Calcium imagining and deep behavior analysis in fentanyl vapor self-administration and relapse

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Introduction

The prevalence of opioid use disorder (OUD) and overdose deaths have reached epidemic proportions, continue to escalate worldwide, and constitute a global crisis. In 2019 synthetic opioids, including fentanyl, were being used by 1.2% of the worldwide population and contributed to more than 70% of the record-breaking number of overdose deaths. Fentanyl, which is often used clinically for anesthesia and analgesia, is commonly administered intravenously or by inhalation (smoking/vaping), which results in rapid drug bioavailability in the brain. Technical challenges have contributed greatly to our lack of understanding of the neurobiology of OUD, including limitations of behavioral models, difficulty tracking individual neurons longitudinally in freely behaving animals, and inadequate behavioral analysis tools. Intravenous drug self-administration is considered the "gold standard" model to investigate the neurobiology of OUD preclinically, but it remains difficult to perform *in vivo* electrophysiology or calcium imaging during drug self-administration due to the tangling of drug catheter and recording cable. This technical challenge was overcome with the development of a noninvasive mouse model of opioid self-administration using vaporized fentanyl that recapitulates key features of OUD. Imaging freely behaving animals is difficult, and conventional single-unit recordings can neither distinguish neuron subtypes nor track individual neurons longitudinally. In contrast, *in vivo* imaging using miniaturized fluorescence microscope (miniscope) systems allows for examining spatially and temporally coordinated activity in hundreds of individual neurons longitudinally in freely behaving animals. Complex behavioral analysis is infrequently incorporated in preclinical models, which likely contributes to limited translational impact. Recent computational advances in convolutional neural networks, pose estimation, and machine learning analysis has overcome these challenges to provide tools for computational neuroethology. We are leveraging these cutting-edge imaging technologies and behavioral analysis tools to gain a deeper insight into the neuronal ensembles that encode opioid-related behaviors during fentanyl self-administration and relapse.

Keywords: Opioid use disorder, self-administration, relapse, fentanyl, imaging

Centers for Disease Control and Prevention. Drug Overdose Surveillance and Epidemiology (DOSE) System. Atlanta, GA: US Department of Health and Human Services, CDC; [April 26, 2022]. Access at: https://www.cdc.gov/drugoverdose/nonfatal/dashboard

Figure 1. Technical challenges

The field currently faces a technical challenge: the drug tether (gray) that connects to the intravenous catheter and the cable (white) of sophisticated technologies tangle during intravenous drug self-administration.

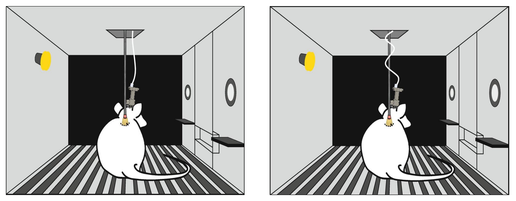


Figure 2. Vape technology

Using vaping equipment that is commercially available increases the translational validity of our preclinical model

Figure 3. Fentanyl vapor self-administration

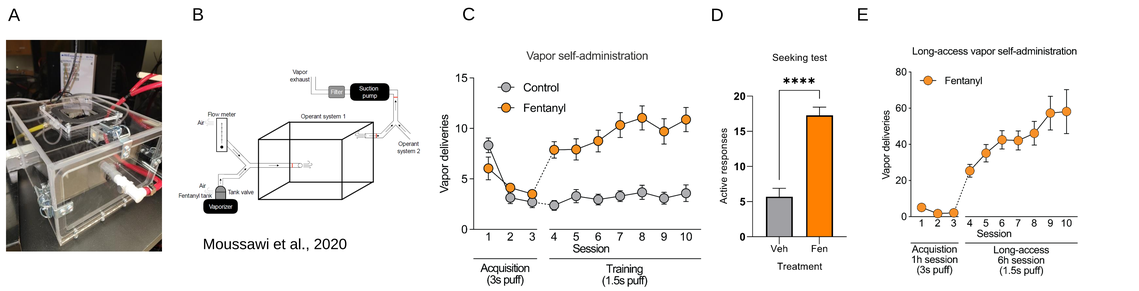


FIGURE 2. (A) Vacuum-sealed operant chambers custom built by LJARI. **(B)** Schematic of airflow dynamics from the operant chamber, to the air compressor, through the HEPA filter, and out the exhaust (Moussawi et al., 2020). **(C)** Vapor Self-administration of mg/kg Fentanyl over 10 days for 1 hour of training, mice receive 3 seconds of delivery within the first 3 days and 1.5 second deliveries from then on. **(D)** Drug seeking test performed 3 days post last vapor delivery **(E)** Fentanyl long access self-administration of 6 hour sessions Note: Inhaled and intravenously infused drugs present similar pharmacokinetics and pharmacodynamics (Dershwitz et al., 2000). This model of fentanyl self-administration recapitulates key features of OUD neurobiology. LJARI: La Jolla Alcohol Research, Inc.; OUD: opioid use disorder.

