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The use of chemogenetic approaches in alcohol use disorder research and treatment

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Abstract

Several novel techniques were developed recently to explore neural circuit mechanisms of neuropsychiatric disorders. These techniques include the Designer Receptors Exclusively Activated by Designer Drugs (DREDD)-based chemogenetic tools, which represent valuable platforms for selective and noninvasive control of neural activity with a high degree of spatial resolution. Among all variants, Gq and Gi-DREDDs are widely used by neuroscientists to dissect out the circuitry and cellular signals. This review is focused on strategies to access a specific neuronal population or circuit using the DREDD technique and summarizes the current knowledge of the DREDDs' application in alcohol use disorder research and therapeutics.

Keywords

Chemogenetic; DREDD; Alcohol use disorder

Introduction

Neuropsychiatric disorders and neurodegenerative diseases often involve maladaptive neuronal and synaptic activity (Gunaydin & Kreitzer, 2016; Volkow & Morales, 2015). The causal relationships between abnormal neuronal activity and behavioral alterations in these diseases remain poorly understood. Experimental approaches that allow for the precise control of neuronal activity are thus emerging as essential tools for the investigation of normal brain function and of disease-associated changes.

In the past, many conventional approaches have been used to excite or suppress neuronal activity in research models and in clinical settings. These include electrical methods such as deep brain stimulation (Perlmutter & Mink, 2006), regional brain lesioning using chemicals such as lidocaine or NMDA (John & Kumar, 1998; Packard & McGaugh, 1996), pharmacological interventions using receptor agonists or antagonists (Wang et al., 2010, 2015), optical uncaging of neurotransmitters such as glutamate (Shoham, O'Connor, Sarkisov, & Wang, 2005), or viral vector-mediated overexpression or downregulation of genes, e.g., adeno-associated virus (AAV)-mediated expression of inwardly rectifying

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potassium channel 2.1 (Cazorla et al., 2014). Unfortunately, most of these traditional methods have disadvantages relating to their lack of selectivity, irreversibility, or low spatiotemporal resolution.

These limitations have been addressed by several recently developed and widely used optogenetic and chemogenetic approaches (Alexander et al., 2009; Boyden, Zhang, Bamberg, Nagel, & Deisseroth, 2005; Deisseroth, 2011; Roth, 2016; Sternson & Roth, 2014; Vardy et al., 2015). These methods employ genetic technology to express specific genes in the targeted neuronal population using either viral vector transduction or animal transgenesis (Alexander et al., 2009; Cheng et al., 2017; Klapoetke et al., 2014; Ma, Barbee, Wang, & Wang, 2017; Ma et al., 2018; Roth, 2016; Sternson & Roth, 2014; Vardy et al., 2015). Optogenetic approaches utilize the expression of engineered opsins, which act as light-sensitive ion channels. Each opsin variant can be stimulated by a specific wavelength of light, allowing selective excitation or inhibition of neuronal activity in real time (Klapoetke et al., 2014; Ma et al., 2017, 2018). Using different optical stimulation protocols, researchers can control neuronal activity over a time scale that ranges from milliseconds up to several days (Kravitz, Tye, & Kreitzer, 2012; Ma et al., 2018, 2017; Nabavi et al., 2014). Although this method has the advantage of providing precise temporospatial control of neuronal activity, its use is limited by being relatively expensive and invasive, and showing distance-dependent, and therefore uneven, light stimulation. These factors have limited its widespread use as a conventional laboratory method. In contrast, because chemogenetic approaches can be used to remotely, and therefore non-invasively, control neuronal activity, these may provide an ideal approach to manipulate specific neurons, glia, or even other cell types in a given brain region.

An overview of the designer receptor exclusively activated by a designer drug (DREADD) method

Chemogenetics, which was previously called pharmacosynthetics or pharmacogenetics (Farrell & Roth, 2013; Sternson & Roth, 2014), refers to the engineering of macromolecules such as kinases or receptors to allow their activation by small drug-like compounds (Roth, 2016; Sternson & Roth, 2014). G protein-coupled receptors are widely used as chemogenetically engineered macromolecules, and a class of these known as DREADDs has been the most widely used chemogenetic platform (Roth, 2016; Urban & Roth, 2015). This review will mainly focus on DREADDs.

