Voltage-gated sodium channel 1.6 as a novel target for amyotrophic lateral sclerosis treatment

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Introduction

Voltage-gated sodium channel (Nav) 1.6 has multiple roles in neurodegenerative diseases. The most common motor neuron disease is **amyotrophic lateral sclerosis (ALS),** and there is a correlation between increased expression and function of NaV1.6 and abnormal hyperexcitability of the motor cortex in ALS pre-clinical models. Furthermore, neuroinflammation mediated by microglia in ALS shows high expression of Nav1.6.

The gold standard drug used to treat ALS patients is Riluzole, a non-selective **inhibitor of Nav channels**. Therefore, the inhibition of Nav channels can be partly involved in the relief of ALS symptoms.

Objective

Selective inhibition of the $Na_V1.6$ subtype for a novel ALS treatment

Venoms from spiders are a source of peptides with exquisite modulatory properties for Navs. They have nanomolar affinity for central nervous system (CNS) Nav channels, however they also target the off-target Nav1.4.

Methods

- Structure–activity relationship investigations on ProTx-III

Single amino acid modifications

Multiple amino acid modifications

Non-canonical amino acids

- Molecular docking

$Na_v1.6$ PDB 8FHD

Alphafold model analogues

- Whole-cell patch-clamp electrophysiology

QPatch 16X

HEK-293 cells expressing $hNa_v1.6/\beta1$

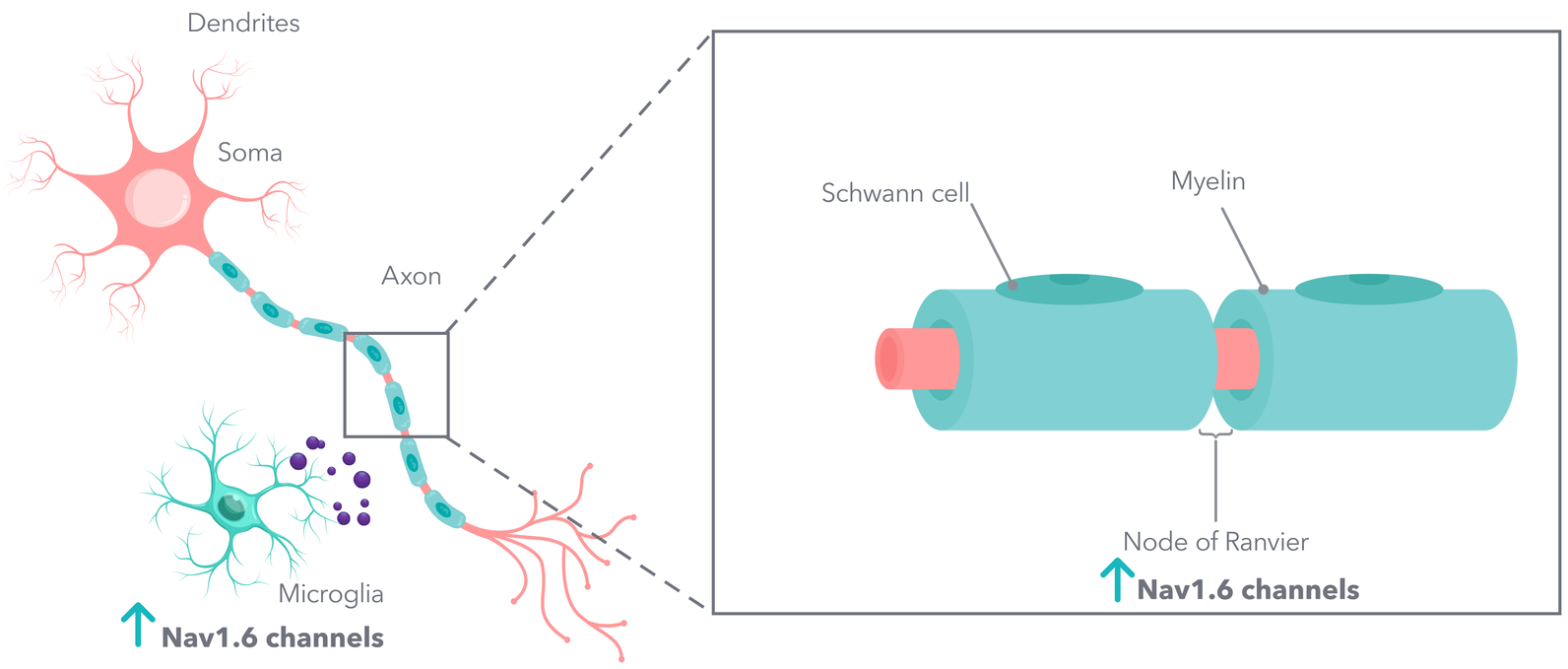

Figure 1: High expression of voltage-gated sodium (Na_V) channel 1.6 on the Nodes of Ranvier and Microglia cells.

Results

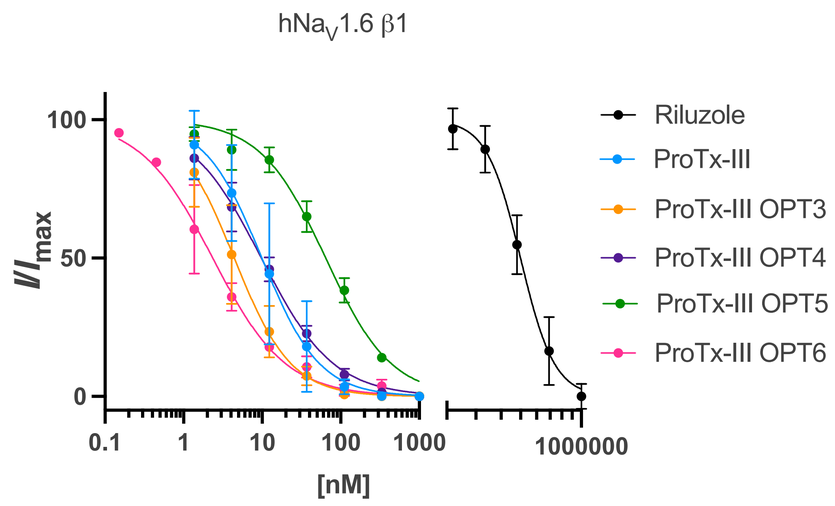

Figure 2: Concentration-response of Riluzole, ProTx-III and the analogues OPT3-OPT6 for the inhibition of $hNa_V1.6$ β1 measured by whole-cell patch clamp electrophysiology.

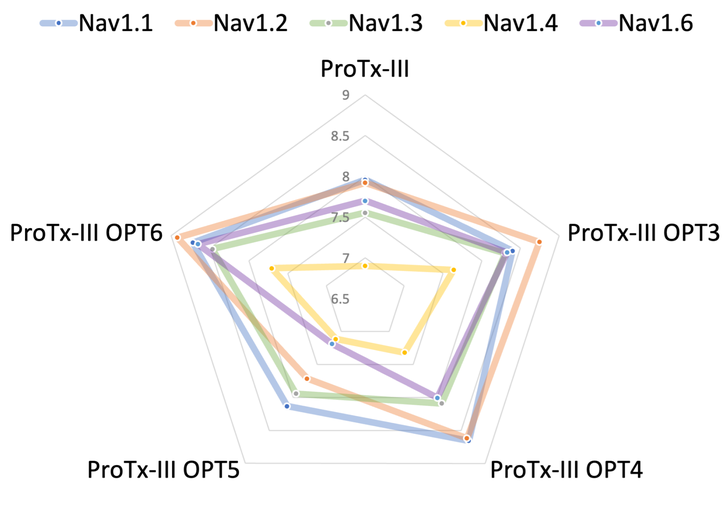

Figure 3: Radar graph of pIC50 values of ProTx-III and the analogues OPT3-OPT6 against $hNa_V1.1$, $hNa_V1.2$, $hNa_V1.3$, $hNa_V1.4$ and $hNa_V1.6$, calculated from the concentration-response curves.

