

Chapter 2

Orthogonal Pharmacological Control of Neuronal Activity

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Abstract

Studying the functional architecture of the brain requires technologies to precisely measure and perturb the activity of specific neural cells and circuits in live animals. Substantial progress has been made in recent years to develop and apply such tools. In particular, technologies that provide precise control of activity in genetically defined populations of neurons have enabled the study of causal relationships between and among neural circuit elements and behavioral outputs. Here, we review an important subset of such technologies, in which neurons are genetically engineered to respond to specific chemical ligands that have no other pharmacological effect in the central nervous system. A rapidly expanding set of these “orthogonal pharmacogenetic” tools provides a unique combination of genetic specificity, functional diversity, spatiotemporal precision and potential for multiplexing. We review the main orthogonal pharmacogenetic technologies that utilize engineered neuroreceptors to control neuronal excitability. We describe the key performance characteristics informing the use of these technologies in the brain, and potential directions for improvement and expansion of the orthogonal pharmacogenetics toolkit to enable more sophisticated systems neuroscience.

Introduction

The brain is a complex system comprising billions of interconnected, specialized cells whose collective function gives rise to mental states and observable behavior, while malfunction leads to neurological and psychiatric disease. Studying this system requires technologies to precisely sense and control the activity of specific neural cells and circuits in model organisms. An important focus of technical development in recent years has been technologies that provide precise control of activity in genetically defined populations of neurons. Such technologies have enabled the study of causal relationships between the functioning of neural circuits and behavior, yielding novel insights into processes such as aggression¹, anxiety² and appetite³. Here, we review an important subset of such technologies, in which exogenous genes introduced into neurons enable them to respond to specific chemical ligands that have no other pharmacological effect in the central nervous system (CNS). An expanding repertoire of such tools provides a powerful combination of genetic specificity, functional diversity, spatiotemporal precision and potential for multiplexing that will be critical in obtaining a systems-level understanding of brain function.

In the past, neuroscientists have modulated neural activity using pharmacology or electrical stimulation, obtaining either molecular or spatial specificity (Table 2-1). Each method is incomplete, since both location and molecular identity are needed to define the functional circuit roles of neurons. Recently, novel technologies have been developed that are capable of controlling neural activity with both spatial and molecular precision. These technologies take advantage of advances in understanding of cell type-specific gene expression in neurons⁴ and methods of targeting transgenes to cells based on their

	Conventional Pharmacology	Electrical Stimulation	Optogenetics	Orthogonal Pharmacogenetics
Cell type specificity	Medium	None	High	High
Temporal precision	Medium	High	High	Medium
Spatial precision	None	High	High	Medium
Multiplexing	Low	Low	Medium	High
Signaling variety	Low	Low	Medium	High
Spatial Coverage	High	Low	Low	High
Requires gene delivery	No	No	Yes	Yes
Requires device	No	Yes	Yes	No

Table 2-1. Capabilities of neural control technologies

genetic properties, location and circuit connectivity⁵. Control is achieved by expressing exogenous actuator proteins that make specific neurons responsive to “orthogonal” stimuli that normally have no effect on nervous system function.

One successful instantiation of this concept, “optogenetics”, uses actuator proteins that are sensitive to visible light, including ion channels, transporters, G-protein coupled receptors (GPCRs) and protein-protein binding domains. Expressing these proteins in neurons makes it possible to control various aspects of their activity with light⁶⁻⁸. In addition to the molecular, spatial and circuit specificity achievable through genetic targeting, optical stimulation provides a high degree of temporal precision, in some cases on millisecond timescales enabling control of neuronal spike timing and frequency⁹ (Table 2-1). Multiplexing is possible with up to 3–4 channels using actuator proteins that respond to different wavelengths. A drawback of optogenetic brain stimulation in mammals is the need for implanted optical fibers to deliver light. In addition to being

burdensome experimentally, the resulting localized illumination makes it difficult to control diffuse signaling networks.

