NOVELTY SEEKING BEHAVIOR AND ENGRAM CELLS: WHERE DO MEMORIES BEGIN

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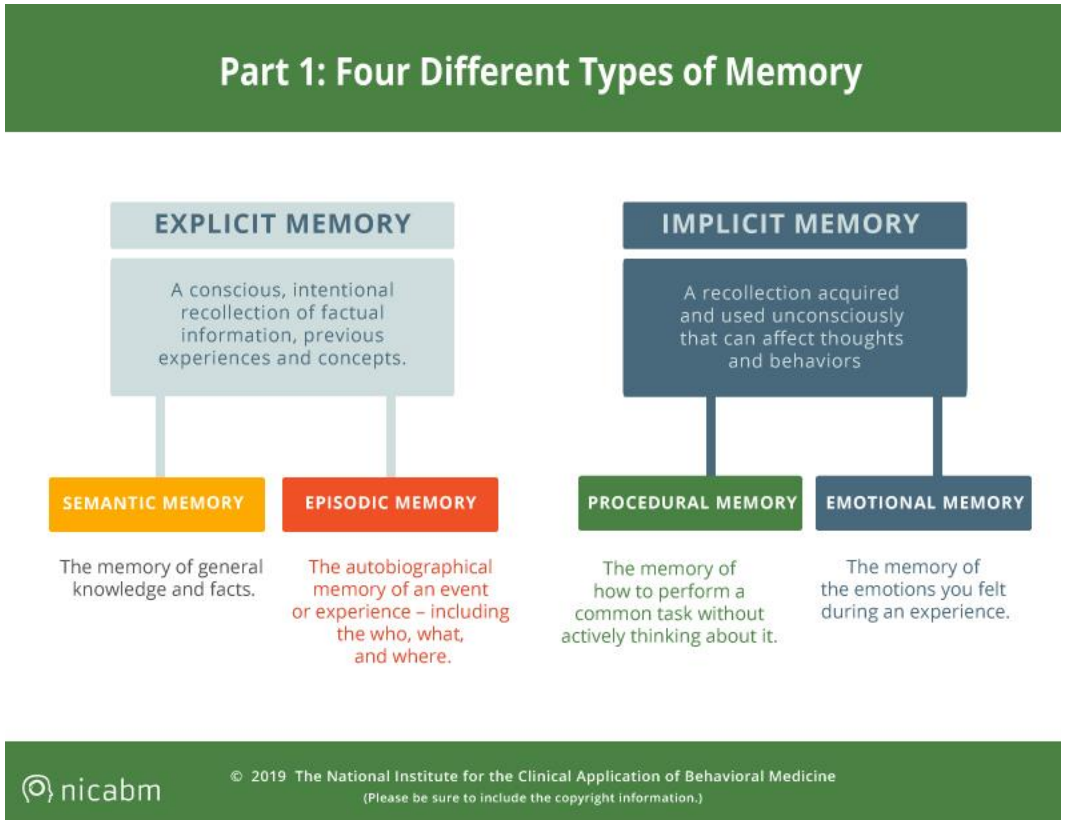
salk
Where cures begin.

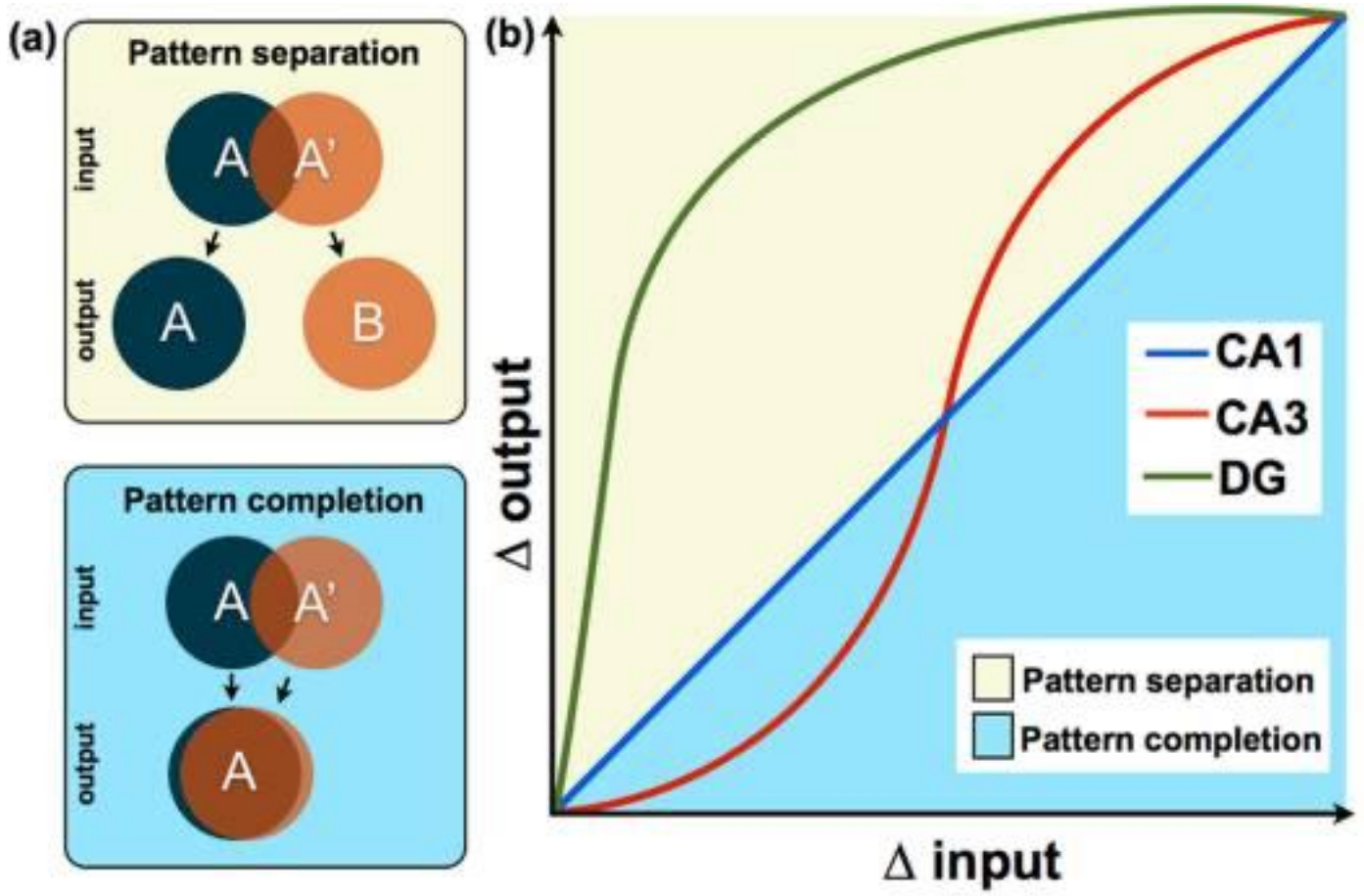
Memory

- Memory is not a single entity but rather a complex system with different forms, including sensory memory, short-term memory (and working memory), and long-term memory. Long-term memory can be further categorized into explicit (declarative:) and implicit (non-declarative) forms, with explicit memory including episodic and
- semantic memory.



Episodic Memories: Pattern Separation and Pattern Completion

Episodic memory is how we remember our lives.
To create accurate memories, we must be able to both distinguish similar experiences from one another, a process called pattern separation,
and recollect the multitude of components of each life event as a unified whole,
a process called pattern completion.



Curiosity enables memory

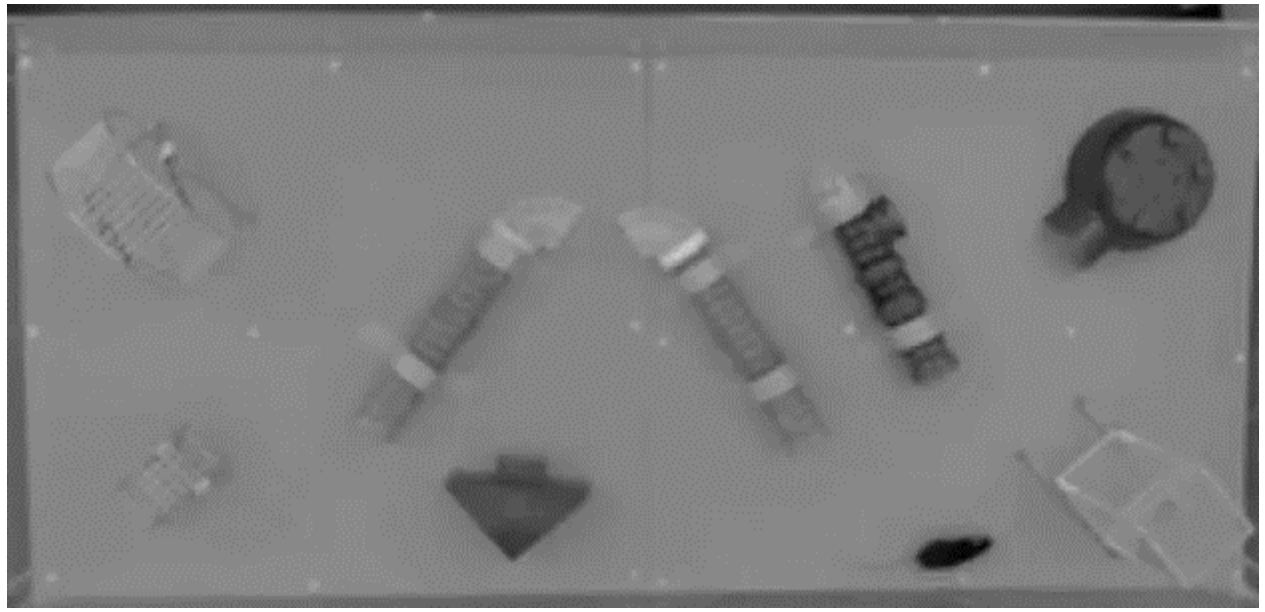
- Novelty-seeking behavior, the tendency to seek out and engage with new experiences, can significantly impact memory development. Experiencing novel environments and stimuli can enhance memory encoding, consolidation, and retrieval.
- Novelty Seeking behavior is a basic genetically determined motivation, driven by Curiosity, analogous to thirst and hunger.

- In order to remember something, you have to have experienced it.

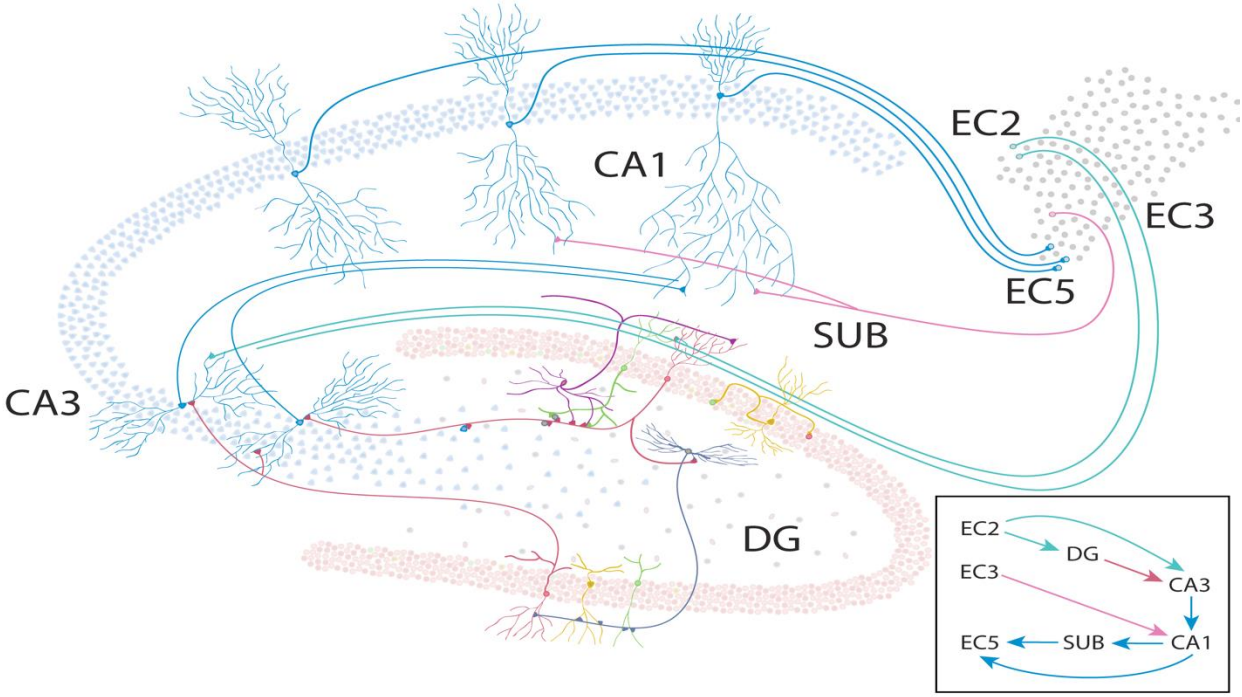
❑ In order to experience something with enough intensity to remember, you must be Curious.

Exploration of a large, complex novel environment

Home cage



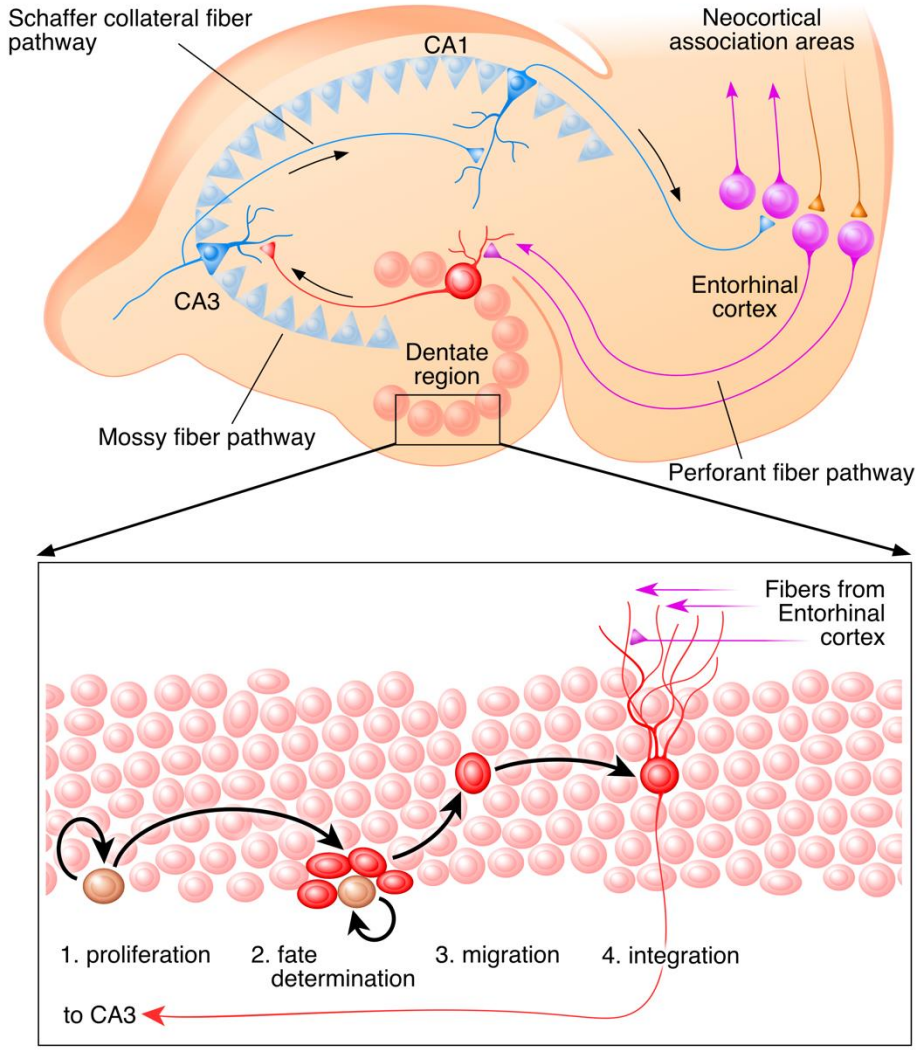
The Hippocampal Network



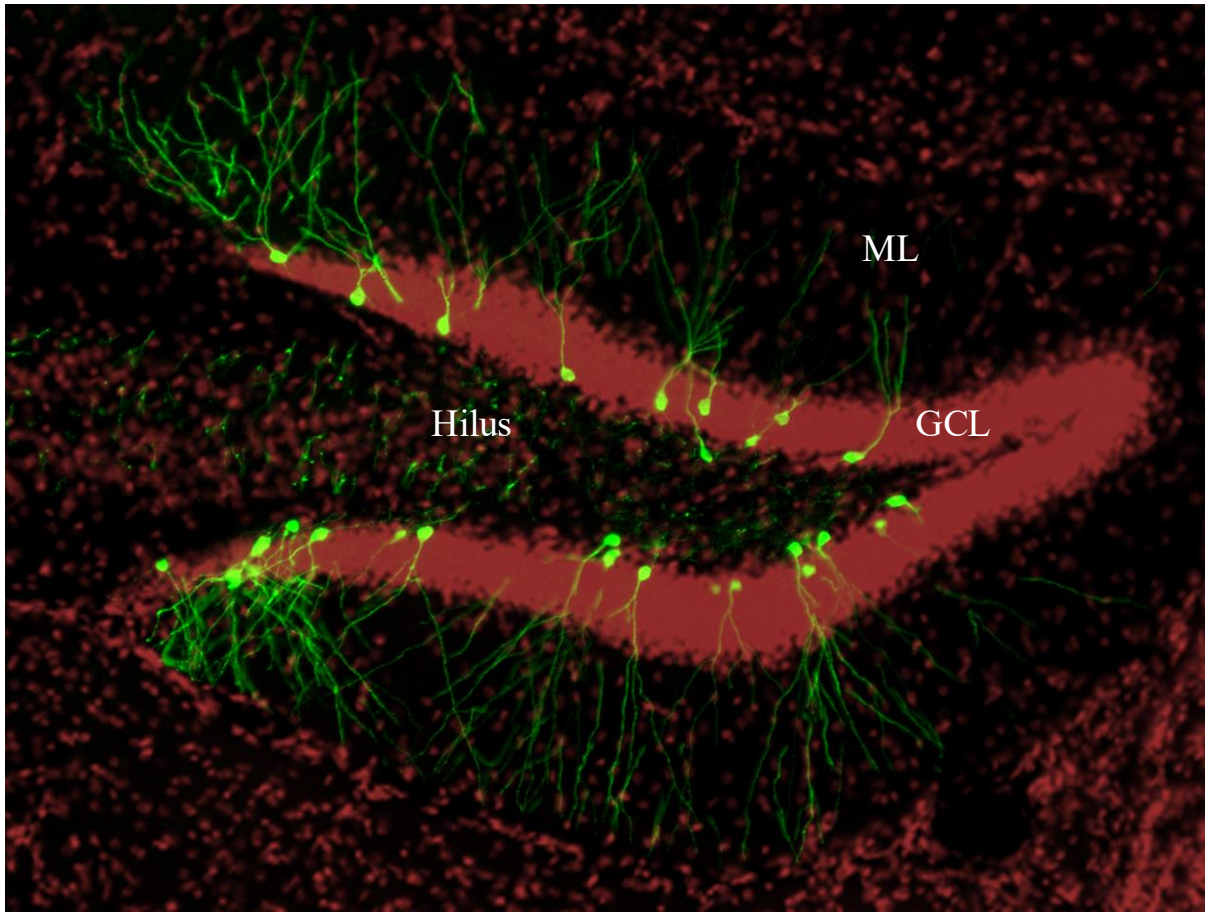
There are Excitatory pathways (glutamate)
But the hippocampus is
Known for inhibitory (GABA) regulation

The Hippocampus is primarily responsible for memory formation and retrieval, particularly for long-term memories and spatial navigation. It's located in the temporal lobe and is a key part of the limbic system, a brain region involved in emotions, learning, and memory.

