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Reduced sensory synaptic excitation impairs motor neuron function via Kv2.1 in spinal muscular atrophy

Emily V Fletcher1,2, Christian M Simon1,2, John G Pagiazitis1,2, Joshua I Chalif1,2, Aleksandra Vukojicic1,2, Estelle Drobac1,2, Xiaojian Wang1,2 & George Z Mentis1–3

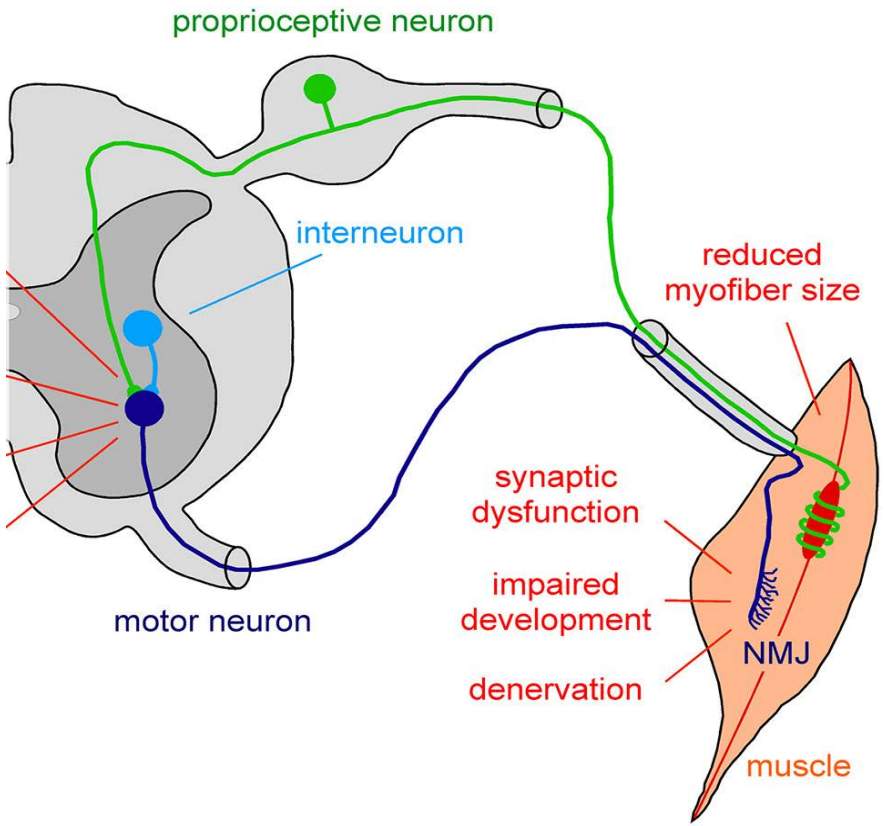
***Students**:*
Eptaminitaki Chrysovalanti Giasemi
Maria Anesti
Andria Vryoni
Xristina Dimitriou

Spinal muscular atrophy (SMA)

- second most common **autosomal recessive disease** that affects **motor neuron**
- **high incidence and severity** (affects 1 of every 6000 to 10,000)
- most common genetic cause **of infant mortality**

SMA causes:
degeneration and **loss of motor neurons**

Progressive muscle weakness, respiratory failure and death



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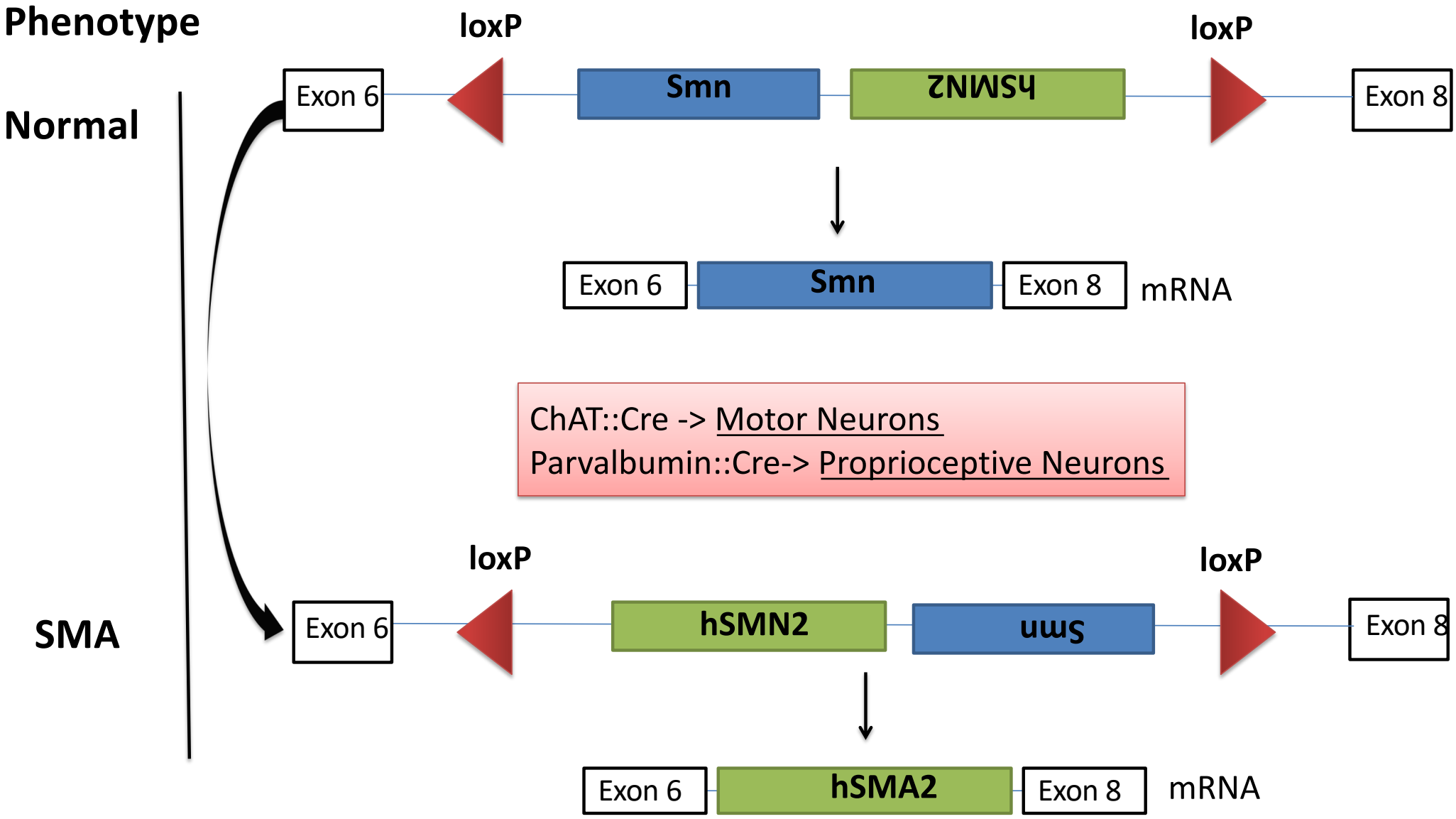
SMA is caused by homozygous mutations:

- **in the *survival motor neuron 1 (SMN1) gene***
- ***retention of at least one copy of the hypomorphic gene paralog SMN2***

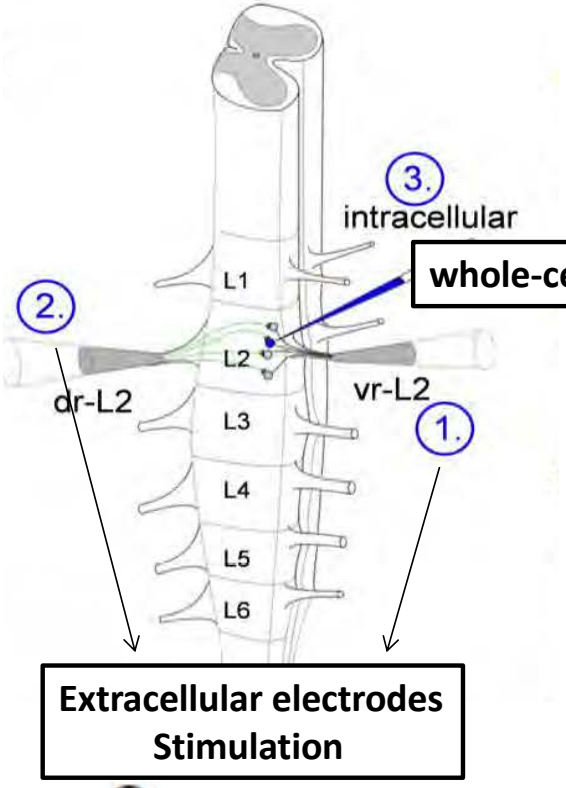
Aim of the study

Is the SMA a motor circuit disease or motor neuron disease?

