How the Brain Remembers Alcohol:

A study on how alcohol-associated memories become embedded in the brain

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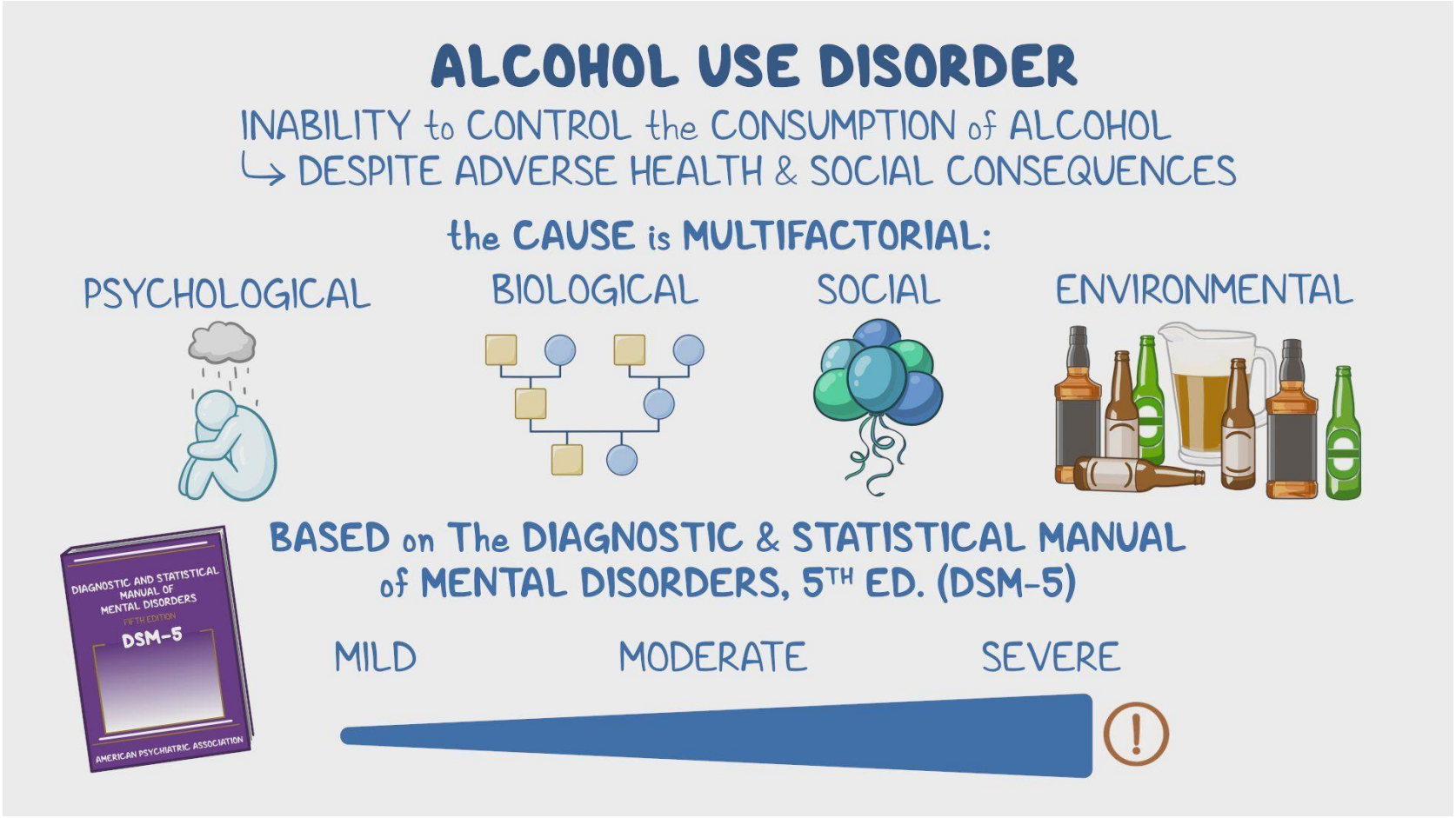
Advised by Dr. Alfredo Zuniga


Your brain forgets names, birthdays... but that one drink? Unforgettable.
...So, what's keeping the memory alive?

Why alcohol? An intro to AUD

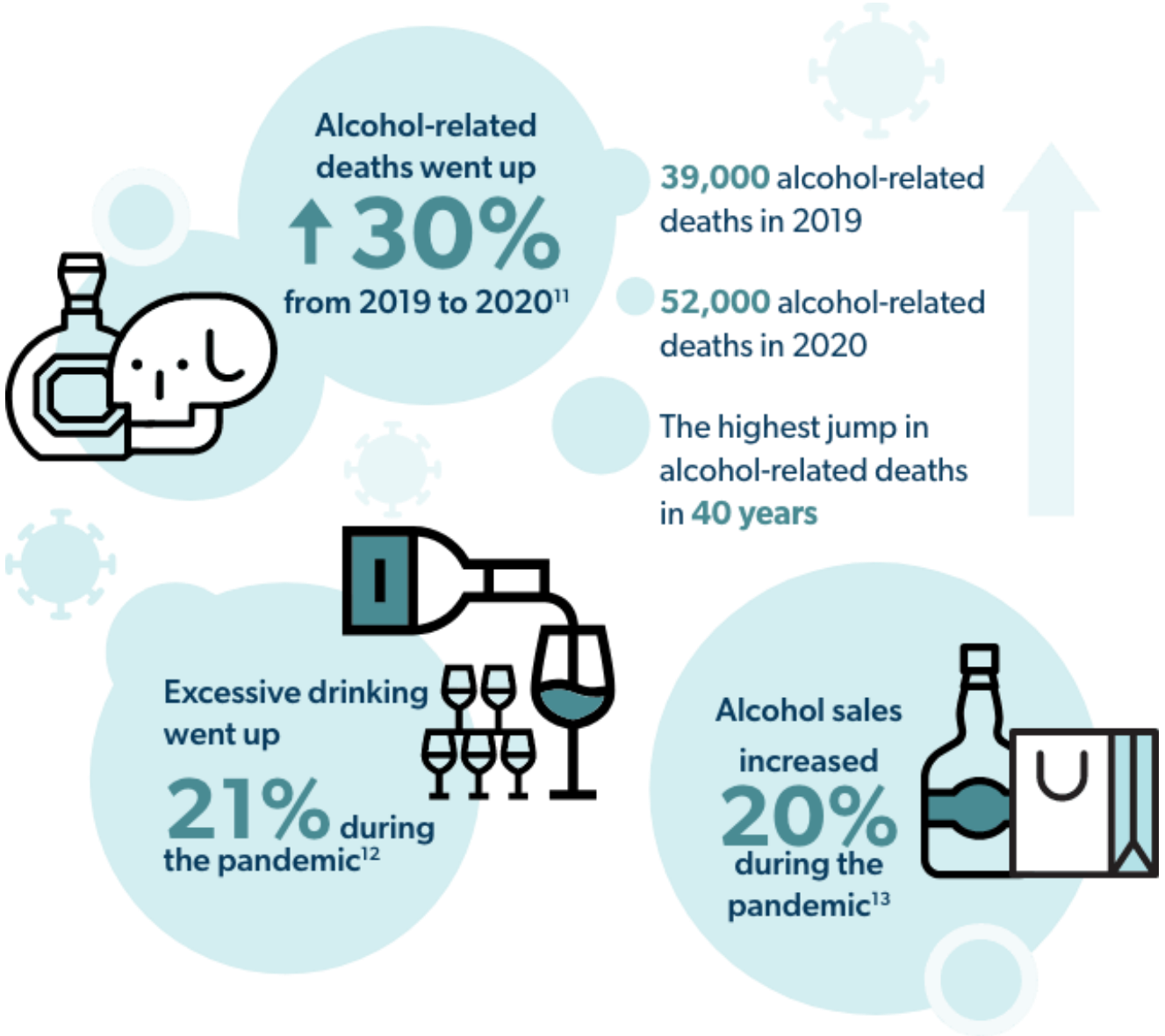


- Alcohol use disorder (AUD) is a medical condition.
- Impaired ability to stop or control alcohol use despite negative consequences.
- Lasting brain changes increases vulnerability to relapse.
- Multiple causes

https://www.osmosis.org/learn/Alcohol_use_disorder:_Clinical_sciences

Some stats on AUD

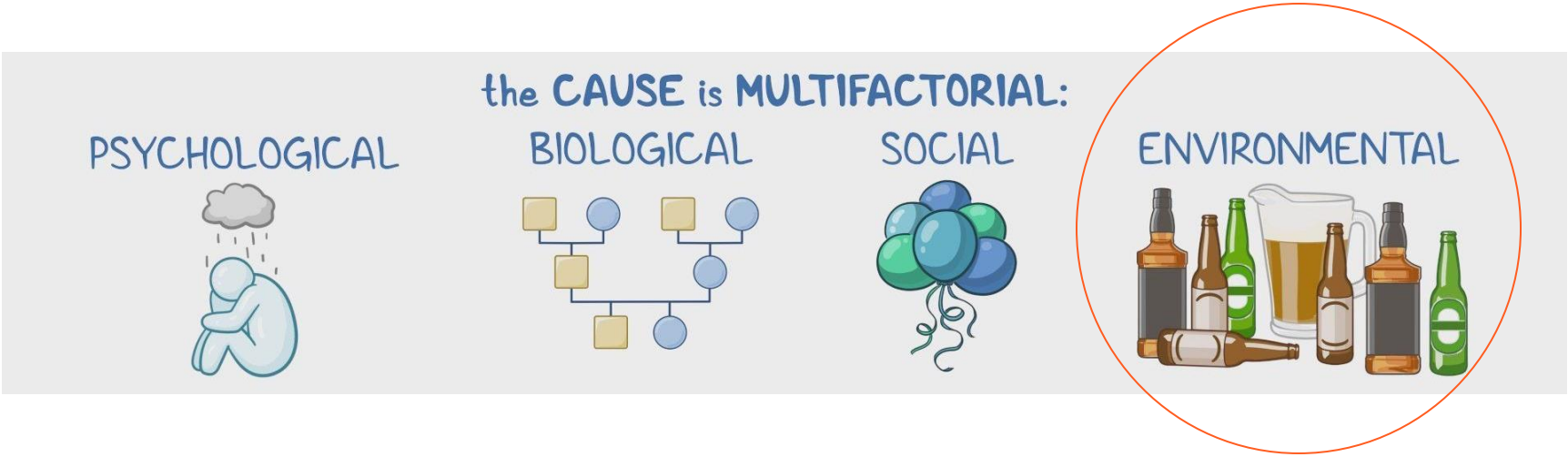
- **28.9 million people ages 12 and older** had AUD in only 2023.
- Worse it has ever been during the pandemic.
- 62.5% of the population reports alcohol use.
- **178,307 deaths** from alcohol misuse
- It continues to rise **every year.**



https://healthesystems.com/rxi-articles/alcohols-impact-across-workers-and-medical-management/

Why is it so hard to stop?

- The brain links alcohol to pleasure and stress relief.
- Changes in brain chemistry make cravings intense and persistent.
- Over time, alcohol **becomes necessary** just to feel "normal," making quitting even harder.



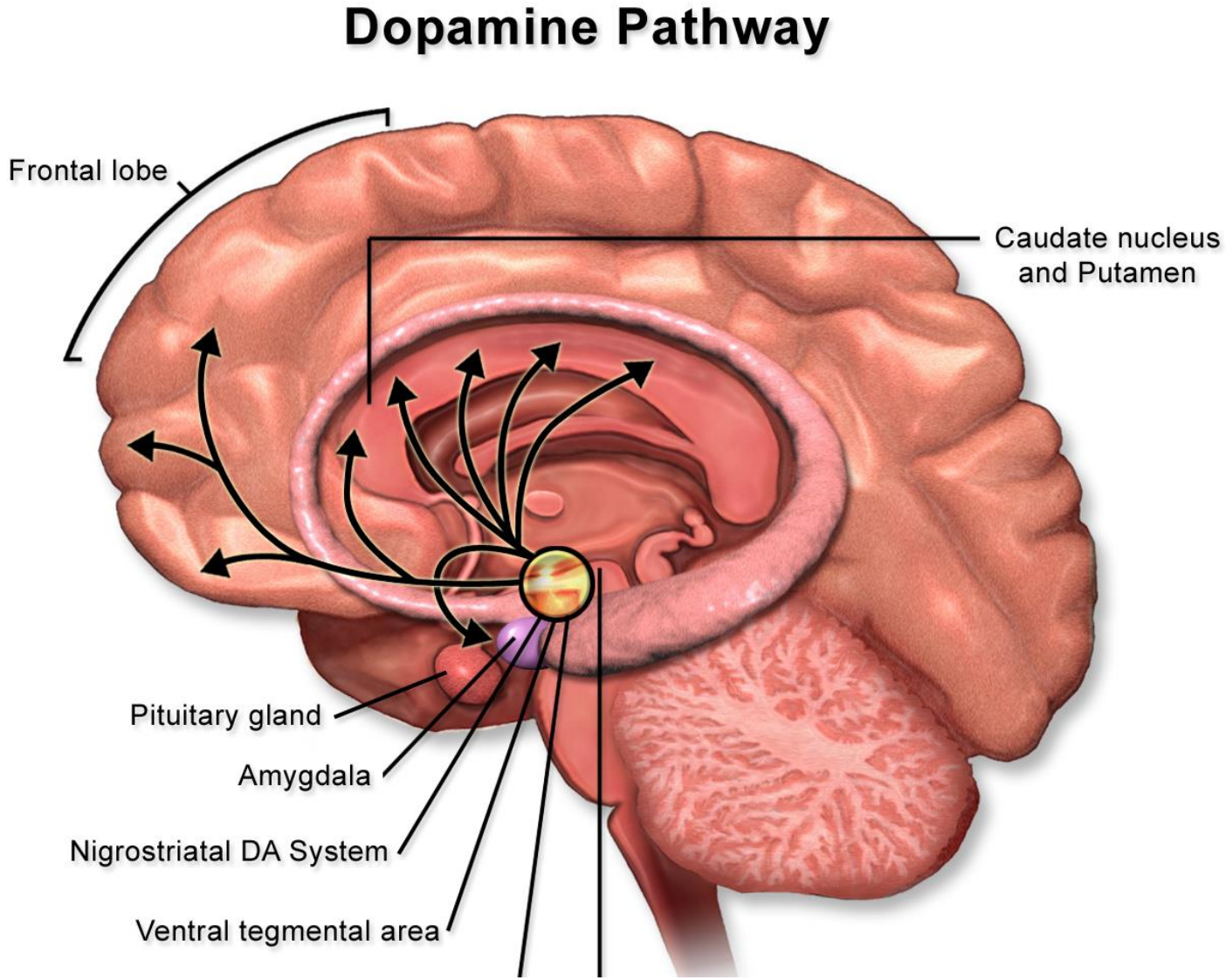
....mediated by associative learning!

