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Cannabinoid type 1 receptors in A2a neurons contribute to cocaine-environment association

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Abstract

Rationale—Cannabinoid type 1 receptors (CB1Rs) are widely expressed within the brain's reward circuits and are implicated in regulating drug induced behavioral adaptations. Understanding how CB1R signaling in discrete circuits and cell types contributes to drug-related behavior provides further insight into the pathology of substance use disorders.

Objective and methods—We sought to determine how cell type-specific expression of CB1Rs within striatal circuits contributes to cocaine-induced behavioral plasticity, hypothesizing that CB1R function in distinct striatal neuron populations would differentially impact behavioral outcomes. We crossed conditional *Cnr1^{fl/fl}* mice and striatal output pathway cre lines (*Drd1a-cre*, *D1*, *Adora2a-cre*, *A2a*) to generate cell type-specific CB1R knockout mice and assessed their performance in cocaine locomotor and associative behavioral assays.

Results—Both knockout lines retained typical locomotor activity at baseline. *D1-Cre* × *Cnr1^{fl/fl}* mice did not display hyperlocomotion in response to acute cocaine dosing, and both knockout lines exhibited blunted locomotor activity across repeated cocaine doses. *A2a-cre* *Cnr1^{fl/fl}* mice did not express a preference for cocaine paired environments in a two-choice place preference task.

Conclusions—This study aids in mapping CB1R-dependent cocaine-induced behavioral adaptations onto distinct striatal neuron subtypes. A reduction of cocaine-induced locomotor

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Brandon D. Turner and Nicholas K. Smith contributed equally to this work.

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activation in the D1- and A2a-*Cnr1* knockout mice supports a role for CB1R function in the motor circuit. Furthermore, a lack of preference for cocaine-associated context in A2a-*Cnr1* mice suggests that CB1Rs on A2a-neuron inhibitory terminals are necessary for either reward perception, memory consolidation, or recall. These results direct future investigations into CB1R-dependent adaptations underlying the development and persistence of substance use disorders.

Keywords

Cannabinoid receptor; Cocaine; Striatum

Introduction

Propensity to relapse to drug use is a hallmark of substance use disorders (SUDs) and is thought to arise via long-lasting changes in neural circuits encoding reward-related associations. Epidemiological (Zuo et al. 2009) and animal model studies (Bystrowska et al. 2014; Caille et al. 2007; Covey et al. 2015; Fourgeaud et al. 2004; Gremel et al. 2016; Grueter et al. 2010; McCutcheon et al. 2011; Mennerick et al. 2008; Orio et al. 2009; Robbe et al. 2002; Thiemann et al. 2008; Touriño et al. 2008; Wiskerke et al. 2008; Wolf 2016) have provided both correlative and causative evidence that neuronal regulation by cannabinoid type 1 receptors (CB1Rs) expressed throughout forebrain associative circuits is necessary for both the formation and persistence of SUDs.

Early efforts using wide-ranging CB1R manipulations demonstrated that pharmacological inhibition or knockout of CB1Rs in mice inhibited the reinforcing effects of psychostimulants in both non-contingent and contingent assays (Orio et al. 2009; Soria et al. 2005; Yu et al. 2011). Subsequently, targeted removal of CB1Rs using conditional knockout mouse lines revealed the impact of CB1R recruitment on drug-associated behaviors differed based on affected brain regions and the neuronal subtypes contained within. Notably, forebrain CB1Rs expressed in glutamatergic and GABAergic neurons have been shown to exert bidirectional control over psychostimulant-induced behaviors; deletion of CB1Rs from GABAergic neurons inhibited the responsiveness to cocaine while removing CB1Rs from glutamatergic neurons inhibited association of drug reward with external stimuli (Martín-García et al. 2016). A similar dichotomy was seen with hedonic feeding behavior (Bellocchio et al. 2010), where removal of CB1Rs in glutamatergic projection neurons attenuated the potentiation of hedonic feeding by THC while removing CB1Rs from GABAergic neurons prevented hypophagia induced by an aversive dose. While the effects of glutamatergic CB1R knockouts can likely be attributed to cortical projection neurons, the effects of GABAergic CB1R knockouts may be stemming from any of several interneuron populations throughout the forebrain and/or medium spiny neurons (MSNs) in the striatum. As such, it is unclear which GABAergic cell types require CB1Rs to permit drug-induced behavioral adaptations.

