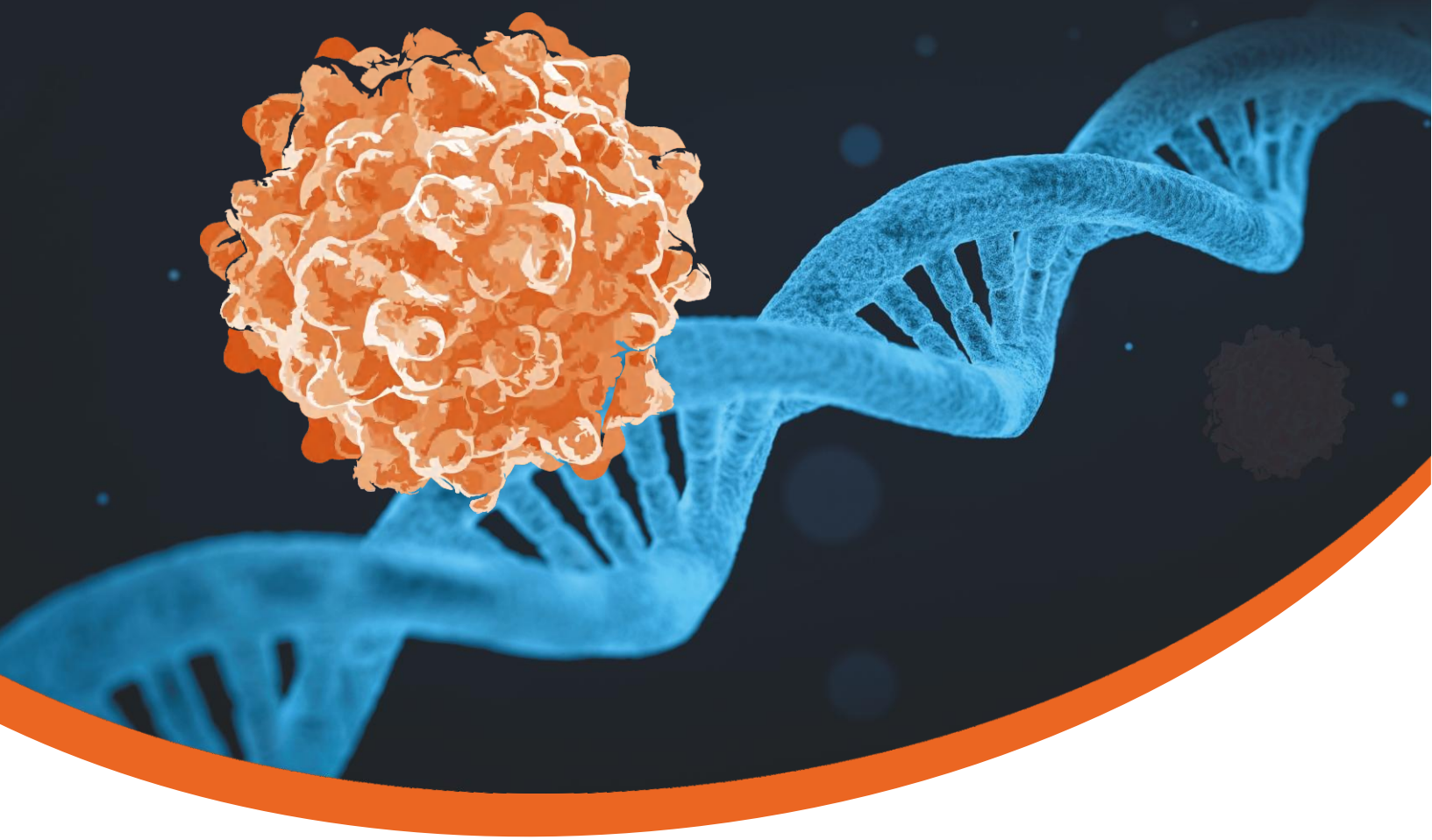
BrainVTA | Genevoyager



Applications of rAAV in Neuroscience Research

Adeno-Associated Virus

What are AAV, rAAV, and scAAV?

Adeno-associated virus, AAV

Adeno-associated virus (AAV) is a member of the Parvoviridae family, and is a small non-enveloped icosahedral virus. It cannot replicate independently. Its diameter is approximately 20-26 nm, and carries a linear single-stranded DNA genome of around 4.7 kb.

