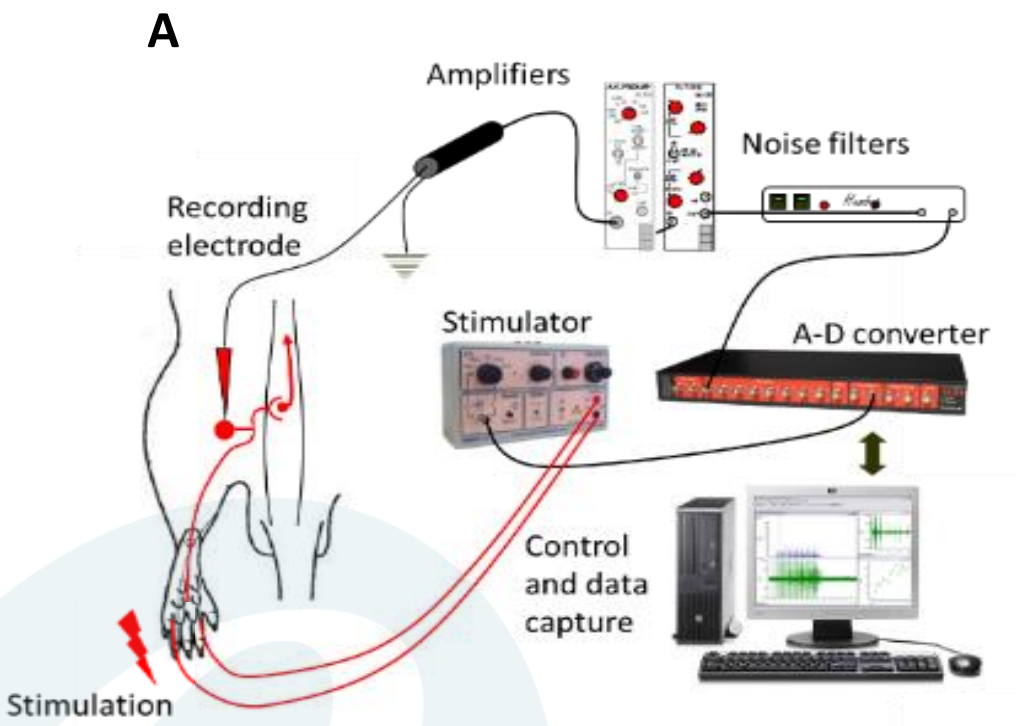
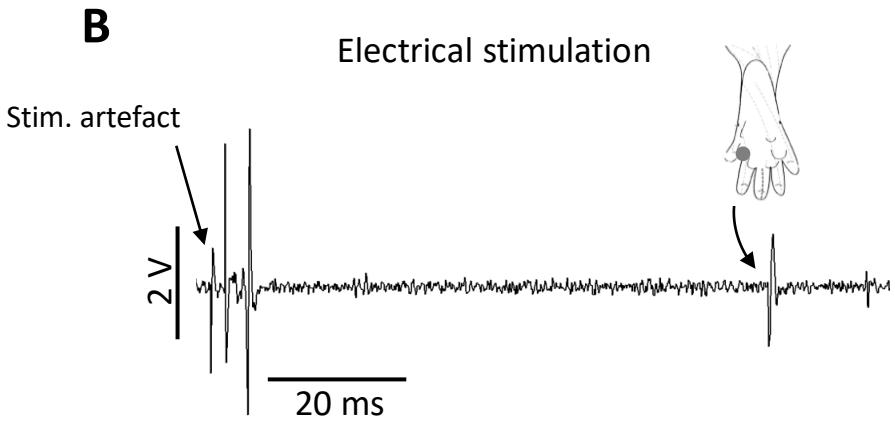
Recording of nociceptors in the anesthetized mice