DREADDs are engineered G protein-coupled muscarinic receptors that have been modified to respond specifically to a synthetic compound such as clozapine-N-oxide (CNO), while not responding to their endogenous ligand, acetylcholine (Urban & Roth, 2015). The DREADD method has advantages over opto-genetic approaches because it is less invasive, relatively easy to employ, and more affordable. Using a DREADD to remotely control animal behavior only requires two steps: 1) a viral infusion to deliver the DREADD gene *in vivo*, and 2) an intraperitoneal injection of CNO to activate the DREADD, which is expressed in the brain.

CNO is a major metabolite of clozapine, which is an antipsychotic drug that acts as an agonist of serotonin and dopamine receptors (Miyamoto, Miyake, Jarskog, Fleischhacker, & Lieberman, 2012; Seppi et al., 2011). CNO has been shown to be pharmacologically and behaviorally inert in rodents at doses of 0.1–3 mg/kg (Roth, 2016). However, CNO has the potential to be metabolized back to clozapine *in vivo*, especially in guinea pigs and humans (Jann, Lam, & Chang, 1994); clozapine production can correspond to 10% (by mass) of the administered CNO. A recent study by Gomez et al. (2017) provided a series of essential details related to the *in vivo* use of the CNO-DREADD system in rodents. This study identified the primary concerns as: 1) CNO can be back-metabolized to clozapine, 2) clozapine has a higher DREADD binding affinity than CNO, and 3) clozapine, but not CNO, can rapidly enter the brain following systemic administration. Gomez et al. therefore suggested using sub-threshold concentrations of clozapine (instead of CNO), with appropriate controls, in studies using DREADDs. Furthermore, a previous study revealed that a low dose of CNO (3 mg/kg) did not result in any detectable clozapine in the cerebrospinal fluid within 1.5 h after the injection, and a limited peak concentration of 5 ng/mL was observed 2.5 h post-injection (Raper et al., 2017). Therefore, the extent of the problem posed by back-metabolized clozapine may depend on the duration of the observed behavior and on the CNO dose (Mahler & Aston-Jones, 2018). Taken together, the present evidence suggests that it is best to examine DREADD effects within a limited test period (<2 h post-injection), to use a low dose of CNO or a sub-threshold dose of clozapine, and to always include appropriate controls (e.g., administering CNO or clozapine to animals expressing only mCherry or a similarly non-responsive protein) (Roth, 2016).

Other chemical agonists such as compound 21 and perlapine have been developed to avoid the problems associated with CNO back-metabolism (Chen et al., 2015; Roth, 2016). These compounds have very similar structures to that of CNO (Fig. 1A) and have high DREADD-binding affinities, without showing back-metabolism (Roth, 2016). Perlapine, which has been approved for the treatment of insomnia, was shown to have >10,000-fold selectivity for a DREADD, versus the endogenous muscarinic receptors (Chen et al., 2015). Like clozapine, more studies are needed to test the lowest effective *in vivo* dose of perlapine when it is used with DREADDs.

To date, five major variants of DREADDs have been engineered using the following G protein-coupled receptor signaling components: Gq, Gi, Gs, G_{olf}, or β -arrestin (Roth, 2016) (Fig. 1B). Among these variants, Gq- and Gi-DREADDs are the most commonly used.

The human M3 muscarinic receptor (hM3) has been engineered as a Gq-DREADD known as hM3Dq; this is an excitatory DREADD that facilitates an increase in overall cellular activity. The Gq-DREADD only responds to CNO, and its activation triggers Gq-mediated signaling in many different cell types. These include embryonic fibroblast cells *in vitro* and neurons and astrocytes *in vitro* or *in vivo*. In neurons, Gq-DREADD activation mainly inhibits the opening of a series of voltage-gated potassium channels encoded by the KCNQ gene family (K_V7.1 – K_V7.5). This inhibition leads to an increase in the resting membrane potential, thereby increasing neuronal excitability and firing (Alexander et al., 2009; Atasoy, Betley, Su, & Sternson, 2012; Krashes et al., 2014), and in neurotransmitter release (Cheng

et al., 2017). In astrocytes, activation of the Gq-DREADD results in an increase in the intracellular Ca^{2+} level (Aguilhon et al., 2013).

