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Probing the role of striatal mu opioid receptors in behavior reinforcement

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

in

Biology

by

Desiree Johnson

Committee in charge:

Professor Matthew Banghart, Chair
Professor Takaki Komiyama, Co Chair
Professor Yimin Zou

2020

The Thesis of Desiree Johnson is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California San Diego

2020

Dedications

I dedicate this work

to my family,

my loved ones,

and friends

for their unconditional support

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ACKNOWLEDGMENTS

I would like to thank Dr. Matthew Banghart for his guidance and support as well as the opportunity to work on an important and exciting project. I have learned so much in his lab and hope to carry on what I've learned in my future endeavors as a scientist. I would also like to thank my mentor Shannan McClain for doing all the surgeries for the behavior experiments and for her guidance and support. I would like to thank Christina Gremel for her support and advice during with the behavior experiments and also Byungkook Lim for providing the rotarod.

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The thesis is coauthored with McClain, Shannan D. The thesis author was the primary author of this chapter.

ABSTRACT OF THE THESIS

Probing the role of striatal mu opioid receptors in behavior reinforcement

by

Desiree Johnson

Master of Science in Biology

University of California San Diego, 2020

Professor Matthew Banghart, Chair

Professor Takaki Komiyama, Co-Chair

Opioid drugs, such as morphine, bind to opioid receptors in the brain and provide an analgesic, rewarding, and euphoric effect. Endogenous opioid peptides also bind to opioid receptors in response to natural rewards such as food and exercise. The hijacking of these natural reward circuits have been hypothesized to lead to addiction. Basal ganglia nuclei are rich in opioid neuropeptides and these opioid neuropeptides and their receptors have been implicated in supporting behavior reinforcement for rewards. The striatum, a major basal ganglia input nuclei, is a mu opioid receptor hotspot. The striatum is implicated in goal directed, habitual and decision-making behaviors, as well as drug addiction, but the role of striatal opioid signaling in the learning and successful completion of operant behaviors and drug reward have yet to be established. To understand how opioid neuropeptides within striatal microcircuits contribute to operant behaviors and opioid drug we used two main approaches. We genetically deleted mu opioid receptors (MORs) from striatal direct pathway neurons, or selectively removed MORs from different striatal regions using AAV-cre. In both cases we found that lever pressing for food rewards was unaffected. In parallel, to test the role of striatal MORs in opioid drug reward, using the same genetic and viral manipulations we ran mice through a morphine conditioned place preference (CPP) assay and found that these animals established a place preference for the morphine-paired chamber. These results indicate that MORs in striatal direct pathway neurons may not be involved in operant behaviors or opioid drug reward.

INTRODUCTION

Opioid Epidemic

Opioids are powerful analgesics used to treat pain (Robbins and Everitt, 1999). According to the CDC opioid related death is the leading cause of injury associated death in the United States. Addiction due to opioid use also poses a huge financial burden for the US (Robbins and Everitt, 1999). This is predominantly due to the misuse of these analgesics potentially leading to opioid use disorder or addiction. With an increased understanding of the effect opioids have on the brain, both endogenous (natural) and exogenous (via drug administration), comes an increased understanding of this disorder and conscious efforts towards eliminating the stigma addiction poses (Buchman and Reiner, 2009). To this day, pain management remains limited to usage of these highly addictive drugs with accompanied education on safe administration (Gilson and Kreis, 2009; Matthes et al 1996). Although addiction doesn't discriminate (i.e addiction is seen in every community regardless of socioeconomic and racial background) and although there have been efforts to alleviate the stigma surrounding addiction there still remains unequal access to proper pain therapy to black and brown patients due to the opinion that this population is likely to abuse (James and Jordan, 2018). This results in the under treatment of pain in this population (James and Jordan, 2018). Yet, black and brown people are not more likely than whites, according to statistics, to use illicit drugs but are more likely to be incarcerated for drug offenses by tenfold (James and Jordan, 2018; Netherlan and Hanson, 2017) which contributes to the negative feedback of people of color not receiving pain relieving drugs for care. Neuroscience has made immense progress in regards to eliminating the stigma of

addiction by revealing the plasticity the brain undergoes when in an addicted state. An indirect way to alleviate the unequal responses to the opioid crisis that affect black and brown communities is to find better pharmaceuticals for pain relief, and in order to do that we need to fully understand the neural pathways that lead to addiction.

Opioids are rewarding (Fields and Margolis, 2015). Reward is defined as subjective hedonic feeling of pleasure which can result in behavior reinforcement (positive reinforcement) where there is an increased likelihood of a continued behavior response (Fields and Margolis, 2015). This can also be seen as appetitive learning which is similar to goal directed learning in that there is a set behavior response in order to receive some distinct reward (Cooper et al, 2011). Rewards, from an evolutionary standpoint, evolved to help people understand what they preferred. This preference would help with survival when it comes to discriminating between nutrients or when presented with limited resources (Cooper et al 2011). Effects of chronic use of opiates such as morphine is a 'hijacking' or taking over natural reward centers in the brain, the centers that evolved to help us obtain natural rewards, eventually leading to addiction (O'brien 2009). In order to understand the role that opioids have in the brain we first need to know the neural circuits responsible for natural rewards. Knowing what circuits in the brain are hijacked can lead the way for better pharmaceuticals that remain potent analgesics yet lack addictive chemical properties.

The striatum

The current mechanisms responsible for the rewarding effects of drugs of abuse are dependent on the neurotransmitter dopamine (Schultz, 1998; Robbins and Everitt, 1999). Opioid drugs, such as morphine or heroin, bind to mu opioid receptors (MORs) in

the midbrain and activate the mesolimbic dopamine pathway, a pathway that has been implicated in addiction (Merrer et al, 2009). These drugs are mu opioid receptor agonists. The neurons activated in this pathway send their projections to the prefrontal cortex and striatum (Robbins and Everitt, 1999). The striatum is an important input nuclei of the basal ganglia, is part of the limbic system, and is home to the majority of mu opioid receptors (Merrer et al 2009). The striatum is one of the main components of the basal ganglia circuit and can be split into two regions, dorsal and ventral. The dopamine that projects to the striatum will act on cells and corresponding receptors within the striatum. There are four cell types within this region including direct and indirect medium spiny neurons (MSN's), cholinergic interneurons, and GABAergic interneurons. The focus here will be on the most prominent, the MSN's. These MSN's project differently within the basal ganglia circuit. In the dorsal striatum, direct pathway MSN's (dMSN) expressing dopamine d1 receptors project directly to the output structures of the basal ganglia the substantia nigra (SNr) and globus pallidus internal (GPi) (Yager et al, 2015). Indirect pathway MSN's (iMSN) have d2 receptors and project indirectly to these same nuclei but take a detour through the globus pallidus external (GPe) and Subthalamic Nucleus (STN) (Yager et al, 2015). Both of these projections act on the thalamus to either increase or decrease stimulation, with iMSN's causing inhibition of thalamus and dMSN's causing excitation (Yager et al, 2015). The result of inhibiting or exciting the thalamus contributes to an inhibited or increased behavior response, respectively (Yager et al, 2015). The nucleus accumbens (NAc), or ventral striatum, also express d1 and d2 receptor and MSN subtypes but projects to different midbrain structures (Yager et al, 2015). The dMSN's in the NAc sends and receives