Adult Neurogenesis (2): Hippocampus



Fate of neural stem cells in the adult hippocampus

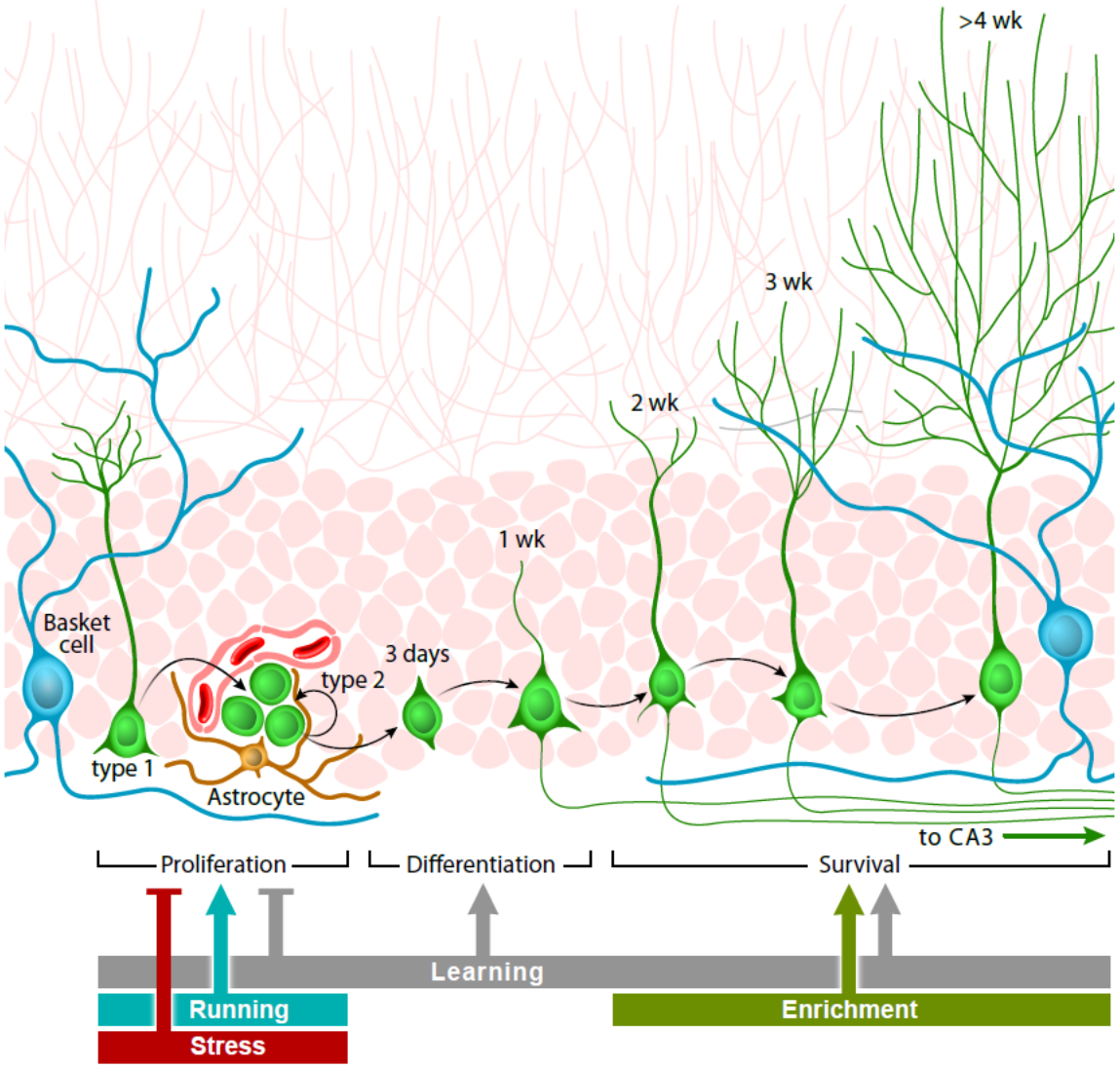


- The vast majority of cells born in the adult dentate gyrus are excitatory, granule cells

- In contrast to olfactory bulb, where most of the neurons are inhibitory

4wpi **CAG-GFP**

Environmental Regulators of Adult Neurogenesis



SCIENCE May 14, 2021

A cell type–specific cortico-subcortical brain circuit for investigatory and novelty-seeking behavior

Mehran Ahmadlou1*†, Janou H. W. Houba1, Jacqueline F. M. van Vierbergen1, Maria Giannouli1, Geoffrey-Alexander Gimenez1, Christiaan van Weeghel1, Maryam Darbanfouladi1, Maryam Yasamin Shirazi1, Julia Dziubek1†, Mejdy Kacem1, Fred de Winter2, J. Alexander Heimel1*

Exploring the physical and social environment is essential for understanding the surrounding world. We do not know how novelty-seeking motivation initiates the complex sequence of actions that make up investigatory behavior. We found in mice that inhibitory neurons in the medial zona incerta (ZIm), a subthalamic brain region, are essential for the decision to investigate an object or a conspecific. These neurons receive excitatory input from the prelimbic cortex to signal the initiation of exploration. This signal is modulated in the ZIm by the level of investigatory motivation. Increased activity in the ZIm instigates deep investigative action by inhibiting the periaqueductal gray region. A subpopulation of inhibitory ZIm neurons expressing tachykinin 1 (TAC1) modulates the investigatory behavior.

Novelty Seeking behavior is a basic motivation driven by Curiosity, analogous to thirst and hunger.

- The Hippocampus is not critical for novelty seeking behavior, or important for curiosity
- But once you have performed novelty seeking behavior, how do you determine whether something is novel or the same, or similar
- The Hippocamps and specifically is important for the determination of novelty and similarity
- One cannot accurately determine novelty or similarity without novelty seeking behavior and curiosity
- First exposure to a novel environment is induced by curiosity and exploration and likely does not require DG
- The second exposure requires curiosity to explore and to determine similarity or difference

Hypothesis and direction

- Once similarity or sameness is determined, curiosity is saited, exploration decreases
- Are Dentate Granule neurons unique in their response to novelty?
- Do DG Neurons change in response to novelty?
- What is the molecular basis for Novelty Detection in the DG?

Dentate Granule Cells are ENGRAM CELLS

Cells active during memory encoding (exploration) are important for later recall (engram cells).

Are there unique features of DG engram cells that might enable pattern separation/novelty detection?

Single active DGCs can be isolated after a novel experience and show a more dramatic transcriptional shift than other hippocampal cells

Home cage

1hr

Dissect hippocampus

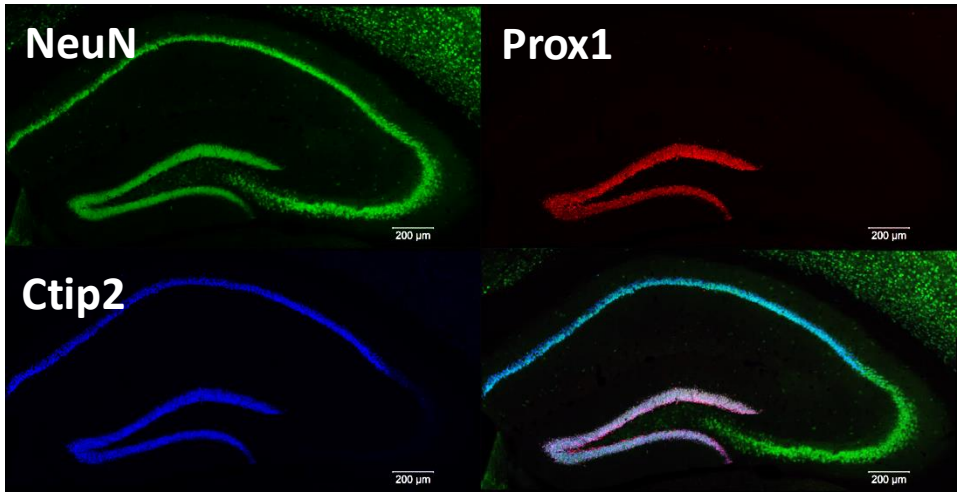
Nuclei dissociation

Antibody staining for cell type, activity

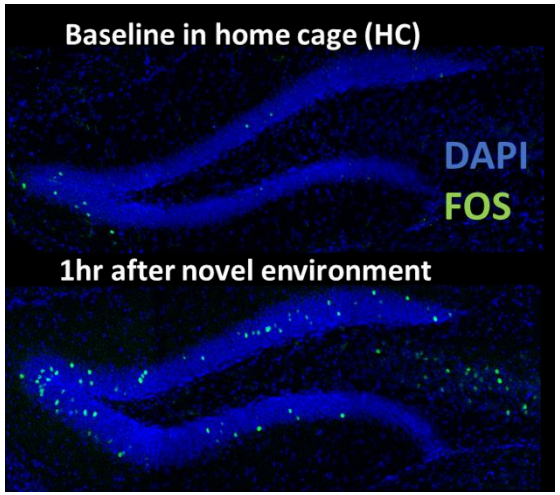
FACS

RNA-Seq via Smartseq2

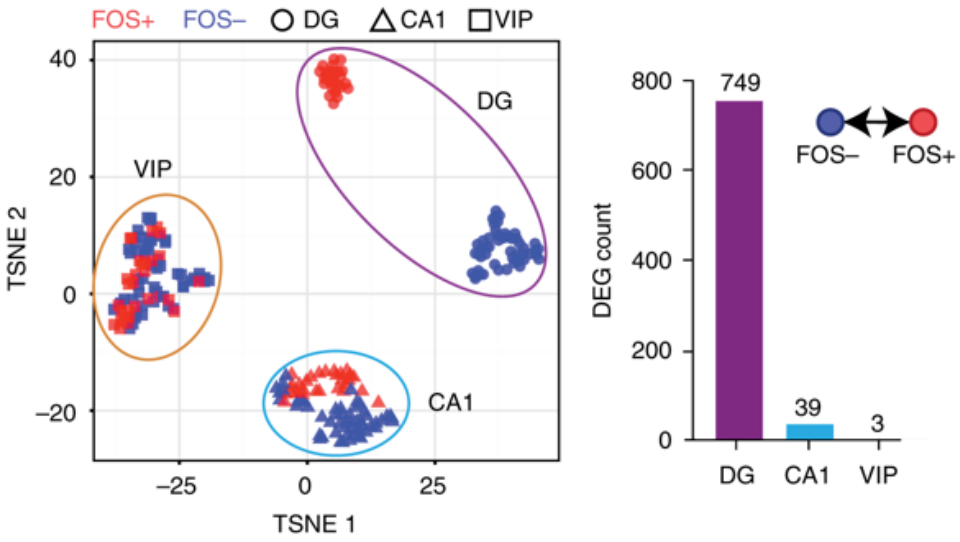
a Cell type markers



b Activity marker: Immediate early gene (IEG) FOS



c Activity-related transcriptional change

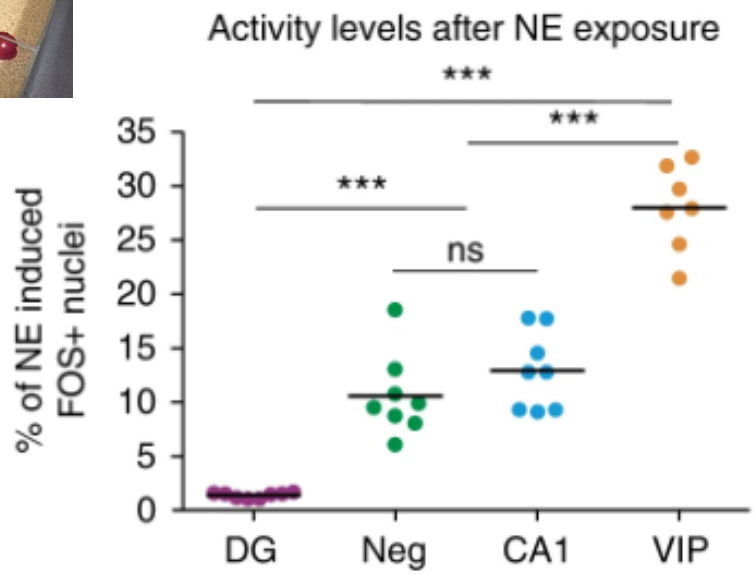


Jaeger, Linker, Parylak et al. 2018, *Nat. Comm.*

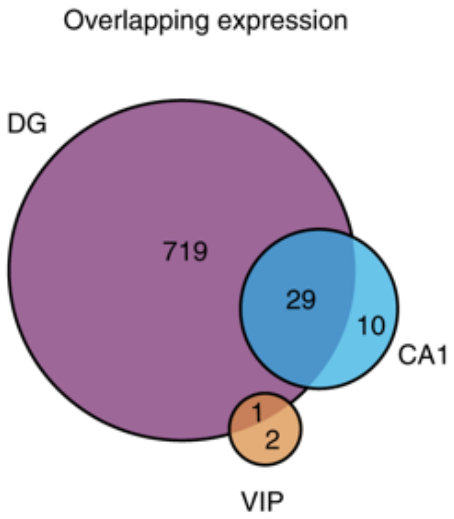
Activity-induced transcriptional change occurs in DGCs despite (or because of?) sparse activation

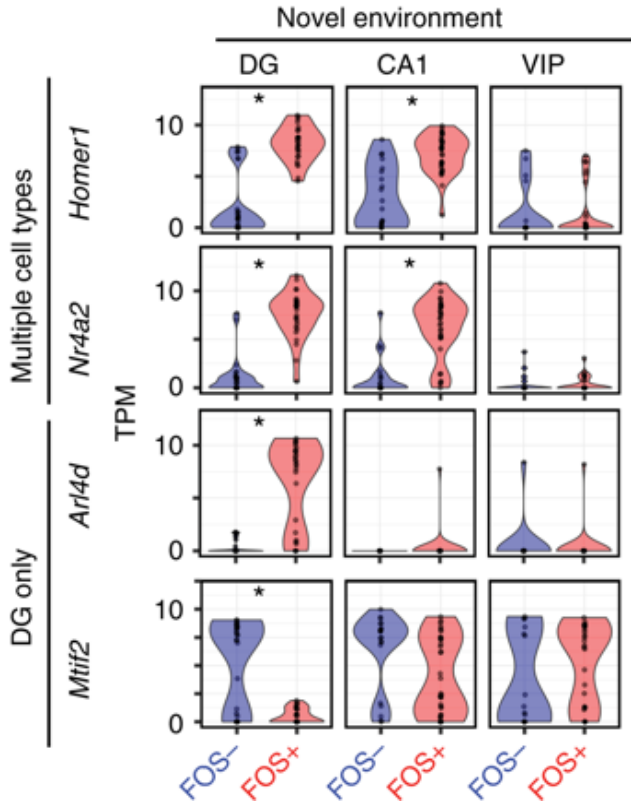
Activation by novel environment

1hr



Shared vs. DG-unique activity-induced genes





Categories of genes responding to activation in FOS+ DGCs

Activity-induced genes (up in FOS+)	Activity-repressed genes (down in FOS+)
Transcriptional regulation	Transcriptional regulation
Poly(A) RNA binding	Endoplasmic reticulum
Phosphoprotein	Mitochondrion
Ubiquitin-dependent catabolic process	Mitochondrial respiratory chain complex
Acetylation	GTP binding
Potassium ion transport	Extracellular exosome
Regulation of translation	rRNA processing
Ubl conjugation pathway	Dbl homology domain
Activation of MAPK activity	Signal transduction
Positive regulation of ERK1 and ERK2	Ceramide biosynthetic process
Cortical actin cytoskeleton	Ubl conjugation pathway
Glycoprotein	

Active DGCs show an additional wave of transcription by 4-5hr

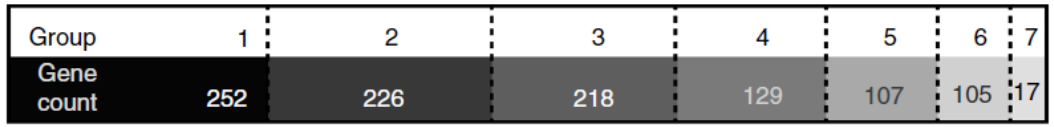
1hr → Fos +/-

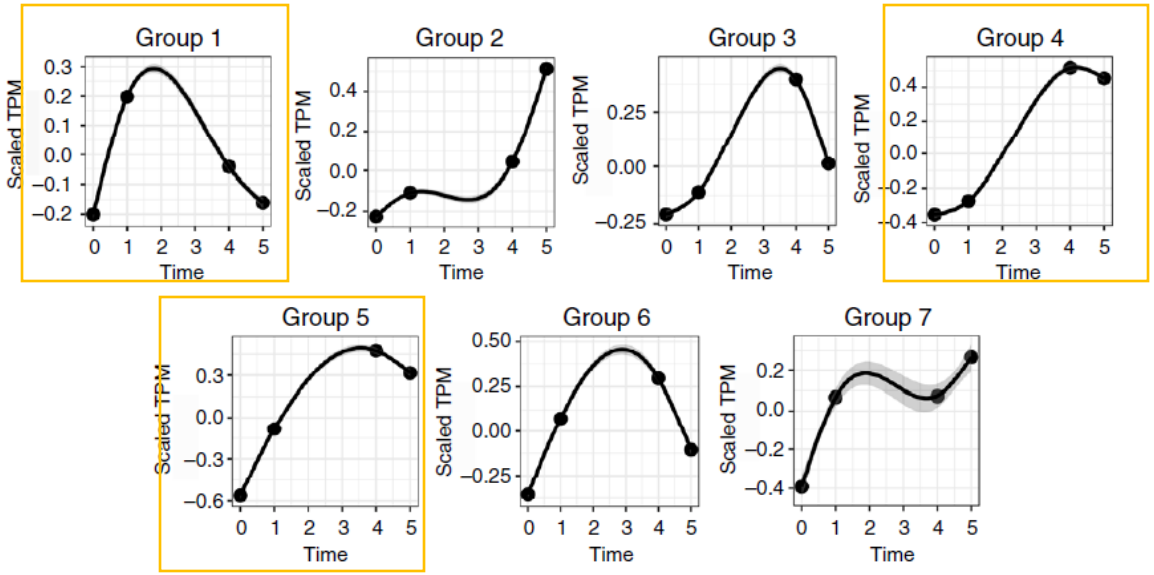
4hr → Arc +

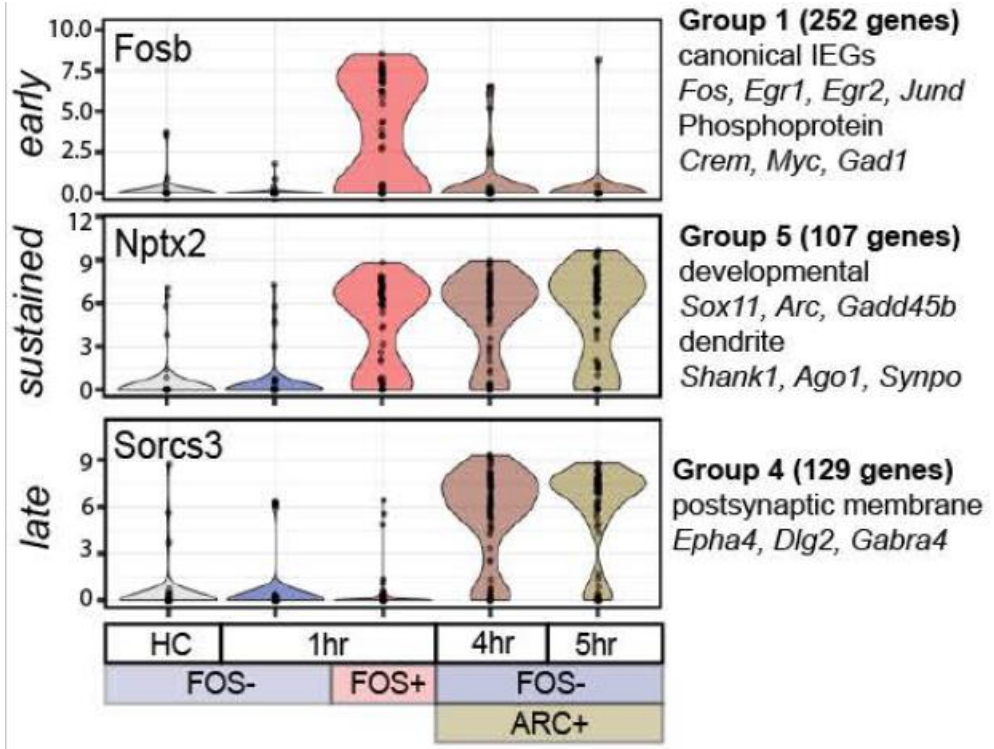
5hr →

Home cage 0hr → Fos -

Activity-induced expression patterns







Group 1 (252 genes)
canonical IEGs
Fos, Egr1, Egr2, Jund
Phosphoprotein
Crem, Myc, Gad1

Group 5 (107 genes)
developmental
Sox11, Arc, Gadd45b
dendrite
Shank1, Ago1, Synpo

Group 4 (129 genes)
postsynaptic membrane
Epha4, Dlg2, Gabra4

Baseline → Early → Late

Summary: Activity in Mature DGCs

- Single-nuclei RNA-Seq enables the study of transcription in rare, heterogeneous DG engram cells whose activation encodes a memorable event

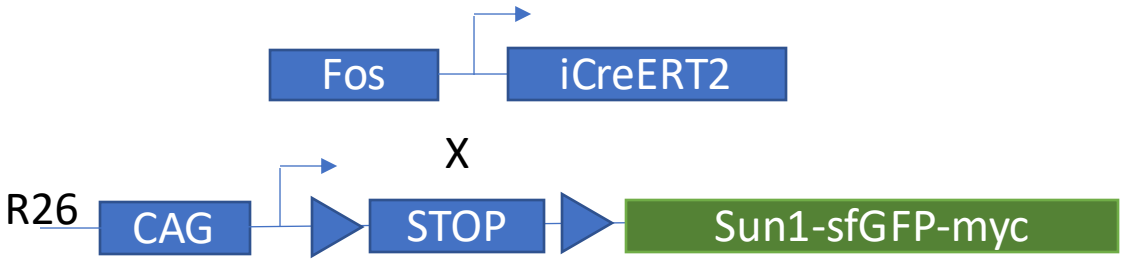
- DGCs respond to a novel environment with much greater transcriptional change than hippocampal CA1 and VIP cells

- DGCs respond to activation with multiple waves of transcription, modulating different at genes 1hr vs. 4-5hr– Longer?

