

Chemogenetics: drug-controlled gene therapies for neural circuit disorders

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Abstract

Many patients with nervous system disorders have considerable unmet clinical needs or suffer debilitating drug side effects. A major limitation of existing treatment approaches is that traditional small molecule pharmacotherapy lacks sufficient specificity to effectively treat many neurological diseases. Chemogenetics is a new gene therapy technology that targets an engineered receptor to cell types involved in nervous system dysfunction, enabling highly selective drug-controlled neuromodulation. Here, we discuss chemogenetic platforms and considerations for their potential application as human nervous system therapies.

INTRODUCTION

Nervous system disorders are among the most debilitating chronic diseases. Small molecule therapeutics that improve core symptoms of neurological and psychiatric disorders have benefited millions of people worldwide. Despite this progress, patients with nervous system disorders have large unmet needs. A minority of patients with neuropathic pain respond to available treatments, and amongst those responsive patients, pain is only relieved by approximately 50% [1]. For epilepsy, a degree of seizure control can be achieved in approximately 60% of patients; however, a third of patients that are treated with antiepileptic drugs still have seizures. Moreover, efficacy can be accompanied with neurocognitive side effects, and a large percentage of patients are either refractory or intolerant to pharmacotherapy [2]. In addition, there are insufficient treatment options for most neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS).

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Other therapeutic modalities are used to address the unmet needs of pharmacotherapy. Surgical nervous system resections are last-resort treatments. Although surgery can yield improvements in neuropathic pain or pharmacotherapy-resistant epilepsy, tissue resection or ablation may require multiple surgeries, can leave permanent deficits, and generally faces resistance from patients and physicians [3]. Moreover, many nervous system disorders are not candidates for surgical resection. Deep brain stimulation (DBS) is an alternative and mostly non-destructive surgical method that implants a stimulating electrode [4]. DBS modulates the activity of cells at the stimulation site but also axons-of-passage, potentially recruiting many different brain areas, thus limiting mechanistic understanding and reducing generalization of the approach [4,5].

Gene therapy is an additional therapeutic option being developed for neurological diseases [6]. Approved gene therapies for neurological disorders target large volumes of the nervous system and are designed to correct the function of a disease-causing underlying genetic mutation, as with Zolgensma[®] for pediatric patients with *SMN1* mutation leading to spinal muscular atrophy. Other gene therapies in development target endogenous enzymes or neuropeptides to specific brain areas, for example to increase dopamine production [7]. All three of the aforementioned approaches to neurologic treatment have strengths that must be balanced with considerable weaknesses (Table 1).

Here, we discuss the potential for a new therapeutic modality, called chemogenetics, which combines the respective advantages of pharmacotherapy, DBS, and gene therapy while minimizing drawbacks (Table 1 & Figure 1). Chemogenetics is a method by which a cell is modified to express an engineered receptor so that it can be selectively activated by its cognate drug [8,9]. The receptor can be targeted to a local region of the nervous system with greater specificity than DBS and with the temporal and scalable neuromodulation of traditional pharmacotherapy. We first briefly discuss the reasons behind the need for new therapeutic approaches to neurological diseases that are most relevant to chemogenetic technologies. Then, we describe some of the background to chemogenetics, before focusing on considerations for translation of chemogenetics into a human gene therapy.

DRUG DEVELOPMENT & TARGET SELECTION IN NERVOUS SYSTEM DISORDERS

The molecular biology revolution of the 1990s led to a better understanding of the gene products targeted by available pharmacological therapies for neurological and psychiatric disorders, most of which had activity at multiple receptors [10]. This transformed the approach to drug discovery and led to a hypothesis that generating highly selective molecules for single molecular targets could potentially achieve efficacy without unwanted side effects. Molecular target-based approaches resulted in small molecules modulators, peptides, or antibodies being directed to a plethora of drug targets, including subtypes of neurotransmitters, their reuptake transporters, ion channels, enzymes, and misfolded proteins. There have been a few notable successes in this single-target approach, such as monoclonal antibodies against CGRP for the prophylactic migraine treatment, 5HT_{2a} partial agonist for psychosis in PD; α 4b2 nicotinic acetylcholine receptor partial agonists

for smoking cessation, and hypocretin (HCRT1/2) receptor antagonists to promote sleep. However, the target-based approach in neuroscience drug development, comes with common failure-modes from attempting to drug the wrong targets or from insufficient target engagement. These problems can be due to: (1) an incorrect biological hypothesis, (2) limited nervous system exposure of the drug, or (3) a need to balance target engagement in the treatment-relevant cells while minimizing adverse effects of target engagement at cells that are unrelated to efficacy.

Choosing the wrong target is a pernicious error that can sometimes become apparent only after attempting to understand the failure of a large clinical trial, and, in some cases, even then the reasons for the failure may be unclear [11]. In addition, achieving sufficient exposure of a drug at the target is associated multiple tradeoffs often requiring structural changes that may reduce affinity or selectivity. However, it is the complexity of the nervous system that is one of the greatest challenges to overcome for developing new therapies.

