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# Functional reorganisation of synaptic activity and intrinsic membrane conductances in dorsal horn neurones of neuropathic rats and the effects of gabapentin *in vitro*.

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## AIM OF INVESTIGATION

In neuropathic pain, genetic and molecular reorganisation and plasticity associated with development of this chronic pain state is matched by functional plasticity including central neural sensitization and 'rewiring' of neural networks. Despite the significant progress made in our understanding of the neural plasticity associated with neuropathic pain, at the level of the dorsal horn, our knowledge and understanding is relatively in its infancy. Here we used whole-cell patch clamp recording techniques in isolated spinal cord slice preparations from adult rat Chung models of neuropathic pain to characterise intrinsic electrophysiological and extrinsic synaptic properties of dorsal horn neurones (lamina I/II) ipsilateral to the site of injury and compared these to neurones of the contralateral spinal cord. The effects of the "gold standard" neuropathic pain treatment gabapentin were also tested.

## METHODS

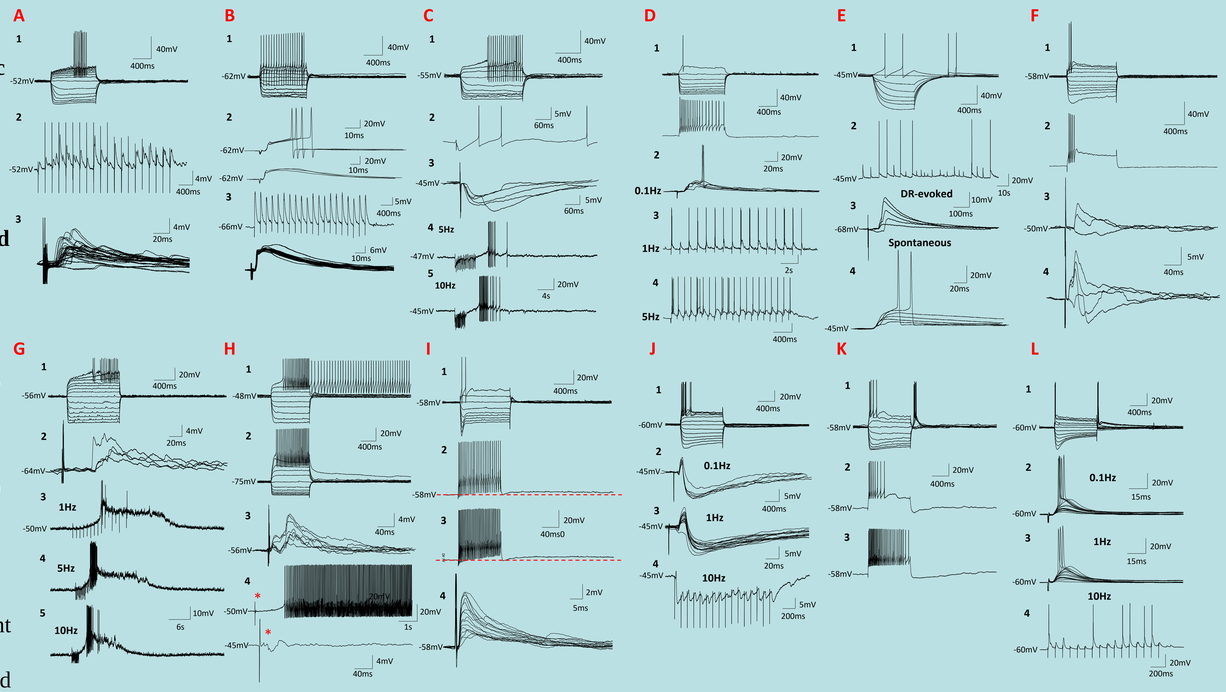
Adult male (8-10 weeks old) Chung model rats were prepared by tightly ligating the L5 spinal nerve. Two weeks following surgery, the status of the neuropathic pain condition was confirmed by monitoring mechanical allodynia using von Frey hairs prior to electrophysiology experiments. Animals were then terminally anaesthetized, the spinal cord and dorsal roots removed, cut into 450-500 µm thick para-sagittal slices and maintained in artificial cerebrospinal fluid (aCSF) of the following composition (mM): 127 NaCl; 1.9 KCl; 1.2 $KH_2PO4$; 2.4 $CaCl_2$; 1.2 $MgCl_2$; 26 $NaHCO_3$; 10 D-glucose, equilibrated with 95% $O_2$-5% $CO_2$. Individual spinal slices with attached dorsal root/DRG were transferred to a custom-built recording chamber continuously perfused with aCSF. Whole-cell recordings were performed at 34-35°C from Lamina I or II neurones in the dorsal horn of spinal cord slices using Axopatch 1D and/or Multiclamp 700B amplifiers using the 'blind' version of the patch-clamp technique. The composition of the intracellular solution was (mM): Glu, 140; EGTA-Na, 1; HEPES, 10; KCl 10; $Na_2ATP$, 4; GTP, 0.3. Biocytin (5 mM) was also included in the pipette solution to facilitate retrospective visualisation of recorded neurones to confirm morphology and localisation in the spinal slice (lamina I/II). Recordings from both lamina I and II neurones are included in this study. Post-synaptic potentials and/or currents (PSP/Cs) were evoked by electrical stimulation of the dorsal roots using a concentric bipolar stimulating electrode positioned on the roots, over a range of frequencies (0.1, 1, 5 and 10 Hz). Aδ and C-fibres were identified based upon their estimated conduction velocities and their ability to follow trains of repetitive stimulation.