Mouse model

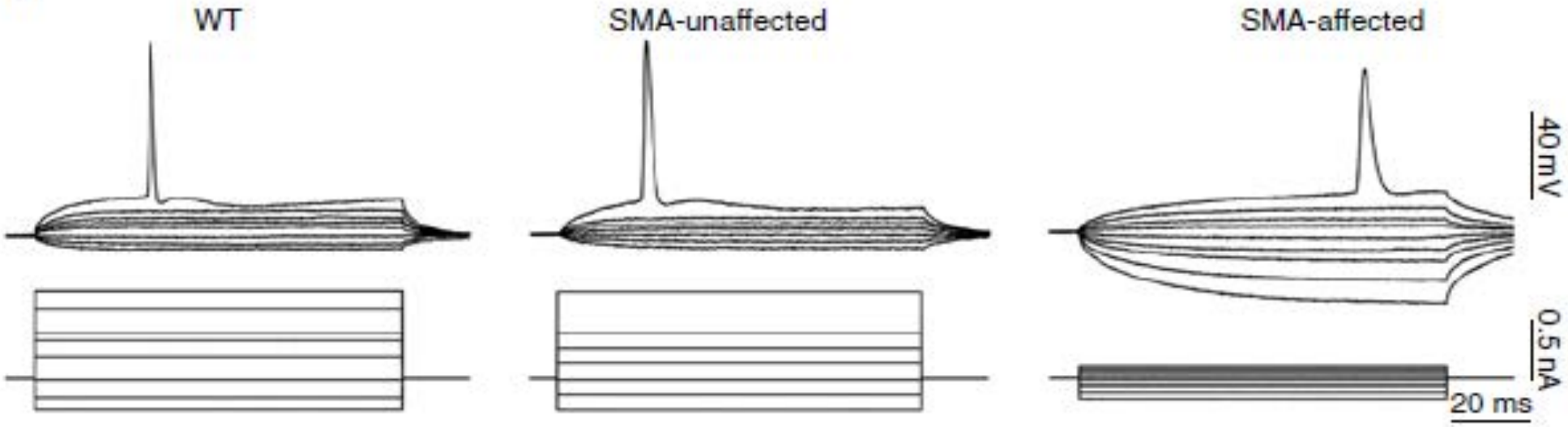

Phenotype
Normal
loxP
loxP
Exon 6
Smn
hSMN2
Exon 8
Exon 6
Smn
Exon 8
mRNA
ChAT::Cre -> Motor Neurons
Parvalbumin::Cre-> Proprioceptive Neurons
SMA
loxP
loxP
Exon 6
hSMN2
Smn
Exon 8
Exon 6
hSMA2
Exon 8
mRNA

SMA-motor neuron pools

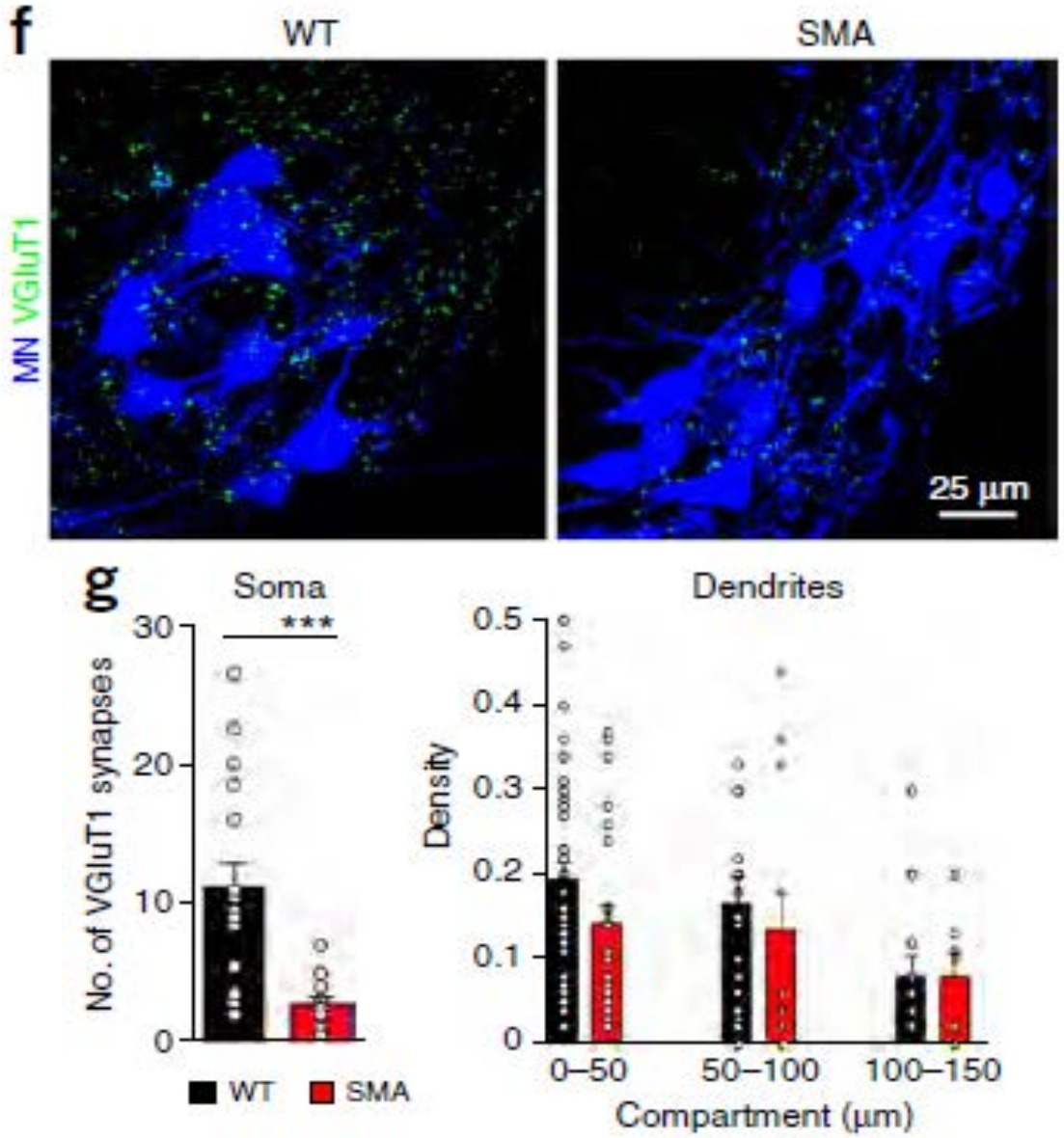


Analysis of the intrinsic membrane properties of SMA MNs revealed two populations:

- **57% were similar to wild-type (WT) MNs** ("SMA-unaffected")
- 43% exhibited signs of dysfunction ("SMA affected")



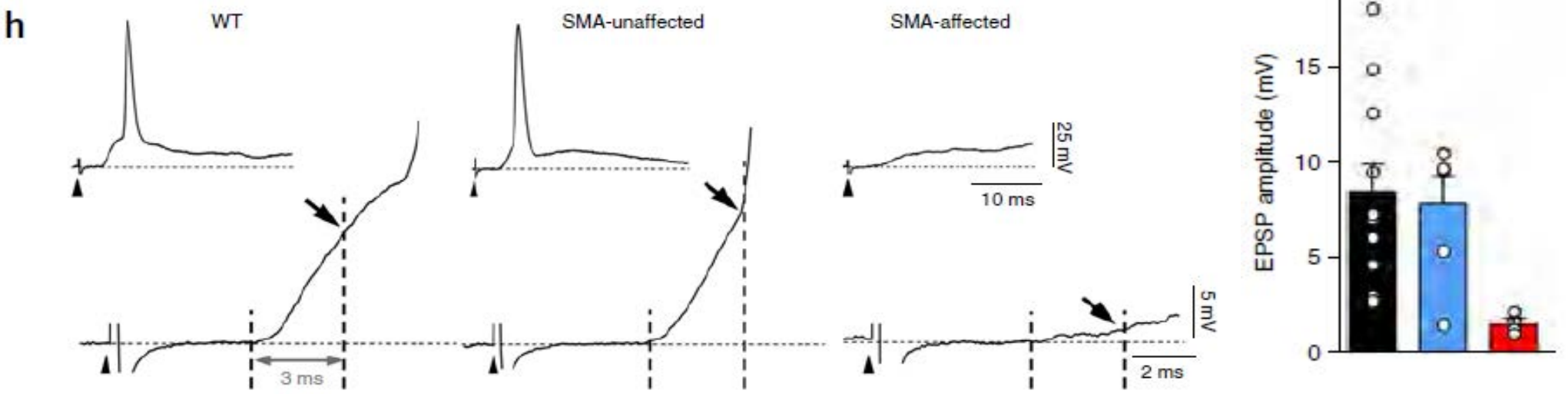
Proprioceptive synapses on MNs exist



Vesicular glutamate transporter 1 (VGluT1) is a marker of proprioceptive synapses.