Recombinant AAV, rAAV

rAAV is a gene vector whose construction is based on the non-pathogenic wild-type AAV. In research, the commonly used rAAV is typically a chimeric form resulting from the combination of the AAV2 genome with various capsid proteins. This combined rAAV is denoted as rAAV2/x, where x represents different serotypes of capsid proteins.

Self-complementary AAV, scAAV

scAAV is built upon the foundation of rAAV by designing its coding region as double-stranded DNA. After infecting cells, the complementary segments of scAAV form double-stranded DNA without waiting for the synthesis of the second DNA strand. This unique feature allows scAAV to reach its expression peak within 3-5 days after infection. However, the double-stranded DNA structure of scAAV also leads to a reduced capacity of approximately 2.1 kb, making it suitable for rapid expression of smaller gene fragments.

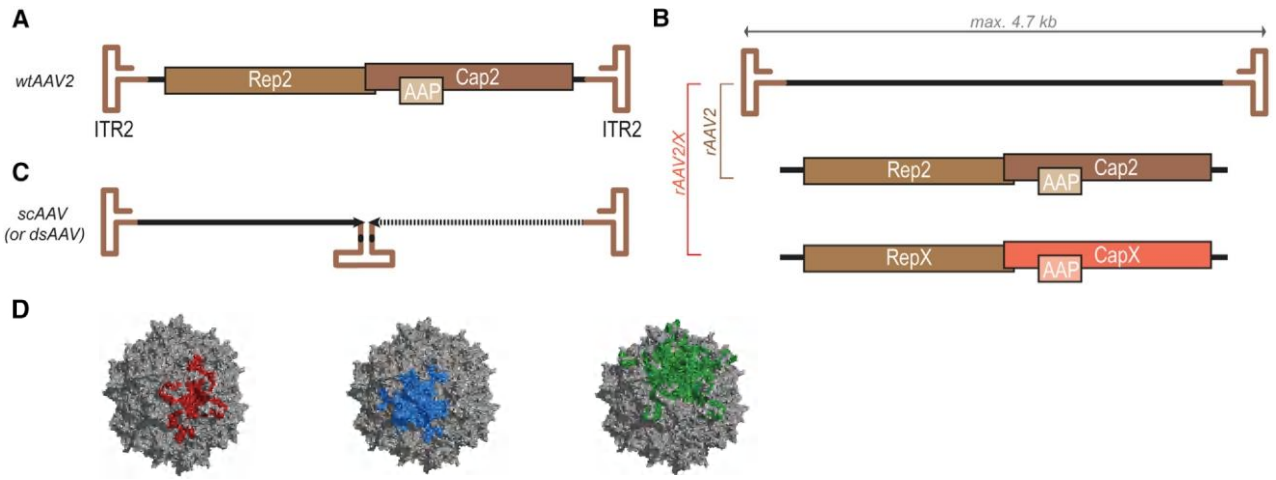


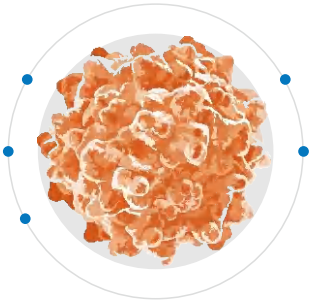
Figure 1. AAV structure

Characteristics of rAAV Vectors

High safety and low immunogenicity

Broad host cell range

Diverse serotypes suitable for various research and applications



Strong diffusion capability

rAAV vectors reach their expression peak in about three weeks, scAAV vectors reach their expression peak in about 3-5 days

Table 1. Partial list of AAV serotypes

AAV Serotype	Tissue Tropism
AAV2/1	CNS (high-titer anterograde transsynaptic), skeletal muscle, cardiac muscle, smooth muscle, retina, vascular endothelial cells
AAV2/2	Retina, central nervous system, liver, vascular smooth muscle, skin, inner ear tissues, kidney
AAV-7m8	Retina
AAV1/2 (Cap1 and Cap2 1:1 mixed serotype)	Oligodendrocytes
AAV-olig001	Oligodendrocytes
AAV2/5	Central nervous system, lungs, retina, liver, synovial joints, smooth muscle
AAV2/7	Muscle, liver
AAV2/6	Nervous system, lungs, muscle, heart
AAV-6m	Microglia
AAV-MG1.2	Microglia
AAV-6.2	Lungs
AAV-6.2FF	Lungs
AAV2/8	Central nervous system, liver, kidney, muscle, adipose tissue, pancreas, retina
AAV-Rec2	Adipose tissue
AAV2/9	Central nervous system, cardiac muscle, retina, skin, lungs
AAV2/retro	Nervous system (retrograde, non-transsynaptic)
AAV-PHP.eB	Blood-brain barrier crossing
AAV-B10	Blood-brain barrier crossing
AAV-PHP.S	Spinal cord, cardiac ganglia, enteric nervous system
AAV-PAN	Pancreas (intraperitoneal injection)
AAV-DJ	Retina, lungs, kidney, liver, in vitro infected cells

