Chemogenetic Tools to Interrogate Brain Functions

Scott M. Sternson[1] and Bryan L. Roth[2]

[1]Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia 20147; email: sternsons@janelia.hhmi.org

[2]Department of Pharmacology and Division of Chemical Biology and Medicinal Chemistry, University of North Carolina Chapel Hill School of Medicine, Chapel Hill, North Carolina 27599; email: bryan_roth@med.unc.edu

Annu. Rev. Neurosci. 2014. 37:387–407

First published online as a Review in Advance on June 16, 2014

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

This article's doi: 10.1146/annurev-neuro-071013-014048

Keywords

DREADDs, designer receptors exclusively activated by designer drugs, PSAM, PSEM

Abstract

Elucidating the roles of neuronal cell types for physiology and behavior is essential for understanding brain functions. Perturbation of neuron electrical activity can be used to probe the causal relationship between neuronal cell types and behavior. New genetically encoded neuron perturbation tools have been developed for remotely controlling neuron function using small molecules that activate engineered receptors that can be targeted to cell types using genetic methods. Here we describe recent progress for approaches using genetically engineered receptors that selectively interact with small molecules. Called "chemogenetics," receptors with diverse cellular functions have been developed that facilitate the selective pharmacological control over a diverse range of cell-signaling processes, including electrical activity, for molecularly defined cell types. These tools have revealed remarkably specific behavioral physiological influences for molecularly defined cell types that are often intermingled with populations having different or even opposite functions.

Contents

INTRODUCTION

In an amazingly prescient article published in 1979, Francis Crick (1979) engaged in a "wish list" of items that would be essential for understanding brain function:

> For example, a method that would make it possible to inject one neuron with a substance that would then clearly stain all the neurons connected to it, and no others, would be invaluable. . . . [Similarly,] a method by which all neurons of just one type could be inactivated, leaving the others more or less unaltered [is also needed].

Today, in 2014, we have tools both to identify (Wall et al. 2010) and to visualize (Chung et al. 2013, Hama et al. 2011, Ke et al. 2013) neuronal connectivity in intact brains. Efficient technologies are also readily available to selectively silence genetically identified neurons using small molecules (Armbruster et al. 2007, Lechner et al. 2002, Magnus et al. 2011) and photons (Li et al. 2005, Zhang et al. 2007).

In a later article, Crick also presaged the need for methods to "turn neurons on" (Crick 1999), and now optical (Boyden et al. 2005, Li et al. 2005), small-molecule (Alexander et al. 2009, Armbruster et al. 2007, Magnus et al. 2011), and photochemical (Callaway & Katz 1993, Kokel et al. 2013, Zemelman et al. 2002) technologies are also available. This review focuses on methods that utilize small molecules to activate (Alexander et al. 2009, Armbruster et al. 2007, Magnus et al. 2011) and inhibit (Armbruster et al. 2007, Lechner et al. 2002, Magnus et al. 2011) neuronal firing. Over the years, a number of terms have been used to describe small-molecule-mediated activation of engineered proteins including pharmacogenetics (Sasaki et al. 2011), pharmacosynthetics (Farrell & Roth 2012), and chemogenetics (Strobel 1998). Here we use the term chemogenetics because it was used first to describe this approach, whereas the term pharmacogenetics is not appropriate given its connotations in pharmacology and genetics (Farrell & Roth 2012). We also highlight new tools that allow the precise modulation of signaling (Armbruster et al. 2007, Farrell et al. 2013, Guettier et al. 2009b, Nakajima & Wess 2012) in genetically defined neurons, glia, and other cell types.

EARLY CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN–COUPLED RECEPTORS

G protein–coupled receptors (GPCRs) represent the largest class of neuronal signal-transducing molecules (Allen & Roth 2011). Depending on the specific downstream effector system initiated,

GPCRs can excite, inhibit, or otherwise modulate neuronal firing (Farrell & Roth 2013). Initial attempts at modulating cellular signaling using chemogenetic approaches utilized GPCRs that were engineered by site-directed mutagenesis to bind nonnatural ligands. In a pioneering study, Strader et al. (1991) designed a mutant β2-adrenergic receptor that was unable to bind the native ligand adrenaline but could be activated by 1-(3′,4′-dihydroxyphenyl)-3-methyl-L-butanone (L-185,870) (**Figure 1**). Although L-185,870 had relatively low potency for the engineered receptor (EC_{50} ~ 40 uM), the results represented an essential proof of concept for this general approach. Further modifications led to a highly engineered β2-adrenergic receptor with even higher potency for L-185,870 (~7 uM) (Small et al. 2001) and nonresponsiveness to native ligands.

The next advance occurred with the creation of a family of engineered receptors dubbed RASSLs (receptor activated solely by synthetic ligand). The initial RASSL was an engineered k-opioid receptor (KOR) that was insensitive to native peptide ligands but could be activated potently by the synthetic KOR agonist spiradoline (Coward et al. 1998). This KOR RASSL (**Figure 1**) was subsequently used in the first chemogenetic study from which remote control of cardiac activity was achieved (Redfern et al. 1999). For these experiments, the KOR RASSL was conditionally and reversibly expressed in cardiac myocytes using tetracycline-inducible expression driven by a myosin heavy chain promoter (αMHC–tTA). Subsequently, RASSL technology was used to unravel the code for sweet and bitter taste (Mueller et al. 2005, Zhao et al. 2003). Several other RASSLs have also been generated (for a review, see Conklin et al. 2008), although their utility

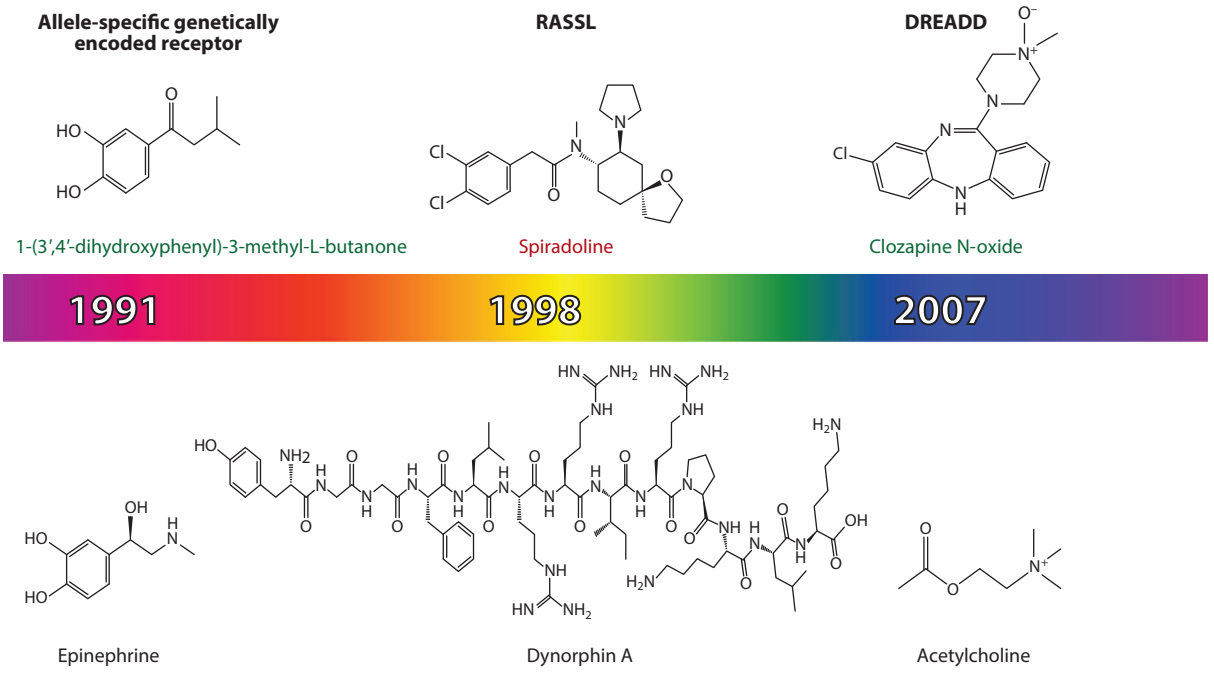


Figure 1

Evolutionary timeline of GPCR-based chemogenetic approaches listing the main corresponding tools starting with allele-specific engineered β-adrenergic receptors, RASSLs, and DREADDs. Relevant structures shown include (*top*) engineered ligands and (*bottom*) endogenous ligands. Green text indicates no pharmacologic activity at the native target; red indicates activity. Abbreviations: DREADD, designer receptor exclusively activated by designer drug; GPCR, G protein–coupled receptor; RASSL, receptor activated solely by synthetic ligand.

in the neurosciences has been hampered owing to the pharmacological activities of the cognate ligands (e.g., spiradoline is a potent KOR agonist) and to the fact that some, but not all (Chang et al. 2007), RASSLs have high levels of constitutive activity (Hsiao et al. 2008, 2011; Sweger et al. 2007).

Since then, researchers have engineered several other receptor-ligand pairs based on 5-HT_{2A} serotonin (Kristiansen et al. 2000, Westkaemper et al. 1999) and adenosine (Gao et al. 2006; Jacobson et al. 2001, 2005) receptors. Optically activated chimeric opsins that can activate canonical GPCR signaling cascades (Airan et al. 2009) have also been created. In each case, and in distinction to RASSLs, the orthologous ligands (with the exception of the opsins) showed greatly attenuated activity at the native receptor and greatly enhanced activity at the engineered receptor. Additionally, the affinities and potencies for the native orthologous ligands were greatly reduced. The chemogenetic and optogenetic tools are important because they demonstrate the potential that many GPCRs could be engineered to bind relatively inactive cognate ligands. However, given the relatively weak potency of synthetic ligands (Kristiansen et al. 2000, Westkaemper et al. 1999) and adenosine (Gao et al. 2006; Jacobson et al. 2001, 2005) or given modest signaling (Airan et al. 2009), they have not been broadly adopted as tools for remotely controlling neuronal signaling.

