# DREADDs (Designer Receptors Exclusively Activated by Designer Drugs): Chemogenetic Tools with Therapeutic Utility

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## Keywords

synthetic biology, G protein–coupled receptors, GPCRs, systems biology, drug discovery

## Abstract

In the past decade, emerging synthetic biology technologies such as chemogenetics have dramatically transformed how pharmacologists and systems biologists deconstruct the involvement of G protein–coupled receptors (GPCRs) in a myriad of physiological and translational settings. Here we highlight a specific chemogenetic application that extends the utility of the concept of RASSLs (receptors activated solely by synthetic ligands): We have dubbed it DREADDs (designer receptors exclusively activated by designer drugs). As we show in this review, DREADDs are now used ubiquitously to modulate GPCR activity noninvasively in vivo. Results from these studies have directly implicated GPCR signaling in a large number of therapeutically relevant contexts. We also highlight recent applications of DREADD technology that have illuminated GPCR signaling processes that control pathways relevant to the treatment of eating disorders, obesity, and obesity-associated metabolic abnormalities. Additionally, we provide an overview of the potential utility of chemogenetic technologies for transformative therapeutics.

## INTRODUCTION

Chemogenetics is a term that was coined to describe the observed effects of mutations on enzyme substrate specificities (1). It is now used to describe the process by which macromolecules can be engineered to interact with previously unrecognized small molecules. Such engineered macromolecules include nucleic acid hybrids (2), kinases (3, 4), a variety of metabolic enzymes (5–7), and G protein–coupled receptors (GPCRs) (8–10). In the field of GPCR research, numerous chemogenetic platforms have been described (for a review, see 11), including allele-specific activation of genetically encoded receptors (8), receptors activated solely by synthetic ligands (RASSLs) (9), engineered receptors (10), neoceptors (12), and designer receptors exclusively activated by designer drugs (DREADDs) (13, 14). Of these chemogenetic GPCR platforms, DREADDs—for example, those that are selectively activated by the inactive clozapine analog clozapine-N-oxide (CNO)—have emerged as the most widely adopted technology, and they are discussed here.

## DREADD TECHNOLOGY

### Gq DREADDs

To date, we have seen the invention of CNO-activated DREADDs that allow for the selective interrogation of various GPCR signaling cascades, including those activated by Gq, Gi, Gs, Golf, and β-arrestin. In this review, each class of DREADDs is described, starting with the Gq DREADD. As previously detailed elsewhere (14), the original Gq DREADD (termed hM3Dq; see below) was created by directed molecular evolution of the human M3 muscarinic receptor (hM3) in yeast (13). In these studies, the hM3 was subjected to multiple cycles of random mutagenesis in yeast expressing a chimeric Gq protein, and mutants were selected for CNO-mediated agonist activity. In this particular example, CNO-induced activation of mutant hM3 receptors elicited growth on selective media. Key features of this yeast-based screen were the identification and subsequent discarding of mutant receptors that displayed constitutive activity. We sought to avoid constitutively active mutants because our goal was to discover engineered receptors that would be relatively silent when expressed in vitro and in vivo (see discussion in 14, 15). Surprisingly, only two point mutations of hM3—Y3.33C and A5.46G—were required to achieve a mutant hM3 with (*a*) nanomolar potency for CNO, (*b*) insensitivity to the endogenous muscarinic acetylcholine receptor (mAchR) ligand acetylcholine, and (*c*) low levels of constitutive activity. This hM3 receptor with the Y3.33C and A5.46G mutations was dubbed hM3Dq (human M3 muscarinic DREADD receptor coupled to Gq). Because Y3.33 and A5.46 are conserved residues, M1 and M5 Gq DREADDs [e.g., hM1Dq and hM5Dq (16, 17)] were also created and validated (13). hM3Dq has been the most frequently used Gq DREADD (**Table 1**).

This hM3Dq DREADD—when activated by CNO—couples to Gq-mediated signaling in many contexts, including transiently transfected HEK cells (14) in vitro, stably transfected mouse embryonic fibroblasts and other cells in vitro (11, 14, 18), neurons in vitro and in vivo (19), astrocytes in vivo (20), hepatocytes in vitro and in vivo (21), and pancreatic β-cells in vitro and in vivo (18, 22). hM3Dq also shows robust coupling to *Drosophila* Gq-like G proteins (23).

Studies by independent groups have documented that the pattern of signaling induced by the CNO-hM3Dq chemogenetic platform faithfully mimics that of native M3 muscarinic receptors activated by acetylcholine (24, 25). Importantly, overexpression of hM3Dq in a variety of in vivo contexts including neurons (19), astrocytes (20), hepatocytes (21), and pancreatic β-cells (18, 22) does not enhance basal activity. Thus, the apparent absence of constitutive activity initially observed in vitro is recapitulated in many in vivo contexts. These results indicate that even when

**Table 1 Representative uses of Gq DREADD**

| Tissue | Effect | Pharmacologic Relevance | Reference |
|---|---|---|---|
| Mrgprb4-expressing neurons | Recapitulates reinforcing activity of light touch | Identifies neuronal population and signaling pathway required for light touch | 93 |
| Hepatocytes | Enhances metabolic activity | Identifies Gq signaling as essential for metabolic control in hepatocytes | 21 |
| Indirect pathway neurons of nucleus accumbens | Enhances cocaine's reinforcing activity | Identifies direction for control of neurons that mediate the addictive properties of cocaine | 94 |
| Suprachiasmatic neurons | Modulates circadian rhythms | Potential neurons and signaling pathway to target for circadian disorders | 83 |
| Pancreatic β-cells | Chronic activation enhances β-cell function | Identifies signaling pathway of potential utility of metabolic disorders | 22 |
| AgRP-expressing neurons | Activation promotes feeding | Identifies signaling pathways for modulation of feeding and potential targets for the treatment of obesity | 27 |
| Various *Drosophila* neurons | Wide range of effects | Validates use of DREADD technology for *Drosophila* and *Drosophila* genetics | 23 |
| Ventral tegmental dopamine neurons | Hyperactivity | Identifies signaling events that can modulate dopaminergic tone | 95 |
| Nucleus accumbens | No effect | Gq signaling does not acutely modify alcohol intake in these neurons | 96 |
| Astrocytes | Large number of physiological actions | Identifies Gq signaling as essential for astrocyte actions for many physiological processes | 20 |
| GABAergic neurons | Regulates sleep-wake cycles | Identifies signaling events essential for regulating sleep-wake cycle | 78 |
| Conjugation of CNO to oligonucleotides | Performs cell type–specific conjugation of oligonucleotides | Novel approach for modulating protein expression using DREADDs | 97 |
| Hypothalamic neurons | Identifies neurons that regulate hunger | Identifies signaling pathways for modulation of feeding and possible ways to treat obesity | 29 |
| Hippocampal neurons | Generates hybrid memories de novo | Identifies Gq signaling as essential for memory | 98 |
| Transfected eukaryotic cells | Modulates allosteric activity | Uses DREADD to validate allosteric model | 17 |
| GABAergic neurons in hypothalamus | Modulates feeding | Identifies Gq signaling in GABAergic neurons as involved in feeding; potential utility in eating disorders and obesity | 28 |
| AgRP-expressing neurons | Induces feeding | Identifies signaling pathways for modulation of feeding and potential treatments of obesity | 26 |
| Pyramidal neurons | Enhances neuronal activity; causes seizures, hyperactivity | Validates Gq DREADD transgenic approach | 19 |
| Orexin neurons | Alters orexin neuron activity | Modulates sleep-wake cycle | 46 |
| Pancreatic β-cells | Enhances insulin release | Validates Gq DREADD in vivo | 18 |
| Transfected cells | Evaluates multiple signaling cascades | Validates Gq DREADD | 25 |
| Transfected cells | Evaluates dimerization | DREADDs do not form functional homo- or heterodimers | 24 |
| Transfected cells | Invention of DREADD technology and validation of Gq signaling | Validates DREADDs in vitro | 14 |

