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Role of the Hippocampus in Adaptation to Reward Loss: Emotionality or Cognitive Flexibility?

By

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A Paper Presented to the
Faculty of Mount Holyoke College in
Partial Fulfillment of the Requirements for
the Degree of Bachelor of Arts with
Honor
Program in Neuroscience and Behavior
South Hadley, MA 01075
May 2022

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This paper was prepared
under the direction of
Professors Marta Sabariego and André O. White
for eight credits

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Acknowledgements

This thesis is a result of immense support from my community and there aren't enough words in this world to describe the gratitude that I feel for each and every single one of them.

Firstly, I would like to extend my gratitude to my thesis advisors Marta Sabariego and André White. I am extremely grateful to have such incredible, dedicated, and caring individuals in my life not only as my thesis advisors but also as my mentors. I want to thank Professor Sabariego for introducing me to the world of research and showing me that it is important to do everything with passion and integrity. I look up to the way she conducts herself as not only a researcher but also a mentor, a professor and a mother. If it wasn't for her, I would not be in this field and I would not be loving what I do as much as I love it. I want to thank Professor White for teaching me the importance of observation. Even though I was only able to make his acquaintance last year, it feels like he has enriched my life with his knowledge and wisdom. He is the most patient teacher and I always admire how well he is able to guide me, while taking into consideration the state of mind that I am in. He has not only helped me grow as a researcher but also guided me through career choices and decisions. Professor Sabariego and White together, have been my pillars of support throughout the past year and I want to thank them for believing in me to see this project through.

I would also like to thank a few other processors whose lessons have made it possible for me to come this far. Professor Schwartzer has been my advisor throughout my college life and he has always encouraged me to follow my passion and believed in the work that I do. Professor Binder and Professor Natasha Matos have also been important elements in guiding me throughout my years at MHC. Thank you to all my other professors that have helped me get through my college years.

I would also like to thank the Neuroscience and Behavior Department's Curtis Award for funding equipment that made this research possible.

Next, I have to thank a group of individuals without whom running the experiments for my thesis would have never been possible. Members of the Sabariego and White Labs: Eleftheria, Grace, Divna, Lucy, Liv, Jennie, Shi, Petra, Gabby, Sarah, Kylee, Zainab, Nafeesah, Zena, and Hiba; thank you for your support and enthusiasm towards this project. I especially want to thank Eleftheria, Grace, Divna, Lucy, Shi, and Liv for your tireless enthusiasm in running three cohorts of animals across various behavioral paradigms. I remained impressed by your grit and determination towards research. Grace and Eleftheria, you are both forces of nature and I have indeed relied on you two the most. To Jennie, Kylee, and Zainab, I want to thank you for supporting me through the data analysis of my project and I would not have wonderful graphs to show without your constant efforts understanding the coding and software aspects of this project. The people that make up both these labs are the most dedicated group of individuals I have ever met and it has been such a pleasure to be able to share my lab experience with you. All of you

have shown immense love and support towards me in this past year and I just want to thank you for always believing in me and lending kind words of encouragement.

To my grandparents, Abu, Ajju, Ajoba, and Vasanti Ajji. The four of you have been the cornerstone of every experience in my life. From teaching me important lessons as a kid, to making unfathomable sacrifices for the entire family so that we can come as far as we have, your capacity to keep giving never ceases to amaze me. To my parents, Geetanjali and Ashish Chitale, thank you for being my support system from day one. From listening to my endless rants, to giving me endless love and support for my achievements and just as much strength in times of struggle. I aspire to be at least half as good of a parent as you both have been. To the rest of my family members, thank you for always keeping faith in me and for being the loudest cheerleaders in my life. Without your blessings and the unforgettable memories that you have given me, I would not find the joy and courage to lead my life!

To my best friends who instantly turned into family. Kareena, Shreya, Aditi, and Kusha, I cannot put into words what your constant support and presence in my life means. The four of you have been witness to every high and every low that I have faced in the last four years of college and all I want to do is thank you for being with me and continuing to support me no matter where I am in life. All four of them are strong, independent, and dedicated women who never cease to amaze me. Especially during this past year, I want to thank you for listening to me when I needed you to be there. This thesis would really not have been possible without the four of you. Here's to a particular alum who has never stopped encouraging me in my academic and career endeavours: Megi. I admire Megi for all the hard work, passion, and dedication with which she does everything. I am lucky to have you as my friend and mentor. To my friends from across the world who have not been with me physically but always encourage me in spirit: Esha, Khushi, Mahek, Abrol, Parthiv. You all have seen me grow in different phases of my life and never stopped showing your support no matter how much physical distance might separate us.

It takes a village, it really does :)

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Abstract

Frustration is defined as an aversive emotional state triggered by reward loss. Despite compelling evidence associating reward loss with the etiology of anxiety and depression, neural correlates underlying adaptation to reward loss remain unidentified. Previous experiments conducted in our lab have demonstrated that hippocampal lesions impair the ability of rats to adjust to reward loss. However, it is unclear whether the impairment was due to a lack of emotionality (i.e. lesioned rats not feeling frustrated after reward loss) or a lack of cognitive flexibility (i.e. lesioned rats unable to modify previously learned responses). In order to investigate these questions, we exposed rats (with active and inactive hippocampi) to a reward-loss paradigm alongside a conditioned place preference (CPP) task designed to assess emotional responses. We found that while hippocampal-inactivated rats did not adapt their response to reward downshifts, they showed signs of negative emotion in the CPP task. This suggests that animals with a dysfunctional hippocampus do not lack emotionality but rather experience cognitive inflexibility. This research could contribute to revealing the function of neural circuits of reward loss, a critical step in developing treatments for anxiety, depression, and stress-related disorders.

Introduction

In the study of learning and memory, the topic of incentives has been a large area of research since the beginning of the twentieth century (Flaherty, 1996). Eliott (1928) demonstrated that rats increased mistakes and time taken to eat a reward when shifted from a regular wet food mixture (more palatable food reward) to a sunflower seed mixture after animals were trained with the wet food mixture. At the time, Eliott concluded that the increase in time and mistakes by the rats to navigate the complex maze was a result of a change in reward, different from what the rats initially "expected." The mismatch between the expectation of a wet food reward mixture and the actual presentation of the sunflower seed mixture led to an increase in the mistakes and time. Other experimenters also noted similar results in primate research where monkeys initially accept a piece of lettuce but reject it after seeing experimenters place a piece of banana, which is an incentive that they prefer over lettuce. These results suggest that animals indeed function under the expectancies of incentives, and there are changes in behavior when the value or expectation of these incentives is not met. This is referred to as incentive relativity and is defined as the incongruence between the absolute value of a reward and its expected value (Torres and Papini, 2017).

Chapter 1: Background Concepts

Incentive here is used as a synonym of reward and appetitive reinforcer. Hence, the words "incentive" and "reward" have been used interchangeably in this document. Over the years, experimenters have studied incentive relativity using protocols that involve manipulating expected rewards - either by upshifting (increasing) or downshifting (decreasing) them. Here is a table clarifying terminology that is widely used in the field of incentive relativity:

	SUCCESSIVE NEGATIVE CONTRAST	
Task	**Consummatory successive negative contrast (cSNC)**	**Instrumental successive negative contrast (iSNC)**
Downshift condition	Initial exposure to a large reward followed by few sessions of exposure to a smaller reward	
Control	Always exposed to a small reward	
Dependent Variable	Reward consumption (fluid intake, lick frequency)	Anticipatory behavior (running, lever pressing)
Effect	Downshifted animals consume less of the small reward than unshifted controls	Downshifted animals respond less for the small reward than unshifted controls

	SUCCESSIVE POSITIVE CONTRAST	
Task	**Consummatory successive positive contrast (cSPC)**	**Instrumental successive positive contrast (iSPC)**
Upshift	Initial exposure to a small reward followed by a few sessions of exposure to a	

condition	large reward	
Control	Always exposed to a large reward	
Dependent Variable	Reward consumption (fluid intake, lick frequency)	Anticipatory behavior (running, lever pressing)
Effect	Upshifted animals consume more of the large reward than unshifted controls	Upshifted animals respond more for the large reward than unshifted controls

Table 1. Most used incentive relativity procedures as typically studied in rats.

Expectancy refers to a prediction of the impending presentation of a particular reward. The terms *nonreward* and *reward loss* are here used interchangeably and refer to the omission, reduction in magnitude, or quality degradation of an appetitive reinforcer (e.g. food). The term *contrast* refers to an apparent exaggeration of reward differences provoked in animals experiencing two rewards in a particular situation (Flaherty, 1999). Contrast implies a comparison of incentives in which one is always present and the other may be remembered, or anticipated (Torres & Papini, 2017). In contrast effects, the animal is exposed to a transition in incentive value from higher to lower (negative contrast) or from lower to higher (positive contrast). The term *successive* refers to the fact that there is usually a single transition in reward that occurs across sessions. In successive contrast effects, the organism is exposed to a transition in incentive value from higher to lower (successive negative contrast, SNC) or from lower to higher (successive positive contrast, SPC). Successive contrasts have been studied in instrumental (iSNC, iSPC) and consummatory (cSNC, cSPC) situations. Consummatory responses require an interaction with the reward, usually in terms of consumption. Typical measures include licking frequency, cumulative time in contact with the reward and fluid intake (Torres & Papini, 2017). Instrumental responses are assessed before the animal comes into direct contact with the reward (e.g. response latency), therefore they are anticipatory. Typically, measures include errors, latency, speed and response frequency (Flaherty, 1999) (Megi Thesis)

Because this thesis work used an instrumental successive negative contrast task, I will focus further on this procedure. We refer to the iSNC effect to describe a deterioration in the response of animals that have experienced a decrease in the size or quality of a reward, in comparison to the response of animals that have always received a low reward. This concept is also referred to as the "contrast effect." There are at least four factors that need to be considered to understand the contrast effect: detection, motivation, emotion, and memory.

2.1 Detection

The iSNC effect size experienced by rats trained to collect food rewards is directly proportional to the difference in size of the reward between pre and post shift. The detection of a disparity between rewards is put forth as the ratio invariance rule, parallel to Weber's law for sensory comparisons. Weber's law suggests that there is a minimum amount or threshold by which a stimulus must be changed in order for there to be a noticeable difference in the sensory experience of the stimuli. Hence, this rule suggests that the ability to detect a change in the stimulus is a function of the ratio before and after the change. This idea is supported by animal research that demonstrates that rats change their behavior only when the ratio between pre and post-shift is high enough, regardless of the reinforcer quantity. Therefore, this suggests that animals adjust their behavior depending on the magnitude of difference in reward rather than just the absolute value (Pellegrini et al., 2008).

2.2. Motivation

The behavior of animals in situations where reward changes are involved also depends on internal factors. A striking internal factor, especially in situations where the reward is appetitive in nature, is the magnitude of food deprivation (Flaherty 1996). In iSNC situations, food deprived animals seem to show a larger contrast effect and the value of the incentive, either sugar pellets or sucrose solutions, varies based on the internal state of the animal, which is dictated by the extent of the food deprivation. In a study conducted by Cuenya et al (2015) animals were exposed to either presession feeding or postsession feeding. It was found that presession feeding reduced the value of the preshifted reward (32% sucrose solution), meaning

that a downshift of reward (to 4% sucrose solution) did not result in a contrast effect. However, for animals that were subjected to postsession feeding, a contrast effect following the reward downshift was observed. In conclusion, the value of a reward comes not only from the absolute properties (e.g. amount of sucrose content, number of sugar pellets) but also on the animal's internal state at the moment of reward consumption (Torres & Papini, 2017).

2.3. Emotion

Incentive relativity has been proposed to be accompanied by a negative emotional response that inhibits approach to the site previously associated with a highly valuable reward and redirects the animal to the search of other sources of reinforcement (Amsel, 1992). Changes in reward, especially unexpected reward downshifts, are linked to negative emotions which are marked by distress vocalizations, increase in stress hormones, aggressive behaviors, etc (Torres and Papini, 2017), in rodents (Dudley & Papini, 1997). Additional evidence of the link between emotion and incentive relativity is physical pain. In 2017, Ortega et al., demonstrated that whereas opioid and cannabinoid antagonists, both of which are used as pain reduction methods, reduce the overall contrast effect, opioid agonists further escalate it. Through various studies in the realm of incentive relativity and emotionality, a view has been established that the negative emotion derived from reward downshift could also be referred to as psychological pain (Papini et al., 2015). (Flaherty, Clarke, & Coppotelli, 1996) Furthermore, treating animals with anxiolytics and ethanol have demonstrated the ability to reduce contrast effect, which further supports that reward downshift causes negative emotion. In agreement with this notion, inbred rat strains that display higher anxiety show enhanced contrast effects (Torres & Sabariego, 2014). Lastly, inactivations or lesions of brain regions related to emotion such as the centromedial amygdala (Kawasaki et al., 2015), corticomedial amygdala, insular cortex, ventrolateral orbital cortex, anterior cingulate cortex (Ortega et al., 2017; Papini et al., 2015) influence the size of the contrast effect.