The striatum is a GABAergic subcortical structure that serves as both the primary input nuclei of the basal ganglia and as a principal site for the generation of goal-directed reward seeking, habitual behaviors, and behavioral adaptations following drug exposure (Everitt and Robbins 2016; Gremel and Costa 2013; Lüscher et al. 2020; Wall et al. 2019). Both MSNs,

the primary projection neurons of the striatum, and local interneuron classes express CB1Rs in the dorsal striatum (Mathur et al. 2013). Notably, MSNs can be separated into two distinct subsets, “direct” and “indirect,” or midbrain and pallidal projecting, which have been shown to have opposing effects on drug-induced behavioral adaptations by promoting and opposing reward learning, respectively (Turner et al. 2017). Based on the abundance of CB1Rs in the dorsal striatum, as well as its well-established role in organizing appetitive behaviors, we hypothesized that CB1Rs expressed within the dorsal striatum are likely mediating previously published effects of removing CB1Rs from GABAergic neurons on drug-induced behavioral adaptations. Due to the anatomical complexity of multiple MSN and interneuron subpopulations, cell type-specific approaches are necessary to disambiguate the impact of striatal CB1Rs on drug-induced behavioral adaptations.

Here, we sought utilize cell type-specific knockouts of CB1Rs to identify how their function in specific populations of striatal neurons, particularly direct and indirect pathway MSNs, impacts cocaine-induced behaviors. D1 dopamine and A2a adenosine receptors are prominently expressed by striatal MSNs (Kravitz and Kreitzer 2012; Kreitzer and Malenka 2008) and have been extensively utilized to target direct and indirect pathway MSNs, respectively (Joffe et al. 2017; Lobo et al. 2010; Lobo and Nestler 2011; Tejada et al. 2017; Wall et al. 2013). Utilizing *Drd1a* (D1) and *Adora2a* (A2a)-Cre mouse lines, we selectively removed CB1Rs from pathway-specific striatal MSN subsets by crossing with transgenic conditional *Cnr1^{fl/fl}* mice, generating direct and indirect knockouts of CB1Rs (D1-*Cnr1* and A2a-*Cnr1*). We find that D1-*Cnr1* mice do not display hyperlocomotion in response to cocaine and both A2a-*Cnr1* and D1-*Cnr1* mice do not sensitize to repeated doses of cocaine. Additionally, A2a-*Cnr1* mice do not develop a significant preference for cocaine in a conditioned place preference (CPP) assay. These results demonstrate that CB1R signaling in striatal MSNs uniquely contributes to discrete aspects of cocaine’s rewarding properties. Our findings suggest that CB1R regulation of striatal MSN output may be an essential node for cocaine induced circuit plasticity that is required for cocaine-induced behavioral adaptations making CB1Rs on striatal MSNs a valuable target for developing SUD interventions.

Methods

Animals

All animals were bred and housed at Vanderbilt under the supervision of the Department of Animal Care. Conditional *Cnr1^{fl/fl}* mice (contributed by Dr. Eric Delpire) were crossed to *Drd1a*- and *Adora2a*-Cre mice to generate cell type-specific knockouts of CB1Rs. Mice were housed on a 12-h light/dark cycle and fed *ad lib*. Breeding cages were given access to 5LOD chow (PicoLab®, 28.7% Protein, 13.4% Fat, 57.9% Carbohydrate) to improve the viability of litters. Upon weaning at P21–28, experimental animals were fed standard chow. Male mice between 8 and 12 weeks of age were used for all experiments.

Behavior

Open field test (OFT), locomotor sensitization, and CPP assays were performed in MedAssociates Activity Test Chambers. All activity and location were recorded using

Noldus Ethovision 10. All mice used in cocaine experiments underwent a 1-h OFT to habituate them to the chambers. During habituation, total locomotor activity and center time were monitored.

Sensitization: Mice in sensitization cohorts received intraperitoneal (IP) injections of saline for 2 consecutive days before being placed in an activity chamber for 15 min. On days 3 through 7, mice were administered 15 mg/kg cocaine IP and similarly allowed to explore the chamber. Cumulative locomotor activity during the 15-min sessions was acquired.

