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The Role of Ciliary Melanin-Concentrating Hormone (MCH) Signaling in the Neurobehavioral Response to Cocaine

Amanda Shelowitz

College of Medicine, University of Florida

Dr. McIntyre, Department of Neuroscience

Abstract

Neuromodulatory peptides that modulate behavioral responses to psychostimulants are known to activate receptors enriched in neuronal primary cilia. Primary cilia are microtubule-based organelles that play a critical role in cellular signaling through their high density of G-protein coupled receptors. One such receptor is the melanin-concentrating hormone receptor 1 (MCHR1). It is currently unclear how MCHR1 ciliary localization and MCH neuron activity modulate psychostimulant responses. This project aimed to identify the role of primary cilia localized MCHR1 in modulating behavioral responses to cocaine. Male and female mice were subjected to conditioned place preference (CPP) to determine the rewarding effect of cocaine and locomotor activity tests. MCHR1 KO and MCHR1^{IFT88} KO strains exhibited increased acute locomotor responses and sensitization to cocaine compared to their control littermates. CPP testing showed that MCHR1 KO mice had a reduced preference for the cocaine-paired chamber compared to control littermates. In contrast, MCHR1^{Ift88} KO mice did not differ from control littermates in the development of CPP. Locomotor tests with MCH^{cre} mice showed that activating MCH neurons 30min before cocaine administration decreased acute locomotor response and sensitization compared to mice receiving saline and cocaine. These findings further support a role for MCH signaling in modulating response to psychostimulants and suggest that receptor loss and cilia loss impact responses differently.

Keywords: primary cilia, MCH, cocaine, reward, mice

Introduction

The problem of drug addiction is one of the most complex issues facing society, and can lead to serious health problems. Drug overdoses are responsible for approximately 100,000 deaths annually in the United States (Friedman & Hansen, 2022). Despite the prevalence of this problem, it is not fully understood how the brain contributes to addiction pathology. Most addictive drugs exert their effect by eliciting dopamine release in the mesolimbic pathway, which regulates motivation and reward signaling (Dunigan & Roseberry, 2022). Less understood is the role of neuropeptides in their contribution to the rewarding properties of psychostimulants. The neuropeptide hormone melanin-concentrating hormone (MCH) has been shown to enhance the stimulatory properties of cocaine (Typhon et al., 2008). This peptide binds to receptors enriched in the neuronal primary cilia membrane (Alhassen et al., 2022). Recent evidence suggests that neuronal primary cilia in the mesolimbic pathway regulate drug responses and drug-related behaviors (Ramos et al., 2021). This project seeks to elucidate the role of primary cilia in regulating MCH action on cocaine responses.

Background

Primary cilia are microtubule-based sensory organelles that protrude from almost all cells, including neurons (Green & Mykytyn, 2014). Primary cilia dysfunction in neurons is associated with deficits in memory function, obesity, sensorimotor issues, motivation changes, and alterations in synaptic plasticity (Waters & Beales, 2011). Cilia play a significant role in cellular signaling via their high G-protein-coupled receptors (GPCRs) concentration. GPCRs use G-proteins to transmit signals to cells to activate a cascade of second messengers that elicit a wide range of physiological functions (Schou et al., 2015). Multiple GPCRs localize to cilia and modulate drug-related behaviors, such as the serotonin receptor 5HT6, the orphan receptor GPR88, and dopamine receptors D1R and D2R. A primary-cilia-enriched GPCR of particular interest is the melanin-concentrating hormone receptor (MCHR) (Chung et al., 2009). Melanin-concentrating hormone (MCH) and its receptor, MCHR1, modulate rodent drug-addictive behaviors (Chung et al., 2009; Macneil, 2013; Schmidt & Pierce, 2006). MCH is a peptide hormone that partially regulates energy homeostasis by modulation of feeding behaviors. (Naufahu et al., 2013). MCHR1 is highly abundant in the primary cilia of GABAergic neurons in

the nucleus accumbens (Engle et al., 2018). MCH is produced by neurons in the lateral hypothalamus, which project axons to the nucleus accumbens (Nahon, 2006), indicating a connection between MCH signaling and the dopamine reward system.