-How do we determine if there are long term changes in DGC after activation?

- How do we determine if activated DGC can be reactivated ?

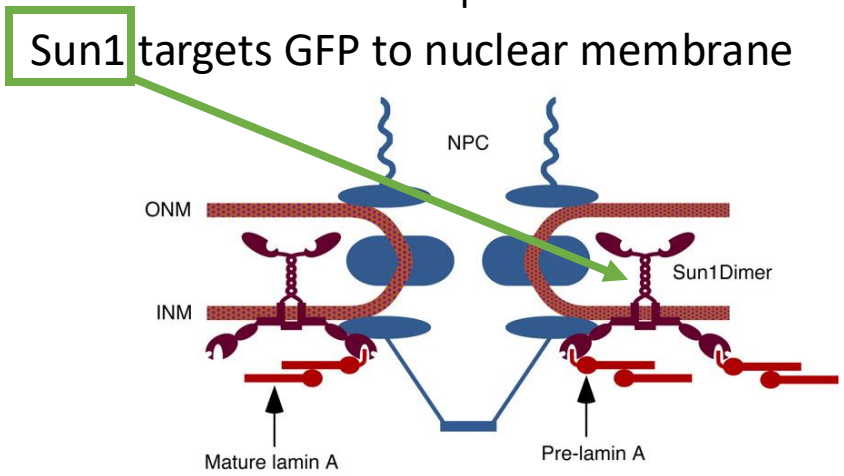
Long-term Cre-regulated tracking of engram neurons in DG



4-hydroxytamoxifen (4-OHT) triggers recombination in active FOS+ cells

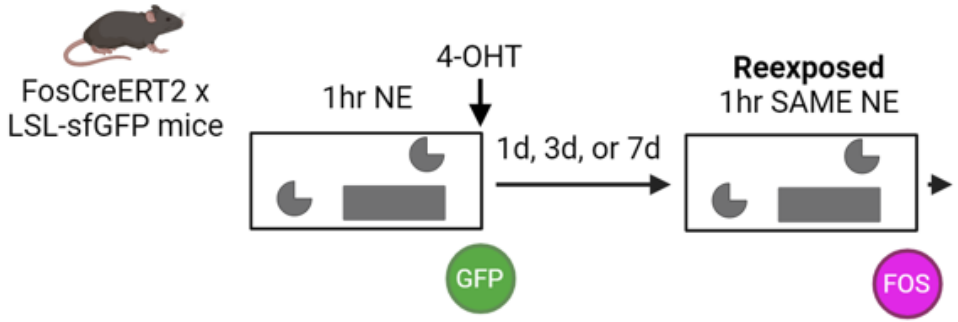
+

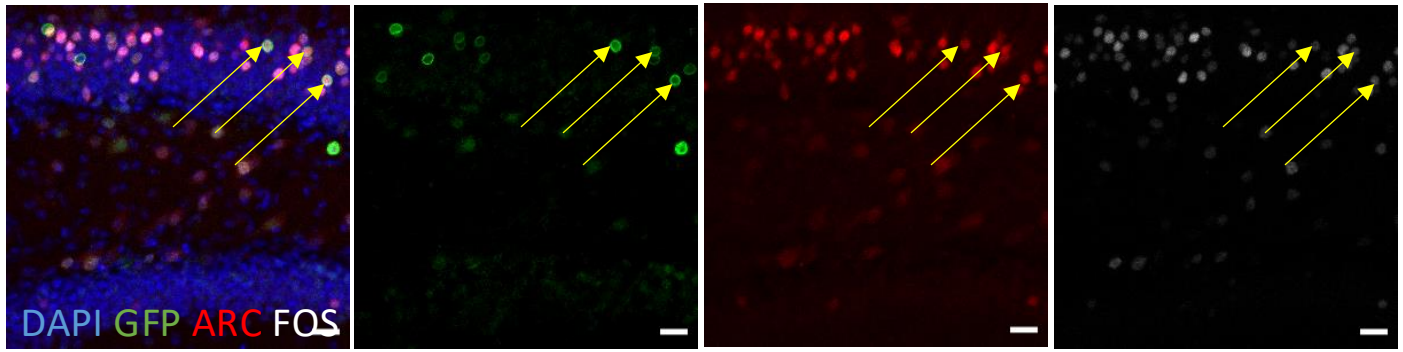
Sun1 targets GFP to nuclear membrane



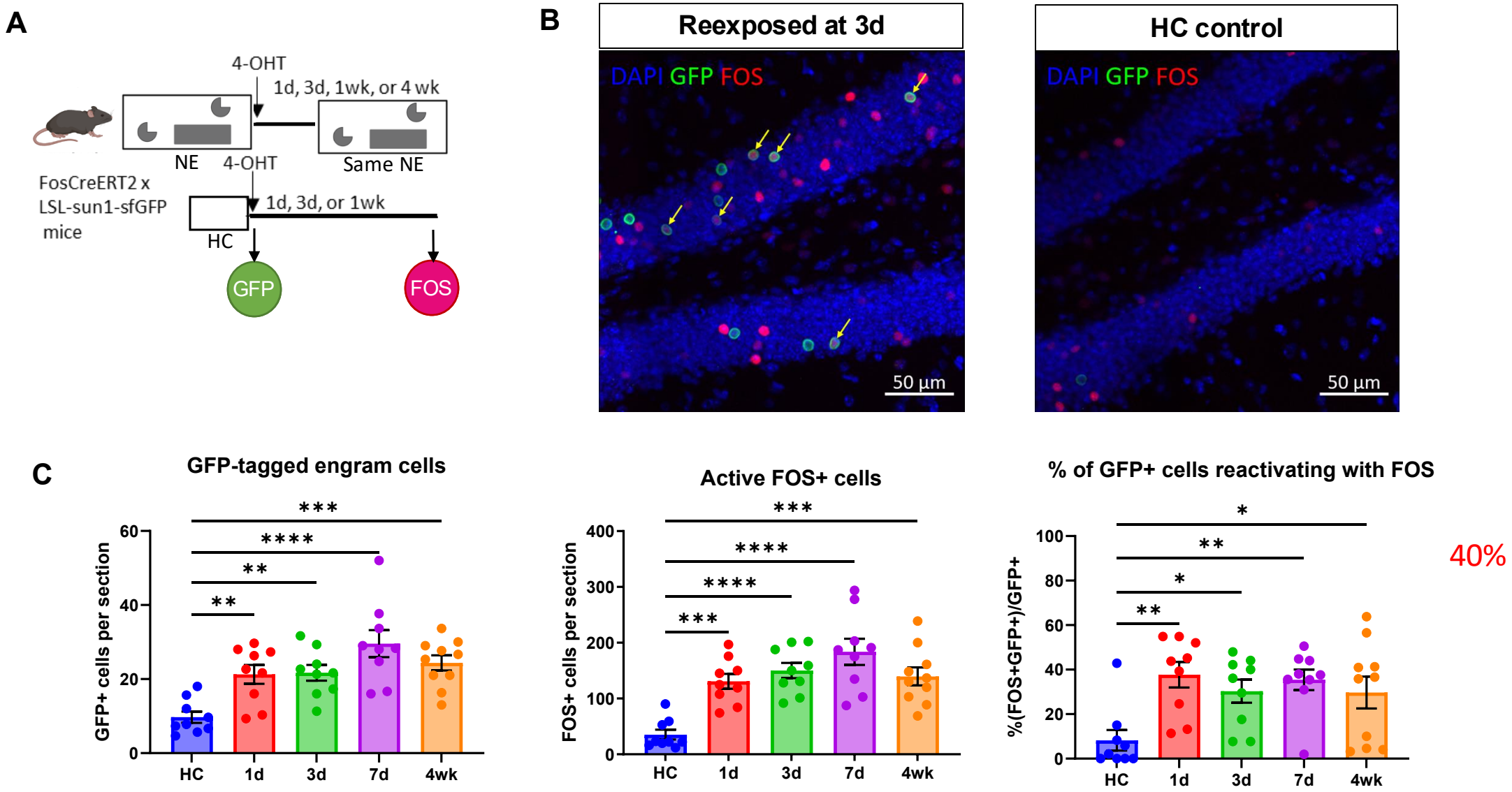
Liu 2007, *Journal of Cell Biology*

Detect 1st activation with GFP and 2nd with ARC/FOS:





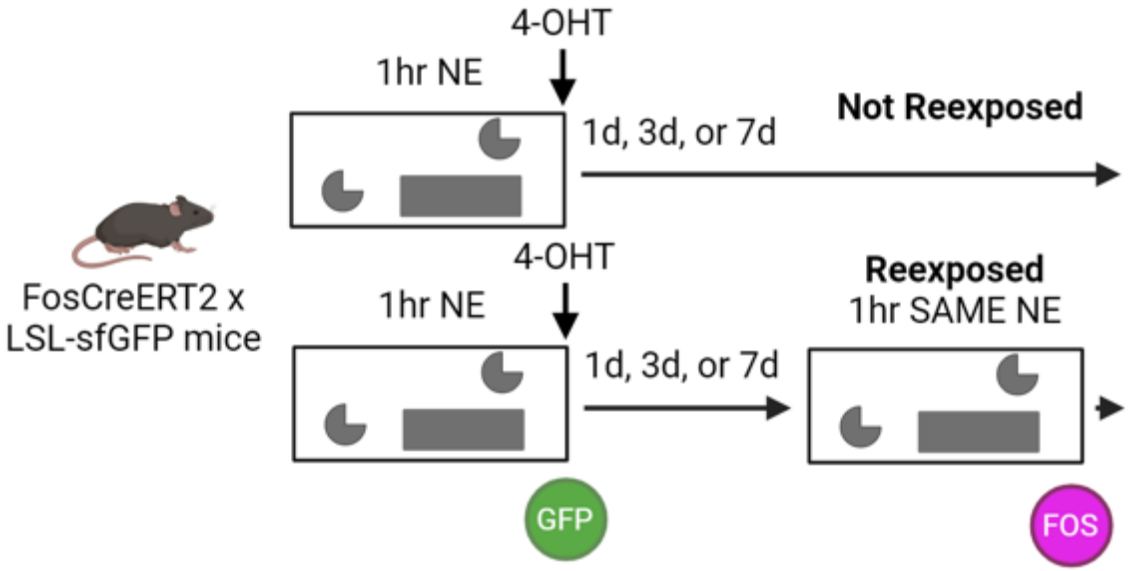
DG engram cells representing a novel environment (NE) reactivate at much higher than chance levels to that SAME environment up to 4 weeks later



How is transcription in DG engram cells related to their function?

Are there lasting transcriptional changes in DG engram cells that enable them to reactivate at high rates over a period of days to weeks?

Characterizing activity-related transcriptional change in DG engram cells

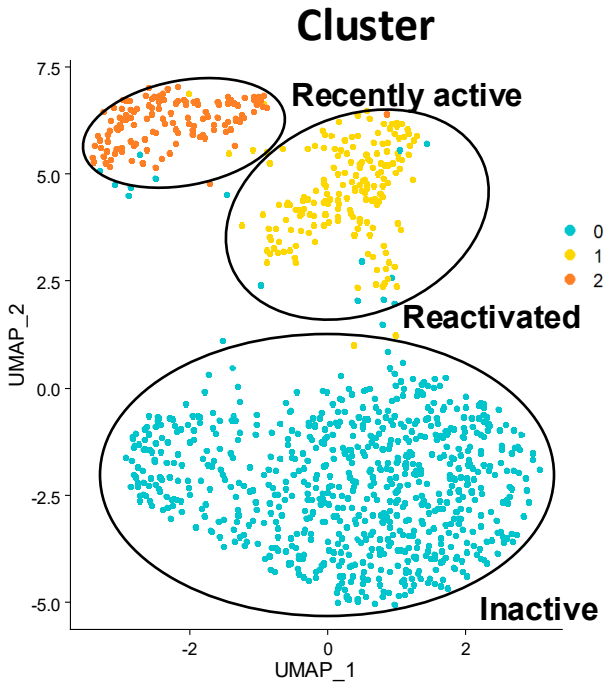


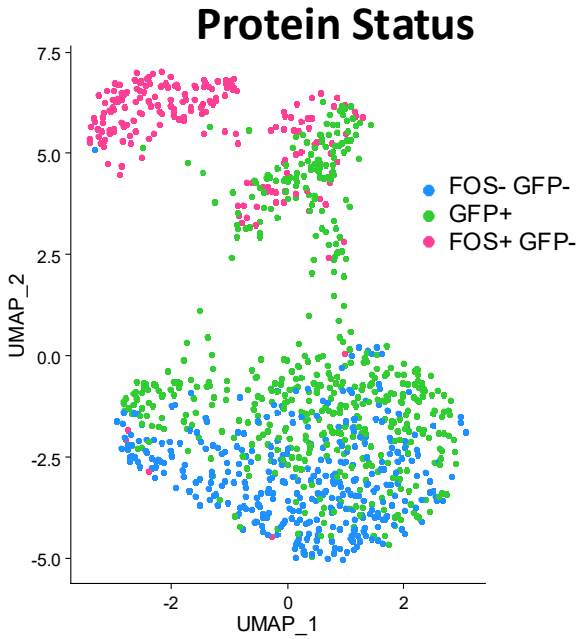
Collect DGC nuclei (PROX1+CTIP2+) for SmartSeq2	
GFP- FOS-	Baseline
GFP+	Remote activation
GFP- FOS-	Baseline
GFP- FOS+	Recent activation DGC
GFP+	Remote activation & Reactivation

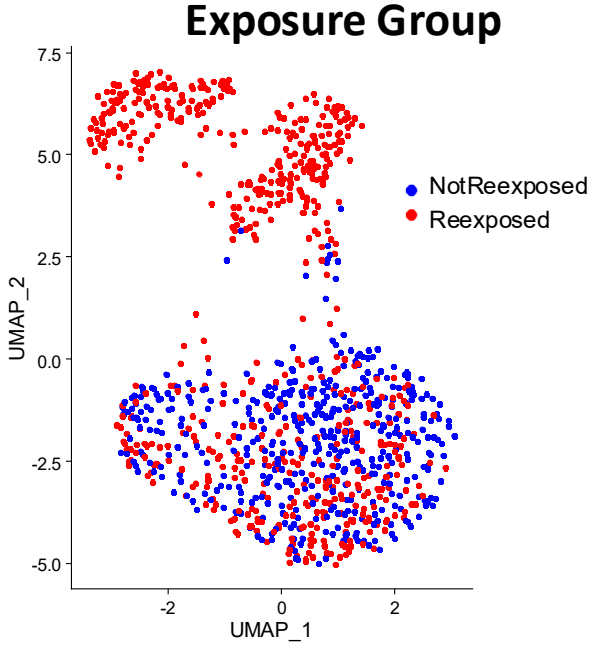
Total nuclei = 1152

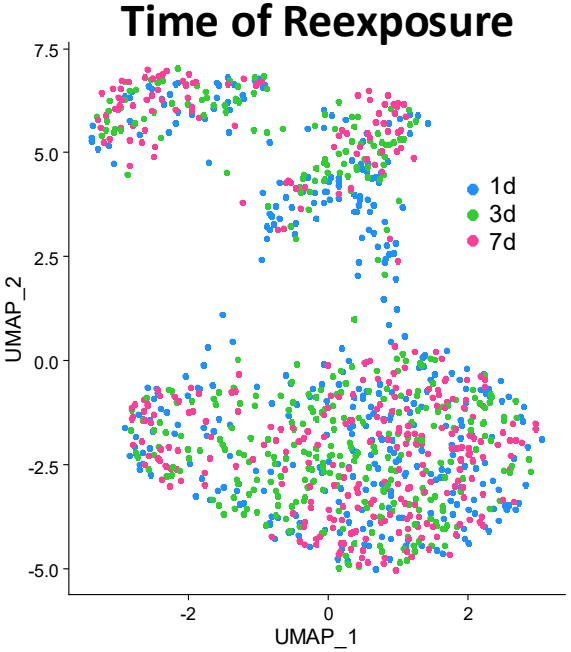
Includes 2-3 male mice per group per time point

Unbiased clustering identifies reactivated DG engram cells

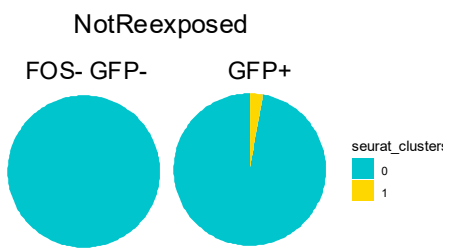


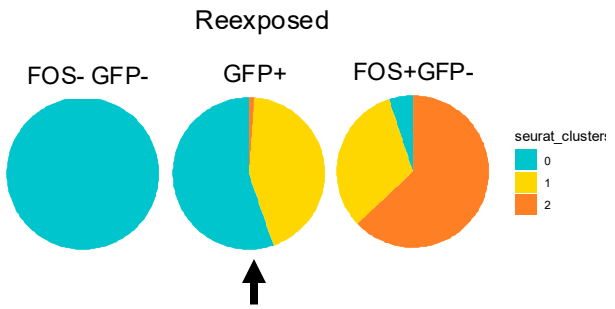






Cluster assignment by protein status and reexposure



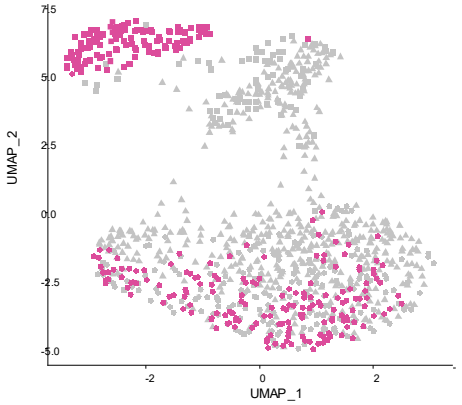


Total nuclei passing QC = 1061

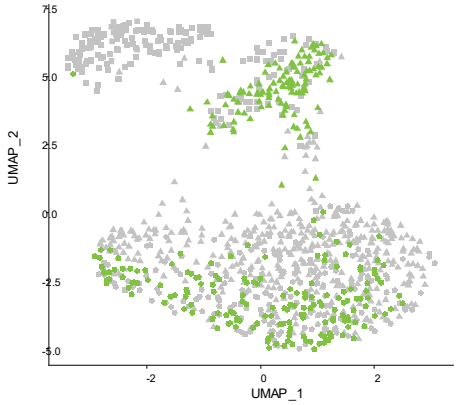
Similar to our histology results, about 40% of the GFP+ cells in the re-exposed group, reactivate.