Abbreviations: AgRP, Agouti-related peptide; CNO, clozapine-N-oxide; DREADD, designer receptor exclusively activated by designer drugs.

hM3Dq is massively overexpressed throughout the lifetime of a mouse, no apparent adverse effect can be detected in the contexts mentioned above.

The outcomes achieved by CNO-induced hM3Dq activation are cell type specific (**Table 1**). For example, this activation in neurons depolarizes them and enhances their excitability (19, 26), which can lead to burst-like firing (19, 26–29). Therefore, not surprisingly, hM3Dq is most frequently used as a tool to enhance neuronal firing (**Table 1**). In astrocytes, an increase in $Ca^{2+}$ release and attendant physiological alterations of the autonomic nervous system have been described (20). In pancreatic β-cells, CNO-induced hM3Dq activation induces insulin release acutely, whereas chronic CNO administration causes an increase in β-cell number (18, 22). In hepatocytes, activation of hM3Dq increases blood glucose levels, perhaps owing to increased glycogen breakdown and gluconeogenesis (21).

Because hM3Dq is apparently subject to the same sorts of canonical regulatory processes as are other GPCRs (e.g., phosphorylation, desensitization, internalization, downregulation) (25), prolonged activation with CNO could lead to an attenuated response owing to desensitization and/or downregulation of hM3Dq. To date, however, no significant attenuation of responses has been reported following chronic CNO administration. For instance, we initially demonstrated that consecutive daily doses of CNO had nearly identical effects on potentiation of locomotor responses and γ-rhythms in intact, freely moving mice in which hM3Dq was overexpressed in hippocampal and pyramidal neurons (19). Similarly, repeated doses of CNO are apparently able to maintain an effective activation of hM3Dq in hypothalamic neurons, as measured by feeding responses in vivo (26, 27) and in pancreatic β-cell responses and as indicated by various metabolic parameters (22). The sustained activity of hM3Dq may be due to the large degree of receptor reserve, which is secondary to high levels of ectopic receptor expression.

## Gi DREADDs

As Y3.33 and A5.46 are conserved among all known mAchRs ranging from *Drosophila melanogaster* to *Homo sapiens* (30, 31), we were able to create a Gi-preferring DREADD by creating the same point mutations in both the M2 and M4 mAchRs (e.g., Y3.33C and A5.46G) (14). The mutant receptors were dubbed hM2Di and hM4Di and correspond to the human muscarinic M2 and M4 Gi-coupled DREADDs, respectively. hM2Di and hM4Di activate Gi-mediated signaling in a variety of cellular contexts (14, 32).

Because muscarinic Gi-coupled GPCRs (e.g., M2 and M4) can activate G protein inwardly rectifying potassium channels (GIRKs) (33, 34), we wondered if hM4Di could similarly activate GIRKs, leading to attenuation of neuronal firing (e.g., silencing). We observed a robust hyperpolarization when hM4Di was coexpressed with GIRK1/2 in HEK-293 cells as a result of GIRK activation, presumably as a consequence of liberation of β/γ subunits (14). In hippocampal pyramidal neurons that overexpressed hM4Di, its activation by CNO not only induced hyperpolarization but also silenced spontaneous and depolarization-evoked firing (14). These results suggested to us that hM4Di might be useful for in vitro and in vivo studies in neurons in which the silencing/attenuation of neuronal firing could be used to deconstruct neural circuitry (14).

The first studies to demonstrate the utility of hM4Di-mediated silencing came from experiments conducted in direct- and indirect-pathway basal ganglia neurons in vivo (35). These studies illuminated a previously unappreciated distinction between these two genetically distinct neuronal populations with regard to behaviors linked to drug addiction and reinforcement (35). Simultaneously, Krashes and colleagues (26) demonstrated that hM4Di-mediated silencing of Agouti-related peptide (AgRP)-expressing neurons in the arcuate nucleus of the hypothalamus (ARN) inhibited feeding. A large number of subsequent studies have documented the efficiency

of hM4Di in silencing neurons noninvasively in vivo (**Table 2**). Not surprisingly, hM4Di is now widely used in both in vitro and in vivo studies when physiologically relevant, noninvasive neuronal silencing is required for the identification of neuronal circuits involved in a particular behavior or neurophysiological response. hM4Di has also been demonstrated to attenuate neuronal firing in *Drosophila* (23).

In addition to hM4Di's well-documented ability to attenuate neuronal signaling, activation of hM4Di also leads to other downstream signaling events, including the activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway (14). This ability of hM4Di to couple to other signaling pathways has also been used to identify signaling pathways involved in carcinogenesis and metastasis (36). Finally, in tantalizing preliminary studies, Sternson's group (37) has reported that hM4Di may also silence neuronal activity via inhibition of neurotransmitter release (e.g., synaptic silencing)—an application that may find widespread use in neuropharmacology for circuitry-based neuroscience investigations.

