bound to DOX and consequently unable to link to the TRE sequence
- With behavioral task and no DOX, the Fos promoter stimulates the synthesis of tTA, which binds the synthetic promoter tetracycline-responsive element (TRE) an allows the expression of the effector gene

→ Not permanent
→ High background due to slow DOX metabolism

Reijmers, Leon G., et al. *Science* 317.5842 (2007): 1230-1233.

MANY similar approaches based on cFos dependent neuronal tagging

- CANE: lock and key strategy for *capturing activated neuronal ensembles with engineered mice and viruses*
- vGATE: mixture of three viruses, *virus-delivered genetic activity-induced tagging of cell ensembles*
- E-SARE: *synaptic activity- responsive elements,* regulating the expression of Arc and effector genes with the employment of 3 activity dependent transcription factors
- RAM: *robust activity marking;* like SARE, using a synthetic promoter to tag active neurons

Franceschini, Alessandra, et al. *Frontiers in Neuroscience* 14 (2020): 1111.

Research Papers about activity tagging

1 Targeted recombination in active populations as a new mouse genetic model to study sleep-active neuronal populations: Demonstration that Lhx6+ neurons in the ventral zona incerta are activated during paradoxical sleep hypersomnia

Hyun-Sook Lee[1,2,3] | Risa Yamazaki[1] | Dianru Wang[1] | Sébastien Arthaud[1] | Patrice Fort[1] | Laura A. DeNardo[4] | Pierre-Hervé Luppi[1]

Journal of Sleep Research

→ TRAP model

2 The Temporal Association Cortex Plays a Key Role in Auditory-Driven Maternal Plasticity

Gen-ichi Tasaka,[1] Libi Feigin,[1] Ido Maor,[1] Maya Groysman,[1] Laura A. DeNardo,[3] Jennifer K. Schiavo,[2] Robert C. Froemke,[2] Liqun Luo,[3] and Adi Mizrahi[1,4,*]

Neuron *107*, 566–579, August 5, 2020

3 A thalamo-amygdalar circuit underlying the extinction of remote fear memories

Bianca A. Silva[1,4], Simone Astori[2], Allison M. Burns[1], Hendrik Heiser[1,3,5], Lukas van den Heuvel[1,6], Giulia Santoni[1], Maria Fernanda Martinez-Reza[1,7], Carmen Sandi[2] and Johannes Gräff[1]

NATURE NEUROSCIENCE

→ cFos circuit analysis

Lhx6+ neurons and paradoxical sleep: background

- Paradoxical sleep is Rapid-eye movement sleep (REM) sleep → called paradoxical because the brain is very active (dreaming) but the body is paralyzed (atonia)

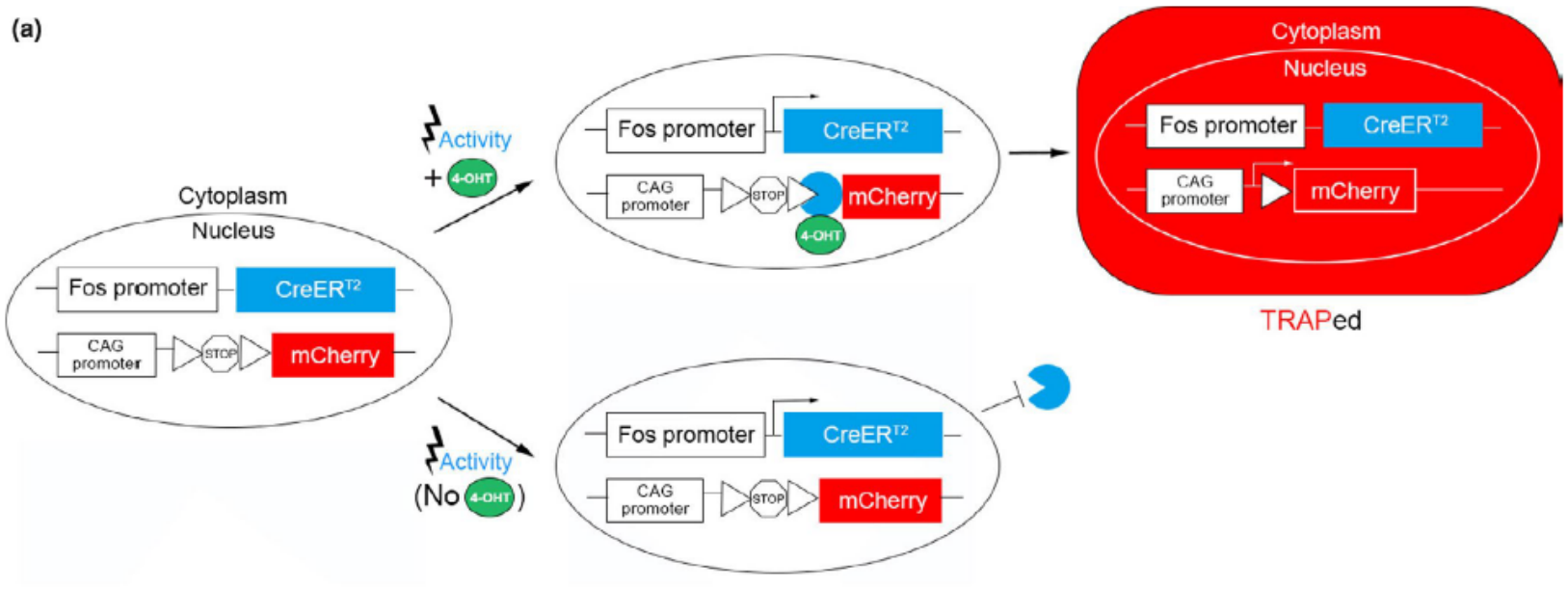
- Melanin-concentrating hormone (MCH) neurons in the lateral hypothalamic area (LHA) and zona incerta (ZI) they are REM promoting

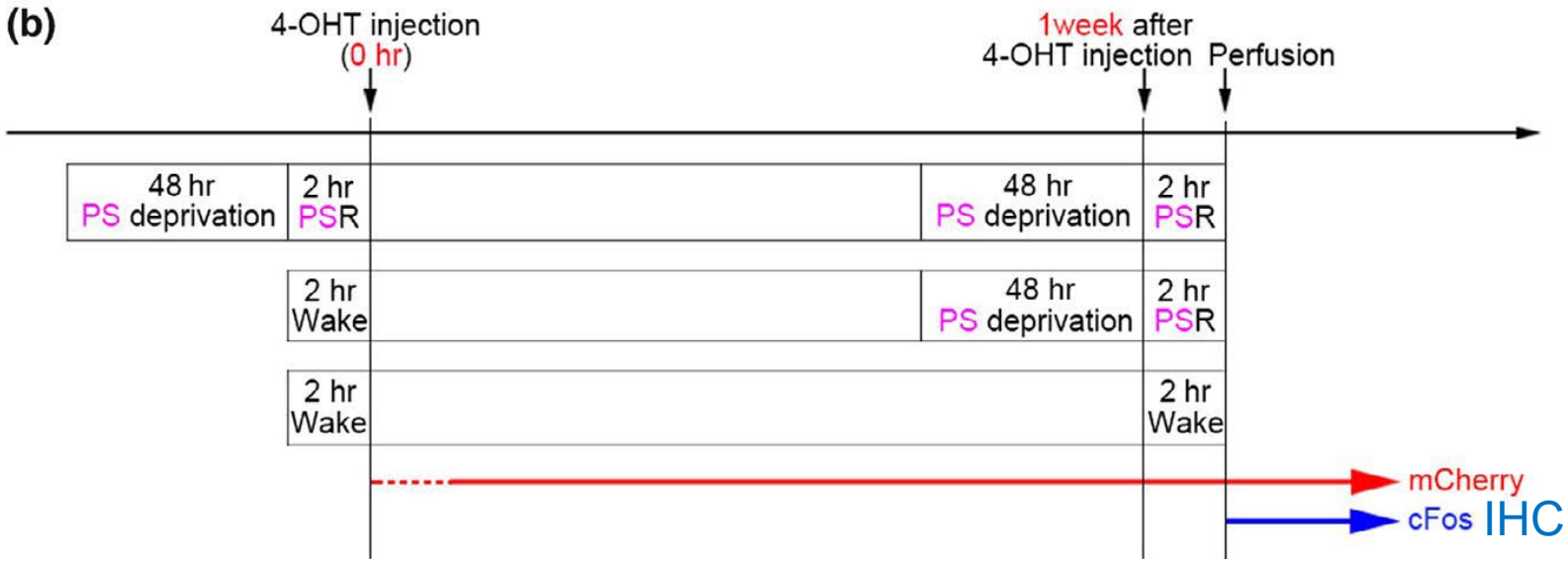
- Neurons expressing LIM homeobox 6 (Lhx6) in the ZI express cFos at the end of dark period or after total sleep deprivation (Liu, Kai, et al. *Nature* 548.7669 (2017): 582-587)

  → If activated they increase NREM and REM sleep

No characterization of REM active neurons in the LHA and ZI has been done

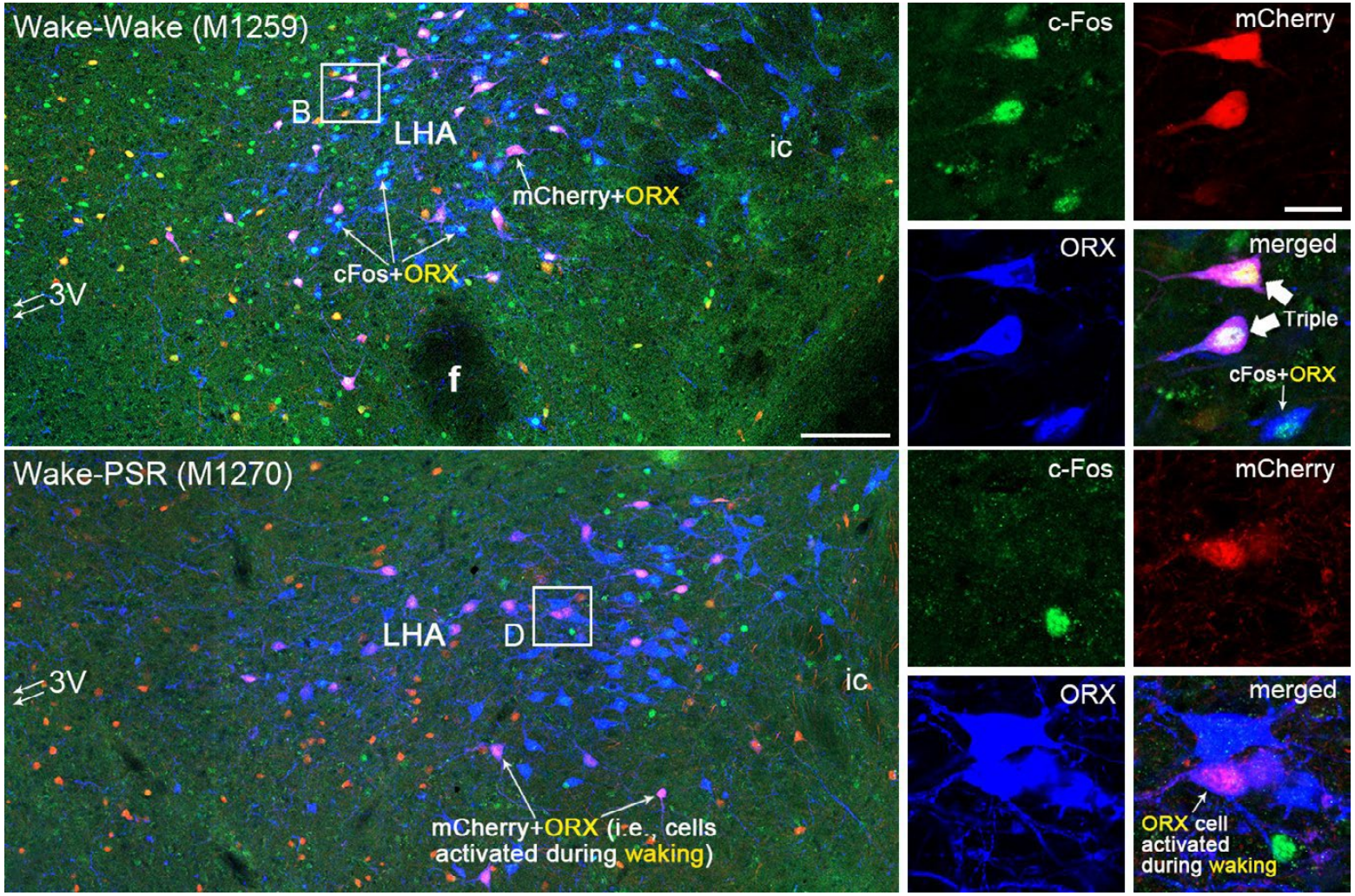
TRAP system and experimental plan





1. Co-labeling of mCherry (TRAP) and cFos IHC after REM sleep rebound
2. No co-labeling of mCherry and cFos IHC
3. Co-labeling of mCherry and cFos after wake (W)