2.4. Memory

Adjustments to reward loss require several memory processes (Papini, 2003). First, for reward loss to have an impact on behavior, devaluations or omissions need to occur in the context of a reactivated reward memory. This is because in order to assign hedonic value to a reward, we need to access memories of our previous incentive history. Second, if the downshift event (transition from high to low reward) is sufficiently aversive, an emotional memory may be encoded. This type of memory is called egocentric memory and it would encourage behavioral suppression (i.e. avoidance). Third, every time the animal is exposed to the downshifted reward a process of memory update would adjust the expectancy learned to a new value. Multiple trials reinforced with a lower reward would promote an expectation adjustment, this is: the animal would no longer expect the initial high reward. This type of memory is called allocentric memory and it would reduce and eventually eliminate the negative discrepancy promoting approach responses (Torres & Papini, 2017). Research has attempted to provide evidence for these memory processes, for example, memory enhancers such as corticosterone (a stress hormone) and D-cycloserine (an NMDA-receptor partial agonist) administered immediately after the reward devaluation prolong the contrast effect (Papini et al., 2015). It is hypothesized that these drugs enhanced the reactivation of the egocentric memory of the reward devaluation (Torres & Papini, 2017).

Out of the four factors that are principal to the contrast effect, this thesis will dive further into emotion. Based on past literature, the negative emotion that arises as a result of reward omission or devaluation is mainly frustration. The next section will examine the role of frustration in the contrast effect and the behavioral adaptations that this emotional experience promotes.

Chapter 2: Frustration Theory

Amsel's and colleagues proposed a frustration theory developed to capture the behavioral effects of surprising nonreward. Nonreward refers to the omission, reduction in magnitude or quality of an appetitive reinforcer. A nonreward is considered to be surprising if the value of the current reward is degraded while cues for the previously higher value reward are present (Amsel, 1992). The following table (2) consists of the three theoretical concepts that are involved in Amsel's frustration theory:

Phenomenon	Mechanism	Requisite	Example
After-effects	Primary Frustration	Negative discrepancy between expectancy and current reward value	ROE = Reinforcement-omission effect
Anticipatory Effect	Secondary Frustration	Approach-avoidance conflict	SNC = Successive negative contrast

Table 2: Elements of Amsels's Frustration Theory (1992). This table has been adapted from Papini et al., 2003.

3.1 After-effects

The first instant of surprising nonreward takes place when there is a mismatch between the actual reward that is received which is lesser than the reward that the animal expects, based on past reward experiences. This unexpected nonreward leads to an internal state called primary frustration, giving rise to immediate behavioral effects and hence acting as an aversive reinforcer (Papini, 2003). As seen in Figure 1A, the primary frustration (Rf) fuels Pavalovian conditioning, as seen in the S→Rf connection given that the reward did not match the expectation (ei). Due to the newly learned association between the stimuli present in the animal's surroundings and the surprising experience of nonreward, in the subsequent trials, exposure to the same stimuli can lead to the memory of primary frustration resurfacing.

3.2. Anticipatory effects

Second, the stimuli that used to be associated with rewards of high value are now also paired with the experience of primary frustration. This leads to the animal experiencing ambiguity when it comes to the original cues, activating opposite expectations of reward and frustration, and this is called secondary frustration (eF) (Figure 1B). There is a conflict that is created due to the apetitive nature of the reward and the aversive nature of the secondary frustration, creating a contesting response between approach and avoidance. This competition between the decision to approach or avoid explains the successive negative contrast effect. When the animals choose to approach the devalued reward, expectations about the reward are re-adjusted and the approach-avoidance conflict is resolved. Only in a situation where the reward is devalued to the extent of omission, do the animals choose to avoid the situation altogether.

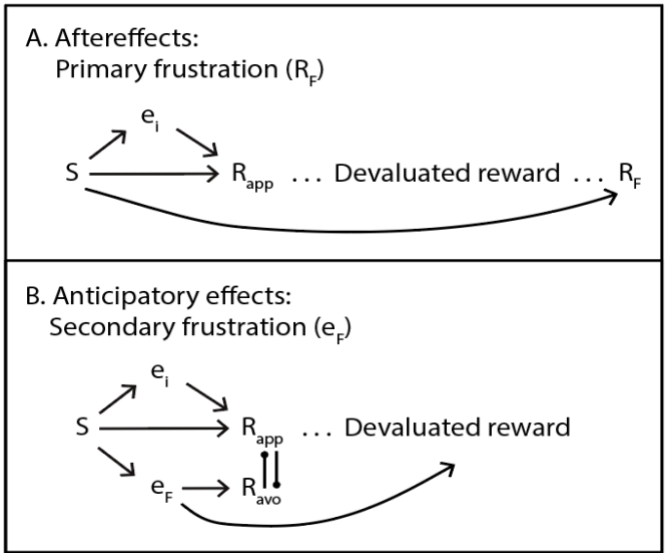

Figure 1: Mechanisms suggested by Amsel's (1992) frustration theory to explain SNC. (A) Primary frustration is an internal unconditioned state induced by the surprising reduction of an incentive/reward. (B) The stimuli acquire the ability to elicit ambivalent expectations of both the incentive and the frustrative response. The latter, referred to as secondary frustration, induces a competing avoidance response. The competing expectations result in a conflict that induces response competition. (Image adapted from Papini et al., 2006)

My thesis will focus on understanding the processing of reward loss as well as the adaptation that arises from experiences of frustration. In order to further strengthen the relationship between reward loss and experience of frustration, the next section will examine literature that deepens understanding of negative affect responses in situations of loss, specifically reward loss.

Chapter 3: Negative Valence Systems

My work seeks to understand the neural systems that support behavioral responses to aversive reward changes. It is hence important to inspect the involvement of negative emotions in conjunction with aversive reward changes. Recently, the National Institutes of Mental Health introduced the Research Domain Criterion (RDoC) to provide a research-based framework that supports better understanding and investigation of mental health conditions. The RDoC provides a comprehensive exploration of factors that regulate mental health conditions, including genes, molecules, cells, circuits, physiology, and behavior (National Institute of Mental Health, n.d). The RDoC can be contrasted with existing tools, such as the Diagnostic Statistics Manual for mental health disorders, which can contain outdated or rigid classifications of mental health conditions. The RDoC describes several aversive events that can activate distinct and overlapping neural systems, including acute threat (fear), potential threat (anxiety), sustained threat, loss, and frustrative nonreward. The RDoC collective defines the neural systems that support responses to these aversive events as Negative Valence Systems.

While the Negative Valence System categorizes loss and frustrative nonreward separately, considering the situational, behavioral, neurochemical, pharmacological, endocrine, molecular and neurobiological commonalities (e.g., Amsel, 1992; Flaherty, 1996; Papini, 2003), they can be assessed together. Instances of both involve deprivation, withdrawal, devaluation or inability to obtain motivationally significant rewards. Loss and frustrative nonreward take place, for humans, when they receive or are exposed to surroundings in which they have experienced worse-than-expected outcomes or rewards (eg. death of a spouse, jail term, divorce, personal injury, being fired from work, retirement, etc.; Scully et al., 2000). There is copious amounts of evidence that links frustration, specifically after loss and nonreward, with aversive emotion, negative affect and suffering, activation of the hypothalamic-pituitary-adrenal axis, limbic-circuit recruitment, and behavioral disturbances, including aggression and drug abuse (Abler, Walter, & Erk, 2005; Papini et al., 2015). Given that the negative valence system categorizes reward loss as a link to frustrative emotion, it is imperative to consider emotionality as an element in behavioral adjustment to reward loss. This classification system and past literature encourages the development of paradigms to assess emotionality after reward loss consequences. Hence, it is of

special interest for this thesis to consider negative valence systems and surrounding literature in developing the experimental protocol to investigate the reason behind inability to adapt to reward loss.

Moreover, in order to investigate the neural correlates of reward loss as well as the brain structures that support adaptation to loss, it is important to evaluate literature surrounding specific brain areas related to reward, appetitive memories and emotionality. The next sections will delve into literature related to the hippocampus as the structure of interest for this thesis.

Chapter 4: The Hippocampus

There is a good indication that the hippocampus could be part of the neural circuit responsible for incentive relativity. While rodents who are around 24 days of age (having a mature hippocampal formation) show signs of the contrast effect, rodents prior to the age of 24 days do not. This shows the importance of a mature hippocampal formation in understanding and adapting to environmental changes in reward. (Amsel, 1992). Second, the hippocampus is crucial for both memory formation and goal-directed behavior. It has also recently been established that there is a specialized group of cells in the hippocampus that is responsible for encoding reward-related information (Gauthier & Tank, 2018). Given that my thesis will expose animals to a reward devaluation paradigm, in this section I will cover the hippocampal functions with respect to incentive relativity, memory, and frustration. First, a brief overview regarding the anatomy of the hippocampus is stated to familiarize the readers with specific terminology that will be referenced throughout this paper. Then, I will focus on specific functions of the hippocampus, thought to be crucial for my thesis experiments. In particular, I will discuss: memory, spatial navigation, VTE and reward assessment, cognitive flexibility, and reward representation.

3.1 Hippocampal Anatomy

The hippocampus is a sea-horse-like structure that resides within the parahippocampal gyrus in the inferior temporal horn of the lateral ventricle. It is a structure with multiple curves which extends into the temporal lobe's medial surface. The hippocampus can be divided mainly into the hippocampus proper and other closely associated regions. As seen in figure 2, CA1, CA2, and CA3 (CA comes from Cornu Ammonis which refers to a seahorse-like or a ram's horn structure) are the three subparts of the hippocampus proper. The "other" regions include the dentate gyrus (DG), subiculum, presubiculum, parasubiculum, and entorhinal cortex (EC). The DC and EC are the primary structures that input information to the regions of the hippocampus proper and the CA1 is one of the major structures that is responsible for information output. The entorhinal cortex receives projections from the CA1 region and is thought to assist the hippocampus with a diverse range of functions. Of particular importance to this thesis, is the dorsal hippocampus, which has been identified as the region that consists of neuronal cell

assemblies specifically responsible for encoding reward (Gauthier and Tank., 2018). Moreover, the dorsal hippocampus CA1 area directly projects to nucleus accumbens (NAc) and enables the behavioral manifestation of place-reward memories (Trouche et al. 2019). Only NAc neurons activated during dorsal hippocampus sharp wave ripples (SWRs) are tuned to spatial reward-related information (Sosa, Joo and Frank, 2020).

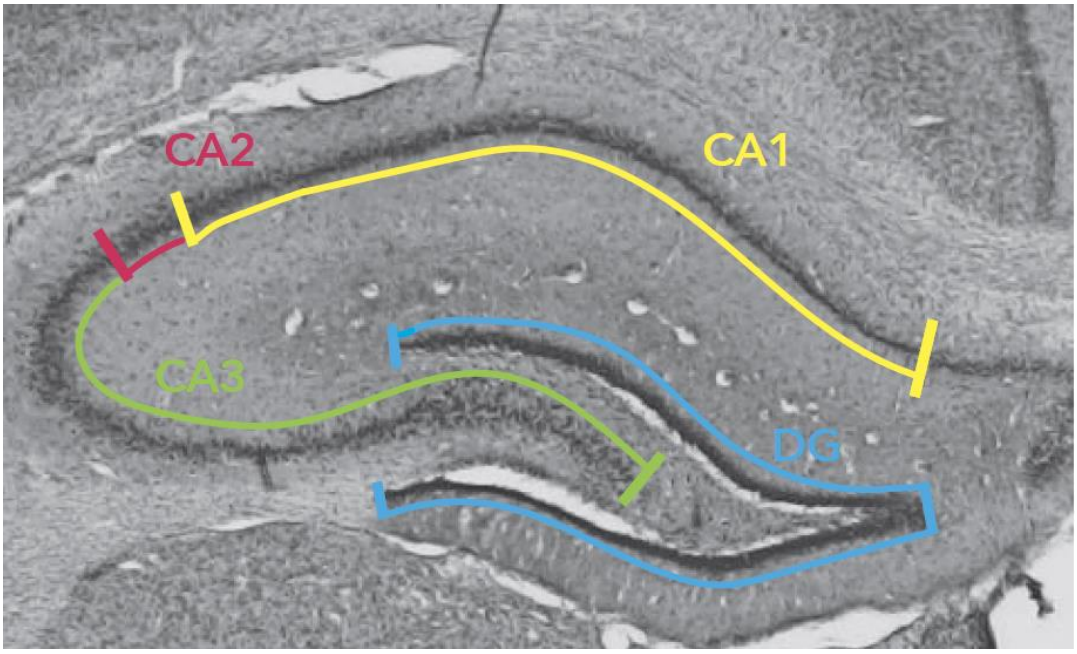

Figure 2: Depiction of a rodent's dorsal hippocampus labeled with structures of importance such as CA1, CA2, CA3, and the Dentate Gyrus (DG).

