SLOW TOUCH AND FAST NOCICEPTION: EXPLORATION OF THE INTERACTIONS BETWEEN NOXIOUS AND INNOCUOUS MECHANICAL TOUCH SENSATION

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ABBREVIATIONS

ANOVA	Analysis of Variance
CCM	Corneal Confocal Microscopy
CDT	Cold Detection Threshold
CNBD	Corneal Branch Density
CNFD	Corneal Nerve Fibre Density
CNFL	Corneal Nerve Fibre Length
CPT	Cold Pain Threshold
CT	C-Tactile
CTPI	C-Tactile Preference Index
fMRI	Functional Magnetic Resonance Imaging
HFA	Hair Follicle Afferent
HPC	Heat-Pinch-Cool
HPT	Heat Pain Threshold
HSAN	Hereditary Sensory and Autonomic Neuropathy
HTMR	High Threshold Mechanoreceptor
IASP	International Association for the Study of Pain
KMO	Kaiser-Meyer-Olkin
LCs	Langerhans Cells
LTMR	Low Threshold Mechanoreceptor
MRGPR	Mas-Related G-protein-Receptor
NCCA	Non-Contact Corneal Aesthesiometry
NDS	Neuropathy Disability Scale
NGF	Nerve Growth Factor
NGFB	Nerve Growth Factor β
NS	Nociceptor Specific
PAR	Protein Activated Receptor
PKCγ	γ isoform of Protein Kinase C
PSDC	Post-Synaptic Dorsal Column
RA	Rapidly Adapting
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex

SA	Slowly Adapting
SEM	Standard Error of the Mean
SF-MPQ	Short Form McGill Pain Questionnaire
SPQ	Situational Pain Questionnaire
STT	Spinothalamic Tract
TDD	Tactile Direction Discrimination
TPD	Two Point Discrimination
TPT	Touch Perception Task
VAS	Visual Analogue Scale
VGLUT3	Vesicular Glutamate Transporter Type 3
WDR	Wide Dynamic Range
WDT	Warm Detection Threshold

CONTRIBUTION

This section is to confirm that Andrew Marshall, the author of this thesis, was actively involved and made a significant contribution to all chapters/studies presented and discussed in this thesis. He was involved in the conception and design as well as analysis in all studies. He consented and recruited all patients undergoing cordotomy and performed all clinical phenotyping and psychophysical assessments. He performed microneurography and analysed the neural data that was generated. For chapter VIII he set up the pain model and performed psychophysical assessments. For Chapter V he performed the neurological assessments. He performed the statistical analysis in this thesis. Finally, he has written all the chapters of this thesis which have been reviewed by his supervisors Professors Francis McGlone and Häkan Olausson.

The following tasks were performed by other members of the research team:

For Chapter IV: Microneurography was also undertaken by Dr Saad Nagi, Adarsh Makdani, Dr Ewa Jarocka and Dr Francis O'Neill; Analysis of neural data generated by microneurography was also undertaken by Dr Saad Nagi and Dr Ewa Jarocka; Psychophysical data was collected by Dr Saad Nagi.

For Chapter V: Ophthalmic examinations were undertaken by Dr Mitra Tavakoli; Behaviour testing and administration of pain questionnaires to participants with HSAN-V and subsequent analysis of this data was performed by Dr Irene Perini; Recruitment of participants with HSAN-V was performed by Dr Jan Minde.

For Chapters VI and VII: Anterolateral Cordotomy procedures were performed by Dr Manohar Sharma, Consultant Pain Physician.

For Chapter VIII: Recruitment of participants, administration of the pain model and psychophysical assessments were also performed by Dr Jaquette Liljencrantz.

ABSTRACT

Human cutaneous somatosensation is a multimodal physiological process by which physical stimuli on the body surface activate neural substrates that consequently give rise to perceptions such as pressure/vibration, temperature, itch, and pain. When considering skin mechanosensation, the canonical view is that low force innocuous mechanical stimuli are encoded by cutaneous low threshold mechanosensitive receptor (LTMR) endings, innervated by fast conducting, thickly myelinated Aβ afferent nerves (Mountcastle, 2005). In contrast, higher force, potentially tissue damaging, mechanical stimuli are, according to this view, encoded by high threshold mechanosensitive receptor (HTMR) endings that receive innervation from slowly conducting thinly myelinated Aδ or unmyelinated C afferent fibres.

The discovery of a system of LTMR afferents that are C-fibres has challenged this view and led to the recognition of a second 'slow' touch pathway, a pathway that has an affective rather than discriminative function (McGlone, Wessberg and Olausson, 2014). Conversely, the recent suggestion that fast-conducting afferent fibres innervating a type of ending previously considered to be an LTMR actually have nociceptive properties in rodents (Bai *et al.*, 2015) has focussed attention on putative Aβ 'fast' nociceptive pathways.

The work in this thesis investigates the neural basis for 'slow' touch and 'fast pain' in the human cutaneous somatosensory system concentrating first in the peripheral nervous system before addressing spinal pathways and higher-level consequences of a pathological lack of specific afferent fibre types. The thesis closes with an assessment of alterations in the perception of tactile sensation in a human model of allodynia.

1 INTRODUCTION

1.1 PRIMARY MECHANOSENSITIVE RECEPTOR AFFERENT FIBRES

The primary afferent fibres of the cutaneous somatosensory system are typically classified according to their size, degree of myelination and conduction velocity into three broad classes, Aβ, Aδ and C. Importantly this classification has functional significance with particular fibre types firing in response to particular types of skin stimulation. These fibre types are discussed below in relation to the encoding of low and high force mechanical skin stimulation. The term encoding in this context is to specify the 'neural code' for a particular stimulus. To do this the fibre must both spike to the relevant stimulus as well as show spiking that is modulated by that stimulus in the relevant range. Subtypes of Aδ and C afferent fibres encode for thermal stimuli with different fibre types responding to a particular stimulus (e.g. innocuous cold, innocuous heat and their noxious range equivalents). These afferent types are not detailed further in this section but will be discussed in relation to second order ascending spinal pathways.

1.2.1 High threshold mechanosensitive receptor afferents - nociception and pain

The International Association for the Study of Pain (IASP) defines a nociceptive stimulus as 'An actually or potentially tissue-damaging event transduced and encoded by nociceptors' and a nociceptor as 'A high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli' (Merskey and Bogduk, 1994). According to IASP definitions a mechanically sensitive nociceptor afferent would (1) have a high mechanical threshold relative to LTMR afferents and (2) exhibit an increase in firing rate in response to the increasing force of the applied mechanical stimulus within the potentially tissue-damaging range.

Multiple distinct types of nociceptor have been revealed by electrophysiological recordings in primates, microneurography studies in humans as well as in animal skin-nerve preparations (Le Bars, Gozariu and Cadden, 2001; Namer and Handwerker, 2009; Schmelz, 2009; Zimmermann *et al.*, 2009). Nociceptor afferents have traditionally been classified on the basis of their neurophysiological properties such as conduction velocity and responsiveness to mechanical, heat, cold and chemical stimuli. However, it is increasingly recognised that nociceptor sub-types exhibit a rich genetic and molecular diversity that correlates with their axonal and receptive, especially chemo-receptive properties (Moqrich, 2014). In humans and other species, the preponderance of nociceptor fibres are unmyelinated and the majority of these are classified as polymodal as they respond to both mechanical, thermal and chemical stimuli (Raja, Meyer and Campbell, 1988; Van Hees and Gybels, 1981). C-fibre nociceptors that under normal conditions are insensitive to mechanical and thermal stimuli have also been identified in humans using electrical stimulation paradigms (Schmidt *et al.*, 1995). These, so called 'silent' nociceptors become sensitised by inflammatory mediators, a phenotypic switch that causes them to become mechanically and thermally responsive (Meyer *et al.*, 1991; Schmidt *et al.*, 1995).The detailed response properties and axonal features of Aδ nociceptors are not well defined in humans. A single study did however show that some do respond to both mechanical and heat stimuli (Adriaensen *et al.*, 1983). This is in line with studies in primates where A-fibre nociceptors can be classified as A-M, A-MH and A-H depending upon their responsiveness to mechanical (M) and heat (H) stimuli (Lewin and Moshourab, 2004; Dubin and Patapoutian, 2010). More recently genetically and neurophysiologically distinct A-fibre nociceptor types that play a specific role in either pain associated with hair pull (Ghitani *et al.*, 2017) or the nociceptive withdrawal reflex (Arcourt *et al.*, 2017) have been described in rodents. Therefore, it is probable that a greater diversity of A-fibre nociceptors exists in humans.

Studies that have used graded force-controlled mechanical stimuli have shown that both Aδ and C-fibre nociceptor afferents can encode the force of mechanical skin stimulation (Garell, McGillis and Greenspan, 1996; Slugg, Meyer and Campbell, 2000; Koltzenburg and Handwerker, 1994; Handwerker, Anton and Reeh, 1987; Andrew and Greenspan, 1999; Khalsa, LaMotte and Grigg, 1997; Khalsa, Zhang and Qin, 2000). Aδ fibres, however, display a greater mechanical sensitivity over the range of stimuli and also are better at

encoding stimulus area (i.e. they have higher firing rates for stimuli with a smaller surface area – a property that suggests the ability to detect sharpness) (Handwerker, Anton and Reeh, 1987; Andrew and Greenspan, 1999; Garell, McGillis and Greenspan, 1996; Slugg, Meyer and Campbell, 2000).

In humans it has been widely accepted that the nociceptive system is comprised of only Aδ and C afferents – Aδ nociceptors for first/fast pain (sharp-stabbing) and C nociceptors for second/slow pain (dull/burning) (Bromm and Treede, 1984; Mackenzie *et al.*, 1975; Marshall, 1953; Ochoa and Torebjörk, 1989; Torebjörk and Hallin, 1973) – Human Aβ afferents are thought to all be low threshold and non-nociceptive under normal conditions. In keeping with this, patients with forms of hereditary sensory and autonomic neuropathy (HSAN) who lack C- and Aδ fibres have congenital insensitivity to pain (Minde *et al.*, 2004; Minde, 2006; Minde *et al.*, 2009; Indo, 2002; Miura *et al.*, 2000). High-frequency electrical stimulation below the activation threshold of Aδ afferents can, however, generate a painful percept and a facilitated nociceptive flexion (spinal) reflex – an effect confirmed by a preferential small-fibre anesthetic block (Willer, Boureau and Albe-Fessard, 1978; Willer, Boureau and Albe-Fessard, 1980; Willer and Albe-Fessard, 1983). Furthermore, high threshold mechanoreceptor (HTMR) afferents with fast conduction have been reported in other species; nociceptors with morphological features and conduction velocities in the thickly myelinated Aβ range, have a prevalence which varies from 18-65% of the myelinated nociceptor population across different species (Lawson, 2002a; Djouhri and Lawson, 2004). For instance, ~12% of the Aβ population innervating monkey hairy skin consist of nociceptors, representing 18% of the myelinated nociceptor population (Treede, Meyer and Campbell, 1998).

In mice selective ablation of post-mitotic sensory neurons expressing NaV1.8, a voltage-gated sodium channel thought to be specific to nociceptor fibres (Abrahamsen *et al.*, 2008), results in a profound loss of noxious range mechanical and cold sensitivity whilst noxious heat responses are spared. Pathologically these deficits were not only associated with a marked loss of small diameter presumed nociceptor afferents but also 13% of neurofilament 200 positive large myelinated neurons. It was suggested that this reflected a minor loss in 'touch' afferents but the precise nature of these afferents and their associated cutaneous ending is not known. It is possible they could be large myelinated nociceptor afferents. Possible insights come from a recent report on circumferential endings in mouse hairy skin (Bai *et al.*, 2015). This suggests that "Aβ field-LTMRs", a class of fast-conducting low-threshold mechanoreceptors (brush-sensitive LTMRs), "exhibit hallmarks of myelinated nociceptors". In line with IASP definition of a nociceptor, mouse Aβ field-LTMRs display features such as a high threshold for monofilament activation and capacity to encode noxious skin indentations. If Aβ field-LTMRs were to have nociceptive properties this would have important implications for pain research and therapy. However, whilst Aβ field-LTMRs have been reported in humans (**see section 1.1.1**), albeit from only a small number of microneurographic recordings in forearm hairy skin, they were shown to display properties of LTMR afferents (Vallbo *et al.*, 1995; Olausson, Wessberg and Kakuda, 2000; Edin, 2001; Loken *et al.*, 2009).

Whilst activity in nociceptors encodes stimuli in the noxious range their activation is not necessarily associated with the perception of pain (Dubin and Patapoutian, 2010; Van Hees and Gybels, 1981). This process is poorly understood and will depend on complex processing of the activity from different classes of afferent, including LTMR afferents, in the central nervous system. The situation appears to be more complex for noxious mechanical stimuli (Dubin and Patapoutian, 2010). For example, the activity in polymodal c-nociceptors in response to radiant heat stimulation shows a strong correlation with the presence and intensity of pain (Van Hees and Gybels, 1981). In contrast the level of mechanical stimulation that evokes firing rates in the range associated with heat-induced pain are typically felt as innocuous. This disparity is very likely to be due to the associated activation of LTMR afferent which exert a systems level suppressive effect on nociceptive inputs (Andrew and Greenspan, 1999). Nevertheless, human capsaicin insensitive A-fibre nociceptor activation, interpreted as being from Aδ fibres, is related to the perception of pinprick stimuli (Magerl *et al.*, 2001). Judgements about pain ultimately reflect a multifaceted higher-level

experience (Morton, Sandhu and Jones, 2016), a process that will depend on expectations of pain resulting from past and learned pain. However, this is highly likely to be shaped by activity in primary afferent fibres. Interestingly, patients with congenital insensitivity to pain, presumed due to HSAN, are still able to perceive and feel empathy for others' pain, despite having an abnormal personal experience of pain (Danziger et al 2005). The relationship between higher-level pain processing, including subjective pain evaluation, and C-fibre density has not, however, been formally assessed.

1.2.2 A-fibre low threshold mechanosensitive receptor afferents - discriminative touch in humans

Aβ fibres have large diameters and are thickly myelinated. These structural characteristics enable fast conduction velocities (approximately 50 m s^{-1})(Kakuda, 1992) and thus facilitate immediate feature detection, a property of significant importance to discriminative aspects of touch such as texture discrimination as well as for sensorimotor control (McGlone and Reilly, 2010; McGlone, Wessberg and Olausson, 2014).

The neural basis of discriminative touch has been most extensively studied in the glabrous skin of the palm (Mountcastle, 2005). Studies in non-human primates as well as recordings of single afferents in humans with the technique of microneurography reveal the existence four types of Aβ LTMR afferent in glabrous skin (Hagbarth and Vallbo, 1967; Johansson and Vallbo, 1979b; Vallbo and Johansson, 1984), Mountcastle, 2005). These afferents are subdivided based on their adaption properties and receptive field characteristics, features that correspond with the anatomical structure and location of the receptor end organ they innervate (McGlone and Reilly, 2010) Mountcastle, 2005). Fibres that spike during the onset and/or offset but not during sustained mechanical skin stimulation are termed rapidly adapting (RA). In glabrous skin these are further sub-classified as rapidly adapting type I (RA1) and rapidly adapting type 2 (RA2 or Pacinian units) which innervate Meissner and Pacinian corpuscles respectively. Aβ LTMR afferents that show sustained firing to a constant mechanical stimulus are termed slowly adapting (SA). These are also sub-divided in to slowly adapting type 1 (SA1 or Merkel cell afferents) and slowly adapting type 2 (SA2) fibres that respectively innervate Merkel cell and Ruffini end organs. Both Merkel cell and Meissner end organs are located superficially at the dermo-epidermal junction and the respective afferents have correspondingly small receptive fields. This contrasts with cutaneous Pacinian and Ruffini endings which are located deeper in the dermis and have larger, often indistinct receptive fields (Fig. 1.1). Stimulation of single Aβ afferents using low (typically 1-5 μA) currents can be performed during the course of human microneurography, a method termed intraneural microstimulation (INMS) (Ochoa and Torebjörk, 1983; Vallbo, 1981; Vallbo *et al.*, 1984; Trulsson *et al.*, 2001; McGlone *et al.*, 2002). INMS enables stimulation of defined afferent types to be linked with an evoked percept. Stimulation of RA1, Pacinian and SA1 afferents can all evoke cutaneous perceptions that match the fibre stimulated both spatially and in terms of their adaptive properties. Stimulation of RA afferents are typically associated with sensations of flutter or vibration whereas stimulation of SA1 fibres are associated with a pressure sensation. Stimulation of SA2 fibres on the other hand has not been found to generate a reproducible percept. These fibres therefore form four channels that are thought to play complementary roles in discriminative touch perception. Meissner and Merkel cell afferents, with their superficial endings and small receptive fields, show a marked concentration towards the finger tips (Mountcastle, 2005). This provides the part of the hand most involved in fine touch discrimination with the equivalent of the fovea in the eye.

The neural basis of tactile sensation in human hairy skin (~90% of the body surface) has received much less attention. Whilst hairy skin is innervated by types of SA LTMR and Pacinian units, RA1 LTMR afferents are not present. Instead, hairy skin receives innervation from two further RA Aβ afferent types, hair follicle and field afferents (Vallbo *et al.*, 1995)(Fig. 1.1). Hair follicle afferent (HFA) fibres innervate hair follicles and spike in response to hair deflection. In the hairy skin of the human forearm each HFA innervates 10-33 hair

follicles (Vallbo *et al.*, 1995) and have receptive fields that span in the region of 16 x 11m in the long and short axes respectively. Human field units have similar receptive field areas but do not respond to hair deflection. It has been debated whether field units are actually HFA and that the lack of response to hair deflection reflects different phases of the hair follicle cycle. There are subtle differences in the receptive field patterns between hair follicle and field unit afferents. Field units, in contrast to the archipelago-like pattern of responsiveness that HFAs have centred around visible follicles within their receptive field, have more irregularly shaped receptive fields containing more confluent hot spots (Vallbo *et al.*, 1995). Further, recent studies in rodents suggest they are genetically and anatomically distinct from HFAs (Bai *et al.*, 2015). Although the end organ associated with human field units is currently unknown in mice they form circumferential endings associated with hair follicles, whereas HFAs terminate in a lanceolate pattern.

