Probing the Role of the Ventral Hippocampus to Nucleus Accumbens Pathway in Individual Differences in Appetitive Learning

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PROBING THE ROLE OF THE VENTRAL HIPPOCAMPUS TO NUCLEUS ACCUMBENS PATHWAY IN INDIVIDUAL DIFFERENCES IN APPETITIVE LEARNING

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ABSTRACT

A critical issue in substance abuse research is why some individuals can actively use addictive drugs and quit with relative ease, while others may only try a small dose before becoming life-long dependent (Fitzpatrick et al., 2016). The use of designer receptors exclusively activated by designer drugs (DREADDs) may shed light on individual differences in learning behaviors and why some individuals seem to be more prone to addiction and relapse than others. Using stereotaxic surgery on rats, an in vivo dual-vector approach was used to bilaterally inject Cre recombinase into the nucleus accumbens (NAc) and an excitatory Cre-dependent, G protein-coupled DREADD into the ventral hippocampus (vHPC). Five weeks after surgery, clozapine-N-oxide (CNO) was injected intraperitoneally to selectively activate this pathway for six days. Rats were then given one day of cross treatment followed by conditioned reinforcement. These procedures allowed determination of whether activation of the vHPC—NAc projection affects acquisition and/or expression of Pavlovian conditioned approach (PCA) behavior. There were no statistically significant differences amongst treatment groups. However, trends in data support a differential role of the vHPC—NAc pathway in sign- and goal-tracking behaviors, suggesting a need for further investigation using larger sample sizes to determine the importance of this pathway. Although this study found no statistically significant evidence for the role of the vHPC-NAc pathway in PCA behaviors, current and future findings may add to an understanding of how learning and neurological activity may play a role in behaviors associated with addiction in human beings.
INTRODUCTION

Addiction has become the leading cause of death, morbidity, and lost productivity (Chen et al., 2003; Fuhrmann-Berger, 2018; Florence et al., 2021). In the United States alone, there are nearly 21 million people currently struggling with at least one addiction (Yerby & Hampton, 2021). According to the Centers for Disease Control and Prevention (CDC, 2022), this issue has led to 93,000 overdose deaths in 2020. Addiction has been a public health concern within the United States in particular for centuries, leading to abundant addiction research regarding its etiology and underlying mechanisms.

Individual Differences in Addiction Development

As the number of individuals suffering from addiction increases, addiction research has become critical in the scientific community. Even more concerning than the sheer number of individuals with addiction, however, is how some individuals can actively use addictive drugs and quit or cut down with relative ease, while others may only try a small dose before becoming life-long dependent (Fitzpatrick et al., 2016). Research has begun to consider that some individuals may respond emotionally to environmental cues, creating emotionally salient events that motivate behavior (Morrow et al., 2011; Meyer et al., 2012; Robinson et al., 2014). Through the use of animal models, it is possible to venture into the study of these considerations and strive to find answers.

Pavlovian Conditioned Approach: Sign- & Goal-Tracking

In order to use animals to model addiction, rats are often classified using a Pavlovian conditioned approach (PCA) procedure. For PCA, rats are placed in a controlled environment in which an illuminated, retractable lever, i.e., the conditioned stimulus (CS), is presented for a few seconds. This is then followed by a food reward, i.e., the unconditioned stimulus (US). Although there is no need for the rats to engage with the lever in any way in order to receive the food reward, they learn to associate the presence of the lever with the acquisition of a reward. As PCA progresses, two learning behaviors are observed as the association between these two stimuli is made. Some rats, referred to as sign trackers (ST), seem to treat the lever itself as an incentive --sniffing, gnawing, and scratching the lever when presented-- as well as accepting the food reward. Goal trackers (GT), on the other hand, are observed to have no interest in the lever itself but prepare for the food reward upon the le-
ver’s presentation. This allows for the researcher to measure incentive salience. Incentive salience is a property that a cue may have in which it has become an attractive, desired stimulus (Berridge, 1996; Beckmann & Bardo, 2012; Pitchers et al., 2015; Dickson et al., 2015). STs are observed to become attracted to reward-paired cues in which the cues seem to become rewarding in themselves, attributing incentive salience to the cue. GTs, on the other hand, seem to learn reward-cue associations, but treat the cues as mere predictors without incentive properties. It is important to note that both STs and GTs accept the food reward, and both types learn the conditioned response at the same rate (Kuhn et al., 2018). However, the interest in the lever-cue and where incentive salience is attributed is the difference between these two learning types (Figure 1).

