UNIVERSITY OF CALIFORNIA SAN DIEGO

Investigating the role of the orbitofrontal cortex in learned modulation of innate olfactory behavior

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Jeremy Stark

Committee in charge:

Professor Cory Root, Chair
Professor Ralph Greenspan
Professor Byungkook Lim

2022

The Thesis of Jeremy Stark is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2022

EPIGRAPH

"Behavior is a consequence of experience. Experience acquired over long periods of evolutionary time can result in **innate** behaviors. Whereas experience over the life of an organism results in **learned** behaviors."

-Richard Axel

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

I want to thank the members of the Root lab that helped make my experiment's completion possible.

Thank you Dr. Root for your continual guidance, K-Dan for your expertise in data analysis and portrayal, and Amanda for your willingness to learn behavior training.

I also want to thank those above in addition to family and friends who supported my endeavor.

ABSTRACT OF THE THESIS

Investigating the role of the orbitofrontal cortex in learned modulation of innate olfactory behavior

by

Jeremy Stark

Master of Science in Biology

University of California San Diego, 2022

Professor Cory Root, Chair

Advantageous innate responses to environmental stimuli are commonplace in bacteria to higher-order animals. Plasticity of these responses allow for adaptation to environmental changes where innate preferences stop conferring survivability and reproductive advantages. The Orbitofrontal cortex (OFC) and other structures related to valence have been implicated in adaptation, but it is unknown if the OFC is required for learned modulation of innate odor preferences. If the OFC is involved in overriding the innate response to odor, then silencing it

should block a learned attraction to aversive odor. To test this prediction, we trained mice by pairing aversive odor with reward. Thirsty mice were presented with a choice between drinking small amounts of water in one port with an attractive odor or drinking more water in a port with an aversive predator odor. Preference was measured as the ratio of time spent in the aversive odor trimethyl-3-thiazoline (TMT) port compared to the attractive odor port, 2-phenylethanol. After several days of training, mice learned to increase their preference for TMT. We used chemogenetic silencing of the OFC on a test day to observe any changes in TMT preference. On the test day, control mice with unimpaired OFCs continued to increase their preference for TMT while experimental mice with silenced OFCs decreased their preference for TMT. Based on experimental findings, the OFC is required for using previously learned changes in preference that go against innate valence in choice-based situations. This indicates that the OFC plays a role in modulating valence perception of innate responses.

INTRODUCTION

Millions of generations of natural selection give rise to emergent traits that may seem too remarkable to have mutated by chance. Many of these traits are visibly seen and have clear benefits to the organism like growth of a protecting bony turtle shell or increased reflection of the retinal choroidal tissue in owls and other nocturnal predators to process more light. Other phenotypes are not visibly seen but drive internal states or even choice behavior. One example of these traits is the natural or innate aversion to predator molecules. This fascinating case can be seen in laboratory bred rodents that had never encountered foxes or any other sort of predator. Yet they still act in an aversive fashion to trimethyl-3-thiazoline (TMT), a component of fox odor that is often used as an aversive stimulus (Fendt et al. 2008). Mice and humans have been observed to have an innate response to certain odors in an attractive or aversive manner. While these innate drives often confer, given an organism's environment, a flexibility of these innate responses must be achieved to be better fit for survival and reproduction in a changing environment.

For the sensation of smell, chemical signals are sensed by olfactory neurons tuned to specific molecular features. Populations of these neurons transduce complex signals to the olfactory bulb, the most anterior part of the rodent brain. From here, among other projections, the signal primarily goes to the piriform cortex, associated with many processes including odor processing and identification (Russo et al. 2020), as well as the cortical amygdala that drives innate responses (Root 2014). The orbitofrontal cortex (OFC) is known to be downstream of the piriform cortex and therefore likely to be an important higher order cortex that uses odor information (Illig, 2005). There have been notable increases seen in response to conditioned odor

stimuli after learning (Rolls, 2008). The OFC sends a variety of cortical projections, most of which are involved in learning and hedonics. The OFC also has projections involving brain circuits for innate behavior that could alter the behavioral response. In monkeys with OFC lesions, incorrect choices were made in behavior testing paradigms that involved aversive or rewarding cues (Rolls, 2004). Furthermore, in monkeys it was found that neurons that fire in relation to a type of food eaten to satiety have selective decreases in firing to that food compared to other foods (Rolls, 2008).

Previous works have shown that the OFC is involved in value coding and choice selection when different tastes are present. The OFC is also known to be involved in the reversal of previously learned behavior, though not required for initial association learning (Stalnaker, 2007). Researchers have studied this area of the brain and observed input and output projections in relation to odor and valence (Ramirez-Lugo et al. 2016). The OFC has been found to encode value after learning (Wang et al., 2020). The OFC is required for reversal learning, further suggesting a role in adaptive value assignment (Schoenbaum, et al., 2002). There is still much to learn, and a causal experiment could shine a light on how we think odors, valence, and memory, interplay involving this area of the prefrontal cortex. Including if the OFC is required for using a learned change in preference for an innate odor.

Researchers have studied causal effects of OFC function in human subjects with temporal lobe epilepsy (Bérard et al. 2021). In a control group, areas of the brain other than the OFC were electrically stimulated with little to no effects. Interestingly, stimulation of the OFC led to the occurrence of pleasant olfactory hallucinations in most of the patients. Olfaction also seems to involve a portion of the OFC in a lexical context, meaning no odor needs to be given for the OFC to be involved (Pomp et al. 2018). Autobiographical memories may also recruit the left posterior

OFC as seen in fMRI studies (Watanabe et al. 2018). More human studies narrowed down the time course of activation in the brain due to olfaction and saw theta and gamma waves in correlation with medial and superior OFC increases in activity (Bae et al. 2021). Researchers saw the need for the OFC in conscious odor identification and as a modulator of consciousness (Fagundo et al. 2015). Disruption of OFC activity was sufficient to impair olfactory-related choice correctness in humans as well (Howard et al. 2019). Lastly, in patients with late-life depression, changes in functional connectivity, including between the left OFC and the left calcarine gyrus, were correlated with worse odor identification scores (Yang et al. 2022). These studies show us the importance of OFC in humans for a variety of functions ranging from simple odor perception and identification to changes in choice-based behavior.