Associative Learning

- Also known as Pavlovian Learning
- In terms of addiction, it mostly relates to context and the environment.

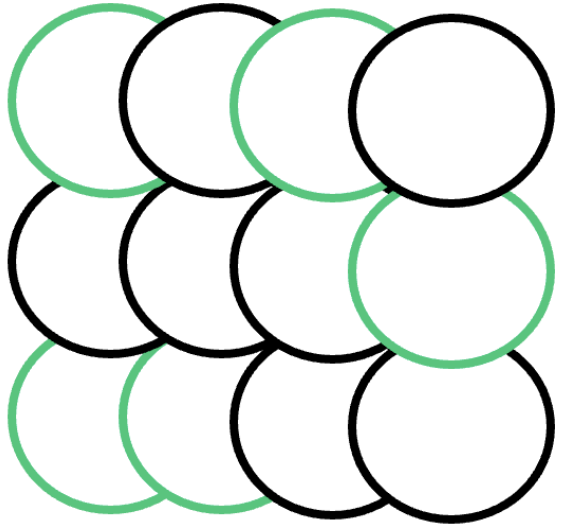
The “want” to drink is partly mediated by dopamine & reward system

- Dopamine, a neurotransmitter, is crucial for reward-seeking behavior.
- Shapes our actions and desires.
- Highly palatable foods, addictive substances, etc.



How and where do we “store” learning?

- When we learn, neurons in certain regions of the brain activate, aka fire action potentials



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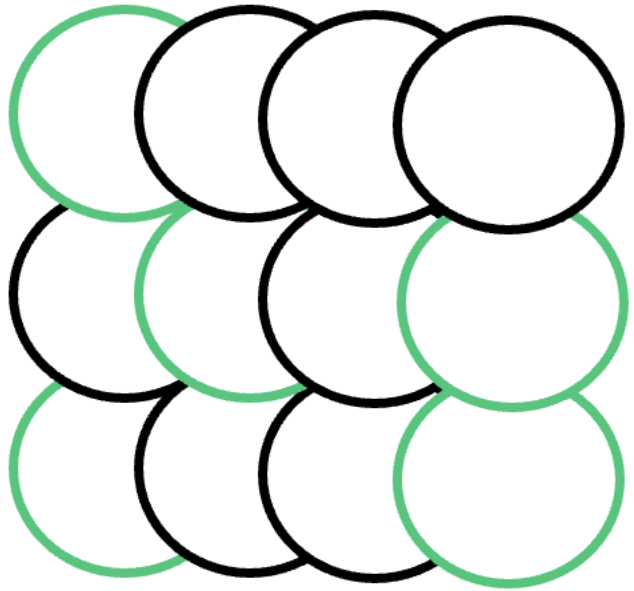
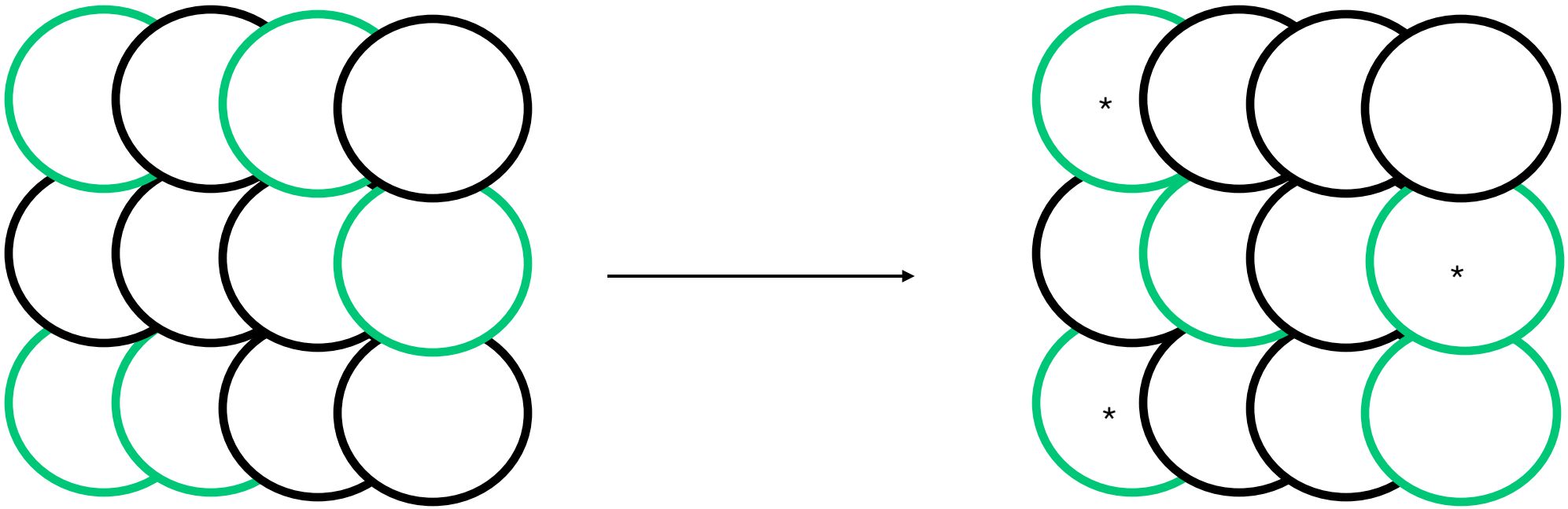
How and where do we “store” learning?

- When these environments/cues are encountered again, an urge to drink is prevalent. However, different ensembles are active, some being the same neurons previously activated!

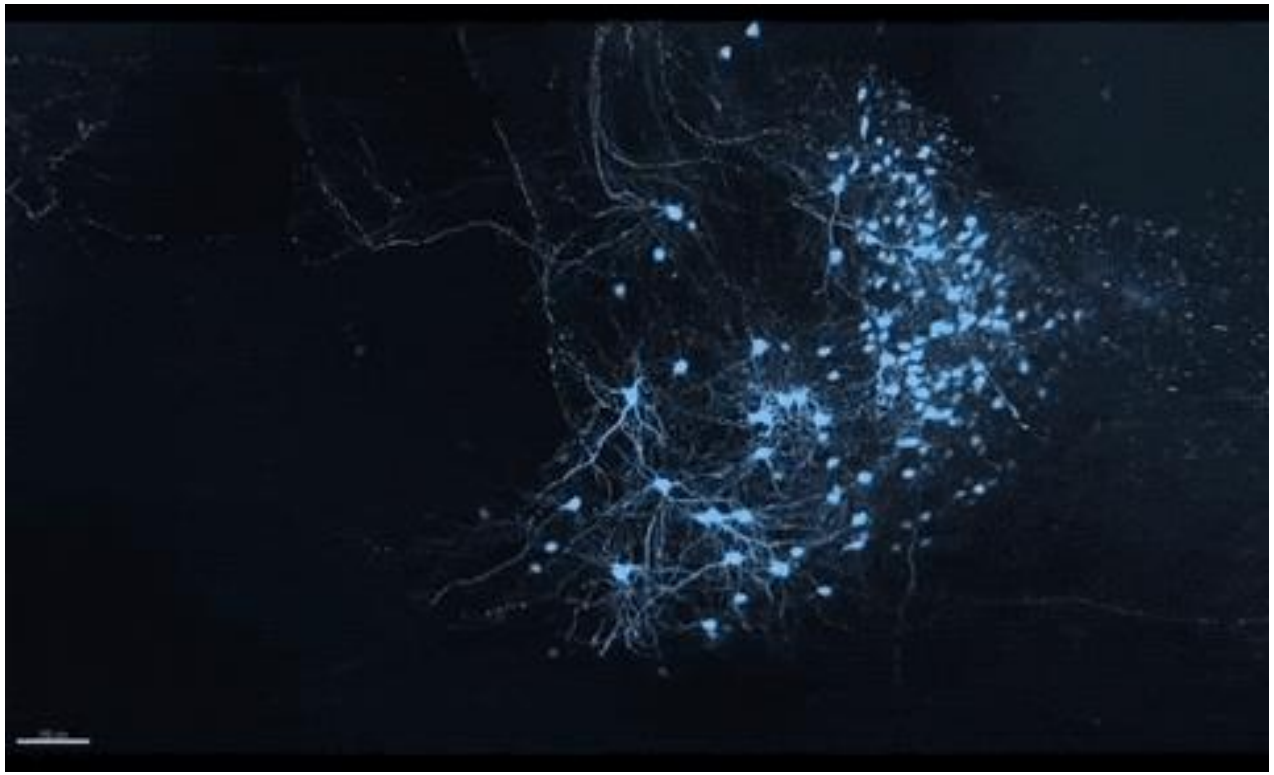


Encoding (learning)

Retrieval (recall)



What are these cells?



- **Engram** neurons are brain cells that store and retrieve memories by forming specific neural circuits.
- *Physical representations of a memory* in the brain.
- Activate when a memory is formed and reactivate when that memory is recalled.
- Strengthens overtime.

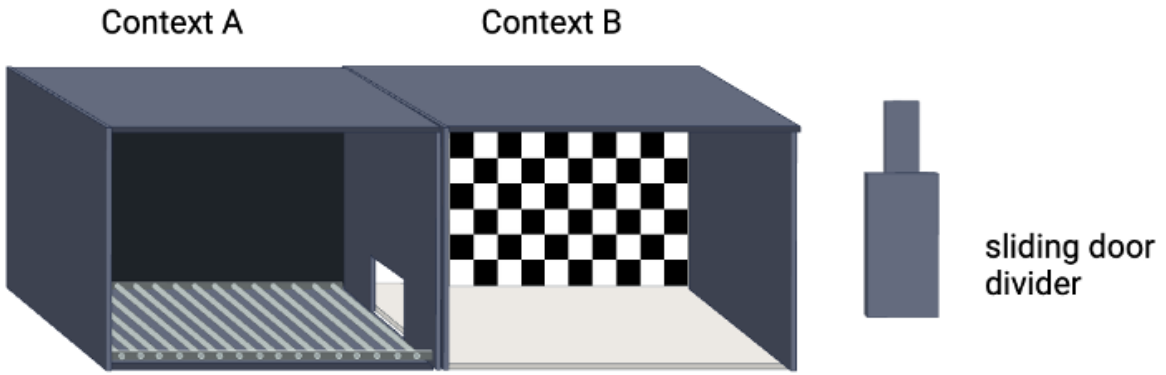
So, how can we capture an alcohol-seeking memory?

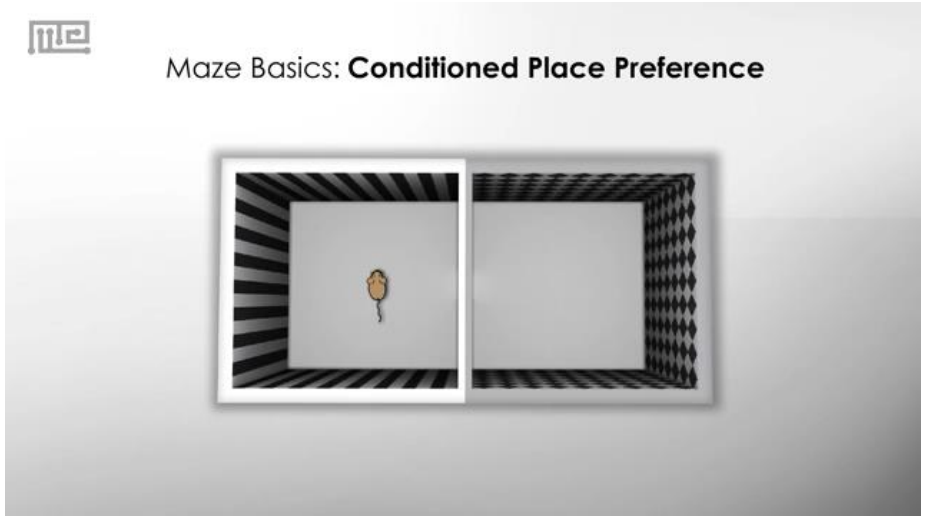
- We can use a **TRAP2** mouse system, to tag (or “TRAP”) engram neurons, using 4-OHT.
  - Will show as a green fluorescent protein.

- Induce alcohol-seeking behavior using **CPP** in this mouse system.

