Neuronal Activity in Prefrontal Cortex Underlying Relapse in Substance Use Disorders

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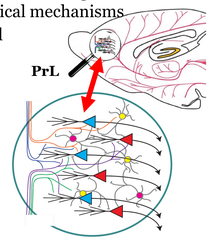
Background

- The opioid epidemic is a critical health crisis in the United States, with limited FDA-approved treatments to prevent relapse
- Identifying more effective treatment targets prevented by lack of understanding of the underlying neurobiological mechanisms
- Dysregulated prefrontal cortex activity is a hallmark of substance use disorders
- Preclinical drug self-administration studies reveal that prelimbic-prefrontal cortex (PrL) activity is necessary for reinstatement of drug seeking

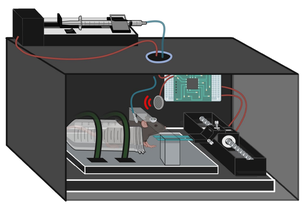


- To identify precise activity underlying relapse, we pair two-photon calcium imaging with drug self-administration
- We hypothesize that discrete, heterogeneous activity dynamics in prefrontal cortex underlie drug-seeking reinstatement

Methods

Subjects: Male & female C57BL/6J mice (8 wks old/20g minimum at study onset), single-housed with *ad libitum* access to standard chow & water in MUSC IACUC approved, AAALAC-accredited facilities

Surgeries: Mice received intracranial AAV virus (CaMKIIa-GCaMP6s, ChRmine) injections & GRIN lenses (4 mm len, 1 mm dia) targeting PrL (AP +1.85, ML +0.5, DV -2.45 mm) & stainless steel head-rings for head restraint. Following 1 week post-operative recovery, animals received intra-jugular catheters for intravenous heroin self-administration



Behavior: Mice underwent 14 days of heroin self-administration (max 1 mg/kg/session) followed by extinction (>10 d, <10% avg presses final 2 acq days) prior to reinstatement testing (drug-associated tone cue, 1 mg/kg heroin ip injection, 1% v/v TMT). Heroin hydrochloride was obtained from NIDA Drug Supply. Experiments were performed in custom-built chambers equipped with head-restraint stations and operated via Arduino and MatLab software. See Vollmer, Doncheck et al., 2021.

Two-photon imaging: Calcium activity in GCaMP6s-expressing PrL neurons were taken throughout heroin self-administration using a Bruker Nano multiphoton microscope equipped with hybrid scanning core with galvanometers and fast resonant scanners (>30 Hz, recorded at 4 frame averaging), a tunable Spectra Physics InSight DeepSee laser (set to 920nm). Data were acquired using PrairieView software, neurons identified using FIJI software, and analyses performed using custom-written Python code in Jupyter Notebooks. See Grant et. al., 2021.

Figure 1: Experimental Design

Resolving neuronal activity during heroin seeking by pairing in vivo two-photon calcium imaging with heroin self-administration in mice

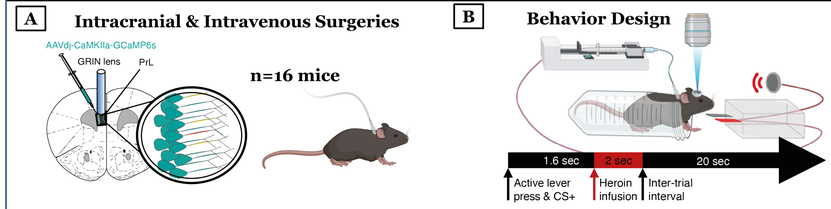


Figure 1A. Surgical design: Animals received intra-PrL CaMKIIa-GCaMP6s virus injections GRIN lenses, and head rings one week prior to intravenous catheterization.

Figure 1B. Heroin self-administration: Animals learned to press one lever, but not the other, to receive an intravenous infusion of heroin paired with an audio cue.

Figure 2: Heroin Self-Administration

Preclinical approach to model heroin use disorder wherein mice readily acquire, extinguish, and reinstate heroin seeking of their own volition

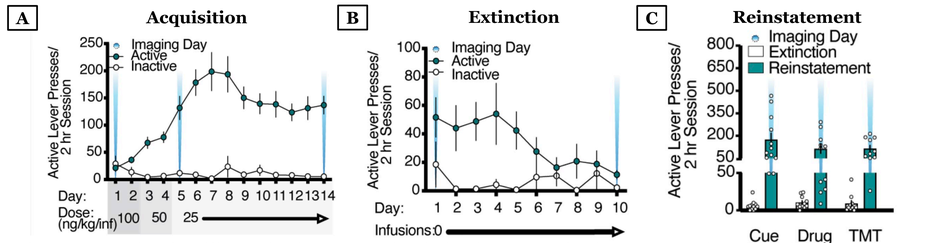


Figure 2A: Animals will acquire lever pressing behavior to receive intravenous heroin infusions. 2B: Once acquired, heroin reinforcement is removed and animals extinguish lever pressing. 2C: Re-exposure to drug-associated cues, the drug itself, or stress (TMT = predator odor), elicits reinstatement.

Figure 3: Two-Photon Calcium Imaging

Visualizing prelimbic cortex pyramidal neuron activity during heroin-seeking reinstatement

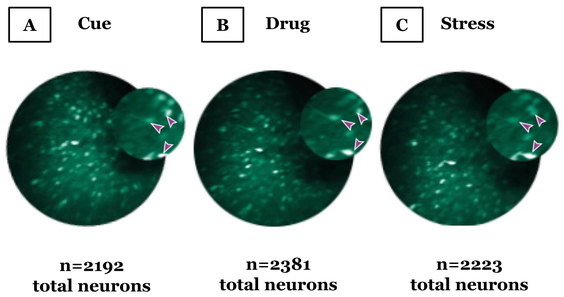


Figure 3 (A-C): Example field of view containing the same cells across different reinstatement tests. The calcium indicator, GCaMP6s, is expressed in prelimbic cortex pyramidal neurons; brighter neurons had greater average calcium activity during the recording.

