Clinical Implications of Basic Neuroscience Research | CIRCUITS AND CIRCUIT DISORDERS

Chemogenetics—A Transformational and Translational Platform

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Neurologic disorders are frequently a result of inappropriate electrical and/or chemical signaling of neurons and glia. Ultimate remediation would necessitate reprogramming these signals. Historically, correcting neuronal and glial signaling is accomplished via drug therapy/administration, although they frequently fail to effectively and fully treat the underlying disorder. Developments in basic research have produced several new classes of potential therapeutics to directly and precisely control neuron activity at the single-cell level. We review one such technology, Designer Receptors Exclusively Activated by Designer Drugs, and suggest its potential as a powerful tool for augmenting neuronal and glial signaling and activity for basic and translational applications.

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One billion people—one-sixth of the world's population—have a neurologic disease or disorder.[1] The treatment options for these disorders are limited despite the great demand for effective therapeutics. A multitude of drugs have been developed to remedy these conditions, but few possess the efficacy and specificity necessary to achieve an effective therapeutic index. Consequently, a variety of technologies, most of which aim to directly control the electrical and chemical impulses that dictate nervous system activity in the brain, are being developed to address this global health issue.

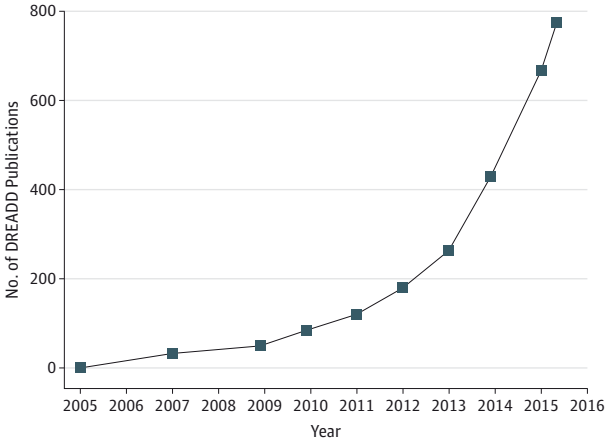
The treatment of neurologic diseases would, ideally, include spatially precise, noninvasive, bidirectional, on-demand control of neurons and glia. To date, the approved therapies for manipulating neuronal activity, such as deep brain stimulation, although useful, are invasive and unidirectional. In addition, deep brain stimulation alters the activity of the target neurons and distant neurons via axons in passage. Conceivably, optogenetic technologies that use light to switch neurons on and off could be used for precise, millisecond control of neuronal activity, although there will likely be difficulties translating this technology because of problems with light diffusion and penetration.[2] An alternative approach with significant translational capacity, which we have named Designer Receptors Exclusively Activated by Designer Drugs (DREADDs),[3] has gained wide utility (**Figure 1**) during the past decade as a means to modulate cellular signaling to turn neuronal circuits on and off. Because DREADDs are based on a chemogenetic[4] platform that relies on small druglike chemical actuators, they are relatively easily translated to large animals, including, perhaps, humans.

DREADD technology has had a major effect on our understanding of neural circuitry in behavior and disease at the bench,[5] and DREADDs have the potential to ultimately be clinically translated. For instance, in rodent models, DREADDs have demonstrated the ability to control neuronal activity to ameliorate disease phenotypes in conditions as diverse as Parkinson disease,[6] Down syndrome,[7] seizures,[8,9] and autism.[10] In addition, DREADD-based approaches modulate behaviors as diverse as addiction,[11,12] sleep,[13] aggression,[14] breathing,[15] and feeding.[16-18] DREADDS have also enhanced and silenced learning and memory and have been used to create artificial memories.[19-21] Preliminary reports[22-24] have also demonstrated successful incorporation of DREADDs into the nonhuman primate brain, accompanied by successful modulation of brain activity and behavior. Given the rapid advances of potential relevance to neurologists and other neuroscientists (perhaps an article per day on DREADD technology is now being published) (Figure 1), we provide a primer for bringing DREADD technology, a powerful tool for targeted control of cellular signaling and neuronal activity, into more therapeutic arenas.

What Are DREADDs?