CPP as a model of alcohol-seeking

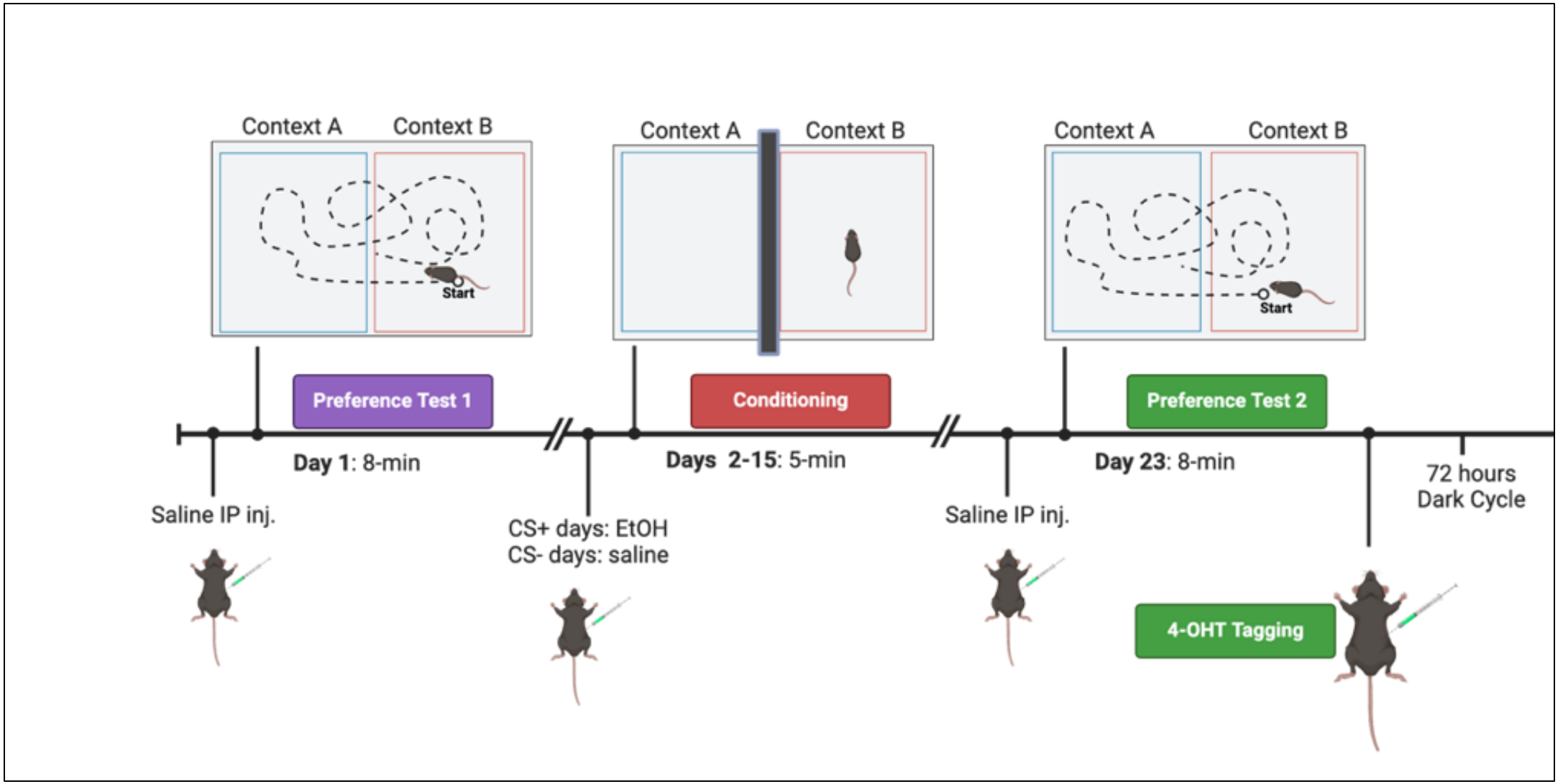
- **Conditioned place preference (CPP)** is utilized to study reward-related learning and addiction.
- Uses Pavlovian learning



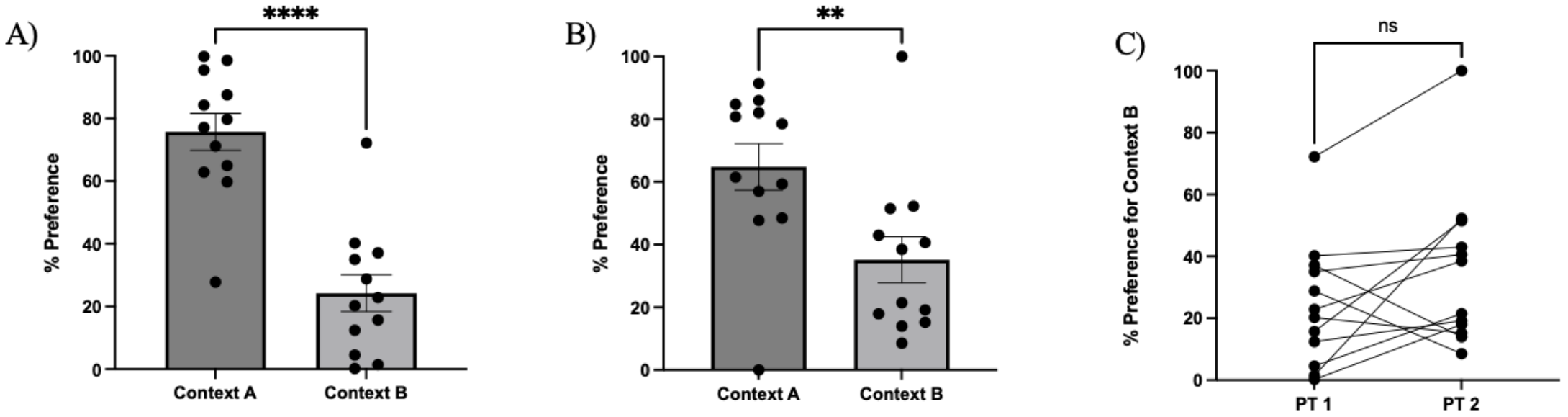


- **Biased approach:** intrinsic bias to assign ethanol context.
  - Least preferred context=ethanol context
- **Unbiased:** Randomly assign either context as ethanol or saline

Ethanol CPP in TRAP2 mice using a biased approach



A biased approach failed to produce CPP in TRAP2 mice



Why? Some previous research provided potential reasoning...

This is the first project of its kind at Wooster!

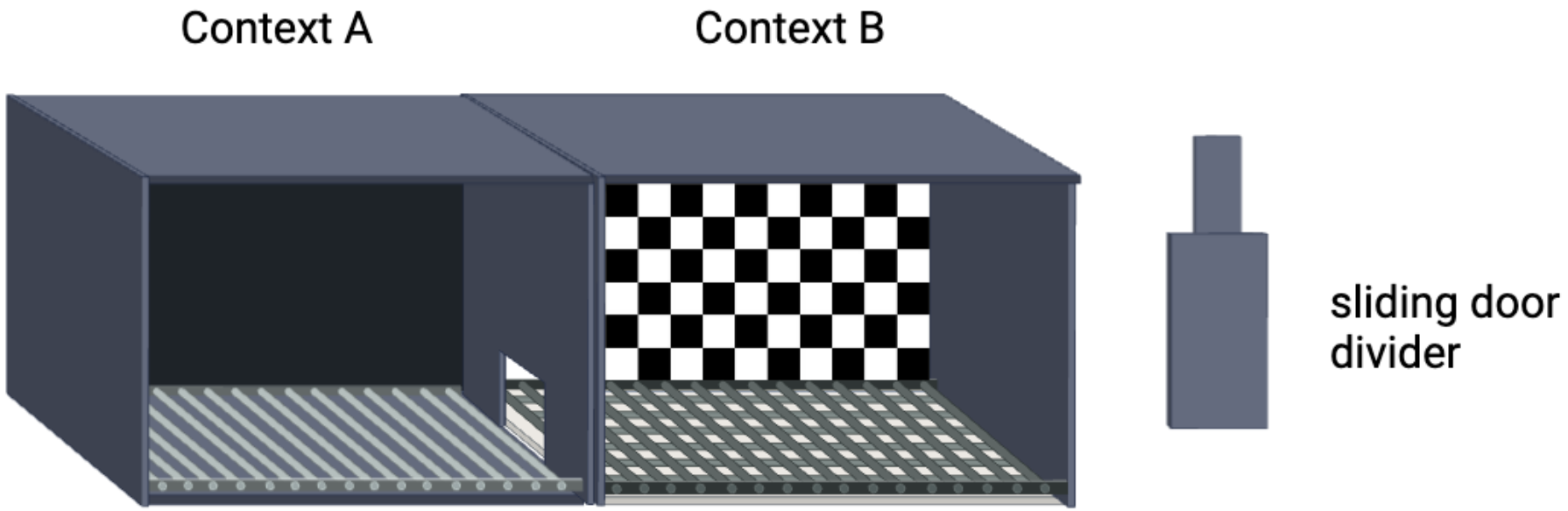
That's science! not everything will work, but we can try to find ways that **could**.

CPP can be impacted by **strain,** dose of the drug/substance**, duration of conditioning and test sessions,** and number of drug-conditioning trials (Le Foll & Goldberg 2005).

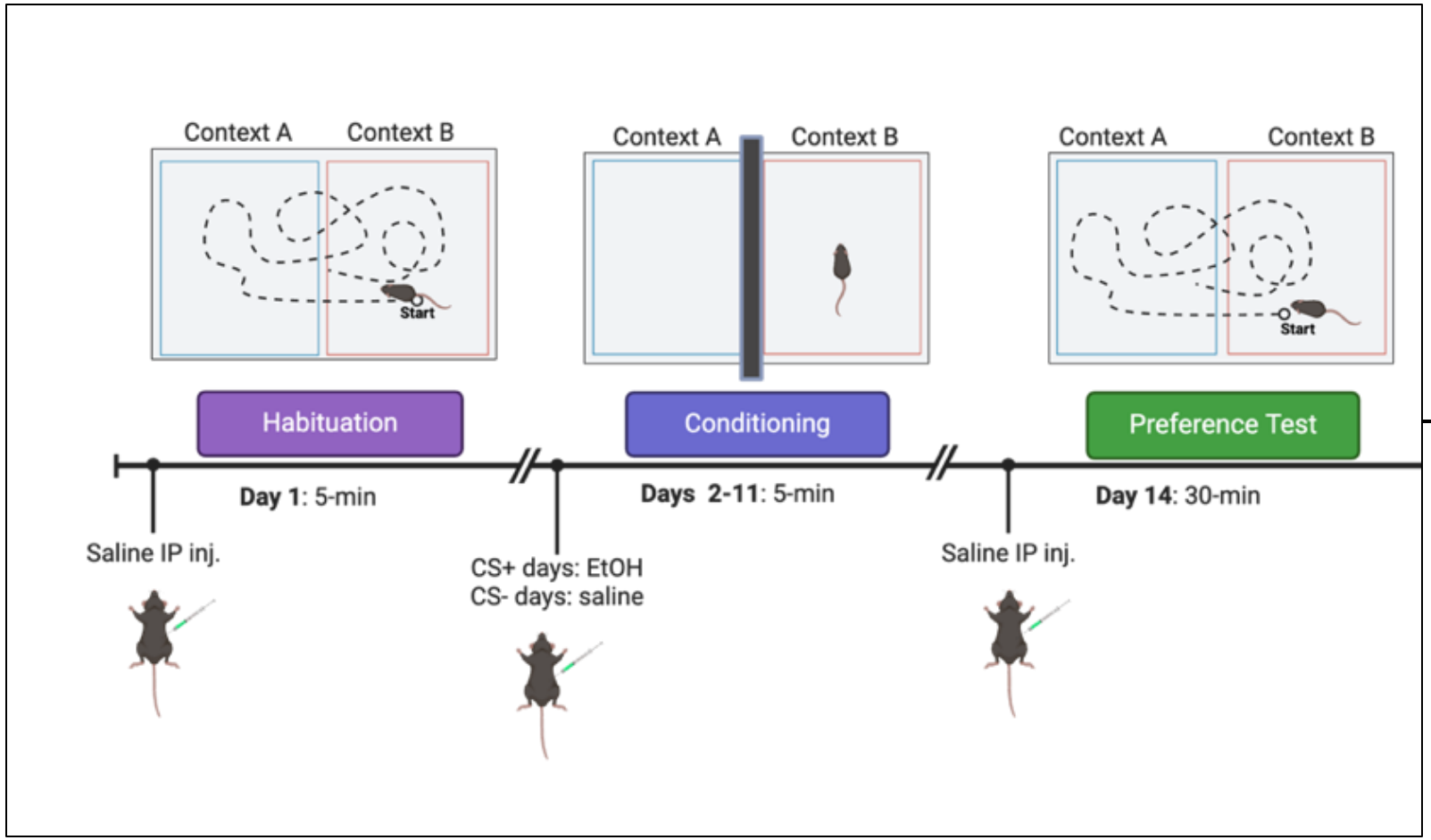
Context cues, may impact behavioral results, where some cues may not be sufficient or favorable enough to induce target behavior (DeNardo et al. 2019, Shimizu et al. 2015)

A **biased procedure** may shift preference due to **reduction of initial aversion** to the non-preferred compartment, rather than ethanol-reward CPP (Thiel et al. 2008)

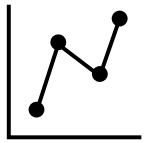
Take 2 ... New parameters and an updated apparatus design



