*Genetic approaches to control living cells: OPTOGENETIC, CHEMOGENETIC and MAGNETOGENETIC

*CHEMOGENETIC: genetically engineered receptors to control brain functions

CHEMOGENETICS has been defined as a method by which proteins are engineered to interact with previously unrecognized small molecule chemical actuators

Over the past two decades, a large number of chemogenetic (also known as “chemical genetic”) platforms have been invented that have been useful for biologists in general and most especially for neuroscientists.

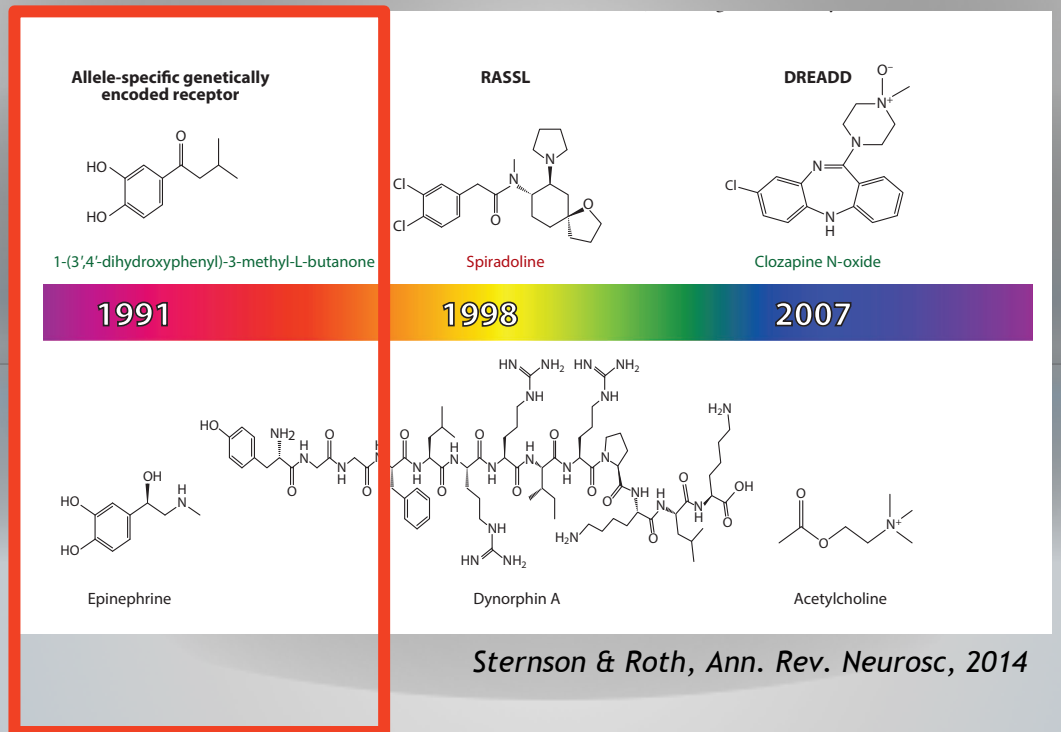
Different protein classes have been chemogenetically engineered including G-protein coupled receptors (GPCRs) and ion channels.

* 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).

* CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN-COUPLED RECEPTORS

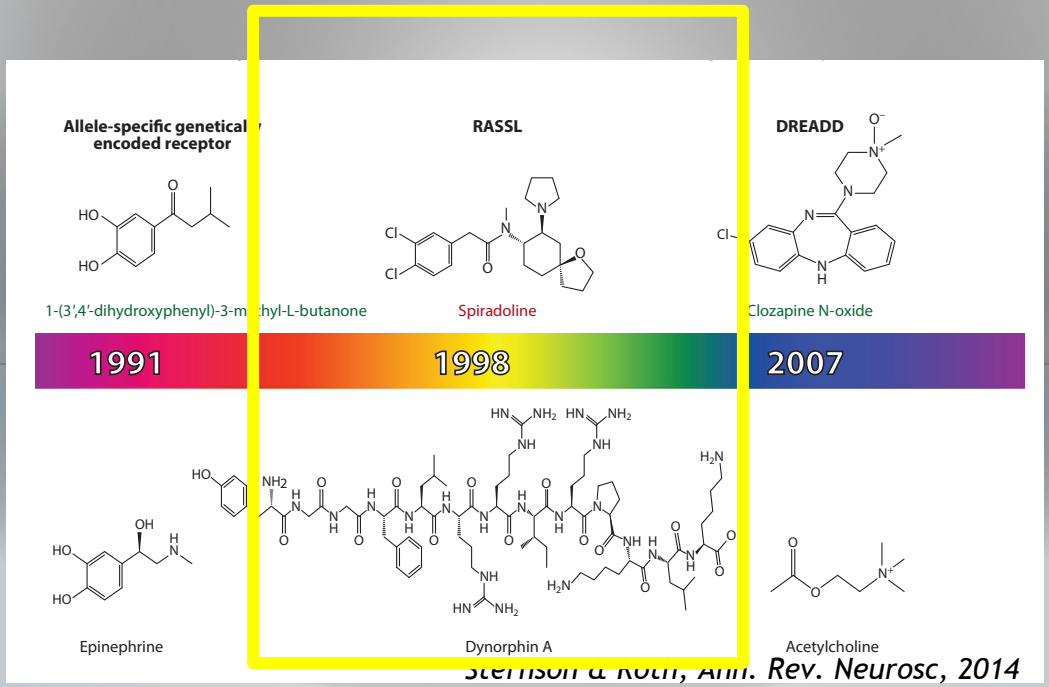
Initial attempts at modulating cellular signaling using chemogenetic approaches utilized **GPCRs** that were **engineered** by site-directed mutagenesis **to bind nonnatural ligands**. In a pioneering study, Strader et al. (1991) designed a mutant ß2-adrenergic receptor that was unable to bind the native ligand adrenaline but could be activated by 1-(3,4-dihydroxyphenyl)-3-methyl-L-butanone (L-185,870). Although L-185,870 had relatively low potency for the engineered receptor (EC50 ~ 40 uM), the results represented an essential proof of concept for this general approach.



Sternson & Roth, Ann. Rev. Neurosc, 2014

* CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN-COUPLED RECEPTORS

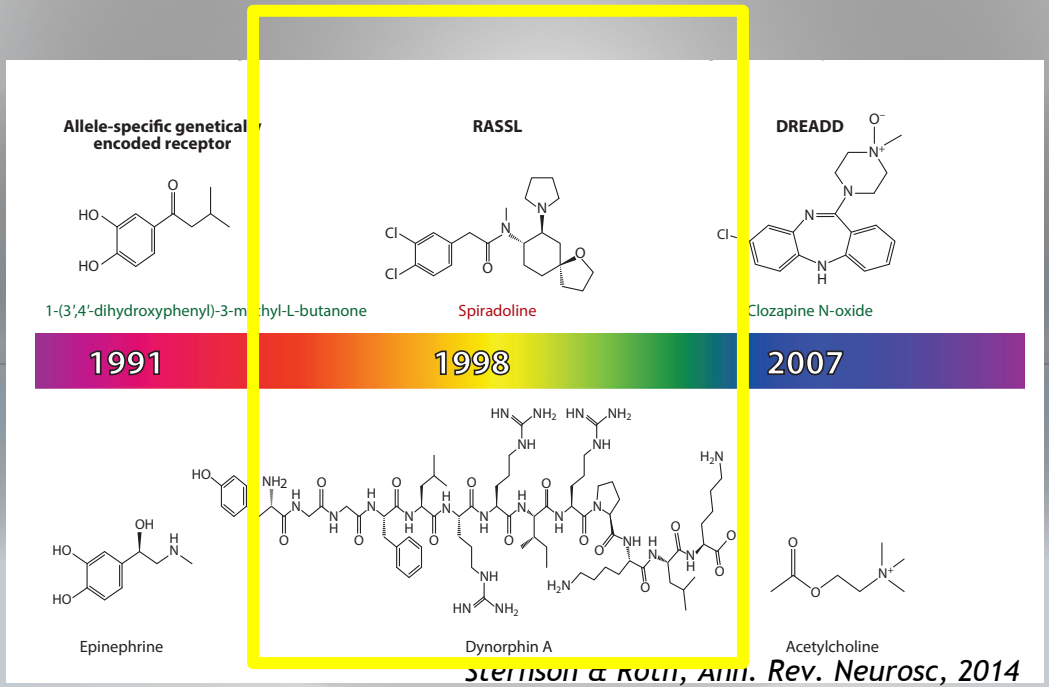
The next advance occurred with the creation of a family of engineered receptors dubbed **RASSLs** (receptor activated solely by synthetic ligand). The initial RASSL was an engineered **k-opioid receptor (KOR)** that was insensitive to native peptide ligands but could be activated potently by the **synthetic KOR agonist spiradoline** (Coward et al. 1998). This KOR RASSL was subsequently used in the first chemogenetic study from which remote control of cardiac activity was achieved (Redfern et al. 1999).



Stern son & Roth, Ann. Rev. Neurosc, 2014

* CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN-COUPLED RECEPTORS

Several other **RASSLs** have also been generated (for a review, see Conklin et al. 2008), although their utility 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).



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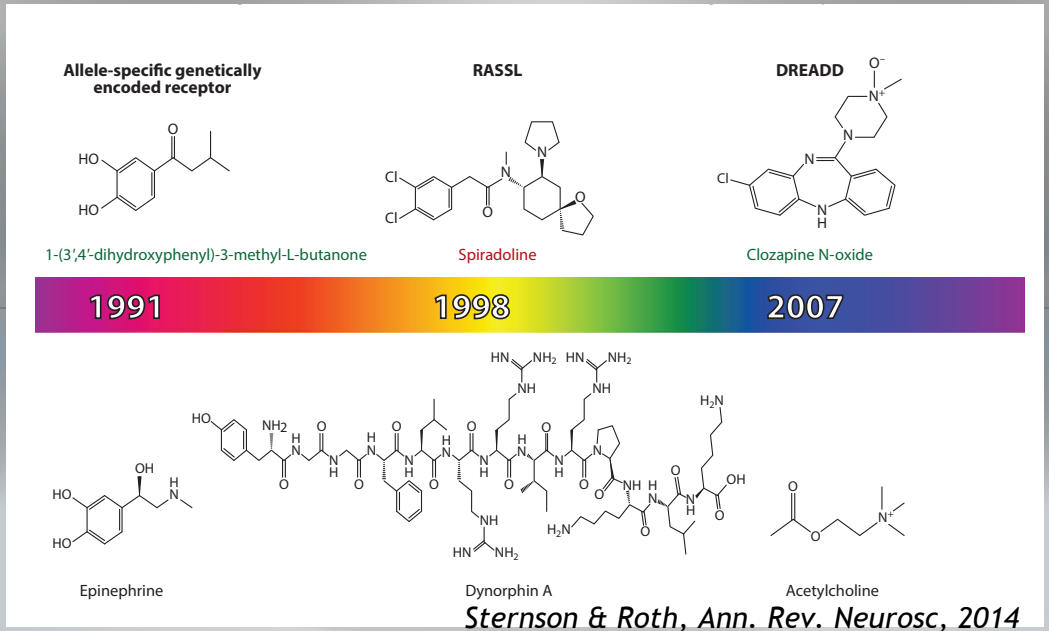
* CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN-COUPLED RECEPTORS

