ADAI
ADDICTIONS, DRUG & ALCOHOL INSTITUTE

SMALL GRANTS PROGRAM

Final Report of Funded Project

Principal Investigator: Kaylin Ellioff

Dept: Pharmacology **Box:** 357750

Title of Project: Vapor administration of cannabinoids to modulate ascending pain circuitry

Budget Number: 68-6830 **Award Period:** 07/01/22 – 06/30/23

Date of this Report: 06/10/24

Please respond to the following areas, using attachments when necessary.

1: Major Research Results:

Over the course of this grant period, significant progress was made toward completing the scientific goals outlined in the grant "Vapor administration of cannabinoids to modulate ascending pain circuitry." Much of our focus has been optimizing head-fixed vaporization as described in Aim 2, and due to our rigorous and fine-tuned optimization, experiments like those detailed in Aim 1 will be straightforward to complete.

Our first objective was to construct and adapt a nosecone outfitted to the vapor apparatus that would fit around a mouse's nose, avoid condensation collection, and achieve temporal control of vapor delivery. We achieved this by custom-designing and 3D printing a nose cone that is attached via flexible tubing to a series of solenoids controlled by MED-PC software (**Figure 1**). This design allows us to control for a variety of crucial variables including but not limited to: number of vapor puffs per trial, vapor puff duration, vapor puff incubation duration, and inter trial interval duration. In addition to this precise control of the vapor delivery, we also included a tone generator as part of this set-up to serve as a cue for experiments requiring Pavlovian conditioning.

Following the construction of this apparatus, we next sought to optimize the vaporization parameters using this nose cone vapor delivery design paired with fiber photometry as a gross read-out of drug reaching the brain. We injected *TH*-Cre mice with a Cre dependent calcium indicator (DIO-GCaMP6s) and implanted a photometry fiber into the ventral tegmental area (VTA) (**Figure 2A**). Mice then underwent a one-week vapor habituation protocol and were recorded each day. On day 7, mice received nicotine vapor (2 mg/mL) to elicit dopaminergic neuronal activity within the VTA. Nicotine was chosen as the drug to pilot this set-up because 1) it has a short plasma half-life (6-7 minutes) in mice and 2) it is known to activate DA neurons within the VTA. We found that mice habituate to the control vapor over time as shown by a reduction in the fluorescent signal by day 6 (**Figure 2B**). After nicotine administration, we don't see a difference in fluorescence.

To look more specifically at dopamine release in the nucleus accumbens core (NAcc) from the VTA, a projection well-known to be activated during rewarding stimuli such as nicotine delivery, we injected WT mice with the novel dopamine sensor (GRAB DA2m) into the NAcc and implanted a photometry fiber (**Figure 3A**). To confirm that this sensor is indeed sensing DA release from the VTA, these mice were also injected with a red, light-activated opsin (ChrimsonR-tdTomato). This allows us the capability to stimulate these projections while measuring GRAB-DA fluorescence in the NAcc as a

positive control. As before, mice underwent a one-week vapor habituation protocol and were recorded daily. On day 8, mice received nicotine vapor (2 mg/mL). In this cohort of mice, we introduced a cue 5 seconds prior to the 2-second vapor puff. Like the VTA neuronal activity, we see that mice habituate to the vapor over days, as shown by a reduction in the decrease in Z-score in response to the vapor puff (**Figure 3B**). The delivery of nicotine again did alter the response. These mice did show increases in GRAB-DA fluorescence when optically stimulated in the VTA, confirming that the lack of response is not due to lack of signal, but may be due to some aversive qualities of the nicotine delivery that is stronger than the expected reward response.

Though technically challenging, these initial experiments, have provided a necessary foundation on which future vaporized drug-delivery/imaging experiments can be built upon. Our thorough optimization and characterization of this novel method will allow us, as well as other researchers to answer fundamental questions regarding neurocircuitry and vaporized compounds.

2: Attraction of Other Funds (please be specific):

This grant provided the necessary training and preliminary data necessary for a pending NRSA F31 titled, "Investigating the role of THC in mediating pain signaling in the BLA."

3: Publications or Presentations: (please send copy):

Publications:

Singh S, **Ellioff KJ** Bruchas MR, Land BB, and Stella N. (*In press*). Analgesic properties of next generation modulators of endocannabinoid signaling: leveraging modern tools for ligand development. *The Journal of Pharmacology and Experimental Therapeutics.*

Oral presentations:

Ellioff KJ, Qiu K, English AE, Piantadosi SC, Stella N, Bruchas MR, Land BB. Characterizing the Effects of Cannabidiol and Terpenes for Pain Relief.