Triple labelling with w-active neuronal markers

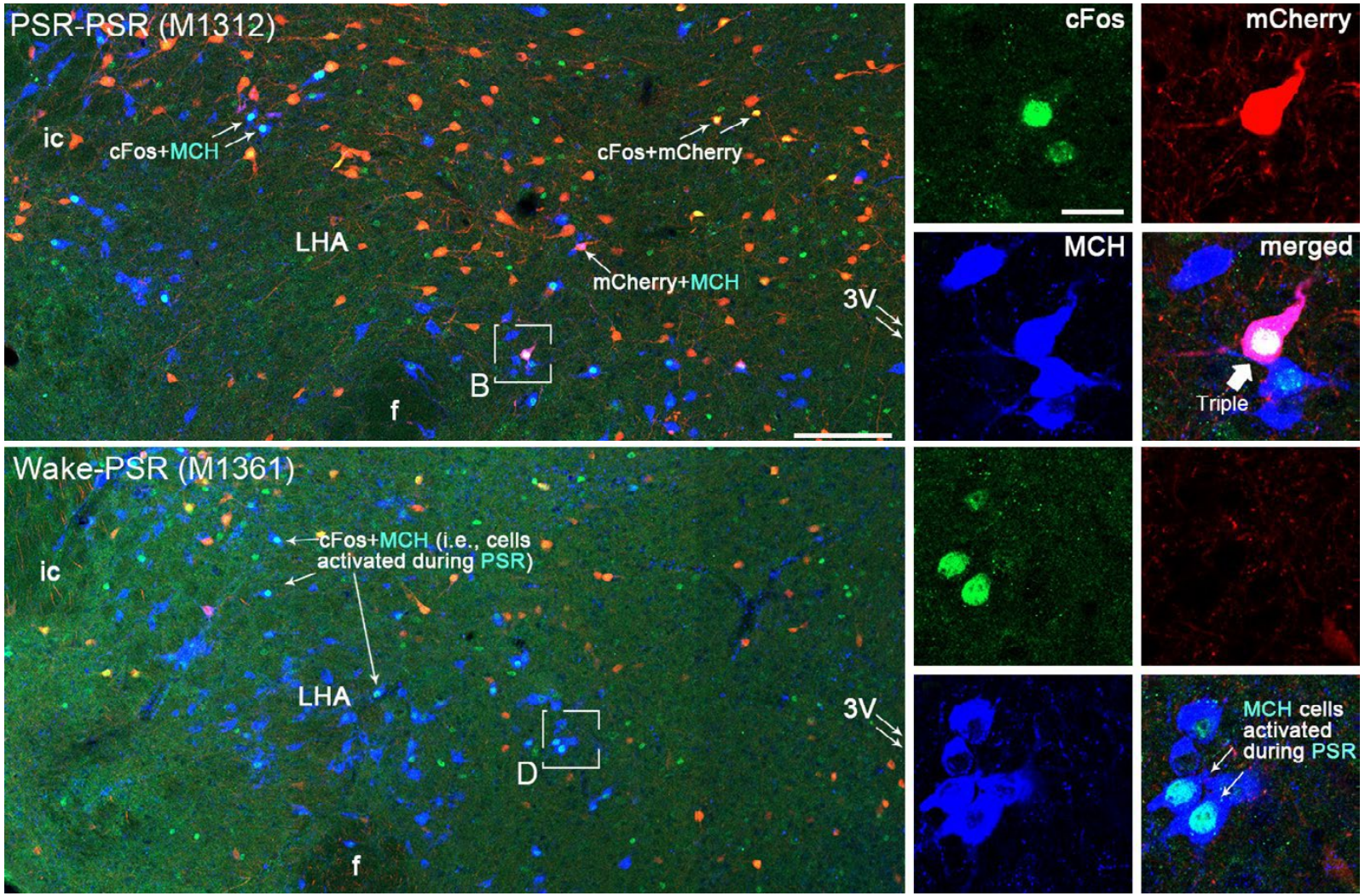


- In LHA if mCherry (TRAP) is activated by wake and perfusion happened after a period of wake : triple labelling mCherry+cFos+Orexin

- If mCherry is activated by wake but perfusion happened after paradoxical sleep rebound (PSR) no triple labeling, only mCherry+orexin

ORX = orexin neurons, fundamental for maintaining wakefulness (see narcolepsy)

Triple labelling with MCH neurons

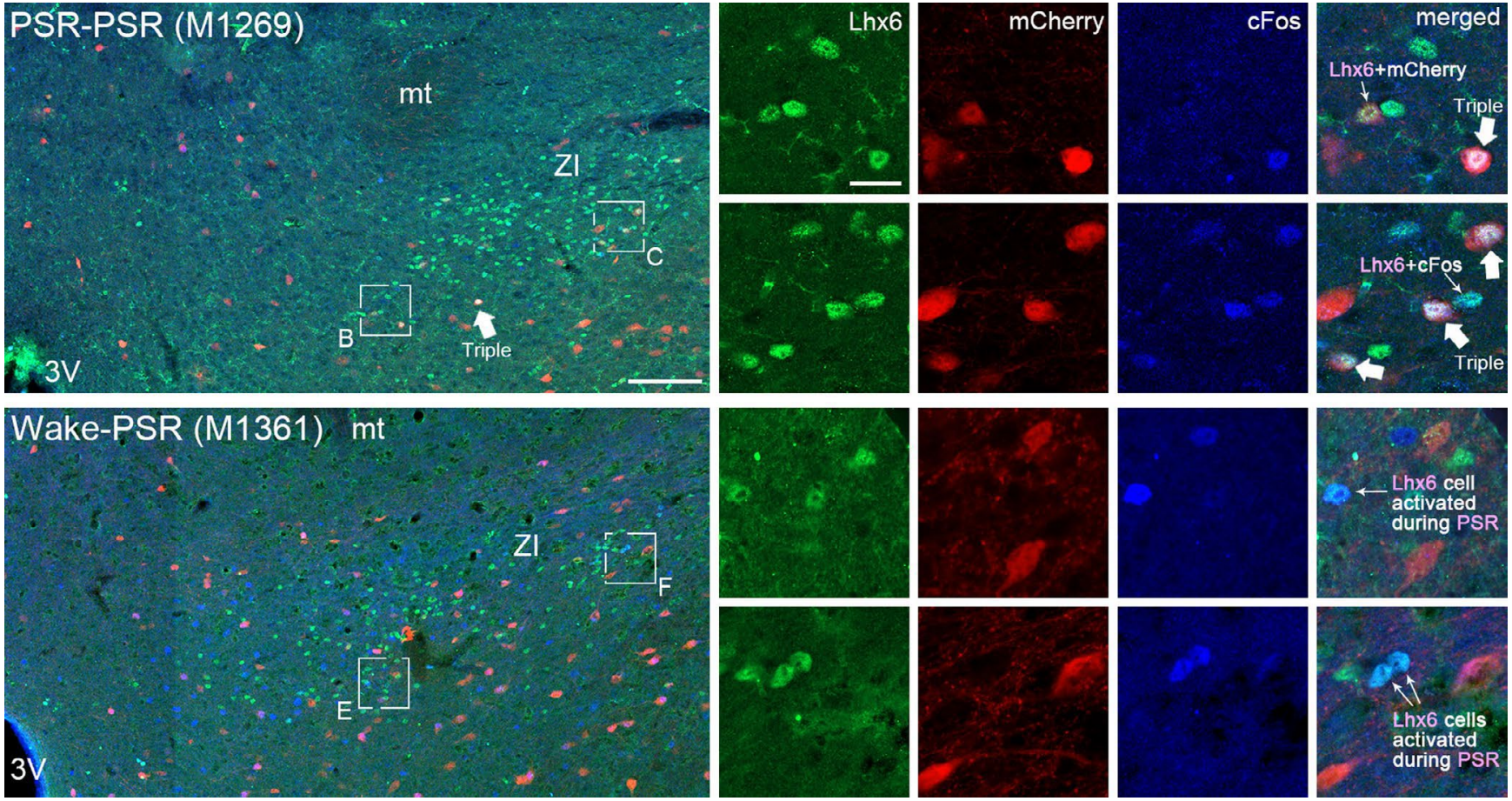


- In LHA if mCherry (TRAP) is activated by PSR and perfusion happened after PSR : triple labelling mCherry+cFos+MCH

- If mCherry is activated by wake but perfusion happened after PSR no triple labeling, only cFos+ MCH

MCH neurons have been shown to be REM active

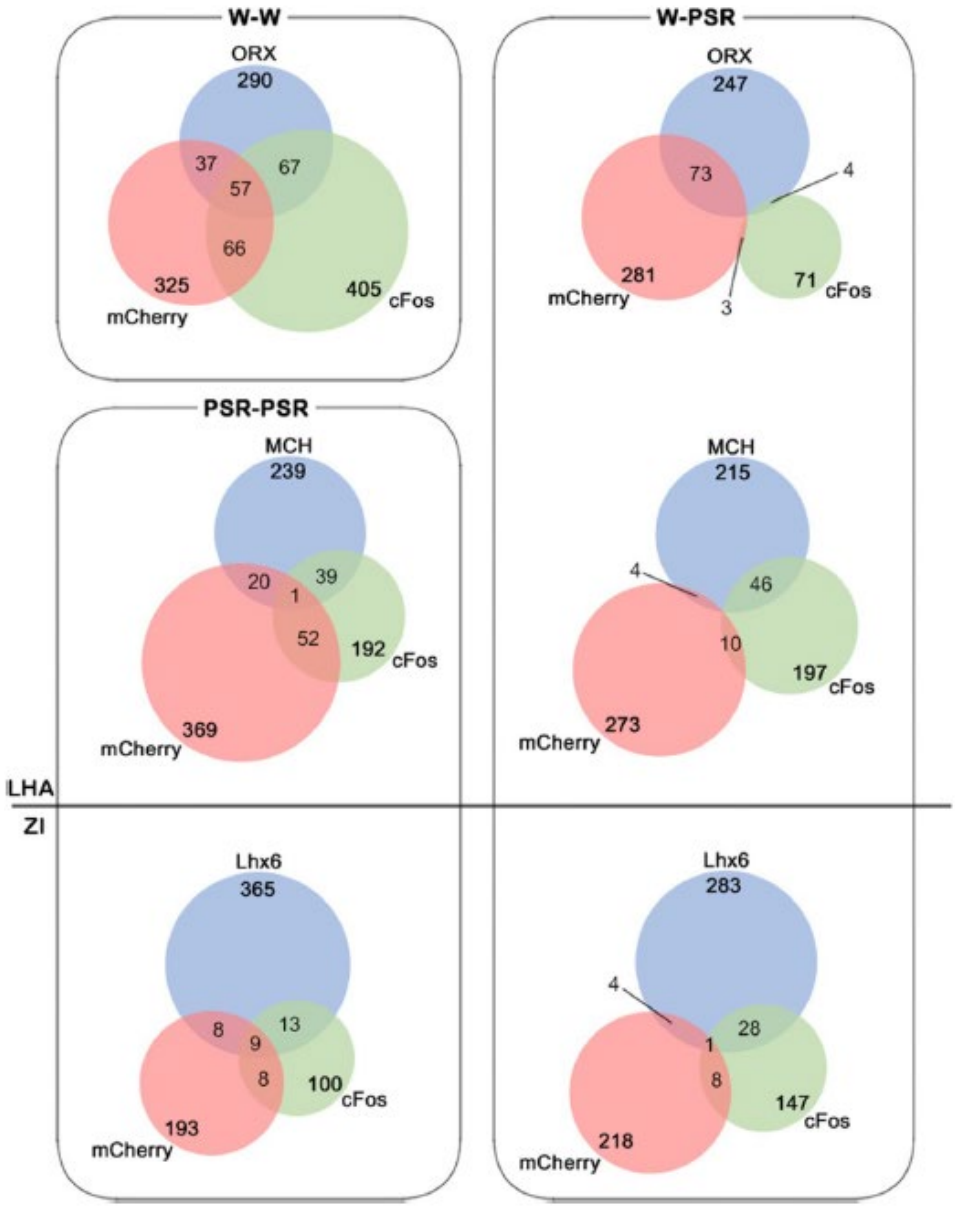
Triple labelling with Lhx6 neurons



- In ZI if mCherry (TRAP) is activated by PSR and perfusion happened after PSR : triple labelling mCherry+cFos+Lhx6

- If mCherry is activated by wake but perfusion happened after PSR no triple labeling, only cFos+ Lhx6

Quantification of the triple labelling



8.8% of ZI neurons labelled with mCherry during PSR expressed Lhx6, in contrast to the W condition, in which only 2.1% of the mCherry+ cells expressed Lhx6

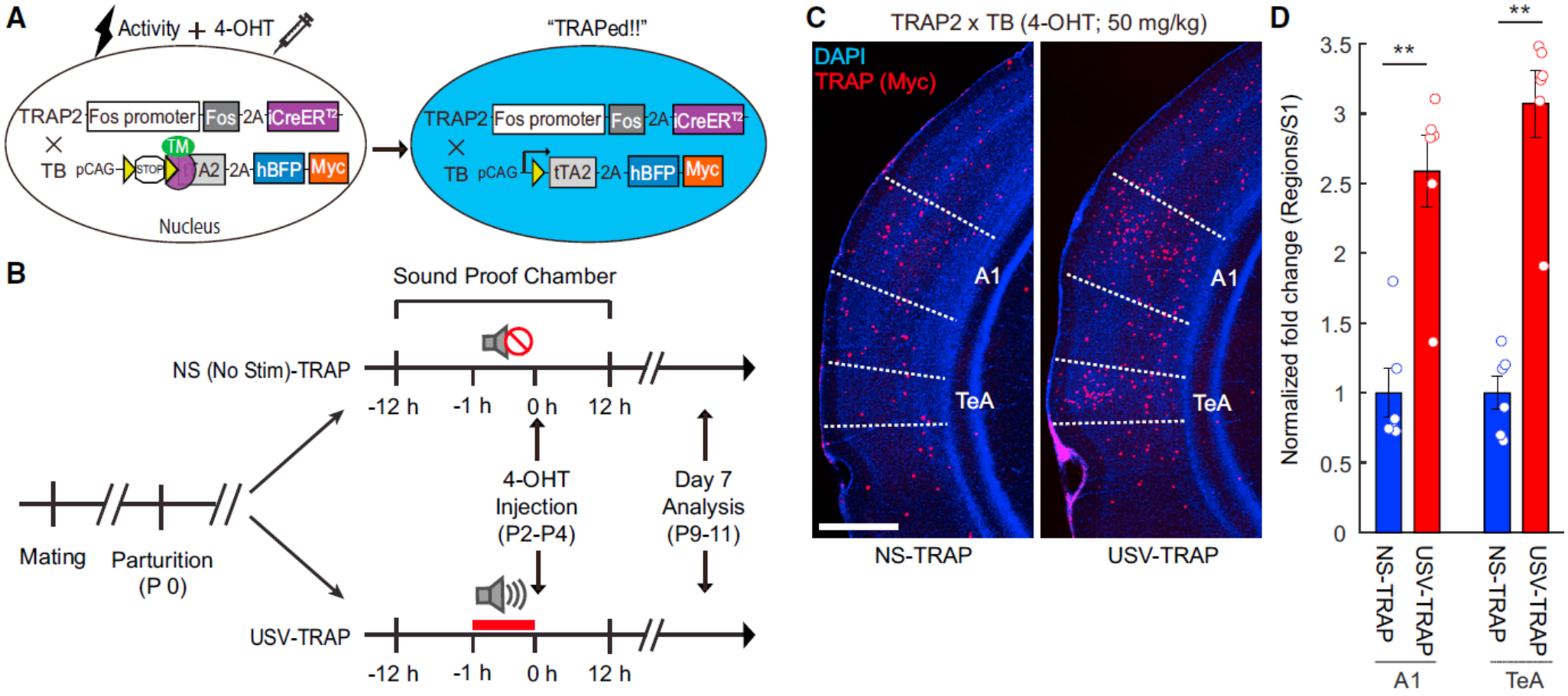
Summary and conclusions

- ORX neurons express cFos during W but not during PSR
- Neurons expressing MCH known to be specifically active during PSR are mCherry+ during PSR and not during W
- A large proportion of the neurons expressing mCherry express cFos when the animals are re-exposed to the same condition, validating the TRAP methodology
- Lhx6+ neurons are specifically activated during PSR and not during W like previously reported

