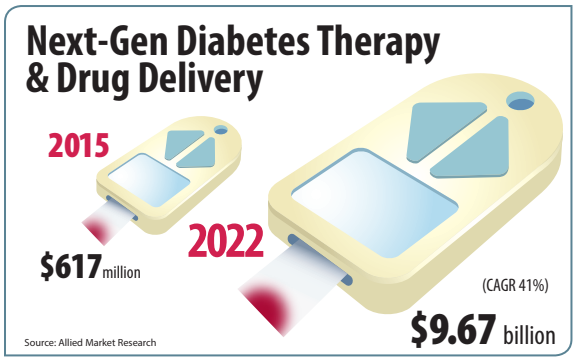
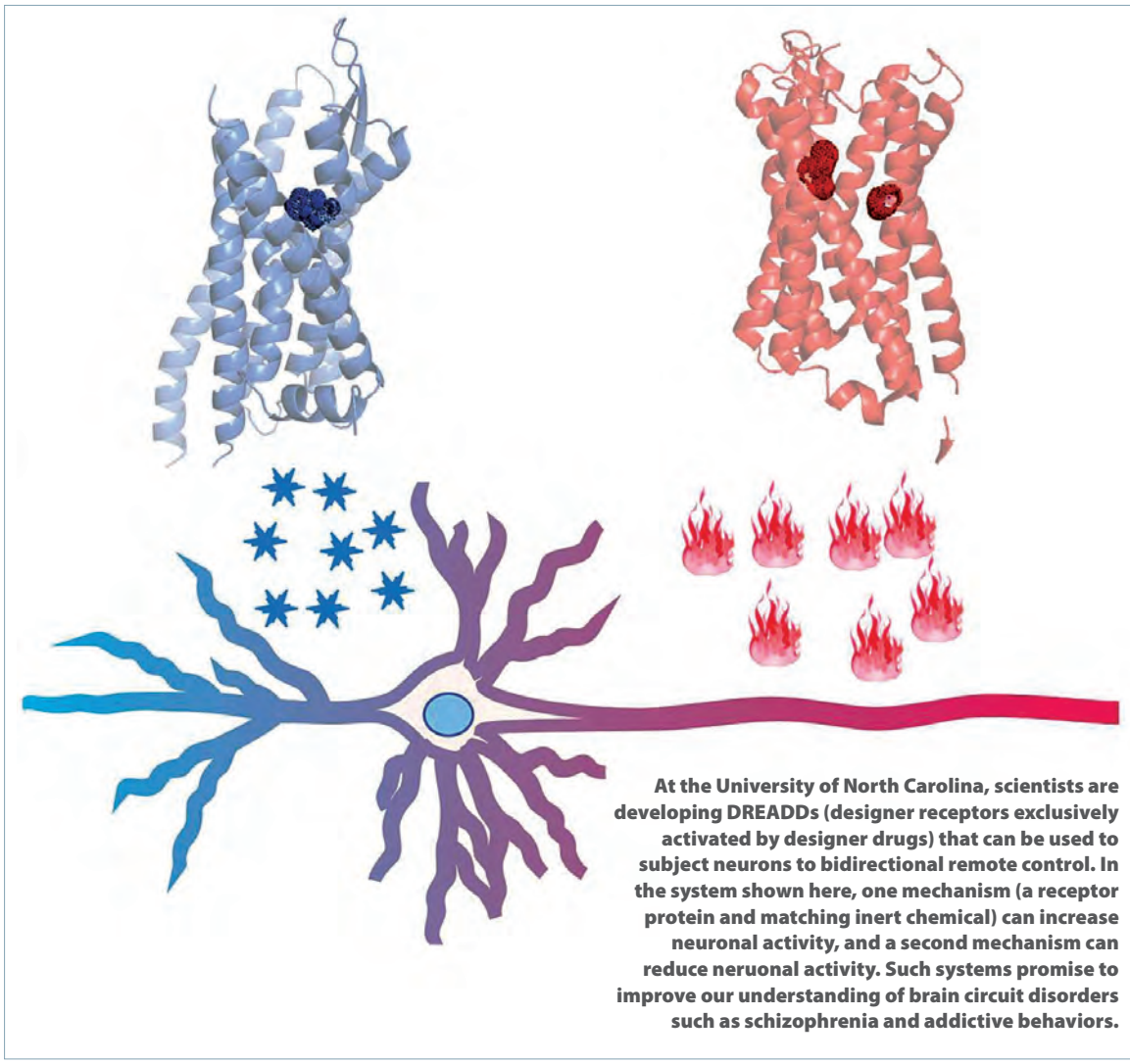
GEN Genetic Engineering & Biotechnology News