- Gordon Research Seminar, Cannabinoid Function in the Central Nervous System. *2023.*

4: Employment of Students: I, Kaylin Ellioff, was partially funded using this award.

5: Improvement of Research Facilities: Funds from this grant were used to establish and optimize a vaporized drug delivery system to be used in conjunction with head-fixed imaging. This is a novel, cutting-edge approach that allows for time-locked vaporized drug delivery while simultaneously measuring neuronal activity. This technology is now available via collaboration for others to use.

6: Enhancement of Course Material: None.

7: Comments and Suggestions: None.

Please send this report to:

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Figure 1

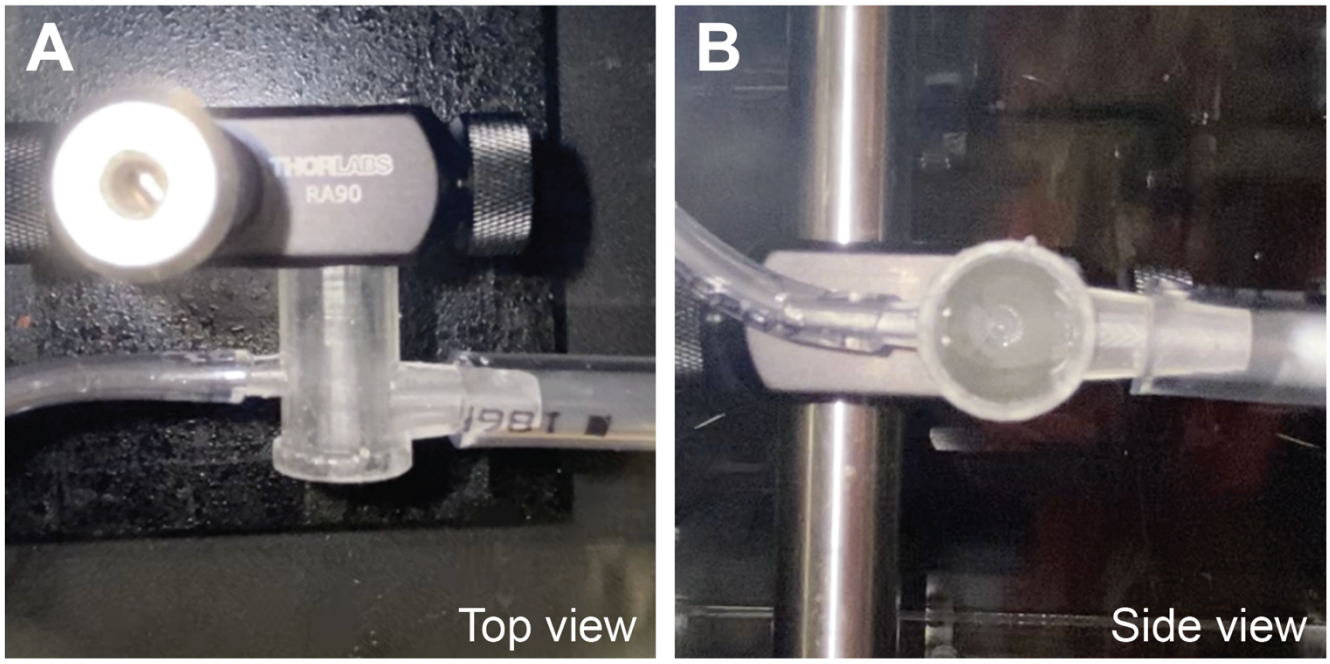


Figure 2

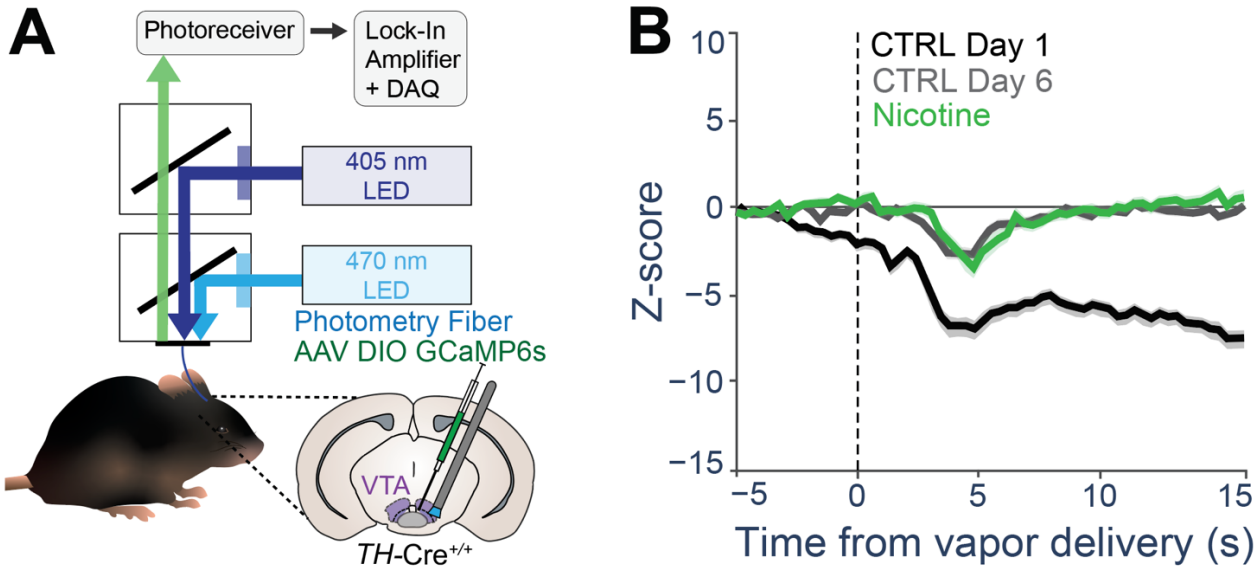
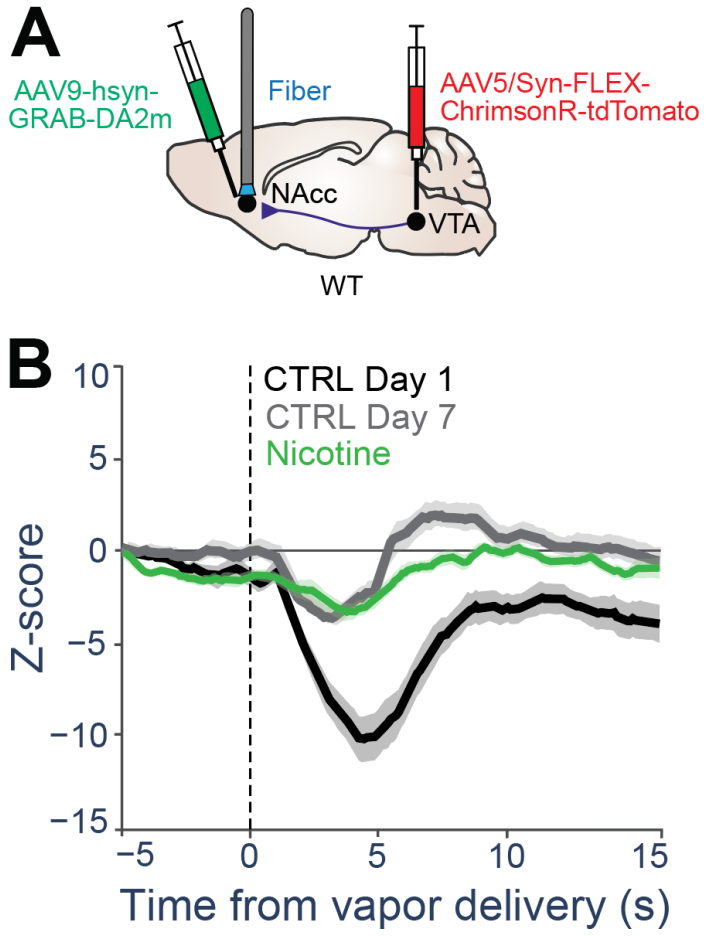


Figure 3


A
AAV9-hsyn-
GRAB-DA2m
Fiber
AAV5/Syn-FLEX-
ChrimsonR-tdTomato
NAcc
VTA
WT
B
CTRL Day 1
CTRL Day 7
Nicotine
Z-score
10
5
0
−5
−10
−15
−5
0
5
10
15
Time from vapor delivery (s)