### Gs DREADDs and β-Arrestin DREADDs

To expand the DREADD toolbox to include Gs/Golf-mediated signaling, a chimeric hM3Dq β-adrenergic receptor DREADD was created and named Gs DREADD (11, 18) (**Table 3**). Gs DREADD couples robustly to both Gs (11, 18) and Golf G proteins (38). Activation of Gs DREADD in striatal neurons led to augmented phosphorylation of DARPP-32 (dopamine- and cyclic AMP–regulated phosphoprotein of 32,000 molecular weight)—a canonical downstream effector of enhanced Golf signaling in striatum (38). Unlike hM3Dq and hM4Di, Gs DREADD has a modest degree of constitutive activity, which can lead to basal phenotypes in some (18) but not all (38, 39) cellular contexts. Gs DREADD also has been overexpressed in *Drosophila* and found to recapitulate Gs signaling when activated by CNO (23, 40).

A β-arrestin-preferring DREADD has also been created (41) by mutation of an amino acid required for G protein signaling in hM3Dq. Although this β-arrestin-preferring DREADD activates β-arrestin pathways in vitro, it requires high CNO concentrations to achieve full activation, which could limit its utility in vivo (41).

## TRANSLATIONAL APPLICATIONS OF DREADD TECHNOLOGY

### Viral Approaches to Achieve Cell Type–Specific DREADD Expression

Several viral approaches that yield cell type–specific DREADD expression have been created, and each is discussed in turn below. Many studies used the flip-excision (FLEX)-switch approach, which was originated by Schnütgen (42) and modified for use with adeno-associated virus (AAV) delivery systems by Sternson's lab (43) and others (44). This FLEX-switch technique utilizes two pairs of heterotypic, antiparallel loxP-type recombination sites, which undergo an inversion of the coding sequence followed by the excision of two sites. As a result, one of each orthogonal recombination site is oppositely oriented and incapable of further recombination (43). Injecting FLEX-switch viruses [also known as double-inverted open reading frame (DIO) viral vectors] that encode DREADDs in mice with cell type–specific Cre recombinase can give cell type–specific expression (**Figure 1*a***). Thus, for the studies outlined in **Figure 1*a***, AgRP-Cre mice were used; these are mice genetically engineered to express Cre recombinase under the control of an AgRP promoter. Mice can then be microinjected in the ARN with FLEX-AAV that expresses channelrhodopsin-2 (ChR2) (29, 45), hM3Dq (26, 27, 29), or hM4Di (26). After recombination in situ, ChR2, hM3Dq, or hM4Di is expressed only in AgRP-expressing neurons (**Figure 1*b*,*c***). Following stimulation by

**Table 2 Representative uses of Gi DREADD**

| Tissue | Effect | Pharmacologic Relevance | Reference |
|---|---|---|---|
| Thalamocortical afferent neurons | Impairs cognition | Potential model of cognitive impairment in schizophrenia | 99 |
| Arcuate nucleus neurons and nucleus tractus solitarius neurons | Inhibits feeding | Identifies neurons involved in regulating feeding | 52 |
| Amygdala neurons | Inhibits conditioned fear | Identifies neurons involved in anxiety and fear responses | 100 |
| Suprachiasmatic neurons | Modulates circadian rhythms | Identifies potential neurons to target for circadian disorders | 83 |
| Cortical parvalbumin interneurons | Identifies circuitry involved in visual discrimination | Identifies neurons to target for certain visual disorders | 85 |
| Neurons projecting to the central nucleus of the amygdala | Inhibits feeding | Identifies neurons and signaling pathway to target for eating disorders and obesity | 49 |
| Orbitofrontal cortical neurons | Alters goal-directed behavior | Identifies neurons and signaling pathway for regulating motivation | 101 |
| Various *Drosophila* neurons | Wide range of effects | Validates use of DREADD technology for *Drosophila* and *Drosophila* genetics | 23 |
| Transfected eukaryotic cells | Causes probe-specific allosteric actions | Validates DREADD technology and potential utility for probe dependency of allosterism | 16 |
| Striatal direct- and indirect-pathway neurons | Validates combined micro-PET and DREADD technology | Validates use of micro-PET imaging to identify circuits involved in behavior | 47 |
| Prodynorphin-expressing neurons | Recapitulates addiction responses | Identifies potential signaling pathways to target for addiction therapy | 48 |
| GABAergic neurons in the basal forebrain | Impairs smell-based sensory discrimination | Identifies signaling process that can modulate discrimination of smell sensation | 102 |
| Nucleus accumbens neurons | Inhibits alcohol intake | Identifies signaling processes and neurons for alcohol self-administration | 96 |
| Circuits connecting amygdala and hypothalamus | Identifies neurons involved in different types of fear | Identifies signaling and neurons to be modulated to affect various types of fearful states | 103 |
| Hypothalamic neurons | Identifies neurons that regulate hunger | Identifies signaling pathways for modulation of feeding, for use in treatment of obesity | 29 |
| Direct- and indirect-pathway neurons | Modulates striatal neuron plasticity | Gi signaling essential for regulating neuronal plasticity | 86 |
| Egr2-expressing neurons | Regulates respiration | Identifies neurons and signaling essential for respiratory control | 50 |
| 5-HT neurons | Regulates respiration and temperature | Identifies neurons and signaling essential for respiratory and thermal control | 51 |
| Orexin neurons | Alters orexin neuron activity | Modulates sleep-wake cycle | 46 |
| Triple-negative breast cancer cells | Modulates metastatic potential | Synthetic biology approach to deconstruct cancer metastasis | 36 |
| Direct- and indirect-pathway neurons | Alters amphetamine sensitization | Validates Gi DREADD in vivo | 104 |
| Transfected cells in culture | Validates DREADD | Validates Gi DREADD in vitro | 32 |
| Transfected cells | Invention of DREADD technology and validation of Gi activity | Validates DREADDs in vitro | 14 |

Abbreviations: 5-HT, 5-hydroxytryptamine; DREADD, designer receptor exclusively activated by designer drugs; PET, positron emission tomography.

**Table 3 Representative uses of Gs DREADD**

| Cell type | Effect | Pharmacologic significance | Reference |
|---|---|---|---|
| Direct-pathway neurons | Promotes reinforcement | Identifies signaling potentially involved in addiction | 39 |
| Suprachiasmatic neurons | Modulates circadian rhythms | Identifies potential neurons and signaling pathway to target for circadian disorders | 83 |
| Various *Drosophila* neurons | Wide range of effects | Validates use of DREADD technology for *Drosophila* and *Drosophila* genetics | 23 |
| *Drosophila* heart | No effect | cAMP not involved in regulating heart rate in *Drosophila* | 40 |
| A2A-expressing adenosine neurons | Inhibits amphetamine sensitization | Identifies neuronal population and signaling that could be important in addiction; validates transgenic approaches for Gs DREADD in neurons | 38 |
| Pancreatic β-cells | Enhances insulin release | Validates Gs DREADD in vivo | 18 |

Abbreviations: cAMP, cyclic adenosine monophosphate; DREADD, designer receptor exclusively activated by designer drugs.

light (ChR2) or CNO (hM3Dq), robust feeding can be induced (26, 27, 29). By contrast, inhibiting AgRP-expressing neuron firing by CNO administration to hM4Di-expressing mice inhibits feeding (26) (**Figure 1*c***).

