Titratable control of neuronal activity using precision genetic neuromodulation

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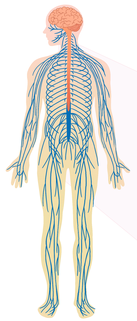
SANIA THERAPEUTICS

Sania pioneers ways to selectively target and safely control neural circuits as an access point to treating prevalent disorders



Neural circuits play an important role in many disease states. Targeting neural circuits enables treatments for many disease states across the body.

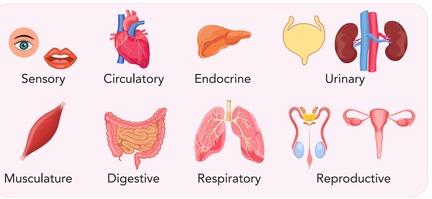


Figure 1. Our central and peripheral nervous system and example organ systems they innervate.

Our novel gene therapy approach combines precision gene delivery with a controllable therapeutic to build gene therapies capable of treating millions

Precision delivery

Cell-type specific AAV vector

Sania's R-Scan recreates human neural circuits to pioneer human-centric capsid evolution.

Circuit control

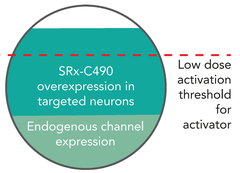
Delivered protein enables selective control of neural circuit with oral small molecule.

- ✔ Translatable R&D
- ✔ Enhanced safety
- ✔ Low dose
- ✔ Scalable & manufacturable

Abstract: 503

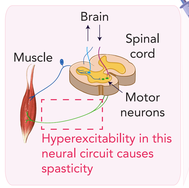
Sania's chemogenetic system (SRx-C490) for controllable gene therapies can be readily translated for hyperexcitable disorders such as spasticity

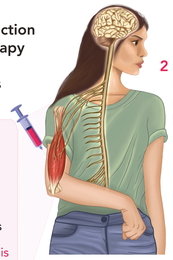
Our chemogenetic system



First clinical use of Sania gene therapy in spasticity

1 **Intramuscular injection of AAV gene therapy**
Motor neurons selectively express SRx-C490

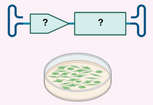
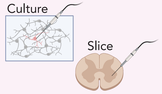
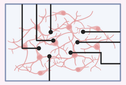




2 **Oral activator** for SRx-C490 to reduce hyperexcitability and treat spasticity in a titratable manner

Abstract: 1140

SRx-C490: from design to *in vitro* assays to proof of concept *in vivo*

Discovery	*In vitro*		*In vivo*
Chemogenetic protein design (HEK cells)	High resolution neuronal screen (electrophysiology)	High throughput neuronal screen (MEA)	Proof of concept animal studies (mouse spasticity)
			
• Identification • Rational mutations • Single channel receptor readout	• Single cell activity • Dose response efficacy in culture and disease model tissue (with Sania AAVs) • Effects on baseline neuronal activity	• Network/population activity • Activator screening • Dose response relationship using Sania AAVs	• Therapeutic window (dose response) in disease state • Efficacy, toxicity, endpoints
Data not shown	*Figures 2 & 4*	*Figure 3*	*Figure 5*

SRx-C490 overexpression across neuron types *in vitro* significantly attentuates neuronal firing when activated, without altering baseline activity

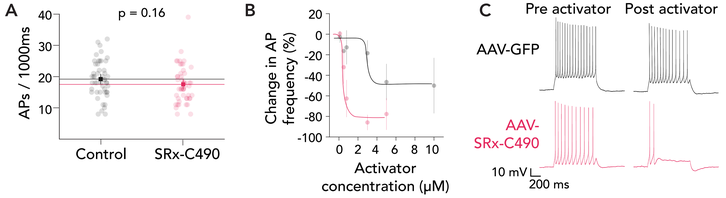


Figure 2. Action potential firing in single human iPSC-derived motor neurons expressing SRx-C490 (pink) or GFP control (black). (A) The number of action potentials (AP) at three times rheobase (minimum current input to evoke a single action potential; difference = -1.7 [95%CI -3.8, 0.69]). ***(B)*** Activator dose dependent percentage change in AP frequency. ***(C)*** Representative traces showing activator-dependent effects on AP firing.
*Sensory neurons show same dose response relationship (data not shown here).