The NIH Brain Initiative[®] (Brain Research through Advancing Innovative Neurotechnologies) and Europe's Human Brain Project are research initiatives aiming to understand the integrated functions of the brain. A key observation from this work has been that there are many functionally specialized networks in the brain that are important for normal brain function. For example, intermingled neuronal subtypes in sensory ganglia are responsible for transmitting information about touch, pain, and itch [12]. Furthermore, the perceptual and emotional qualities of these distinct sensory modalities are established in separate brain regions by distinct downstream circuit connections of these cell types [13]. The different functional specializations of intermingled cell types and their connections points to an organizational framework that offers a solid mechanistic foundation for understanding brain function. In principle, any of the nodes (cells) or wires (axons) in these neural circuits can experience dysfunction that can lead to neurological disease. The development of maladaptive synaptic circuits resulting from neuroanatomical and/or neurochemical changes have implicated hyperactivity or hypoactivity of specific neuronal circuits in epilepsy [14,15], pain [16], AD [17], PD [18], schizophrenia [19] and addiction [20].

Each cellular circuit node in these networks expresses thousands of gene products that are potential targets for pharmacotherapy. However, brain-localized differential gene expression is restricted to fewer than 100 genes, distributed across neurons, glia, and other nervous system cell types [21,22]. Moreover, brain-wide analysis of gene expression distribution reveals few genes that are limited to a single brain region [22,23]. In addition, effective nervous system therapies often attempt to increase or decrease neuron activity, thus target selection is further limited to druggable, neuron-activity-modulating gene products. Thus, the major limitation of traditional pharmacotherapy is that most molecular drug targets are not localized to one circuit, or even to the brain. A systemically administered drug, distributed throughout the body and brain, will likely exhibit additional interactions that will interfere with efficacy and produce dose limiting side effects. Despite the successful generation of new chemical entities that are highly potent and very selective for their intended target, regional specificity is not achieved.

In light of these limitations, deep brain stimulation has been adopted to control neuronal circuit activity focally in the nervous system. DBS is FDA approved for medically refractory PD, essential tremor, dystonia, and obsessive-compulsive disorder [4]. However, the mechanism by which DBS works is unclear. For example, there remains mechanistic uncertainty about whether DBS activates or inhibits circuits. This makes it difficult to predict what sites would be effectively treated by DBS, and treatment design is largely empirical [4]. Moreover, DBS is not sufficiently region-specific as it stimulates neurons as well as long-range axons by modulating a local electric field. Because of these issues, clinical outcomes from DBS are difficult to predict and may also have tolerability issues.

Thus, despite the enormous investments in small molecule drug discovery for neuroscience, there have been few examples of novel targets showing efficacy in the past two decades. As such, it is necessary to adopt alternative strategies for nervous system diseases. Importantly, these novel strategies should limit the risk of testing an unvalidated target, increase the selectivity of neural circuit control, and address the fundamental issues of hyper- or hypo-excitability in nervous system disorders.

CHEMOGENETICS: BACKGROUND

Chemogenetics inverts traditional drug discovery. Typically, drugs are developed for an endogenous cellular receptor that modifies neuronal activity to ameliorate a disorder. Chemogenetics determines a clinically approved drug upfront based on its bioavailability, stability, pharmacokinetics, and tolerability. This drug is then used as the target around which the receptor is designed [24]. Although this can be difficult, protein engineering offers a diverse range of options. The underlying receptor platform is chosen by considering its functional consequences when expressed in a cell, potential for modularity to achieve different functional effects, and the simplicity with which it can be delivered by gene therapy.

Chemogenetics is widely used in biological research for altering the activity of defined cell populations [8,25]. In neuroscience, there is a ubiquitous experimental approach that aims to selectively activate or inhibit specific neuronal subtypes in the brains of model organisms. This permits gain-of-function (gof) or loss-of-function (lof) perturbations in neural circuits to examine the sufficiency or necessity, respectively, of a neural circuit node in a particular aspect of nervous system function [26]. For example, chemogenetic activation or silencing of Agouti regulated protein-expressing (AGRP) neurons in the hypothalamus dramatically increase or decrease food consumption [27], while perturbation of nearby cells influence aggression, sex, emotion, sociality, and thermoregulation [28]. This highlights the impressive selectivity of chemogenetics in the brain for cell type-specific evaluation of brain functions.

Chemogenetics generalizes chemical control of cellular pathways by engineering a limited set of tunable, modular, and selective receptor/ligand systems that can be installed in virtually any cell population. Optimal chemogenetic tools possess two core properties: 1) the engineered receptor actuator is normally non-perturbative to cells, i.e. it has low constitutive

activity and low responsiveness to endogenous ligands; 2) the exogenously applied ligand is non-perturbative to cells that lack the actuator transgene.