Figure 4. Calcium imaging via miniature fluorescence microscopy

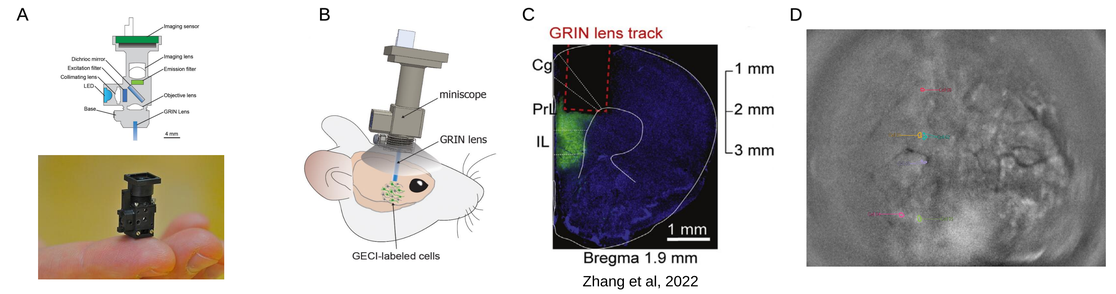
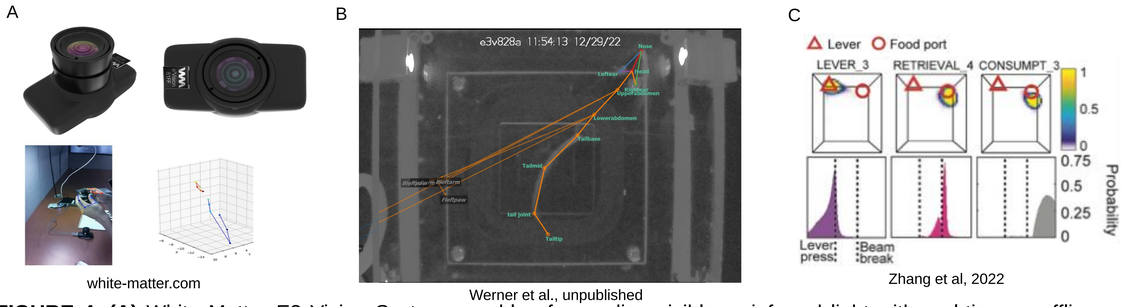


FIGURE 3. (A) The components of a miniscope and the miniscope itself. **(B)** Schematic for a mounted miniscope with the GRIN lens aimed at GECI-labeled cells in a deep brain structure. **(C)** Sample histological image showing GCaMP6f expression (green) with a GRIN lens track (red) aimed at the PrL. Scale bar: 1 mm. **(D)** Identification of neurons in the Pfc *in-vivo*

Figure 5. Deep behavior analysis



white-matter.com

Werner et al., unpublished

Zhang et al, 2022

FIGURE 4. (A) White Matter E3 Vision System capable of recording visible or infrared light with real-time or offline processing (Watch Tower System). A single-cable system and GoPro-style mounts allow for maximum utility. **(B)** Pose estimation with SLEAP for behavioral analysis using open-source software packages that utilize transfer learning, an application of machine learning, to encode and label animal behaviors (Pereira et al., 2022). **(C)** Lever press, reward retrieval, and consumption behavioral microstates (LEVER_3, RETRIEVAL_4, CONSUMPT_3, respectively) identified during a food self-administration task using Deep Behavior Mapping (DBM; Zhang et al., 2022).

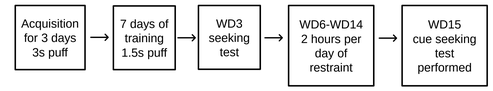
Future Directions

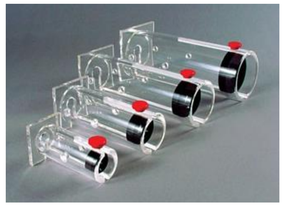
Our lab will leverage a multi-faceted approach to perform miniscope calcium imaging and record behavior during fentanyl vapor self-administration in mice.

Our aims are to:

- Record calcium transients from neurons in the prelimbic cortex.
- Record calcium transients from neurons in the nucleus accumbens.
- Identify sequences of behavioral microstates.

- **Examine how stress during drug abstinence affects relapse.**





Our overall goal is to examine the neuronal ensembles that encode drug-related behaviors to gain a deeper insight into the neurobiology that underlies OUD.

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