

August 2018

Chemogenetics

Introduction

Major advances in neuroscience methods have allowed researchers to selectively manipulate neural systems in conscious animals, with two emerging techniques; optogenetics and chemogenetics. Both these techniques enable the exploration of neural circuitry underlying complex behaviors in health and disease.

Optogenetics and chemogenetics have similarities in their method of altering neuronal expression, in that they both require the introduction of engineered receptors or ion channels in specific brain areas, via viral vector or plasmid expression systems. Optogenetics requires the expression of bacterial, light-sensitive ion channels and the subsequent use of fiber optics to activate or inhibit neuronal activity *in vitro* or *in vivo* (Boyden *et al*, 2005; Zhang *et al*, 2007). While this technique provides excellent temporal control of *in vivo* neuronal activity, it is inherently invasive, requiring cerebral implantation of fiber optics. In contrast, chemogenetics does not require a chronic implant but retains the ability to manipulate neuronal activity. This is achieved through administration of ligands, selective for engineered receptors/ion channels, which are otherwise inert (Armbruster *et al*, 2007; Campbell & Marchant, 2018). The key features of optogenetics and chemogenetics are summarized in Table 1.

Tocris provides an innovative range of high performance life science reagents for use in chemogenetic experiments and neuroscience research, including DREADD ligands and PSEM compounds.

Table 1 | Chemogenetics vs Optogenetics

	Chemogenetics	Optogenetics
Method of intervention	Inert, small molecule ligands selective for genetically engineered receptors/ion channels	Light-sensitive ion channels activated by implanted fiber optics
Is the intervention 'physiological'?	Yes – uses conserved, intracellular signaling pathways, or changes in ion channel conductance, to alter neuronal activity	No – patterns of excitation/inhibition are artificially synchronized by light stimulation pattern
Is the intervention inert?	Yes – receptors/ion channels lack pharmacological activity without ligands and ligands are pharmacologically inert without specific receptors/ion channels	No – the fiber optic light source can create heat and bacterial light-sensitive channels used can be antigenic
Is this method invasive?	Minimally to no – ligands can be given by intracerebral infusion, intraperitoneal injection or in drinking water, dependent on specific ligand	Yes – inherently invasive due to implantation of fiber optics
Is specialized equipment required?	No	Yes – requires implantable fiber optics as light source

History and Development

RASSLs

As previously mentioned, chemogenetics refers to the use of genetically engineered receptors or ion channels and the selective ligands that activate those receptors to enable manipulation of neuronal activity. G-protein coupled receptors (GPCRs) have been at the forefront of chemogenetic development, with the first paper outlining GPCRs that respond only to synthetic ligands published in 1998. These receptors, called Receptors Activated Solely by a Synthetic Ligand (RASSLs), were successfully used *in vivo* to enable remote control of cardiac activity, for example. However, the use of RASSLs in neuroscience was hampered by the pharmacological activity of the ligands *in vivo* and endogenous activity of receptors in the absence of their specific ligand (Coward *et al*, 1998; Sternson & Roth, 2014).

DREADDs

More recently, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) have been developed. The first of these were mutated human muscarinic receptors activated solely by inert ligands (Armbruster *et al*, 2007). Multiple rounds of mutagenesis and screening against the biologically inert Clozapine *N*-oxide (CNO), identified muscarinic receptors coupled to the G_{α_q} intracellular signaling pathway, which can activate neuronal activity in response to CNO. All three G_{α_q} -DREADDs (hM_1D_q , hM_3D_q and hM_5D_q) are activated by low concentrations of CNO (Roth, 2016). Additionally, the same study identified hM_2D_i and hM_4D_i as capable of inhibiting neuronal activity through their coupling to G_{α_i} intracellular signaling pathways (Armbruster *et al*, 2007). These inhibitory DREADDs also respond to CNO (Figure 1).

Binding of CNO to G_{α_q} -DREADDs results in stimulation of phospholipase C (PLC), which catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). Both IP_3 and DAG have second messenger functions: IP_3 binds to its receptors to elicit Ca^{2+} release from intracellular stores, while DAG activates multiple forms of protein kinase C (PKC). Binding of CNO to G_{α_i} -DREADDs results in inhibition of adenylyl cyclase (AC), leading to a decrease in intracellular cAMP levels. cAMP activates protein kinase A (PKA) and EPAC, therefore the action of CNO at G_{α_i} -DREADDs inhibits PKA and EPAC downstream signaling (Figure 1).