There are two main platforms for modular chemogenetics in neurons: ligand gated ion channels (LGICs) and G-protein coupled receptors (GPCRs). GPCRs were used to develop the first chemogenetic system at Merck in 1991 [29]. Additional improvements led to the development of designer receptors exclusively activated by designer drugs (DREADDs). This was first achieved by directed evolution of an improved interaction of the $G\alpha_q$ -coupled muscarinic acetylcholine receptor 3 (hM3) with clozapine-*N*-oxide (CNO), which is an ostensibly inactive metabolite of the antipsychotic drug clozapine [24]. The same mutations also enhanced clozapine potency for this engineered receptor [24], which was named hM3Dq. Conversely, the potency of acetylcholine, the endogenous hM3 agonist, was reduced by >50,000-fold for hM3Dq. Heterologous expression of hM3Dq in cortical neuron cultures did not significantly affect membrane potential in the absence of CNO [24]. In the presence of CNO, neurons were rapidly depolarized due to $G\alpha_q$ →protein lipase C (PLC)-mediated closure of M-current (Kcnq) potassium conductances. A neuronal silencer DREADD was generated by applying the hM3Dq mutations at homologous residues in hM4, which is an endogenous $G\alpha_i$ -coupled receptor. Expression of hM4Di in neurons rendered cells sensitive to CNO-induced hyperpolarization due to activation of G-protein inwardly rectifying (GIRK) potassium conductances and resulted in reduction of neuron activity [24,30,31]. hM4Di-mediated neuronal silencing has also been found by inhibition of synaptic release [30,31], which may involve additional pathways that are modulated by G-protein signaling. Importantly, neuromodulation is dependent on the effectiveness of the G-protein signaling pathways coupling to a specific set of ion channels or synaptic proteins that must be present in the targeted cell type, which may be unknown in human neurons under disease conditions. The modularity of this chemogenetic design was extended using previously established structure-function analyses of GPCRs, which were applied to hM3D receptors to produce receptors that selectively signal through $G\alpha_s$ or β -arrestin [32,33]. Thus, the discovery of mutations that produce new pharmacological selectivity has been exploited in conjunction with GPCR structure-function information to produce a range of chemogenetic research tools for control of different cellular signal transduction pathways, many of which couple to ion channels (Figure 2).

The primary limitation of the DREADD/CNO system is that CNO has been found to be excluded from the brain, in part due to P-glycoprotein pump activity, and the *in vivo* activity of CNO in the brain is due to metabolic CNO conversion to clozapine [34]. The hypnotic drug perlapine, which is used in Japan, has been shown to be an DREADD agonist [35,36], although perlapine shows similar binding affinity for DREADDs as endogenous receptor, suggesting little selectivity [35]. One potential alternative is a set of DREADD mutations that has been applied to the kappa opioid receptor [37], but the agonist, Salvinorin B, is poorly water soluble and also a P-glycoprotein pump substrate [38]. Thus, DREADDs are an expanding research toolbox that enables selective pharmacological control over distinct signaling pathways and offer a new capability for sophisticated analysis of cellular and circuit functions. Additional work is needed, but progress is being made to identify selective, stable, and brain penetrant agonists aside from clozapine [39–41].

The second major chemogenetic system uses LGICs for direct pharmacological control over ion conductance. The functional properties of ion channels are primarily dictated by their ion selectivity. Inward flux of cations or outward flux of anions depolarizes cells, and correspondingly inward flux of anions or outward flux of cations leads to cellular hyperpolarization [42]. Several LGIC families have been developed as chemogenetic tools, including the large superfamily of Cys-loop receptors.

To utilize the functional diversity of the Cys-loop ion channel family, a set of chemogenetic technologies was created based on chimeric LGICs derived from $\alpha 7$ nicotinic acetylcholine receptor (nAChR) and other Cys-loop family members. Importantly, the extracellular ligand binding domain (LBD) of $\alpha 7$ nAChR is transferrable to the transmembrane ion pore domains (IPDs) of other members of the Cys-loop LGIC family [43–45]. This property allows the pharmacology of the $\alpha 7$ nAChR to be maintained while accessing the ion conductance properties of other LGICs, such as the cation-selective serotonin receptor 3 (5HT3) or the anion-selective glycine receptor (GlyR).

To leverage these characteristics, mutagenesis of the $\alpha 7$ nAChR LBD conferred selective agonist activity to structurally distinct small molecules while reducing endogenous agonist potency of acetylcholine (ACh) [45]. The mutated LBDs were termed pharmacologically selective actuator modules (PSAM, pronounced as sam) [45]. PSAMs and their cognate agonists solve the LGIC pharmacology problem once; then they can be used to achieve a variety of functional effects on cells depending on what IPD they are spliced with to form chimeric LGICs.

PSAMs have been used to construct a variety of chimeric ion channels that directly control neuron electrical activity. PSAM-5HT3 provides prolonged depolarizing currents in the presence of the corresponding agonist [45] and results in sustained neuron activation. PSAM-GlyR has large chloride-selective conductance with a long steady state window current to maintain silencing as long as the agonist is present [45]. Cells expressing PSAM-GlyR channels have similar electrical properties as those that lack the channels, but in the presence of one of the cognate pharmacologically selective effector molecule (PSEM) agonists, neurons dramatically reduce input resistance, silencing the neurons by making it difficult to fire action potentials (Figure 3) [45].

Multiple PSAM LBDs have been developed using combinations of mutations that confer selectivity to different small molecules. For example, distinct PSAMs have been produced for the clinically used drugs tropisetron (an anti-emetic) and varenicline (an anti-smoking drug), as well as chemical derivatives of those molecules [46]. These modular chimeric LGICs offer potent, bi-directional control over neurons activity.