AAV Serotype	Tissue Tropism
AAV-ShH10	Retina, Müller glial cells
AAV-Rh10	Lungs, liver, heart, central nervous system, blood, in vitro infected cells
AAV-Anc80L65	Inner ear, retina, skeletal muscle, liver
AAV-BR1	Brain microvascular endothelial cells
AAV-BI30	Vascular endothelial cells
AAV-Ark313	T cells
AAV-SCH9	Subventricular zone (SVZ) neural stem cells
AAV-r3.45	Neural stem cells from mice, rats, and humans
AAV-MaCPNS1/ AAV-MaCPNS2	Peripheral nervous system of rodents (mice and rats), central and peripheral nervous system of non-human primates (rhesus macaques and marmosets)
AAV-Mac	Whole brain of non-human primates (rhesus macaques, grivets, and marmosets)
AAV-DSS	Bone
AAV-EC71	Cardiac vascular endothelial cells

Note There may be variations in tissue tropism for the same serotype across different animals and injection routes. This information from publications is provided for reference only.

rAAV in Gene Expression Regulation

Gene Over-expression

Conventional overexpression: The coding region of the target gene is inserted into the rAAV vector, and gene over-expression is achieved by infecting cells with the virus.

Endogenous gene overexpression: Also known as CRISPRa, endogenous gene over-expression involves utilizing dCas9 technology to achieve overexpression of endogenous genes (transcriptional activation).

Gene Knockdown

RNA Interference: Refers to the highly conserved phenomenon in the evolutionary process where homologous mRNA is efficiently and specifically degraded by double-stranded RNA. Through RNAi technology, it is possible to selectively eliminate or suppress the expression of specific genes, offering advantages such as specificity and high efficiency.

CRISPRi: Utilizing dCas9, CRISPRi achieves transcriptional down-regulation at the DNA level (endogenous gene transcriptional inhibition).

Gene Knockout

Conditional gene knockout: By utilizing a rAAV viral vector carrying a specific promoter and Cre enzyme, injecting it into a specific brain region of floxed mice can achieve the targeted gene knockout in a specific brain area.

Advantages

- Manipulation of genes in specific cell types
- Achieves regional specificity
- Enables postnatal knockout, minimizing compensation issues
- Relatively easy virus production and injection
- Short timelines, low costs

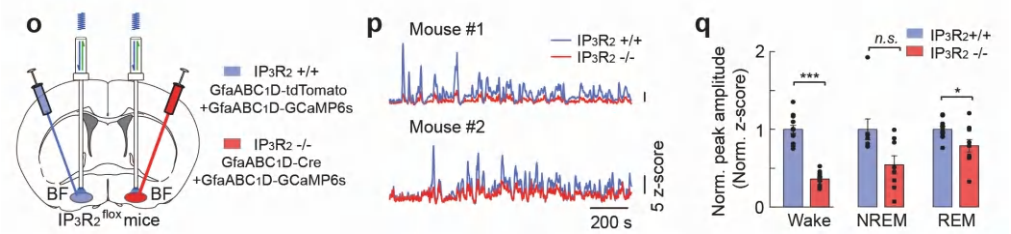


Figure 2. IP_3R_2 knockout (KO) reduced astrocytic Ca^{2+} activity during wakefulness and REM sleep

Injecting AAV2/5-GfaABC1D-Cre into the basal forebrain (BF) of $IP_3R_2^{flox}$ mice resulted in specific astrocytic knockout of IP_3R_2, leading to reduced Ca^{2+} activity during both wakefulness and rapid eye movement (REM) sleep (client's article, IF=38.079, Peng W, Liu X, Ma G, et al. Cell Discov. 2023).