## RESULTS

**1 Neurones were characterised on the basis of their intrinsic electrophysiological properties including the expression of subthreshold active conductances, action potential firing properties in response to depolarising current injection and dorsal root afferent-mediated synaptic inputs.**

Sub-threshold active conductance's included **instantaneous inward rectification ($I_{IR}$)** observed as a decrease in neuronal input resistance at membrane potentials more negative than around -70 mV (A, B, C, D, F); **hyperpolarisation-activated, time-dependent inward rectification ($I_H$)** observed as a depolarising sag in the membrane response to negative current injection (I, J, K, L); **A-like transient outward rectification ($I_A$)**, observed as a delay in reaching threshold for firing in response to depolarising current injection (C, G, H) and **low threshold T-type calcium conductances ($I_T$)** observed as a depolarising hump at the offset to negative current injection or depolarising current injection from negative holding potentials (E, K).

Firing patterns were characterised as **adapting** (F, J, L), based on the slowing or cessation of firing in response to depolarising current injection; **non-adapting** (B, C, E, G, H) or **pseudo-adapting** the latter characterised by adaptation of firing in response to a threshold depolarising current that could be superseded to non-adapting in response to higher amplitude current injection (D, I, K).

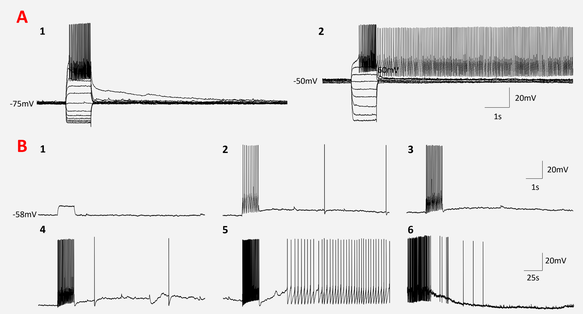



Electrical stimulation of dorsal roots evoked fast EPSPs (A, B, D, G, I, L) IPSPs (C) and combinations of these (E, F, H, J) and repetitive stimulation could give rise to delayed slow EPSPs (G).

**2 AFTERDEPOLARISATION POTENTIALS - A prominent feature of dorsal horn neurones in Chung models of neuropathic pain was the presence of depolarising conductances activated in response to suprathreshold current injection. Thus action potential firing persisted after termination of depolarising current injection. These afterdepolarisation potentials (ADP) could be further divided into instantaneous and delayed ADPs.**

**(A) Instantaneous ADP's** were observed as a continuation of action potential firing after termination of a depolarising current sufficient to induce action potential firing. This ADP was also voltage-dependent, terminating rapidly at negative holding potentials but failing to inactivate at potentials closer to threshold.