Further reading

Campbell & Marchant (2018) The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. *Br J Pharmacol.* **175**, 994.

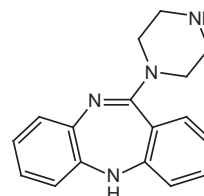
Roth (2016) DREADDs for Neuroscientists. *Neuron.* **89**, 683.

Featured DREADD ligands

DREADD agonist 21

Cat. No. 5548

Potent hM_3D_q DREADD agonist

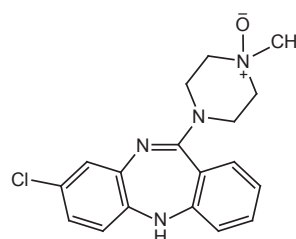


DREADD agonist 21 is a potent muscarinic DREADD agonist (pEC_{50} values are 8.91, 8.48 and 7.77 for hM_1D_q , hM_3D_q and hM_4D_i *in vitro*, respectively). It exhibits over 10-fold higher affinity for hM_1D_q and hM_4D_i compared to wild type receptors and displays little to no activity at wild type M_3 receptors. *In vivo*, DREADD agonist 21 has been shown to activate neurons expressing hM_3D_q and inhibits activity in neurons expressing hM_4D_i and displays excellent brain permeability.

Clozapine *N*-oxide dihydrochloride

Cat. No. 6329

Water soluble activator of muscarinic DREADDs

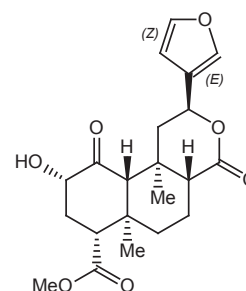


Water soluble version of CNO (Cat. No. 4936). CNO binds human muscarinic DREADDs; it activates neurons expressing hM_1D_q , hM_3D_q and hM_5D_q , and silences neurons expressing hM_4D_i *in vitro* and *in vivo*. CNO has also been shown to be a P-glycoprotein (P-gp) efflux pump substrate.

Salvinorin B

Cat. No. 5611

Activator of KORD



Salvinorin B is a potent and selective κ -opioid DREADD (KORD) activator ($EC_{50} = 11.8$ nM). It shows selectivity for KORD over endogenous κ opioid receptors. Salvinorin B is brain penetrant and induces neuronal hyperpolarization and silences neurons expressing KORD.