projections from the VTA and the iMSN's project to the ventral pallidum (Yager et al, 2015). NAc stimulation results in a differing phenotype affecting the motivation and emotional aspects of behavior (Yager et al, 2015). The NAc is also part of the mesolimbic dopamine circuit which is activated in response to drugs of abuse (Robbins and Everitt, 1999). When an opioid binds to a midbrain dopamine neuron this circuit activates at the VTA and sends dopaminergic projections to the striatum (Robbins and Everitt 1999). When the brain is exposed to drugs chronically the brain undergoes plasticity or strengthening of synapses in the striatum (Yager et al, 2015). This rewiring takes over the associate learning processes that occur when receiving natural reward. On the path to addiction the user transitions from a state of goal directed drug seeking to habitual drug taking and dependency (Robbins and Everitt, 1999). From past

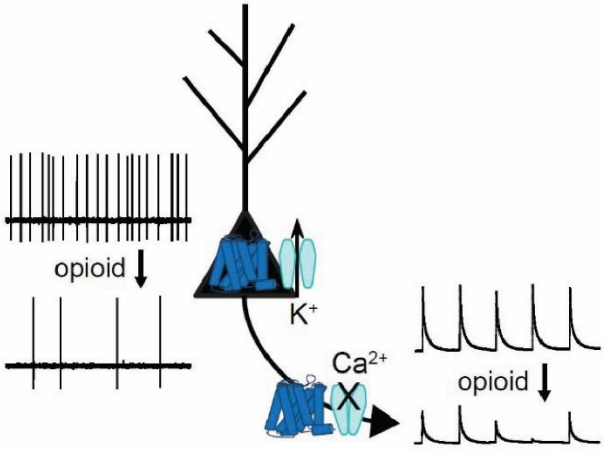

Figure 1: Physiological outcomes of opioid binding. These vary depending on what type of neuron is being targeted. With few exceptions, opioid tend to inhibit neural output either by directly suppressing action potential firing, for example by opening potassium channels, or they can suppress neurotransmitter release from synaptic terminals in response to action potentials by inhibiting calcium channels for example. Post-synaptic ionic currents recorded from a downstream cell.

literature we know that the dopamine projecting to the striatum contributes to learning

and reward but the contribution of striatal mu opioid receptors to this circuit remains unknown.

The striatum in behavior reinforcement

Endogenous opioid neuropeptides and their receptors within the striatum is an area and loci of interest for opioid action because of their role in natural rewards such as goal directed, habitual, and motivational behavior. We know that the net result of an opioid binding to its receptor is inhibition which provides its rewarding and analgesic effects by either opening potassium channels or closing voltage gated calcium channels (Figure 1). The discovery of the opioid system stemmed from the discovery of opium found in the seeds of the poppy plant and its most active ingredient is the opioid drug, morphine (Merrer et al, 2009). Endogenous opioids include neuropeptides our body naturally produce. There are various types of endogenous neuropeptides but here the focus will be on endorphin, enkephalin, and dynorphin which have high affinities for mu

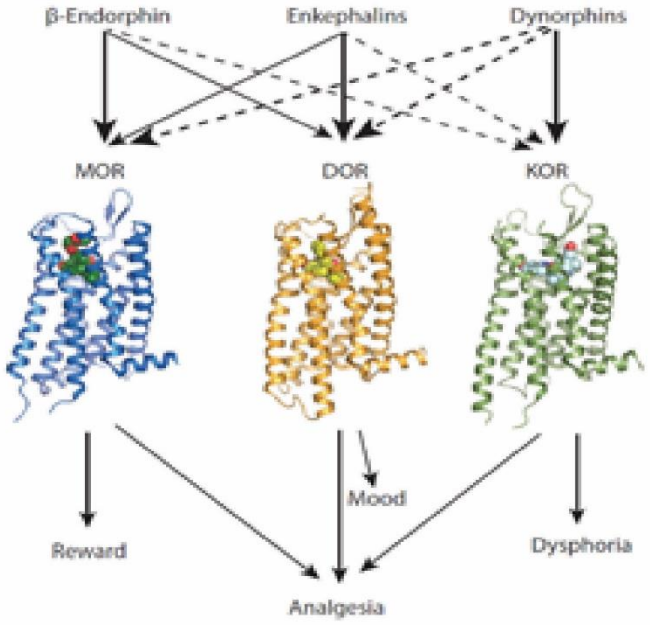

Figure 2: Endogenous opioid neuropeptides. Beta endorphin, enkephalins, and dynorphins are all peptides that produce rewarding or aversive affects when bound to their receptor. Endorphins have a high affinity for MOR, enkephalins have a high affinity for DOR and dynorphins have a high affinity for KOR. Adapted from Corder et al, 2018 and Kieffer, 2009.

opioid receptor (MOR), delta opioid receptor (DOR) and kappa opioid receptor (KOR) (Figure 2). The striatum is rich in opioid neuropeptides but these neuropeptides and their receptors remain an area of interest. Direct and indirect pathway MSN's in the