We studied the role of OFC in overwriting innate responses. The OFC was experimentally manipulated with chemogenetic designer receptors activated by designer drugs (DREADDS) to test its role in overwriting innate responses. The most similar previous research, using optogenetics, yielded promising yet inconclusive results so I have tried using a different experimental approach of chemogenetics and an altered training paradigm in mice. Through training, mice learned to associate the naturally aversive TMT odor with a greater water reward. At the end of training, once the mice showed a greater preference for TMT, Clozapine-N-Oxide (CNO) was administered to activate the hM4Di receptors within the OFC. The mice had their TMT preference tested again to observe any changes in response from the learned TMT response.

METHODS

Twenty C57BL6 mice were selected and thirteen were placed into the experimental group while seven were placed into the control group. Bilateral stereotaxic viral injections were carried out targeting the OFC at anterior, ventral, and medial coordinates of +2.7, -2.4, and +/- 1 respectively. Coordinates are given in millimeter distances with respect to the bregma. The experimental group was injected with 500nl of AAV-synaptophysin-hM4Di-mCherry while the control group was injected with AAV-humansynaptophysin-EYFP with titers of 8.6x10 12 and 4.3x10 13. This was done for a first cohort of 8 and 4 mice respectively. In a second cohort of mice, 5 and 3 experimental and control mice respectively received the same viral injections with the only difference being a reduction of the titers to 1x10 12 for both. Each set of mice were given three weeks to recover and viral expression.

After three weeks, mice were deprived of water to make them thirsty and thus motivated to drink. Henceforth it should be noted that mice had their normal source of water in their cage removed for the duration of the experiment, making the port/waterspout during training the only source of water. After one day of complete water deprivation, mice underwent water training for two days. Water training was conducted to condition mice to drink water from waterspouts in the training chambers. During water training, mice were placed into a chamber with access to two water ports that equally dispensed a small, 15ul, droplet of water with a short interval time between following droplets when the spout was licked. Each mouse received one of these sessions a day that lasted 20 minutes (Figure 1.1). All mice learned to sample the spouts and received water on the first day of water training. By the end of the second water training day, each mouse spent similar amounts of time in each port. Chamber dispensation and data retrieval were controlled with a Labview program. Time in port was measured with an IR beam

positioned at the opening of the port, in a space necessary to be crossed to access the waterspout. When the IR beam was broken for a period of time by the mouse, the collection of these durations were summated and recorded to yield a time in port value.

Odor training began after water training and lasted seven days. Odor training was similar to water training. The difference was the pairing of TMT odor dispensation to a short minimum interval of one second between water dispensation while neutral odor, 2-phenylethanol (2PE) dispensation, was paired with a long interval of ten seconds between water dispensation in respective ports (Figure 1.2). This was done to increase preference for TMT through training since the TMT paired spout is more rewarding to the mouse. The port with paired TMT was alternated to be the left or right port each day for the mice. Days 1, 4, 7, 8, and 9 had water access turned off for the first 5 minutes while still fluxing odor at the respective port when the IR beam was broken indicating mouse entry, to create a probe trial period. Day 1 was the first time that the mice had ever been exposed to either odor and provided a baseline value. Days 2, 3, 5, and 6 had water immediately available including the first 5 minutes and were used to ensure that mice continually sampled ports for water. This was done to limit time that could be extinction training. Days 4 and 7 functioned as classic probe trials one and two respectively (Figure 2a, b). A saline injection probe on day 8 controlled for how an injection may influence choice behavior. The saline injection was given 1 hour prior to placement of each mouse in the chamber and was given as X ul per gram intraperitoneally (i.p.). Lastly, day 9 was the test date where each mouse was injected with CNO. Each injection was given 1 hour prior to placement of each mouse in the chamber and was given as X ul per gram i.p. Days 8 and 9 did not have water dispensation for the first 5 minutes to gather information on TMT preference.

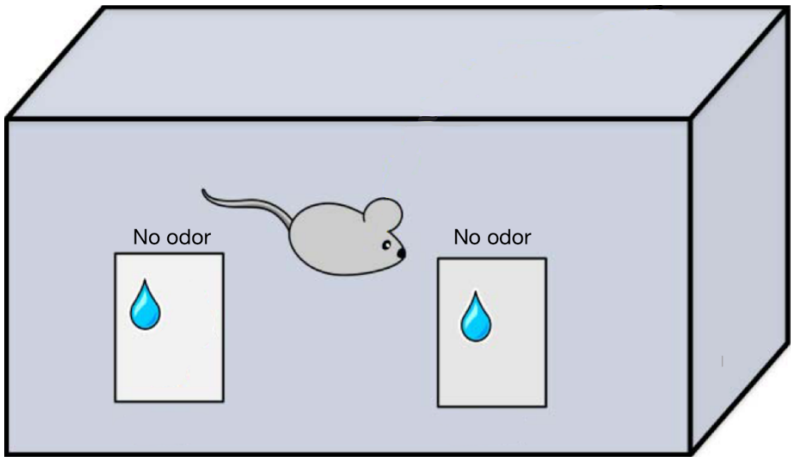

Figure 1.1: Depiction of the water training mouse chamber indicating the two ports of choice with associated odor and equal water dispensation disparity. The TMT and 2PE port each dispensed water at a 1Hz frequency.

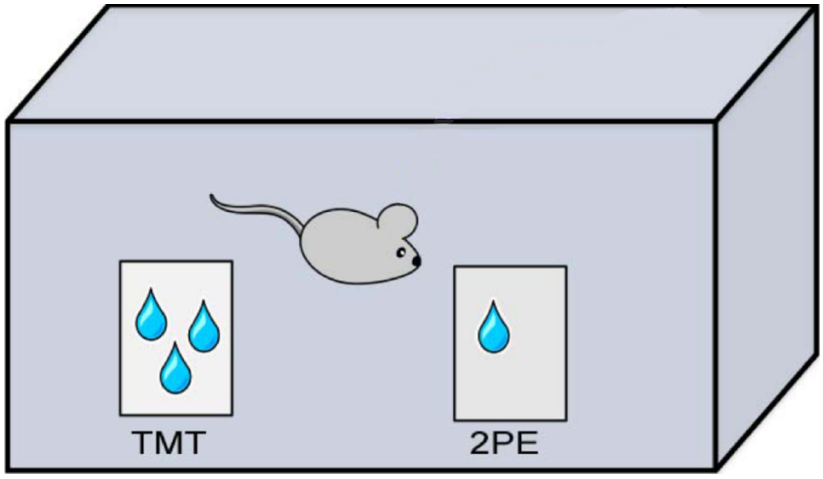

Figure 1.2: Depiction of the odor training mouse chamber indicating the two ports of choice with associated odor and respective water dispensation disparity. The TMT port dispensed water at a 1 Hz frequency while the 2PE port dispensed water at a 0.1 Hz frequency.