CHEMOGENETIC CONTROL OF NEURONAL AND NON-NEURONAL SIGNALING USING DREADD TECHNOLOGY

Fundamental problems associated with these early attempts to control GPCR signaling, as stated above, were that the ligands were not particularly well suited for in vivo studies because of the effects on cognate and noncognate receptors and that the engineered receptors occasionally had high levels of constitutive activity. To overcome these problems, Armbruster & Roth (2005) developed a platform they termed DREADD (designer receptor exclusively activated by designer drug) in which directed molecular evolution in yeast was used to activate GPCRs via pharmacologically inert, drug-like small molecules (Alexander et al. 2009, Armbruster et al. 2007). As initially described (Armbruster et al. 2007, Dong et al. 2010, Rogan & Roth 2011), an engineered human M3-muscarinic receptor was subjected to random mutagenesis, expressed in genetically engineered yeast (Schmidt et al. 2003), and grown in media containing clozapine N-oxide (CNO) (**Figure 1**). CNO was chosen because of its excellent ability to penetrate the central nervous system (Bender et al. 1994), favorable pharmacokinetics in mice (Bender et al. 1994) and humans (Jann et al. 1994), and inert pharmacology (Armbruster et al. 2007).

Under screening conditions, only yeast that express a mutant M3-muscarinic receptor that can be activated by CNO survive. After several cycles of selection and mutagenesis as well as comprehensive bioinformatics and pharmacological characterization, researchers selected an M3-muscarinic receptor with two mutations (Y149C, A239G) that fulfilled the following criteria:

- Nanomolecular potency for activation by CNO
- Relative insensitivity to acetylcholine (the native ligand)
- No detectible constitutive activity.

The resulting Y149C, A239G M3-muscarinic receptor was the first DREADD and is now known as hM3Dq to indicate its selectivity for Gαq-mediated signaling pathways (**Figure 2**). Because these two residues (e.g., Y149C and A239G) are conserved among all muscarinic receptors throughout evolution from *Drosophila* to humans, they can create an entire family of DREADD-based muscarinic receptors (vis hM1Dq, hM2Di, hM3Dq, hM4Di, hM5Dq), all of which are potently activated by CNO, insensitive to acetylcholine, and devoid of constitutive activity (Armbruster et al. 2007). M1-, M3-, and M5-DREADDs all couple to Gαq, whereas M2- and M4-DREADDs couple to Gαi-G proteins (**Figure 2**). Subsequently, Guettier et al. (2009)

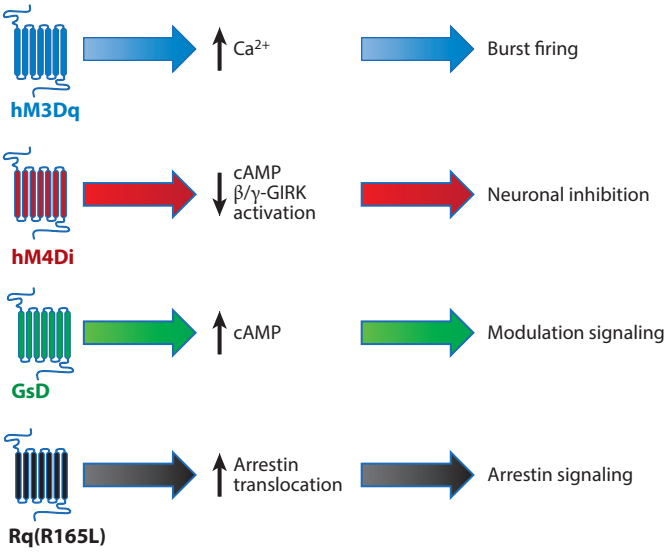


Figure 2

Main DREADD-based tools currently available as well as their typical uses in neuroscience.

created a chimeric muscarinic-adrenergic receptor DREADD (GsD) that selectively activates Gαs and activates neuronal cAMP-mediated signaling (Farrell et al. 2013).

In the initial study Armbruster et al. (2007) reported that hM4Di-DREADD could also induce neuronal silencing via Gαi-mediated activation of G protein inwardly rectifying potassium channels in hippocampal neurons in vitro. Armbruster et al. (2007) also predicted that hM4Di would also be useful for silencing neuronal activity in vivo. Subsequently, many groups have independently reported the successful attenuation of neuronal firing by CNO-mediated activation of hM4Di (Atasoy et al. 2012; Brancaccio et al. 2013; Ferguson et al. 2011; Kozorovitskiy et al. 2012; Krashes et al. 2011; Parnaudeau et al. 2013; Ray et al. 2011, 2012; Sasaki et al. 2011). In every instance, the attenuation of neuronal firing was accompanied by striking behavioral and/or physiological consequences (**Table 1**) and the imputation of distinct populations of genetically identified neurons as mediators of behavior and/or physiology. hM4Di has also been used to deconstruct signaling pathways involved in the migration of tumor cells (Yagi et al. 2011) and to interrogate orthosteric (Alvarez-Curto et al. 2011a,b) and allosteric (Abdul-Ridha et al. 2013, Nawaratne et al. 2008) signaling modes of Gαi-mediated receptors.

With regard to hM3Dq and other Gαq-DREADDs, Alexander et al. (2009) discovered that activating genetically encoded hM3Dq in hippocampal principal cells by CNO induced slow depolarization and burst firing. Since then, many groups have independently reported successful activation of neuronal firing by CNO-mediated activation of hM3Dq in a variety of contexts (Atasoy et al. 2012, Brancaccio et al. 2013, Garner et al. 2012, Kong et al. 2012, Krashes et al. 2011, Sasaki et al. 2011, Vrontou et al. 2013). Additionally, hM3Dq has been used to interrogate the consequences of acute and chronic activation of Gαq-mediated signaling in pancreatic β-cells (Guettier et al. 2009, Jain et al. 2013). In every reported instance, activation of Gαq signaling led to striking behavioral and/or physiological consequences (**Table 1**).

Gαs-DREADD (GsD) was initially used in studies of pancreatic β-cells in vitro and in vivo to deconstruct the signaling pathways essential for insulin secretion (Guettier et al. 2009). Subsequent studies demonstrated that CNO-mediated activation of GsD potently and efficaciously

Table 1 Representative recent publications using DREADD technology

DREADD	Experiment	Result	Reference(s)
hM3Dq +/− hM4Di	Remote control of feeding	Identification of neurons that encode hunger	Atasoy et al. (2012), Kong et al. (2012), Krashes et al. (2011)
hM3Dq	Generation of a synthetic memory trace	Memory encoded sparsely	Garner et al. (2012)
hM4Di	Alteration in neuronal plasticity	Altered striatal connectivity	Kozorovitskiy et al. (2012)
hM4Di	5-HT neuron silencing	Behavior and physiological consequences	Ray et al. (2011)
hM3Dq	Identification of neurons responsible for pleasurable sensation	DRG neurons identified as target of MGPR4 orphan receptor	Vrontou et al. (2013)
GsD	Modulation of cAMP	Modulates circadian clock; regulates insulin secretion	Brancaccio et al. (2013), Guettier et al. (2009)

Abbreviations: DREADD, designer receptor exclusively activated by designer drug; DRG, dorsal root ganglion; GsD, Gαs-DREADD.

augments cAMP-mediated signaling in a variety of neuronal contexts (Brancaccio et al. 2013, Farrell et al. 2013, Ferguson et al. 2011). Given the central role of cAMP-mediated signaling in reward (Carlezon et al. 1998), memory (Kida et al. 2002), and psychoactive drug actions (Carlezon et al. 1998, Pliakas et al. 2001), GsD will likely prove useful for deconstructing the role of cAMP-mediated signaling pathways in genetically defined neurons in vivo (for a recent example, see Ferguson et al. 2011).

GPCRs signal not only via G proteins but also by activating β-arrestin-mediated signaling pathways (Luttrell et al. 1999; for a review, see Allen & Roth 2011). Arrestin-mediated signaling has been implicated in the actions of many psychoactive drugs (Beaulieu et al. 2009) including opioids (Bohn et al. 1999), lithium (Beaulieu et al. 2008), and antipsychotics (Allen et al. 2011). Thus, the creation of a DREADD that specifically activates β-arrestin signaling would be valuable for delineating the role(s) of arrestin-ergic signaling in a variety of cellular contexts (Allen & Roth 2011). Recently, Nakajima & Wess (2012) reported that a mutant M3-muscarinic receptor designated Rq(R165L) could selectively activate arrestin signaling without perturbing G protein–mediated pathways (**Figure 2**). Although the potency of CNO is likely too low to be of great utility for studies in vivo, Rq(R165L) serves as a nice proof of concept for the selective activation of arrestin-ergic signaling by DREADDs.

Importantly, in every reported instance, the expression of either hM4Di or hM3Dq DREADDs has no apparent effect on baseline behaviors, neuronal function, or morphology (see Alexander et al. 2009). Additionally, CNO administration in the absence of DREADD expression has no measurable effect on any of the many monitored behavioral and physiological outcomes (see Alexander et al. 2009; Ray et al. 2011, 2012). In Guettier et al. (2009), a small effect on pancreatic β-cell activity was noted when GsD was overexpressed, although other studies have reported no effect of baseline GsD expression in a variety of neuronal contexts (Brancaccio et al. 2013, Farrell et al. 2013, Ferguson et al. 2011).

Loffler et al. (2012) have raised a concern that some of the effects of CNO could be mediated by the relatively inefficient conversion of CNO to clozapine. A formal pharmacokinetic study in

mice, however, disclosed no apparent conversion of CNO to clozapine—at least following acute administration (Guettier et al. 2009). In addition, and as summarized above, in every experiment reported to date, CNO has no effect on any observed phenomenon in either mice or rats when administered in the absence of DREADD expression. However, a small fraction of CNO is interconverted to clozapine (~10% by mass) in guinea pigs and humans (Jann et al. 1994). Thus, investigators contemplating the use of CNO in humans (or other primates or guinea pigs) will need to design experiments so that the dose of CNO is kept relatively low and that appropriate controls are performed (e.g., CNO administration in the absence of DREADD expression).