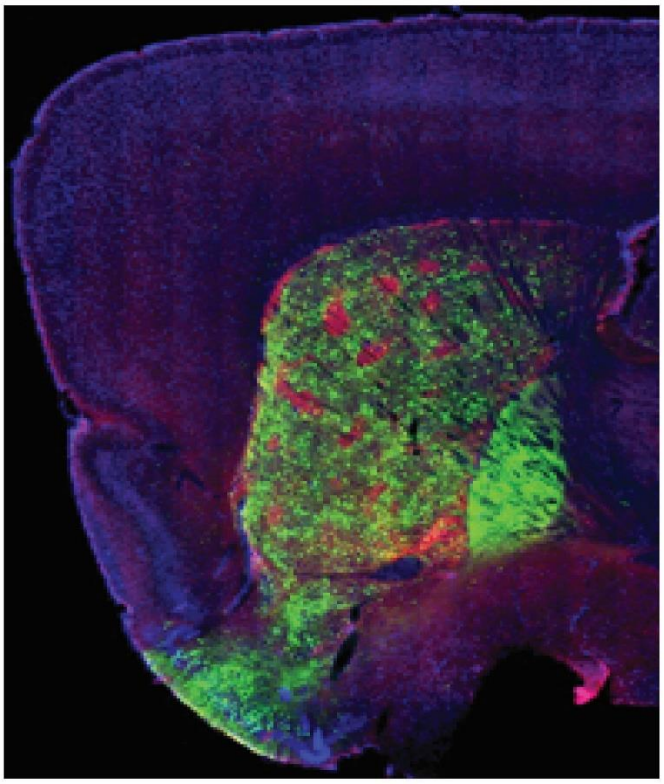

Figure 3: The striatum is rich in mu opioid receptors. The mu opioid receptors on MSN's in the striatum are expressed in a patchy pattern. These patches (red) which have high MOR expression are thought to be self contained limbic microcircuits. All of the enkephalin is in the surrounding matrix (green)

striatum are rich in MORs and these neurons are organized in patches often referred to as striosomes or simply patches. The surrounding matrix compartment is filled with these endogenous neuropeptides but it is unknown what the role of these receptors in the striatum are and how they contribute to behavioral processes mediated by the striatum (Figure 3).

Previous literature has implicated the striatum in coordinating rewarding events. The dorsolateral striatum (DLS) and the dorsomedial striatum (DMS) have been used to

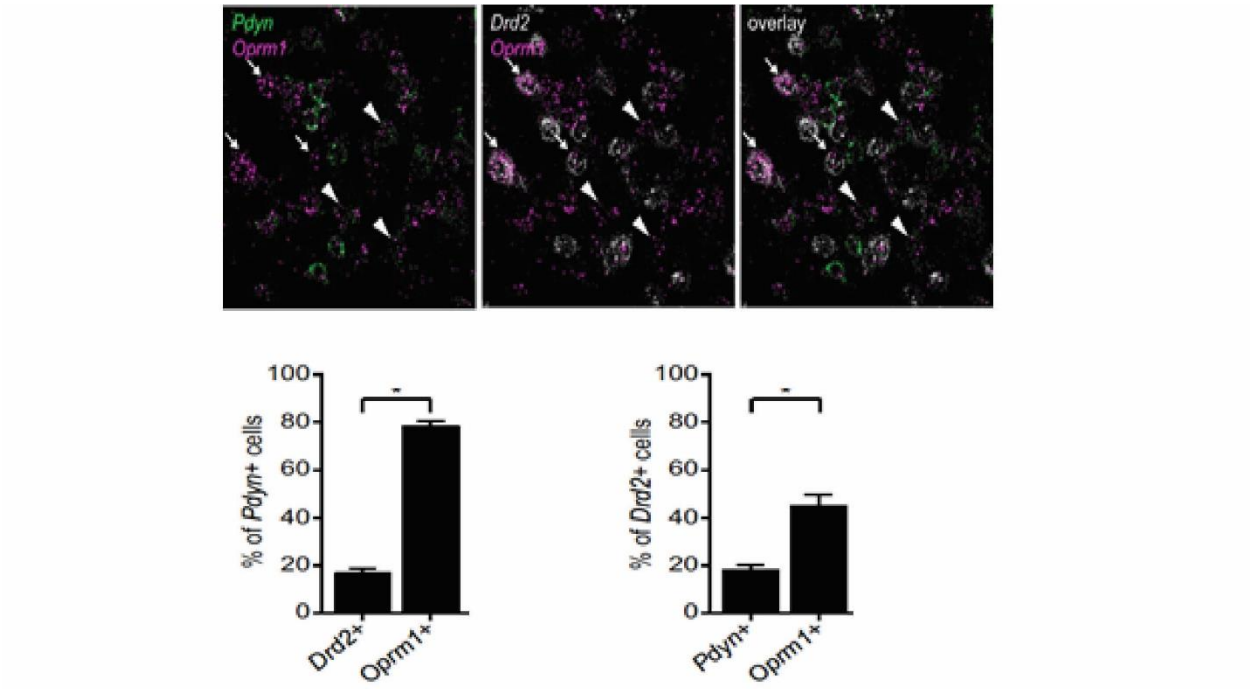

Figure 4: Direct pathway and indirect pathway striatal neurons colocalized with MOR. Top: fluorescent in-situ hybridization showing colocalization with direct pathway neurons (pdyn labeled mRNA) and the mu opioid receptor (Oprm1 labeled mRNA) and indirect pathway neurons (Drd2 labeled mRNA) and mu opioid receptor Bottom: Quantification data indicating 80% co-localization in direct pathway cells versus 50% colocalization in indirect pathway cells. Banghart et al, 2015

study habitual and goal directed behavior, respectively. Previous studies have found that when lesioning the DMS and running mice through both goal directed and habitual behavior paradigms the mice tend to act in a habitual manner (Gremel and Costa, 2013). When Lesioning the DLS mice tend to act in a goal directed manner as compared to sham treated animals (Gremel and Costa, 2013). This study indicates the countering roles of these two regions of the dorsal striatum and possible compensatory mechanisms at play when one region is 'off-line'. When running mice through a conditioned place preference assay, an indirect measure of drug reward and seeking, and stimulating the VTA terminals that project to the NAc- mice displayed a conditioned place preference (Koo et al, 2012). This phenotype occured when stimulated with either

vehicle or brain derived neurotrophic factor (BDNF), which has been known to reverse the plasticity seen resulting from drug usage (as a positive control) (Koo et al, 2012). Berridge and colleagues through a collection of experiments found that there is a hotspot in the rostrodorsal region of the NAc shell. When running rats through a conditioned place preference assay, and stimulating the rostrodorsal hotspot resulted in a significant place preference as opposed to stimulation at any other part of the medial shell of the nucleus accumbens. These results indicate an anatomical specification responsible for reward behavior. Infusing the Nucleus accumbens shell with Naloxone, a mu opioid receptor antagonist, decreased palatability of a sucrose reward although motivation to work for the reward was unchanged (Wassum et al, 2009). Using optogenetic tools to parse out differences in reinforcement salience of dMSN's and iMSN's researchers found that mice will press more and display a place preference for side of stimulation when dMSN's are stimulated yet mice show an aversion when iMSN's are stimulated (Kravitz et al, 2012). Using a novel rescue strategy and transgenic mice line lacking mu opioid receptors globally and rescuing the mu opioid receptors solely in the striatum researchers found *in vivo* evidence that this cell population is needed for opioid award (Cui et al, 2014). In a study where researchers infused morphine into the dorsal and ventral striatum they found that mice will self-administer morphine into the ventral striatum significantly more than the dorsal striatum and dorsal striatum infusion was similar to control group (David and Cazala, 2000). The collection of this literature implements striatal circuits in the acquisition of operant learning and further hints to its responsibility in opioid reward. Using fluorescent in-situ hybridization (fish) researchers identified that although mu opioid receptors are present

in both direct and indirect pathway MSN's, there are vastly more in direct (80%) versus indirect (50%) (Figure 4).

