# Recording of nociceptors In the anesthetized mice

Minor effect of a single dose of 6 mg/kg oxaliplatin at day 3 and 4 post-injection on the activity of unmyelinated DRG neurons

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# METHOD

## Recording of DRG neurons

- Isoflurane anaesthesia, artificial ventilation, control of core body temperature and blood pressure.
- Electrode inserted in the DRG;  search based on orthodromic electrical stimulation of the hind paw.
- All neurons with conduction velocity <2.5 m/s are included.
- Measure of spontaneous activity, and activity induced by non-noxious and noxious mechanical and thermal stimulations of the receptive field on the hind paw.
- Mechanical stimulations: camel and hog brush (10 strokes each); VF 25, 50 , 100 and 200 mN (6 s); pinch with 2 different micro haemostat clamps (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 pinch post-responses; whole duration for thermal responses (from 2 to 50 s).
- Blood gas analysis at completion of the experiment.

## Experimental design

- Eight-9 weeks old male C57BL6 mice injected with 6 mg/kg oxaliplatin or the corresponding vehicle (5 % glucose).
- Von Frey test before injection (baseline) and at day 3-4 post-injection. DRG recording at day 3-4 post-injection.
- Sixteen vehicle and 20 oxaliplatin-treated mice were experimented.
- Experimenters were blind to the treatment.
- Goal was to characterize 3 unmyelinated nociceptors in each mice