Another approach to orthogonal control of genetically specified neurons uses actuator proteins that respond to unique chemical ligands that have no other pharmacological activity in the CNS. This approach, to which we refer as orthogonal pharmacogenetics (OP), has been used for some time to control gene expression (e.g. using tetracycline-dependent transcriptional promoters). Recently, novel actuator proteins have been developed that enable chemical control of neuronal firing, second-messenger signaling and synaptic function. Like optogenetics, OP can use genetic targeting to achieve molecular, spatial and circuit specificity. In addition, ligands with different pharmacokinetic properties can be used to specify the timescale of neural control, ranging from minutes to days. This temporal resolution is not so high as with optogenetics. However, it is fully satisfactory in many cases where circuits play modulatory roles or the objective of the perturbation is long-term inhibition. Unlike optogenetics, OP does not require invasive implants, and both local and diffuse groups of neurons can be controlled depending on where the actuator gene is expressed (Table 2-1). In theory, OP also has the capacity for virtually unlimited multiplexing, as long as a sufficient number of unique ligand-receptor pairs can be developed. Importantly, such multiplexing can be both within a cell type (e.g., by expressing inhibitory and excitatory ion channels controlled by different ligands) and between multiple cell types (Figure 2-1).

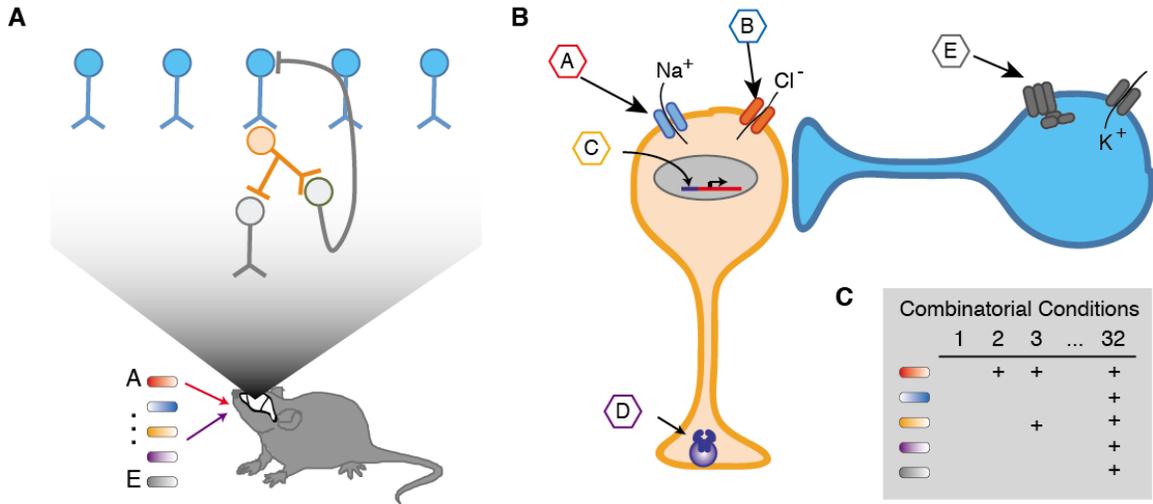


Figure 2-1. Illustrated example of multiplexed orthogonal pharmacogenetics. *A.* Two cell types (blue and orange) involved in a particular neural circuit (top) are genetically modified to express orthogonal actuators responding to several distinct ligands that can be administered orally to the model organism (bottom). *B.* One neuron (orange) expresses four distinct OP constructs, enabling temporally specific, multiplexed control of excitation (ion channel controlled by ligand A), inhibition (ion channel controlled by ligand B), gene transcription (transcriptional transactivator controlled by ligand C) and decreased presynaptic transmitter release (vesicle protein multimerization controlled by ligand D). A second neuron (blue) has an orthogonal GPCR coupled to an endogenous potassium channel, enabling orthogonal inhibition under control of ligand E. *C.* Using the five ligands corresponding to different orthogonal actuators, it is possible to test 32 binary (ligand on or off) experimental conditions in this system.

OP systems have been engineered to provide chemical control over various aspects of neural activity, including ion channel and GPCR signaling, gene transcription and synaptic function. In addition, OP actuators have been developed providing control over gene translation and enzymatic activity that could be adapted to neurons. Below, we highlight the major categories of recently developed OP systems and their applications in neuroscience. We evaluate them with reference to a common set of performance characteristics applicable to functional actuators (orthogonality, compatibility, modularity and deliverability) their chemical effector ligands (molecular specificity and

deliverability), and the combination of ligand and actuator (temporal response, dose response), as defined in Table 2-2.