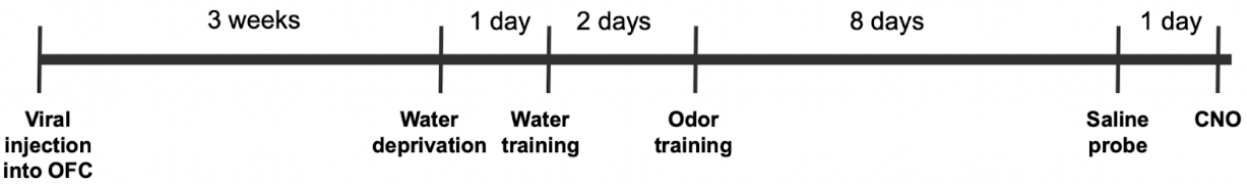

Figure 1.3: Timeline showing onsets and durations of time for mouse manipulation and behavior training, culminating in CNO testing.

To confirm that chemogenetic silencing worked, histological analysis of c-fos was used as a proxy for neuronal activity. Following the testing day, mice were injected with CNO i.p. 1 hour prior to exposure to TMT in a chamber with minimal ventilation for 5 minutes to induce c-fos expression. Intracardiac perfusions with 10 ml 4% paraformaldehyde were carried out 1 hour after odor exposure. The brains were harvested and sectioned at 100um width coronally. Immunostaining was carried out to visualize c-fos expression. Under fluorescence microscopy, c-fos expression was analyzed for neuronal activity. hM4Di-mCherry (experimental) and EYFP (control) signal were also analyzed for accuracy and spread of stereotaxic targeting and viral construct expression.

An exclusionary criterion was adopted to ensure only mice that successfully learned through training were used. The criterion considered the baseline TMT preference and the highest expected levels of TMT preference at the end of training. Mice that had an increase of at least 1.25x preference for TMT over baseline compared to the average of probe day 2 (day 7 of training) and the saline probe (day 8 of training) were kept. Of the initial 13 experimental mice, 8 continued while of 7 initial control mice, 5 continued. Using the one sample kolmogorov-smirnov test, experimental and control data on the saline probe and test day were both found to not be normally distributed. For this reason, the Wilcoxon statistical test for nonparametric data sets were used over sample t tests.

RESULTS

To investigate the contribution of the OFC to learned modulation of innate response, a 2-port choice assay was used. In this paradigm, water restricted mice were presented with two water/odor ports. The learning involved several stages: first they were presented with equal water and no odor at both ports. Next, they were presented with equal water but odor of opposite valence (aversive TMT and the attractive 2PE) in the two ports, to measure their baseline preference. Lastly, the port with attractive odor was devalued to have 10x less water dispensed, causing them to prefer the port with aversive odor and more water.

To test if the OFC is necessary for overwriting innate aversion, twenty mice underwent eight days of training followed by one test day. Of these mice, 8 experimental and 5 control mice continued after passing the exclusionary criterion. Clozapine-N-Oxide (CNO) was administered intraperitoneally to the remaining control and experimental groups of mice. CNO, acting as a ligand, activated hM4Di in the OFC of experimental mice and caused silencing of the OFC while control mice did not have hM4Di.

Their preference between the two ports was quantified by taking measurements of port entry, duration, and upon licking of water dispensing spouts within the port. Measurements of experimental significance were taken during an initial 5-minute period of baseline, probe, and test days where no water dispensation was given. This allowed for gauging of changes in preference for TMT across training days. The averaged ratio of time spent in the TMT port compared to the 2PE port can be seen in Figure 2.1; this can be thought of as TMT preference. A value of 1 would indicate an equal preference for TMT and 2PE, a value less than 1 would indicate lower preference for TMT compared to 2PE, and a value above 1 would indicate higher

preference for TMT. As expected, there was a general positive trend seen in TMT port preference through training seen in the control and experimental groups.

