# Developing a model to evaluate C-tactile fibre contribution to allodynia and for testing new topical medications

## Background

Mechanical allodynia, characterised by an extremely painful unpleasant response to light touch, is common in neuropathic pain conditions. Sufferers can be unable to wear even the lightest of clothes against affected areas. Our understanding of the mechanisms underlying both mechanical allodynia and its pharmacotherapy are incomplete.

Previously it was thought that mechanical allodynia was caused by activity in large fast conducting myelinated nerves, called A-beta fibres, that respond to gentle touch. This activity then in some way gained access to nociceptive, or 'pain sensing', pathways with the resulting cross-talk being responsible for the allodynia. This view has been challenged by the recent discovery of a system of small, slowly conducting unmyelinated nerve fibres that also respond to gentle touch. These so-called C-tactile (CT) afferents under normal conditions signal pleasant touch sensations and induce analgesia, but under chronic pain conditions change their role to augmenting pain. Interestingly CT afferents respond preferentially to gentle brushing touch, precisely the stimulus that typically evokes mechanical allodynia. CT afferents are tuned to the velocity of brushing touch and fire preferentially to slow as opposed to fast stroking. This velocity dependence is used as a 'marker' of CT afferent function. However, this stimulus will also always significantly activate large fast conducting mechanically sensitive nerve fibres. This makes it difficult to dissect out which nerve fibres are predominantly causing allodynia. It would be extremely useful to selectively block CT afferent fibres so that their role in the symptoms of mechanical allodynia can be further investigated and understood.

The aim of this project was to develop a model to understand the importance of C-tactile fibres in the development of allodynia. This was addressed using two parallel lines of investigation:

Using rigorous sensory, or psychophysical, testing the function of C-tactile fibres, A-beta mechanoreceptor fibres and nociceptors was investigated under conditions of differential nerve block with sodium channel blocking local anaesthetic agents.

The neural basis of the psychophysical tests was examined in detail using microneurography to record the activity of single nerve fibres (C-tactile, nociceptor and A-beta) before and following administration of topical local anaesthetic agents.

## Psychophysical studies

Psychophysical studies were performed in 20 healthy volunteer participants. Lidocaine was

**Mass of drug delivered (in µg) =**

**(Time x current x molecular wt) / (Electron charge x Avogadro Constant)**

administered non-invasively using iontophoresis. Iontophoresis allows a rapid non-invasive delivery of polar molecules. Importantly it provides controllable and verifiable dose delivery that can be calculated using the following formula:Lidocaine and adrenaline iontophoresis was delivered to the volar forearm on one side with adrenaline only contralaterally. The iontophoresis

was delivered over a large area so that brushing touch could be adequately assessed. The adrenaline only iontophoresis acted as a control. The presence of adrenaline resulted in a blanched region (due to the vasoconstrictive effects) which demarcated the area of iontophoresis. This was also noted during the microneurography-iontophoresis study (see figure 4).

Lidocaine iontophoresis resulted in significant impairment in detecting warm and cold as well as mechanical stimuli (figure **1**). The effect was most marked for mechanical detection. The detection of painfully cold and warm stimuli was not significantly altered.

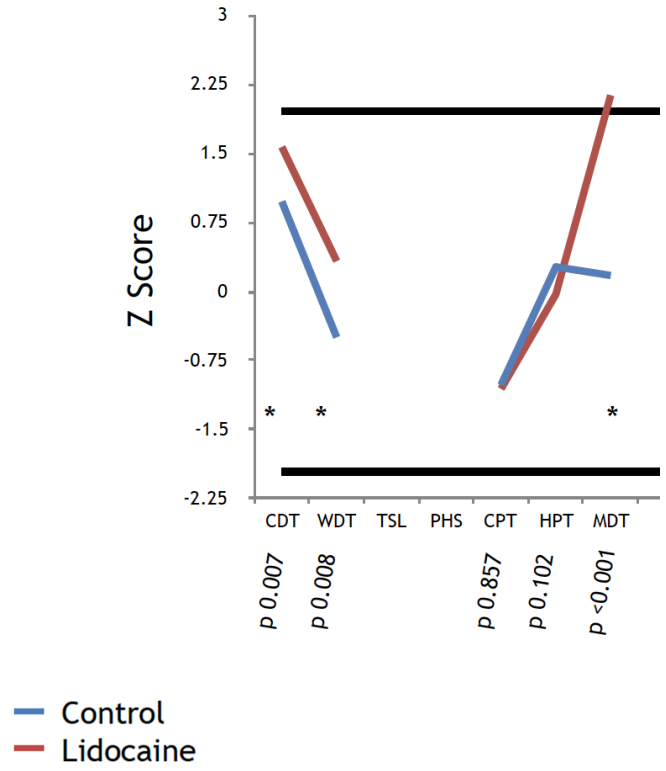



**Figure 1**
Results of quantitative sensory testing in control and lidocaine treated areas. CDT=cold detection threshold, WDT=warm detection threshold, CPT=cold pain threshold, HPT=heat pain threshold, MDT=mechanical detection threshold.