CRISPR/Cas9 gene knockout:

Utilizing Cas9-sgRNA to induce double-strand breaks in DNA, cells may subsequently repair the DNA through non-homologous end joining (NHEJ), leading to gene mutations in the cleaved region and resulting in the knockout of the target gene.

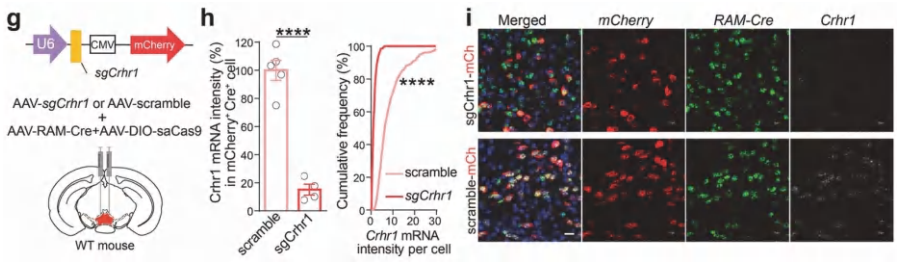


Figure 3. CRISPR-Cas9-mediated specific knockout of Crhr1 in VTA's Mor-Ens

Researchers infected mice' s VTA (ventral tegmental area) with AAV-sgCrhr1-mCherry, AAV-RAM-tTA-TRE-Cre, and Cre-dependent AAV-DIO-saCas9. Single-molecule fluorescence in situ hybridization experiments targeting Crhr1 indicated specific knockout of Crhr1 in the Mu-Opioid Receptor-Expressing Neuron Cluster (Mor-Ens) upon morphine activation (client' s article, IF=15.992, Jiang C, Yang X, He G, et al. Mol Psychiatry. 2021).

DIO/DO System

Built upon the principles of the Cre-loxP system, it enables specific cell gene manipulation.

DIO

Namely Cre-on, where gene expression occurs in Cre-positive cells.

DO

Namely Cre-off, where gene expression occurs in Cre-negative cells, while Cre-positive cells do not express the gene.

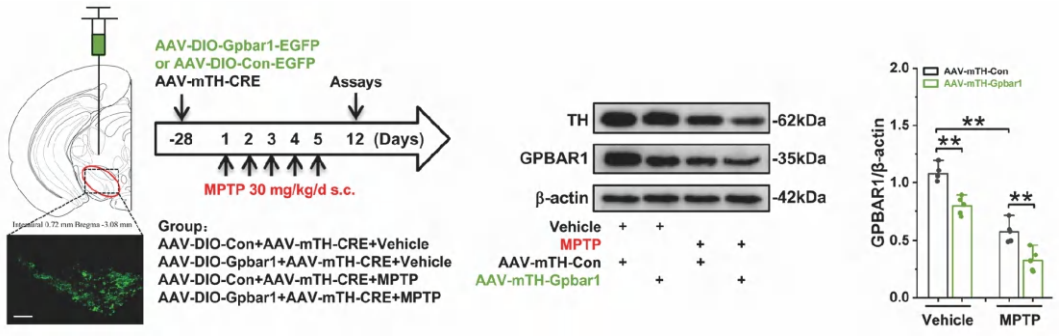


Figure 4. Gpbar1 knockout in DA neurons (modified)

Researchers injected a mixture of rAAV2/9-mTH-NLS-CRE and rAAV2/9-CMV-DIO-EGFP-shRNA (Gpbar1) in a 1:1 volume ratio into the mouse midbrain, achieving specific knockdown of Gpbar1 in midbrain dopamine (DA) neurons (client' s article, IF=10.334, Zhang Y, Sun X, Zhang Y, et al. Pharmacol Res. 2022).

Inducible Expression System

CreERT2 system (Tamoxifen-inducible): Cre recombinase can induce gene recombination only in the presence of Tamoxifen, allowing temporal control of gene recombination by regulating the timing of Tamoxifen administration.

Tet-on/Tet-off system (Doxycycline-inducible): In this system, gene expression requires the binding of tTA or rtTA to the Tet-operon (TRE promoter).

Tet-on System

Upon administration of Doxycycline (Dox) to animals, the complex formed by Dox and rtTA initiates gene expression.

Tet-off System

Under physiological conditions, tTA actively binds to the TRE promoter; however, if cells are exposed to Dox, gene expression is prevented.

