Remote Control of Cellular Signaling Using DREADD Technology

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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).

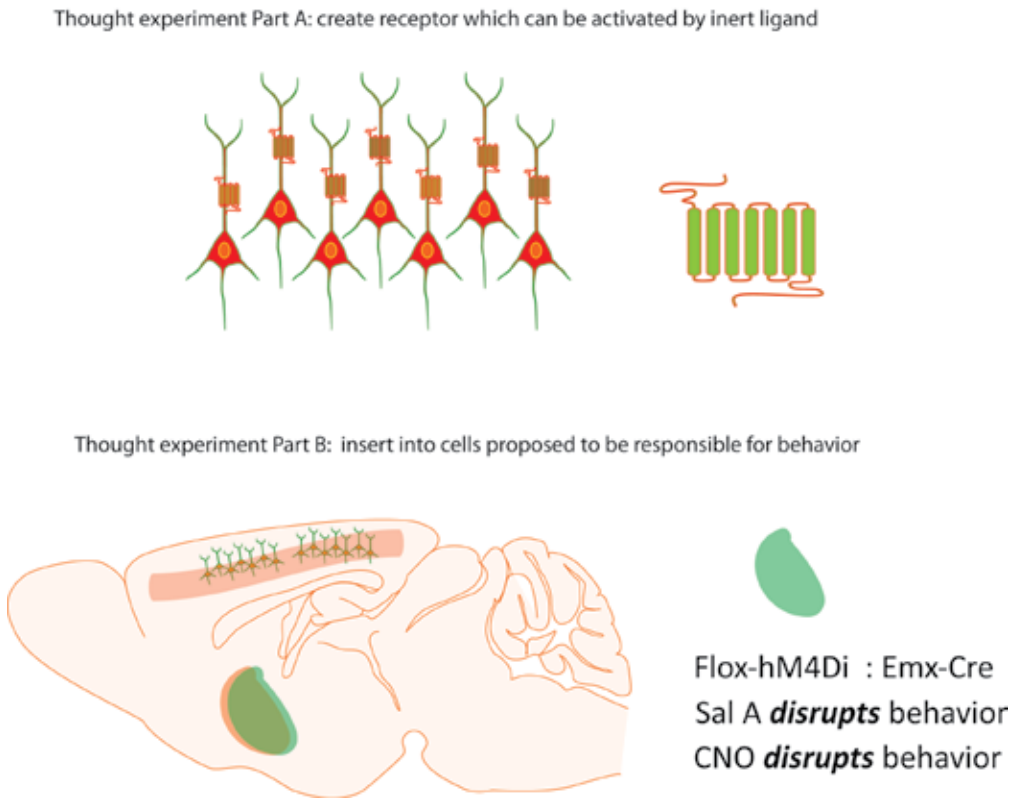

Figure 1. "Thought experiments" for using engineered GPCRs inserted into specific cells to interrogate signaling processes essential for behavior. Ideally, by inserting an engineered G_i-coupled receptor into cortical neurons via the Cre-Lox system, one can induce a behavior reminiscent of that induced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

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

Technology		Ligand (s)	Outcome	Reference
Allele-specific control of GPCR signaling via engineered β-adrenergic receptor–ligand pair	β2-adrenergic receptor Asp^{113}->Ser^{113} mutant	1-(3',4'-dihydroxyphenyl)-3-methyl-L-butanone (L-185,870)	Reversible activation of G_s canonical signaling	Strader et al., 1991
RASSL–G_i (receptors activated solely by synthetic ligands)	κ-opioid chimeric receptor	Spiradoline (small-molecule κ-opioid agonist)	Reversible activation of canonical G_i signaling	Coward et al., 1998
Engineered receptor–ligand pairs to reversibly inactivate signaling	5-HT_{2A} serotonin receptor Phe^{340}->Leu^{340} mutant receptor	Inactive ketanserin analogues	Reversible inhibition of G_q signaling	Westkaemper et al., 1999
TREK (therapeutic receptor–effector complex) β-adrenergic receptor mutant	Extensive modifications of β2-adrenergic receptor	L-158,870	Reversible G_s activation	Small et al., 2001
Neoceptors	Engineered adenosine receptors	Inactive adenosine receptor ligands	Reversible activation of canonical adenosine signaling	Jacobson et al., 2001
RASSL-G_s	Melanocortin-4 receptor mutants	Small-molecule MC4 agonists	Reversible activation of G_s signaling	Srinivasan et al., 2003
G_i and G_q-DREADD	M_1, M_2, M_3, M_4, M_5-muscarinic receptor mutants	Inactive clozapine metabolite clozapine-*N*-oxide (CNO)	Reversible activation of G_i or G_q signaling	Armbruster et al., 2007
G_s-DREADD	Engineered M_3-muscarinic receptor	Inactive clozapine metabolite CNO	Reversible activation of G_s signaling	Guettier et al., 2009
Arrestin-DREADD	Engineered M_3-muscarinic receptor	Inactive clozapine metabolite CNO	Reversible activation of arrestin signaling	Nakajima and Wess, 2012

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 various 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.

Figure 2. Point mutations essential for creation of DREADD receptors. Shown are the locations of the two-point mutations (Y149C$^{(3.33)}$, A239G$^{(5.46)}$) that are conserved residues within all acetylcholine muscarinic receptors, including *Drosophila*.

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

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Table 2. Representative experiments using DREADDs to modulate behavior by remote cell-type-specific control of neuronal signaling

DREADD	Experiment	Result	References
hM_3D_q +/– hM_4D_i	Remote control of feeding	Identification of neurons that encode hunger	Krashes et al., 2011; Atasoy et al., 2012
hM_3D_q	Generation of a synthetic memory trace	Memory encoded sparsely	Garner et al., 2012
hM_4D_i	Alteration in neuronal plasticity	Altered striatal connectivity	Kozorovitskiy et al., 2012
hM_4D_i	5-HT neuron silencing	Behavior and physiological consequences	Ray et al., 2011
hM_3D_q	Identification of neurons responsible for pleasurable sensation	DRG neurons identified as target of MGPR4 orphan receptor	Vrontou et al., 2013
G_sD	Modulation of cAMP	Modulates circadian clock	Brancaccio et al., 2013

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_4G_i) (Armbruster et al., 2007), G_q (e.g., hM_3G_q) (Armbruster et al., 2007), G_s (e.g., G_sD) (Guettier et al., 2009) 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_3D_q by CNO induces neuronal depolarization and burst firing (Alexander et al., 2009; Krashes et al., 2011; Atasoy et al., 2012);
- Activation of hM_4D_i by CNO induces neuronal hyperpolarization and silencing (Armbruster et al., 2007; Krashes et al., 2011; Atasoy et al., 2012);
- Activation of G_sD by CNO enhances neuronal Gs 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_4D_i 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.

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Summary

DREADD technology has emerged as a facile approach for remotely and noninvasively controlling neuronal and nonneuronal signaling. CNO-induced activation of hM_3D_q triggers neuronal burst firing and, accordingly, hM_3D_q is frequently used to remotely activate neurons. The activation of hM_4D_i by CNO can silence neurons and, accordingly, hM_4D_i 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.

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