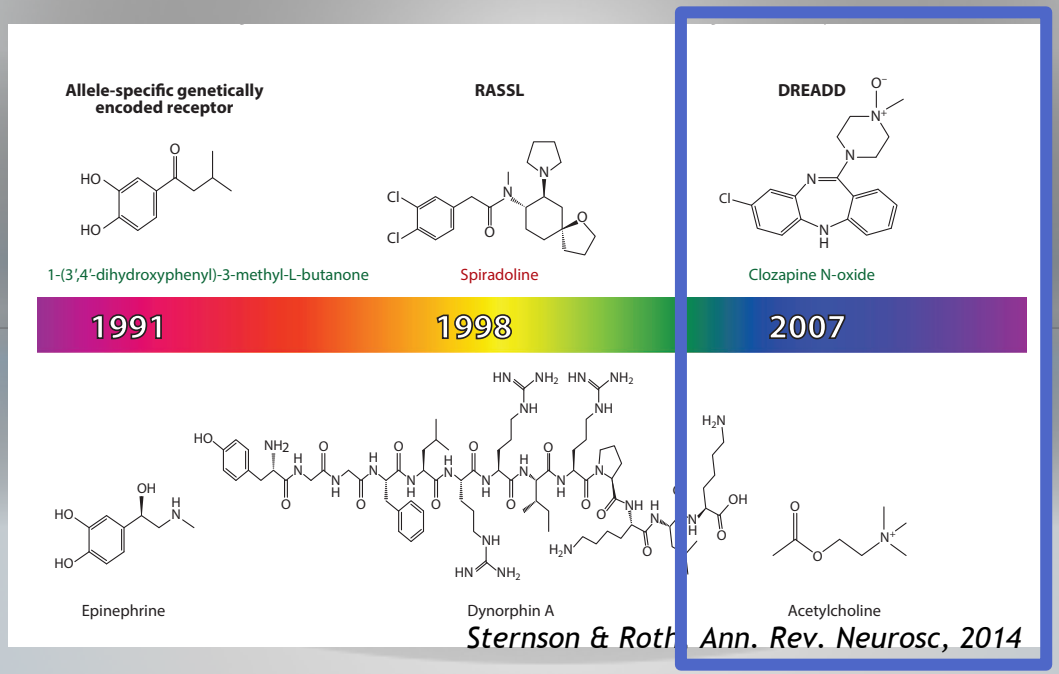
Fundamental **problems** associated with these **early attempts** to control GPCR signaling, as stated above, were that the **ligands** were **not** particularly **well suited for in vivo studies** because of the effects on cognate and non cognate receptors and that the engineered receptors occasionally had high levels of constitutive activity



Sternson & Roth, Ann. Rev. Neurosc, 2014

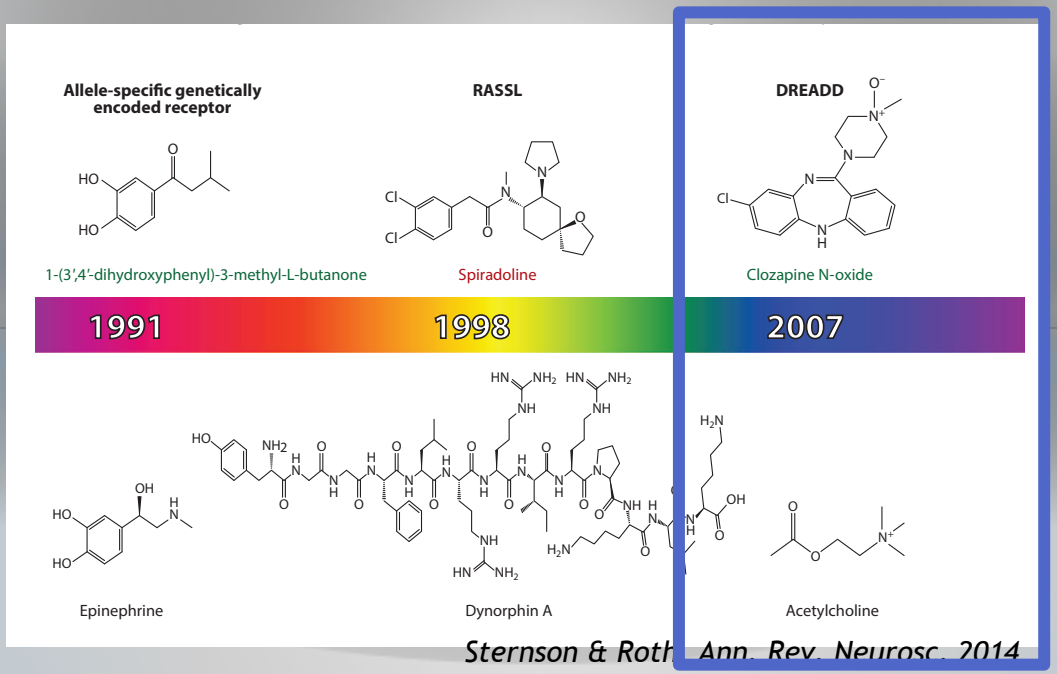
* CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN-COUPLED RECEPTORS

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).



* CHEMOGENETIC TECHNOLOGIES BASED ON G PROTEIN-COUPLED RECEPTORS

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).** 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).



Sternson & Roth Ann. Rev. Neurosc. 2014

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

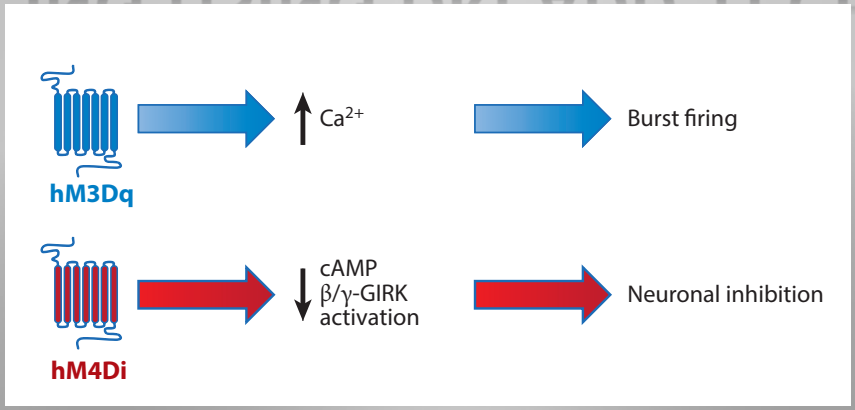
After several cycles of selection and mutagenesis as well as comprehensive bioinformatics and pharmacological characterization, researchers selected an **M3-muscarinic receptor** with two mutations **(Y149C, A239G)** that fulfilled the following criteria:

Nanomolecular potency for **activation by CNO**

Relative **insensitivity to acetylcholine** (the native ligand)

No detectible constitutive activity.

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

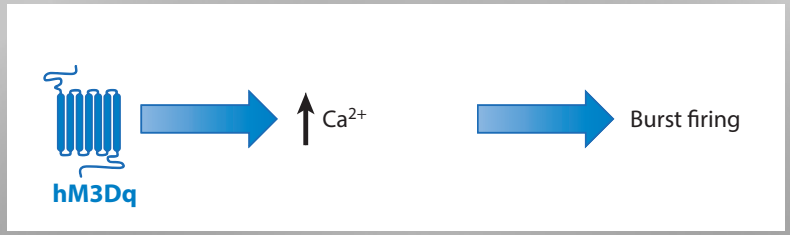


Sternson & Roth, Ann. Rev. Neurosc, 2014

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.

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.**

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



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).

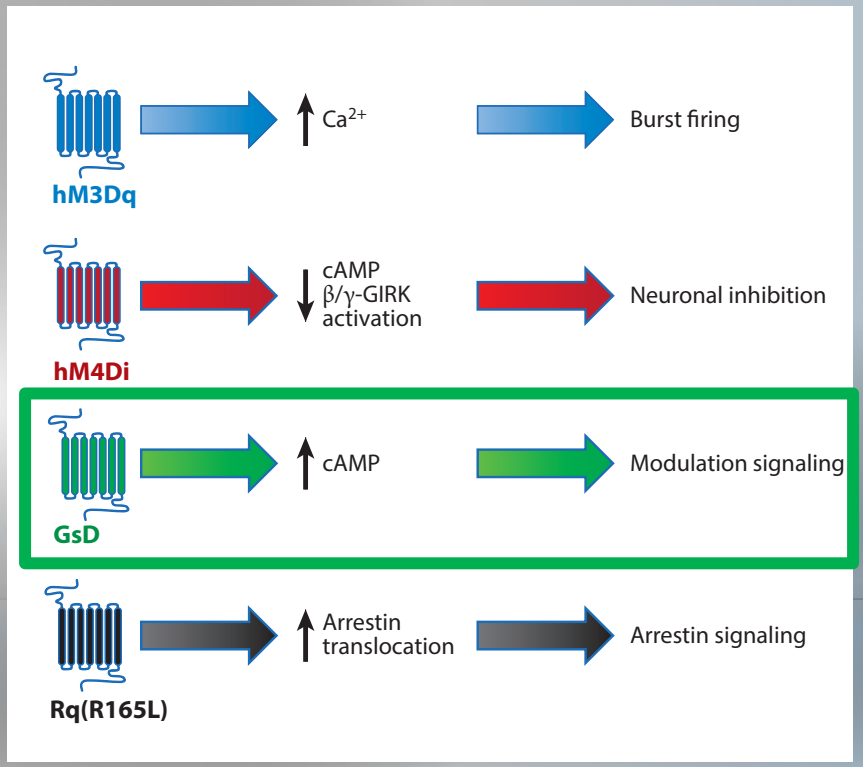
Additionally, hM3Dq has been used to interrogate the consequences of acute and chronic activation of Gαq-mediated signaling in pancreatic ß-cells (Guettier et al. 2009, Jain et al. 2013). In every reported instance, activation of Gαq signaling led to striking behavioral and/or physiological consequences.

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

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 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).

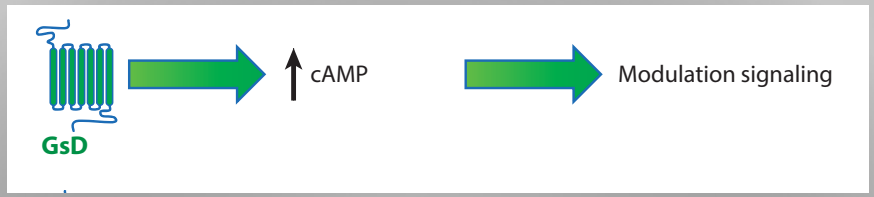
CHEMOGENETIC CONTROL OF NEURONAL AND NON-NEURONAL SIGNALING USING DREADD TECHNOLOGY

Subsequently, Guettier et al. (2009) created a chimeric muscarinic-adrenergic receptor DREADD **(GsD)** that selectively activates Gαs and activates neuronal cAMP-mediated signaling (Farrell et al. 2013).



Sternson & Roth, Ann. Rev. Neurosc, 2014

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

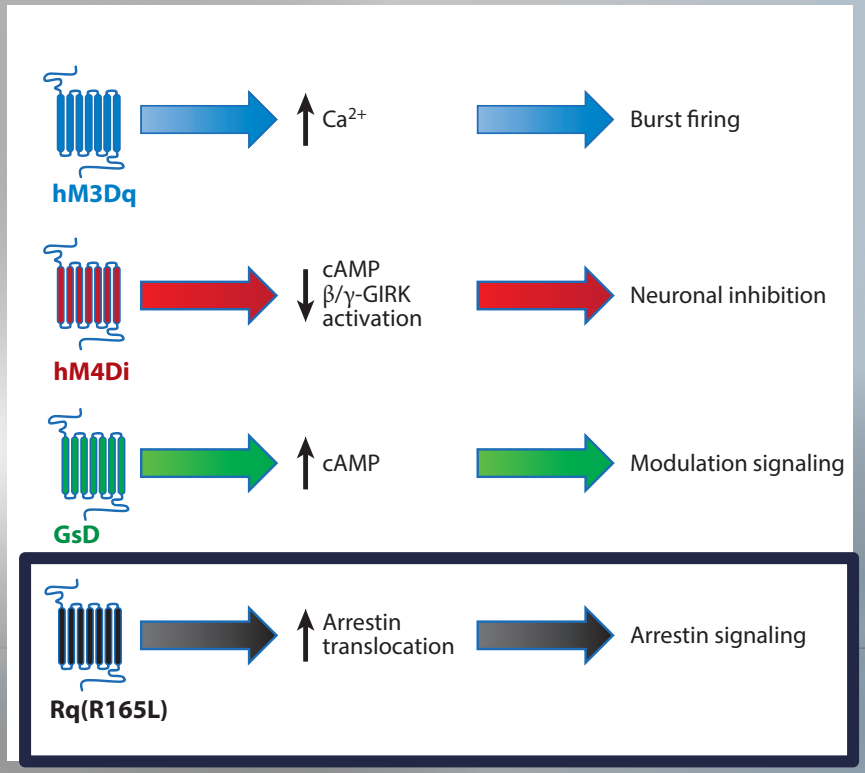


Gαs-DREADD (GsD) was initially used in studies of pancreatic β-cells in vitro and in vivo to deconstruct the signaling pathways essential for insulin secretion (Guettier et al. 2009). Subsequent studies demonstrated that CNO-mediated activation of GsD potently and efficaciously

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

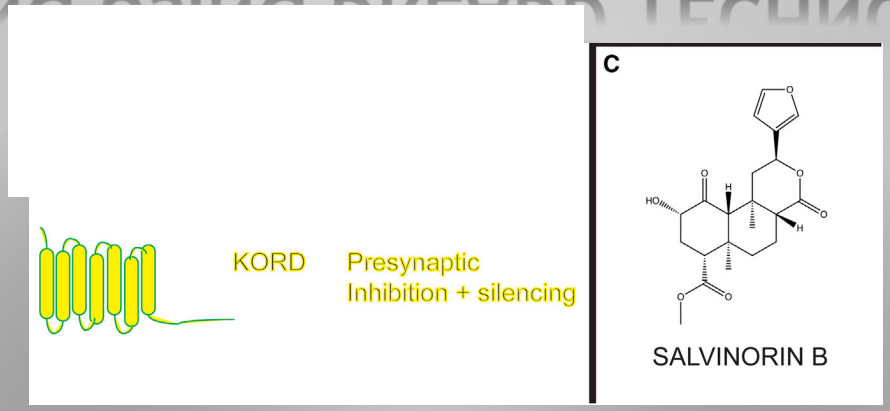
GPCRs signal not only via G proteins but also by activating ß-arrestin-mediated signaling pathways. Arrestin-mediated signaling has been implicated in the actions of many psychoactive drugs including opioids, lithium, and antipsychotics.