These striatal subregions remain difficult to study because of the interconnectedness of the circuit. In order to efficiently study the role of the mu opioid receptors in the striatum sophisticated tools in order to parse out the regions less invasively yet efficiently is needed. The striatum has been explored in regards to natural rewards but there is still much to be known about the direct role natural and opioid rewards in the striatum have on behavior such as the ones mentioned previously. We want to know the anatomical and cellular aspects responsible for these reward phenotypes. The aim of this research is to understand the striatal role in hedonic reward and rewarding properties of opioid drugs.This work will focus on endogenous opioids that are released in response to rewarding stimuli such as food rewards after completion of an operant task. The results of studying natural rewards will lead us to studying morphine, a commonly prescribed opioid, and the effect of striatal mu opioid receptors in behavior reinforcement.

We utilized pharmacological manipulations and observations using behavior. Behavior is defined as response to external stimuli that strays from the organism's natural responses (Krakauer et al, 2017). Since reward is closely connected to the subjective experience, understanding how pharmacologic manipulations influence behavior is vital to understanding the mechanisms involved. Our approach is to use a mu opioid receptor conditional knockout transgenic mouse line and observe their behavior phenotypes on several operant behavior and drug seeking behavior tasks, such as CPP. We will also use cre dependent viral injections to selectively lesion

regions of striatum and run mice through the same behavior paradigms in order to further dissect out anatomical differences in the responses to opioid reward and natural reward.

RESULTS

To investigate the role of endogenous opioids in learning and motivation we systemically shut down opioid signaling in food restricted mice using naloxone (NLX) a nonselective opioid receptor antagonist. We measured administration of NLX's effect on performance in an operant lever pressing task. Using a between subjects design with administration of NLX at concentration 3mg/kg in food restricted mice- mice receiving NLX during the nine training days displayed a significantly lower rate of pressing (P=0.005, 2 way ANOVA) (Figure 5A). Interestingly, on devaluation test days (Figure 5B) both conditions were sensitive to reward devaluation. This suggests the mice ability

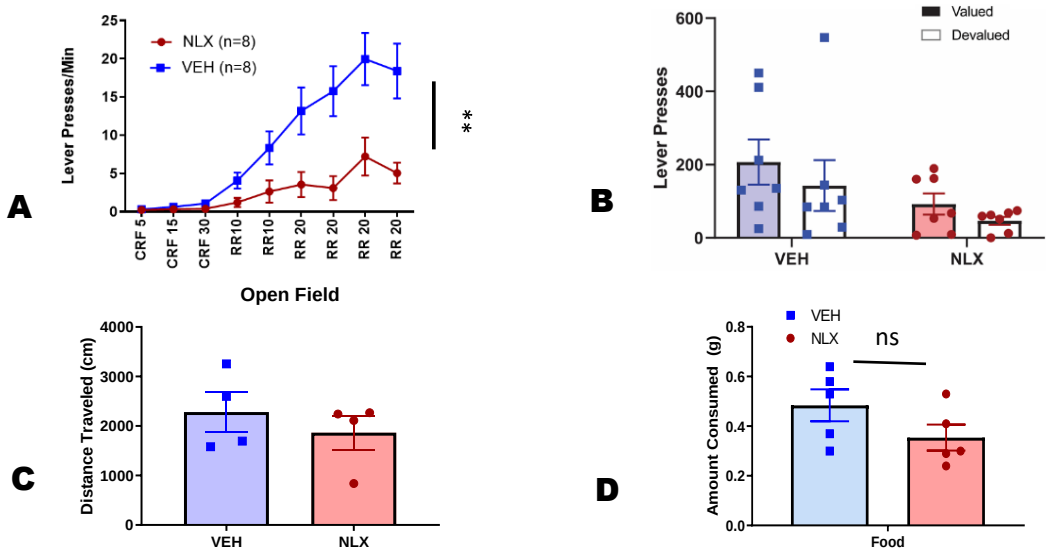

Figure 5: Systemic naloxone suppresses operant lever pressing. (A) Rate of lever pressing for wild type mice administered either NLX at 3 mg/kg (n=8) or vehicle (n=8). Rate of pressing in NLX administered animals was significantly less than VEH treated animals (p=0.005, two way ANOVA) **(B)** Devaluation test day measuring NLX and VEH group tendency to press in goal directed manner. **(C)** Open field test showed no significant difference in gross locomotion **(D)** Free Feeding test in vehicle treated (n=5) or NLX treated (n=5) showed animals in both conditions consumed similar amounts of food when given ad lib access (p=0.15, unpaired t-test).

to learn in a non-significant trend towards goal directed fashion regardless of successful acquisition of task. Performance in open field and free feeding assay (Figure 5C, D) were similar in both conditions implying that differences seen were not due to impairment in gross locomotion or in feeding circuits by causing satiety sooner.

Next to investigate mice lacking striatal direct pathway MORs acquisition and performance in an operant behavior task we utilized conditional knockout (PdynCre MOR-cKO) mice and ran them through our operant behavior paradigm. Compared to their littermate controls (*Oprm1 fl/fl mice*, male n=5 female n=4), PdynCre MOR-cKO (male n= 2; female n=6) mice pressed at a comparable rate during the nine training sessions (Figure 6A) and during devaluation testing (Figure 6B) mice displayed a significant trend towards goal direction when reward was valued versus when it was devalued (*Oprm1 fl/fl* p=0.06; Pdyncre MOR- cKO p=0.03, Mann-Whitney test). Mice did not display a difference in gross locomotion as tested with open field (Figure 6C). A significant difference in pressing was seen between genders in each condition (Figure 6A) in which we note in the graph. Wild type animals do not display this type of gender difference when put through a similar operant behavior assay. The gender differences seen could be due to unknown differences in sensitivities with transgenic animals to food restriction or to any conditions in behavior assay that don't affect wild type animals, further investigation would provide better insight into this. Next in a separate cohort of PdynCre MOR-cKO and corresponding control *(Oprm1 fl/fl*) mice were administered a 3mg/kg concentration of either NLX or VEH and put through the operant behavior task. Results reveal that lever pressing was attenuated in both cKO and control animals when treated with NLX (Figure 6D). This implies that endogenous signaling independent of

mu opioid receptors in striatal direct pathway neurons are needed for complete learning and motivation in this operant task. Next, in order to understand the role mu opioid receptors play in different regions of the striatum we selectively removed MORs from specific regions (Dorsomedial striatum (DMS), Dorsolateral striatum (DLS) and measured their performance on the goal directed operant behavior task. Results revealed no attenuation in lever pressing for reward during the acquisition phase (Figure 7A). All animals displayed sensitivity to reward devaluation, indicating they still learned in a goal directed manner regardless of lack of striatal mu opioid receptors (Figure 7B) ($p=0.24, 0.34, 0.39$ for GFP ctrl, CRE DLS, and CRE DMS respectively; Mann-Whitney test).