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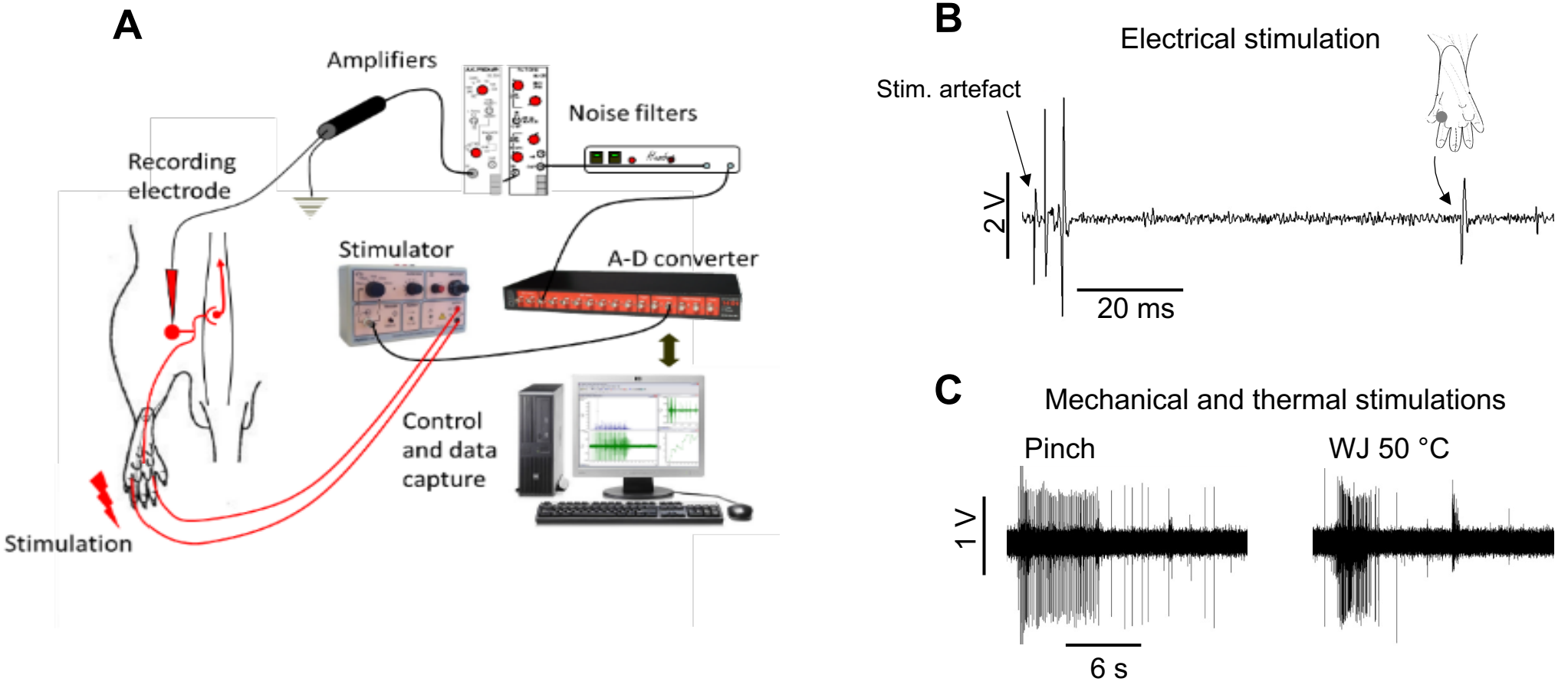
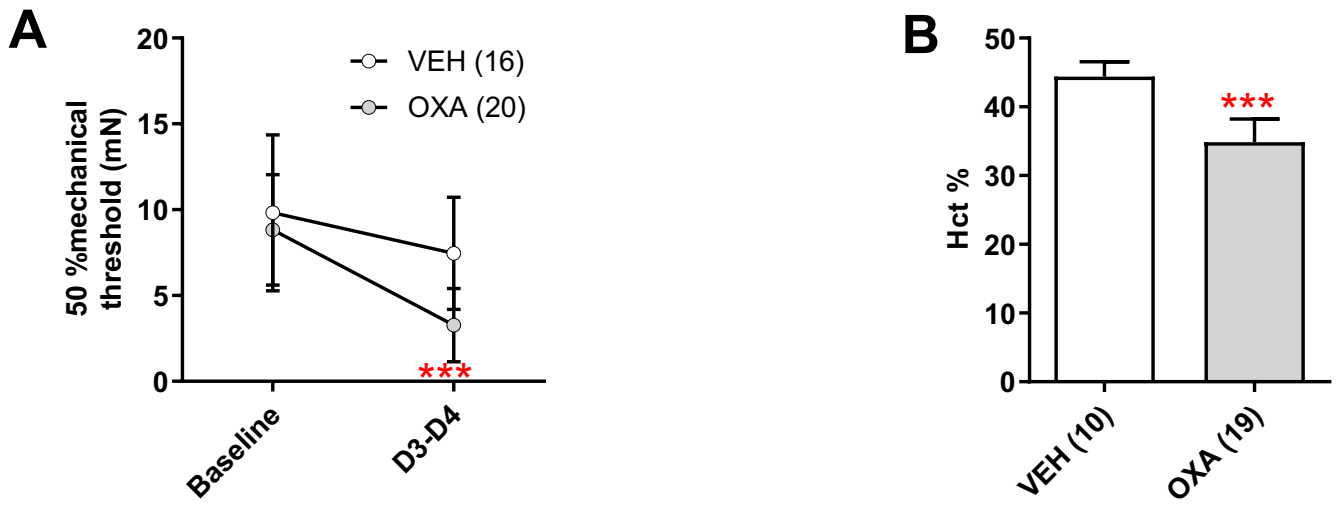
# SET UP AND BASIC RECORDING ILLUSTRATION




- **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.

# OXALIPLATIN INDUCES ALLODYNIA AND ANAEMIA




**All data expressed as mean ± SD**

**A: Von Frey**

- All mice underwent VF testing.
- The 50% mechanical threshold was significantly decreased in oxaliplatin-treated mice (2 way ANOVA with repeated measures, $p<0.001$).

**B: haematocrit (Hct)**

- Hct was measured in 10 vehicle-treated and 19 oxaliplatin-treated mice after DRG recording.
- Hct was significantly decreased in oxaliplatin-treated mice ($p<0.001$, Mann-Whitney rank sum test).