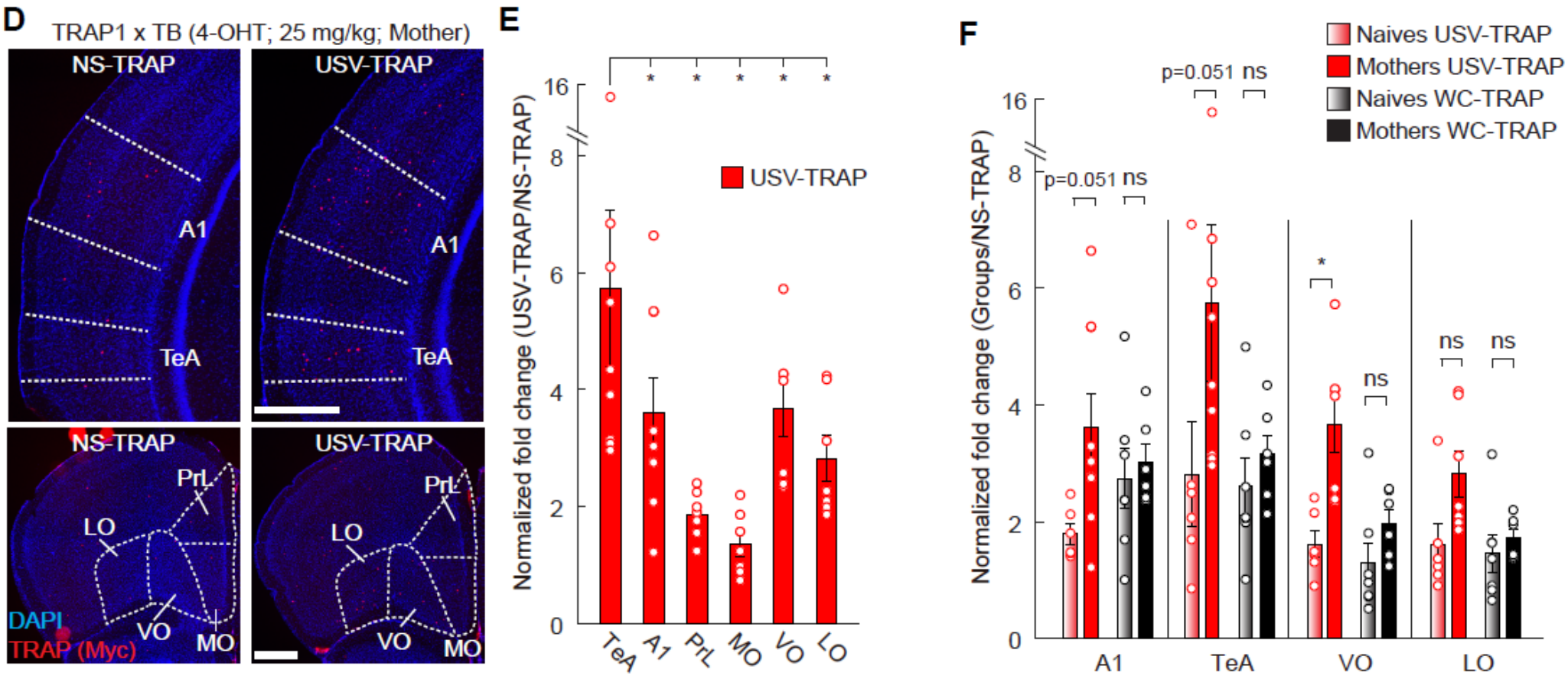
Temporal association cortex and auditory-driven maternal plasticity

- Study how ultrasonic vocalizations (USVs) affect mothers' behaviour → focus on cortical circuits
- Using TRAP technique, the temporal association cortex (TeA) shows high activity in mothers exposed to USVs
- Using tracing techniques, dense extracellular recordings and neuronal activity manipulation (DREADD receptors) to study TeA active cells

Activation of TeA neurons after USVs exposure



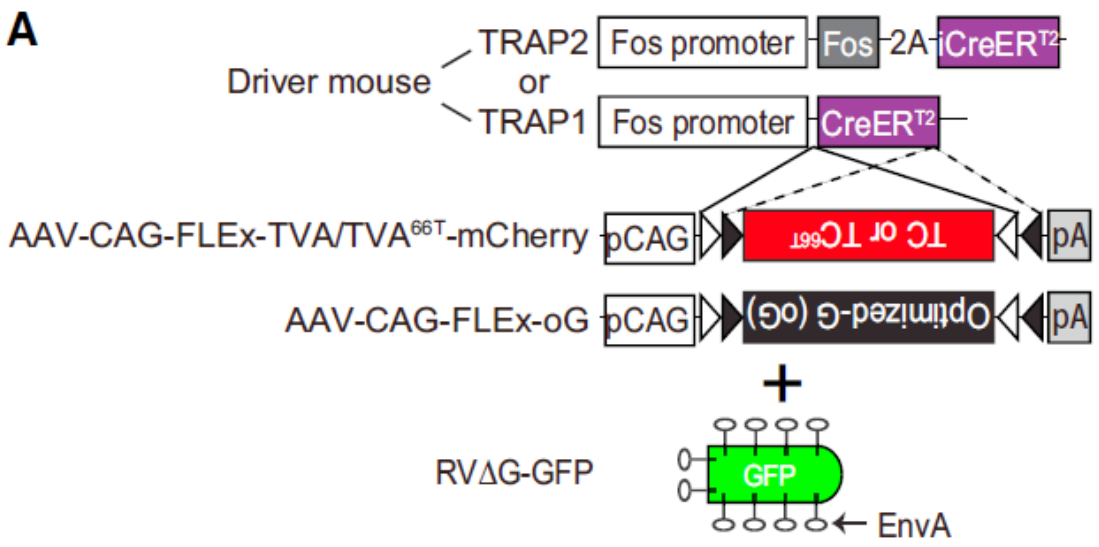
USVs activated neurons in other brain regions and naïve female

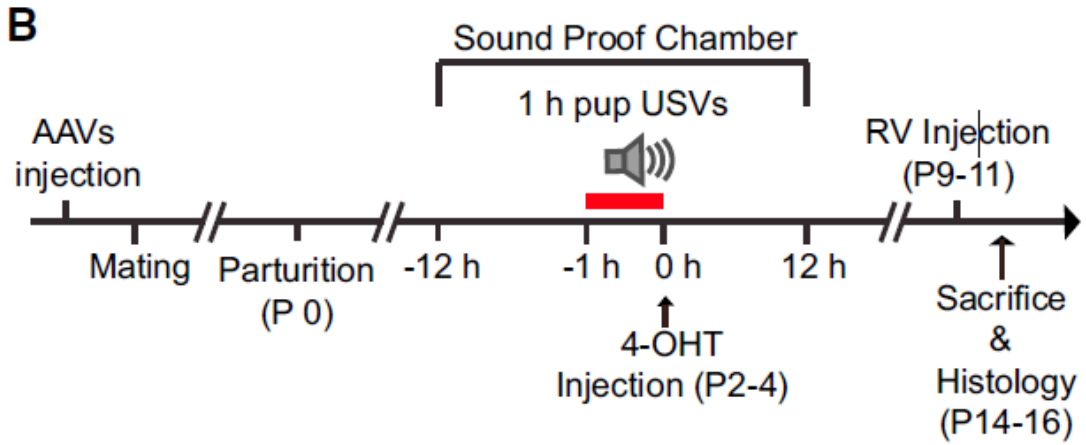


- Ventral and lateral orbitofrontal cortex also showed TRAPed cells
- Compared to other regions, USV activated more cells in TeA and more in mother than in naïve females

→ **TeA might be important for processing USVs in mothers**

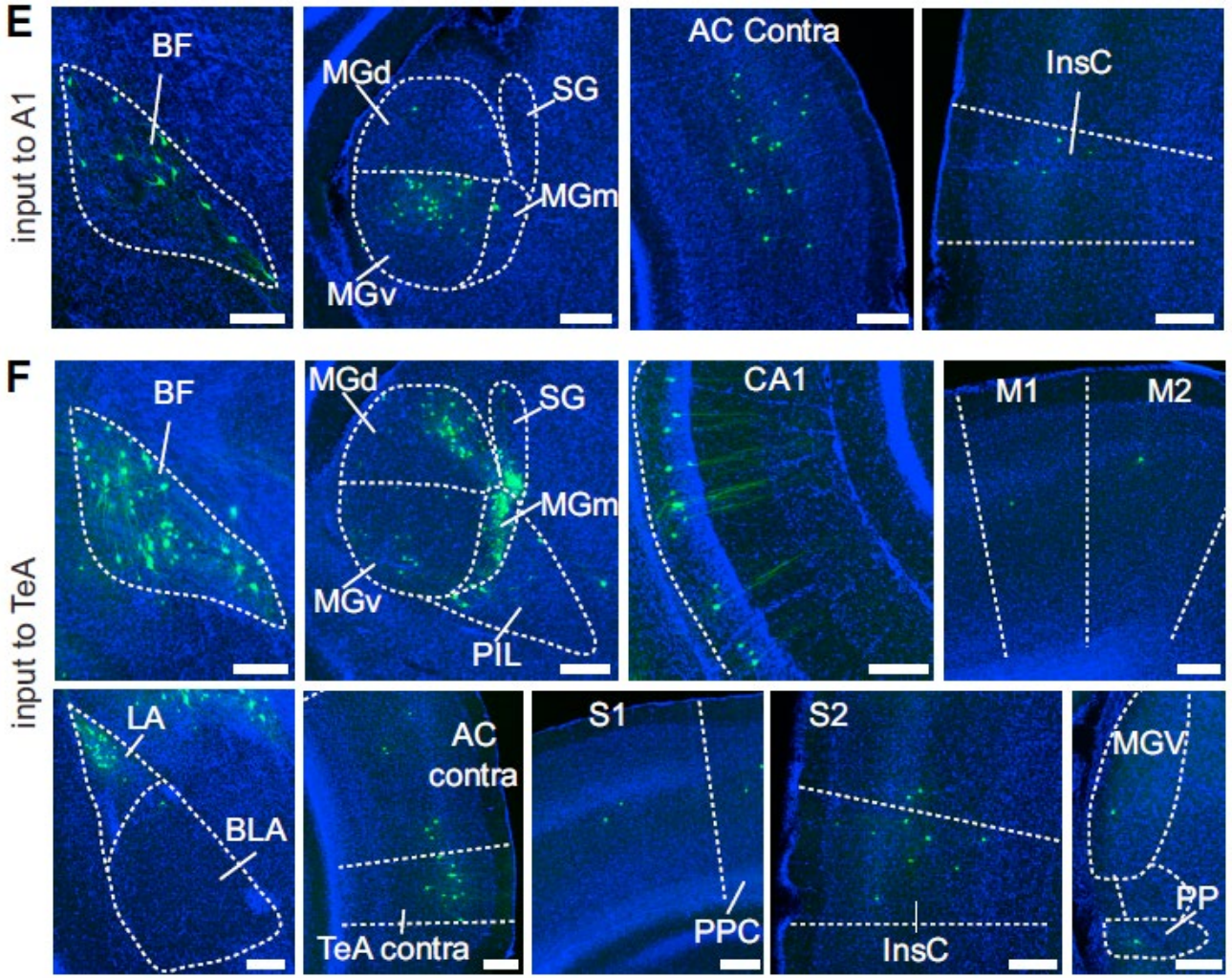
Combination of monosynaptic *trans*-synaptic rabies with TRAP

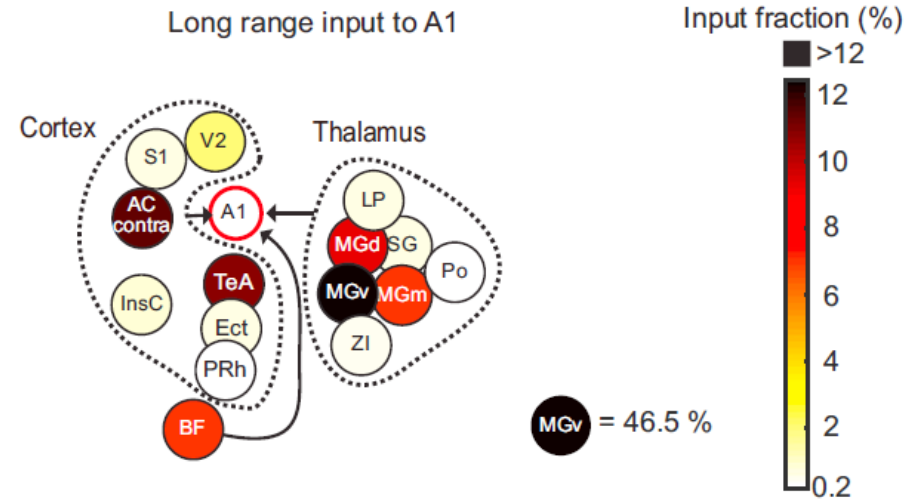


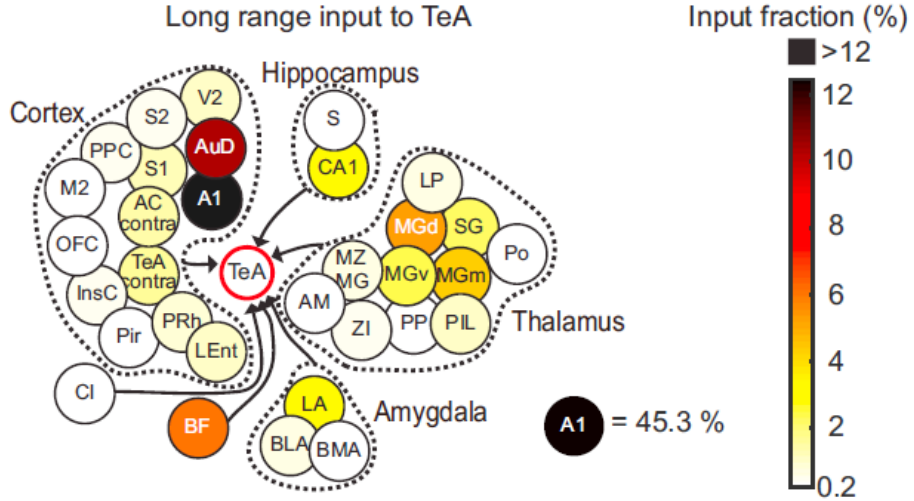


- Optimized rabies glycoprotein oG was cloned in a AAV and co-injected with receptor viruses (avian sarcoma and leukemia derived)
- Mice were trapped with USVs
- One week mice were injected again with a pseudotyped G-deleted rabies virus
- IHC 5 days after