Actuator characteristics	
Orthogonality	Actuator is insensitive to endogenous ligands or other signaling elements. Actuator inactive until triggered by ligand (or inactive in presence of ligand in a switch-off system).
Compatibility	Endogenous machinery needed for actuator performance is present in target cells. Actuator does not interfere with normal cell function unless it is activated by ligand.
Modularity	Actuator can be modified to produce different signaling effects upon ligand binding.
Deliverability	Actuator can be delivered to target cells by viral vectors and through transgenesis. Ideally, the essential genetic payload should be a single gene smaller than 1.5kb to enable single AAV construct delivery.
Effector ligand characteristics	
Molecular specificity	At the effective dose, ligand acts only on its corresponding actuator.
Deliverability	Ligand is bioavailable, preferably <i>per orum</i> , and penetrates CNS.
System characteristics	
Temporal response	On and off kinetics for cellular and behavioral response after administration as determined by ligand pharmacokinetics and receptor activation, inactivation and second-messenger signaling.
Dose response	Dependence of cellular and behavioral response on ligand dose.

Table 2-2. Performance characteristics of orthogonal pharmacogenetic systems

Orthogonal neuroreceptors: LGICs and GPCRs

The most active recent area of development in OP has focused on neuroreceptors. Both ligand-gated ion channels (LGICs) and GPCRs have been developed as orthogonal actuators by identifying or engineering receptors with minimal sensitivity to endogenous neurotransmitter agonists and strong activation by specific exogenous ligands that have no other significant pharmacological effect in the CNS. Targeted expression of these orthogonal receptors permits temporally controlled excitation or inhibition of neurons through the administration of their cognate ligands.

The first orthogonal GPCR and LGIC systems for use in neuroscience were based on receptors from nonmammalian organisms. The Callaway group developed a system based on the *Drosophila* allatostatin receptor (AlstR) and its cognate neuropeptide ligand allatostatin (AL), neither of which is expressed in mammals¹⁰. AL does not cross-activate endogenous mammalian GPCRs, nor is AlstR activated by mammalian GPCR ligands¹¹. Activation of heterologously expressed AlstR by AL leads to Gi-coupled activation of endogenous mammalian G protein-gated inward rectifier K⁺ (GIRK) channels, leading to a reduction in cell excitability (Figure 2-2). Virally targeted expression of AlstR in cortical and thalamic neurons and intracranial administration of AL produce neuronal silencing on a timescale of minutes in several species¹².

Around the same time, the Lester group adapted the *C. elegans* glutamate-gated chloride channel (GluCl) for silencing of mammalian neurons by administration of the anthelmintic GluCl agonist ivermectin (IVM). GluCl was rendered insensitive to its native ligand glutamate by a single point mutation and codon-optimized to achieve

greater expression in mammalian cells^{13,14}. IVM activation of GluCl α and β subunits expressed in neurons elicits a Cl^- conductance across the membrane that effectively shunts action potential generation¹⁵ (Figure 2-2). The GluCl/IVM system later became the first to be used for neuronal silencing with a systemically administered ligand in awake, behaving animals¹⁶.

More recently, versatile orthogonal neuroreceptor systems have been established by modifying mammalian GPCRs and LGICs. A collection of modified GPCRs called DREADDs, “designer receptors exclusively activated by designer drugs”, were developed using a combination of directed evolution and rational protein engineering¹⁷. Building on previous efforts to engineer the ligand selectivity of GPCRs¹⁸, the first DREADDs were generated from the human M3 muscarinic receptors (hM3). Survival screens based on the yeast pheromone response¹⁹ were used to evolve this receptor for activation by the small molecule clozapine-*N*-oxide (CNO) and lack of activation by the native ligand acetylcholine. CNO is a normally inactive metabolite of the atypical antipsychotic clozapine. CNO activation of the mutant hM3D triggers Gq-coupled signaling leading to membrane depolarization through phospholipase C β (PLC β)/PIP₂ mediated inhibition of KCNQ channels²⁰ (Figure 2-2). Following a similar design scheme, a second CNO-activated DREADD, hM4D, was generated that couples to Gi, leading to activation of GIRK channels and neuronal silencing similar to that elicited by AlstR/AL (Figure 2-2).