Unlike the situation with the palm the precise role that Aβ fibres innervating hairy skin have in touch perception is far from clear. Other than in the trigeminal system (Trulsson and Essick, 2010) there are no INMS data to suggest whether selective stimulation of defined afferent types is associated with a particular percept. Recent studies in rodents have suggested that the various classes of LTMR afferents terminate in a highly organised manner within the dorsal horn of the spinal cord. Here they integrate with other afferent inputs within a complex interneuronal rich network (Abraira *et al.*, 2017; Abraira and Ginty, 2013). Therefore, rather than having distinct receptor-based channels, the ascending spinal outputs relevant to touch perception in hairy skin is likely to contain partially processed information. Clearly, while the organisation of afferent inputs in human and non-human primates is unlikely to be less complex, it should be appreciated that there may be significant species differences. For instance, the intricate organisation of the innervation of mouse hairy skin cannot fully apply to humans because of species differences in hair follicle sub-types (Li *et al.*, 2011; Abraira and Ginty, 2013; Buffoli *et al.*, 2014).

Non-human hairy skin is also prominently innervated by LTMR Aδ fibres (Djouhri, 2016). These fibres, termed d-hair units, innervate awl/auchene and zigzag hair follicles (Li *et al.*, 2011; Abraira and Ginty, 2013) in mice and spike in response to hair deflection ((Li *et al.*, 2011; Abraira and Ginty, 2013; Rutlin *et al.*, 2014; Djouhri, 2016). There is only a single report describing the electrophysiological properties of what were thought to be d-hair units recorded in human radial nerve innervated skin on the dorsum of the hand (Adriaensen *et al.*, 1983). It is unclear whether they play a significant role in human tactile perception (e.g. vibration is a strong stimulus to non-human d-hair units and yet individuals with selective Aβ fibre denervation cannot feel a vibratory stimulus) (Olausson *et al.*, 2008a). Nevertheless d-hair units are of importance to this thesis at least insofar as the upper limit of their conduction velocity is often taken as the cut-off between Aδ and Aβ fibres (Djouhri and Lawson, 2004).

Until recently little was known about how cutaneous mechanical stimuli induce action potential generation in primary afferent fibres. However, the discovery of a family mechanically sensitive ion channels, termed piezo proteins, has significantly advanced understanding of this process of mechano-transduction. Piezo ion channels exist in two forms, Piezo 1 and Piezo 2. Piezo 1 is primarily of importance in detecting changes in pressure internally and is involved in the regulation of red blood cell volume for example (Murthy, Dubin and Patapoutian, 2017). Piezo 2 shows high expression in dorsal root ganglia (Coste *et al.*, 2010). Genetically engineered mice who lack Piezo 2 show profound deficits in proprioception and touch sensation (Ranade *et al.*, 2014). Similarly, loss of function mutations of Piezo2 in humans are associated with profound proprioceptive deficits as well as marked abnormalities in touch discrimination (Chesler *et al.*, 2016). These individuals have elevated punctate touch detection thresholds and perform at chance level during two-alternative forced choice vibration detection and two-point discrimination tasks, stimuli that neurologically intact individuals detect with 100% accuracy. However, Piezo2 cannot be the only mechanism of mechano-transduction as carriers have normal detection of higher threshold, including potentially noxious range, mechanical stimulation of the skin and deep tissues.

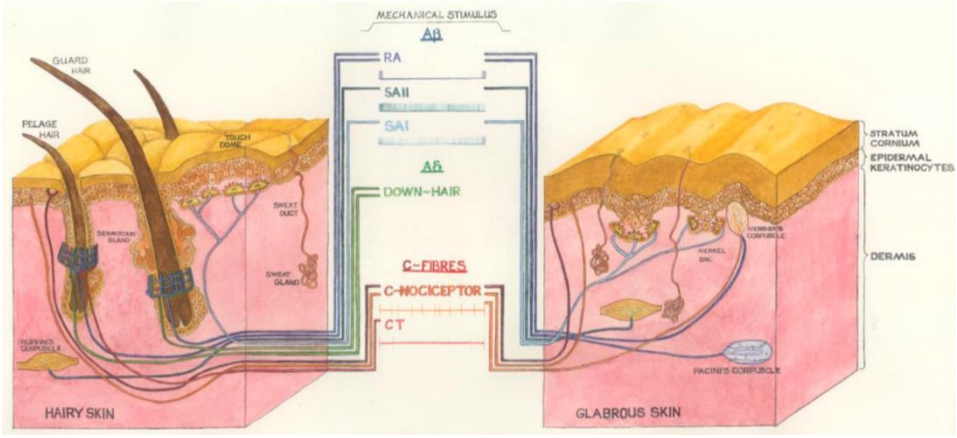

Fig. 1.1.

The Innervation of Hairy and Glabrous Skin Showing the Types of Nerve Fibres and Receptors: Both skin types are innervated by Aβ LTMR afferents, which encode the discriminative aspects of touch. CT-afferents are limited to hairy skin. Abbreviations: SA, slowly adapting; RA, rapidly adapting; CT, C-tactile afferent. Image taken from (McGlone, Wessberg and Olausson, 2014). Permission to reproduce this figure has been granted by Cell Press.

1.2.3 Unmyelinated low threshold mechanosensitive receptor afferents - C-Tactile afferents and affective touch

Slowly conducting unmyelinated C-fibres, termed C-low threshold mechanoreceptor (C-LTMR) afferents, that fire in response to low force static and dynamic mechanical stimuli were originally recorded in cats (Zotterman, 1939) and then subsequently reported in many mammalian species including primates (Douglas and Ritchie, 1957; Iggo, 1960; Bessou *et al.*, 1971; Iggo and Kornhuber, 1977; Kumazawa and Perl, 1977b; Lynn and Carpenter, 1982; Shea and Perl, 1985; Sugiura, Lee and Perl, 1986; Leem, Willis and Chung, 1993; Liu *et al.*, 2007; Seal *et al.*, 2009; Obreja and Schmelz, 2010; Li *et al.*, 2011; Abraira and Ginty, 2013; Delfini *et al.*, 2013; Vrontou *et al.*, 2013). A number of lines of evidence, including an apparent complete loss of tactile sensation under conditions of myelinated nerve block (Kelso *et al.*, 1975; Kumazawa and Perl, 1977b) led, erroneously, to the conclusion that they had been lost during evolution in humans. C-fibres with low mechanical thresholds were eventually reported in the human infra-orbital nerve in 1988 (Johansson *et al.*, 1988). In humans these fibres are termed C-tactile (CT) afferents. CT afferents have been reported in hairy skin sites in the upper and lower limb as well as in the trigeminal system (Fig. 1.1). They have, however, never been recorded in the glabrous skin of the palm (Johansson and Vallbo, 1979a; Johansson and Vallbo, 1979b; Johansson and Vallbo, 1980; Johansson, Vallbo and Westling, 1980; Vallbo and Johansson, 1984) and thought unlikely to significantly innervate glabrous sites, a conclusion that concurs with studies in rodents (Liu *et al.*, 2007; Li *et al.*, 2011).

The role that CT afferents play in human tactile sensation and perception has been a matter of debate. Quantification of the density of CT afferents innervation is not possible currently because of the lack of a specific cytological marker in humans. However, at least in the hairy skin of the forearm, they are encountered frequently, indeed as often as Aβ fibres (Vallbo, Olausson and Wessberg, 1999), a finding that would make a redundant role in touch unlikely. Two genetically distinct populations of C-LTMR afferent, one expressing the Mas-related G-protein-receptor MRGPR-B4 (Liu *et al.*, 2007) and the other both Tyrosine Hyroxylase (Li *et al.*, 2011; Reynders *et al.*, 2015) and the vesicular glutamate transporter type 3 (VGLUT3) have been described in mice (Seal *et al.*, 2009). Both of these exclusively innervate hairy skin and show peripheral endings closely associated with hair follicles. The peripheral endings in humans are not currently known. The association with hairy skin and potentially hair follicles is of interest as CT-afferents

have not been shown to fire in response to hair deflection during human microneurography studies (Olausson *et al.*, 2010).

Unlike C-nociceptor fibres, CT afferents have low mechanical thresholds to punctate skin stimulation (0.3-2.5mN) (Vallbo, Olausson and Wessberg, 1999) and fire vigorously in response to gentle slow stroking stimuli (Loken *et al.*, 2009; Nordin, 1990; Vallbo, Olausson and Wessberg, 1999). CT afferents do fire in response to both blunt and sharp skin stimulation but unlike C-nociceptors they do not discriminate between these stimuli (Nordin, 1990; Vallbo, Olausson and Wessberg, 1999). CT afferents also have a different pattern of activity dependent slowing than C-nociceptor fibres (Watkins *et al.*, 2017) and do not fire significantly after capsaicin application (Olausson *et al.*, 2010). Therefore, CT afferents are distinct from nociceptors and do not encode for noxious range mechanical stimulation. Furthermore, their slow conduction velocities along with other neurophysiological characteristics such as a tendency to fatigue in firing to repeated stimuli (Nordin, 1990; Vallbo, Olausson and Wessberg, 1999), limited response to vibration (Iggo, 1960; Kumazawa and Perl, 1977b) and delayed acceleration (Vallbo, Olausson and Wessberg, 1999) mean that CT afferents are poorly suited to a role in touch discrimination.

A characteristic property of CT-afferents is that their firing rates are strikingly dependent on the velocity of a gentle stroking stimulus, spiking most vigorously to velocities of 1-10 cm s^{-1} and significantly less so for faster and slower stimuli (Loken *et al.*, 2009; Essick *et al.*, 2010). Remarkably, this inverted U-shaped neural stimulus-response function shows strong positive correlation with psychophysical ratings of touch pleasantness (Essick *et al.*, 2010; Essick, James and McGlone, 1999). This has led to the hypothesis that CT-afferents form a specific coding channel that carries information pertinent to pleasurable aspects of gentle touch (Morrison, Löken and Olausson, 2010; McGlone, Wessberg and Olausson, 2014) that have emotional, affiliative and social relevance. This has been termed the social or affective touch hypothesis, a hypothesis that would imply the importance of skin-to-skin contact. Amongst an increasing body of indirect evidence supporting the importance of human-to-human touch (McGlone, Wessberg and Olausson, 2014) CT afferent firing is tuned not only to the velocity of a stoking stimulus but also to the temperature of the human hand (Ackerley *et al.*, 2014a).

Evidence supporting the hypothesis of a role of CT afferents in affective touch, distinct from one that is discriminative, also comes from psychophysical and neuroimaging studies under conditions of selective CT and Aβ activation. The study of selective CT-afferent activation is challenging because of the dual A- and C-fibre innervation of human hairy skin; A-fibres are unavoidably activated by tactile stimulation that also activate CT-afferents. Studies have, however, been performed in individuals who suffered a rare neuronopathy syndrome that resulted in complete loss of Aβ LTMR afferents below cervical level C2 but that spared thinly myelinated and unmyelinated afferents (Forget and Lamarre, 1987; Cole and Sedgwick, 1992). Although these individuals were initially thought to lack innocuous range tactile sensation, gentle stroking touch of hairy skin sites actually resulted in a faint, barely perceptible poorly localised cutaneous sensation without qualities of pain or temperature (Olausson *et al.*, 2002; Cole *et al.*, 2006; Olausson *et al.*, 2008a). This stimulus was also associated with a normal appearing sympathetic skin response (Olausson *et al.*, 2008a). In contrast, tactile stimulation directed at Aβ fibres such as vibration and stimulation of glabrous skin were not detected. Importantly, CT-targetted stimulation in this pathological Aβ denervated state did not activate primary somatosensory cortex (in fact there was significant fMRI deactivation). In contrast there was significant activation of dorsal posterior insular cortex. The dorsal posterior insula receives a strong spinothalamic tract input via the ventromedial posterior nucleus of the thalamus (Craig, 2002). It is thought to play a major role as a gateway for the processing of emotionally salient sensory information that represent the physical condition of the body including sensations of pain, itch, temperature as well as viscero receptive activity (Craig, 2002). In contrast to the findings in Aβ deafferented individuals, gentle stroking touch in patients with Hereditary Sensory and Autonomic Neuropathy type V (HSAN-V), who show reduced C-fibre innervation density (Minde *et al.*, 2004), presumably affecting CT

afferents, is perceived as less pleasant than in controls and is associated with flattening of the inverted U-shaped psychophysical pleasantness rating stimulus-response function (Morrison *et al.*, 2011). Furthermore, this is associated with reduced activation of dorsal posterior insula cortex (Morrison *et al.*, 2011). Finally, whilst pleasantness ratings for gentle stroking of the palm, likely lacking significant CT-afferent innervation, are similar to those seen for hairy skin stimulation, descriptor ratings in the touch perception task imply greater salience for sensory as compared to emotional information. Commensurate with this palmar stimulation showed differential activation of somatosensory compared to insula cortex, the opposite to the activations seen for CT innervated skin (McGlone *et al.*, 2012) .

Together these imply not only that primary cortical targets for Aβ and CT afferents are distinct but also that CT cortical inputs are placed within a wider system that is strongly implicated as a neural substrate for human awareness of feelings from the body - including pain, temperature and itch (Craig, 2002). In this respect CT afferents, as with other small diameter afferent fibre classes, can be viewed as serving a function that is more interoceptive than exteroceptive, and one that is primarily protective. There is therefore increasing evidence for the existence of a dual system for tactile sensations on hairy skin with affective and discriminative pathways – as there is for pain. A prediction of a dual touch system, for which there is good evidence for distinct peripheral as well as cortical neural substrates, is the existence of distinct second order spinal projection pathways for the 'relay' of cutaneous CT inputs (Fig 1.2.).

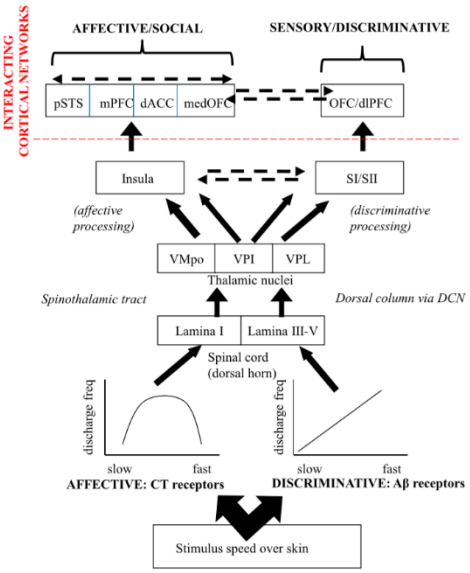

Fig 1.2.

Schematic Diagram of the Dual Pathways Model for Discriminative and Affective Touch on Hairy Skin

CT afferent fibres show an inverted U-shaped stimulus-response to the velocity of stroking and are as such tuned to touch having affiliative/affective importance. In contrast, Aβ fibres respond in a linear fashion to the physical properties of the stimulus. The two types of fibre then show distinct ascending pathways. CT afferent inputs are thought to ascend in a Lamina I spinothalamocortical pathway to insula cortex (see section 1.2.2) whereas Aβ discriminative pathways reach primary sensory cortex via the dorsal columns. The affective processing stream shows prominent forward connections with social brain networks and regions implicated in emotional regulation. Mutual modulation between affective and discriminative processing streams is enabled by reciprocal cortical connections. Abbreviations: VMpo, ventromedial posterior nucleus; VPI, ventroposterior inferior nucleus; VPL, ventral postlateral nucleus; medOFC, medial orbitofrontal cortex; OFC, orbitofrontal cortex; dlPFC, dorsolateral prefrontal cortex; S1/S11, primary and secondary somatosensory cortex (Francis *et al.*, 1999; Craig, 2002; Petrides, 2005; Dum, Levinthal and Strick, 2009; McGlone *et al.*, 2012; McGlone, Wessberg and Olausson, 2014; Morrison, Löken and Olausson, 2010). Figure is adapted from McGlone et al. (2014). Permission to reproduce this figure has been granted by Cell Press.

1.3 SPINAL PROCESSING AND PATHWAYS FOR MECHANOSENSORY AFFERENT INPUTS

There has been increasing recognition that the ascending pathways, rather than being a mere passive conduit for inputs from primary afferent fibres, convey partially processed sensory information (Ma, 2010; Abraira *et al.*, 2017). This processing must occur prior to final output from ascending projection neurons and therefore take place in the primary receiving area for afferent inputs in the spinal cord, the dorsal horn. The grey matter of the spinal cord is organised in to anatomically distinct Rexed laminae (Fig. 1.3). The dorsally located laminae form the major receiving zones for primary afferent inputs. Here more superficial layers (I and II) receive major input from small diameter fibres, including from low threshold C-fibres (Light and Perl, 1979; Sugiura, 1996; Todd, 2010; Li *et al.*, 2011; Abraira and Ginty, 2013). Deeper dorsal horn layers receive input from large diameter fibres and are thought to involved discriminative somatosensation (Sewards and Sewards, 2002). Recent elegant work in rodents shows that the primary LTMR afferents supplying hairy skin terminate in an organised although overlapping manner in the dorsal horn with C- and Aβ fibre endings synapsing in superficial and deeper laminae respectively (Li *et al.*, 2011; Abraira and Ginty, 2013).