MCH signaling in the brain can be manipulated through genetic manipulations or infusion of MCHR1 agonists and antagonists directly into the brain. In previous work, co-administration of MCH with cocaine enhanced locomotor responses compared to cocaine administration alone (Brabant et al., 2010; Chung et al., 2009). Mice lacking MCHR1 (MCHR1 KO) exhibited reduced sensitization to cocaine-induced locomotion compared to wild-type mice. Similarly, the rewarding properties of cocaine were also altered in MCHR1 KO mice. Using a conditioned place-preference (CPP) assay of cocaine reward, MCHR1 KO mice showed a reduced preference for the cocaine-associated chamber compared to controls (Chung et al., 2009). Another study examined the locomotor activity of mice with MCHR1 selectively removed from GABAergic cells and in the presence of a dopamine transporter (DAT) inhibitor to elucidate the contribution of MCHR1 activity to GABAergic and dopaminergic regulation of locomotor-based behavioral outcomes. It was found that MCHR1 KO mice exhibited more locomotor activity and hyperactivity than the wild-type (WT) mice (Chee et al., 2019). Previous work showed that primary cilia contribute to behavioral responses to amphetamine. Mice lacking cilia on dopaminergic and GABAergic neurons showed differences in amphetamine-stimulated locomotor activity compared to wild-type mice (Ramos et al., 2021). Although this data explains some effects of MCH and cilia on the rewarding properties of drugs, it is still unclear how specific MCHR1+ cilia and MCH neuron activity mediate responses to psychostimulants.

Aims and Hypotheses

This project aims to determine the role of cilia in MCHR1 signaling in cocaine responses. These experiments test how the loss of the primary cilium, compared to the loss of MCHR1, influences cocaine-induced locomotor activity and cocaine CPP. Mice with intact cilia and MCHR1 signaling are predicted to show more significant cocaine-induced locomotor activity and place preference. In addition, it is hypothesized that MCH neuron stimulation would result in increased locomotor activity.

Methods

Animal Models

Mice of both sexes and between the ages of 10 and 12 weeks were used. During the light cycle, behavioral testing was conducted between 8 a.m. and 4 p.m. To assess the role of MCHR1 signaling in the modulation of cocaine response, two different models were used. The first model tested the impact of selective removal of primary cilia from MCHR1+ cells. MCHR1-Cre mice (expressing Cre recombinase under the control of the MCHR1 promoter (Jax Labs strain number, #021582)) were crossed with mice harboring floxed *Ift88* alleles (Berbari et al., 2014; Haycraft et al., 2007; Ramos et al., 2021). Breeding was established for behavior experiments that consisted of mice that were heterozygous for the Cre allele ($MCHR1^{cre}$) and either heterozygous ($Ift88^{+/F}$) or ($Ift88^{F/F}$) for the floxed Ift88 allele. $MCHR1^{cre}$:$Ift88^{+/F}$ mice are phenotypically wild-type and called $MCHR1^{cre}$:IFT88 WT. $MCHR1^{cre}$:$IFT88^{f/f}$ mice are cilia knockouts referred to as MCHR1:Ift88 KO. The second model specifically targeted the role of MCHR1 in neurobehavioral responses to cocaine via MCHR1 ablation. In this model, the *Mchr1* start codon was deleted using the CRISPR-Cas9 system, resulting in the loss of expression of the receptor (Jasso et al., 2021).

To determine the effect of MCH neuron activity on modulating cocaine responses, MCH^{cre} mice were used to express Cre recombinase in MCH-producing neurons. MCH^{cre} mice were used to express a virally-encoded receptor to activate MCH neurons. All experiments in this study were approved by the University of Florida Institutional Animal Care and Use Committee and followed NIH guidelines.