As expected pleasantness ratings to gentle brushing stimuli was significantly affected by the stroking velocity. Slow brushing stimuli were rated as more pleasant than fast brushing. This was not affected by lidocaine (i.e. there is no evidence that lidocaine blocks the preference for CT afferent targeted stroking touch) (figure 2). In contrast brushing touch with both a soft and harsh stimulus was rated as less intense at all velocities following lidocaine iontophoresis (figure 3).

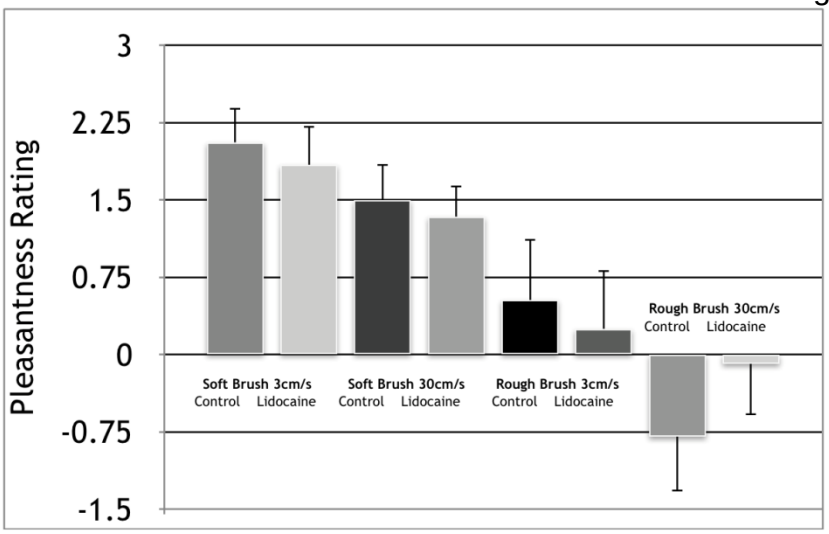



**Figure 2**
Pleasantness ratings for stroking stimuli applied to the forearm. Slow brushing is rated significantly more pleasant for soft brushing but this is not affected by lidocaine.

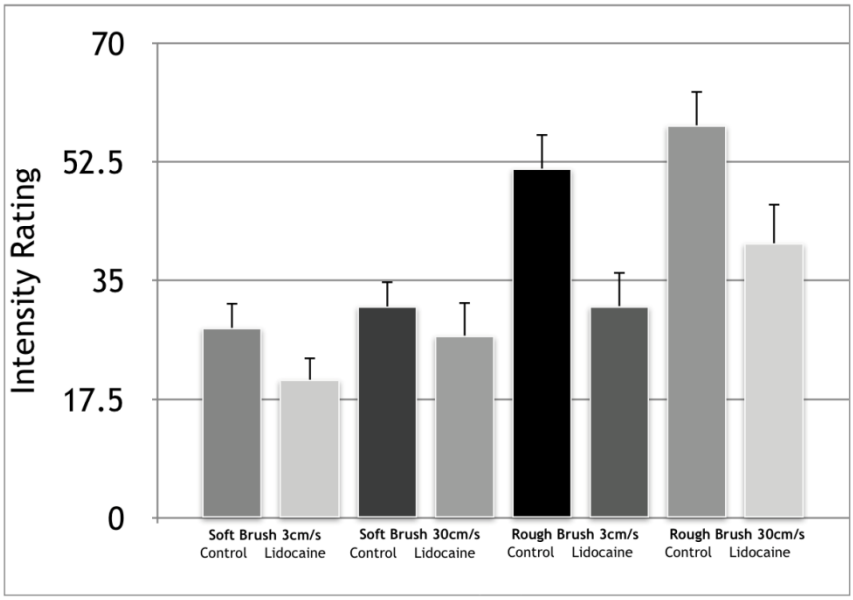
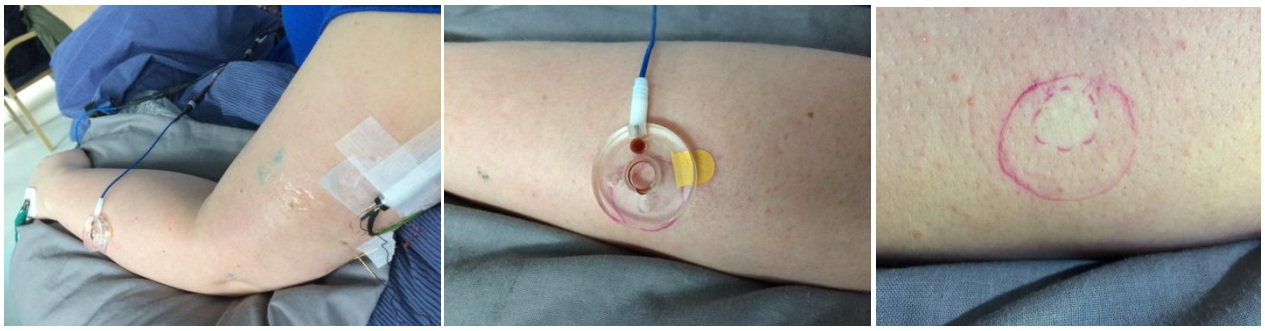