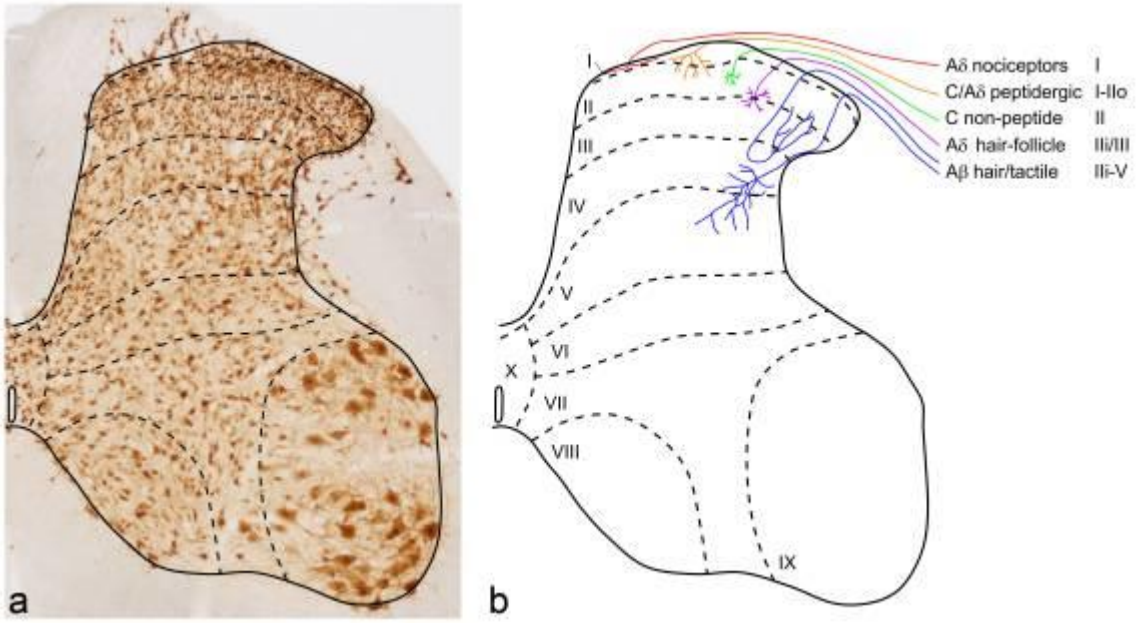

Fig. 1.3. Transverse section of rat mid-lumbar spinal cord and schematic showing Rexed laminae and recipient zones for afferent fibre types. (a) Based on variations in the size and density of neurons Rexed (Rexed, 1952) divided the grey matter of the spinal cord into a series of parallel laminae, the boundaries of which are indicated by the dashed lines. Neurons are labelled by immunostaining with the antibody NeuN. The schematic (b) also shows that the laminar terminations of cutaneous primary afferents are dependent upon fibre diameter and function. Small diameter, nociceptive fibres terminate in Laminae I/II (Light and Perl, 1979; Todd, 2010). Aδ hair follicle afferents show arborisation around the lamina II/III border (Li *et al.*, 2011; Abraira and Ginty, 2013). Aβ LTMR afferents terminate in deeper layers (Brown *et al.*, 1981; Li *et al.*, 2011; Abraira and Ginty, 2013). CT afferent fibres are not specifically show but arborise in lamina II (Light and Perl, 1979, Sugiura et al., 1986, Li et al., 2011, Abraira and Ginty, 2013, Lu and Perl, 2003, Kumazawa and Perl, 1977a). Refs at end. The image is taken from (Todd, 2010). Permission to reproduce this figure has been granted by Nature Publishing Group.

The major ascending somatosensory tracts are the spinothalamic tract and dorsal column pathways. Further ascending pathways projecting to spinal, brainstem/cerebellar and regulatory nuclei that have fibres which respond to noxious and innocuous mechanosensory stimuli have been identified (Willis and

Westlund, 1997). However, the significance or indeed presence of these pathways are not clearly defined in humans. For example, the spinocervical pathway, which is prominent in cats, appears to be absent or vestigial in humans (Morin and Catalano, 1955). Investigations of the spinal processing and relay of somatosensory information in humans have almost exclusively been in the form of studies in patients with discrete lesions affecting the spinal tracts. This, unavoidably, tends to provide 'contaminated' or nonuniform data due to variable lesion location and higher-level compensatory mechanisms (Nathan, Smith and Cook, 1986; Lahuerta *et al.*, 1994). More detailed anatomical, neurophysiological and recently also cellular/molecular information can be obtained in animal studies. However, whilst this can be correlated with behavioural measures, studies in animals lack comparison with conscious perception.

1.3.1 Dorsal Column Pathways

The dorsal column medial lemniscal pathway is known to transmit information from fast conducting Aβ LTMR afferents (Willis and Coggeshall, 1991). The pathway contains directly and indirectly projecting fibres which both ascend ipsilateral to the primary afferent inputs. The direct pathway consists of the central branches of primary afferents that project directly to dorsal column nuclei (Willis and Coggeshall, 1991). Fibres from the lower limbs synapse in the gracile nucleus, and those from the upper limb synapse in the cuneate nucleus. This direct pathway is thought to mediate fine discriminative touch, especially from the hand. The indirect projection, which forms the post-synaptic dorsal column (PSDC) pathway, arises from dorsal horn projection neurons that have cell bodies concentrated in laminae IV and V (Abraira *et al.*, 2017). The receptive field and neuronal firing patterns of PSDC neurons are consistent with relay of processed primary afferent inputs (Whitsel *et al.*, 1972; Nathan, Smith and Cook, 1986; Noble and Riddell, 1988), Indeed, the PDSC appears to be the output of a complex dorsal horn network. This network is overwhelmingly dominated by intrinsic interneurons, the various inhibitory and excitatory sub-classes of which receive and process information from multiple but distinct LTMR inputs as well as descending modulatory fibres (Abraira *et al.*, 2017).

The dorsal column pathway is not thought to convey information relating to noxious range mechanical stimulation. Indeed, dorsal column stimulation is a recognised treatment for chronic pain (Sdrulla, Guan and Raja, 2018). Patients with dorsal column lesions have deficits dominated by profound abnormalities in proprioception as well as spatial and temporal aspects of discriminative mechanosensation (Nathan, Smith and Cook, 1986). In contrast, pain evoked by noxious mechanical stimulation is unaffected (Wall and Noordenbos, 1977). However, a minority of direct dorsal column projection neurons in humans, cats and rats are unmyelinated (Patterson *et al.*, 1989; Patterson *et al.*, 1990; Patterson, Chung and Coggeshall, 1992), many of which are peptidergic and presumably nociceptors (Patterson, Chung and Coggeshall, 1992). Furthermore, Fos protein expression is seen in PSDC neurons following both noxious cutaneous and visceral stimulation (Palecek, Paleckova and Willis, 2003). Overall it is thought that these fibres, particularly those in the PSDC pathway, are important in signaling visceral rather than cutaneous pain (Palecek, Paleckova and Willis, 2003; Palecek and Willis, 2003). This may explain why midline myelotomy, which interrupts the dorsal column pathway, is occasionally used successfully to treat visceral but not somatic pain (Kim and Kwon, 2000). No systematic assessment of affective touch has been made in patients with dorsal column lesions.

1.3.2 The Spinothalamic Tract

The spinothalamic tract ascends in the anterolateral funiculus of the spinal cord. It is sometimes sub-divided into the lateral spinothalamic tract and ventral spinothalamic tract on the basis of distinct cells of origin and their response properties. The latter ascends in the ventrolateral and venteromedial spinal cord in cats (Jones *et al.*, 1987) although whether this is a distinct tract or forms part of the spinothalamic tract in humans has not been established. The lateral spinothalamic tract has long been established as the major

ascending nociceptive and thermoreceptive pathway (Spiller and Martin, 1912). Stimulation of the anterolateral funiculus in humans results in pain, usually with a thermal quality (Mayer, Price and Becker, 1975). Its disruption results in pain relief and deficits in thermoreception as well as nociception (King, 1957; Vierck and Luck, 1979; Peschanski, Kayser and Besson, 1986; Vierck, Greenspan and Ritz, 1990; Lahuerta *et al.*, 1990; Lahuerta *et al.*, 1994; Vierck and Light, 1999; Bain, Hugel and Sharma, 2013).

The axons ascending in the lateral spinothalamic tract almost exclusively have their origin from output cells in lamina I of the spinal cord dorsal horn (Craig, 2002; Jones *et al.*, 1987). These axons decussate, typically 1-2 spinal segments above their origin, to ascend contralateral anterolateral funiculus (Willis and Coggeshall, 1991) (Fig. 1.4.). Within the lateral spinothalamic tract fibres are arranged somatotopically with the upper body being represented antero-medially and more caudal regions postero-laterally (Lahuerta *et al.*, 1994).

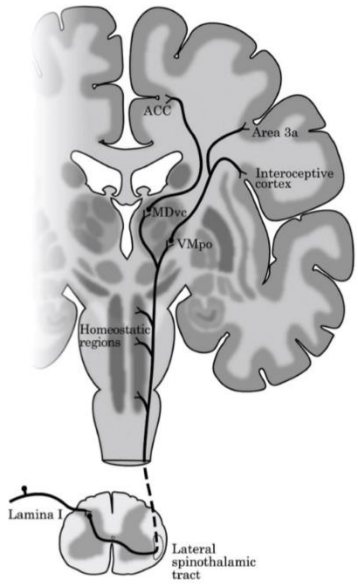

Fig. 1.4.

Schematic of the lamina I spinothalamocortical pathway. Abbreviations: ACC, Anterior Cingulate Cortex; VMpo, Ventromedial Posterior Nucleus; MDvc, Ventrocaudal Portion of the Medial Dorsal Nucleus The image is taken from (Craig, 2003). Permission to reproduce this figure has been granted by Annual Reviews.

There is evidence of functional-anatomical organisation of STT projection neurons within lamina I of the dorsal horn (Craig, 2003; Han, Zhang and Craig, 1998; Craig and Dostrovsky, 2001). The lamina I output cells receive both monosynaptic and polysynaptic inputs from small diameter primary afferent fibres (Craig, 2003; Todd, 2010). A number of subtypes of lamina I projection neuron have been identified in cats and rodents, classified according to their response characteristics (Craig, Krout and Andrew, 2001; Craig and Andrew, 2002; Andrew and Craig, 2002b; Christensen and Perl, 1970). These neuronal subtypes can also be distinguished on the basis of their morphology and membrane properties (Han, Zhang and Craig, 1998; Craig, Krout and Andrew, 2001). Lamina I cells have been identified that specifically respond to warm or cool stimuli (Andrew and Craig, 2001; Craig, Krout and Andrew, 2001; Dostrovsky and Craig, 1996). In addition, there are at least two types of nociceptive cell types, nociceptive-specific (NS) and polymodal nociceptive, also termed HPC cells (standing for heat, pinch and cold)(Andrew and Craig, 2002a; Andrew and Craig, 2002b). NS cells are better than HPC cells at encoding force and stimulus surface area (Andrew and Craig, 2002a) as well as maintaining firing to prolonged mechanical stimulation (Andrew and Craig, 2002b). These features are in keeping with predominant Aδ nociceptor inputs (Handwerker, Anton and Reeh, 1987; Garell, McGillis and Greenspan, 1996; Andrew and Greenspan, 1999; Slugg, Meyer and Campbell, 2000) and suggest that activity in NS cells strongly contribute to the sharp/pricking nature of first

pain (Andrew and Craig, 2002b) and pain during sustained noxious range mechanical stimulation. In contrast HPC cells, which predominantly receive C-fibre inputs, exhibit temporal summation to repeated heat stimuli, a feature that correlates with a build-up of burning pain in the equivalent human psychophysical studies.

This dissociation in the function of lamina I projection neurons has led to the suggestion that there exist distinct functionally specific channels for first (sharp) and second (burning) pain within the lateral spinothalamic tract (Craig, 2003). Further supporting this 'labelled line' theory, lamina I projection neurons that respond to a single specific stimulus, for example NS cells that specifically respond to noxious heat (Craig and Kniffki, 1985), have also been identified. Indeed, there is some evidence in humans that pathways mediating ascending relay of noxious mechanical and heat show anatomical segregation within spinothalamic pathways and indeed the thalamus itself (Lenz *et al.*, 1993a; Lenz *et al.*, 1993b; Lenz *et al.*, 1994; Friehs, Schröttner and Pendl, 1995; Bowsher, 2005).

The cells of origin of the ventral spinothalamic tract are, in contrast to those of the lateral spinothalamic tract, located in deep dorsal horn laminae (IV-V and VII-VIII)(Meyers and Snow, 1982). These cells have large, occasionally bilateral, receptive fields and are responsive to innocuous mechanical stimuli. The same cells also respond to noxious cutaneous stimuli as well as input from visceral and proprioceptive afferents. In view of this response to a variety of innocuous and noxious stimuli they are often termed wide dynamic range (WDR) neurons. Transmission of innocuous mechanosensory information in the ventral spinothalamic tract could account for the observation that touch sensation remains partially intact, albeit with reduced spatial acuity, after lesions that disrupt the dorsal column and other ascending pathways (e.g. spinocervical pathway in the cat) but spare the anterolateral funiculus (Vierck, 1977; Wall and Noordenbos, 1977; Nathan, Smith and Cook, 1986; Danielsson and Norrsell, 1989). Deficits in tactile sensation are more profound or ostensibly complete if the anterolateral tracts are also interrupted.

The lateral spinothalamic tract has not been considered to relay innocuous tactile information. Lamina I output cells under normal circumstances[1] rarely, if ever, respond to electrical stimulation of Aβ fibres (Craig, 2010). However, it is increasingly recognised that the lamina I spinothalamocortical pathway plays a major role in homeostasis and interoception (Craig, 2002) and, crucially, that affective/pleasant aspects of touch form an integral part of this system (McGlone, Wessberg and Olausson, 2014). In this respect CT-afferents would be expected to follow the route of other C-fibre inputs (Fig. 1.2). Stimulation of CT-afferents is associated with activation of the dorsal posterior insula (Olausson *et al.*, 2002). The major somatosensory input into dorsal posterior insular cortex in primates is from posterior ventral medial nucleus of thalamus (Craig *et al.*, 1994; Craig and Zhang, 2006). Spinal inputs to this relay derive almost exclusively from dorsal horn lamina I via the anterolateral, or spinothalamic, tract (Craig and Zhang, 2006). In rodents, cats and primates, the central terminals of C-LTMR afferents synapse in Laminae II/IIIi of the dorsal horn (Light and Perl, 1979; Sugiura, Lee and Perl, 1986; Li *et al.*, 2011; Abraira and Ginty, 2013; Lu and Perl, 2003; Kumazawa and Perl, 1977a). Lamina II cells activated by C-LTMR afferents include vertical neurons (Grudt and Perl, 2002) which, in turn, have axons that arborise in Lamina I (Maxwell *et al.*, 2007) and contact projection neurons (Lu and Perl, 2005). C-LTMR afferents in rodents express vesicular glutamate transporter 3 (VGLUT3) (Seal *et al.*, 2009; Reynders and Moqrich, 2015; Reynders *et al.*, 2015). The terminals of VGLUT3 neurons form dense connections with a specific form of Lamina II excitatory interneuron that expresses γ isoform of protein kinase C (PKCγ) (Seal *et al.*, 2009). Therefore, the first synapse of C-LTMR may be with PKCγ excitatory interneurons[2]* which, when excited, activate lamina I projection neurons (Lu *et al.*, 2013). Indeed, recordings from rat spinoparabrachial tract have identified a sub-population of L1 projection neurons that, in keeping with them receiving C-LTMR afferent input, not

[1] Polysynaptic pathways have been identified that relay inputs from Aβ LTMRfibres to lamina I (Cheng et al., 2017).
[2] Indicating a greater degree of complexity, a recent characterisation of diverse classes of dorsal horn interneuron has, however, shown that other sub-types are also contacted by C-LTMRs (Abraira et al., 2017).

only fire to gentle stroking touch but also show preferential tuning to slow velocities (Andrew, 2010). Interestingly all slow touch responsive neurons responded also to noxious mechanical and noxious heat stimuli, thus form a second population of mechanoreceptive WDR neurons.

There is therefore a deal of indirect evidence from rodent studies suggesting that information salient for pleasant/affective touch, including the velocity dependent tuning curve associated with pleasant touch, will, similar to that from other small diameter inputs, ascend in the spinothalamic tract rather than classical discriminative dorsal column-medial lemniscal touch pathways. Potential deficits in affective touch after spinothalamic tract lesioning were intimated by Otfrid Foerster's eloquent clinical observations of patients who underwent surgical sectioning of the anterolateral funiculus (Foerster, 1932)

'*Except for the pain- and temperature sensations, also other sensory qualities were spoiled after the anterolateral transection. First of all, the feelings of tickle and itch were included, but so were all other feelings of pleasure and displeasure as well*.' (p. 43, translated from the original German)

A subjective reduction in erotic touch has also been reported following anterolateral cordotomy (Lahuerta *et al.*, 1990). However, the second order pathway for CT-afferents is not clearly established and in humans direct evidence of a dedicated spinothalamic second order pathway for CT inputs exists is lacking.

1.4 TOUCH-PAIN INTERACTIONS

There is a complex relationship between tactile sensation and pain. Touch is often used to sooth pain. However, in some circumstances, such as neuropathic pain, even the slightest touch can be painful. Adding to the complexity, just as touch can affect pain, pain can affect the perception of tactile stimuli.

1.4.1. Soothing touch

In experimental settings tactile stimulation, in general, has the effect of reducing the pain intensity of a concomitantly applied noxious stimulus. The mechanisms underlying the pain inhibition are likely to be manifold in terms of the afferent fibre types and neural networks involved. Pain inhibition by tactile stimulation was initially believed to be due to activation of Aβ afferents. Indeed vibrotactile stimulation, which strongly activates Aβ afferents, have been shown to reduce experimental pain (Wall and Cronly-Dillon, 1960). However, recent evidence suggests that CT afferent activity is strongly linked to the analgesic effect of touch.

CT-afferent targetted touch is effective in reducing thermally induced pain (Liljencrantz *et al.*, 2017; Habig *et al.*, 2017) in neurologically intact individuals but this effect is impaired in patients with acquired small fibre neuropathy (Habig *et al.*, 2017). Interestingly the analgesic effect of CT targetted touch also occurred when the tactile and noxious stimuli were separated in time (Liljencrantz *et al.*, 2017), an effect also seen in neonates undergoing heel prick (Gursul *et al.*, 2018). The beneficial effects of tactile stimulation on pain were less marked or absent with Aβ targetted stimulation (Liljencrantz *et al.*, 2017). Animal studies also support an analgesic role for C-LTMR fibres. For instance, a specific monosynaptic inhibitory pathway has been identified in the sustantia gelatinosa (dorsal horn lamina II) in which C-LTMR impulses suppress those from C-nociceptors (Lu and Perl, 2003). Also, the intrathecal administration of the chemokine TAFA4, which is a selective marker of Tyrosine Hydroxylase positive C-LTMRs, markedly reduces inflammation associated mechanical hypersensitivity in mice (Delfini *et al.*, 2013). Conversely TAFA4-null mice show the opposite pattern, namely enhanced mechanical and chemical hypersensitivity, an effect which is reversed by application of TAFA4. This process involves reinforcement of inhibitory synaptic transmission within spinal networks through activation of GABAergic transmission and an increase in the number of inhibitory synapses on lamina IIi somata (Kambrun *et al.*, 2018). As well as spinal mechanisms (Salter and Henry, 1990; Dougherty, Willis and Lenz, 1998; Lu and Perl, 2003; Delfini *et al.*, 2013) neural networks at cortical (Inui, Tsuji and Kakigi, 2006) and sub-cortical levels (Mancini *et al.*, 2015) are also thought to play an important role in the inhibitory effect of tactile stimulation on pain.

