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Dopamine D1 receptor agonist treatment attenuates extinction of morphine conditioned place preference while increasing dendritic complexity in the nucleus accumbens core

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HIGHLIGHTS

- D1R agonist given after extinction training attenuates extinction of morphine CPP.
- D1R agonist given after extinction increases dendritic complexity in accumbens core.
- D1R agonist effect on accumbal dendritic morphology depends on previous conditioning.

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ABSTRACT

The dopamine D1 receptor (D1R) has a role in opioid reward and conditioned place preference (CPP), but its role in CPP extinction is undetermined. We examined the effect of D1R agonist SKF81297 on the extinction of opioid CPP and associated dendritic morphology in the nucleus accumbens (NAc), a region involved with reward integration and its extinction. During the acquisition of morphine CPP, mice received morphine and saline on alternate days; injections were given immediately before each of eight daily conditioning sessions. Mice subsequently underwent six days of extinction training designed to diminish the previously learned association. Mice were treated with either 0.5 mg/kg SKF81297, 0.8 mg/kg SKF81297, or saline immediately after each extinction session. There was a dose-dependent effect, with the highest dose of SKF81297 attenuating extinction, as mice treated with this dose had significantly higher CPP scores than controls. Analysis of medium spiny neuron morphology revealed that in the NAc core, but not in the shell, dendritic arbors were significantly more complex in the morphine conditioned, SKF81297-treated mice compared to controls. In separate experiments using mice conditioned with only saline, SKF81297 administration after extinction sessions had no effect on CPP and produced differing effects on dendritic morphology. At the doses used in our experiments, SKF81297 appears to maintain previously learned opioid conditioned behavior, even in the face of new information. The D1R agonist's differential, rather than unidirectional, effects on dendritic morphology in the NAc core suggests that it may be involved in encoding reward information depending on previously learned behavior.
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1. Introduction

Misuse of prescribed opioids is an increasingly frequent clinical and societal problem that leads to opioid use disorder (OUD)[1]

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[1] Abbreviations: Opioid Use Disorder (OUD), conditioned place preference (CPP), nucleus accumbens (NAc), medium spiny neuron (MSN), dopamine D1 receptor (D1R), dopamine D2 receptor (D2R), mu opioid receptor (MOR), ventral tegmen-

in an estimated 8–12% of individuals with chronic pain [1]. OUD is a chronic, relapsing condition that produces tremendous morbidity and mortality. Even with currently available opioid substitution and psychosocial treatments, less than 30% of patients achieve long-term, relapse-free abstinence [2]. Relapse is caused by multiple factors, including exposure to "triggers," or stimuli that have become associated with opioids because they repeatedly co-occur with opioid use. For example, the cash used to purchase a drug or the needle used for administration are examples of drug cues and can induce drug craving and relapse, even in an abstinent individual [3,4]. A better understanding of how stimuli become associated with opioid reward and an approach to modify these associations could alter the course of OUD.

Conditioned place preference (CPP) is an animal model that can be used to study the associative learning aspects of drug reward [5–7]. In the CPP model, animals show a preference for spending time in an environment that has previously been associated with a drug of abuse [8]. The extinction of this preference occurs if the association is weakened by repeated exposure to the drug-associated context in the absence of the drug [5,6]. Just as CPP provides an animal model for the formation of drug-associated cues in humans, CPP extinction serves as a model for cue exposure therapy. In this therapy, the association between a trigger and a drug of abuse is weakened after drug-related cues and contexts are repeatedly presented to a patient in the absence of the drug [9,10]. Exposure therapy reduces stimulus-elicited drug craving for at least 2 months when combined with biofeedback in heroin addiction [11]. The combination of such behavioral approaches with novel pharmacotherapy has the potential to improve treatment efficacy.

Dopamine is thought to be responsible for the powerful reward that causes drug use to become associated with co-occurring contexts, as in morphine CPP. Opioids produce reward by binding the μ opioid receptor (MOR) on ventral tegmental area (VTA) interneurons, thereby disinhibiting the VTA neurons that release dopamine into the nucleus accumbens (NAc) [12]. This dopamine release can imbue salience to cues and contexts that are sensed at the time of opioid reward [13–15]. The dopamine 1 and 2 receptors (D1R and D2R) are necessary for CPP, as knocking out these receptors or antagonizing them pharmacologically prevents the acquisition of morphine CPP [16–19]. In contrast, adding D1R agonist to heroin enhances low-dose heroin self-administration [20].