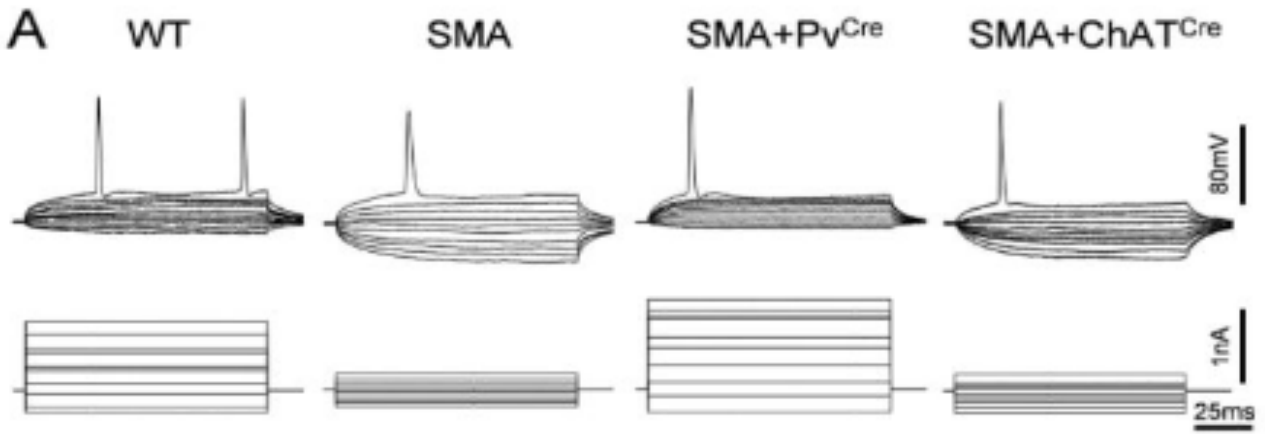
Hyperexcitable MNs, but hypoactive

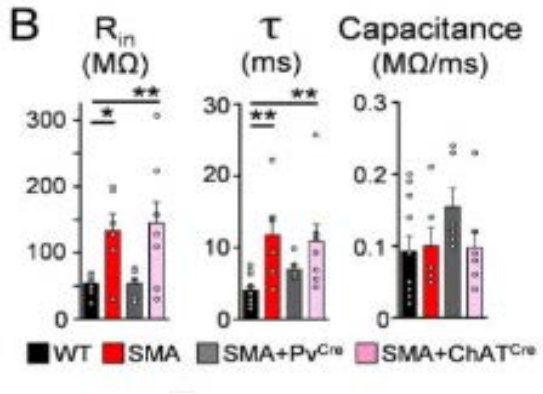
Analysis of monosynaptic induced excitatory postsynaptic potentials (EPSPs)



Selective upregulation of SMN in proprioceptive neurons alone normalizes MN membrane

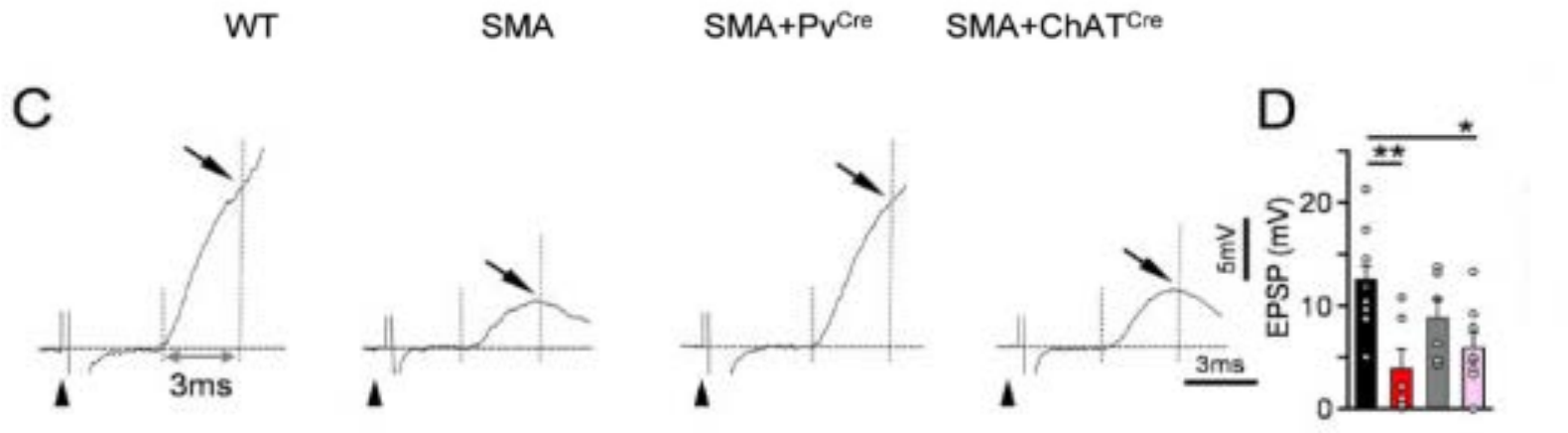
P4





- SMA MNs: increased input resistance and time constant, no difference in capacitance
- In SMA+PvCre mice: increased input resistance was corrected to WT levels, while restoration of SMN in only MNs (SMA+ChATCre) had no effect

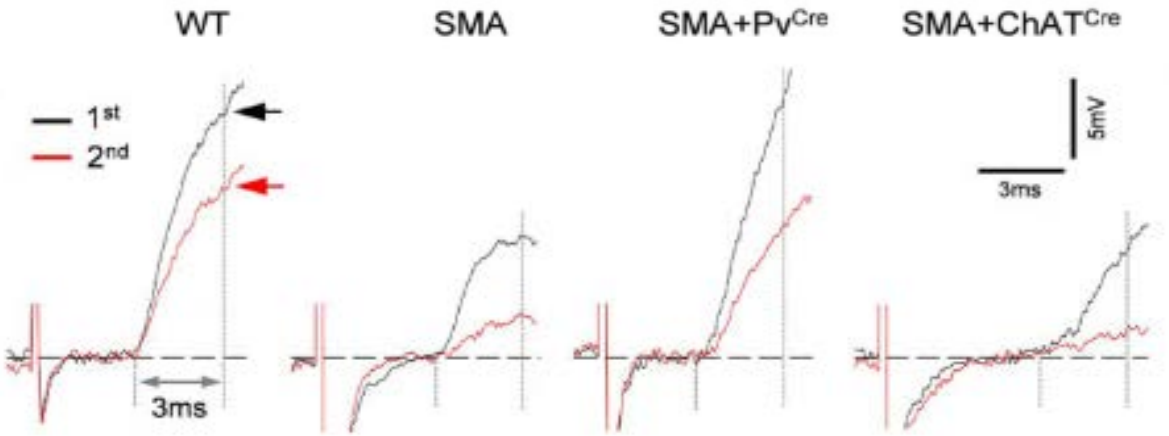
Selective upregulation of SMN in proprioceptive neurons alone normalizes MN membrane