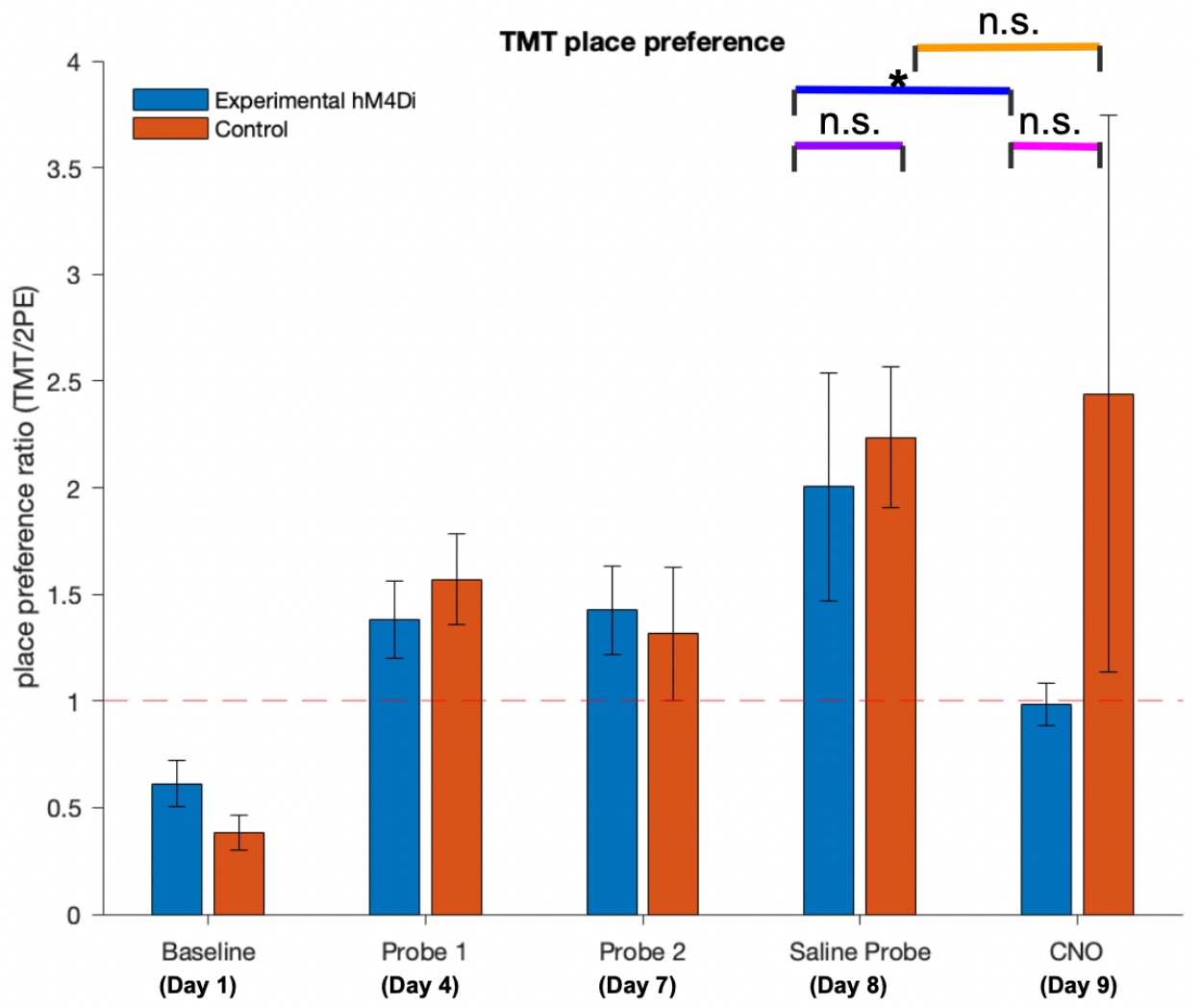

Figure 2.1: The averaged ratio of time spent in the TMT port divided by time spent in the 2PE port during probe trials constituting the first 5 minutes of behavioral sessions where dispensation of water was withheld. 8 experimental mice had hM4Di-mCherry while 5 control mice had EYFP virally expressed in the OFC.

TMT preference as ascertained through lick data as seen in Figure 2.2 further supports this observation. Using the Wilcoxon statistical test, though there was a trend for higher licking

in the control group, there was no statistically significant difference in TMT preferences between control (mean: 2.235 +/- 0.330) and experimental mice (mean: 2.004 +/- 0.534) during the saline probe for measured time in port (p-value: 0.354). Using the Wilcoxon test, there was also no statistically significant difference between control (mean: 2.503 +/- 0.4043) and experimental mice (mean: 1.619 +/- 0.262) TMT preferences for measured time licking (p-value: 0.065). This indicates that through training, experimental and control mice were able to learn the association between TMT and water commensurately.

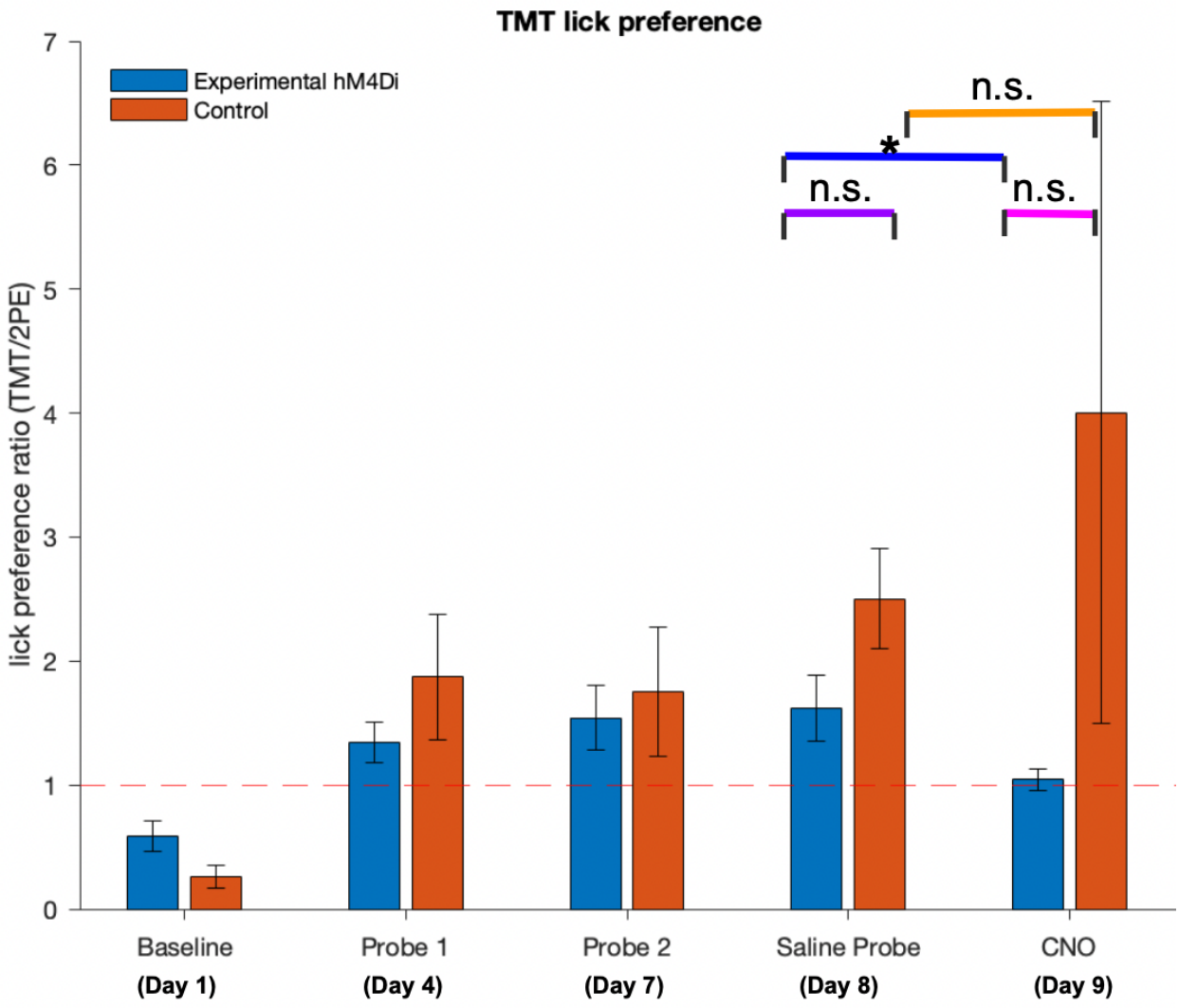

Figure 2.2: The averaged ratio of time spent licking the TMT port divided by time spent licking the 2PE port. Data was retrieved from the same set of 13 mice and on the same day.