1.4.2 Painful touch

Mechanical allodynia, characterised by an extremely painful unpleasant response to light touch, provides a striking example of how the processing of innocuous somatosensory stimuli is altered in the context of pain. Mechanical allodynia provides a protective role, such as in the guarding of an injury, but is also a common maladaptive accompaniment of neuropathic pain conditions. Sufferers can be unable to wear even the lightest of clothes against affected areas (Lolignier, Eijkelkamp and Wood, 2015).

The precise mechanisms underlying this phenotypic switch from innocuous to noxious are not fully understood. The classical view is that under conditions of central sensitisation Aβ inputs gain access to and excite nociceptive pathways in the spinal cord (Woolf, 1993). This view is supported by most although not all studies in human subjects in which selective blockade of myelinated afferents abolishes allodynia (Torebjörk, Lundberg and LaMotte, 1992; Koltzenburg, Lundberg and Torebjörk, 1992; Cervero and Laird, 1996; Landerholm and Hansson, 2011; Nagi *et al.*, 2011). Indeed, tactile allodynia does not develop in patients with sensory neuronopathy who lack Aβ fibres (Liljencrantz *et al.*, 2013). Furthermore, studies in rodents have documented spinal polysynaptic pathways by which Aβ inputs gain access to lamina I projection neurons (Cheng *et al.*, 2017). However, there is a conspicuous similarity in the stimulus that

characteristically evokes mechanical allodynia, namely a gentle stroking touch, and that which preferentially activate CT afferents (Vallbo, Olausson and Wessberg, 1999; Loken *et al.*, 2009; McGlone, Wessberg and Olausson, 2014). Additionally, the relationship between Aβ activation and the perception of pain and unpleasantness of tactile stimulation in allodynia is less than straightforward.

Whilst pain intensity ratings positively correlate with the velocity, and presumably also Aβ firing frequency, of a gentle stroke applied to sensitised hairy skin, the perceived unpleasantness of touch actually decreases with increasing stimulus velocity (Löken, Duff and Tracey, 2017). Although they do not develop mechanical allodynia, Aβ denervated individuals do perceive touch applied to an experimental allodynic area as less intense when compared to untreated skin (Liljencrantz *et al.*, 2013). Moreover, implying a change in central processing of CT afferents in the allodynic state, both Aβ denervated as well as neurologically intact individuals show differences in activation of the posterior insula cortex to stimulation of the allodynic versus control areas. These findings are consistent with both a suppression of CT-afferent inputs and prioritisation of nociceptive signalling under conditions of central sensitisation.

1.4.3 Pain and hypoesthesia

It has been well-documented that a pain can be associated with tactile hypoesthesia. Patients with pain often remark on subtle numbness around the painful site. Indeed, deficits in touch discrimination have been described in patients around an area affected by chronic pain (Hollins, Sigurdsson and Morris, 2001; Maihöfner *et al.*, 2006), an effect that can be reversed with pain relief (Nathan, 1960). In experimental models of pain, such as those using capsaicin, there often develops an area of secondary tactile hypoesthesia, which can occur even in the absence of dynamic mechanical allodynia (Magerl and Treede, 2004; Geber *et al.*, 2008). Paradoxically, in both pain models and in patients, tactile hypoesthesia can co-exist with mechanical hyperalgesia (Geber *et al.*, 2008). The deficits are thought to represent plasticity in the processing of tactile input although this is not directly related to ongoing pain intensity (Geber *et al.*, 2008). Potential mechanisms acting at a spinal segmental (Geber *et al.*, 2008; Dougherty, Willis and Lenz, 1998) as well as supraspinal level (Brüggemann, Shi and Apkarian, 1998; Apkarian *et al.*, 2000) have been proposed. Whether reduced processing of CT afferent inputs contributes to tactile hypoesthesia in painful states is unclear although the putative suppression of CT-afferent inputs in allodynia (Liljencrantz *et al.*, 2013; Liljencrantz and Olausson, 2014) may be relevant in this respect.

2 AIMS

The overall aim of this thesis is to further define the neural basis of human noxious and innocuous range mechanosensation at the level of both the primary afferent fibre, spinal cord and higher cortical function. To do this several gaps in the current knowledge base will be addressed.

Using the method of microneurography to record from individual afferent fibres **Paper I** aims to address the following questions:

1) Do human field units have mechano-nociceptive properties?

2) If not, are there other fast conducting myelinated fibres that that are high threshold and encode cutaneous mechanical stimuli in the noxious range?

3) If present, do human Aβ fibres with mechano-nociceptive properties contribute to conscious perception of cutaneous noxious range mechanical stimuli?

4) Are there identifiable deficits in the perception of noxious range mechanical stimulation in individuals who lack Aβ fibres?

5) Are there identifiable preservations in the perception of noxious range mechanical stimulation in individuals who lack small diameter fibres because of a rare nerve growth factor-β (NGFB) mutation (HSAN-V)?

By quantifying small fibre denervation and relating this to pain evaluation measures in patients with HSAN-V **paper II** aims to address the following questions:

1) Is there evidence of small fibre denervation in the cornea of individuals heterozygous and homozygous for the rare nerve growth factor-β (NGFB) mutation?

2) Does the degree of small fibre denervation correlate with clinical measures of disease severity?

3) Is there a functional relationship between small-fibre density and higher-level pain experience?

Using psychophysical testing in patients undergoing therapeutic ablation of the spinothalamic tract **Papers III and IV** address the following questions

1) What is the 2nd order pathway for CT afferents in humans?

2) Does lesioning of the presumed 2nd order CT pathway produce identifiable deficits in affective touch using the standard psychophysical tests of CT function?

3) Does lesioning of the spinothalamic tract produce a similar pattern of deficit to that seen in C-fibre denervated individuals with HSAN and congenital insensitivity to pain?

4) Is there further evidence to suggest segregation of ascending pathways relaying information relating to noxious range thermal and noxious range mechanical stimuli in the human spinal cord?

Using psychophysical testing in a human experimental pain model **Paper V** addresses the following questions:

1) Is there evidence of abnormal processing of CT as well as Aβ LTMR afferent inputs in a model of tactile allodynia?

2) Is there evidence that CT afferent activation mediates tactile allodynia?

3) Does the area of secondary tactile hypoesthesia relate to the degree of suppression of CT and Aβ processing?

3 METHODOLOGICAL CONSIDERATIONS

3.1 STUDY APPROVAL AND ETHICS

All testing was preformed according to the Declaration of Helsinki. Informed and written consent was obtained.

For **Paper I**, studies performed in Sweden were approved by the local ethics committee of the medical faculty, University of Linköping, Sweden (dnr 2015/305-31). Reimbursement was provided at SEK 200 per hour. Studies performed in Liverpool were approved by the local ethics committee for Liverpool John Moores University (14/NSP/039). Reimbursement was provided at £10 per hour.

For **Paper II** and **Paper V** studies were approved by the local ethics committee of the medical faculty, University of Gothenburg, Sweden. Reimbursement was provided at SEK 200 per hour.

For **Paper III** and **Paper IV** studies were approved by the Health Research Authority National Research Ethics Service (study reference 14/NW/1247).

3.2 PARTICIPANTS

For **Paper I**, 89 healthy participants (63 males, 26 females; range: 20-49 years) were recruited for microneurography studies. Some individuals were recruited more than once with an interval of at least 3 months (97 experimental sessions in total). Psychophysical testing for mechanical pain was performed on two large-fibre deafferented subjects (one male, 64 years; one female 66 years) and 3 age- and gender-matched controls (range: 58-65 years), in addition to 16 healthy participants from the microneurography sample (20-30 years).

For **Paper II**, 19 individuals (11 males, 3 homozygous, mean age 50 ± SEM 4.9) with a mutation of the NGFB gene were investigated. 19 healthy age-matched controls for the corneal confocal microscopy investigation and 12 healthy age-matched controls for the SPQ questionnaire were included in the analysis. The homozygous individuals did not share parents and were only distantly related. Each presented with painless fractures, osteochondritis, bone necrosis and neuropathic joint destruction. Compared to the homozygous carriers, the symptomatic heterozygote carriers in this sample had later onset of symptoms (20-70 years) and less severe clinical signs. Although not suffering from painless fractures, they often manifested Charcot arthropathies at single or multiple joints, particularly in the lower extremities. All individuals had normal autonomic and cognitive functions and considered themselves to have normal touch sensibility with no history of allodynia or hyperalgesia (Minde *et al.*, 2004; Minde, 2006; Minde *et al.*, 2006; Minde *et al.*, 2009).

For **Paper III** and **Paper IV**, all patients were recruited following admission to the Walton Centre for Neurology and Neurosurgery, Liverpool, UK. All suffered from intractable unilateral cancer related pain below the cervical level C4 with an expected lifespan of less than 12 months. The patients' demographic and clinical details are shown in table 6.1. The majority of patients carried a diagnosis of pleural mesothelioma with treatment resistant chest wall pain. No patient had symptoms or signs of neurological impairment in the region of sensory testing. All patients were medicated with regular and *pro re nata* opioids as well as a variety of non-opioid analgesia. The most common descriptor terms on the Short Form McGill Pain Questionnaire (SF-MPQ) (Melzack, 1987) for the cancer related pain were aching, shooting and stabbing (Fig. 6.1). The median and range for numeric rating scale of average 4 hours' pain, maximum pain in the past 4 hours and current pain were 76 (20-90), 98 (79-100) and 50 (10-81) respectively. A large number (13/19) of patients had previously received chemotherapy with potential peripheral neurotoxicity although no patient described ongoing symptoms potentially attributable to this.

For **Paper V** 40 healthy subjects (median age 25 years, range 19–43 years, 20 men) were studied.

3.3 PAPER I - STIMULATION PARADIGMS AND EXPERIMENTAL DESIGN

3.3.1 Nerve recording and search procedure

Recordings from single afferents were obtained using the microneurography technique (Hagbarth and Vallbo, 1967; Vallbo *et al.*, 1979; Vallbo, Hagbarth and Wallin, 2004; Vallbo, 2018). Recordings were taken from the peroneal nerve proximal to the fibular head (Fig. 3.1). In some cases, recording was made from the radial nerve main branch at mid-humeral level.

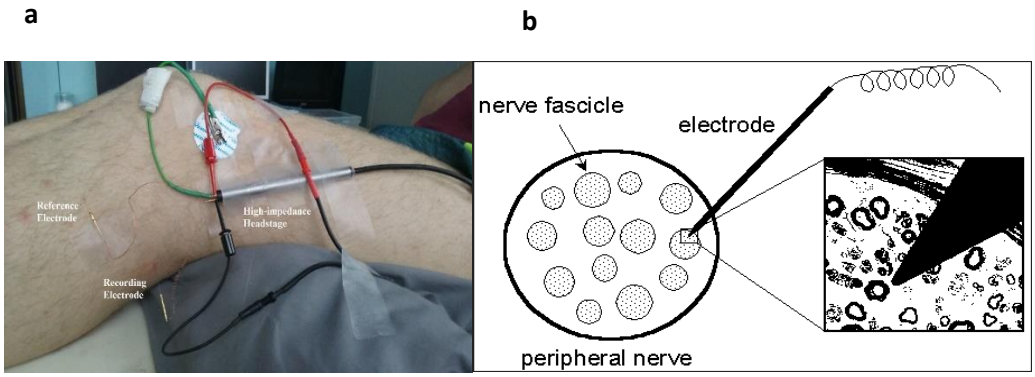

Fig. 3.1.

(a) Experimental set up for peroneal microneurography. (b) Schematic showing the intrafascicular location of the tungsten microelectrode.

The course of the peroneal nerve at the level of the knee was approximated by surface electrical stimulation (1-4 mA, 0.2 ms, 1 Hz) using a 2-mm-diameter probe connected to an optically isolated constant-current stimulator (Setup 1: FE180 Stimulus Isolator; ADInstruments, Oxford, UK). This was followed by precise localization of the nerve by percutaneous insertion of a high-impedance tungsten recording microelectrode to deliver weak electrical pulses (0.02-1 mA, 0.2 ms, 1 Hz; FHC, Inc. Bowdoin, ME, USA). For the radial nerve stimulation was also employed but the initial approach to the nerve was guided by high frequency ultrasound imaging. The electrode was insulated, except for the ~5 µm bare tip, with a typical length of 20 mm for peroneal and 40mm for the radial nerve. The shaft diameter was 0.2 mm. An uninsulated subdermal electrode in an adjacent area served as the reference. A high-impedance pre-amplifier was taped to the skin near the recording electrode (Setup 1: MLT185 headstage, ADInstruments, Oxford, UK), which was used in conjunction with a low-noise high-gain amplifier (Setup 1: FE185 Neuro Amp EX; ADInstruments, Oxford, UK). Once the electrode tip was intra-fascicular, indicated by subject's reports of cutaneous sensations to weak electrical stimulation (≤ 0.02 mA), the neural activity was amplified and single LTMRs were searched by soft-brush stroking (1-10 cm s^{-1}), and single HTMRs by coarse-brush stroking (1-10 cm s^{-1}) or pinching, the fascicular innervation zone while making small adjustments to the electrode position. A subset of experiments was performed using Setup 2 – in which the data was digitally collected using a bespoke amplifier and sampled using SC/ZOOM, both developed by the Physiology Section, Department of Integrative Medical Biology, Umeå University – following the aforementioned protocol.

3.3.2 Unit identification

Individual Aβ LTMRs (all soft-brush sensitive) were separated into rapidly adapting (RA) and slowly adapting (SA) types on the basis of their adaptive responses to ramp-and-hold indentation of the skin, as per the criteria used in Vallbo et al (1995). Three groups of RA units were identified: Aβ RA1-LTMR (hair unit),

responsive to deflection of individual hairs and light air puffs – air puff responses were abolished after hair removal; Aβ field-LTMR, comprising multiple spots of high sensitivity with no response to hair displacement or remote tapping of the skin; Aβ RA2-LTMR (Pacinian unit), comprising a single spot of maximal sensitivity and robust response to remote tapping/vibration. Two groups of SA-LTMRs (type 1 and 2) were identified where several features were examined including spontaneous firing, stretch sensitivity, and receptive field characteristics. Additionally, inter-spike interval pattern to sustained indentation (100 mN for 30-60 s) was tested, where possible, with a skewed and broad pattern for SA1 and normal distribution for SA2. Coefficients of variation of inter-spike intervals were 0.80 for SA1 (median; range: 0.32-3.53; n=16) and 0.29 for SA2 (median; range: 0.21-0.60; n=6) (Edin, 1992; Vallbo *et al.*, 1995).

Mechanical threshold and receptive field size of individual units were determined using Semmes-Weinstein monofilaments (nylon fibre; Aesthesio, Bioseb, Pinellas Park, FL, USA). If a unit responded to the same (weakest) monofilament in at least 50% of trials, it was taken as the mechanical threshold. For receptive field mapping, pre-determined monofilament forces of 100 and 600 mN were used for LTMR and HTMR, respectively. The receptive field area was estimated by treating it as an ellipse. Latency responses to surface electrical stimulation of the receptive field were captured which, together with the distance from the stimulation site to the recording electrode, were used to calculate the conduction velocity (m s^{-1}). Electrically and mechanically evoked spikes were carefully compared on an expanded time scale to ensure that the electrically stimulated unit was the same as the one that was mechanically probed. In one instance, the response latency was estimated by rapid mechanical tapping, using an electronic filament, of a receptive field spot.

3.3.3 Mechanical skin stimulation

The punctate force-coding function of individual units was tested with a range of forces, applied to high-sensitivity spots in the receptive field, using commercially available monofilaments (nylon fibre; Aesthesio, Bioseb, Pinellas Park, FL, USA), in addition to custom-built electronic monofilaments that allowed high-resolution (1 ms) force measurements using capacitive sensors (nylon fibre; Physiology Section, Department of Integrative Medical Biology, Umeå University). Each monofilament trial-set comprised of eight consecutive applications of different forces, applied for 5 s each: 4, 10, 20, 60, 100, 260, 1000 and 3000 mN (corresponding values in pressure: 16, 24, 27, 53, 68, 106, 193 and 292 g/mm^2). The trial commenced when the filament came into contact with the skin and lasted until it was withdrawn. When using the commercial apparatus, the start and end of the filament-skin contact were indicated with a push-button by the experimenter. Where the recording was stable enough, data for replicate trials of each monofilament force were collected.

3.3.4 Single-unit intra-neural microstimulation

The same recording electrode was also used to stimulate that same axon using low-current electrical pulses (1 and 30 Hz; µA range, starting from zero in small increments). That the same recorded afferent was stimulated electrically depends on the spatial alignment of the evoked percept, i.e. the projected field, with the physiological receptive field. This was determined by questioning the participant about the location of the projected field relative to the location of mechanical touch applied to the receptive field. Where a receptive field-projected field overlap was found, sensory qualities of the resulting sensation were noted by asking the participant to describe what they felt followed by a questionnaire (Fig. 3.2).

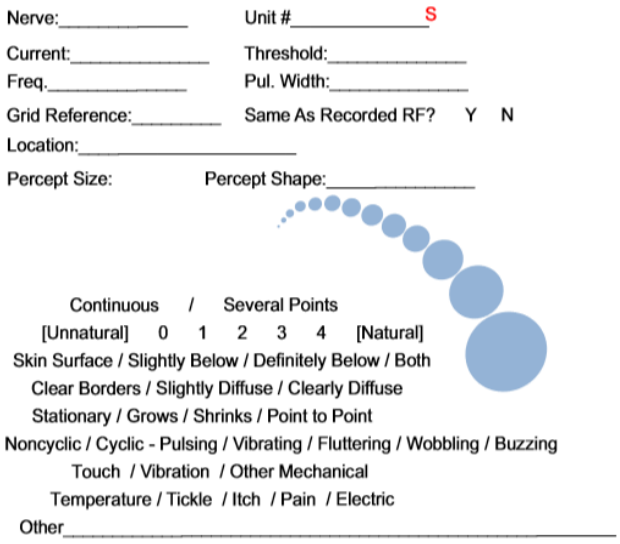

Fig. 3.2.

Questionnaire for sensory qualities. Activation of single units by intra-neural microstimulation produced conscious percepts that were captured by administering this questionnaire (modified from previous studies (Ochoa and Torebjörk, 1983; Vallbo *et al.*, 1984; Macefield, Gandevia and Burke, 1990)).