Apoptotic System

taCasp3-TEVp: Caspase-3 (Casp3) protein is a key molecule in the apoptotic pathway, and its activation involves protein cleavage. TEVp is a specific serine protease derived from Tobacco Etch Virus, capable of cleaving Casp3 in mammalian cells, thereby activating Casp3 and triggering a cascade of reactions leading to apoptosis.

DTA: Diphtheria Toxin A subunit is the A subunit of the diphtheria toxin, which can inhibit the synthesis of eEF-2 and induce cell death.

Inhibition System

TetTox (TeNT): The light chain of Tetanus toxin, which acts as a protease to specifically degrade VAMP2, inhibiting synaptic glutamate vesicle release and interrupting neural signal transmission.

Kir2.1: An inward rectifying potassium ion channel; overexpression of Kir2.1 leads to cellular hyperpolarization, reducing neuronal activity.

iβARK: A 122-residue inhibitory peptide derived from β-adrenergic receptor kinase 1, selectively blocking Gαq GTP activation, and inhibiting Ca^{2+} signaling mediated by the Gq-GPCR pathway in astrocytes, thus affecting mouse behavioral activity.

Activation System

NaChBac: A bacterial voltage-gated sodium ion channel. Cellular overexpression of NaChBac can activate neuronal activity, enhancing neuronal excitability.

rAAV in Circuit Function Research

Optogenetics

Optogenetics is a technology that combines optics and genetics to precisely control the activity of specific types of neurons in live animals, including freely moving animals, within the brain, spinal cord, and peripheral nerves.

Table 2. Common photosensitive protein elements

Function	Photosensitive Protein	Excitation Wavelength (nm)	Properties
Optogenetic activation	ChR2	470	Wild-type photosensitive protein
	ChR2 (H134R)	470	Mutant of ChR2, with channel opening speed half as fast as wild-type ChR2
	ChETA	470-490	ChR2 mutant with an open time of only 4ms, allowing for high-frequency stimulation at 200Hz in certain neurons
	ChR2 (E123T/T159C)	470	Inward current stronger than ChETA, inward current similar to hChR2 (H134R), faster channel closing speed, and increased sensitivity of the channel to changes in light signals
	ChR2 (C128S/D156A)	470 (activ) 590 (inact)	Also known as SSFO, activated by blue light, the channel can remain open for up to 30 minutes and can be rapidly closed by subsequent exposure to yellow light
	ChIEF	470	Less inactivation of the channel under light exposure, suitable for prolonged stimulation
	C1V1(E162T)	540	Channels can be opened using 540-580 nm laser, facilitating two-photon excitation
	ReaChR	590	Excitation wavelength of 590-630 nm, where red light has greater penetration ability in tissues, allowing manipulation of larger and deeper tissue areas
	ChrimsonR	590	Faster channel closing speed, suitable for high-frequency stimulation scenarios
	Chronos	470	Faster channel opening and closing speeds, capable of operating at a frequency of at least 40Hz

Function	Photosensitive Protein	Excitation Wavelength (nm)	Properties
Optogenetic inhibition	Arch	570	Activation by yellow light around 570 nm, can inhibit almost all electrophysiological activity
	NpHR3.0	570	Typically driven by light around 570 nm, with short response times and high sensitivity, making it possible to be used simultaneously with hChR2 (H134R)
	jaws	632	Red light has better tissue penetration, allowing manipulation of larger and deeper tissue areas
	GtACR1	515	Suitable for situations with short light exposure times and brief behavioral effects
	GtACR2	470	Blue light inhibition with faster kinetics as compared to GtACR1

Chemogenetics

DREADDs is a technology that involves altering the structure of G-protein coupled receptors, specifically acetylcholine receptors. After modification, these receptors can only be activated or inhibited by a specific compound called Clozapine-N-oxide (CNO). Such modified receptors selectively influence different GPCR-coupled signaling pathways, including Gq, Gi, Gs, Golf, and β-arrestin. Among these, the most widely used variants are Gq-DREADD (hM3Dq) and Gi-DREADD (hM4Di).

Table 3. Common chemogenetics elements

Name	Description
hM3D(Gq)	Activation element
hM4D(Gi)	Inhibition element
KORD	Inhibition element
CNO	Regulatory compound

Calcium Imaging

A method used to monitor changes in calcium ion concentrations within neurons, which serves as an indicator of neuronal activity. In practical research, this involves utilizing viral vectors to express calcium indicators like GCaMP within the animal's brain. Once expression is achieved, techniques such as fiber-optic recording, two-photon microscopy, or endoscopy are employed to observe neuronal activity in specific brain regions. Animals are often examined to study changes in cellular activity associated with tasks and population-level neuronal responses.