The human M4 muscarinic receptor (hM4) has been engineered as a Gi-DREADD (hM4Di); this is an inhibitory DREADD that facilitates an overall reduction in cellular activity. Gi-DREADD only responds to CNO, and its activation triggers Gi- and $\text{G}\beta/\gamma$ -mediated signaling cascades. These result in the activation of $\text{G}\beta/\gamma$ -mediated inwardly rectifying potassium channels, which suppresses neuronal firing activity and synaptic transmission (Armbruster, Li, Pausch, Herlitze, & Roth, 2007; Cheng et al., 2017; Ferguson et al., 2011; Krashes et al., 2014; Stachniak, Ghosh, & Sternson, 2014). In addition, a different Gi-DREADD has been developed using the k-opioid receptor (KORD-DREADD) (Vardy et al., 2015). The KORD-DREADD responds to salvinorin B, but not to other endogenous activators of this receptor such as dynorphin. The KORD-DREADD has been successfully used in mice to hyperpolarize neurons and silence synaptic activity (Vardy et al., 2015).

The rat M3 muscarinic receptor (rM3) has been engineered as a Gs-DREADD (rM3Ds) (Guettier et al., 2009). Binding of CNO to the Gs-DREADD activates the Gs signaling cascade. This increases the level of the cyclic AMP, which activates protein kinase A (Farrell et al., 2013). However, Gs-DREADD was reported to show some constitutive activity in pancreatic β cells *in vitro* (Guettier et al., 2009), and this limits its use *in vivo*.

The rM3 has also been engineered to produce a β -arrestin-DREADD (rM3Darr) (Nakajima & Wess, 2012). However, this type of DREADD requires a very high concentration of CNO to achieve full activation, and this limits its use *in vivo* (Nakajima & Wess, 2012; Raper et al., 2017; Urban & Roth, 2015).

Investigation of neuronal circuits using DREADDs

To employ chemogenetics in a specific brain region or neuronal population, AAV has often been used to transduce DREADD genes into brain cells (Roth, 2016). DREADD expression can be driven by either Cre or flippase (Flp), which are DNA recombinases. For example, to express a Gi-DREADD (hM4Di) in dopamine D2 receptor-expressing medium spiny neurons (D2-MSNs) in mice, an AAV encoding Cre-dependent hM4Di was injected into the striatum of transgenic mice expressing Cre recombinase driven by the D2 receptor promoter or adenosine 2a receptor promoter (Bock et al., 2013; Cheng et al., 2017). The cell type-specific promoters used to generate these mouse lines ensure that Cre is selectively expressed in D2-MSNs. The viral DREADD construct includes a gene encoding a fluorescent protein (mCherry) to enable confirmation of the expression pattern, and the hM4Di gene is reversed and flanked by two Cre-recognized loxP sites; this arrangement is known as double-floxed inverse orientation (DIO). In wild-type animals, the Cre-driven DREADD AAV can also be infused with an AAV that expresses Cre (AAV-Cre) in order to achieve high local Cre expression. For example, a previous study introduced a 2:1 mixture of AAVDIO-hM4Di-mCherry and AAV-Cre into the nucleus accumbens (NAc) core of wild-type C57BL/6 J mice, and this resulted in high local expression of hM4Di (Cassataro et al., 2014). DREADD expression can also be induced by Flp, which selectively acts at flippase recognition target (FRT) sites (Fenno et al., 2014). For instance, AAV-FLEX^{FRT}-hM4Di