3.4 PAPER II - STIMULATION PARADIGMS AND EXPERIMENTAL DESIGN

3.4.1 In vivo corneal confocal microscopy

In vivo corneal confocal microscopy was performed using a Heidelberg Retina Tomograph (HRT III, Rostock Cornea Module) based on an established protocol (Tavakoli and Malik, 2011). The subject's eyes were anesthetized using 0.4% benoxinate hydrochloride, and Viscotears were applied on the front of the eye for lubrication. Participants fixated on a target with the eye that was not being examined. A drop of viscoelastic gel was placed on the tip of the objective lens, and a sterile disposable Perspex cap was placed over the lens. The gel optically couples the objective lens to the cornea. Several scans of the entire depth of the cornea were recorded by turning the fine focus of the objective lens backwards and forwards for approximately 2 minutes to acquire satisfactory images of all corneal layers providing *en face* two dimensional images with a lateral resolution of approximately 2 µm/pixel and final image size of 400 pixels x 400 pixels. High quality images of the sub-basal nerve plexus of the cornea were obtained from each patient and control subject. This layer is of particular relevance for defining neuropathic changes, since it is the location of the main nerve plexus that supplies the overlying corneal epithelium. These nerve fibre bundles contain unmyelinated fibers, which run parallel to the Bowman layer before dividing and turning upwards toward the surface to terminate as individual axons underneath the surface epithelium. Six images per subject from the centre of the cornea were selected and examined in a masked and randomized fashion using purpose-written, proprietary software (CCMetrics; M. A. Dabbah, Imaging Science, University of Manchester). Three corneal nerve parameters were quantified: (i) Corneal nerve fibre density (CNFD) - the total number of main fibres per mm2 of corneal tissue; Corneal nerve branch density (CNBD) - the number of branches emanating from all major nerve trunks/mm2 of corneal tissue; (iii) Corneal nerve fiber length (CNFL) - the total length of all main fibres and branches (mm/mm2) within the area of corneal tissue. Examination of stromal layer images were also performed to assess the presence of Aδ fibres.

3.4.2 Situational Pain Questionnaire

A Swedish translation of the original 30-item Situational Pain Questionnaire (SPQ) was administered to the subjects (Clark and Yang, 1983). The SPQ consists of a series of situations describing low-painful (“I have been bitten by a mosquito”) or high-painful events (“The dentist drills in one of my teeth without anesthesia”). Subjects rate the amount of pain they would feel in those imaginary situations on a scale going from 1 “non-noticeable”, to 10 “worst possible pain”. The SPQ addresses the ability of the subjects to discriminate imaginary painful from non-painful situations and also assesses the amount of pain they think they would experience if presented with those situations. The interpretation of the SPQ is based on a model of Signal Detection Theory that allows the quantification of the ability to discriminate between the two categories of events and the amount of pain perceived, via two scores (Danziger, Prkachin and Willer, 2006). The first score P(A) looks the ability of the subject to discriminate between high-painful and low-painful situations, whereas the (B) score reflects the degree to which the situations are considered as painful. In Signal Detection Theory a response to a double choice task can be categorized as hit or false alarm, whether it is a response to signal or noise. In this case signal refers to high-painful situations and noise to the low-painful ones. A correct discrimination between low and high-painful situations (i.e. high pain ratings for high-painful events and low pain ratings for low-painful events) means a high rate of responses to signal (hits) P(S/s) and a low rate of responses to noise (false alarms) P(S/n). P(A) is obtained by calculating the area under the receiver operating characteristic curve, obtained by plotting P(S/s) against P(S/n). The P(A) value can range from 0.5 to 1, where 1 reflects perfect discrimination. The score B indicates the extent of pain perceived and is calculated by looking at the median response rating for hits and false alarms (P(S/s) + P(S/n) = 1. A high B score reflects a low degree of pain perceived (Danziger, Prkachin and Willer, 2006).

3.5 PAPER III and PAPER IV - STIMULATION PARADIGMS AND EXPERIMENTAL DESIGN

In **Paper III** affective and discriminative as well as noxious range mechanical sensation, itch and innocuous and noxious temperature sensation were assessed. Stroking skin stimulation was also used for the Touch Perception Task (TPT). All stimuli were delivered on the hairy skin of the right and left dorsolateral forearm both before and after cordotomy. For all tactile stimuli patients were prevented from seeing the stimulated limb. Temperature sensation and noxious mechanical stimulation were also assessed in **Paper IV**, including sites in the lower limb and trunk.

3.5.1 Anterolateral Cordotomy

The antero-lateral cordotomy was performed at the C1/C2 level contralateral to the cancer related pain (Bain, Hugel and Sharma, 2013). In brief, the procedure was performed with sedation and local anesthesia. Following dural puncture with a 20G spinal needle the cordotomy electrode was advanced into the antero-lateral quadrant of the spinal cord (fig 3.3). Positioning in the spinothalamic tract was verified by eliciting cold, heat or other painful sensations, encompassing the region of cancer related pain, using 50Hz electrical stimulation through the cordotomy electrode. Motor twitch threshold using 10Hz stimulation was also performed to assess for proximity to the corticospinal tract. Adjustments of the electrode were made to maximise location with the spinothalamic tract and minimise proximity to motor pathways. The spinothalamic tract was disrupted using a radiofrequency current which produces a heat induced lesion. This was performed in steps, typically starting at 65°C for 25-30s, with a maximum temperature of 85°C. Lesioning of the spinothalamic tract was confirmed in the operating theatre by demonstrating a contralateral loss of temperature sensation. Operative details for all cases are shown in table 6.1.

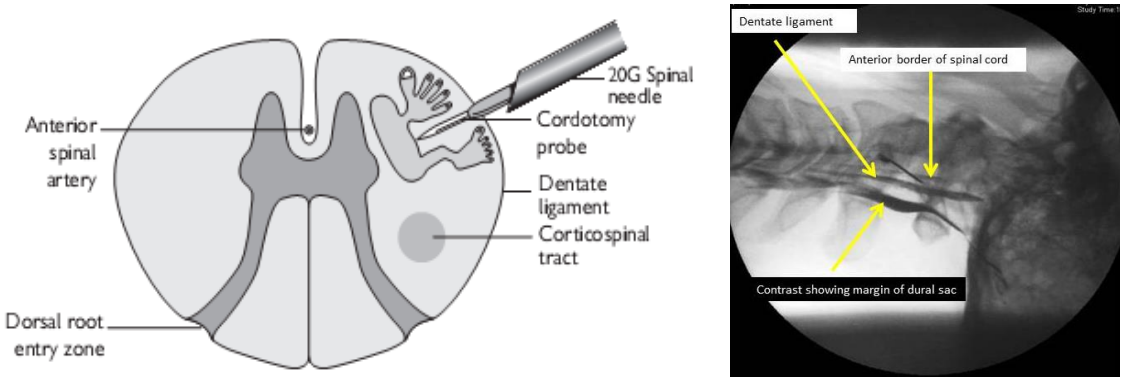

Fig. 3.3.

(a) Schematic of the neuroanatomical approach during percutaneous anterolateral cordotomy. (b) Intraoperative myelogram performed in the supine position. The contrast dye can be clearly seen to outline the dentate ligament. The spinal needle is inserted in to the spinal cord anterior to the dentate ligament.

3.5.2 Discriminative touch

Mechanical sliding calipers were used to assess two-point discrimination. Discrimination thresholds were measured using an ascending-descending method. Mechanical detection thresholds were measured using a standardized set of von Frey monofilaments (Optihair2- Set Nervtest, Germany), which bend at forces

between 0.25 and 512mN (von Frey, 1896) according to the 'method of limits'. Graphesthesia was measured by assessing whether participants could identify numbers drawn the skin using the blunt end of a Neurotip (Owe Mumford Ltd, UK).

3.5.3 Affective touch

Skin stimulation was delivered manually over a 10 cm distance using a 70mm wide goat's hair artist brush with an indentation force of approximately 0.3 N. Stimuli were given at three different velocities (0.3, 3 and 30 cm s^{-1}). The experimenter (AM) was trained in the delivery of the stimuli. Also, a display on a computer monitor, not visible to the patient, with a stripe moving representing the appropriate velocity in each trial was used to guide stimulation. Patients were asked to rate both the pleasantness and intensity of the stimulation using a 20 cm paper visual analogue scale. Anchor points for touch intensity were no sensation (0) and very intense (10). For pleasantness anchor points were 'unpleasant' (−10) and 'pleasant' (10) with 0 representing a neutral stimulus.

3.5.4 Noxious mechanical skin stimulation

Assessment of pinprick sensation on the right and left dorsolateral forearm was made using a Neurotip (Owe Mumford Ltd, UK).

3.5.5 Itch

Assessment of itch sensation was made using cowhage. Cowhage spicules contain the pruritogen mucunain (Reddy *et al.*, 2008; Davidson and Giesler, 2010) and on contact with the skin induce a histamine independent itch via activation of proteinase-activated receptors-2 and -4 (Reddy *et al.*, 2008; Davidson and Giesler, 2010). Recordings in primates have shown that cutaneous application of cowhage activates ascending spinothalamic projection neurons (Davidson *et al.*, 2012). Approximately 20 cowhage spicules were collected onto a cotton bud and rubbed directly on a $1cm^2$ skin site for 20 seconds. Spicules were then removed with a strip of lightly-adhesive paper tape (Micropore, 3M, USA) immediately after application. Assessments were made post-cordotomy only, on the right and left side. Patients were asked to rate the intensity of itch on a numeric rating scale (0-100). If no perception of itch was elicited cowhage application was repeated up to a maximum of three times before the sensation was judged to be absent.

Cowhage was used because of availability and the lack of need for specialist equipment for administration in a hospital setting. Also, cowhage induces a much more intense itch sensation compared to histamine (Papoiu *et al.*, 2011).

3.5.6 Temperature sensation

Assessment of cold sensation detection threshold, warm sensation detection threshold, cold pain threshold and heat pain threshold was made using the method of limits with the MEDOC TSA II (Medoc, Ramat Yishai, Israel) on the dorsal aspect of the right and left forearm. The thermode had a surface area of 9.0cm2 and baseline temperature of 32°C. Thresholds were obtained using ramped stimuli of 1°C/s, the patient terminating the ramp with a button press. The mean of three consecutive temperature thresholds was calculated.

3.5.7 Touch Perception Task

The Touch Perception Task TPT) was developed as a validated descriptive scale for touch perception (Guest *et al.*, 2011; Ackerley *et al.*, 2014b). The full TPT consists of 26 sensory and 14 emotional descriptors that provide information about differing aspects of touch in relation to specific tactile stimulations. For the purpose of the current study a shortened form of the TPT consisting of 28 descriptors was administered omitting seven sensory (firm, gritty, jagged, lumpy, rubbery, sticky and vibrating) and five emotional (sexy, thrilling, enjoyable, soothing and relaxing) descriptors (table 6.2). Patients were asked to rate the degree to

which each word described the skin stimulus on a rating scale consisting of 5 choices: “not descriptive,” “slightly descriptive,” “moderately descriptive,” “highly descriptive,” and “very highly descriptive.” Stimuli were administered using a manual tactile stimulator that delivers a force-controlled stimulus at a pressure of 0.22N. To this either sandpaper or artificial fur were attached to give an application dimension of 80 × 50 mm. Brushing was delivered at 3 cm s^{-1} over a 10cm distance in a proximal to distal direction.

3.6 PAPER V - STIMULATION PARADIGMS AND EXPERIMENTAL DESIGN

In **Paper V** stimuli were delivered to two sites on the left dorsal forearm, a control site and a test site. The heat/capsaicin model was applied to the test site to induce primary and secondary hyperalgesia (Petersen and Rowbotham, 1999). Following the model application subjects participated in tactile direction discrimination testing as well as stroking pleasantness and pain testing.

3.6.1 Heat/capsaicin sensitisation

A Peltier thermode (3 × 3 cm, Medoc, TSA 2001, Thermosensory Analyzer, Rimat Yishai, Israel) was used to deliver a 5min 45°C stimulus thus inducing a mild thermal burn. Then capsaicin cream (Capsina, 0.075%, Hants, UK) was applied to that same, preheated, skin area for 30 min. Primary hyperalgesia develops within the application area and is surrounded by a secondary zone. Dynamic tactile allodynia and punctate hyperalgesia typically develop in the secondary zone.

3.6.2 Tactile direction discrimination

Stimuli were delivered using a hand-held stimulator (half cylinder contact surface covered in fine woven fabric, diameter 4 mm × length 15 mm), vertical load 16 g, stimulation velocity 1 cm s^{-1}. Participants were instructed to verbally report the direction (distal or proximal) after each movement. The test started with motion over an 18 mm distance: three consecutive correct responses shortened the distance whereas one incorrect response increased it. The paradigm consisted of 32 trials in each zone (Olausson, Wessberg and Kakuda, 2000).

3.6.3 Stroking pleasantness and touch evoked pain

Tactile stimuli were delivered manually to the model and control zones. To account for a potentially small area of allodynia stroking was delivered using a small soft goat's hair brush (0.5 cm wide, 3 cm long) over a distance was 5 cm, approximate application force 0.3 N. Stroking was delivered at two velocities, 3 and 30 cm s^{-1}. To control for differences in stimulus duration 10 consecutive strokes were applied at 30 cm s^{-1}. A single stroke stimulus of 30 cm s^{-1} was also included. The participants were instructed to rate their subjective perception of each stroking on a computerized visual analogue scale with the endpoints unpleasant and pleasant (0–10). Similarly, a pain rating for each stroking stimulus was recorded using a VAS with the endpoints no pain and worst pain imaginable (0–10). The area of allodynia and punctate hyperalgesia as well as tactile hypoesthesia were mapped following the main test protocols. The SF-MPQ was also administered.

3.7 STATISTICAL CONSIDERATIONS

Statistical analyses were carried out with SPSS (version 23; IBM, Armonk, NY), Excel 2010 (Microsoft™) and Graphpad Prism. Data was analyzed using standard parametric and non-parametric statistical methods (t-tests, ANOVA, Kruskal-Wallis, Mann-Whitney, Spearman's and Pearson's correlation tests). Regression analysis was employed to address the relationship between the different brushing velocities and ratings of pleasantness. The independent variable, velocity, was transformed to log 10 values. To test for significance of a quadratic regression term, the curve fit of a linear regression (reduced regression model) was tested against the fit of a quadratic regression (full regression model), with an F-test for significant reduction of the error sum of squares in the full compared to the reduced model (Chatterjee and Hadi, 2000). When the quadratic regression term provided a significant fit, it is generally described as having an inverted U-shape, which means that the function (pleasantness ratings) peaked at intermediate velocities.

For Paper III since the population studied were substantially older than in previous studies (Guest *et al.*, 2011; Ackerley *et al.*, 2014b) and that an abbreviated version of the TPT was used in the current study, a further principal components analysis using information obtained in the pre-cordotomy state and healthy control participants was performed to reduce the number of variables into fewer numbers of factors. Given that the aim was to separate sensory and emotional aspects of touch scores from sensory and emotional descriptors were entered in separate factor analyses to yield sensory and emotional factors respectively. The approach was similar to that used in previous studies (Guest *et al.*, 2011; Ackerley *et al.*, 2014b). Further details are given in Chapter 7.

4 PAPER I – Aβ PRIMARY AFFERENT NEURONS SIGNAL MECHANICAL PAIN IN HUMANS

Saad Nagi*, Andrew G. Marshall*, Adarsh Makdani, Ewa Jarocka, Jaquette Liljencrantz, Mats Trulsson, Francis O´Neill, Sumaiya Shaikh, Jonathan Cole, M. Catherine Bushnell, Francis McGlone, and Håkan Olausson

*Co-first author

4.1 ABSTRACT

In humans, the canonical view is that nociception is signalled by thinly myelinated (Aδ) and unmyelinated (C) primary afferent neurons. However, it has long been known that other mammals have Aβ afferents signalling nociception, and a recent study in mice found that it is particularly the Aβ field mechanoreceptors that have nociceptive properties. We performed single-unit axonal recordings (microneurography) – from 129 afferents of the peroneal nerve in 89 healthy participants – to test whether humans have Aβ afferents with nociceptive properties. Contrary to the mouse, human Aβ field mechanoreceptors did not display nociceptive properties. However, we identified a hitherto unreported class of high-threshold mechanoreceptors (HTMRs) that was insensitive to gentle brushing, had conduction velocities in the Aβ range, and encoded noxious skin indentations. Intra-neural microstimulation of single Aβ HTMRs evoked painful percepts. Furthermore, we found that mechanical pain perception was abnormal in two subjects with selective Aβ deafferentation, yet normal in subjects with congenital loss of small diameter afferents. We have thus demonstrated a role for Aβ fibres in human pain, implying that the classical neurological view that mechanical-pain examination specifically assesses thin-fibre function needs to be re-appraised. Furthermore, the demonstration of human Aβ nociceptors opens up novel therapeutic targets in pain disorders.

4.2 INTRODUCTION

The canonical view is that the human nociceptive system comprises of Aδ and C afferents only – Aδ nociceptors for first/fast pain (sharp-stabbing) and C nociceptors for second/slow pain (dull/burning) (Torebjörk and Hallin, 1973; Mackenzie *et al.*, 1975; Ochoa and Torebjörk, 1983; Marshall, 1953; Bromm and Treede, 1984) – while human Aβ afferents are thought to be low threshold and non-nociceptive. Consistent with that, the consensus protocol for somatosensory testing in neuropathic pain (Rolke *et al.*, 2006a) recommends the assessment of Aδ nociceptors on the basis of mechanical pain function; by implication, this suggests that large-fibre neuropathies are devoid of pain disturbances. This fails to recognize that most other species contain nociceptors in the thickly myelinated Aβ range, the prevalence of which varies from 18-65% of the myelinated nociceptor population across different species (Djouhri and Lawson, 2004). In the monkey hairy skin, for instance, ~12% of the Aβ population consists of nociceptors, representing 18% of the myelinated nociceptor population (Treede, Meyer and Campbell, 1998). Furthermore, in humans, high-frequency electrical stimulation below the activation threshold of Aδ afferents can generate a ***painful*** percept and a facilitated nociceptive flexion (spinal) reflex – an effect confirmed by a preferential small-fibre anesthetic block (Willer, Boureau and Albe-Fessard, 1978; Willer, Boureau and Albe-Fessard, 1980; Willer and Albe-Fessard, 1983).

