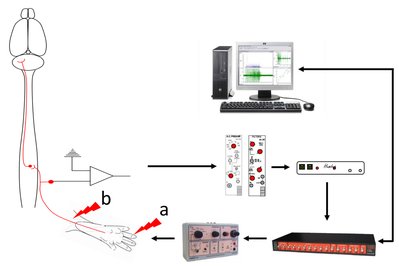
Independent contribution of recovery cycle and repetitive firing to activity dependent slowing of conduction velocity in the distal axon of unmyelinated nociceptors in the anaesthetized mouse

Julien Allard, E-Phys, Saint Genes Champanelle, France
Julien.allard@e-phys.com

Aim

Nav1.8 has been implicated in the activity dependent slowing of conduction velocity (**ADS$_{cv}$**) of action potentials in nonhuman primate unmyelinated nociceptors (**nociceptors** herein)[1]. This raises the possibility of establishing a Nav1.8 channel blocker assay based on ADS$_{cv}$ in rodents. As a first step in this direction, the present study explored the basal characteristics of ADS$_{cv}$ of nociceptors in the mouse.

Set up and method



Experiments were performed in artificially ventilated C57BL/6 mice under isoflurane-anaesthesia. Core body temperature, blood pressure, blood gas and pH were monitored.
A medio-lateral laminectomy was performed over the L4 vertebra to expose the L4 DRG. The distal portion of the sciatic nerve was exposed at the level of the trifurcation into the sural, tibial and peroneal branches, and the branches freed from surrounding connective tissue. The activity of nociceptors was measured at the level of the L4 DRG using a conventional rig for single-unit extracellular recording fitted with extra-fine tip tungsten-in-glass electrodes (Kation Scientific) .
Electrical stimulations (2 ms square wave pulses) were delivered at the level of the receptive field using a pair of 30 G needles (a), or on the relevant branch of the sciatic nerve using a pair of platinum wire hook electrodes (b).
Recordings were acquired and processed using Spike 2.0 (Cambridge Electronic Design).

Discussion

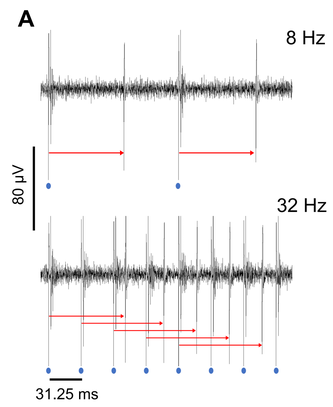
ADS$_{cv}$ induced by repeated stimulations of the receptive field was significantly increased compared to that obtained from sciatic nerve branch stimulations. This was not the case for the conduction velocity slowing related to the recovery cycle, which was similar when measured from receptive field and sciatic nerve branch stimulations.
Assuming that Nav1.8 is essential for the ADS$_{cv}$ induced by repeated firing[1,2], the greater contribution of the distal portion of the axon to the ADS$_{cv}$ correlates nicely with the reported "*differential spatial distribution of Nav1.8 within C-fiber axons, being functionally more prominent in the most distal axons and terminal regions*"[3]. Yet, the smaller diameter and lower temperature of the terminal axon compared to the more proximal portion might be sufficient to explain the greater ADS$_{cv}$ related to repeated firing measured from the receptive field[2,4].
Regarding recovery cycle, all units were subnormal at the combination of frequencies of stimulations used herein, in agreement with data generated in the rat[5]. It is accepted that the mechanisms underlying ADS$_{cv}$ related to repeated firing and recovery cycle are different (increase intra-axonal sodium concentration versus Nav inactivation, respectively). Indeed, the similarity of the ADS$_{cv}$ related to the recovery cycle obtained from stimulation of the receptive field and sciatic nerve branch effectively rules out explanations based on the specific characteristics of nociceptor axon terminals.

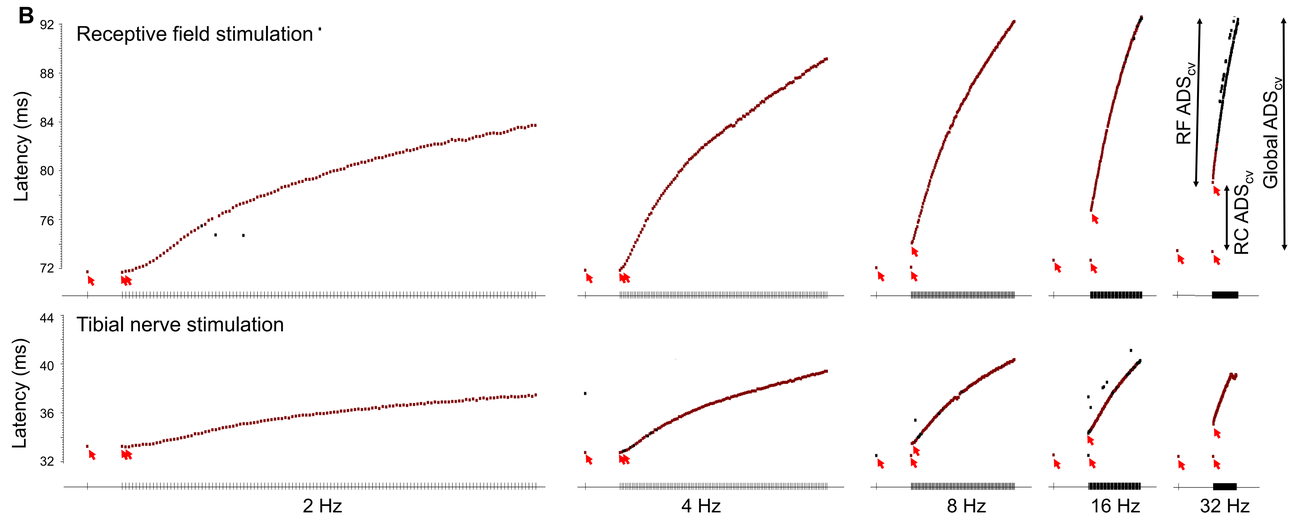
References

1. Vardigan et al., Pain, 2025, 166:631-643.
2. Tigerholm et al., J Neurophysiol, 2014, 111:1721-1735.
3. Klein et al., J Neurosci, 2017, 37:5204-5214.
4. Thio et al., PLoS Comput Biol, 2024, 20:e1012475.
5. George et al., J Physiol, 2007, 578:213-232.

