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Using DREADDs to investigate addiction behaviors

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Abstract

Drug addiction is characterized by compulsive drug-seeking and drug-taking, and a high propensity for relapse. Although the brain regions involved in regulating addiction processes have long been identified, the ways in which individual cell types govern addiction behaviors remain elusive. New technologies for modulating the activity of defined cell types have recently emerged that are allowing us to address these important questions. Here, we review how one such technology, DREADDs (Designer Receptors Exclusively Activated by Designer Drugs), can be used to refine our knowledge of addiction circuitry. These engineered receptors modulate cellular activity by acting on G protein coupled signaling cascades and in this review we pay particular attention to how this slower-onset modulation preferentially regulates behaviors that develop over time.

Drug addiction is a tremendously costly public health problem that has profound medical consequences to individuals, as well as serious social and economic impacts on our society. Unfortunately, relapse rates are high and treatment outcomes remain poor, in part due to our incomplete understanding of the complex nature of this destructive disease. The progression from initial drug exposure to regular drug use and ultimately to compulsive habitual behavior and loss of inhibitory control involves a sequential series of cellular and molecular adaptations throughout the brain, although concentrated in the cortico-basal ganglia-thalamic circuitry. In addition to regulating motivation and reward, this system is involved in cognitive and motor processes. Accordingly, along with addiction, dysfunction of processing within cortico-basal ganglia-thalamic loops has been implicated in many other neuropsychiatric disorders, including ADHD, obsessive-compulsive disorder and Parkinson's disease. Thus, the adaptations involved in addiction may interfere with optimal neurocognitive function across several important domains, and therefore it is essential that any new interventions to prevent relapse to drug-seeking not interfere with critical brain functions involved in motivation, decision making and motor function for desired outcomes of daily living.

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Conflict of interest statement

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Although in their infancy, new technologies have emerged in the past decade that are revolutionizing our ability to understand the cells and circuits that are engaged by drugs of abuse and that regulate the behaviors that contribute to addiction. These tools are particularly powerful because they are now allowing us to isolate the function of targeted neurons; which has the potential for providing us with meaningful findings for advancing the field of addiction through a better understanding of how select components of neural circuits, including subsets of cells, govern behavior. In this review, we will describe how one such technology, DREADDs (Designer Receptors Exclusively Activated by Designer Drugs), is refining our understanding of addiction-related behaviors.

DREADDS and manipulation of circuit function

DREADDS are a powerful new chemogenetic approach for reversible modulation of neuronal activity; and accordingly, there are many potential applications for this technology [1,2]. For example, DREADDs have recently been coupled with metabolic mapping techniques (e.g. DREADD-assisted metabolic mapping; DREAMM) for *in vivo* functional imaging [3,4•]. Although DREADD techniques are most commonly used in mice and rats, non-human primate studies are now underway. DREADDs consist of a family of engineered G protein coupled muscarinic receptors that have been modified so they are no longer activated by their endogenous ligand acetylcholine but are instead potentially activated by the otherwise inert synthetic ligand, clozapine-*n*-oxide (CNO) [5••]. Currently, DREADDs are available that modulate cellular activity through activation of G_s-coupled signaling cascades (rM_3Ds), G_q-signaling cascades (hM_3Dq), $G_{i/o}$-coupled signaling cascades (hM_4Di) and most recently β-arrestin-mediated signaling cascades (rM_3Darr) (Figure 1) [2]. Although these receptors have frequently been portrayed as primarily activating or inhibiting cells through alterations in neuronal firing, which they do, it may be more accurate to think of them as each engaging distinct intracellular signaling cascades. For example, $G_{i/o}$ signaling may do more than inhibit neuron firing, and each of these G protein mediated pathways are complex and vary to some extent between cell types [6•].

DREADDS and psychomotor sensitization

Psychomotor sensitization is a progressive and persistent increase in the psychomotor activating effects (i.e., locomotion and stereotypy) induced by repeated, intermittent exposure to a drug [7]. Sensitization is a useful paradigm for studying addiction processes because it is an easily observable behavioral output of the neural circuitry thought to underlie the incentive-motivational aspects of drug-seeking that facilitate the transition to addiction [8–10]. Using $G_{i/o}$-coupled DREADDs that are expressed under cell-type specific promoters, we have examined the role of subtypes of medium spiny projection neurons (MSNs) in the dorsomedial striatum in the development of amphetamine-induced psychomotor sensitization. We found that increasing $G_{i/o}$ signaling in indirect pathway MSNs (i.e., those that express the neuropeptide enkephalin and indirectly project to the substantia nigra (SN) via the globus pallidus external (GPe) and subthalamic nucleus (STN) [11]) enhances the development of locomotor sensitization to amphetamine whereas increasing $G_{i/o}$ signaling in direct pathway MSNs (i.e. those that express the neuropeptides dynorphin and substance P and directly project to the SN [11]) impairs the persistence of