Place conditioning: Activity chambers were modified to create two distinct zones based on visual and textural cues. Following habituation to handling, mice were placed into the chamber and allowed to explore freely for 20 min to establish an initial preference (pre-test). On subsequent conditioning sessions, mice were given once daily, counterbalanced injections of vehicle (saline) or cocaine (15 mg/kg) IP immediately prior to being placed in the chamber pertaining to the contextual cues. Cocaine was paired with the less preferred context of each individual mouse. Overall, no context bias within the activity boxes was observed in pre-tests. Post-tests were performed following a single pairing, three additional pairings, and 2 and 4 weeks after the last cocaine exposure. Time spent in cocaine-paired context was determined.

Drugs

Cocaine hydrochloride was obtained from Sigma-Aldrich.

Data analysis

Behavior activity was tracked using Noldus Ethovision 10 software and analyzed in Microsoft Excel and GraphPad Prism6. Open field locomotion was assessed by comparing distance traveled over time across genotype using a two-way repeated measures ANOVA and a Tukey's multiple comparisons test. Overall distance, center time, and center entries were compared using a one-way ANOVA with Dunnet's multiple comparisons test to compare knockout groups to controls. Cocaine hyperlocomotion was assessed by comparing cocaine day 1 locomotion to saline day 2 locomotion using a two-way repeated measures ANOVA and a Dunnet's multiple comparisons test. Cocaine locomotor sensitization was assessed by comparing cocaine day 1 locomotion to all subsequent cocaine day locomotion values using a two-way repeated measures ANOVA followed by a Dunnet's multiple comparisons test. CPP was determined by subtracting the pre-test value from post-test values of the time spent on the drug-paired side (time spent on paired side). CPP were compared using one-way ANOVA with Dunnet's multiple comparisons test to compare knockout groups to controls. For all analyses, alpha was 0.05 and applicable tests were two tailed.

Results

Removal of CB1Rs from D1 and A2a expressing cells does not impair basal locomotor function

Previous findings have demonstrated that CB1R expression and/or function, particularly in the forebrain, is necessary for the acquisition and expression of psychostimulant-associated behaviors (Caille et al. 2007; Martín-García et al. 2016; Mereu et al. 2015; Orio et al. 2009; Ramiro-Fuentes et al. 2010; Susana Ramiro-Fuentes and Fernandez-Espejo 2011). Particularly, results have shown knockout of CB1Rs in GABAergic neurons alters the sensitivity to cocaine (Martín-García et al. 2016). To further delineate the contribution of CB1R function in GABAergic neurons, specifically striatal MSNs, to cocaine-associated behaviors, we generated cell type-specific CB1R knockouts by crossing *Cnr1^{fl/fl}* animals to D1-Cre and A2a-Cre mice (D1-*Cnr1^{fl/fl}* and A2a-*Cnr1^{fl/fl}*, hereafter referred to as D1-*Cnr1* and A2a-*Cnr1* mice, Fig. 1a).

As the striatum is heavily involved in locomotor function, our first objective was to determine if removal of CB1Rs from D1 or A2a-expressing MSNs impacted basal locomotion using a 1-h open field test (OFT). No differences in total distance traveled were observed when comparing A2a-*Cnr1* and D1-*Cnr1* mice to littermate controls (Fig. 1b, c). Manipulations of forebrain CB1Rs can precipitate or alleviate anxiety like behavior (Bedse et al. 2017; Hill et al. 2010; Shonesy et al. 2014). Therefore, we also measured time spent in the center of chamber as a means to assay basal levels of anxiety. Neither A2a-*Cnr1* or D1-*Cnr1* mice differed significantly from controls in time spent or frequency of entries into the center of the chamber (Fig. 1d, e). These results suggest cell type-specific knockout of CB1R striatal MSNs does not interfere with locomotor activity or alter baseline anxiety.

