Chronic constriction injury of the sciatic nerve results in limited changes of polymodal spinal convergent neurons responses in the anaesthetized rat

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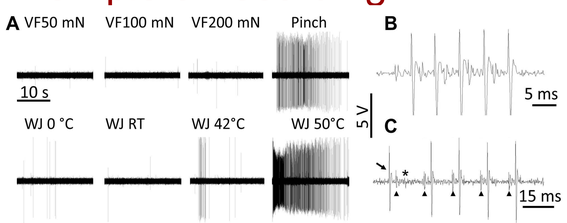
Introduction

- The use of appropriate preclinical pathophysiological model is essential for the design of efficient analgesics. Chronic Constriction Injury (CCI) of the sciatic nerve is a preclinical model of neuropathic condition[1], usually used in rats in behavioural experiments.
- Electrophysiological measures can overcome the limitations of behavioural experiments, namely the mismatch between supposedly pain-related behavioural responses and pain perception[2], the impossibility to exert and record responses to highly and sustained noxious stimuli, and the difficulty to measure spontaneous pain.
- The activity of spinal projection neurons involved in pain process reflects the balance of peripheral noxious input and descending activation/inhibition from the brain. The activity of spinal neurons in CCI rats has been characterised in a number of studies, without establishing clear quantitative correlation between spinal neuron activity and behavioural responses[3]. The aim of the present work was to characterise the activity of polymodal spinal neurons in CCI rats in order to compare results from behavioural and electrophysiological experiments.

Experimental design

- Neuropathic condition was induced by loose ligation of the sciatic nerve in male rats. SHAM surgery was used as control.
- Behavioural testing was performed 6 days after surgery. Activity of spinal neurons innervating the glabrous skin of the hind paw were recorded under anaesthesia 10-15 days after surgery in the same animals, on both the ipsilateral and contralateral side to the surgery.
- Two independent, similar, experiments were performed successively including 10 CCI and SHAM rats each (i.e. 40 rats in total). Surgery and behavioural evaluations were identical in the 2 experiments.
- Electrophysiological recording focussed on wide dynamic range (WDR) and nociceptive specific (NS) neuron in experiment 1 and 2, respectively. Projection neurons (PN) included in experiment 2 were identified by antidromic stimulation from the cervical cord.

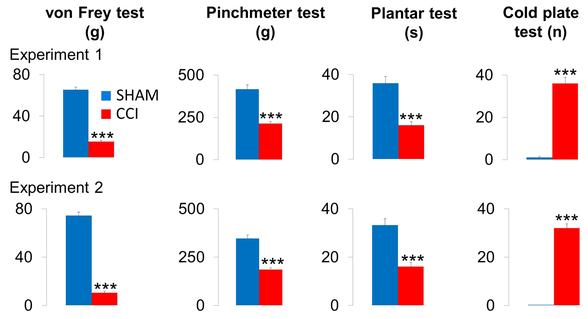
Example of recording



- **A**: responses of a nociceptive specific neuron to mechanical and thermal stimulation. **B**: 1-for-1 following of a train of 5 antidromic electrical stimuli at 200 Hz. **C**: collision of a pulse in a train of 5 (60 Hz) when an orthodromic impulse (arrow) occurred within the critical interval (arrow heads, antidromic stimulus artefacts; star, theoretical position of the collided antidromic action potential).
- VF: von Frey hair; WJ: water jet.

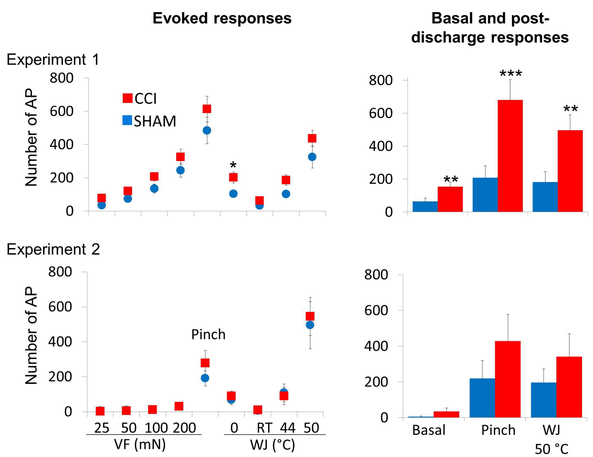
Results

Figure 1 : CCI rats displayed significant hypersensitivity to mechanical and thermal stimuli compared with SHAM rats