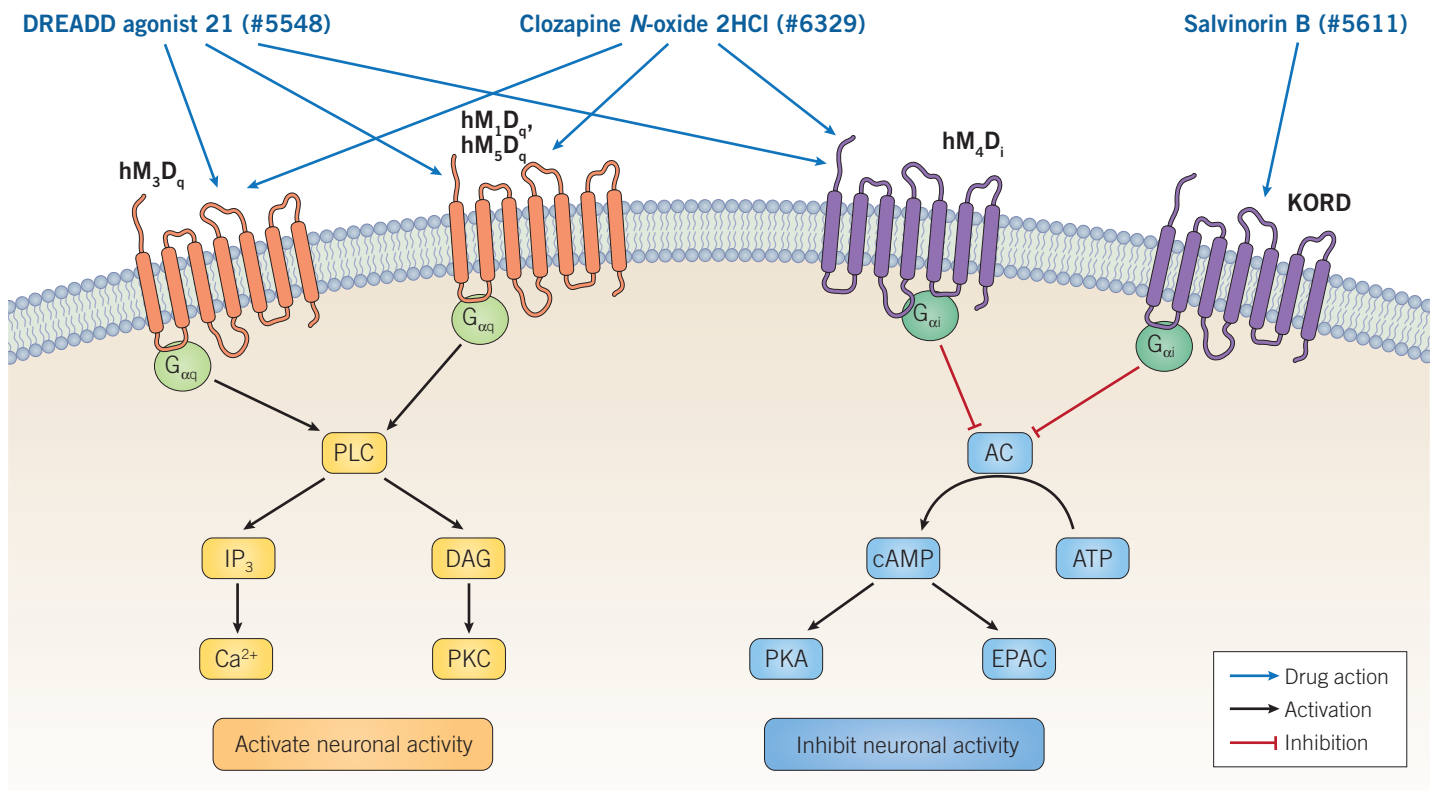


Figure 1: Mechanism of action of DREADD ligands. Binding of DREADD ligands to $G_{\alpha q}$ -DREADDs provokes neuronal firing, whereas binding to $G_{\alpha i}$ -DREADDs results in inhibition of neuronal activity. Clozapine *N*-oxide dihydrochloride and DREADD agonist 21 are non-selective muscarinic DREADD agonists and so can activate or inhibit neuronal activity, depending on the specific receptor being expressed. Salvinator B is selective for the KORD receptor, which is coupled to $G_{\alpha i}$ signaling; consequently binding results in inhibition of neuronal activity.

CNO is a metabolite of clozapine, however, research suggests that this conversion is bidirectional and that CNO may undergo reverse metabolism to clozapine. Clozapine is then able to activate endogenous receptors when CNO is given at the concentrations required for DREADD activation (Gomez *et al*, 2017). Clozapine is an atypical antipsychotic, acting at a range of targets and leading to a variety of behavioral effects. The reversible metabolism of CNO to clozapine has been shown in mice, rats, guinea pigs, non-human primates and humans (Gomez *et al*, 2017; Manvich *et al*, 2018).

The potential reverse metabolism of CNO has led to structure-activity relationship studies to develop alternative, stable ligands. DREADD agonist 21 is a potent DREADD ligand that was initially examined for activity against hM_3D_q . The same research also identified the approved drug perlapine as a potent hM_3D_q agonist; Perlapine is approved as a hypnotic and sedative in Japan (Chen *et al*, 2015). Subsequently, both DREADD agonist 21 and perlapine have been shown to be potent agonists of hM_1D_q , hM_3D_q and hM_4D_i , with little to no off-target activity. DREADD agonist 21 has also

been tested *in vivo*, where it has been shown to activate neurons expressing hM_3D_q and inhibit the activity of neurons expressing hM_4D_i (Thompson *et al*, 2018).