The Stimulus-Response-Outcome (SRO) pilot assay (Figure 8A, B) confirmed habitual behavior can be established in mice. Habitual behavior is characterized by no change in lever pressing for rewards in the valued or devalued states (Figure 8B) whereas animals displaying pressing in a goal directed fashion would press significantly less in the devalued state, demonstrating sensitivity to devaluation. In order to investigate whether MORs in striatal direct pathway neurons are important for the development of habits we put our cKO mice through the SRO lever pressing assay. Results revealed that cKO mice have similar rates of pressing during training (Figure 8C) and devaluation (Figure 8D) as control mice and both groups have similar pressing and rates of devaluation as seen in the pilot experiment (Figure 8A, B).

Knowing that goal-directed and habitual actions to obtain natural rewards are intact in our conditional knockout mice we tested whether the same is true when exposed to opioid drug rewards.

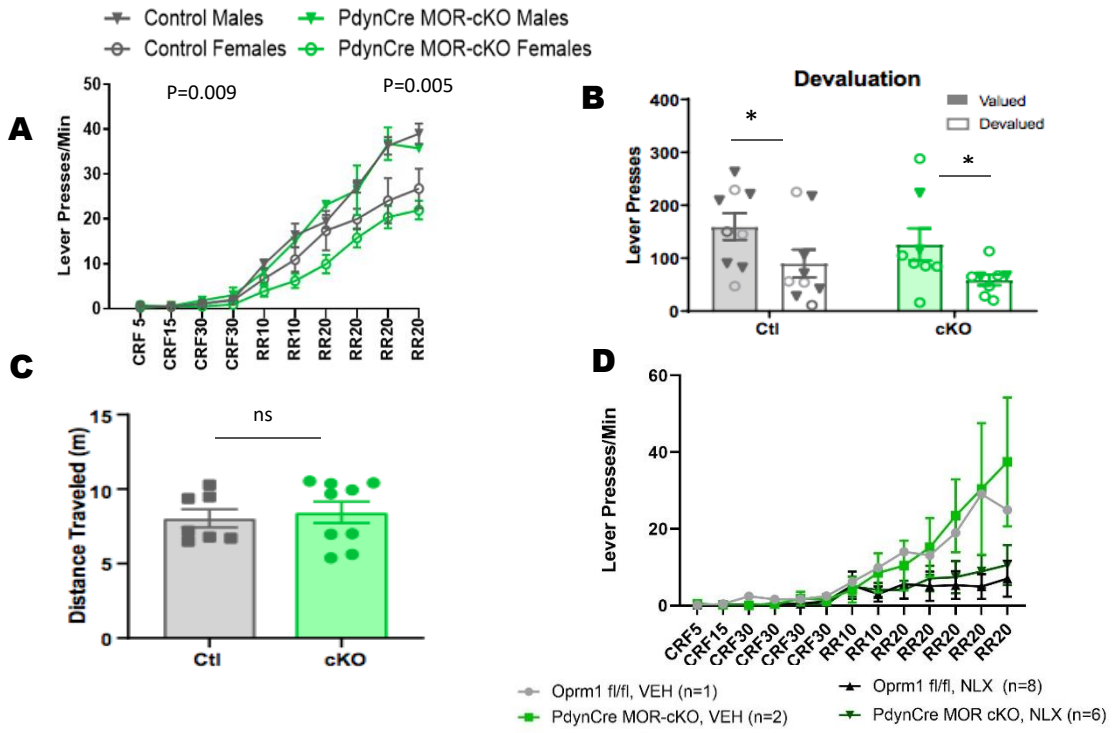

Figure 6: Direct Pathway mu opioid receptors are not necessary for goal directed operant task. (A) Acquisition of lever pressing intact in mice lacking mu opioid receptors in direct pathway neurons (PdynCre MOR-cKO). Significant difference between males and females rate of pressing. (**B)** Devaluation (test day) showed that both control and PdynCre MOR-cKO mice performed in a goal directed manner. Shaded bars are valued days, where mice received 1hr free access to no-reward (sucrose) and non-shaded bars are devalued days where mice received 1hr free access to reward. (**C)** Open field assay showed no attenuation in gross locomotion that could explain any differences seen measured by distance travelled (p=0.47,). (**D)** PdynCre MOR-cKO and *Oprm1 fl/fl* (ctrl) mice performance and motivation in operant lever pressing task is attenuated upon administration of NLX (ctrl VEH vs ctrl NLX p=0.009; cko VEH vs cKO NLX p=0.03, two way ANOVA)

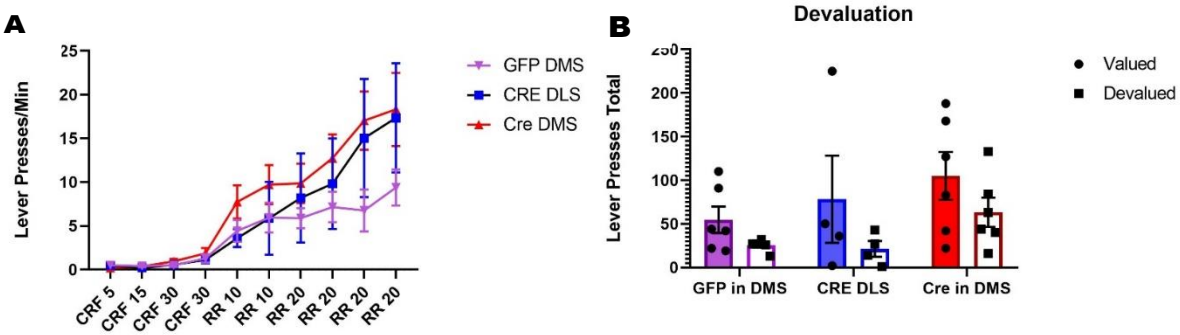

Figure 7: AAV deletion of mu opioid receptors in specific striatal subregions reveal no behavioral phenotype. (A) AAV cre injected in the DMS or DLS had no effect on the acquisition of operant lever pressing task. **(B)** Devaluation testing revealed lever pressing phenotype was goal directed in which lever pressing in all conditions were lower on the devalued day of testing.