Surgery and Virus Injection

MCH^{cre} mice underwent surgery to inject an excitatory designer receptor exclusively activated by designer drugs (DREADD), hM3Dq, into the brain. The purpose of this was to stimulate MCH neurons selectively. The mice were anesthetized with isoflurane and underwent surgery to inject a virus into the lateral hypothalamus of their brains using an Adeno-Associated Virus (AAV) approach. hM3Dq was micro-injected using bregma as a point of reference. The MCH promoter is used to target those neurons specifically. Mice were used in behavioral studies three to four weeks post-surgery.

Locomotor Activity

Locomotor activity was recorded to determine how manipulations to the MCH system in the mouse models affect physiological responses to cocaine. Over five days, locomotor activity, specifically distance traveled and stereotypic counts, were recorded for two hours on the first day and one hour on the following days, as previously described (Ramos et al., 2021). Locomotor activity was tested on $Mchr1^{Cre}$:Ift88, MCHR1 KO, and MCH^{cre} mouse models. In the first hour of the experiment, mice were placed in locomotor boxes to record baseline distance traveled and stereotypic counts. For the second hour of the first day and every hour on the following days, mice were injected with 10 mg/kg of cocaine, and the distance traveled and stereotypic counts were recorded for an hour. In MCH^{cre} mice, a modified paradigm combined chemogenetic activation of MCH neurons with cocaine administration. Mice were first injected with either saline or 1 mg/kg of the DREADD agonist, Compound-21, followed by 10 mg/kg cocaine 30 minutes later. Activity was measured for 120 minutes on the first day of locomotor testing and 90 minutes on days 2-5.

Conditioned Place Preference

Conditioned Place Preference was used to model the rewarding properties of cocaine in mice lacking cilia on MCHR1-expressing neurons ($Mchr1^{Cre}$:Ift88 mice) and mice lacking MCHR1 (MCHR1 KO). During this 10-day trial, infrared beams were used to measure time and position in a three-chambered box. Day 1 consisted of a baseline measurement of preference. Cocaine association occurred on Days 2-9, known as training days. On alternating days mice were injected with either cocaine (10mg/kg) or saline (10mL/kg) and placed in associated chambers for 20min. CPP was analyzed on the 10th day. On this day, the mice had access to the entire box, and time spent in the cocaine vs saline chamber was recorded.

Statistics

The primary measure of interest was time spent in the cocaine- vs. the saline-paired compartment. Data from the initial CPP test was analyzed using a multi-factor ANOVA, with compartment (saline vs. cocaine) and day, as within-subjects variables and strain, genotype, and sex as between-subjects variables. A two-way ANOVA test was performed for locomotor data to measure distance traveled vs. time.

Results

MCHR1 Signaling Disruption Increases Locomotor Responses to Cocaine

To investigate the role of MCHR1 function in modulating responses to cocaine, MCHR1 knockout mice were used (Jasso et al., 2021). Baseline activity was recorded on the first day, and no differences in basal activity were detected. Following cocaine administration, MCHR1 KO mice showed increased locomotor activity (two-way ANOVA, main effect genotype, $F(1, 28) = 8.122$, $p<0.01$) (Figure 1A). MCHR1 KO animals also showed a significant enhancement in sensitization to cocaine over the five testing days (two-way ANOVA, main effect genotype, $F(1,28)=8.8$ $p=0.01$) (Figure 1B). Investigating the effects on stereotypic behaviors as determined by repeated beam breaks without lateral movement, the MCHR1 KO cohort showed a significant increase in acute response to cocaine (Two-way ANOVA, main effect genotype $F(1,28)=5.223$, $p<0.05$) and sensitization (Two-way ANOVA main effect genotype, $F(1,28)=5.70$, $p<0.05$) (Figure 1C, D).