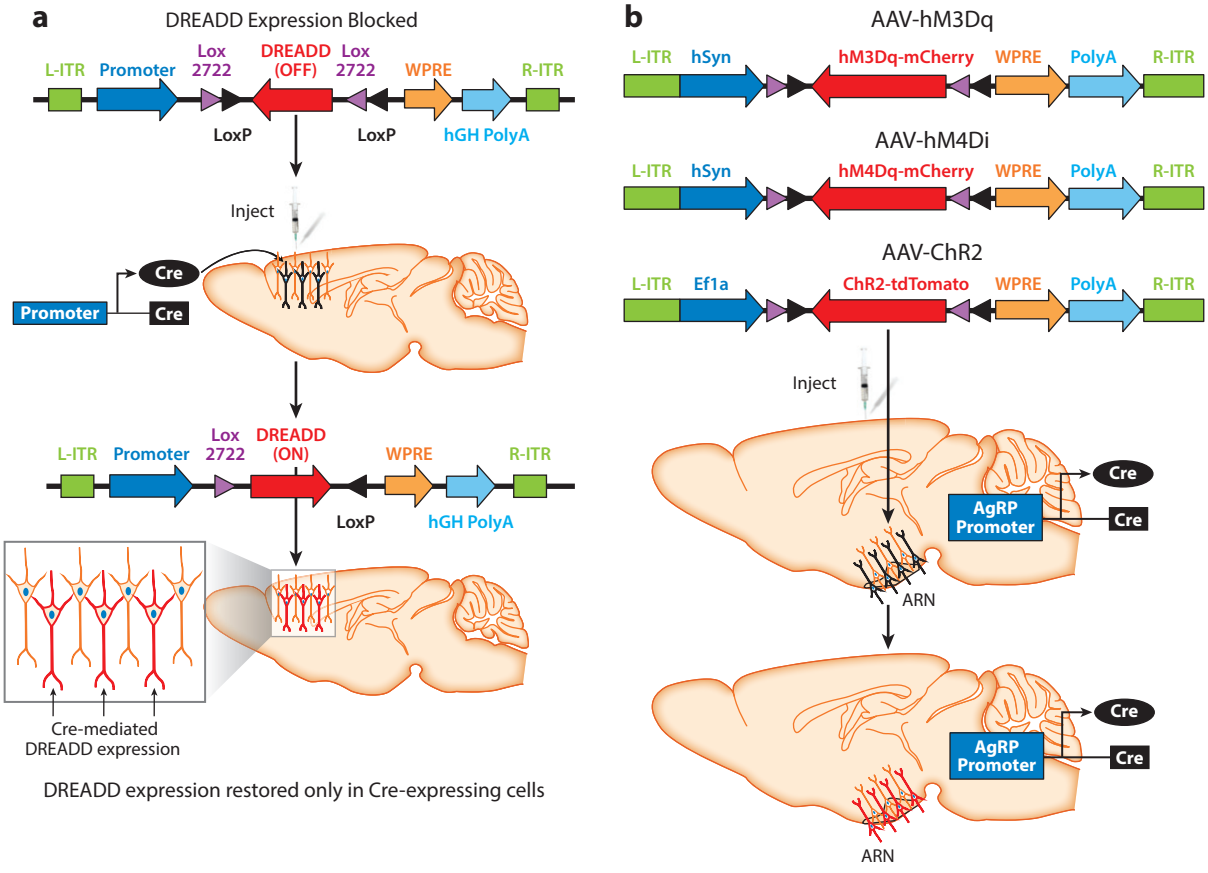
Other viral approaches to achieve cell type–specific DREADD expression include the use of cell type–specific promoters in both AAVs (46) and modified herpes simplex viruses (35, 39, 47, 48) (**Figure 2*a***). An additional viral approach likely to gain widespread application combines the use of retrogradely transported canine adenovirus (CAV) cargoes (e.g., CAV-Cre) with a FLEX-based AAV (e.g., AAV-FLEX-hM3Dq; see **Figure 2*b***) (49). CAV-Cre can be injected into areas of axonal projections, and CAV-Cre particles are then retrogradely transported to cell bodies that have been infected with a FLEX-DREADD construct. Following Cre-mediated recombination, neurons that project only to a specific area can be either activated (e.g., by hM3Dq) or inhibited (e.g., by hM4Di). The retrograde labeling approach is an exciting emerging technology that will allow for precise control over neurons that project to specific brain regions (49).

## Transgenic Approaches for Cell Type–Specific DREADD Expression

Several transgenic approaches have been developed to achieve cell type–specific control of DREADDs. These include creating transgenic mice using cell type–specific promoters. For instance, constitutive astrocyte-specific expression has been achieved using a glia-specific modified glial-fibrillary acidic protein (GFAP) promoter (20). Pancreatic-β-cell-specific expression and hepatocyte-specific expression of hM3Dq were accomplished using a rat insulin promoter (18) and a mouse albumin promoter (21), respectively. Finally, hM3Dq expression in A2A adenosine receptor (A2AR)-expressing neurons in mouse striatum was achieved using an A2A-bacterial artificial chromosome (A2A-BAC) (38). Other transgenic approaches include the use of tetracycline-regulated expression systems (19) and the use of intersectional genetic approaches (50, 51).