Next, CNO was administered to test if silencing the OFC affected the expression of learned behavior. There was no diminishment of TMT preference of the control mice after the administration of CNO. This can be seen with a Wilcoxon test of time in port preference between control animals on saline day (mean: 2.2352 +/- 0.3303) and CNO testing day (p-value: 0.421). The Wilcoxon test of lick preference was also not statistically significant (p-value: 0.421). This indicates that the CNO is not having off-target effects in the control group which validates CNO effects if seen in the experimental group.

With the administration of CNO, TMT preference was diminished in the experimental group towards baseline. Using the Wilcoxon test to compare place preference of the experimental mice between saline day and CNO day yielded a statistically significant difference (p-value: 0.015). Similarly, using the Wilcoxon test to compare lick preference of the experimental mice yielded a statistically significant difference (p-value: 0.038). This indicates that there was a difference in the experimental mice from the administration of CNO. In this case there was a significant diminishment in TMT preference from time in port data as well as licking data. The above statistical analyses indicate that control and experimental sets of mice that learned to increase their TMT preference through rewarding operant training. Looking from saline probe to administration of CNO on the testing day, control mice did not have a statistically significant change while experimental mice did. However, there was not a significant difference between values of the control and experimental TMT preference on the CNO testing date for time (p-value: 0.622) nor licking (p-value: 0.724). This lack of significance between the two groups may be due to high variability in the control group. Nonetheless, a significant decrease in preference within the experimental group but not the control group indicates a real effect of silencing the OFC in overwriting innate aversive cues.

The baseline TMT preference were higher in the experimental group than in the control group. The baseline mean TMT preferences for experimental mice were 0.612 for time in port and 0.583 for licking while mean TMT preferences for control mice were 0.383 for time in port and 0.260 for licking. For time in port the difference was not statistically significant (p-value: 0.284) nor was it for licking data (p-value: 0.222) using Wilcoxon tests.

The number of entries made by mice into either the TMT or 2PE ports was measured as a ratio of TMT entries divided by 2PE entries (Figure 2.3). After baseline ratio values of less than 1, from probe 1 the ratio of entries for control and experimental were close to values of 1. A diminishment in ratio within control and experimental groups can be seen from saline probe to CNO test day with a lesser diminishment.

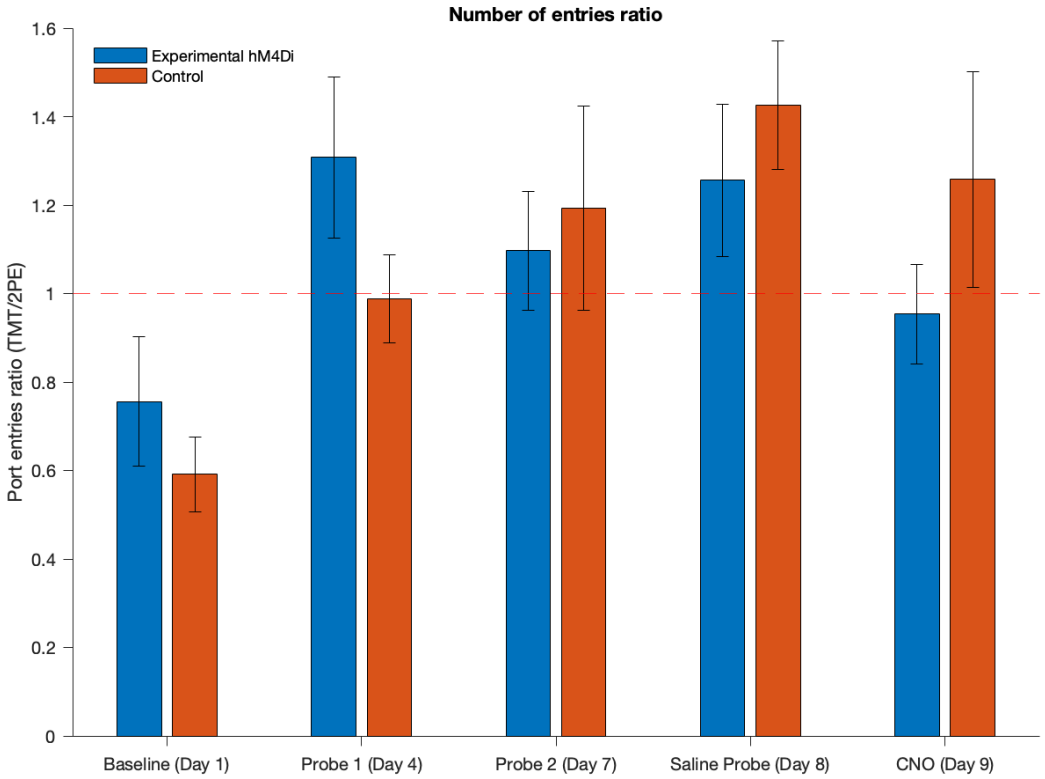

Figure 2.3: The averaged ratio of TMT port entries divided by 2PE port entries during the first 5 minutes of probe trials. Data was retrieved from the same set of 13 mice and on the same day.

The averaged total water consumption of mice in both groups was recorded from minute 5 to 15 of sessions. Overall, there were no significant differences in daily water consumption between control and experimental groups, indicating that both groups drank similar amounts of water across days.