At the University of North Carolina, scientists are developing DREADDs (designer receptors exclusively activated by designer drugs) that can be used to subject neurons to bidirectional remote control. In the system shown here, one mechanism (a receptor protein and matching inert chemical) can increase neuronal activity, and a second mechanism can reduce neruonal activity. Such systems promise to improve our understanding of brain circuit disorders such as schizophrenia and addictive behaviors.

This is Your Brain on DREADDs

Caroline Seydel

The phrase "mind-control drugs" probably conjures up some terrifying images, but in the case of chemogenetics, it could be cause for rejoicing. To study the brain in any useful level of detail requires precise targeting of neural circuits, no easy task in an organ that's basically a thicket of long, interconnected cells—cells that send and receive a profusion of electrical and chemical signals.

The idea behind chemogenetics is simple: create a receptor that reacts only to a pharmacologically inert ligand, that doesn't do anything in the body. Then, stick that receptor into the particular neurons you want to influence. Once the cells start expressing the receptor, inject the ligand to activate the neurons, or inhibit them, depending on your receptor, with no unintended effects in other cells.

Receptors that exemplify the chemogenetic principle are called DREADDs (designer receptors exclusively activated by designer drugs). DREADDs sidestep the major issue of off-target effects, because they aren't found anywhere in the body except where the researcher puts them. The "designer drug" that activates them is usually clozapine-*N*-oxide, or CNO—or rather the CNO metabolite clozapine. CNO has minimal side effects at the dosages used for chemogenetics.

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Bispecific Playgrounds? No, Factory Floors

Patricia Fitzpatrick Dimond, Ph.D.

Bispecific antibodies (bsAbs) represent a relatively new and clinically validated class of therapeutic molecules. Three bsAbs have been approved for different therapeutic indications, and over 50 bsAbs are currently in clinical development.

Scientists have been particularly inventive in creating solutions to the fundamental problem of combining two antibody specificities into one molecule, with protein engineering playing a significant role in bsAb development. So many solutions have been devised that therapeutic developers may choose from a vast array of bsAb formats.

Different bsAb formats have distinct characteristics and support unique modes of action. When choosing or working

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In the mAb Race, Don't Stumble at Characterization

Vivienne Raper, Ph.D.

Monoclonal antibody (mAb)-based therapeutics are emerging as one of the fastest-growing categories of drugs being developed today. In fact, according to a recent analysis,[1] the market for mAbs has doubled in the last five years. Much of this growth is due to the emergence of new mAb-derived products, such as antibody-drug conjugates (ADCs) and bispecific antibodies (bsAbs).

These new biotherapeutics have a wide range of applications, including applications in cancer treatments and in antiviral treatments for acute and chronic diseases. Essentially, mAbs are multiple copies of the same lab-produced molecule. They work as substitutes for antibodies within the body, binding to antigens on the surface of a cell. By doing this, they can flag cancer cells for the immune system or eliminate infected cells displaying viral antigens on their surface. bsAbs, which constitute a class of newer and more complex mAb-related biotherapeutics, have two mAbs within the same drug to target two types of antigen.

Getting Competitive

As with most therapeutics, mAbs are developed through extensive selection of lead candidates, and the subsequent characterization of their size, shape, stability,

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This is Your Brain on DREADDs

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Chemogenetic Ups and Downs

DREADD pioneers include Bryan L. Roth, M.D., Ph.D., a professor in the Division of Chemical Biology and Medicinal Chemistry and in the Department of Pharmacology at the University of North Carolina Chapel Hill. Dr. Roth's first DREADD-related paper languished for two years before it was finally published in 2005. At the time, DREADD technology was a curiosity, but it's everywhere now.