Following the development of muscarinic DREADDs, an inhibitory DREADD has been developed from the κ -opioid receptor, named KORD. This inhibitory DREADD is activated by binding of the ligand Salvinator B, leading to inhibition of neuronal activity via $G_{\alpha i}$ signaling and AC inhibition. KORD can be used alongside activating DREADDs such as hM_3D_q to enable bidirectional control of neuronal activity (Vardy *et al*, 2015).

All DREADDs have some common features that make them ideal for use in neuroscience experiments. Firstly, DREADDs exhibit no response to endogenous ligands due to genetic mutations within their ligand binding sites that abolish binding. This means that any activity of the DREADD will be solely due to the specific DREADD ligand applied. Secondly, expression of DREADDs *in vitro* or *in vivo*, has no effect on cellular activity, neuronal function or baseline behaviors, prior to the addition of the DREADD ligand (Sternson & Roth, 2014).

PSAMs/PSEMs

While RASSLs and DREADDs are based on GPCRs, modified ion channels, termed Pharmacologically Selective Actuator Modules (PSAMs), have also been used to modulate neuronal activity. PSAMs are based on research demonstrating that the extracellular ligand binding domain of the $\alpha 7$ nicotinic ACh receptor (nAChR) can be transplanted onto the ion pore domain of other ligand-gated ion channels. Splicing of the $\alpha 7$ nAChR ligand binding domain with the ion pore domain of the 5-HT₃ receptor generates an ion channel with $\alpha 7$ nAChR pharmacology but 5-HT₃ cation conduction properties (Eiselé *et al*, 1993). Similarly, the $\alpha 7$ nAChR ligand binding domain has been spliced with the ion pore domain of the chloride selective glycine receptor (GlyR) to generate an ACh responsive chloride channel (Grutter *et al*, 2005). Selective mutation of the $\alpha 7$ nAChR ligand binding domain has subsequently produced PSAM ion channels, which show no ACh binding, but are selectively bound by compounds termed pharmacologically selective effector molecules (PSEMs).

Chimeric ion channels, or PSAMs, enabling control of cation or anion conductance have been generated by the combination of the mutated $\alpha 7$ nAChR ligand binding domain (harboring a single, or two mutations) with the ion pore domain of several different ligand gated ion channels. These PSAM chimeras are named according to their mutations and linked ion pore domain: PSAM^{L141F}-GlyR, PSAM^{L141F,Y115F}-GlyR, PSAM^{L141F,Y115F}-5-HT₃ and PSAM^{L141F,Y115F}-GABA_C. 5-HT₃ containing chimeras allow activation of neuronal activity, while GlyR and GABA_C containing chimeras are inhibitory (Figure 2) (Magnus *et al*, 2011; Sternson & Roth, 2014).

Further reading

Magnus *et al*. (2011) Chemical and genetic engineering of selective ligand-ion channel interactions. *Science*. **333**, 1292.

Sternson & Roth (2014) Chemogenetic tools to interrogate brain functions. *Annu Rev Neurol*. **37**, 387.