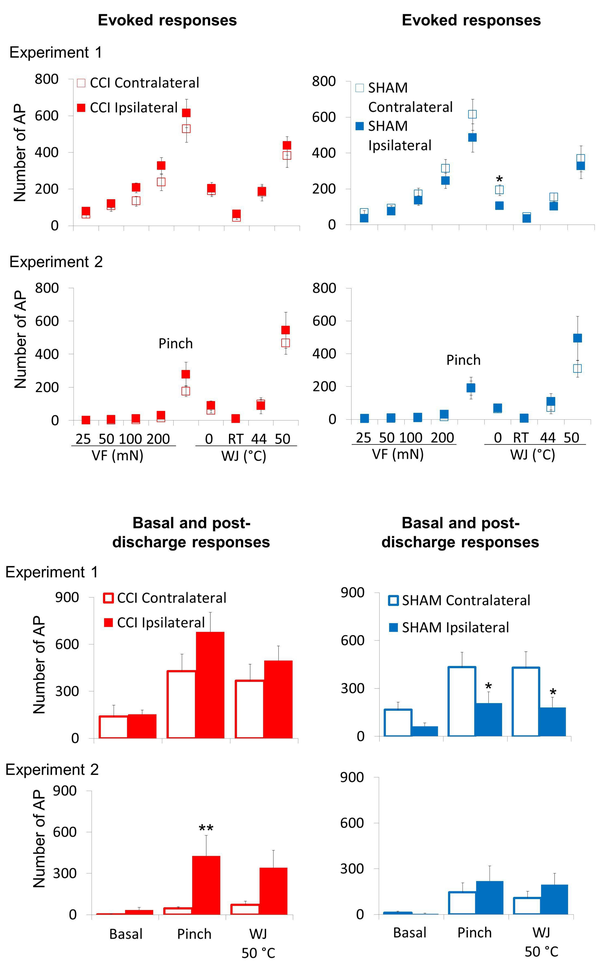
- Measures: force inducing withdrawal responses (in g equivalent) for the von Frey and pinchmeter tests, latency of the withdrawal responses for the plantar test (s), and number of withdrawal responses for the cold plate test.
- There was virtually no difference for all measures between the ipsilateral and contralateral paw in SHAM rats, and between the contralateral paws in CCI and SHAM rats (not shown).
- N=10 for all measures. ***: p<0.001, t-test.

Figure 2: post-discharge responses of spinal neurons were increased in CCI rats compared to SHAM rats



- *, p<0.05; **, p<0.01; ***, p<0.001. Mann-Whitney rank sum test.
- Number of neurons: experiment 1, CCI, n=23 and SHAM, n=19; experiment 2, CCI, n=20 (9 PN) and SHAM, n=22 (11 PN).

Figure 3: comparison of the ipsilateral versus contralateral side responses within CCI or SHAM rats did not evidence differences for evoked spinal neurons responses



- *, p<0.05; **, p<0.01. Mann-Whitney rank sum test.
- Number of neurons in experiment 1: CCI ipsilateral/contralateral, n=23/20; SHAM ipsilateral/contralateral, n=19/21.
- Number of neurons in experiment 2: CCI ipsilateral/contralateral, n=20 (9 PN)/21 (6 PN); SHAM ipsilateral/contralateral, n=22 (11 PN)/20 (6 PN).

Methods

- Tactile allodynia and hyperalgesia were performed using electronic von Frey and pinchmeter tests respectively; thermal allodynia and hyperalgesia were evaluated using cold plate and plantar tests respectively[4].
- Extracellular recordings were obtained with tungsten microelectrodes, 2-5 MΩ impedance.
- Rats were anaesthetized with pentobarbital and isoflurane in experiment 1 and 2, respectively. A laminectomy was performed at the lumbosacral level for recording. In experiment 2, a second laminectomy was performed at the level of the cervical enlargement for antidromic stimulation.
- Rats were paralysed with gallamine triethiodide and artificially ventilated. End tidal carbon dioxide, blood pressure and body temperature were monitored throughout the experiments.
- Mechanical stimuli consisted of application of VF at 25, 50, 100 and 200 mN or pinch with mini haemostats clamp (applied for 6 s). Thermal stimuli consisted of WJ (10 ml) at room temperature (RT), 0, 44 and 50 °C. Evoked responses were quantified for 5 s, and basal and post-discharge responses for 1 min.
- Up to 6 spinal neurons were characterized per rat.

Conclusions

- CCI rats displayed a marked behavioural mechanical and thermal hypersensitivity on the treated side compared to SHAM rats. In contrast, evoked responses of spinal neurons on the treated side in CCI rats were only marginally increased when compared to SHAM rats, or when compared to the contralateral side in the same rat.
- In experiment 1 (WDR neurons), basal and post-discharge responses were increased in CCI rats compared with SHAM rats but there was little difference when comparing the ipsilateral and contralateral side. In experiment 2 (NS neurons), post-discharge responses of spinal neurons were more clearly increased in CCI rats on the ipsilateral side compared to the contralateral side.
- The mismatch between behavioural and electrophysiological responses cannot be explained by an increase of the receptive field size of spinal neurons (which has not been observed), or by the sensitization of supraspinal structures involved in the pain process (which has not been demonstrated yet, see reference 5 for such a demonstration in the model of spinal nerve ligation).
- A possible explanation is that responses of projection neurons involved in pain perception in CCI rats are indeed significantly increased compared with SHAM rat[3,6], but that the present results were blurred by the mixing of data obtained from projection neurons and interneurons.
- We plan to conduct additional experiments studying the correlation of electrophysiological and behavioural responses by focusing on the characterisation of clearly identified projection neurons.

References

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