## Deconstructing Feeding Circuits and the Pharmacologic Regulation of Feeding

As is clear from **Tables 1–3**, DREADD technology over the past few years has had a major impact on our understanding of the neural circuitry's regulation of many behaviors. Here we highlight recent studies that interrogate feeding behavior, and we show how the results from these studies


a
DREADD Expression Blocked
L-ITR
Promoter
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DREADD (OFF)
Lox 2722
WPRE
R-ITR
LoxP
LoxP
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Inject
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Promoter
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DREADD (ON)
L-ITR
Promoter
WPRE
R-ITR
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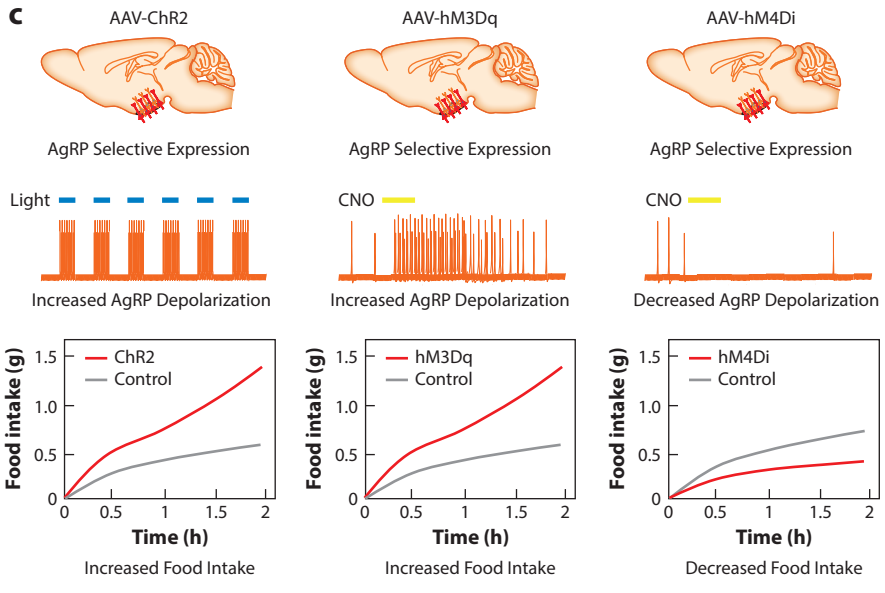
c
AAV-ChR2
AAV-hM3Dq
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AgRP Selective Expression
AgRP Selective Expression
AgRP Selective Expression
Light
CNO
CNO
Increased AgRP Depolarization
Increased AgRP Depolarization
Decreased AgRP Depolarization
Food intake (g)
ChR2
Control
hM3Dq
Control
hM4Di
Control
Time (h)
Increased Food Intake
Increased Food Intake
Decreased Food Intake

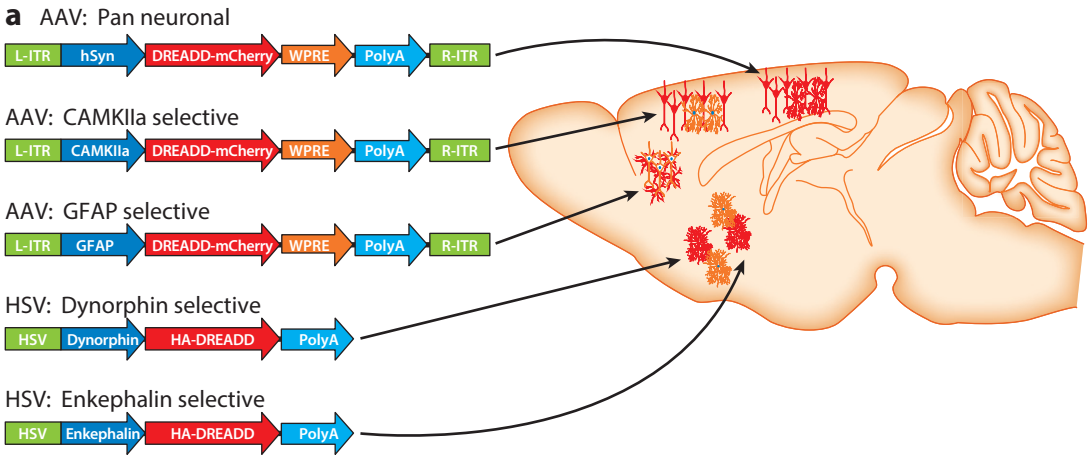
could accelerate the discovery of novel medications for eating disorders, obesity, and obesity-related metabolic disorders. Several studies have demonstrated that activating AgRP-expressing neurons in the ARN induces feeding behavior acutely using optogenetic ChR2 (29, 45) and chemogenetic hM3Dq (26, 27, 29) approaches (**Figure 1**). As AgRP-expressing neurons are GABAergic, the net effect of activating them is to inhibit the activity of neurons innervated by AgRP-expressing neurons. Because AgRP-expressing neurons release GABA, neuropeptide Y (NPY), and AgRP, it has been unclear which of these neurotransmitters are essential for modulating feeding by AgRP-expressing neurons. Krashes and colleagues (27) recently found that release of both NPY and GABA is responsible for the rapid effects of the activation of AgRP-expressing neurons on feeding, whereas the prolonged actions of the activation of AgRP-expressing neurons are mediated by AgRP acting on MC4 melanocortin GPCRs (**Figure 3*a***). These studies are important because they reveal a previously unappreciated complexity with regard to the temporal texture responsible for neuron subtype–specific control of feeding. In a related paper (29), Sternson's lab found that blockade of NPY1-NPY and GABA-A GABA ionotropic receptors blocked the acute feeding behavior response induced by the activation of AgRP-expressing neurons by hM3Dq.