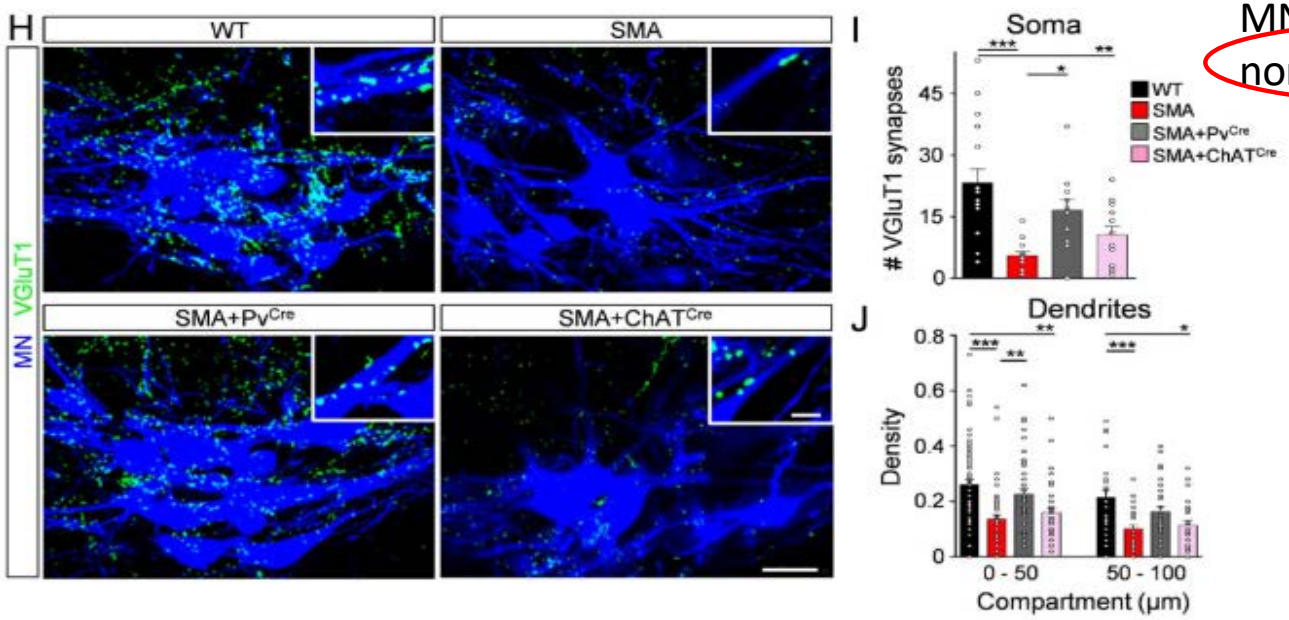


- reduction of the EPSP amplitude in SMA MNs, following proprioceptive fiber stimulation at P4, and this decrease was restored in SMA+PvCre but not in SMA+ChATCre

Selective upregulation of SMN in proprioceptive neurons alone normalizes MN membrane hyperexcitability and VGluT1 synapses

presynaptic or postsynaptic reduction?

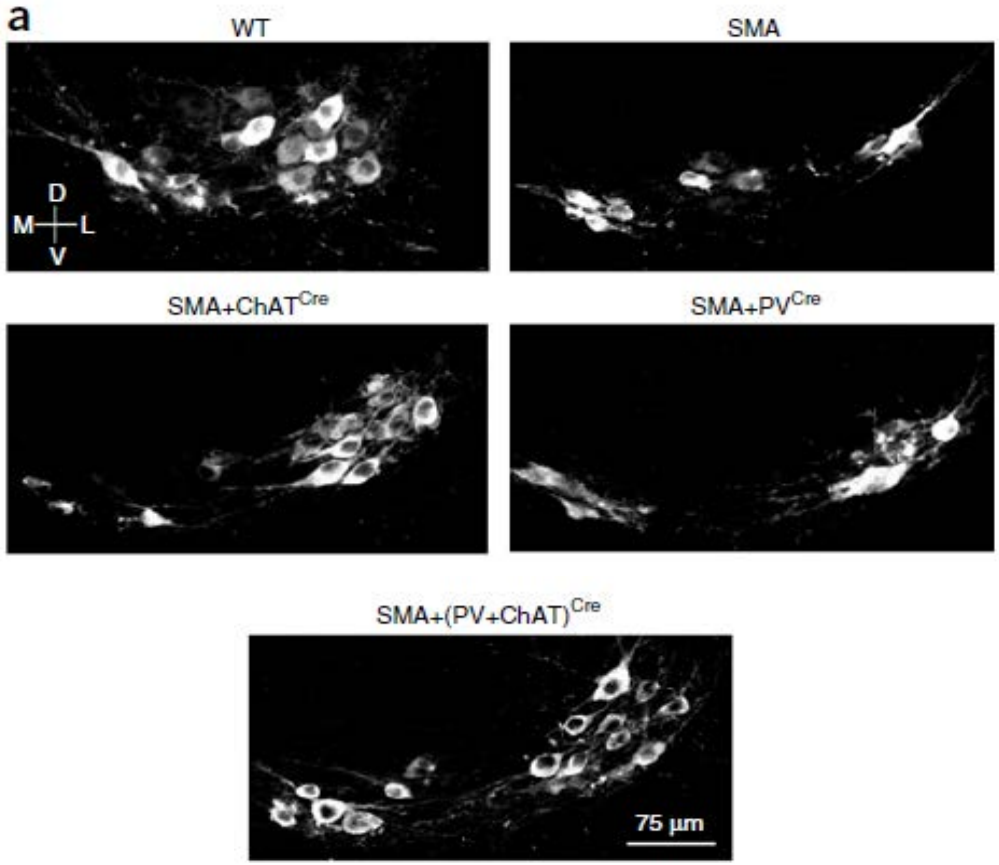


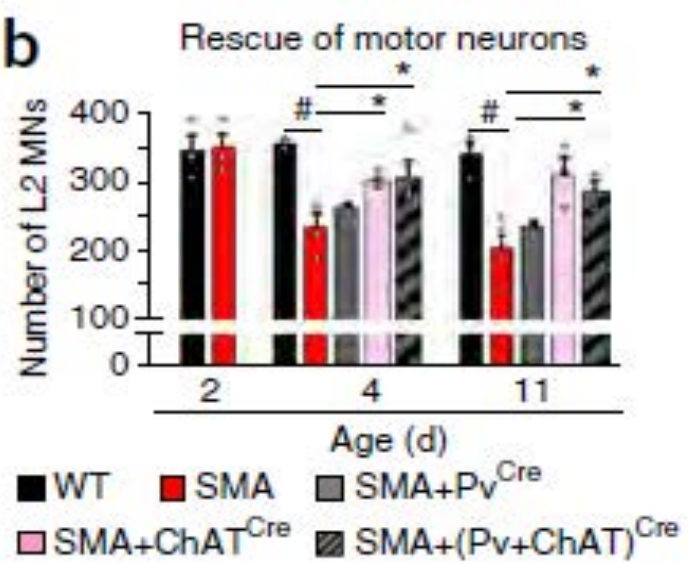


MN dysfunction is a cell-autonomous or non-cell-autonomous consequence?

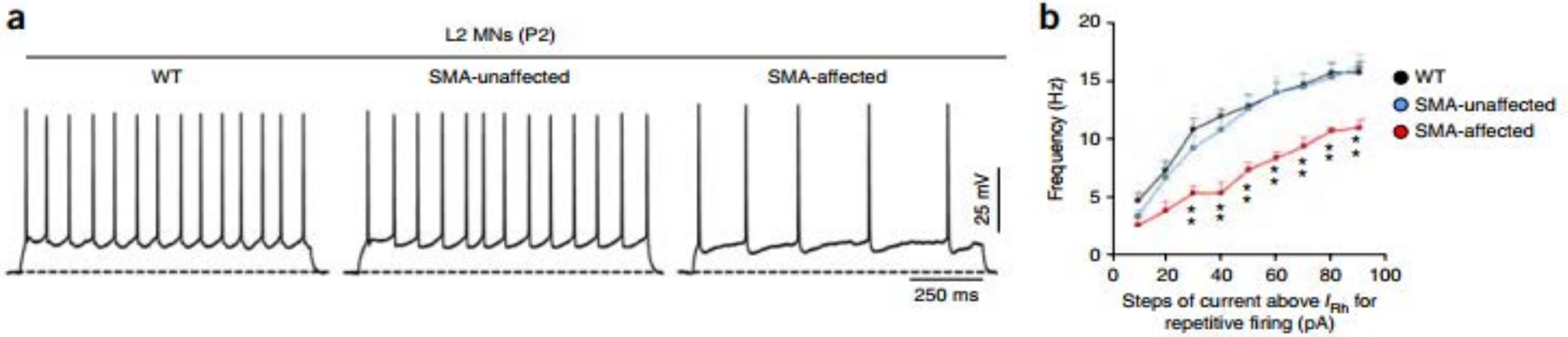
Dysfunction and death of MNs are two independent events in SMA