(produced by Stanford Gene and Viral Vector Core) was injected into the globus pallidus externus of transgenic mice expressing Flp driven by the parvalbumin promoter; this achieved selective expression of hM4Di in the parvalbumin neurons in this brain region (Beier et al., 2017). Since Cre and Flp do not have overlapping recognition sites, Flp- and Cre-dependent vectors can be used together, thus providing more research flexibility (Beier et al., 2017). In addition, selective expression of DREADDs can be achieved using cell type-specific promoters. For example, D2-MSNs were targeted in rats by injecting a vector that induced enkephalin promoter-driven expression of hM4Di into the dorsal striatum (Ferguson et al., 2011); this approach took advantage of the fact that enkephalin is exclusively synthesized by striatal D2-MSNs. This specific promoter-driven expression thus provides an excellent alternative strategy when the relevant Cre or Flp transgenic rat lines are not available. However, because the level of promoter-driven DREADD expression is highly dependent on promoter activity, this does not provide an ideal strategy to achieve high levels of expression in wild-type animals. A dual-viral strategy can be used to circumvent this limitation. For example, a Cre-inducible DREADD (AAV-DIO-hM3DqmCherry) was infused into the ventral tegmental area (VTA), which contains the cell bodies of dopaminergic neurons, and the retrograde canine adenovirus-2 expressing Cre was simultaneously infused into the NAc, which contains the dopaminergic fibers (Boender et al., 2014). The canine adenovirus-2 virus is retrogradely transported from the NAc terminals, and thus selectively expresses Cre in the VTA neurons that project to this region. This approach facilitates the selective expression of Cre-inducible hM3Dq at relatively high levels in the VTA-NAc circuit. The disadvantage of this dual-viral technique is that it can be difficult to match the VTA injection site with the area showing NAc-derived retrograde Cre expression. If the area showing retrograde expression does not overlap with the DREADD virus injection site, the hM3Dq expression level can be low. More interestingly, a battery of retrograde AAV-DREADD viruses (e.g., AAVretro-hSyn-DIO-hM4Di/hM3DqmCherry) has been developed by Dr. Bryan Roth and are now available from Addgene. Since the retrograde expression of AAVretro-DIO-DREADD does not depend on the injection location of a separate Cre-expressing virus, this potentially solves the mismatch problem, but it needs to use a Cre mouse line. For example, injection of AAVretro-hSyn-DIO-hM4Di-mCherry into the NAc of DAT-Cre mice can achieve selective expression of hM4Di in the NAc-projecting VTA neurons. However, further investigation is required to determine whether these retrograde DREADD viruses can achieve high expression levels, and the duration of the expression achieved. Another strategy to achieve circuit-specific manipulation is to express a DREADD in the cell body area and apply CNO into the axonal terminal field. For instance, to manipulate glutamatergic activity in the anterior insular cortex → the central amygdala pathway, hM4Di was expressed in the anterior insular cortex and CNO was then micro-infused into the terminal field, which is the central amygdala (Venniro et al., 2017).

Many DREADD transgenic animals have also been developed and are commercially available; these include Cre- or tetracycline-inducible lines (Alexander et al., 2009; Roth, 2016; Zhu et al., 2014, 2016). For example, LSL-hM4Di and LSL-hM3Dq mouse lines have been developed based on a previous Rosa-Ai9 construct (Zhu et al., 2016). In these lines, CAG promoter-driven DREADD expression is blocked by an upstream STOP codon; this is flanked by loxP sites, and expression is therefore activated in the presence of Cre. The

constructs also include labels to facilitate visualization – the HA tag can be detected using antibodies and the mCitrine tag generates green fluorescence. After mating these mice with other lines expressing Cre driven by regional- or cell population-specific promoters, robust specific DREADD expression was observed in double transgenic offspring (Zhu et al., 2016). These conditional DREADD mice displayed behavioral changes after the systemic administration of CNO, including altered fear conditioning and locomotor activity (Zhu et al., 2016), indicating that these transgenic DREADDs were expressed and functioned as expected. However, further electrophysiological verifications are required in these animals. The use of transgenic DREADD reporter mouse lines in conjunction with either viral infusion or Cre/Flp driver lines can provide brain-wide cell-specific expression of DREADDs; this facilitates the investigation of circuit questions in a large scale.

Use of the DREADD method in alcohol use disorder research

According to a recent global status report on alcohol and health published by the World Health Organization, alcohol use disorder (AUD) causes an average of 3.3 million deaths per year; this accounted for 5.9% of all fatalities and imposed a substantial global disease burden (World Health Organization, 2014). In addition, approximately 16% of the drinkers aged 15 years worldwide engage in heavy episodic drinking (World Health Organization, 2014). AUD can be characterized by several behavioral patterns, such as loss of control of alcohol consumption, abnormal motivation to seek alcohol, and the emergence of adverse emotional or physiological effects associated with alcohol withdrawal, including anxiety, hyperalgesia, and social withdrawal (Ron & Barak, 2016).