DREADDs represent engineered G-protein–coupled receptors (GPCRs) that can be activated by inert, druglike small molecules to provide remote control of cellular signaling, neuronal activity, and behavior. G-protein–coupled receptors are integral membrane proteins that mediate nearly all physiologic processes in the body by responding to a variety of endogenous and exogenous ligands, including neurotransmitters and chemokines.[25] It is for this reason that at least one-third of approved medications target GPCRs,[25] including many neuropsychiatric drugs.[26] However, because many of the most effective medications are promiscuous,[26,27] they typically have adverse effects and toxic effects due to off-target actions. Prominent examples relevant to neurologists are the antiparkinsonian drugs cabergoline and lisuride,[28] which can cause life-threatening valvular heart disease via off-target activation of serotonin receptor 2B.[28,29] These frequently unpredictable adverse effects pose a significant challenge when attempting to develop small molecule–based approaches for modulating specific neuronal circuits (but see the article by Keiser et al[30]). To circumvent these inherent problems with GPCR-based approaches for modulating neuronal activity in a therapeutic manner, DREADDs were developed in 2005.[4]

Figure 1. Annual Rate of Designer Receptors Exclusively Activated by Designer Drug (DREADD) Publications



To create DREADDs, we used a process called directed molecular evolution whereby we were able to create mutant human muscarinic acetylcholine receptors (M1, M2, M3, M4, and M5) that did not respond to the endogenous ligand acetylcholine but were activated by the clozapine metabolite clozapine-N-oxide (CNO).[3] Because CNO has excellent druglike properties and has been safely administered to humans[31] and because we used human GPCRs, we envisioned that DREADD technology might ultimately be useful for human therapeutics. We also ensured that processes by which we engineered DREADDS rendered the mutant GPCR silent (eg, minimal constitutive activity) in untreated individuals and potently active in individuals treated with the designer drug for therapeutically useful periods (eg, minutes to hours).

DREADDs are applied as a system (**Figure 2**), providing great potential for highly flexible experimental and translational applications. Tapping into this flexibility, however, requires an understanding of the system's component parts and their applications. The initial step in using the DREADD system is to select the appropriate DREADD for the task at hand. Does the desired intervention in the brain region of interest require activation, suppression, or bidirectional control? DREADDs derived from the human muscarinic acetylcholine receptors (hMDREADDs) silence[3] or enhance[32] neuronal firing in the presence of the inert and orally available CNO.[3] Although CNO has been administered to humans with no apparent effect,[31] it can be back-metabolized to clozapine in guinea pigs, nonhuman primates (unpublished data), and humans. This metabolic conversion limits the dosing ranges and utility of hMDREADD. Significant efforts were accordingly made to identify new chemical actuators for the muscarinic DREADDs, which culminated in the discovery that the relatively innocuous, safe, and central nervous system (CNS)–penetrant antihistamine perlapine[33] is a potent hMDREADD activator.

The hMDREADD suite of receptors has been used extensively to provide unidirectional control of brain activity. However, to achieve bidirectional control, another designer receptor or alternative chemogenetic approach[34] would need to be used. Thus, a new DREADD was developed based on the human κ opioid receptor (KOR) and named KOR-DREADD (KORD).[18] Thus, KORD, for the first time, facilitates the bidirectional chemogenetic control of neurons.

The KORD silences neuronal firing in the presence of the inert salvinorin A metabolite salvinorin B (SalB). Currently, SalB is limited in its oral availability but after parenteral administration is highly brain penetrant.[35] Together, the hMDREADD and KORD receptors can be used to toggle the activity of specific neurons on and off simultaneously. The rate at which these neurons can be switched on and off is also adjustable, providing kinetic flexibility. Neuronal silencing with DREADDs can be prolonged (hours) or attenuated (minutes). The onset of CNO-modulated neuronal firing occurs within 5 to 10 minutes after intraperitoneal injection, with a peak electrophysiologic response 45 to 50 minutes after injection[5,32] and persistent activity detected several hours after injection.[5,35] In contrast, SalB enters the brain in seconds and rapidly decreases thereafter,[35] with KORD-mediated behavioral effects ceasing 1 hour after injection.[18] Efforts are also under way to generate new DREADD ligands with varying biological half-lifes capable of expanding this kinetic window.