Mapping inputs onto A1 and TeA

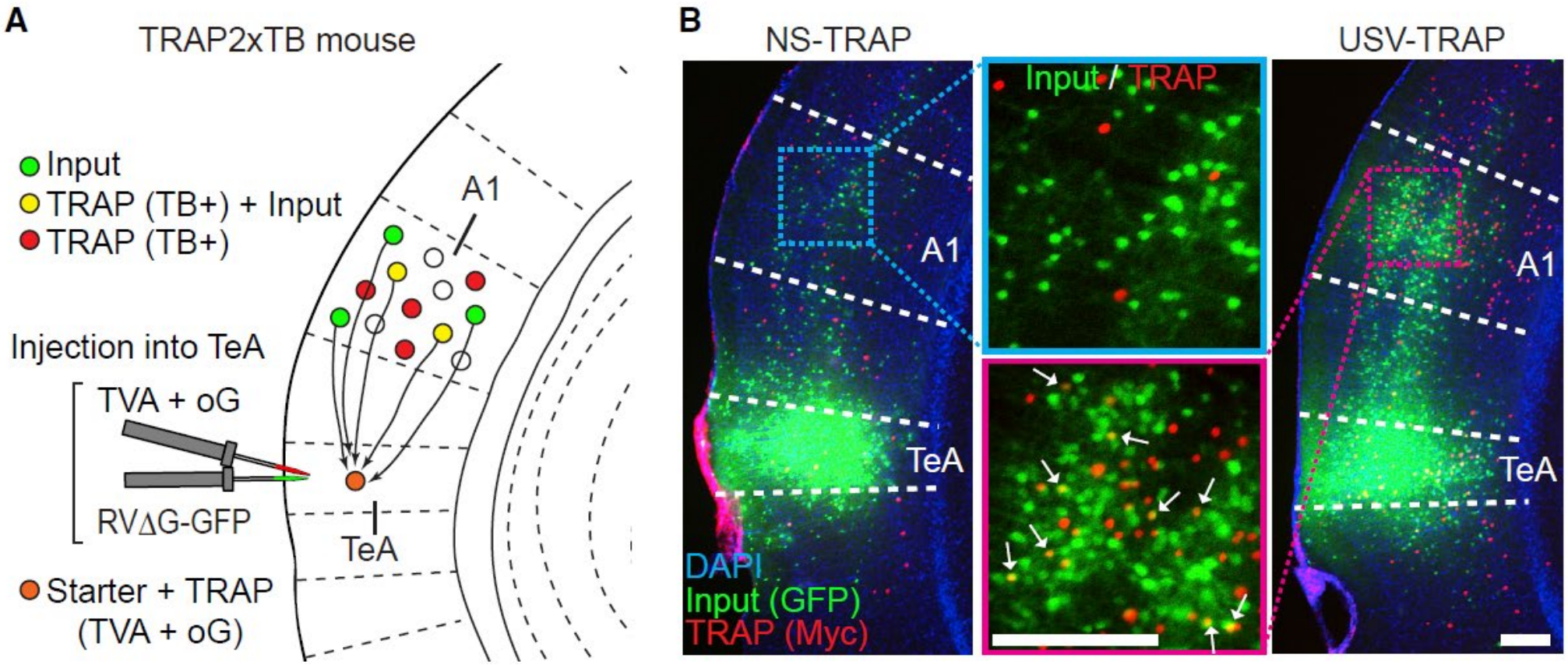






45.3% of the inputs into TeA arise directly from A1

USV-responsive neurons in TeA and A1 are interconnected

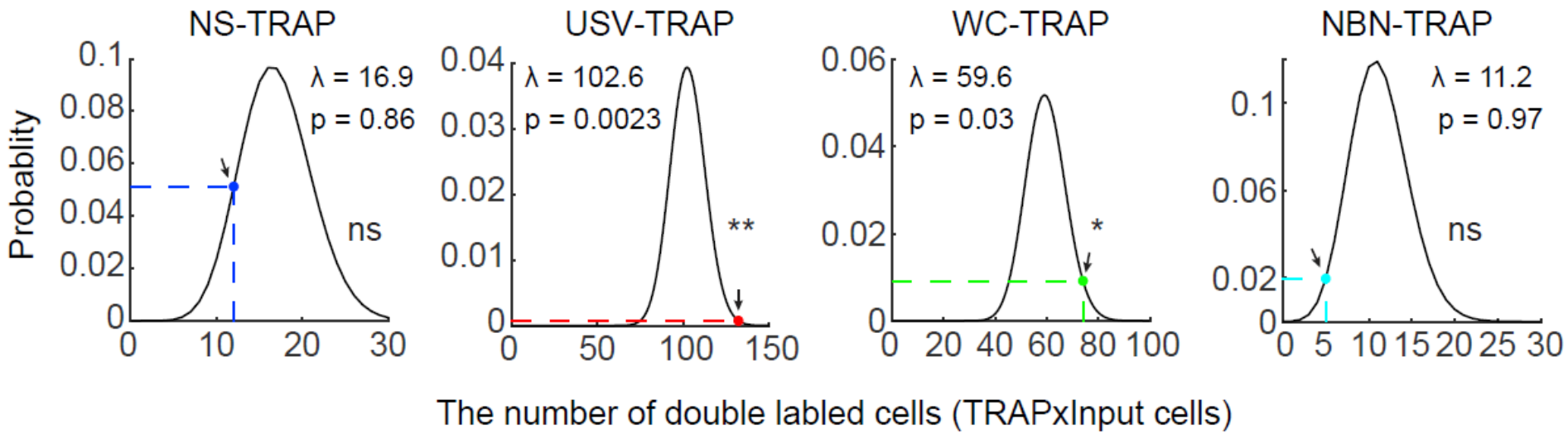


- They TRAPed and traced neurons from the TeA and observed how many cell were also TRAPed in A1

Probability of interconnection between active neurons in TeA and A1

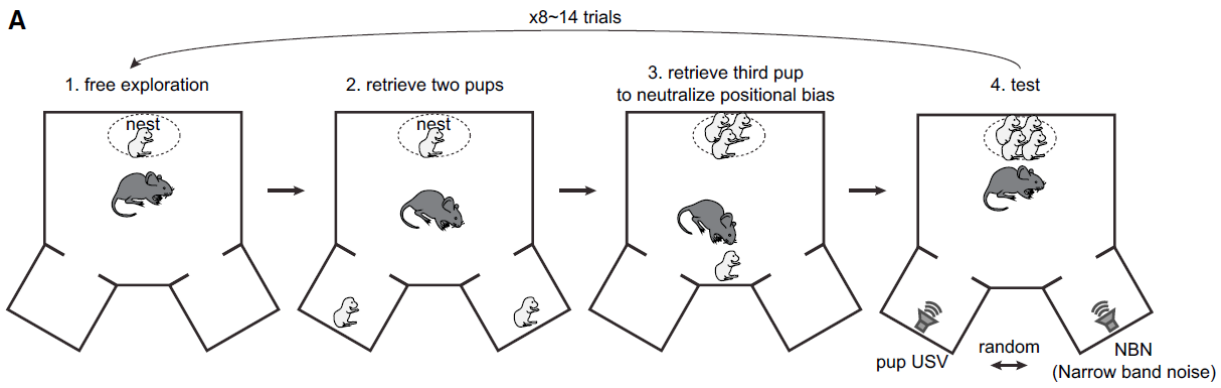
- They measured the density of TRAP-only cells, input-only cells and TRAP-input-cells (double labelled, DL)
- Then computed the probability of finding a DL neurons with this estimation:
- The number of DL neurons was significantly <u>larger than expected</u> in USV and WC stimulated groups → **neurons responding to noise bursts are preferentially connected**

$$\textit{Expected number of double-labeled cells} = [\textit{Cell density in A1}] \times [\textit{Volume of ROI}] \times \Pr[\textit{TRAP cells}] \times \Pr[\textit{Input cells}]$$

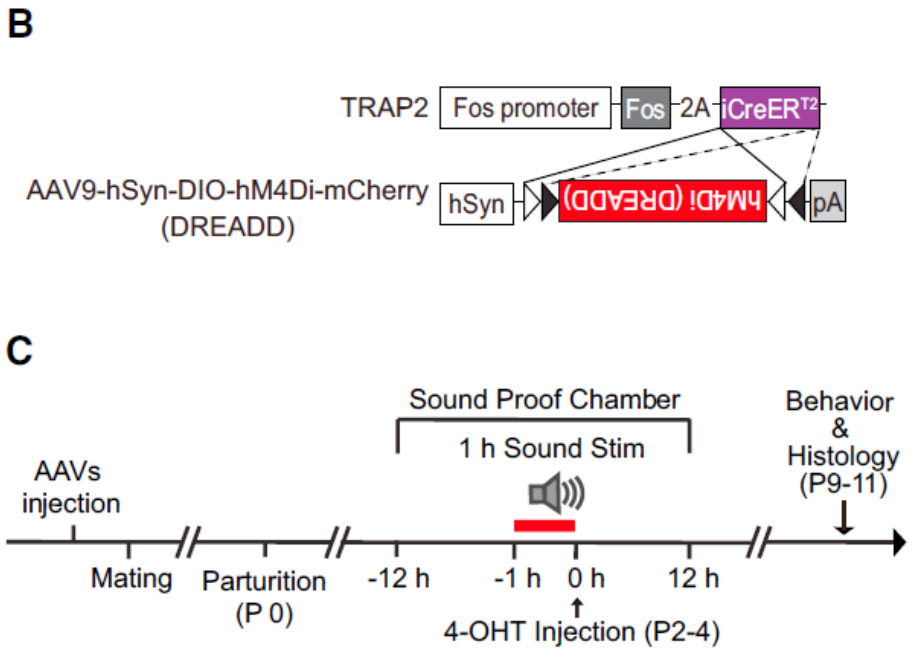


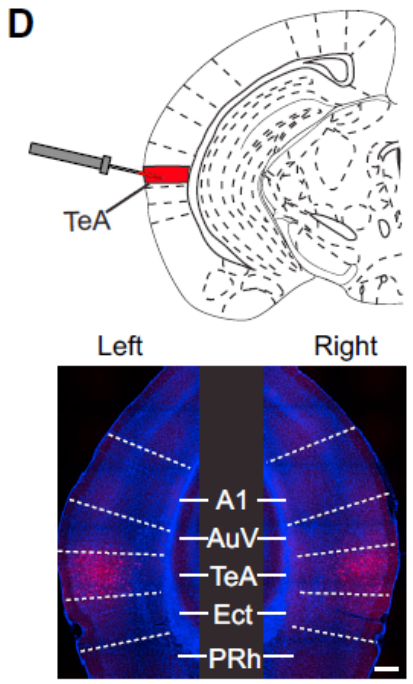
USV-TRAPed TeA cells control maternal behavior

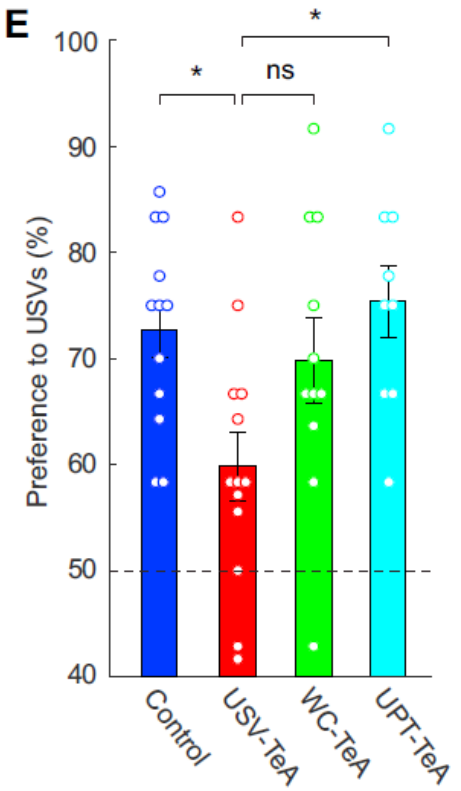
- Forced-choice preferential task of pup retrieval
- Combination of TRAP and inhibitory chemogenetics (DREADD receptors)