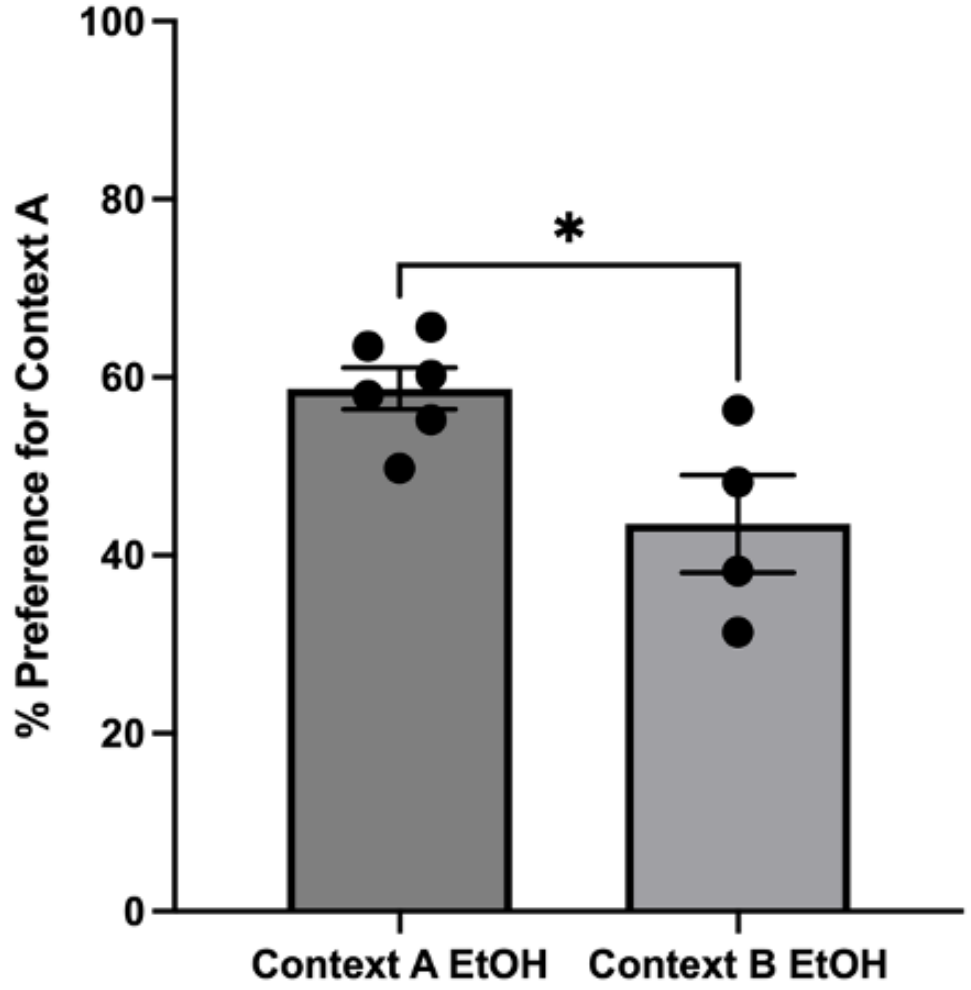
Ethanol CPP in TRAP2 mice using an unbiased approach



Live scoring + CPP scores analyzed



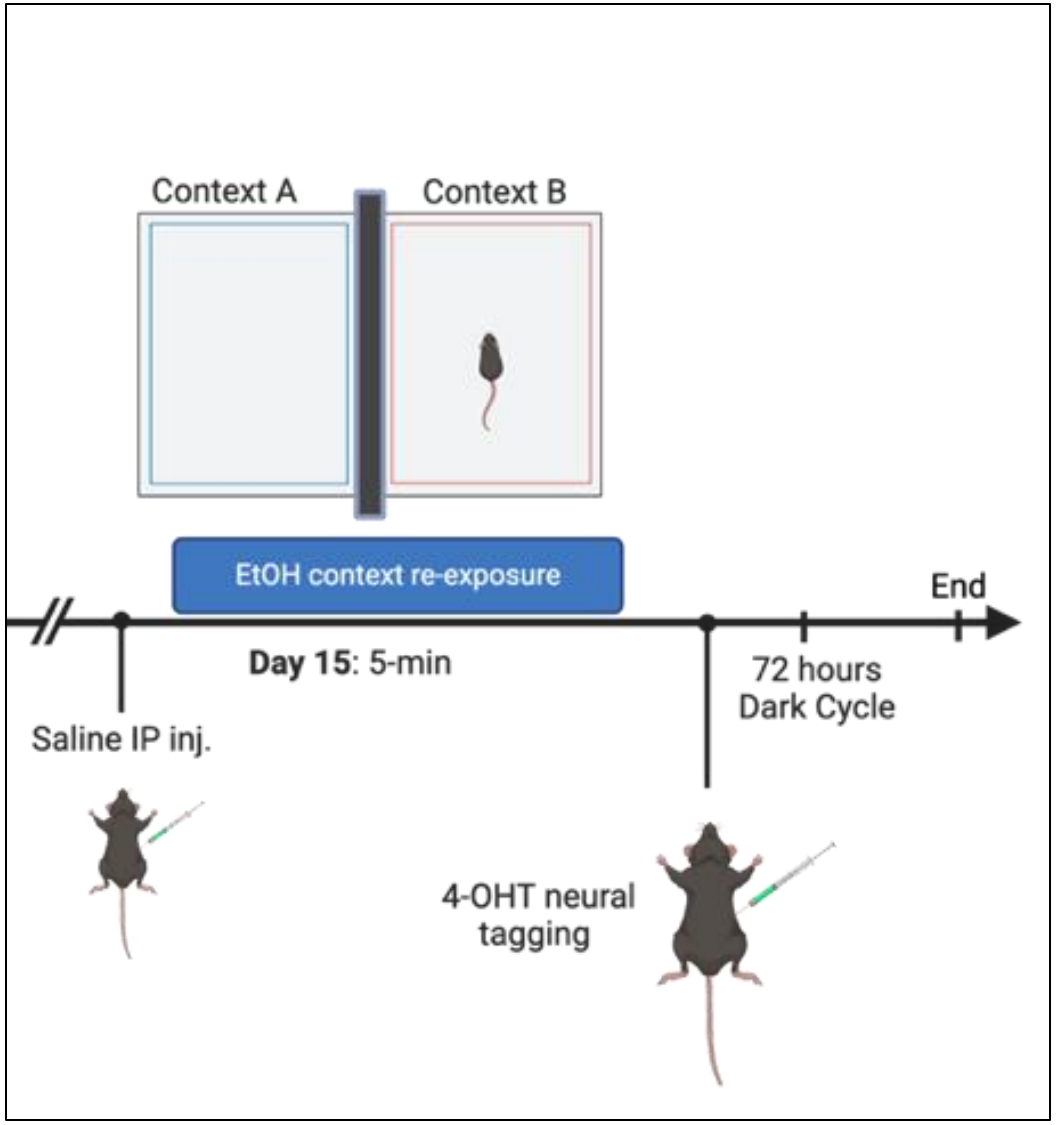
Successful ethanol CPP in TRAP2 mice!



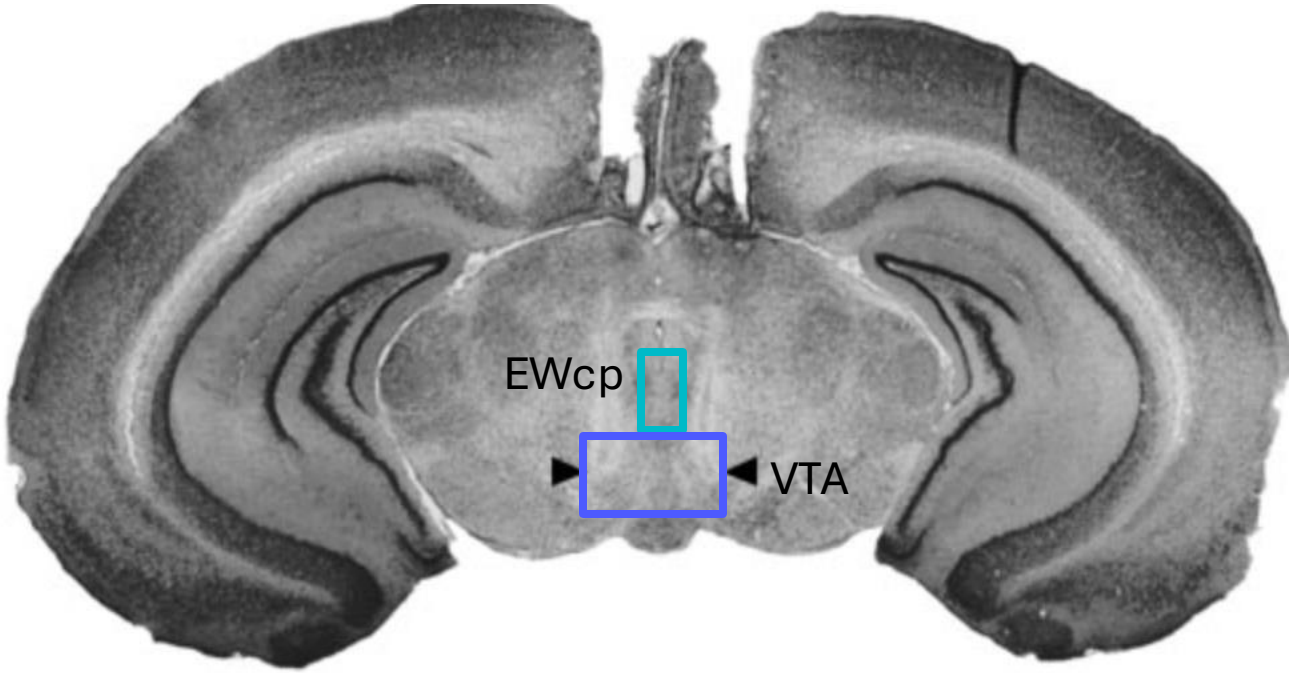
- If ethanol was paired with context A, preference for context A was greater than for context B.
- Vise versa with context B!

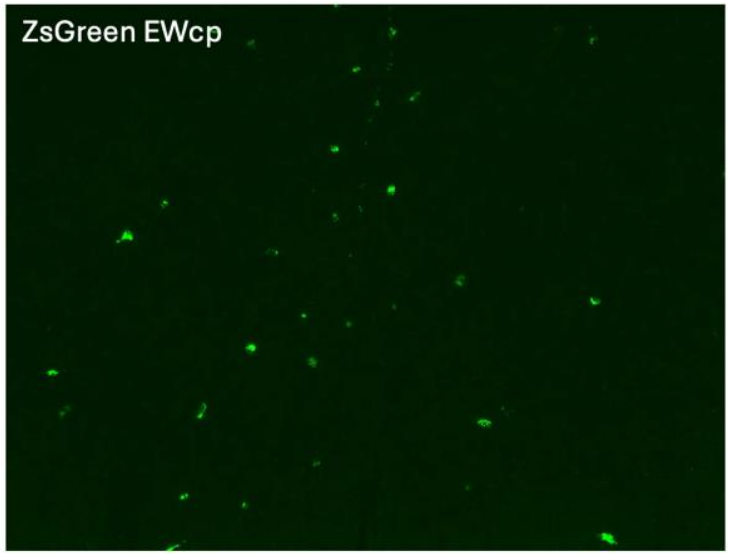
Now we tag engram neurons!

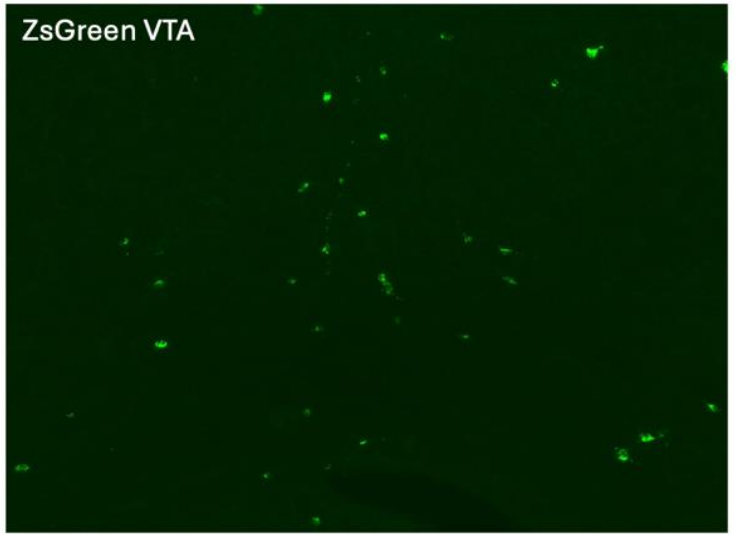
- We have successfully modeled alcohol seeking.
- Now, let's see which neurons reactivate when reinstated in the ethanol-associated chamber.