To date, several DREADDs have been successfully employed in AUD-related research studies (Table 1); these included investigations of binge or excessive alcohol intake (Cassataro et al., 2014; Cheng et al., 2017; den Hartog et al., 2016; Li et al., 2017; Navarro et al., 2016; Pleil et al., 2015), alcohol-seeking behavior (Bull et al., 2014; Cheng et al., 2017; Jaramillo et al., 2018; Pina & Cunningham, 2017; Pina, Young, Ryabinin, & Cunningham, 2015; Prasad & McNally, 2016), alcohol sensitization (Jaramillo et al., 2017), alcohol-induced reinstatement and extinction (Marchant et al., 2016; Prasad & McNally, 2016), as well as alcohol-induced anxiety (Kang et al., 2017), hyperalgesia (Smith, Walcott, Heinricher, & Ryabinin, 2017), and over-eating (Cains, Blomeley, Kollo, Racz, & Burdakov, 2017).

Excessive alcohol consumption can be modeled experimentally using either the drinking in the dark (DID) procedure, which models binge-like drinking, or the intermittent-access to alcohol in a two-bottle choice procedure, which models chronic alcohol drinking (Ron & Barak, 2016). Using the DID paradigm, Cassataro et al. (2014) showed that Gi-DREADD (hM4Di)-mediated NAc inhibition produced a reduction in mouse alcohol consumption, whereas Gq-DREADD (hM3Dq)-mediated NAc activation did not alter this behavior. This study infused a mixture of AAV-Cre and Cre-inducible AAV encoding DREADDs into the NAc, leading to high levels of DREADD expression. The DID procedure takes 2–4 h/session and was therefore thought to occur within the effective duration of DREADD activation, based on earlier reports (Alexander et al., 2009; Ray et al., 2011) indicating that the *in vivo* effects of DREADD activation lasted for 5–10 h. A series of controls were employed to

control for the non-specific effects of the CNO-DREADD system; these were designed to test the impact of hM4Di on the consumption of water, 1% sucrose, and 5% sucrose using the same DID paradigm, as well as testing locomotor effects. However, this first study to use the CNO-DREADD system in the alcohol research field did not conduct any verification of CNO-DREADD function at the cellular level. In a separate study, the role of the bed nucleus of the stria terminalis (BNST) in binge drinking was examined (Pleil et al., 2015). This 2-h DID study in transgenic mice found that inhibition of the BNST using the hM4Di Gi-DREADD decreased alcohol intake, whereas activation of the BNST using the hM3Dq Gq-DREADD increased alcohol intake. In this study, the transgenic mice expressed Cre under the control of the corticotrophin-release factor promoter; Cre then drove DREADD expression. Importantly, this study verified the function of the CNO-DREADD (hM4Di) system using *ex vivo* whole-cell patch-clamp recording. The authors found that bath application of 10- μ M CNO caused hyperpolarization of BNST neurons. Importantly, in their recent paper (Mazzone et al., 2018), they found that Gq-DREADD activation promoted long-term depression (LTD) in the BNST, which highlights additional functional changes associated with the CNO-DREADD system.

We recently employed Cre-inducible Gq (hM3Dq)- and Gi (hM4Di)-DREADDs to test the roles of the direct and indirect pathway neurons of the dorsomedial striatum in excessive chronic alcohol-drinking behavior (Cheng et al., 2017). We infused either AAV-DIO-hM4Di or AAV-DIO-hM3Dq into the dorsomedial striatum of transgenic mice expressing Cre under the control of either the D1 or D2 receptor promoter in order to achieve selective expression of hM4Di or hM3Dq in D1- or D2-MSNs. The mice were trained to consume alcohol using the intermittent-access two-bottle choice procedure. During the test session, we injected a relatively low dose of CNO (3–5 mg/kg) intraperitoneally to activate the DREADDs, and then tracked the effects of this on alcohol drinking over 24 h. We demonstrated that the impact of CNO-mediated DREADD activation declined in a time-dependent manner, and had completely disappeared by 24 h; this indicated that the back-metabolized clozapine may not contribute significantly to the behavior observed. This research also conducted electrophysiological studies, which revealed that both Gq- and Gi-DREADDs could activate or silence (respectively) synaptic transmission in the dorsomedial striatal direct and indirect pathways. This finding demonstrates that DREADDs can be used to dissect the circuitry and mechanisms underlying behaviors related to addiction.