CONSIDERATIONS FOR THERAPEUTIC CHEMOGENETICS

The broad utility of chemogenetics for research purposes, including in preclinical models of human neurological disorders, has generated interest in extending chemogenetics as a human gene therapy. Optimally, a chemogenetic system should have several characteristics for therapeutic use in the nervous system:

1. The introduced receptor should be activated by low agonist doses;
2. Human use will be facilitated by a chemogenetic agonist with an established human tolerability profile at a dose sufficient to modulate the chemogenetic receptor;
3. If the brain target is the central nervous system, then the drug must cross the blood-brain barrier;
4. The receptors should activate or inhibit neurons efficaciously, durably, and reversibly;
5. Chemogenetic modulation should be restricted to the therapeutically relevant region and cell types;
6. Involvement of a cell type or brain region in a nervous system disease should be well-validated, for example by prior surgical resection or nerve-block studies;
7. The site and level of expression of the chemogenetic receptor should be measurable noninvasively.

Chemogenetic receptors are locally delivered by viral injections, primarily using AAV vectors, which have been demonstrated to be suitable for human gene therapy applications [6,7]. The relatively short coding sequences of both DREADDs and PSAM channels allow efficient packaging into viral vectors. For therapeutic applications, a small amount of an AAV that carries DNA encoding the chemogenetic construct is delivered by direct injection to the affected part of the nervous system [6,7]. An array of AAV serotypes must be screened and optimized for each tissue target in rodent and non-human primate models as well as human explant tissue if it is available [6]. Small promoters (<1 kb) are preferable for AAV gene therapies due to small genome packaging size of AAV. Within the brain, cell type-specific promoters for neurons (*Synapsin*) or glia (*Gfap*) are often used [47]. This is important to restrict expression to the cell type of interest in the nervous system.

Chemogenetic receptors are expressed on the cell surface where they are ideally inert. This can be achieved by using receptors with low constitutive activity and lacking response to endogenous agonists. It is preferable to find the lowest receptor expression level that is compatible with functional efficacy to minimize the potential for protein interactions that may be perturbative to the targeted cell [48]. Both DREADDs and PSAMs are well-suited for low expression levels because GPCRs utilize an amplification signal transduction cascade and PSAM-based ion channels have high single channel conductance.

One of the most important considerations for chemogenetic therapeutics is selection of the small molecule agonist. Human use is facilitated by a chemogenetic agonist that is an approved drug with suitable pharmacokinetics and that crosses the blood-brain barrier. In addition, the potency of the drug should be similar to or even better than at the endogenous target for which the drug is already approved. For DREADDs, the atypical antipsychotic drugs clozapine and olanzapine have been suggested as the best candidates because of their high affinity and potencies at DREADD receptors [49,50]. However, these molecules also have high affinity interactions with a large range of targets [10]. The use of clozapine is limited by potentially fatal side effects and contraindications that requires frequent

monitoring [51]. Olanzapine also has many contraindications and produces iatrogenic weight gain [52]. It remains to be seen whether these antipsychotics are sufficiently tolerable to be used as components of long-term chemogenetic gene therapy.

PSAM chimeric LGICs that are potently activated by the FDA-approved anti-smoking drug, varenicline, were developed for human clinical use [46]. The potency of varenicline for the chemogenetic silencer channel is > 100-fold more potent (~2 nM) than the anti-smoking target of varenicline, which is partial agonism at the $\alpha 4\beta 2$ nAChR. Varenicline is particularly attractive for potential therapeutic applications because it shows limited metabolism, durable pharmacokinetics, and high oral and brain bioavailability. Because the engineered PSAM has such high potency for varenicline, lower systemic exposures of varenicline will likely be sufficient for activation of the engineered ion channels than what is currently used in the clinic for smoking cessation. This is a crucial consideration because it exceeds the basic design requirement of therapeutic chemogenetic systems. In addition, with PSAMs, chemogenetic inhibition or activation was sustained for at least 2–3 weeks of continual exposure to varenicline, indicating suitability for chronic use [46]. Robust responses to chemogenetic silencing of neurons using low doses of varenicline have also been demonstrated in rodent and nonhuman primate models [46].

An additional consideration for chemogenetic applications in the brain is the need for precise and quantitative methods to establish the expression level and distribution of the chemogenetic receptor in patients. Positron emission tomography is well-suited to this because it uses micro-doses of a radiolabeled ligand for the target receptor, and it permits localization of the chemogenetic receptor to be accurately localized with MRI overlay of the underlying tissue. DREADD localization in the brain can be detected by radiolabeled clozapine and other ligands by PET [34,39,41,53] and the functional consequences of DREADD activation can be monitored by functional MRI [54]. Likewise, expression of PSAM ion channels was visualized noninvasively using positron emission tomography with ^{18}F -ASEM [46], a ligand previously used in people [55]. For both chemogenetic platforms, PET has been used for noninvasive measurement of the expression and anatomic site of chemogenetic receptors.

APPLICATIONS OF CHEMOGENETICS IN NERVOUS SYSTEM THERAPEUTICS

The first therapeutic applications of chemogenetics are likely to meet at least 5 criteria:

1. A localized disease focal point at which neuromodulation will provide clinical benefit;
2. Disorders of hypo- or hyper-excitability that are well suited for the mechanism of action of chemogenetic receptors;
3. Significant unmet medical need;
4. Existing surgical procedures that are part of the standard of care that can be adopted for targeting the affected tissue with AAV; and

5. Requirement for tunable control over the activity in the affected tissue by adjusting the dose of the chemogenetic agonist.