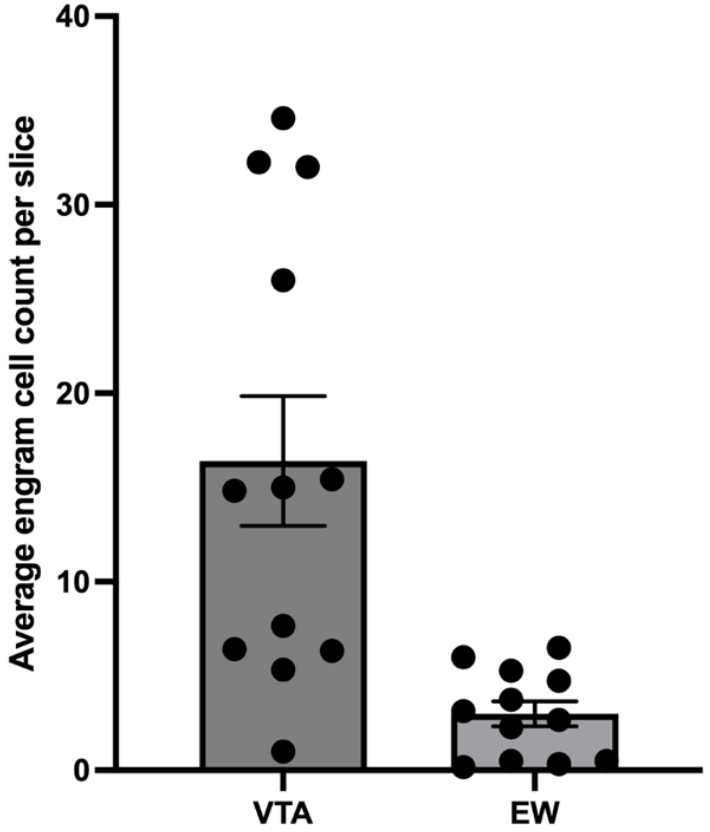
Analyzing engrams using fluorescent microscopy

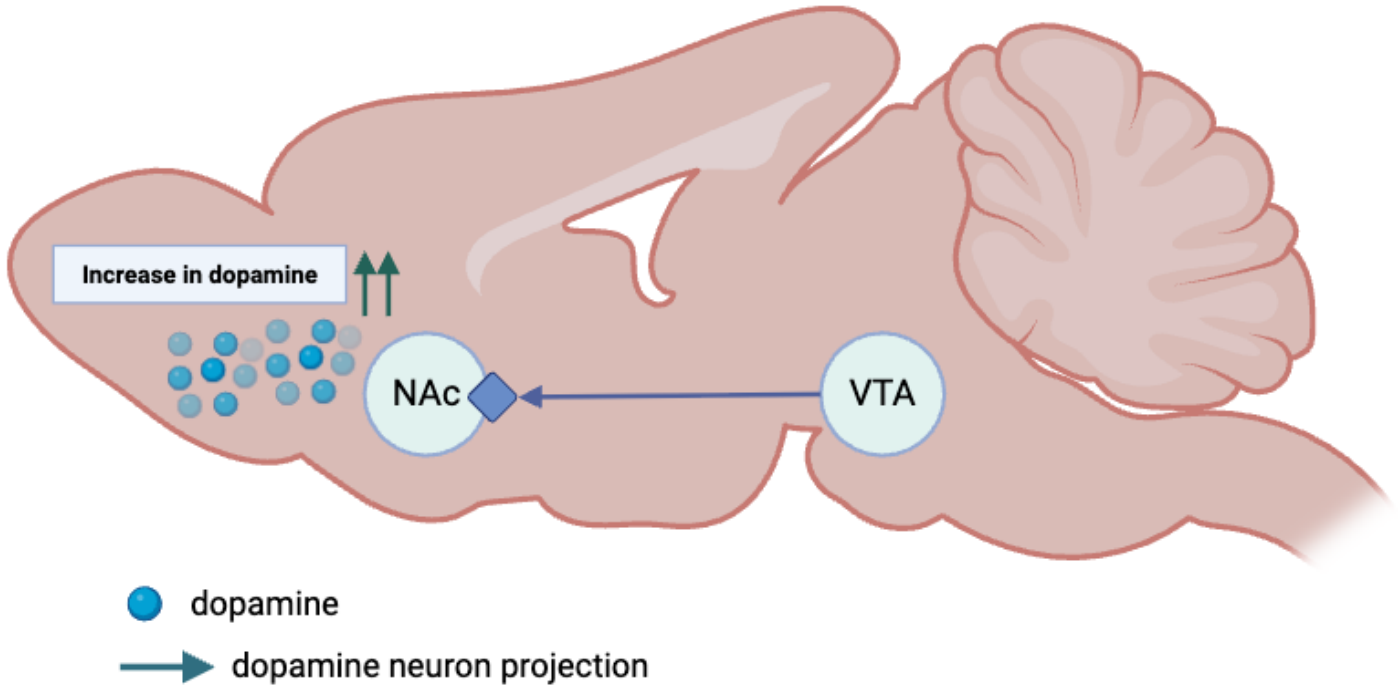






The VTA and Ewcp are involved in reward

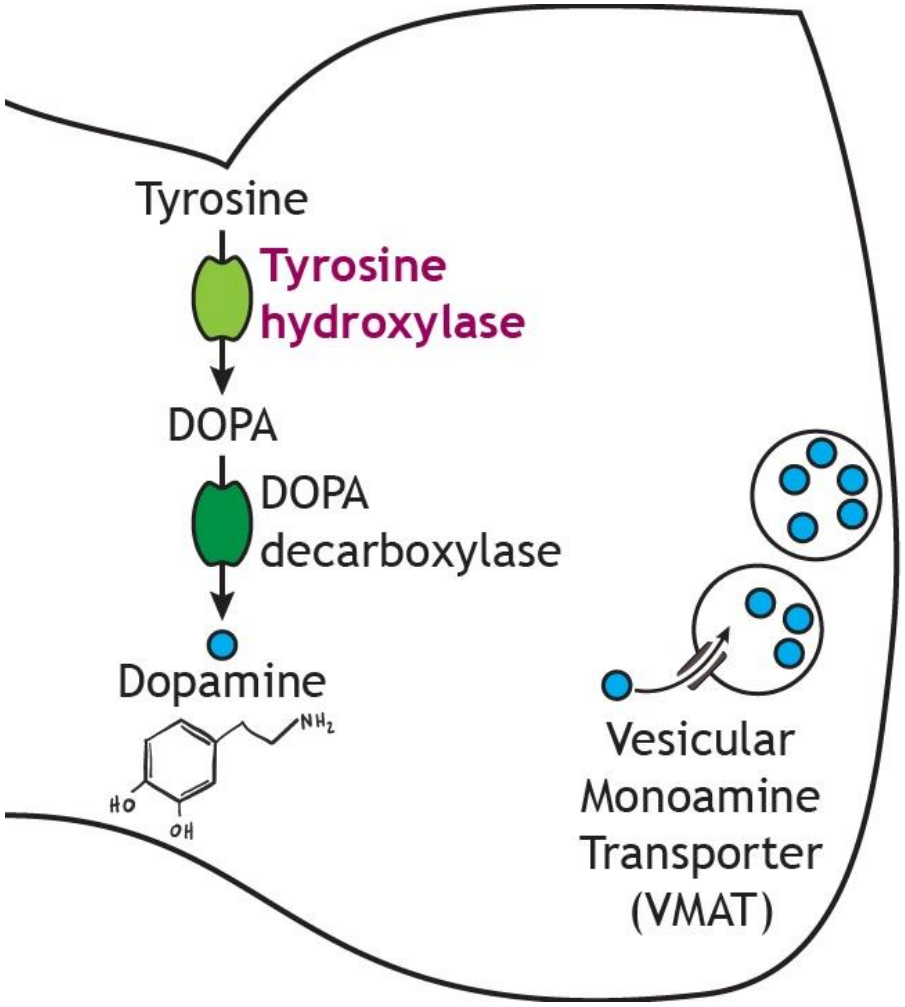




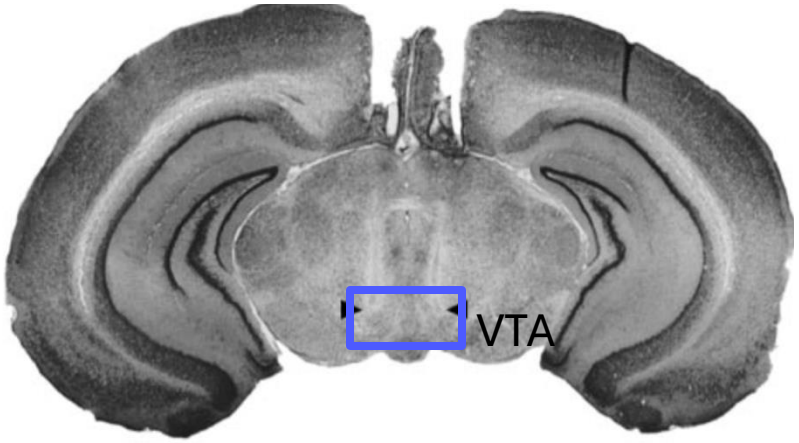
Are these VTA engram neurons dopaminergic?

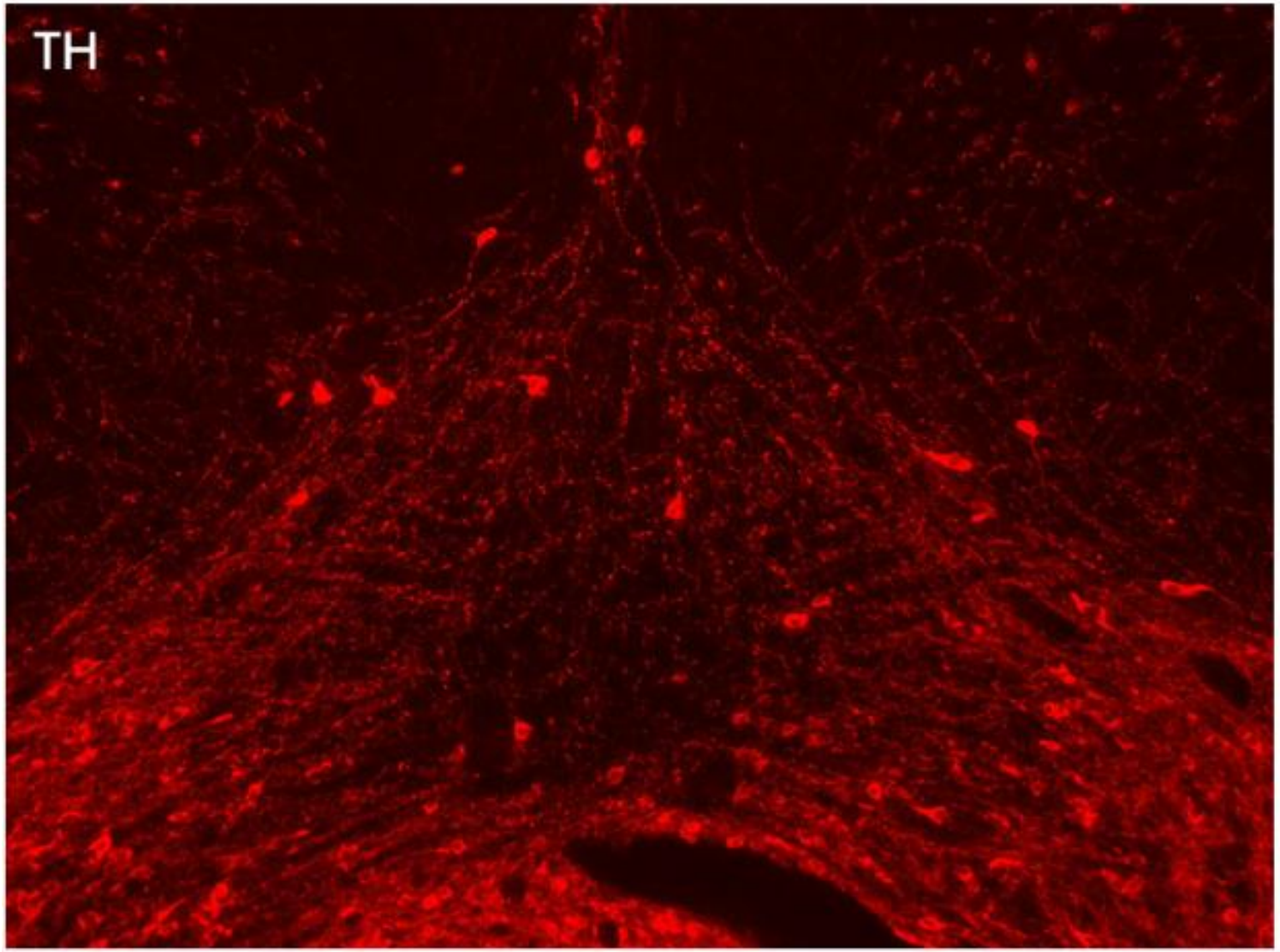
<u>How did we do this</u>?

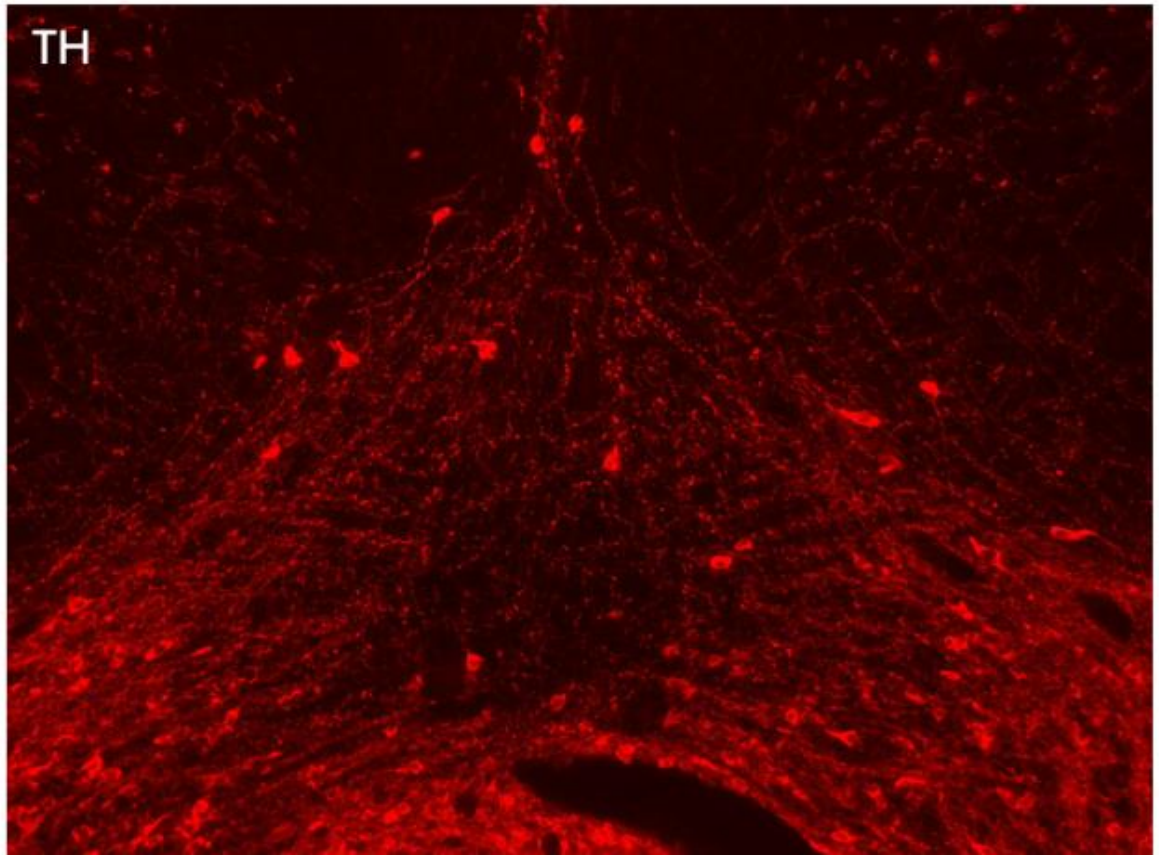
- Used **immunohistochemistry (IHC)** for **tyrosine hydroxylase (TH)**
- Used as a marker for dopaminergic neurons.