**Figure 1:** STs are first attracted to the illuminated lever before accepting the food reward. GTs, however, show no interest in the lever, going only to the food dispense area (Tomie & Morrow, 2018).

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**Sign Trackers & Goal Trackers: Addiction**

Several observations seem to indicate that STs are more prone to addiction-like behaviors than GTs. In studies that focus on drug seeking behavior, STs are observed to show greater cue-induced drug-seeking behaviors (Saunders & Robinson, 2011). STs are also found to have more susceptibility to the cognitive-motivational learning of drug-seeking behaviors, while having a limited understanding of contextual cues (Flagel et al., 2021). In terms of drug use, exposure to stimuli previously associated with a drug may cause drug-seeking behaviors in STs, making them more prone to addiction. STs have been observed in various studies to
have more cue-driven motivation for food, cocaine, opioid, and nicotine, supporting the implications of this type of response for the etiology of addiction (Robinson & Flagel, 2009; Flagel et al., 2010; Saunders & Robinson, 2010; Saunders & Robinson, 2013; Yager & Robinson, 2010; Saunders et al., 2013; Yager et al., 2015; Nasser et al., 2015). Similarly, these sign-tracking behaviors seem to be long-lasting and resilient, suggesting a resistance to extinction and a greater inclination for relapse (Tomie et al., 2008; Flagel et al., 2021; Colaizzi et al., 2020). Finally, sign-tracking animals are prone to spontaneous recovery as well as rapid reacquisition of sign-tracking behaviors even when provided extensive drug-taking elimination training (Tomie et al., 2008; Tomie et al., 2016).

**Sign-Tracking in Humans**

The behaviors of sign-tracking and goal-tracking may look extremely similar for human beings. For example, Garofalo and di Pellegrino (2015) trained human participants to associate a previously neutral visual cue (CS) with a monetary reward (US), tracking the participants’ eye movement towards the sign (CS) or the goal (US). This determined whether an individual was categorized as a ST or GT. STs had a greater likelihood of responding to the CS even after the US was unavailable (Colaizzi et al., 2020). In other studies of goal-tracking and sign-tracking human beings, participants were trained to associate a visual distractor cue to a reward. Once trained, if participants looked towards the distractor, the reward was omitted, requiring the participant to suppress their urge to look at the cue in order to receive the reward. Despite this expectation, participants frequently looked towards the distractors, particularly in cases where the distractor was related to a high-value reward (Failing et al., 2015; Le Pelley et al., 2015; Pearson et al., 2015; Pearson et al., 2016; Colaizzi et al., 2020). Studies such as these indicate the parallels of rat models and human behaviors, highlighting the issue of impulse control in particular.

**PREVIOUS EXPERIMENTS**

**Self-Administration of Cocaine**

In studies such as Saunders and Robinson (2011), researchers sought to better understand the cues in the environment associated with addiction. In these experiments, groups of rats were trained in a PCA environment to associate the retracted lever cue with food reward,
allowing for determination of STs and GTs phenotypes. From there, a self-administration experiment was conducted. Both STs and GTs were given an intravenous catheter surgery to provide an administration port for cocaine. In the self-administration box, there is both an active and inactive nose poke. If a rat’s nose poked in the PCA active nose port, an intravenous infusion of cocaine was administered. The rats were then placed on a schedule in which the number of nose pokes needed to receive the cocaine increased after each infusion. Through this schedule, ST rats were found to work harder for the administration of cocaine. Finally, after the use of an extinction procedure, rats were tested for cocaine-induced reinstatement, revealing that STs were more motivated to receive the cocaine and more readily reinstated than GTs. These findings support the conclusion that STs are more prone to drug-seeking behaviors in general as well as have a greater inclination for reinstatement of drug-seeking behaviors, translating to a greater inclination of relapse. This lab has used studies such as these to reinforce the importance of sign- and goal-tracking behaviors in this addiction research.

