

Chemogenetics: Beyond Lesions and Electrodes

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The field of chemogenetics has rapidly expanded over the last decade, and engineered receptors are currently utilized in the lab to better understand molecular interactions in the nervous system. We propose that chemogenetic receptors can be used for far more than investigational purposes. The potential benefit of adding chemogenetic neuromodulation to the current neurosurgical toolkit is substantial. There are several conditions currently treated surgically, electrically, and pharmacologically in clinic, and this review highlights how chemogenetic neuromodulation could improve patient outcomes over current neurosurgical techniques. We aim to emphasize the need to take these techniques from bench to bedside.

KEY WORDS: Chemogenetics, Chemogenetic neuromodulation, Designer receptor activated by designer drugs (DREADD), Translational medicine, Viral vector

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The last 5 decades have seen a gradual shift in functional neurosurgery away from lesioning techniques, which caused irreversible damage to the nervous system, toward the use of implanted devices for targeted delivery of drugs and electric current. Hence, contemporary functional neurosurgical therapies are nondestructive and dynamic. Nonetheless, the introduction of implanted devices increased cost and introduced a set of device-related complications. Moreover, the capacity of electric current to act promiscuously on multiple neuronal and even glial cells limited the specificity of neural control in any given neural target. A new technology stands poised to replace device mediated neuromodulation.

ABBREVIATIONS: **AAV**, adeno-associated virus; **BBB**, blood brain barrier; **CNO**, clozapine N-oxide; **CNS**, central nervous system; **CRPS**, complex regional pain syndrome; **DREADD**, designer receptor exclusively activated by designer drugs; **GPCR**, G-protein coupled receptor; **GluCl**, glutamate-gated chloride channel; **GlyR**, glycine receptor; **HSV**, herpes simplex virus; **KOR**, kappa opioid receptor; **LGC**, ligand gated channel; **mAChR**, muscarinic acetylcholine receptor; **PD**, Parkinson disease; **PSAM**, pharmacologically selective actuator module; **PSEM**, pharmacologically selective effector molecule; **SalvA**, salvinorin A; **SCS**, spinal cord stimulation; **STN**, subthalamic nucleus

Chemogenetics refers to the modulation of neural activity through neurotransmitter receptors that are genetically engineered to bind specific exogenous ligands, usually biologically inert small molecule drugs. In this regard, it represents an inversion of the normal method of pharmacology that engineers ligands to bind specific receptors. The fact that the nervous system may use the same receptor to affect different functional roles in different neuroanatomical locations has always limited the capacity of this approach to achieve real specificity. In other words, even an infinitely specific drug binding the same receptor in different parts of the brain will cause off-target side effects. In contrast, the ability to surgically deliver designer receptors to various focal anatomical targets of the nervous system is opening the door to a heretofore unattainable degree of specificity while lacking the complications associated with implanted devices.

Chemogenetics has been widely used as a research tool in neuroscience and applied in animal models; however, no chemogenetic techniques are currently utilized in human therapies or clinical trials. Currently, pharmacological treatment is the standard for first line treatment of movement disorders, pain, epilepsy, spasticity, and psychiatric conditions, with device mediated neuromodulation providing a means to treat “medically refractory” disease. Safe chemogenetic approaches that allow for device-free targeted neuromodulation coupled with

complication-free drug therapy may become frontline therapies for many of these disorders, hence dramatically expanding the role of functional neurosurgery while better serving our patients. Two fledgling companies have emerged recently to develop safe and practical tools for clinical chemogenetic neuromodulation.

MECHANISMS OF CHEMOGENETIC NEUROMODULATION

G-protein coupled receptors (GPCRs) are ubiquitous and facilitate most signal transduction processes in the mammalian brain.¹ GPCR activity can be difficult to study in Vivo. Endogenous activation of GPCRs can confound experiments pharmacologically targeting GPCRs. Any ligand used to activate a GPCR will do so systemically and cannot be targeted to a particular brain area or cell type.² By genetically altering specific GPCRs, researchers can selectively activate a particular cell type in a predictable manner.³ Engineered GPCRs called designer receptors exclusively activated by designer drugs (DREADDs) can facilitate neuronal excitation and inhibition with both spatial and temporal control. Different cellular signaling cascades are altered depending on the class of G-protein coupled with the receptor, which include the G_s, G_q, and G_i proteins.⁴ The G_s- and G_q-coupled proteins activate neurons through the activation of adenylyl cyclase and phospholipase C, respectively. Activation of G_i proteins inhibits cellular activity by reducing intracellular levels of cAMP. The most utilized GPCR mutation targets muscarinic acetylcholine receptors (mAChRs), which are engineered to have affinity for clozapine *N*-oxide (CNO), a drug that is thought to have minimal effects when administered systemically. One of the most extensively studied excitatory DREADD platforms utilizes a mutant human M3 acetylcholine receptor associated with a G_q-coupled receptor (hM3Dq) to induce neuronal activation. The most used inhibitory DREADD is a mutated human muscarinic M4 receptor that works through the G_i pathway (hM4Di) to hyperpolarize cells. In addition to mAChRs, kappa opioid receptors (KORs) have also been utilized as a chemogenetic platform. KORs are G_i-coupled receptors that bind salvinorin A (SalvA), a potent hallucinogen.⁵ Vardy et al⁶ developed an engineered KOR (KORD) that responds to a metabolite of SalvA, salvinorin B that is biologically inert in clinical dosages. This inhibitory KORD can be coexpressed with excitatory DREADDs in the same cell population to provide precise bidirectional neuronal control.^{7,8} These DREADDs are a powerful tool currently utilized in chemogenetic research both in Vivo and in Vitro, and this technique shows promise for clinical translation.⁹ The 2 characteristics of DREADDs that provide this specific activation are (1) a biologically inert ligand that will only act on engineered receptors (eg, CNO), and (2) a modified GPCR that is activated by this ligand but not endogenous neurotransmitters and preferably no other drugs.¹⁰ Not all neurotransmitter receptors are G-protein coupled. Ion channels directly facilitate the flow of cations or anions across the cellular membrane to either depolarize or hyper-

