An Exogenous Cannabinoid Decreases Olfactory Sensitivity in Non-Fasted Mice

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Do cannabinoids influence olfactory perception?

The endocannabinoid system is a widespread neuromodulatory system involved in a variety of processes including appetite, memory, motor activity, and pain perception.

Endogenous cannabinoids (2-AG and Anandamide) and exogenous cannabinoids (Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and WIN-55,212) are inhibitory retrograde neurotransmitters that bind to cannabinoid receptor type 1 (CB1) or cannabinoid receptor type 2 (CB2) on the presynaptic cell.

CB1 receptors are present in the mouse main olfactory bulb (MOB); most densely within the cells of the granule cell layer (GCL) and internal plexiform layer (IPL)[1].

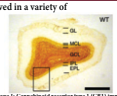


Figure 1: Cannabinoid receptor type 1 (CB1) immunohistochemistry in the main olfactory bulb of a wildtype mouse. The granule cell layer (GCL) and the internal plexiform layer (IPL) have the greatest density of CB1 receptors. (Figure from Soria-Gómez & Bellocchio et al., 2014).

Proposed model of the cannabinoid system in the MOB (Soria-Gòmez & Bellocchio et al., 2014)

Figure 2: Previous research on the behavioral effects of THC from marijuana consumption has shown that elevated cannabinoid levels promote increased food intake (i.e. "the munchies")[2]. A proposed model of this process has been put forth by Soria-Gómez & Bellocchio et al., 2014, claiming that the endocannabinoid system controls food intake via endocannabinoid system signaling within the main olfactory bulb (MOB)[2].

1. AM251 (CB1 receptor antagonist) infusion into the MOB decreases food intake.

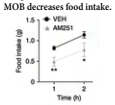


Figure 3: Mice were bilaterally infused into the MOB with AM251 (CB1 antagonist) or vehicle (4μg per bulb) after fasting for 24 hours. Food intake was measured for 2 hours after infusion (Figure from Soria-Gómez & Bellocchio et al., 2014).

2. WIN-55,212 (CB1 receptor agonist) infusion into the GCL of the MOB blocks the effect of inhibitory cortical feedback to mitral cells.

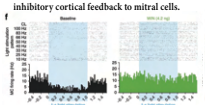


Figure 4: Channelrhodopsin-2 expressing axon terminals of the anterior olfactory nucleus (AON) and anterior piriform cortex (APC) when optogenetically stimulated, inhibit mitral cell firing. Local infusion of WIN-55,212 induced disinhibition of mitral cells by blunting the inhibitory cortical feedback from the AON and APC (Figure from Soria-Gómez & Bellocchio et al., 2014).

3. CB1 agonist intraperitoneal (IP) injection increases odor exploration.

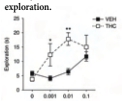


Figure 5: Odor exploration (of almond extract or isoamyl acetate) after an IP injection (1 mg/kg) of a CB1 agonist compared to vehicle in C57BL/6N mice (*$p < 0.05$, **$p < 0.001$). (Figure from Soria-Gómez & Bellocchio et al., 2014).

Do elevated cannabinoids influence odor sensitivity or only exploration?

Research Goal

Since behavioral threshold is thought to be set by the highest affinity receptor[3], our goal was to explore the mechanism by which elevated cannabinoid levels might influence olfactory sensitivity.

Experimental Timeline

Figure 6: Bilateral MOB cannula implantation and head-bar surgeries were performed on C57BL/6J mice (5 female, 5 male). Mice were given three days to recover then underwent a 2-week water restriction protocol. Water restricted mice were trained to report the detection of odor (isoamyl acetate or IAA) in a Go/No-Go head-fixed operant conditioning paradigm. Mice were then tested each day on progressively lower concentrations of IAA versus a blank (mineral oil solvent) to measure their odor detection threshold (ODT). Mice were infused with vehicle, WIN.55,212, or AM251 and behavioral performance was evaluated at the threshold concentration of IAA. The location of the cannula was subsequently verified using histology.

Measuring Olfactory Behavioral Sensitivity

Figure 7: Images of our semi-automated, Arduino-controlled, head-fixed, behavioral rig setup and flow dilution olfactometers. Mice were first acclimated to head-fixation and trained to lick for water. Mice were then trained to discriminate between the isoamyl acetate odor and the mineral oil solvent.

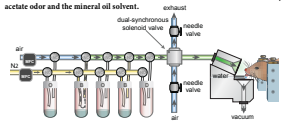
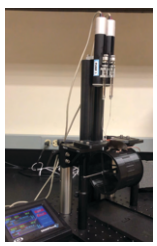


Figure 8: Odors are delivered using a custom flow-dilution olfactometer. A dual-synchronous solenoid valve allows the odor to reach equilibrium before presentation to the animal[4].