3.2 Memory

Historically, the hippocampus has been at the forefront of memory-related work. Knowledge about the functions of the hippocampus was uncovered in 1957, when Brenda Milner worked with patient H.M, whose medial temporal lobe (which included the hippocampus) was bi-laterally lesioned to resolve severe epileptic episodes. After the live-saving procedure, H.M suffered from temporally graded retrograde amnesia, meaning that he could not retain new memories formed after his accident and also suffered memory loss of some memories that occurred one or two years prior to the accident. Older memories were well conserved. This case was pivotal in showing the importance of the hippocampus in memory function and inspired

many researchers to also study hippocampal functions through animal models. (Clark and Squire, 2010). Over the years, researchers developed multiple memory-based tasks such as DNMS (delayed non-matching to sample), NOR (novel object recognition), to name a few, in order to test the behavioral responses of rodents with hippocampal lesions. Rodents with hippocampal lesions tended to have impaired performance in such memory tasks, highlighting the importance of the hippocampus in memory functions. (Clark et al., 2001; Winters, Saksida, & Bussey, 2008). Over the years, research has delved into more specific features of hippocampal memory. Memory, along with other more specific functions of the hippocampus are important to rodents when engaging the experimental paradigms that my thesis utilizes.

5.3 Spatial navigation

While the hippocampus serves to support declarative memories, it also plays an important role in spatial navigation. In 1971, O'Keefe and Dostrovsky discovered place cells in the CA1 region of the hippocampus. By recording neurons in the hippocampus, it was observed that certain cells fire at specific physical locations, giving rise to the idea that some cells in the CA1 region of the hippocampus help us form a cognitive map and navigate around space. These cells were thus named place cells. Before the idea of spatial functions of the hippocampus was formalized, in 1948, Tolman observed a peculiar trend in rodents who were put through varying maze shapes. He trained animals to recognize the location of a reward on one type of maze. He then tested animals by keeping the location of the reward constant while changing the shape of the maze. Despite the change in maze patterns, animals were efficient at locating the reward. This suggested the formation of a general cognitive map when the animals were exposed to various maze-patterns (O'Keefe & Nadel., 1978). Research on spatial navigation functions of the hippocampus has been executed through various methods such as behavioral assays as well as electrophysiological recordings. In more recent years, models and protocols have also been proposed to improve the study of real time place cell firing and make it easier to analyze place cell firing within the hippocampus (McClain et al., 2019). Since my experimental paradigm involves a maze task that requires rodents to rely on their spatial navigation ability, it is

imperative to acknowledge the importance of the hippocampus under the umbrella of spatial navigation.

3.4 VTE and Reward Assessment

In order to further delve into involvement of the hippocampus in spatial navigation, researchers have used multiple maze designs with decision points that require animals to choose between two or more possible paths. When a rodent arrives at such a point, multiple researchers have reported that animals pause and look back and forth, as if reflecting an indecision between possible options and thus imagining future potential outcomes; this behavior has been defined as Vicarious Trial and Error (VTE) behavior (Muenzinger, 1938; Tolman, 1939; Muenzinger & Gentry, 1931). Along with spatial navigation and decision-making, VTE behavior has also been linked to assessing emotion in anxiety-based tasks such as the elevated plus maze (Redish, 2016).

As rodents move through a maze, hippocampal place cells fire at certain specific locations. Neural recordings from rodent maze studies suggest that sequential hippocampal place cell firing at the theta frequency corresponds to future planning and deliberation of possible paths that rodents can choose. These firing patterns coincide with moments during which an animal is engaged in VTE behavior. Thus, hippocampal place cells reactivate sequentially to chart out all possible outcomes and potential end goals, helping an animal develop a spatial representation of the choices that they are faced with (Foster & Wilson, 2007). More specifically, it appears that cells in the dorsal hippocampus (dHPC) contribute to VTE behavior in rodents since inactivations of the dHPC lead to disturbances in VTE behavior (Meyer-Muller et al., 2020). While a large body of research on VTE behavior has been done using alternation tasks (assessing working memory), little is known about how this behavior manifests when animals have a choice between two possible outcomes that differ in value. Here, I investigate the role of the hippocampus on adaptation to a change in reward quantity and therefore VTE behavior will be analyzed as a measure of hesitation when evaluating incentive value.

3.5 Cognitive Flexibility

Animals employ various strategies to adapt to reward changes, and one such strategy is cognitive flexibility (Miyake et al., 2000; Jurado & Rosselli, 2007). In animal models, cognitive flexibility generally refers to the ability to switch a behavioral response according to the context of a situation (Scott, 1962) to update a strategy to optimally obtain a reward or adjust to a change in reward. Amongst other brain regions, cognitive flexibility appears to involve the hippocampus communicating with other brain regions. In a study conducted by Garthe et al (2009), it was found that suppression of neurogenesis in the DG led to significantly impaired spatial learning on the Morris Water Maze (MWM) task as compared to rodents with normal hippocampal neurogenesis ability. The MWM is thought to assess cognitive flexibility because animals are required to use allocentric cues to find the updated location of a platform submerged in cloudy water. When the location of the platform is changed, animals must assess these environmental cues, the previous location of the platform and their own spatial location, requiring the ability to flexibly think and assess multiple spatial elements. This study hence demonstrated the important role that the hippocampus plays in cognitive flexibility. Other studies focusing on neural circuitry involved in cognitive flexibility have demonstrated strengthening of the vHPC and medial prefrontal cortex (mPFC) connection in situations of novelty. Additionally, blocking dopamine D1 receptors on vHPC cells that are active when rodents are exploring novel (or updated) situations prevented the display of novelty-associated behavioral cues. These observations further indicate the importance of the hippocampus associated with novel situations that require rodents to think flexibly (Park et al., 2021). Cognitive flexibility is critical for survival as it allows animals to adapt to the changing environment. In my experiments, rats needed to adapt to reward loss and manipulations to the hippocampus possibly prevented behavioral adaptations that would typically involve neural computations in this brain area.

3.6 Reward Representation In The Hippocampus

Remembering reward locations and the cues that are associated with them is paramount. The hippocampus, as discussed in earlier sections, has important functions relating not only to memory but also spatial navigation, location and imagining the future. Along with these functions, there is emerging literature supporting the idea that the hippocampus is also involved

in reward representation. Hippocampal place cells fire in specific locations that relate to the "presence" or "absence" of rewards during active exploration (Singer & Frank, 2009).

There are hippocampal cell sequences associated with rewarding experiences. These cell sequences replay during sharp wave ripples (SWRs) which are spontaneous neuronal population events that occur in the hippocampus during sleep and quiet restfulness, and are thought to play a critical role in the consolidation of reward memories (Jiang et al., 2018; Singer & Frank, 2009; Buzsáki, 1986; Wilson and McNaughton, 1994; Sutherland and McNaughton, 2000). Hippocampal reactivation during pauses in behavioral activity leads to sequential firing of place cells leading to and from rewards and reflecting the animal's path. (Foster & Wilson, 2006; Diba & Buzsaki, 2007; Karlsson & Frank, 2009; Davidson et al., 2009). Since the SWRs occur after the animal has completed the trajectory of its path, the reactivation of the hippocampus can assist the animal in learning the relationship between the path and its result (Johnson & Redish, 2005; Foster & Wilson, 2006; Diba & Buzsaki, 2007). Hence, by extension, we can speculate that a rewarding experience at the end of a task would lead to hippocampal reactivation after the reward has been attained at a particular location. In their study, Frank & Singer demonstrated that SWR activity was closely associated with animals learning that rewards were associated at the end of a certain path. This was evidenced by higher SWR activity in reward-associated trials as opposed to non-reward associated ones. Studies like these led to the conclusion that hippocampal replay during SWRs assists the animal in learning information about the path to the reward or the location of the reward, especially with high firing activity in the CA1 region of the hippocampus (Frank & Singer, 2009; Grieves et al., 2016). However, more recent research has demonstrated another connection in relation to reward representation in the hippocampus. In 2018, Gauthier & Tank developed a virtual maze task, observed electrophysiological recordings from the hippocampus and reported a dedicated population of cells that encode reward information. These reward cells in the hippocampus fire irrespective of place cell activity and are specifically dependent on reward information in the animal's surroundings. Gautier & Tank's research was hence instrumental in identifying that place cell firing which is thought to encode reward location is separate from reward cell firing which indicates the presence of reward independently of space. Since then, there has been growing evidence in the field which suggests

reward-specific neuronal activity in the hippocampus (Jin and Lee, 2021; Jarzebowski et al., 2021). Due to the overwhelming evidence that links the hippocampus and reward encoding, we have chosen to manipulate the hippocampus in order to study behavioral responses to reward loss.

Chapter 5: Past Research, Future Outcomes

Previous experiments conducted in our lab (Hoxha et al., 2021) have demonstrated that hippocampal lesions impair the ability of rats to flexibly adapt to changes in reward. In this past experiment, all animals underwent stereotaxic surgery to either receive excitotoxic hippocampal lesions (using ibotenic acid) or sham (control) lesions. After the animals had recovered, they were trained to recognize two types of rewards - high (12 sugar pellets) and low (2 sugar pellets) - on opposite sides of a figure 8 maze. This led animals to develop a preference for the side of the maze that contained a bigger reward. After four days of free choice trials, the high reward was downshifted to 2 pellets to equal that of the low reward site (i.e.both rewards were then the same in terms of absolute value).

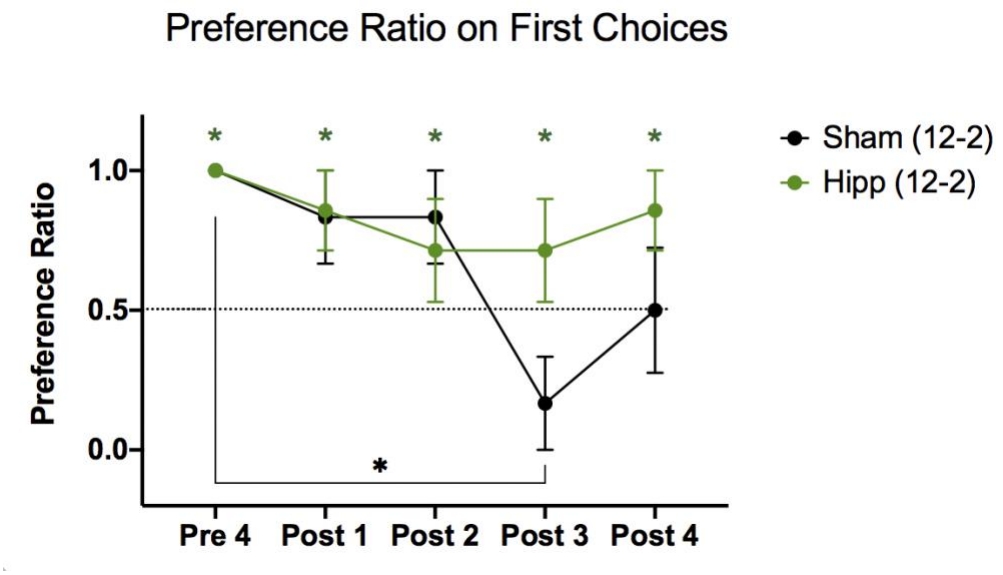

Figure 3: Preference ratio on first choices made between the two reward sides. Preference ratios above 0.5 indicate a preference for the high/devalued side. Preference ratios below 0.5 indicate a preference for the low/unshifted side.

Control animals demonstrated the ability to switch their preference to the unshifted reward when the reward was devalued on one side of the maze (choice reversal indicating frustration). After this temporary switch rats adjusted their response and equally chose either side of the maze equally, indicating an adaptation after reward devaluation (Figure 3). However,

while hippocampal lesioned animals persisted on their choice of the devaluated reward side, they had no trouble acquiring the preference for the size paired with the large reward.

While this study did demonstrate that the hippocampus is necessary for animals to flexibly adapt to changes in reward, there were certain limitations that need to be addressed and improved upon. These changes and improvements are the opportunities that my thesis will capitalize on in order to understand why animals with hippocampal malfunction are unable to flexibly adapt their actions to changes in the environment.

Firstly, only female subjects were used, hence in my Thesis experiments, I also included male rats. There might be differences in sex which could not be demonstrated in previous experiments and that could potentially come up if a mixed sex cohort is used. Furthermore, for this experiment, animals underwent excitotoxic permanent hippocampal lesions before the animals learned the maze task. Furthermore, lesioning out the structure also meant that the connections to other structures such as the medial entorhinal cortex could have been indirectly affected. Given that animals learned the task with a damaged hippocampus, there could be behavioral differences in responses to reward adaptation if the hippocampus was intact at the time of initial learning. In order to address this issue, I have used chemogenetic methods to inactivate the hippocampus to ensure that the hippocampus is intact during the time that the animal learns the devaluation task, and the inactivation only takes place during the reward devaluation days. This allows finer control and would enable us to understand exactly how the reward downshift is perceived with an inactive hippocampus.