To summarize, various DREADDs allow for chemogenetic activation of various canonical (e.g., Gαs, Gαq, Gαi) and noncanonical (e.g., β-arrestin) signaling pathways in essentially any cellular context (**Figure 2**). In all neurons examined to date, activation of these pathways with CNO leads to burst firing with hM3Dq and attenuation/silencing of neuronal firing with hM4Di. Activation of GsD and arrestin-biased DREADD in neurons likely modulates neuronal activity contexts (Brancaccio et al. 2013, Farrell et al. 2013, Ferguson et al. 2011).

The advantages of DREADDs over other approaches such as optogenetics are as follows:

- CNO can be administered orally and noninvasively (e.g., via drinking water).
- CNO kinetics predict a relatively prolonged duration of neuronal activation, inhibition, or modulation (e.g., minutes to hours).
- CNO-mediated activation of DREADDs requires no specialized equipment.
- CNO is readily available.
- CNO diffuses widely following administration, allowing for the modulation of signaling and activity in distributed neuronal populations.
- CNO has been administered to humans and is a known metabolite of widely prescribed medication.

The main disadvantage of the DREADD system is the lack of precise temporal control as is achieved with light-mediated systems such as optogenetics and optopharmacology. This disadvantage could soon be overcome with photocaged CNO (B.L. Roth, manuscript in preparation). Another useful tool that may soon be available involves having additional GPCR-ligand pairs available to allow for multiplexing control over signaling (E. Vardy and B.L. Roth, submitted manuscript). Finally, it may also be useful to obtain more potent control over arrestin signaling than is currently afforded by arrestin-biased DREADD; such technology is also in development (Y. Gotoh and B.L. Roth, manuscript in preparation).

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE

Ion channels are especially well suited for manipulating neuronal activity because they directly control the electrical properties of cells. Early work in manipulating the electrical properties of neurons involved genetically targeted expression of ion channels that chronically alter neuronal conductance. For example, neuronal overexpression of inward rectifier potassium channels resulted in suppression of neuronal excitability, but prolonged expression led to toxicity and could also result in compensatory responses (Ehrengruber et al. 1997, Nadeau et al. 2000). By allowing rapid, remote control over different ion conductances, ligand-gated ion channels (LGICs) are better suited for temporal control over neuronal activity. LGICs have been widely exploited for neuronal stimulation or silencing to examine causal relationships between electrical activity and animal behavior, primarily by intracranial administration of agonists for glutamate (Stanley et al. 1993) and GABA (Hikosaka & Wurtz 1985) receptors. However, to perturb a localized subset of neurons in the brain, small molecules must be locally targeted, typically through a cannula that

destroys overlying neural tissue. A greater drawback is that these perturbations are not cell type specific owing both to the widespread expression of glutamate and GABA receptors on neurons and to the absence of pharmacologically distinct LGICs on most cell types.

More recently, several LGICs that are optimized primarily for use in mammals have been developed for cell type–specific pharmacological control of neuron electrical activity. LGICs suitable as ectopically expressed tools for neuronal activity perturbation also require a selective ligand that does not activate endogenous ion channels. Three categories of LGIC tools have been developed for cell type–selective neuron perturbation: (*a*) invertebrate LGICs, (*b*) ectopic expression of endogenous mammalian LGICs in the context of a global knockout background for that channel, and (*c*) engineered ligand and ion channel systems.

Invertebrate LGICs

Invertebrate LGICs with pharmacological properties distinct from mammalian ion channels have been exploited to perturb electrical activity in genetically targeted neuron populations via transgenic expression in the mammalian brain. Glutamate-gated chloride (GluCl) channels from the roundworm *Caenorhabditis elegans* have been developed as selective neuronal silencers (Slimko et al. 2002). GluCl channels are high conductance chloride channels formed as heteromers of GluClα and GluClβ subunits, both of which must be expressed to produce functional channels (Slimko et al. 2002). GluCl conductance can be activated by the antiparasite drug ivermectin (IVM), which is a high potency allosteric agonist. IVM is commonly administered at low doses to mammals as an antiparasite medication without obvious neurological side effects, implying selective action on the GluCl channels of parasites over endogenous mammalian LGICs. In neurons, IVM gating of GluCl channels results in the suppression of neuronal activity (**Figure 3**), primarily through a large drop in input resistance across the neuronal membrane (Slimko et al. 2002). The effect on neuronal activity can be understood using Ohm's law ($V = IR$), where the voltage change (V) is directly proportional to the current (I) across the membrane as a function of the membrane resistance (R) of the cell. Reduction of neuronal input resistance by opening the GluCl channels acts as an electrical shunt, thereby reducing the influence of an injected or synaptic current on the membrane potential and, hence, decreases neuronal excitability.

An additional consideration for the use of GluCl channels in mammals regards their affinity for glutamate, which is an abundant neurotransmitter in mammalian brains. Activation of exogenous GluCl by endogenous glutamate has been minimized by a single-point mutation (Y182F) in the glutamate binding pocket of GluClβ, which substantially reduces the potency of glutamate activation of GluCl (Li et al. 2002). IVM sensitivity is only weakly changed, likely owing to the distinct GluCl binding sites for IVM and glutamate (Hibbs & Gouaux 2011).

This modified GluCl channel is useful for silencing neurons in behaving mice in conjunction with minimally invasive intraperitoneal IVM administration. Targeted delivery of GluClα/β subunits to defined neuron populations by viral vectors and transgenic overexpression has been used to suppress neuronal function in the striatum, amygdala, and hypothalamus. Inhibition of striatal neurons on one side of the brain during amphetamine administration resulted in rotation during locomotion (Lerchner et al. 2007). Given this behavioral readout, dosing and pharmacokinetic properties have indicated that IVM has a slow onset of action, requiring dosing one day prior to behavioral test, and that IVM clearance requires multiple days. This is likely due to the high lipophilicity of IVM, which presumably aids brain penetration, but can also result in accumulation in fat depots in the body (McKellar et al. 1992), reducing brain access and acting as a reservoir for IVM after the initial dose. Using these dosing parameters, researchers have also used the IVM/GluCl system to suppress PKCδ-expressing neurons in the central nucleus of the amygdala,

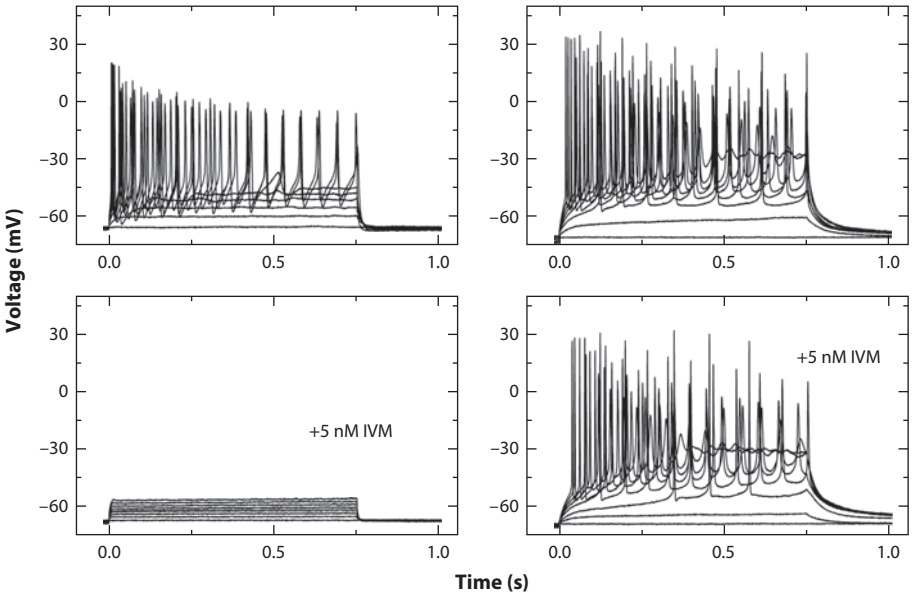


Figure 3

Neuronal silencing with invertebrate ligand-gated ion channels. (*Left*) Coexpression of GluClα and GluClβ (subunits of glutamate-gated chloride channels) in neurons does not reduce cellular excitability, but electrical activity is strongly suppressed in the presence of ivermectin (IVM). (*Right*) Untransfected cells are not silenced by IVM. Figure modified from Slimko et al. (2002).

which increases fear responses (freezing) to a conditioned stimulus previously paired with a foot shock (Haubensak et al. 2010). In addition, GluCl/IVM-mediated suppression of neurons in the ventromedial hypothalamic nucleus, a region that can induce aggressive behavior when activated, reduces aggression of male mice toward male intruders (Lin et al. 2011).

Further modifications to GluCl channels that improved protein trafficking and IVM sensitivity have also been reported (Frazier et al. 2013), and these may prove useful for reducing potential toxicity or off-target effects associated with high IVM doses that are required for neuron silencing in the brain. As an alternative method, the mammalian glycine receptor, another chloride channel, has been recently engineered for neuronal perturbation via the introduction of mutations that render the channel sensitive to IVM allosteric activation and that reduce glycine sensitivity (Lynagh & Lynch 2010). These channels require only a single subunit to be delivered to neurons to achieve neuronal silencing. In principle, chemical modifications to IVM could also provide variants with faster pharmacokinetic properties, which would facilitate more acute perturbations.

Mammalian LGICs

Tools for selective perturbation of neuronal activity have also been developed using mammalian LGICs, which enables use of an extensive range of selective small-molecule ligands for these channels. By ectopically targeting the LGIC to the cell type of interest, researchers use these tools to adapt nonessential mammalian LGICs for selective neuronal activation or silencing. Because these LGICs are also expressed endogenously, selective channel expression is performed on a global knockout background for the endogenous LGIC gene to avoid activation of endogenous channels.