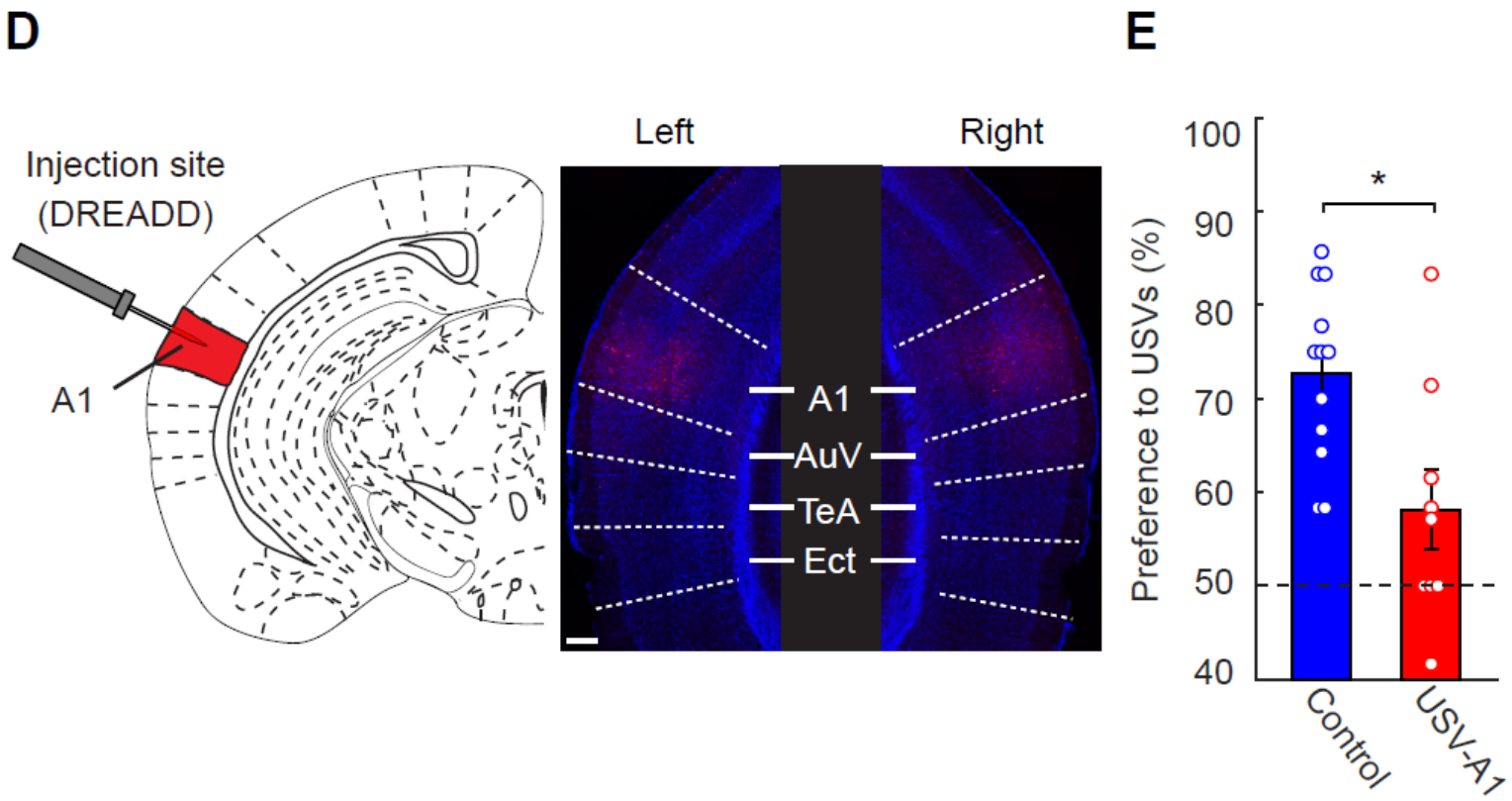
→ Chemogenetic silencing of USV-TRAP cells in TeA decreased the maternal preference for USVs





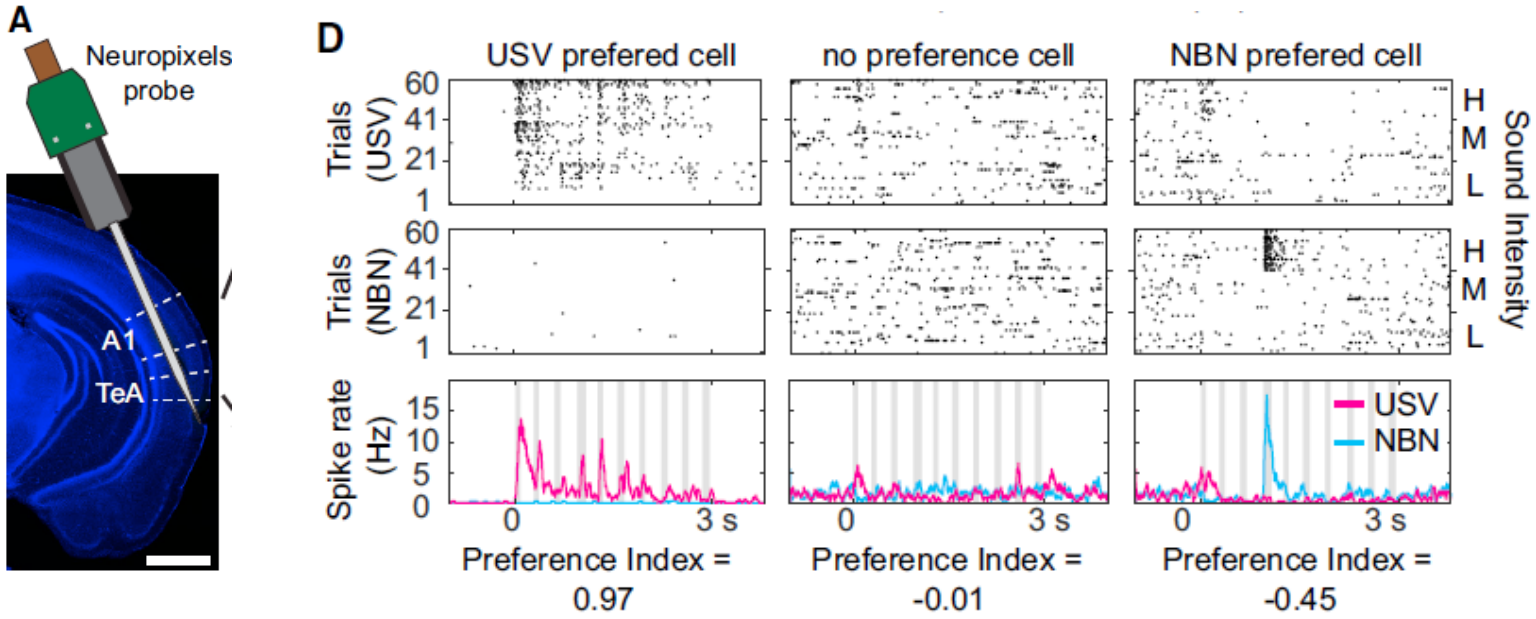


Silencing USV-TRAPed A1 neurons decrease maternal responses as well



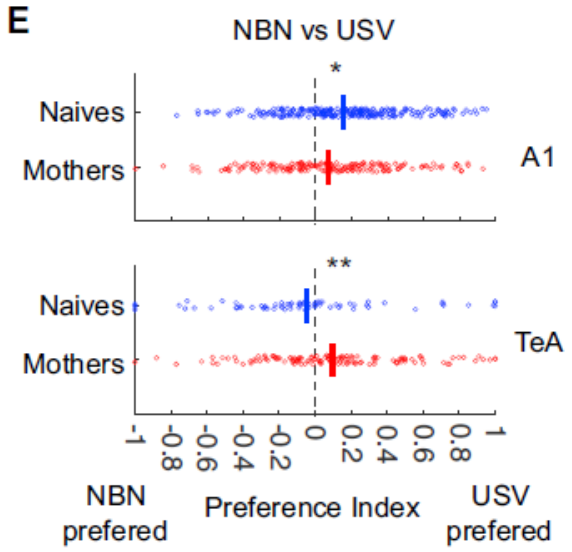
- USV-responsive neurons in A1 are supposed to drive USV neurons in TeA
- TRAPing and chemogenetic inhibition of USV-responsive TeA neurons reduces pup retrieval similarly to inhibition of TeA neurons

USV cellular response in mothers and naïve females



- Recorded spiking activity using high-density microelectrode arrays in awake head-restrained animals

- Preferential index (PI) for firing rate to USVs Vs NBN



→ **In TeA motherhood caused higher responses to USVs**

Summary and conclusions

TeA plays a role in encoding pup cries during motherhood

- Because of its long-range inputs, the TeA is not merely a high-order auditory cortex but rather a site that integrates sounds with other information
- TeA and A1 USVs responsive neurons are functionally connected in mothers
- Inhibition of these same neurons causes reduction of maternal pup retrieval
- TeA USVs neuronal responses increase when females become mothers

→ TRAP+chemogenetics enabled the homogeneous manipulation of several thousand functionally tagged neurons

→ TRAP+rabies can be useful to elucidate the anatomy and physiology of any new brain region

Thalamo-amygdalar circuit and fear memories

- Understanding the neuronal circuit behind remote fear and trauma memories
- Previous data only focused on the neural correlates of extinction protocols applied shortly after the encoding of the traumatic memory
- Traumatic memories undergo a systems consolidation process over time

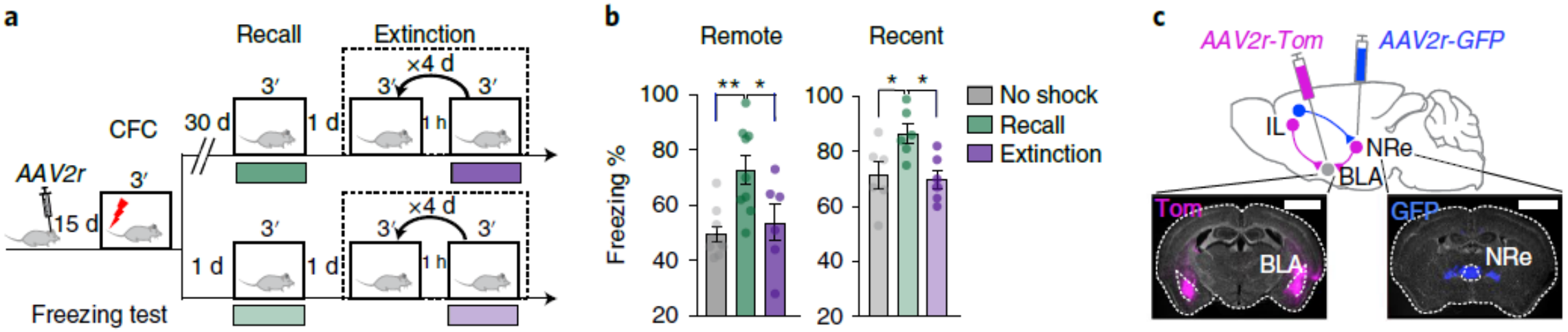
→ **Fear extinction for remote memories might not rely on the same canonical brain networks as for recent time points**

→ **Combination of viral tracing, neuronal activity mapping (no TRAP though!), fiber photometry, chemogenetic and optogenetic (basically everything!)**

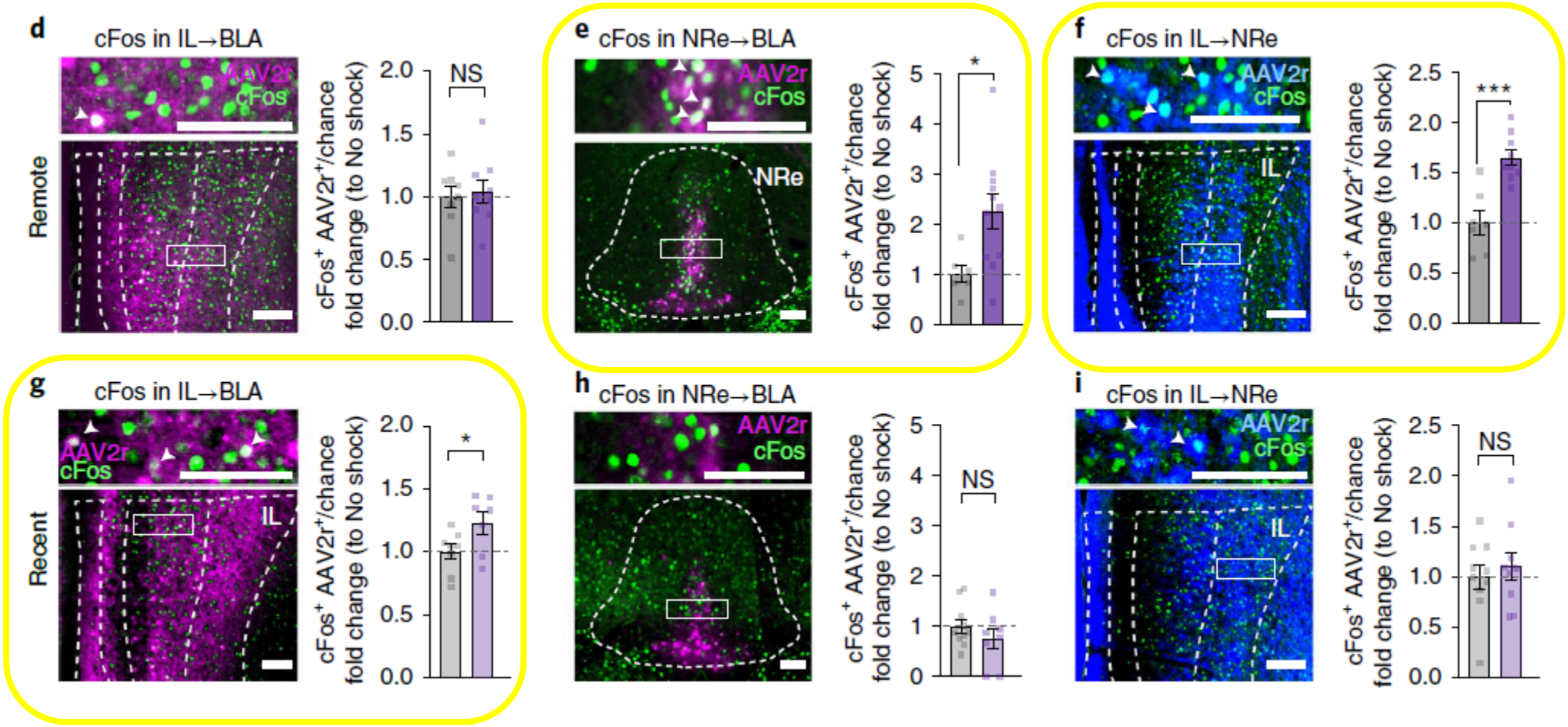
Remote fear extinction activates IL→NRe→BLA pathway

IL = infralimbic cortex
NRe = thalamic nucleus reuniens
BLA = basolateral amygdala

- Connections between IL and BLA are active during fear attenuation induced by exposure therapy extinction protocols (on rodents and on recent memories only)
- Retrograde tracing and cFos expression analysis upon contextual fear conditioning (CFC) test
- Retrograde viruses injected in BLA and NRe to observe connections with the IL