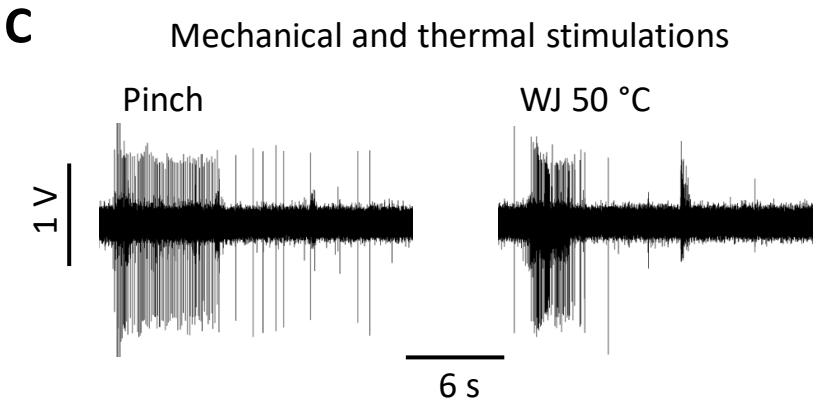
Measure of responses to repeated noxious mechanical or thermal stimulations, and electrical stimulations, over 40 min

Evidence of desensitization of unmyelinated DRG neurons to noxious repetitive stimulations

Set up and basic recording illustration





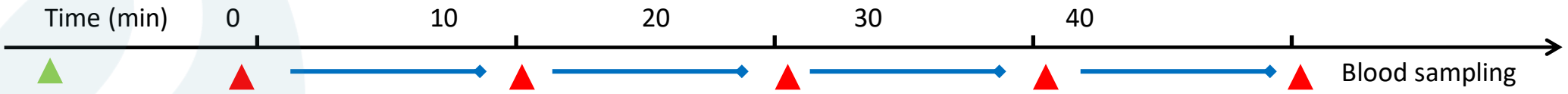


- **A**: set up (identical to that used for recording of single unit in the spinal cord).
- **B**: response to electrical stimulation of the receptive field appears as a single action potential with stable latency. **C**: responses to mechanical and thermal stimuli appear as trains of action potentials. Amplitude after 20 k gain.

Method

- Basic DRG neurons recording
  - Isoflurane anaesthesia, artificial ventilation, control of core body temperature and blood pressure.
  - Lateral laminectomy to access the L4 DRG for recording of nociceptors.
  - Placement of a pair of stimulating electrodes connected to an electrical stimulator on each side of the paw.
  - Recording electrode inserted in the DRG; search of neurons based on orthodromic electrical stimulation of the hind paw.
  - All neurons with conduction velocity <2.5 m/s are included.
  - Mechanical stimulations: hog brush (10 strokes); VF 25, 50, 100 and 200 mN (6 s); pinch with micro haemostat clamp (6 s). Thermal stimulations: water jet (10 ml) at 0, 24, 42, 46 and 50 °C (approx. 3 s).
  - Quantification of responses as number of action potentials (#AP; 10 strokes for brush, over 5 s for VF and pinch; whole duration for thermal responses (from 2 to 30 s).

- Experimental design:

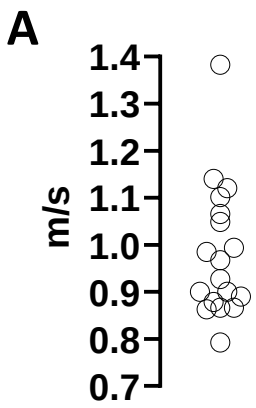


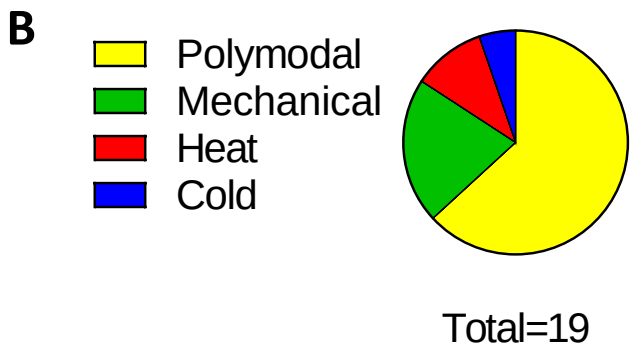
▲ : response to mechanical and thermal stimuli
- brush, VF25, 50, 100, 200 mN and pinch
- Water Jet (10 ml): 0, 24, 42, 46 and 50 °C

