Which subset of C-fibres contributes to the TTX resistant component of the spinal evoked field potential in rodents?

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Introduction

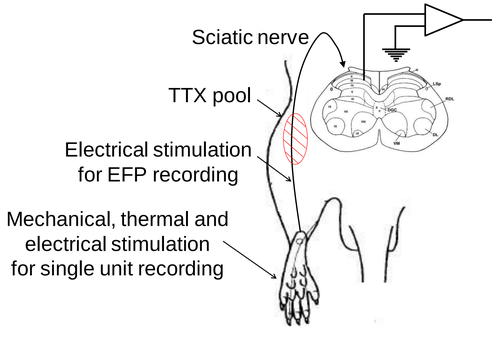
The expression of the voltage gated sodium channel 1.7 (Nav1.7) in nociceptors is mandatory for the generation of pain sensation[1]. Nav1.7 is supposed to play a role in the generation and transport of action potential at the peripheral end, and in synaptic transmission at the central end[2].

Compound action potentials measures performed in vitro and in vivo suggest that the conduction of action potentials in C-fibres along the sciatic nerve is essentially tetrodotoxin (**TTX**) sensitive[3,4]. This is in agreement with the view that Nav1.7 is essential for action potential propagation in C-fibre, as Nav1.7 is the predominant TTX sensitive Nav in nociceptors.

In contrast, Steffens and co-workers have demonstrated that the spinal C-fibre induced evoked field potential (**EFP**) in response to electrical stimulation (**ES**) of the sciatic nerve was insensitive to TTX applied on the sciatic nerve[5].

The present work attempted to explain how TTX, when applied on the sciatic nerve, can suppress the C-fibre compound action potential triggered by a distal ES while leaving intact the corresponding spinal EFP.

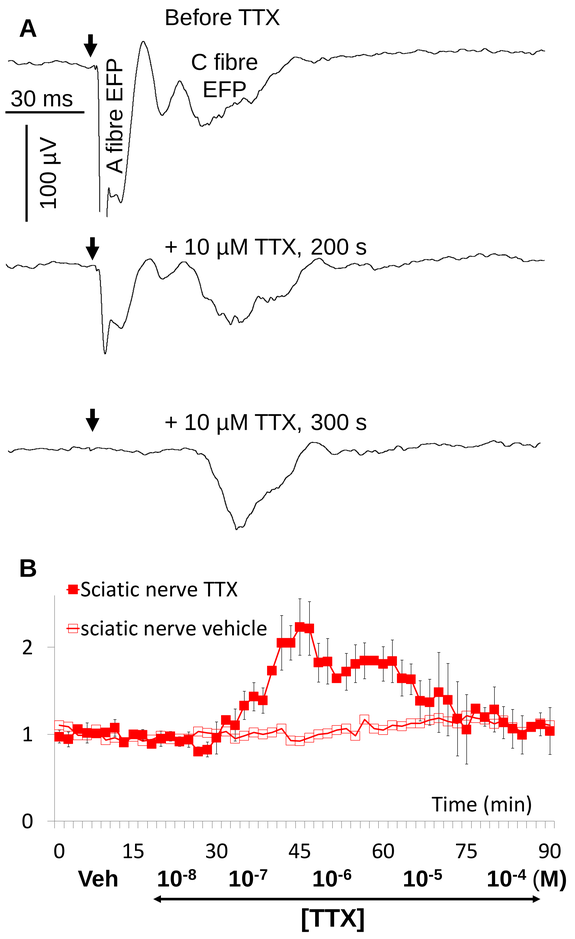
Experimental set up



- EFP and single unit activity in the spinal cord are measured using the same set up with different band pass filters.
- EFP is measured in response to ES of the sciatic nerve; single unit activity is measured in response to thermal and mechanical as well as well as ES of the receptive field on the hind paw.