"Nobody understood what this would possibly be useful for," he laughs. "It's nice to see that it has turned out to be a useful technology."

These days, Dr. Roth and his team are working to develop new DREADDs, with an eye to multiplexing. "We'd like to be able, if they're going to be used in humans, to have activating and inhibiting DREADDs in the same neuron," Dr. Roth says. This could be a way to exert fine control over treatment of symptoms that vary throughout the day, or to manipulate multiple neuronal circuits simultaneously.

Chemogenetics isn't the only way to target brain cells for activation: optogenetics allows researchers to activate or suppress neuron activity with pulses of light. The two technologies have different strengths, Dr. Roth points out, and many researchers use both.

"Optogenetics is very good if you want millisecond control," he notes. Chemogenetics, on the other hand, is easier to use, and more practical for activating larger populations of neurons. Instead of implanting light fibers all over the brain, "you can put the drug in the drinking water," Dr. Roth explains, and simultaneously activate all the cells containing your DREADD, wherever they are located.

Once the utility of DREADDs caught on in the neuroscience community, Dr. Roth's lab was inundated with requests for the plasmids. To keep up with demand, he deposited them with Addgene, a nonprofit organization whose mission is to make it easier for labs to share genetic engineering tools.

Chemogenetic Resources

"Any scientist from anywhere in the world can send their DNA to us," says Leila Haery, Ph.D., senior research scientist at Addgene. "Our role is to minimize the time that scientists spend dealing with the logistics of sharing their materials."

Addgene offers many chemogenetic plasmids attached to different promoters, which can be used for different types of experiments. Addgene doesn't create any new constructs, Dr. Haery remarks, but researchers will often request plasmids, stick a new promoter on the DREADD, and then send the resulting plasmid back to Addgene so that it can be shared with others.

After obtaining a plasmid from Addgene, researchers face the challenge of getting it into the specific neurons they want to study. To help streamline this process, Addgene offers some constructs as viral preparations.

"One of the main challenges of using viruses is getting delivery into specific cells," Dr. Haery points out. "It's really important to know that you're activating or inhibiting specific neurons."

To this end, Addgene offers many of the chemogenetic plasmids in several different viral serotypes. "We have a few different serotypes which have tropism for specific cell types," Dr. Haery notes. Of the approximately 100 chemogenetic plasmids Addgene currently offers, the organization packages 12 of them in viruses, and each of those might be available in up to five serotypes.

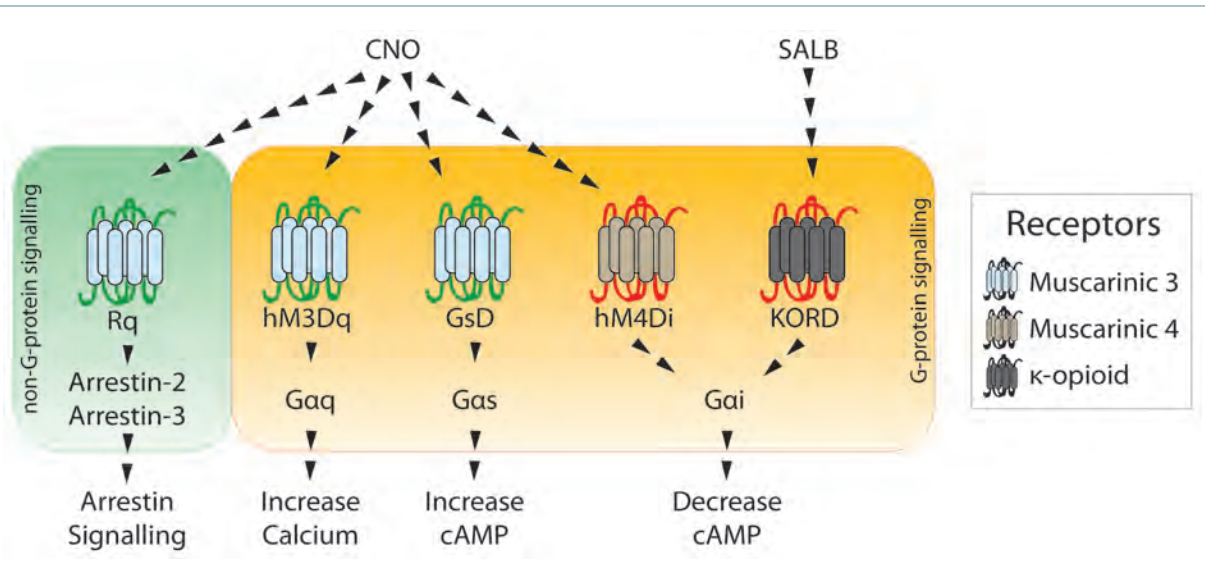
Another common way to control which cells express the DREADD is by the promoter that drives its expression. Certain promoters target glial cells, for instance, or particular neurons. Addgene is expanding the selection of promoters available to request, as researchers create new plasmid constructs and redeposit them with Addgene. Finally, some plasmids available through Addgene contain Cre-dependent constructs, limiting DREADD expression to cells that express Cre recombinase.