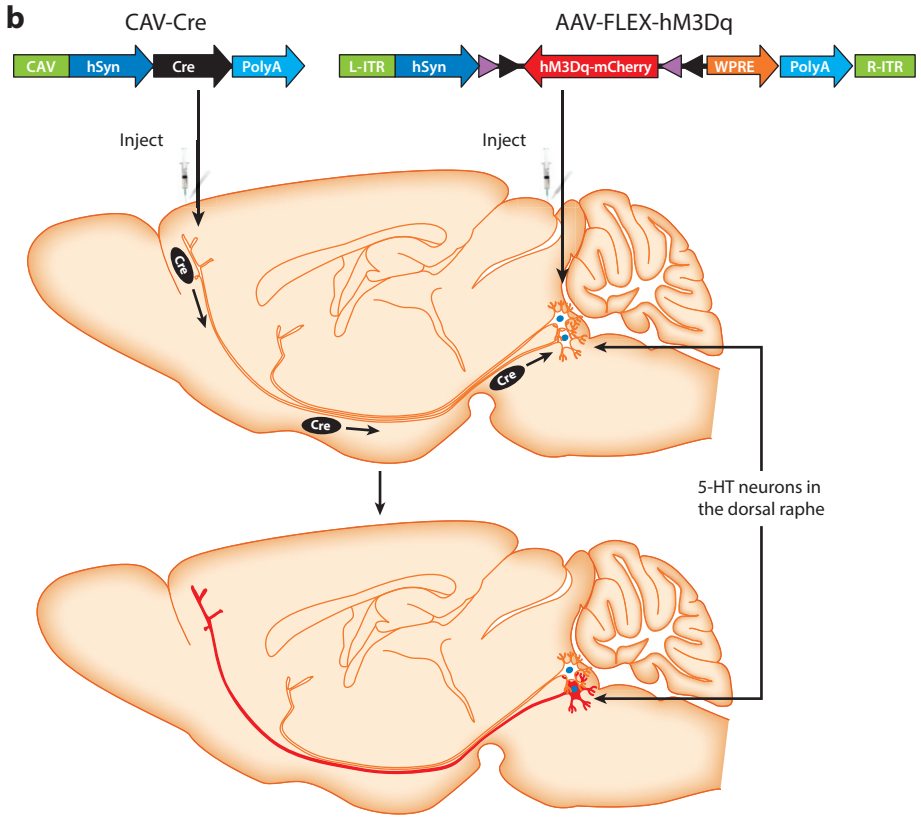
A more recent series of studies by Kong et al. (28) targeted a distinct group of GABA-expressing neurons in the ARN referred to as rat insulin promoter (RIP)-expressing neurons [which are genetically distinct from both AgRP-expressing and pro-opiomelanocortin (POMC)-expressing neurons]. For these studies, RIP-Cre mice were used and interrogated with both chemogenetic and optogenetic approaches (28). The authors found that activating these RIP/Cre-expressing neurons with hM3Dq had no effect on feeding behavior, although robust alterations in energy expenditure were induced (28). These hM3Dq-mediated effects on energy expenditure were abolished when GABA synthesis was blocked, thereby implicating GABA as an energy-expenditure regulator that does not alter feeding behavior. The authors also demonstrated that these effects were mediated by direct projections to the paraventricular hypothalamus (**Figure 3*a***).

A third group, Carter et al. (49), has used DREADD technology to identify the role of parabrachial nucleus (PBN)-expressing neurons (**Figure 3*a***) in the regulation of feeding. The authors found that hM4Di-mediated inhibition of PBN activity promoted feeding by suppressing an apparently aversive circuit mediated by connections with the central nucleus of the amydgala (CeA). An independent group reported that activation of POMC-expressing neurons by hM3Dq inhibits feeding (52) (**Figure 3*a***). Taken together, these results provide information on a rich and

**Figure 1**

Cell type–specific control using FLEX-switch DREADD viruses. (*a*) Schematic of a FLEX-switch DREADD virus in which the DREADD is in an inverse orientation until the virus infects neurons that express Cre recombinase (*black*). The FLEX-switch viral construct contains L-ITRs and R-ITRs, a promoter (usually hSyn), the DREADD gene in an inverse orientation flanked by two repeating lox sites (Lox2722 and LoxP), a WPRE, and hGH PolyA. To obtain cell type–specific expression, the FLEX-switch virus is microinjected into mice that express the Cre recombinase gene under the control of a cell type–specific promoter. The Cre recombinase cuts both lox sites, thus correcting the orientation of the DREADD for proper expression and allowing for the selective expression of the DREADD receptor only in Cre-expressing neurons (*red*). (*b*) Selective expression of hM3Dq, hM4Di, or ChR2 in AgRP-containing neurons was achieved by microinjecting the FLEX-switch virus into the ARN in AgRP-Cre mice. These mice express Cre recombinase only in AgRP-containing neurons (*black*) and thus, after infection, selectively express hM3Dq, hM4Di, or ChR2 only in those cells (*red*). (*c*) Demonstration of cell type–specific control of AgRP-containing neurons by increasing (ChR2 and hM3Dq) or decreasing (hM4Di) AgRP-specific depolarization after light- or CNO-induced stimulation, resulting in a robust increase (ChR2 and hM3Dq) or decrease (hM4Di) of food intake by these mice. Abbreviations: AAV, adeno-associated virus; AgRP, Agouti-related peptide; ARN, arcuate nucleus of the hypothalamus; ChR2, channelrhodopsin-2; CNO, clozapine-N-oxide; DREADD, designer receptor exclusively activated by designer drugs; FLEX, flip-excision; hGH PolyA, human growth hormone poly A tail; hM3Dq, human M3 muscarinic DREADD receptor coupled to Gq; hSyn, human synapsin; L-ITR, left inverted terminal repeat; R-ITR, right inverted terminal repeat; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element. Figure based on data from References 26 and 45.




DREADD expression induced only in 5-HT neurons projecting to the prefrontal cortex

complex circuitry that regulates feeding behavior and energy balance, thereby providing many potential sites for pharmacological interrogation (**Figure 3*a***). Importantly, these circuits were all deconstructed using FLEX-switch-based DREADD technology in combination with cell type–specific Cre-driver lines (**Figure 1**).

Can this information obtained by deconstructing feeding and metabolic circuits through the artificial activation of Gq or Gi signaling be used to explain the actions of anorectic agents as well as to design more effective antiobesity drugs? The answer is yes, and the data specifically implicate serotonergic neurotransmission as a key factor that regulates feeding. For many decades, selective 5-hydroxytryptamine$_{2C}$ (5-HT$_{2C}$) serotonin receptor agonists such as MK-212 as well as drugs such as fenfluramine have been known to effectively suppress feeding behavior in animals (53–56). Fenfluramine has demonstrable antiobesity actions in humans—particularly when combined with the amphetamine derivative phentermine (57–59). Although the effects of fenfluramine have been attributed in the past to its serotonin-releasing activity (55), it is now appreciated that fenfluramine is a prodrug and that its product norfenfluramine is the 5-HT$_{2C}$ agonist responsible for its anorectic activity (58, 60). Unfortunately, the 5-HT$_{2B}$ agonist actions of norfenfluramine were responsible for life-threating valvular heart disease (61–65) and resulted in the worldwide withdrawal of fenfluramine. These observations, however, implied that selective 5-HT$_{2C}$ agonists might be safe and effective antiobesity agents and led to the successful development of lorcaserin as a 5-HT$_{2C}$-preferring agonist for obesity-related disorders (59, 66–68).