Figure 4: Population-Level Activity

Identifying activity of all neurons underlying heroin-seeking reinstatement

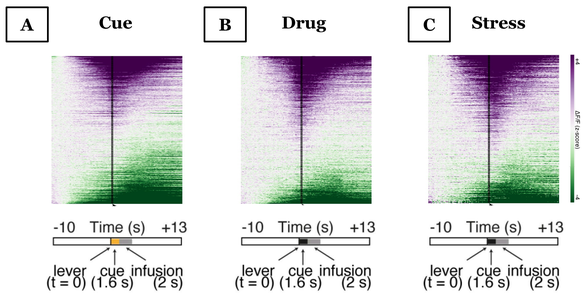


Figure 4 (A-C): Heat maps show differences in activity around the time that animals press the active lever (black line) during reinstatement tests. More excitatory neurons are in purple, more inhibitory neurons are in green. The white line represents the population average, which belies the great heterogeneity observed when broken apart by single cells.

Figure 5: Cluster-Level Activity

Identifying unique clusters of neuronal activity dynamics during heroin-seeking reinstatement

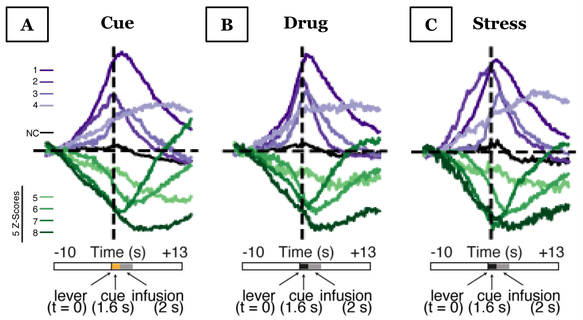


Figure 5 (A-C): Polyline graphs depict discrete clusters emerge around the time of the lever press during reinstatement tests. Four excitatory (1-4, purple) and four inhibitory (5-8, green) clusters peak in unique waves, and one cluster exhibited no significant change (NC, black) in activity.

Figure 6: Tracking Cluster Stability

Determining whether clusters are made up of the same cells regardless of reinstatement trigger

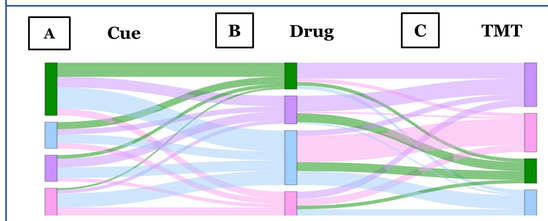


Figure 6: Shows that cells do switch clusters between tests, indicating that the same neurons may exhibit different activity in response to different triggers. Depicted clusters are analogous to excitatory clusters 1 (blue), 2 (pink) 3-4 combined (purple), and inhibitory cluster 8 (green) shown in Figure 5.

Figure 7: Tracking Single-Cell Activity

Longitudinally tracking individual neurons to identify whether they exhibit the same activity dynamics in response to different reinstatement triggers

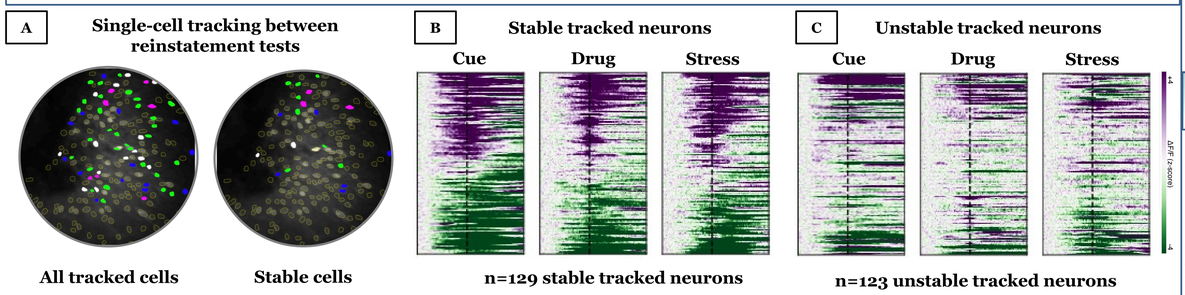


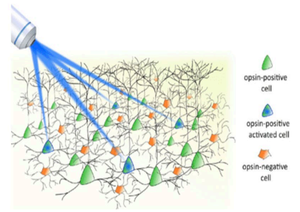
Figure 7A: Gives an overview of ongoing experiments. We will test whether all neurons within specific clusters (color-coded here to match the four clusters depicted in Figure 5: pink = cluster 1, blue = cluster 2, white = cluster 3, green = inhibitory cluster)

Conclusions

- Population-level PrL neuronal activity dynamics are highly heterogenous during reinstatement of drug seeking & can be subdivided into different cell clusters based on activity dynamics.
- Single-cell tracking reveals that some neurons switch clusters between modes of reinstatement, whereas others exhibit the same activity.

Future directions

- Tests are currently underway to assess the *functional relevance* of neuronal clusters and individual neurons for behavior.
- This involves the use of single-cell optogenetics, a technique visualized in the illustration below.



- Single-cell optogenetic manipulations involve using a second laser (Spectra Physics Femtotrain laser) that can be split into beamlets to target neurons of choice. These neurons express an opsin, a protein which is activated by a specific wavelength of light, which allows for ion flow that can activate the neurons. We can simultaneously visualize these changes in activity using the calcium indicator.

References

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Acknowledgments

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Some illustrations were made using BioRender.