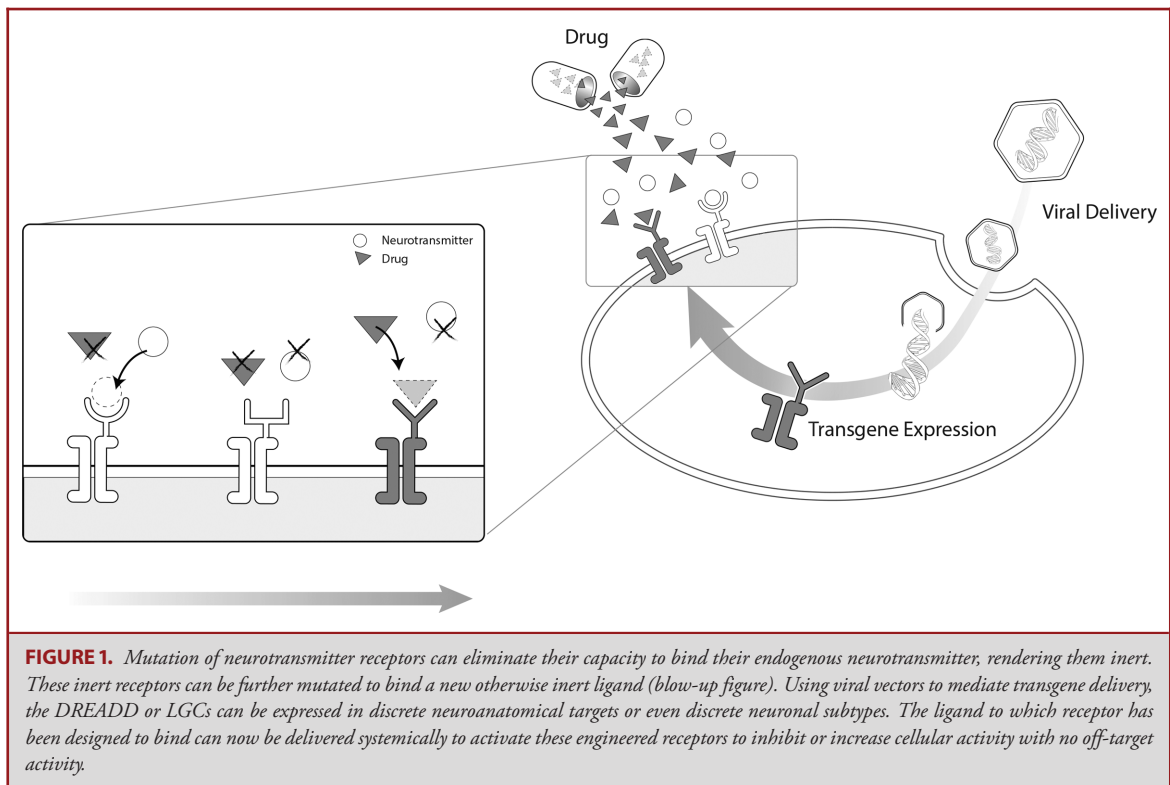
polarize cells.¹¹ Direct ligand gated channels (LGCs) can also be altered through genetic engineering to bind specific otherwise inert drugs. Although now used less frequently than metabotropic mechanisms, LGCs can be chemogenetically manipulated to directly control cell membrane potential.⁴ The anthelmintic drug ivermectin can silence neuronal populations that express a modified glutamate-gated chloride channel (GluCl).¹² Neuronal firing can also be reduced by expressing an engineered glycine receptor (GlyR) activated by ivermectin.¹³⁻¹⁵ However, the clinical potential for ivermectin-based systems is limited by the unwanted binding of ivermectin to unintended receptors in the brain, most notably, GABA_A.¹⁶

