Remote Control of Cellular Signaling Using DREADD Technology

Bryan L. Roth MD, PhD

Department of Pharmacology
University of North Carolina Chapel Hill Medical School
Chapel Hill, North Carolina
Introduction

In his visionary review of 1979, Francis Crick suggested that a major goal of neuroscience is to identify “which features (of the brain) it would be most useful to study and in particular to measure” (Crick, 1979). To identify and perturb these features in a productive way, it would be necessary to invent a method “by which all neurons of just one type could be inactivated, leaving the others more or less unaltered” [emphasis mine] (Crick, 1979). Sometime later, he expanded this wish list to include the ability “to turn the firing of one or more types of neuron on and off in the alert animal in a rapid manner” (Crick, 1999). The idea Crick proposed, then, was that in order to begin to construct a wiring diagram of neuronal circuits involved in regulating particular behaviors, there was a pressing need for a way to reversibly regulate neuronal activity in a cell-type-specific manner.

During the past 10 years, a number of technologies have been developed to achieve the cell-type-specific and reversible modulation of neuronal activity he envisioned. These include the following:

- Light-activated channels for activating (Nagel et al., 2002, 2003, 2005; Boyden et al., 2005) and silencing (Li et al., 2005; Zhang et al., 2007) neurons;
- Photochemical activation of neurons (Zemelman et al., 2002, 2003; Kokel et al., 2013);
- Chemogenetic or pharmacogenetic activation of neurons via engineered receptor–ligand pairs (Alexander et al., 2009); and
- Chemogenetic or pharmacogenetic inactivation of neurons via insect receptor–ligand pairs (Lechner et al., 2002) or engineered receptor–ligand pairs (Armbruster et al., 2007).

In a similar way, in order to understand how signaling processes in neuronal and nonneuronal cells regulate behavior, we will need tools that allow for precise spatiotemporal control of neuronal and nonneuronal signaling in a reversible, temporally controllable fashion. Thus, the aim of this research is to insert engineered receptors into specific neuronal populations and then to activate or inactivate them to discover how signaling processes regulate behavior in freely moving animals (Fig. 1).

![Figure 1](image.png)

**Figure 1.** “Thought experiments” for using engineered GPCRs inserted into specific cells to interrogate signaling processes essential for behavior. Ideally, by inserting an engineered G_\text{i}-coupled receptor into cortical neurons via the Cre-Lox system, one can induce a behavior reminiscent of that induced by the κ-opoid–selective ligand salvinorin A. CNO, clozapine-N-oxide; Sal A, salvinorin A.

© 2013 Roth
Activating G-Protein Coupled Receptors

During the past 20 or more years, a number of tools have been developed that allow for the reversible activation of G-protein coupled receptors (GPCRs) (Table 1) (Conklin et al., 2008; Rogan and Roth, 2011). These have been variously dubbed "allele-specific genetically engineered receptors" (Strader et al., 1991); "receptors activated solely by synthetic ligands" (RASSLs) (Coward et al., 1998); "engineered receptors" (Westkaemper et al., 1999); "therapeutic receptor–effector complexes" (TREKs) (Small et al., 2001); "neoceptors" (Jacobson et al., 2001); and "designer receptors exclusively activated by designer drugs" (DREADD) (Armbruster et al., 2007). Among these variations on the theme of engineered GPCR–ligand pairs, DREADDs have emerged as the most frequently used tool for remotely controlling neuronal signaling. This chapter focuses on the specific application of DREADD technology.

Designer Receptors Exclusively Activated by Designer Drugs—DREADDs

DREADDs were originally invented by modifying muscarinic acetylcholine receptors to be activated by the inert ligand clozapine-N-oxide (CNO) via directed molecular evolution in genetically engineered yeast (Armbruster et al., 2007). In the process, two-point mutations of highly conserved amino acids (Y3.33C and A5.46G) via the Ballesteros and Weinstein numbering convention; Ballesteros and Weinstein, 1995) rendered all 5 human muscarinic receptors both unable to be activated by the native agonist but activating by CNO (Armbruster et al., 2007). Figure 2 shows the locations of the two-point mutations (Y149C (3.33), A239G (5.46)) that are conserved residues within all acetylcholine muscarinic receptors, including Drosophila.