Removal of CB1Rs from striatal MSNs reduces cocaine-induced locomotor activity

We next examined whether A2a-*Cnr1* or D1-*Cnr1* animals differed in locomotor responding to cocaine in a cocaine-sensitization assay. A locomotor activity baseline (I.P. saline) for 2 consecutive days was followed by five (5)-day cocaine (I.P. 15 mg/kg) administration and locomotor activity was assessed in a 15-min OFT (Fig. 2a). Locomotor activity of control mice robustly increased over the 5-day sensitization period (Fig. 2b), consistent with cocaine locomotor sensitization shown previously (Joffe and Grueter 2016; Kashima and Grueter 2017; Turner et al. 2018). Although locomotor activity was greater than baseline, neither D1-*Cnr1* nor A2a-*Cnr1* mice exhibited significant sensitization (Fig. 2b) when compared to the first day of cocaine. Comparing a genotype $\times$ day interaction on the first day of cocaine administration, while both controls and A2a-*Cnr1* animals exhibited significant elevations in locomotion compared to saline day 2, D1-*Cnr1* mice did not reach statistical significance (Fig. 2c). Similarly, only littermate controls exhibit a statistically significant increase locomotion between the first and final administration of cocaine (Fig. 2d), likely due to a cocaine-induced increase in distance traveled across all groups from days 1 to 14. These findings demonstrate that CB1R function in both D1 and A2a cells contributes to the acute locomotor stimulating effects of cocaine, blunting behavioral plasticity associated with repeated cocaine exposure.

CB1Rs in A2a neurons are necessary for cocaine conditioned place preference

In order to investigate the specific contribution of CB1Rs in D1 and A2a MSNs to cocaine associative learning and memory, we next sought to determine whether D1- or A2a-*Cnr1* mice formed cocaine context associations measured using a conditioned place preference (CPP) assay. Initially, we performed a subthreshold place-conditioning assay to determine if there was a hypersensitivity to cocaine learning. Following a pre-test to assess basal preference for the different sides of the chamber, we performed a single pairing of saline/cocaine with the non-preferred side of the chamber. Neither D1- or A2a-*Cnr1* groups displayed a difference in preference when compared to littermate controls (Fig. 3a, c). Mice were then subjected to three additional pairings with saline/cocaine. A2a-*Cnr1* animals did not form a significant preference to the cocaine paired side when compared to pre-test (Fig. 3b) and were significantly different from littermate controls (Fig. 3d). Conversely, D1-*Cnr1* animals did form a significant preference for the cocaine-paired chamber and were similar to littermate controls (Fig. 3b, d). These results indicate that CB1Rs on A2a MSNs are required for either the formation or expression of cocaine CPP.

Discussion

Our findings provide evidence that CB1Rs expressed on D1 and A2a cells differentially modulate components of cocaine-induced behavioral adaptations. We found both D1- and A2a-targeted knockouts of CB1Rs disrupted cocaine locomotor. However, only D1-specific knockout of CB1Rs significantly interfered with initial cocaine hyperlocomotion. Conversely, CB1R knockout in A2a but not D1 neurons prevented the development and/or expression of activity cocaine conditioned place preference. This is likely due to a shift in reward perception, behavior acquisition, and/or disruption of reward memory recall. Overall, these data suggest specific cocaine-induced behavioral adaptations, particularly locomotor adaptation and context associations, are attributable to CB1R expression in GABAergic neuron subpopulations: specifically, direct and indirect pathway striatal MSNs.

Numerous studies have linked CB1R expression and function to the development of reward associations including mal-adaptive associations to drugs of abuse. Notably, the striatum contains the highest concentration of CB1Rs in the brain where they are robustly expressed on MSNs and interneurons (Mathur et al. 2013; Van Waes et al. 2012). We chose to utilize two Cre-recombinase mouse lines, *D1*- and *A2a*-Cre, to target direct and indirect pathway MSNs and knockout CB1Rs in a cell type-specific manner. These cre-lines have been used repeatedly to target direct and indirect MSN subtypes within striatal circuits (Joffe et al. 2017; Lobo et al. 2010; Lobo and Nestler 2011; Shonesy et al. 2018) and are reported to lack germline recombination (Luo et al. 2020). However, some non-striatal expression is likely to occur. While A2a-expression is limited largely to the striatum (Lee et al. 2019; Weaver 1993), *D1*-Cre can target other neurons within the central nervous system including *D1*-expressing principal neurons in the cortex (Land et al. 2014). Despite this, the non-overlapping effects seen in previous publications where D1 and glutamatergic neuron-specific-Cre lines were used to generate specific *Cnr1* knockout mice (Monory et al. 2007) suggest the phenotypes arising from removing CB1Rs from D1 neurons observed here are not likely due to cortical D1 neurons.