This strategy has been used to demonstrate cell type–selective chemical activation of neurons via targeted expression of the TRPV1 ion channel. TRPV1 is a nonselective cation channel that is gated by the small molecule capsaicin (the molecule in chili peppers that renders them spicy), resulting in neuronal depolarization (Arenkiel et al. 2007, Zemelman et al. 2003). Because capsaicin and other TRPV1 agonists can act on endogenous channels, TRPV1 must be targeted to specific cell types in mice in which endogenous *Trpv1* has been genetically inactivated (**Figure 4*a***). This has been carried out by ectopically targeting TRPV1 to dopamine neurons in *Trpv1*$^{-/-}$ mice. In these mice, capsaicin results in robust activation of dopamine neurons, elevated release of dopamine in the striatum, and increased locomotor activity (Guler et al. 2012). Although the use of this chemogenetic strategy for neuronal activation requires extensive mouse breeding, it also offers the convenience of no surgical procedures to selectively express TRPV1 or to deliver its agonist capsaicin.

A related strategy has been developed for neuron inhibition using cell type–selective activation of $GABA_A$ receptors by the allosteric agonist zolpidem. *Gabrg2* encodes the $GABA_A$ receptor $\gamma 2$ subunit, which is essential for $GABA_A$ receptor function and is critical for zolpidem sensitivity. However, zolpidem sensitivity in $GABA_A$ receptors is eliminated in mice with a targeted mutation of the $\gamma 2$ subunit at amino acid position 77 from isoleucine to phenylalanine (I77F), while retaining GABA responsiveness. To utilize zolpidem as a selective neuronal silencer, mice were engineered to be zolpidem insensitive in every neuron except the cell type that was targeted for silencing (**Figure 4*b***). This was accomplished by replacing exon 4 of *Gabrg2*, which encodes I77 of the $\gamma 2$ subunit, with an I77F mutation. The modified exon 4 was flanked by loxP sites so that it could be removed cell type selectively by crossing to a Cre recombinase–expressing mouse line (Wulff et al. 2007). Then, mice carrying a transgene encoding the native *Gabrg2* cDNA sequence under a cell type–specific promoter could be bred onto this background to add zolpidem-sensitive $\gamma 2$ subunits only to the cell populations associated with Cre recombinase removal of the I77F-encoding exon. The result was zolpidem sensitivity only in the Cre-expressing cell type of interest. Applying this strategy to cerebellar Purkinje cells resulted in pronounced ataxia in the presence of zolpidem that was not apparent in I77F mutant mice (Wulff et al. 2007). A key aspect of this silencing approach is that neurons are not directly inhibited by zolpidem, but instead the efficacy of endogenous GABA-releasing synaptic input is potentiated in the zolpidem-sensitive neuron populations.

Engineered LGICs and Ligands

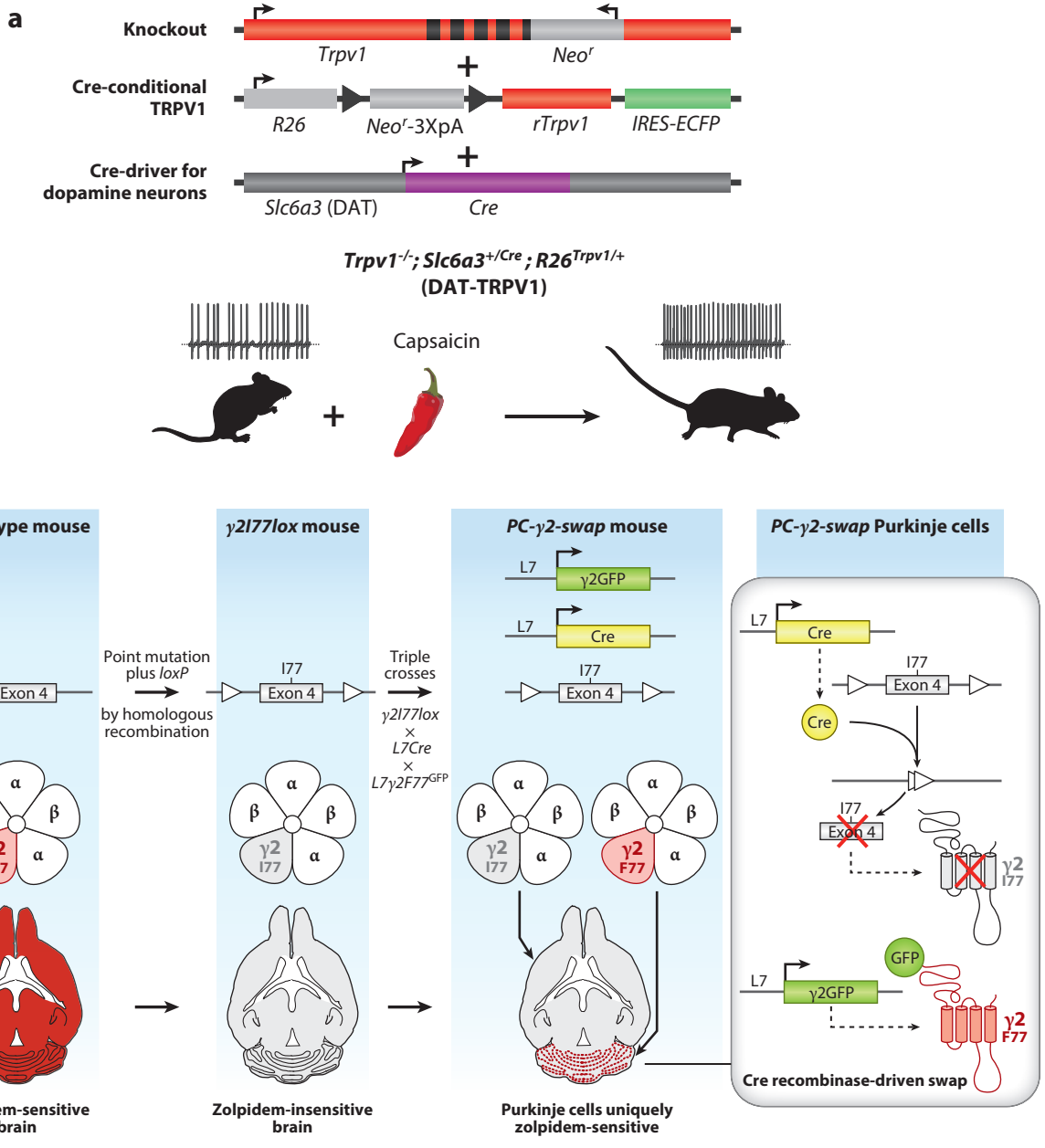
A newer approach to chemogenetic manipulation of neuron electrical activity is based on chimeric ion channels that were developed using concerted genetic and chemical engineering of selective interactions between ion channels and small-molecule agonists (Magnus et al. 2011).

Figure 4

Electrical activity perturbation strategies using mammalian ligand-gated ion channels. (*a*) Scheme for neuronal activation that involves cell type–selective Cre recombinase–dependent expression of TRPV1 on a global *Trpv1* knockout background. *Slc6a3*$^{+/Cre}$ restricts ectopic TRPV1 expression to dopamine neurons. Intraperitoneal delivery of the TRPV1 agonist capsaicin results in dopamine neuronal activation and increases locomotion in mice. Panel modified from Guler et al. (2012). (*b*) Scheme for silencing of neuronal activity using the $GABA_A$ receptor allosteric agonist zolpidem. The endogenous zolpidem sensitivity (mediated by the $GABA_A$ receptor $\gamma 2$ subunit at position F77) is eliminated with global knockin of a loxP-flanked exon encoding F77I. In a cell type of interest (in this case, Purkinje neurons targeted selectively using the L7 promoter), Cre recombinase is expressed to remove the loxP-flanked exon. The same cell type–specific promoter is also used to transgenically express the zolpidem-sensitive $\gamma 2$ subunit. Neurons in mice with all these components can be selectively silenced with zolpidem. Via this system, Purkinje neuron silencing was shown to affect motor behavior. Abbreviation: GFP, green fluorescent protein. Panel modified from Wulff et al. (2007).

These engineered LGICs overcome restrictions of earlier LGIC-based tools such as limited characterization of invertebrate channels, the need to knock out endogenous mammalian ion channel genes, and the generally limited capability to optimize either channel properties or the pharmacokinetic properties of ligands.

This chemical and genetic engineering strategy for cell type–specific control over ion conductance is based on classic experiments demonstrating that the extracellular ligand binding domain (LBD) of the α7 nicotinic acetylcholine receptor behaves as an independent actuator module that can be transplanted onto the ion pore domains (IPDs) of other members of the large Cys-loop



receptor ion channel family. Thus, splicing the α7 nAChR LBD to the IPD of the serotonin receptor (5HT3a) produces a channel (α7-5HT3) with α7 nAChR pharmacology and 5HT3a conductance properties (Eisele et al. 1993). An analogous engineered channel has been developed by fusing the α7 nAChR LBD to the chloride-selective glycine receptor (GlyR) IPD, which renders an acetylcholine-responsive chloride channel (α7-GlyR) (Grutter et al. 2005). This modular property is a strong foundation for optimizing functional characteristics. Moreover, because α7 nAChR LBDs assemble into homomeric pentamers, chimeric LGICs based on this motif self-assemble without needing to express additional cofactors, which facilitates their use as tools targeted to molecularly defined neuronal populations.