ChAT staining at P2, P4 and P11



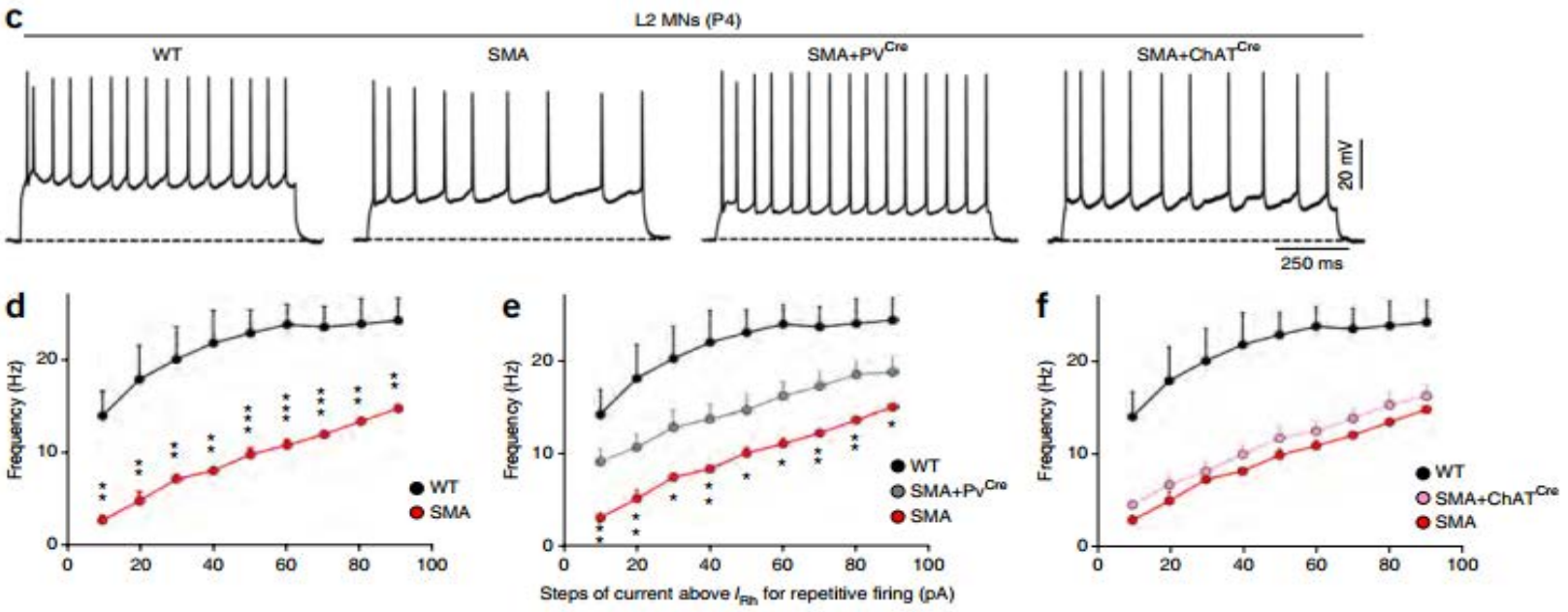


Improvement of reduced firing frequency in SMA MNs following selective upregulation of SMN in proprioceptive neurons only



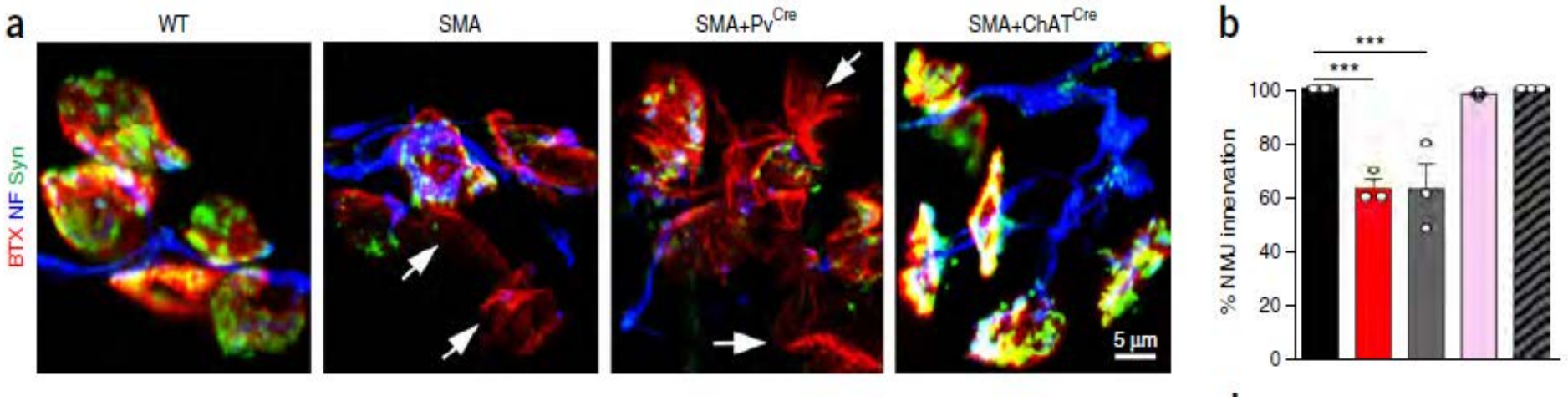
SMA-affected MNs, despite exhibiting increased input resistance paradoxically displayed reduced firing rates compared to controls at P2

Improvement of reduced firing frequency in SMA MNs following selective upregulation of SMN in proprioceptive neurons only



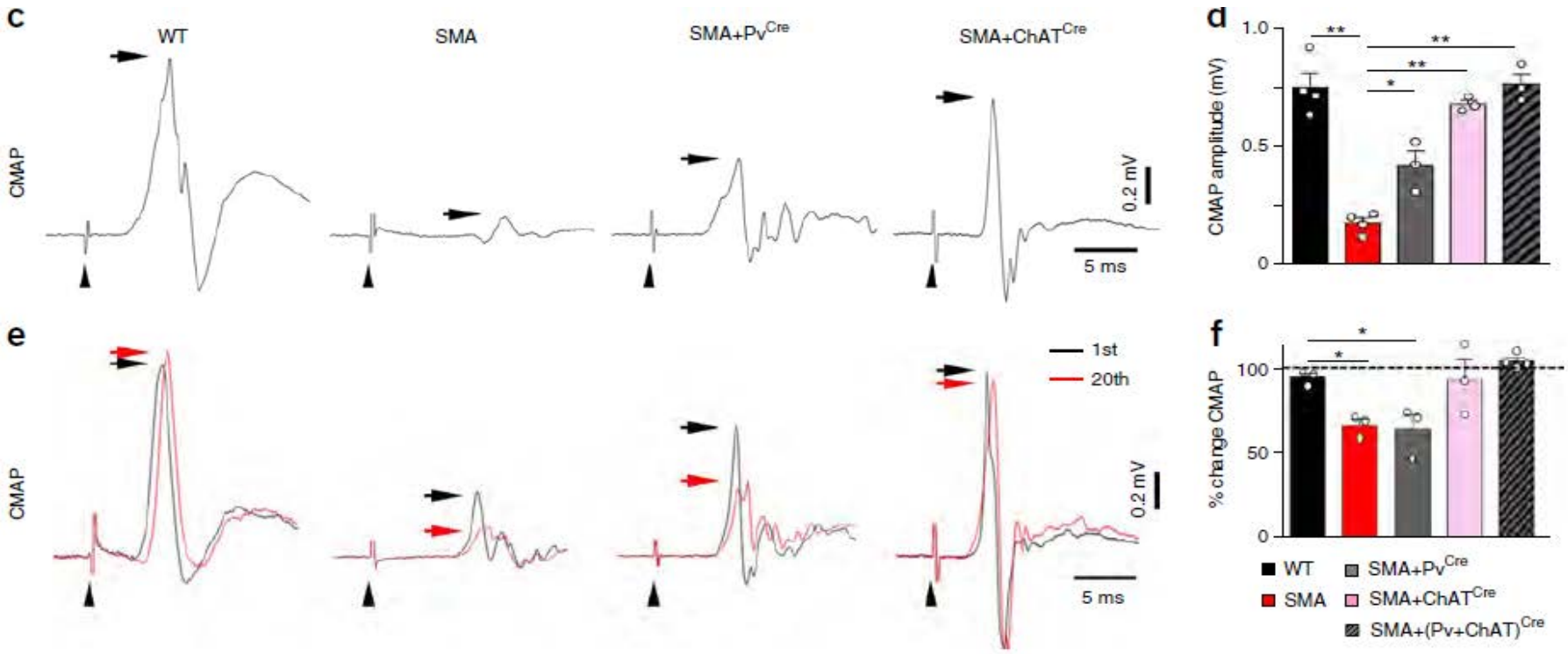
Thus, SMA MNs fire at reduced frequencies and this reduction may be triggered by the dysfunction of sensory–motor synapses.