Table 4. Common calcium indicators

Category	Name	Characteristics	Function
GCaMP6	GCaMP6s	High sensitivity	Suitable for indicating low-frequency signals
	GCaMP6f	Rapid kinetics	Fastest dissociation, suitable for indicating high-frequency signals
	GCaMP6m	Moderate activity	The widest applicable range
jGCaMP8	GCaMP8s	High sensitivity	Higher sensitivity than GCaMP7s
	GCaMP8f	Rapid kinetics	Response rate is 4 times that of jGCaMP7f, with a decay time 2.6 times that of jGCaMP7f
	GCaMP8m	Moderate activity	Response rate is 4 times that of jGCaMP7m, with a decay time 3.5 times that of jGCaMP7m
Red-shifted calcium imaging	jRGECO1a	Red-shifted calcium imaging	Excitation wavelength is red-shifted, allowing for co-use with green GCaMP
	jRCaMP1a	Red-shifted calcium imaging	Excitation wavelength is red-shifted, allowing for co-use with green GCaMP
	jRCaMP1b	Red-shifted calcium imaging	Excitation wavelength is red-shifted, allowing for co-use with green GCaMP
GCaMP-X	GCaMP6m-XC	Non-invasive calcium ion sensor	Cytoplasmic localization, not concentrated in the nucleus, suitable for long-term (≥4 weeks) calcium signal detection
	GCaMP6m-Xn	Non-invasive calcium ion sensor	Nuclear localization
XCaMPs	XCaMPs	Four-color GCaMP sensors	XCaMP-Y, XCaMP-B, XCaMP-R, XCaMP-G, enabling simultaneous detection of activities in different neuron types
CaMPARI	CaMPARI	Convert green to red	Under ultraviolet and high-calcium conditions, a permanent and irreversible shift from green to red fluorescence occurs

Neurotransmitter Sensors

Li Yulong' s lab from Peking University develops cutting-edge research tools, namely advanced imaging probes (GRAB sensors), and deposited sensor plasmids at BrainVTA for sharing with the research community. All GRAB sensors (including ACh, DA, NE, 5-HT, Ado, ATP, VIP, CCK and eCB) are available!

Table 5. Features of published sensors

Name	Neuro-transmitters	Version	Color	Backbones (From human)	Affinity	Signal Response Amplitude	Dynamics	Downstream Signal Coupling
Ach3.0	Acetylcholine	Second generation	Green	M3 receptor	EC50~2μM	ΔF/F0~280%	τon~112ms, τoff~580ms	Hardly
Ach3.0-mut	Acetylcholine	Control of Second generation	Green	M3 receptor	EC50~0μM (W200A mutation)	ΔF/F0~1.8%	/	/
DA2m (DA4.4)	Dopamine	Second generatio	Green	D2 receptor	EC50~90nM Medium affinity	ΔF/F0~340%	τon~40ms, τoff~1300ms	Little
DA2h (DA4.3)	Dopamine	Second generatio	Green	D2 receptor	EC50~7nM High affinity	ΔF/F0~280%	τon~50ms, τoff~7300ms	Little
DAmut (2nd)	Dopamine	Control of Second generation	Green	D2 receptor	EC50~0μM ($C118^{3.36}$A and $S193^{5.42}$N mutation)	No effect	/	/
rDA1m (rDA2.5m)	Dopamine	/	Red	D2 receptor	EC50~95nM Medium affinity	ΔF/F0~150%	τon~80ms, τoff~770ms	Little
rDA1h (rDA2.5h)	Dopamine	/	Red	D2 receptor	EC50~4nM Medium affinity	ΔF/F0~100%	τon~60ms, τoff~2150ms	Little
rDAmut (rDA2.5 mut)	Dopamine	Control	Red	D2 receptor	EC50~0μM ($C118^{3.36}$A and $S193^{5.42}$N mutation)	No effect	/	/
NE1m (NE2.1)	Norepinephrine	/	Green	a2A receptor	EC50~930nM Medium affinity	ΔF/F0~230%	τon ~70ms, τoff~ 750ms	Uncoupled
NE1h (NE2.2)	Norepinephrine	/	Green	a2A receptor	EC50~83nM High affinity	ΔF/F0~130%	τon ~30ms, τoff~ 2000ms	Uncoupled
NEmut	Norepinephrine	Control	Green	a2A receptor	EC50~0μM (S5.46A mutation)	No effect	/	/
Ado1.0	Adenosine	/	Green	a2A receptor	EC50~60nM	ΔF/F0~130%	τon~36ms, τoff~1890ms	Hardly
Ado1.0 mut	Adenosine	Control	Green	a2A receptor	EC50~0μM (F168A mutation)	No effect	/	/
5-HT1.0	Serotonin	/	Green	5-HT2C receptor	EC50~22nM	ΔF/F0~250%	τon~0.2s, τoff~3.1s	Uncoupled
5-HTmut	Serotonin	Control	Green	5-HT2C receptor	EC50~0μM ($D134^{3.32}$Q mutation)	No effect	/	/

