Lamina I spinoparabrachial neurons: sensitization to noxious stimuli in rats with chronic constriction injury of the sciatic nerve (Bennett/CCI model) and poor response to pregabalin

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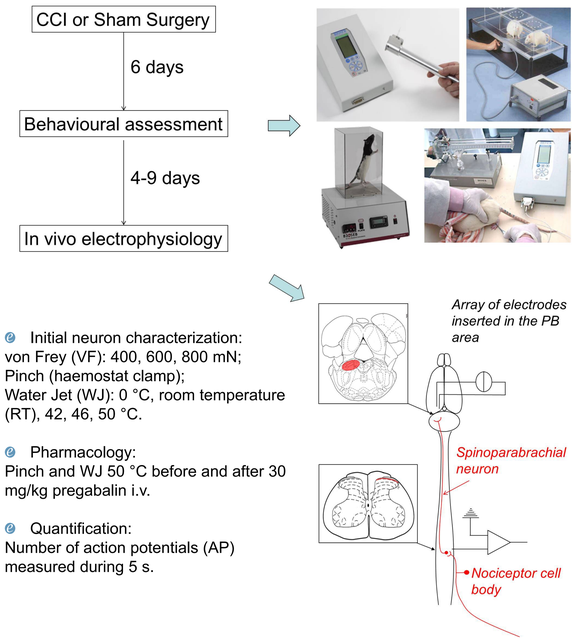
Introduction

- Lamina I of the spinal cord contains a population of projection neurons apparently essential for the generation of acute pain sensation[1]. In contrast to the Wide Dynamic Range neurons (WDR) classically located in deeper laminae, most of these neurons are selectively activated by noxious stimuli (nociceptive specific, NS), and some are modality specific. The relative importance of lamina V WDR versus lamina I NS neurons in the coding of pain sensation is a matter of intense debates (see discussion in 2).
- A number of publications have highlighted the implication of lamina I spinal projection neurons in chronic pain state (see for example 3 and 4). Most of these studies used behavioural experiments and in vitro/ex vivo preparations. Few studies tackled the arduous task of recording lamina I projection neurons in vivo[5-7].
- Our objective is to establish in vivo electrophysiological models for the screening of drugs aiming at treating neuropathic pain. In our previous study, when WDR or NS neurons were "randomly" sampled in the spinal cord, there was no difference in the responses obtained between rats with Chronic Constriction Injury (CCI[8]) or SHAM operation of the sciatic nerve. The present work aimed to characterise the activity of a more defined population of spinal neurons in CCI and SHAM rats, namely the lamina I spinoparabrachial (SPB) neurons, and to assess the effect of pregabalin on these neurons.

Experimental design



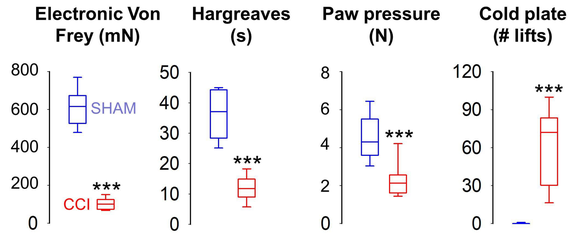
- Initial neuron characterization:
von Frey (VF): 400, 600, 800 mN;
Pinch (haemostat clamp);
Water Jet (WJ): 0 °C, room temperature (RT), 42, 46, 50 °C.

- Pharmacology:
Pinch and WJ 50 °C before and after 30 mg/kg pregabalin i.v.

- Quantification:
Number of action potentials (AP) measured during 5 s.

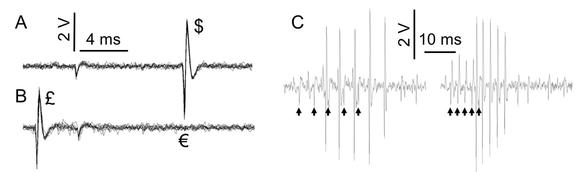
Results

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

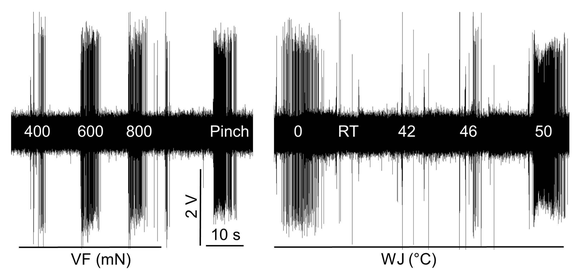


- Quantifications: force inducing withdrawal response for the von Frey and paw pressure tests, latency of the withdrawal response for the Hargreaves test, and number of paw lifts for the cold plate test.
- There was no difference for all measures between the ipsilateral and contralateral paw in SHAM rats or between the contralateral paw in CCI and SHAM rats (not shown).
- Graphs: median and 10th, 25th, 75th and 90th percentiles for data in each group. N=20 for all measures. ***: p<0.001, Mann-Whitney Rank Sum test.