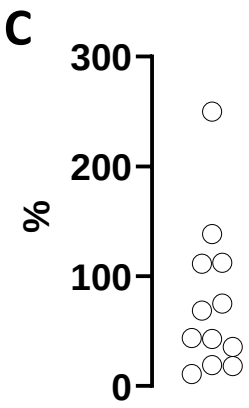
▲ : pinch or 0 °C or 50 °C

→ : peripheral electrical stimulation
- 1 pulse every 10 s

Conduction velocity and modality distribution

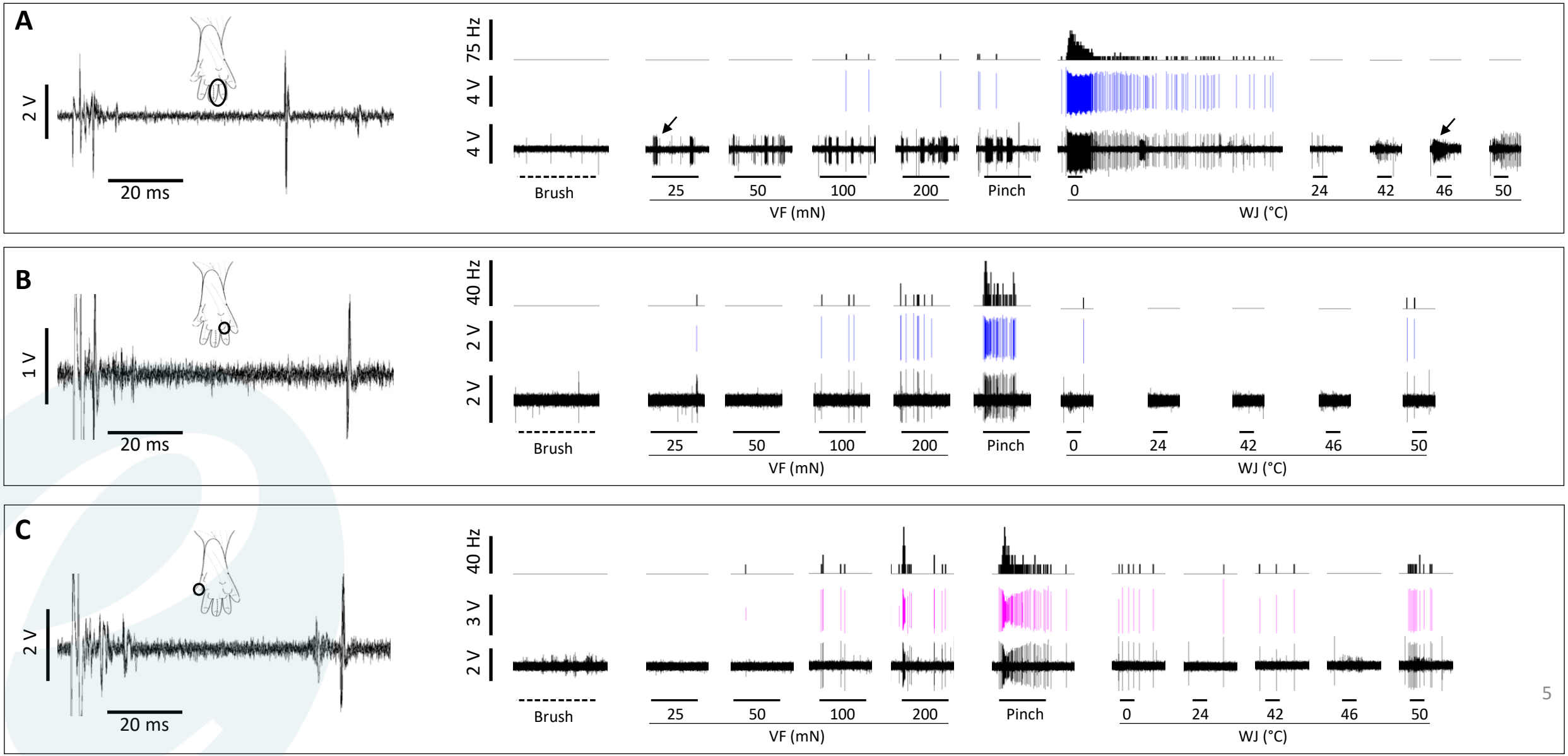






- Success rate
  - Twenty three mice were initially experimented. One mice was lost during the surgical preparation. A fit for purpose signal to noise ratio was obtained in 19 of the 22 remaining mice.
- **A**: conduction velocity
  - One DRG neuron displayed a conduction velocity of 3.1 m/s (Aδ, not shown). The conduction velocity of all other neurons was <2.5 m/s (25^{th}, median and 75^{th} percentile: 0.88, 0.97 and 1.10 m/s).