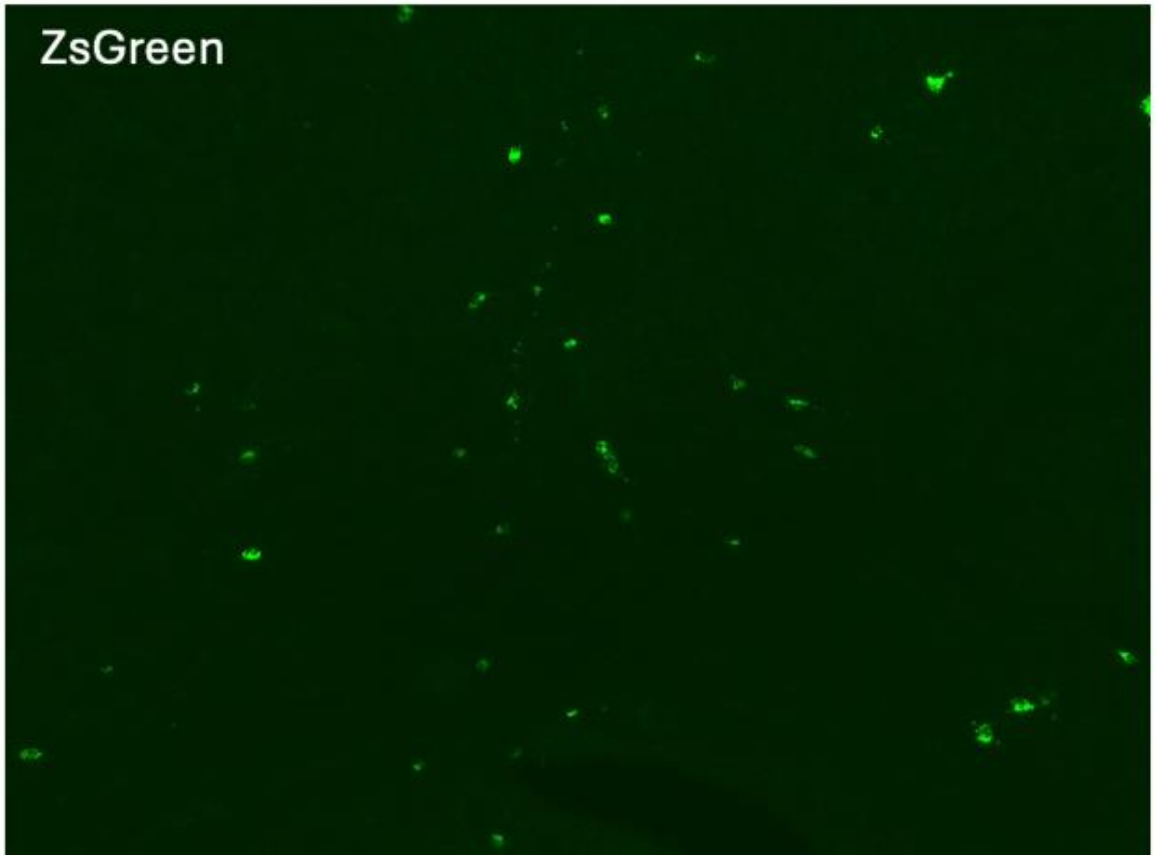


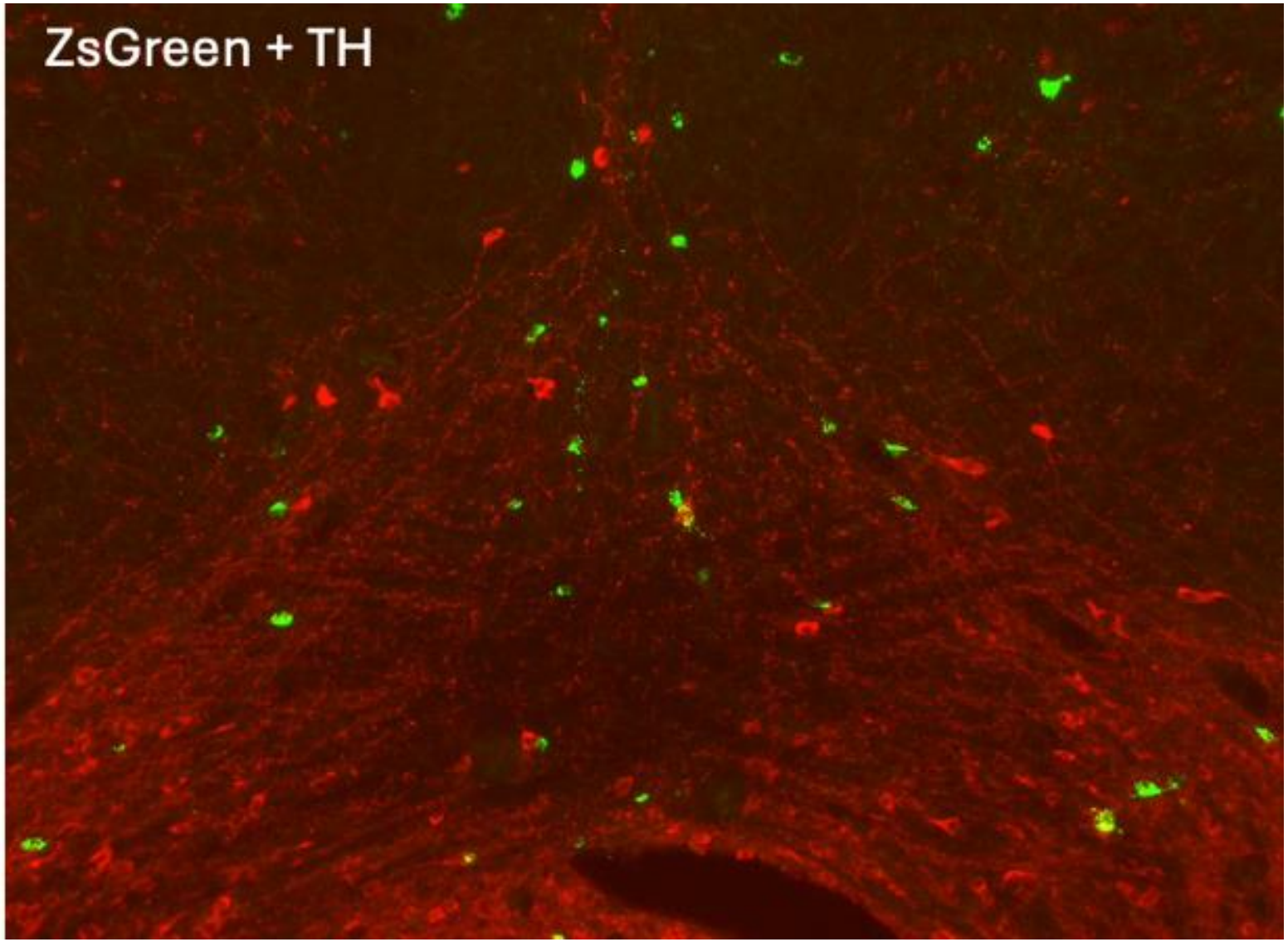
TH expression in the VTA



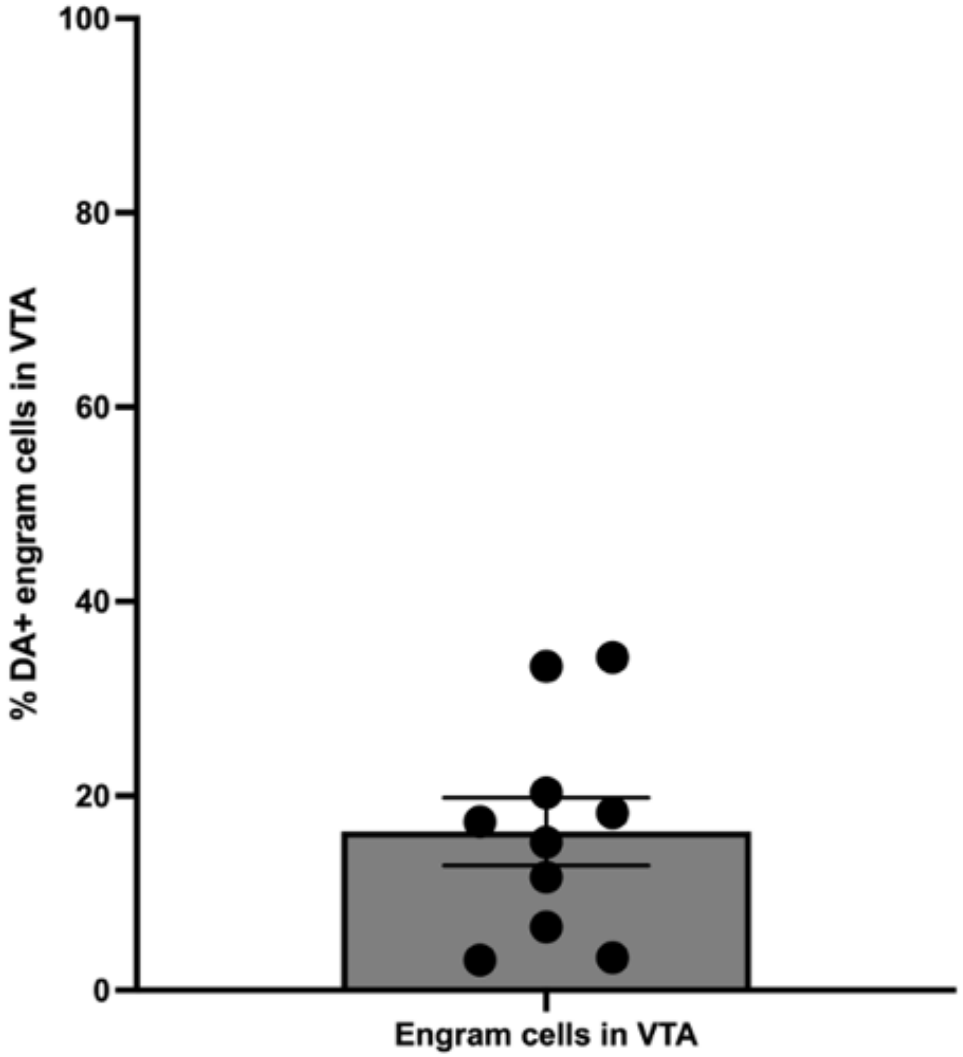



TH

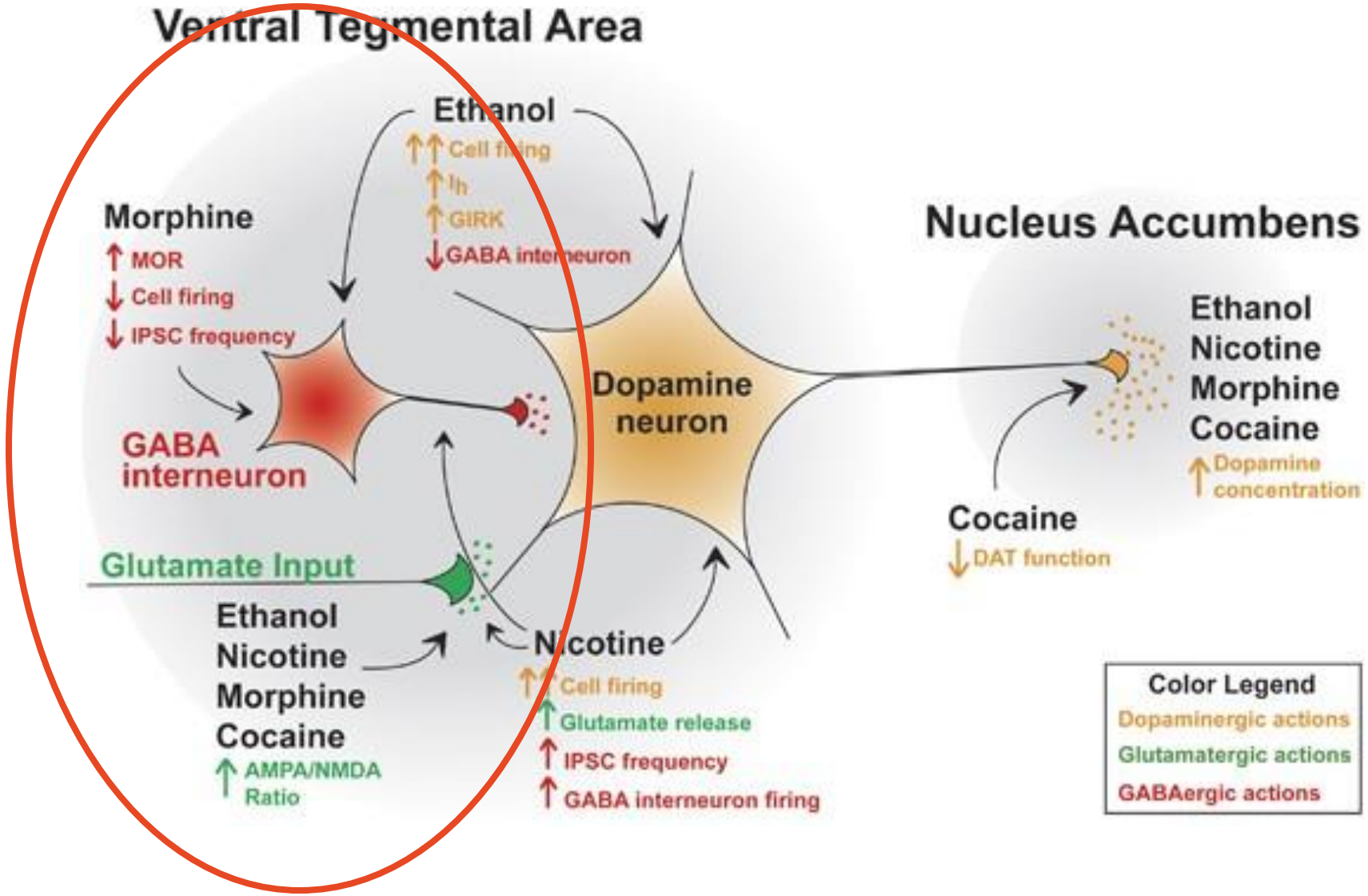

ZsGreen


ZsGreen + TH

Only a minority of VTA engrams are dopaminergic



The VTA is made up of more than just dopamine neurons



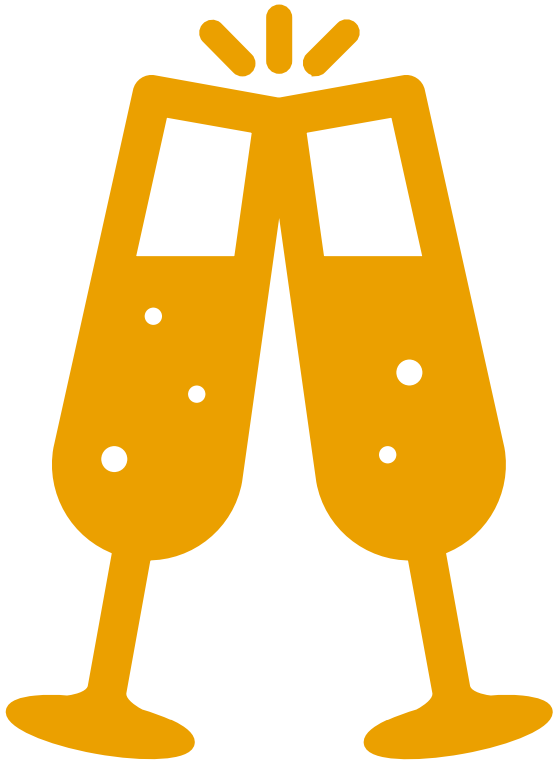
GABA plays a role in ethanol reward

- Alcohol is an indirect GABA agonist

- GABA activation slows down the CNS, making you feel less anxious and more relaxed

- **VTA GABA neurons** send signals to **cholinergic neurons in the NAc**, lowering acetylcholine levels. This helps shape learning based experiences (Creed et al. 2014).

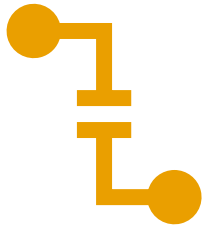
- **GABA from the VTA** helps **signal when a reward is expected**, by reducing the brain's response to the actual reward. (Cohen et al. 2012).



What does this mean?

Alcohol-seeking behavior is **not solely mediated by dopamine signaling.**