Conditioned place preference is a behavior assay that measures drug seeking behavior. The paradigm consists of a three-chamber apparatus with hallway separating the two chambers. After measuring the mouse baseline preferences for the two sides, the week-long assay involves injecting mouse with drug of interest and placing mouse on one side of the apparatus on one day and the next day injecting mouse with saline and placing them on the other side, repeated twice (Figure 9B). Each side differs based on color, pattern, and textile cues (Figure 9A). On the last day mice are placed in the chamber and allowed to freely explore the entire apparatus and time spent on each side is reported. In naive c57 animals with all receptors intact the mice should highly prefer the side that they were injected with opiate, in this case morphine.

Because the role of MOR in the NAc in opioid drug reward is not clear, we selectively removed MOR from the medial shell of the NAc (NAc-mSh) by injecting Cre-AAV in *oprm1fl/fl* mice and ran the mice through the CPP assay. The results indicate that mice lacking MOR in NAc-mSh still have a strong preference for the morphine-paired chamber. We also selectively removed MOR from the DMS as a control and as expected saw that these animals also had an established place preference for the drug paired zone ($p=<0.001$, 0.002, 0.02 difference in vehicle and morphine zone for ctrl, DMS injected and NAc-mSH injected, respectively. Mann-Whitney test) (Figure 9D).

To determine if MORs expressed in striatal direct pathway neurons anywhere in striatum support morphine reward, we tested for CPP at 5mg/kg morphine in PydnCre MOR-cKO mice. We found that in mice lacking mu opioid receptors (MORs) in direct pathway neurons, morphine CPP is not attenuated (Figure 10A; ctrl $p=0.008$, cKO $p=0.004$ Mann- Whitney test). This phenotype was not due to impairment of gross

locomotion as measured in an open field assay (Figure 10B). Deletion of most of the MOR in striatal direct pathway neurons indicates that the MOR's in this region may not be responsible for drug reward.

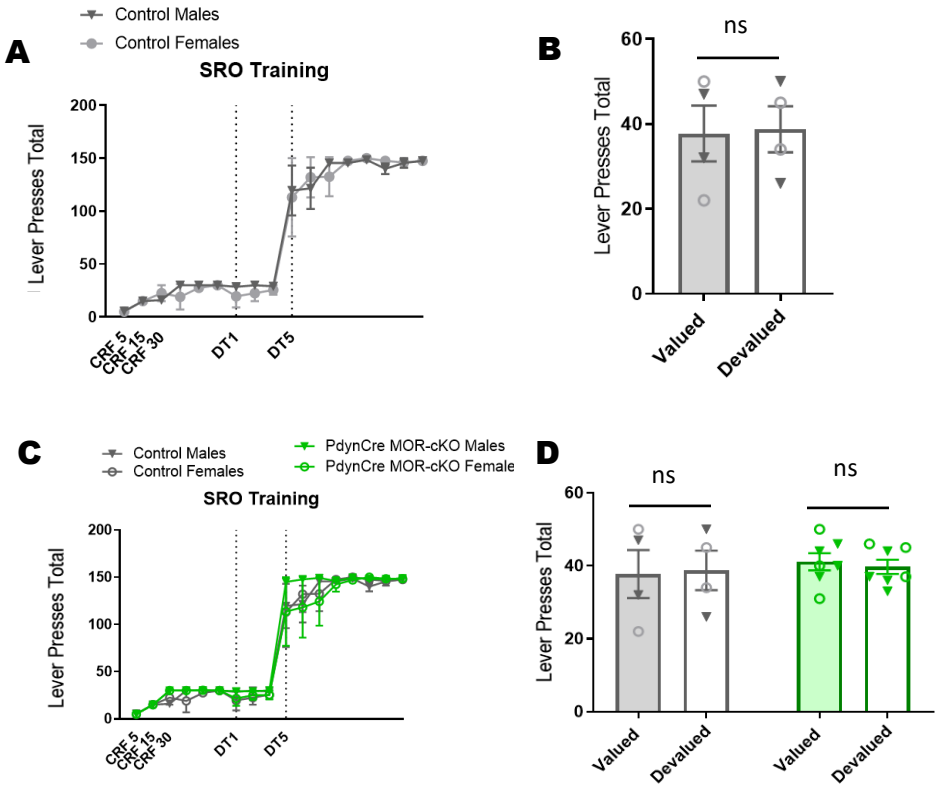

Figure 8: Stimulus response outcome (SRO) established habit behavior in conditional knockout mice. (A) SRO training days with wild type mice shows successful acquisition of behavior task. **(B)** Devaluation test days revealed no difference in pressing between valued and devalued days indicating mice behavior was habitual. **(C)** MOR cKO mice learn habit SRO behavioral assay similar to control animals **(D)** Both control and MOR cKO animals show no sensitivity to reward devaluation