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.



Sternson & Roth, Ann. Rev. Neurosc, 2014

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



Roth, Neuron, 2016

The k-opioid-derived **DREADD (KORD)** is a new chemogenetic GPCR that is activated by the pharmacologically inert compound. Thus, **salvinorin B** has no activity at any other tested molecular target (>350 GPCRs, ion channels, transporters, and enzymes evaluated) and thus has no apparent off-target activity (Chavkin et al., 2004; Vardy et al., 2015).

Salvinorin B does retain modest affinity for KOR (>100 nM) so that investigators using the KORD should use the lowest dose possible and verify no effects of salvinorin B in the absence of KORD. Several labs have reported successful inhibition of neural activity with KORD (Marchant et al., 2016; Vardy et al., 2015; Denis et al.,2015)

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

It has to be noted 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, antagonist of 5-HT_{2A} subunit, (~10% by mass) in guinea pigs and humans (Jann et al. 1994).

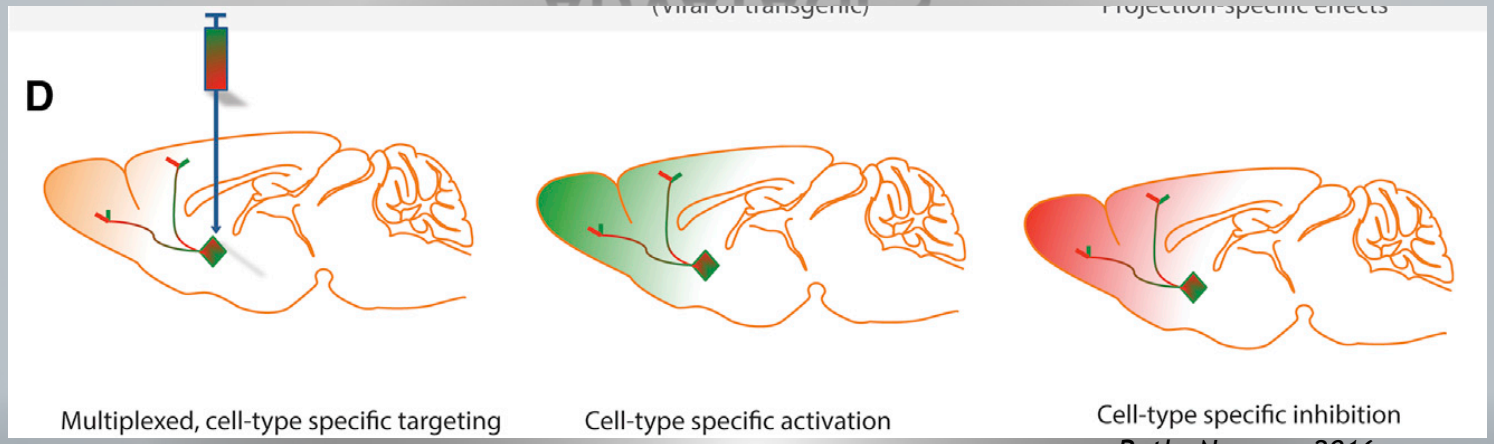
Thus, investigators contemplating the use of CNO in humans (or other primates or guinea pigs) will need to design experiments so that the dose of CNO is kept relatively low and that appropriate controls are performed (e.g., CNO administration in the absence of DREADD expression).

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

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.
* Unlike bacterial opsins, which silence neurons via a strong hyperpolarization and with millisecond precision, DREADDs induce a modest hyperpolarization and an apparently strong inhibition of axonal release of neurotransmitter in the seconds-minutes-hours time frame.

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



Roth, Neuron, 2016

The availability of a new inhibitory DREADD—KORD—activated by a ligand orthogonal to CNO now allows for the **multiplexed and bidirectional chemogenetic modulation of neural activity and behavior** (Vardy et al., 2015). Thus, we recently demonstrated that KORD may be expressed simultaneously with hM3Dq to allow for the sequential chemogenetic activation (with hM3Dq and CNO) and inhibition (with SalB and KORD) (Vardy et al., 2015) of neuronal activity. It is likely that KORD and hM3Dq could be combined in a **combinatorial fashion with various opsins** and other chemogenetic tools to afford highly multiplexed control of neuronal activity with millisecond precision (e.g., with opsins) and for long periods of time for behavioral studies.

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

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

Another useful tool that may soon be available involves having additional GPCR-ligand pairs available to allow for multiplexing control over signaling.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE

Ion channels are especially well suited for manipulating neuronal activity because they directly control the electrical properties of cells.

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 were 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.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE

Three categories of LGIC tools have been developed for cell type-selective neuron perturbation:

* invertebrate LGICs
* Ectopic expression of endogenous mammalian LGICs in the context of a global knockout background for that channel
* engineered ligand and ion channel systems.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: 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 GluClß 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.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: INVERTEBRATE LGICS

In neurons, IVM gating of GluCl channels results in the suppression of neuronal activity.

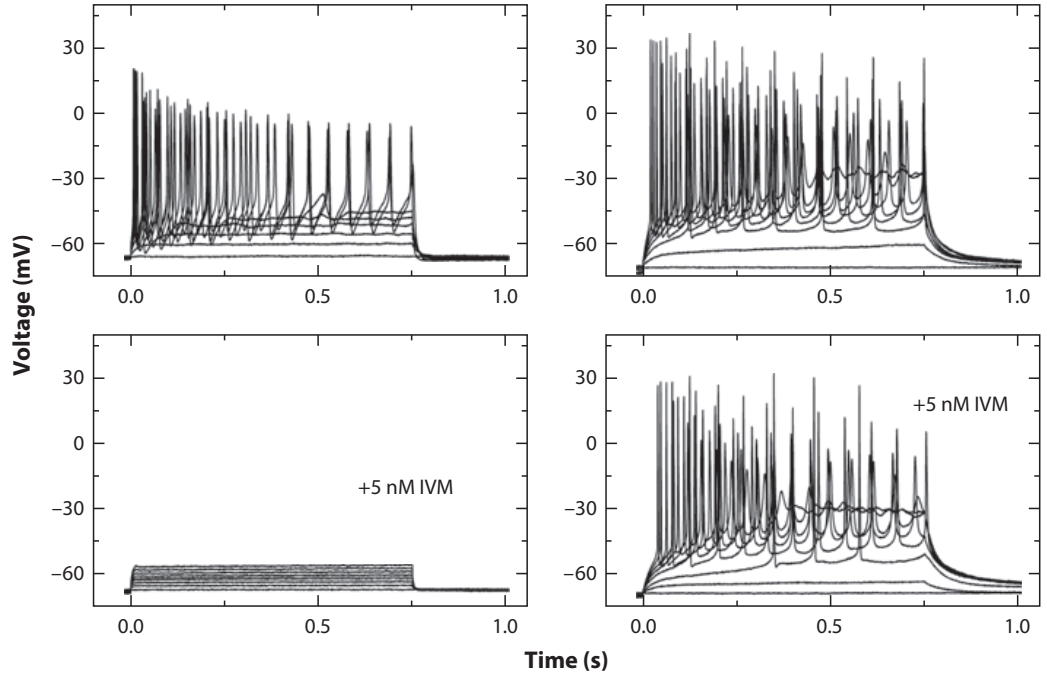


Figure 3

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).

Sternson & Roth, Ann. Rev. Neurosc, 2014

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: INVERTEBRATE LGICS

Activation of exogenous GluCl by endogenous glutamate has been minimized by a single-point mutation (Y182F) in the glutamate binding pocket of GluClß, 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.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: 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.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: MAMMALIAN LGICs

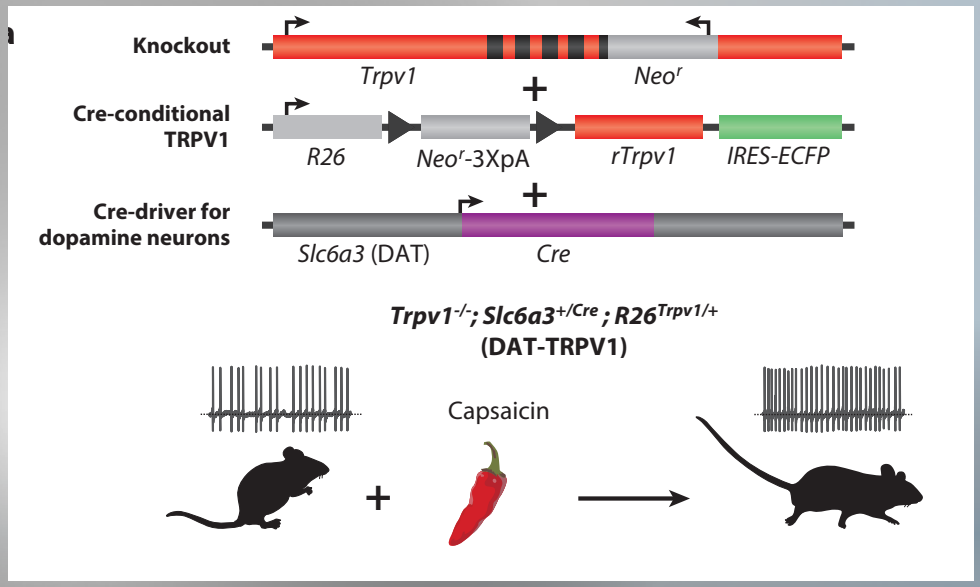
This strategy has been used to demonstrate cell type–selective chemical activation of neurons via targeted expression of the TRPV1 ion channel. TRPV1 is a nonselective cation channel that is gated by the small molecule capsaicin (the molecule in chili peppers that renders them spicy), resulting in neuronal depolarization (Arenkiel et al. 2007, Zemelman et al. 2003).

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: MAMMALIAN LGICs

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.

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).