While we know that hippocampal lesions prevent animals from adapting to reward downshifts, the reason for the difference in behavior is not clear. One explanation could be that hippocampal lesions interfere with the reward memory. We have refuted this hypothesis because rats are able to form an initial preference for the large reward. A second possibility is that animals with hippocampal lesions do not feel negative affect/frustration following the reward downshift and therefore do not require a behavioral adjustment. A final possibility is that despite feeling negative affect, hippocampal damage precludes rats from flexibly modifying their behavior to adapt to the reward change. In order to explore these two possibilities, we have developed a conditioned place preference protocol to go in conjunction with the reward loss

maze paradigm. In this protocol, we paired a cocaine injection with a particular context and a saline injection with the other context. After animals demonstrate an association between the drug and context by spending time in the cocaine associated context, we eliminated the cocaine and exposed rats to extinction sessions. During the extinction sessions, the animals were allowed to freely explore both available contexts. The animals underwent the devaluation task alongside the extinction phase of the CPP task; immediately after the daily maze session, animals were placed in the CPP apparatus. During extinction, animals are exposed to (/undergo) new learning to understand that cocaine is not actively being administered before exposure to a specific context anymore. Extinction of the context-drug association is demonstrated by the animal spending almost equal amounts of time in the drug associated context and the non-drug associated context. Of particular interest is the activity of the rodents in the CPP apparatus after they have experienced a reward loss in the devaluation task. If hippocampal inactivation precludes the ability to feel frustration, then a reward devaluation (on the maze paradigm) should not cause any behavioral change in the CPP paradigm. However, if the problem is linked to cognitive flexibility then we should observe signs of frustration and a concomitant increase in preference for the previously cocaine-associated context. In this manner, the introduction of the CPP protocol in conjunction with the devaluation task would help understand if lack of emotionality is a reason behind the inability of rodents to flexibly adapt to changes in reward.

My work will examine (1) whether the function of excitatory neurons in the hippocampus is required for animals to adapt their response to a change in reward, and (2) whether the inactivation of the hippocampal circuitry influences drug-seeking behavior after an experience with reward loss, indicative of the involvement of emotionality. Hence, we hypothesize that rats with hippocampal inactivation will fail to adapt to the reward loss: exhibit a preference for the devalued reward arm of the maze and will not display an increase in preference for the cocaine-associated context in the CPP task after experiencing reward devaluation on the maze.

Materials and Methods

I. Subjects

The subjects were 15 experimentally naive, female (8) and male (7) Long–Evans rats weighing between 240g and 470g at the beginning of the experiment. They were housed individually on a reversed 12-hr light/dark cycle with continuous access to water. The hippocampus of the rats that belonged to the experimental group was stereotaxically infused with pAAV-hSyn-hM4D(Gi)-mCherry (inhibitory DREADD) (hM4Di; n = 8). The hippocampus of the rest of the rats was infused with pAAV-hSyn-GFP, to serve as the control group (eGFP; n =7). All rats underwent the same initial surgical procedure. During testing, rats were food restricted and maintained at 80–85% of their weight ad libitum**.** Throughout this paper, the experimental group is either referred to as the hM4Di group or the group with hippocampal inactivations and the control group is referred to as the eGFP group or the sham group.

II. Surgery

All surgeries were performed using aseptic procedures. Anesthesia was maintained throughout surgery with isoflurane gas (0.8–2.0% isoflurane delivered in O2 at 1 L/min). The animal was positioned in a Kopf stereotaxic instrument, and the incisor bar was adjusted until Bregma was level with Lambda. The bone overlying the target site was removed using a high-speed drill. After completion of each viral injection, the wounds were closed, and the animal was allowed to recover from anesthesia in their cages while being fed ibuprofen in drinking water. Behavioral testing began 2 weeks after surgery.

Bilateral hippocampal microinjections of either pAAV-hSyn-hM4D(Gi)-mCherry or pAAV-hSyn-eGFP was administered using a 10 μL Hamilton (Reno, NV) syringe mounted on a stereotaxic frame and held with a Kopf model 5000 microinjector (the Hamilton syringe and the virus was able to be obtained thanks to the Curtis-Smith Award). The viral vector was dissolved in 0.01 M phosphate-buffered saline to provide a solution with a concentration of 10 mg/mL and was injected at a rate of approximately 0.1 μL/min. The syringe needle was lowered to the target and left in place for 1 min before beginning the injection. After the injection, the syringe needle was left in place for 2 min to reduce the spread of the virus up the needle tract. The virus was

injected into 12 sites (total volume 2.78 μL) within each hippocampus (all coordinates are in millimeters and relative to Bregma): anteroposterior (AP) μ2.4, mediolateral (ML) ± 1.0, dorsoventral (DV) μ3.5; AP 3.2, ML ± 1.4, DV μ3.1, μ2.3; AP μ3.2, ML ± 3.0, DV μ2.7; AP μ4.0, ML ± 2.5, DV μ2.8, μ1.8; AP μ4.0, ML ± 3.7, DV μ2.7; AP μ4.8, ML ± 4.9, DV μ4.4, μ3.9. Once awake and responsive, each rat was returned to its home cage for a 14-day recovery period.

III. Behavioral testing

Section A: Animal Handling

After a 2-week recovery from surgery, rats were food-restricted and maintained at 80-85% of their weight *ad libitum*, and before any behavioral assessment, rats received reward pellets in their home-cage in order to prevent neophobia. Three days prior to any behavioral testing, all experimenters handled the rats in two testing rooms for 1-3 mins followed by scruffing next to the conditioned place preference (CPP) apparatus in order to acquaint the rats with the intraperitoneal cocaine injection administration procedure.

Section B: Devaluation Task

Devaluation Task Apparatus:

The devaluation task testing was conducted in a figure-8 shaped maze (Figure 4). The maze was constructed from sanded hard polyvinyl chloride plastic runways that were 10.3 cm wide with 3 cm tall walls on each side of the runway. The center stem of the maze was 141 cm long and the cross pieces at each end of the central stem were 55.5 cm long. The maze was positioned on top of four legs, elevating the base of the maze 68.5 cm off of the ground. At one end of the central stem, two movable barriers were positioned 26.3 cm apart serving as the delay area. The side walls between the two movable doors were 21.6 cm high. The black circles on the maze design in Figure 4A displays the site on the maze on which the sugar pellet rewards were placed. The maze diagram with the measurement is also depicted in Figure 4A. There were visual cues around the testing room and the experimenter always stood at the same place in the room relative to the maze. Overhead lighting provided dim illumination in the room.

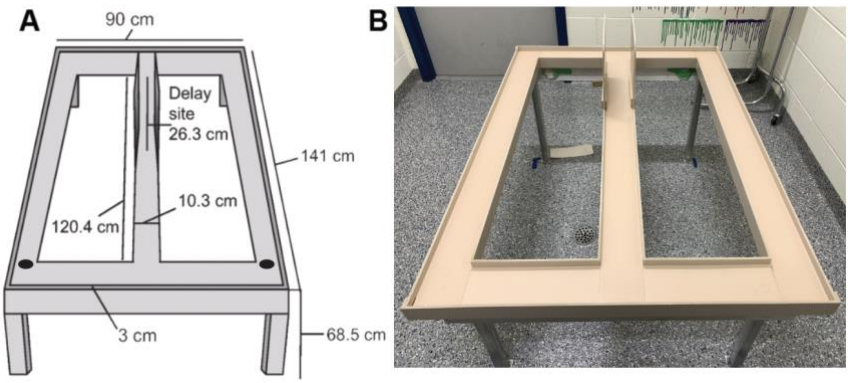

Figure 4: The Figure-8-Maze apparatus used for the reward devaluation behavioral paradigm. A) Illustration of the maze indicating measurements of each section (Image adopted from Tenney et al, 2021). B) Image of the maze used for the experiment.

Task Description:

The devaluation task designed to induce frustration through reward devaluation can be understood in 4 phases in chronological order. Phase 1 is the habituation phase where animals are allowed to freely explore the maze (without any barriers) for a period of 10 minutes. Sugar pellets were sprinkled around the maze as animals freely explored the area. Phase 2 is known as the pre-shift training phase. During this phase, animals are exposed to two levels of rewards - 12 sugar pellets or 2 sugar pellets - on opposite sides of the maze, as seen in figure 5A. Animals undergo forced choice sessions where one side of the maze is blocked and they are forced to take the unblocked route. In every training session, there are an equal number of forced trials to the high and low reward sites. Phase 3 is known as the pre-shift testing phase where animals are exposed to 6 forced choice trials to either side of the maze and then undergo 6 free choice trials. During the free choice trials, animals can make their independent choice of maze side - higher reward or lower reward paired side. Animals generally develop a preference for the side of the maze that contains the higher reward. The last phase, phase 4, of the devaluation task is called the post-shift test phase where the higher reward is devalued to 2 sugar pellets, to equal the absolute value of the smaller reward (Figure 5B). Animals are exposed to 6 forced choice trails and 6 free choice trials per session during the post-testing phase as well.

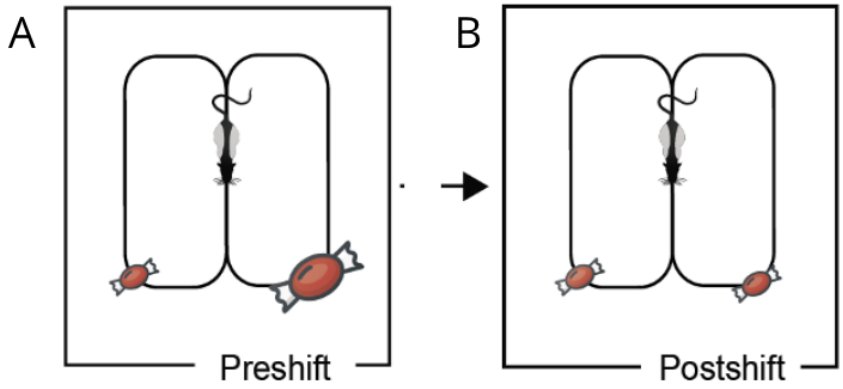

Figure 5: This figure represents the reward allotment on either side of the maze in different phases of the devaluation task. (A) Panel A shows the reward allocation in the maze during the pre-shift phases (testing or training - phase 2 and 3 respectively). There is a larger reward - 12 sugar pellets - on one side of the maze and a smaller reward - 2 sugar pellets - on the other side. (B) Panel B represents the reward allotment during the post-shift phase (phase 4). The rewards on either side of the maze are now equal in absolute value - 2 sugar pellets.

Section C: Conditioned Place Preference Task

Apparatus

The conditioned place preference box designed by MED Pc Associates has 3 distinct sections. There are two 7.4 cm X 12.7 cm X 12.7 cm compartments colored in black and white (Figure 6(A) A and B), separated by a gray compartment measuring 9.8 cm X 12.7 cm X 12.7 cm (Figure 6(A) C). All three compartments can be secured shut using the transparent lids placed on the ceiling of the compartments, locked using the steel buckle (figure 6(A) D). Installed within the top lids are circular LED lights that are set to a constant dull-yellow brightness level for all the compartments (Figure 6(A) E). There are U-shaped entryways connecting the white compartment to the gray compartment and the gray compartment to the black (Figure 6(B) F). The doorways between compartments can be shut by lowering the steel rods labeled G in figure 6(B), separating the compartments from one another. The floor of the black and white compartment is made of steel and varies from one another. The black compartment has a grid-like steel floor while the white compartment consists of a steel-rod flooring. The gray compartment has a detachable wooden floor plank which is also gray in color. Below the

flooring of the apparatus, removable steel compartments hold corn bedding to collect rodent feces and can be periodically changed.

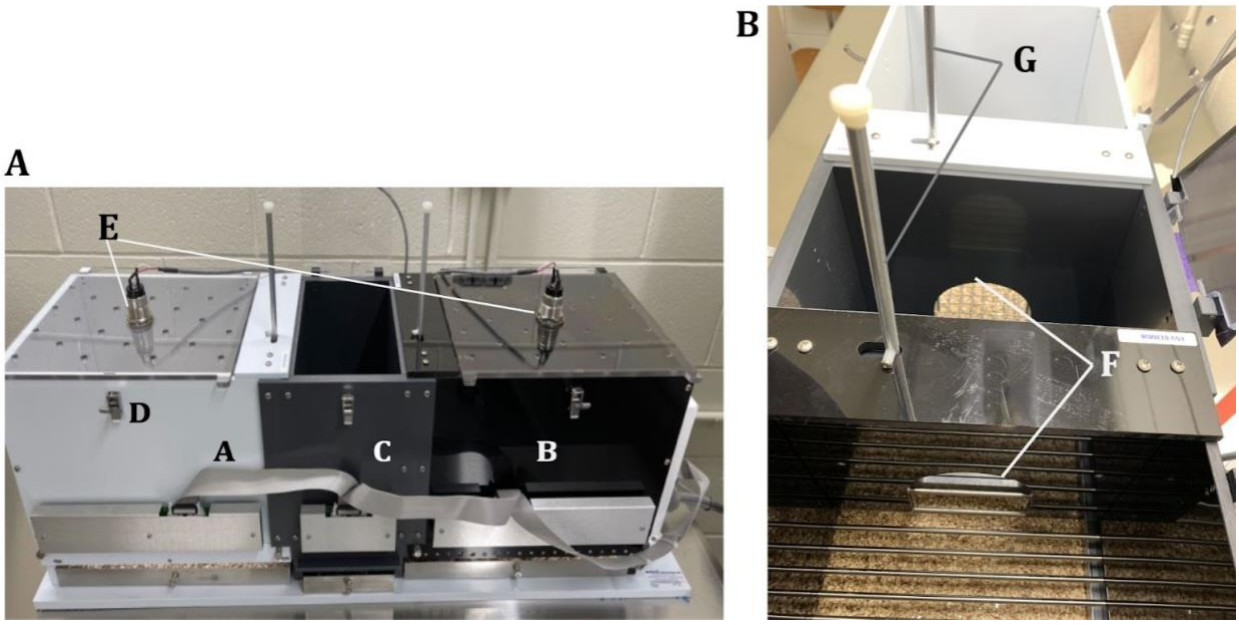

Figure 6: Panel A: A) White Compartment B) Black Compartment C) Gray Compartment D) Lock that keeps plastic lids in place E) LED lights to dimly illuminate the compartments
Panel B: F) Entryways that connect all three chambers G) Rods that can lower down and seal the U-shaped entryways shut.