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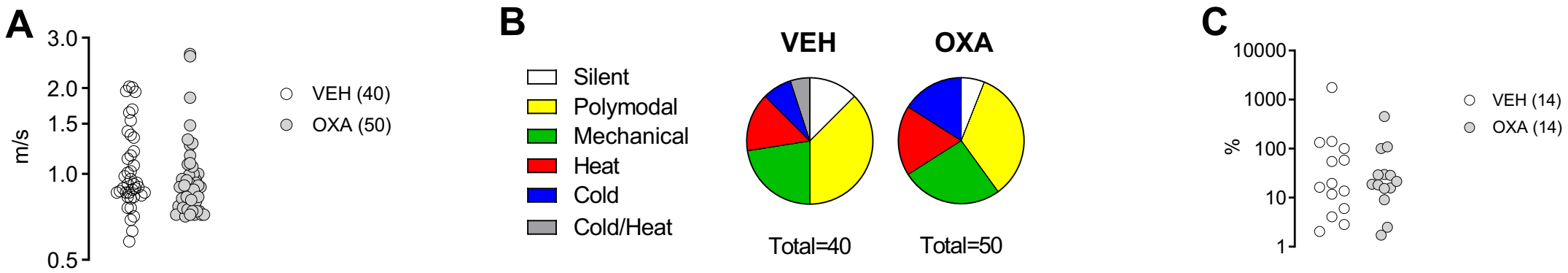
# CONDUCTION VELOCITY AND MODALITY DISTRIBUTION




## A: conduction velocity

- Conduction velocity was <2.5 m/s but for 2 mice in the oxaliplatin group (2.58 and 2.63 m/s).
- The median value of the CV was 0.92 and 0.91 m/s in the vehicle and oxaliplatin group, respectively.

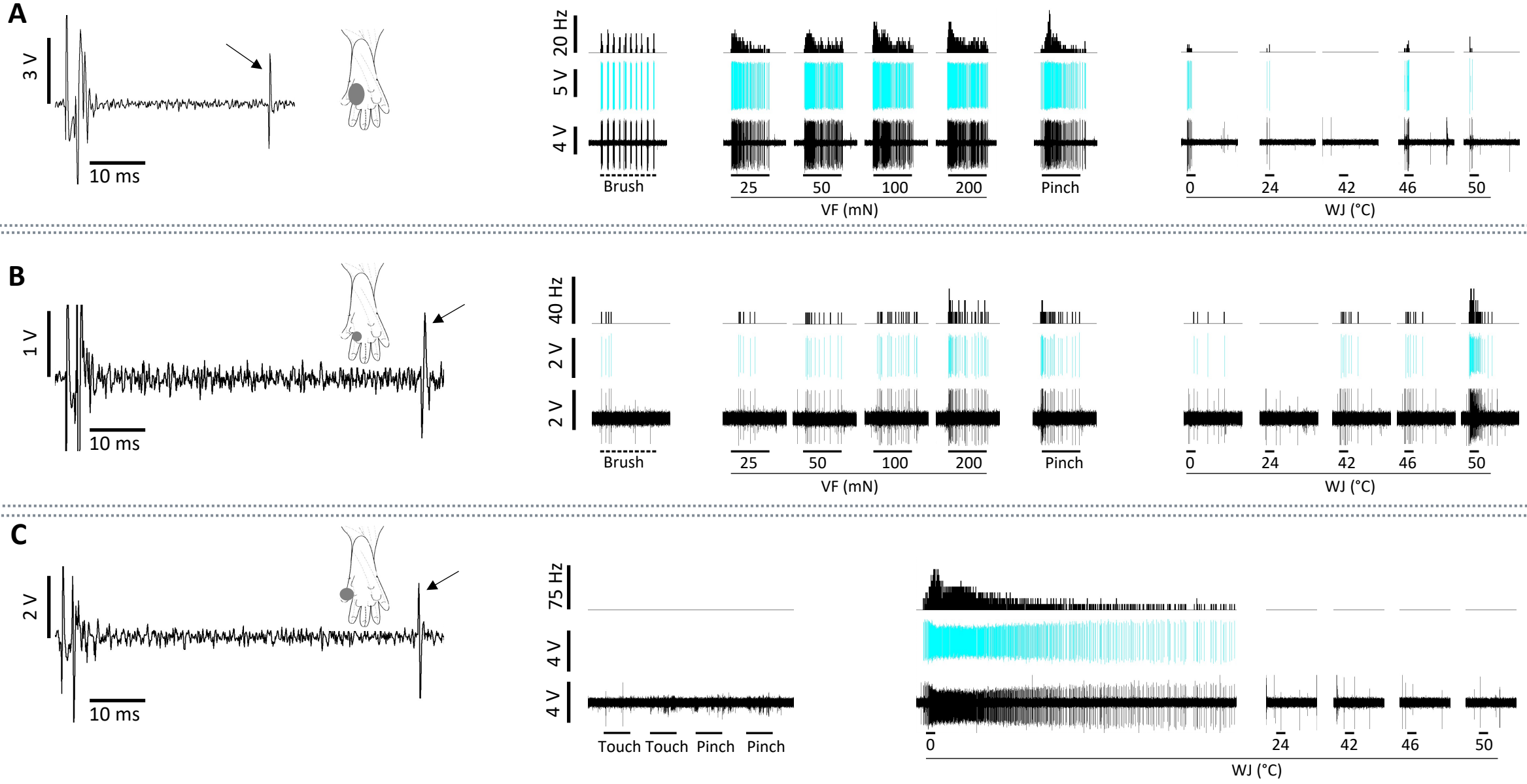
## B: modality distribution of nociceptors