Riding the (Sound) Wave

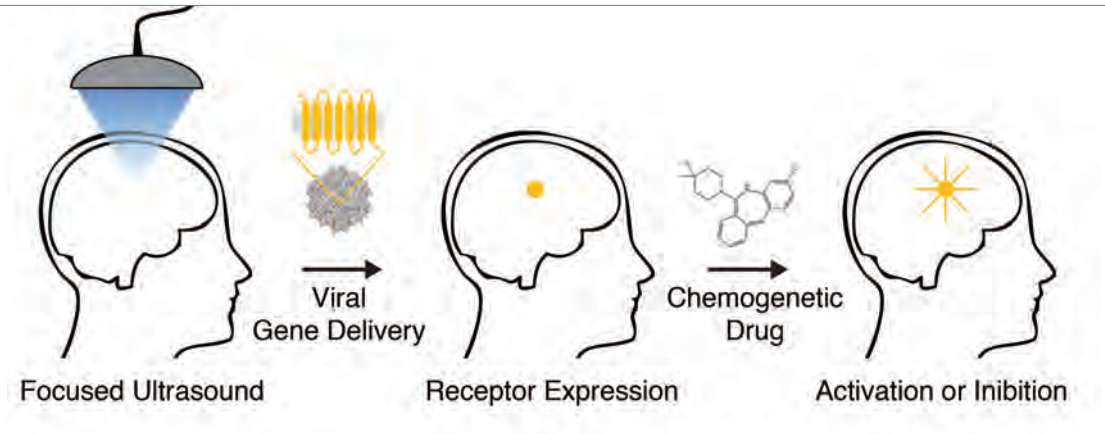
All of these methods—viral serotypes, promoters, and Cre-dependent constructs—can work together to ensure the chemogenetic receptor is expressed in a specific cell type. But what about targeting a particular area of the brain? Currently, the best method is injecting virus directly into the brain, which works well but can cause damage.

Mikhail G. Shapiro, Ph.D., assistant professor of chemical engineering at the California Institute of Technology, turned to ultrasound to develop a noninvasive technique for getting viruses into specific brain regions. First, micrometer-size bubbles are sent into the bloodstream, then focused ultrasound is applied to the area of interest, with millimeter precision.

"Wherever we're applying the ultrasound, these bubbles expand and contract with the ultrasound wave," Dr. Shapiro says. As the bubbles bump against the blood–brain barrier,



Chemogenetics resources provided by Addgene include this schematic, which shows various chemogenetic receptors and their signaling properties. Five types of chemogenetic receptors (Rq, hM3Dq, GsD, hM4Di, and KORD) have been genetically engineered from muscarinic or opioid receptors (as indicated by corresponding colors in the legend). Each receptor is specifically activated by its ligand (clozapine-*N*-oxide [CNO] or salvinorin B [SALB]) to signal to downstream effectors (arrestin-2/arrestin-3 or G protein α subunits Gαq, Gαs, or Gαi). Activation of these effectors then leads to unique physiological outputs, as listed. Some receptors signal canonically through G proteins (yellow box), whereas others have been engineered to signal through noncanonical pathways (green box).