In a recent paper on circumferential endings in mouse hairy skin (Bai *et al.*, 2015), it is shown that "Aβ field-LTMRs", a class of fast-conducting low-threshold mechanoreceptors (*brush-sensitive* LTMRs), "exhibit hallmarks of myelinated nociceptors". Indeed, mouse Aβ field-LTMRs display features such as a high threshold for monofilament activation and capacity to encode noxious skin indentations. These are characteristics of a nociceptor according to the definition by the International Association for the Study of Pain: "A ***high-threshold*** sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and ***encoding noxious stimuli***" **13**. That Aβ field-LTMRs have nociceptive properties has important implications for pain research and therapy; however, the difficulty in translating rodent findings to humans has thwarted many such advances in the past (Fudge *et al.*, 2016; Hackam and Redelmeier, 2006).

Aβ field-LTMRs have only been sparsely reported in humans with no more than a dozen single-fibre recordings (mainly in forearm skin) (Vallbo *et al.*, 1995; Olausson, Wessberg and Kakuda, 2000; Loken *et al.*, 2009), and no documented account of single field-LTMR stimulation for testing any perceptual attributes. In the current study, we used the *in vivo* electrophysiological technique of microneurography (Hagbarth and Vallbo, 1967) to test if humans have Aβ afferents with nociceptive properties (high threshold for monofilament activation and capacity to encode noxious force). Given the recent finding in mice (Bai *et al.*, 2015), it was hypothesized that such afferents might be the Aβ field-LTMRs. In addition, we used the technique of intra-neural microstimulation (Ochoa and Torebjörk, 1983; Vallbo *et al.*, 1984) to selectively activate single field units to determine the quality of any resulting sensation. We found a "preponderance" (Bai *et al.*, 2015) of Aβ field-LTMRs in the human skin, but they did ***not*** have nociceptive properties. In fact, they shared many similarities with other Aβ LTMR types, and thus likely contribute to discriminative aspects of touch. However, we did identify a hitherto unknown type of Aβ high-threshold mechanoreceptor with nociceptive properties. This first demonstration of human Aβ nociceptors has implications for clinical diagnostics, pain research and therapy.

4.3 METHODS

Single-unit axonal recordings (microneurography) were performed from the left peroneal nerve of 89 healthy participants (63 males, 26 females; range: 20-49 years), with some of them recruited more than once with an interval of at least three months (97 experimental sessions in total). In 16 participants from the microneurography sample, psychophysical testing for mechanical pain was also performed. In addition, psychophysical testing for mechanical pain and cold detection was performed in two Aβ deafferented subjects (IW: male, 64 years; GL: female, 69 years) (Treede and Cole, 1993; Cole *et al.*, 1995; Cole *et al.*, 2006) and six matched controls (range: 50-64 years; 4 females, 2 males). All participants provided informed consent in writing before the start of the experiment. The study was approved by the ethics committees of Linköping University (dnr 2015/305-31) and Liverpool John Moores University (14/NSP/039) and National Institutes of Health's Combined Neuroscience Ethics Committee (16-AT-0077), and complied with the revised Declaration of Helsinki. All participants were seated on a chair with both legs stretched out and supported with vacuum pillows. Care was taken to ensure that each participant was comfortably seated, and well-adjusted to room temperature (22°C), before starting the experiment. If subjectively cold, the participants were covered in a blanket except for the test region. Two microneurography setups were used, henceforth referred to as Setup 1 and Setup 2.

4.3.1 Microneurography

The course of the peroneal nerve at the level of the knee was approximated by surface electrical stimulation (1-4 mA, 0.2 ms, 1 Hz) using a 2-mm-diameter probe connected to an optically isolated constant-current stimulator (Setup 1: FE180 Stimulus Isolator; ADInstruments, Oxford, UK). This was followed by precise localization of the nerve by percutaneous insertion of a high-impedance tungsten ***recording*** microelectrode to deliver weak electrical pulses (0.02-1 mA, 0.2 ms, 1 Hz; FHC, Inc. Bowdoin, ME, USA). The electrode was insulated, except for the ~5 μm bare tip, with a typical length of 20 mm and shaft diameter of 0.2 mm. An uninsulated subdermal electrode in an adjacent area served as the reference. A high-impedance pre-amplifier was taped to the skin near the recording electrode (Setup 1: MLT185 headstage, ADInstruments, Oxford, UK), which was used in conjunction with a low-noise high-gain amplifier (Setup 1: FE185 Neuro Amp EX; ADInstruments, Oxford, UK). Once the electrode tip was intra-fascicular, indicated by subject's reports of cutaneous sensations to weak electrical stimulation (≤ 0.02 mA), the neural activity was amplified and single LTMRs were searched by soft-brush stroking (1-10 cm/s), and single HTMRs by coarse-brush stroking (1-10 cm/s) or pinching, the fascicular innervation zone while making small adjustments to the electrode position (Watkins *et al.*, 2017). A subset of experiments were performed using Setup 2 – in which the data was digitally sampled using SC/ZOOM developed by the Physiology Section, Department of Integrative Medical Biology, Umeå University – following the aforementioned protocol.

4.3.2 Unit identification

Individual Aβ LTMRs (***all*** soft-brush sensitive) were separated into rapidly adapting (RA) and slowly adapting (SA) types on the basis of their adaptive responses to ramp-and-hold indentation of the skin, as per the criteria used in Vallbo et al (Vallbo *et al.*, 1995). Three groups of RA units were identified: ***Aβ RA1-LTMR (hair unit)***, responsive to deflection of individual hairs and light air puffs – air puff responses were abolished after hair removal; ***Aβ field-LTMR***, comprising multiple spots of high sensitivity with no response to hair displacement or remote tapping of the skin; ***Aβ RA2-LTMR (Pacinian unit)***, comprising a single spot of maximal sensitivity and robust response to remote tapping/vibration. Two groups of ***SA-LTMRs*** (***type 1*** and ***2***) were identified where several features were examined including spontaneous firing, stretch sensitivity, and receptive field characteristics. Additionally, inter-spike interval pattern to sustained indentation (100 mN for 30-60 s) was tested, where possible, with a skewed and broad pattern for SA1 and normal distribution for SA2. Coefficients of variation of inter-spike intervals (Vallbo *et al.*, 1995; Edin, 1992) were 0.80 for SA1 (median; range: 0.32-3.53; n=16) and 0.29 for SA2 (median; range: 0.21-0.60; n=6).

Mechanical threshold and receptive field size of individual units were determined using Semmes-Weinstein monofilaments (nylon fibre; Aesthesio, Bioseb, Pinellas Park, FL, USA). If a unit responded to the same (weakest) monofilament in at least 50% of trials, it was taken as the mechanical threshold. Measurement of mechanical threshold was the minimal criterion for inclusion in the sample for all Aβ LTMR types, except RA1-LTMRs whose preferred stimulus is hair movement and thus responsiveness to light air puffs was determined. For receptive field mapping, pre-determined monofilament forces of 100 and 600 mN were used for LTMR and HTMR, respectively. The receptive field area was estimated by treating it as an ellipse. Latency responses to surface electrical stimulation of the receptive field were captured which, together with the distance from the stimulation site to the recording electrode, were used to calculate the conduction velocity (m/s). Surface electrical stimulation was performed using bipolar stimulation with the cathode on or just proximal to the receptive field and the anode distal to the receptive field. Occasionally the position of the anode and cathode were switched to enable a cleaner recording. Stimulus duration was 0.2ms. Stimulus intensity was slowly increased from 0.1mA until a consistent response, defined as three consecutive stimuli being followed by a spike, were seen. Electrically evoked spikes were overlaid with mechanically induced spikes over the receptive field to ensure they were of the same waveform. Electrically and mechanically evoked spikes were carefully compared on an expanded time scale to ensure that the electrically stimulated unit was the same as the one that was mechanically probed (Figure 1C). In one instance, the response latency was estimated by rapid mechanical tapping, using an electronic filament (details as follows), of a receptive field spot.

4.3.2 Mechanical stimulation

The punctate force-coding function of individual units was tested with a range of forces, applied to high-sensitivity spots in the receptive field, using commercially available monofilaments (nylon fibre; Aesthesio, Bioseb, Pinellas Park, FL, USA), in addition to custom-built electronic monofilaments that allowed high-resolution (1 ms) force measurements using capacitive sensors (nylon fibre; Physiology Section, Department of Integrative Medical Biology, Umeå University). Each monofilament *trial-set* comprised of eight consecutive applications of different forces, applied for 5 s each: 4, 10, 20, 60, 100, 260, 1000 and 3000 mN (corresponding values in pressure: 16, 24, 27, 53, 68, 106, 193 and 292 g/mm2). The trial commenced when the filament came into contact with the skin and lasted until it was withdrawn. When using the commercial apparatus, the start and end of the filament-skin contact were indicated with a push-button by the experimenter. Where the recording was stable enough, data for replicate trials of each monofilament force were collected.

4.3.3 Single-unit intra-neural microstimulation

The same recording electrode was also used to stimulate that same axon using low-current electrical pulses (1 and 30 Hz; µA range, starting from zero in small increments). That the same recorded afferent was stimulated electrically depends on the spatial overlap of the evoked percept, i.e. the projected field, with the physiological receptive field (Ochoa and Torebjörk, 1983; Vallbo *et al.*, 1984). This was determined by questioning the participant about the location of the projected field relative to the location of mechanical touch applied to the receptive field (Sanchez Panchuelo *et al.*, 2016). Where a receptive field-projected field overlap was found, sensory qualities of the resulting sensation were noted by asking the participant to describe what they felt followed by a questionnaire (Figure S2B).

4.3.4 Psychophysics

In 16 healthy participants from the microneurography sample, psychophysical pain ratings to graded monofilament stimulation were collected (dorsal foot: n=8; dorsal toe: n=8). Eight monofilament forces were applied for 5 s each, as used in the neural recordings, in random order (three repeats of each force) guided by a custom-written script in MATLAB (R2014b, The MathWorks Inc., Natick, MA, USA). Participants were asked to rate the pain intensity on a computerized visual analogue scale, ranging from 0 (no pain) to 100 (most intense pain), using the arrow keys of a computer keyboard.

4.3.5 Psychophysical testing in Aβ deafferented subjects and Hereditary Sensory and Autonomic Neuropathy-V

To test if human Aβ afferents contribute to pain perception, we collected psychophysical data for monofilament stimulation in two well-characterized Aβ deafferented subjects (IW and GL). These subjects suffer from a rare sensory neuronopathy syndrome – a disorder that selectively affects large primary sensory neurons' cell bodies in the dorsal root ganglia (Sterman, Schaumburg and Asbury, 1980) – resulting in a complete and permanent loss of Aβ afferent fibres below C3. However, the Aδ system, which is normally thought to be the substrate for mechanical pain (Rolke *et al.*, 2006a), is intact, as documented in previous clinical and neurophysiological studies (Cole *et al.*, 1995; Cole *et al.*, 2006; Olausson *et al.*, 2008a).

At the start of the experiment, we tested the cold detection threshold – a function of Aδ system – at the dorsal foot/distal leg (TSA-II, Medoc Ltd., Ramat Yishai, Israel), and found it to be normal in IW and GL (mean ± SEM): IW = 26.8 ± 0.3°C; GL = 27.0 ± 0.5°C; Controls = 27.2 ± 0.5°C; Baseline = 32.0°C. In IW and matched controls, the cold detection threshold was measured in triplicate trials, while in GL a more extensive set of ten trials was used (interspersed with warming stimuli). To test mechanical pain function, the same monofilament protocol (applied to dorsal foot) was followed in Aβ deafferented subjects as in healthy participants. The only exception was that the Aβ deafferented subjects could not operate the keyboard themselves to rate pain intensity on a computerized visual analogue scale due to their proprioceptive deficits. Therefore, they were asked to point at the screen and the experimenter pressed the keyboard keys accordingly.

The capacity of Aβ afferents to provide sufficient afferent information to cause pain was assessed in subjects with hereditary sensory and autonomic neuropathy (HSAN) type V. HSAN-V is a congenital sensory neuropathy due to the R221W mutation in the nerve growth factor β gene and is associated with selective loss of C-fibre and Aδ fibre afferents (Minde *et al.*, 2004; Einarsdottir *et al.*, 2004; Larsson *et al.*, 2009; Minde *et al.*, 2009). Two heterozygous subjects (both males, 74 and 86 years) were selected since they could not detect non-painful cold or heat pain within the test range (dorsal foot; triplicate trials; test range: 20-50°C). When tested with von Frey, their ratings were no different from matched controls. Both were able to detect vibration – tested bilaterally at the great toe using a 128 Hz tuning fork. Therefore, they had evidence of profound Aδ deficits but Aβ function was intact.

4.4 RESULTS

Using the microneurography technique (Hagbarth and Vallbo, 1967), we performed single-unit axonal recordings from the cutaneous fascicles of the peroneal nerve in 89 awake healthy participants (97 experimental sessions). We recorded from a total of 129 units including all five Aβ LTMR types16: field-LTMR (n=59); rapidly adapting type 1 LTMR (RA1, hair unit: n=15); RA2-LTMR (Pacinian unit: n=3); slowly adapting type 1 LTMR (SA1: n=25); SA2-LTMR (n=10). All Aβ LTMR types responded vigorously to soft-brush stroking (Loken *et al.*, 2009).

Besides Aβ LTMRs, we recorded from 14 myelinated ***high-threshold mechanoreceptors*** (HTMRs, Fig. 1A) – representing ~11% of the A-fibre sample. All myelinated HTMRs were ***insensitive*** to soft-brush stroking (except 1, see Discussion) and showed no response to joint movement, but were activated by coarse-brush stroking. Myelinated HTMRs were further classified into Aβ and Aδ groups on the basis of their conduction velocity; the ***slowest Aβ LTMR*** (field unit, ***26.5 m/s***) in the sample was taken as the Aβ/Aδ divider, such that any A fibre slower than this was classified as Aδ (for more details, see Supplementary Information). Thus, 2 Aδ and 11 Aβ HTMRs were identified; for one myelinated HTMR, conduction velocity could not be measured. Within the Aβ HTMR population, the slowest and fastest units were conducting at 29.5 and 43.7 m/s, respectively.
We also recorded from three unmyelinated HTMRs that, akin to myelinated HTMRs, were insensitive to soft-brush stroking, with mechanical thresholds of 10.0, 14.0 and 40.0 mN and conduction velocity of 0.9 m/s each.

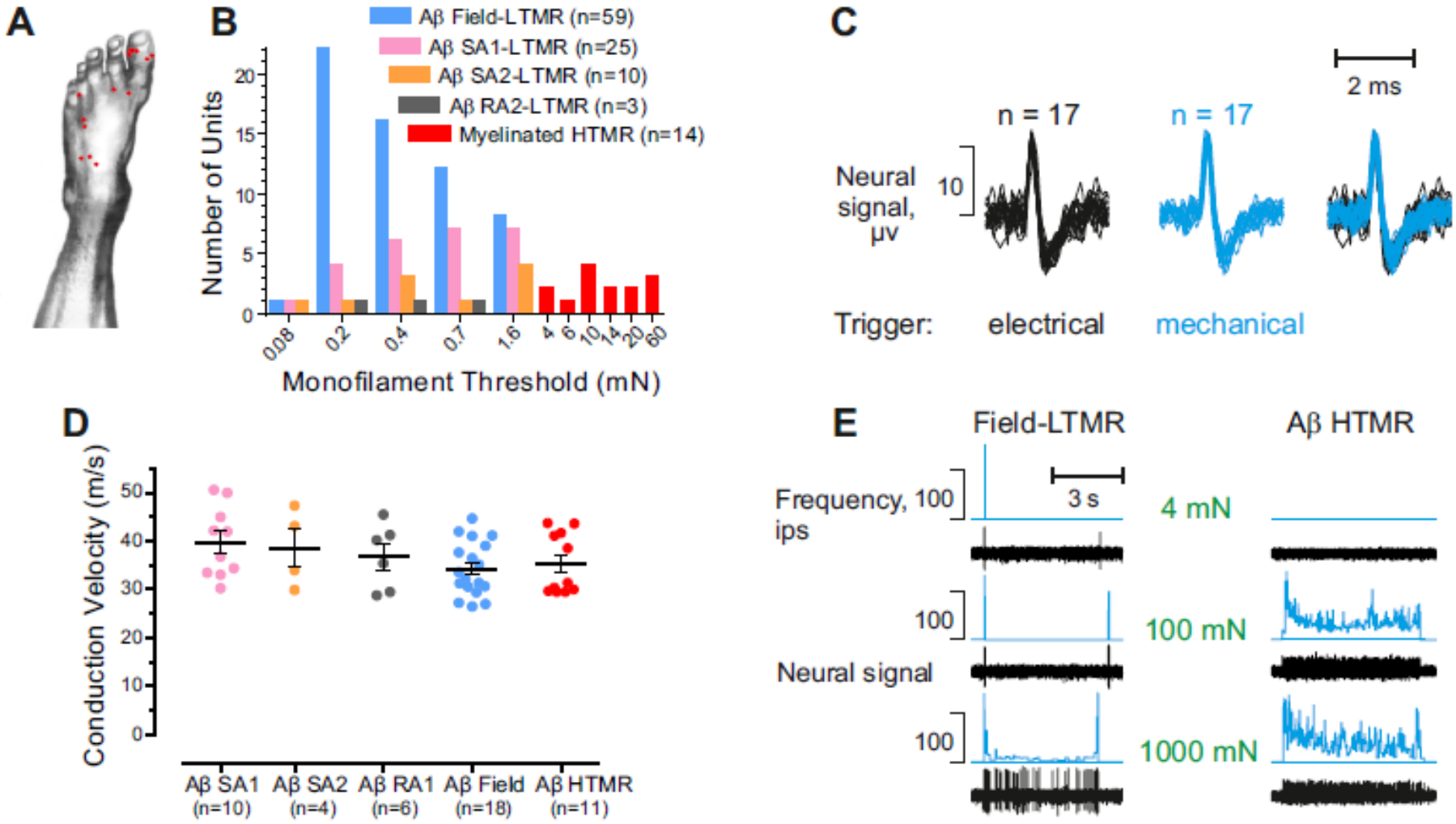
A
B
Aβ Field-LTMR (n=59)
Aβ SA1-LTMR (n=25)
Aβ SA2-LTMR (n=10)
Aβ RA2-LTMR (n=3)
Myelinated HTMR (n=14)
Number of Units
Monofilament Threshold (mN)
C
2 ms
n = 17
n = 17
Neural signal, μv
10
Trigger:
electrical
mechanical
D
Conduction Velocity (m/s)
Aβ SA1 (n=10)
Aβ SA2 (n=4)
Aβ RA1 (n=6)
Aβ Field (n=18)
Aβ HTMR (n=11)
E
Field-LTMR
Aβ HTMR
Frequency, ips
100
3 s
4 mN
100
100 mN
Neural signal
100
1000 mN

Fig. 4.1.