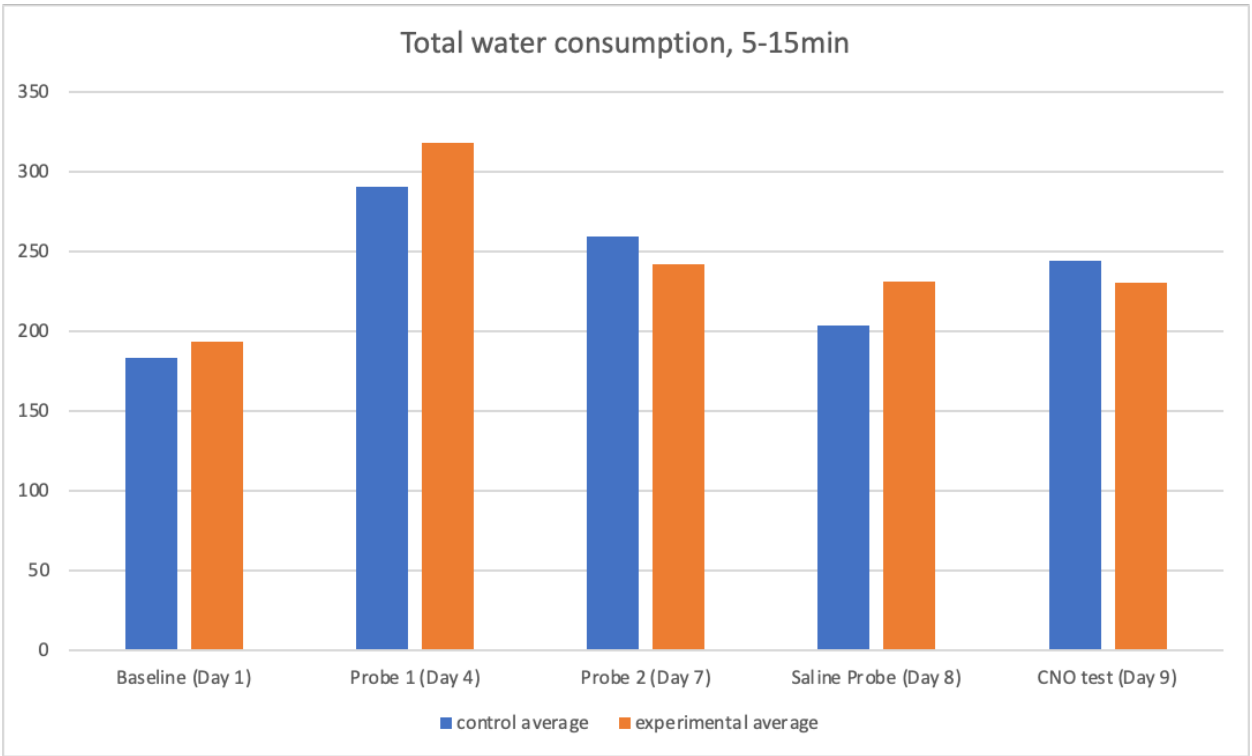

Figure 2.4: Summated amount of water consumed from TMT and 2PE ports between control and experimental mouse groups, measured in droplets (15ul each).

The efficacy of viral targeting and chemogenetic silencing was assessed by histology. Histology showed accurate targeting of the OFC at the coordinate of +2.7, -2.4, and +/- 1 (Figure 3.1) in all animals. To determine whether expression of hM4Di decreased neuronal activity, the immediate early gene, c-fos, was used as a proxy for neuronal activity. Mice were administered CNO and exposed to TMT for five minutes, before harvesting their brains one hour later (Figure 3.2). Analysis of c-fos expression revealed a dramatic reduction of c-fos positive neurons in the area labeled with hM4Di-mCherry. Thus, hM4Di appears to have been effective in silencing the OFC.

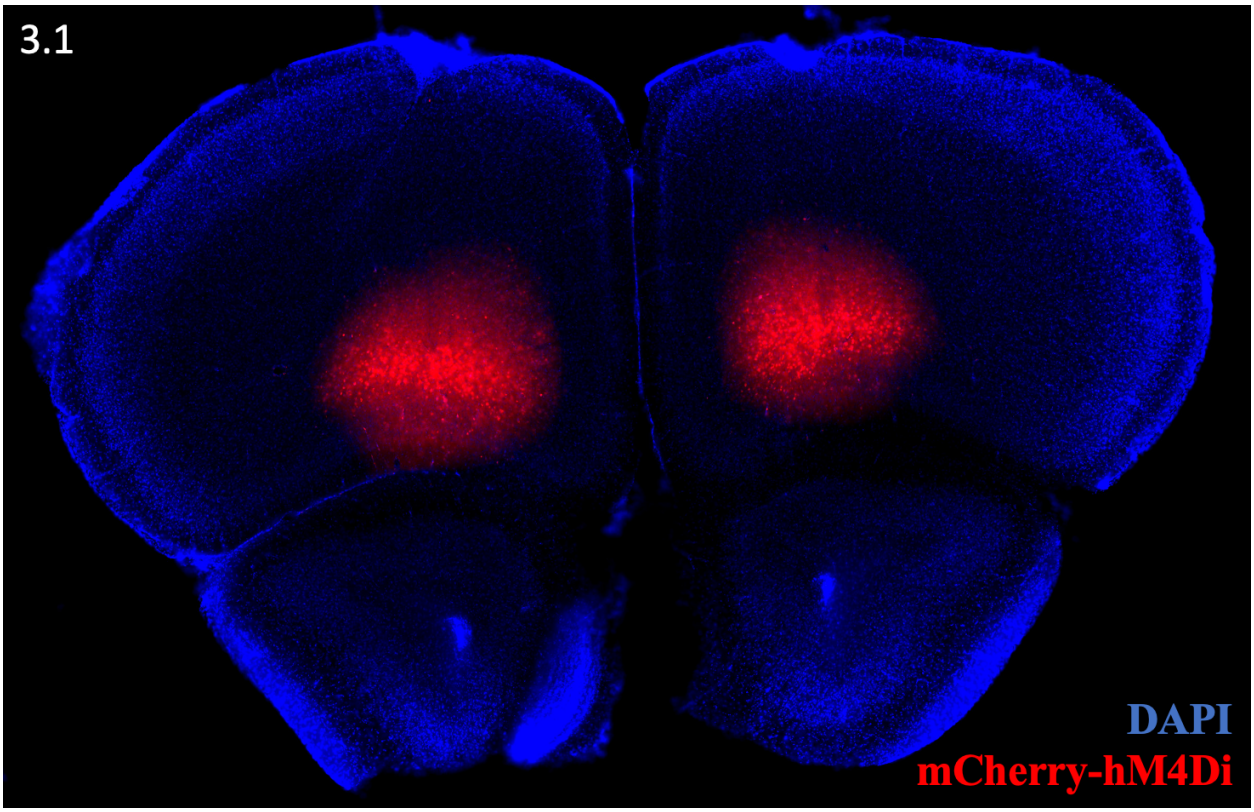

Figure 3.1: Representative histology showing a coronal section of an experimental mouse brain. Located 2.7mm anterior to the bregma, the viral injection was given 1mm lateral from the median and with a depth of 2.4mm ventrally, targeting the OFC. Blue staining is from DAPI and indicative of nuclei, red staining is from mCherry within the experimental viral construct that was injected and indicative of hM4Di positive neurons.