Task Description:

The rats first undergo a pre-test day in order to establish which side of the Conditioned Place Preference (CPP) Box they naturally prefer. On pre-test day, rats were allowed to explore the entire apparatus freely and time spent in each compartment was recorded. In the conditioning phase of the experiment, cocaine was paired with the side that the rat initially preferred less (spent less time exploring) and a saline control was paired with the naturally preferred side (spent more time exploring). Rats were given intraperitoneal (IP) cocaine injections and then restricted to the compartment chosen to be paired with cocaine. On alternating days, IP saline injections were administered and the rats were restricted to the non-cocaine paired side for 30 mins. The conditioning phase lasted 4 days for the first cohort (animals 33-37), 2 alternating days of cocaine and saline injections each. The conditioning phase lasted 6 days for the second

cohort (animals 46-51), where 3 alternating days are cocaine injections and the other 3 days are saline injections. The conditioning phase helps establish a drug-associated memory to the context that has been paired with cocaine.

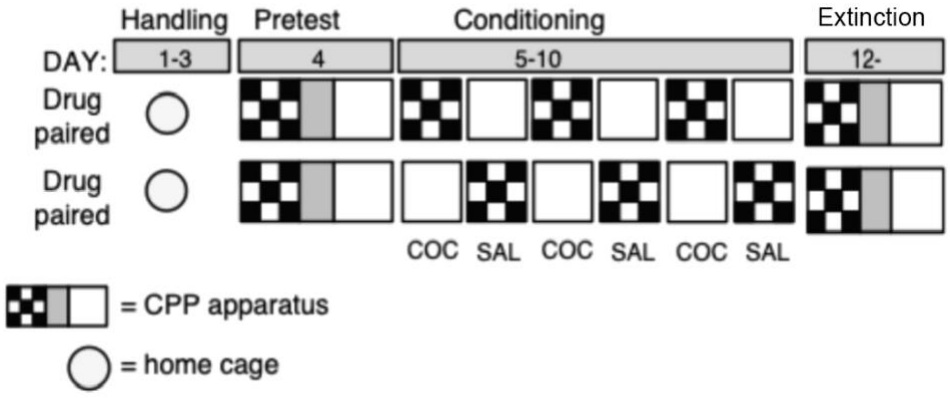

Figure 7: Timeline for assessing drug-associated memories through conditioned place preference

As seen in figure 7, after the conditioning phase in the CPP, the extinction phase, which takes place alongside the devaluation task, begins. After each (training or testing) session on the maze, daily, the rats are taken to the CPP box and allowed to freely explore both the contexts (drug-paired and saline paired) for 15 minutes. This period serves as extinction for the rat since its preference for both the contexts is expected to equalize. On all post-shift testing days, after the maze session is completed, the rat is also placed in the CPP box for 15 mins to examine if a preference for the drug-paired side returns after experiencing a devalued reward on the maze.

Experiment Overview:

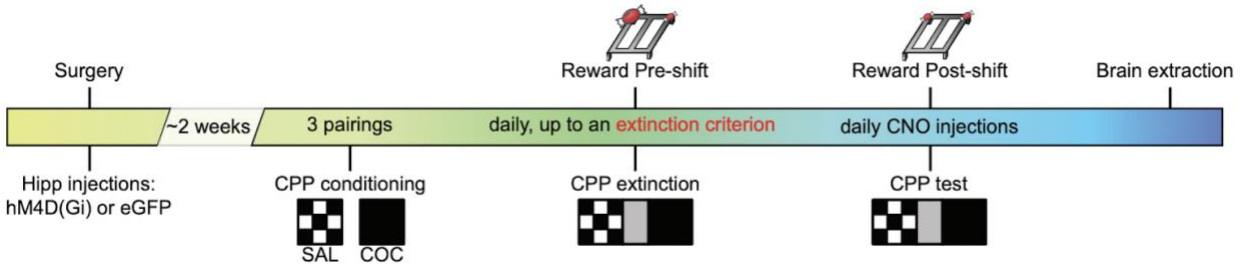

Figure 8: This figure represents a comprehensive timeline of the entire experiment, combining the CPP task and the devaluation task. This figure shows how the different elements of the experimental design fit into one another in the form of a timeline.

Figure 8 explains the experimental timeline and depicts how the devaluation task and the CPP task work alongside each other. After initial animal handling and pre-testing on the CPP, conditioning days begin where animals are restricted to cocaine or saline paired compartments on alternating days after receiving a cocaine or saline intraperitoneal injection. After the condition phase is over, the extinction phase of the CPP task begins alongside daily sessions of the devaluation task. Extinction in the CPP apparatus simply means that the animal is free to explore all contexts for the duration of 15 mins. After daily sessions on the maze, the animals are always taken to the CPP apparatus for 15 mins of free-exploration time. On the last day of the pre-shift training phase, animals are injected with a vehicle, acting as a CNO control, 20 mins prior to spending the daily maze session. On all 6 of the post-shift days, animals are injected with CNO 20 mins prior to testing on the maze. A total of 10 animals were included in the CPP data analysis. 5 animals had to be excluded because they either did not develop a preference for the cocaine-paired side after conditioning or failed to extinguish their preference to the cocaine-paired side altogether.

IV. Drugs

Clozapine N-oxide (CNO):

On the last day of pre-shift testing and through all six days of post-shift testing, the animals were subcutaneously injected with a vehicle and CNO (respectively) 20 mins prior to the start of the maze session. The CNO stock was made using saline and DMSO until the solution was clear. The final DMSO concentration was 4% (vol/vol). Rats were given a dose of 5 mg/kg depending on daily weight prior to the start of the behavioral experiments of the day. The vehicle had the same ratio of sterile saline and DMSO (4%) without the CNO, acting as a control injection administered subcutaneously on the last pre-shift testing day.

Cocaine:

Cocaine-HCL procured from MilliporeSigma as a salt then a stock solution was generated using sterile saline (vehicle). Rats were administered cocaine at a dose of 7.5 mg/kg at the start of the conditioning session. We administered cocaine via intraperitoneal injections, delivered on alternating days with saline.

V. Behavioral coding

The ethogram BORIS was used to score the animal responses pertaining to six behaviors. (1) choice point VTE, the rat pauses at the choice points and looks back and forth over its possible trajectories, (2) left search, the rat engages in search behavior by dipping its head outside of the left side of the maze, (3) right search, the rat engages in search behaviors like dipping its head outside of the right side of the maze, and (5) central search, the rat engages in search behavior like dipping its head out of the maze anywhere in the center arm on the maze, including the far end of the choice point. Videos were coded by two individuals in the Sabariego lab in order to control and lower bias and obtain more accurate results. In the end, the average of results from two coders, who were blind to the experimental conditions, were used to plot the data to explore various behavioral trends.

The behavioral analysis software ETHOVISION was used to analyze the amount of time that animals spent in each zone - exterior, middle or center - of the open field (figure 9B). The rodents' nose point and center point in the body were tracked over the entire time and an average value of time spent in each zone based on each of the points was considered as the total amount of time spent in each zone.

VI. Open Field

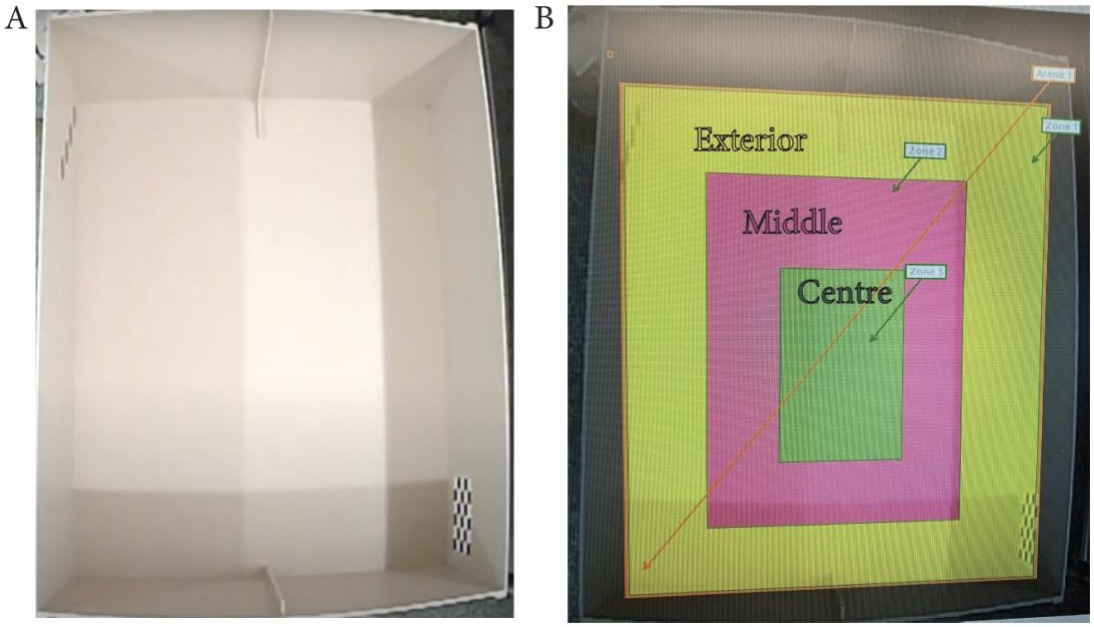

Figure 9: (A) Image of the actual open field used in the experiments. (B) Image of the open field divided into 3 zones - exterior, middle, and center - as coded in Ethovision to measure time spent in each zone.

Apparatus Description:

As seen in Figure 9A, a 100 cm X 100 cm cream colored box was placed on a steel stand with a recording camera placed perpendicularly above it, hanging by the ceiling. 2 sides of the empty box had visual cues (checkers on the right and circles on the left as seen in Figure 9A) pasted on them to assist the animal with orientation. The box served as an empty open field for the animal to explore when placed inside it and the video camera captured the movement of the animal while it explored the different areas of the open field.

Task Description:

A smaller sub-cohort of animals, particularly rodents 33-37, was exposed to the open field task. Twenty minutes prior to being exposed to the open field, animals were injected with CNO as per the aforementioned dosage. Animals were then allowed to freely explore the open field for a period of 20 mins and a video was recorded of the animals exploring the plain. These videos

were later analyzed to understand the amount of time that the animals spent in different zones of the open field using Ethovision. The open field zone was broken down into 3 zones: the exterior, middle, and interior, as seen in Figure 9B.

VII. Light Dark Box

Apparatus Description:

The CPP apparatus above (Figure 6) was modified to build a dark side and a light side, with no barriers between the two zones (Figure 10).

Task Description:

A small cohort of animals (46-51) was tested on the light-dark box. Each animal tested on the light-dark box was injected with CNO according to the aforementioned dosage, 20 mins prior to being tested inside the box. Animals were lowered into the light compartment and then allowed to explore the apparatus for 10 mins. Time spent in the light and the dark zone was recorded and the time taken for animals to transverse to the dark side after starting the experiment in the light side was also recorded.

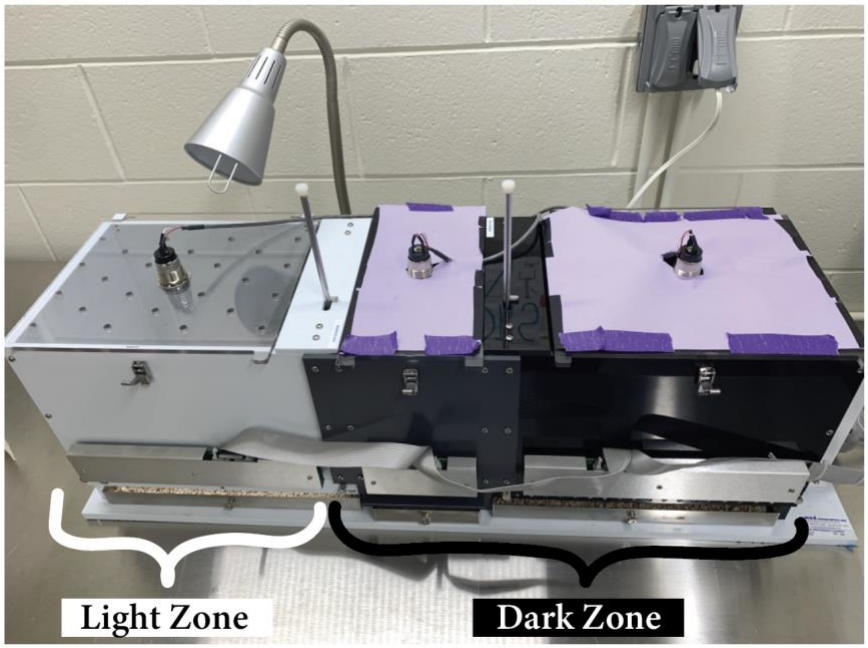

Figure 10: A depiction of the CPP apparatus modified to build a light-dark box. The left side where the white compartment lies depicts the light zone. The right side, with purple papers covering the tops for total darkness represents the dark zone.