Effects at the neuromuscular junction through selective restoration of SMN in proprioceptive neurons.



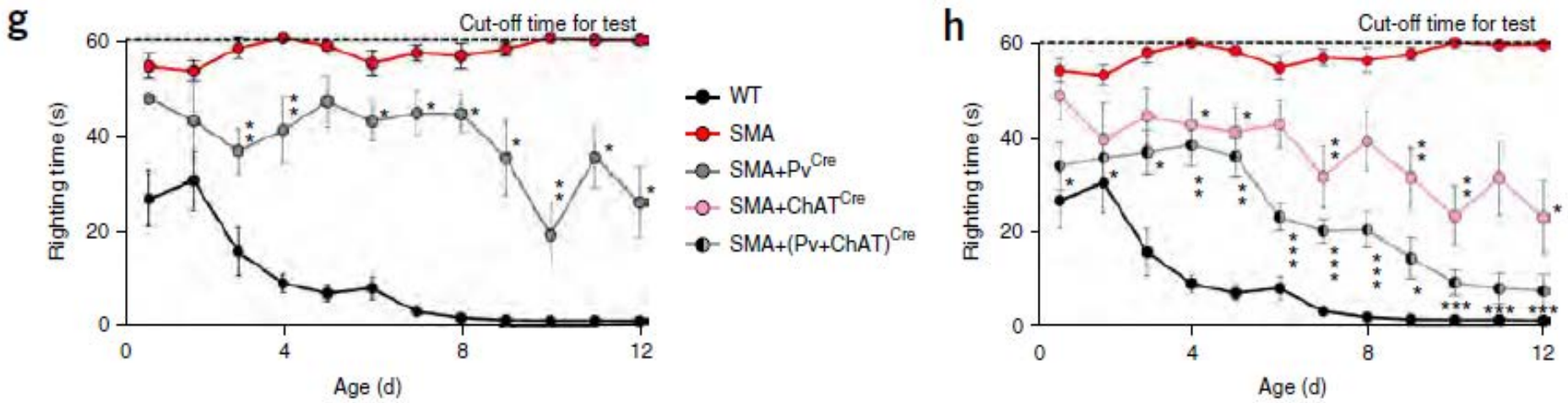
Proprioceptive neurons are responsible for both survival of MNs and Neuromuscular Junctions.

Measurement of NMJs through CMAP (Compound Muscle Action Potential)



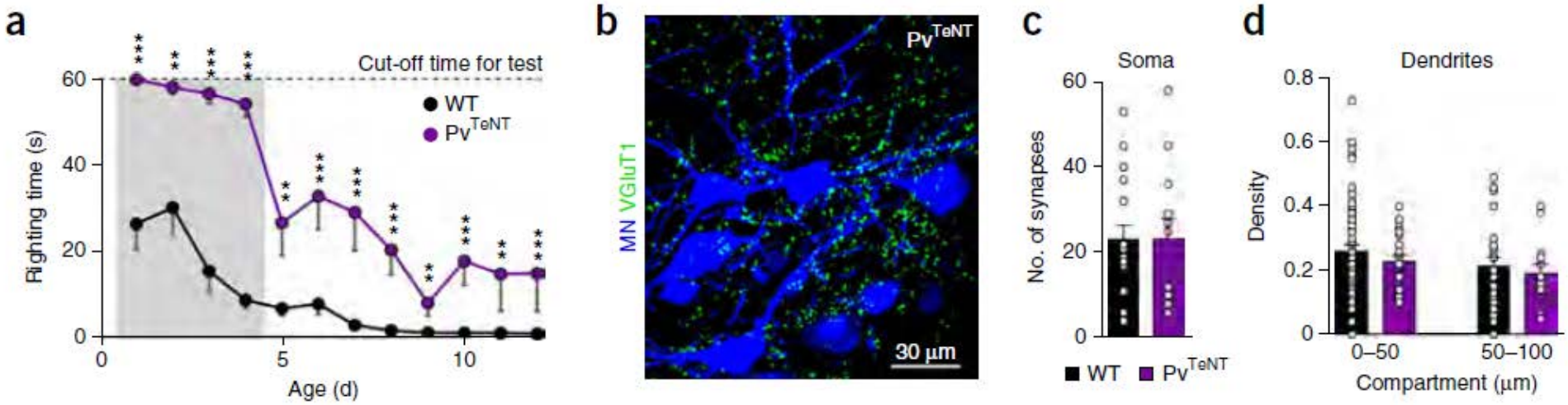
SMN restoration in proprioceptive neurons improves the **function** of Neuromuscular junction and **not** the **denervation**.

Behavioral benefits of selective SMN restoration in propreoceptive and Motor Neurons



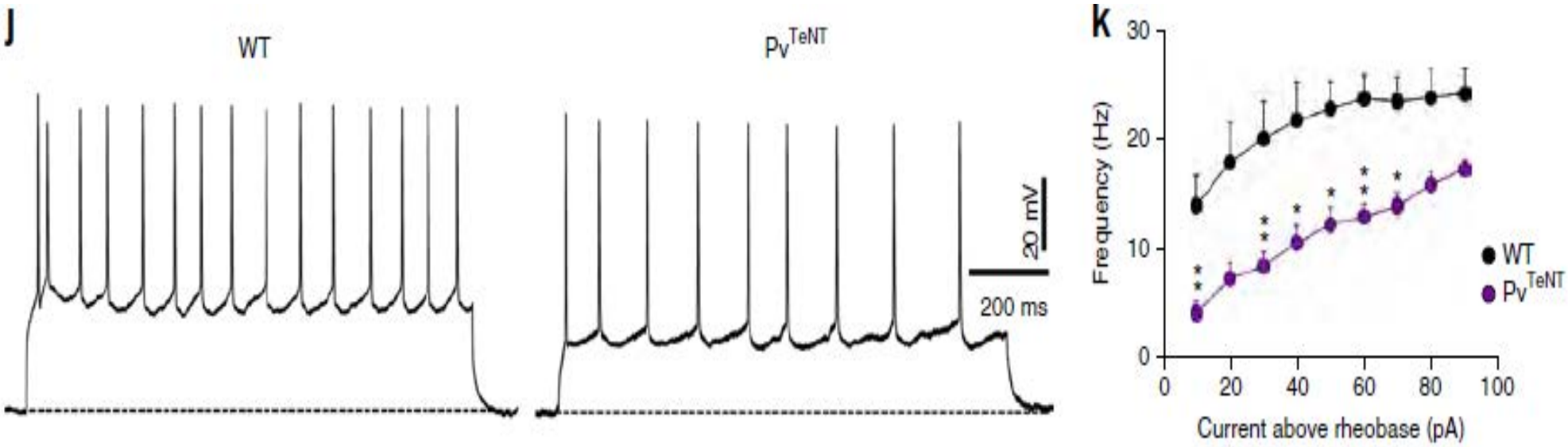
Highest reduction of righting time in SMA+(Pv+ChAT) cre mice.

Blocking of neurotransmitters released by propreoceptive neurons cause MN dysfunction



Reduction of righting ability after P4.
The number of proprioceptive synapses didn't change.