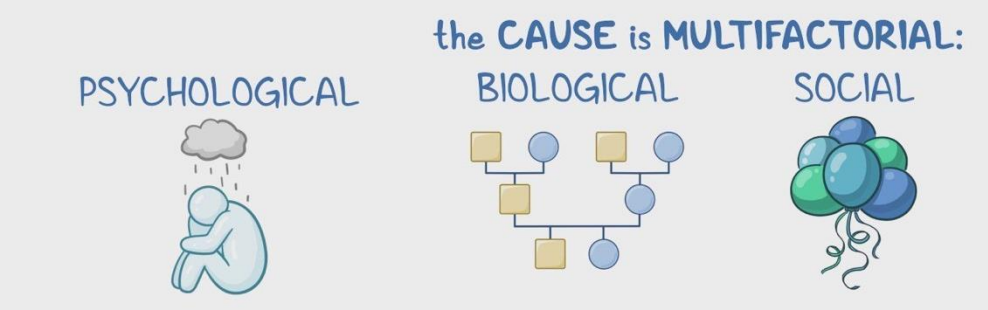
VTA **GABA**ergic neurons **may** contribute to alcohol-seeking behavior.

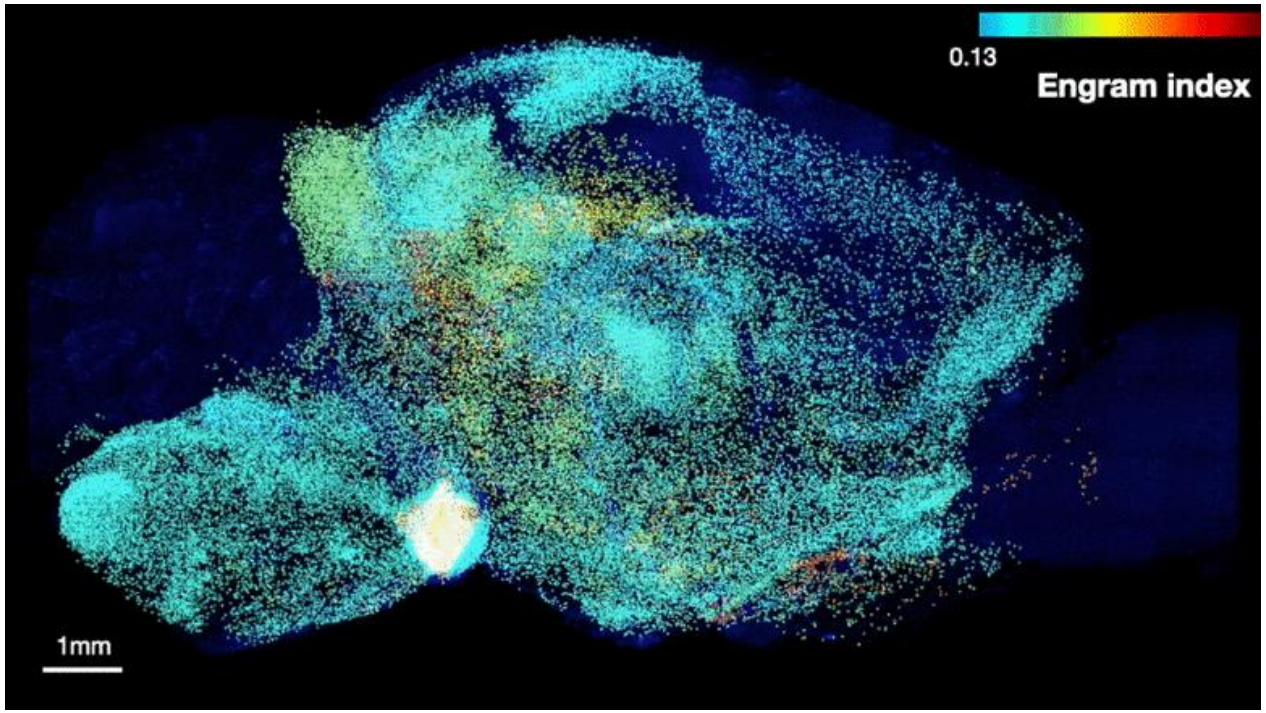


Behavior is encoded in a **distributed neural network** rather than a single neurotransmitter system/region.

Next steps

- Characterize VTA and EWcp engram neurons.
- **Optogenetics or chemogenetics** to silence these engrams, do we still see the same behavior?
- **Map** whole-brain engrams, investigate circuitry

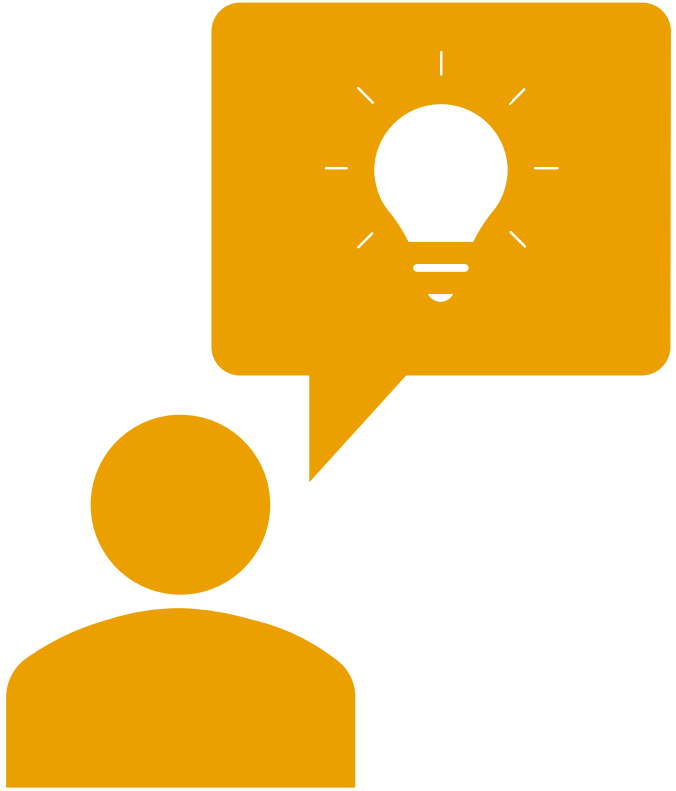




https://scitechdaily.com/innovative-brain-wide-mapping-reveals-a-single-memory-is-stored-across-many-connected-brain-regions/

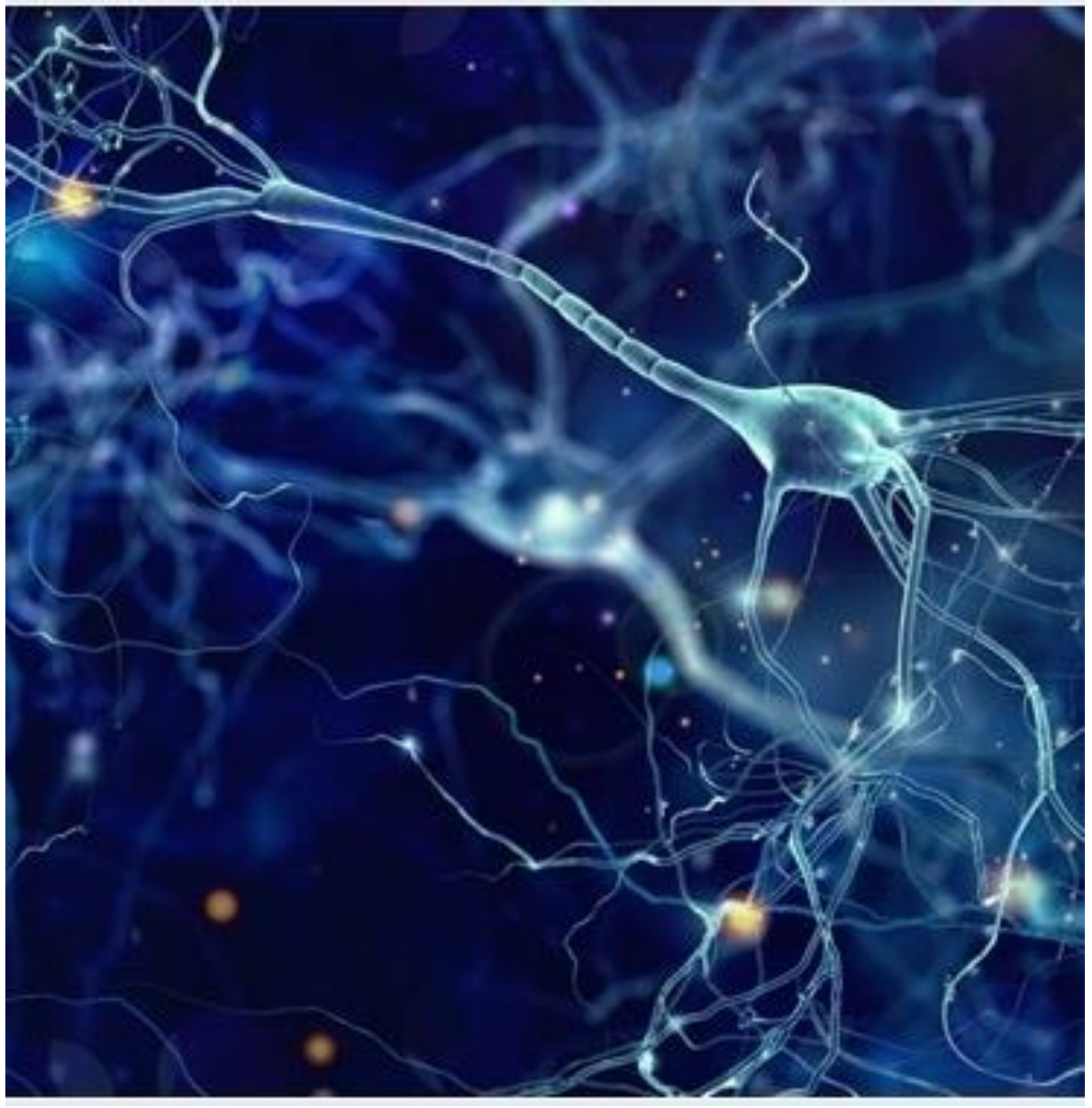
- Social or psychological contributors?
- Biological -- What **genes** drive these behaviors?
  - Evidence shows AUD is hereditary, is there really one gene that drives this susceptibility?

...and maybe one day, understanding a single memory could change the course of someone's life.



Overall, here's what I did...