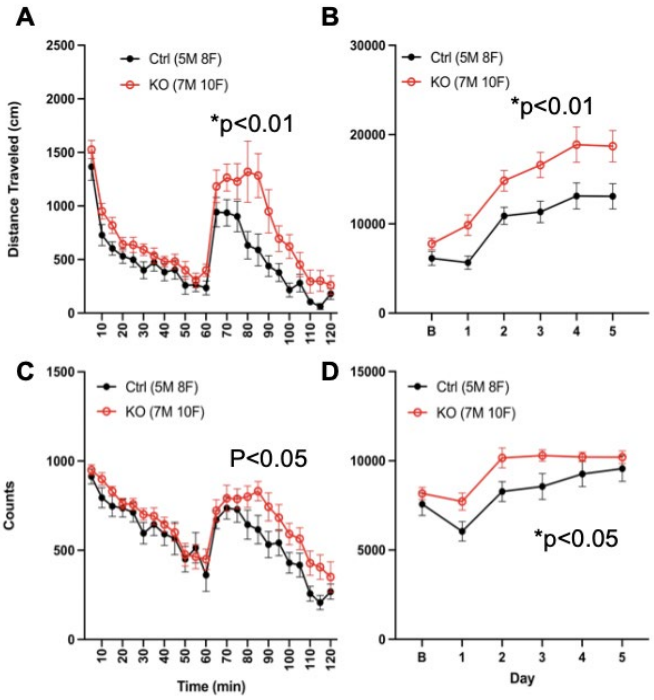


Figure 1. Loss of MCHR1 increases locomotor responses to cocaine. (A) Baseline locomotor activity did not differ between genotypes; however, acute locomotor responses to cocaine were enhanced in MCHR1 KO mice (Two-way ANOVA, main effect genotype, $F(1, 28) = 8.122$, $p<0.01$). (B) Locomotor sensitization to cocaine was enhanced in MCHR1 KO mice compared to WT (Two-way ANOVA, main effect genotype, $F(1,28)=8.8$ $p=0.01$). (C) There was a significant increase in acute cocaine-induced repeated beam break counts in MCHR1 KO mice compared to its control littermates (Two-way ANOVA, main effect genotype $F(1,28)=5.223$, $p<0.05$). (D) A significant increase in repeated beam break counts via sensitization in MCHR1 KO mice was also detected (Two-way ANOVA main effect genotype, $F(1,28)=5.70$, $p<0.05$).

This study also investigated the effect of cilia loss from MCHR1+ neurons on physiological responses to cocaine. In contrast to MCHR1 KO mice, baseline activity of MCHR1^{Ift88} KO mice showed a significant increase in distance traveled compared to control littermates (two-way ANOVA, main effect genotype, $F(1, 43)= 5.774$, $p<0.05$) (Figure 2A). Following cocaine administration, MCHR1^{Ift88} KO mice showed increased locomotor activity compared to WT littermates (two-way ANOVA, main effect genotype, $F(1, 43)= 5.616$, $p<0.05$) (Figure 2A). MCHR1^{Ift88} KO animals also showed a significant enhancement in sensitization to cocaine over the five testing days (two-way ANOVA, main effect genotype, $F(1, 43)=12.1$, $p<0.005$) (Figure 2B). Investigating the effects on stereotypic behaviors as determined by repeated beam breaks without lateral movement, MCHR1^{Ift88} animals showed no changes in acute response to cocaine or sensitization (Figure 2C-D).