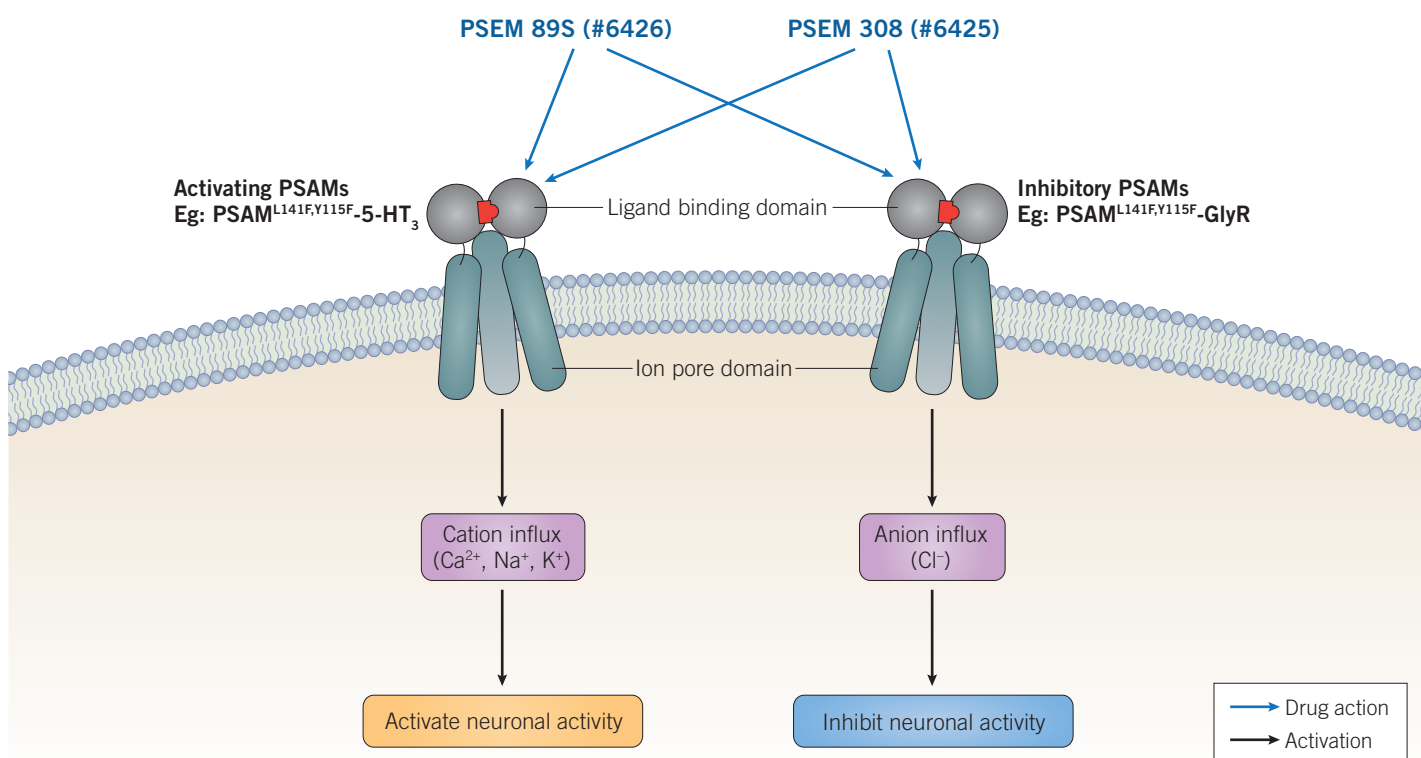


Figure 2: Mechanism of action of PSEMs. Activating PSAMs are composed of a mutated $\alpha 7$ nAChR ligand binding domain spliced with the ion pore domain of a cation selective channel, such as 5-HT₃. Binding of PSEMs to activating PSAMs results in influx of cations and activation of neuronal activity. Inhibitory PSAMs are composed of a mutated $\alpha 7$ nAChR ligand binding domain spliced with the ion pore domain of an anion selective channel, such as GlyR. Binding of PSEMs to inhibitory PSAMs results in influx of anions and inhibition of neuronal activity.

Uses of Chemogenetic Compounds in Research

DREADDs and their ligands have been utilized in a range of different neural network experiments including, but not limited to, investigation of the circuitry underlying repetitive behaviors (Rapanelli *et al*, 2017); the role of temporal cortex pathways in context-induced drug seeking behaviors (Ge *et al*, 2017); the involvement of cholinergic signaling in pain-induced anxiety (Jiang *et al*, 2018) and the role of orexigenic neurons in the regulation of the sleep/wake cycle (Sasaki *et al*, 2011). Additionally, DREADDs allow for the interrogation of GPCR signaling, to further understanding of the physiological roles of GPCRs. DREADDs can also be used to investigate different aspects of GPCR signaling, such as downstream pathways and biased agonism/functional selectivity (Bradley *et al*, 2018; Bradley & Tobin, 2016).

In their paper outlining the development of KORD, Vardy *et al* also showed that DREADDs can be multiplexed to allow bidirectional control over neuronal activity. They co-expressed KORD and hM₃D_q in neurons expressing the vesicular GABA transporter (VGAT), specifically in the ventral tegmental area (VTA) (VTA^{VGAT} neurons). Previous optogenetic investigations have shown that manipulations of VTA^{VGAT} neuron activity are able to modify locomotion. Application of CNO alone reduced

distance travelled, as an indicator of locomotor activity, in mice expressing KORD and hM₃D_q, however, this was reversed when Salvinorin B was given (Figure 3). This paper demonstrated, for the first time, that two different DREADDs and their ligands can be used with the same mouse for bidirectional control of behavior (Vardy *et al*, 2015).