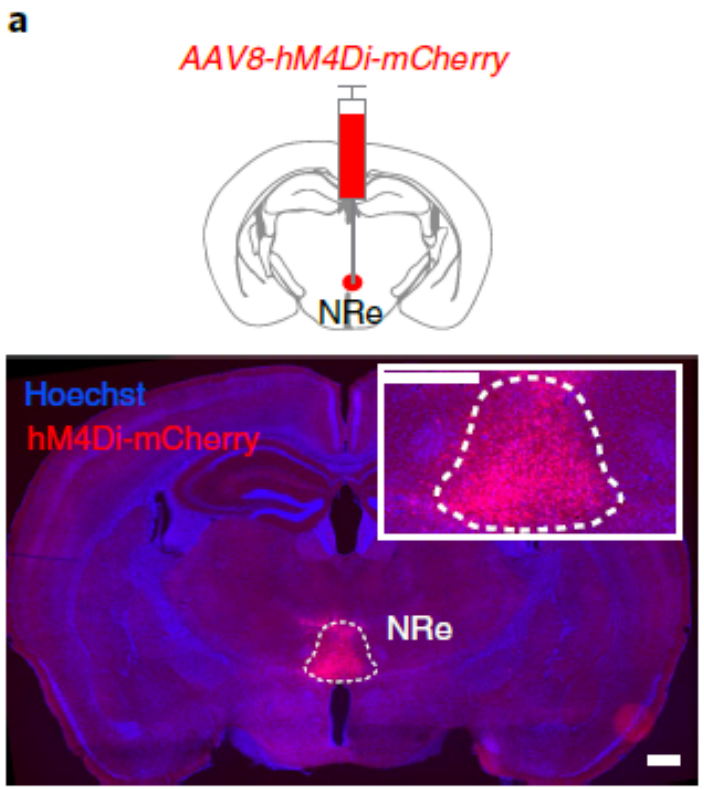


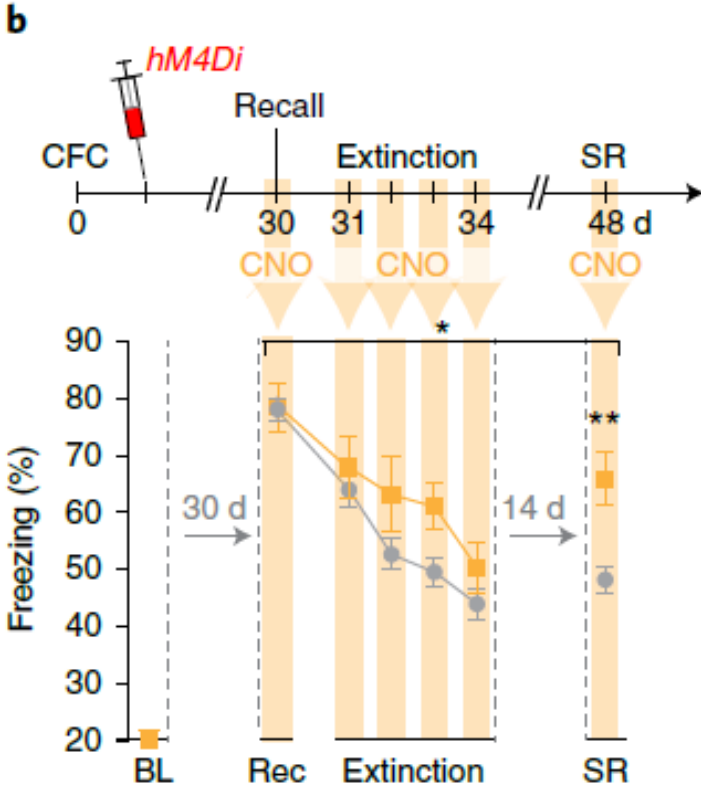
Validation of the IL→NRe→BLA pathway



- cFos staining after last session of fear memory extinction
- The NRe projects to the BLA and could be a node between IL and BLA controlling remote fear memory extinction

NRe is directly participating in remote fear memory extinction



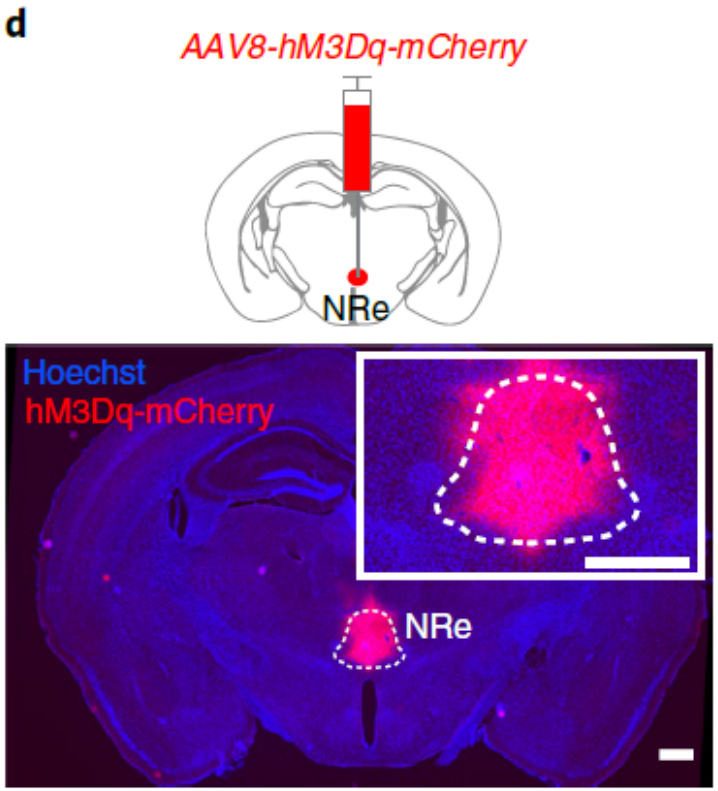


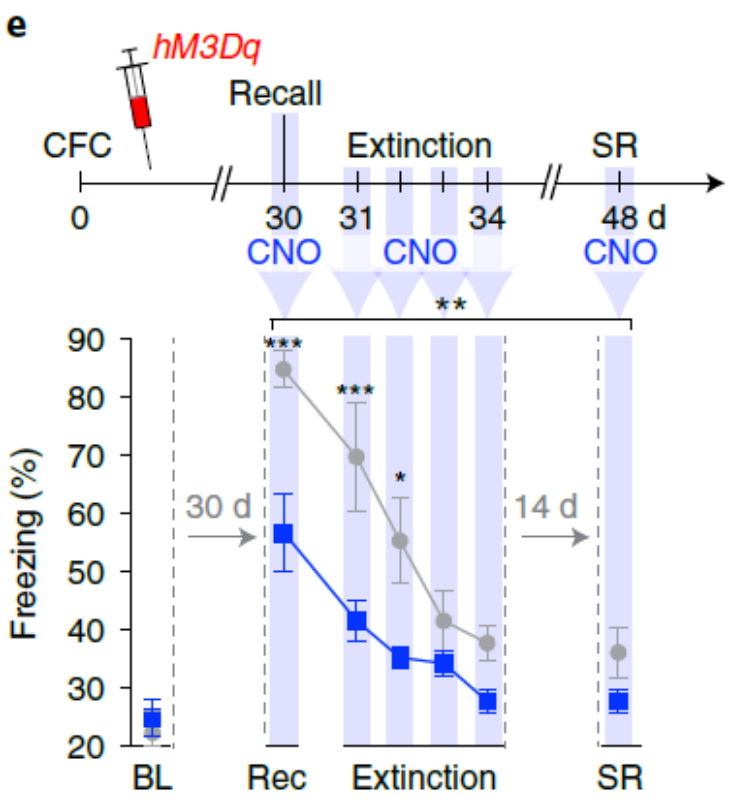
Rec = recall
SR = spontaneous recovery of the fear

- Daily CNO administration at remote memory recall and during the extinction paradigm
- CNO-treated animals retained significantly higher freezing levels

→ Persistent impairment of fear extinction upon NRe inactivation

NRe is directly participating in remote fear memory extinction



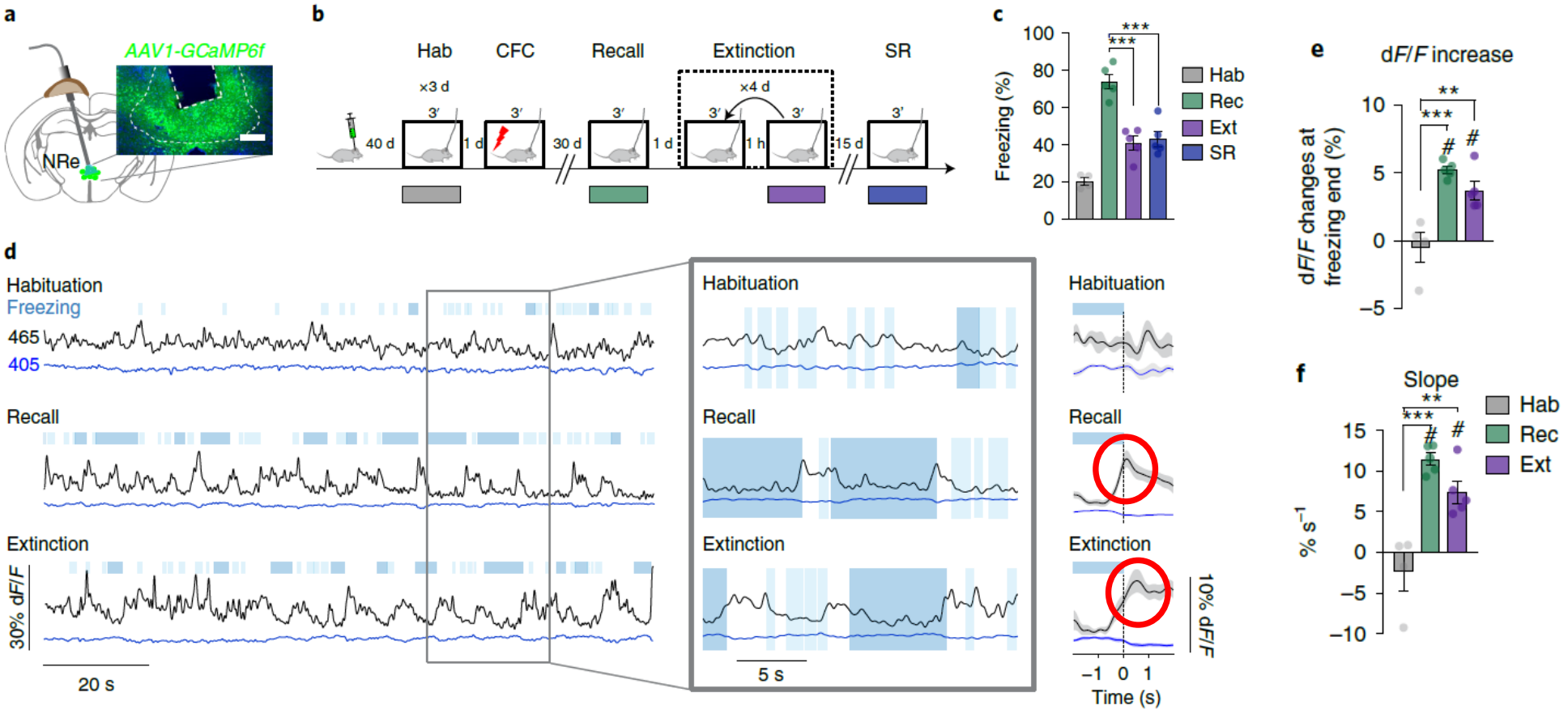


Cellular specificity?

- Gain of function of NRe activity is beneficial for remote fear memory extinction

→ activation of the NRe reduces freezing behavior during memory extinction and contextual re-exposure

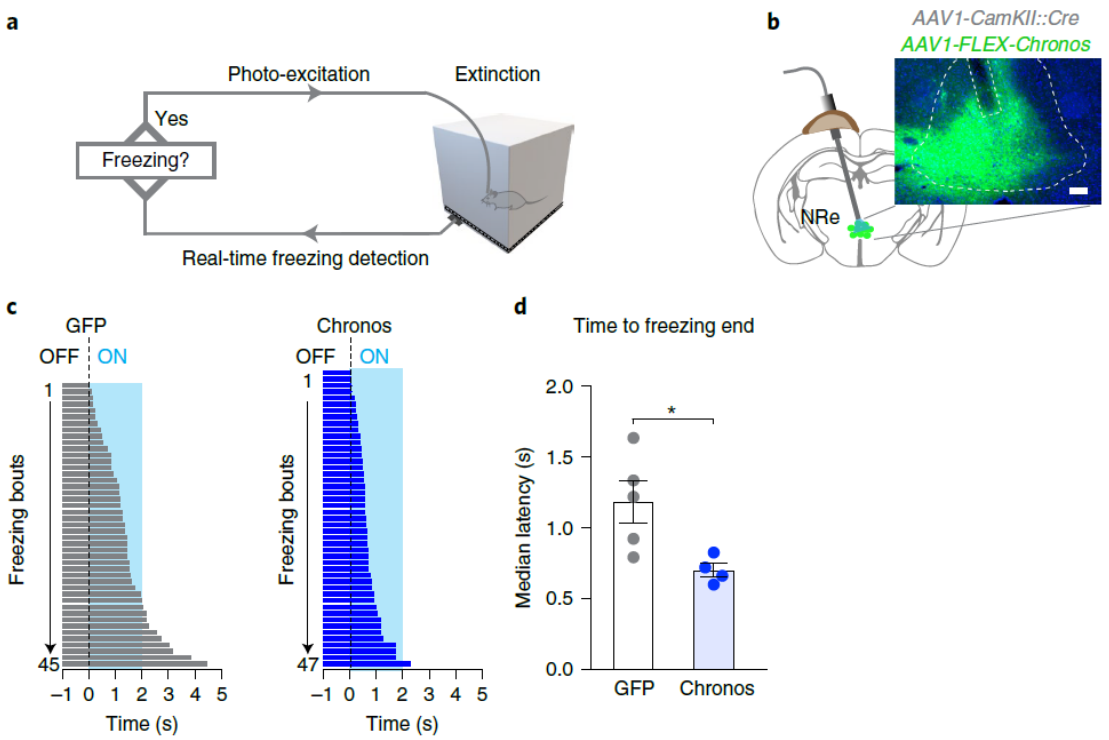
NRe neurons are more active when freezing stops