**Ventral Hippocampus, Nucleus Accumbens, & Dopamine**

Once PCA and self-administration behaviors had been observed, the next consideration was what pathways within the brain seem to be involved in this process. In a 2016 experiment, Fitzpatrick et al. considered how the hippocampus, as well as its subregions, may be involved in the acquisition and expression of sign-tracking and goal-tracking behaviors. The hippocampus is associated with a variety of memory types such as spatial navigation and encoding of contextual information (Tomie & Morrow, 2018). This may play an important role in addiction and the environmental aspects of usage. Also of interest was the increased release of dopamine for STs in the nucleus accumbens (NAc). It has previously been found that the ventral hippocampus (vHPC) is critical for dopaminergic activity in the NAc, and lesions in the vHPC region decrease dopamine levels within the NAc (Lipska et al., 1992; Lipska et al., 1991; Fitzpatrick et al., 2016). Similarly, drugs of abuse are known to elevate dopamine transmission, and many individuals with addiction predisposition are found to have relatively high dopaminergic activity in general (Hyman et al., 2006; Morrow & Flagel, 2016). Previous research in this lab has reinforced this, finding that lesions in the vHPC may cause a decrease in dopamine levels in the NAc (Fitzpatrick et al., 2016). This suggests that because STs release more dopamine in the NAc, sign-tracking behaviors are dopamine-dependent (Fitzpatrick et al., 2016). In order to
score ST versus GT, Fitzpatrick et al. (2016) used a PCA index score that averaged responses, incorporating the number, latency, and probability of lever presses and magazine entries. In this study specifically, lesions of the vHPC yielded a decrease in sign-tracking behaviors and an increase in goal-tracking behaviors (Figure 2). Similarly, lesions of the vHPC were found to prevent learning of the sign-tracking response, which was associated with a decrease in the concentration of dopamine metabolite, homovanillic acid, in the NAc. These findings suggest that the vHPC and NAc play an important role in the acquisition of sign-tracking behaviors.

**Figure 2:** PCA Index Scores of rats that have undergone vHPC lesions before the acquisition of PCA training were found to be more likely to GT (score ≤ -0.5) than ST (score ≥ 0.5), in comparison to those given sham surgeries (Fitzpatrick et al., 2016).

It is then possible that increased vHPC activity may be related to regulations in the dopamine of the NAc in STs and is a critical component to motivation involved in these behaviors (Fitzpatrick et al., 2016). Therefore, the vHPC and NAc pathway is considered to be involved in the acquisition of sign-tracking behavior due to its involvement in dopamine and motivation processes (Chang et al., 2012). With these findings in mind, this lab wanted to further investigate how manipulation of the activity within this area of the brain may influence sign- and goal-tracking behaviors.
DREADDs

Chemogenetics are an engineered method for researchers to interact with the molecular chemicals of the brain (Forkmann & Dangelmayr, 1980; Sternson & Roth, 2014; Strobel, 1998; Roth, 2016). Of the many classes of genetically engineered proteins, designer receptors exclusively activated by designer drugs (DREADDs) have become one of the most widely used methods for manipulating neuronal activity (Roth, 2016). DREADDs are G protein-coupled muscarinic receptors that have been genetically modified to respond specifically to particular compounds foreign to the body. A virus that will cause DREADD proteins to be expressed is injected into a specific brain region to allow experimenters to excite or inhibit the downstream pathway. For example, an hM3Dq DREADD is typically used to excite firing within a particular neuronal pathway; this is the excitatory DREADD used in the experiments conducted in this lab. This is strictly activated by clozapine-N-oxide (CNO), a synthetic compound that is not naturally produced by the body (Roth, 2016; Morrow & Flagel, 2016). Once CNO is injected, there is an increase in neuronal excitability and firing for approximately two hours (Alexander et al., 2009; Cheng & Wang, 2019).

Cre Recombinase

Cre recombinase is a tyrosine recombinase enzyme derived from the P1 bacteriophage, this is used for its site specific recombination of DNA (Van Duyne, 2015). A Cre-dependent DREADD, then, will only express in the presence of Cre, allowing for the pathway of interest to be the only pathway in which the DREADD will be expressed. This is done by injecting a DREADD-expressing virus into the input region and injecting a retrograde Cre-expressing virus into the output region to selectively activate this pathway. For this study specifically, rather than exciting or inhibiting any projection of the vHPC, this allows for targeting of the vHPC-NAc pathway by requiring that Cre be present in order for the DREADD to be activated.

Hypothesis

This lab anticipated seeing an increase in sign-tracking behavior in response to this excitatory DREADD due to the theorized role of increased activity within the vHPC-NAc pathway leading to an increase in sign-tracking behaviors. A previous inhibitory experiment in this lab conducted by Cristina Maria-Ríos, found no significant differences in the lever presses and food-cup entry number between treatment groups.
(Figure 3). This suggested that the vHPC to NAc pathway may not be necessary for the acquisition of goal- and sign-tracking behaviors. However, this lab theorized that there may be a more complex and selective role in the performance of sign-tracking behaviors. Given the findings of Fitzpatrick et al. (2016) in particular, we expected that the excitatory DREADD would cause an increase in sign-tracking behaviors.