**Figure 3**
Intensity ratings for stroking stimuli applied to the forearm. Lidocaine significantly reduces the perceived intensity of stroking touch for both soft and rough stimuli.

To explore the neural mechanism behind these perhaps surprising results recordings from single afferent fibres pre- and post-lidocaine/adrenaline iontophoresis were made using the technique of microneurography.

**Microneurography studies**

Microneurography of the radial, lateral antebrachial or dorsal antebrachial nerve, all of which supply the hairy skin on the forearm, was performed in 30 healthy participants in 40 recording sessions lasting 4-8 hours. The experimental set up is shown in figure 4.



A B C

Figure 4
Experimental setup
A – Microneurography pre-amplifier on the posterior upper arm with electrode inserted to record from radial nerve. The iontophoresis chamber is on the forearm
B – Iontophoresis chamber close up placed over the receptive field of a hair follicle afferent
C – Area of blanching of the skin at the site of iontophoresis (marked by dotted line) within the receptive field of the hair follicle afferent (marked by solid line)

Initial studies had a high failure rate due to difficulties in locating and piercing the main nerve trunk. Over time this improved greatly due in large part to the use of ultrasound scanning to visualise both the nerve and microneurography electrode.

Useful unitary data, meaning that single nerve firing was recorded pre- and post-lidocaine/adrenaline iontophoresis to allow comparison, were obtained from 30 afferent fibres. The breakdown of nerve fibre types and effect of lidocaine/adrenaline iontophoresis on their firing is shown in the table.

| **Sensory Afferent Type** | **Number** | **Effect of Lidocaine on unit firing** |
|---|---|---|
| Hair Follicle Afferent | 10 | No effect |
| Field Afferent | 2 | No effect |
| Slowly Adapting Type 1 | 7 | Reversible reduced firing during sustained indentation in 6 fibres, 2 of which showed temporary elevation in mechanical threshold. Fibre showed a rapidly adapting 'phenotype' post-lidocaine. |
| Slowly Adapting Type 2 | 9 | No effect on firing in 8, reversible reduced firing during sustained indentation with preserved mechanical threshold in a single fibre |
| C-Tactile Afferent | 1 | No effect |
| C-Polymodal Nociceptor | 1 | Blocked |

In brief there was no observable effect of lidocaine on nerve fibres that supply hair follicles. All but one slowly adapting type 2 (SA2) afferents did not show clear alteration in firing. A single, well-documented, SA2 fibre showed a reduction in spontaneous firing during iontophoresis and a temporary change in character, namely reduced firing during sustained indentation over the receptive field, lasting approximately 25 minutes. The mechanical threshold for firing was maintained. Most markedly, and intriguingly so, all slowly adapting type 1 (SA1) afferents except one showed a change in character with a marked reduction in firing during the sustained indentation period. Therefore SA1 fibres showed a change from slow to fast adapting (Figure **5**).