Pain and epilepsy are two canonical hyperexcitability disorders of the nervous system, and chemogenetics is effective in rodent models of pain and epilepsy (Table 2). One of the major goals in pain research is to control nociceptive afferent excitability. Viral injections of the inhibitory DREADD, hM4Di, into sciatic nerve were used to target sensory neurons that carry pain signals, which showed CNO-dependent inhibition and increases in mechanical and thermal pain thresholds [56]. Using PSAM chimeric LGICs, pain responses can be modulated bidirectionally with opposite neuron activity perturbations in D1R and D2R neurons in the mouse striatum [57]. These studies showed that D2R neuron activation increases pain while D2R neuron inhibition increases pain sensitivity. Activation of a different population called POMC neurons in the mouse hindbrain also reduced thermal pain withdrawal responses, presumably due to endogenous (β -endorphin release [58]. The magnitude of the analgesic effect was similar to morphine in the same assay [58]. Thus, there is considerable potential to use chemogenetics to suppress chronic pain conditions, by neuromodulation of specific localized sensory ganglia or by suppressing central representations of the negative emotional qualities of pain.

Chemogenetics also has promise as a focal epilepsy therapy. Epileptic foci can be mapped by electrophysiology and then targeted with stereotactic delivery of the chemogenetic AAV. Chemogenetic inhibition of motor cortex in rats has been shown to reduce seizure frequency in a focal epilepsy model [59]. Intracortical injections of the muscarinic receptor agonist, pilocarpine or the GABAA receptor antagonist, picrotoxin evoke acute seizures in rats. For rats expressing the DREADD hM4Di receptors in motor cortex delivered focally by AAV's, seizures could be reduced when CNO was administered to the animals following either pilocarpine or picrotoxin intracortical injections. Similarly, spontaneous seizures elicited in a tetanus toxin model of neocortical epilepsy were also suppressed by CNO in rats expressing hM4Di receptors [59]. These findings suggest that there was sufficient expression of the chemogenetic transgene to modulate circuit-based hyperexcitability and therefore highlights the potential for AAV-based chemogenetics in control of seizures.

PD, which is caused by cell death of dopamine neurons that innervate the basal ganglia, leads to severe motor impairment, in part due to excessive inhibition of the motor thalamus by the basal ganglia. Disruption of components of the basal ganglia using lesions, traditional pharmacology, and DBS improve the motor symptoms of PD [60–62]. Chemogenetics has been demonstrated to improve motor symptoms in rodent PD models by inhibiting the Globus Pallidus, internal part (GPi) and Substantia Nigra reticulata (SNr) [63]. Chemogenetics using PSAM-GlyR and varenicline have been shown to strongly suppress SNr in mice and GPi in a monkey [46]. Thus, chemogenetics is a promising approach to 'release the brake' on motor functions in PD [18]. The advantage of this approach over DBS, which is currently used for treating PD symptoms, is that it eliminates the need for a permanent stimulating electrode implant, while maintaining scalable control over neuromodulation controlled by the dosage of the chemogenetic effector drug.

The application of chemogenetic neuromodulation to slow the causes of neurodegenerative diseases has only been lightly explored. One study investigated an amyotrophic lateral sclerosis (ALS) mouse model expressing mutated superoxide dismutase (SOD) to test a hypothesis that the level of activity in motor neurons was associated with cell death. Brief periods of chemogenetic activation with PSAM-5HT3 chimeric LGICs resulted in a sharp reduction in motor neuron loss and retention of motor neuron innervation of associated muscle fibers [64]. It is not known if a similar principle can be applied to other neurodegenerative diseases. However, a related phenomenon was found in a study from the same research group using chemogenetic neuromodulation in a schizophrenia mouse model with chromosome 16 deletions that mimic 22Q11 deletion syndrome [65]. Transient activation with PSAM-5HT3 of parvalbumin-expressing interneurons in the prefrontal cortex or ventral hippocampus was effective at improving cognitive function and restoring gamma oscillation activity in the prefrontal cortex of the mice [65]. Additional studies are needed to establish how often neuromodulation needs to be applied, with what intensity, and whether the neuromodulation procedures can be extended to the natural disease state.

Anxiety disorders can be debilitating in some patients, and a considerable number of individuals are resistant to anxiolytic pharmacotherapy or cannot abide the side effects of high doses of these drugs. A large number of brain regions have been identified with optogenetic or chemogenetic neuromodulation in rodent models to produce anxiolytic or anxiogenic responses [66–68]. However, these brain regions, which include the prefrontal cortex, amygdala, and hippocampus, are also needed for other critical human functions. The unmet need in these patients makes consideration of chemogenetic approaches to therapy appealing. Localized, scalable control over the level of activity in anxiogenic or anxiolytic brain regions could be implemented with patient-controlled dosing to influence anxiety-specific responses without the considerable side effects associated with systemic treatment using traditional pharmacotherapy.

Obesity is typically a life-long struggle, especially when associated with genetic causes. Morbid obesity can be associated with defined monogenic mutations or of unknown cause and is associated with dysfunction in appetite-controlling neural circuits [69,70]. Most anti-appetite drugs have serious dose limiting side-effects that result in insufficient weight-lowering efficacy and major post-approval adverse health events leading to a series of high profile drug withdrawals [71–73]. In principle, an approach that selectively targets appetite-control circuits would circumvent many adverse outcomes that are due to drug-effects in other brain circuits and tissues. Regionally targeted chemogenetic neuromodulation therapies could circumvent the extensive side effects associated with past efforts at appetite control.