Sternson & Roth, Ann. Rev. Neurosc, 2014

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

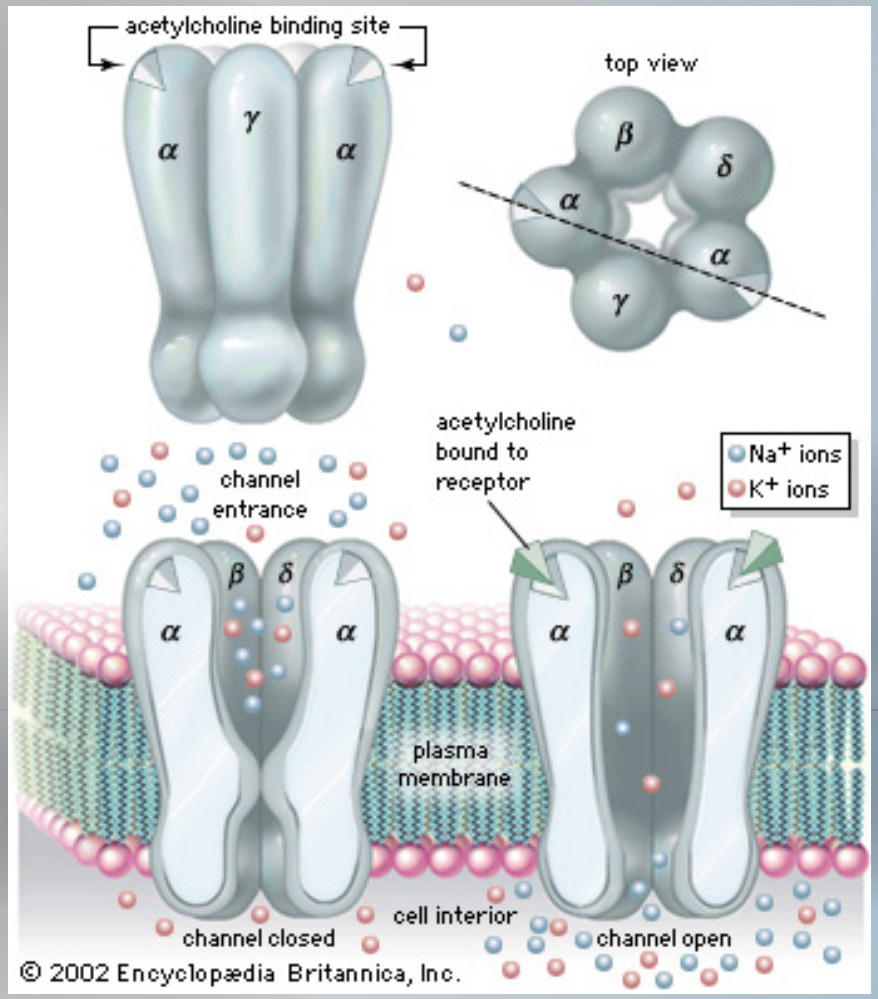
A newer approach to chemogenetic manipulation of neuron electrical activity is based on **chimeric ion channels that were developed using concerted genetic and chemical engineering** of selective interactions between ion channels and small-molecule agonists (Magnus et al. 2011).

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.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

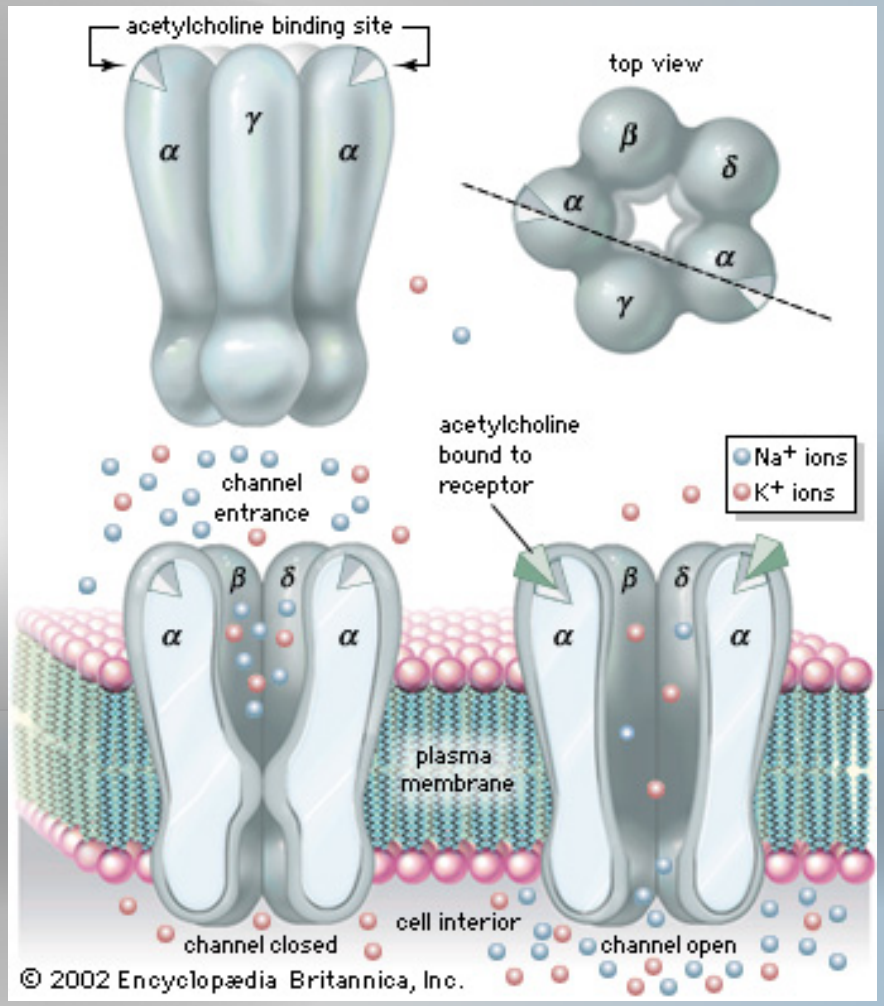
This chemical and genetic engineering strategy for cell type-specific control over ion conductance is based on classic experiments demonstrating that the extracellular **ligand binding domain (LBD)** of the α7 nicotinic acetylcholine receptor behaves as an independent actuator module that can be transplanted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

In mammals, the Cys-loop superfamily comprises both **cationic** [**nicotine** and 5-HT (5-hydroxytryptamine) receptors (i.e. **5-HT3 receptors**)] and **anionic** (GABA (γ - aminobutyric acid) receptors (i.e. **GABAA and GABAC receptors**) and **glycine receptors**] ion channels



CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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)**



CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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.

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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.**

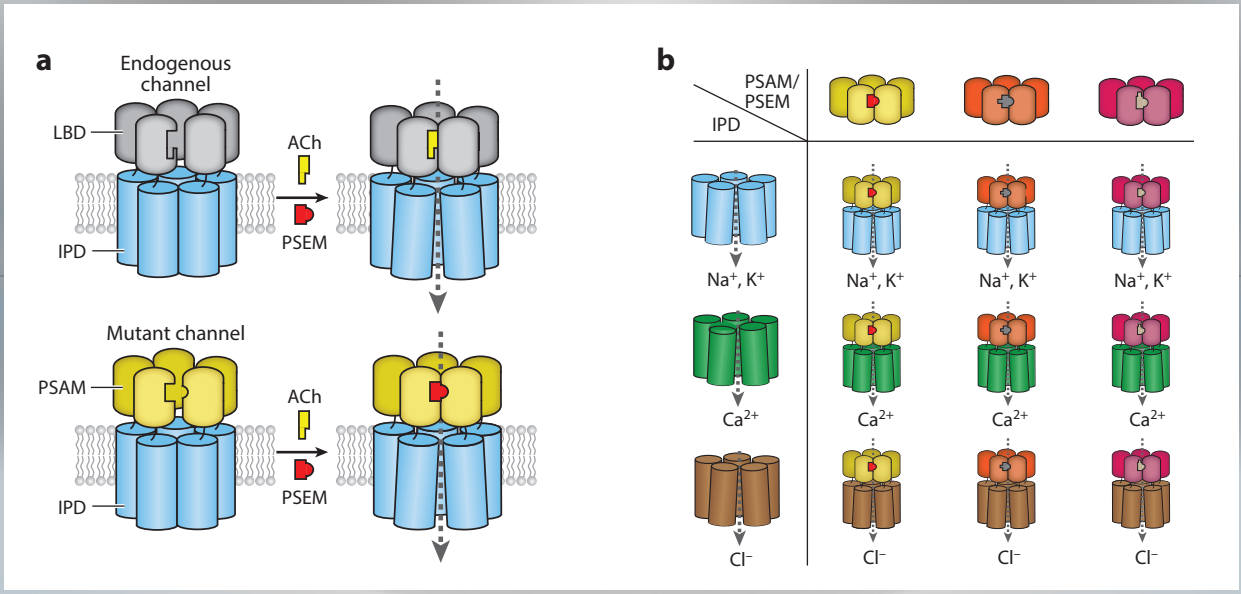
An α7 nAChR **agonist, quinuclidinyl benzamide PNU-282987**, was used as a starting point for agonist design because it crosses the blood-brain barrier (Walker et al. 2006); is highly selective for α7 nAChR over other isoforms; and is highly selective against a broad panel of vertebrate ion channels, 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

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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., PSAML141F.

The cognate agonists were called **pharmacologically selective effector molecules (PSEM;** pronounced “sem”) and are referred to with specific numbers, e.g., PSEM89S



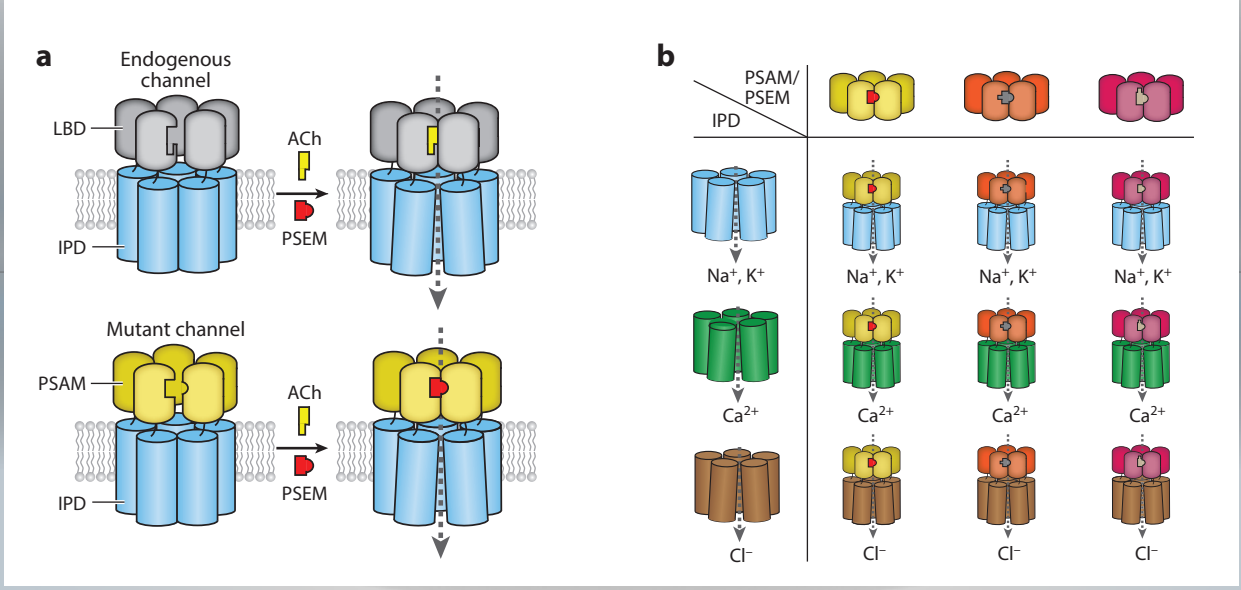
Sternson & Roth, Ann. Rev. Neurosc, 2014

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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 activating either the endogenous α7 nAChR or other PSAM-containing channels.

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).