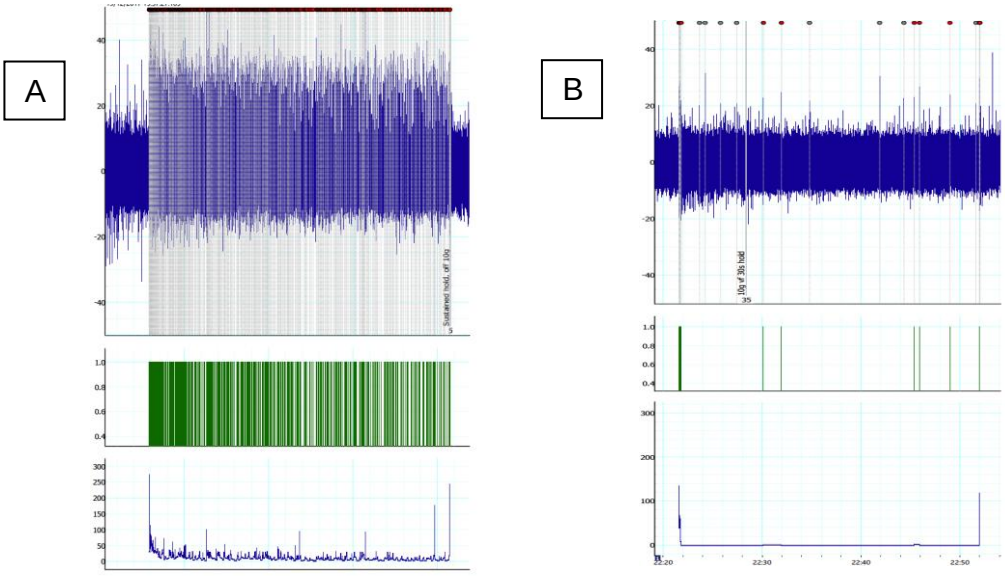



**Figure 5**
Firing of a single SA1 fibre to sustained indentation to a 10g monofilament for approximately 30 seconds. A=Pre-lidocaine, B=Post lidocaine. Top traces are raw spiking activity, middle traces show timing of individual spikes and lower trace instantaneous firing rate. Note that post-lidocaine firing only occurs at the onset and offset of the mechanical stimulus

Firing to the onset and offset of indentation was relatively preserved. Importantly firing recovered over time to yet again become slowly adapting. This return in firing temporally correlated with the return of mechanical detection threshold to ‘normal’ levels (i.e. the hypoaesthesia/reduced ability to detect a mechanical skin stimulus induced by lidocaine is linked to blocking of SA1 fibre firing but only during the sustained firing phase). Recordings from Field afferents as well as CT and polymodal nociceptor fibres were too few to provide useful insights. However, again intriguingly and contrary to the canonical view, the single CT fibre recorded was not blocked by lidocaine iontophoresis. This may be in line with unpublished observations by collaborators in Sweden that suggest that transmission in C-tactile fibres may be resistant to block with lidocaine.

**Conclusions, future directions and relevance to the Pain Relief Foundation**

The study was designed to develop a model of ‘selective’ block of unmyelinated low threshold mechanosensitive afferent fibres so that it could serve in future studies exploring the mechanism of CT afferents in dynamic mechanical allodynia. The psychophysical data did not show any effect on velocity dependent ratings for the pleasantness of gentle stroking and therefore no clear deficit in CT function. Furthermore, in the single CT afferent nerve recorded firing was not blocked by low dose lidocaine. The developed model however did markedly affect mechanosensation with tactile detection thresholds becoming elevated and ratings for the intensity of brushing touch being reduced. On the basis of the microneurography this is most easily explained on the partial blocking of SA1 fibres. This is a potentially highly important finding. At a cellular level firing in SA1 afferents is dependent on two factors. Firing observed at onset and offset of mechanical stimulation can be induced by activation of the nerve fibre itself by the mechanical stimulus. However the sustained phase requires a functioning interaction between the nerve fibre and a specialised mechanosensitive skin cell called the Merkel cell. That the sustained phase was affected by lidocaine but the onset and offset preserved suggests that the predominant effect of the blockade is due to disruption of the Merkel cell - nerve fibre transduction mechanism. This is dependent on Piezo 2, a mechanically sensitive channel. It has been suggested that Piezo 2 blockers might be useful in treating mechanical allodynia. The microneurography - iontophoresis model that has been developed as a result of this study is well placed to serve as a way of testing topical medications for superficial neuropathic pain.

Future studies are planned to further investigate the potential lidocaine resistance of CT afferents. For this we will need to record from more CT fibres during application of lidocaine. This might be followed up in collaboration with Swedish colleagues. Future studies assessing other anti-neuropathic pain medications are also planned.

Although not strictly related to the grant the microneurography arm of the project resulted in a significant by-product, namely the recording of hitherto unrecognised high threshold fast conduction mechanoreceptor afferents in humans. This exciting finding documents the presence of A-beta fibres with nociceptor properties which is likely to be highly relevant to pain. A manuscript for this has been submitted to Nature Neuroscience.