Differentially expressed genes by cell state

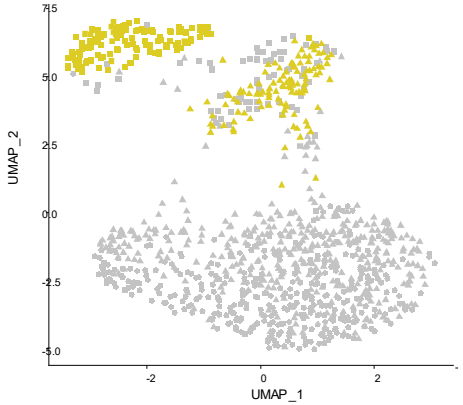
Recent Activation
Vs Baseline



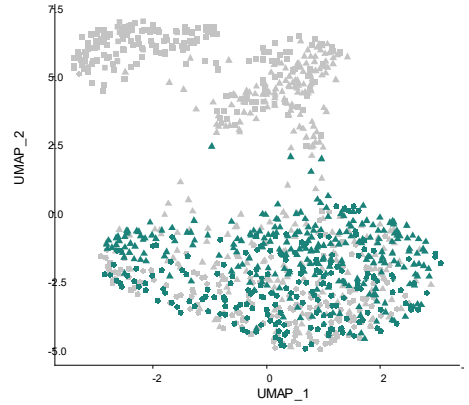
Reactivation
Vs Baseline

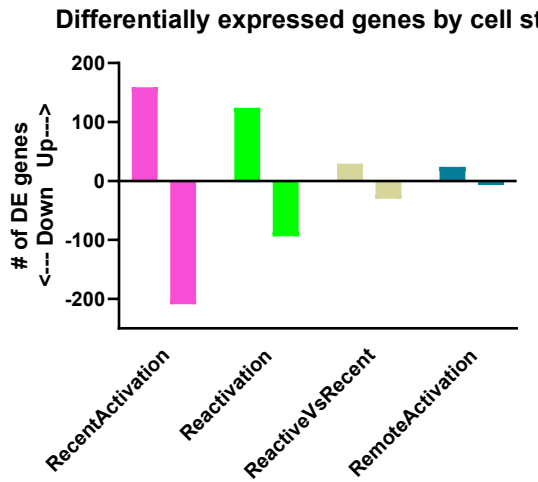


Reactivated
Vs Recent Activation



Remote Activation
Vs Baseline

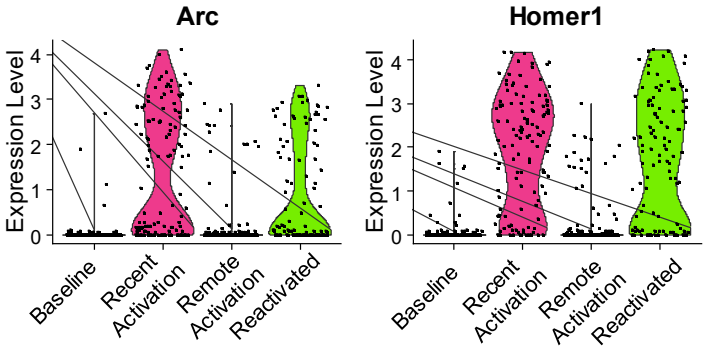




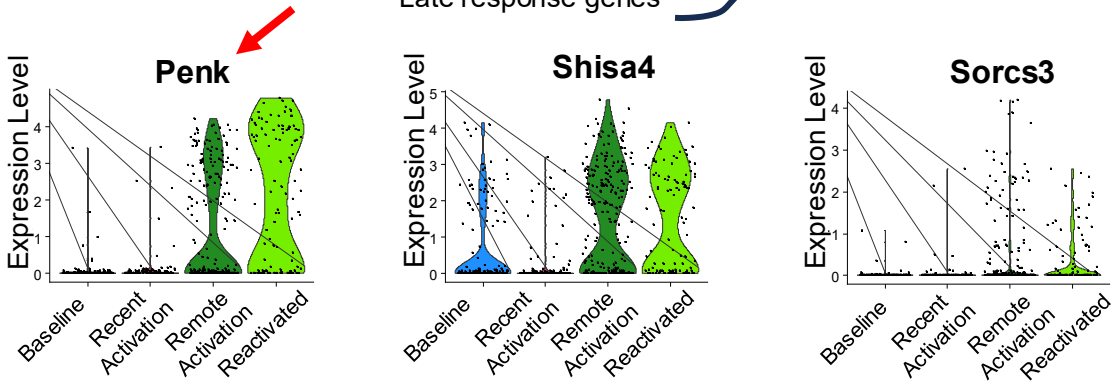
Top 8 GO terms ranked by p value:

neuron to neuron synapse	neuron to neuron synapse	glutamatergic synapse	negative regulation of synaptic transmission
learning	neuronal cell body	muscle tissue development	synaptic signaling via neuropeptide
regulation of neuron projection development	synaptic signaling via neuropeptide	molecular sequestering activity	neuron to neuron synapse
rhythmic process	cell morphogenesis	response to inorganic substance	regulation of striated muscle cell differentiation
enzyme-linked receptor protein signaling pathway	regulation of synapse structure or activity	protein localization to synapse	hormone secretion
associative learning	learning	calcium ion binding	protein serine/threonine kinase activity
cellular response to calcium ion	regulation of nervous system development	SMAD binding	response to nerve growth factor
response to corticotropin-releasing hormone	synaptic signaling	chemical synaptic transmission	positive regulation of apoptotic process

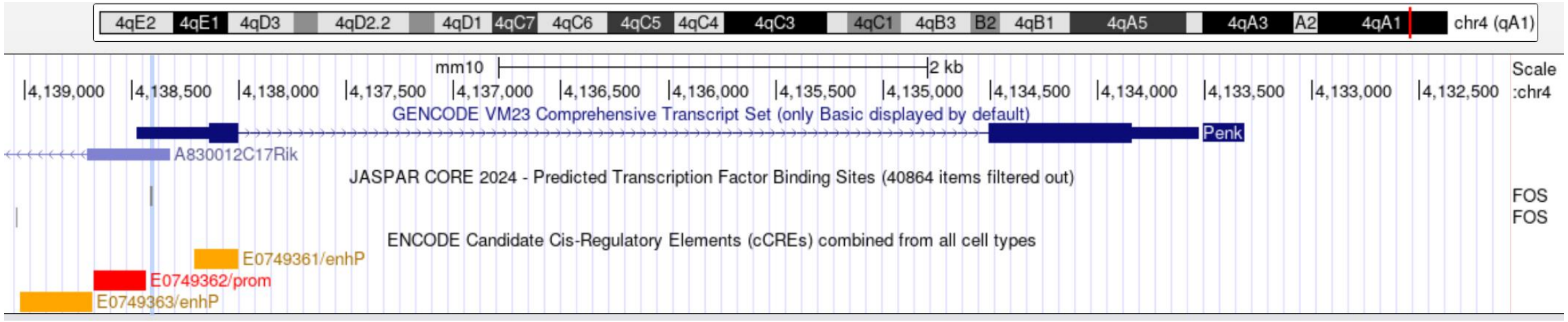
Rapid activity-related genes



Late response genes



Penk has a FOS binding site near the promoter region



UCSC Genome Browser

Differential comparison summary

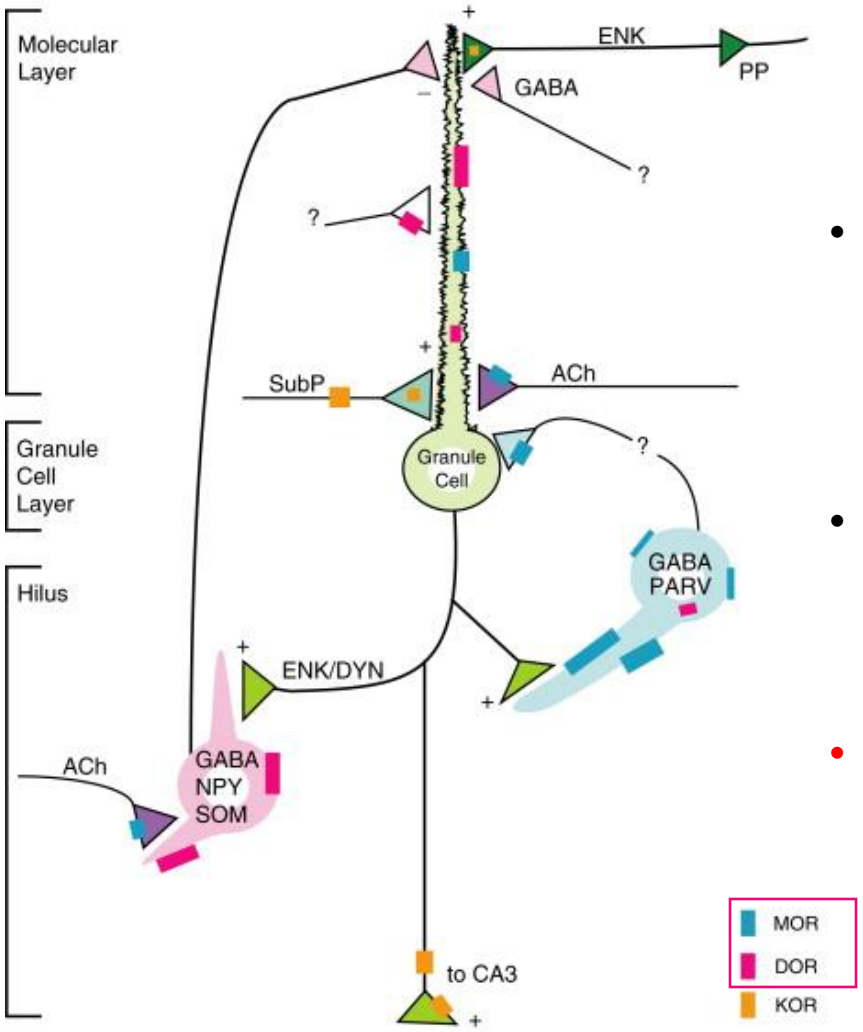
- A single recent activation of DGCS (FOS+ cluster 2 vs. baseline cluster 0) initiates a strong transcriptional response, with hundreds of DE genes

- Reactivation is associated with a distinct transcriptional response compared to the first activation (FOS+GFP+ cluster 1 vs. FOS+ cluster 2) - 40% reactivation

- Engram cells that responded to a 1st exposure and were never given the opportunity to activate again show differences in synaptic transmission and neuropeptide-related genes- Some respond to the second exposure – PENK zbs

- We conducted further studies explore the role of these gene candidates in the strikingly high reactivation rate among engram DGCs

Penk generates endogenous opioids called enkephalins, which tend to disinhibit DGCs



- *Penk* produces a preproprotein that is cleaved to form **Met and Leu-enkephalins**
  - Enkephalins are stored in dense core vesicles, **requiring more prolonged, intense neuronal stimulation** than release of glutamate/GABA
  - Enkephalins bind to **mu and delta opioid receptors** (MORs and DORs)

- **MOR and DOR activation tends to increase DG excitability and facilitate LTP in the molecular layer- Inhibition of inhibitory cells cause increase in excitability due to Disinhibition**

- **c-FOS our indicator of Activation, binds to and activates Penk!**

Drake 2007

Penk in the DG – activity-related or a DGC subtype?

Cell Reports

CellPress
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Report

A Sparse, Spatially Biased Subtype of Mature Granule Cell Dominates Recruitment in Hippocampal-Associated Behaviors

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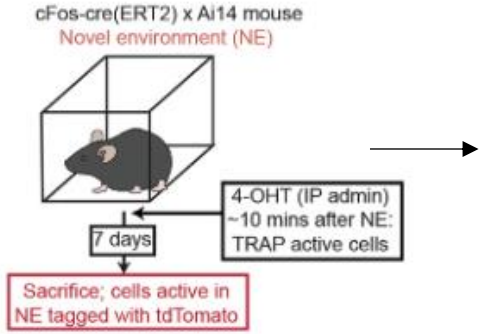
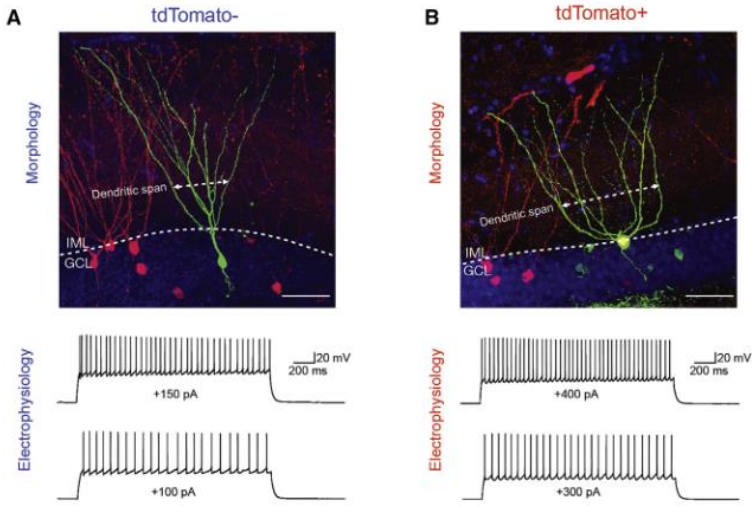
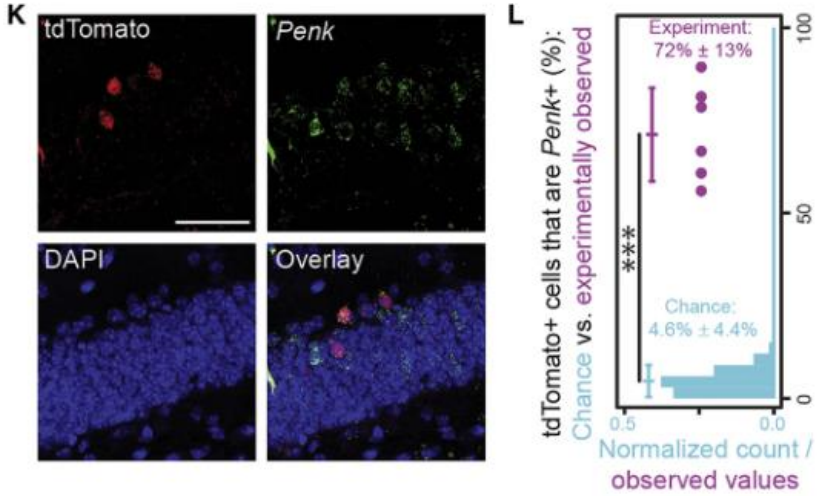
https://doi.org/10.1016/j.celrep.2020.107551

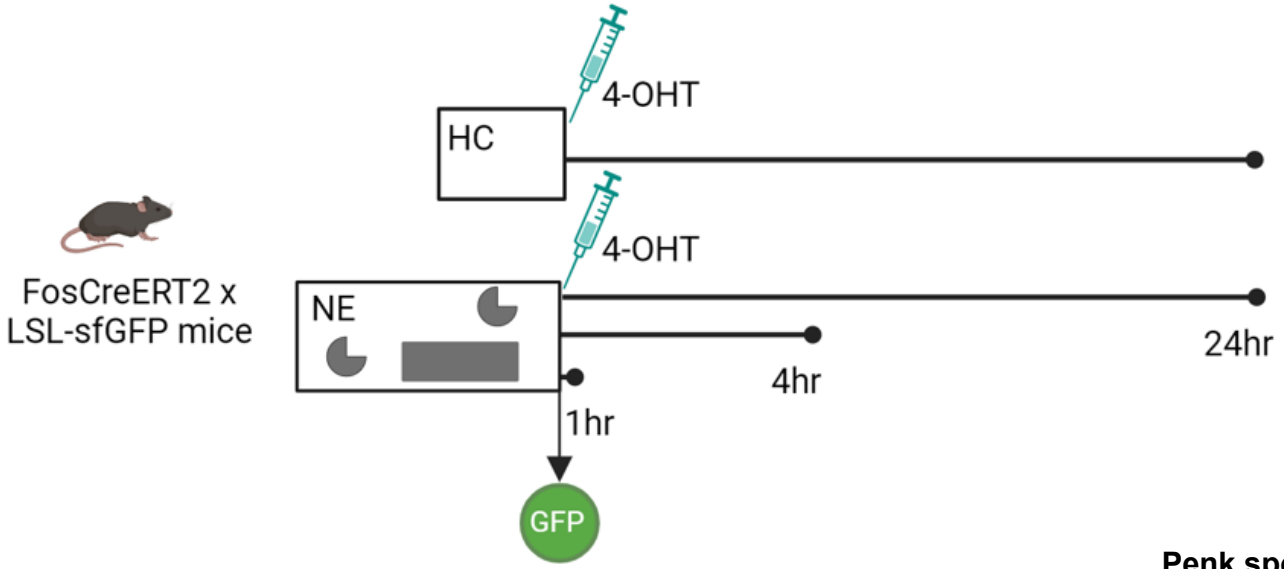


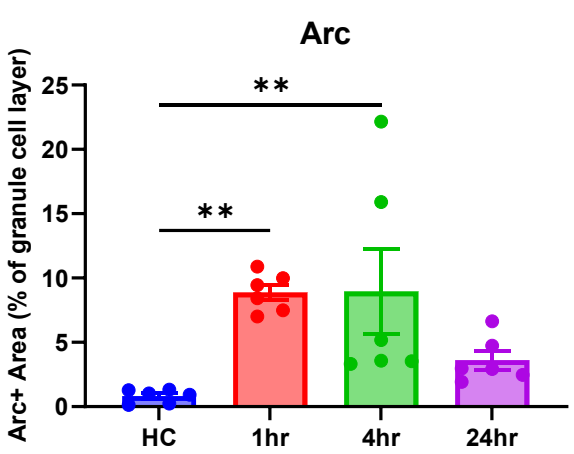


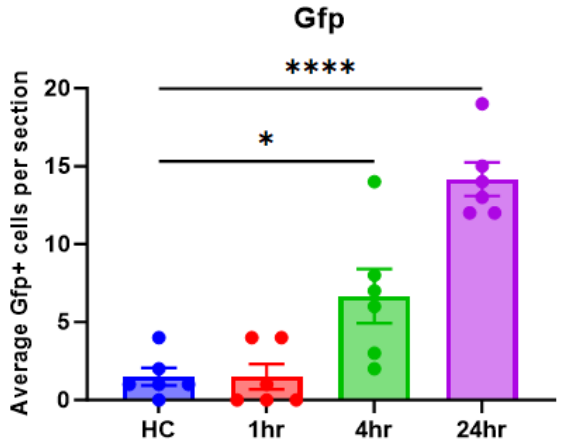
They conclude that Penk-expressing cells are a distinct DGC subtype that is preferentially recruited into active ensembles