The Sternson laboratory developed a technique to engineer the ligand-binding domain of the alpha 7 nicotinic acetylcholine receptor to abate affinity to endogenous acetylcholine and instead bind various selected pharmacologically selective effector molecules (PSEMs). These engineered binding sites fused to ion channels are referred to as pharmacologically selective actuator modules (PSAMs).¹⁷ Depending on the ion pore utilized in this technique, cell activation or inhibition can be achieved. Two of the most notable combinations are PSAMs spliced with the serotonin receptor 3a (5-HT₃) and the GlyR. PSAM-5HT₃ activation results in cation influx inducing neuronal depolarization. PSAM-GlyR activation allows anion intake, resulting in hyperpolarization and neuronal inhibition. Various agonists of the drug varenicline have been developed as “ultrapotent” PSEMs and have been very effective neuronal modulators in animals, including primates.¹⁷ Varenicline is well tolerated in low doses and has excellent central nervous system penetrance, thus making this platform desirable for clinical translation. Another therapeutic advantage of this system is that multiple PSAM/PSEM complexes can be utilized at once to provide bidirectional neuromodulation. Although promising, limitations to this technique such as limited ligand bioavailability and functional changes to native synaptic and axonal circuits must be addressed before human application.^{4,18,19} See Figure 1 for a visualization of chemogenetic engineering.

VIRAL GENE THERAPY FOR CHEMOGENETIC RECEPTOR DELIVERY

For DREADD techniques to be used in Vivo without the use of a transgenic model, the genes for modified receptors must be expressed in the host tissues. There are several methods of viral delivery that can achieve this result. The purpose of these viral vectors is to integrate therapeutic genes (in this case, a modified receptor) into a desired cell type within a confined anatomic region of interest which is dependent on characteristics of the virus.^{20,21} The principle viral vectors used for gene delivery to the nervous system include adeno-associated virus (AAV), lentivirus, and herpes simplex virus (HSV).

Lentivirus is a type of retrovirus that can stably integrate into host deoxyribonucleic acid (DNA).^{22,23} Lentiviruses infect



both dividing and nondividing cells, which allows for sustained transgene expression and viral replication.^{24,25} However, there is evidence of inflammatory response and mutagenesis with lentiviral delivery in humans.²⁶ More recent gene editing techniques have allowed researchers to reduce this risk in lentiviral delivery.²¹ Lentiviral vectors could be a useful tool for the delivery of chemogenetic receptors, especially considering lentiviral vectors can be pseudotyped with envelope glycoproteins that allow for effective transduction in desired cell types (eg, neurons).²⁷⁻²⁹ Lentivectors are in use in human Parkinson disease (PD) trials.³⁰ Because they stably insert transgenes into the genome, lentiviral vectors have also been used for the delivery of genes to therapeutic stem cell lines used in human clinical trials, such as the phase I trial conducted by Clive Svendsen and Robert Baloh at Cedars-Sinai Medical Center (NCT02943850).³¹

HSV-1 is a DNA virus that has naturally evolved to be an enticing candidate for gene delivery into the central nervous system (CNS), as it is inherently neurotrophic. The virus infects peripheral tissues (epithelial cells of skin or mucous membranes) and lytically multiplies. De-enveloped virus can then enter sensory neurons that innervate the affected area. The virus invades sensory ganglia (DRG or trigeminal ganglion) via retrograde transport and remains latent in those cells until subsequent secondary infections occur.²⁴ Modified HSV-1 viral vectors are replication deficient and insert a therapeutic gene into the viral genome without damage to the cell from viral proliferation.³²

HSV-1 can also be used to create amplicon vectors. These have no lytic function, require the use of a helper virus, and can deliver large genes into human cells.³³ Newer amplicon vector production techniques yield higher viral titers of large genetic packages.³⁴ HSV-1 vectors have several characteristics that make them desirable for neurological clinical application: the viral DNA does not integrate into the host chromosome reducing the risk of mutagenesis, the virus can infect both quiescent and proliferating cells (glia and neurons), it can carry relatively large genetic packages, it is neurotrophic, and it can spread across synapses in both a retrograde and anterograde fashion.³⁵ HSV delivery of ligand-dependent neuromodulatory tools has been displayed in Vivo. The Glorioso Lab peripherally injected an HSV vector to express the alpha subunit of a glycine receptor (α 1) in sensory afferents. Local administration of glycine reduced nociception in multiple models of pain in a controlled and reversible manner.³⁶

AAVs have been used in most contemporary neural gene therapy trials not involving brain tumors. These vectors are derived from dependoviruses that require helper viruses to replicate. Lacking any original viral genes, they cannot replicate within host cells, but they can produce long-term gene expression without insertion into the host genome.³⁷⁻³⁹ This reduces complications associated with insertional mutagenesis as seen with the use of other viral vectors, such as lentivirus. Using AAVs, long-term gene expression is possible without the risk of cytotoxicity.