- Developed the first functional CPP model in the TRAP2 mouse system at the College of Wooster.
- Identified and analyzed 'TRAPed' engram neurons.
- Characterized dopamine neurons in the VTA.
- Contributed to the research of two students who have made significant strides in the field, using my TRAP2 mice.



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A big thank you to Dr. Zuniga and Dr. Kelly for all their guidance and support throughout this independent study process.

THE COLLEGE OF WOOSTER

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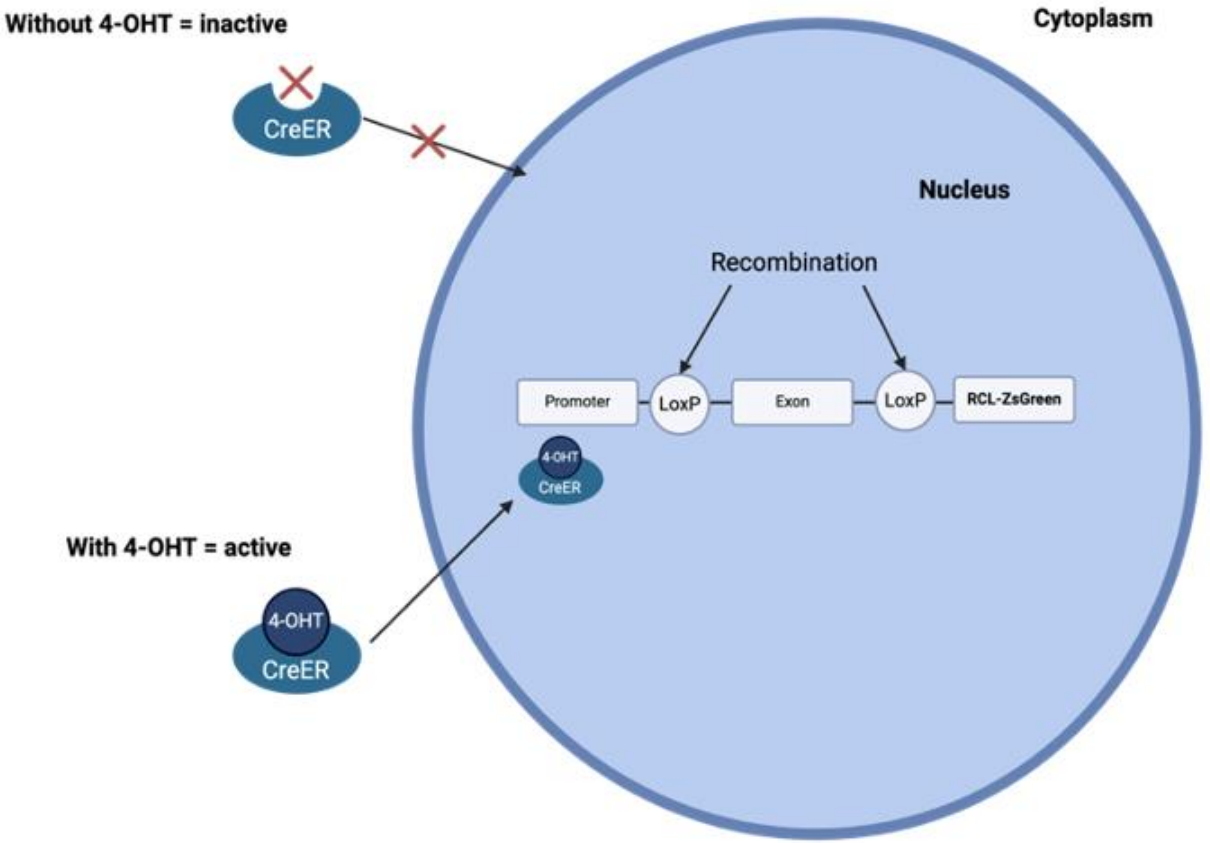
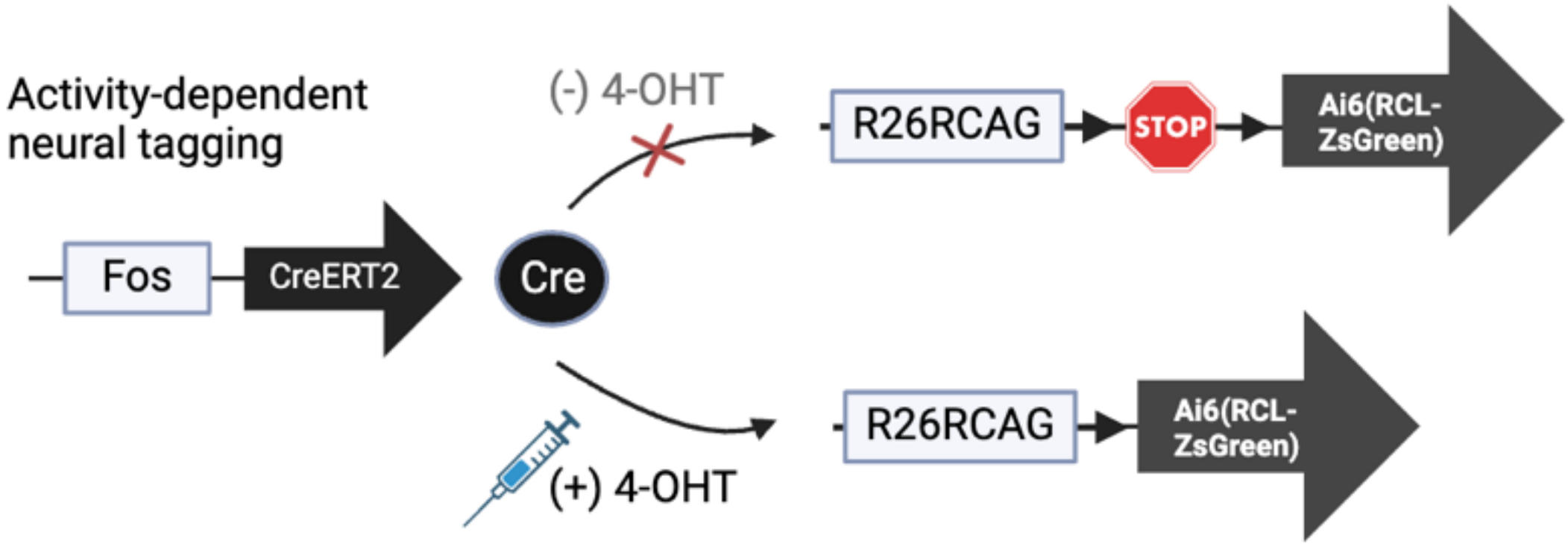
Supplementary slides

The TRAP2 transgenic mouse system

How It Works

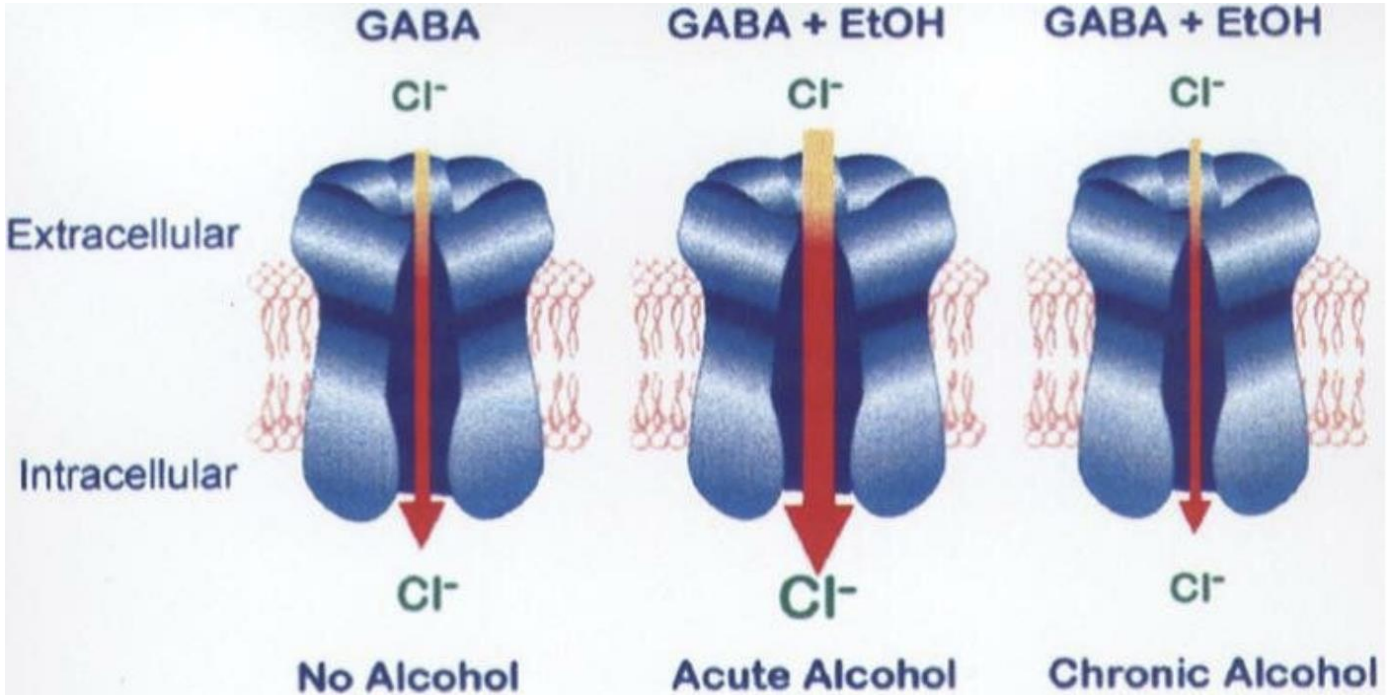
- Uses an activity-dependent genetic tagging system:
  - Neurons that are active during a specific event express a reporter gene (like *tdTomato, ZsGreen, o*r GFP, a fluorescent marker).
  - This tagging is controlled by the IEG *c-Fos* promoter, which is activated in response to neuronal activity.
- With drugs like tamoxifen (4-OHT), researchers can precisely control when neurons get tagged, linking them to specific memories.
- When 4-OHT is administered, these active neurons are permanently tagged, allowing us to observe these activated neurons.




Activity-dependent
neural tagging
(-) 4-OHT
R26RCAG
STOP
Ai6(RCL-
ZsGreen)
Fos
CreERT2
Cre
R26RCAG
Ai6(RCL-
ZsGreen)
(+) 4-OHT

Alcohol is an indirect GABA agonist

- GABA activation slows down the CNS, making you feel less anxious and more relaxed.
- Hence why it is termed a depressant.
- Alcohol initially increases GABA's sedating effects through positive allosteric interactions and by increasing inhibitory chloride flux.
- Over time, heavy drinking depletes GABA receptors through loss of GABAergic neurons, reduced GABA synthesis, and decreases in high-affinity GABAA receptor subtypes.



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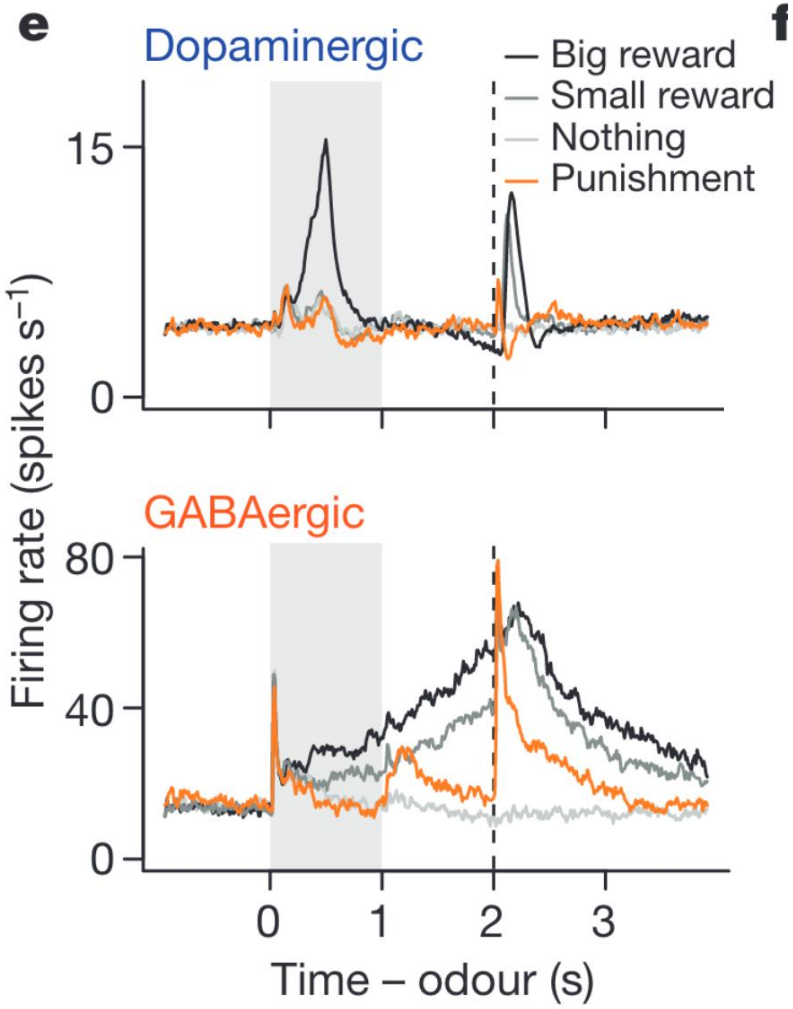
GABA mediates prediction of reward

- GABAergic neurons in the VTA showed persistent activity during delay period before receiving reward, which parametrically encoded the value of upcoming outcomes.
- This suggests that these neurons **encode expectation about rewards**. If this is the case, one prediction is that the activity of these neurons is **not modulated by delivery or omission of reward.**
- This indicates that VTA GABAergic neurons provides a significant inhibitory input that counteracts excitatory drive from primary reward when **the reward is expected.**



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