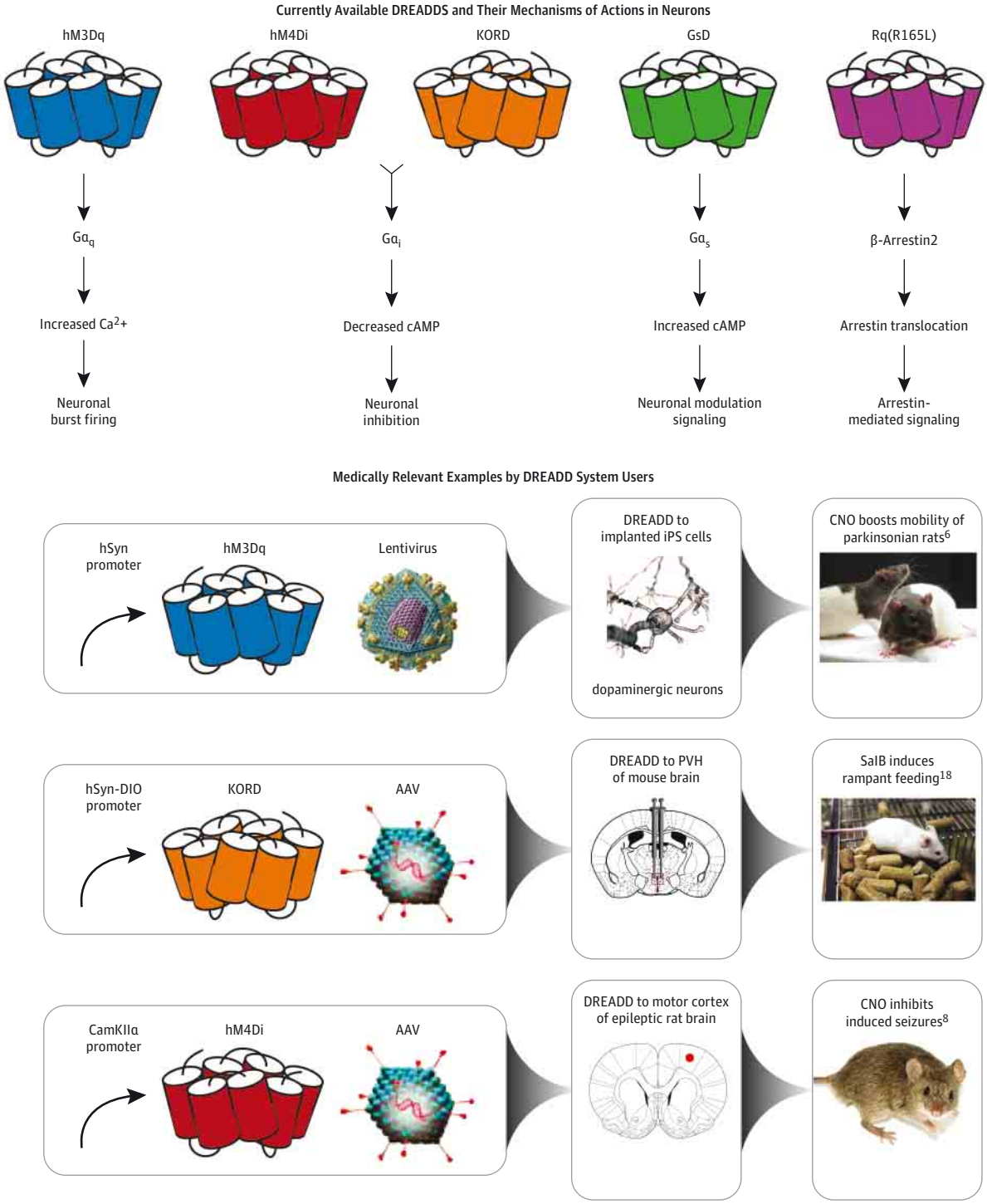
DREADDs can augment neuronal firing in multiple brain regions as observed in studies of mice, rats, and other mammals.[5] This augmentation is possible because DREADDs have been designed to couple to the signaling machinery of the cell via the transactivators $G\alpha_q$, $G\alpha_i$, $G\alpha_s$, or β-arrestin2.[3,32,36,37] These DREADD-coupled transactivators exhibit strong signaling activity when the DREADD binds to its designer drug, resulting in activation of various downstream signaling pathways. Selection of the correct transactivator and subsequent signaling pathway is of critical importance when considering the DREADD system for therapeutically relevant experiments. Examples of the biological activities born from activating these signaling paradigms have been extensively reviewed[5]; however, the ultimate phenotypic effect for each pathway should be assessed on a tissue-by-tissue basis. The safe and routine application of chemogenetic approaches, such as DREADDs, in humans will ultimately require extensive safety and efficacy studies assessing how activation of each pathway augments neuronal activity, behavior, and symptoms. As a cursory overview, Figure 2 highlights the consequences of activating these pathways, as determined using DREADDs, in numerous mammalian brain regions.