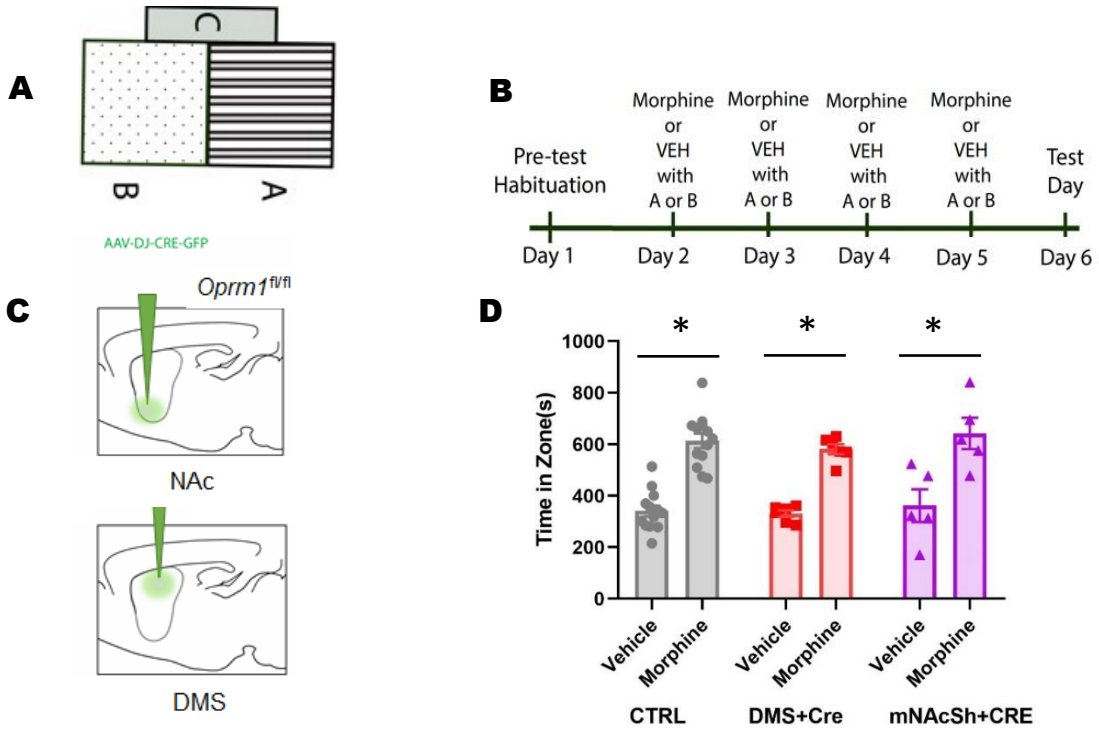

Figure 9: Conditioned place preference (CPP) intact in mice lacking mu opioid receptors in direct pathway neurons (A) Morphine conditioned place preference assay schematic. Mice are injected intraperitoneally (ip) with either morphine or vehicle (VEH). Conditioned place preference three chamber apparatus. Sides differ based on tactile (touch) and visual (dots vs stripes) cues **(B)** CPP behavior assay design indicating each day of behavior protocol. **(C)** AAV coordinate schematic for CRE injected *Oprm1 fl/fl* mice in nucleus accumbens (NAc) and dorsomedial striatum (DMS) **(D)** AAV-cre deletion of mu opioid receptors from the medial shell of NAc and DMS showed no change in establishment of a CPP phenotype

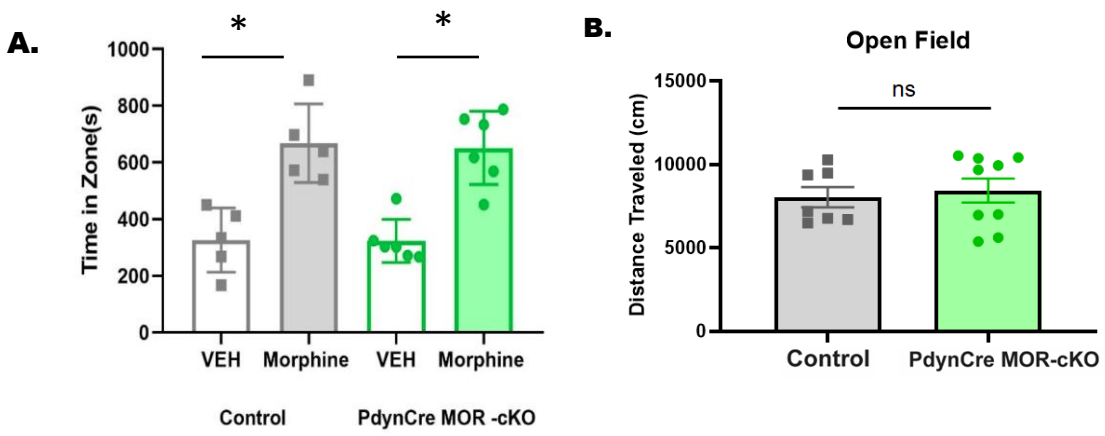

Figure 10: Conditional knockout mice display conditioned place preference. (A) Mice lacking mu opioid receptors in striatal direct pathway neurons display an attenuated conditioned place preference **(B)** Open field showed no differences in gross locomotion between conditions measured as total distance travelled.

DISCUSSION

Previous literature implicated the dorsolateral striatum to be instrumental in habits and the dorsomedial striatum to be instrumental in goal directed behavior (Gremel and costa, 2013). To ensure that mu opioid receptors were involved in behavioral reinforcement we ran wild type mice through our operant goal directed behavior paradigm under systemic naloxone (NLX), a nonselective opioid receptor antagonist. Previously it was shown in rats that NLX treated animals when put through a similar goal directed behavior assay pressed similarly to control animals (Wassum et al, 2009) and that NLX administration produced habit behavior in their rats. Our study in mice contradicted that results. We IP injected NLX systemically into wild type mice and ran them through our GDA behavior paradigm. We saw that in devaluation there was no significant difference between the naloxone treated animals and vehicle treated. Yet in acquisition of the task we saw that the NLX treated animals had a lower rate of

pressing, this was not because of attenuation in their locomotion or coordination. As well as not due to an interruption in their feeding circuits. We wanted to test whether deleting mu opioid receptors in direct pathway neurons would result in attenuation of goal directed behavior, and if this was the reason for our initial naloxone study results. We ran transgenic mice (PdynCre MOR-cKO) through our GDA assay and saw that goal directed behavior was intact and there was no difference in the acquisition of the task. The lack of phenotype in these operant tasks, with the attenuation/ elimination of the striatal mu opioid receptors imply that successful completion of these operant behaviors occurs independently of these striatal circuits.

Although the results of MORs on striatal direct pathway neurons don't seem necessary in order to achieve a behavioral phenotype in regards to natural and drug rewards, it is possible that they are still important for the acquisition of drug seeking behavior. We tested this next. Utilizing conditioned place preference assay and transgenic cre mouse line that conditionally floxxed out (removed) mu opioid receptors from direct pathway medium spiny neurons (PdynCre MOR-cKO mice) our results showed that striatal mu opioid receptors in these neurons are not necessary for developing drug seeking behavior when tested with morphine at 5 mg/kg. This indicates that the mu opioid receptors on these neurons are not needed for the development of drug seeking behavior, which was further confirmed when mice lacking MORs in NAc-mSh also had an established place preference.