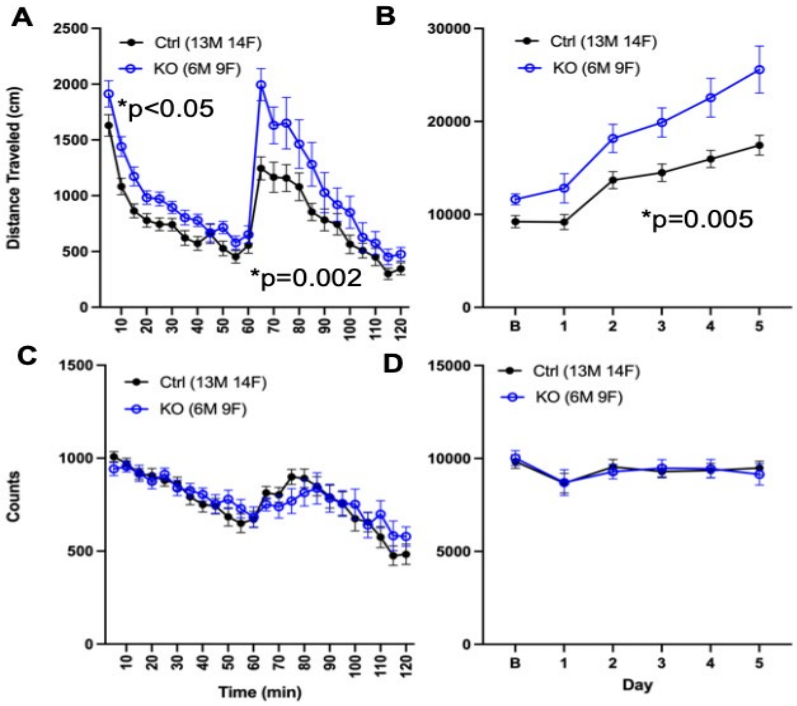
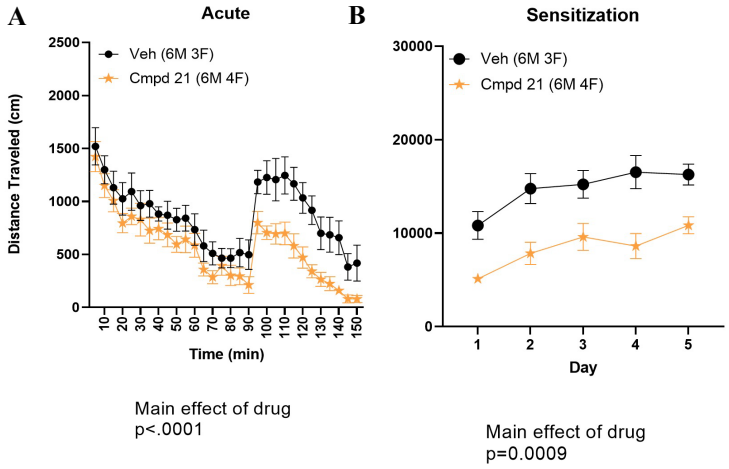


Figure 2. Removal of cilia from MCHR1 neurons increases locomotor responses to cocaine (A) MCHR1^{IFT88} KO mice showed both increased baseline locomotor activity (Two-way ANOVA, main effect genotype, $F(1, 43)= 5.774$, $p<0.05$) and cocaine-induced locomotion compared to its control littermates (Two-way ANOVA, main effect genotype, $F(1, 43)= 5.616$, $p<0.05$). (B) MCHR1I^{FT88} KO showed a significant increase in sensitization via distance traveled compared to its control littermates (Two-way ANOVA, main effect genotype, $F(1, 43)=12.1$, $p<0.005$). (C) There was no difference in baseline repeated beam breaks between genotypes or after cocaine injection. (D) There was no significant difference in sensitization via stereotypic counts between MCHR1^{IFT88} genotypes.

Activating MCH Neurons Decreases Locomotor Responses to Cocaine.

To assess the effect of MCH neuron activity on modulating behavioral responses to cocaine, the activating DREADD receptor hM3Dq in MCH neurons was expressed in the lateral hypothalamus. For these experiments, a modified locomotor protocol was used where mice were pre-treated 30min before cocaine administration. All mice expressed hM3Dq and control animals were injected with saline while experimental animals received the DREADD agonist compound-21. MCH^{cre} DREADD mice treated with compound-21 and cocaine showed a significant decrease in both acute locomotor responses (Figure 3A) ($p<.0001$) and sensitization via distance traveled (Figure 3B) ($p=0.0009$) compared to MCH^{cre} DREADD with saline and cocaine.

