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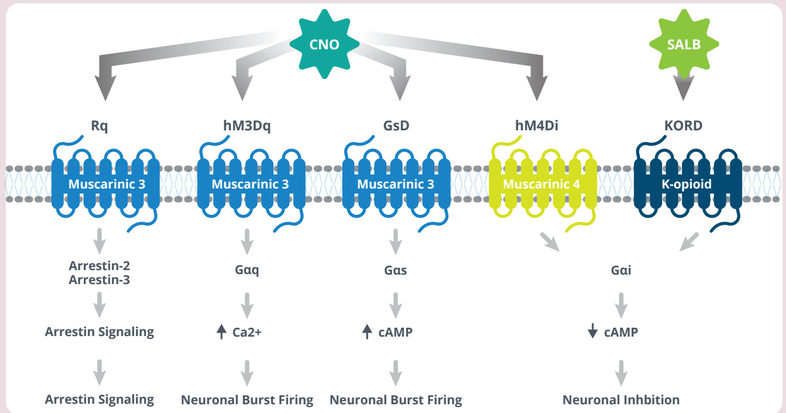
Chemogenetics Toolbox

To understand the brain, it's critical to be able to control signalling in specific neuronal and non-neuronal cells. Chemogenetics--a method by which proteins are controlled with small molecule chemical actuators--is used in neuroscience to noninvasively control neural circuits, enabling neuroscientists to identify how these circuits specify behavior. Designer receptors exclusively activated by designer drugs (DREADD) and pharmacologically selective actuator module (PSAM) are two classes of chemogenetic receptors and both are typically introduced into cells through viral delivery using AAV vectors. The specific cell targeting and expression patterns of chemogenetic receptors can be modified by using different AAV vector serotypes and gene promoters.

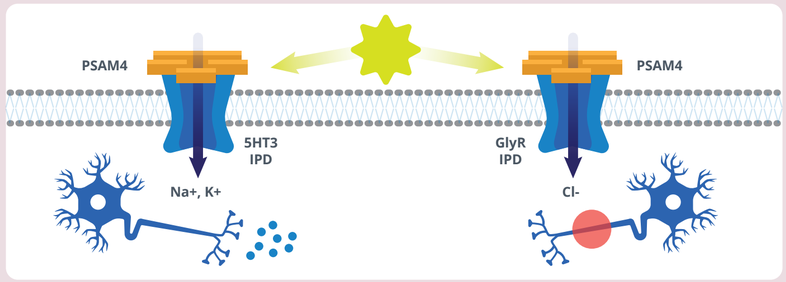
Receptors

There are many classes of chemogenetic tools. The two specific types of engineered chemogenetic receptors described here are both activated by different ligands and signal through different pathways. These chemogenetic receptors represent noninvasive and nontoxic methods for selectively activating or inhibiting brain activity.

Designer receptors exclusively activated by designer drugs (DREADD) are modified G-protein-coupled receptors (GPCRs) that are activated by inert chemical ligands. These receptors were reverse engineered to respond preferentially to specific ligands, which can thus be used to recapitulate the signalling output of the naturally-occurring receptors. The different DREADD receptors signal through specific intrinsic G-protein signaling pathways, enabling either neuronal activation or inhibition, as well as β-arrestin signaling.



Pharmacologically selective actuator module (PSAM) is a modified ligand binding domain that responds to nanomolar concentrations of FDA-approved drugs. When this receptor is coupled to various ion-pore domains, the resulting chimeric protein is a type of modular receptor that can control brain activity through ligand-gated ion channels. Based on the identity of the ion channel, activation of these complexes results in neuronal inhibition or activation. PSAM complexes can be used to investigate neuronal activity and behavior. [7]



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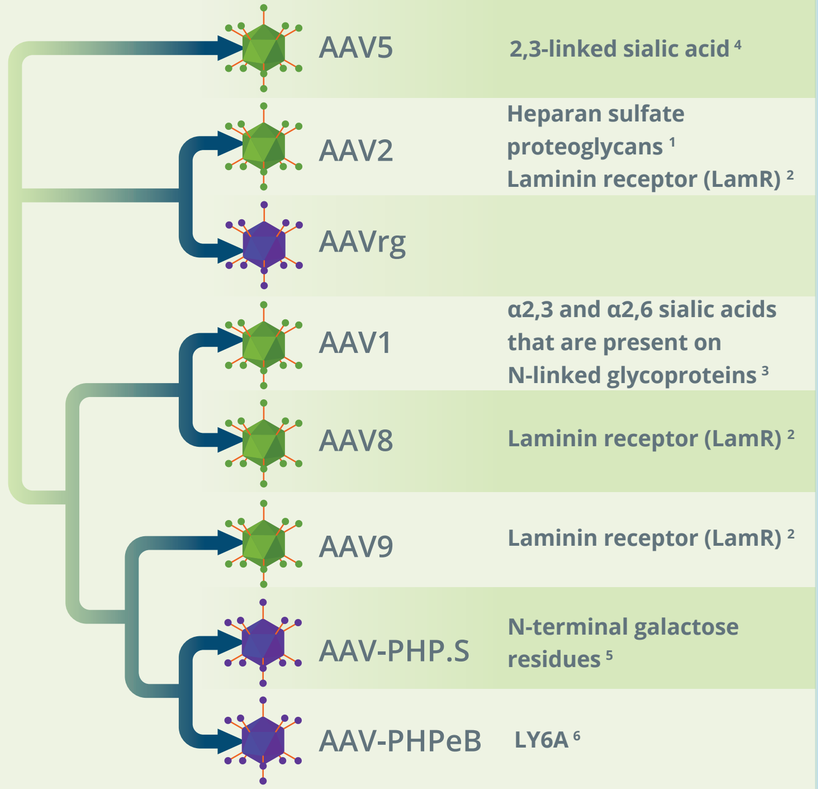
Serotypes

AAV entry into cells is mediated by the interaction between viral capsid proteins and protein receptors on cells. Through this mechanism, different AAV serotypes can lead to different patterns of gene expression. Choosing the viral serotype is an important step in experimental design, and can be used to target AAV vectors to specific cells of interest. In addition, protein-protein interactions dictate the degree of viral spread within injected tissue, which can further impact gene expression patterns. Understanding how closely related AAV capsid proteins are can give insight into the tropism of different AAV serotypes. Here, we use protein homology to show the relationship between several naturally-occurring and engineered AAV capsids.

- Engineered Serotype
- Naturally-Occuring Serotype



Sequences from Addgene plasmids 112862, 104963, 104964, 112864, 112865, 81070, 103005, and 103006 were used to generate this figure. Geneious Prime 2019.1.3. (https://www.geneious.com)

Promoters

Promoters can direct either cell type-specific expression or general, broad and ubiquitous expression. General promoters typically drive higher expression levels than cell type-specific promoters. For longer promoters, fragments or minimal binding domains are preferred over the full-length promoter because they use less space in the limited AAV genome. Enhancers are also used to direct specific expression patterns to specific groups of cells.

Promoter	Description	Tropism	Length
hSyn1	Human synapsin 1 promoter fragment	Neurons	495bp [8]
CaMKIIa	Ca2+/calmodulin-dependent protein kinase II promoter	Neurons	400bp [9] [10]
GFAP	Glial fibrillary acidic protein gene promoter	Glia	681bp [11]
CD68	Human CD68 promoter	Microglia	666bp [12]
EF1a	Human elongation factor 1 alpha promoter	General expression	1200bp [13]
CAG	Strong hybrid mammalian promoter	General expression	1300bp [14]

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