VIII. Neurohistological methods

At the completion of all behavioral testing, animals were euthanized and tissues were collected for biological assays. Rats will be either anesthetized with 2-4% isoflurane (inhalation) followed by decapitation using a guillotine. The brains were then sliced using a cryostat and 50µM slices were obtained. The slides were then stained using DAPI and were coverslipped immediately. ~24 hrs after coverslipping, the slides were imaged using the confocal microscope.

IX. Statistical Analysis

All data presented are expressed as mean ± SEM. Group comparisons were evaluated with a Two-Way ANOVA and considered statistically significant at $p<0.05$. All statistical analyses were conducted in GraphPad Prism 8.

Results

Confocal Images representing viral infusions

Figure 11 represents the confocal images of brain sections of hM4Di and eGFP animals. In Panel A and B, image 1 and 4 show the DNA in neurons stained using DAPI. Image 2 shows the hM4Di DREADDs that carries the mCherry and image 5 shows the control animal brain section that carries the GFP reporter. Images 3 and 6 are the merged images of all dyes combined together. While figure 11, panel A represents the expression of the DREADDs in the dentate gyrus area of the hippocampus, figure 11, panel B represents the eGFP in the dentate gyrus area of control animals.

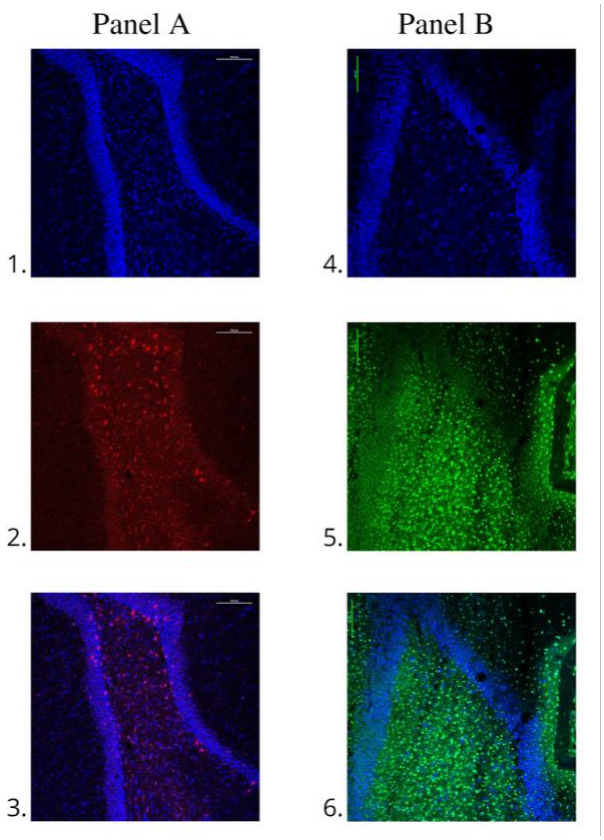

Figure 11: Panel A represents the confocal images that capture the DREADDs infused and fluorescing using mCherry. Panel B represents the confocal images that capture the eGFP controls.

Hippocampal inactivation prevented adaptation to reward devaluation

After animals developed a preference for the high reward location, the high reward was downshifted to equal the small reward. Twenty minutes before animals were exposed to the reward downshift, all animals received a subcutaneous injection of CNO. As shown in figure 12, rats with an inactivated hippocampus failed to adapt to the reward change and perseverate on their previously acquired preference for the now devalued side. On the other hand, control eGFP rats slowly adapted their behavior by increasing the number of visits that they made to the non-devalued side. In particular, the preference ratio measures the total number of choices made to the high (during pre-shift) and devalued (during post-shift) divided by the total number of free choice trials (6) per session and is represented on the y-axis. The x-axis marks the different experimentation days. Analysis of the preference ratio on the last pre-shift testing day and all the following post-shift days was carried out for rats in both groups. A Two-Way ANOVA of condition (eGFP or hM4Di) and session (last preshift day along with 5 days of post-shift testing), revealed a main effect of condition $F(1, 13) = 6.261$, $P = 0.0265$, as well as a main effect of session, $F (5, 65) = 10.44$, $P < 0.0001$. Specifically, on post-shift day 4, there was a significant difference in the preference ratio between the hM4Di group and the eGFP group. Additionally, there was a significant difference between the last day of pre-shift and the post-shift day 3 ($p = 0.0070$), post-shift day 4 ($p < 0.001$), and post-shift day 5 ($p = 0.0004$) for the eGFP group animals. These results suggest that both groups developed a preference for the higher reward side by the last day of pre-shift testing. As seen in figure 12, overtime, the control eGFP group significantly reduced visits to the devalued reward site during post-shift testing but the hM4Di group generally persevered on their choices to the side with the devalued reward. As compared to the last day of pre-shift, the post shift 3, 4, and 5, preference ratio of the eGFP group is also significantly lower, whereas there is no significant difference over time for the hM4Di group. This adds to the evidence pointing to the explanation that the eGFP group varied on their choices to reward sites but the hM4Di group persevered on choosing the devalued side.

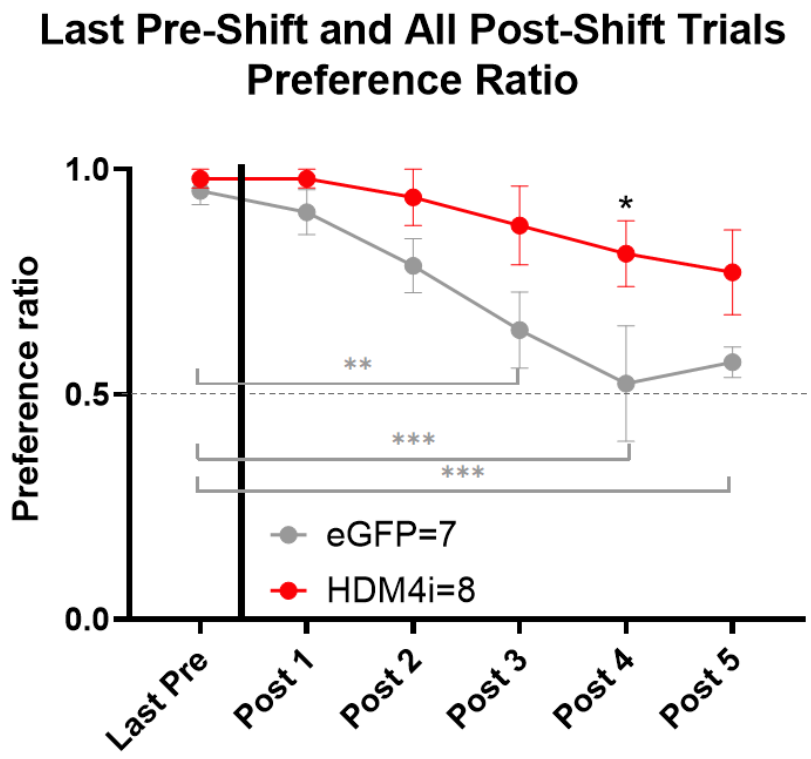

Figure 12: Preference ratio on the last day of pre-shift testing and 5 days of post-shift testing. This data is depicted as the mean ± SEM (n=8, hM4Di, n=7, eGFP). A Two-Way ANOVA revealed a significant main effect of session (preference ratio across testing days) and a main effect of group (eGFP vs hM4Di). There was a significant difference in the preference ratio between the last day of pre-shift testing and post-shift day 3, 4, 5 for the eGFP group. Additionally, the eGFP group had a significantly lower preference ratio on post-shift day 4 as opposed to the hM4Di group.

Control animals adapt on day 4 while the experimental group remains impaired.

In order to take a closer look at the preference ratio between groups on post-shift day 4, we performed a Two-Way ANOVA between session (last day of pre-shift testing and post-shift day 4) and group (eGFP and hM4Di). The y-axis represents the preference score and the x-axis displays the session, as seen in figure 13. The results revealed a main effect of session, $F(1, 13) = 15.59$, $p = 0.0017$, and a main effect of group, $F(1, 13) = 4.783$, $p = 0.0476$. In particular, when comparing the preference ratio of eGFP and hM4Di on post-shift day 4, the eGFP group had a significantly lower preference ($p = 0.0205$) for the side with the devalued reward. This suggests

that while the animals in the control eGFP group adapted to the reward loss by making equal visits to both reward sites, the hM4Di group made significantly higher visits to the devalued reward side, unable to adapt to the reward devaluation.

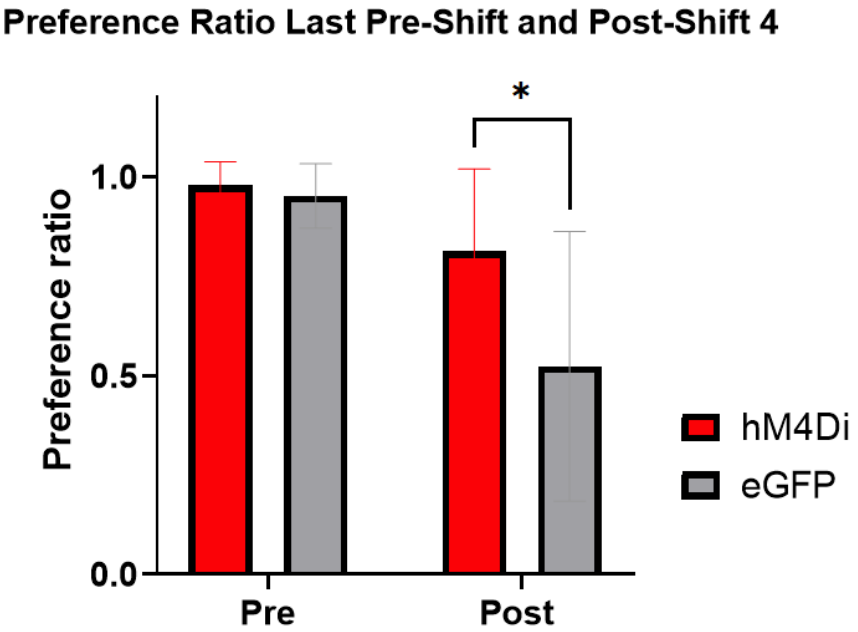

Figure 13: Bar graph representing the preference ratio for the last day of pre-shift testing and the post-shift day 4 for both eGFP and hM4Di groups (n=8, hM4Di, n=7, eGFP). There was a significant main effect across session (last pre-shift day vs post-shift day 4) and of group (eGFP vs hM4Di). eGFP animals had a significantly lower preference ratio as compared to hM4Di animals on post-shift day 4.

Reward loss promoted drug-induced reinstatement.

The CPP score is calculated by subtracting the time spent in the saline-paired compartment from the time spent in the cocaine-paired compartment. A positive value on the CPP score indicates a preference of the cocaine-paired side while a negative value indicates a preference of the saline-paired side. The y-axis represents the averaged CPP scores of the eGFP group and the hM4Di group across experimental days, as seen in figure 14. A Two-Way ANOVA of session (experimental day) X group (eGFP vs hM4Di) revealed a significant main effect of session $F(6, 48) = 8.314$, $p < 0.0001$ but no main effect of group ($p = 0.3746$). Specifically, for the hM4Di

group, there is a significant increase in the CPP score between the last day of pre-shift to the first day of post-shift, p = 0.0271. These data and the graph suggest that there was a significant difference in the CPP score between the last day of pre-shift and the first day of post shift in the hM4Di group. The preference for the cocaine paired side of the CPP apparatus significantly returned after a reward devaluation in the maze. The eGFP group follows a very similar pattern, but the statistical analysis failed to show a significant difference in CPP score between the two days. This shows that both the eGFP and the hM4Di group demonstrated frustration after reward devaluation in the maze.