Multiple AAV serotypes infect a myriad of cell types and have distinct tropism.^{40,41} This increases cellular specificity, which provides a more tailored approach to therapy. Naturally occurring AAV serotypes show different tropisms for unique cellular types by binding to different cell-surface receptors.⁴² AAV1, AAV2, AAV4, AAV5, AAV6, AAV8, and AAV9 are the most common serotypes studied in the CNS.⁴¹ Each serotype has a distinct viral capsid that elicits a unique antigenic response and selectively binds cells expressing particular receptors. AAV2, the most extensively studied AAV serotype, primarily binds heparin sulfate proteoglycan.⁴³ Via this mechanism, AAV2 effectively transduces the CNS, kidney cells, and photoreceptor cells in the eye. Targeted virus delivery of AAV2 into the CNS can further refine transduction to avoid unwanted gene expression outside of the area surrounding the injection, making this serotype ideal for stereotactic delivery into the CNS. Other serotypes can effectively transduce glial cells such as ependymal cells,⁴⁴ oligodendrocytes,⁴⁵ or astrocytes.⁴⁶ Recombinant AAV capsids can also be engineered to combine proteins of multiple serotypes to create a hybrid virus.^{28,47} Rational capsid design involves inserting a known peptide sequence into the viral capsid in order to control ligand binding, but this requires extensive knowledge of the capsid structure and what receptor it binds.^{40,48} The primary and secondary receptor sites for some AAVs are currently unknown.⁴¹ Capsid shuffling is an alternative to peptide insertion. In this technique, capsid genes of multiple AAVs are digested, combined, and reassembled at random to create chimeras of AAV capsid genes. These capsids are then tested in Vitro or in Vivo to determine if they selectively and effectively transduce desired target tissues.^{24,49} In addition, a new “targeted evolution” technique can be used to select novel capsids from libraries of random peptide inserts that have particular binding and distribution properties. Additionally, transgene expression can be more precisely targeted by use of neuronal or glial promoters that can drive or increase gene expression in a specific cell subpopulation of interest. In addition to cell-specific transduction, certain AAV capsid modifications can also circumvent immune responses to avoid the need for immunosuppression.^{50,51}

DREADD expression in this context can be restricted to the CNS with promoters like preproenkephalin, neuronal specific endolase, and glial fibrillary acidic protein.⁵² Transcapsidization of viral vectors, utilization of cell-specific promoters, and targeted injection of viral vector into a chosen brain area can all be combined to achieve expression of chemogenetic tools with a level of specificity that is far superior to current electrical or pharmacological therapies.^{46,53}

A large constraint of gene delivery for neurological disorders is that many viral vectors have limited blood brain barrier (BBB) penetrance, thus many proposed DREADD treatments would require invasive surgery. AAV9 can effectively cross the BBB, allowing for widespread CNS gene delivery from blood or cerebrospinal fluid (CSF), but systemic delivery of AAV9 would mitigate the benefit of anatomical specificity that makes chemogenetic treatment so appealing.⁵⁴ There are certain pathologies

that may benefit from more generalized transduction of receptors throughout the brain and spinal cord. In this case, CSF-mediated delivery of viral vector would be warranted. However, transduction of viral vectors can vary widely with CSF administration, and this limits any penetrance of vector into the deeper parenchyma of the brain.^{40,55} For targeted transduction to dorsal root ganglia (discussed in detail later), intrathecal delivery appears to be the most promising route of administration.⁵⁶ Additionally, focused ultrasound can mechanically disrupt the BBB to allow for the delivery of therapeutics.⁵⁷ Hyperosmolar solutions like mannitol and vasoactive drugs are also being investigated for improved drug delivery to the CNS via arterial infusion.⁵⁸ One of the benefits of chemogenetic neuromodulation is the ability to affect discrete areas, which would require a direct injection approach, whether into the brain, spinal cord, or peripheral nervous system. This need is likely to keep chemogenetic neuromodulation in the purview of neurosurgeons for the foreseeable future.

BENEFITS OVER OPTOGENETICS

Previously, we reviewed the potential application of optogenetics in neurosurgery.⁵⁹ Optogenetic techniques modulate individual receptors engineered with light sensitive chromophores. The most used receptors are the excitatory channelrhodopsins and the inhibitory halorhodopsins. Optogenetics can excite cells with the introduction of light; however, there is much to be desired from this technique in human translation. Although instant effects can be beneficial, the therapeutic effect of optogenetics requires a sustained light source for treatment. These light sources usually must be implanted, which is invasive and requires maintenance on the light source. On top of that, light can increase local temperatures and cause parenchymal injury. In addition, although light can be administered to discrete areas of the nervous system, there is evidence of light scattering and diffusion in Vivo.⁶⁰ Additionally, optogenetic modulation depends on the ability to administer light to the area of interest. For example, it is easier to implant fiber optic devices into areas like cerebral cortex but presents more complications when targeting deeper nuclei. It should be noted, though, that optogenetics have the advantage of a more precise temporal resolution when compared to chemogenetic neuromodulation, as the latter is dependent on the bioavailability of the ligand and delivery through the systemic circulation.

Because of the aforementioned features of chemogenetics, it may prove to be a powerful neuromodulation tool in a wide array of applications. Binding of a synthetic designer drug to DREADDs or LGCs can induce cell-specific activation or inhibition. The benefit of “wireless neuromodulation” makes this approach attractive when compared to the traditional deep brain stimulation (DBS) and optogenetic stimulation systems.⁵⁹ In contrast to optogenetic neuromodulation, chemogenetics do not generally provide a very high temporal resolution. This,

however, is not necessarily considered a limitation, but rather renders chemogenetics more suitable for a variety of disease processes that do not require millisecond time scale control. Of note, the study of neuroplasticity underlying both learning and memory as well as functional disorders extensively implicates intracellular second messenger cascades with associated protein phosphorylation. Because DREADDs work through G-proteins, they may prove to be a tool to affect therapeutic neuroplasticity.