this behavior [12••]. Consistent with these findings, Farrell *et al.* [6•] found that increasing G_s signaling in all indirect pathway MSNs through generation of a transgenic mouse with rM3Ds expression under control of the adenosine2A (adora2a) receptor promoter blocked the development of amphetamine-induced locomotor sensitization. Although MSNs regulate motor behaviors and increasing G_s signaling in all indirect pathway MSNs decreased novelty-induced locomotion [6•], the observed behavioral changes following amphetamine treatment are unlikely to be a result of merely changing motor behaviors because these manipulations did not affect the acute locomotor responses to amphetamine. Further, increasing $G_{i/o}$ signaling in a subset of indirect pathway neurons was sufficient to modulate amphetamine behaviors but had no effect on basal locomotor activity [6•,12••]. Therefore, the preferential effects of DREADDs on the plasticity associated with this time and drug-dependent plasticity model suggest that DREADD activation has a more subtle impact than simply activating or silencing neurons, but rather acts to enhance or diminish the plasticity associated with repeated drug administration. Consistent with this idea, we have recently found that altering G protein coupled signaling cascades in direct pathway MSNs with DREADDs modulates the plasticity associated with retaining decision-making strategies in a high versus low reward-discrimination task [13•]. In particular, G_s versus $G_{i/o}$ activation by DREADDs during training produced opposite effects on retention of a decision-making strategy over time, but had no effect on responding during acquisition of the task nor on task performance following acquisition [13•].

Cell-type specific $G_{i/o}$-coupled DREADDs have also been used to examine the role of glutamatergic neurons in the basolateral nucleus of the amygdala (BLA) in the development of locomotor sensitization to cocaine. It was found that increasing $G_{i/o}$ signaling in the BLA during repeated cocaine treatment attenuated the development of locomotor sensitization without altering basal levels of locomotion [14•]. This manipulation was also sufficient to block cocaine-induced increases in the frequency of miniature excitatory post-synaptic currents (mEPSCs) in dopamine D1 MSNs in the nucleus accumbens shell, suggesting that BLA regulation of MSN plasticity is probably an important mechanism regulating sensitization [14•].

DREADDS and drug self-administration

In addition to behavioral sensitization, DREADDS have been used successfully in drug self-administration models to examine the circuitry underlying drug-taking behaviors, including motivation to take drugs under a progressive ratio schedule of reinforcement. Interestingly, using targeted injections of a conditional hM_4Di viral vector into adora2a-Cre mice, Bock *et al.* [15••] found that increasing $G_{i/o}$ signaling in indirect pathway MSNs in the nucleus accumbens core had no effect on responding for cocaine when it was available under low effort conditions (fixed ratio 1; FR1) but enhanced motivation for cocaine as evidenced by higher breakpoints in progressive ratio schedules. This effect was region-specific, as the same manipulation in the dorsal striatum had no impact on motivation for cocaine. In addition, these results cannot be attributed to non-discriminative effects on motivation, as increasing $G_{i/o}$ signaling in indirect pathway MSNs in nucleus accumbens core had no effect on breakpoints for food reward [15••]. Thus, together with the sensitization findings, this series of DREADD experiments demonstrates that the plasticity that occurs in indirect

pathway MSNs following drug use likely regulates the processes that govern the transition to addiction.

Although most work with DREADDs has centered on understanding behaviors produced by psychostimulant drugs, DREADDs have also been utilized as an effective tool for studying addiction processes in other drug classes. For example, although increasing G_q signaling throughout the nucleus accumbens had no effect on ethanol consumption in a limited access paradigm, increasing $G_{i/o}$ signaling in the same region reduced ethanol consumption without altering either water or sucrose intake or effecting basal levels of locomotor activity [16]. Thus, as with the psychostimulant experiments, the differential regulation of consumption of drugs versus natural rewards as well as motor behavior further suggests that DREADDs modulate plasticity with more subtlety than an 'off and on' model would predict.