Drug-seeking behavior, as well as motivation and relapse, can be demonstrated more effectively using operant self-administration paradigms. The effect of DREADD-based inhibition on the motivation to seek alcohol was examined in rats using operant self-administration with a progressive ratio schedule (Bull et al., 2014). Expression of a Gq-DREADD (hM3Dq) in astrocytes within the NAc core was driven by the glial fibrillary acidic protein promoter. Administration of CNO (3 mg/kg) activated hM3Dq and reduced the motivation for alcohol seeking. *Ex vivo* calcium imaging was used to validate hM3Dq function in astrocytes because activation of the DREADD increases the intracellular calcium concentration (Aguilhon et al., 2013). Furthermore, Jaramillo et al. (2018) used a Cre-dependent Gi-DREADD (hM4Di) to inhibit two distinct cortical inputs to the NAc core in rats: the medial prefrontal cortex input, and the insular cortex input. Inhibition of the medial pre-frontal cortex-NAc core circuit using hM4Di decreased alcohol-seeking behavior,

whereas inhibition of the insular cortex-NAc core circuit using hM4Di increased alcohol-seeking behavior. Sucrose-seeking behavior was not altered by manipulation of either circuit. More importantly, no behavioral changes were observed in mCherry-expressing controls following the intraperitoneal injection of CNO (3 mg/kg), providing further evidence that administration of a low dose of CNO is less likely to produce a functionally relevant concentration of back-metabolized clozapine *in vivo*. Another fascinating study used a KORD-DREADD and a Gs-DREADD (rM3Ds) to examine the relapse and extinction of alcohol-seeking behavior (Marchant et al., 2016). This investigation found that activation of KORD by salvinorin B inhibited neuronal firing in the ventral subiculum and reduced relapse-associated lever presses, whereas salvinorin B did not produce any behavioral changes in the group infused with the Gs-DREADD.

Anxiety disorders are common during alcohol withdrawal, and these often drive relapse (Sinha, 2001). The lateral habenula has been associated with anxiety disorders (Zhao-Shea et al., 2015). Kang et al. (2017) found that withdrawal from alcohol consumption increased the activity of neurons in this region by reducing the number of M-type K⁺ channels. Activation of these channels in lateral habenula neurons decreased their firing and rescued the anxiety-like behavior during withdrawal. To mimic the inhibitory effect of M-type K⁺ channels, Kang et al. (2017) used the CNO-hM4Di system, which was shown to inhibit the firing of lateral habenula neurons in brain slices. This study demonstrated that DREADD-mediated inhibition of neurons in the lateral habenula *in vivo* attenuated rat anxiety-like behavior during withdrawal from alcohol.

In addition, DREADDs have been used to modulate AUD-related behaviors by altering activity in many other brain regions; these include the orbitofrontal cortex (den Hartog et al., 2016), ventral pallidum (Prasad & McNally, 2016), anterior cingulate cortex (Smith et al., 2017), and lateral hypothalamus (Cains et al., 2017; Navarro et al., 2016). Given that a low dose of CNO (around 3 mg/kg) is enough to alter most of the AUD-related behaviors, and that the durations of several relevant behavioral paradigms were compatible with the time window of CNO activity *in vivo*, DREADDs provide highly effective tools for prospective alcohol research studies.

Conclusions and potential therapeutic application in AUD

Many studies have suggested that a particular brain region, a specific neuronal population, or a unique neuronal circuit controls alcohol-taking or -seeking behavior. As discussed in this review, DREADDs have shown significant potential in the regulation of neuronal or circuit activity *in vivo*. Several interesting reports have demonstrated that DREADDs can be safely expressed in non-human primates, where they successfully modulate circuit activity and behavior (Eldridge et al., 2016; Nagai et al., 2016). In addition, AAV is an FDA-approved gene delivery platform in humans and it has been used in several gene therapies (Pignataro et al., 2018), indicating that it would be possible to deliver DREADDs in humans. Given that CNO may undergo back-metabolism to clozapine in humans, perlapine might be the best candidate agonist because this compound has been approved for use in humans. Importantly, perlapine can activate CNO-based DREADDs in a very similar manner to CNO.