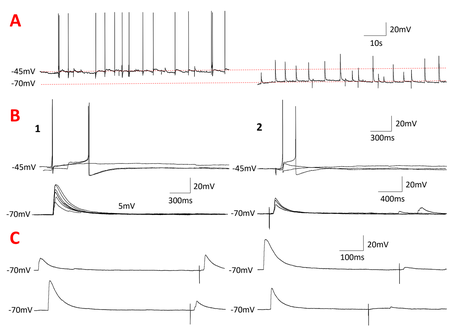
**(B) Delayed ADP's** were characterised by a distinct time-dependent delay in reaching a peak, action potential firing ceasing at termination of current injection followed by a slow subsequent depolarisation. The magnitude of this depolarisation was dependent on the number of action potentials evoked in response to current injection and membrane potential. This ADP could persist for minutes after initiation and inactivated slowly over time.




**3 Spontaneous Dorsal-Root Afferent-Mediated Excitatory Post-Synaptic Potentials (EPSPs)**

Spontaneous EPSPs were a feature of dorsal horn neurones in Chung models of neuropathic pain, presumably resulting from spontaneous ectopic discharge in dorsal root afferents.

A. Samples of a continuous record showing spontaneous sub- and suprathreshold EPSPs at different membrane potentials.
B. Same neurone in A showing both spontaneous EPSPs and EPSPs evoked by electrical stimulation of the dorsal roots on a faster time-base. Note the similarity in rise-time and waveform of evoked and spontaneous events.
C. Samples of the record shown above. Note EPSPs electrically evoked proximal to the time of spontaneous events were occluded whereas those electrically evoked at time-points independent of spontaneous events mimicked the spontaneous events.

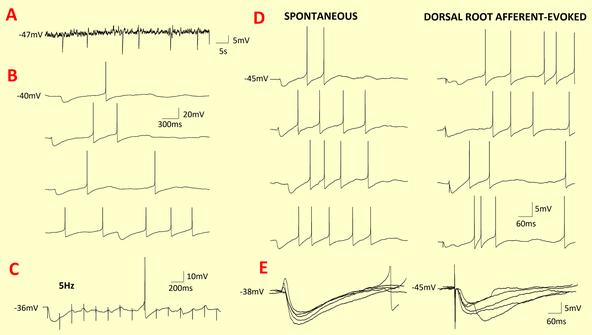



**Taken together these data indicate spontaneous and evoked potentials were generated at the level of the dorsal roots.**

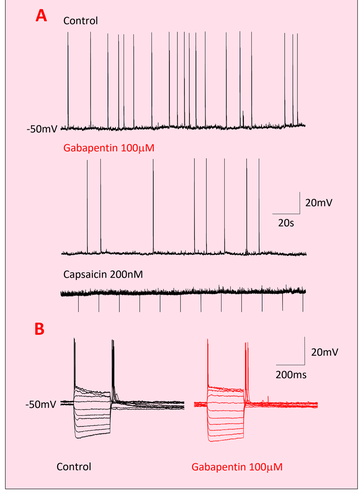
**4 Spontaneous Dorsal-Root Afferent-Mediated Inhibitory Post-Synaptic Potentials (IPSPs)**

Spontaneous IPSPs were a feature of dorsal horn neurones in Chung models of neuropathic pain, resulting from spontaneous ectopic discharge in dorsal root afferents.

A. Samples of a continuous record showing spontaneous IPSPs in a dorsal horn.
B. Same neurone in A showing spontaneous IPSPs on a faster time-base and rebound excitation with associated firing at the termination of IPSPs.
C. High frequency electrical stimulation of dorsal root afferents evoked IPSPs.
D. Samples of the same record comparing spontaneous IPSPs (left) with IPSPs evoked by stimulation of dorsal root afferents.
E. Same neurone as above with spontaneous and dorsal-root stimulation evoked IPSPs shown superimposed on a faster time-base.




**5 Gabapentin suppresses spontaneous activity on dorsal horn neurones and modulates postsynaptic conductances.**




**CONCLUSIONS - Neuropathic pain associated with Chung model rats is associated with significant pre- and postsynaptic functional plasticity at the level of the dorsal horn. Changes in post-synaptic intrinsic membrane conductances is a feature of this model, with changes in functional expression of $I_{IR}$, $I_H$ and an ADP. The ADP persists for tens of seconds in ipsilateral dorsal horn neurones and may contribute to the persistent pain observed after cessation of the insult. GBP suppresses pre- and post-synaptic activity in dorsal horn neurones suggesting more than one mechanism of action of this compound.**