PSAMs and PSEMs have also been used in similar investigations into neuronal circuits and the behaviors they control. For example, Atasoy *et al* have used PSAM^{L141F}-GlyR with PSEM308 to investigate pathways responsible for hunger signals. Agouti related protein (AGRP)-expressing neurons in the hypothalamic arcuate nucleus (ARC) are sensory neurons that are activated by circulating hormones, such as ghrelin. The paraventricular nucleus (PVN) of the hypothalamus receives AGRP neuronal inputs from ARC and has an established role in feeding behavior, however its role in the short term (< 1 hour) feeding evoked by AGRP neuron activation is unclear. By silencing neurons of the PVN with PSAM^{L141F}-GlyR and PSEM308, Atasoy *et al* showed rapid induction of feeding, confirming a role for PVN inhibition in AGRP neuron-evoked feeding behavior (Atasoy *et al*, 2012).

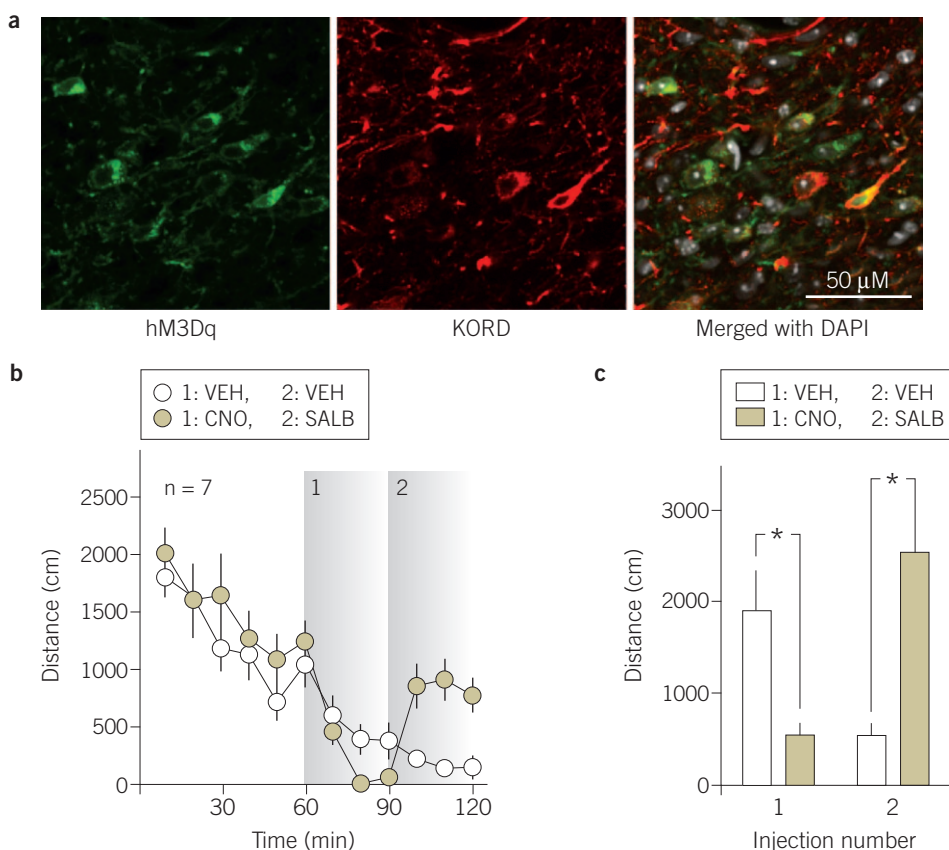


Figure 3: Effect of bidirectional chemogenetic manipulation of VTA^{VGAT} neurons. A) Representative immunofluorescent images show co-expression of hM₃D_q and KORD in VTA^{VGAT} neurons. B) Injection of CNO (at 60 mins) inhibits locomotor activity. This effect is reversed by Salvinorin B injection (SALB at 90 mins). C) DREADD ligand administration has a significant effect on locomotor activity, when compared to vehicle treated mice at the same time point. * $p < 0.05$. Adapted from Vardy *et al*, 2015.