Recently, a systematic engineering approach was also taken to the development of a modular system of orthogonally controlled Cys-loop ion channels with distinct ligand sensitivity and ion conductance properties³. The modularity of this system is based on

fusing the $\alpha 7$ nicotinic acetylcholine receptor (nAChR) ligand-binding domain onto the ion pore domain of either a cation-selective serotonin 5-HT₃ receptor ($\alpha 7$ -5HT₃) or anion-selective glycine receptor ($\alpha 7$ -GlyR) to produce functional channels with the same pharmacological profile but different ion permeability^{21,22}. Novel ligand recognition properties were engineered through a “bump-hole” approach, which uses structural models to generate libraries of predicted ligand-receptor pairs that are then synthesized and screened for selective functional activity. Structural analogs of the $\alpha 7$ -specific synthetic agonist PNU-282987 were tested for selective activation of mutant, but not wild-type, channels. At the same time, mutant channels were screened for lack of activation by acetylcholine and nicotine. The resulting mutant ligand binding domains are dubbed ‘pharmacologically selective actuator modules’ (PSAMs). Each PSAM is exclusively activated by a cognate synthetic agonist, called a “pharmacologically selective effector molecule” (PSEM). Three specific PSAM/PSEM tools have been designed, each with different ion conductance properties for controlling neuronal excitability³. These include the cation-selective activator, PSAM^{Q79G,Q139G}-5HT₃HC/PSEM^{22S}, the anion-selective silencer, PSAM^{L141F,Y115F}-GlyR/PSEM^{89S}, and a third Ca²⁺-selective channel, PSAM^{Q79G,L141S}-nAChR V13’T/PSEM^{9S}.

Another orthogonal LGIC system is based on the transient receptor potential ion channel TRPV1, an endogenous mammalian receptor predominantly expressed in the peripheral nervous system. TRPV1 is a nonselective cation channel activated by noxious heat, pH and exogenous ligands including the hot chili pepper compound capsaicin²³. Targeted neuronal expression of TRPV1 in the mouse brain leads to capsaicin-activated currents and action potentials²⁴. To use TRPV1 for orthogonal control of specific

neurons, the host organism can be modified to knock out endogenous TRPV1 expression. On this TRPV1^{-/-} background one can reintroduce TRPV1 into target cells as an exogenous OP actuator²⁵.