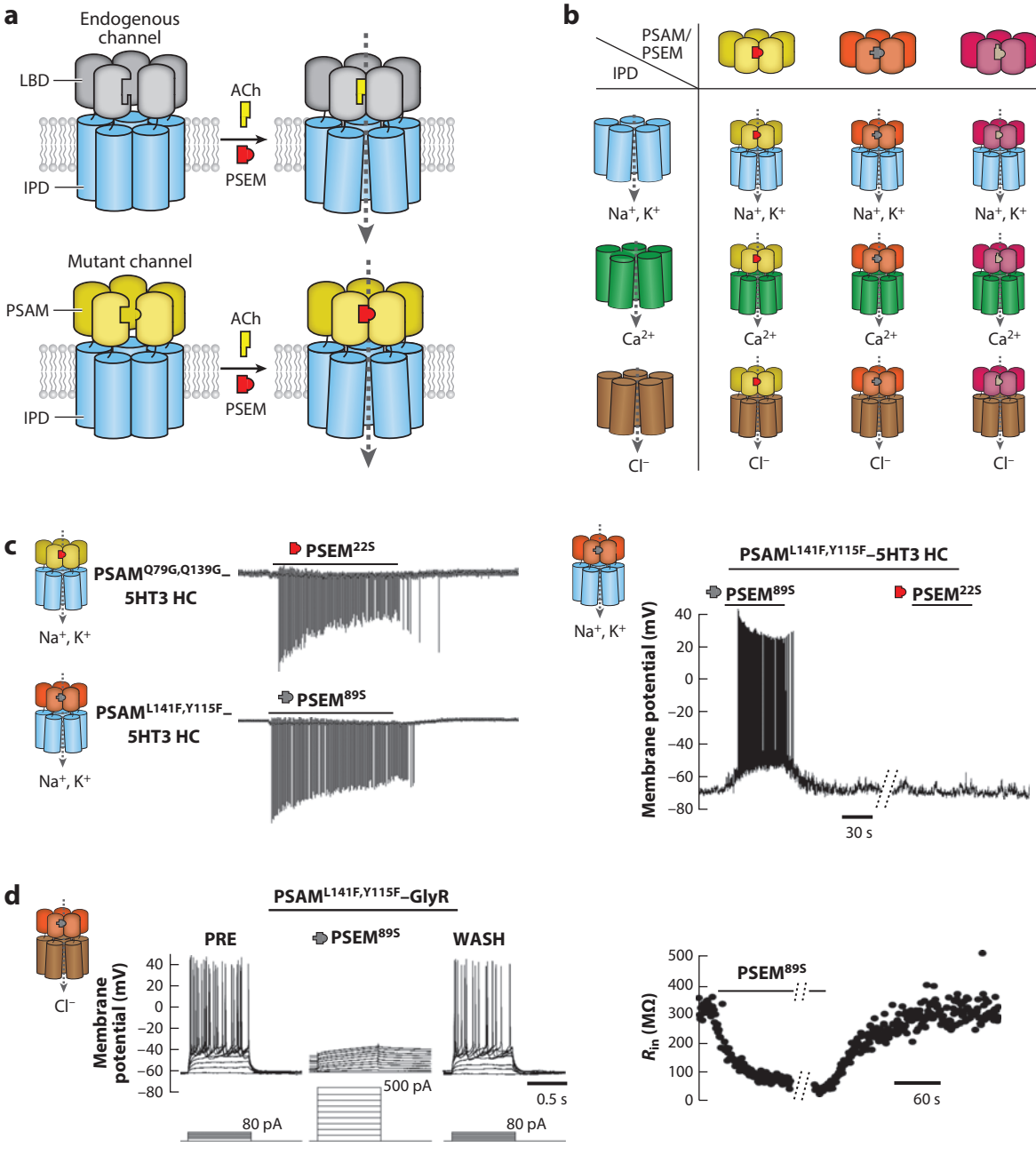
The major challenge to use these chimeric ion channels and their ligands as cell type–selective perturbation tools is that α7 nAChR is endogenously expressed in many neuron populations and α7 nAChR agonists can perturb these other cell groups. As described above, this problem has been typically addressed by eliminating the endogenous allele, which usually requires expensive and time-intensive mouse breeding approaches. For chimeric channels using the extracellular LBD of the α7 nAChR, an alternative solution was used. The ligand recognition properties of the α7 nAChR LBD were engineered using a "bump-hole" strategy (Bishop et al. 2000, Hwang & Miller 1987, Lin et al. 2001, Westkaemper et al. 1999) in which LBD mutations generate "holes" that allow binding of bulky ("bumped") chemical analogs of ligands that would not otherwise bind the endogenous LBD. An α7 nAChR agonist, quinuclidinyl benzamide PNU-282987, was used as a starting point for agonist design because it crosses the blood-brain barrier (Walker et al. 2006); is highly selective for α7 nAChR over other isoforms; and is highly selective against a broad panel of vertebrate ion channels, GPCRs, and transporters (Bodnar et al. 2005). A library of mutated ion channels was tested in an activity-based screen against a library of "bumped" quinuclidinyl benzamides. From this screen, multiple mutated ion channel and complementary agonist combinations were identified with ligands that did not activate the unmodified receptor. Furthermore, several combinations of agonist/mutated LBDs were engineered to be orthogonal to each other, allowing their use in concert. These mutated LBDs were called pharmacologically selective actuator modules (PSAM; pronounced "sam"), and distinct PSAMs are represented by the specific mutation that renders their selectivity, e.g., $PSAM^{L141F}$. The cognate agonists were called pharmacologically selective effector molecules (PSEM; pronounced "sem") and are referred to with specific numbers, e.g., $PSEM^{89S}$ (**Figure 5*a***).

A variety of PSAM/PSEM combinations allowed for the generation of pharmacologically selective ion channels that have distinct ion conductance properties and that can be gated without

Figure 5

Pharmacologically selective actuators and effectors for control of ion conductance. (*a*) Design scheme for chimeric LGICs composed of LBD and IPD modules. LBD mutations yield a PSAM that selectively binds PSEMs (*red*) but not the endogenous ligand (ACh) (*yellow*). PSEMs do not bind the unmodified LBD. (*b*) Combinatorial generation of pharmacologically selective LGICs with diverse conductance properties by joining PSAM and IPD modules. (*c*) (*Left*) Chimeric LGICs for neuronal activation channels built from a nonspecific cation-selective IPD (5HT3 HC, a high-conductance variant of the serotonin 3 receptor) and two pharmacologically distinct PSAMs. Application of the appropriate PSEM leads to sustained neuronal activation (PSEM application for 120 s; traces are cell-attached recordings). (*Right*) The pharmacological selectivity of one of the chimeric channels is shown in a neuron expressing $PSAM^{L141F,Y115F}$-5HT3 HC, which leads to neuronal activation in the presence only of $PSEM^{89S}$ but not of $PSEM^{22S}$. (*d*) Using the same PSAM as in panel *c* with the IPD from GlyR results in a pharmacologically selective chloride channel. This channel suppresses neuronal activity even in response to high current injection (*left*) owing to the shunting properties of the open channel that strongly reduces neuronal input resistance (R_{in}). Figure modified from Magnus et al. (2011). Abbreviations: ACh, acetylcholine; GlyR, glycine receptor; IPD, ion pore domain; LBD, ligand binding domain; LGIC, ligand-gated ion channel; PSAM, pharmacologically selective actuator module; PSEM, pharmacologically selective effector molecule; WASH, washout of ligand with artificial cerebrospinal fluid.

activating either the endogenous α7 nAChR or other PSAM-containing channels (**Figure 5*b***). PSAMs were fused to IPDs from several members of the Cys-loop LGIC family: serotonin, glycine, GABA C, and nicotinic acetylcholine receptors. Because the IPD determines the ionic conductance properties, PSAM-IPD chimeric channels activated with the corresponding PSEMs provided pharmacological control of ion conductance for either nonspecific cations, chloride or calcium (Magnus et al. 2011).



Multiple pharmacologically selective cation channels were generated by fusing different PSAMs to the 5HT3 IPD (**Figure 5*c***). Neurons expressing these channels depolarized and fired action potentials for minutes during PSEM application. Action potential activity ceased shortly after PSEM removal. The same PSEM molecules could also be used to activate chimeric chloride channels by fusion of the cognate PSAM LBDs with GlyR or GABA C IPDs (**Figure 5*d***). These channels sharply reduced the input resistance of neurons and strongly inhibited neuronal excitability in the presence of the appropriate PSEM (**Figure 5*d***). PSAM-GlyR-expressing neurons were electrically shunted by PSEM application and could not be activated even with injection of hundreds of picoamps of depolarizing current, but washout of the PSEM restored neuron excitability within minutes.

Because of the strong shunting properties of PSAM-GlyR silencing, these channels are especially useful for suppressing neuronal activity, even during strong, concerted excitatory synaptic input. This was strikingly demonstrated in experiments dissecting the role of two interneuron cell types in CA1 hippocampal circuits. Strong synaptic drive into CA1 by patterned optical stimulation of Schaeffer collateral axon projections activates a local circuit involving multiple interneurons that shapes the output of principal CA1 pyramidal neurons. PSAM^{L141F,Y115F}-GlyR channels selectively silenced molecularly defined subpopulations of CA1 interneurons during strong synaptic stimulation. This allowed precise dissection of the relative contribution of these two interneuron circuit components to the input-output properties of hippocampal principal cells (Lovett-Barron et al. 2012).

The in vivo efficacy of PSAM-GlyR silencing with a PSEM was demonstrated under especially challenging conditions designed to test silencing capability during concerted depolarizing input from activation of the light-activated ion channel channelrhodopsin-2 (ChR2). ChR2 was expressed in hypothalamic neurons that induce feeding behavior when optically stimulated along with PSAM^{L141F,Y115F}-GlyR. During optical stimulation of hypothalamic neurons with an implanted optical fiber and in the absence of the cognate PSEM ligand, mice consumed food rapidly within minutes of photoactivation. Intraperitoneal delivery of PSEM^{89S} led to suppression of ChR2-evoked feeding. This effect was completely reversed the following day when ChR2 activation once again was sufficient to evoke feeding (Magnus et al. 2011). These experiments demonstrated that this selective ligand and LGIC system can serve as a powerful neuronal silencer in vivo. Subsequent studies have confirmed the efficacy of PSAM^{L141F,Y115F}-GlyR and a related channel, PSAM^{L141F}-GlyR, for neuronal silencing in vivo by suppression of contextual fear learning (Lovett-Barron et al. 2014) and inhibition of neurons that are critically important in a skilled reaching task (Esposito et al. 2014). Furthermore, an additional study applied PSAM^{L141F,Y115F}-GlyR for neuronal silencing and PSAM^{L141F,Y115F}-5HT3 HC for neuronal activation to bidirectionally control hippocampal interneuron activity to reduce or enhance foreign object recognition learning, respectively (Donato et al. 2013). These experiments highlight the flexibility of PSAM-IPD ion channels for gain-of-function and loss-of-function neuronal activity manipulations and also illustrate the in vivo efficacy of their cognate PSEM ligands.