rAAV in Circuit Tracing

Anterograde Trans-monosynaptic Labeling

rAAV2/1 vectors with a titer of 10^{13} possess the ability for anterograde trans-monosynaptic tracing. It is commonly amplified with recombinase (Cre/Flp) system to initiate robust transgene expression.

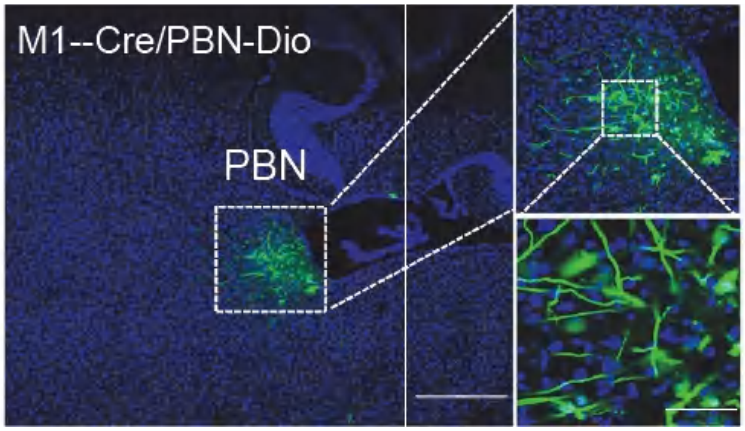


Figure 5. Projection from M1 to PBN

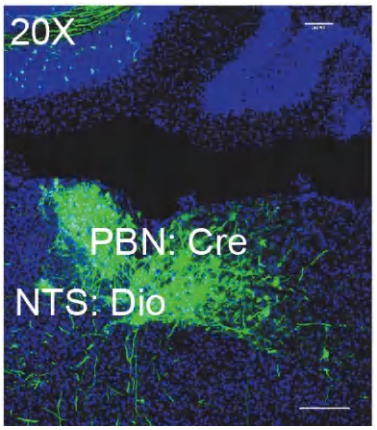


Figure 6. Projection from PBN to NTS

The researchers injected anterograde trans-monosynaptic AAV (AAV2/1-hSyn-Cre) into M1 (primary motor cortex) and AAV2/9-CAG-Dio-EGFP into PBN (parabrachial nucleus). The results demonstrated the presence of EGFP-labeled neurons in the PBN, confirming monosynaptic connections between M1 and PBN (*Figure 5, client' s article, IF=17.694, Yao L, Ye Q, Liu Y, et al. Nat Commun. 2023*).

The researchers injected anterograde trans-monosynaptic AAV (AAV2/1-hSyn-Cre) into the PBN and AAV2/9-CAG-Dio-EGFP into the NTS (nucleus of the solitary tract). The results demonstrated the presence of EGFP-labeled neurons in the NTS, confirming monosynaptic connections between the PBN and NTS. This suggests a potential regulatory influence of the PBN on the NTS (*Figure 6, client' s article, IF=17.694, Yao L, Ye Q, Liu Y, et al. Nat Commun. 2023*).

Retrograde Non-transsynaptic Labeling

rAAV2/retro is a commonly used retrograde non-transsynaptic viral vector. The viral particles are taken up at axon terminals and transported in a retrograde manner along the cellular scaffolding to the nucleus for expression.

WGA-based Transsynaptic Labeling

WGA binding with recombinase and fluorescent proteins is used for anterograde and retrograde tracing.