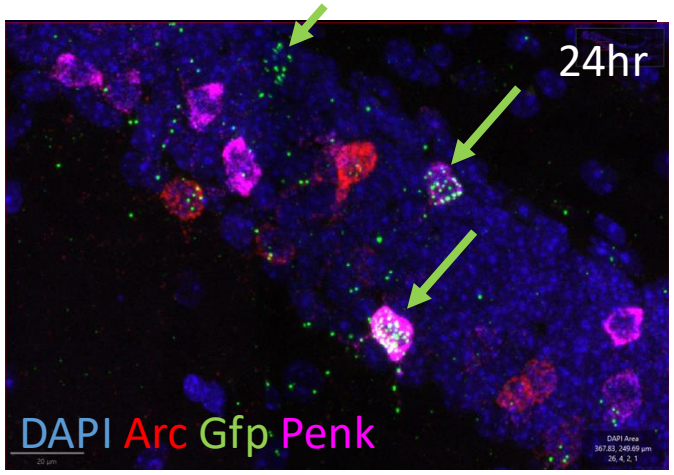


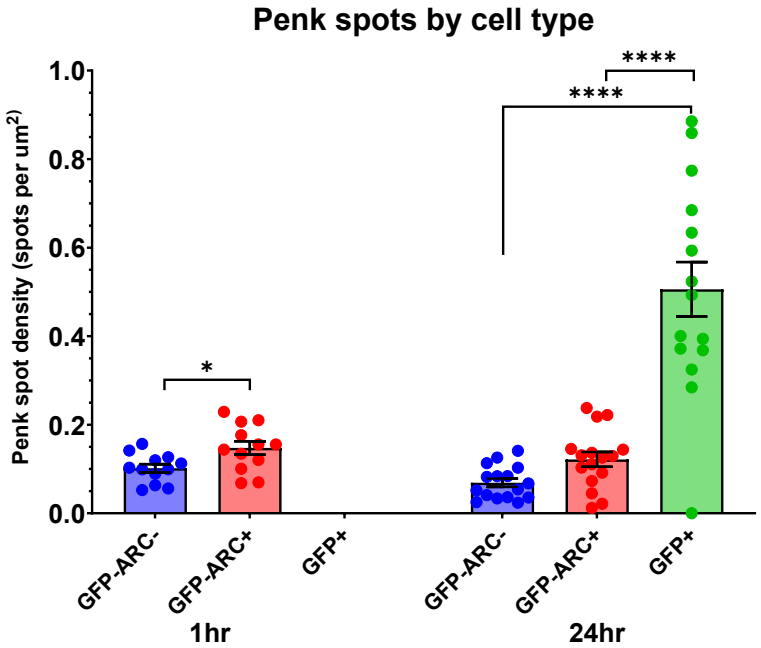
RNAscope validation: *Penk* is increased significantly in *Gfp+* cells at 24hr post-NE and only minimally at 1hr post-NE in *Arc+* cells





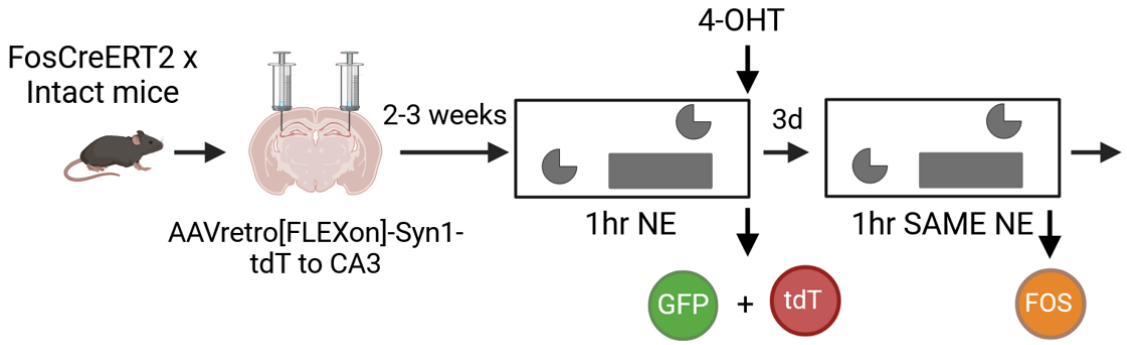


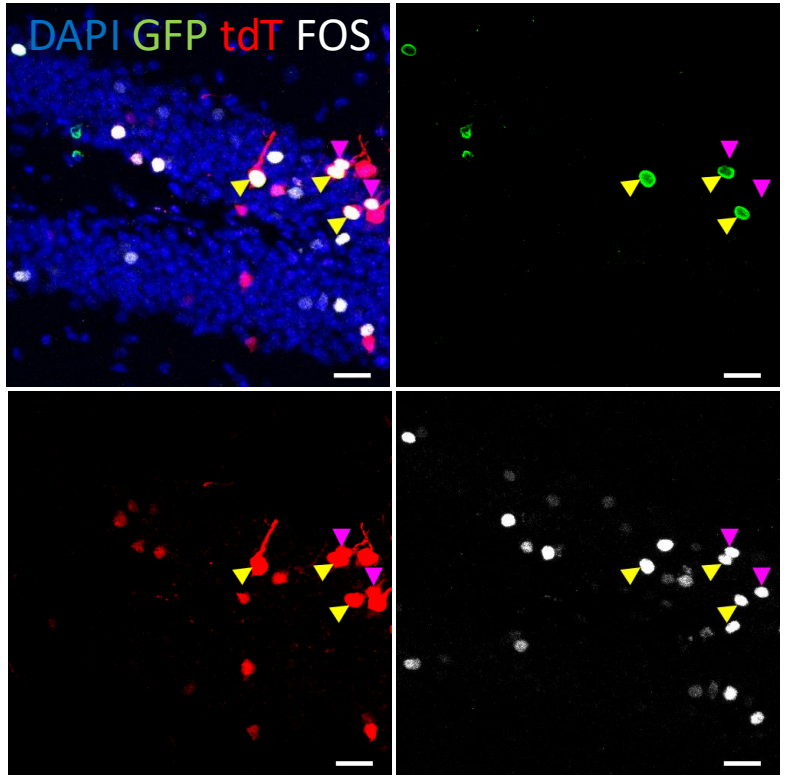


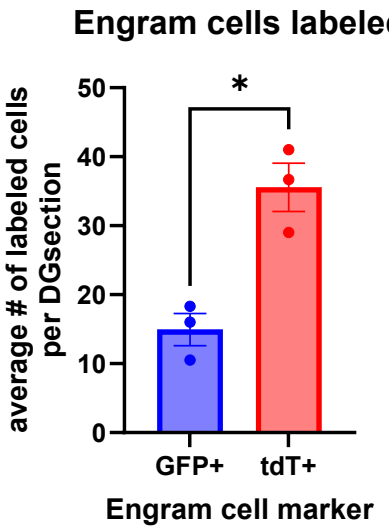


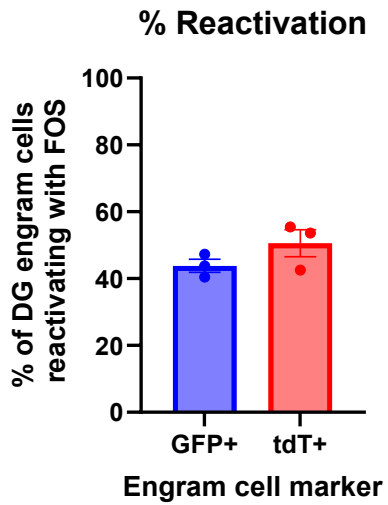
In our model system, *Penk* appears more consistent with activity-related induction than a separate cellular subtype

DG engram cells also reactivate at high rates to the same environment using a complementary Cre-dependent viral labeling strategy

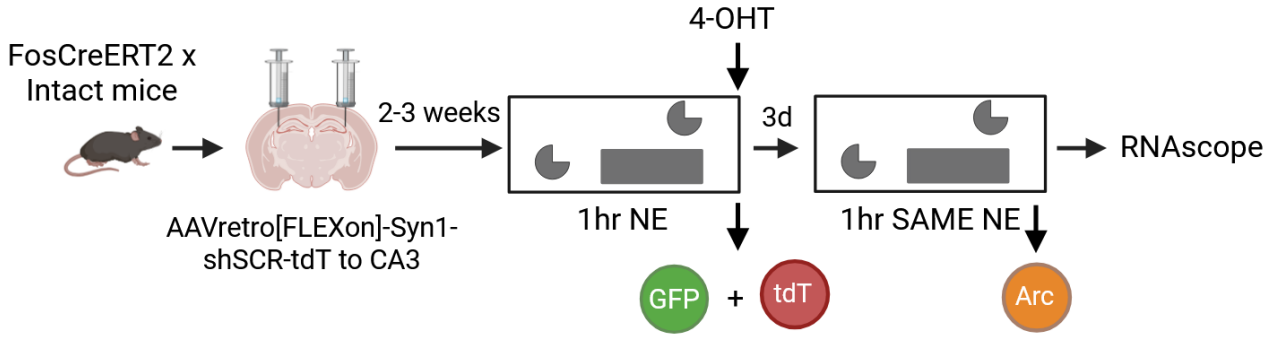


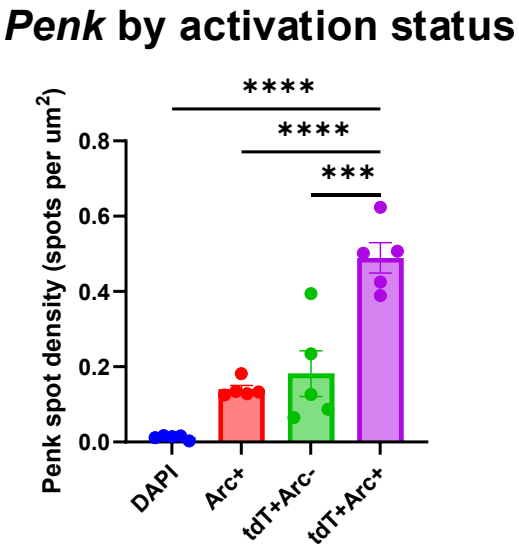


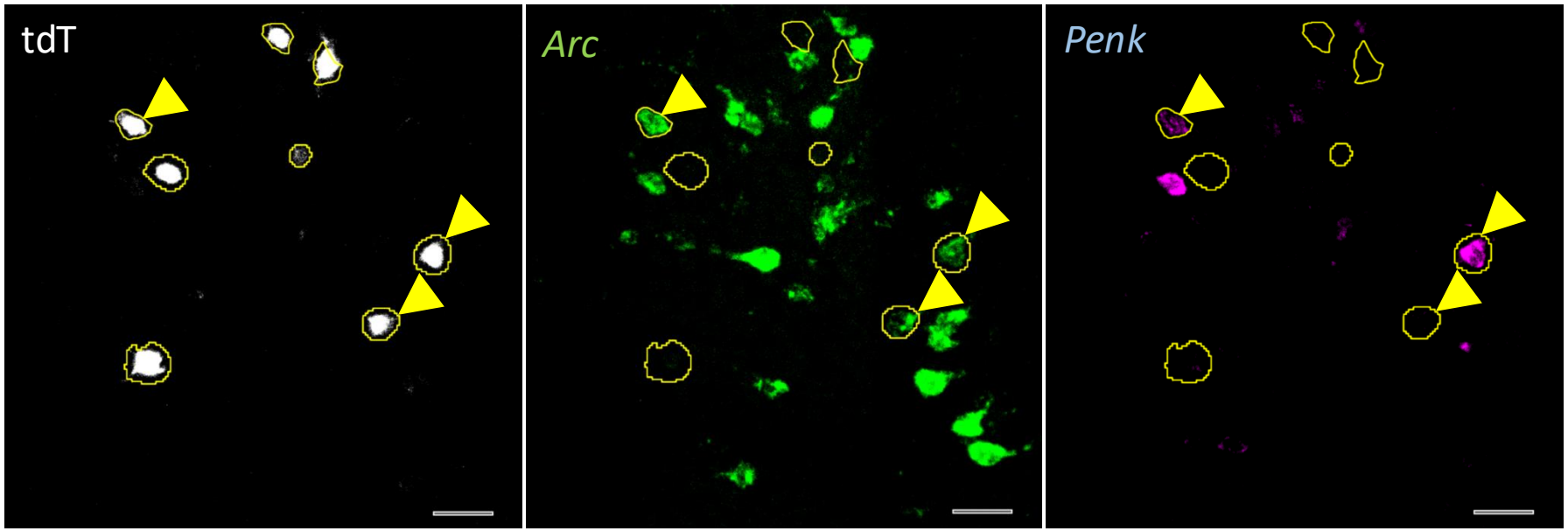




Reactivated engram cells have higher *Penk* than non-reactivated cells



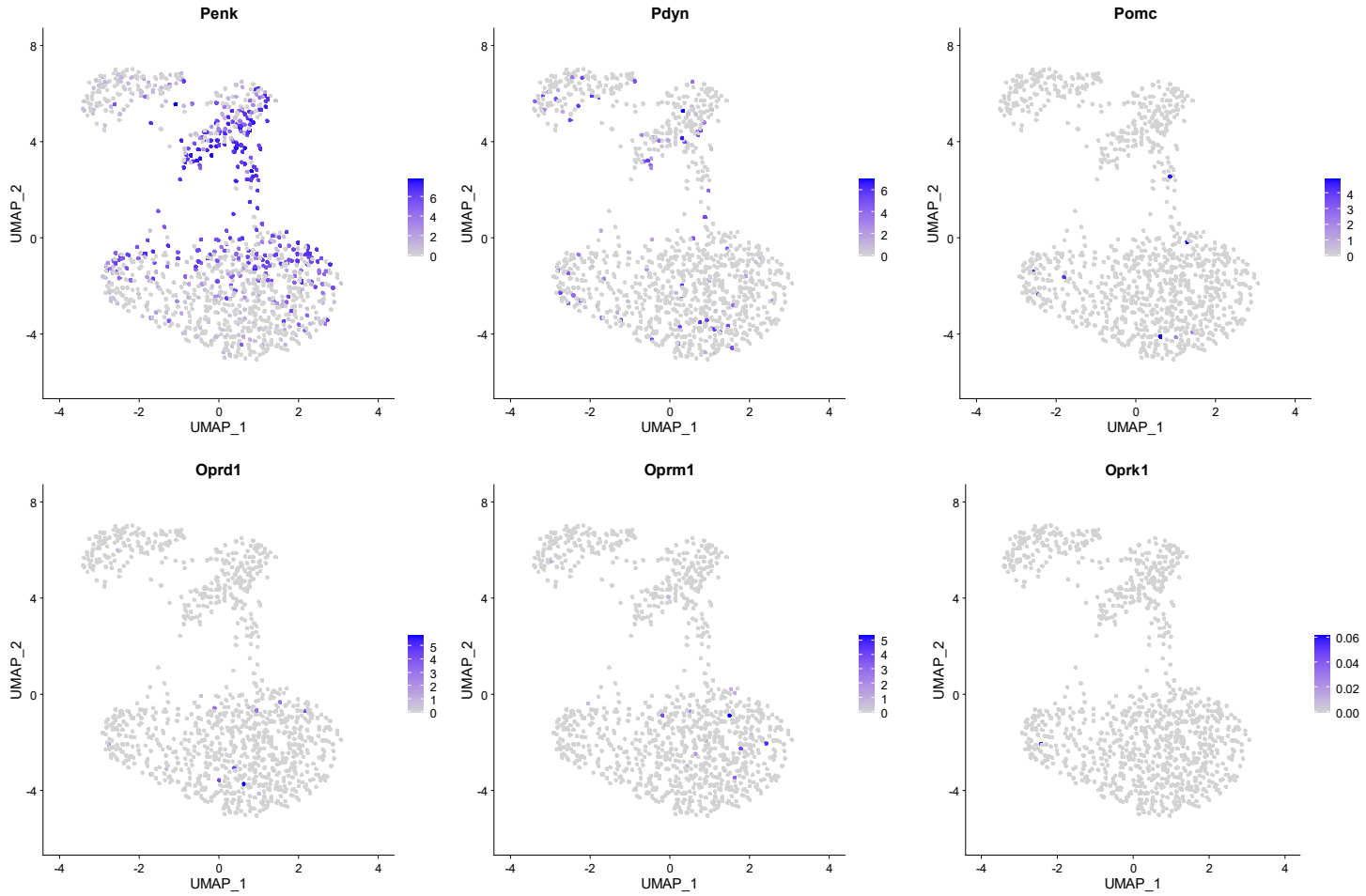




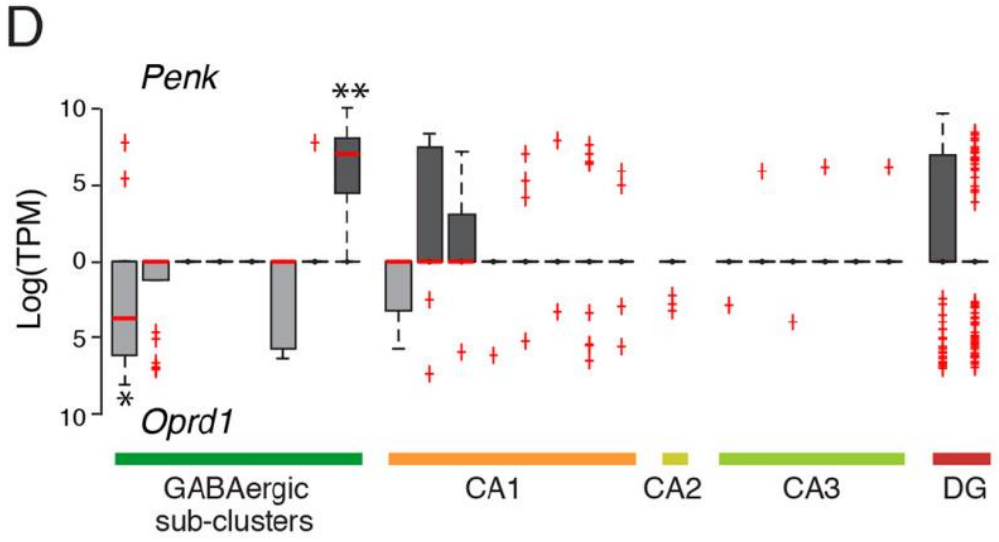
Of opioid-related genes, only *Penk* shows substantial expression in DGCs in snRNA-Seq data