- Expression of genetically encoded Ca^{2+} indicator GCaMP6f in NRe excitatory neurons to record neuronal activity
- Transient elevation in NRe activity shortly before the termination of freezing bouts during recall extinction sessions
- No increase in NRe activity during habituation → activation specific to fear responses

NRe mediates freezing cessation during remote extinction

PHOTOACTIVATION



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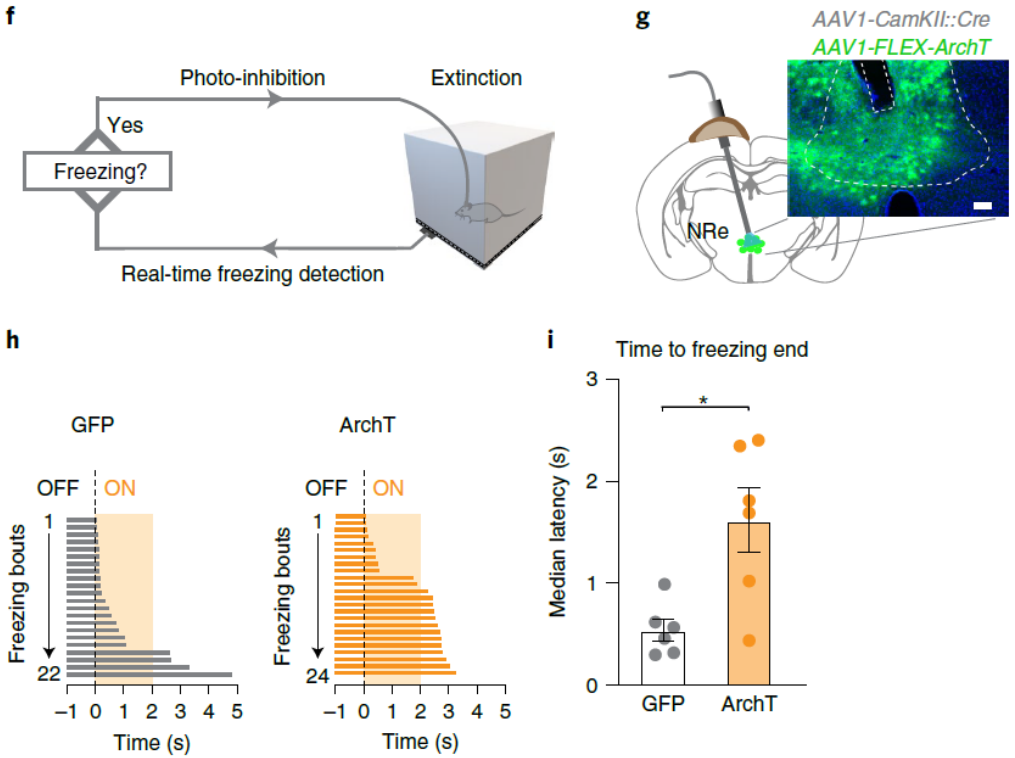
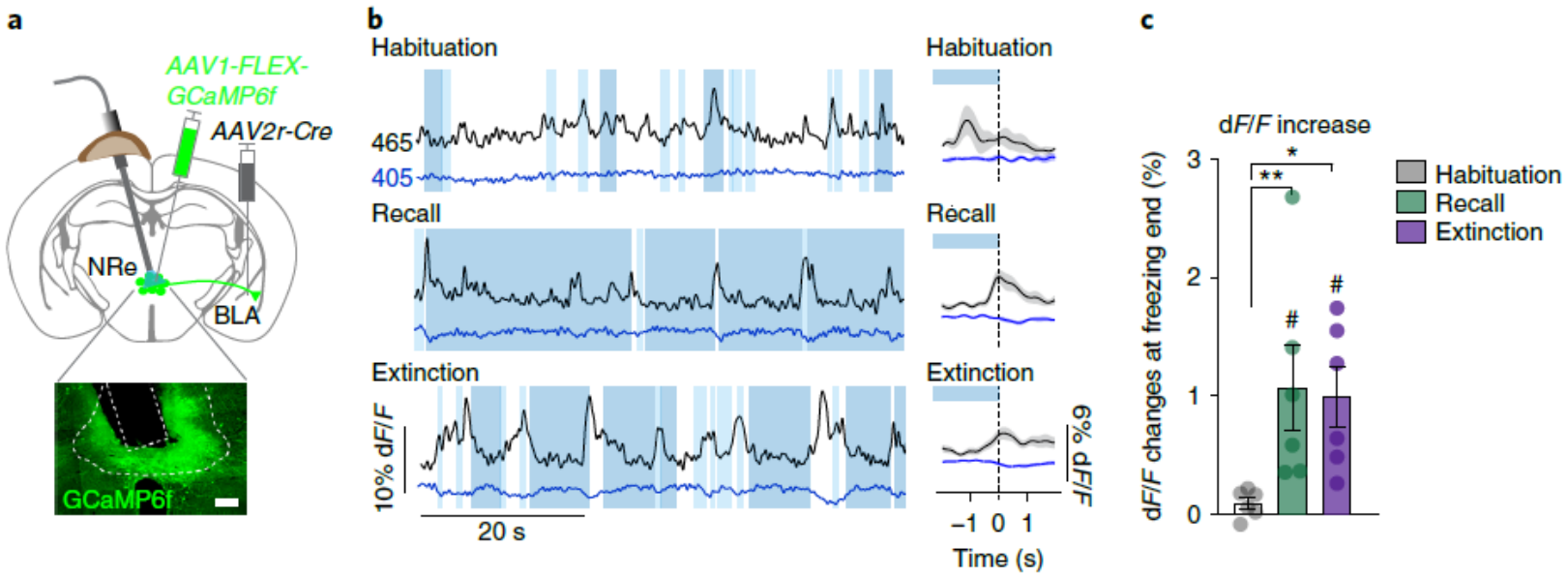
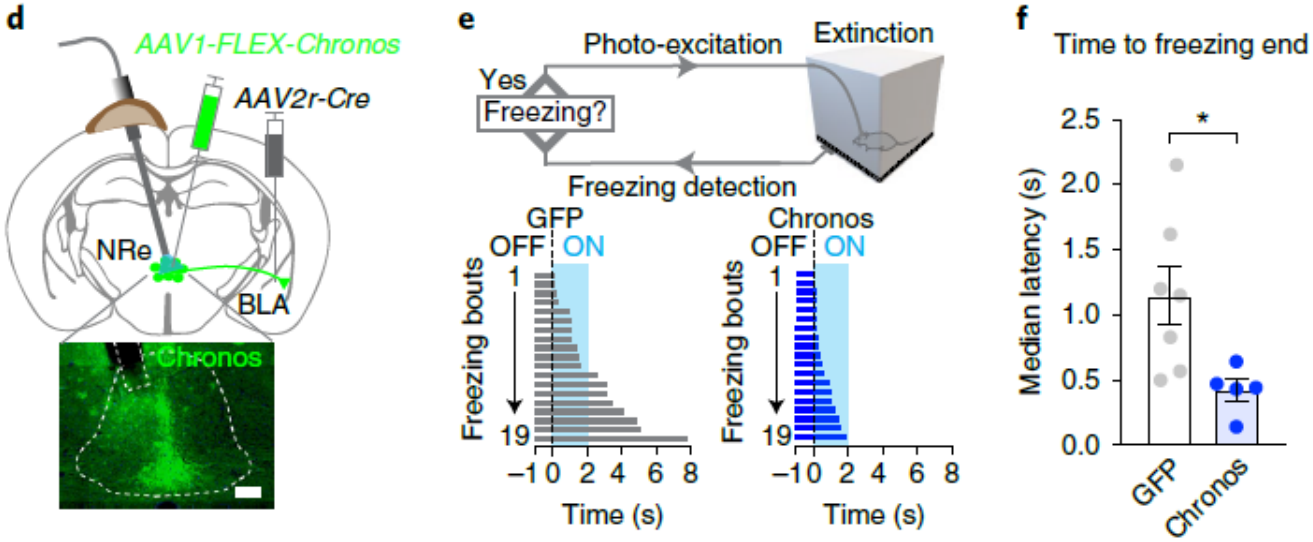


- Online freezing detection system coupled to a laser driver → photostimulation only upon freezing
- Excitation using Chronos of excitatory NRe neurons reduced freezing time during remote fear extinction
- Inhibition using ArchT increased freezing duration

NRe to BLA projections regulate freezing cessation



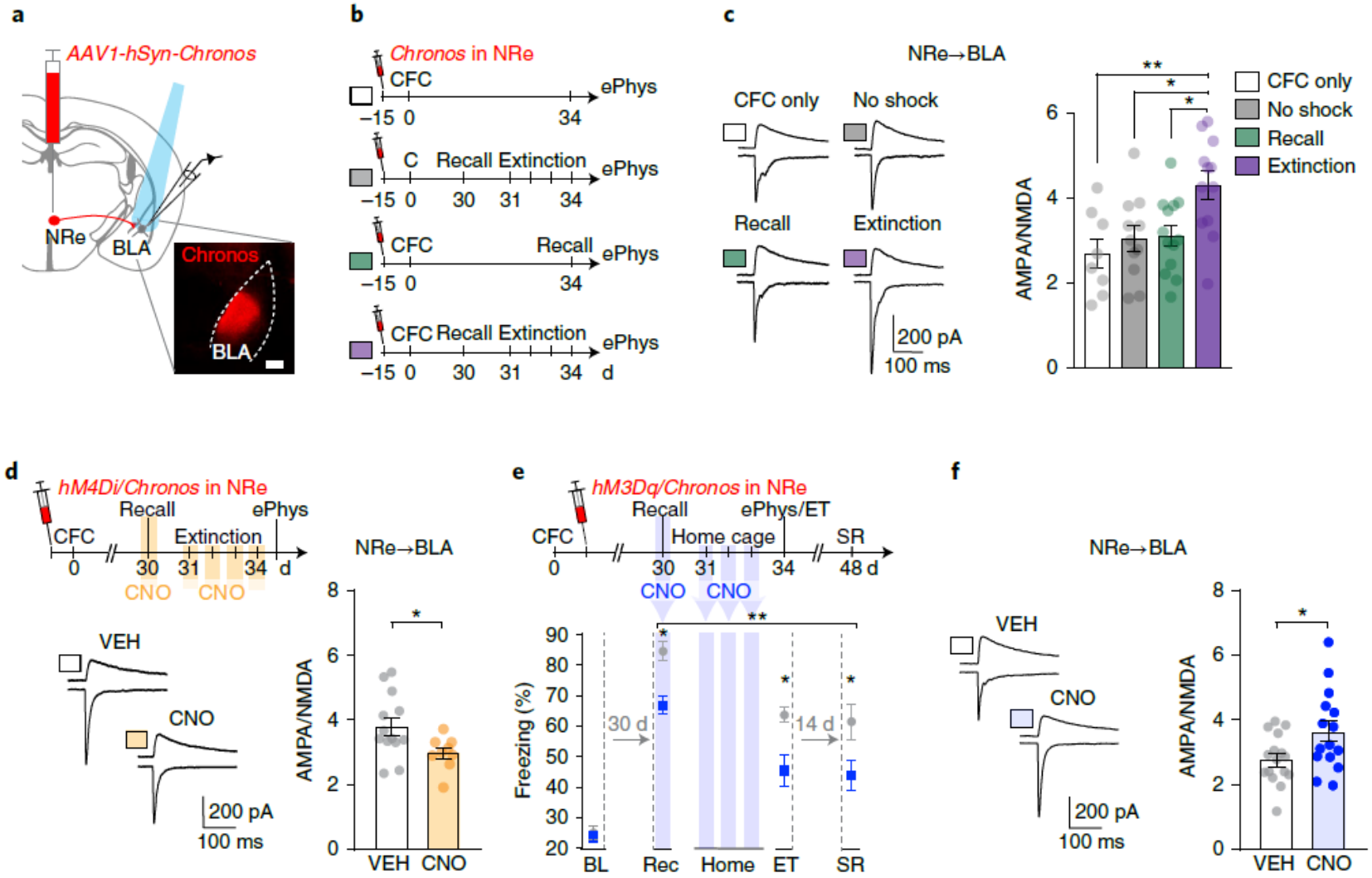


- Using retrograde virus carrying the Cre recombinase to express either GCaMP6 or Chronos only in the NRe neurons projecting to the BLA