Results

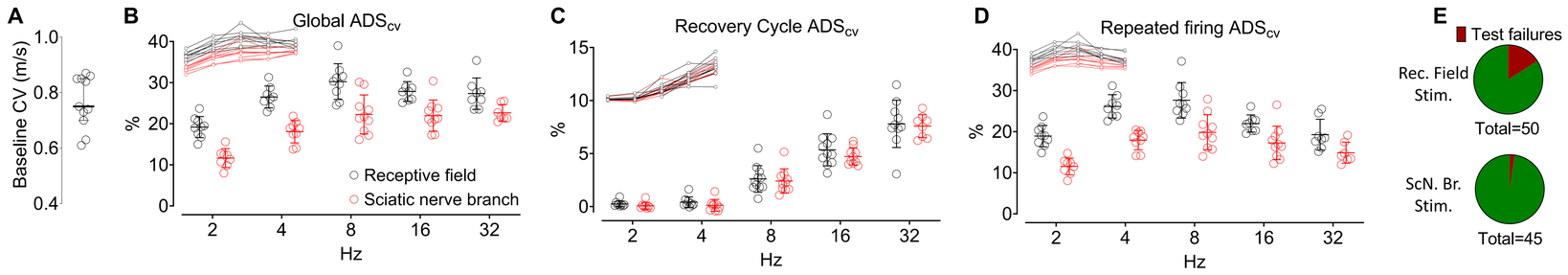
1 Recording and raster plot





A : example of recordings obtained with stimulations of the receptive field. Blue dots point to the stimulus artefact. Red arrow marks the latency between the action potential and the parent stimulus artefact.
B : example of raster plots for the 5 successive test sequences (120 stimulations each, at 2, 4, 8, 16 and 32 Hz; baseline at 0.2 Hz) obtained with stimulation of the receptive field and sciatic nerve branch (tibial nerve in this case). Points obtained for latencies immediately before and for the 1st and 2nd stimulations in the test sequences are pointed by red arrows. These last 2 points allow a measure of the variation of conduction velocity related to the recovery cycle for each frequency. Marked subnormality appears at frequency ≥8 Hz. Note that global ADS of conduction velocity reaches a plateau around 8 Hz.
RF ADS$_{cv}$ and RC ADS$_{cv}$: ADS$_{cv}$ related to repeated firing and recovery cycle, respectively.

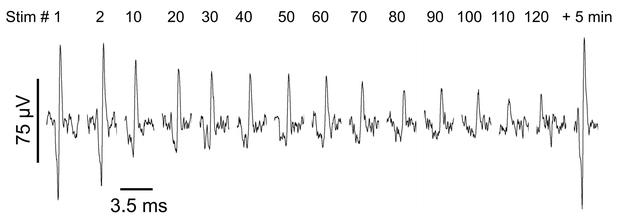
2 ADS$_{cv}$ quantification



A : baseline conduction velocities of the 11 nociceptors included in the study (computed from receptive field stimulations, travelling distance estimated to 6.0 cm).
Global ADS$_{cv}$ (**B**) and recovery cycle-related ADS$_{cv}$ (**C**) were quantified as (increase in latency)/(baseline latency)*100. Repeated firing-related ADS$_{cv}$ (**D**) was computed by subtracting recovery cycle-related ADS$_{cv}$ to global ADS$_{cv}$. n=7-11 for **B**, **C** and **D**. **E** : proportion of test sequences in which action potential generation/conduction failures occurred. These were excluded from the analysis (such failures were probably due to conflicts between terminal branches within the receptive field). Rec. Field and ScN. Br. Stim., receptive field and sciatic nerve branch stimulation.
For frequencies of stimulations ≥8 Hz, note 1) the plateau of the global ADS$_{cv}$, 2) the steady increase of RC ADS$_{cv}$ and 3) the decrease of the RF ADS$_{cv}$.
Global and repeated firing-related ADS$_{cv}$ obtained from receptive field and sciatic nerve branch stimulations were statistically different at all frequencies tested (multiple t-test comparisons, 0.0001<p<0.01).
At completion of the experiments, mean (SD) of blood pH and partial CO_2 pressure were 7.44 (0.04) and 21.9 (2.0) mmHg, respectively (n=8). Blood pressure was 61.7 (6.9) mmHg (n=10).

3 Action potential amplitude

Example of decrease in amplitude of action potential observed upon successive stimulations at 32 Hz. Amplitude was fully recovered after 5 min rest.
Amplitude was stable at frequencies of stimulation ≤16 Hz.
Baseline conduction velocity for this unit was 0.59 m/s.



4 Firing frequency of DRG neurons (historical data)

The mean instantaneous frequencies (IF) measured for 4 consecutive action potentials (AP) and over 1 or 0.1 s were obtained for 55 nociceptors and 17 mechanoreceptors.
Cold and heat : water jet at 0 and 50 °C, respectively.