DREADDS and relapse

Drug relapse is a recurring problem among addicts, even following long periods of drug abstinence. This behavior can be modeled in animals using either extinction training or withdrawal paired with drug prime-, cue and/or stress-induced reinstatement tests. Recently, Mahler *et al.* [17•] used DREADDs to examine the contribution of subregions of the ventral pallidum (VP) in cue and cocaine prime-induced reinstatement following withdrawal. They found that increasing $G_{i/o}$ signaling in rostral VP neurons decreased cue-induced but not cocaine prime-induced reinstatement whereas the same manipulation in caudal VP had the opposite effect; that is it attenuated cocaine prime-induced but not cue-induced reinstatement [17•]. Additionally, both activation of inhibitory hM_4Di DREADDs in rostral VP terminals in the VTA and functional disconnection of the rostral VP from dopamine neurons in the VTA (via unilateral expression and activation of hM_4Di in rostral VP combined with contralateral expression and activation of hM_4Di only in tyrosine hydroxylase (TH+) of the VTA) attenuated cue prime-induced reinstatement, demonstrating that rostral VP connectivity to dopamine neurons in the VTA is crucial for driving this form of reinstatement [17•].

Another recent study using DREADDs to investigate the cell types that modulate ethanol-seeking following self-administration [18••]. They found that increasing G_q signaling selectively in astrocytes in the nucleus accumbens core following a 3-week period of abstinence decreased motivation for ethanol, as assessed by decreases in breakpoints in a progressive ratio schedule of reinforcement. This manipulation also facilitated responding for intracranial self-stimulation but had no effect on motor activity [18••].

Conclusions and future considerations

The studies described in this review demonstrate how new technologies, such as DREADD receptors, are being implemented in order to understand the circuitry and intracellular signaling processes underlying the different phases of addiction. These techniques are allowing us to answer circuit-mapping questions that have previously been unaddressable due to technical limitations. For example, it has been difficult to isolate the contribution of subsets of MSNs or astrocytes in addiction-related behaviors because the neurons are

physically intermingled and pharmacological approaches are limited due to multiple cell types expressing the same receptor (e.g. $G_{i/o}$-coupled dopamine D_2 receptors are expressed in indirect MSNs as well as cholinergic interneurons in the striatum). As described above, we can circumvent these issues by expressing DREADDs under the control of selective promoters in order to achieve cell-type specific manipulations. These strategies can also be used to examine terminal regions, and new combinatorial approaches with Cre-recombinase dependent DREADDs are allowing us to examine select afferent and efferent connections while preserving normal connectivity elsewhere in the circuit.

Because DREADDs are a new technology, much of the work of these pioneering studies has been to establish and describe new methodologies. Nonetheless, these studies are already giving us insights into the brain regions and component behaviors that mediate various aspects of addiction. For example, this work raises the intriguing possibility that the circuits that regulate motivation and reward for drugs, and can be modeled by psychomotor sensitization and drug self-administration paradigms, are distinct from the circuits that regulate motivation for natural rewards or those that govern motor behavior. However, the plasticity underlying drug addiction may be homologous to that which underlies other types of reward and motor output and whether it is mediated by distinct sets of neurons or distinct sets of synapses by the same neurons is not yet clear. No doubt this will be a focus of future DREADD work, especially since it is important that effective treatments that can modulate seeking of drugs but not natural rewards be developed. Nonetheless, given that DREADDS can induce subtle yet long-lasting changes in neuronal plasticity by engaging G protein signaling pathways, DREADD technology is particularly well-suited for studying addiction processes and may one day itself represent a viable treatment for preventing addiction or relapse.

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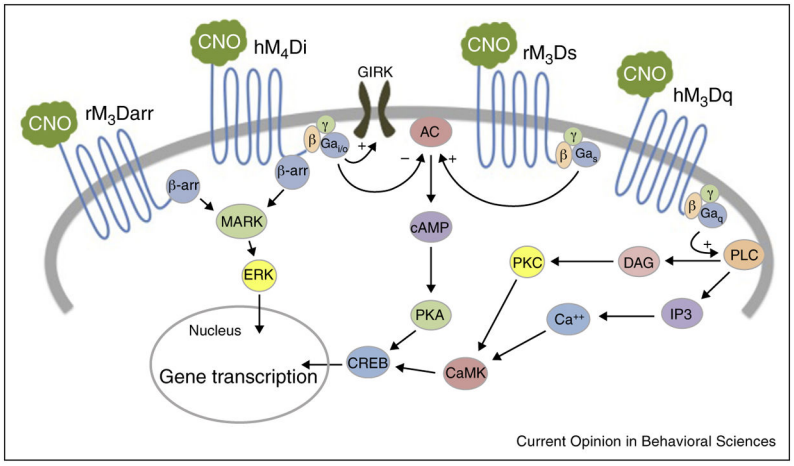

Figure 1.

DREADD receptors. This illustration depicts the subtypes of DREADD receptors that are currently available as well as the primary intracellular signaling cascades that are engaged by each DREADD receptor following activation by clozapine-*N*-oxide (CNO).