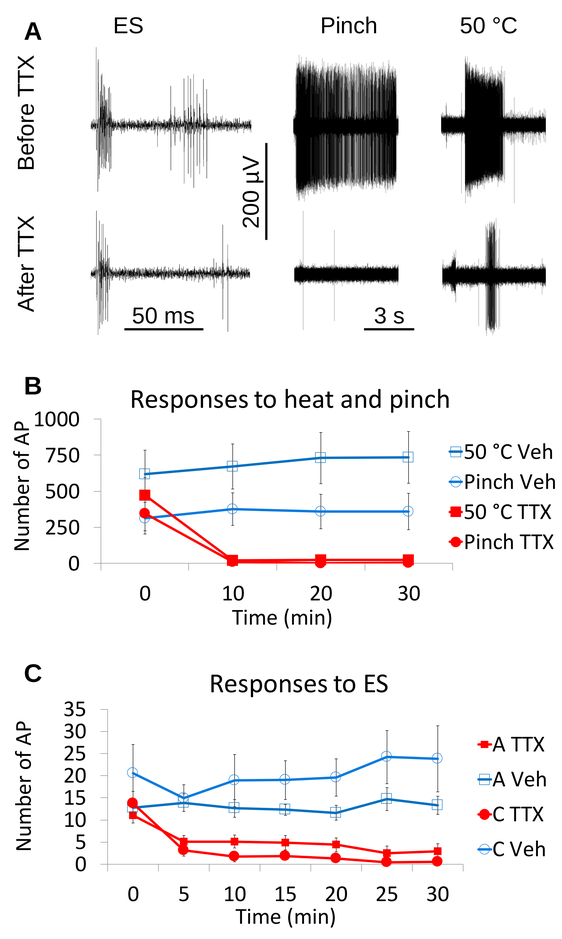
Results

Figure 1 : Spinal cord EFP induced by ES of the sciatic nerve



- **A**: spinal EFP recordings obtained before and at 2 different time points after application of 10 µM TTX on the sciatic nerve. Arrow: time of ES.
- **B**: quantification of the area under the curve of the C-fibre related EFP upon application of increasing TTX concentration every 15 min on the sciatic nerve (TTX, n=3; Vehicle, n=2).
- Note in **A** the persistence of the C-fibre related deflexion, in contrast with the disappearance of the A-fibre related deflexion.

Figure 2: Spinal cord single unit response to noxious stimuli of the receptive field



- **A**: response of a spinal single unit to noxious stimuli (ES, pinch and 50 °C water jet) before and 15 min after 1 µM TTX application on the sciatic nerve. Note in this example the existence of a residual response after TTX application.
- **B**: mean responses to 50 °C water jet and pinch every 10 min after application of 1 µM TTX on the sciatic nerve (TTX, n=6 ; Vehicle, n= 7). **C**: mean responses to ES of the receptive field in the same experiment. A and C refer to the action potentials generated by A- and C-fibres, respectively.

Methods

- Male C57BL6/J mice (20-30 g, Janvier Labs) were terminally anesthetized with urethane, paralyzed, and artificially ventilated.
- End tidal CO_2, blood pressure and body temperature were monitored throughout the experiments.
- Electrical activity was measured using a Neurolog set up (Amplification, NL100AK and NL104A; Filter, NL125/126) connected to 2 MΩ tungsten electrode.
- Recording parameters were 1 and 20 K gain, 1 Hz-1 KHz and 3-5 KHz band pass for EFP and single unit recording, respectively.
- For EFP, ES of the sciatic nerve were performed with bipolar Ag wire electrodes (0.5 ms, 0.1 to 2.0 mA). ES were conducted at 1/20 Hz and the average of 5 successive responses used for quantification.
- For single units, mechanical stimuli consisted of pinch with mini haemostats clamp (applied for 5 s), and thermal stimuli of water jet at 50 °C. Electrical stimulations (single square wave pulse, 2 ms, 5 mA) were delivered with 2 stainless steel needles inserted in the receptive field.

Conclusions

- In anesthetized mice, the C fibre component of the electrically induced EFP was insensitive to TTX applied on the sciatic nerve, confirming the original finding of Steffens and co-workers obtained in rat. The same treatment dramatically reduced or abolished responses to noxious stimuli of all single units recorded in the spinal cord.
- We hypothesize that ES of the sciatic nerve recruits a subset of TTX resistant fibres that are not recruited by stimulation of the receptive field on the glabrous skin.
- The physiological function of the TTX resistant fibre innervating the hind paw and contributing to the C fibres EFP remains to be determined.

References

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