LIMITATIONS OF CHEMOGENETIC NEUROMODULATION

A few limitations of chemogenetic neuromodulation have been highlighted in the preceding sections; however, it is important to highlight these limitations in the context of translation to human therapy. Although there is a vast potential to chemogenetic neuromodulation, several limitations need to be further studied and addressed. A major concern of this therapy is the potential for unwanted retrograde viral spread of the vector; this could lead to significant off-target effects. This is less of a concern with AAV vectors, but further research is needed to confirm that there is no transient or unwanted spread of the chosen viral vector for each given application. Another source of off-target effects could be related to the PSEM or designer drug chosen for a given therapy. Varenicline is a drug currently being studied in the context of chemogenetic modulation, and it is noted that it is already Food and Drug Administration approved and therefore easier for translation into humans. However, the long-term studies for this drug do not extend past 52 wk. Chemogenetic modulation would theoretically require lifetime administration of medication for any incurable pathology, including ones that are medically refractory. Any chemomodulatory agents would need additional validation to show a low adverse effect profile, especially with lifetime administration. Long-term studies of these drugs would also need to prove that there is no activation of endogenous or native receptors, which would also cause off-target effects. Additionally, if there are genetic and functional changes to endogenous neural circuits in response to chemogenetic neuromodulation, these would need to be further defined and characterized. Substantial changes to native circuits could be extremely detrimental in cases of limited patient compliance or medication scarcity. Although no longer in its infancy, gene therapy is still a new technology with many challenges, including the long-term stability of gene expression, immune response to foreign genes, and unpredictable spread leading to off-target effects. However, the fact that chemogenetic transgenes are inert until coupled to their paired ligands adds a margin of error. There are very few large animal and translational studies being conducted to explore chemogenetic neuromodulation and address these limitations. We hope to highlight why it is important for the medical community to shift the narrative that chemogenetic modulation is an exploratory tool and instead a therapeutic one.

APPLICATIONS OF CHEMOGENETICS IN NEUROSURGERY

Pain

Intractable neuropathic pain syndromes represent a promising field with a variety of applications of chemogenetic technology. Weir et al⁶¹ showed that use of chemogenetic technology silenced aberrant activity and reversed allodynia in a peripheral nerve injury mouse model. In addition, they reported that the delivered GluCl was sustainably expressed in sensory neurons.⁶¹ Our group and others have targeted the trigeminal ganglion with implanted stimulators for the management of intractable facial pain.^{62,63} However, this approach is associated with a relatively high complication rate associated with the implanted stimulation system, causing lead erosions and infections.⁶² Similarly, electrical stimulation of the DRG for the management of a variety of pain syndromes has already demonstrated a pain alleviating effect.^{64,65} There are a variety of indications for DRG stimulation, including thoracic neuralgia, postherpetic neuralgia axial back pain, perineal pain, diabetic neuropathy, phantom limb pain, and complex regional pain syndrome (CRPS). However, electrical stimulation lacks spatial resolution, and current dissemination can lead to activation of the motor root and cause unwanted side effects. Also, like the trigeminal ganglion stimulation, the implanted system is prone to infections and a number of complications. In addition, dorsal root entry zone lesioning is a neurosurgical procedure commonly indicated for intractable nerve root avulsion pain. However, this is a destructive irreversible procedure with associated neurologic morbidity. Similarly, sympathectomies for CRPS or other indications – though not commonly used – are destructive and associated with a number of complications. In this section, chemogenetic applications for all these procedures will be discussed. Delivery of chemogenetic viral vectors to sensory ganglion will be the first wave of clinical trials by the new biotechnology companies because of the inherent accessibility of these structures and the fact that ligands need not penetrate the BBB. For example, viral vector delivery directly to the trigeminal ganglion can be accomplished easily through an approach similar to glycerol rhizolysis or percutaneous trigeminal ganglion stimulation. These standard of care approaches to trigeminal neuralgia are destructive, causing numbness that can ultimately result in anesthesia dolorosa and corneal anesthesia with its ocular complications. Similarly, the anticonvulsant medication used to treat trigeminal neuralgia often affects mentation, arousal, and balance complicating treatment of working adults and the elderly. Trigeminal chemogenetics could provide a nondestructive, adjustable, highly specific pharmacological treatment for both classic trigeminal neuralgia and other forms of facial neuropathic pain currently being treated with implanted devices with limited success. Because chemogenetic inhibition can be adjusted, a higher level of inhibition can be achieved without concern for permanent corneal anesthesia.