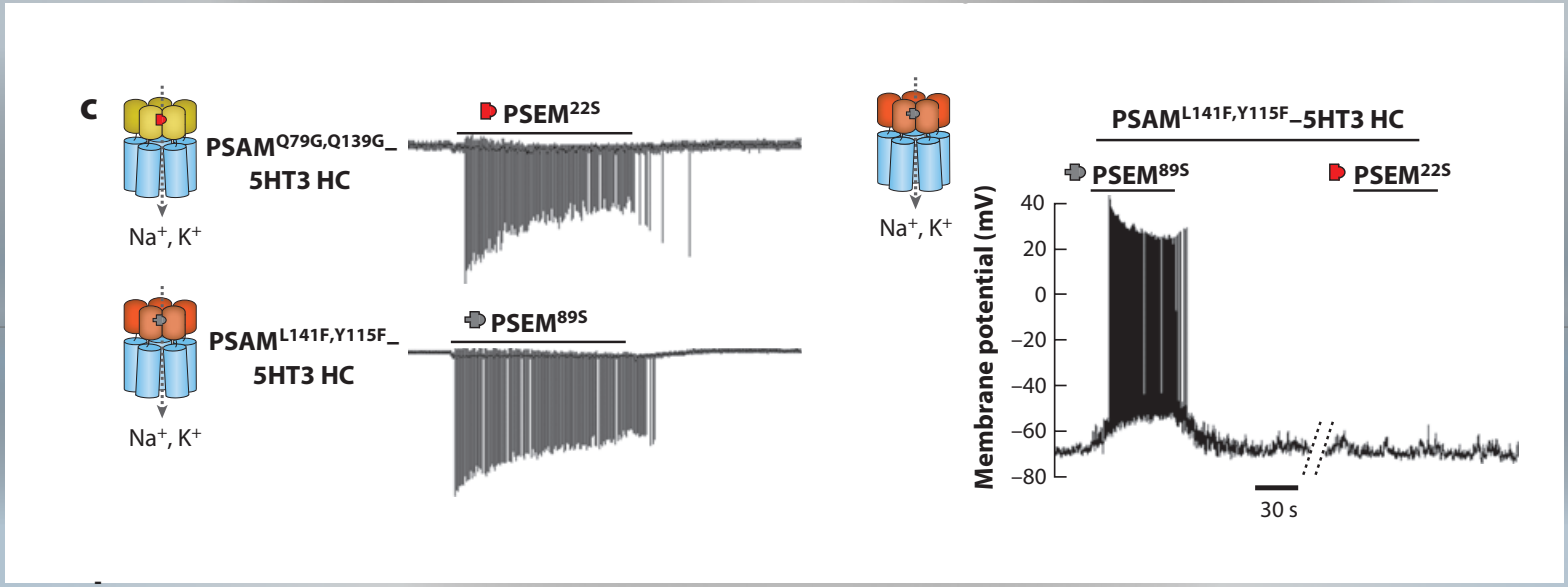


Sternson & Roth, Ann. Rev. Neurosc, 2014

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

Multiple pharmacologically selective cation channels were generated by fusing different PSAMs to the 5HT3 IPD.

Neurons expressing these channels depolarized and fired action potentials for minutes during PSEM application. Action potential activity ceased shortly after PSEM removal.



Sternson & Roth, Ann. Rev. Neurosc, 2014

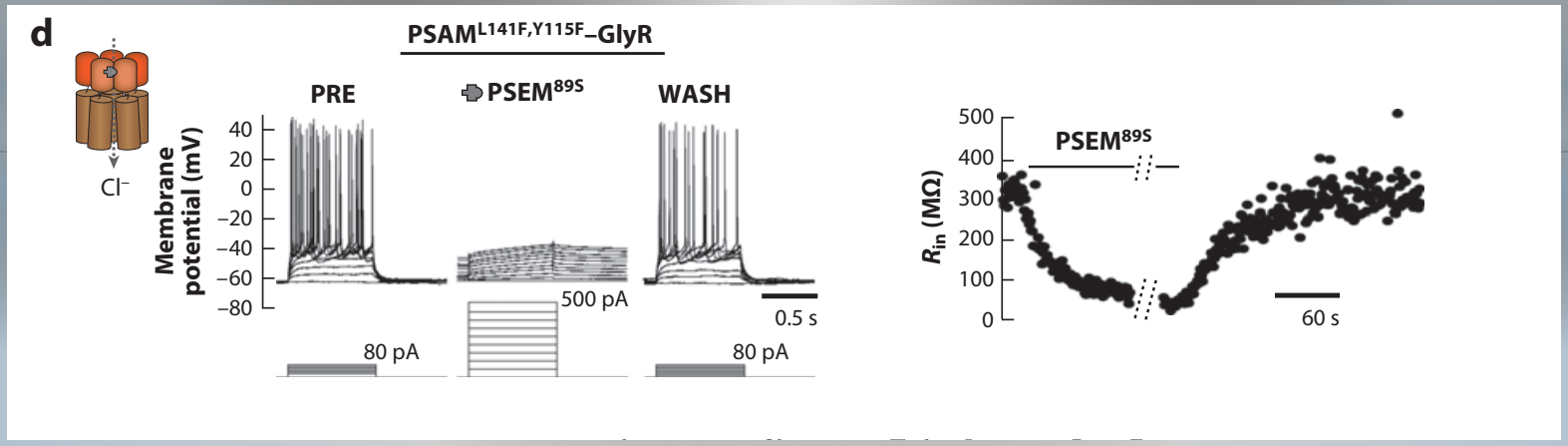
CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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.

These channels sharply reduced the input resistance of neurons and strongly inhibited neuronal excitability in the presence of the appropriate PSEM.

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.



Sternson & Roth, Ann. Rev. Neurosc, 2014

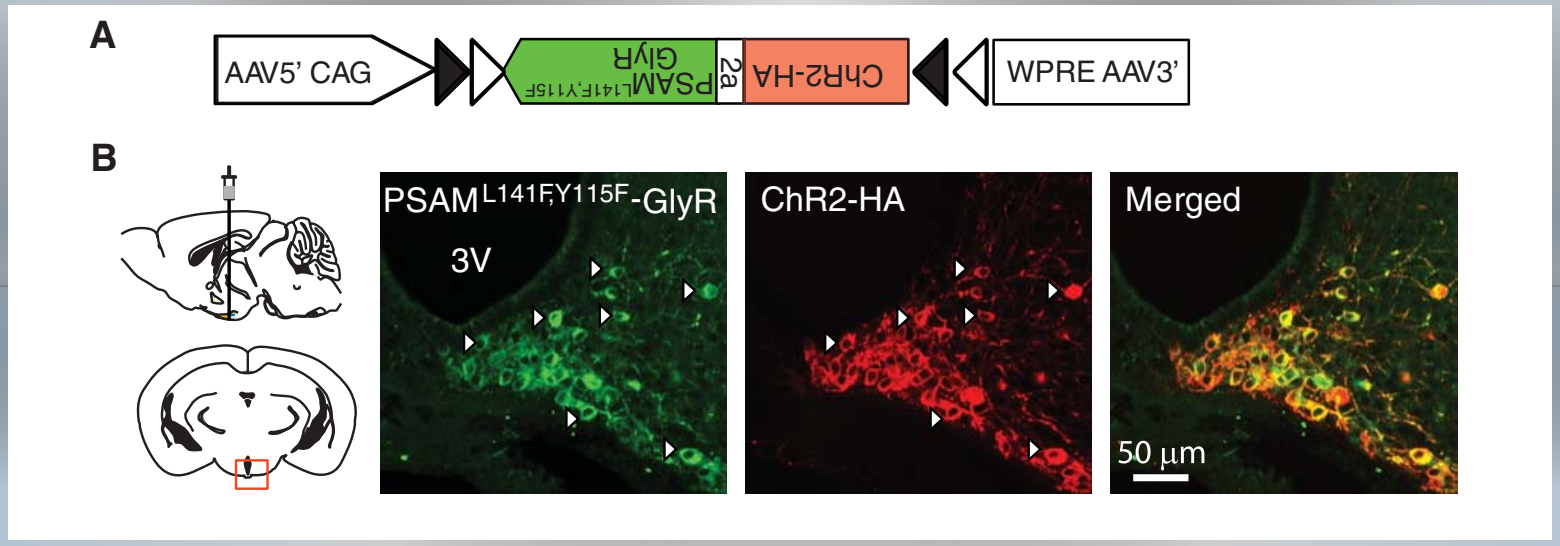
CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

To test the effectiveness of a PSEM–PSAMIPD system in vivo, it was examined the capacity of a neuronal silencer to influence behavior in mice.

The authors used PSAML141F,Y115F-GlyR and PSEM89S in hypothalamic Agouti-related protein–expressing (AGRP) neurons to suppress voracious eating evoked by photostimulation of these neurons coexpressing the light-activated cation channel, channelrhodopsin-2 (ChR2)

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

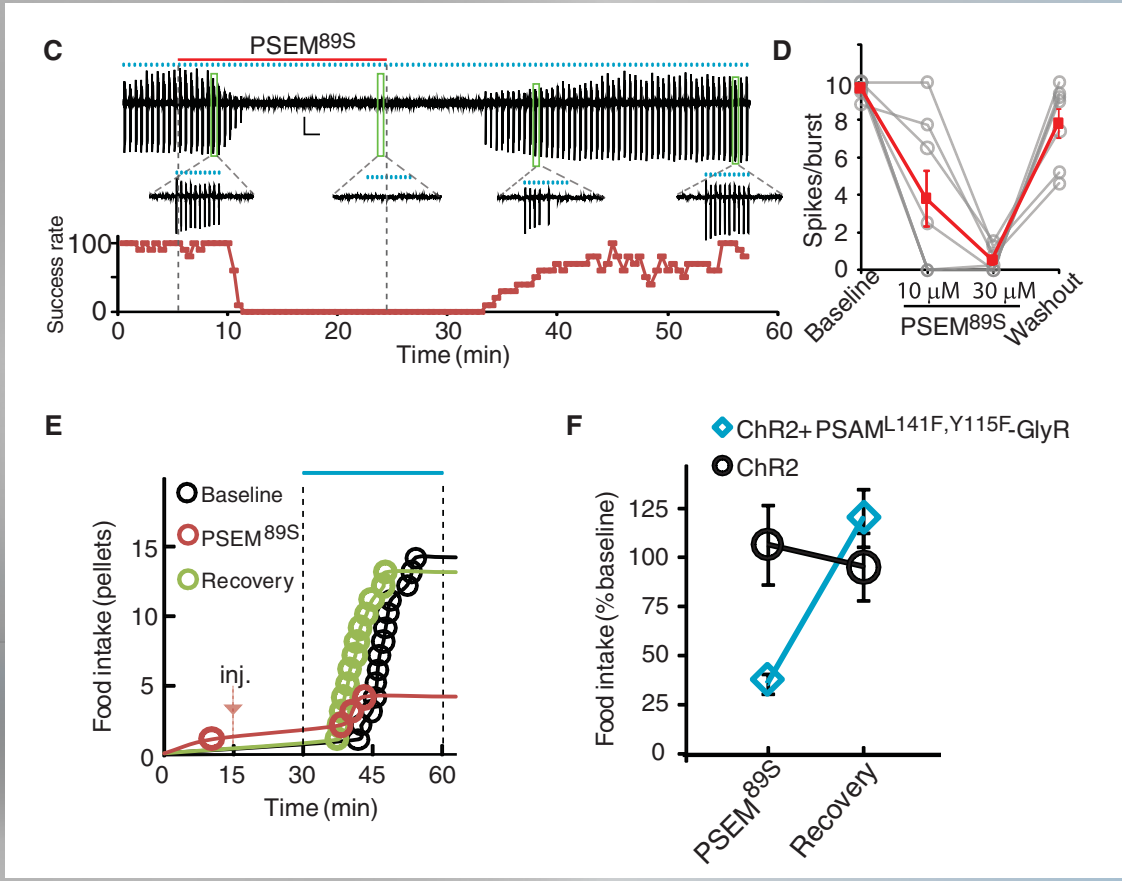
AGRP neurons in Agrp-cre mice were transduced using a bicistronic Cre recombinase (Cre)-dependent viral vector. AGRP neurons coexpressing ChR2 and PSAML141F,Y115FGlyR (Fig. 4B) could be activated with light and were reversibly silenced byPSEM89S during photostimulation in brain slices



Magnus et al., Science, 2011

CHEMOGENETIC CONTROL OF IONIC CONDUCTANCE: ENGINEERED LGICS AND LIGANDS

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 PSEM89S 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., Science, 2011

*MAGNETOGENETIC: ELECTROMAGNETIC REMOTE CONTROL OF BRAIN FUNCTIONS

While these new technologies have provided significant advances, **chemogenetics** doesn't provide instant time- and location dependent control, and **optogenetics** is inherently invasive and requires permanent brain implants.

An alternative that holds the potential to by-pass many of these limitations is the idea of using magnetic fields as a way to remotely control neurons.

