Using Optogenetics and Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)

Summary: Emerging techniques that allow researchers to control the activity of a subset of neurons are revolutionizing our understanding of how the central nervous system works. Whether to use optogenetics (light) or DREADDs (drugs) as a means to control neuronal activity depends on which question you wish to answer. One of our series of Reports on Progress.

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The ability to manipulate the activity of specific subsets of neurons in the brains of living animals is leading to significant new insights into how the central nervous system works. The recently developed yet already well-established approaches of optogenetics and pharmacosynthetics use light or small molecules, respectively, to control the activity of neurons in the brains of laboratory animals. Using these techniques, we can see how distinct groups of neurons contribute to normal behavioral states or to abnormal behavior similar to those associated with neuropsychiatric disorders.

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Optogenetics describes a technique by which light-sensitive ion channels (opsins) that gate either negatively (halorhodopsin) or positively (channelrhodopsin-2) charged ions across the neuronal membrane and thereby inhibit or stimulate neuronal activity, respectively, are expressed in neurons. By implanting an optical fiber near a set of neurons, we can then deliver light of the appropriate wavelength to inhibit or activate these neurons and watch for any change in behavior that results. An analogous pharmacosynthetics approach, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), mutates muscarinic acetylcholine receptors (mAChRs) so they do not respond to their cognate agonist (acetylcholine) but are instead activated by the otherwise biologically inert ligand clozapine-*N*-oxide (CNO). Expression of DREADD receptors in neurons enables us to control their activity simply by delivering CNO to the animal. The hM3Gq DREADD receptor is derived from the excitatory M3 mAChR and its activation by CNO results in increased neuronal activity. Conversely, the hM4Gi DREADD receptor is derived from the inhibitory M4 mAChR and its activation by CNO decreases neuronal activity.

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Both techniques involve injecting a virus that expresses the opsin or DREADD, usually tagged with a fluorescent protein, in the reverse orientation (so expression cannot occur). When cells expressing the enzyme Cre recombinase are infected with these viruses, Cre re-orients the inserted opsin or DREADD and expression occurs. In the hundreds of genetically modified mouse lines in which Cre is expressed only in discrete neuronal populations, the activity of these neurons can be selectively controlled using optogenetics or DREADDs. Lines of Cre-expressing rats are also becoming available.

The experimental applications of optogenetics and DREADDs are revolutionizing neuroscience. For example, using optogenetics, some researchers have identified the specific neuronal circuits in the amygdala that mediate fear; others have altered the actions of interneurons in the nucleus accumbens that are part of the neural pathway responsible cocaine addiction. Using DREADDS, researchers can change taste preferences in tongue receptors to influence what a mouse will drink.

The major strength of optogenetics and DRE-

ADDs is that, when used in combination with mouse behavioral genetics, they can bring the activity of defined populations of neurons under the direct control of the experimenter. These approaches do have limitations, though. For example, viruses used to deliver opsins and DREADDs depend on the activity of the gene promoters that drive Cre expression; Cre levels can vary depending on the activity of that promoter, which means the amount of opsin or DREDD expressed also will vary. Moreover, it may be that inserting the virus itself or aspects of the experiment, such as the animal engaging in particular behaviors or being treated with a class of drug, changes the activity of the gene promoter, which then would affect the levels of opsin or DREADD receptor expression during an experiment. Also, the virus or the transgenic mouse that has altered Cre expression under a given gene promotor may also influence other gene(s) and unexpectedly change the animal's behavior.

Another consideration is the fact that viruses even in perfect circumstances will infect only a small percentage of targeted neurons, meaning that only a small fraction will typically express the opsin or DREADD. Therefore, we must be careful when interpreting negative findings using these approaches; perhaps the findings would have been positive if all the neurons had been altered. It's also important to remember that neurons can produce and release more than a single neurotransmitter. Attributing the effects of optogenetic or DREADD manipulation on defined populations of neurons to an action on a particular neurotransmitter known to be produced by those neurons can be complicated by the fact that new evidence suggests that many classes of neurons in the brain in fact release more than a single neurotransmitter. Finally, since opsins and DREADDs become integrated into the neuronal membrane after virus transduction, it is possible that they also may disrupt the expression or function of endogenous receptors. If so, experiments combining optogenetics or DREADDs with pharmacological manipulations of endogenous receptors may result in an atypical representation of neuronal function or behavior compared with native conditions.

