A rodent *in vitro* sciatic nerve DRG and dorsal root preparation in naïve and CCI model animals

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❶ INTRODUCTION

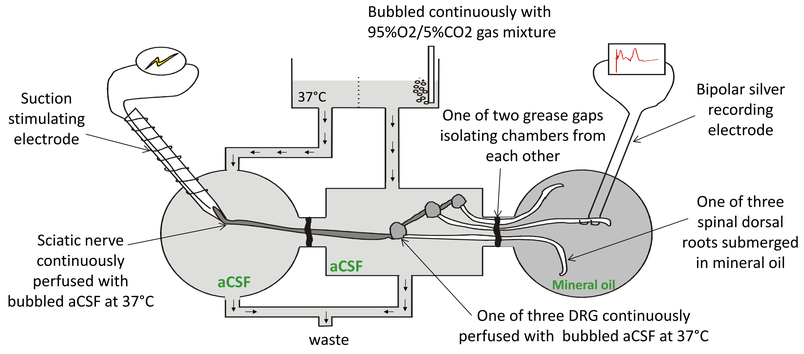
Pain remains a huge and largely unmet challenge worldwide and channelopathies that underlie a large number of pain conditions thus present an important target for the development of novel treatments acting at cell surface ion channels. Ion channels that regulate neuronal excitability (K), propagate action potentials (Na), control neurotransmitter release (Ca) and are involved in the immune responses (P2X) and sensory transduction (TRPV1) have all been demonstrated to be selective analgesic targets in animal models of pain (Cregg *et al*., 2010). For example, some sodium channels are thought to play a significant role in nociceptive information processing and there is evidence that the TTX-resistant sodium channel NaV 1.8 is necessary for the generation of ectopic discharge in damaged sensory axons (Roza *et al*., 2003), whilst decreased expression of this channel has also been shown to inhibit neuropathic pain (Lai *et al*., 2002). Further research is therefore necessary to fully elucidate the role of ion channels in pain states. We have developed an *in vitro* model to examine the contribution of ion channels to an electrically evoked compound action potential (CAP) recorded from naïve and chronic constriction injury (CCI) model mice and rats.

❷METHODS

Experiments were carried out in compliance with the U.K. Animals (Scientific Procedures) Act, 1986. Adult male BL6 CBF1 mice and adult male Sprague-Dawley rats were used in this study. The CCI model was prepared as described by Bennett and Xie (1988) and verified by measurement of paw withdrawal threshold (PWT) with a series of graduated von Frey hairs. Only those animals with significantly decreased PWT (≤ 0.16 g), indicating mechanical allodynia, were selected for electrophysiological experiments.

Animals were decapitated after being deeply anaesthetised with sodium pentobarbitone (80 mg/kg, i.p.). The left sciatic nerve including the tibial, sural and common peroneal peripheral branches, L4-L6 DRG and associated dorsal roots was surgically isolated in ice-cold (<4°C) artificial cerebrospinal fluid (aCSF) and once dissected the main trunk of the sciatic nerve was desheathed, the DRG capsules were opened and, where required, surgical disruption of the injury site neuroma was performed (in CCI model animals). The complete nerve preparation was then placed in a purpose built chamber (see below) for a minimum recovery period of one hour before commencing recording.

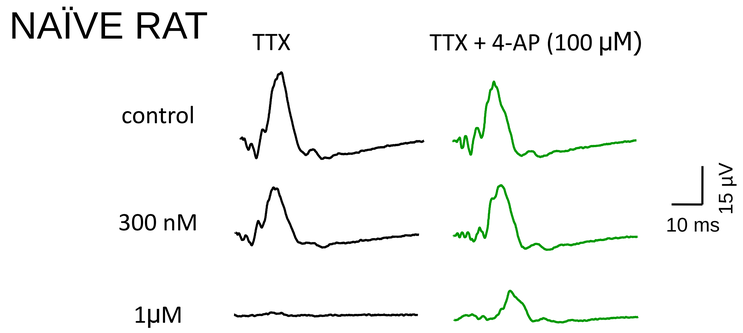
Recordings were optimised, a stimulus intensity/response input-output curve generated and stimulation was then set and maintained at a level that evoked a CAP of approximately 75% of maximum. At the end of recordings, lidocaine (1 mM) was administered to demonstrate the contribution of stimulus artefact to the recorded CAP.



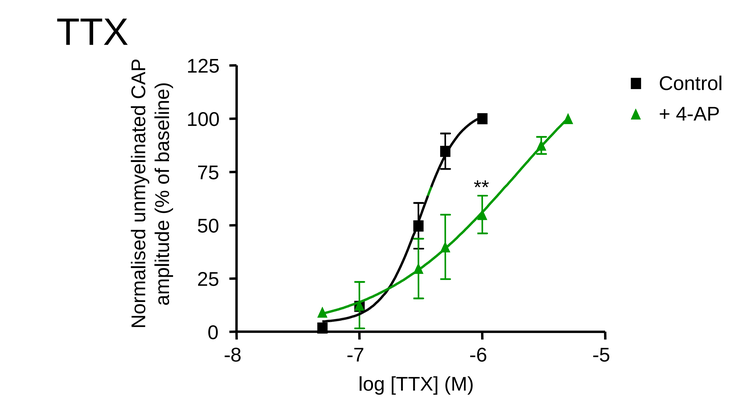
Schematic of the chamber during recording with the peripheral end of the sciatic nerve and DRG perfused with warmed aCSF and the dorsal roots placed on the contralateral side of a grease gap and immersed in mineral oil. Sciatic nerve stimulation (10-40 V, 1 ms, 0.2-0.5 Hz) evoked compound action potentials (CAPs) recorded from dorsal roots using a bipolar silver electrode.

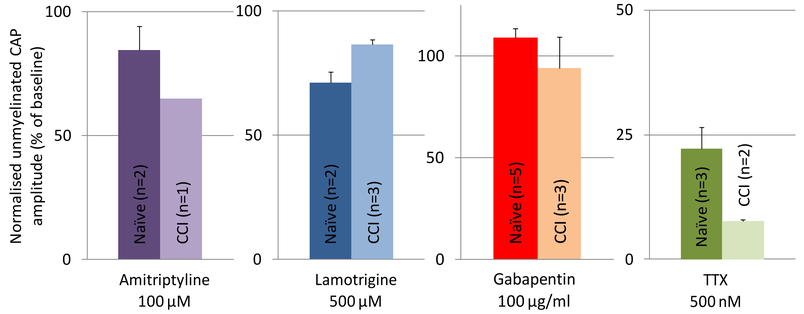
❸ RESULTS

Typical examples of electrically-evoked CAPs



Summarised group data





Top: a line graph showing the concentration-dependent depression in amplitude of electrically-evoked unmyelinated CAPs induced by application of TTX alone and during co-application with 4-aminopyridine (4-AP). Bottom: histograms showing the depression of the unmyelinated CAP response induced by the administration of a series of standard test compounds in naïve and CCI model animals

❹ SUMMARY

- We have identified a novel *in vitro* model for examining the contribution of ion channels to an electrically-evoked compound action potential
- We have demonstrated the validity of this model isolated from both naïve and pain model animals
- We have examined the effects of administration of a range of standard compounds in this model

❺ REFERENCES

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