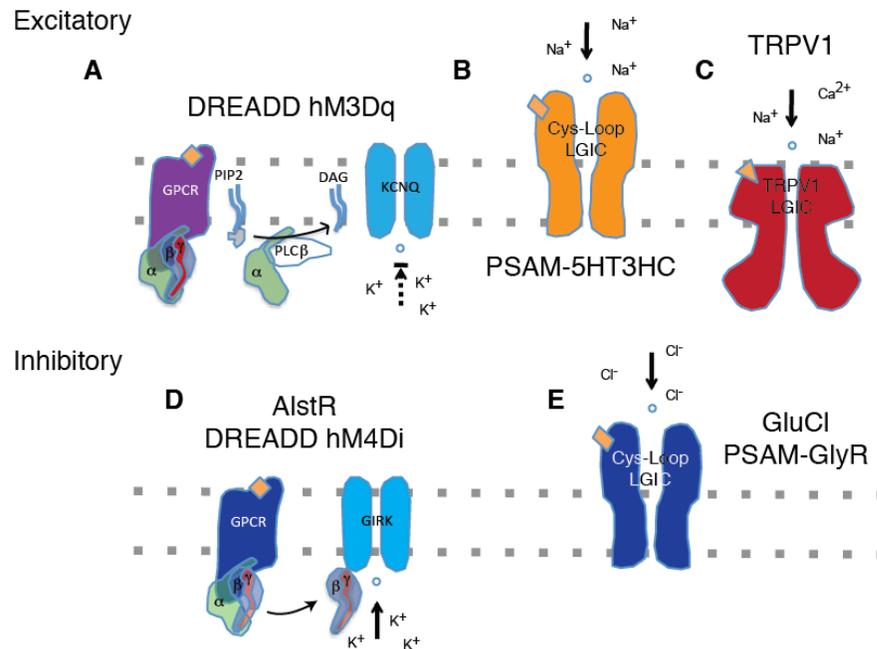


Figure 2-2. Mechanisms of orthogonal neuroreceptors. GPCRs form the basis for both excitatory and inhibitory OP systems (*A*, *D*) based on interactions with different endogenous G proteins. GPCR signaling cascades leading to excitation and inhibition are described in the text. Cys-loop LGICs (*B*, *E*) are also used to effect inhibition and excitation based on pore domain ion selectivity. TRPV1 (*C*) excites cells through a nonselective cation conductance.

Performance Characteristics

The set of available OP neuroreceptor tools is summarized in Table 2-3. Their specific performance characteristics inform their ability to fulfill the unique objectives of a neuroscience study. As defined in Table 2-2, key performance characteristics depend on the properties of actuators, effectors, or both.

Actuator orthogonality, compatibility, modularity and deliverability

GPCR and LGIC architectures of orthogonal receptors confer distinct functional properties. Neural control using GPCR-based systems depends on second messenger signaling cascades. Although these secondary effectors are generally present in neurons, their precise quantity and subcellular localization could impose limits on actuator function. Conversely, expression of heterologous receptors could sequester second messenger molecules, disrupting endogenous receptor activity²⁶. G-protein-mediated cascades may also have undesirable effects beyond altering neuronal firing (e.g., affecting gene expression), especially with sustained activation^{27,28}. In contrast to GPCRs, LGIC actuators are self-contained membrane proteins with ligand-dependent ionic conduction directly affecting membrane excitability. They require no intermediary molecules. However, close attention must be paid to their ionic selectivity. The high Ca²⁺ permeability of TRPV1, for example, is likely to trigger Ca²⁺-mediated cell signaling events in addition to exciting cells.

Both LGICs and GPCRs are functionally modular. The PSAM/PSEM system described above illustrates the relative ease of generating new chimeric channels based

on the modularity of Cys-loop receptors. Ligand-binding domains developed and tested while connected to one transmembrane domain were transplanted onto other transmembrane domains, resulting in constructs with completely different ionic conductance. Structure-function studies support further potential for altering ion selectivity, single-channel conductance, and open channel duration (reviewed in²⁹⁻³¹). When modifying Cys-loop receptors, one must ensure that mutant channels have minimal leak current in the resting state. GPCRs are modular with regard to their second messenger coupling. Domain swapping and point mutations of intracellular loops can alter G-protein specificity, allowing modulation of Gi-, Gs-, and Gq-coupled signaling pathways³².

Engineered receptors can be delivered into the CNS via transgenic modification or viral vectors. With coding sequences of approximately 1.7 kb for the M3 muscarinic receptor, 1.2 kb for AlstR, 1.4 kb for GluCl, 1.5 kb for PSAMs and 2.5 kb for TRPV1, each receptor construct can be accommodated by lentiviral vectors; in addition, GluCl, AlstR and PSAMs can be delivered by adeno-associated virus (AAV). Most of these tools require the delivery of only one genetic construct, except GluCl, which requires α and β subunits. The requirement for two constructs permitted GluCl to be used with intersectional genetic targeting³³. Codon optimization and signal peptide fusions can improve translation and membrane trafficking of nonnative receptors^{14,34-36}. Receptors can also be regionally targeted to somato-dendritic, axonal, or postsynaptic sites³⁷⁻³⁹.

