Demonstration of the peripheral analgesic potency of the Nav1.7 channel blocker GNE-3565 using distal electrical threshold tracking of unmyelinated nociceptors in vivo

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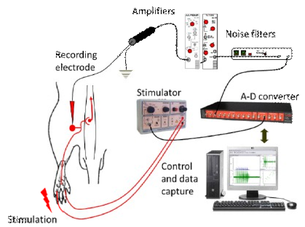
Aim

- Nav1.7 blockade results in slowing of conduction velocity and apparent inhibition of action potential generation within unmyelinated nociceptors.
- The “on target” nature of these effects has been robustly demonstrated. Yet, the distinction between inhibition of action potential generation and axonal conduction failure is problematic.
- The aim of the present study was to assess whether GNE-3565 inhibited action potential generation using a threshold tracking method measuring the distal electrical excitability of DRG neurons in vivo.

Method

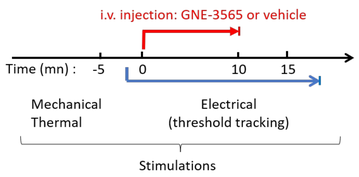
Set up



- Electrical DRG neuron activity was measured in artificially ventilated male Swiss mice under isoflurane anaesthesia using conventional extracellular single-unit recording technique.

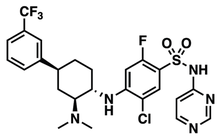
Protocol

- Initial characterization using mechanical and thermal stimulations.
- For threshold tracking, electrical stimulations of the receptive field (2 ms square wave) were delivered at 1/5 Hz. Failure to generate action potential upon 2 successive stimulations led to incremental increase of the stimulation intensity (0.05 mA for 0.1-1.0 mA; 0.5 mA for 1-10 mA).



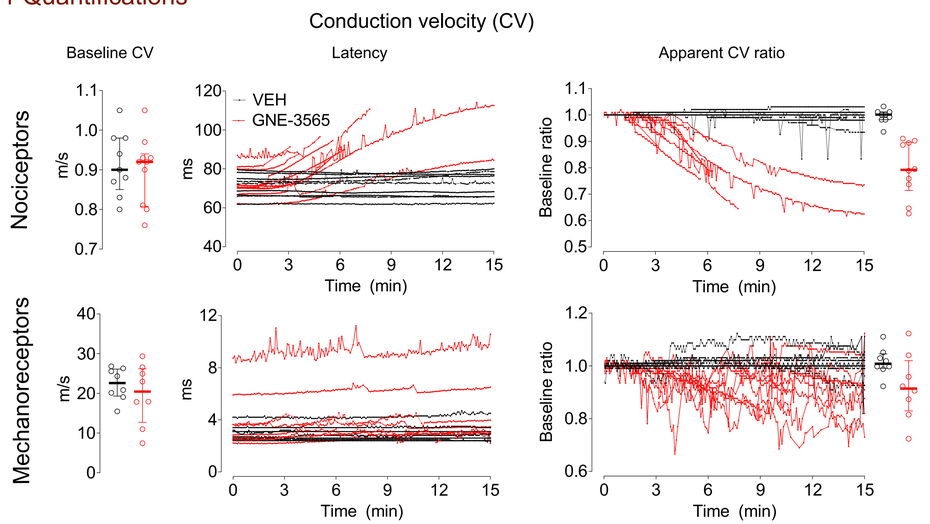
Nav1.7 channel blocker

- GNE-3565 (kind gift of Genentech Inc.) in 10 % DMSO, 35 % PEG-400, 55 % H2O, pH 3.0-3.2 (2.5 mg/ml). I.v. infusion, 9 mg/kg over 10 min.