Figure 3. Activating MCH neurons reduces locomotor responses to cocaine. (A) MCH^{cre} mice that received compound 21 and cocaine showed a significant decrease in acute locomotor activity compared to MCH^{cre} who



received saline and cocaine ($p<.0001$). (B) MCH^{cre} mice who received compound 21 and cocaine showed a significant decrease in sensitization compared to MCH^{cre} mice who received saline and cocaine ($p=0.0009$).

MCHR1 Activity Modulates the Rewarding Properties of Cocaine.

To determine the effect of MCHR1 signaling on the rewarding properties of cocaine, a conditioned place preference test was used. Both MCHR1 and MCHR1^{Ift88} mice did not exhibit a pre-biased preference for either white or black chambers on the baseline day (Figure 4A, B). On the CPP test day, MCHR1 control mice showed an increased preference for the cocaine-paired chamber compared to their pre-test preference ($p<0.001$) (Figure 4A). In contrast, MCHR1 KO mice did not show a preference for the cocaine-paired chamber compared to the pre-test day (Figure 4A), which was also significantly less compared with their control littermates ($p<0.05$). This study then tested the role of cilia on MCHR1 neurons in CPP development.

MCHR1^{Ift88} KO ($p<0.0005$) and control mice ($p<0.005$) both showed an increased preference for the cocaine-paired chamber compared to the pre-test (Figure 4B). However, unlike MCHR1 KO mice, there was no difference in CPP between MCHR1^{Ift88} KO mice and control littermates.

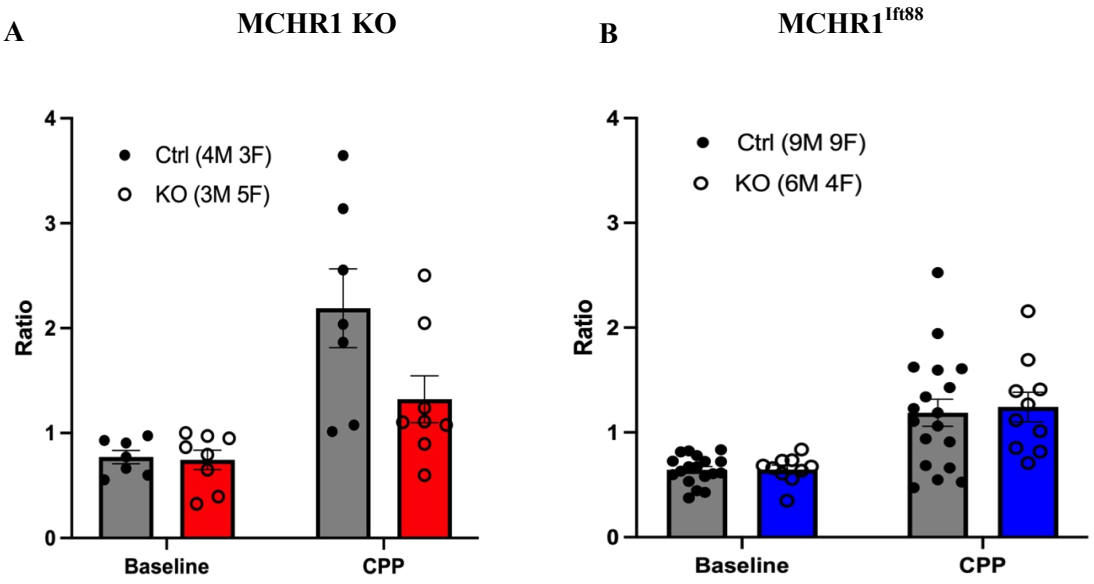


Figure 4. CPP is differentially affected by MCHR1 manipulations. For the baseline days, both strains of mice showed no significant difference in initial preference for one chamber over the other between genotypes. (B) Both control ($p<0.0005$) and Mchr1^{Cre}:Ift88 KO ($p<0.005$) mice displayed an increase over the baseline of the ratio of time spent in the cocaine-paired chamber on the CPP test day but did not differ between groups. (A) MCHR1 control mice displayed a significant induction of CPP over the baseline preference following cocaine pairing ($p<0.001$), whereas MCHR1 KO animals did not show a significant induction of CPP compared to baseline. The ratio of time spent was significantly different between control and KO mice ($p<0.05$)