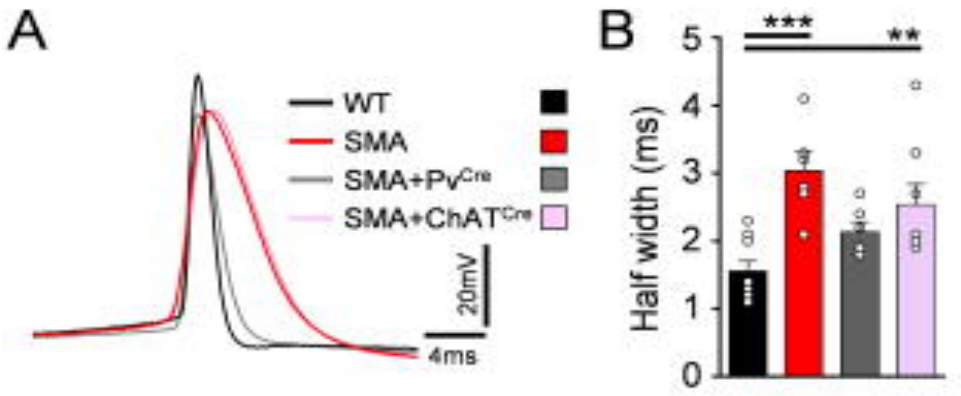
Changes in firing frequency- current plot



Reduction of firing frequency.

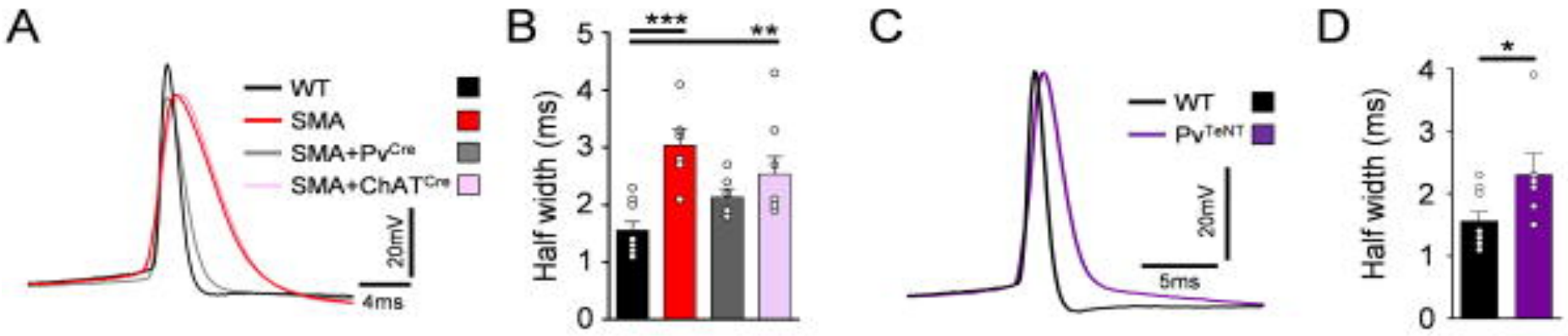
Genetic emulation of the disease regarding to the intrinsic properties of the Motor Neurons.

Sensory–motor synaptic dysfunction in SMA leads to a widening of the MN spike waveform



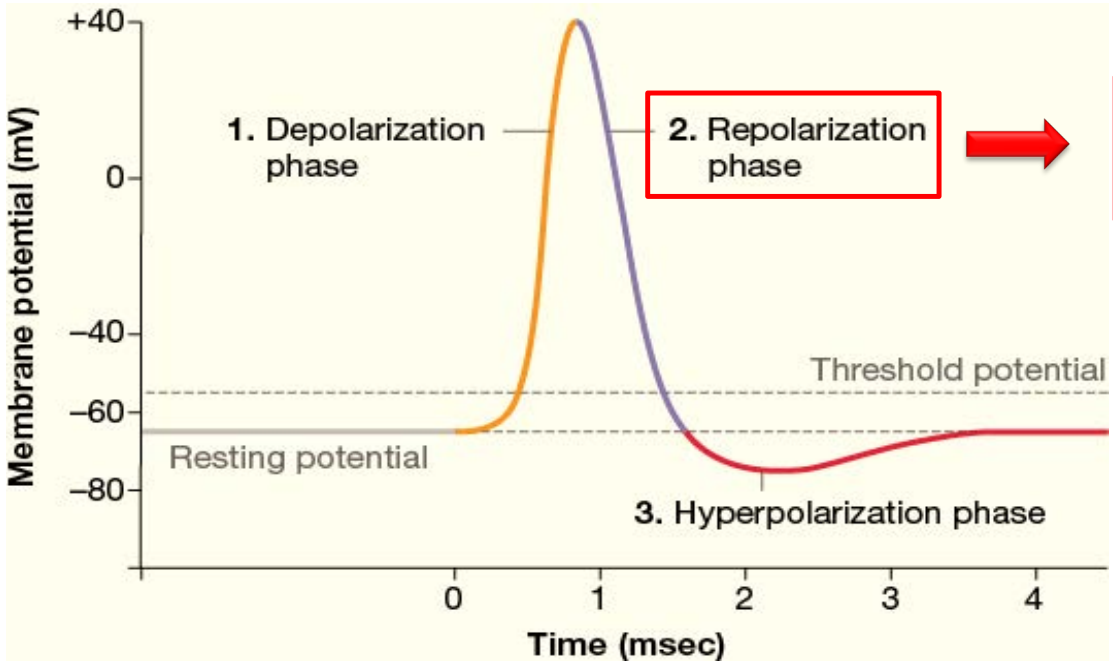
- Prolonged action potentials in SMA MNs compared to WT controls.
- Selective restoration of SMN in **proprioceptive neurons** results in action potential similar to **WT.**
- Selective restoration of SMN in **motor neurons** results in action potential similar to **SMA**.

Sensory–motor synaptic dysfunction in SMA leads to a widening of the MN spike waveform



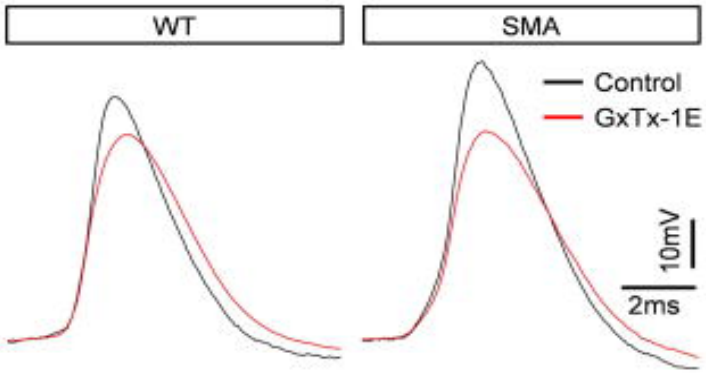
- Prolonged action potentials in SMA MNs compared to WT controls.
- Selective restoration of SMN in **proprioceptive neurons** results in action potential similar to **WT.**
- Selective restoration of SMN in **motor neurons** results in action potential similar to **SMA**.
- Prolonged action potentials in Pv^{TeNT} MNs similarly to SMA MNs.

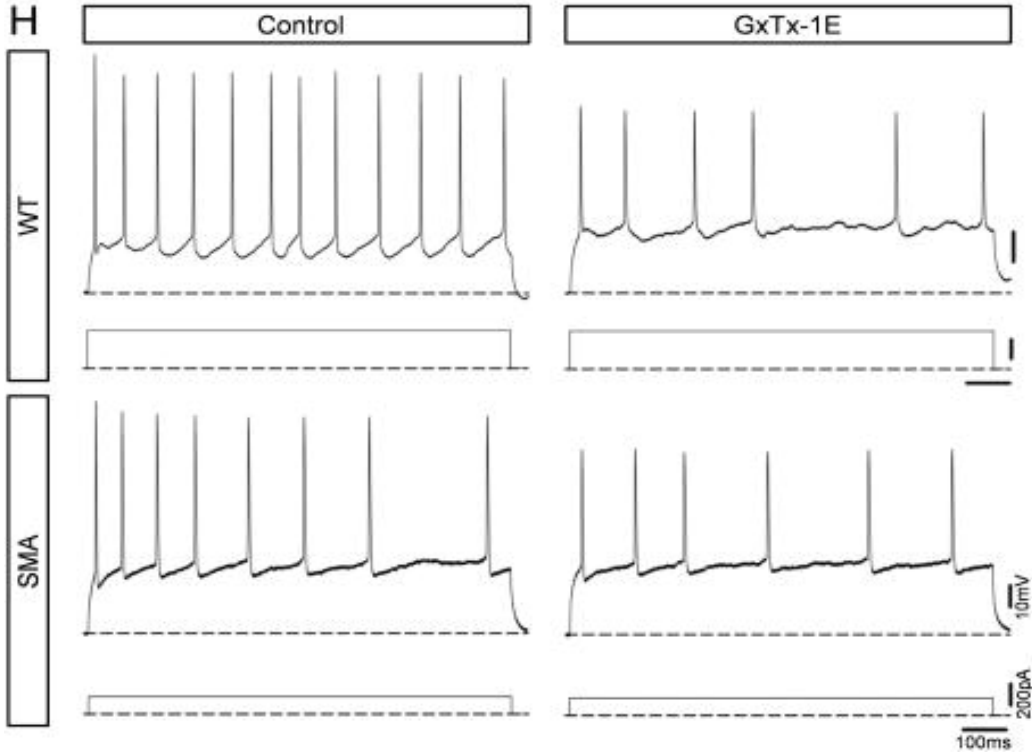
Repolarization is mediated by potassium channels