Humans are equipped with high-threshold Aβ primary afferent neurons. (A) Location of myelinated HTMR receptive fields from recordings in the peroneal nerve. Each dot represents the location of an individual myelinated HTMR (n=14). The data include 11 Aβ HTMRs, 2 Aδ HTMRs and one myelinated HTMR with unknown conduction velocity. All of them were found in the foot and toe regions, with none in the more proximal areas. (B) Mechanical threshold distribution of myelinated HTMRs and four types of Aβ LTMRs. (C) Spike activity of an Aβ HTMR to electrical and mechanical stimulation of the receptive field. Individual electrically and mechanically evoked spikes were superimposed on an expanded time scale to show that the electrically stimulated spike (used for latency measurement) was from the same unit as the one that was mechanically probed at the receptive field. (D) Conduction velocities of Aβ HTMRs and four types of Aβ LTMRs to surface electrical stimulation (and monofilament tapping in case of one Aβ HTMR). (E) SA-like properties of Aβ HTMR at higher indentation forces. Spike activity of an Aβ HTMR and an Aβ field-LTMR during monofilament stimulation at three different forces – applied using electronic filaments with force feedback. Compared to the Aβ field-LTMR, the Aβ HTMR showed a sustained response at a lower indentation force. See also Figures 2E, S1E and S1F. ips: impulses per second.

4.4.1 Human field-LTMRs do not have nociceptive properties

The mechanical thresholds of human Aβ field-LTMRs were no different to other Aβ LTMR types: Mean ± SEM (*median and quartiles – 25th and 75th percentiles – in parentheses*); field-LTMRs = 0.5 ± 0.06 mN (*0.4 mN, Q 0.2-0.7 mN, n=59*); SA1-LTMRs = 0.8 ± 0.1 mN (*0.7 mN, Q 0.4-1.6 mN, n=25*); SA2-LTMRs = 0.9 ± 0.2 mN (*0.6 mN, Q 0.4-1.6 mN, n=10*); RA2-LTMRs = 0.4 ± 0.1 mN (*0.4 mN, Q 0.2-0.7 mN, n=3*); 1-way ANOVA: F (3, 93) = 2.080, P = 0.1082 (*Kruskal-Wallis test: P = 0.1727*; Fig. 1B).
The responsiveness of human field units plateaued as the indentation force increased and no correlation was found with corresponding psychophysical pain ratings – for details on that, and other properties of human field units, see Supplementary Information and Figs. S4.1A-G. Collectively, these observations argue against a nociceptive function for human field units.

4.4.2 Myelinated high-threshold mechanoreceptors

The mechanical thresholds of Aβ HTMRs were significantly higher than for Aβ LTMRs: Mean ± SEM (*median and quartiles in parentheses*); 18.4 ± 6.3 mN (*10.0 mN, Q 6.0-14.0 mN, n=11*); 1-way ANOVA: F (4, 103) = 18.29, P < 0.0001; Dunnett's multiple comparisons test: P < 0.001 for all Aβ HTMR-LTMR comparisons (*Kruskal-Wallis test: P < 0.0001; Dunn's multiple comparisons test: P < 0.05 for all Aβ HTMR-LTMR comparisons*).

The conduction velocities of Aβ HTMRs (mean ± SEM: 35.4 ± 1.9 m/s, n=11) were statistically *indistinguishable* from those of Aβ LTMRs , thus representing a ***hitherto unreported*** class in humans (SA1-LTMRs: 39.8 ± 2.3 m/s, n=10; SA2-LTMRs: 38.6 ± 4.0 m/s, n=4; RA1-LTMRs: 36.8 ± 2.8 m/s, n=6; field-LTMRs: 34.3 ± 1.3 m/s, n=18; 1-way ANOVA: F (4, 44) = 1.371, P = 0.2595; Figures 4.1C and 4.1D).
The receptive field of Aβ HTMRs comprised of multiple small high-sensitivity spots (Figure S4.3). Their receptive field size (mean ± SEM: 26.8 ± 6.9 mm2, n=9), mapped using a filament force six times higher than that for an Aβ LTMR, was significantly smaller than field-LTMRs (P < 0.0001) but no different from the SA1-LTMRs (P > 0.05: Dunnett's multiple comparisons test).

The Aβ HTMRs showed RA-like responses at forces near threshold, but seven (out of nine) of them exhibited SA-like responses at higher forces (starting from 4-5 times threshold). The field-LTMRs too showed some sustained response, but at forces that were several hundred times above their threshold (Fig. 1E).

Force encoding of HTMRs was performed using a single high-sensitivity spot, chosen on the basis of the greatest response (apparent number of spikes) at the units' mechanical threshold. The Aβ HTMRs encoded

force in the perceptibly noxious range of indentation: Peak frequency responses at the highest and most painful indentation force of 3000 mN – rated, on average, as ~50/100 on the pain scale – were significantly higher ($P < 0.05$) from all other forces tested (Dunnett's multiple comparisons test; Figs 2A and 2B); A significant linear fit was displayed between peak frequency responses and indentation forces (linear regression: $R2 = 0.6981$, $P = 0.0098$) and a strong positive correlation was found with psychophysical pain ratings (Pearson correlation coefficient: $r = 0.8944$, $P = 0.0027$; Figs 2C). A comparison between Aβ HTMRs and field- LTMRs revealed a significant effect of unit type on peak frequency responses to punctate forces (2-way ANOVA: $F (1, 251) = 36.47$, $P < 0.0001$). For mean firing and spike numbers, see Figures 2D-F.
In two Aβ HTMRs where noxious thermal (4°C and 50°C) stimulation was tested, no response to change in temperature was observed. Generally, any response to hair movement or pulling was often not possible to test (the receptive field was often devoid of any visible hairs); in one case, we shaved the receptive field, but this had no effect on the unit's mechanical sensitivity.

To test if human Aβ afferents are important for pain perception, we collected psychophysical data for monofilament stimulation in two rare Aβ deafferented subjects (initials IW and GL) who have preserved Aδ fibres (Cole *et al.*, 1995; Cole *et al.*, 2006; Olausson *et al.*, 2008a)(Fig. 4.3). A comparison of the pain ratings between Aβ deafferented subjects and six age-matched healthy participants revealed a significant effect of condition – healthy versus Aβ deafferented (2-way ANOVA: $F (1, 198) = 26.68$, $P < 0.0001$). Furthermore, while significant pain, defined as a pain rating of > 10 numeric rating scale, emerged at 260 mN ($P < 0.01$) in age-matched healthy participants with significantly ($P < 0.0001$) increasing psychophysical pain ratings to increasing forces of indentation, significant pain in Aβ deafferented subjects only emerged at the highest indentation force of 3000 mN ($P < 0.05$). At this (highest) force level, the magnitude of pain in Aβ deafferented subjects was significantly lower than those of age-matched controls ($P < 0.0001$; Tukey's multiple comparisons test for all post hoc comparisons). In comparison, psychophysical data in two patients with HSAN-V, who have selective loss of C-fibre and Aδ afferents (Einarsdottir *et al.*, 2004; Minde *et al.*, 2004; Minde *et al.*, 2009), did not, despite the presence of congenital insensitivity to pain, differ significantly from healthy participants.

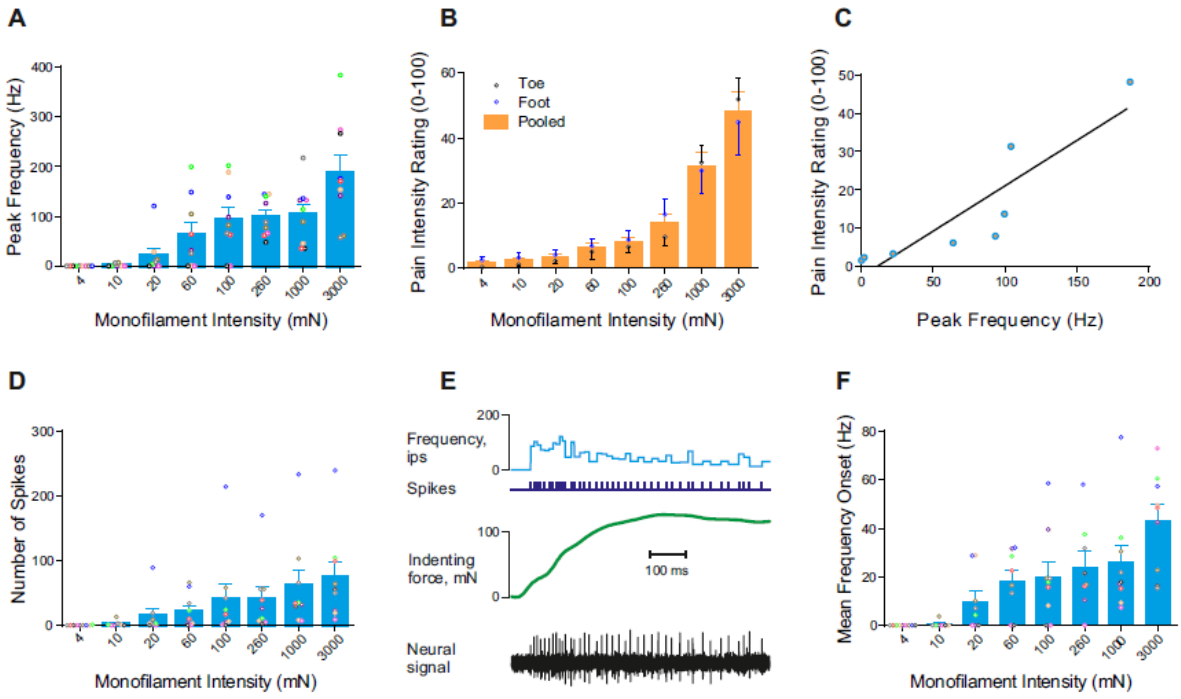

Fig 4.2.

Aβ HTMRs encode noxious punctate forces and signal mechanical pain. (A) Peak discharge rates of human Aβ HTMRs for monofilament stimulation. The data show individual and average (± SEM) responses of nine Aβ HTMRs to 5-s monofilament stimulation at eight different indentation forces. A significant linear fit was displayed ($R2 = 0.6981$, $P = 0.0098$), and the response at the highest indentation force (3000 mN) was significantly more than responses to all weaker indentation forces. Individual units (n=9) are color-coded.
(B) Psychophysical data for pain intensity to monofilament stimulation. Psychophysical pain ratings were collected from 16 healthy participants (dorsal foot: 8 participants, 12 trial-sets; dorsal toe: 8 participants, 9 trial-sets) from the microneurography sample. The monofilament force had a significant effect on psychophysical pain ratings (2-way ANOVA: $F (8, 171) = 29.96$, $P < 0.0001$), with a significant pain of ~14 out of 100 emerging at 260 mN – and higher pain ratings with increasing forces (Tukey's multiple comparisons test). No difference in pain ratings was found between the foot and toe stimulation sites (2-way ANOVA: $F (1, 171) = 0.1733$, $P = 0.6777$), hence the data were pooled for subsequent analysis. (C) Psychophysical pain ratings as a function of neural discharge in Aβ HTMRs. A comparison of the average peak discharge rates of nine Aβ HTMRs and average psychophysical pain ratings for skin indentations (eight forces, 4-3000 mN) revealed a significant linear correlation (Pearson's linear correlation: $r = 0.8944$, $R2 = 0.8$, $P = 0.0027$). (D) Number of spikes produced by monofilament stimulation in human Aβ HTMRs. Individual and average (± SEM) responses of nine Aβ HTMRs to 5-s monofilament stimulation at eight different indentation forces. A significant linear fit was displayed ($R2 = 0.6361$, $P = 0.0177$). Individual units (n=9) are color-coded. See also Fig. S1E. (E) Spike activity of an Aβ HTMR during the first 0.5 s of monofilament stimulation. Top panel: neural discharge rates; middle panel: spike markers and indentation force markers; bottom panel: neural recordings. The first 0.5 s was selected as the onset period (dynamic phase) of monofilament stimulation (total duration: 5 s). ips: impulses per second. (F) Mean discharge rates of human Aβ HTMRs for monofilament onset. Individual and average (± SEM) responses of nine Aβ HTMRs to the onset period (0.5 s) of monofilament stimulation at eight different indentation forces. A significant linear fit was displayed ($R2 = 0.6784$, $P = 0.0120$). Individual units (n=9) are color-coded.

4.4.3 Perceptual responses to microstimulation of single Aβ LTMR and Aβ HTMR

Using single-unit intra-neural microstimulation (Ochoa and Torebjörk, 1983; Vallbo *et al.*, 1984), a technique where the same recording electrode can be used to electrically stimulate that same single neuron – confirmed by a spatial overlap between the receptive and projected fields – a match was found for 9 Aβ field-LTMRs in 7 participants. This is the *first* demonstration that microstimulation of single field units can produce a conscious percept.

Notably, microstimulation of single field units never produced a painful percept and were associated with reports of "vibrating", "fluttering" or "buzzing" (Fig. S4.4A) – an observation that was consistent with the non-painful (matched) percepts associated with the activation of other Aβ LTMRs in our sample (n=5: 1 RA1 (fluttering/vibrating); 1 RA2 (vibrating); 3 SA1 (pressure, n=2; buzzing/vibrating, n=1). This observation further argues against the role of field units in human nociception.

In contrast to Aβ LTMRs, a ***painful*** percept was reported for Aβ HTMRs with a receptive field-projected field overlap in three microstimulated units, each in a separate individual (Fig. S4.4A). For unit 1, a "sharp" pain sensation was reported, and it was rated as 80 on a 0-100 pain scale. For unit 2, the pain sensation was described as "pinprick", and rated as 20 on a 0-100 pain scale. For unit 3, the pain sensation was described as "sharp" and "pinprick" – pain ratings were not collected for this unit.

There was no difference in the electrical threshold for intra-neural microstimulation between Aβ HTMRs (mean ± SEM: 6.3 ± 2.8 µA, 30 Hz, n=3) and Aβ LTMRs (6.3 ± 1.1 µA, 1 and 30 Hz, n=14).

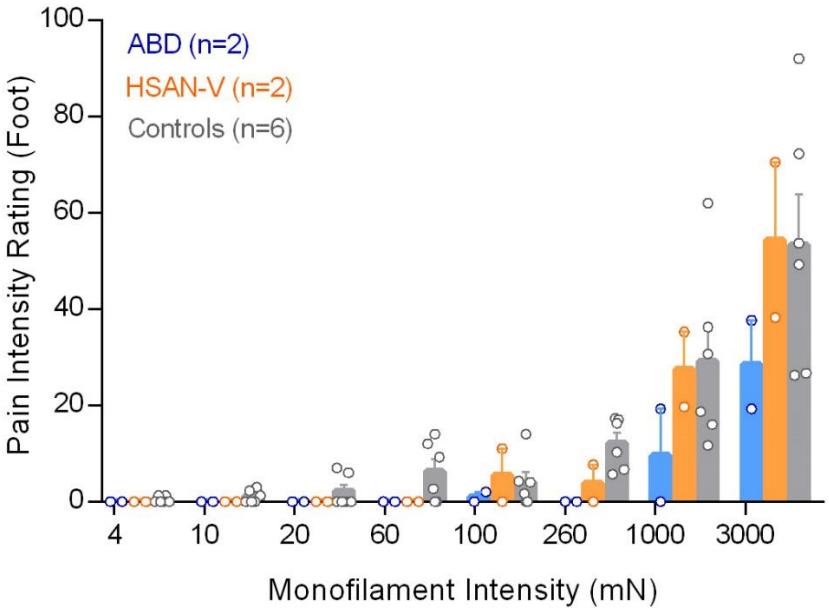

Fig. 4.3.

Abnormal mechanical pain perception in the absence of Aβ afferents. Individual and mean (±SEM) psychophysical pain ratings to monofilament stimulation (4-3000 mN, tested in triplicates) of dorsal foot for two Aβ deafferented subjects (IW and GL), two subjects with HSAN-V who could not detect non-painful cold or noxious heat and pain ratings from six age-matched healthy participants. A significant effect of condition – healthy versus Aβ deafferented – was found on the pain ratings (2-way ANOVA: $P < 0.0001$, $F(1, 198) = 26.68$). In healthy participants, significant pain was reported from 260 mN upwards, whereas in Aβ deafferented subjects significant pain was only reported at 3000 mN. The magnitude of pain at 3000 mN was significantly lower in Aβ deafferented subjects than healthy participants (Tukey's multiple comparisons test). There was no significant difference between subjects with HSAN-V and healthy participants. Abbreviations: ABD, Aβ deafferented; HSAN, hereditary sensory and autonomic neuropathy.

4.5 DISCUSSION

The properties of human Aβ HTMRs were in accord with the characteristics of a ***nociceptor*** – that is, high threshold for mechanical activation (no overlap with LTMRs) and capacity to encode force in the noxious range (Burgess and Perl, 1967; Perl, 1968). Intra-neural microstimulation of single Aβ HTMRs produced a painful sensation having a sharp/pinprick quality. Contrary to Aβ LTMRs, all myelinated HTMRs, except one, were *insensitive* to soft-brush stroking. The sole exception had a relatively low mechanical threshold (4.0 mN), which was nonetheless higher than all Aβ LTMRs, and displayed other nociceptive properties including coding of noxious force and pain sensation to intra-neural microstimulation. The same differential effect vis-à-vis soft-brush stroking is known for unmyelinated afferents where C-tactile fibres respond vigorously and C nociceptors are insensitive (Vallbo, Olausson and Wessberg, 1999; Watkins *et al.*, 2017).

When comparing the properties of mouse and human Aβ field-LTMRs, it is evident that human Aβ field-LTMRs do ***not*** have nociceptive properties. That the human Aβ field-LTMRs, contrary to their mouse namesake (Bai *et al.*, 2015), have similar mechanical thresholds to other LTMRs and fail to encode skin indentations in the perceptibly noxious (force) range is clear from our current observations, which are based on the largest sample of field unit recordings in humans. Field units in the cat (Burgess, Petit and Warren, 1968), though a more heterogeneous population, have mechanical thresholds in the range of 0.2-0.7 mN, and indeed 51 out of 59 human field units in our sample had thresholds ≤ 0.7 mN. In contrast, the mechanical thresholds of mouse field units were an order of magnitude higher (Bai *et al.*, 2015). Even in the rat foot (hairy) skin, the field units have an average mechanical threshold of 0.6 mN (Leem, Willis and Chung, 1993), which is similar to that of human field units (0.5 mN) as reported in the current study. The first-ever demonstration of intra-neural microstimulation of single field units, though producing conscious percepts of mechanical character (like other LTMRs), never produced a painful sensation, thus further arguing against their role in human nociception.