- Objective classification of the modality of the nociceptors encountered should be discussed (e.g. many “bloody obvious mechano-specific nociceptors” display a minor response to cold). Chi-square test did not show significant difference in the distribution between the 2 groups.

## C: ratio of heat/pinch response of polymodal nociceptors

- Polymodal nociceptors (i.e. responding to noxious mechanical and thermal stimuli) represent a highly heterogeneous population. As an illustration, the ratio of the response to WJ 50 °C over response to hard pinch was plotted for polymodal nociceptors for vehicle and oxaliplatin-treated mice. Note that the response to heat represented a very variable fraction of the response to pinch.

# EXAMPLES OF RECORDINGS (legend on following slide)



# EXAMPLES OF RECORDINGS: LEGEND

## General description

- **A**, **B** and **C** correspond to 3 different recordings obtained in the present study.
- The recording on the left hand side shows the action potential 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 filtered from the raw electrical activity (expressed after 20 k gain); upper line, peristimulus histogram showing the firing frequency in 0.1 s bin.

## Comment

- Recording in **A** (unit V39) was obtained from a vehicle treated mice and corresponds to a C (conduction velocity, 1.93 m/s), low threshold mechanoreceptor (C LTMR). Note the distinct firing to each brush stroke, the sustained and marked response to VF 25 mN, and the poor firing rate  increase in the 25 mN-pinch range (the set of mechanical stimuli presently used is designed to characterize nociceptors). Responses to thermal stimuli are poor (one may question whether the response to WJ 0 °C is due to cooling or due to the force of the water jet applied on the RF).
- Recording in **B** (unit V20) corresponds to a C (conduction velocity, 0.96 m/s) polymodal nociceptor. Note the erratic response to brush (compare with **A**), and the progressive increase of the response with the noxiousness of the stimulus applied (mechanical or thermal).
- Recording in **C** (unit V22) corresponds to a C (conduction velocity, 0.92 m/s) cold-specific nociceptor. 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 50 s), but no response to any other stimuli. The exact location of the receptive field on the digit could not be determined. However, touching and pinching at different locations along the digit failed to elicit any action potential.