Although we looked at what the mu opioid receptors located on the direct pathway medium spiny neurons do in regards to natural and drug rewards and saw no phenotype, there are still several anatomical and cellular components of the striatal

circuitry that have yet to be explored. It is possible that another cell type within the striatum plays a larger role in this shift between natural and drug rewards, such as indirect pathway medium spiny neurons or interneurons (Banghart et al, 2015). We know that the brain under chronic drug use undergoes plasticity (Yager et al, 2015). We also know that the dorsomedial and dorsolateral striatum compensate for one another when one is 'offline' (Gremel and Costa, 2014), does the rest of the striatum (ie. ventral striatum) respond similarly and what tools can we utilize in order to better control for the compensatory mechanism at play in the striatum. The conditioned place preference assay is extremely sensitive. It is possible that much like seen in delta opioid receptor deficient mice that cue associated place preference with drug occurs and place preference with drug alone does not occur (Merrer et al, 2012). Similarly it is possible that our mu opioid receptor conditional knockout mice are sensitive to the same cue induced place preference that interfered with our paradigm which did not control for cues. Further repeating these experiments with that control would be worthwhile given the overlapping nature and function of opioid receptors. Another way to alleviate this could be investigation with self-administration that is arguably a better presentation of drug seeking behavior representative of human drug addiction (Gerrits et al 2003). Self-administration can also be a good alternative for the stress that intraperitoneal injections have on the animal when run through a conditioned place preference assay. Stress has been shown to make animals more susceptible to the actions of drugs of abuse (Koob and Moal, 2001).

The results give insight into a needed niche of addiction research. It has been hypothesized for a long time that natural reward circuits governed by the midbrain

structures are responsible for the addicting action of drugs (Gerrits et al, 2003). We know that endogenous signaling is important for the acquisition of lever pressing tasks but that MORs in striatal direct pathway neurons are not the target. Future directions would include exploring the locus responsible for the acquisition of a goal directed lever pressing task. This can include transgenic knockout mice lacking delta opioid receptors in medium spiny neurons or knockout line missing mu or delta opioid receptors in cholinergic interneurons, another cell player within the striatum. We can explore the loci of morphine CPP in specific subregions of the nucleus accumbens, a region that receives innervation from dopamine neurons coming from the ventral tegmental area or revisit the dorsal striatum. We can look at the striosome and matrix compartments and attempt to understand the interconnectedness of the region and its possible compensatory mechanisms.

MATERIALS AND METHODS

Animals

All procedures approved by the institutional animal care committee (IACUC). All animals for the NLX experiments were male and female c57bl/6j. Animals used for goal directed operant lever pressing and conditional place preference were PdynCre/*Oprm1 fl/fl* and *Oprm1 fl/fl* both male and female.

Behavioral Procedures

Operant conditioning was performed using Med-Associates behavior boxes and sound attenuating chambers. In the goal directed operant behavior assays the mice were first trained on a 15 minute random time (RT) schedule with no levers present and

20mg pellet delivered on average every minute. To introduce lever pressing, continuous reinforcement (CRF) training followed RT in which the mice were challenged to press for 5, 15, and 30 rewards (CRF5, 15, 30). Following CRF training, the mice are trained in a random ratio where on average of 10 lever presses the mice receive a reward (RR10). Following two days of RR10 training the mice undergo RR20 training for 4 days where the mice receive a pellet on average after 20 presses. After concluding the acquisition phase, the mice then undergo devaluation testing in two consecutive days utilizing a 5 minute extinction schedule in which the lever was extended and inactive. Order of devalued (mice receive 1hr ad-lib access to reward) and valued (mice receive ad-lib access to 20% sucrose solution) were counterbalanced across days. House light was active and left lever used for all goal directed operant lever pressing behaviors. Session would time out when either mice received a max number of rewards (15 for all programs except RT, CRF, and devaluation) or when 60 minutes lapsed. Intraperitoneal injections were performed to administer NLX to mice during NLX experiment. 100 uL of 3mg/kg NLX or VEH was injected IP 15 minutes before being placed into operant behavior boxes. Open field tests were done once in a 30 minute session, while maintaining NLX and VEH groups. Open field tests were performed in a grey box below a stable camera, data was analyzed using video tracking software.

Stimulus response outcome (SRO) procedures were performed under food restricted conditions and in the same Med-Associates behavior boxes as the goal directed lever pressing assays. Day one began with random time (RT) training to expose mice to the box and reward port. SRO reward was 20% sucrose solution. The following 3 days mice were introduced to the lever in a continuous reinforcement

schedule (CRF 5,15, 30) each day corresponding to lever presses giving reward in a fixed ratio fashion. Following CRF training mice were trained in distinct trial (DT) schedule in which one lever press signaled the retraction of the lever and delivery of reward (DT1). After learning DT1 for three days the mice underwent DT5 which corresponds to lever retraction and reward after 5 lever presses. Mice underwent DT5 acquisition for 10 consecutive days. Following DT5 mice underwent testing via devaluation similar as described in the goal directed behavior assay except the devalued day was preceded with an hour ad lib access to 20% sucrose (reward condition). Number of lever presses was recorded using Med-PC software.

Conditioned Place Preference

Conditioned place preference was performed in 3 chamber conditioned place preference box (Harvard Apparatus). Each chamber differed based on visual and tactile cues separated by an acrylic hallway. The box was placed inside a sound attenuating chamber with fan on during the duration of the experiment. Conditioning began with a habituation day where mice were given free access to the entire box and time in each zone was calculated. Based on habituation day mice were assigned vehicle and morphine zone based on more preferred side and least preferred side, respectively. Morphine Sulfate provided by National Institute on Drug Abuse (NIDA). The next 4 days mice were IP injected with either morphine or vehicle and placed in assigned chamber with the rest of the box closed off. This continued for 4 days with each mice receiving morphine and vehicle twice. On day 6 mice were placed in CPP box without injection of drug or vehicle and given free access to apparatus for 20 minutes and time spent on each side was recorded. Videos were analyzed using tracking software smart 3.0.

Food restriction

Mice were weighed daily and kept at 85% of their baseline weight. Food restriction was within guidelines of IACUC and animal care facility (ACP) at UCSD. All food restricted animals were given ad-lib access to water. Animals were fed at the same time everyday and given on average 2-4 g pellets depending on weight.

Stereotaxic injections of AAV

Stereotaxic injections of AAV were performed as follows. Mice were anesthetized under isoflurane and injected with AAV in the following coordinates, for NAc AP 1.6; ML +- 0.6; DV −4.25 and AP 1.3; ML +- 0.6; DV −4.2, 2 injections billaterally, 300 nl, for DMS, AP 0.5 mm, L±1.5 mm and V −2.5 mm from the skull and AP 0.7 , L±1.5 mm and V −2.5 mm and DLS (B: AP 0.5 mm, L±2.65 mm and V −3.3 mm from the skull) DLS (.62 L±2.65 mm and V −3.3 mm from the skull. Mice were allowed to recover, and virus was allowed to express for 3 week after surgery before testing.

The thesis is coauthored with McClain, Shannan D. The thesis author was the primary author of this chapter.

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