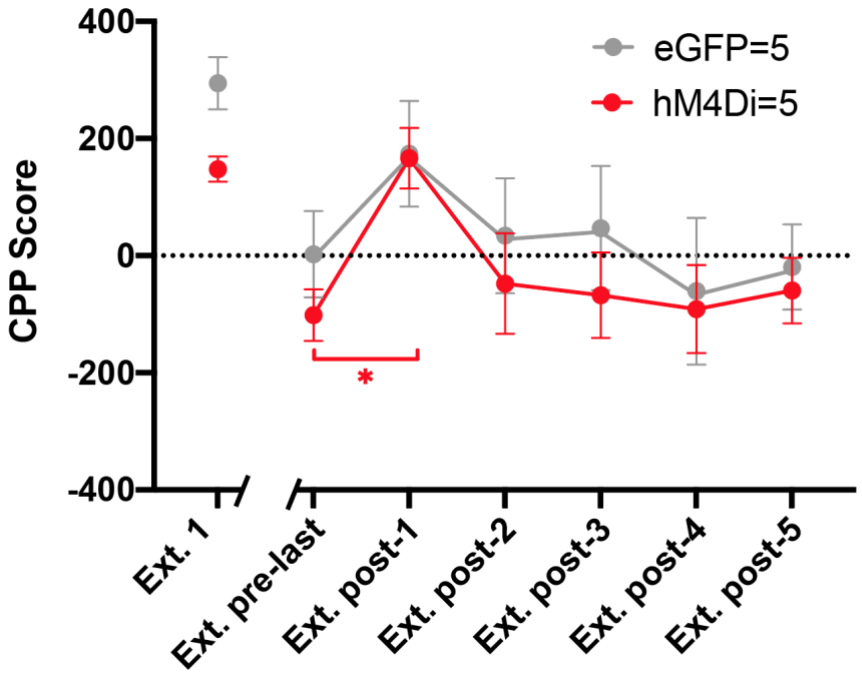

Figure 14: This graph shows the progression of the CPP score of both groups (n=5, hM4Di, n=5, eGFP) over sessions, meaning the experimental period (first day of extinction, extinction on the last day of pre-shift testing, extinction on 5 days post-shift testing). A Two-Way ANOVA revealed a main effect of session and specifically between the last day of pre-shift testing to the first day of post-shift testing for the hM4Di group, analogous with the reward devaluation on the maze. While it did not reach significance, the eGFP group followed a similar pattern of CPP scores between the last day of pre-shift testing and the first day of post-shift testing.

Vicarious Trial Error Events across groups.

We also investigated the average number of VTE events during pre-shift and post-shift phases. A Two-Way ANOVA of session (pre-shift vs postshift) and group (eGFP vs hM4Di) revealed a main effect of session $F(1, 26) = 21.40$, $p < 0.0001$ but no main interaction for group. This means that overall, there was an increase in the VTE events between pre-shift and post-shift testing days but no significant difference in the groups in either of those sessions. While there seems to be a trend of lower number of average VTE events in the hM4Di group during post-shift testing days, this difference is not significant and the error bars demonstrate a large variability between subjects on VTE behavior. A larger n might help understand if there lie differences in VTE behavior between eGFP and hM4Di groups during post-shift testing sessions.

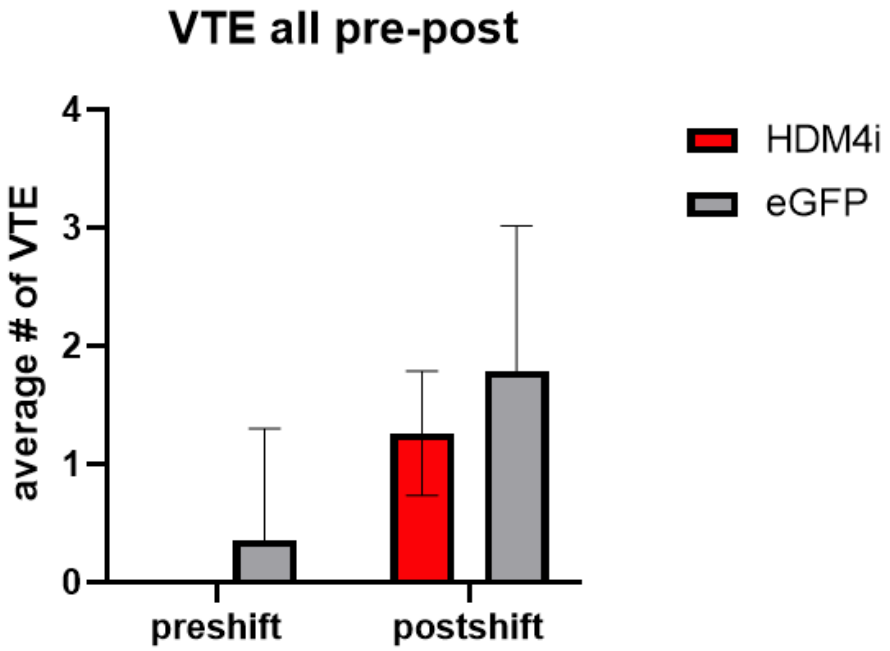

Figure 15: Bar graph showing the average number of VTE events across both groups (n=8, hM4Di, n=7, eGFP) during the pre-shift and post-shift testing phases. A Two-Way ANOVA of session and group revealed a main effect of session but no main effect of group.

Intact emotionality after hippocampal inactivation.

In order to examine the performance of the rodents on the open field task, the average time spent in each zone of the open field (exterior, middle, and center) was measured. A Two-Way

ANOVA between group (eGFP vs hM4Di) and zone (exterior, middle, center) revealed a significant main effect of zone $F(2, 12) = 690.3$, $p < 0.0001$, however no significant main effect of group. Overall, both groups of animals spent significantly more time in the exterior zone rather than the middle and the center zones. Since there was no significant difference between groups in the amount of time spent in each zone, we can conclude that both groups of animals displayed behavior that was indicative of similar levels of anxiety.

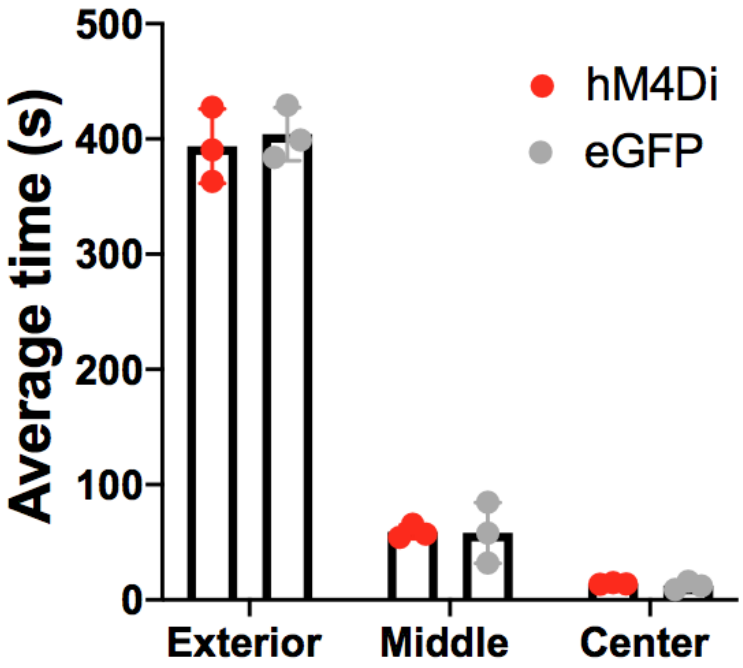

Figure 16: Bar chart representing the average amount of time that each group of animals spent in one of three zones of the open field. A Two-Way ANOVA of group and zone revealed a main effect of zone, where a significantly high amount of time was spent in the exterior zone by both groups of animals. There was no main effect of group, moreover, both groups displayed similar levels of anxiety.

The performance of rats in the light-dark box was measured as the amount of time animals spent in the black and the white compartment. We assessed the results using a Two-Way ANOVA of group (hM4Di, eGFP) and zone (black, white) and it revealed a significant main effect of zone $F(1,8) = 37.98$, $p = 0.0003$, but no main effect of group. This means that overall, both groups of

animals spent significantly higher time in the black compartment as opposed to the white one, indicating that both groups displayed similar levels of anxiety.

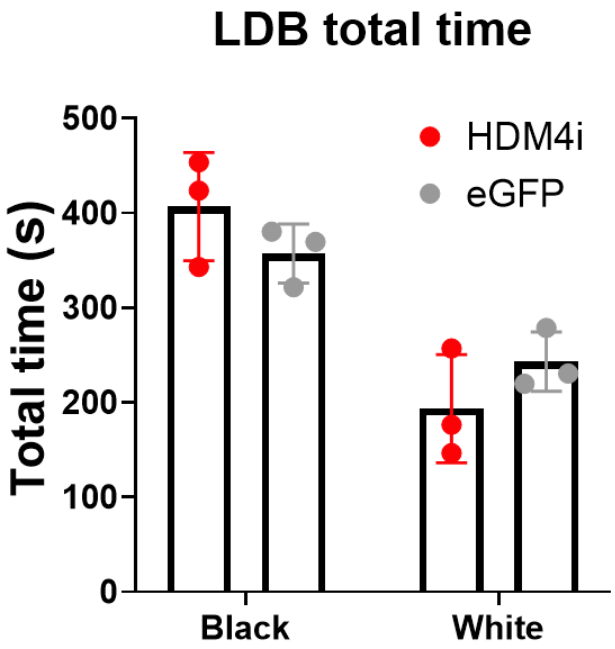

Figure 17: Bar graph indicating total amount of time spent in the back and white compartment by both groups of animals.

Discussion

Understanding the neural correlates of reward loss can provide insight into why some aversive experiences drive adaptive behaviors in some individuals and maladaptive behaviors in others. Because there is mounting evidence demonstrating the existence of neuronal cell assemblies in the hippocampus that encode reward (Gauthier & Tank, 2018; Jin and Lee, 2021; Jarzebowski et al., 2021), in this thesis I explored the role of the hippocampus in the behavioral adaptation to reward loss. I found that hippocampal silencing through inhibitory DREADD activation prevented rats from adapting to a reward devaluation. In addition, I explored whether the hippocampus was involved in the emotional processing immediately following a reward loss event by examining reinstatement of a cocaine-associated behavior. We observed that rats failed to adapt to a reward downshift in the devaluation task. However, they reinstated their previously extinguished drug-associated behaviors in the CPP task. Together, these results suggest that the hippocampus plays an integral role in behavioral adaptation after reward loss but in not the emotional response to the aversive event.

Instrumental behavior can be driven by goal-driven or habit-dependent behavior. Goal-directed behavior stems from rodents making behavioral choices that are motivated by the end goal or reward that they will achieve (Balleine & Dickenson, 1998). Lesions studies of the baslo-lateral amygdala (BLA) (Blundell et al., 2001; Balleine et al., 2003), the nucleus accumbens (NAc) (Corbit et al., 2001) and the medial prefrontal cortex (Passingham and Wise, 2012) are found to be the major neural correlates involved in goal-directed behavior. Specifically, in instrumental tasks such as lever pressing responses, animals with lesions to the BLA and NAc display higher instrumental responses to levers that were associated with highly valued rewards as opposed to no rewards, particularly when a reward downshift takes place. In studies where brain regions in goal-directed behavior are involved, there is impaired performance in the post-devaluation phase, meaning that rodents engage in similar behaviors towards differently valued stimuli (Blundell et al., 2001; Balleine et al., 2003; Corbit et al., 2001). It is known that these brain regions receive several projections from the hippocampus and disrupting circuitry that links these regions to the hippocampus has also resulted in impaired performance on goal-directed

tasks (Numan, 2015; Manella et al., 2013; Pennartz et al., 2011). The hippocampus, hence, is a structure of interest in experiments that use instrumental tasks to study goal-directed behavior.

When a certain action is performed regularly, the environmental cues linked to the repetitive action are strengthened (stimulus-response association), making them easily accessible with every iteration of the repetitive action. This process can be understood as habit formation (Dickinson, 1985). Well-ingrained habits can be identified as those that have lost sensitivity to consequence or change (Graybiel, 2008). Several studies have implicated the dorso-lateral striatum (DLS) in formation of habit-based behaviors (Apicella et al., 1991; Barnes et al., 2005; Cromwell et al., 2005; Fuji & Graybiel, 2003; Hikosaka et al., 1989; Hollerman et al., 1998; Jin & Costa, 2010; Jog et al. 1999). Lesion studies (Daw et al., 2005; Dolan & Dayan, 2013; Packard, 2009; Yin & Knowlton, 2006) and inactivations (Yin et al., 2004; Balleine et al., 2009) of DLS have provided further evidence that the DLS is critical for habit behaviors. However, it is important to note that the goal-directed system interacts with the habit-formation process to update information from environmental inputs and changes to the value of a habit-based outcome (Gillan et al., 2015).

Given that my experiments utilize a task that could lead to habit formation before the reward devaluation, it is important to assess the results of the devaluation task through this lens. Additionally, the manipulation to the hippocampus could help us distinguish between animals that engage in habit-directed vs goal-directed behavior. The results from our experiment show that hippocampal inactivation prevents rat from modifying their behavioral responses in accordance to a reward downshift. This is in contrast with control rats that are able to alter their behavioral patterns after such reward devaluation. These results suggest that the hippocampus is a key region that supports adaptation to reward loss. We observe that rats with an inactivated hippocampus are unable to adapt to the reward loss that they experience and persevere on maladaptive responses. One explanation for this could be that animals with an inactivated hippocampus rely on a more habit-oriented instrumental response. This is because they engage in behaviors that are not adaptive by perseverating on their choices to the devalued reward side even though there is a mismatch of expectation. The failure in adaptation to reward devaluation shows the lack of executive control and repeated engagement in maladaptive behaviors. In our

experiment, while the hippocampus is inactivated, habit-based systems involving the DLS are still intact. It can thus be inferred that systems responsible for habit formations, such as the DLS possibly take over in animals with an inactivated hippocampus to compensate for the deficits of learning and memory (Hales et al., 2014; Kosaki & Watanabe, 2012; Abela, 2013). On the other hand, animals with an intact hippocampus adapt their behavior after reward devaluation. This could be because the hippocampus as well as other structures that have been deemed responsible for goal-directed behavior are still intact. This indicates that information can be updated with the new reward outcomes that rodents experience on the maze (Gillan et al., 2015). Hence, understanding the different neural systems involved in habit-based and goal-directed behavior could assist in explaining the lack of adaptation to reward loss in animals with an inactivated hippocampus.