- **B**: modality distribution of nociceptors
  - Note that the classification of the modality of the nociceptors encountered is often arbitrary.
- **C**: ratio of heat/pinch response
  - The ratio of the number of action potentials obtained in response to WJ 50 °C over that obtained in response to pinch was computed for the 12 polymodal neurons. Note the wide range of the ratio, indicating that polymodal neurons represent an heterogeneous population.

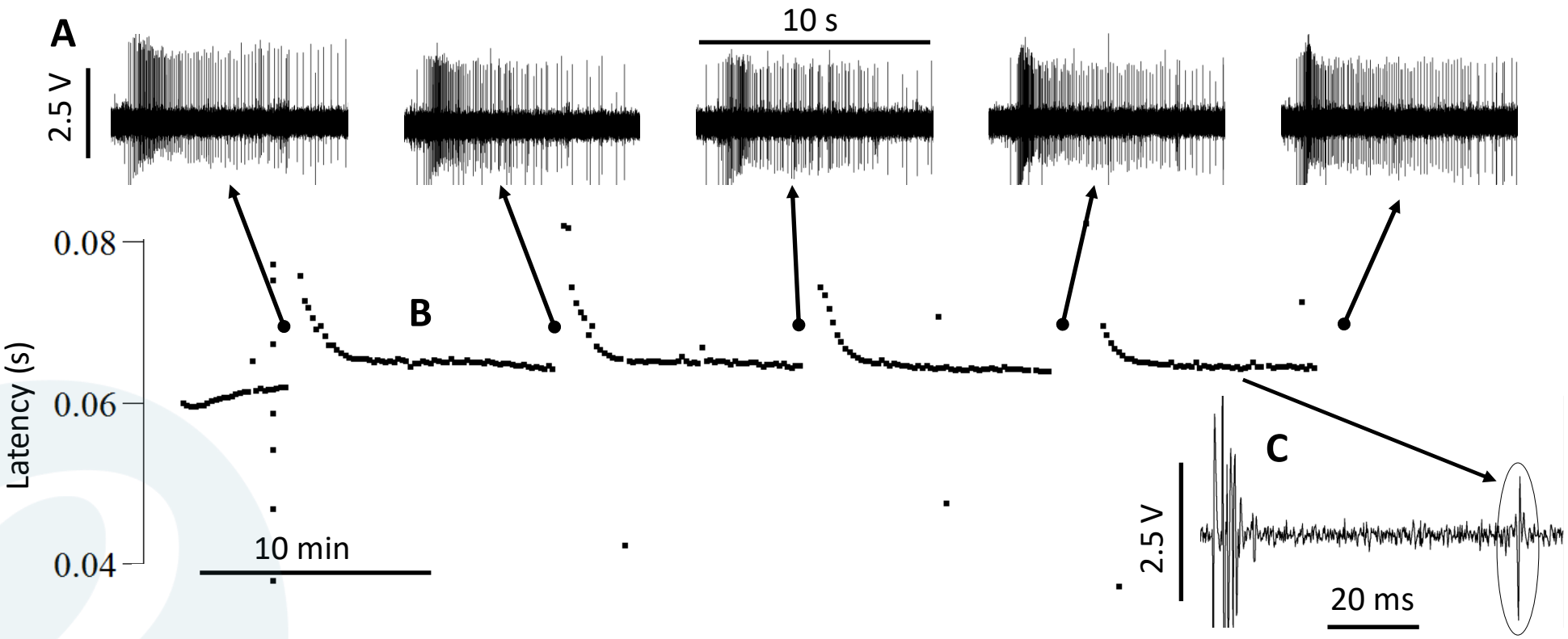
Examples of initial characterization (legend on following slide)