TECHNICAL REPORTS

Remote regulation of glucose homeostasis in mice using genetically encoded nanoparticles

Sarah A Stanley[1,4], Jeremy Sauer[2,4], Ravi S Kane[2], Jonathan S Dordick[2] & Jeffrey M Friedman[1,3]

LETTER

doi:10.1038/nature17183

Bidirectional electromagnetic control of the hypothalamus regulates feeding and metabolism

Sarah A. Stanley[1], Leah Kelly[1], Kaamashri N. Latcha[1], Sarah F. Schmidt[1], Xiaofei Yu[1], Alexander R. Nectow[1], Jeremy Sauer[2], Jonathan P. Dyke[3], Jonathan S. Dordick[2] & Jeffrey M. Friedman[1,4]

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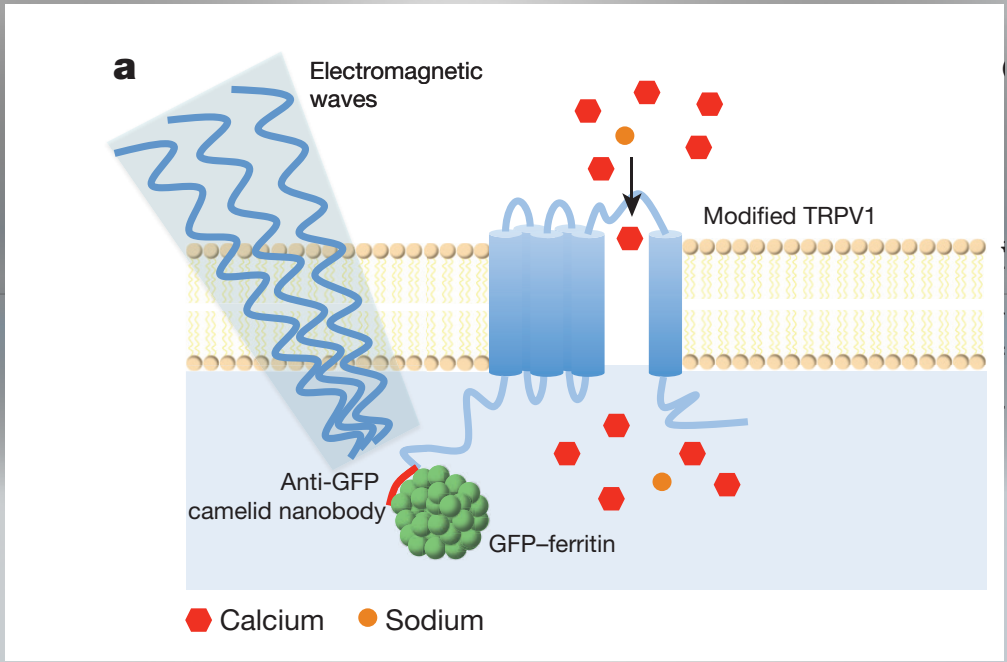
Exogenous magnetic nanoparticles have recently been shown to regulate gene and protein expression

Here we report the development of a genetically encoded system for remote regulation of gene expression by low-frequency radio waves (RFs) or a magnetic field. **Iron oxide nanoparticles** are synthesized intracellularly as a GFP-tagged ferritin heavy and light chain fusion.

The ferritin nanoparticles associate with a camelid **anti-GFP-transient receptor potential vanilloid 1 fusion protein, aGFP-TRPV1**, and can transduce noninvasive RF or magnetic fields into channel activation, also showing that TRPV1 can transduce a mechanical stimulus.

This, in turn, **initiates calcium-dependent transgene expression**.

In mice with stem cell or viral expression of these genetically encoded components, remote stimulation of insulin transgene expression with RF or a magnet lowers blood glucose. This robust, repeatable method for remote regulation in vivo may ultimately have applications in basic science, technology and therapeutics.



one key question remained unanswered: can genetically encoded magnetic matter synthesized in the neurons be adapted to manipulate excitatory and inhibitory synaptic transmission in the brain?

Building on their own prior work, Stanley et al. have now done just that to neurons in a hypothalamic subnucleus, the VMH

LETTER

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Bidirectional electromagnetic control of the hypothalamus regulates feeding and metabolism

Sarah A. Stanley[1], Leah Kelly[1], Kaamashri N. Latcha[1], Sarah F. Schmidt[1], Xiaofei Yu[1], Alexander R. Nectow[1], Jeremy Sauer[2], Jonathan P. Dyke[3], Jonathan S. Dordick[2] & Jeffrey M. Friedman[1,4]

The paper describes **an innovation that successfully controls glucose-sensing neurons in the ventromedial nucleus of the hypothalamus (VMH)** to bidirectionally regulate feeding behavior and glucose metabolism, representing a powerful methodological bedrock for future exciting discoveries in neuroscience.

Neuroendocrinology: Electromagnetogenetic Control over Feeding and Glucose Metabolism

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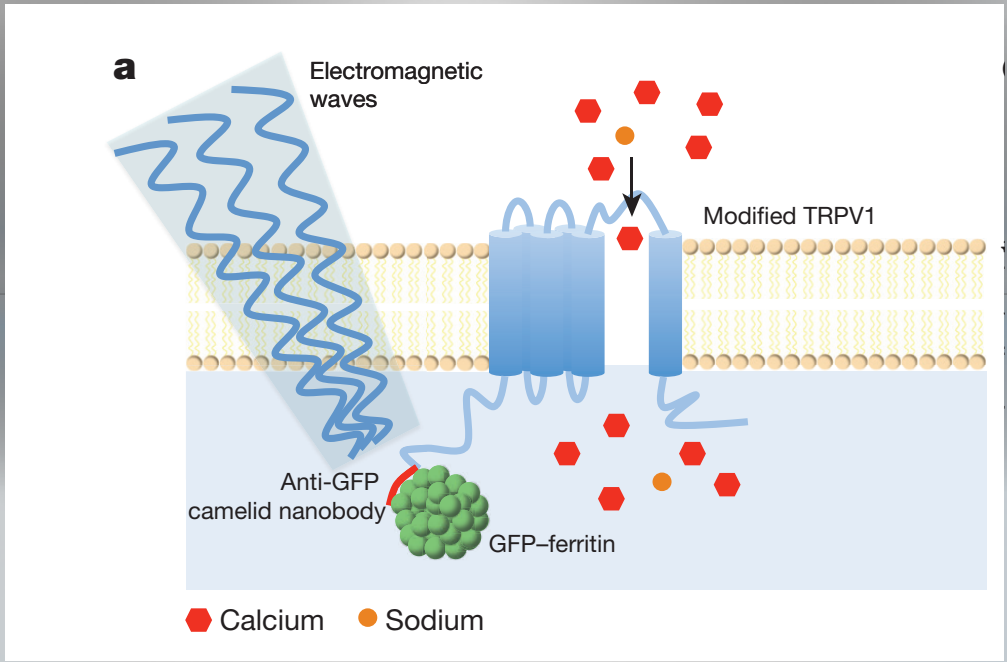
http://dx.doi.org/10.1016/j.cub.2016.04.044

Early lesioning studies revealed that the VMH is involved in the recognition of satiety because damage to the VMH resulted in voracious eating and obesity. While those experiments defined the ventromedial hypothalamus (**VMH) as a satiety center**, recent data have assigned this nucleus a much broader role in the **control of energy homeostasis**, and chronically altering VMH neuron activity is now known to affect peripheral glucose metabolism.

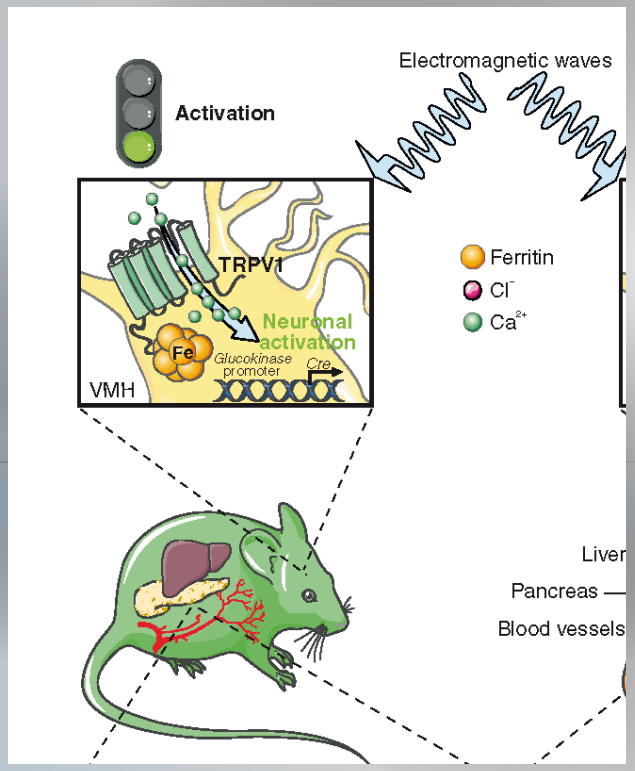
Distinct VMH neurons work as glucose sensors and balance the release of hormones that defend against hypoglycemia when glucose drops too low. While all neurons utilize glucose as fuel, VMH neurons quickly change their firing rate in response to changes in brain glucose levels.

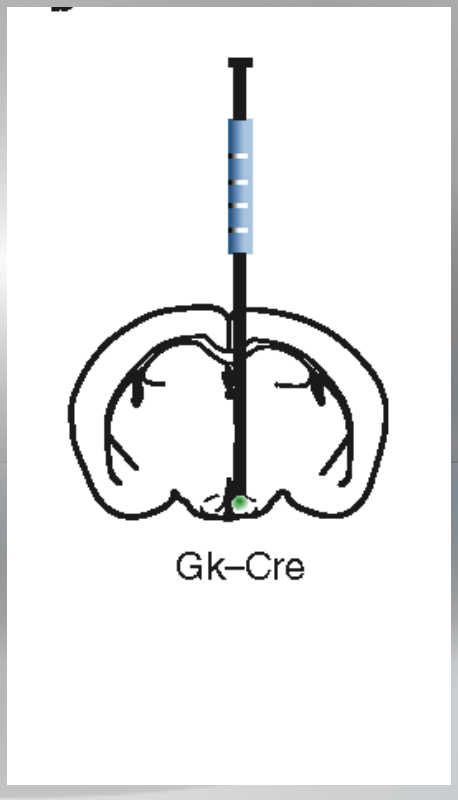
According to their fundamental role in glucose monitoring, and **given that precise control of the levels of glucose** — the important energy substrate for neurons — is critical for the organism, **VMH neurons represent an ideal candidate to evaluate the relevance of remotely controlling a biological response using a radio.**

The concept relies on coupling a temperature-detecting TRPV1 (commonly known as the receptor for the irritating compound in chilli pepper) to a form of ferritin (an iron-storing protein) that has been modified to respond to electromagnetic waves. The energy of the waves, which travel readily through tissues, is absorbed by the ferritin, heating it and causing it to undergo motion. This movement is transferred to TRPV1, by virtue of its fusion to ferritin, changing the confirmation of TRPV1 and triggering influx of calcium, leading to neuronal activation.

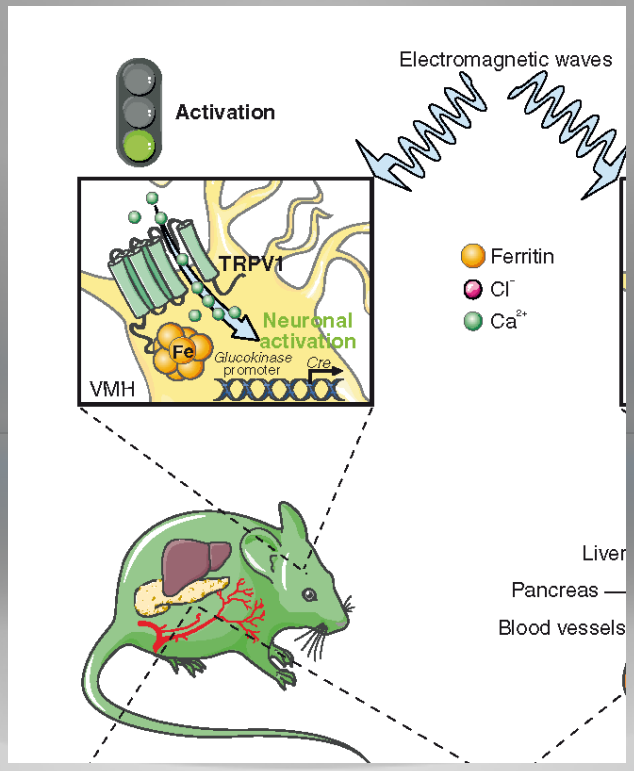


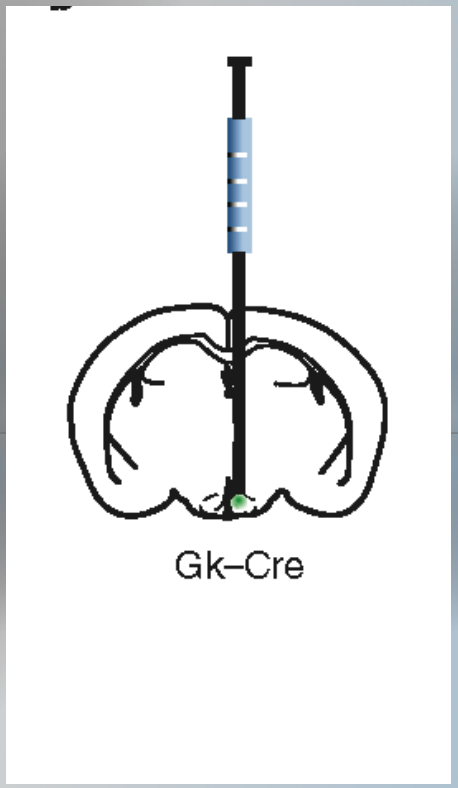
A replication-deficient adenovirus with Cre-dependent expression of anti- GFP–TRPV1/GFP–ferritin (Ad-FLEX-anti-GFP-TRPV1/GFP-ferritin) was injected unilaterally into the ventromedial hypothalamus (VMH) of glucokinase–Cre (GK–Cre) mice, which express Cre in glucosesensing neurons, targeting ~2,000 neurons.