2 lamina I SPB neuron recording

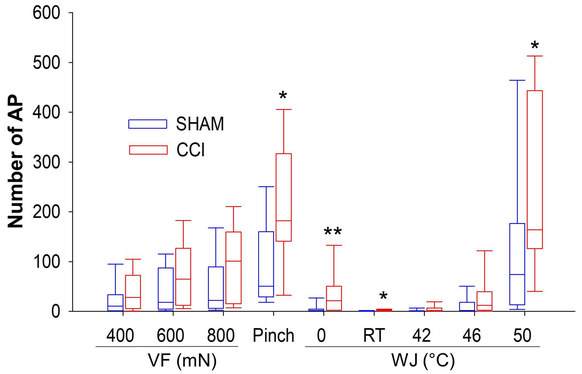


- A: superimposition of responses to 10 "direct" stimulations of the PB area ($: antidromic AP). B: superimposition of responses to 10 stimulations of the PB area triggered by an orthodromic AP (£): antidromic AP is no longer recorded (€). C: 1-for-1 following of a train of 5 antidromic stimulations at 200 and 400 Hz.



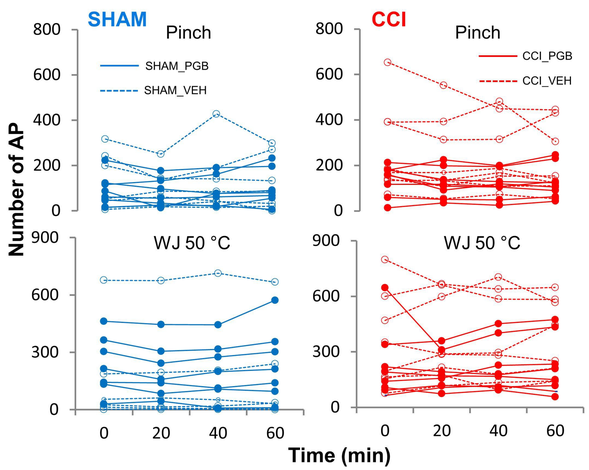
- Example of responses to mechanical and thermal stimuli.

3 Lamina I SPB neurons in CCI rats displayed enhanced responses to mechanical and thermal noxious stimuli



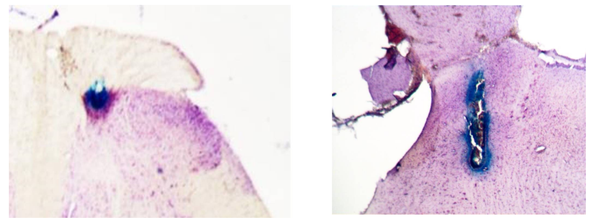
- The number of AP generated by pinch and WJ at °0 C, RT and 50 °C was significantly enhanced in CCI rats compared with SHAM rats.
- Graphs: median and 10th, 25th, 75th and 90th percentiles for data in each group. N=20 and 17 for CCI and SHAM rats. *: p<0.05, **: p<0.01, Mann-Whitney Rank Sum test.

4 Lamina I SPB neuron responses were insensitive to 30 mg/kg pregabalin (PGB)



- Rats were injected i.v. with 30 mg/kg pregabalin or vehicle after T0. Pinch and WJ 50 °C were applied every 20 min.
- Lines represent data obtained for each neuron studied.

5 Histological control



- Recording and stimulation sites were revealed using Perls Prussian blue staining. 23/29 of the recovered recording sites were located in lamina I.

Method essentials

- Male Wistar rats, isoflurane anaesthesia, monitoring of end tidal CO_2 and cardiovascular parameters, areflexia, vecurorium bromide paralysis, ventilation, stereotaxic frame, antidromic search, 2 MΩ recording microelectrode, open study.

Conclusions

- Evoked responses of lamina I SPB neurons to pinch and WJ 50 °C, i.e. prolonged suprathreshold noxious stimuli, were significantly increased in CCI rats compared to SHAM rats. Similar results were obtained in other vivo electrophysiological studies focussed on the impact of CCI on lamina I SPB neurons[6,7].
- Responses of lamina I SPB neurons to pinch and WJ 50 °C were unaffected by i.v. pregabalin at 30 mg/kg in CCI and SHAM rats. This may be in contradiction with the recognised analgesic activity of pregabalin. Nevertheless, this statement should be balanced by data showing that pregabalin failed to display analgesic activity in Von Frey and Hargreaves' test[9] and did not act directly at the spinal level[10,11].
- Other drug standards should be tested (e.g. tramadol, duloxetine) in the present electrophysiological paradigm to confirm that it can accurately predict the activity of drug candidates aiming at treating neuropathic pain.

References

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