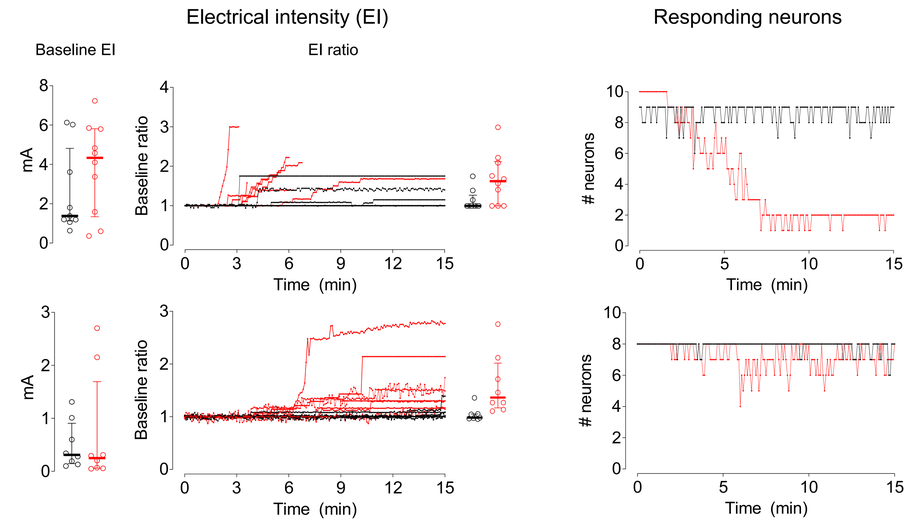


Results

1 Quantifications

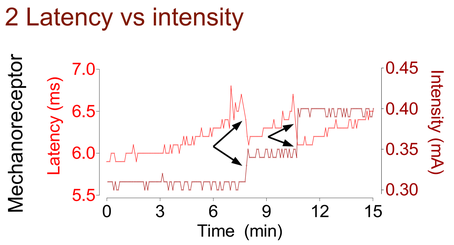


- The variation of the latency of the action potentials and the corresponding computed apparent conduction velocities showed distinct patterns for nociceptors and mechanoreceptors during GNE-3565 or vehicle infusion. Compound-dependent decrease in conduction velocity was evident in nociceptors but confounded by latency shifts in mechanoreceptors (see below).

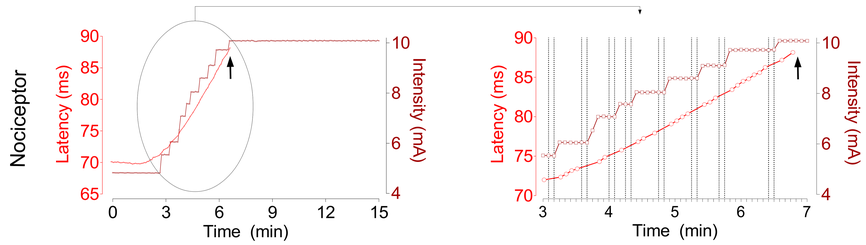


- Compound-dependent decrease in distal electrical excitability was observed for both nociceptors and mechanoreceptors. The difference in baseline EI for the 2 nociceptor groups is unfortunate.
- There was a compound-dependant decrease of the number of nociceptors responding to electrical stimulations.

2 Latency vs intensity

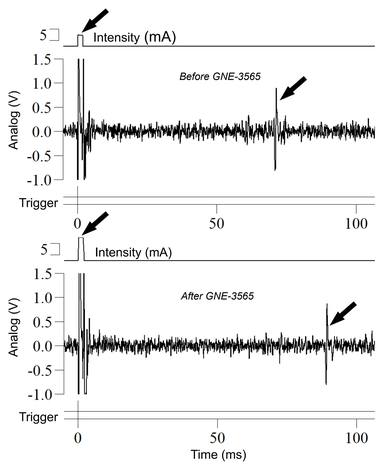


- Increases in electrical intensity following action potential generation failure led to marked decreases in AP latency in mechanoreceptors (arrows).



- This example of successful threshold tracking sequence illustrates 1) that AP latency was barely affected by variations of the electrical intensity of the stimulations and 2) the eventual “loss” of the action potential at the highest intensity of electrical stimulation which could be delivered (arrow).

3 Recording



- Top to bottom channels: electrical intensity of the stimulation, electrical activity measured in the DRG, and trigger of the electrical stimulations.
- Note the change before and after GNE-3565 infusion.

Conclusions

- Electrical threshold tracking of action potential generation at the nerve terminals of nociceptors and mechanoreceptors can be performed in vivo. We suspect that threshold tracking is highly dependent on the placement of the stimulating electrode relative to the receptive field and axon position.
- In unmyelinated nociceptors, GNE-3565-dependent increase in distal electrical threshold and marked decrease in conduction velocity demonstrate a role for Nav1.7 in transduction and conduction. In mechanoreceptors, increase in distal electrical threshold and corresponding compensation of apparent decrease in conduction velocity upon increased electrical intensity (and decrease responses to mechanical stimulations, not shown) suggest some role for Nav1.7 at the very distal end of the axon.
- Essential reading : Deng et al., 2023, Neuron, 83, 2239–2259 and Zheng et al., Neuron, 103, 598-616.

"Who'd ever have guessed that mice do actually have rights?!"