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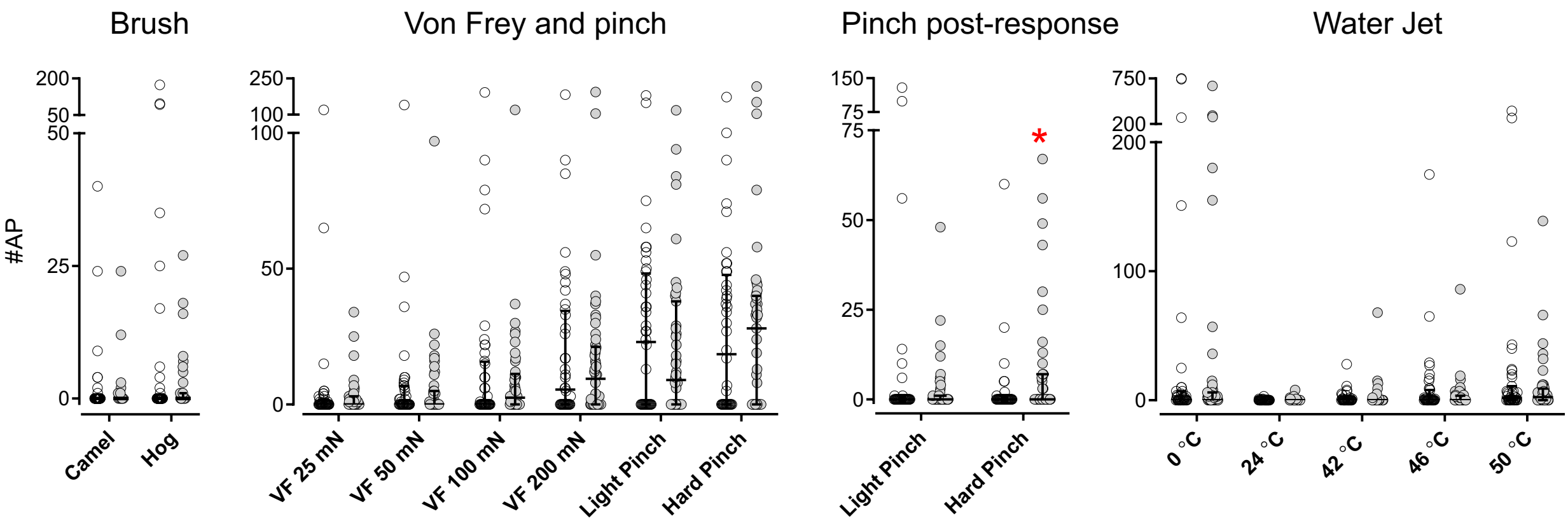
# POOLED RESPONSES




- Responses of all characterized neurons are plotted; dot plots with 25$^{th}$, median and 75$^{th}$ percentile.
- n=40 and 50 in the vehicle and oxaliplatin groups, respectively.
- Spontaneous activity was present in 1 polymodal neuron in the vehicle group, and in 3 cold and 1 mechanical neuron in the oxaliplatin group.
- Post-response to hard pinch was significantly increased in the oxaliplatin group ($p<0.05$, Mann-Whitney rank sum test).
- Note that the comparison is hampered by the mix of neurons with different specific modalities. Yet, this is an unbiased comparison.