Figure 3: Inhibition of the vHPC does not significantly affect the acquisition of sign- and goal-tracking behaviors.

Review

From the findings of the research studies discussed, rat models suggest that STs may be more likely to become addicted as well as more prone to relapse. These rat behaviors parallel human behaviors, allowing for rat models to possibly provide insight into addiction behaviors in human beings. Considering the parallel between animal and human behaviors, it can be assumed that for some individuals with a substance use disorder, cues previously associated with a drug may cause craving and relapse (Grüsser et al., 2004; Colaizzi et al., 2020). This then led to the interest in the brain pathways to better understand the causes of these behaviors. Previous research has found that the vHPC and NAc, specifically this pathway, plays an important role in the acquisition of sign-tracking behaviors due to its involvement in dopamine and motivation processes (Chang et al., 2012). With all of these findings in mind, this lab tested the effects of these manipulations, and tested the hypothesis that the vHPC-NAc pathway is involved in sign-tracking behaviors. This evidence could provide insight into the neurological process of addiction in human beings.
**METHODS**

**DREADDs and Cre**

In this study specifically, a procedure injecting a Cre-dependent DREADD into the vHPC and Cre recombinase into the NAc using a viral vector was used. This allows for the pathway to carry the gene to make the desired receptor. This gene is then taken up by the cells and integrated into the genome to produce the desired receptor. This allows for the vHPC to NAc pathway to be specifically targeted in the experiment.

**Animals**

The participants for this study originally included 32 Sprague Dawley rats, 16 females and 16 males. These rats were approximately 7 to 8 weeks old, purchased from Charles River Breeding Laboratories, and were put on a 12:12- dark/light cycle (lights on 7pm). Following surgery, the study included male (n=16) and female (n=15) rats, one of the rats having been excluded from PCA due to an adverse reaction to CNO.

**Surgery & Recovery**

Through stereotaxic surgery, an in vivo dual-vector approach was used to bilaterally inject Cre recombinase [pENN.AAVrg.hSyn.H1.eG-FP-Cre.WPRE.SV40 (Retrograde)] into the NAc (mm from Bregma: 0.5 mL; 7.8 DV, ± 0.8 ML, -1.7 AP) and an excitatory Cre-dependent, G protein-coupled DREADD (pAAV-hSyn-DIO-hM3D(Gq)-mCherry) into the vHPC (mm from Bregma: 1.0 mL; 8.4 DV, ± 4.8 ML, -6.0 AP) at a rate of 0.1µl/min, to selectively target the vHPC–NAc projection. Twenty-six rats were given this surgery, and the other six received a control surgery in which Cre was injected into the NAc, while an mCherry control (does not contain DREADD; pAAV-hSyn-mCherry) was injected into the vHPC. While undergoing surgery, each rat was subjected to 1-5% of isoflurane as well as an injection of carprofen (5mg/kg S.C.). For two days post-operation, each rat was again injected with carprofen for pain relief. The rats were then given a period of five weeks prior to undergoing the PCA in order for the cells to take up the DREADD gene, integrate this into the genome, and produce the desired receptors.

**Pavlovian Conditioned Approach (PCA): Machinery**

The Pavlovian conditioning chamber consisted of 24.1 cm width x 20.5 cm depth x 29.2 cm height walls, a fan for ventilation and white
noise, a pellet magazine, a retractable lever, and a red house light. During the experiment, the retractable lever would pop out and be illuminated, followed by the delivery of a banana-flavored food pellet. This chamber also consisted of metal bars serving as a floor for the rats to stand on.

**PCA: Procedure**

Prior to the experiment, the rats were familiarized with the banana-flavored food pellets in their home cages. After five weeks post-operation, rats were placed into the chambers and were pre-trained to the lever and food reward pairing process for one day. Then, during the six PCA rounds, each rat was injected with clozapine-N-oxide (CNO; 3mg/kg, dissolved in 6% DMSO) or vehicle (6% DMSO) intraperitoneally 25 minutes before being placed in the PCA chamber. Six control surgery and fourteen DREADD surgery rats were given CNO, while twelve DREADD surgery rats were given the vehicle injection. Each PCA session consisted of 25 presentations of the retractable lever that remained extended for 10 seconds, and 25 deliveries of the banana-flavored pellets. The intertrial interval (ITI) ranged from 30-60 seconds. This means that the period of time between lever-food pairings varied randomly from 30 to 60 seconds throughout the session. All rats consumed the entirety of the food pellets administered. All animals were then classified according to their conditioned responses towards the lever cue (ST), or towards the magazine (GT). The rats were then given a day for a crossover treatment test to measure expression of the learned behavior (expression), followed by conditioned reinforcement.