Class	Actuator	Effector	Effect on neurons	Signaling and Endogenous Partners	Design & Proof-of-concept Refs.	Application Refs.
GPCR	AlstR <i>drosophila</i>	AL	Inhibition	G _i -coupled; activates GIRK K ⁺ channel	10, 12, 46	56-58
GPCR	DREADD hM4Di <i>human</i>	CNO	Inhibition	G _i -coupled; activates GIRK K ⁺ channel	17	59, 61-62
GPCR	DREADD hM3Dq <i>human</i>	CNO	Excitation	G _q -coupled; inhibits KCNQ K ⁺ channel	17, 20	60-62
LGIC	GluCl α & β <i>C. elegans</i>	IVM	Inhibition	Cl ⁻ channel	13-16	1, 33
LGIC	PSAM-5HT3HC <i>human-mouse</i>	PSEM ^{22S}	Excitation	Cation channel (Na ⁺ \approx K ⁺ > Ca ²⁺)	3	
LGIC	PSAM-GlyR <i>human</i>	PSEM ^{89S}	Inhibition	Cl ⁻ channel	3	3
LGIC	PSAM-nAChR V13T <i>human-rat</i>	PSEM ^{9S}	Not shown	Ca ²⁺ channel	3	
LGIC	TRPV1 <i>rat</i>	Capsaicin	Excitation	Cation channel (Ca ²⁺ > Na ⁺ \approx K ⁺)	24-25	

Table 2-3. Orthogonal neuroreceptors. Far-right columns indicate corresponding references.

Ligand deliverability and specificity

Ligands with good pharmacokinetics, including oral bioavailability and brain penetration, allow manipulation of deep brain structures and dispersed neuronal populations. The ability to conveniently deliver effector ligands is a key advantage of the DREADD/CNO, GluCl/IVM, and the PSAM/PSEM systems (Table 2-4). No specialized equipment is necessary, as the exogenous activating ligands of these tools allow convenient systemic administration of rapidly diffusible agonists orally or by intraperitoneal or intravenous injection. The bioavailability (i.e., degree to which the drug becomes available to the target tissue after administration) depends on its ability to cross the BBB. On the other hand, neuronal manipulation using AlstR/AL or TRPV1/capsaicin (in a wild-type background) requires localized application of their effector ligands via parenchymal or

Ligand	Origins	Specificity	CNS penetration	Bioavailability	Kinetics	Refs.
Clozapine-N-oxide (CNO)	Inactive metabolite of clozapine	No known activity at effective dose	Yes	Oral	On: 5-10m Clearance: 2h	20
Allatostatin (AL)	Natural neuropeptide	No known activity in mammals	No	Injection only	On: 1-3m Clearance: 40-60m* (ICV)	12, 46, 56
Ivermectin (IVM)	Anthelmintic	Specific up to 10X effective dose	Yes	Oral	On: 4-12h* Clearance: 2-4d*	16, 33
PSEM ^{89s}	Synthetic derivative of nAChR agonist PNU-282987	Minimal binding to endogenous nAChRs	Yes	Oral	On: 15m Clearance: 1-2h	3
Capsaicin	Pepper ingredient, natural TRPV1 agonist	Acts on native TRPV1 receptors unless they are knocked out	Yes	Oral	On: 2-5min Clearance: <15min	25

Table 2-4. Key effector ligands used in orthogonal pharmacogenetic systems. The * indicates timescales inferred from behavioral or signaling response. Far-right column indicates corresponding references.

intracerebroventricular administration. AL is a neuropeptide that cannot cross the BBB. In wild-type background, systemically administered capsaicin would elicit unwanted effects via endogenous TRPV1 receptors.

To achieve truly orthogonal control, effector ligands must have no significant activity in cells not expressing their partner actuator at doses used for actuation. IVM is known to activate or potentiate other Cys-loop receptors present in the CNS, but with much lower sensitivity⁴⁰⁻⁴³. PSEMs were screened for ligand binding by radioligand displacement against a number of other LGICs, GPCRs and transporters³, revealing weak to moderate binding of PSEM89^S to the $\alpha 4\beta 2$ neuronal nAChR receptor; off-target functional activation remains to be assayed. Conversely, undesired on-target effects can result from agonism by endogenous ligands. For example, endogenous TRPV1 ligands including the endocannabinoid anandamide and N-arachidonoyl-dopamine are expressed

in the CNS^{44,45} and could possibly allow capsaicin-independent enhancement of neuronal activity. For each system it is important to determine an effective dosage range for optimal control with minimal side effects.