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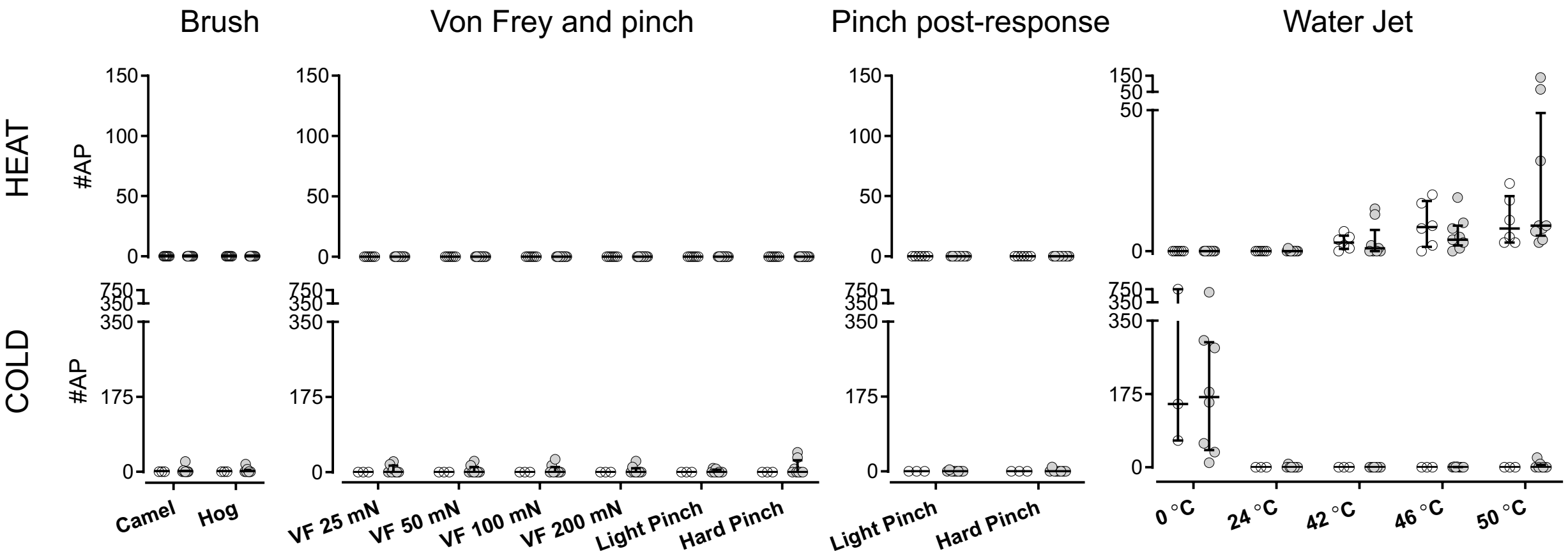
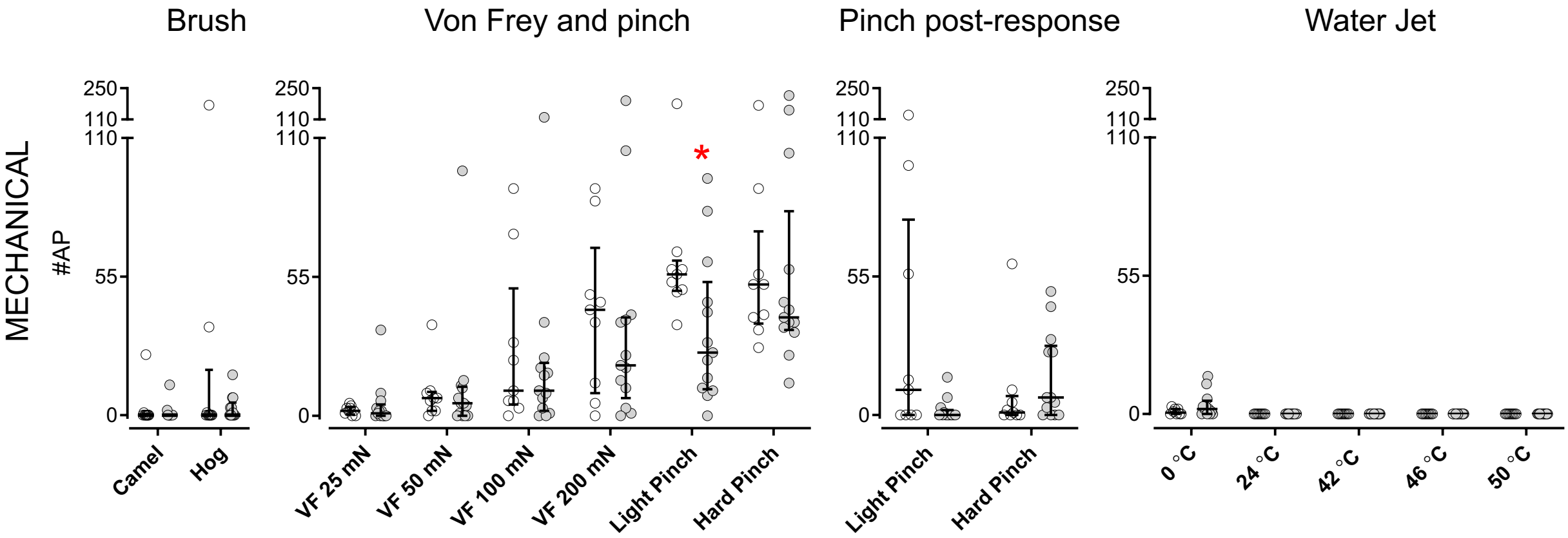
# MODALITY SPECIFIC NEURONS: COLD AND HEAT