**Measures**

PCA behavior was given an index score using the average of response bias \( \frac{(\text{lever presses number} - \text{magazine entry number})}{(\text{lever presses number} + \text{magazine entry number})} \), latency score \( \frac{(\text{magazine entry latency} - \text{lever press latency})}{\text{time CS is present}} \), and probability difference \( \frac{(\text{lever press probability} - \text{magazine entry probability})}{100} \) for lever presses and magazine entries. This scored behavior from a +1.0 (absolute ST) to -1.0 (absolute GT), with 0 being no bias. STs were indicated as those with an index score \( \geq 0.5 \), GTs were those with index scores \( \leq -0.5 \), and intermediate (IR) responders were those with \(-0.5 < \) score < 0.5.
RESULTS

Effect of CNO Drug on Behavior

Previous studies have discussed a concern that CNO may metabolize into clozapine, causing an effect on the brain (Gomez et al., 2017). In order to control this, a control surgery was used with CNO injections to ensure CNO would not influence other areas of the brain. The findings concluded that injections of CNO did not significantly alter behavior in control rats (mCherry/CNO) for lever press number (Figure 4A; 2way ANOVA; effect of group; F(1,15)=0.1279, p=0.7256) or magazine entry number (Figure 4B; 2way ANOVA; effect of group; F(1,15)=0.01281, p=0.9114).

Figure 4: No statistically significant differences in behavior for lever press number or magazine entry number between mCherry/CNO and DREADD/Vehicle groups.

Effects on Acquisition

At the end of the acquisition period, the pool of rats consisted of 4 ST, 4 GT, and 5 IR in the DREADD/CNO group; 5 ST, 0 GT, and 6 IR in the DREADD/vehicle group; and 2 ST, 1 GT, and 3 IR in the control group. Data showed no statistically significant differences amongst treatment groups for lever press numbers (Figure 5A; 2way ANOVA; effect of treatment; F(1,22)=1.508, p=0.2325), latency (Figure 5A; 2way ANOVA; effect of treatment; F(1,22)=3.523, p=0.0738), and probability (Figure 5A; 2way ANOVA; effect of treatment; F(1,22)=2.964, p=0.0992) in comparison to magazine entry numbers (Figure 5B; 2way ANOVA; effect of treatment; F(1,22)=0.1738, p=0.6782), latency (Figure 5B; 2way ANOVA; effect of treatment; F(1,22)=0.01610, p=0.9002), and probability (Figure 5B; 2way ANOVA; effect of treatment; F(1,22)=0.00186, p=0.9660).
across all training days. These findings concluded that DREADD/CNO excitation of the vHPC-NAc pathway had no statistically significant effect on acquisition of sign- or goal-tracking. However, although there were no statistically significant effects, data suggested that the DREADD/CNO group tends to sign-track less frequently than the DREADD/vehicle group. This suggests that there may be a difference in sign-tracking behaviors, requiring further investigation using an increased sample size to determine significant effects.

**Figure 5**: No statistically significant differences amongst treatment groups for lever press numbers, latency and probability in comparison to magazine entry numbers, latency, and probability.

Effects on Expression

Following PCA testing, a crossover treatment was used to determine expression. After learning sign- and goal-tracking behaviors, we
wanted to see if the rats would express the learned behavior differently in response to different treatments. DREADD/CNO rats would then receive a vehicle and DREADD/Vehicle would then receive CNO. Results revealed no significant differences in sign-tracking lever presses (Figure 6A; Paired t-test; effect of treatment; t(11)=0.5525, p=0.5916) and goal-tracking magazine entries (Figure 6B; Paired t-test; effect of treatment; t(12)=0.1046, p=0.9184) behaviors in response to the cross treatment when DREADD/CNO received vehicle. In addition, there were no statistically significant differences in sign-tracking lever presses (Figure 6A; Paired t-test; effect of treatment; t(9)=1.428, p=0.1869) and goal-tracking magazine entries (Figure 6B; Paired t-test, effect of treatment; t(10)=0.6970, p=0.5017) when DREADD/Vehicle received CNO. These findings suggested that expression of sign- and goal-tracking behavior is not affected by different treatments following acquisition.