Ligand precursors

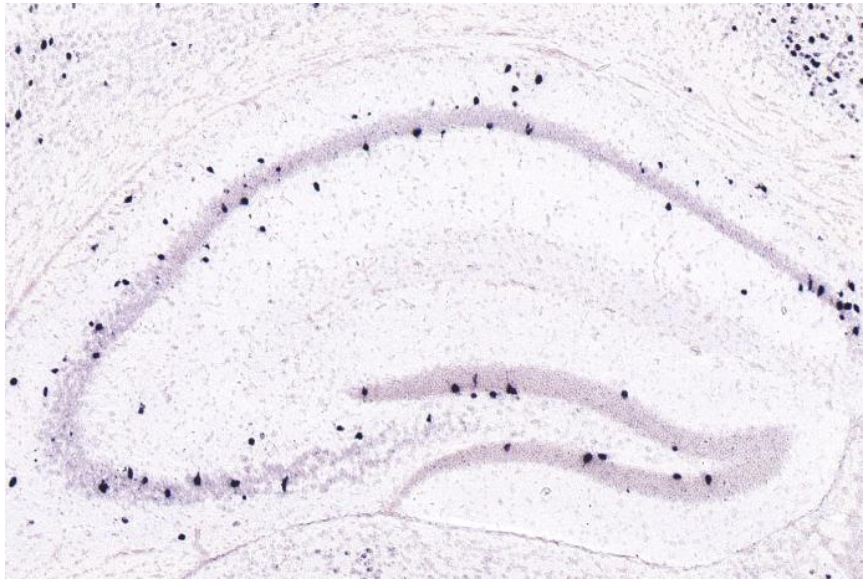
Receptors



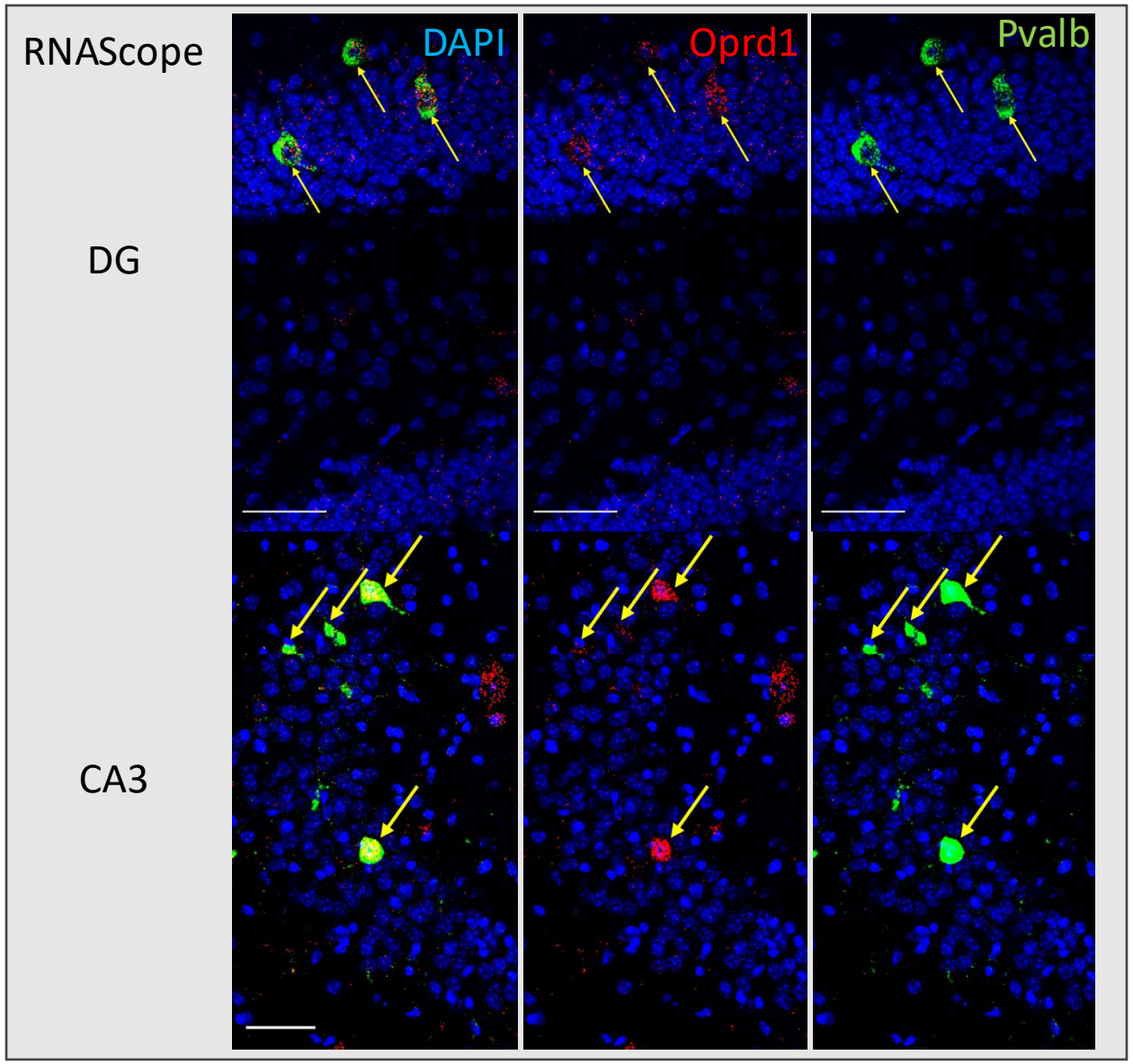
Potential target of released enkephalins: Delta opioid receptor (*Oprd1*) expression in DG and CA3 PV+ cells



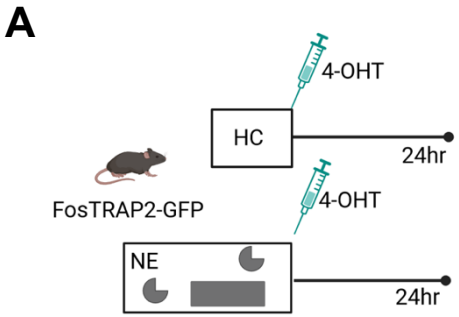
Habib et al 2016, *Science*

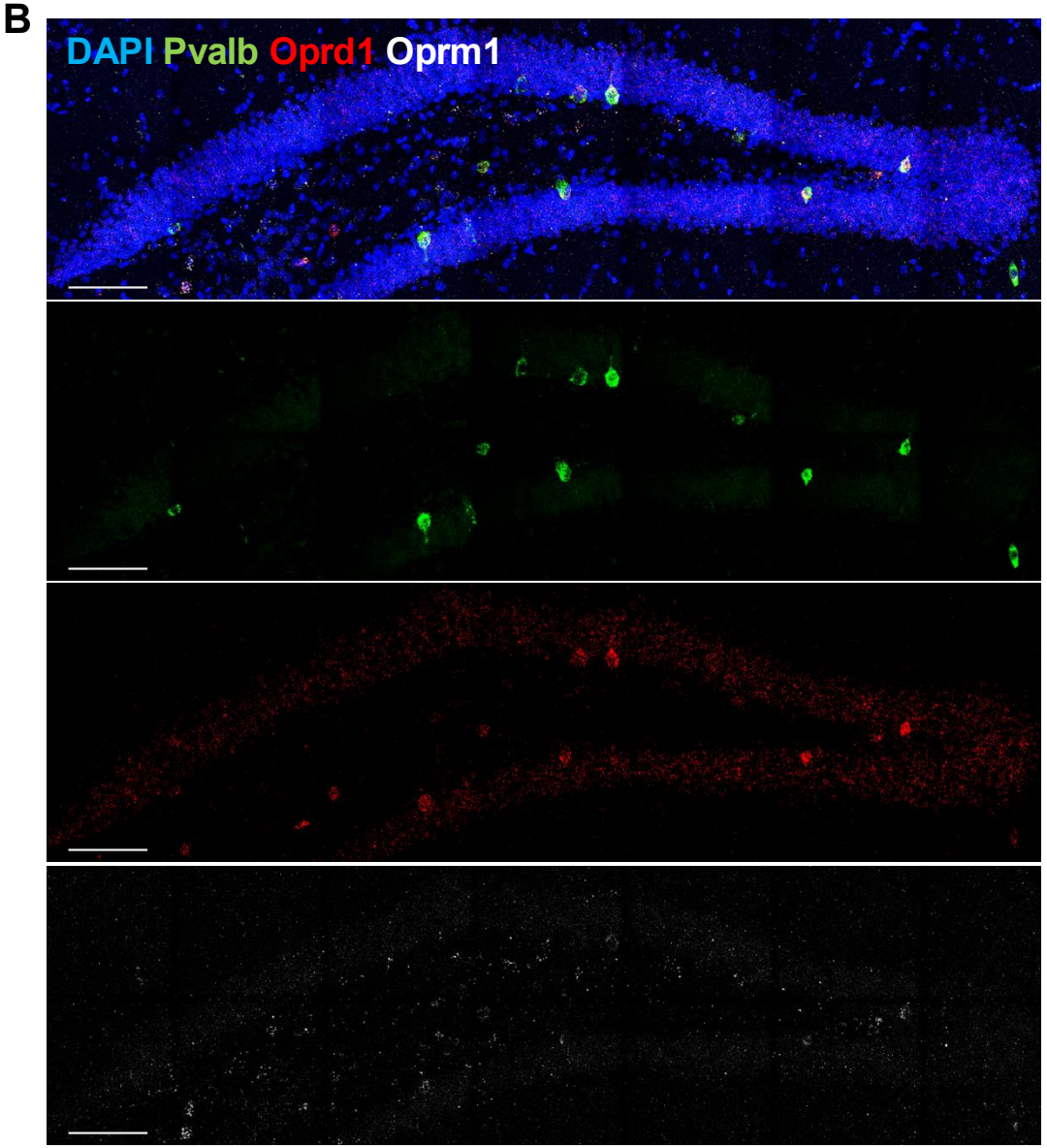


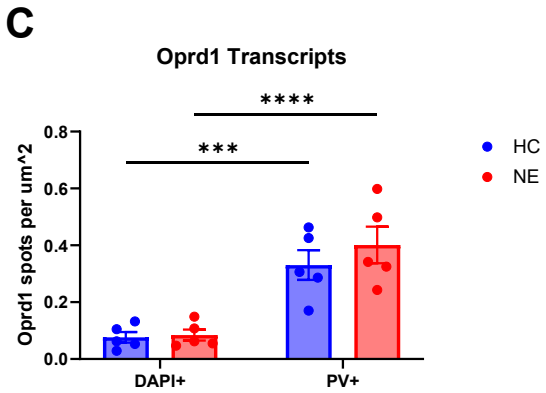
Allen Brain Atlas, *Pvalb*

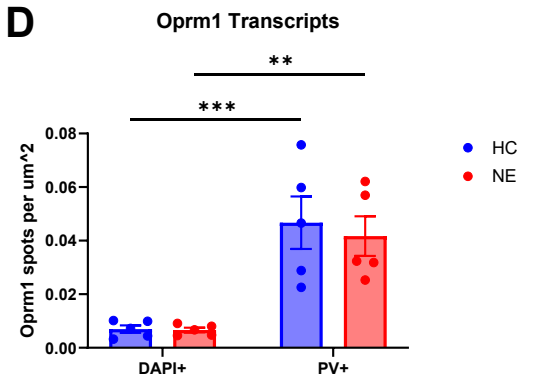


Opioid receptor expression is significantly higher in DG PV+ cells than in surrounding granule cell layer and unchanged by NE

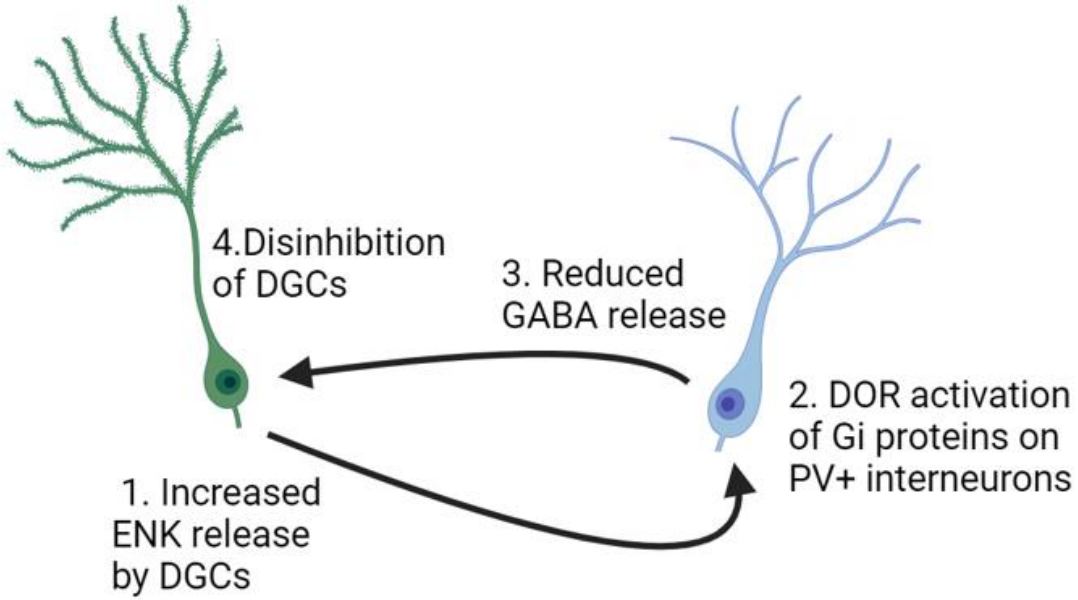








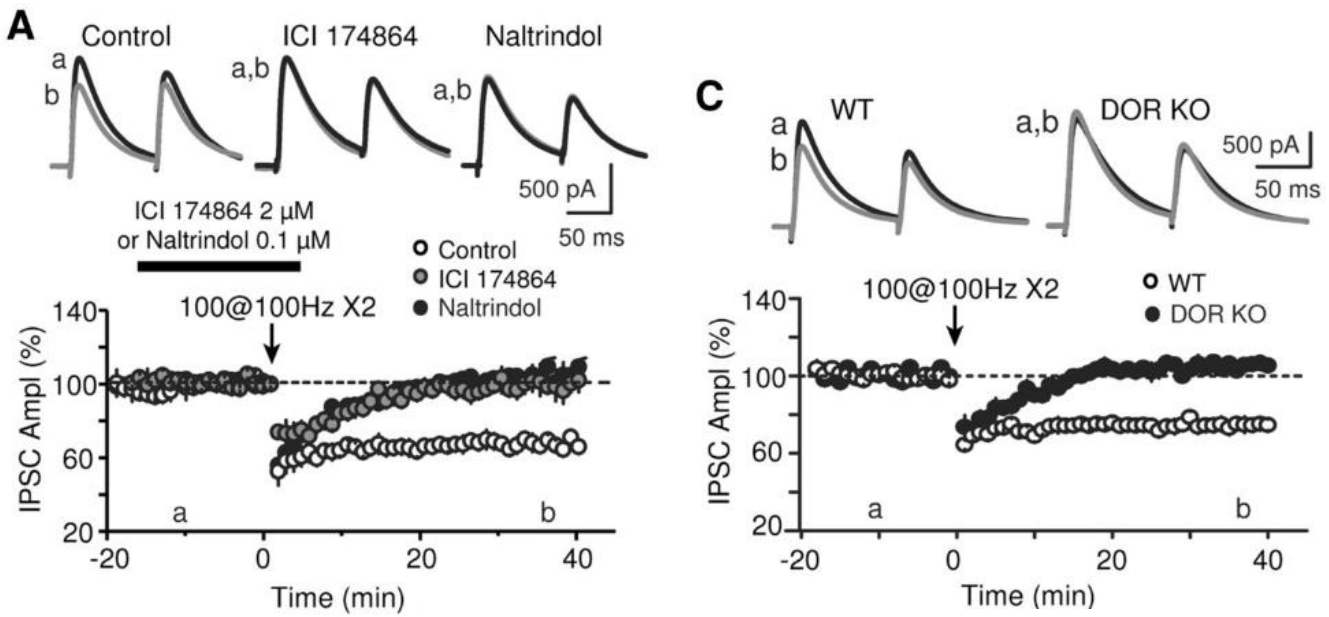
Working model for Penk action: disinhibition of DGCs via PV basket cells



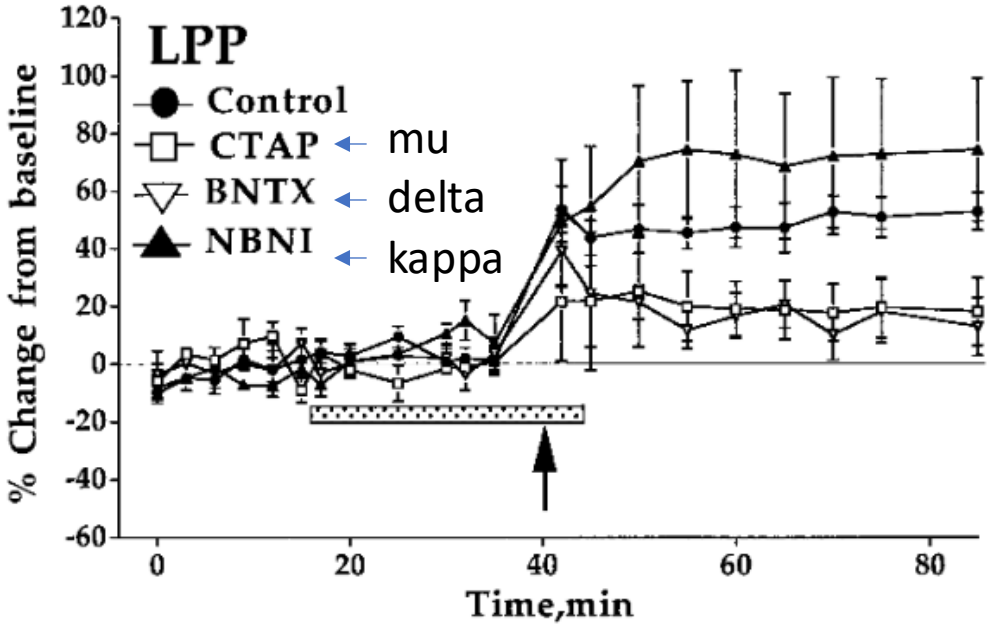
Opioid-mediated plasticity in the hippocampus

CA2: Delta opioid receptor activation is critical for long-term <u>depression of inhibition</u> after stimulation of Schaffer collaterals

DG: Delta opioid receptors, via <u>suppression of inhibition</u>, are critical for LTP induction in the lateral perforant path

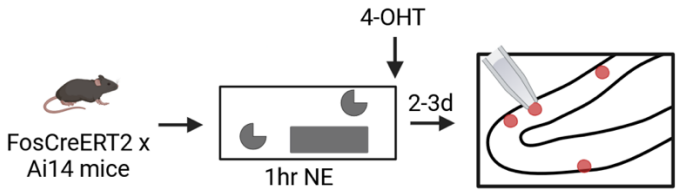


Piskorowski and Chevaleyre, 2013, *J Neurosci*

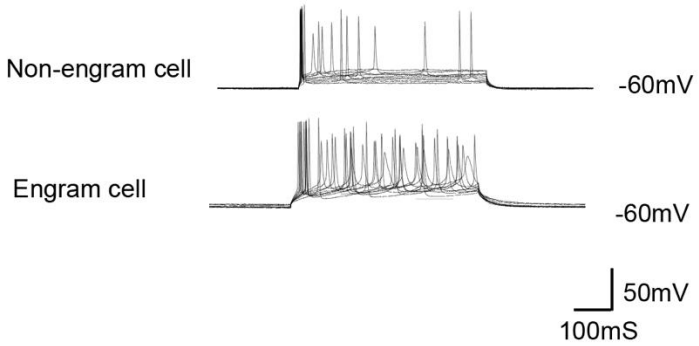


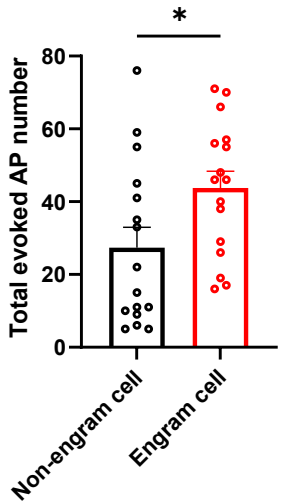
Bramham and Sarvey, 1996, *J Neurosci*

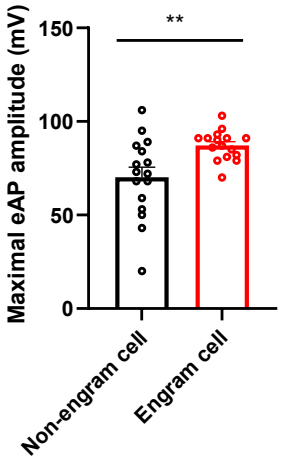
Patch clamp recordings reveal engram neurons are hyperactive vs. non-engram neurons

