Alcohol use disorder is a global health issue with dire social and economic costs, understanding the neuronal pathways and its effect on neurotransmitter-signaling systems, and how they are altered, will help to design new gene targeting drugs to suppress alcohol addiction and alleviate withdrawal symptoms.

**Biological question**: Acute alcohol administration increases the firing of VTA dopamine neurons. My aim is to investigate the role of D1 dopamine receptors during alcohol relapse and to identify significant dopamine signatures during relapse[1].

**Research model**

- To study the activity across the dopamine system, we will visualize dopamine release using the GPCR indicator dLight during alcohol-seeking period.
- Laboratories animals will be Th-Cre rats[2] and will receive the genetically encoded CAG promoter pAAV-CAG-dLight1.1[3] . LEDS will generate two excitation wavelengths at 405 nm (isosbestic control signal) and 465 nm ($Ca^{2+}$ dependent signal).
- Optical measurements will be measured by femtowatt photoreceivers at various locations of the nucleus accumbens (core, above medial accumbens sheel, lateral accumbens shell).
- Signals will be downsampled and processed:
    - ratio $\Delta F/F_0$ will be calculated where:
      $\Delta F = F - F_0$, F: $Ca^{2+}$ dependent signal, $F_0$: isosbestic signal
    - $\Delta F/F_0$ will be low-pass filtered
    - $\Delta F/F_0$ within a time-window around events will be compiled and be averaged
    - 95% CI will be calculated for each event recording and used to filter events[4]
- The rats will be placed in a chamber with a pump located on the wall of the chambers. Activating the dispenser extinguishes a blue light, and triggers a syringe which delivers alcohol. The rats will be trained and tested following a context induced reinstatement procedure[5]: to use context as a factor, two contexts with different olfactory and tactile and visual properties will be created.
- Rats will be initially trained in the context where an activated pump by the rat, stops the light and dispenses alcohol. Then the rats are trained in an alcohol-missing context where the same pump when activated stops the light but doesn't deliver alcohol. The training phase is followed by a testing phase, the rats are self-controlling the nose: they are first in the alcohol-missing context then they are in the alcohol-context. Signals will be recorded at each stage of this procedure.
- We will use (RSA), for data analysis to compare similarities between brain activity and the measurements. A first order representational dissimilarity matrix (RDMs)[6] will be constructed. From the pairwise correlation distances, indicating the degree to which each pair of waveforms are similar or dissimilar across experiment sessions. A second-order Brain RDM across the firs-order RDM for each brain region will report dissimilarity between brain regions.
- Compared to small molecule probes, genetically encoded indicators G-protein coupled receptors (GPCR), are small, easy to express in an AAV, can target specific cells, and can be expressed over long periods of time. In addition, dLight indicators have reported faster kinetics than many similar probes.
- In this model we want to detect in the least evasive manner dopamine activity and we do not think this activity is well understood enough to simulate it with optogenetic actuators and indicators.

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[1] Relapse being defined as humans going back to drinking after stopping, and similar behavior has been observed in rodents, humans re-exposed to alcohol return to pre-abstinence levels of drinking

[2] TH-Cre rat is the animal used in research requiring tissue specific expression

[3] From addgene

[4] Compute CI for each recording and keep events which are significant and not too similar: https://statisticsbyjim.com/hypothesis-testing/confidence-intervals-compare-means/

[5] https://pubmed.ncbi.nlm.nih.gov/27612655/

[6] https://www.frontiersin.org/articles/10.3389/neuro.06.004.2008/full

- We may want to confirm the dLight probe measurements with an indirect measure of DA release by measuring presynaptic calcium release in VTA neurons using the genetically encoded fluorescent probe GCaMP6f that shows high sensitivity to $Ca^{2+}$.

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