Temporal resolution and dose response

The activation and deactivation kinetics of *in vivo* neuronal manipulation using OP systems can range from minutes to hours and depend on the pharmacokinetic properties of the ligand such as absorption, distribution, metabolism and excretion, as well as receptor properties including affinity for agonist, desensitization and internalization. The TRPV1/capsaicin tool allows the most rapid, transient neuronal activation, with excitatory responses occurring within minutes of administration and lasting approximately 10 minutes, attributed to rapid capsaicin metabolism²⁵. Activation of DREADDs by CNO can also be observed within 5-10 minutes of drug administration, with induced behavior lasting from minutes to many hours. GPCRs are especially sensitive to desensitization and/or internalization with prolonged ligand exposure. These processes can either terminate a pharmacologically induced signal prematurely or facilitate sustained signaling or hyperexcitability⁴⁶ as endocytosis of GPCRs does not always terminate the signal⁴⁷. CNO itself is cleared after approximately 2 hours²⁰. IVM-induced GluCl currents activate over several hours and remain open for times on the order of 8 hours, presumably because neither desensitization nor ligand dissociation occur. Silencing effects by GluCl/IVM can last for 2–4 days; postsilencing recovery may actually require receptor turnover¹⁶. Long periods of enhanced or silenced activity can be

beneficial in some experiments, but present the risk of adaptive, compensatory, or plastic changes at the cellular or network levels. PSAMs are activated by their ligands within 15 minutes and recovery is observed after 24 hours.

Where temporal response depends on desensitization kinetics, it may be possible to modify it at the actuator level. Mutations in the ligand binding domain, transmembrane domains and the large cytoplasmic domain of Cys-loop receptors have all been shown to affect desensitization⁴⁸⁻⁵¹. For TRPV1, a point mutation that reduces Ca²⁺ permeability also abolishes desensitization⁵². Phosphorylation is also known to effect desensitization of many membrane receptors⁵²⁻⁵⁴. The removal of phosphorylation sites in the C-terminus of a heterologously expressed GPCR produced receptors that were resistant to internalization and less prone to desensitization, resulting in prolonged signaling⁵⁵. For applications requiring more defined endpoints, it may be possible to design synthetic antagonists or selective pore blockers for controlled termination of manipulated activity. Thus, there would be both an “on” ligand and an “off” ligand.

Dose-dependence of behavioral responses has been reported for Alst/AL⁵⁶ and GluCl/IVM¹⁶ and dose-dependent increases in neuronal activity have been demonstrated with hM3Dq/CNO²⁰ and TRPV1/capsaicin^{24,25}. There is no *in vivo* dose-response info for the PSAM/PSEMs.

Applications of Orthogonal Neuroreceptors

Several orthogonal neuroreceptor systems have been used *in vivo* to study neural circuitry (Table 2-3). Viral-mediated expression of AlstR has been targeted to somatostatin-expressing neurons of the ventrolateral medulla to study pathological breathing patterns of adult rats⁵⁶. Transgenic mouse lines expressing AlstR have been used to examine locomotor activity in V1 and V3 spinal cord neurons^{57,58}.

GluCl/IVM-induced silencing has been used in conjunction with channelrhodopsin-2 (ChR2) mediated activation to define an inhibitory microcircuit within the amygdala involved in mouse fear conditioning³³. Because the GluCl channel requires co-expression of α and β subunits, an intersectional approach was used to restrict the expression of GluCl to specific GABAergic neurons within an anatomically defined amygdala subregion.

Viral vectors bearing different gene promoters have been used for targeted expression of the hM4Di/CNO DREADD silencer in striatonigral vs striatopallidal neurons to study the opposing roles of direct and indirect pathways in regulating adaptations from repeated psychostimulant drug exposure⁵⁹. Recently, the hM3Dq/CNO activator was expressed in an activity-dependent manner to examine how artificial reactivation of a stimulated network affects the encoding of contextual fear memory in mice⁶⁰. The hM4Di/CNO silencer and hM3Dq/CNO activator tools have also been used in parallel experiments to study the opposing impact of activation and silencing of agouti-related protein (AgRP) neurons of the hypothalamus on feeding patterns and energy expenditure⁶¹. Controlled activation and inhibition of orexinergic neurons in the

hypothalamus elucidated their role in controlling sleep and wakefulness⁶². Because CNO activates both excitatory and inhibitory DREADD actuators, opposite effects had to be studied in separate cohorts of animals.