It is now well established that 5-HT$_{2C}$ agonism modulates feeding behavior—at least in mice—via direct actions on POMC-expressing neurons of the ARN (**Figure 2*b***). For several years, serotonin via 5-HT$_{2C}$ receptors has been known to directly activate POMC-expressing neurons (69). Subsequent studies wherein 5-HT$_{2C}$ receptors were selectively ablated in POMC-expressing neurons demonstrated that the anorectic and metabolic actions of fenfluramine were mediated by POMC 5-HT$_{2C}$ receptors (70, 71) (**Figure 3*b***). Given that the 5-HT$_{2C}$ receptor is coupled to Gq (72) and that activation of 5-HT$_{2C}$ receptors depolarizes POMC-expressing neurons (69), the anorectic actions of lorcaserin are likely mediated via 5-HT$_{2C}$-induced depolarization of POMC-expressing neurons, thereby attenuating feeding behavior (**Figure 3*b***). Not surprisingly, activation of hM3Dq in POMC-expressing neurons suppresses feeding behavior (52), thus

**Figure 2**

Alternative cell type–specific expression methods. (*a*) Utilization of different cell type–selective promoters in combination with either the AAV or the HSV. The hSyn promoter (*top set of arrows*) can be used for DREADD expression in all neuronal subtypes [e.g., both cortical neuronal subtypes (*red*)]. The CAMKIIa promoter (*second set of arrows*) leads to expression only in neurons that express CAMKIIa [e.g., cortical pyramidal neurons (*red*) but not interneurons (*orange*)]. The GFAP promoter (*third set of arrows*) expresses mainly in nonneuronal glial cell types [e.g., astrocytes (*red*)]. HSV viral vectors containing dynorphin (*fourth set of arrows*) and enkephalin (*bottom set of arrows*) promoters allow for selective DREADD expression in either of the two main populations of medium spiny neurons in the striatum. (*b*) Selective modulation of neurons on the basis of their projection paths can be achieved by utilizing the CAV that expresses Cre recombinase in combination with the FLEX-switch DREADD AAVs. In this example, CAV-Cre is microinjected into the PFC, and the FLEX-switch hM3Dq DREADD is microinjected into the dorsal raphe. After infection, the CAV-Cre viral particles are retrogradely transported back along the axon to the soma of the infected serotonin (5-HT) neuron. In the soma, the Cre recombinase flips the DREADD into the correct orientation, allowing for the selective expression (*red*) and control of specific neurons that project to specific areas in the brain—in this case, 5-HT neurons that project to the PFC. Abbreviations: 5-HT, 5-hydroxytryptamine; AAV, adeno-associated virus; CAMKIIa, $Ca^{2+}$/calmodulin-dependent protein kinase II alpha; CAV, canine adenovirus; DREADD, designer receptor exclusively activated by designer drugs; FLEX, flip-excision; GFAP, glial-fibrillary acidic protein; HA, human influenza hemagglutinin; hM3Dq, human M3 muscarinic DREADD receptor coupled to Gq; HSV, herpes simplex virus; hSyn, human synapsin; L-ITR, left inverted terminal repeat; PFC, prefrontal cortex; PolyA, poly A tail; R-ITR, right inverted terminal repeat; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element.

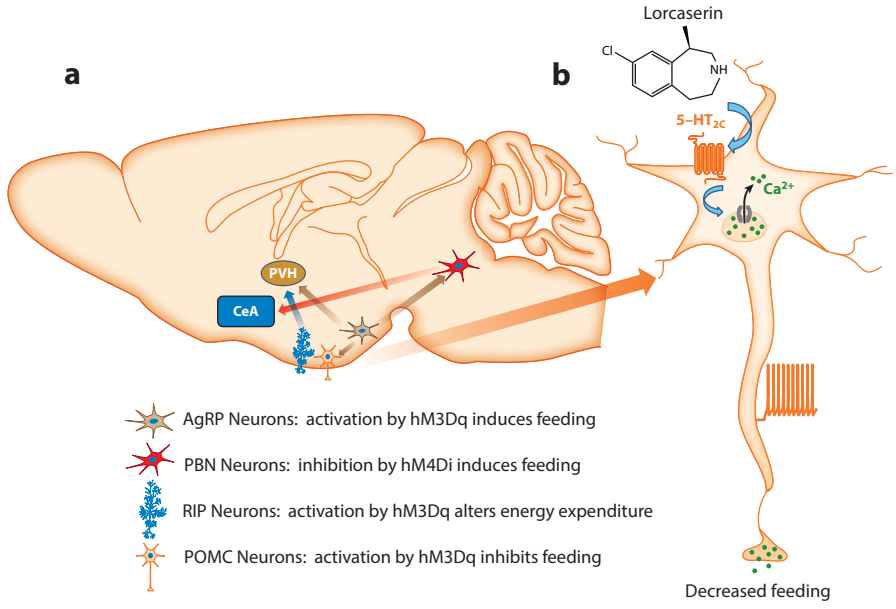



**Figure 3**

Cell type–specific control of neuronal function reveals potential therapeutic targets. (*a*) An illustration of the recently elucidated complexity underlying the regulation of feeding and energy expenditure. Activation of AgRP-expressing neurons (*brown*) induces feeding, whereas activation of the POMC-expressing neurons (*orange*) inhibits feeding. Additionally, inhibition of the PBN-expressing neurons (*red*) induces feeding by suppressing the PBN's activity in the CeA, and activation of the RIP-expressing neurons (*blue*) alters energy expenditure. (*b*) Lorcaserin, a 5-$HT_{2C}$ agonist, increases POMC neuronal excitability by activating the Gq-coupled 5-$HT_{2C}$ receptor, thus leading to increased rates of neuronal firing and suppression of feeding behavior, similar to the results arising from hM3Dq activation of POMC-expressing neurons. Abbreviations: 5-HT, 5-hydroxytryptamine; AgRP, Agouti-related peptide; CeA, central nucleus of the amygdala; hM3Dq, human M3 muscarinic DREADD receptor coupled to Gq; PBN, parabrachial nucleus; POMC, pro-opiomelanocortin; PVH, paraventricular hypothalamus; RIP, rat insulin promoter.

directly implicating GPCR-mediated Gq activation as a central regulator of feeding behavior in POMC-expressing neurons (**Figure 3*b***).

Taken together, these results suggest that it may be possible to discover anorectic drugs that target several canonical GPCR signaling pathways in a variety of hypothalamic nuclei. These include Gq-coupled GPCRs enriched in POMC-expressing neurons as well as Gi-coupled GPCRs enriched in AgRP-expressing neurons. Indeed, in support of these predictions, a recent study identified Gpr17 as enriched in AgRP-expressing neurons (73). Gpr17 couples to Gq (74), and the latter's activation of Gpr17 potentiates food intake, whereas Gpr17 antagonists inhibit food intake (73). Another study reported that the Gi-coupled GPR171 is enriched in ARN-expressing neurons and that knocking down GPR171 expression potentiated feeding (and thereby relieved a brake on Gi signaling) (75). As technologies for the profiling of cell type–specific expression of GPCRs become widely adopted, it should be possible to identify the entire complement of GPCRs within discrete neuronal subtypes within the brain (76–78) and peripheral tissues (79, 80). GPCRs that are enriched in a specific neuronal subtype and that couple to the appropriate signal cascade (e.g., Gi in ARN-expressing neurons and Gq in POMC-expressing neurons) thus become potential targets for therapeutic drug discovery efforts aimed at obesity-related disorders.