- Dot plots with 25th, median and 75th percentile.
- Heat, n=6 and 9 in the vehicle and oxaliplatin group, respectively; Cold, n=3 and 8, respectively.
- Heat neurons are strictly modality specific, whereas cold neurons are characterized by extreme response to WJ 0 °C compared to any other stimulus.
- There was no statistical difference for corresponding responses between groups.

# MODALITY SPECIFIC NEURONS: MECHANICAL




- Dot plots with 25th, median and 75th percentile.
- n=9 and 13 in the vehicle and oxaliplatin groups, respectively.
- Mechanical neurons do not respond to warm and heat, but a minor response to cold is occasionally present.
- Response to light pinch was significantly decreased in the oxaliplatin group ($p<0.05$, Mann-Whitney rank sum test).

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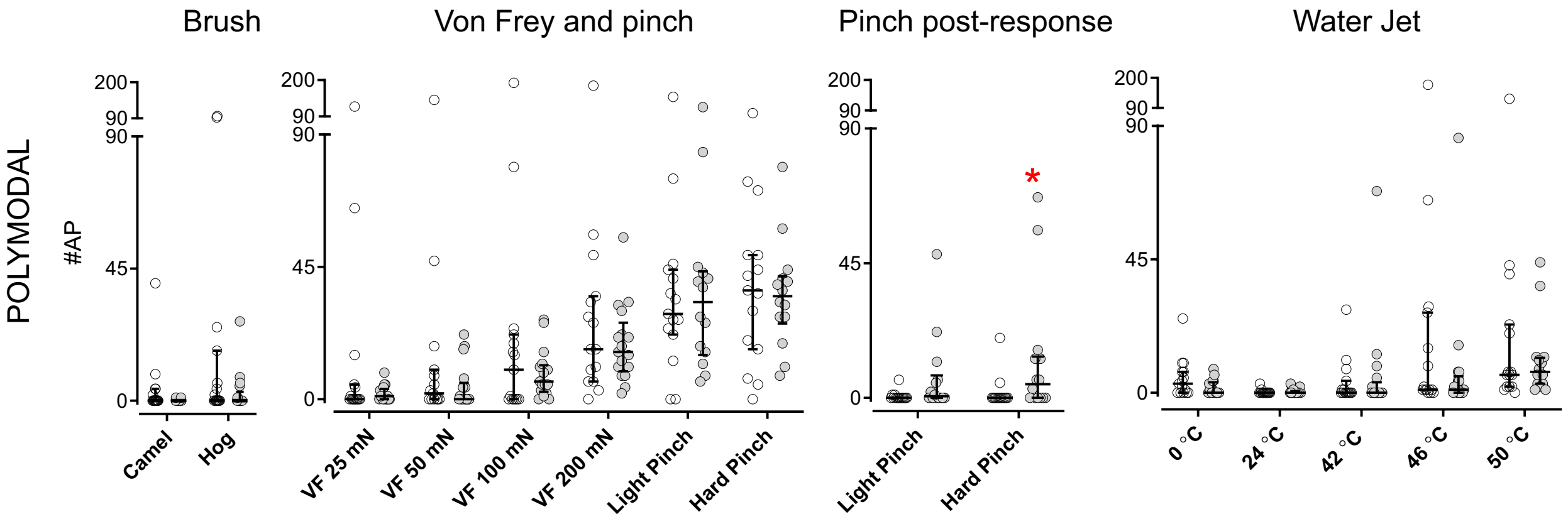
# MODALITY SPECIFIC NEURONS: POLYMODAL




- Dot plots with 25th, median and 75th percentile.
- n=15 and 17 in the vehicle and oxaliplatin groups, respectively
- Compared to mechanical neurons, polymodal neurons are characterized by the additional response to warm and heat.
- Post-response to hard pinch was significantly increased in the oxaliplatin group ($p<0.05$, Mann-Whitney rank sum test).