Table 1. Representative chemogenetic technologies for the remote control of cellular signaling

<table>
<thead>
<tr>
<th>Technology</th>
<th>Ligand(s)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele-specific control of GPCR signaling via engineered β-adrenergic receptor–ligand pair</td>
<td>β2-adrenergic receptor Asp113→Ser113 mutant</td>
<td>Reversible activation of Gs canonical signaling</td>
<td>Strader et al., 1991</td>
</tr>
<tr>
<td>RASSL–Gi (receptors activated solely by synthetic ligands)</td>
<td>κ-opioid chimeric receptor</td>
<td>Spiradoline (small-molecule κ-opioid agonist)</td>
<td>Reversible activation of canonical Gi signaling</td>
</tr>
<tr>
<td>Engineered receptor–ligand pairs to reversibly inactivate signaling</td>
<td>5-HT2A serotonin receptor Phe340→Leu340/mutant receptor</td>
<td>Inactive ketanserin analogues</td>
<td>Reversible inhibition of Gi signaling</td>
</tr>
<tr>
<td>TREK (therapeutic receptor–effector complex) β-adrenergic receptor mutant</td>
<td>Extensive modifications of β2-adrenergic receptor</td>
<td>L-158,870</td>
<td>Reversible Gs activation</td>
</tr>
<tr>
<td>Neoceptors</td>
<td>Engineered adenosine receptors</td>
<td>Inactive adenosine receptor ligands</td>
<td>Reversible activation of canonical adenosine signaling</td>
</tr>
<tr>
<td>RASSL–Gs</td>
<td>Melanocortin-4 receptor mutants</td>
<td>Small-molecule MC4 agonists</td>
<td>Reversible activation of Gi signaling</td>
</tr>
<tr>
<td>Gi and Gq-DREADD</td>
<td>M1, M2, M3, M4, M5-muscarinic receptor mutants</td>
<td>Inactive clozapine metabolite clozapine-N-oxide (CNO)</td>
<td>Reversible activation of Gi or Gq signaling</td>
</tr>
<tr>
<td>Gq-DREADD</td>
<td>Engineered M3-muscarinic receptor</td>
<td>Inactive clozapine metabolite CNO</td>
<td>Reversible activation of Gi signaling</td>
</tr>
<tr>
<td>Arrestin-DREADD</td>
<td>Engineered M3-muscarinic receptor</td>
<td>Inactive clozapine metabolite CNO</td>
<td>Reversible activation of arrestin signaling</td>
</tr>
</tbody>
</table>
activated by acetylcholine (their endogenous agonist) and exquisitely sensitive to CNO (Fig. 2).

To date, DREADDs suitable for remotely activating the designer receptors $G_i$ (e.g., hM$_3$G$_i$) (Armbruster et al., 2007), $G_q$ (e.g., hM$_3$G$_q$) (Armbruster et al., 2007), $G_s$ (e.g., hM$_3$G$_s$) (Armbruster et al., 2007) and arrestin (e.g., Arr-DREADD) (Nakajima and Wess, 2012) signaling have been reported. These are activated using the pharmacologically inactive compound and clozapine metabolite CNO and have been extensively validated (Table 1). In all neuron types reported to date:

* Activation of the hM$_3$D$_q$ by CNO induces neuronal depolarization and burst firing (Alexander et al., 2009; Krashes et al., 2011; Atasoy et al., 2012);
* Activation of hM$_3$D$_q$ by CNO induces neuronal hyperpolarization and silencing (Armbruster et al., 2007; Krashes et al., 2011; Atasoy et al., 2012);
* Activation of G$_i$D by CNO enhances neuronal G$_s$ signaling (Brancaccio et al., 2013; Farrell et al., 2013); and
* CNO has no effect on baseline firing (Alexander et al., 2009; Krashes et al., 2011; Atasoy et al., 2012) or signaling in neurons not expressing DREADDs (Brancaccio et al., 2013; Farrell et al., 2013).