## LIMITATIONS AND POTENTIAL CONFOUNDS WHEN USING DREADDs

As with every tool, DREADDs have potential limitations that researchers should consider when utilizing them experimentally. A major potential limitation arises when neuronal manipulation requires precise millisecond-level temporal control. Neuronal modulation with DREADDs takes on the order of minutes, whereas other optical methods provide millisecond-level control in the activation or silencing of neurons. Thus, CNO can take 2 h to be cleared from plasma (18), delaying the ability to halt neuronal modulation. Although this pharmacokinetic property is an advantage in terms of therapeutics and simple neural circuitry, the lack of precise temporal control can cause complications when attempting to dissect the complex neuronal circuits involved in certain behaviors. Examples are social behaviors in which silencing or activating neurons requires specific and reversible timing during the behaviors.

Another potential limitation in the use of DREADDs is the difficulty to differentially deliver specific and reversible amounts of neuronal modulation to a given circuit. Whereas CNO concentrations can always be decreased or increased, the amount of stimulation required to elicit certain behaviors may be difficult to titrate precisely. Optical approaches provide more precise control over the amount of stimulation delivered to a neuron and can simultaneously record how the neuron responds to the stimulation (81). This type of control is better suited for investigating certain circuits in which different behaviors can result from varying levels of stimulation (82). Because of these limitations and because therapeutic applications will most likely require longer-term modulation of neuronal circuits, DREADDs are ideally suited to this task. Additionally, the fact that DREADDs are engineered GPCRs that provide a potentially rich resource is emphasized by the observation that many FDA-approved drugs target GPCRs.

An additional potential confound arises when DREADDs are used to investigate therapeutic targets. DREADDs, when overexpressed, may exceed the physiological levels of endogenous receptors. Conceivably, this overexpression could lead one to incorrectly target endogenous GPCRs in specific cell types in an attempt to mimic these results and thus lead one to misleadingly implicate these targets for pharmacological intervention. Quantifying DREADD expression levels and comparing them to levels of endogenous receptors can provide insight into whether this physiological outcome will translate when endogenous receptors are directly targeted.

## TRANSLATIONAL APPLICATIONS OF DREADDs AND AVAILABLE DREADD TECHNOLOGIES

As is clear from the foregoing, the DREADD-based chemogenetic toolbox has been widely adopted, and we have seen results that have clear translational ramifications for many therapeutic areas. In the areas of obesity-related disorders and the regulation of feeding, DREADD technology has allowed investigators to implicate specific GPCR-linked pathways in individual cell types with predicted effects on feeding behavior and centrally mediated metabolic consequences (**Figures 1–3**). Additionally, DREADDs have begun to illuminate the roles that GPCRs play in modulating numerous other disorders, including addiction-related disorders (35, 38, 39, 48), seizures (19), disorders relating to circadian rhythms (83) and sleep-wake cycles (46, 84), peripheral metabolic disorders (18, 21, 22), disorders relating to respiration and thermoregulation (50, 51), and disorders relating to perception (85) and synaptic plasticity (86). To further facilitate such translational approaches, numerous mouse genetic and viral technologies have been created (**Figure 1**).

With regard to the translational applications of DREADDs, we envision using DREADDs and CNO in humans via techniques whereby DREADDs are expressed in particular cell types

implicated in certain disorders. Thus, for instance, one might want to express DREADDs in serotonergic neurons, activate them, and thereby treat depression-related disorders. Additionally, as the circuits responsible for other psychiatric disorders are illuminated, one could turn them "on" or "off," thereby ameliorating circuitry-based disorders. The use of CNO-based DREADDs in such translational contexts could be facilitated by the fact that CNO has already been administered to humans without apparent effect (87); this lack of effect is consistent with its pharmacological inertness (14). Although CNO is relatively metabolically inert in rodents (18, 88), it can be metabolized to clozapine in guinea pigs and humans (87), although the extent of this metabolism is on the order of a few percent. Thus, when this particular technological platform is used in humans, low doses of CNO will likely be needed to avoid this potential problem. Alternatively, non-CNO-based DREADDS or non-CNO ligands could be developed. Such tools, which are already in hand (E. Vardy & B.L. Roth, manuscript in preparation), should minimize the potential problems with the current CNO-based DREADDs. Clearly, such technology in humans would require cell type–specific expression of DREADDs likely via virally mediated gene transduction technology. Alternatively, induced pluripotent stem cells stably expressing DREADDs would represent another potential way to harness both tissue engineering and synthetic biology technologies for ameliorating human disease. Such approaches would likely be piloted in subhuman primates before being applied to humans, and such experiments are currently in progress.

## CONCLUSIONS

Since the introduction of the DREADD-based chemogenetic approach in 2007 (14), it has been widely adopted and adapted by many investigators. The major uses of DREADDs, at least for now, are in the neurosciences, where they are broadly used to enhance or inhibit neuronal activity in a cell type–specific and noninvasive manner. What has been relatively unappreciated by those using DREADD technologies is that DREADDs not only enhance or inhibit neuronal activity, but they also illuminate endogenous signaling pathways in the neurons interrogated and signaling pathways that are responsible for this modified activity. Thus, DREADDs—when active—illustrate the importance of GPCR signaling processes for regulating neuronal activity. Because DREADDs serve as surrogates for endogenous GPCRs, they perforce modulate neuronal systems in a manner identical to the modulation performed by those receptors in vivo. One potential issue with the current suite of DREADDs, however, is the lack of engineered GPCRs that selectively activate G12/13-mediated signaling; thus, DREADDs might not be well suited as molecular machines for mimicking the actions of all GPCRs. DREADDs induce physiologically relevant, noninvasive, and reversible patterns of neuronal modulation—unlike many other approaches that artificially activate (89, 90) or inhibit (91, 92) neurons. Although DREADDs lack the amazingly precise temporal control afforded by optical technologies (89, 90), they may prove to be well suited for translational applications, which typically occur via chronic modulation of GPCR signaling.

## DISCLOSURE STATEMENT

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