Dopamine in the NAc may also have a role in maintaining drug-cue associations, as conditioned drug stimuli themselves cause dopamine release in the NAc core and activate D1R-containing medium spiny neurons (MSNs) in the NAc core during expression of CPP [21,22]. Additionally, downregulation of D1R MSN transmission prevents reinstatement of morphine CPP [23]. We are not aware of any studies examining the effects of D1R agonists on morphine CPP extinction and accumbal neuronal structural plasticity, but dopaminergic agonist has been shown to alter drug-cue associations when administered during a period of memory reconsolidation [24].

Changes in NAc MSN morphology appear to be important in encoding opioid reward and morphine CPP and its extinction. Seminal work has shown that withdrawal from morphine is associated with a reduction in NAc MSN spine density and dendritic complexity [25,26]. Our group has shown that morphine CPP is correlated with an increase in NAc core MSN dendritic complexity, while morphine CPP extinction is associated with a decrease in the same [6,7]. Because morphology determines the electrotonic properties of neurons, changes in dendritic complexity may affect their electrical signaling. For example, dendrites filter post-synaptic potentials as current flows through them toward the soma, which in turn affects action potential generation [27].

If neuronal morphology is involved in encoding CPP in the NAc, then any pharmacologic intervention that impacts CPP could also impact morphology. Being involved in drug reward, drug-cue association, and morphine CPP specifically, the D1R is an ideal candidate for pharmacologic manipulation that could affect morphine CPP extinction. Dopamine directly affects the morphology of neurons, as D1R and D2R agonists alter dendritic complexity and spine density of striatal neurons *in vitro* [28]. Interestingly, dopamine depletion also alters accumbal neuronal morphology, and does so differentially in the NAc core and shell [29]. Thus dopamine receptors could potentially modulate reward by alteration of the structure of NAc MSN neurons [30].

We hypothesize that opioid-induced release of dopamine contributes to the formation of morphine-conditioned behavior through effects on MSN dendritic complexity. In this study, the effect of D1R activation on morphine CPP extinction, and on associated neuron morphology in the NAc was examined. We chose the D1R due to its consistent role in morphine reward and CPP. Because it is necessary for CPP acquisition and enhances reinstatement of CPP, we hypothesized that D1R activation immediately after extinction training would attenuate CPP extinction and enhance reward responses. Based on our past work, if D1R activation results in the maintenance of CPP expression, we would expect an associated increase in the dendritic complexity of NAc core MSNs.

In our study, groups of mice that acquired morphine CPP were treated with different doses of D1 agonist SKF81297 after extinction sessions while controls were treated with saline. We determined the effect of extinction training on CPP by comparing preference scores between treatment groups. After extinction training, brain samples were processed using Golgi-Cox staining to identify single MSNs in the NAc core and shell and then image analysis derived morphology measures that were compared between groups. We performed a separate study to control for exposure to SKF81297 in the absence of morphine. In the control study, dendritic morphology and CPP behavior were compared between two groups of saline conditioned mice treated with SKF81297 or saline after extinction sessions.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice were used because they display measurable indications of reward in response to morphine [6,7]. Mice were 6–8 weeks of age, housed in groups of 1–4 mice per cage. They were acclimated to a 12 h light/dark cycle for at least 7 days before the study. Animal testing was performed in a facility approved by the Association for the Assessment and Accreditation of Laboratory Animal Care and approved by the Institutional Animal Care and Use Committee of the VA Boston Healthcare System. The experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Because of the modest effect size and variability in CPP behaviors, larger numbers of subjects were required for each treatment group. A total of 163 mice were used for behavioral studies with 10–65 mice per behavioral experimental group, and a subset of these mice was used for neuron morphology studies with 3–6 mice per group.

tal area (VTA), extinction (Ext), After each extinction training session, one group was injected with saline (Morphine/Saline), a second group was injected with dopamine D1 receptor agonist SKF81297 0.5 mg/kg (Morphine/SKF 0.5), and a third group was injected with SKF81297 0.8 mg/kg (Morphine/SKF 0.8). A control group (Saline/Saline) was injected with only saline s.c. during conditioning and extinction and never developed a CPP.