(There have been no reports on the utility of the arrestin-specific DREADD for remotely controlling neuronal arrestin signaling.)

The mechanism(s) responsible for these alterations in neuronal activity are unknown. However, the hyperpolarization of neurons and inhibition of firing by hM$_3$D$_q$ is likely caused in part by the activation of G-protein inwardly rectifying potassium channels (Armbruster et al., 2007). To date, a large number of investigators have reported success in using DREADD technology to selectively modulate neuronal signaling and firing (Table 2).

### Pros and Cons of DREADD Technology

DREADDs are now widely used in neuroscience to remotely control neuronal signaling. DREADDs offer the following advantages over other, more invasive technologies such as optogenetics:

* They are able to noninvasively control neuronal and nonneuronal signaling, as CNO can be administered peripherally via injection (Alexander et al., 2009) or through drinking water (D.J. Urban and B.L. Roth, unpublished observations) (protocols available at http://dreadd.org/);
* They can modulate signaling and activity of widely dispersed neurons (Garner et al., 2012);
* They can modulate signaling and activity of optically inaccessible neurons (Vrontou et al., 2013);
* They can be used to modulate activity of neurons early in development in a noninvasive manner (Kozorovitskiy et al., 2012);
* They are appropriate for long-term studies (e.g., days to weeks) (Farrell et al., 2013); and
* CNO-modulated activity can last hours after a single injection (Alexander et al., 2009).

The main disadvantage DREADD technology as compared with optical technologies is the lack of precise, millisecond control of activity. Although it is likely that “caging” CNO is possible (B.L. Roth, unpublished observations) so that millisecond control can be achieved by photochemically uncaging CNO, optical technologies will likely remain the most useful under conditions in which precise millisecond control of neuronal activity is needed.

<table>
<thead>
<tr>
<th>DREADD</th>
<th>Experiment</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>hM$_3$D$_q$ +/- hM$_3$D$_i$</td>
<td>Remote control of feeding</td>
<td>Identification of neurons that encode hunger</td>
<td>Krashes et al., 2011; Atasoy et al., 2012</td>
</tr>
<tr>
<td>hM$_3$D$_q$</td>
<td>Generation of a synthetic memory trace</td>
<td>Memory encoded sparsely</td>
<td>Garner et al., 2012</td>
</tr>
<tr>
<td>hM$_3$D$_q$</td>
<td>Alteration in neuronal plasticity</td>
<td>Altered striatal connectivity</td>
<td>Kozorovitskiy et al., 2012</td>
</tr>
<tr>
<td>hM$_3$D$_i$</td>
<td>5-HT neuron silencing</td>
<td>Behavior and physiological consequences</td>
<td>Ray et al., 2011</td>
</tr>
<tr>
<td>hM$_3$D$_i$</td>
<td>Identification of neurons responsible for pleasurable sensation</td>
<td>DRG neurons identified as target of MGPR4 orphan receptor</td>
<td>Vrontou et al., 2013</td>
</tr>
<tr>
<td>G$_i$D</td>
<td>Modulation of cAMP</td>
<td>Modulates circadian clock</td>
<td>Brancaccio et al., 2013</td>
</tr>
</tbody>
</table>

© 2013 Roth
Summary

DREADD technology has emerged as a facile approach for remotely and noninvasively controlling neuronal and nonneuronal signaling. CNO-induced activation of hM3Dq triggers neuronal burst firing and, accordingly, hM3Dq is frequently used to remotely activate neurons. The activation of hM4Di by CNO can silence neurons and, accordingly, hM4Di is frequently used to remotely inactive neuronal activity. The development of additional DREADDs, as well as DREADDs that selectively activate distinct downstream effectors, will greatly expand our ability to remotely control and interrogate neuronal signaling in both health and disease.

References