Examples of initial characterization: legend

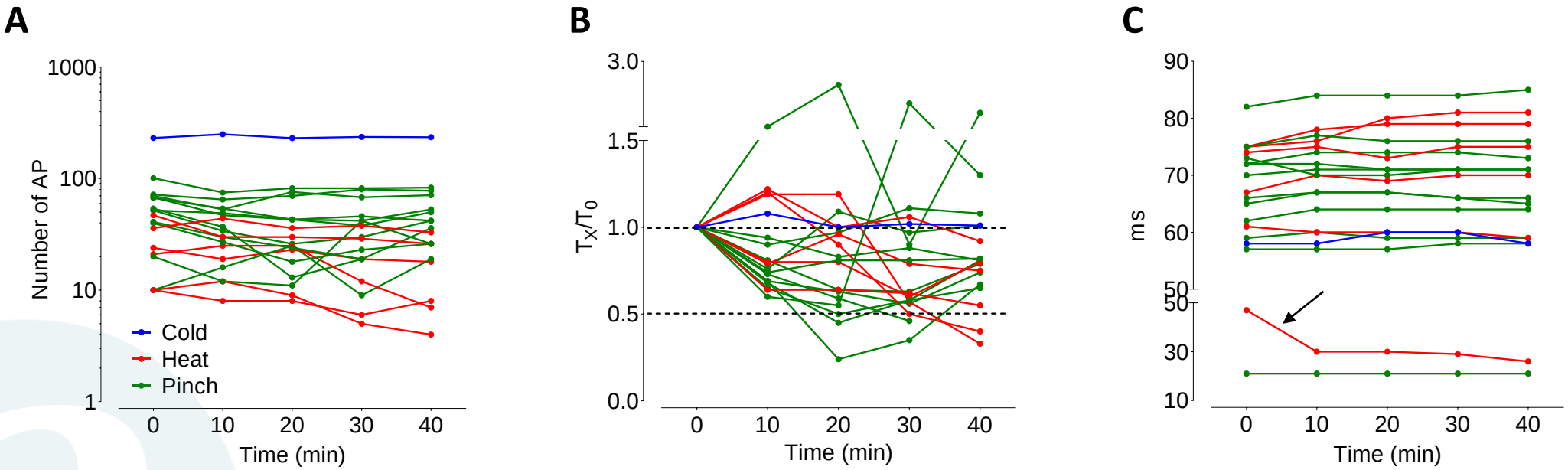
- General description
  - **A**, **B** and **C** correspond to the initial characterization of 3 different DRG neurons recorded in the present study.
  - The recording on the left hand side shows the overlay of 5 successive action potentials generated by an electrical stimulation of the receptive field, and the location of the receptive field on the hind paw.
  - The illustration on the right hand side shows the response to mechanical (10 strokes with hog brush, Von Frey (VF) and pinch with micro haemostat clamp applied for 6 s) and thermal stimulations (10 ml water jet (WJ)) for the considered DRG neuron.
  - Bottom line, raw electrical activity (expressed after 20 k gain); middle line, action potentials generated by the considered DRG neuron "spike sorted" from the raw electrical activity (expressed after 20 k gain); upper line, peristimulus histogram showing the firing frequency in 0.1 s bin.
  - Additional visual check by the operator and correction when necessary is always performed to ensure accuracy of the automated spike sorting from the raw (bottom line) to the filtered (middle line) signal. Such correction is not possible on this illustration.
- Comment
  - **A**: cold-specific nociceptor (conduction velocity, 1.1 m/s). There is a marked response to WJ at 0 °C (the duration of the WJ is approximately 2-3 s, whereas the duration of the response exceeds 20 s). There is no response to any other stimuli. Note that mechanical and thermal stimulations induced responses from other neurons close to the recording site (e.g. arrows for VF 25 mN and WJ 46 °C). Yet, the response of the cold nociceptor could be unambiguously quantified.
  - **B**: mechanical nociceptor (conduction velocity, 0.86 m/s). Only Von Frey at 200 mN and pinch elicited noticeable responses. The 2 action potentials generated by WJ 50 °C is not considered sufficient a response to classify the neuron as polymodal.
  - **C**: polymodal nociceptor (conduction velocity, 0.88 m/s). There was a marked response to VF 200 mN, pinch and WJ 50 °C. Note the difference in magnitude of the response to pinch (most noxious mechanical stimulus) and WJ 50 °C (most noxious thermal stimulus).