In their paper from 2017, Schwartz *et al* used hM_4D_i to investigate the neuronal circuitry that regulates choice during approach-avoidance behavior (Schwartz *et al*, 2017). Following recording of neuronal activity in the infralimbic cortex (IL) and nucleus accumbens (NAc), this pathway was disrupted by selective expression of hM_4D_i and CNO administration. In a pain-predictive cue (PPC) avoidance task, chemogenetic or pharmacological inactivation of IL resulted in reinstatement of PPC-avoidance (Figure 2). This establishes a role for IL-NAc circuits in regulating approach-avoidance behavior in a PPC avoidance task (Schwartz *et al*, 2017).

Inhibitory DREADDs have also been used in the investigation of memory processes. Varela *et al* utilized hM_4D_i and CNO

to study the involvement of the hippocampus in retrieval of remote memories (Varela *et al*, 2016). The hippocampus is critical for memory storage; following encoding in the hippocampus, memories undergo system level consolidation and over time they develop greater stability and increased reliance on neocortical areas for retrieval. Using hM_4D_i and CNO to inactivate the entire hippocampus during contextual fear conditioning in mice, Varela *et al* showed remote, but not recently encoded memories, can be recalled after CNO administration (Figure 3). This indicates a time-dependent role for the hippocampus in contextual memory retrieval (Varela *et al*, 2016).

Life Science Literature from Tocris

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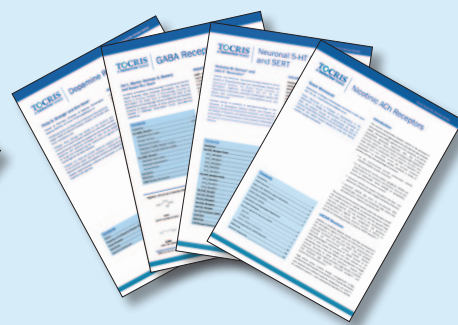
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Chemogenetic Compounds Available from Tocris

Category	Product Name	Cat. No.	Primary Action
Adenylyl Cyclase			
	Forskolin	1099	Adenylyl cyclase activator
	SQ 22536	1435	Adenylyl cyclase inhibitor
cAMP			
	cAMPS-Rp	1337	cAMP antagonist
	Dibutyryl-cAMP	1141	Cell-permeable cAMP analog
DREADD ligands			
	Clozapine <i>N</i> -Oxide	4936	Activator of muscarinic DREADDs
	Clozapine <i>N</i> -Oxide dihydrochloride	6329	Activator of muscarinic DREADDs; water soluble version of Clozapine <i>N</i> -Oxide (Cat. No. 4936)
	DREADD agonist 21	5548	Potent activator of muscarinic DREADDs
	DREADD agonist 21 hydrochloride	6422	Potent muscarinic DREADD agonist; water soluble version of DREADD agonist 21 (Cat. No. 5548)
	Perlapine	5549	Potent muscarinic DREADD agonist
	Salvinorin B	5611	Activates the κ -opioid DREADD (KORD)
G Protein (Heterotrimeric)			
	ESI 09	4773	EPAC inhibitor
	Gallein	3090	Inhibitor of $\beta\gamma$ signaling
	Melittin	1193	Inhibits G_s and stimulates G_i activity
	NF 023	1240	Selectively inhibits α -subunit of $G_{\alpha i}$
Inositol Lipids			
	2-APB	1224	IP_3 receptor antagonist. Also TRP channel modulator
Phospholipase C			
	<i>m</i> -3M3FBS	1941	Phospholipase C activator
	U 73122	1268	Phospholipase C inhibitor
PSEMs			
	PSEM 89S	6426	PSAM ^{L141F} -GlyR and PSAM ^{L141F,Y115F} -5-HT ₃ chimeric ion channel agonist
	PSEM 308	6425	PSAM ^{L141F} -GlyR and PSAM ^{L141F,Y115F} -5-HT ₃ chimeric ion channel agonist

Plasmid vectors for the transfection of cells with DREADDs and PSAMs are available from Addgene, www.addgene.org/chemogenetics.

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