Striatal circuits are essential for locomotor function. Notably, phytocannabinoids, such as tetrahydrocannabinol (THC), can disrupt locomotor function via putative action in striatal circuits (Monory et al. 2007). Other groups have demonstrated striatal eCB signaling is important for movement (Catlow and Sanchez-Ramos 2015; Sañudo-Peña et al. 1999) as well as in the generation of movement disorders such as Huntington's and Parkinson's (Blázquez et al. 2011; Kreitzer and Malenka 2007; Mieviss et al. 2011). Surprisingly, CB1R knockout from either D1 or A2a MSNs did not impact locomotor behavior (Fig. 1b, c). These findings are consistent with an earlier report using glutamate-, GABA-, CaMKII-, and D1-specific CB1R knockout lines which similarly showed no difference in locomotor activity at baseline (Bellocchio et al. 2010; Monory et al. 2007). Together, these findings suggest that while CB1Rs are highly abundant on MSNs throughout striatal circuits, their function in *MSNs* may not contribute strongly to basal locomotive function.

Previous studies had shown that global reduction in CB1R function suppresses cocaine-induced hyperlocomotion. Global CB1R knockout mice display reduced hyperlocomotion following cocaine dosing (Corbillé et al. 2007) but mice still sensitize to repeated doses of cocaine. Similarly, administration of the CB1R antagonist SR14176 prior to cocaine injection prevented cocaine locomotor sensitization, but not when administered prior to testing with a priming dose (Yu et al. 2011). Others have observed similar outcomes using the CB1R antagonist AM251 which likewise reduced cocaine-induced hyperlocomotion when given prior to cocaine injection (Tozzi et al. 2012). It is possible that these varied effects on sensitization may be explained by the activation of CB1Rs on differing classes of neurons throughout appetitive and motor circuits. For instance, cocaine suppresses lateral inhibition onto D1-MSNs arising from D2-MSNs. Disinhibition of D1-MSNs allows for enhanced locomotion (Dobbs et al. 2016). Interestingly, we found that D1-*Cnr1* animals did not exhibit significant elevation of locomotor activity on the first day of cocaine administration (Fig. 2b). Furthermore, statistical analysis indicates neither D1-*Cnr1* nor A2a-*Cnr1* groups sensitized to cocaine (Fig. 2c). The similarity of our findings to studies using global targeting of CB1Rs suggests that these global effects on cocaine-induced hyperlocomotion may be attributable to CB1R function in MSNs throughout striatal circuits. Notably, a similar inhibition of cocaine sensitization can be accomplished by antagonizing CB1Rs specifically within the nucleus accumbens (NAc), a key node in the ventral striatum (Caille et al. 2007; Mereu et al. 2015; Susana Ramiro-Fuentes and Fernandez-Espejo 2011). However, NAc MSNs do not primarily express CB1Rs (Winters et al. 2012) and are unlikely to be driving the lack of sensitization seen in the D1-*Cnr1* and A2a-*Cnr1* animals; the effect of intra-NAc infusion may be due to CB1Rs on glutamatergic afferents or interneurons.

In addition to effects on the acute hyperlocomotive effects of cocaine, CB1Rs can also contribute to the development and expression of cocaine reward-seeking and associative behaviors. Particularly, global CB1R knockouts exhibit lower motivation for cocaine self-administration as measured by progressive ratio breakpoint (Soria et al. 2005) without impacting self-administration rates; similar effects have been demonstrated using AM251 (Xi et al. 2008). In a non-contingent cocaine CPP task, systemic administration of CB1R antagonist prior to drug delivery during acquisition inhibits the development of methamphetamine and cocaine CPP (Yu et al. 2011). From these studies, it was not clear what population of cells expressing CB1Rs are recruited by cocaine to induce changes

in behavioral responding. Using our cell type-specific knockout approach, we found that D1-*Cnr1* mice were able to form a preference for the cocaine paired chamber similar to littermate controls. However, A2a-*Cnr1* mice did not form a significant preference when compared to pre-test exploratory behavior and were significantly different from controls when comparing change in time spent on the drug paired side (Fig. 3b, d). This points to CB1R function in A2a neurons as an important factor potentially determining the rewarding properties of cocaine, which can alter acquisition of cocaine CPP, the neuronal mechanisms engaged during CPP acquisition, or expression of cocaine-environment associations; due to the constitutive nature of our knockout, it is not possible to discern which. Notably, others have demonstrated that infusions of CB1R antagonists into the NAc are similarly capable of inhibiting cocaine-conditioned learning and reward seeking (Soria et al. 2005; Xi et al. 2008). However, as with the effect on cocaine sensitization, these infusions can disrupt CB1R function on NAc interneurons or glutamatergic inputs as NAc MSNs do not express CB1Rs (Winters et al. 2012). Interestingly, GABA-specific knockouts of CB1Rs were previously shown to exhibit *enhanced* operant self-administration of cocaine (Martín-García et al. 2016), suggesting CB1Rs on GABAergic neurons functionally oppose drug-associated behaviors in some contexts. Our findings using cell type-specific deletion of CB1Rs in D1- and A2a-expressing neurons, however, suggest CB1R recruitment at GABAergic neurons impacts behavioral outcomes in a manner dependent on the neuronal population affected.