→ NRe neurons projecting to the BLA are active during freezing cessation

→ If activated they reduce freezing time

NRe→BLA synaptic plasticity in remote fear extinction



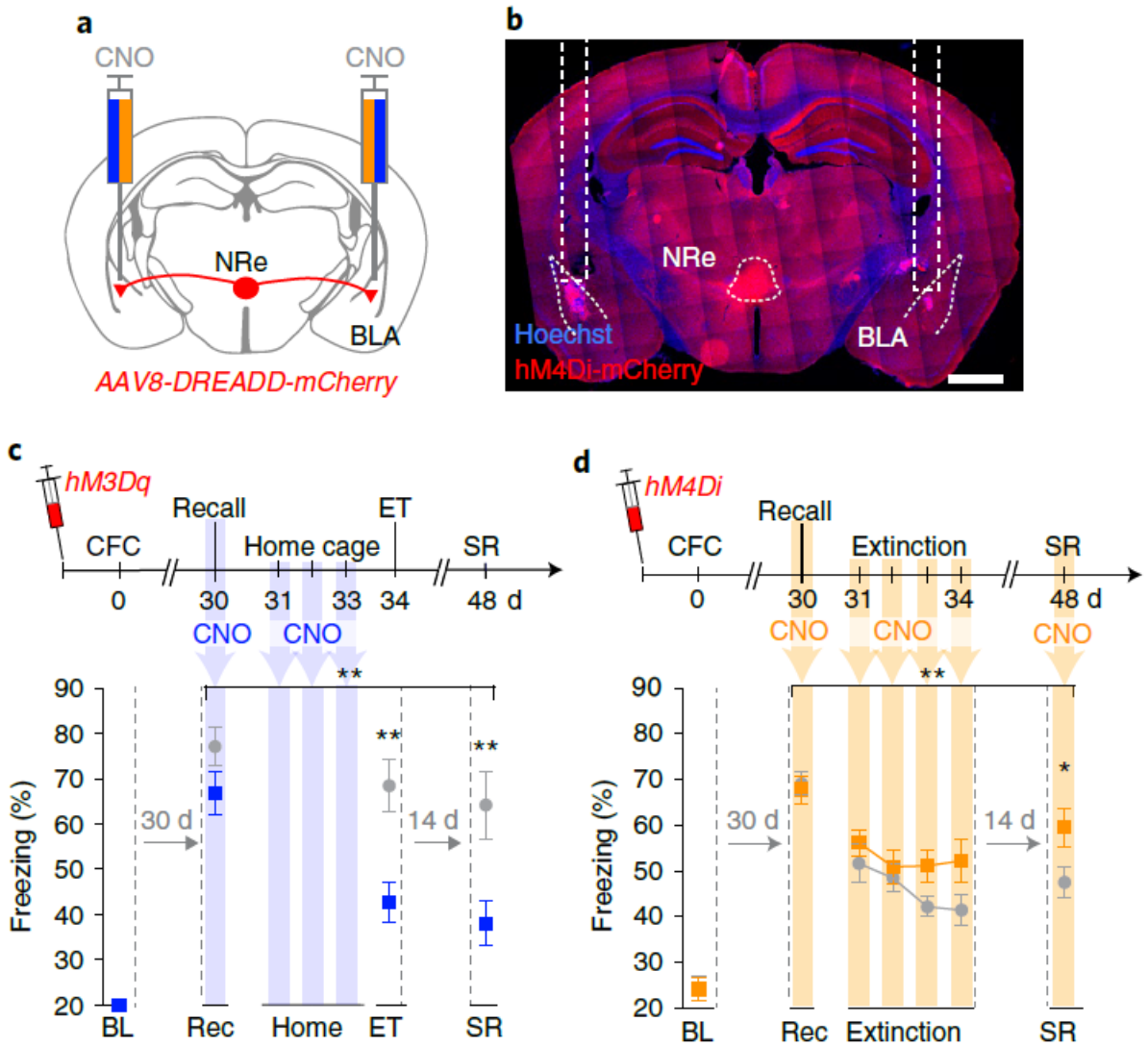
- Ex vivo patch clamping on NRe terminals in the BLA activated by light pulses

→ Increase in AMPA/NMDA ratio after remote fear memory extinction

→ Chemogenetic NRe inhibition under fear extinction prevented synaptic potentiation

→ Chemogenetic activation of NRe neurons during a suboptimal fear extinction reduced freezing behavior and increased synaptic potentiation

NRe→BLA projections mediate remote fear extinction



- Repeated intra-amygdalar infusions of CNO in a suboptimal extinction paradigm

→ CNO improved fear extinction after re-exposure to the same context 1 d after the suboptimal extinction paradigm

→ Repeated inhibition of NRe→BLA neurons during the extinction paradigm impaired remote fear extinction

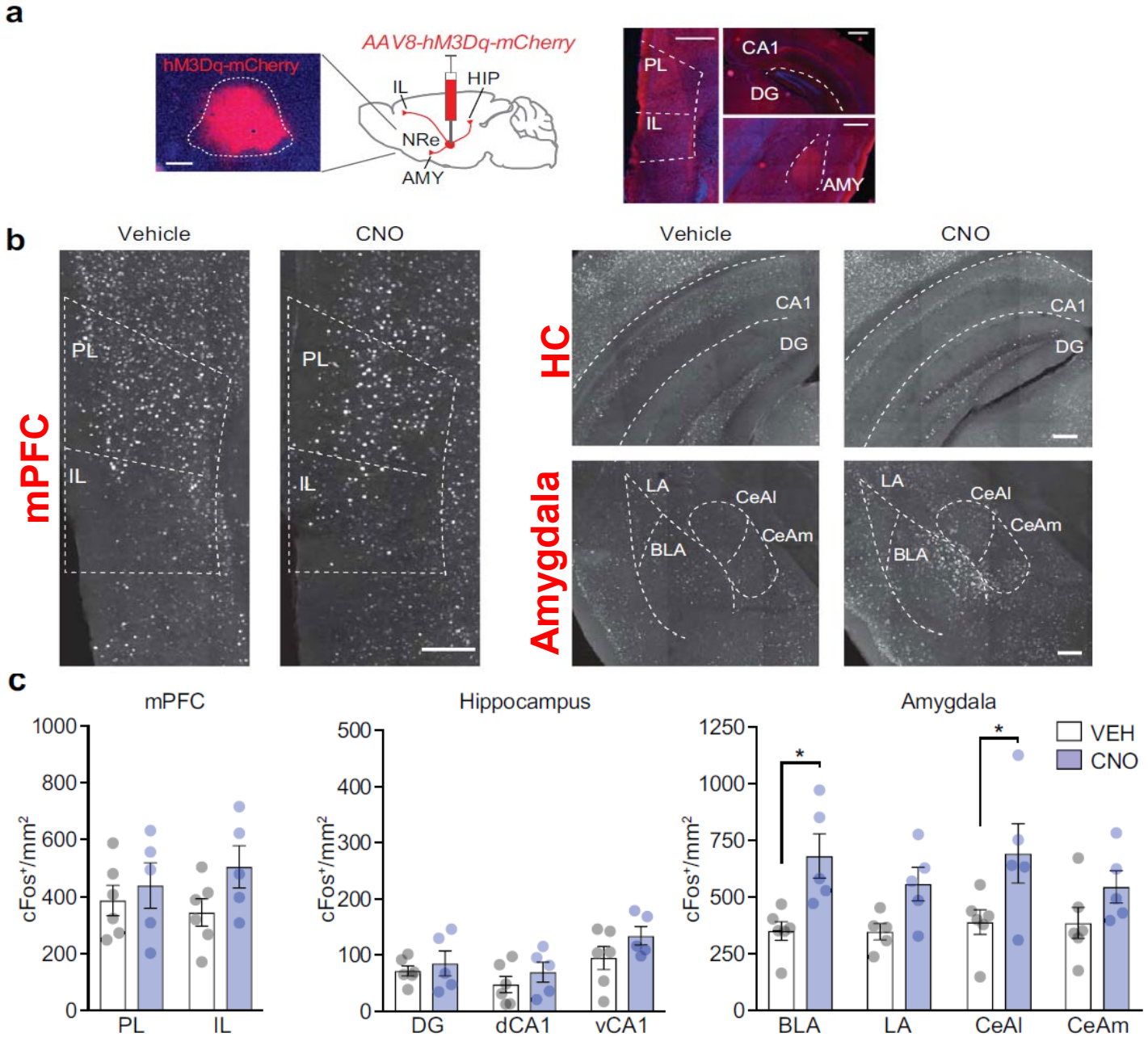
Summary and conclusions

- First functional description of a neuronal circuit underlying remote fear memory extinction
- The NRe activity is increased before the end of freezing epochs during remote fear memory extinction
- The NRe activity is sufficient and necessary to regulate freezing length during extinction
  - → Chemogenetic NRe activation at remote recall immediately triggers an extinction-facilitating effect
  - → Chemogenetic inhibition has no effect on remote fear recall per se but impairs fear attenuation during later stages of the extinction paradigm
- Downstream of the NRe, we found that remote fear memory extinction is mediated by excitatory monosynaptic projections to the BLA
- This connection shows an increased AMPA/NMDA ratio only after extinction training

→ No used of actual activity tagging but interesting method to see how to use IEG expression to analyze circuits activity and clcium imaging to match them with a specific temporal response to a behaviour

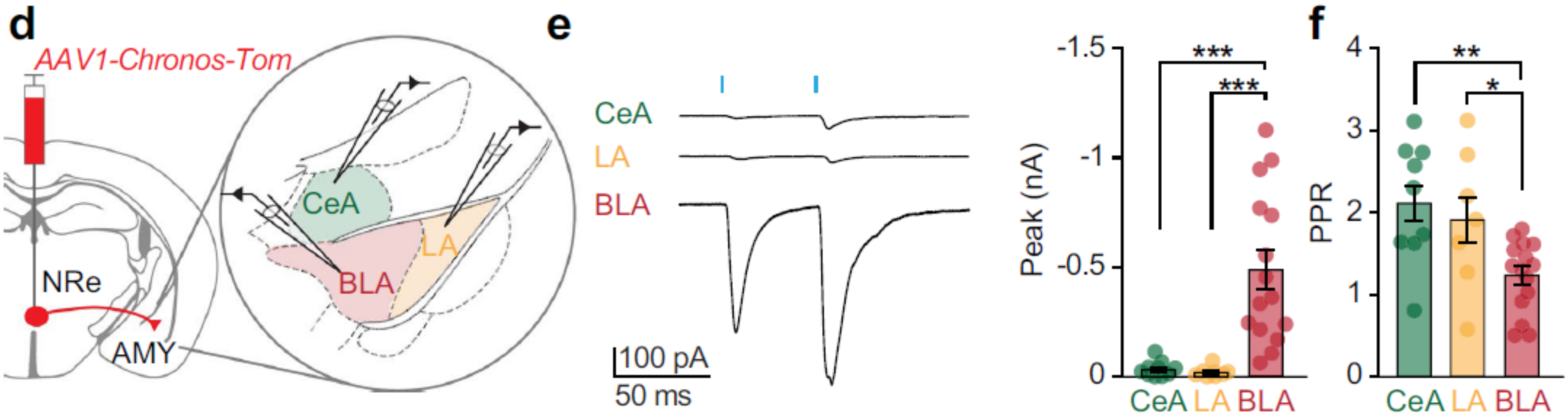
Thank you for your attention! ☺

Dissecting NRe connections through neuronal activation



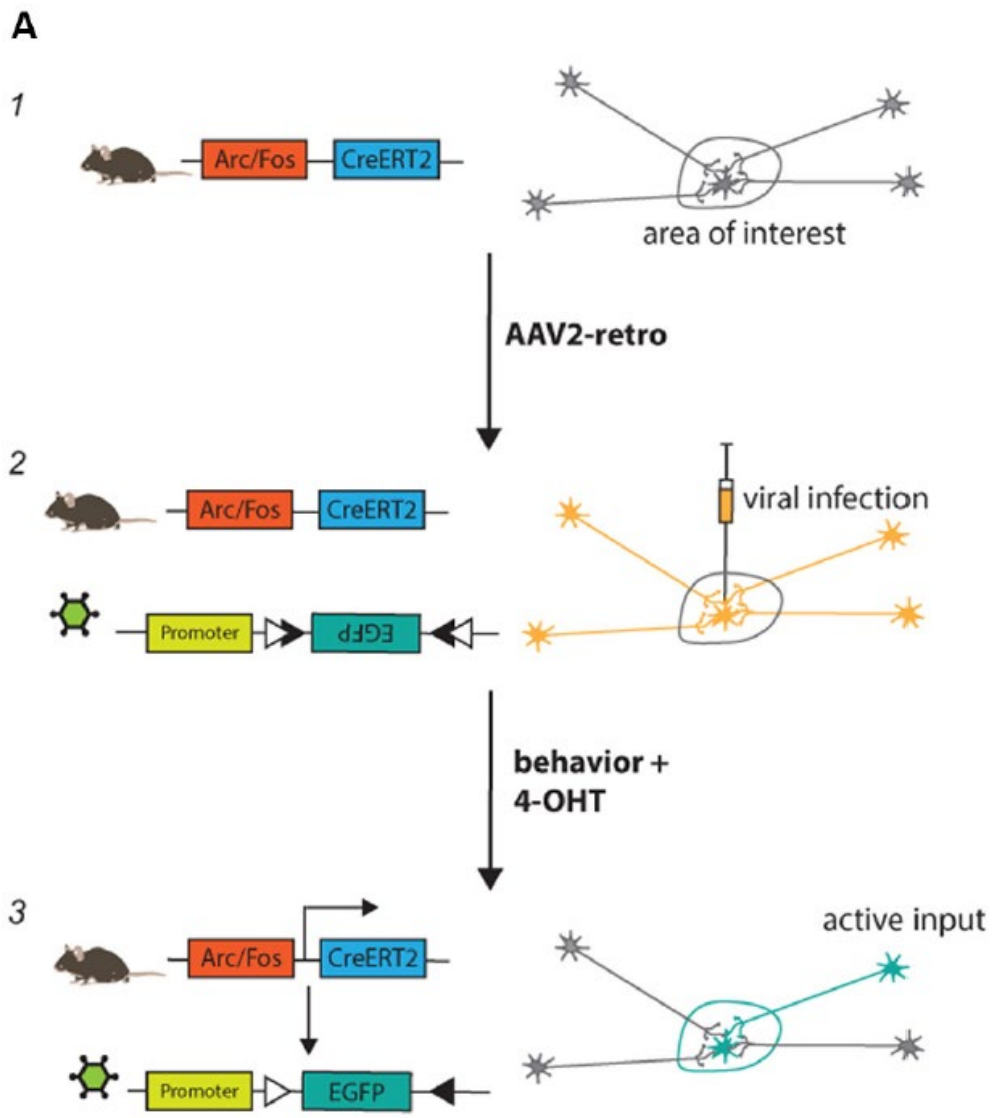
- Using DREADD receptors in the NRe to activate downstream neurons
- Upon CNO injections neurons expressing the hM3 channel will fire and activate downstream neurons

The BLA specifically responds to inputs from the NRe



- Optogenetic activation onto NRe fibers to record excitatory postsynaptic currents (EPSCs) in downstream targets
- The BLA is the only nucleus in the amygdala functionally connected to the NRe

Tagging transiently active inputs: TRACE



Tracing Retrogradely the Activated Cell Ensemble (TRACE) method:

- a tg mouse expresses the tamoxifen-inducible CreERT2-recombinase under the Arc or cFos promoter
- The AAV2-retro infects the cells in the area of interest and carries a floxed EGFP
- Upon 4-OHT injection, the CreERT2 recombination occurs in active cells and EGFP is expressed in all active cells infected with the virus

→ **This causes labeling of active cells in the area of interest as well as in their respective inputs**

Krauth, Nathalie, et al. *Frontiers in Cellular Neuroscience* 14 (2020): 114.