Taken together, CNO-based DREADD systems have many advantages over other *in vivo* techniques used to manipulate neuronal activity because they are more affordable, can produce a range of effects with a relatively high temporospatial resolution, and are non-invasive. Although multiple versions of DREADDs are available, those acting via Gq (hM3Dq) and Gi (hM4Di) remain the most commonly used in the alcohol research field. The unique features of DREADDs will ensure their continued use in alcohol research and provide a promising therapeutic potential for the clinical treatment of AUD.

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Fig. 1.

Available DREADDs and chemical agonists. (A) The chemical formula of CNO, CNO-like agonists for CNO-based DREADDs, and Salvinator B (SalB) for KORD. (B) The diagram shows all available DREADDs and their downstream signaling and consequent neuronal effects. RMP: resting membrane potential.

Table 1

Using DREADDs in alcohol research.

Alcohol-related Behavior	Animals	Regions	DREADDs	CNO (mg/kg)	Validation	Reference
Drinking behavior	Mice	NAc core and shell	FLEX-hM4Di	1, i.p.		Cassaturo et al. (2014)
			FLEX-hM3Dq			
Seeking motivation	Rats	NAc astrocyte	GFAP-hM3Dq	3, i.p.	<i>In vitro</i> Ca ²⁺ imaging	Bull et al. (2014)
Drinking behavior	Mice	BNST	DIO-hM4D(Gi)	3, i.p.	mIPSCs	Pleil et al. (2015)
			DIO-m4D(Gs)			
CPP	Mice	BNST	hM4d	10, i.p.		Pina et al. (2015)
Drinking behavior	Mice	LH	DIO-hM3Dq	3–5, i.p.		Navarro et al. (2016)
			DIO-hM4Di			
Drinking behavior	Mice	OFC	hM4d	10, i.p.	Spike firing	den Hartog et al. (2016)
Relapse and extinction	Rats	VP→STN	hM3Dq, hM4Di	3 or 20, i.p.		Prasad and McNally (2016)
		VP→VTA				
Relapse and extinction	Rats	vSub→NAc	DIO-KORD	15, s.c.	Spike firing	Marchant et al. (2016)
			DIO-m3D(Gs)			
Sensitization	Rats	IC→Acbe	DIO-hM4Di	1, i.p.	Spike firing	Jaramillo et al. (2017)
Withdrawal-induced anxiety	Rats	LHb	hM4Di	5, i.p.	Spontaneous spiking	Kang et al. (2017)
			hM3Dq			
CPP	Mice	BNST→VTA	DIO-hM4Di	10, i.p.		Pina and Cunningham (2017)
Drinking behavior	Mice	BNST→VTA	DIO-hM4d	3, i.p.		Rinker et al. (2017)
Drinking behavior	Mice	DMS	DIO-hM3Dq	3–5, i.p.	eIPSCs	Cheng et al. (2017)
			DIO-hM4Di			
Drinking behavior	Rats	LHb	hM4Di	10, i.p.		Li et al. (2017)
Withdrawal-hyperalgesia	Mice	ACC	hM3Dq	1, i.p.		Smith et al. (2017)
			hM4Di			
Alcohol-induced overeating	Mice	hypothalamus	DIO-hM4Di	5, i.p.	Membrane potential	Cains et al. (2017)
Seeking behavior	Rats	mPFC→NAc	DIO-hM4Di	3, i.p.		Jaramillo et al. (2018)
		IC→NAc				

CPP: conditioned place preference; BNST: bed nucleus of the stria terminalis; LH: lateral hypothalamus; OFC: orbitofrontal cortex; VP: ventral pallidum; STN: subthalamic nucleus; vSub: ventral subiculum; IC: insular cortex; AcbC: nucleus accumbens core; LHb: lateral habenula; DMS: dorsomedial striatum; ACC: anterior cingulate cortex; mIPSCs: miniature inhibitory postsynaptic currents; eIPSCs: evoked inhibitory postsynaptic currents; i.p.: intraperitoneal; s.c.: subcutaneous injection.