Further extension of the chemogenetic toolbox can be based on PSAM/PSEM selectivity modules that are transferable to functionally diverse IPDs, which provide access to a combinatorial array of LGICs based on these components (**Figure 5*b***). The combinations of PSAM/PSEMs and IPDs enable production of additional cell type–selective tools for pharmacological control over LGICs with multiple ionic conductances. These chimeric ion channels can also be further elaborated by applying extensive prior work on IPD structure-function relationships within the Cys-loop receptor superfamily, including mutations that modify channel conductance (Kelley et al. 2003), ion selectivity (Bertrand et al. 1993, Galzi et al. 1992, Gunthorpe & Lummis 2001,

Keramidas et al. 2000), intracellular interactions (Jansen et al. 2008, Temburni et al. 2000, Xu et al. 2006), and desensitization (Breitinger et al. 2001, Galzi et al. 1992, Revah et al. 1991).

OTHER CHEMOGENETIC APPROACHES FOR NEUROBIOLOGY

Chemogenetic methods have also been developed to control other aspects of neuronal function. Cell type–selective pharmacological control of synaptic release has been demonstrated with ligand-induced dimerization technologies. After fusion of the small protein FKBP to the synaptic vesicle–associated protein synaptobrevin, a dimeric FKBP-binding molecule (AP20187) could reversibly oligomerize these critical proteins for synaptic vesicle fusion and neurotransmitter release. In mice engineered to express Synaptobrevin-FKBP in Purkinje neurons, delivery of the ligand directly to the brain results in reversible motor deficits (Karpova et al. 2005). This pioneering work on molecular inhibitors of synaptic transmission highlights the value of considering approaches to selectively suppress synaptic release, which may be especially useful in applications involving suppression of specific axon projections that define a subset of the circuit interactions of a particular cell type.

Kinases are critical for many cell signaling pathways in neurons, but most lack highly selective inhibitors to block activity. To selectively inhibit kinase activity, kinases have been engineered to bind to modified ATP binding-site inhibitors that do not bind to endogenous kinase (Bishop et al. 2000). Selective inhibition of kinases in the brain using pharmacologically selective alleles has provided insight into a number of areas including neurotrophin signaling (Chen et al. 2005), dendritic spine development (Ultanir et al. 2012), and epilepsy (Liu et al. 2013). Targeting pharmacologically selective kinase alleles to specific neuron populations affords cell type–specific modulation of these signaling pathways with a high degree of temporal control.

Another promising area for further development is cell type–specific enzymatic targeting of small molecules (**Figure 6**). In this approach, a small-molecule fluorophore or drug-like molecule is chemically derivatized with a "masking" group that renders it inactive. The masking group is selected to be inert to endogenous enzymatic degradation pathways, but it is labile to an exogenous enzyme that can be targeted as a transgene to specific cell populations. This approach targets fluorophores masked with an ester that is inert to endogenous neuronal esterases. Only specific subsets of neurons expressing a transgene for the enzyme porcine liver esterase showed accumulation of the small-molecule dye (Tian et al. 2012). The effectiveness of this method has also been

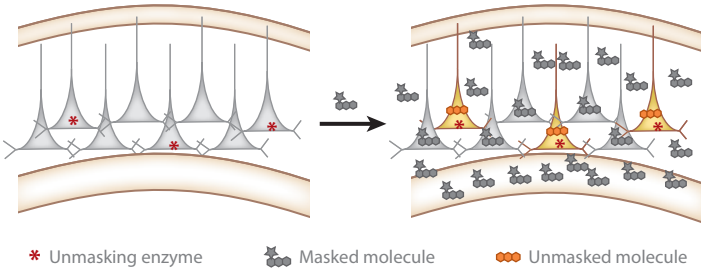


Figure 6

Scheme for cell type–selective targeting of small molecules to a specific subpopulation of neurons within a brain area via targeted unmasking enzyme expression. A subset of neurons expresses an unmasking enzyme (*red asterisk*). A masked molecule (*gray*) that is pharmacologically inert is applied to the entire region and diffuses into every cell. Only the neurons that express the unmasking enzyme liberate the active form of the molecule (*orange*).

demonstrated for a pharmacological inhibitor targeted to specific cells. In addition, a similar approach has been applied in vivo in a zebrafish model (Pisharath 2007, Pisharath et al. 2007). These methods have the potential to enable cell type–specific pharmacology for intracellular signaling pathways in the context of complex heterogeneous tissues such as the brain.

PERSPECTIVES FOR INTEGRATED USE OF VARIOUS TECHNOLOGIES

The growing diversity of pharmacologically orthogonal ion channels, DREADDs, and optogenetic tools can also be used for multiple cell type–specific perturbations in the same animal (Krashes et al. 2014, Magnus et al. 2011). Moreover, because these tools have been rationally developed by concerted chemical and genetic engineering approaches, new variants of these tools can be tailored for specific experimental requirements. One key consideration for applying chemogenetic and optogenetic methods either individually or in concert is the timescales for control of neuronal activity. For example, optogenetic tools provide millisecond precision but require considerable levels of energy to be delivered to the brain for longer timescale perturbations; with PSAM-IPD systems, the pharmacokinetics of PSEMs show rapid onset and brain clearance in one hour (Magnus et al. 2011); and, for DREADDs, the ligand CNO persists for several hours and can be applied for days (Krashes et al. 2011). Therefore, selection of the most suitable tools can now be based on both the mechanism of action for perturbing neuronal function (e.g., ion conductance or G protein signaling) as well as the temporal dynamics of the circuit functions that are under investigation. In the future, we envision that these various technologies will be increasingly used in combination to identify how multiple types of neuronal and nonneuronal perturbations can result in distinctive alterations in circuit dynamics and animal behavior.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

Abdul-Ridha A, Lane JR, Sexton PM, Canals M, Christopoulos A. 2013. Allosteric modulation of a chemogenetically modified G protein–coupled receptor. *Mol. Pharmacol.* 83:521–30

Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K. 2009. Temporally precise in vivo control of intracellular signalling. *Nature* 458:1025–29

Alexander GM, Rogan SC, Abbas AI, Armbruster BN, Pei Y, et al. 2009. Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron* 63:27–39

Allen JA, Roth BL. 2011. Strategies to discover unexpected targets for drugs active at G protein–coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* 51:117–44

Allen JA, Yost JM, Setola V, Chen X, Sassano MF, et al. 2011. Discovery of β-arrestin-biased dopamine D2 ligands for probing signal transduction pathways essential for antipsychotic efficacy. *Proc. Natl. Acad. Sci. USA* 108:18488–93

Alvarez-Curto E, Prihandoko R, Tautermann CS, Zwier JM, Pediani JD, et al. 2011a. Developing chemical genetic approaches to explore G protein-coupled receptor function: validation of the use of a receptor activated solely by synthetic ligand (RASSL). *Mol. Pharmacol.* 80:1033–46

Alvarez-Curto E, Ward RJ, Pediani JD, Milligan G. 2011b. Ligand regulation of the quaternary organization of cell surface M3 muscarinic acetylcholine receptors analyzed by fluorescence resonance energy transfer (FRET) imaging and homogeneous time-resolved FRET. *J. Biol. Chem.* 285:23318–30

Arenkiel BR, Peca J, Davison IG, Feliciano C, Deisseroth K, et al. 2007. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron* 54:205–18

Armbruster B, Roth B. 2005. Creation of designer biogenic amine receptors via directed molecular evolution. *Neuropsychopharmacology* 30:S265

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL. 2007. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc. Natl. Acad. Sci. USA* 104:5163–68

Atasoy D, Betley JN, Su HH, Sternson SM. 2012. Deconstruction of a neural circuit for hunger. *Nature* 488:172–77

Beaulieu JM, Gainetdinov RR, Caron MG. 2009. Akt/GSK3 signaling in the action of psychotropic drugs. *Annu. Rev. Pharmacol. Toxicol.* 49:327–47

Beaulieu JM, Marion S, Rodriguiz RM, Medvedev IO, Sotnikova TD, et al. 2008. A β-arrestin 2 signaling complex mediates lithium action on behavior. *Cell* 132:125–36

Bender D, Holschbach M, Stocklin G. 1994. Synthesis of n.c.a. carbon-11 labelled clozapine and its major metabolite clozapine-*N*-oxide and comparison of their biodistribution in mice. *Nucl. Med. Biol.* 21:921–25

Bertrand D, Galzi JL, Devillers-Thiery A, Bertrand S, Changeux JP. 1993. Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal α7 nicotinic receptor. *Proc. Natl. Acad. Sci. USA* 90:6971–75

Bishop AC, Ubersax JA, Petsch DT, Matheos DP, Gray NS, et al. 2000. A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* 407:395–401

Bodnar AL, Cortes-Burgos LA, Cook KK, Dinh DM, Groppi VE, et al. 2005. Discovery and structure-activity relationship of quinuclidine benzamides as agonists of α7 nicotinic acetylcholine receptors. *J. Med. Chem.* 48:905–8

Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. 1999. Enhanced morphine analgesia in mice lacking β-arrestin 2. *Science* 286:2495–98

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. 2005. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8:1263–68