Extrapolating the impact of striatal CB1R signaling on behavior is complex; striatal CB1Rs are expressed on glutamatergic afferents onto MSNs (Chen et al. 2011; Kreitzer and Malenka 2007; Lerner and Kreitzer 2012; Shonesy et al. 2018), MSN-MSN collaterals, and inhibitory projections from interneurons (Mathur et al. 2013). Our results showing differential effects of behavioral outcomes in D1-*Cnr1* and A2a-*Cnr1* animals indicate potential differences in striatal MSN CB1R recruitment by cocaine. Removing CB1Rs from the MSNs themselves likely disrupts MSN-MSN collateral modulation by eCB signaling *or* prevents eCB signaling on MSN terminals in downstream brain regions (Caballero-Florán et al. 2016; Davis et al. 2018). With respect to the former, removal of CB1Rs from a single MSN subpopulation would prevent eCB-mediated reduction of inhibitory collaterals and potentially increase lateral inhibition from MSNs lacking CB1Rs; This shift may result in an imbalance in direct and indirect pathway MSN activity. Such an imbalance could disrupt locomotion as both MSN populations are active during ambulatory behaviors (Cui et al. 2013) with disinhibition of collateral inhibition onto D1 MSNs being important for cocaine-induced hyperlocomotion (Dobbs et al. 2016). In both our approach and previous studies perturbing eCB signaling in striatal MSNs (Shonesy et al. 2018), there were no observed differences in basal locomotion. However, it is possible that during periods of high striatal activity, as would be expected during hyperactivity induced by cocaine and other psychostimulants, that small imbalances between MSN pathway activity would be exacerbated; As such, lacking eCB signaling at inhibitory striatal collaterals may underlie our observations in D1 and A2a-KO animals which exhibit a relatively blunted locomotor response across multiple days of cocaine administration. Notably, similar observations regarding the importance of striatal pathway balance have been made when disrupting glutamatergic and GABAergic signaling onto direct and indirect pathway MSN subpopulations (Dobbs et al. 2016; Joffe et al. 2018).

Unique to the A2a-*Cnr1* mice was a decrease in cocaine CPP. While inputs onto indirect-pathway A2a MSNs are not canonically thought to undergo long-term changes with respect to cocaine exposure (Turner et al. 2017), our results indicate that CB1R-dependent plasticity of their *outputs*, i.e., collaterals or their downstream terminals, may be a necessary site of plasticity in the formation of cocaine CPP.

Notably, several of the results noted here have been achieved through disrupting endocannabinoid signaling in more ventral striatal regions such as the NAc. This is not unsurprising as many disruptions of ventral and dorsal striatal circuitry, which is highly interconnected through ascending cortico-striatal-thalamic loops, can impinge upon behavioral performance (Everitt and Robbins 2016; Lüscher et al. 2020). Interestingly, our findings are counter to those using CB1R knockouts which broadly target forebrain GABAergic neurons (Bellocchio et al. 2010; Martín-García et al. 2016), where GABAergic CB1Rs engender aversive responses. These differences point to potentially unique engagement of CB1Rs on MSNs and various GABAergic interneuron populations throughout forebrain circuits during drug- and non-drug-associated reward learning that merit further study.

While not explored here, sex is an important variable differentially contributing to SUDs and related behaviors (Becker and Koob 2016; Carroll and Lynch 2016; Kiraly et al. 2018; Perry et al. 2016; Zachry et al. 2019). Additionally, CB1 receptor function in reward behavior is sexually dimorphic. For instance, female CB1 receptor knockout mice show a shorter latency to natural reward acquisition but no effect on cocaine self-administration. In contrast, male CB1 knockout mice showed a reduced motivation to cocaine (Ward and Walker 2009). Thus, our findings of circuit-specific function of CB1 receptors in males and lack of information on females warrant future studies on sex- and circuit-specific function of CB1 receptors in reward behaviors.