Table 1: Ratio between the IC50 of peptides towards $hNa_V1.4$ and the other Nav channels ($hNa_V1.1$, $hNa_V1.2$, $hNa_V1.3$, and $hNa_V1.6$).

Ratio Nav1.4/Nav1.?	ProTx-III	ProTx-III OPT3	ProTx-III OPT4	ProTx-III OPT5	ProTx-III OPT6
$hNa_V1.1\beta1$	11.5	5.8	21.8	10.4	10.4
$hNa_V1.2\beta1$	10.5	12.8	20.0	4.0	16.4
$hNa_V1.3\beta1$	4.5	4.6	5.9	6.8	5.8
$hNa_V1.6\beta1$	6.3	4.9	4.8	1.2	9.0

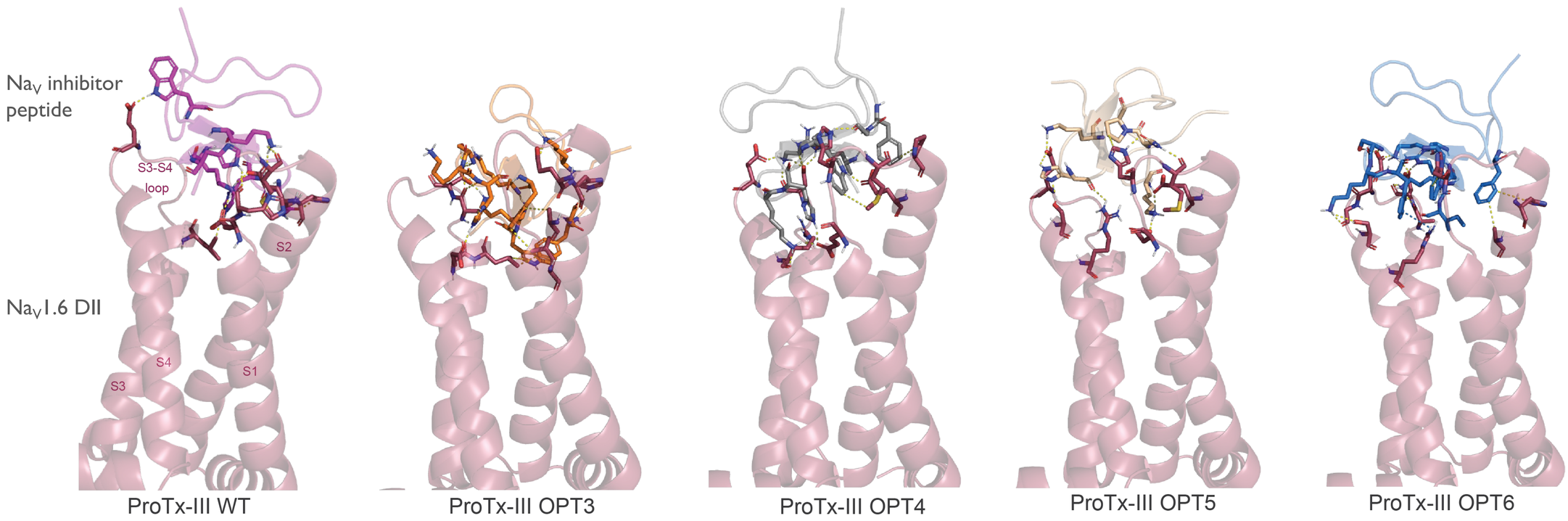

Figure 4: Molecular model of ProTx-III and the analogues OPT3-OPT6 docked on the human $Na_V1.6$ channel DII.

Conclusion

Therefore, ProTx-III OPT4 and OPT6 were selected for further evaluation using *ex vivo* electrophysiology in primary motor neurons from ALS rodent model SOD1. We hope our research will deliver new drug candidates to treat ALS via modulation of neuroinflammation and abnormal hyperexcitability induced by Nav1.6 dysfunction in ALS.

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