Brancaccio M, Maywood ES, Chesham JE, Loudon AS, Hastings MH. 2013. A Gq-Ca^{2+} axis controls circuit-level encoding of circadian time in the suprachiasmatic nucleus. *Neuron* 78:714–28

Breitinger HG, Villmann C, Becker K, Becker CM. 2001. Opposing effects of molecular volume and charge at the hyperekplexia site α1(P250) govern glycine receptor activation and desensitization. *J. Biol. Chem.* 276:29657–63

Callaway EM, Katz LC. 1993. Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proc. Natl. Acad. Sci. USA* 90:7661–65

Carlezon WA Jr, Thome J, Olson VG, Lane-Ladd SB, Brodkin ES, et al. 1998. Regulation of cocaine reward by CREB. *Science* 282:2272–75

Chang WC, Ng JK, Nguyen T, Pellissier L, Claeysen S, et al. 2007. Modifying ligand-induced and constitutive signaling of the human 5-HT4 receptor. *PLoS ONE* 2:e1317

Chen X, Ye H, Kuruvilla R, Ramanan N, Scangos KW, et al. 2005. A chemical-genetic approach to studying neurotrophin signaling. *Neuron* 46:13–21

Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, et al. 2013. Structural and molecular interrogation of intact biological systems. *Nature* 497:332–37

Conklin BR, Hsiao EC, Claeysen S, Dumuis A, Srinivasan S, et al. 2008. Engineering GPCR signaling pathways with RASSLs. *Nat. Methods* 5:673–78

Coward P, Wada HG, Falk MS, Chan SD, Meng F, et al. 1998. Controlling signaling with a specifically designed G_i-coupled receptor. *Proc. Natl. Acad. Sci. USA* 95:352–57

Crick F. 1999. The impact of molecular biology on neuroscience. *Philos. Trans. R. Soc. B* 354:2021–25

Crick FH. 1979. Thinking about the brain. *Sci. Am.* 241:219–32

Donato F, Rompani SB, Caroni P. 2013. Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 504:272–76

Dong S, Rogan SC, Roth BL. 2010. Directed molecular evolution of DREADDs: a generic approach to creating next-generation RASSLs. *Nat. Protoc.* 5:561–73

Ehrengruber MU, Doupnik CA, Xu Y, Garvey J, Jasek MC, et al. 1997. Activation of heteromeric G protein-gated inward rectifier K^+ channels overexpressed by adenovirus gene transfer inhibits the excitability of hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 94:7070–75

Eisele JL, Bertrand S, Galzi JL, Devillers-Thiery A, Changeux JP, Bertrand D. 1993. Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. *Nature* 366:479–83

Esposito MS, Capelli P, Arber S. 2014. Brainstem nucleus MdV mediates skilled forelimb motor tasks. *Nature* 508:351–56

Farrell MS, Pei Y, Wan Y, Yadav PN, Daigle TL, et al. 2013. A Gαs DREADD mouse for selective modulation of cAMP production in striatopallidal neurons. *Neuropsychopharmacology* 38:854–62

Farrell MS, Roth BL. 2013. Pharmacosynthetics: reimagining the pharmacogenetic approach. *Brain Res.* 1511:6–20

Ferguson SM, Eskenazi D, Ishikawa M, Wanat MJ, Phillips PE, et al. 2011. Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat. Neurosci.* 14:22–24

Frazier SJ, Cohen BN, Lester HA. 2013. An engineered glutamate-gated chloride (GluCl) channel for sensitive, consistent neuronal silencing by ivermectin. *J. Biol. Chem.* 288:21029–42

Galzi JL, Devillers-Thiery A, Hussy N, Bertrand S, Changeux JP, Bertrand D. 1992. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature* 359:500–5

Gao ZG, Duong HT, Sonina T, Kim SK, Van Rompaey P, et al. 2006. Orthogonal activation of the reengineered A3 adenosine receptor (neoceptor) using tailored nucleoside agonists. *J. Med. Chem.* 49:2689–702

Garner AR, Rowland DC, Hwang SY, Baumgaertel K, Roth BL, et al. 2012. Generation of a synthetic memory trace. *Science* 335:1513–16

Grutter T, de Carvalho LP, Dufresne V, Taly A, Edelstein SJ, Changeux JP. 2005. Molecular tuning of fast gating in pentameric ligand-gated ion channels. *Proc. Natl. Acad. Sci. USA* 102:18207–12

Guettier JM, Gautam D, Scarselli M, de Azua IR, Li JH, et al. 2009. A chemical-genetic approach to study G protein regulation of β cell function in vivo. *Proc. Natl. Acad. Sci. USA* 106:19197–202

Guler AD, Rainwater A, Parker JG, Jones GL, Argilli E, et al. 2012. Transient activation of specific neurons in mice by selective expression of the capsaicin receptor. *Nat. Commun.* 3:746

Gunthorpe MJ, Lummis SC. 2001. Conversion of the ion selectivity of the 5-HT_{3A} receptor from cationic to anionic reveals a conserved feature of the ligand-gated ion channel superfamily. *J. Biol. Chem.* 276:10977–83

Hama H, Kurokawa H, Kawano H, Ando R, Shimogori T, et al. 2011. Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain. *Nat. Neurosci.* 14:1481–88

Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, et al. 2010. Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* 468:270–76

Hibbs RE, Gouaux E. 2011. Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature* 474:54–60

Hikosaka O, Wurtz RH. 1985. Modification of saccadic eye movements by GABA-related substances. I. Effect of muscimol and bicuculline in monkey superior colliculus. *J. Neurophysiol.* 53:266–91

Hsiao EC, Boudignon BM, Chang WC, Bencsik M, Peng J, et al. 2008. Osteoblast expression of an engineered G_s-coupled receptor dramatically increases bone mass. *Proc. Natl. Acad. Sci. USA* 105:1209–14

Hsiao EC, Nguyen TD, Ng JK, Scott MJ, Chang WC, et al. 2011. Constitutive G_s activation using a single-construct tetracycline-inducible expression system in embryonic stem cells and mice. *Stem Cell Res. Ther.* 2:11

Hwang YW, Miller DL. 1987. A mutation that alters the nucleotide specificity of elongation factor Tu, a GTP regulatory protein. *J. Biol. Chem.* 262:13081–85

Jacobson KA, Gao ZG, Chen A, Barak D, Kim SA, et al. 2001. Neoceptor concept based on molecular complementarity in GPCRs: a mutant adenosine A_3 receptor with selectively enhanced affinity for amine-modified nucleosides. *J. Med. Chem.* 44:4125–36

Jacobson KA, Ohno M, Duong HT, Kim SK, Tchilibon S, et al. 2005. A neoceptor approach to unraveling microscopic interactions between the human A_{2A} adenosine receptor and its agonists. *Chem. Biol.* 12:237–47

Jain S, Ruiz de Azua I, Lu H, White MF, Guettier JM, Wess J. 2013. Chronic activation of a designer G_q-coupled receptor improves β cell function. *J. Clin. Investig.* 123:1750–62

Jann MW, Lam YW, Chang WH. 1994. Rapid formation of clozapine in guinea-pigs and man following clozapine-*N*-oxide administration. *Arch. Int. Pharmacodyn. Ther.* 328:243–50
Jansen M, Bali M, Akabas MH. 2008. Modular design of Cys-loop ligand-gated ion channels: functional 5-HT3 and GABA Rho1 receptors lacking the large cytoplasmic M3M4 loop. *J. Gen. Physiol.* 131:137–46
Karpova AY, Tervo DG, Gray NW, Svoboda K. 2005. Rapid and reversible chemical inactivation of synaptic transmission in genetically targeted neurons. *Neuron* 48:727–35
Ke MT, Fujimoto S, Imai T. 2013. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nat. Neurosci.* 16:1154–61
Kelley SP, Dunlop JI, Kirkness EF, Lambert JJ, Peters JA. 2003. A cytoplasmic region determines single-channel conductance in 5-HT_3 receptors. *Nature* 424:321–24
Keramidas A, Moorhouse AJ, French CR, Schofield PR, Barry PH. 2000. M2 pore mutations convert the glycine receptor channel from being anion- to cation-selective. *Biophys. J.* 79:247–59
Kida S, Josselyn SA, Pena de Ortiz S, Kogan JH, Chevere I, et al. 2002. CREB required for the stability of new and reactivated fear memories. *Nat. Neurosci.* 5:348–55
Kokel D, Cheung CY, Mills R, Coutinho-Budd J, Huang L, et al. 2013. Photochemical activation of TRPA1 channels in neurons and animals. *Nat. Chem. Biol.* 9:257–63
Kong D, Tong Q, Ye C, Koda S, Fuller PM, et al. 2012. GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. *Cell* 151:645–57
Kozorovitskiy Y, Saunders A, Johnson CA, Lowell BB, Sabatini BL. 2012. Recurrent network activity drives striatal synaptogenesis. *Nature* 485:646–50
Krashes M, Koda S, Ye CP, Rogan SC, Adams A, et al. 2011. Rapid, reversible activation of AgRP neurons drives feeding behavior. *J. Clin. Investig.* 121:1424–28
Krashes MJ, Shah BP, Madara JC, Olson DP, Strochlic DE, et al. 2014. An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. *Nature* 507:238–42
Kristiansen K, Kroeze W, Willins D, Gelber E, Savage J, et al. 2000. A highly conserved aspartic acid (D155) anchors the terminal amine moiety of tryptamines and is involved in membrane targeting of the 5-HT_{2A} serotonin receptor but does not participate in activation via a "salt-bridge disruption" mechanism. *J. Pharmacol. Exp. Ther.* 293:735–46
Lechner HA, Lein ES, Callaway EM. 2002. A genetic method for selective and quickly reversible silencing of mammalian neurons. *J. Neurosci.* 22:5287–90
Lerchner W, Xiao C, Nashmi R, Slimko EM, van Trigt L, et al. 2007. Reversible silencing of neuronal excitability in behaving mice by a genetically targeted, ivermectin-gated Cl- channel. *Neuron* 54:35–49
Li P, Slimko EM, Lester HA. 2002. Selective elimination of glutamate activation and introduction of fluorescent proteins into a *Caenorhabditis elegans* chloride channel. *FEBS Lett.* 528:77–82
Li X, Gutierrez DV, Hanson MG, Han J, Mark MD, et al. 2005. Fast noninvasive activation and inhibition of neural and network activity by vertebrate rhodopsin and green algae channelrhodopsin. *Proc. Natl. Acad. Sci. USA* 102:17816–21
Lin D, Boyle MP, Dollar P, Lee H, Lein ES, et al. 2011. Functional identification of an aggression locus in the mouse hypothalamus. *Nature* 470:221–26
Lin Q, Jiang F, Schultz PG, Gray NS. 2001. Design of allele-specific protein methyltransferase inhibitors. *J. Am. Chem. Soc.* 123:11608–13
Liu G, Gu B, He X-P, Joshi RB, Wackerle HD, et al. 2013. Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. *Neuron* 79:31–38
Loffler S, Korber J, Nubbemeyer U, Fehsel K. 2012. Comment on "Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition." *Science* 337:646; author reply 646
Lovett-Barron M, Kaifosh P, Kheirbek MA, Danielson N, Zaremba JD, et al. 2014. Dendritic inhibition in the hippocampus supports fear learning. *Science* 343:857–63
Lovett-Barron M, Turi GF, Kaifosh P, Lee PH, Bolze F, et al. 2012. Regulation of neuronal input transformations by tunable dendritic inhibition. *Nat. Neurosci.* 15:423–30, S1–S3
Luttrell LM, Ferguson SS, Daaka Y, Miller WE, Maudsley S, et al. 1999. β-arrestin-dependent formation of β2 adrenergic receptor-Src protein kinase complexes. *Science* 283:655–61
Lynagh T, Lynch JW. 2010. An improved ivermectin-activated chloride channel receptor for inhibiting electrical activity in defined neuronal populations. *J. Biol. Chem.* 285:14890–97