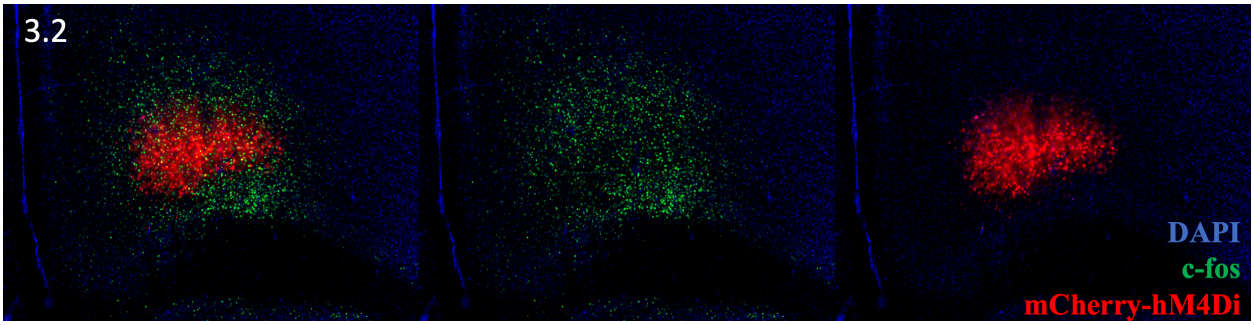

Figure 3.2: Representative histology showing a zoomed in coronal section of the same experimental mouse brain as in Figure 3.1. Zoomed into the injection site, Blue staining is from DAPI, green staining is from antibody fluorescence tagging c-fos, a marker of neural activity, and red staining is from mCherry staining.

DISCUSSION

Mice were taught to increase their preference for an aversive odor to see if they required the OFC for this learned change in preference for innate aversive odors. We showed that mice had a low baseline preference for fox odor, TMT, with respect to 2PE. Observed innate mouse behavioral preference in a novel assay, is consistent with previous reports of innate preference including response to TMT (Root et al. 2014). Through operant rewarding training, TMT preference rose in the control and experimental group in an apparent override of innate response. In this case, all mice overcame their aversion to TMT in the presence of abundant water via operant learning. A continuation of increased TMT preference was also seen in the control group after CNO administration. Indicating that sustained training should not lead to a d ecrease in preference. There was a decrease in TMT preference seen among the experimental mice when CNO administration activated hM4Di to silence the OFC. This decrease indicates that the OFC is necessary for behavioral choice responses of overwritten innate aversion. This shows that upon contact with a naturally aversive odor that has a learned overwritten positive valence, the OFC is required for reversal of response as though the odor wasn't innately aversive.

For mice in general, innate representations of predator odors in the cortex, like TMT, in an aversive fashion can convey greater chances of survivability if a predatory fox were in the environment. However, in the natural environment animals must balance potentially competing drives. For instance, the necessity of food or water may compete with the instinct drive to avoid predation. Our data indicate that the OFC affords plasticity in responding to changes in our environment when one must act oppositely from natural instinct.

In line with studies that intersect innate sensation with OFC function, unilateral recruitment of the OFC in those with the inability to consciously identify or assign valence to odors was sufficient to evoke physiologically appropriate responses (Li, 2010). This study was performed in humans where right OFC impairment led to anosmia. It would be interesting to study unilateral silencing of the OFC for any differences in behavior. In a model where mice make decisions based on the outcome of previous trials, researchers found that OFC impairment led to inability to use previously learned trial outcomes (Baltz, 2018). However, mice were still able to associate value according to their changing motivational/internal state with impaired OFCs. Though this study explored modes of sensation other than olfaction, the ability to use sensory information in choice-making decisions and the inability to use previously learned experiences mirrors results seen in our experiment.

Throughout training there was not extreme variability in mouse preferences. A depiction of this can be seen in the standard error of mean (SEM) error bars in Figure 2.1. A larger range of error can be seen in the time in port place preference by the saline day while both time in port and licking preferences share a great range of error in the control group on the CNO testing day. This seemed to be due to a single control mouse on the CNO testing day that gave a TMT preference ratio value of 7 while most other control mice were in the range of 1.5 to 2.5. Though this one outlier-like value out of 5 control mice increases the SEM, it fits the general narrative of increased TMT preference through learning. Concurring licking data of Figure 2.2 dispels the notion that the mouse simply broke the IR beam indicating mouse placement and was actively licking the port.

The number of entry ratios from Figure 2.3 shows that mice of different groups sampled both ports with similar frequency. There were higher sampling rates of TMT on the baseline and

probe 1 day in the experimental groups which may correlate with higher place and lick preference ratios for TMT on these days. There was also a greater decrease in sampling rate of TMT for experimental mice after the CNO injection compared to that of control mice. Future direction can include larger sample size that specifically studies why there is a higher baseline preference and sampling rate for TMT among experimental mice compared to control mice.

The total water consumption from Figure 2.4 shows that mice of different groups drank similar amounts of water. This means changes in preference were likely not caused by differences in water consumption.

The increased day to day variability in preference of experimental mice may be due to left versus right preference. There was also an abnormally higher mean preference for TMT seen in the experimental group compared to the control group though it was not statistically significant. This coupled with a lessened degree of learning preference for TMT points to the experimental mice having toxicity caused by high titer viral expression of hM4Di in mice (Goossens et al. 2021). Having a control where mice still receive hM4Di and are injected with saline instead of CNO may help determine if hM4Di influenced variability even before CNO administration.

Randomization of training between box 1 and 2 as opposed to alteration may solve the possible confounding variable of left versus right preference, even as alternation was initially proposed as a way to limit left versus right preference. Alternatively, though the OFC may be necessary for overwriting of innate aversion, a projection upstream or downstream may be the locus of overwriting. Connectomics to find the locus could be a potential future experiment. Future experiments can change the methodology to allow for testing of overwriting cues that are

innately appetitive rather than aversive. Experiments could also test the ability for recovery after the silencing effect of CNO and hM4Di have faded, to see if the manipulation is reversable. Experimentation could continue past the CNO test day for control mice, experimental mice without further CNO injections, and experimental mice with further CNO injections as a positive control. I hope more studies can target the OFC as a central component for plasticity of innate odor responses. Such experiments could find interventions to treat human disorders including patients with late-life depression who have poor odor identification (Yang et al. 2022).