Therapeutic Delivery of the DREADD System

After selection of a suitable DREADD, the DREADD must be delivered to and expressed inside the specific neuronal tissue of interest to elicit the desired translationally relevant response. Off-target or weak expression of the DREADD is obviously undesirable. Controlled DREADD delivery and expression can be regulated through many conventional and emerging delivery and expression systems. DREADDs are typically expressed via virally mediated gene transduction,[5] although various transgenic approaches have also been used.[32,38,39]

For virally mediated transduction, the most direct method to control DREADD expression is to identify a gene promoter sequence that is potently and uniquely active in the cell type of interest. DREADDs have been fused to the calmodulin-dependent protein kinase IIα,[32,40] human synapsin,[12,41] glial fibrillary acidic protein,[42] dynorphin,[43] and enkephalin[43] promoters. The calmodulin-dependent protein kinase IIα promoter is specific to neurons where it is expressed, namely, excitatory glutamatergic

Figure 2. The Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) System



AAV indicates adeno-associated virus; CamKIIα, calmodulin-dependent protein kinase IIα; cAMP, cyclic adenosine monophosphate; CNO, clozapine-N-oxide; DIO, double-floxed inverted open reading frame; GsD, $Gα_s$-coupled DREADD; hM3Dq, $Gα_q$-coupled DREADD; hM4Di, $Gα_i$-coupled DREADD; hSyn, human synapsin; iPS, induced pluripotent stem; KORD, DREADD based on the κ opioid receptor; PVH, paraventricular nucleus of hypothalamus; Rq(R165L), hM3Dq $Gα_q$-coupled DREADD with the R165L mutation; and SalB, salvinorin A metabolite salvinorin B. Brown mouse image by George Shuklin (CC BY-SA 1.0; Wikimedia Commons [http://creativecommons.org/licenses/by-sa/1.0]). Feeding mouse image by Rama (CC BY-SA 2.0 fr; Wikimedia Commons [http://creativecommons.org/licenses/by-sa/2.0/fr/deed.en]). Hood rat photograph by Jason Snyder (CC BY 2.0; Wikimedia Commons [http://creativecommons.org/licenses/by/2.0]).

neurons (eg, cortical pyramidal neurons but not interneurons). The human synapsin promoter can express DREADDs in all neuronal subtypes. The glial fibrillary acidic protein promoter expresses primarily in nonneuronal glial cells. The dynorphin and enkephalin promoters are active in either of the 2 main populations of striatal medium spiny neurons. Many other promoters have been tested for expression in adrenergic neurons[44] and primate neurons.[45,46] In addition to these validated promoters, many additional promoters with potential for use in neuronal gene therapy have been highlighted through proteomic analysis of the human body,[47] deep RNA sequencing of the developing human brain,[48] and single-cell RNA sequencing of individual neurons.[49] The promoters that regulate each DREADD can be easily exchanged to augment the selective expression of the DREADD as necessary.

To achieve high levels of delivery, DREADDs are routinely expressed using adeno-associated virus (AAV), although lentivirus and herpesvirus approaches have also been used. Because of the multiple guided targeting systems available to assist in precise injection, AAV is a widely used viral delivery vector in the clinic.[50] In using AAV, the viral genome is replaced with DNA that encodes the DREADD of interest and packaged into the AAV capsid. Multiple AAV capsid serotypes are available for the targeted delivery of DNA cargos to the central nervous system,[51-54] including the primate CNS.[55,56] These viral delivery vectors are nonvirulent and do not replicate within the host. In addition, because of the inherent infectivity of the viral capsid, gene delivery is tightly localized to the site of injection, allowing for precise targeting of neuronal subregions. To assist researchers in delivering DREADDs to the CNS, all DREADDs are available from the University of North Carolina Vector Core facility in multiple CNS-validated AAV serotypes. Because this is an active field, AAV serotypes with unique patterns of tissue tropism and delivery appear frequently. Powerful methods of in vivo capture screening have produced viruses with specific cell-targeting tropisms.[57,58] One can imagine how use of these directed evolution techniques with the clinically tested AAVrh10, found to transduce a large portion of the CNS through intravenous delivery,[59] could hold great promise for the routine delivery of DREADDs in larger brains, such as nonhuman primates and, perhaps, humans.