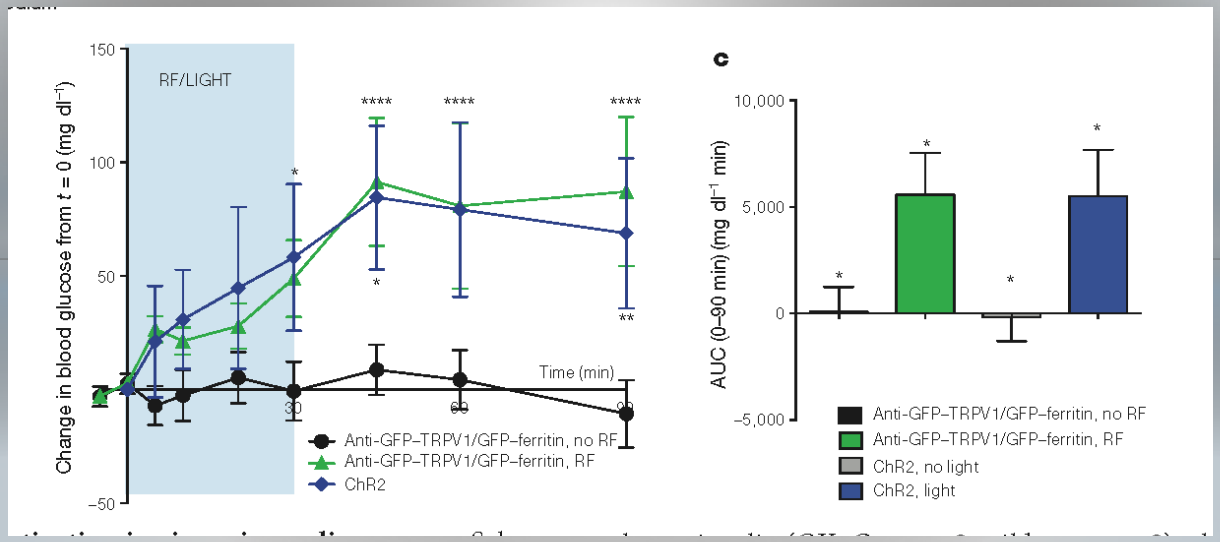
For expression in the VMH, they packed replication-deficient adenoviral particles with the construct and injected them into the VMH of transgenic glucokinase– Cre mice, in which Cre is expressed in glucose-sensing neurons. Although the virus will infect many neurons, the ferritin–TRPV1 fusion will only be expressed in glucokinase-expressing neurons, representing a Cre-dependent magnetogenetic system and eliminating the use of exogenously delivered iron nanoparticles.

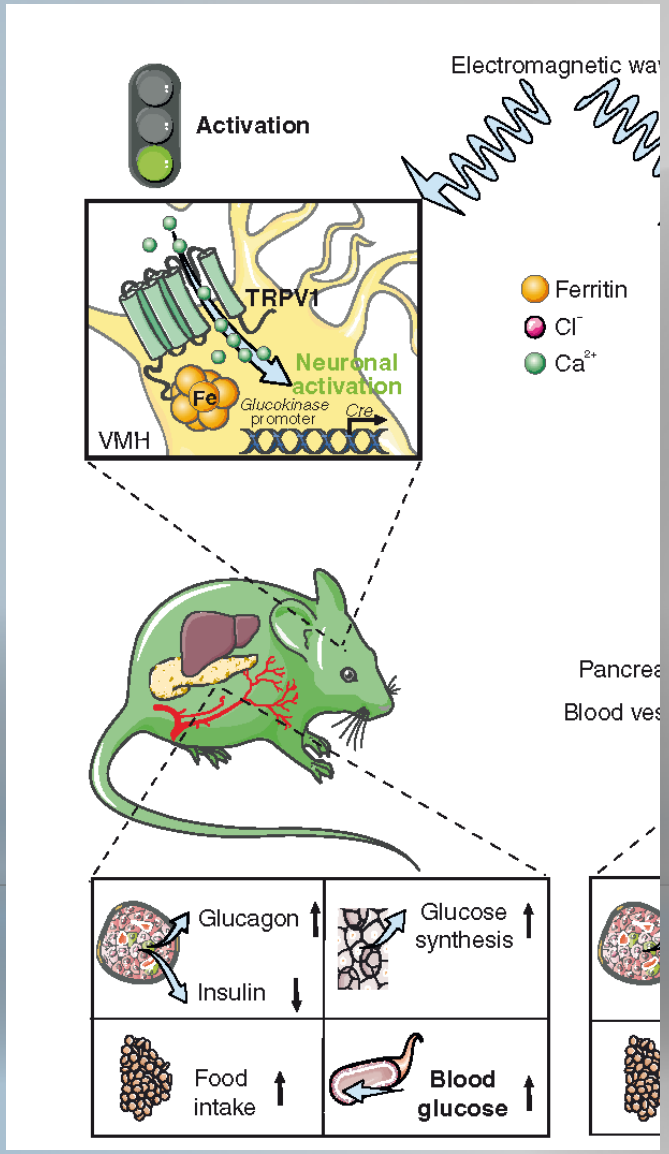




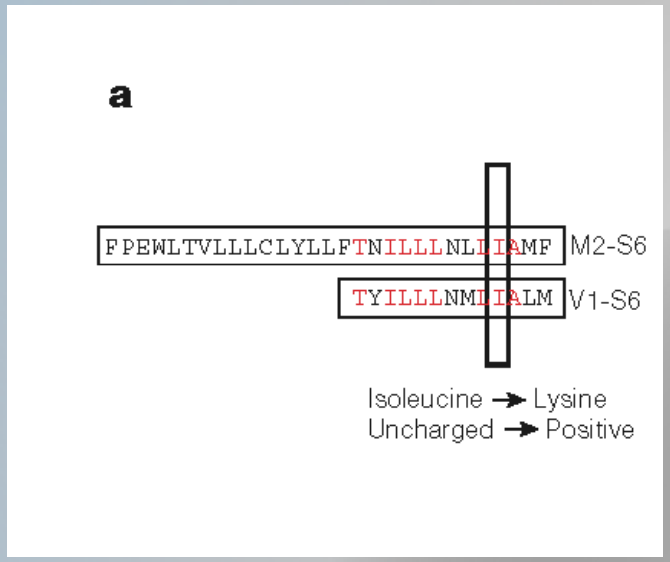
Radio frequency (RF) treatment (465 kHz) of these mice significantly **increased blood glucose** (change in blood glucose at 30 min: RF-treated, 48.9 ± 16.9 mg dl−1 versus untreated, −0.7 ± 12.9 mg dl−1; $P < 0.05$; at 45 min: RF-treated, 91.3 ± 28.2 mg dl−1 versus untreated, 8.7 ± 11.1 mg dl−1; $P < 0.05$) and the cumulative change in blood glucose (area under the curve (AUC; 0–90 min): RF-treated, 5,562 ± 1,977 mg dl−1 min versus untreated, 62 ± 1,184 mg dl−1 min; $P < 0.05$) (Fig. 1b, c).

The time course and extent of glucose changes after RF treatment were **almost superimposable with those after optogenetic activation** of VMH GK-Cre neurons, albeit with a slight delay (Fig. 1b, c).





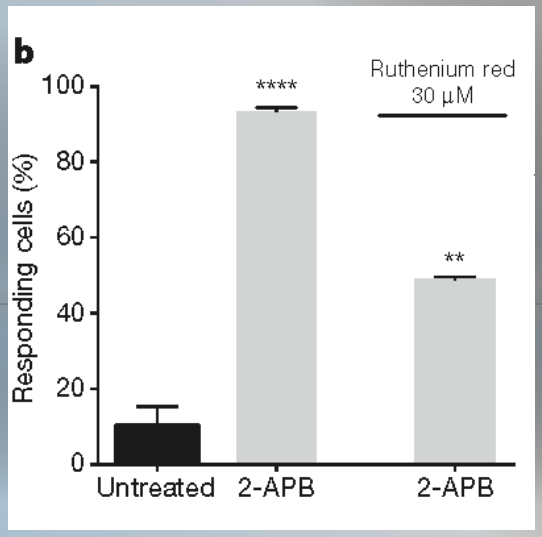
As a compelling verification of the method's efficacy, exposure of living mice expressing the construct in the VMH to radio waves robustly increased peripheral blood glucose. The authors also observed that activation of VMH neurons **halved circulating insulin concentrations**, while **increasing plasma glucagon** levels **and the expression of genes involved in glucose synthesis in the liver**, suggesting that **activating the glucoresponsive neurons regulated the abundance of key glucoregulatory hormones from the pancreas and activated gluconeogenesis.** Thus, it seems that activating this subpopulation of VMH neurons **makes the brain think it's low on sugar.**

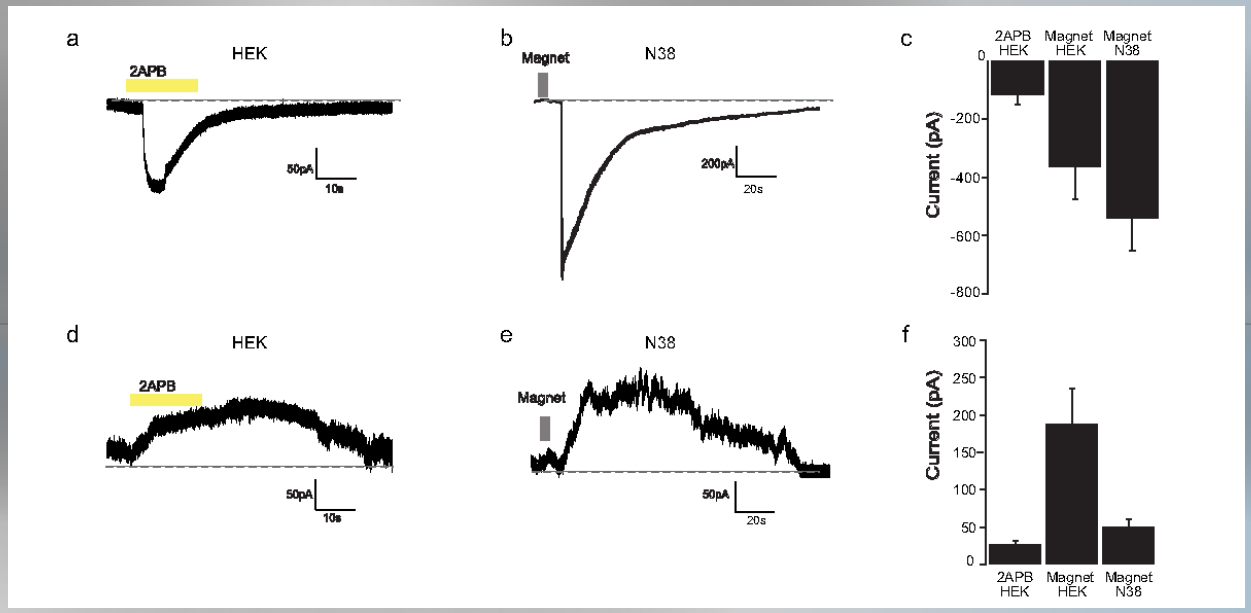


Non-invasive neural inhibition would provide a valuable research tool and potentially offer an alternative to deep brain stimulation which may act by neural inhibition. **An amino acid substitution**, from isoleucine to lysine in the **S6 pore region of TRPM2 and M8** changed ionic selectivity from cations to chloride (Cl^-) ions.