Whole-brain Labeling

AAV-PHP.eB

In mice, tail vein injection with a viral titer of 1E+11 vg achieves blood-brain barrier crossing and results in widespread brain expression (infecting 69% of cortical neurons and 55% of striatal neurons).

AAV-CAP.B10

In mice, tail vein injection with a viral titer of 1E+11 vg achieves targeted neuronal expression across the entire brain while exhibiting low liver targeting. Intravenous injection in non-human primates also achieves blood-brain barrier crossing and widespread brain expression.

Peripheral Labeling

AAV-PHP.S

In mice, tail vein injection with a viral titer of 1E+12 vg achieves whole peripheral nervous system expression across the blood-brain barrier (infecting the spinal cord, cardiac ganglia, and enteric nervous system).

AAV-MaCPNS1/2

In mice, tail vein injection with a viral titer of 3E+11 vg shows better expression in the peripheral nervous system as compared with the same dose of AAV-PHP.S. Intravenous injection in non-human primates achieves both peripheral and central nervous system expressions.

Sparse Labeling

Based on the single virus system

VSV, SFV, RV and CAV have a high efficiency of infection. A small amount of infection can express an abundance of fluorescent protein. Therefore, rapid, sparse and bright cell labeling of the complete morphology of local neurons can be achieved by reducing the viral titer. These tools enable fast bright cell labeling but is cytotoxic, so it is suitable for sparse labeling of local dendrites but not for long-term projection.

Based on Cre/FLP recombinase

This method controls the number of bright neurons in the target area between 10-50 by adjusting the injection volume of the virus. The cells labeled with this method are exquisitely shaped and can trace the morphology of the whole brain to a certain extent.

Based on recombinase system-dependent copackaging strategy

The recombinase system-dependent (including Cre-loxp/Flp-FRT) viral copackaging strategy improves mutual inhibition and enhances compatibility among different rAAVs. Additionally, the author found that it was ~5-fold and over 10-fold more sensitive than the mixture of independently packaged rAAVs, enabling us to capture massive morphological details of individual neurons.

Subcellular Localization

The fusion expression of a signal peptide with fluorescence tags or target genes, which possess intracellular localization functions, allows the gene to be expressed at specific locations guided by the signal peptide.

Table 6. Subcellular localization elements

Subcellular /Targeting Element	Description
H2B/his	Nuclear localization
NLS	Nuclear localization
soma	Cellular localization
NES	Cytoplasmic localization
LCK	Membrane localization
m	Membrane localization
mito	Mitochondrial localization
axon	Axonal localization
nrxn1a rev	Axonal localization
Synaptophysin	Presynaptic localization
PSD-95	Excitatory synaptic localization
Pre-mGRASP	Presynaptic localization
Post-mGRASP	Postsynaptic localization

RV/HSV helper System

AAV-helper expresses RVG and TVA, assisting in retrograde trans-monosynaptic labeling with RV.

AAV-TK assist HSV progeny virus transport to the synapse, and then crosses the synapse into the downstream neuron.

Shipping, Storage, and Dilution

rAAV Transportation

Virus is shipped with dry ice and typically arrives within 1-2 days after shipment.

rAAV Storage

- 4°C for short-term storage and -80°C for long-term storage; each freeze-thaw cycle reduces virus titer by 10%, repeated freeze-thaws should be avoided.
- When storing the virus at -80°C for more than 6 months, it is recommended to reevaluate the viral titer or conduct pre-experimental tests of product activity before use.

rAAV Dilution

- Begin by thawing the rAAV at 4°C and then proceed with dilution.
- Saline solution or PBS can be used for dilution.
- Use the diluted virus immediately after dilution; storing the diluted solution is not recommended.

About Us

BrainVTA (Wuhan) Co., Ltd. was established in 2014 in Wuhan Optics Valley, China. Since its establishment 9 years ago, BrainVTA has been dedicated to virus vector technology and translational medical research services. We have provided high-quality, efficient, and cutting-edge viral tools and technical support services to over 200 pharmaceutical companies, 600 top research groups, and 3,000+ scientific researchers both at home and abroad. As a result, our clients have published over 900 high-quality articles. Our high-quality viral vector products and experimental research services are available in translational medicine fields such as neuroscience, neurological diseases, gene function research, genetic diseases, gene therapy, tumors, and stem cell therapy, etc., and have gained high recognition from frontline researchers.

BrainVTA

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