Discussion

These findings further support the role of MCH signaling in modulating responses to psychostimulants but also show that receptor loss and cilia loss have different effects on these behaviors. In this study, both strains of Mchr1^{Cre}:Ift88 and MCHR1 KO mice demonstrated a preference for the cocaine-paired chamber during the CPP test. Mice lacking the MCHR1 receptor displayed a reduced tendency for the cocaine-paired chamber compared with their littermates with intact MCHR1 receptors. This finding is consistent with another study, which tested CPP on this strain of mice and found a reduction in preference in MCHR1 knockout mice (Chung et al., 2009). This finding suggests that the loss of MCHR1 inhibits the ability to associate cocaine with reward, likely because MCHR1 regulates dopamine release in the brain, a

neuromodulator associated with motivation and reward. When MCHR1 signaling is disrupted, cocaine may not increase dopamine levels in the brain, decreasing its rewarding effects (Chee et al., 2019). Mice lacking cilia did not differ in their induced preference compared to control littermates. It is possible that the loss of MCHR1+ cilia would result in the loss of multiple reward signaling pathways generating a net zero effect. Therefore, the absence of cilia may not play a significant role in the rewarding properties of drug-seeking behavior, or some ciliary receptors may promote drug reward while others inhibit it.

It has been determined that cocaine effects on locomotor activity vary based on the genotypes of different strains. $Mchr1^{Cre}$:Ift88 KO mice demonstrated a greater acute response and increased sensitization to cocaine, as indicated by increased distance traveled and stereotypical counts compared to their control littermates. This suggests that the absence of cilia on MCHR1+ neurons enhances cocaine-induced motor effects by modulating ciliary receptors that regulate locomotor activity. MCHR1 KO mice also showed an increased acute response and sensitization to cocaine compared to their control littermates, represented by the high distance traveled and stereotypic counts. Based on the findings of this study, it may be concluded that when the MCHR1 receptor is lost, the effects of cocaine are potentially increased within the nigrostriatal pathway, a neuronal circuit that determines locomotor output (Chung et al., 2011). According to locomotor and CPP data, MCHR1 signaling inhibits locomotor responses but facilitates reward responses. This effect is thought to be mediated by the regulation of dopamine release and synaptic plasticity in response to cocaine (Thomas, Kalivas, & Shaham, 2008).

Driving MCH activity with a chemogenetic approach resulted in a significant decrease in acute locomotor activity and sensitization over time compared to control mice. This result suggests that stimulating MCH release in the nigrostriatal pathway reduces cocaine behavioral effects, although separating this from other co-released neurotransmitters is an important future step. It is possible that this effect was caused by activating the entire MCH system instead of only activating the production of MCH to project to the nucleus accumbens, the brain's reward center. Chemogenetic activation of MCH neurons projecting only to the striatum will be necessary to determine the role of MCH in affecting cocaine behavior (Terrill et al., 2020).

These results illustrate the complex roles of the MCH system in mediating addictive processes in the brain. This project is impactful in clarifying the dynamics between genotype and psychostimulant-induced behavioral responses to provide a stronger foundation for more

effective personalized treatment for a disease as complex as addiction. Gene therapy/pharmaceuticals targeting MCHR1 and primary cilia may have therapeutic potential for combating the effects of illicit drugs and helping drug addicts recover from their addictions.

Further testing is necessary to determine if cocaine is unique or, like other drugs of abuse, including amphetamines or opioids, in their response to MCHR1 signaling. In future studies, it would be interesting to test these strains of mice under a progressive ratio test with self-administered cocaine to gain a deeper understanding of the MCH system's role in motivation.

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