In general, chemogenetics will have the most unique advantage over current drugs, devices, and surgical approaches if it can be reliably coupled with selective promoter elements that can target specific neuronal subtypes within a targeted brain region. Short promoter elements that target excitatory and inhibitory neuronal subtypes have been demonstrated to be selective in rodents, nonhuman primates, and human explant tissues. Systematic approaches to identifying these short synthetic promoter elements are under development [74–76]. However, locally targeting specific neuronal subtypes has the potential to revolutionize

nervous system treatments because it would allow perturbations at the level of molecularly defined circuits that are thought to be used by the brain to serve distinct functions.

CONCLUSIONS

Pharmacological treatment of nervous system diseases is often stymied by insufficiently selective receptor expression. Chemogenetics offers a generalizable therapeutic strategy by engineering a receptor-drug interaction and then using gene therapy to deliver the chemogenetic receptor to the affected tissue. This permits highly effective neuromodulation that combines a neural circuit-based understanding of brain function with familiar principles of pharmacotherapy. By solving the ‘pharmacology problem’ once, chemogenetic gene therapies can be applied to an extraordinarily diverse set of disease symptoms caused by localized dysfunction of hypo- or hyper-activity in neural circuits.

The potential therapeutic advantages of selective chemogenetic neuromodulation could be transformative for many nervous system disorders. Initial therapeutic applications of chemogenetics will likely target tissues by modifying surgical approaches that are already standard of care for tissue ablation or resection. In addition, the commercial framework of current gene replacement therapies with a very small number of patients receiving an exceptionally high-priced therapy would likely change with a chemogenetic treatment. The indications envisioned for chemogenetics are larger patient populations with debilitating but not life-ending diseases. This democratization of gene therapy would be facilitated by the small amounts of viral vectors required for focal targeting, which are 100–1000s of times less than what is required for most current gene therapies. The long-term clinical potential of chemogenetics can potentially fill a significant gap in treatment options to improve the lives of millions of people that are afflicted by debilitating neurological and neuropsychiatric diseases.

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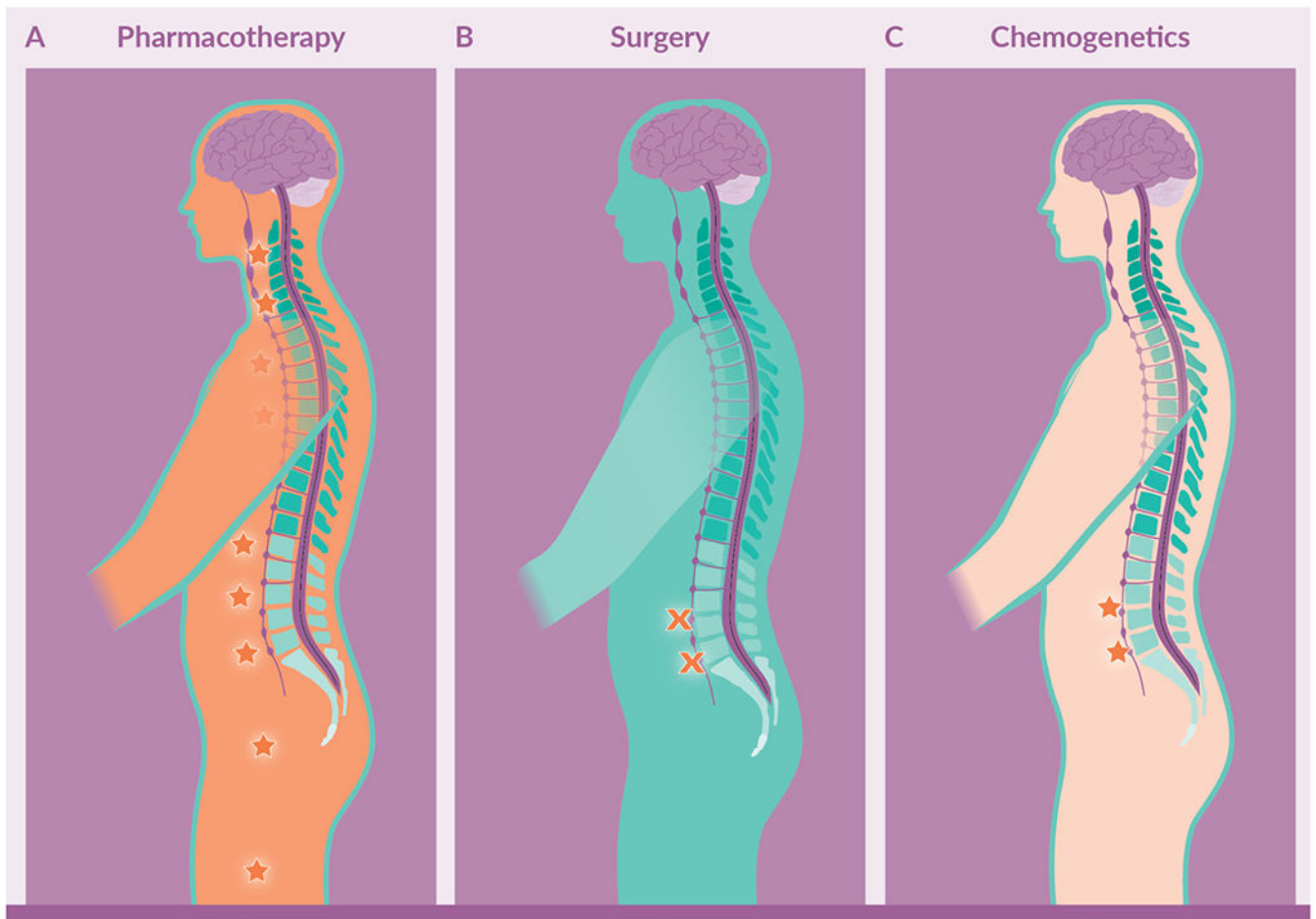