Simultaneous bidirectional control of neuronal activity has been demonstrated by OP and optogenetic actuators in the same set of cells. A bicistronic Cre-dependent AAV was used to co-express the PSAM^{L141F,Y115F}-GlyR silencer and the light-activated channel ChR2 in AgRP neurons. Voracious feeding behavior evoked from continuous photostimulation was strongly suppressed by intraperitoneal administration of PSEM^{89S3}. Such bidirectional modulation will be most informative for deciphering neuronal networks and their role in behavior.

Prospects for Further Engineering of Orthogonal Neuroreceptors

The systems described above represent a promising start for the use of OP to control neural activity, demonstrating actuation of various aspects of neuronal signaling over a range of timescales, triggered conveniently by peripheral ligand administration. Substantial further work is needed to enact the vision presented in Figure 2-1. Multiplexed control over a significant number of cell types will require a larger set of orthogonal ligand-receptor pairs. Investigators should be able to choose among OP systems with various of temporal profiles to meet experimental requirements. More precise control over cellular signaling also necessitates greater “cassette” modularity of ligand interaction and signaling domains.

Further development of OP neuroreceptor systems will be aided by increasing knowledge about receptor structure. The three-dimensional structures of a number of GPCRs and Cys-loop receptors have now been resolved, including the M3 muscarinic receptor⁶³ and the GluCl channel⁶⁴. Structures have also been solved for various conformational states, mutant forms and ligand complexes⁶⁵. Growing availability of structural data along with homology modeling and docking programs will be useful in optimizing current tools and in rational construction of new ones. Already the PSAM/PSEM system has demonstrated the utility of homology-based structural information.

A major goal of future OP receptor engineering efforts should be to expand the repertoire of ligand-receptor pairs. Most ligands used to date are either active on the native receptor or are close relatives of known agonists (Table 2-4). Many molecules with

desirable properties (lack of activity on endogenous targets, high CNS penetration, rapid PK) exist outside of this constrained chemical space. Antimicrobial medications and inactive drug metabolites, for example, are sizeable categories of compounds with characterized pharmacokinetics and lack of activity in mammals. An even larger repository of potential effector ligands may be found among inactive analogs of drug candidates synthesized and characterized by pharmaceutical firms during lead compound optimization.

Engineering receptors that respond to effectors dissimilar from their native ligands could build on previous accomplishments using directed evolution¹⁷ and structure-guided modification³. Directed evolution, in particular, has been successful in altering the chemical substrate and ligand specificity of enzymes and allosteric switches^{66,67}. Directed evolution requires efficient high-throughput screens, which are available for both GPCR signaling¹⁷ and ion channel conductance⁶⁸. Furthermore, directed evolution libraries based on structure-guided recombination between homologous proteins (or domains) have been shown to enhance evolution efficiency⁶⁹. The substantial homology of receptors and ligand-binding domains within and among organisms could enable the use of homologous recombination in OP receptor engineering.

Conclusions

Systems neuroscience research is now more tractable than ever thanks in part to molecular technologies enabling precise sensing and control of neural activity. We have reviewed an important class of such technologies, which provides a chemically addressable orthogonal dimension for neural control, and whose development is a highly active area of research. While a number of orthogonal pharmacogenetic tools have been used in neuroscience to great effect, many more (including those originally developed for use outside the brain) are ready for application. Future engineering efforts are expected to increase the variety of neuronal signaling pathways that can be manipulated. In addition, we believe it is particularly important to expand the repertoire of CNS-compatible ligands used in OP to enable multiplexed interrogation within and across cell types. Here, we have focused on the use of OP tools in neurons, but other relevant cell types in the brain such as glia and endothelial cells may also be targets for application.

A key feature of this class of technologies is the ability of many OP tools to be triggered noninvasively through peripheral ligand administration. The use of these tools together with new technologies for high-resolution noninvasive molecular imaging will make it possible to create complete noninvasive neural input/output systems to study brain-wide neural circuits, complementing more localized research using optical techniques. Furthermore, as gene and cell therapy make progress towards clinical acceptance, it may be possible for genetically encoded OP and noninvasive imaging technologies to help diagnose and treat neurological disease. Thus, orthogonal approaches for interfacing with the brain point in an exciting direction for both basic and clinical neuroscience.

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