Magnus CJ, Lee PH, Atasoy D, Su HH, Looger LL, Sternson SM. 2011. Chemical and genetic engineering of selective ion channel-ligand interactions. *Science* 333:1292–96
McKellar QA, Midgley DM, Galbraith EA, Scott EW, Bradley A. 1992. Clinical and pharmacological properties of ivermectin in rabbits and guinea pigs. *Vet. Rec.* 130:71–73
Mueller KL, Hoon MA, Erlenbach I, Chandrashekar J, Zuker CS, Ryba NJ. 2005. The receptors and coding logic for bitter taste. *Nature* 434:225–29
Nadeau H, McKinney S, Anderson DJ, Lester HA. 2000. ROMK1 (Kir1.1) causes apoptosis and chronic silencing of hippocampal neurons. *J. Neurophysiol.* 84:1062–75
Nakajima K, Wess J. 2012. Design and functional characterization of a novel, arrestin-biased designer G protein-coupled receptor. *Mol. Pharmacol.* 82:575–82
Nawaratne V, Leach K, Suratman N, Loiacono RE, Felder CC, et al. 2008. New insights into the function of M4 muscarinic acetylcholine receptors gained using a novel allosteric modulator and a DREADD (designer receptor exclusively activated by a designer drug). *Mol. Pharmacol.* 74:1119–31
Parnaudeau S, O'Neill PK, Bolkan SS, Ward RD, Abbas AI, et al. 2013. Inhibition of mediodorsal thalamus disrupts thalamofrontal connectivity and cognition. *Neuron* 77:1151–62
Pisharath H. 2007. Validation of nitroreductase, a prodrug-activating enzyme, mediated cell death in embryonic zebrafish (*Danio rerio*). *Comp. Med.* 57:241–46
Pisharath H, Rhee JM, Swanson MA, Leach SD, Parsons MJ. 2007. Targeted ablation of β cells in the embryonic zebrafish pancreas using *E. coli* nitroreductase. *Mech. Dev.* 124:218–29
Pliakas AM, Carlson RR, Neve RL, Konradi C, Nestler EJ, Carlezon WA Jr. 2001. Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J. Neurosci.* 21:7397–403
Ray RS, Corcoran AE, Brust RD, Kim JC, Richerson GB, et al. 2011. Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition. *Science* 333:637–42
Ray RS, Corcoran AE, Brust RD, Soriano LP, Nattie EE, Dymecki SM. 2012. Egr2-neurons control the adult respiratory response to hypercapnia. *Brain Res.* 1511:115–25
Redfern CH, Coward P, Degtyarev MY, Lee EK, Kwa AT, et al. 1999. Conditional expression and signaling of a specifically designed G_i-coupled receptor in transgenic mice. *Nat. Biotechnol.* 17:165–69
Revah F, Bertrand D, Galzi JL, Devillers-Thiery A, Mulle C, et al. 1991. Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature* 353:846–49
Rogan SC, Roth BL. 2011. Remote control of neuronal signaling. *Pharmacol. Rev.* 63:291–315
Sasaki K, Suzuki M, Mieda M, Tsujino N, Roth B, Sakurai T. 2011. Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice. *PLoS ONE* 6:e20360
Schmidt C, Li B, Bloodworth L, Erlenbach I, Zeng FY, Wess J. 2003. Random mutagenesis of the M3 muscarinic acetylcholine receptor expressed in yeast. Identification of point mutations that "silence" a constitutively active mutant M3 receptor and greatly impair receptor/G protein coupling. *J. Biol. Chem.* 278:30248–60
Slimko EM, McKinney S, Anderson DJ, Davidson N, Lester HA. 2002. Selective electrical silencing of mammalian neurons in vitro by the use of invertebrate ligand-gated chloride channels. *J. Neurosci.* 22:7373–79
Small KM, Brown KM, Forbes SL, Liggett SB. 2001. Modification of the β 2-adrenergic receptor to engineer a receptor-effector complex for gene therapy. *J. Biol. Chem.* 276:31596–601
Stanley BG, Ha LH, Spears LC, Dee MG 2nd. 1993. Lateral hypothalamic injections of glutamate, kainic acid, D,L-α-amino-3-hydroxy-5-methyl-isoxazole propionic acid or N-methyl-D-aspartic acid rapidly elicit intense transient eating in rats. *Brain Res.* 613:88–95
Strader CD, Gaffney T, Sugg EE, Candelore MR, Keys R, et al. 1991. Allele-specific activation of genetically engineered receptors. *J. Biol. Chem.* 266:5–8
Strobel SA. 1998. Ribozyme chemogenetics. *Biopolymers* 48:65–81
Sweger EJ, Casper KB, Scearce-Levie K, Conklin BR, McCarthy KD. 2007. Development of hydrocephalus in mice expressing the G_i-coupled GPCR Ro1 RASSL receptor in astrocytes. *J. Neurosci.* 27:2309–17
Temburni MK, Blitzblau RC, Jacob MH. 2000. Receptor targeting and heterogeneity at interneuronal nicotinic cholinergic synapses in vivo. *J. Physiol.* 525(Pt. 1):21–29
Tian L, Yang Y, Wysocki LM, Arnold AC, Hu A, et al. 2012. Selective esterase-ester pair for targeting small molecules with cellular specificity. *Proc. Natl. Acad. Sci. USA* 109:4756–61

Ultanir SK, Hertz NT, Li G, Ge WP, Burlingame AL, et al. 2012. Chemical genetic identification of NDR1/2 kinase substrates AAK1 and Rabin8 uncovers their roles in dendrite arborization and spine development. *Neuron* 73:1127–42

Vrontou S, Wong AM, Rau KK, Koerber HR, Anderson DJ. 2013. Genetic identification of C fibres that detect massage-like stroking of hairy skin in vivo. *Nature* 493:669–73

Walker DP, Wishka DG, Piotrowski DW, Jia S, Reitz SC, et al. 2006. Design, synthesis, structure-activity relationship, and in vivo activity of azabicyclic aryl amides as α7 nicotinic acetylcholine receptor agonists. *Bioorg. Med. Chem.* 14:8219–48

Wall NR, Wickersham IR, Cetin A, De La Parra M, Callaway EM. 2010. Monosynaptic circuit tracing in vivo through Cre-dependent targeting and complementation of modified rabies virus. *Proc. Natl. Acad. Sci. USA* 107:21848–53

Westkaemper R, Glennon R, Hyde E, Choudhary M, Khan N, Roth B. 1999. Engineering in a region of bulk tolerance into the 5-HT_{2A} receptor. *Eur. J. Med. Chem.* 34:441–47

Wulff P, Goetz T, Leppa E, Linden AM, Renzi M, et al. 2007. From synapse to behavior: rapid modulation of defined neuronal types with engineered GABAA receptors. *Nat. Neurosci.* 10:923–29

Xu J, Zhu Y, Heinemann SF. 2006. Identification of sequence motifs that target neuronal nicotinic receptors to dendrites and axons. *J. Neurosci.* 26:9780–93

Yagi H, Tan W, Dillenburg-Pilla P, Armando S, Amornphimoltham P, et al. 2011. A synthetic biology approach reveals a CXCR4-G13-Rho signaling axis driving transendothelial migration of metastatic breast cancer cells. *Sci. Signal.* 4:ra60

Zemelman BV, Lee GA, Ng M, Miesenbock G. 2002. Selective photostimulation of genetically chARGed neurons. *Neuron* 33:15–22

Zemelman BV, Nesnas N, Lee GA, Miesenbock G. 2003. Photochemical gating of heterologous ion channels: remote control over genetically designated populations of neurons. *Proc. Natl. Acad. Sci. USA* 100:1352–57

Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, et al. 2007. Multimodal fast optical interrogation of neural circuitry. *Nature* 446:633–39

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, et al. 2003. The receptors for mammalian sweet and umami taste. *Cell* 115:255–66

Annual Review of Neuroscience

Volume 37, 2014

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Errata

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