**Figure 6:** No statistically significant differences in sign-tracking lever presses or goal-tracking magazine entries for either the DREADD/CNO or the DREADD/Vehicle group.

### Conditioned Reinforcement Findings

Condition reinforcement uses an active and inactive nose-poke port in which the lever is presented in response to only an active nose-poke. Typical data suggests that rats will have preference to the active nose-poke port. STs have particular interest in the active nose-poke port due to the incentive salience associated with the lever. Data showed that DREADD/CNO rats had a statistically significant preference for the active nose-poke port (Figure 7; 2way ANOVA; effect of nose poke;
F(1,22)=8.915, p=0.0068). Interestingly, however, DREADD/vehicle animals, consisting of only ST and IR rats, did not show preference to active nose-pokes during conditioned reinforcement (Figure 7; 2way ANOVA; effect of treatment x nose poke; F(1,22)=5.558, p=0.0277).

Figure 7: DREADD/CNO group showed preference for the active nose-poke port. DREADD/Vehicle group did not show preference for the active nose-poke port.

**DISCUSSION**

Despite the lack of statistically significant evidence to support the role of the vHPC-NAc pathway in PCA behavior, there does seem to be a trend of DREADD/CNO rats sign-tracking less than the DREADD/vehicle group during acquisition. These findings suggest that there may be a trend of increased activity in the vHPC-NAc pathway leading to a decrease in sign-tracking behaviors. This does not seem to parallel the Fitzpatrick et al. (2016) study in which lesions of the vHPC decreased sign-tracking behaviors. However, this data does more specifically support previous findings in which lesions of the HPC increased sign-tracking behaviors (Ito et al., 2005), as well as a previous inhibitory experiment.
in this lab that found a statistically significant decrease in sign-tracking behaviors when looking at expression of the learned behavior (Figure 8). These findings suggest that further investigation is necessary to determine the connection between the vHPC-NAc pathway and sign-tracking behaviors.

**Figure 8:** Discontinuing inhibition of the vHPC-NAc during the crossover treatment test session resulted in a reduction in sign-tracking behaviors for STs.

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**Ventral Hippocampus**

Depending on the context, cues may have different behavioral or craving responses. This differentiation may be due to the vHPC, as it is considered to be a vital player in contextual information and learning. Research has shown that STs are not as engaged in contextual learning
and lack mediation within the vHPC for behavioral responses to cues (Robinson et al., 2014). This suggests that STs may have an impaired contextual association, leading to particular cues inducing the same response regardless of the context (Morrow et al., 2011). In drug use, this means that a cue previously associated with a drug may cause drug seeking behaviors for STs despite the context the individual may be in. This may lead to drug cravings and seeking even in situations in which it may be more problematic. In this study specifically, it seems as though artificially engaging the vHPC may affect sign-tracking behaviors, possibly leading to a decrease in sign-tracking and an increase in goal-tracking. In other words, due to the vHPC’s involvement in contextual learning, as well as mediation of behavioral responses, increased activity in the vHPC may lead to a decrease in sign-tracking behaviors. This supports previous theories of the vHPC’s involvement in sign-tracking.

**Human Translations**

As previously discussed, animal models show excellent promise for paralleling human addictive behaviors (Garofalo & di Pellegrino, 2015; Failing et al., 2015; Le Pelley et al., 2015; Pearson et al., 2015; Pearson et al., 2016; Colaizzi et al., 2020). Findings suggest that there may be a connection between increased activity in the vHPC-NAc pathway and a decrease in sign-tracking behaviors. Should there be an association, whatever it may be, this may offer insight into the neurological causes of sign-tracking behaviors. Since sign-tracking has shown a higher propensity for addiction and relapse. Through translation, this may offer an opportunity to research neurological aspects of addiction and allow for manipulation or treatment of learning and behavioral styles as research progresses.

**LIMITATIONS AND FUTURE RESEARCH**

There are a variety of limitations to this project to be discussed. One limitation that was quickly identified was the study pool. Due to the limited number of rats involved in the study, there was also a lack of GTs in the DREADD/vehicle group. This may skew data and limit the statistical significance of the data. Future research in this lab will consist of a greater pool of rats to increase the chance of a more successful and well balanced study. Furthermore, limitations in time before publishing limit the completion of all observations. This lab will soon be observing brain slices to ensure proper injection sites and observe the spread of the DRE-
ADD. Further analysis will be conducted and published at a later date.

REFERENCES