FIGURE 1. Three treatment approaches to nervous system therapy, example of chronic neuropathic pain.

Systemic pharmacotherapy results in widespread distribution of the drug (orange), which modulates (red stars) the affected sensory ganglia as well as all other sensory ganglia, the brain, and other peripheral organs that also express the target for the drug. Surgery procedures permanently disrupt the sensory ganglia (red x's) to block sensory transmission. Chemogenetics achieves local, tunable, and reversible neuromodulation by targeting a chemogenetic receptor solely to the neuropathic pain-causing ganglia. Ultrapotent chemogenetic receptors use low doses (light yellow) of the chemogenetic drug.

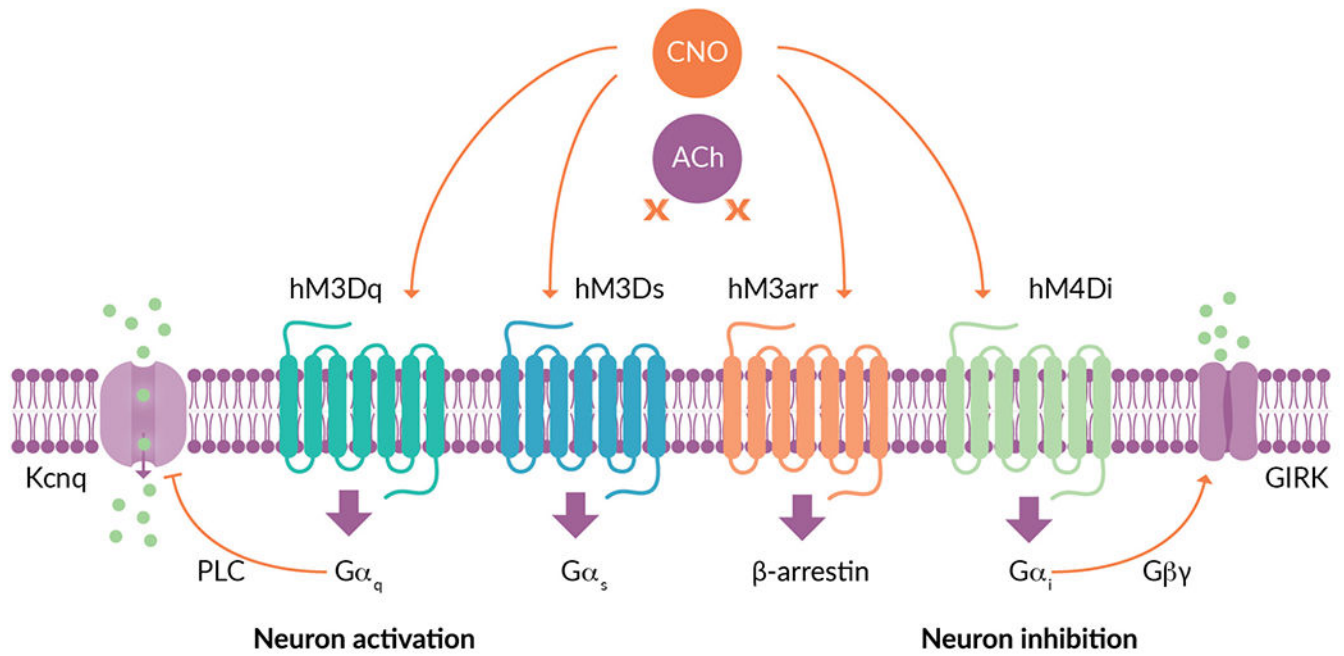


FIGURE 2. DREADD family of chemogenetic receptors.

Receptors based on modifications to the human muscarinic receptors are activated by CNO and have greatly attenuate responsiveness to ACh. hM3Dq activates neurons by inhibiting Kcnq potassium channels. hM4Di inhibits neurons by opening GIRK channels. Other DREADDs, based on modifications of hM3D, engage additional G-protein signaling pathways.