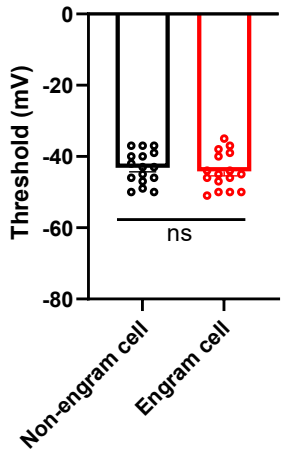


Evoked APs

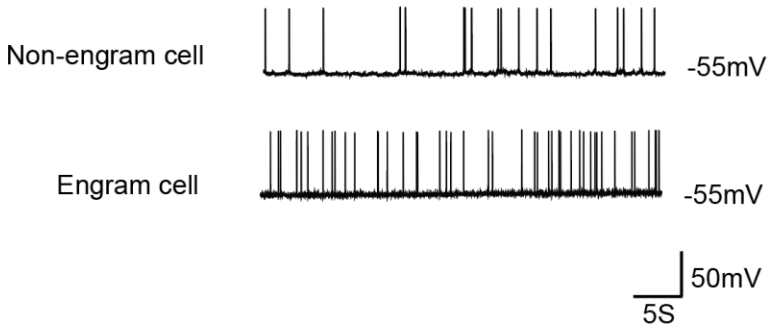


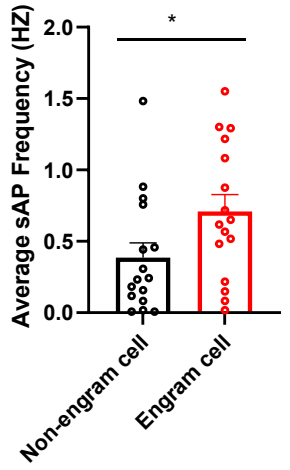


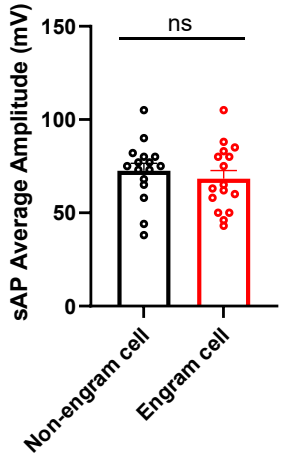




Spontaneous APs

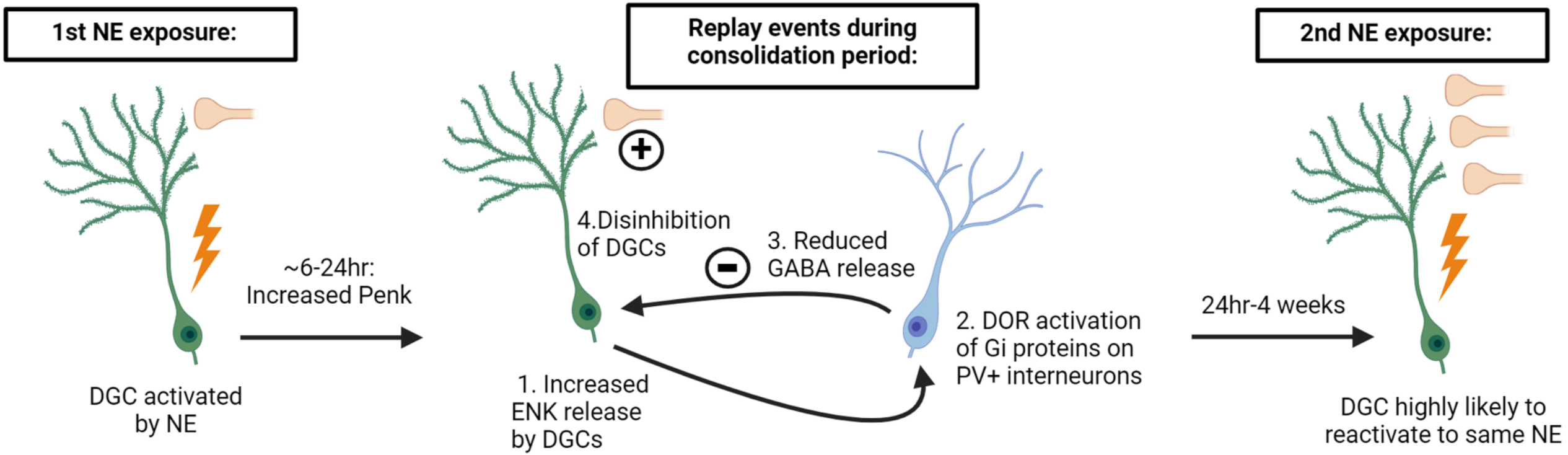






w/ Qiuwen Wang

Timeline of opioid-related disinhibition of DGCs via PV basket cells?

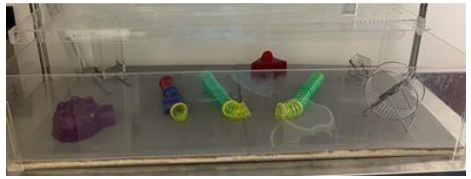


1. How selective are the Engram cells as the difference between the first exposure and second become more different?

2. How does exercise and increased adult neurogenesis influence the selectivity of the DG engram cells??

1. Varying environments to test the selectivity of DG engram cells

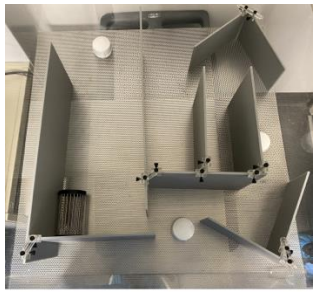
ORIGINAL/ SAME:

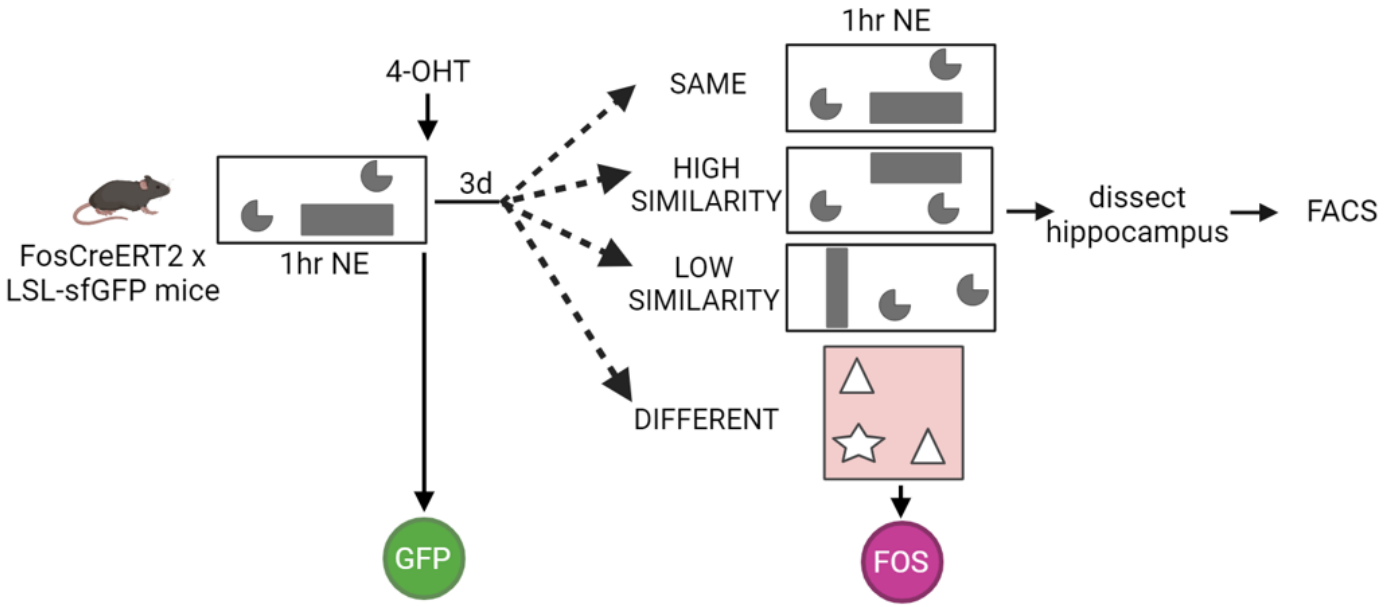


HIGH SIMILARITY:

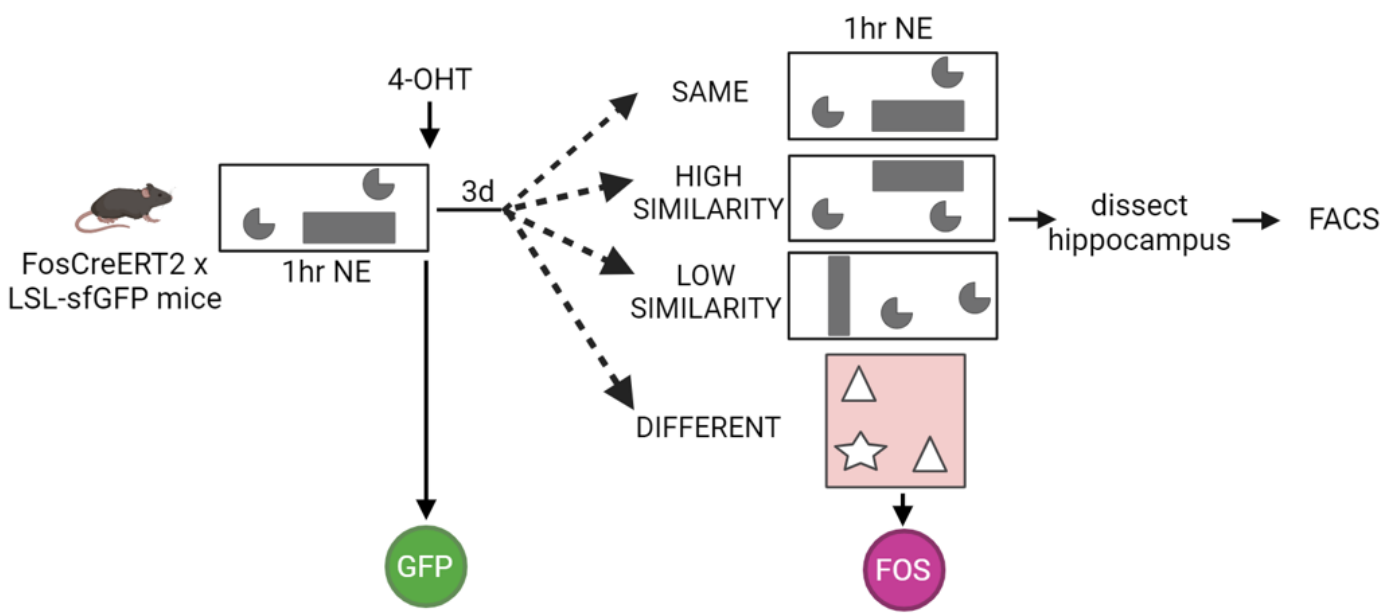
LOW SIMILARITY:

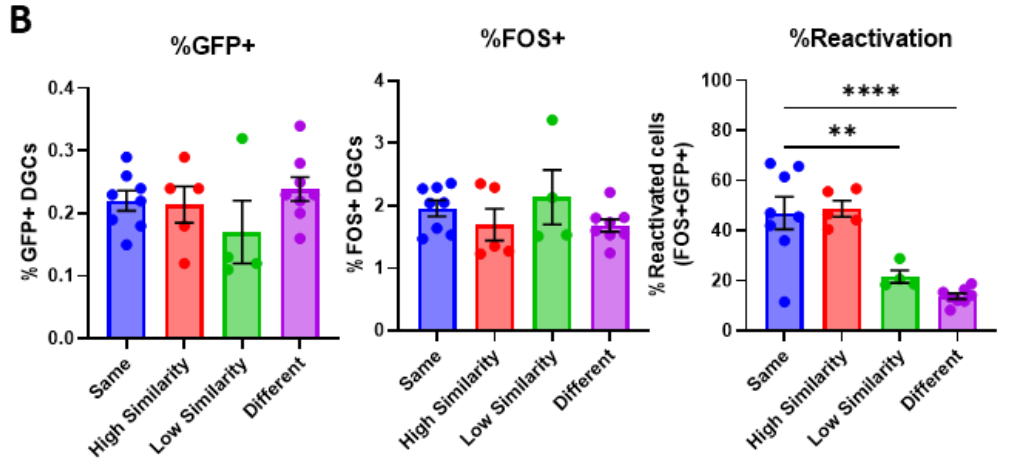
DIFFERENT:





1. Highly-reactivating DG engram cells are still context-specific: reactivation in a second NE depends on level of similarity to the first

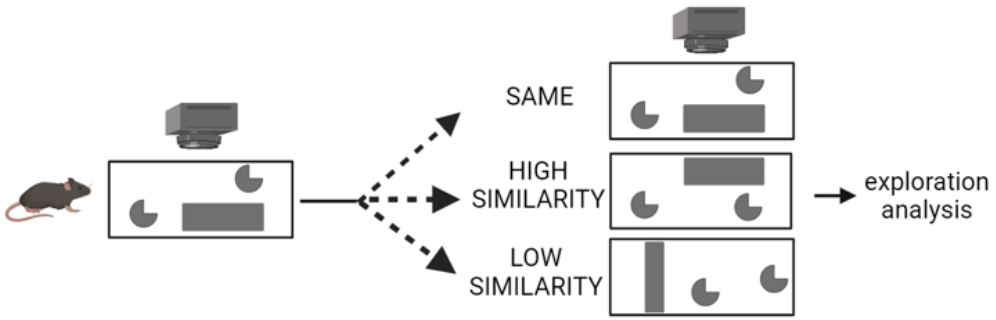


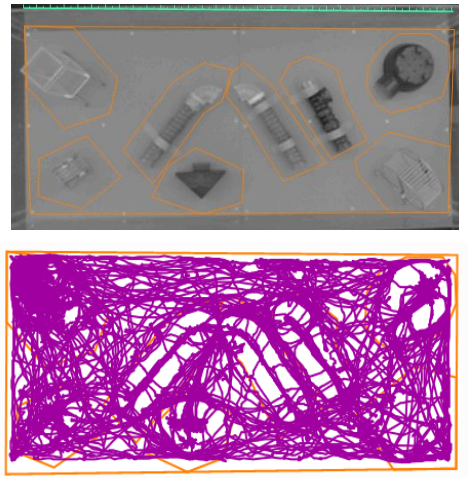


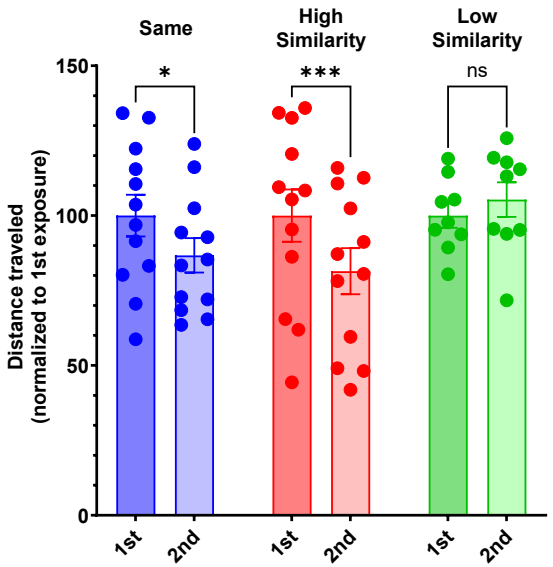
Number of active DGCs in each NE doesn't differ

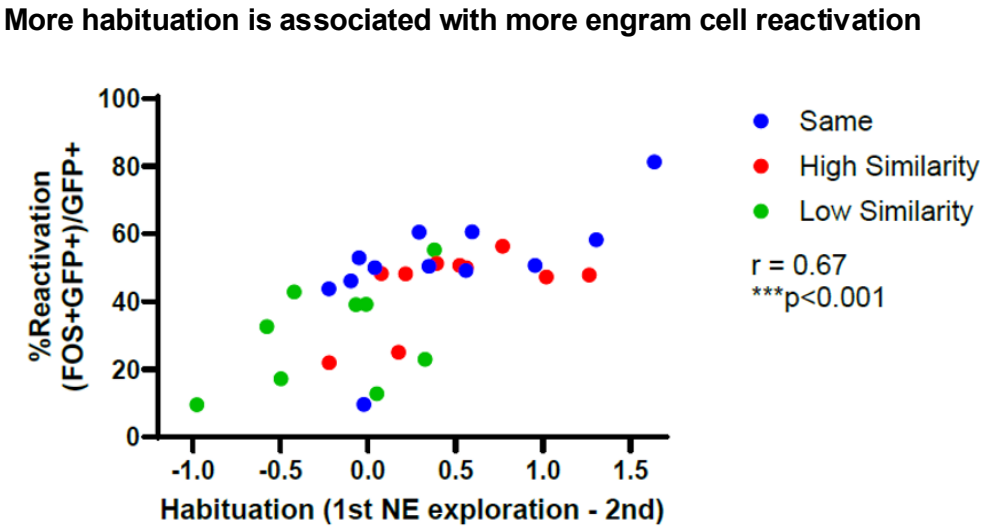
Greater change in NE compared to original NE yields reduced reactivation

1.Mice habituate when re-exposed to the same or a highly similar NE but continue to explore more when the NE is sufficiently novel

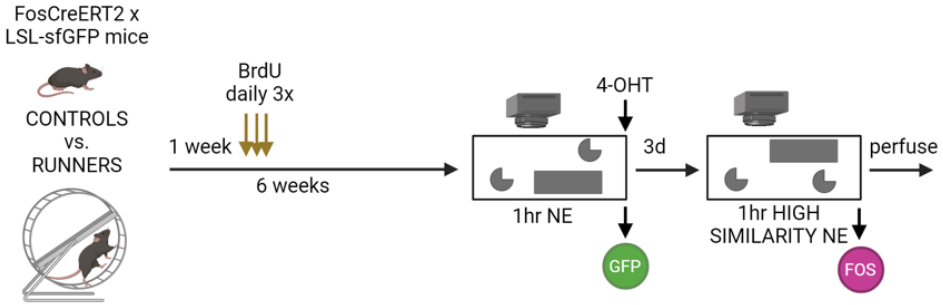


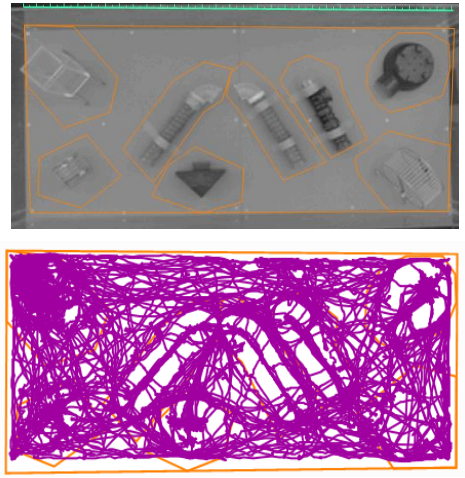


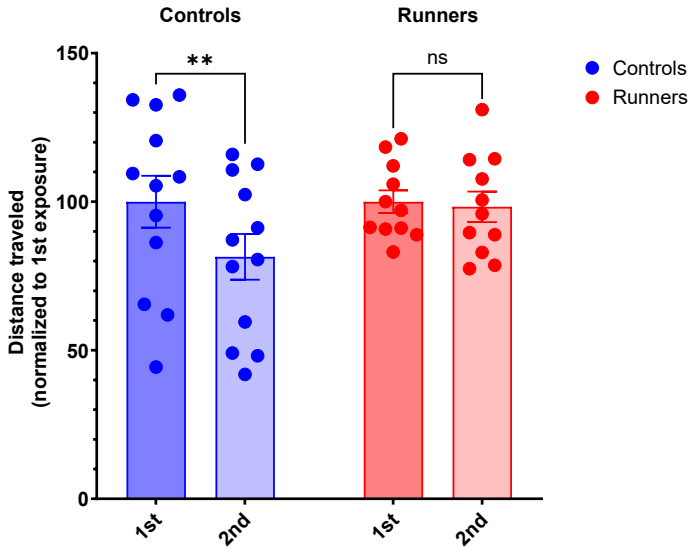




2. Running alters exploratory behavior in a high similarity NE

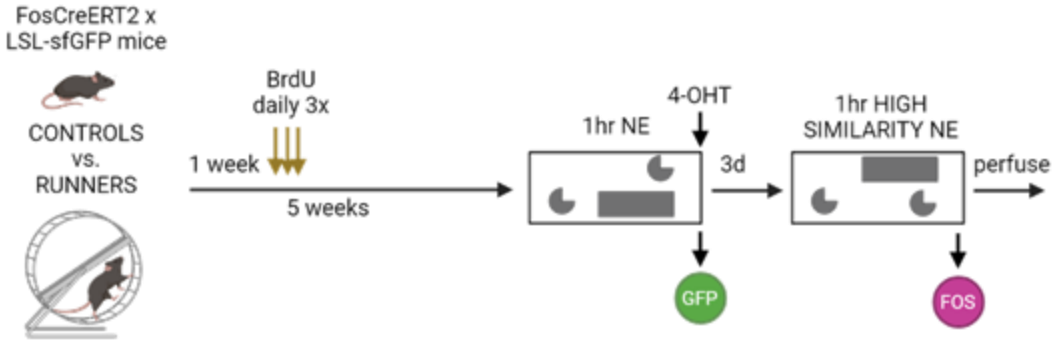


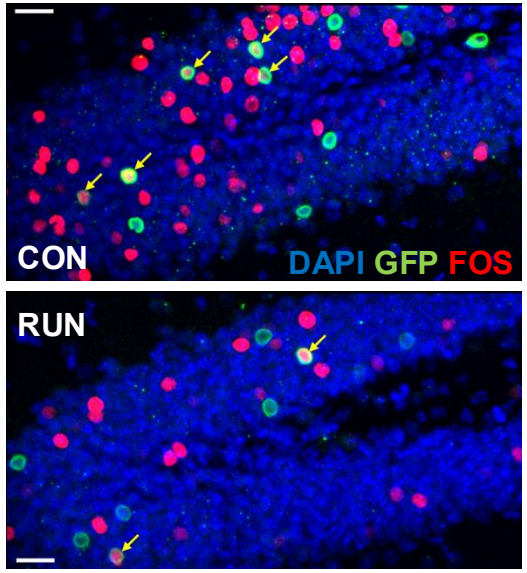




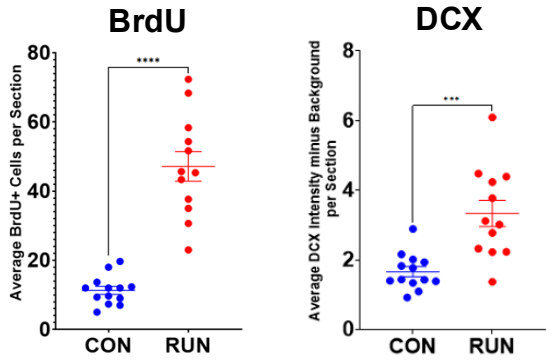
Controls habituate to a high similarity environment, runners don't. Runner's see the similar NE as Different!