Intrabulb Drug Infusions into the GCL of the MOB



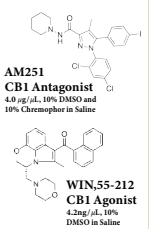


Figure 9: Custom designed injection rig . This rig was designed to infuse drugs into the brain via a cannula system in an awake mouse. Anesthetic agents such as isoflurane can interfere with olfactory perception[5] which created the need for this custom injection rig. Mice were infused with 1.0 μL per MOB at 100 nL per minute. Mice were given 10 minutes before the injectors were removed from the guide cannulae then 20 minutes in their home cage prior to behavioral testing.

Injection Site Tracing Confirmed to the GCL of the MOB

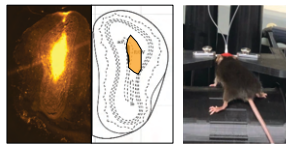


Figure 10: After all data collection was complete, DiI was bilaterally infused (1.0 μL per MOB at 100 nL per minute) using the custom injection rig. After 30 minutes, the mouse was transcardially perfused, the brain was removed, and sectioned on a cryostat (20μm sections) to confirm the targeting of the cannula.

Cannula Surgery Did Not Affect Behavioral Sensitivity

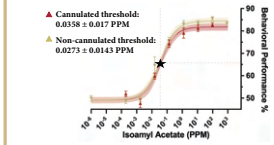
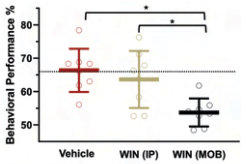


Figure 11: Unmanipulated baseline behavioral performance threshold prior to any drug manipulations. Isoamyl acetate (IAA) odor detection threshold (ODT) for these cannulated mice was not statistically different from the ODT for IAA of a separate cohort of mice without cannulae run in a separate rig. This shows that cannulae implantation does not affect olfactory perception. Shaded region shows 95% confidence interval error.

CB1 Agonist Intrabulb Infusion Significantly *Decreased* Olfactory Sensitivity



Mice were tested at their average ODT concentration (star in Figure 11).

Dashed line indicates the average behavioral performance of unmanipulated mice at ODT concentration.

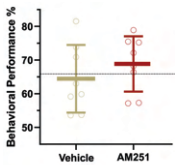


Figure 12: Behavioral performance on odor detection task after infusion of vehicle (10% DMSO in saline) into the GCL of the MOB, after an intraperitoneal injection of WIN,55-212 (1mg/kg of body weight), and after infusion of of WIN,55-212 (4.2ng/μL, 10% DMSO in saline) into the GCL of the MOB. All manipulations were performed on separate days. Behavioral performance was significantly decreased only in the WIN infusion into the MOB manipulation compared to vehicle, IP injection, and unmanipulated ODT (*$p < 0.001$).

Figure 13: Behavioral performance on odor detection task after infusion of vehicle (10% DMSO and 10% chremophor in saline) into the GCL of the MOB, and after infusion of AM251 (4.0 μg/μL, 10% DMSO, and 10% chremophor in saline) into the GCL of the MOB. All manipulations were performed on separate days. Behavioral performance was not significantly different between these two manipulations.

Conclusions and Interpretations

Intraperitoneal injection of WIN-55,212 has no significant effect on olfactory sensitivity in trained mice.

Intrabulb infusion of WIN-55,212 significantly decreases olfactory sensitivity in trained mice.

Intrabulb infusion of AM251 has no significant effect on olfactory sensitivity in trained mice.

***Interpretations*: Exogenous cannabinoids may only influence olfactory sensitivity in naïve (untrained) fasted mice. Or perhaps these data suggest a different interpretation of the findings from Soria-Gómez & Bellocchio et al. 2014. Exogenous cannabinoids may increase odor exploration due to an elevated hunger state. However, this process may be unrelated to olfactory sensitivity.**

Future Directions

To fully understand the mechanism behind our data on a cellular level, in the future we intend to perform a physiological complement to these behavioral data. We plan to measure neuronal firing in the MOB in response to infusion of WIN,55-212.

Due to the strong link between CB1 signaling and feeding behavior, coupled with the findings of Soria-Gómez & Bellocchio et al. 2014; future projects focusing on the role of physiological state in feeding behavior are necessary to understand the role of olfaction in the process of CB1 signaling causing increased food intake.

The olfactory habituation assay performed by Soria-Gómez & Bellocchio et al. 2014 (Figure 15) found that a CB1 agonist prevents mice from habituation to odor, providing some evidence for maintained or elevated odor interest.

Future experiments will also assess if the effect of cannabinoids on olfactory sensitivity is state dependent (i.e., fasted versus non-fasted mice), or if CB1 signaling disrupts olfactory habituation independent of hunger state.

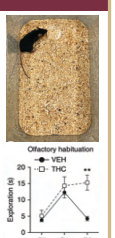


Figure 15: A mouse exploring odor (top). Exploration of an almond or isoamyl acetate after vehicle or WIN,55-212 injection (bottom). Figure from Soria-Gómez & Bellocchio et al. 2014.

Acknowledgements

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