Just as the trigeminal ganglion is accessible via standard percutaneous techniques, revision spine surgery often exposes the DRG. Recurrent radicular pain following discectomy often results from intrinsic damage to the nerve root or scar related entrapment. The current surgical frontline option for neuromodulation in these patients with persistent radicular pain is spinal cord stimulation (SCS).^{66,67} DRG stimulation was developed, in part, to increase the spatial resolution of other neuromodulatory treatments (ie, attempting to target individual dermatomes with epidural SCS is challenging). During fusion or redo discectomy, the affected DRG can be exposed, allowing the operating neurosurgeon to inject a chemogenetic vector, giving the surgeon and patient a means to control persistent pain from that DRG without an implanted stimulator. Alternatively, computed tomography (CT) or magnetic resonance imaging-guided DRG injection of chemogenetic vectors would achieve a high spatial resolution by modulating the pain efferent neurons in the DRG through a “wireless” system.^{68,69} At the same time, unwanted side effects associated with electrical DRG stimulation such as current dissemination and activation of the adjacent motor root would be avoided.

Although the first generation of chemogenetic vectors will likely utilize ubiquitous promoters that drive gene expression in all DRG neurons, recent work has identified proteins uniquely expressed in pain neurons (TrpV1).⁷⁰ Pain neuron specific promoters may provide enhanced specificity of therapy allowing for selective elimination of pain without effects on proprioception or tactile sensation. Indeed, promoter-based selective expression of chemogenetic receptors can allow for higher doses of the ligand with a resulting higher level of neuronal silencing eliminating general reduction in sensation in favor of selective analgesia. Similarly, DRGs have proven to take up a variety of AAV serotypes with particular avidity following CSF injection.^{71,72} If chemogenetic receptors can be selectively expressed in pain neurons, using selective promoters, a simple lumbar puncture may allow for chemogenetic control of a wide range of pain syndromes from failed back syndrome to diabetic neuropathy.

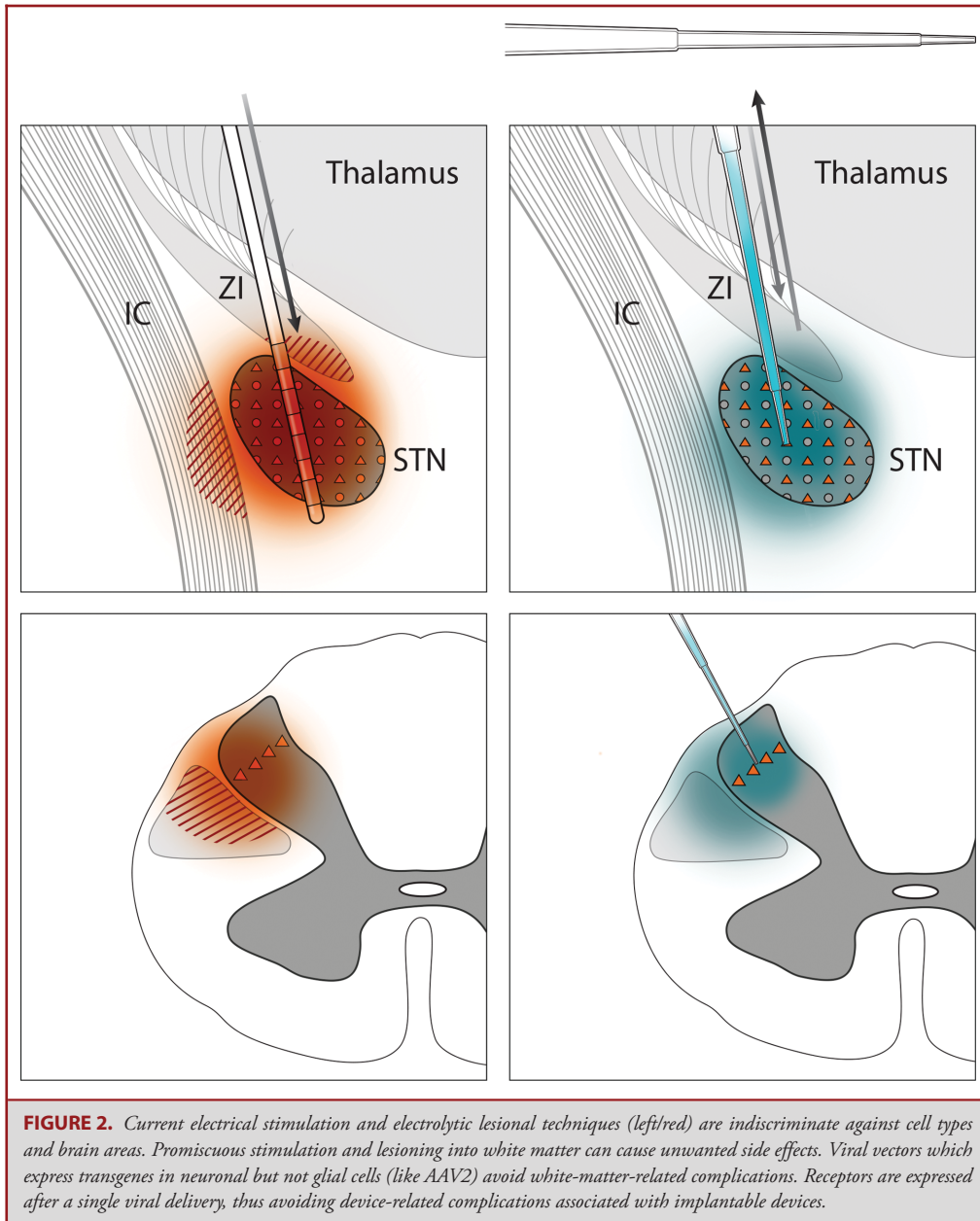
Brachial plexus injuries accompanied with root avulsions can cause a characteristic constant pain syndrome, often intractable to multiple therapies.⁷³ It is not uncommon for these patients to need neurosurgical interventions; currently DREZ lesioning is an option with durable pain relief in the majority of the patients.^{74,75} However, this destructive procedure induces irreversible changes and has a risk for major neurological morbidity. Common complications of this procedure are leg weakness and loss of proprioception and vibration due to the proximity of the dorsal horn to the corticospinal tract and dorsal columns, respectively.⁷⁶⁻⁷⁸ Chemogenetic injections with smaller-gauge needles in the dorsal horns could prove to be safer than DREZ lesioning, devoid of the major neurological morbidity. In addition, with chemogenetics, expression of the GluCl receptors could be achieved only in the pain-afferent neurons in the dorsal horn by using a specific promoter, achieving accurate inhibition of these neurons when the synthetic drug binds this receptor. This would be a wireless