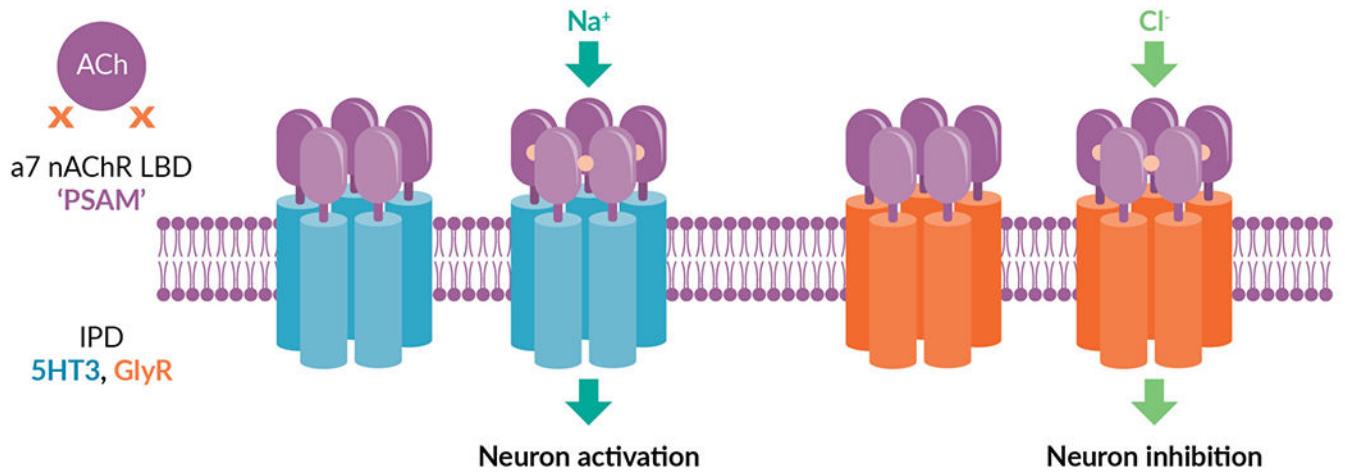


FIGURE 3. PSAM chimeric ion channels.

PSAMs developed from the ligand binding domain (LBD) of the $\alpha 7$ nAChR are spliced to either the IPD of 5HT3 or GlyR to produce chimeric channels for neuron activation or inhibition, respectively. The same PSAM and its cognate agonist (yellow circle) are used for both types of channel. Mutations in the LBD increase drug-potency and reduce ACh sensitivity. PSAM chimeric channels are homomeric pentamers.

TABLE 1

Treatment approaches for nervous system disorders.

	Advantages	Disadvantages
Pharmacotherapy	<ul style="list-style-type: none"> ▶ Molecular targeting ▶ Dose-dependent dynamic range ▶ Reversible 	<ul style="list-style-type: none"> ▶ Systemic treatment of focal disorders ▶ CNS access of drugs ▶ Typically, indirect modulation of neuron electrical activity ▶ May only work in a specific patient population
Surgical resection	<ul style="list-style-type: none"> ▶ Eliminate disease tissue 	<ul style="list-style-type: none"> ▶ Permanent tissue loss ▶ Post-operative side effects ▶ Patient and physician stigma ▶ Need for repeated surgeries
DBS	<ul style="list-style-type: none"> ▶ Local targeting ▶ Scalable ▶ Reversible ▶ Real-time control 	<ul style="list-style-type: none"> ▶ Surgery with permanent implant ▶ Local targeting reduced by activation axons-of-passage ▶ of or of mechanism of neuromodulation is unclear ▶ Hardware-related complications
Gene therapy (traditional)	<ul style="list-style-type: none"> ▶ Replacement of missing or dysfunctional gene product ▶ Can be locally or broadly targeted 	<ul style="list-style-type: none"> ▶ Static effect, no additional control over neuromodulation ▶ Irreversible ▶ Usually cannot non-invasively assess localization and expression
Chemogenetic gene therapy	<ul style="list-style-type: none"> ▶ Cell-type-specific targeting ▶ Dose-dependent dynamic range ▶ Pharmacologically reversible ▶ Local targeting ▶ Mechanistically straightforward ▶ PET to non-invasively assess localization and expression 	<ul style="list-style-type: none"> ▶ Best suited for local brain disorders ▶ Non-natural elements in engineered proteins ▶ Three components (small molecule, receptor, AAV)

TABLE 2

Nervous system disorders potentially suitable for chemogenetic gene therapy.

Nervous system disorder	Circuit pathophysiology	Neuromodulatory focus	Conditions/unmet medical need
Neuropathic pain	Ectopic firing and hyperexcitability of ganglionic cell bodies	Peripheral neuronal ganglia e.g. trigeminal and dorsal root ganglia	Pharmacotherapy-resistant trigeminal neuralgia, lower back pain, osteoarthritic joint pain, intractable sciatic nerve pain
Epilepsy	Electrophysiologically mapped cortical hyperexcitability leading to focal seizure	Cortical tissue at site of epileptic foci Options for increasing the activity of inhibitory interneurons or decreasing the activity of excitatory neurons	Treatment-refractory epilepsy
Parkinson's disease	Loss of dopamine neurons leads to hyperactivity of the striatum. The internal segment of the globus pallidus (GPi) and subthalamic nucleus (STN) becomes over-activated, inhibits motor thalamus, and suppresses movement	Inhibition of hyperactivity of the GPi and/or STN	Parkinson's disease movement initiation
Obesity	Disruption of hypothalamic signaling in neurons that regulate appetite	Inhibition of appetite-promoting neurons or activation of appetite-suppressing neurons	Pharmacotherapy-resistant monogenetic obesity disorders, morbid obesity