Together, these findings point to CB1Rs expressed on striatal MSNs as necessary for the development or expression of psychostimulant-associated locomotor and associative learning. Specifically, our results suggest that modulation of MSN synaptic transmission by eCB signaling, either at local collaterals or downstream projections, may serve as a novel site of striatal output regulation during the formation of SUD-like behaviors.

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Fig. 1.

Cell type-specific deletion of CB1Rs does not alter locomotor activity. **a** Schematic of genetic approach to generate cell type-specific deletions of CB1Rs. **b** Distance traveled over the course of open field assay in 5-min bins. No significant interaction between time and genotype (Interaction $F(22, 374) = 0.7003$, $p > 0.05$). **c** Cumulative distance traveled in 60-min open field assay. Distance did not vary with genotype (Genotype $F(2, 34) = 0.3347$, $p > 0.05$). **d** Cumulative center time did not differ by genotype (Genotype $F(2, 34) = 0.1363$, $p > 0.05$). **e** Number of entries into the center of the arena was not different across genotypes (Genotype $F(2, 34) = 0.4133$, $p > 0.05$). Error bars represent standard error of the mean

Fig. 2. Deletion of CB1Rs in either D1 or A2a+ MSNs blunts cocaine locomotor sensitization. **a** Diagram outlining experimental timeline. **b** Distance traveled per session in cocaine (15 mg/kg) sensitization assay. *A2a-Cnr1* and *D1-Cnr1* mice did not sensitize to cocaine, while *Cnr1^{fl/fl}* controls exhibited significant elevation in locomotion by cocaine day 2 compared to cocaine day 1. Two-way repeated measures ANOVA found a significant interaction between cocaine administration day and genotype (Interaction $F(14, 189) = 3.018$, $p < 0.05$) and multiple comparisons comparing cocaine day 1 to all subsequent days found only *Cnr1^{fl/fl}* locomotor activity increased past cocaine day 1. **c** *Cnr1^{fl/fl}* animals and *A2a-cnr1* both exhibit significant cocaine hyperlocomotion. Two-way repeated measures ANOVA found a significant interaction between treatment and genotype (Interaction $F(14, 189) =$

3.018, $p < 0.05$), and multiple comparisons revealed significant differences between saline and cocaine for *Cnr1*^{fl/fl} and A2a-*Cnr1* but not D1-*Cnr1*. **d** Only *Cnr1*^{fl/fl} animals exhibit significant increases in locomotion when comparing the first day of cocaine to the final (14th) day of cocaine treatment. Two-way repeated measures ANOVA found a significant effect of cocaine treatment day ($F(1,27) = 18.9$, $p < 0.05$), and multiple comparisons revealed this difference to be due to the difference between *Cnr1*^{fl/fl} animals by treatment day. **e** Representative activity diagrams of the final cocaine exposure session (+ 14). Traces represent minutes 11–20 of the session. (* $p < 0.05$). Error bars represent standard error of the mean

Fig. 3.

Lacking CB1Rs in A2a+ neurons impairs cocaine conditioned place preference. **a** Time spent on the drug paired side in either pre-test (outlined) or after a single cocaine exposure (filled) did not vary by genotype, two-way repeated measures ANOVA (Interaction $F(2,28) = 2.047$, $p > 0.05$). **b** Time spent on the drug paired side in either pre-test (outlined) or after three cocaine exposures (filled) increased significantly in both *Cnr1^{fl/fl}* and *D1-Cnr1* but not *A2a-Cnr1*. Two-way repeated measures ANOVA found a significant interaction (Interaction $F(2,28) = 5.575$, $p < 0.05$) and multiple comparisons revealed significant increases for both *Cnr1^{fl/fl}* and *D1-Cnr1*. **c** Change in time spent on the drug paired side after a single pairing of cocaine did not vary by genotype, one-way ANOVA (Genotype $F(2, 29) = 1.757$, $p > 0.05$). **d** *A2a-Cnr1* animals developed a significantly reduced preference for the cocaine paired side when compared to *Cnr1^{fl/fl}* controls. One-way ANOVA found a significant effect of genotype (Genotype $F(2, 29) = 3.688$, $p < 0.05$), and multiple comparisons

revealed this difference to be between *Cnr1*^{fl/fl} and A2a-*Cnr1*. Error bars represent standard error of the mean