neuromodulation option with accurate cell-specificity, inducing irreversible changes in the spinal cord and low risk for damage to the adjacent structures (Figure 2).

The sympathetic chain is another potential target in which chemogenetic technology could be applied to several diseases. More specifically chemogenetic sympathetic neuromodulation for palmar and axillary hyperhidrosis, CRPS, and diseases, causing vasoconstriction due to excessive sympathetic activation, could prove to be beneficial.⁷⁹ Thoracic endoscopic sympathectomy has shown high efficacy in patients with palmar and axillary hyperhidrosis; however, this destructive and irreversible procedure is associated with a 55% rate of compensatory sweating.⁶⁹ Targeting the same thoracic sympathetic levels to deliver viral vectors either thoracoscopically or through a CT-guided approach could potentially provide the same benefit but without inducing irreversible changes to the sympathetic chain.⁸⁰ In addition, the pathophysiology of CRPS is also thought to involve the autonomic nervous system and, more specifically, the thoracic and lumbar sympathetic chain.⁸¹ This is also supported by the fact that sympathetic blocks appear to be efficacious in a subset of patients with CRPS.^{82,83} With that in mind, a similar approach to palmar and axillary hyperhidrosis could be implemented to modulate sympathetic outflow and manage CRPS. It is important to note that sympathetic blocks are a transient pain relief method, whereas chemogenetic neuromodulation could achieve pain relief every time the synthetic drug is ingested and bound to the DREADD or LGC. Additionally, chemogenetic modulation may also have a role in autoimmune diseases, inducing excessive vasoconstriction through overexcitation of the sympathetic nervous system.^{79,84} For instance, Raynaud disease and generalized scleroderma associated with digital ulcers have been managed with stellate ganglion blocks that provide transient benefits owing to increased blood flow, which leads to ulcer healing.⁸⁵ Chemogenetic modulation through transgene delivery to the stellate ganglion could modulate its activity, increase blood flow to the limbs, aid in digital ulcer healing, and obviate the need for multiple procedures that would be needed with stellate blocks.

Epilepsy

Drug-resistant epilepsy is another disorder which could benefit from potential chemogenetic applications. Several experimental gene therapies have been proposed for the treatment of drug-resistant epilepsy; however, chemogenetics have the advantage of on-demand modulation of the epileptogenic zone.⁸⁶ A number of neuronal transduction options have been proposed including the use of an inhibitory (Gi) DREADD that renders neurons less excitable in the presence of the synthetic ligand or the use of an autoregulatory receptor (eg, eGluCl) that increases chloride conductance when extracellular glutamate is elevated.⁸⁶ The potential benefit of these applications in experimental studies is well-established. Seizures have been sustainably suppressed by activating DREADDs in different animal models (ie, acute chemoconvulsant rodent model, chronic epilepsy rodent model,



mouse intrahippocampal model, and mouse intraperitoneal pilocarpine model).⁸⁷⁻⁹⁰

From a clinical standpoint, mesial temporal lobe sclerosis, gray matter heterotopia, and other lesional epilepsy types often warrant destructive and ablative procedures.⁹¹⁻⁹⁵ Even though epilepsy surgery is associated with acceptable seizure free survival rates, the authors believe that chemogenetics could provide a superior safety and efficacy profile in the management of drug-resistant epilepsy. Temporal lobectomy and selective amygdalohippocampectomy could be replaced by viral vector delivery in these struc-

tures, inducing cell-specific inhibition.^{96,97} Similarly, invasive surgeries could also be avoided in the case of gray matter heterotopias, and instead of resections, transgene expression in these structures could prove to be of benefit and eliminate lesional seizures.⁹⁸ In addition, the potential chemogenetic counterparts of epilepsy surgery would not only suppress seizures by targeting the lesions where seizures originate but also greatly reduce the risk for major neurological morbidity associated with a destructive and irreversible surgery (see Figure 2). However, it should be noted that chemogenetics face the same challenges

as resection/ablation if there is uncertainty in identifying the epileptic focus. In the case that the focus is clearly identified, targeted injection would be required for possible sustained elimination of seizures. Therefore, implementation of precise stereotactic techniques would be required for on-target injections, as injections close to but not within the epileptogenic focus would fail to eliminate seizures. Epilepsy is probably the most extensively studied neurosurgical application in chemogenetics and owing to the increased number of gene therapy trials for other diseases, a gene therapy trial implementing chemogenetic technology for drug-resistant epilepsy is warranted.⁸⁶