Combined illustrations for an experiment



- All illustrations are extracted from one experiment following the protocol in the previous slide.
- **A**: analogue recordings showing the responses to the 5 pinches applied every 10 min.
- **B**: raster plot of the latency of the action potential generated by the electrical stimulations applied between the pinches (1 pulse every 10 s). Note the activity-dependent slowing of the conduction velocity after each pinch.
- **C**: analogue recording showing the response to an electrical stimulation.
- Amplitude after 20 k gain.

Desensitization to repetitive mechanical and thermal stimuli



- Each neuron was tracked for 40 min in 18 experiments, and 30 min in 1 experiment.
- **A**: number of action potentials generated by the successive stimulations in 19 experiments. Either pinch, WJ at 0 °C or 52 °C was applied (same stimulus repeated 5 times for each neuron).
- **B**: ratio of the response over baseline (i.e. computed from the data in **A**). Note that there was a marked run down of the response for some neurons.
- **C**: latency of the action potential generated by the electrical stimulations applied between the noxious stimuli. Except in one experiment (arrow), the latency (i.e. the conduction velocity) was remarkably stable throughout the duration of the recording.

Physiological parameters

pH	pCO_2 mmHg	pO_2 mmHg	Hct %	BP mmHg
7.44 (0.07)	30 (4)	169 (33)	42 (3)	76 (13)

- Blood gas analysis was performed at completion of the experiment.
- Data expressed as mean (SD), n=19.
- Parameters are within physiological range, but pO_2, because of the adjunction of extra O_2 in the gas carrier. The current notion is that a pO_2 above physiological range is acceptable, whereas a pO_2 below physiological range is detrimental.

Conclusion

- Summary
  - Recording of DRG neurons for 40 min can be achieved with a reasonable success rate (at least 80 % as we assume that there is room for improvement in our technique).
  - Response to noxious stimuli (mechanical or thermal) tended to decrease upon repeated applications every 10 min for some neurons.
  - Responses to electrical stimulations were stable throughout the experiment.

- Desensitization of unmyelinated DRG neurons: physiology or phenomenology?
  - We noticed a marked desensitization of lamina I spinoparabrachial neurons upon repeated noxious stimulations (pinch and WJ 50 °) in identical experimental conditions (Allard, J Physiol, 2019). In contrast, a class of lamina III-V “dynamic but not necessarily wide range” neurons with restricted receptive fields (which we routinely use for acute pharmacological testing) display rather stable responses to repeated noxious stimulations in the same experimental conditions.
  - A tentative explanation would be that the main component of the response to *acute* noxious stimuli is generated by thinly myelinated Aδ DRG nociceptor (not measured here). Unmyelinated DRG neurons would play a more important role in the generation of long term *pathophysiological* pain.
  - A comparison of the response of C and Aδ DRG nociceptors upon repeated stimulations is mandatory to test this hypothesis.

julien.allard@e-phys.com; +33 (0)645071316; www.e-phys.com