# PHYSIOLOGICAL PARAMETERS

| | pH | $pCO_2$ mmHg | $pO_2$ mmHg | BP mmHg |
|---|---|---|---|---|
| Vehicle | 7.37 (0.06) | 35 (5) | 158 (63) | 76 (13) |
| Oxaliplatin | 7.42 (0.05) | 33 (5) | 164 (52) | 73 (11) |

- Blood gas analysis was performed at completion of the experiment.
- Data expressed as mean (SD).
- For pH, $pCO_2$ and $pO_2$, n=10 and 19 in the vehicle and oxaliplatin groups, respectively.
- For blood pressure (BP), n=38 and 49 in the vehicle and oxaliplatin groups, respectively.
- Results for haematocrit are shown in slide #4.
- There was a significant difference in blood pH in the 2 groups ($p<0.05$, t-test). This might be related to the difference in $pCO_2$ in the 2 groups (in normal physiological conditions, pH is inversely correlated with $pCO_2$).

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# CONCLUSION

## Summary

- Forty and 50 unmyelinated DRG neurons were characterized in vehicle and oxaliplatin-treated mice, respectively.
- Apart from anecdotal observations (presence of marked spontaneous activity in 3 cold neurons and significant increase of hard pinch post-response in the oxaliplatin group ), there was no effect of oxaliplatin on the activity of unmyelinated DRG neurons.

## No effect or inability to detect an effect of oxaliplatin?

- The widespread location and the extremely small size of the receptive field of nociceptors make a “reproducible/comparable” application of the mechanical stimuli from one nociceptor to another uncertain (e.g. application of Von Frey on a fold of skin on the edge of the paw versus the centre of the palm of the paw). There is less uncertainty with the application of water jet.
- The natural phenotypic variation of DRG neurons makes sampling of some phenotypes and hence their comparison between groups limited (only 3 cold neurons were sampled out of the 40 neurons characterized in the vehicle group!).
- Sensitization of lamina I spinoparabrachial neurons upon chronic inflammation has been shown in identical experimental conditions (overall animal preparation, type of anaesthetic, use of artificial ventilation, stimuli application, etc…), suggesting that the present experimental conditions are appropriate for the study of peripheral and central pain pathways (but may be not to assess the effects of oxaliplatin…).

## Literature perspective

- Regarding response to cold, the present result indirectly confirms the selective activation by oxaliplatin of myelinated (Aβ or Aδ?), previously cold-insensitive nociceptors (see for example Deuis et al, Pain, 2013 and MacDonald et al., Brain, 2021; albeit not measured here, cold allodynia is a robust outcome of the present oxaliplatin treatment in mice in our hands).
- We do not know whether oxaliplatin-induced mechanical allodynia is due to the selective activation/modification of myelinated nociceptors (as seems to be the case for cold allodynia). Should mechanical allodynia be induced by selective changes in the activity of unmyelinated nociceptors (e.g. decrease mechanical threshold), this would point to some limitations of the present approach.

## Next

- Perform a similar experiment focused on myelinated DRG neurons.

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# Contact us


**Raymond Price, PhD, EMBA**
**Chief Business Officer**
Raymond.price@neuroservices-alliance.com
Mobile - +1 (858) 649 9403



**Julien Allard, PhD**
**CSO,**
**In Vivo Spinal Cord & DRG Electrophysiology**
julien.allard@neuroservices-alliance.com
Mobile - +33 (0) 6 45 07 13 16


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