Running enhances DG neurogenesis and causes more selective DGC reactivation to a similar environment

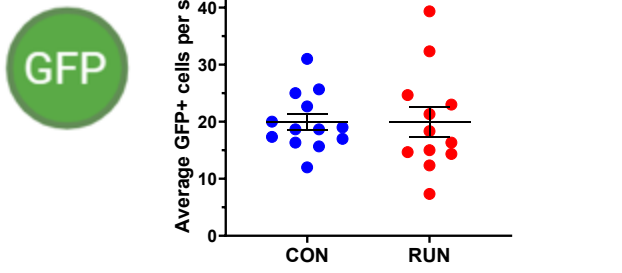




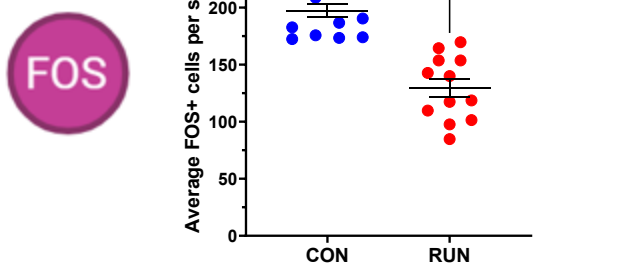
Enhanced neurogenesis:



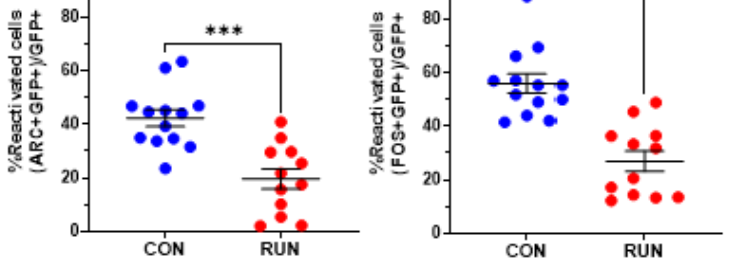
Equivalent activation to 1st NE:



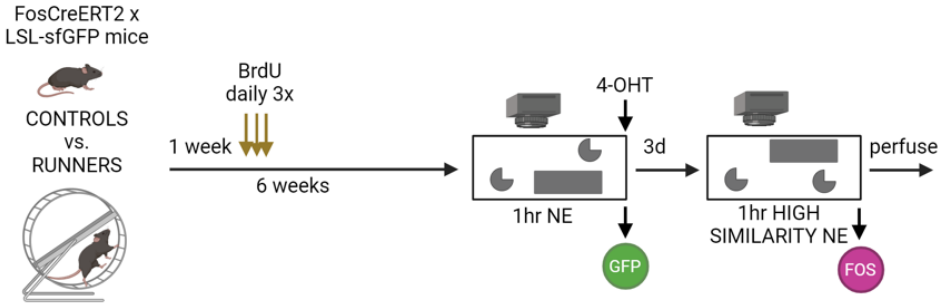
Runners activate fewer DGCs in 2nd NE:

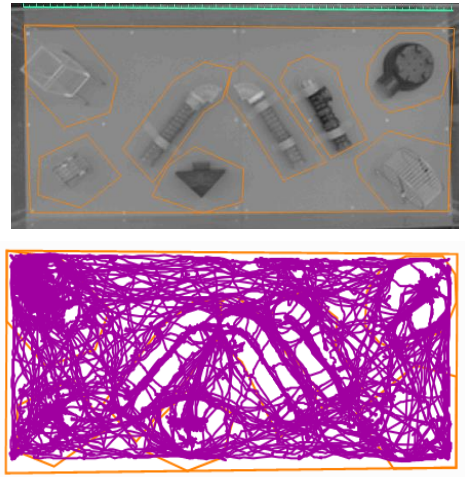


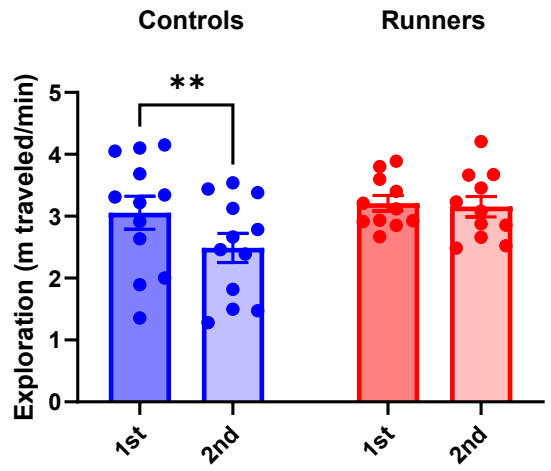
Increased engram cell selectivity:



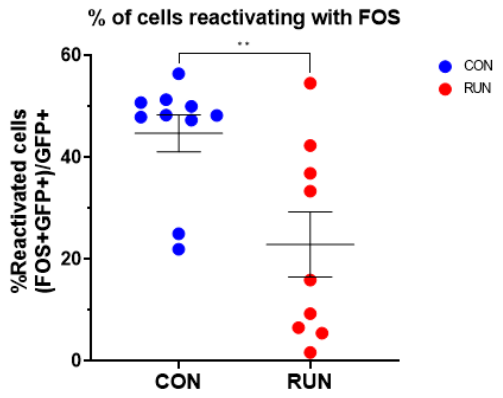
Running-induced change in exploration of a high similarity NE is correlated with DGC reactivation

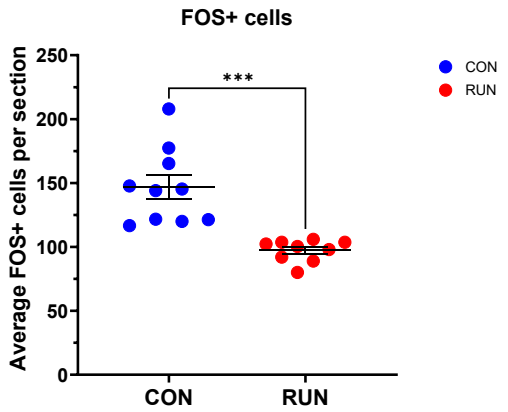




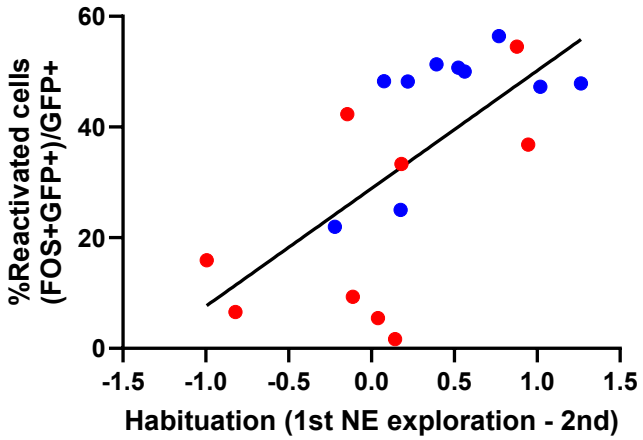


Runners reactivate fewer DGCs and have less total FOS activation to the 2nd exposure:





More habituation is associated with more engram cell reactivation

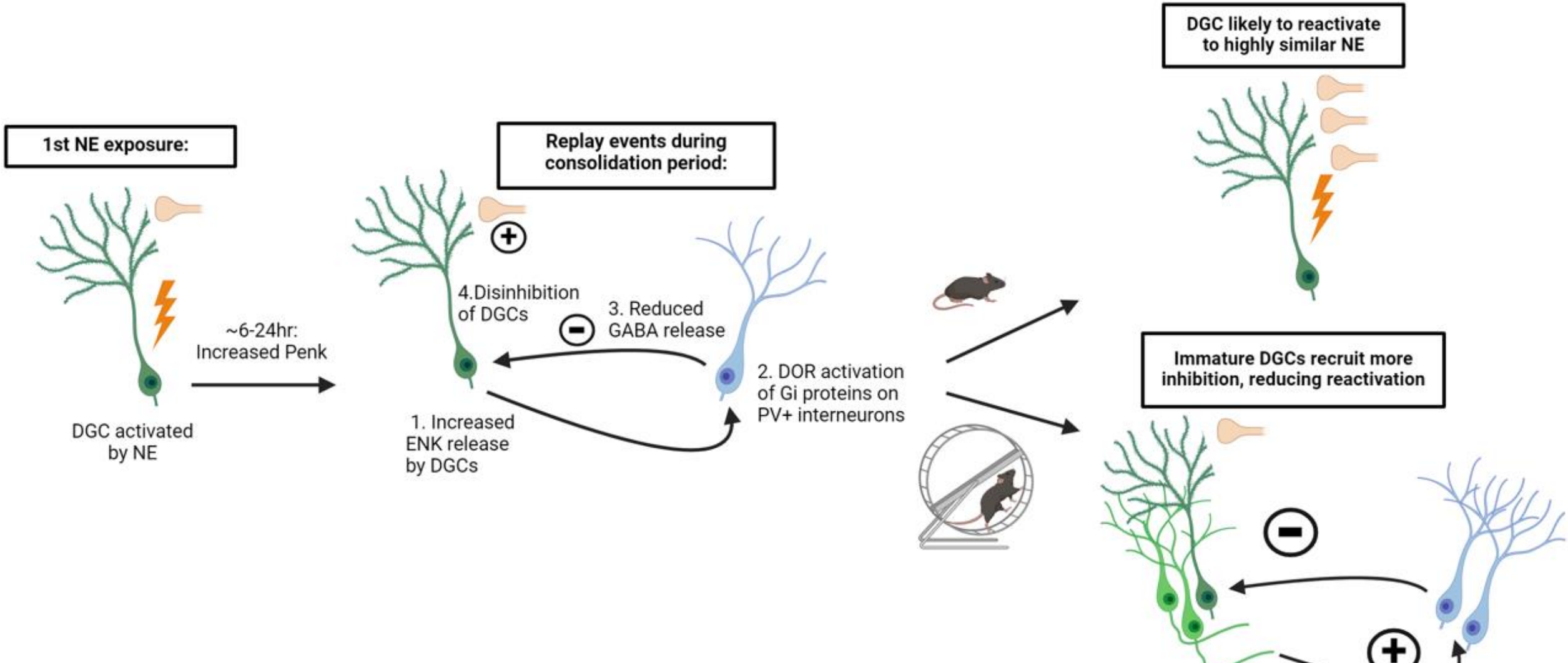


r^2 = 0.44
**p = 0.0018

Interim summary

- DGCs reactivate selectively, responding with greater reactivation to more similar environments
- Running alters exploration of similar environments: runners can distinguish environments that controls interpret as the same
- Running also alters the IEG response in DGCs:
  - Fewer DGCs reactivate to a similar environment in running mice
  - Paradoxically the total IEG activity is reduced in runners when exploring a similar environment

Model for DG engram cell circuitry



General **Summary** and Speculations

Novelty Seeking Behavior, driven by Curiosity, leads to c-fos expression, which enhances Penk expression, in selected DG neurons

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- Episodic, Pattern Separation, Memory, occurs in the hippocampus
- Dentate Granular neurons are engram cells that encode memory
- PENK is activated in Engram cells and leads to enhanced reactivity to similar experiences (memory)
- Exercise and increased adult neurogenesis lead to better memory with less effort/energy expenditure! (On Going)

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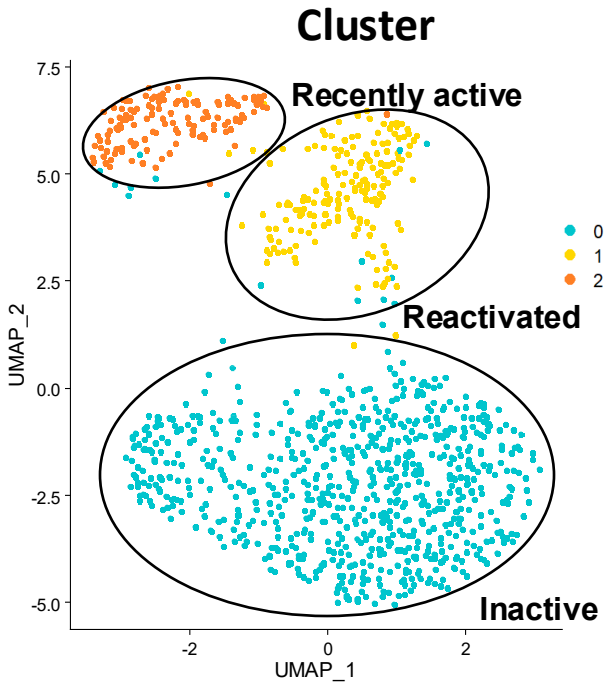
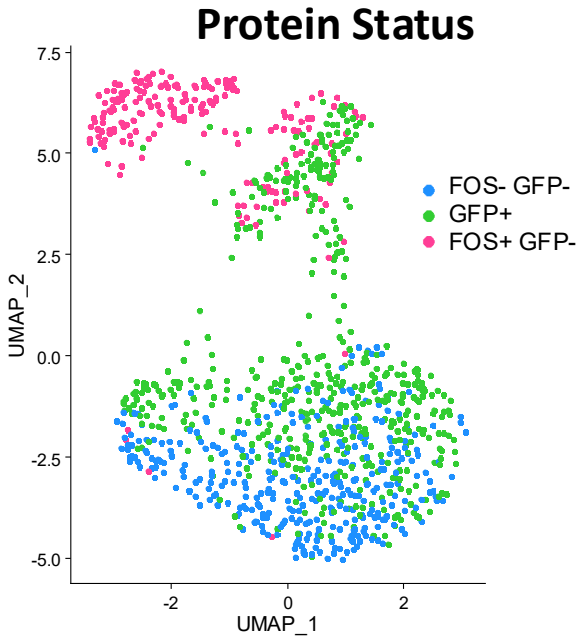
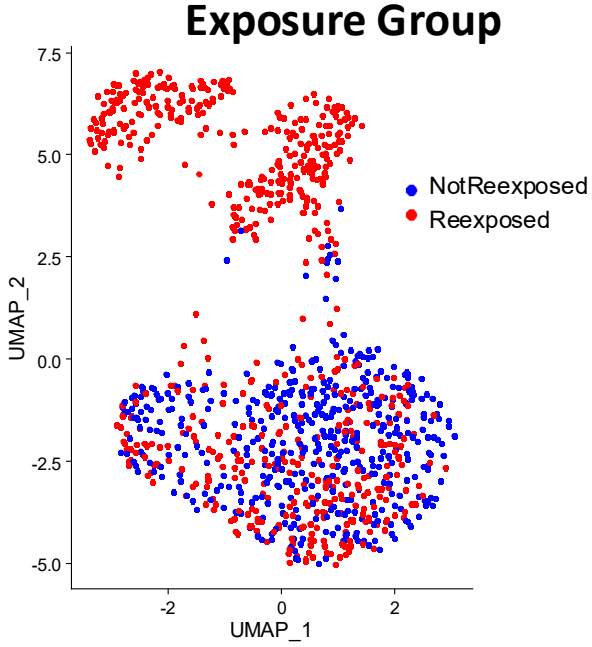
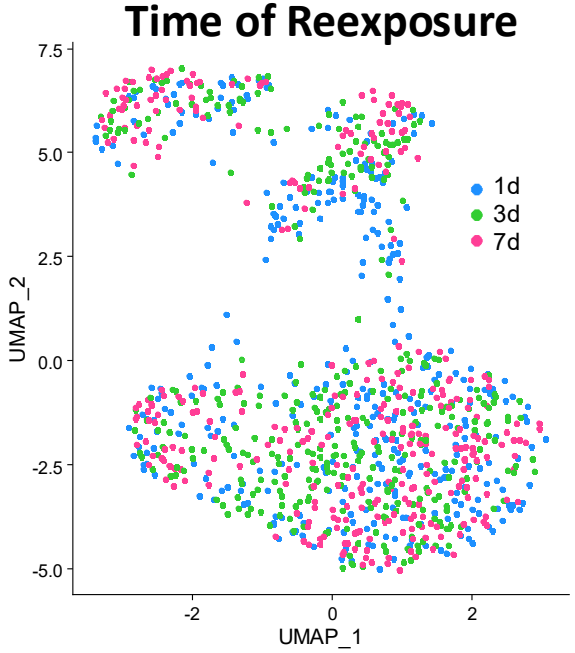
Unbiased clustering identifies reactivated DG engram cells



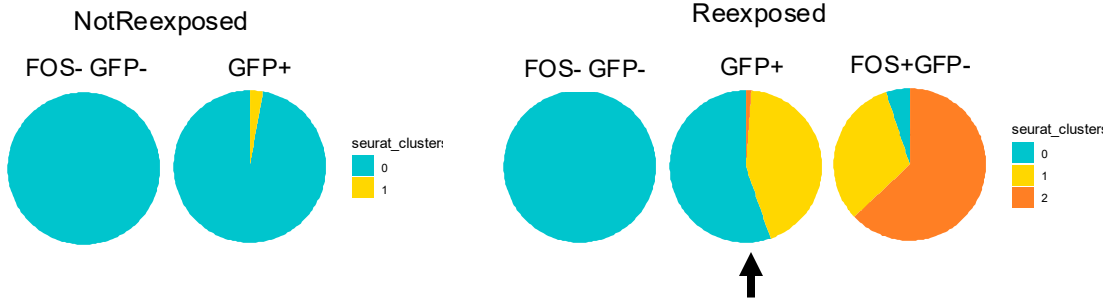




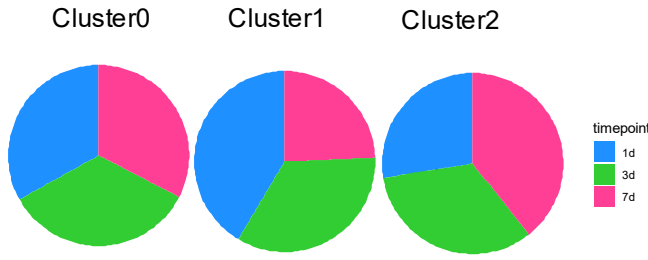


Cluster composition by protein status

Cluster assignment by protein status and reexposure



Cluster composition by collection day



Total nuclei passing QC = 1061

Similar to our histology results, about 40% of the GFP+ cells in the reexposed group reactivate.