DBS

Movement disorders are another group of diseases that could benefit from chemogenetic applications. PD, one of the most common movement disorders, can be managed with bilateral subthalamic nucleus (STN) stimulation, showing superior outcomes compared to unilateral pallidotomy in a randomized trial.^{99,100} In a systematic literature review by Hamani et al,⁹⁹ bilateral STN stimulation was associated with a variable rate of improvement in tremor, dyskinesias, rigidity, bradykinesia, gait, and postural instability. However, their study also reported a 19% adverse effect rate related to the stimulation itself and a 9% complication rate related to the implanted hardware.⁹⁹ Chemogenetic injections in bilateral STN could provide the best of both worlds by having at least the same efficacy rate but with a significantly superior safety profile. The high spatial resolution, along with the avoidance of permanently implanted hardware, would eliminate stimulation and hardware-related complications. Technically, this procedure would include all the steps performed during STN DBS implantation, except viral vectors would be injected once instead of lead implantation (Figure 2). The same concept could be applied when targeting globus pallidus interna for PD and dystonia or the ventral intermediate nucleus of the thalamus for essential tremor.¹⁰¹⁻¹⁰³

Indications for the use of DBS in psychiatric disorders are currently expanding.¹⁰⁴ A multitude of DBS targets have been proposed for the management of treatment-resistant depression, eating disorders, obsessive-compulsive disorder, and addiction.^{104,105} Prior to the introduction of DBS for these indications, destructive neurosurgical procedures had been implemented in a subset of patients. For example, anterior capsulotomy, cingulotomy, and limbic leukotomy were utilized for depression; however, these procedures are considered high-risk for permanent neurological morbidity.¹⁰⁶ DBS has been used to modulate dysfunctional regions and, namely, the nucleus accumbens, subcallosal cingulate cortex, medial forebrain bundle, inferior thalamic peduncle, lateral habenula, and STN for depression, OCD, anorexia, bulimia, schizophrenia, and addiction.^{104,107} Of note, transgene delivery to these regions would be simpler in neuronal targets vs white matter fibers and bundles. Transgene expression in nuclei could be achieved by any virus with appropriate tropism, but modulation of white matter tracts would

require retrograde axonal transport capabilities of the vector in order to inhibit or activate the region of interest. From a surgical standpoint, targeting the aforementioned regions would involve the same DBS procedure with the goal to deliver the viral vector but without implanting the stimulation system. In theory, chemogenetic neuromodulation could provide high spatial resolution that electrical stimulation lacks and no side effects of the synthetic drug, which can be a major issue with psychiatric medications.

CONCLUSION

Chemogenetic neuromodulation has the potential to completely change the face of neurological and neurosurgical treatment. Although there are some drawbacks to chemogenetics, refining and implementing this technique would have significant benefit to patients and practitioners. By targeting distinct anatomical regions and cellular populations with chemogenetic control, patients could avoid systemic side effects of medication, surgical complications, and the complications that arise from indiscriminating electrical stimulation. Chemogenetics are mostly viewed as a research tool to discover nuances of brain anatomy and circuitry, but the entire field would benefit from changing the perspective to a more translational approach.

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COMMENT

Neuromodulation has traditionally relied upon electrical stimulation as the method for altering neural activity. This approach has achieved great success in the clinical arena, although there are issues related to hardware complications and elucidating mechanisms of action. Recent transformational developments in the basic sciences suggest the possibility of alternative strategies for performing neuromodulation that circumvent some of these problems. This review focuses on one such approach, the use of designer receptors and ligands for chemogenetic neuromodulation. The authors provide a thorough introduction to the science of chemogenetics for the neurosurgical audience, including an important discussion of the theoretical advantages and current limitations of the technology. They then describe how chemogenetics could be applied clinically in the major domains of functional neurosurgery. When considering how best chemogenetics could one day be incorpo-

rated into clinical practice, it is important to keep in mind its strengths and weaknesses relative to other techniques. Chief among its advantages is the ability to modulate large areas of the nervous system in a cell-specific manner. This feature lends itself well to the treatment of pain disorders, as the authors aptly discuss. On the other hand, the temporal resolution of stimulation is less than electrical or optical techniques, and thus indications that require specific patterns of stimulation (e.g., theta bursts) may not be appropriate for chemogenetics. We believe that different molecular, cellular, and electrical methods will be complementary, and ultimately hybrid, tools in the functional neurosurgeon's arsenal, paving the way for a bright future in functional restoration.

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