Which technique to use depends on what question(s) you wish to answer. One of the main benefits of optogenetics is the ability to modify neuronal activity rapidly by activating and deactivating the light source. If a researcher is interested in modulating a particular behavioral state such as the "freezing" typically seen when a rodent is fearful it is possible to time-lock cellular excitation/inhibition in relation to that event. It is often possible to mimic the typical firing rates or patterns of firing (e.g., burst versus pause) of neurons using light stimulation, thereby better recapitulating the activity of neurons that occurs naturally during behavioral states. For example, when an animal unexpectedly encounters a rewarding stimulus this usually results in the burst firing of dopamine neurons, which is thought to help the animal to learn about the circumstances that resulted in this unexpectedly positive outcome and seek that same stimulus again in future under similar circumstances. Additionally, based on the placement of the implanted cranial optical fiber, researchers can manipulate only the postsynaptic, presynaptic, and/or axonal membrane. Together, the high level of spatiotemporal resolution of optogenetics, coupled with the ability to regulate specific populations of neurons, offers a unique advantage compared with more traditional approaches to neuromodulation and also compared with DREADDs (see below).

A limitation of the optogenetic approach is the need to deliver photons into the brain, which most commonly occurs via an implanted optical fiber extending from a light source through the skull and into targeted brain regions. This may limit the movement of the animal, confounding the expression and/or measurement of complex behaviors. The optical fiber also is vulnerable to damage caused by activity of the animal, and its implantation may result in tissue damage in precisely the brain sites that the investigator is interested in. Recent advances in wireless neural headstages for optical stimulation have the potential to overcome these limitations, although the size of the headstage may remain a concern for small animals like mice. Additional limitations and considerations when using optogenetics include the chance that the light induces modulation of fibers of passage as opposed to acting solely on the axons or terminals of interest; antidromic stimulation (when the signal travels opposite to the usual direction of the axon), and "rebound" excitation or inhibition during periods when the light stimulation is turned off. Also, the technique could cause light-induced or heat-associated damage to the tissue. Researchers are making progress on creating opsins sensitive to light in the far-red spectrum, which reduces the possibility of light-induced tissue damage and would provide an additional benefit of delivering photons through the skull and into sites of interest without the need for optical fibers.

For research questions that don't depend on millisecond timing, such as animals responding continuously for food or drug rewards during a one or

two hour testing session, DREADDs may be more appropriate than optogenetics. DREADDs offer the ability to stimulate or inhibit neuronal activity in a more persistent manner without the need for constant light delivery. This permits the animals to perform in complex behavioral tasks, such as vigorously pressing levers and collecting rewards or transitioning between different environments in a behavioral apparatus, without being constrained by having an implant attached to their heads. DREADD receptors are activated via injection of CNO, which can be administered peripherally; the effect lasts from minutes to hours. Thus, DREADDs can modulate neuronal activity less invasively and more persistently compared with optogenetics. If a research question requires selective manipulation of pre- versus post-synaptic membranes, CNO can also be delivered locally into brain via intracranial microinjection.

Another attractive property of DREADDs is that DREADD receptors have known intracellular signal transduction mechanisms, so researchers can selectively modulate intracellular signaling cascades. This contrasts with the more general intracellular signaling achieved by most optogenetic approaches (the exception being OptoXR modulation of G-protein signaling). However, it is important to understand that this apparent advantage of DREADDs can be a limitation if the target neurons do not contain sufficient levels of those signaling molecules. Also, as with optogenetics, one must still consider the possibility of antidromic stimulation and rebound excitation.

In addition to modulating neural activity in live animals, optogenetics and DREADDs can also be used in combination with whole-cell recordings to learn more about basic aspects of neuronal physiology *in vitro*. When using *in vitro* recordings to understand mechanisms of synaptic activity, time resolution is of the essence. In this case, optogenetics excels and has a clear advantage over DREADDs. Channelrhodopsin-2, an opsin that can stimulate neuronal activity, is an ion channel that opens within 1-2 milliseconds after light stimulation, allowing sodium ions to enter and depolarize the neuron. Conversely, halorhodopsin and archaerhdopsin, opsins that inhibit neuronal activity, pump chloride ions into the neuron within 10-15 and 8-10 ms, respectively, after light activation, thereby hyperpolarizing the neuron and rendering it silent. This millisecond-scale precision allows experimenters to mimic endogenous single-spike action potential patterns and synaptically evoked events.

In summary, optogenetics and DREADDs, and related emerging technologies to control neuronal activity, are revolutionizing our understanding of the CNS. Optogenetics has the advantage of temporal precision yet requires a light source and optic fibers. DREADDs do not offer the same levels of temporal precision but do not require optical fibers and have a longer duration of action. Understanding the advantages and disadvantages of optogenetics and DREADDs, and tailoring their use to best address the specific research question under consideration, is key to maximally leveraging these technologies.

For further reading:

Aston-Jones G & Deisseroth K. (2013) Recent advances in optogenetics and pharmacogenetics. Brain Research 1511: 1-5.

Farrell MS & Roth BL. (2013) Pharmacosynthetics: Reimagining the pharmacogenetic approach. Brain Research 1511: 6-20.

Stuber GD & Mason AO. (2013) Integrating optogenetic and pharmacological approaches to study neural circuit function: current applications and future directions. Pharmacol Rev. 65(1): 156-70.