Another line of explanation also lies in understanding the importance of the hippocampus in cognitive flexibility. Cognitive flexibility refers to the ability to switch a behavioral response according to the context of a situation (Scott, 1962) to update a strategy to optimally obtain a reward or adjust to a change in reward. Past literature has explored the role of the hippocampus in responding to situations that require rodents to recognize updated locations of rewards (Garthe et al., 2009; Burghardt et al., 2012) and therefore behavioral adjustment to such environmental changes. In particular, Garthe and collaborators (2009) found that suppression of adult neurogenesis with temozolomide led to an impaired performance in a reference memory task that required spatial memory updating in mice, the Morris watermaze. In agreement with this, Burghardt et al., 2012, reported that ablation of adult neurogenesis did not impair the ability of mice to learn an initial location associated with a shock but that the impairment became apparent when mice had to flexibly modify such learning due to the switch of the shock zone to another location. Given that our results demonstrate the inability of animals with an inactivated hippocampus to flexibly adapt their behavioral responses to reward devaluation, future studies could involve the use of tasks directly targeted toward exploiting cognitive flexibility.

Previous research from the Sabariego lab suggests that the hippocampus plays a critical role in adapting to a reward loss event. Rats with hippocampal lesions are able to form an initial preference for a higher reward but they are not able to modify their responses after the reward is

devalued. On the other hand, sham rats adjust to the reward devaluation by decreasing their preference for the devalued site and increasing their preference for the non-devalued site. The previous study in the Sabariego Lab provided intriguing evidence that the hippocampus was required to adapt to reward loss. However, several open questions remained due to the use of lesions. Did lesion animals learn the task differently from their sham counterparts? Does the dorsal and the ventral hippocampus play different roles in adaptation to reward changes? Did excitotoxic lesions of the hippocampus indirectly alter other neural circuits? The permanent lesions could have led to scar-forming astrocyte recruitment or increased cytokine signaling, which could have altered behavior.

To mitigate the risk of off-target effects influencing our results and to gain temporal control on the loss and gain of function of the hippocampal circuit, we used an inhibitory DREADD system to inactivate the dorsal hippocampus in a temporally and spatially controlled manner. The hM4Di is a commonly used DREADD that has been shown to silence hippocampus activity after CNO administration (Smith, Bucci, Luikart, and Mahler, 2016). These DREADDs can be expressed via the CaMKII promoter, preferentially expressed in cortical glutamatergic neurons and some subcortical GABAergic cells (Smith, Bucci, Luikart, and Mahler, 2016). However, we selected the neuronal-specific hSyn promoter because it more closely matched the broad-spectrum hippocampal perturbations caused by a lesion without the same off-target effects. Also, using an inhibitory DREADD approach eliminated the possibility that hippocampus function disrupted memory acquisition during the CPP conditioning or pre-shift training. Therefore, I inhibited the dorsal hippocampus (dHPC) after rats formed strong drug-associated and location-specific reward memories.

The contributions of the dorsal, immediate (iHPC), and ventral (vHPC) hippocampal activity to reward encoding are still a topic of debate in the field. Recordings taken from the dorsal CA1 and subiculum of the hippocampus have been associated with specific reward information encoding (Gauthier & Tank, 2018). Additionally, other studies have reported that silencing the dorsal hippocampus of rodents significantly impaired performance on a fixed reward location task despite having learned the task with a fully active hippocampus (Zaremba et al., 2017). Our results are consistent with these findings since we observed impairments on a

reward-related behavioral task with dorsally focused hippocampal inactivations. However, it has recently been reported that while dHPC cells increase firing in anticipation to reward, the pools of cells active in each reward varies depending on the location whereas in the iHPC-vHPC, the same set of cells are active in anticipation to a reward, regardless of the location. This might suggest that the dedicated neuronal population of cells that encode reward might reside in the iHPC-vHPC as opposed to the dHPC (Jarzebowski et al., 2021). Additionally, other studies that investigated reward value reported that iHPC-vHPC cells showed greater activity in the CA1 region with regards to changes in palatability of rewards when compared to the dHPC (Jin & Lee, 2021). Previous experiments in the Sabariego lab have found that the hippocampus is not required to assess palatability since both hippocampal and sham lesioned rats develop a strong preference for the high reward before the devaluation. However, understanding the specific contributions of the immediate and ventral hippocampus to adaptation to loss remains to be understood and so a future direction could be to compare the performance of rodents with exclusively-dorsal or exclusively-intermediate/ventral inactivation, on the figure-8 maze.

Even though the data from our lab's lesion study suggested that the hippocampus plays a role in responding to reward devaluation, there remained the open question as to the reason for the differences observed between both groups of rats. Namely, do rats fail to adapt to reward loss because they have altered emotional processing, or are they cognitively inflexible? Previous studies have found that reward loss events cause animals to self-medicate (Manzo et al. 2014, Manzo et al. 2015). In 2017, Hogarth & Hardy found that a significantly higher percentage of participants who read negative-mood inducing statements chose alcohol-seeking responses (rather than food-seeking responses) as opposed to participants who had read positive statements (Hogarth & Hardy, 2017). In another study, researchers used a behavioral economics paradigm that showed an increased value placed on alcohol when personalized stress factors were induced (Owens et al., 2014). We used the CPP task to assess frustration induced by reward loss in accordance with above literature.

Our CPP data suggests that both groups of rats experience frustration after reward devaluation that leads to the return of previously extinguished drug-seeking behaviors. There is a significant increase in the CPP score of hM4Di animals from the last day of pre-shift to the first

day of post-shift testing. Therefore, while these animals extinguished their preference for the cocaine-associated context by the last day of pre-shift testing, they reinstated their preference for the cocaine-associated context when they experienced a reward downshift on the devaluation task. These results suggest that all rats feel frustration after reward-loss, regardless of their hippocampal activity. It is also interesting to note that while the behavioral sign of negative affect is seen on the first day of post-shift in the CPP, the preference for the devalued side is maintained for another two days in the control group (and for the whole experimental phase in the experimental group). Together, our results indicate that hippocampal inactivations do not have an effect on emotionality and therefore the reason why animals do not adapt to reward downshifts is not their inability to feel frustration.

Moreover, we examined the effect of hippocampal activity on baseline anxiety, which could confound the interpretation of measures of emotional processing. The open-field task assesses the anxiety level of rodents depending on the amount of time spent in different zones of an arena. More time spent in the outer region, close to the walls, suggests heightened anxiety, whereas more exploration towards the center of the open field indicates otherwise (Seibenhener & Wooten, 2015). Our results show that both groups, hM4Di and eGFP, spent similar amounts of time in the outer region, closest to the walls, suggesting similar levels of general baseline anxiety for both groups. As for the light-dark box (LDB), a higher amount of time spent in the darker compartment suggests enhanced anxiety (Bourin & Mascoët, 2003). The results of the LDB also suggest that both groups acted similarly and spent the most amount of time in the darker environment. Together, these results indicate that inhibition of the hippocampus through the hM4Di DREADD did not alter baseline anxiety-like behavior.

It is crucial that future research explores the neuronal correlates of frustration-induced changes in drug-associated behaviors. Research that links emotionality and reward loss points to reward loss events involving hypothalamic-pituitary and adrenal axis activity, hinting towards their contribution to the etiology of substance use disorders. Stressful events, particularly reward loss events as established before, lead to the activation of the HPA-axis. A rise in stress levels have been linked to instances of substance use and ineffective abstinence from substances such as opium (Hassanbeigi et al., 2013). Reduction in reward, especially measured in terms of

reduction of sucrose content from 32% to 4%, has been linked to the activation of the HPA axis, and lead to an increase in corticosterone levels in rats (Pecoraro et al., 2009; Flaherty et al., 1985). Instances of reward loss that promote high levels of cortisol could hence be linked to increase in behavior linked to substance use. For example, Manzo et al. 2014 found that rats increased preference for ethanol after sessions of appetitive extinction, but not after acquisition (reinforced) sessions. Because ethanol has anxiolytic properties in tasks involving reward loss, these results were interpreted as anti-anxiety self-medication. In the same line, Manzo et al. 2015, reported that rats enhanced voluntary consumption of chlordiazepoxide (a prescription anxiolytic) during periods of emotional distress triggered by reward loss.

Limitations

In order to determine the right balance of dosage and conditioning days for cocaine, we had to vary those two parameters across our cohorts in order to achieve the right balance. In our first cohort which consisted of animals 27-30, the cocaine dosage utilized was 10 mg/kg and this resulted in the cohort developing a preference for cocaine that was never extinguished. In our second cohort, the dosage was adjusted to 7.5 mg/kg while keeping the conditioning days to 4 which resulted in one animal not developing a preference for the cocaine-paired side. In our final cohort, it was agreed that we would maintain a 7.5 mg/kg dose but increase the conditioning days to 6. This seemed to strike the right balance meaning that all the animals did develop a preference for the cocaine-paired side but also demonstrated extinction of preference towards the end of the pre-shift testing phase as desired. While we were able to analyze the CPP data for all the animals that displayed this pattern to evaluate CPP score post-devaluation, the fact that the cohorts were conditioned with different doses and conditioning days might have been a drawback. In the future, the experiment will have to be repeated with 6 conditioning days and a 7.5 mg/kg dose in order to strengthen the pattern that is observed in the CPP task.

Significance

Frustration is defined as an aversive emotional state triggered by situations involving reward loss, either by omission or devaluation. There is compelling epidemiological and clinical evidence

associating reward loss with the etiology of anxiety and depression (Hammen, 2005; Kessler, 1997). Furthermore, as per the Social Readjustment Rating Scale, an instrument that has been used for five decades as a tool for ranking stressful life events, some of the most stressful experiences in life such as death of a partner, divorce, being fired, etc, involve a component of loss (Scully, Tosi, & Banning, 2000). Additionally, in 2017, the World Health Organization (WHO) identified depression as the leading cause of poor health and disability, with more than 300 million people living with the mental disorder. Nearly the same number of people is affected by anxiety disorders. Alcohol abuse results in 3.3 millions deaths each year, and 155 to 250 million people use other psychoactive substances (including prescription medication). The WHO's Mental Health Action Plan 2013-2020 also indicates that people with major mental illness have a 40% to 60% greater chance of dying prematurely than the general population, owing to physical problems, drug abuse and suicide. Considering the growing incidence of SUD, anxiety, and depression disorders (Collins et al. 2013), the coincident rise in suicidal behavior (Mee et al. 2006), and the compelling links between reward loss and the risk of developing these neuropsychiatric disorders (Huston et al. 2013; Mee et al. 2006; Papini et al. 2015), it is extremely important to understand the neural mechanisms that underlie reactions to reward loss as well as the effects of drugs of abuse on the brain. The findings from this thesis help shed light on the brain structures involved in reward loss and also explore neural correlates that could be associated to drug-seeking behaviours through the conditioned place preference task, especially after reward loss.

Future Directions

In the future, we aim to understand the neural correlates that underlie reward processing and develop tasks to assess further explanations for the lack of adaptation to reward loss events. We want to identify firing patterns of neuronal cell assemblies that underlie reward expectations as well as adaptation to incentive loss. Additionally, we want to evaluate hippocampal replay and VTE events in the representation of reward and study changes, if any, in firing pattern after reward changes. These aims would be achieved by taking in-vivo electrophysiological recordings from animals while they are actively carrying out reward devaluation tasks. Additionally, we

also want to expose animals with hippocampal inactivations on tasks such as the dig task to understand if cognitive inflexibility is the reason behind the lack of adaptation to reward loss. Furthermore, we would like to repeat the same experiment with another mixed (male and female) cohort of rats with a conditioning schedule of 6 days at 7.5 mg/kg cocaine dose in order to further strengthen the conclusions that this research draws. We would also plot data comparing the performance of animals with lesions and DREADDs to understand if severing connections to other areas of the hippocampus have an effect on the maze performance during the devaluation task.

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Supplemental Information

ALL 2 pellets **POST-SHIFT TEST**

RAT NUMBER:

DATE:
DAY 1

TRIAL	REWARD	LEFT	RIGHT
1	2		
2	2		
3	2		
4	2		
5	2		
6	2		
7	2		
8	2		
9	2		
10	2		
11	2		
12	2		

DATE:
DAY 2

TRIAL	REWARD	LEFT	RIGHT
1	2		
2	2		
3	2		
4	2		
5	2		
6	2		
7	2		
8	2		
9	2		
10	2		
11	2		
12	2		

DATE:
DAY 3

TRIAL	REWARD	LEFT	RIGHT
1	2		
2	2		
3	2		
4	2		
5	2		
6	2		
7	2		
8	2		
9	2		
10	2		
11	2		
12	2		

DATE:
DAY 4

TRIAL	REWARD	LEFT	RIGHT
1	2		
2	2		
3	2		
4	2		
5	2		
6	2		
7	2		
8	2		
9	2		
10	2		
11	2		
12	2		

Supplemental Figure 1: Example of Devaluation Task Behaviour Sheet