Our combined AAV gene therapy significantly reduced motor neuron population activity at low doses, providing a large therapeutic index

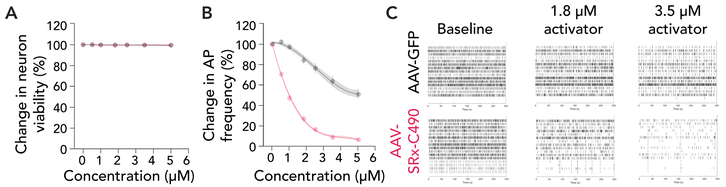
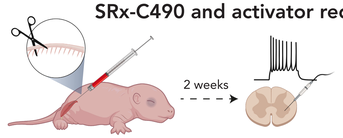


Figure 3. Network properties of human iPSC-derived motor neurons expressing SRx-C490 (pink) or GFP control (black) measured using multielectrode array (MEA). (A) Neuronal viability as measured by electrode impedence at increasing doses of activator. ***(B)*** Percantage change in AP number at increasing concentrations of activator relative to baseline recording (no activator). ***(C)*** Representative MEA raster plots showing activator-dependent effects on AP firing frequency.

SRx-C490 and activator reduce hyperexcitability in mouse motor neurons at low doses



Neonatal mice received a spinal cord injury (to induce motor neuron hyperexcitability and spasticity) and a simultaneous injection of AAV2-Retro packaged with SRx-C490 (pink) or GFP control (black). Spinal cord patch clamp recordings measured activator efficacy on individual motor neurons affected by spasticity.

3μM activator significantly reduces AP frequency at 2x rheobase in SRx-C490 transduced motor neurons (-50%), but not control motor neurons (-10 %).

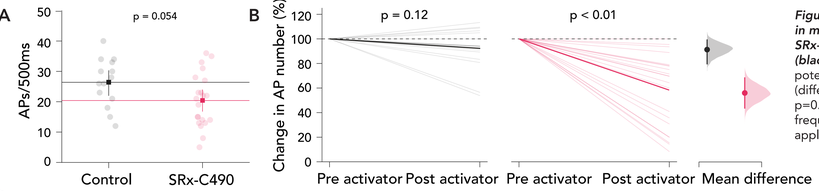


Figure 4. Action potential frequency in mouse motor neurons expressing SRx-C490 (pink) or GFP control (black). A) The number of action potentials (AP) at two times rheobase (difference = -6 [95%CI -11, -0.4], p=0.054). ***B)*** Percentage change in AP frequency before and after application of activator.

SRx-C490 and activator reduce spasticity in a neonatal mouse spinal cord inury model

Neonatal mice recieved a spinal cord injury (to induce spasticity) and a simultaneous injection of AAV2 retro carrying SRx-C490 (pink) or GFP control (black). Electromyography (EMG) recordings measured activator efficacy for reducing muscle spasms.

Low dose of activator (2mg/kg) significantly reduces spasticity in SRx-C490, but not control mice.

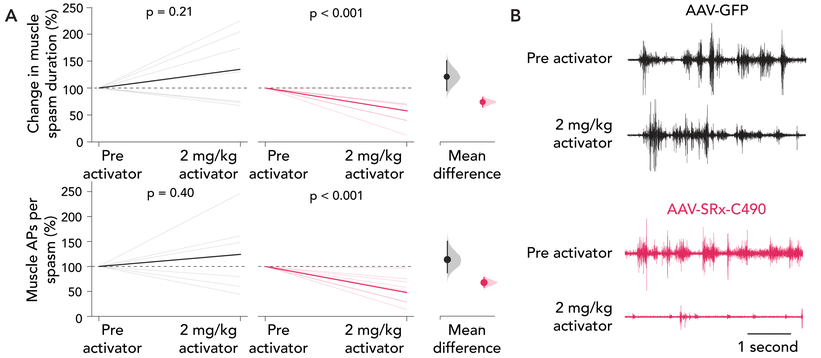


Figure 5. Electromyography (EMG) muscle recordings in a neonatal spasticity model treated with SRx-C490 (pink) or GFP control (black). (A) Activator efficacy for reducing spasm duration and intensity at 2mg/kg. Spasm duration is measured from the onset and offset of EMG activity during spasm (top graphs). Number of compound muscle APs per spasm reflect the intensity of the spasm (lower graphs) ***(B)*** Representative EMG traces showing spontaneous spasms before and after IP administration of activator (2mg/kg) in AAV-GFP and AAV-SRx-C490 treated mice. Activator significantly reduces muscle spasm duration and intensity in AAV-SRx-C490 treated mice, but not control mice.

Conclusions & next steps

- **Conclusions:** *In vivo* electrophysiology and EMG confirm potent effect of SRx-C490 in spasticity model. MEA highlights Sania capsid and SRx-C490 efficacy in human motor neurons.
- **Next steps:** adult mouse experiments assessing the efficacy of our novel gene therapy in treating spasticity.

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