The ability to deliver DREADDs to a localized brain region, coupled with cell type–specific promoters, has allowed researchers unfettered control of neuronal activity. Lacking in this delivery and expression system was the ability to target a subset of neurons from the same subtype class (eg, serotonin neurons projecting specifically from the dorsal raphe to the prefrontal cortex). This limitation was overcome in recent work[60] using canine adenovirus, a retrograde virus capable of traversing neuronal axons to deliver gene cargo to the soma.[61] This feature was seized on to deliver Cre recombinase (capable of flipping DNA sequences flanked by precisely oriented[62,63] loxP nucleotide sequence pairs) to projecting neurons at their synapses in the prefrontal cortex.[60] Injection of loxP-flanked DREADDs at the dorsal raphe ensured that only neurons that acquired Cre recombinase from their projections into the prefrontal cortex would successfully flip and express the delivered DREADD. This spatially restrictive and gated method of expression allows DREADD system users to precisely target a handful of highly specific, functionally relevant neurons for modulation. Canine adenovirus elicits a minimal immunogenic response and could potentially be a translationally relevant delivery system.[64] Use of canine adenovirus will facilitate the implementation of complex on and off switch systems for regulation of neuronal dysfunctions spanning multiple brain regions.

In addition to the aforementioned technologies, which have successfully delivered DREADDS to the CNS, numerous gene delivery techniques exist with unique therapeutic advantages for DREADD delivery. For example, a major limitation of viral vector–based gene therapy is the limit on gene cargo size. For AAV, this limit falls to approximately 6000 nucleotides.[65] This size limitation puts restrictions on the complexity of the delivered DREADD system. For example, large genetic regulatory elements could be used to finely tune cell type–specific DREADD expression but are too large to package in AAV. It is also currently impossible to deliver multiple DREADDs simultaneously encoded within the same AAV viral particle. These limitations could be overcome by nanoparticle-based gene delivery systems, which can package and deliver numerous DNA- and protein-based cargos simultaneously to the CNS.[66] In addition to delivering DREADDs into native CNS cells, DREADDs could also be integrated into induced pluripotent stem cells. Genes of a size similar to that of DREADDs have been integrated into pluripotent stem cells with high efficiency.[67] These DREADD-containing, induced, pluripotent stem cells could then be selectively differentiated through activation of a targeted GPCR-activated pathway[68] or provide postoperative control of neuronal activity after grafting of the stem cells to lesion sites. Indeed, such a study[6] has already been performed in a Parkinson disease rat model, with activation of DREADDs in induced dopaminergic neurons greatly enhancing the beneficial effects of the transplanted tissue.

Hurdles to Potential Clinical Application of DREADDs and Other Chemogenetic Technologies

The component parts and principles necessary to deliver DREADDs to the clinic are currently in place. A multitude of viral and promoter pairs have been tested in human[69-71] and nonhuman primate[45,46,72] brains in preparation for this form of therapeutic intervention. Furthermore, DREADDs have been successfully introduced and activated in nonhuman primate brains.[22-24] Studies in nonhuman primates will continue to advance, addressing the details of potential applications and interventions; however, the major hurdle that needs to be addressed is establishing the first DREADD pilot study in human patients.

Two neurologic disorders are exceptional candidates for DREADD-based intervention: Parkinson disease[44] and seizures.[16,17] For both diseases, deep brain stimulation is performed when first-line interventions fail. It would therefore be possible to deliver DREADDs to patients at the time of deep brain stimulation. The therapeutic ideal for these diseases is to suppress spurious electrical signals propagating from the overactive brain regions of the patient—a task at which the KORD excels. The inherent difficulties to overcome for this approach include those associated with gene therapy and drug delivery, so considerable hurdles exist to ultimately translate this technology to humans. Nonetheless, DREADDs are uniquely positioned at the precipice of bench to bedside translation. They are human receptors that can be delivered to and thus far appear to be well tolerated in nonhuman primates. They are activated by cheap, safe, and biologically available chemical actuators. With a small nudge, they could emerge as a way to potentially treat a variety of neuropsychiatric disorders.

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