potassium channels

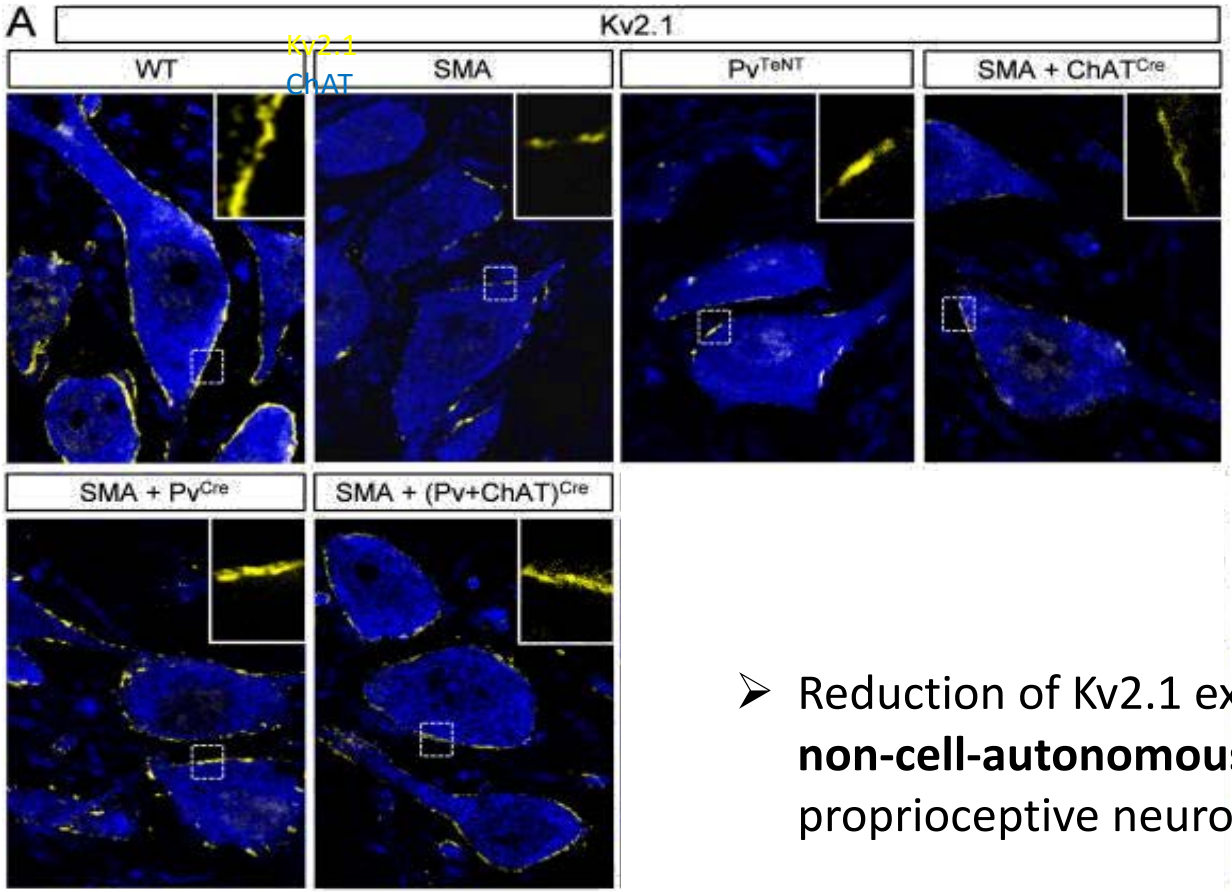
Loss of SMN from proprioceptive neurons reduces the surface expression of Kv2.1 in MNs

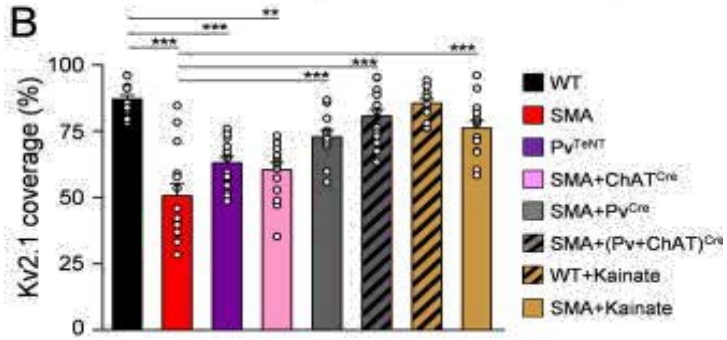




- **Wider action potentials** in WT MNs compared to SMA MNs, after exposure to GxTx-1E
- **Reduced firing frequency** in WT MNs compared to SMA MNs, after exposure to GxTx-1E

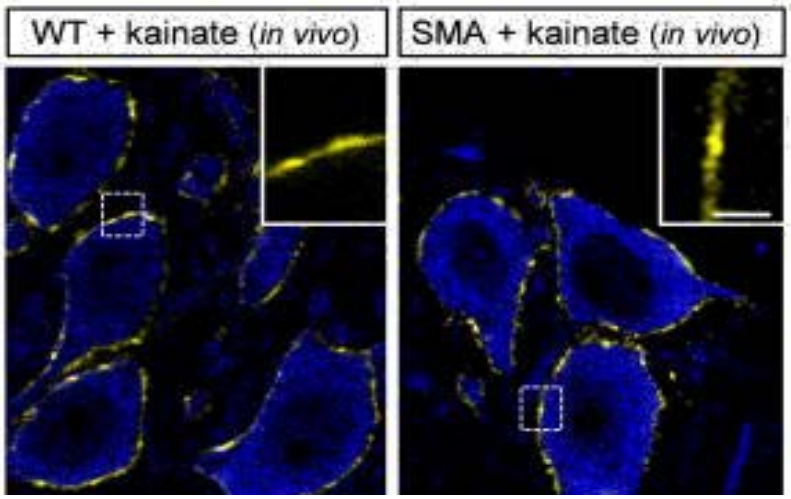
Loss of SMN from proprioceptors reduces the surface expression of Kv2.1 in MNs

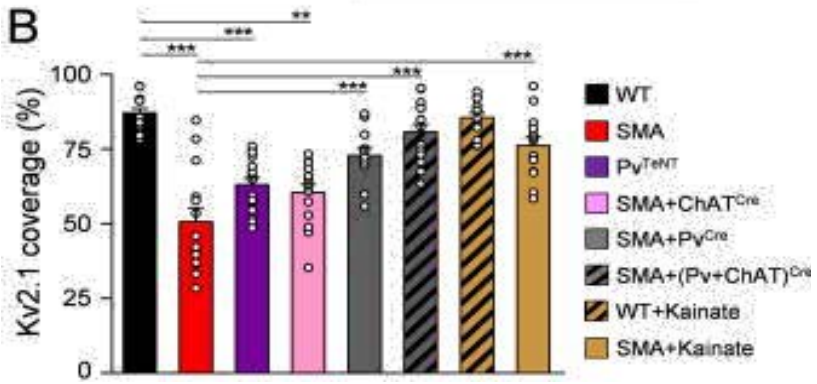




- Reduction of Kv2.1 expression in SMA MNs is a **non-cell-autonomous** consequence of proprioceptive neuron dysfunction.

Chronic postnatal kainate treatment restores normal Kv2.1 surface expression and improves motor function in SMA mice





Conclusions…

1. Reduction of firing frequency of Motor Neurons in SMA.

2. Less glutamate release from proprioceptive neurons.

3. Reduction of glutamate release results in the reduction of Kv2.1 channel expression.

4. These effects can be alleviated by pharmacological treatment, *in vivo*, demonstrating a novel target for therapeutic treatments.

Thank you for your attention!