At the California Institute of Technology, researchers Jerzy O. Szablowski, Ph.D., and Mikhail G. Shapiro, Ph.D., are developing acoustically targeted chemogenetics. This approach begins by using focused ultrasound to open up the blood-brain barrier in a specific geographic region of the brain. Viruses carrying chemogenetic elements can then enter the brain and install engineered receptors that respond to a chemogenetic drug and activate neural pathways.

he explains, they create an opening that allows virus particles to enter the brain, just in that location.

"This allows us to specify, based on where we're applying the ultrasound, what spatial part of the brain we want to modulate," Dr. Shapiro continues. The blood–brain barrier stays open about two hours, and during that time viral vectors containing chemogenetic elements can be injected in the bloodstream.

The technique, which Shapiro calls "acoustically targeted chemogenetics," or ATAC, eliminates certain downsides to setting up a chemogenetic system in animals with larger brains. For instance, the study of a larger region of the brain, or multiple different regions, typically involves dozens of injections of virus and repeated piercings of the brain. With ATAC, the ultrasound beam can easily be shifted around to target the desired areas. "From the point of view of both convenience, and also, how much are you perturbing the brain through the surgical approach, I think this noninvasive technique has some advantage," asserts Dr. Shapiro.

Someday, the technique could make it easier to treat neurological diseases, particularly those originating in a defined area of the brain. "One example of that is epilepsy," Dr. Shapiro points out. "For some patients, you can identify a seizure focus, a region of the brain where the seizures originate."

Instead of administering drugs, which could affect the whole brain, or resorting to surgery to remove just that part of the brain, one could use ultrasound to guide the delivery of a chemogenetic treatment, Dr. Shapiro suggests. Ultrasound-guided delivery could allow specific neurons to be turned off, completely noninvasively.

Uncovering Mechanisms of Addiction

Chemogenetic tools also provide a real boost to researchers studying complex neurological problems, such as addiction. Jun Wang, M.D., Ph.D., assistant professor in the Department of Neuroscience and Experimental Therapeutics at the Texas A&M College of Medicine, uses chemogenetics in mice to control the neural circuits involved in alcohol dependence.

The neurology of alcoholism is, of course, complex. Most people who indulge in alcohol don't become addicted, and identifying the brain characteristics that lead to addiction has been difficult. "The general idea is that drinking too much alcohol changes your brain," Dr. Wang says. Some of these changes increase cravings, resulting in alcoholism. Those are the changes he wants to study. "We want to find a way to specifically reverse or normalize activity in this circuit," he points out.

Acoustically targeted chemogenetics could someday make it easier to treat neurological diseases, particularly those originating in a defined area of the brain.

Dr. Wang and his colleagues inserted DREADDs into neurons in mice that express certain dopamine receptors. The brain's reward system relies on two opposing pathways, which work against each other to train the animal to seek out pleasurable experiences, such as food, and to avoid unpleasant ones.

Dr. Wang refers to the cells expressing dopamine receptor D1, the cells that are activated by dopamine, as "go" neurons. He refers to the cells expressing the D2 receptor, the cells that are inhibited by dopamine, as "no-go" neurons. Using chemogenetics, investigators may specifically target just the go or the no-go circuit. "This is the beauty of chemogenetics," Dr. Wang declares.

By activating just the D2, no-go neurons, Dr. Wang's team caused the animals to drink less alcohol. Turning off the D1 neurons had the same effect, reducing the amount of alcohol the mice consumed. Conversely, exciting the D1 neurons or inhibiting the D2 neurons spurred the mice to drink more alcohol.

The effect, however, is short-lived. "This is not a cure for alcoholism," Dr. Wang cautioned. Still, he envisions a possibility that chemogenetic therapy could one day help alleviate alcohol cravings when they're at their worst. GEN