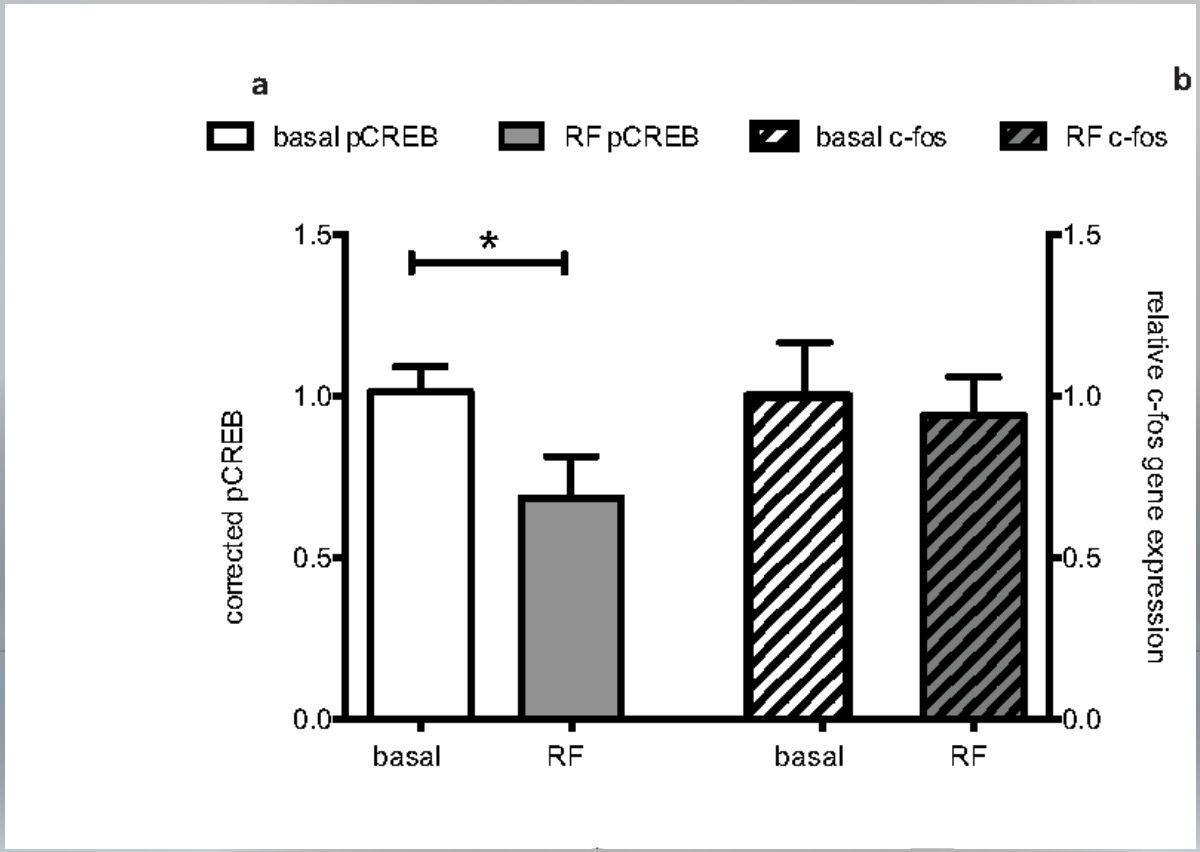
We introduced and tested an analogous mutation in **TRPV1 S6 region (I679K)** (Fig. 2a) to create the TRPV1mutant channel.

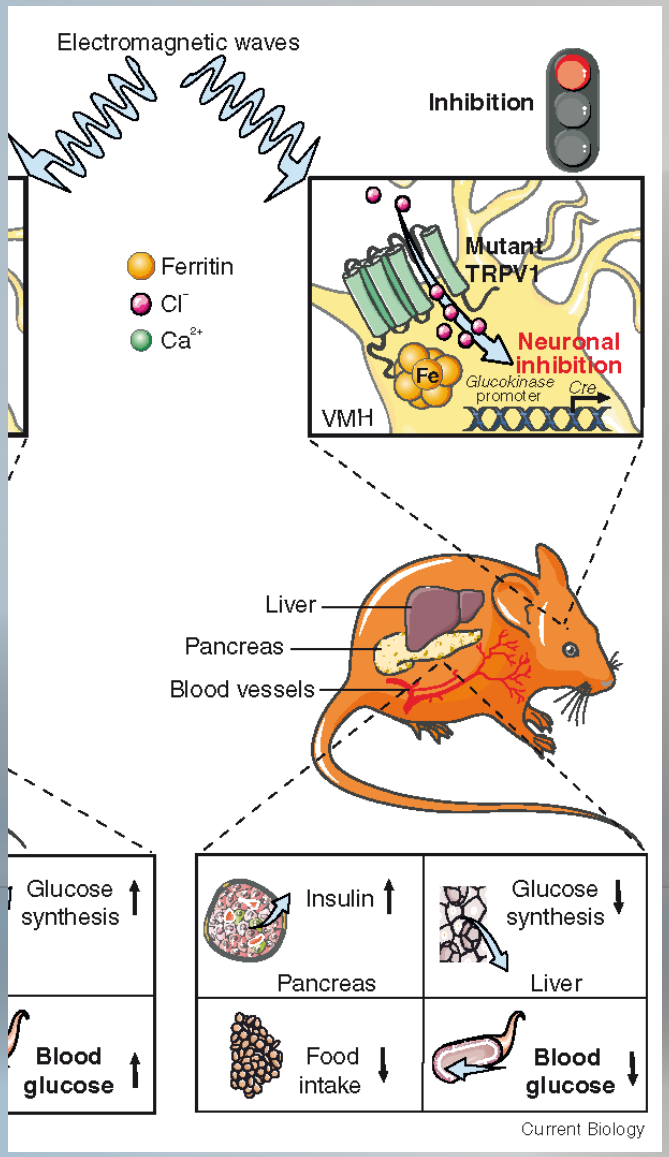
In N38 cells stably expressing anti- GFP–TRPV1mutant/GFP–ferritin, the TRP agonist 2-aminoethoxydiphenyl borate (2-APB) significantly increased intracellular Cl– levels measured by MQAE quenching. This effect was blocked by ruthenium red (Fig. 2b and Extended Data Fig. 8).



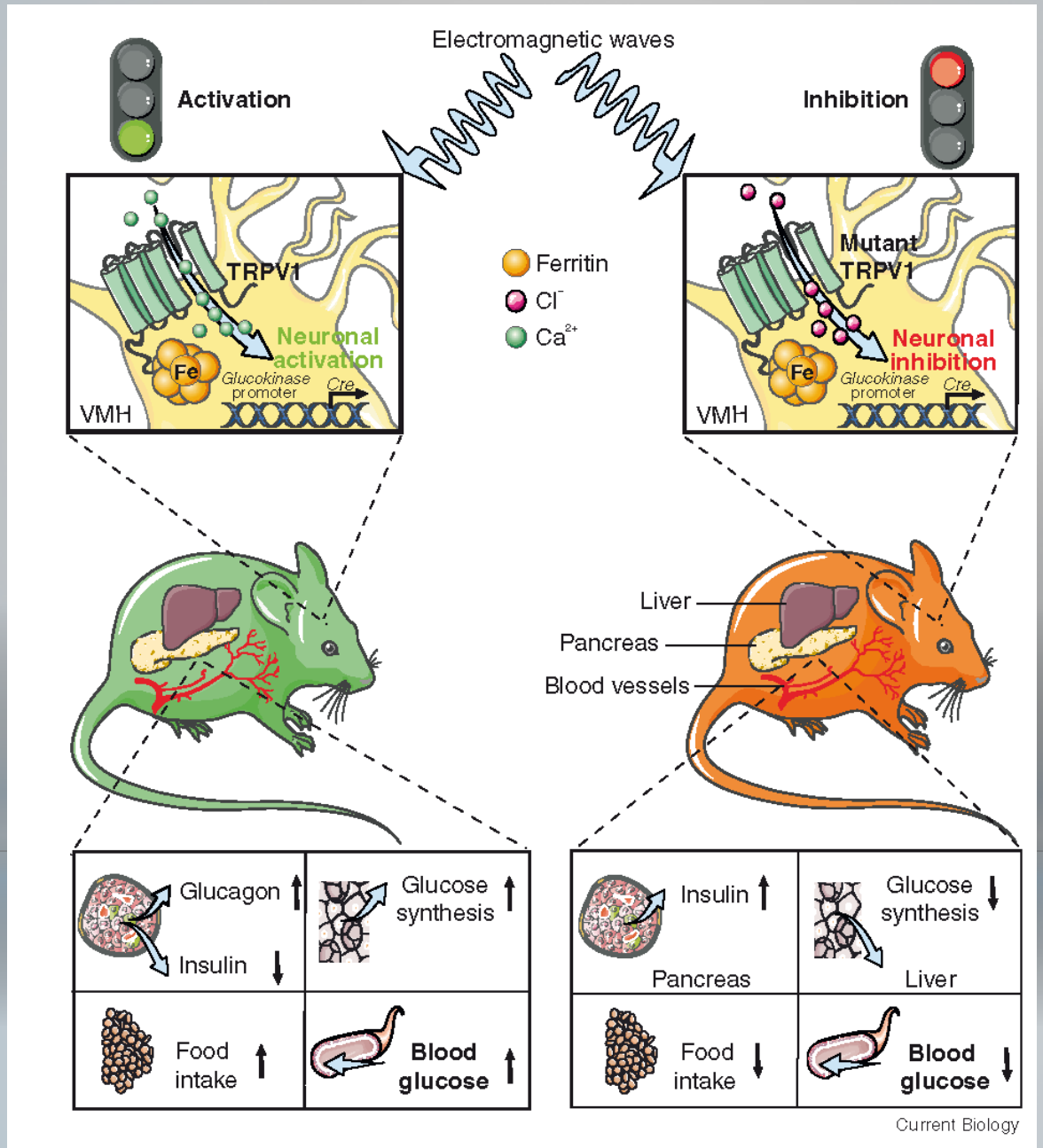


RF treatment of N38 cells expressing anti-GFP–TRPV1mutant/GFP–ferritin significantly reduced pCREB levels and failed to increase c-Fos expression (Extended Data Fig. 7a).





Inhibiting the VMH neurons elevated plasma **insulin** levels whilst conversely **suppressing** the expression of liver **gluconeogenic genes**, concomitant with markedly **lower blood glucose**. This finding suggests that keeping blood glucose levels constant depends on the **tonic activity of hypothalamic neurons** and further substantiates the capability of VMH neurons to directly influence the secretion of hormones with the purpose of maintaining the glucose equilibrium.


Electromagnetic waves
Activation
Inhibition
TRPV1
Neuronal activation
Fe
Glucokinase promoter
Cre
VMH
Ferritin
Cl⁻
Ca²⁺
Mutant TRPV1
Neuronal inhibition
Liver
Pancreas
Blood vessels
Glucagon
Insulin
Glucose synthesis
Food intake
Blood glucose
Pancreas
Liver
Current Biology

CONCLUSIONS REMARKS

* The activity of any cluster of even dispersed cell types could logically be made accessible.
* Furthermore, it may extend beyond TRPV1, a large protein widely distributed throughout the brain.
* Tethering ferritin to another substrate converting the electromagnetic radiation to other intracellular signals, or tuning ion selectivity, may open doors to the study of a range of neuronal populations and processes.
* The fact that the amount of calcium influx varied with the energy supplied suggests that the physiological response can be modulated, which could allow for the control of cells differentially regulated at different ion concentrations, while optimized to respond to magnetic fields only and not to the endogenous ligands.
* An additional interesting observation is how rapidly radio wave treatment activated the fusion protein and took over the control of systemic glucose levels, offering fast interrogation of neural activity, similar to Fe Glucokinase.

* The paper by Stanley et al. and recent articles on the same timely subject are important.
* Electromagnetogenetics requires multiple and unique components, however, and time will tell if it will be applied outside the original laboratories.
* We hope that this state-of-the-art method will become a broadly applicable tool for neuroscientists, used by more than the exclusive number of comic book superheroes born with the power to control metal.