REFERENCES

1. Axel, Richard. “Mind Brain Behavior Symposium - Keynote: Catherine Dulac with Opening Remarks by Richard Axel.” YouTube, YouTube, 6 May 2019, https://www.youtube.com/watch?v=z-IGJH1XhPc. Accessed 5 June 2022.

2. Bae J, Kim K, Moon SA, Choe HK, Jin Y, Kang W-S, Moon C. Time Course of Odor Categorization Processing. Cereb Cortex Commun. 2021;2(4):tgab058. Oct 5. doi:10.1093/texcom/tgab058

3. Baltz ET, Yalcinbas EA, Renteria R, Gremel CM. Orbital frontal cortex updates state-induced value change for decision-making. eLife. 2018;7:e35988. doi:10.7554/eLife.35988

4. Bérard N, Landis BN, Legrand L, Tyrand R, Grouiller F, Vulliemoz S, Momjian S, Boex C. Electrical stimulation of the medial orbitofrontal cortex in humans elicits pleasant olfactory perceptions. Epilepsy Behav. 2021;114(Pt A):107559. doi:10.1016/j.yebeh.2020.107559

5. Fagundo AB, Jiménez-Murcia S, Giner-Bartolomé C, Islam MA, del la Torre R, Pastor A, Casanueva FF, Crujeiras AB, Granero R, Banos R, Botella C, Fernandez-Real JM, Fruhbeck G, Gomez-Ambrosi J, Menchon JM, Tinahones FJ, Fernandez-Aranda F. Modulation of Higher-Order Olfaction Components on Executive Functions in Humans. PLoS One. 2015;10(6):e0130319. Published 2015 Jun 17. doi:10.1371/journal.pone.0130319

6. Fendt M, Endres T. 2,3,5-Trimethyl-3-thiazoline (TMT), a component of fox odor - just repugnant or really fear-inducing?. Neurosci Biobehav Rev. 2008;32(7):1259-1266. doi:10.1016/j.neubiorev.2008.05.010

7. Goossens MG, Larsen LE, Vergaelen M, Wadman W, Van den Haute C, Brackx W, Proesmans S, Desloovere J, Christiaen E, Craey E, Vanhove C, Vonck K, Boon P, Raedt R. Level of hM4D(Gi) DREADD Expression Determines Inhibitory and Neurotoxic Effects in the Hippocampus. eNeuro. 2021;8(6):ENEURO.0105-21.2021. Published 2021 Nov 4. doi:10.1523/ENEURO.0105-21.2021

8. Howard JD, Reynolds R, Smith DE, Voss JL, Schoenbaum G, Kahnt T. Targeted Stimulation of Human Orbitofrontal Networks Disrupts Outcome-Guided Behavior. Curr Biol. 2020;30(3):490-498.e4. doi:10.1016/j.cub.2019.12.007

9. Illig K. R. (2005). Projections from orbitofrontal cortex to anterior piriform cortex in the rat suggest a role in olfactory information processing. The Journal of comparative neurology, 488(2), 224–231. doi:10.1002/cne.20595

10. Li W, Lopez L, Osher J, Howard JD, Parrish TB, Gottfried JA. Right orbitofrontal cortex mediates conscious olfactory perception. Psychol Sci. 2010 Oct;21(10):1454-63. doi: 10.1177/0956797610382121. Epub 2010 Sep 3. PMID: 20817780; PMCID: PMC3627397.

11. Pomp J, Bestgen AK, Schulze P, Muller CJ, Citron FMM, Suchan B, Kunchinke L. Lexical olfaction recruits olfactory orbitofrontal cortex in metaphorical and literal contexts. Brain Lang. 2018;179:11-21. doi:10.1016/j.bandl.2018.02.001

12. Ramírez-Lugo, L., Peñas-Rincón, A., Ángeles-Durán, S., & Sotres-Bayon, F. (2016). Choice Behavior Guided by Learned, But Not Innate, Taste Aversion Recruits the Orbitofrontal Cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience, 36(41), 10574–10583.https://doi.org/10.1523/JNEUROSCI.0796-16.2016

13. Rolls ET. The functions of the orbitofrontal cortex. Brain Cogn. 2004;55(1):11-29. doi:10.1016/S0278-2626(03)00277-X

14. Rolls ET. Functions of the orbitofrontal and pregenual cingulate cortex in taste, olfaction, appetite and emotion. Acta Physiol Hung. 2008;95(2):131-164. doi:10.1556/APhysiol.95.2008.2.1

15. Russo MJ, Franks KM, Oghaz R, Axel R, Siegelbaum SA. Synaptic Organization of Anterior Olfactory Nucleus Inputs to Piriform Cortex. J Neurosci. 2020;40(49):9414-9425. doi:10.1523/JNEUROSCI.0965-20.2020

16. Schoenbaum, G., Nugent, S. L., Saddoris, M. P., Setlow, B. (2002). Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. Neuroreport, 13(6), 885-890. 10.1097/00001756-200205070-00030

17. Thomas A. Stalnaker, Theresa M. Franz, Teghpal Singh, Geoffrey Schoenbaum, Basolateral Amygdala Lesions Abolish Orbitofrontal-Dependent Reversal Impairments, Neuron, Volume 54, Issue 1, 2007, Pages 51-58, ISSN 0896-6273, doi:10.1016/j.neuron.2007.02.014.

18. Watanabe K, Masaoka Y, Kawamura M, Yoshida M, Koiwa N, Yoshikawa A, Kubota S, Ida M, Ono K, Izumizaki M. Left Posterior Orbitofrontal Cortex Is Associated With Odor-Induced Autobiographical Memory: An fMRI Study. Front Psychol. 2018;9:687. Published 2018 May 11. doi:10.3389/fpsyg.2018.00687

19. Wang PY, Boboila C, Chin M. Higashi-Howard A, Shamash P, Wu Z, Stein N P, Abbott L F, Axel R. Transient and Persistent Representations of Odor Value in Prefrontal Cortex. Neuron, 108(1), 209–224.e6. doi:10.1016/j.neuron.2020.07.033

20. Yang M, Chen B, Zhong X, Zhou H, Mai N, Zhang M, Wu Z, Peng Q, Wang Q, Liu M, Zhang Si, Lin G, Hummel T, Ning Y. Disrupted olfactory functional connectivity in

patients with late-life depression. J Affect Disord. 2022;306:174-181. doi:10.1016/j.jad.2022.03.014