The importance of the Aβ system for human pain perception was suggested by the abnormal pain ratings to noxious punctate stimuli in two rare Aβ deafferented subjects, IW and GL. Furthermore, subjects with congenital loss of Aδ and C-fibres rated pain in a pattern the same as healthy participants. According to conventional theories, a participant with a selective large-fibre neuropathy should have intact mechanical pain sensibility, as this is widely believed to be a function of the Aδ system. For example, the quantitative sensory testing protocol of the German Research Network on Neuropathic Pain recommends cold detection and mechanical pain testing for assessment of the Aδ system (Rolke *et al.*, 2006b). Yet, IW and GL have normal cold detection but abnormal mechanical pain perception and individuals with HSAN-V abnormal cold detection and normal mechanical pain detection. We have shown that mechanical pain is not exclusively signalled by the small-fibre system, with the clinically important implication that large-fibre neuropathies can also present with disturbances in pain perception.

Perhaps the reason why Aβ nociceptors have been largely ignored in the animal literature (and almost entirely in human work) is that their proportion relative to the overall myelinated nociceptor population decreases from rodents to primates. Another reason, as postulated by Lawson (Lawson, 2002b), is that the thickly myelinated low-threshold mechanoreceptors likely dominate, by sheer numbers, the Aα/β wave of the compound action potential.

The Aβ-afferent system seems well-suited for execution of withdrawal reflexes given the short latency of signal transmission, as indeed was postulated almost forty years ago (Willer, Boureau and Albe-Fessard, 1978; Willer, Boureau and Albe-Fessard, 1980; Willer and Albe-Fessard, 1983). In comparison to C HTMRs, the Aβ HTMRs display a higher firing rate, a more finely grained receptive field with multiple small high-sensitivity spots and relative insusceptibility to fatigue during sustained noxious mechanical stimulation (tested up to 1 min). These characteristics likely ensure a large, continuous transfer of nociceptive information to the central nervous system with content rich on stimulus quality, localization and such – features that need not be limited to Aβ HTMRs, but may apply, to varying extents, to other types of

myelinated nociceptors (Adriaensen *et al.*, 1983; Ghitani *et al.*, 2017; Arcourt *et al.*, 2017; Garell, McGillis and Greenspan, 1996; Slugg, Meyer and Campbell, 2000). Clearly under normal circumstances noxious mechanical skin stimulation will co-activate multiple mechanosensitive afferent types, both low and high threshold. The resulting perceptual and behavioural consequences will depend on the relative strength and spatio-temporal pattern of inputs from these fibre subtypes (Arcourt et al., 2017, Browne et al., 2017). Recent evidence in rodents clearly suggests that Aβ co-activation modulates reflex nociceptive withdrawal and prevents overflow of the motor response (Arcourt et al., 2017). Also, in humans, mechanically evoked pain increases under conditions of Aβ blockade (Andrew and Greenspan, 1999) suggesting that the net effect of Aβ stimulation is anti-nociceptive.

In the current study, we recorded from the cutaneous fascicles of the peroneal nerve, but it may be that Aβ HTMRs (or their functional homologues) exist in other body domains as well. The fastest "high-threshold A-δ-fiber" in the radial nerve (cutaneous branch) recordings of Adriaensen et al (1983) is 33 m/s. Although it is not possible to determine the precise division between Aβ and Aδ conduction velocities as no comparative large-fibre data were provided, it now seems likely that it was an Aβ HTMR. In peroneal-nerve recordings from human muscle nociceptors, Simone et al (Simone *et al.*, 1994) reported a "Group II" nociceptor, producing "sharp pain" to intra-neural microstimulation, with its receptive field located in a tendon at the base of the big toe. This unit had a conduction velocity of 32 m/s; in comparison, the fastest Group III afferent in their sample had a conduction velocity of 13.5 m/s (mean: 6.7 m/s).

The average Aδ conduction velocity using laser or "epidermal electrical" evoked potentials is typically in the 10-15 m/s range (Tran *et al.*, 2001; Inui *et al.*, 2002). The latencies of pinprick-evoked potentials are shorter than laser-evoked potentials, thus suggesting a possible contribution of Aβ HTMRs to pinprick-evoked potentials. Human Aδ nociceptors in the radial nerve (Adriaensen *et al.*, 1983) show a greater specificity for mechanical stimulation relative to unmyelinated nociceptors which are often polymodal – an observation that is consistent with other species; cat and monkey skin, for instance (Burgess and Perl, 1967; Perl, 1968). By implication, the human Aβ HTMRs may be highly specific for mechanical stimulation, and while we have no observation to the contrary, this needs to be systematically tested in a larger population.

4.5.1 Conclusions

We have, for the first time, identified a class of Aβ afferents, the Aβ HTMRs, which display nociceptive properties and likely contribute to human pain perception. Though consistent with observations in other mammals, their existence in humans had hitherto remained a mystery. Further investigations are warranted into the response properties, molecular signatures, and central projections of human Aβ HTMRs, given the implications for understanding nociception, nocifensive behavior, and clinical pain states.

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4.7 SUPPLEMENTAL INFORMATION

4.7.1 Human field-LTMRs do not have nociceptive properties (continued)

We recorded from 59 Aβ field-LTMRs with the following spatial distribution: Toes 40.7%; Foot 47.4%; Ankle 8.5%; Leg 3.4% (Fig. S1A). These afferents displayed "expansive" (Bai *et al.*, 2015) receptive fields (mean ± SEM; field-LTMRs: 100.5 ± 7.2 mm2, n=51; *cf.* SA1-LTMRs: 41.6 ± 7.5 mm2, n=17; two-tailed t-test with Welch's correction: $P < 0.0001$).

All field units were activated by soft-brush stroking, but they were unresponsive to hair deflection and air puff. Hair were removed by shaving from the receptive field of five field units, but this had minimal effect on their responses (example shown in Fig. S4.1B). No particular preference for the direction of stroking was observed. The spike activity of field units increased with faster brushing, which is consistent with the known brushing profile of all Aβ LTMR types in human hairy skin (Loken *et al.*, 2009).

We tested the on- and off-step responses of Aβ field-LTMRs to mechanical indentation, given that mouse field units were found to "lack the pronounced off-step responses of the Aβ RA-LTMRs"1. The human field-LTMRs displayed both on- and off-step responses to mechanical indentation, even at very low forces (Fig. S4.1C).

The responsiveness of human field units plateaued as the indentation force increased (Fig. S4.1D). Significant pain emerged at 260 mN (pain rating > 10 out of 100; Figure 4.2B) with increasing ratings to higher forces of indentation (displaying a significant linear fit: $R2 = 0.9139$, $P = 0.0002$). In contrast, the peak firing data of field units revealed no significant differences in their responses to indentation forces in the range of 20-3000 mN (Tukey's multiple comparisons test; for *spike numbers* and *mean firing*, see S4.1E-G). Consistent with a plateaued response, a linear fit was not displayed between peak frequency responses and indentation forces ($R2 = 0.1364$, $P = 0.3679$), and no significant correlation was found with psychophysical pain ratings ($r = 0.5608$, $P = 0.1482$).

In two field units where we compared responses to sharp and blunt stimulations, no obvious differences were observed; for instance, their peak firing to a sharp 512 mN filament was 125.7 and 90.1 Hz, and to a corresponding blunt filament was 121.2 and 99.0 Hz, respectively. Taken together, these observations argue against a nociceptive function for human field units in contrast to observations in mice (Bai *et al.*, 2015).

4.7.2 Classification of Aβ and Aδ afferents based on conduction velocity

The classification of afferents into Aβ and Aδ groups on the basis of conduction velocity is not clear-cut. It is suggested that the "upper border of the main part of the D hair CV range can indeed provide a good indication of the upper end of the Aδ CV range" (CV: conduction velocity) (Djouhri and Lawson, 2004). The limitation of this, however, is in its extrapolation to humans where no detailed account of D hair units exists. In a microneurography study on afferents in the radial nerve, Adriaensen et al (Adriaensen *et al.*, 1983) mention three LTMRs, *en passant*, that are excited by single-hair movement but individual conduction velocities are not provided. To avoid the ambiguity concerning Aβ and Aδ groups, we chose the slowest Aβ LTMR (field unit) in our sample to set the divider as 26.5 m/s, such that any myelinated fibre slower than this was classified as Aδ. Based on this, two units from the HTMR population were in the Aδ range and eleven units in the Aβ range with the fastest conducting at ~44 m/s (the overall fastest was a SA1-LTMR conducting at ~51 m/s).
In electrophysiological investigations in the monkey, Treede et al (Treede, Meyer and Campbell, 1998) found two distinct peaks in the conduction velocity distribution, at 15 and 43 m/s, and the Aβ/Aδ boundary was placed "at the minimum between these two peaks, which was 30 m/s". Extrapolating this to our sample would yield a similar outcome to our current approach: Eight Aβ HTMRs were conducting ≥ 30 m/s and three in the 29-30 m/s range. Notably, the single-afferent conduction velocity values, measured using

electrical stimuli (or mechanical in one case) at the receptive field, are lower than those obtained by electrical stimulation of the nerve trunk with factors such as axonal tapering, branch points and mechanical/electrical coupling time contributing to the conduction delay; furthermore, conduction velocities are slower in the lower than in the upper limb in humans (Macefield, Gandevia and Burke, 1989; Kakuda, 1992; Krarup and Trojaborg, 1994).

4.7.3 C-fibre HTMRs

We also recorded from five C-HTMRs, confirmed by the 2-Hz electrical stimulation protocol (Serra *et al.*, 1999; Watkins *et al.*, 2017). These C-HTMRs, akin to A-HTMRs, were insensitive to soft-brush stroking (Fig. S4.2). For A-HTMR, the median mechanical threshold was 10 mN. This was significantly higher than the mechanical thresholds of all tested A-LTMR types, but no different from that of C-HTMRs.

4.7.4 Microneurography data collection and processing

For Setup 1, the neural activity was sampled at 20 kHz, and all data were recorded and processed using the data acquisition and analysis program (LabChart Pro software v8.1.5 and PowerLab 16/35 hardware PL3516/P; ADInstruments, Oxford, UK). Threshold crossing was used to distinguish action potentials from background noise with a signal-to-noise ratio of at least 2:1, followed by template matching to confirm spike morphology.

For Setup 2, the neural signal and forces recorded from the electronic monofilaments were digitally acquired at 19.2 and 1.2 kHz, respectively, using SC/ZOOM (Physiology Section, Department of Integrative Medical Biology, Umeå University). Single action potentials were identified semi-automatically under visual control (Edin, Bäckström and Bäckström, 1988). Before any further data processing, recordings were discarded if the analyses indicated the presence of multiple units or the signal-to-noise ratio prevented secure action potential identification.

The instantaneous discharge rates were generated as time series with values corresponding to the inverse of inter-spike intervals. For Setup 2, force signals, timing of individual spikes, and the instantaneous discharge rate were exported at 1 kHz from SC/ZOOM to MATLAB (R2014b, The MathWorks Inc., Natick, MA, USA).

4.7.5 Microneurography data analysis

For monofilament stimulation, the 500-ms window following the first spike was defined as the trial *onset*, and similarly, the *offset* of a trial was defined as the 500-ms window preceding the last spike. In addition, the total period (onset-hold-offset) from the first to the last spike was also analyzed. For brushing stimulation, the trial duration was calculated from the first to the last spike.

For each trial, the number of spikes were counted. The corresponding mean frequency was defined as the number of inter-spike intervals divided by the sum of the inter-spike interval duration, and the peak frequency was defined as the maximum value of the instantaneous frequency during the period. All data were entered in GraphPad Prism for statistical comparisons (version 6.07, GraphPad Software, Inc. La Jolla, CA, USA). The type of statistical test used for each comparison is described alongside all statistical data. Figures were generated using Corel Draw (X8, version 18.1.0.661, Corel Corporation, Ottawa, ON, Canada).

SUPPLEMENTAL FIGURES

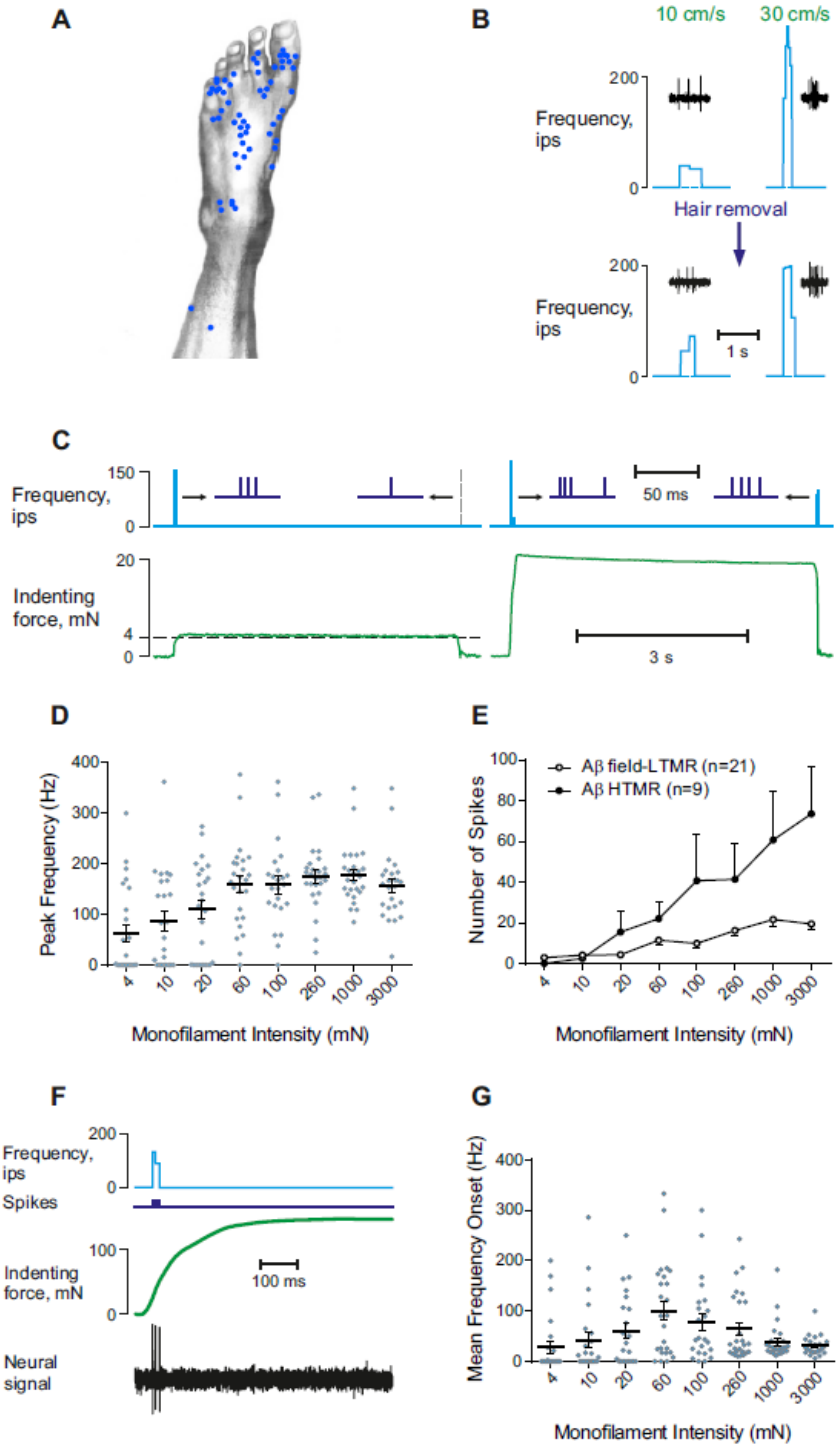

Fig. S4.1.

Human Aβ field-LTMRs do not display nociceptive properties, Related to Figure 1 and 2. (A) Location of Aβ field-LTMR receptive fields from recordings in the peroneal nerve. Each dot represents the location of an individual field unit (n=59). Most of the field units were located in the foot and toe regions, with less than one-eighth in the ankle and more proximal areas. (B) Spike activity of an Aβ field-LTMR to soft-brush stroking before and after hair removal. Using

a soft goat hair brush (30-mm bristle length), a 10-cm area of skin centred on the receptive field of the recorded afferent was stroked at velocities of 10 and 30 cm/s, guided by a moving strip on a computer screen. High sensitivity to brushing was displayed by the field unit with an increase in neural discharge rate to the faster stroking velocity. The removal of hair by shaving had no effect on the responses. ips: impulses per second. (C) Aβ field-LTMR showed on- and off-step responses to mechanical indentation, even at very low forces. Representative responses of a field unit for 5-s monofilament stimulation (with force feedback) at indentation forces of 4 and 20 mN. The upper panel shows the neural discharge rates (inset, spike markers) and the lower panel shows the indentation force markers. ips: impulses per second.
(D) Responses of Aβ field-LTMRs plateaued during increasing indentation forces. Individual and average (± SEM) responses of 21 field units to monofilament stimulation at eight different indentation forces (26 trial-sets). A linear fit was not displayed and no differences were found in the peak frequency between 20 and 3000 mN. (E) Different number of spikes produced by 5-s monofilament stimulation in Aβ field-LTMRs and Aβ HTMRs. Average (± SEM) response for each unit type and monofilament intensity. Both monofilament intensity (2-way ANOVA: $P < 0.0001$, $F(8, 283) = 12.52$) and unit type ($P < 0.0001$, $F(1, 283) = 31.96$) had a significant effect on spike numbers, with 1000 and 3000-mN responses of Aβ HTMRs significantly higher than all Aβ field-LTMR responses ($P < 0.01$; Tukey's multiple comparisons test). Spike numbers for Aβ HTMRs were taken from Figure 2D for comparison with Aβ field-LTMRs. (F) Spike activity of an Aβ field-LTMR during the first 0.5 s of monofilament stimulation. Top panel: neural discharge rates; middle panel: spike markers and indentation force markers; bottom panel: neural recordings. The first 0.5 s was selected as the onset period of monofilament stimulation (total duration: 5 s). ips: impulses per second. (G) Mean discharge rates of human Aβ field-LTMRs for monofilament onset. Individual and average (± SEM) responses of 21 field units to the onset period (0.5 s) of monofilament stimulation at eight different indentation forces (26 trial-sets).

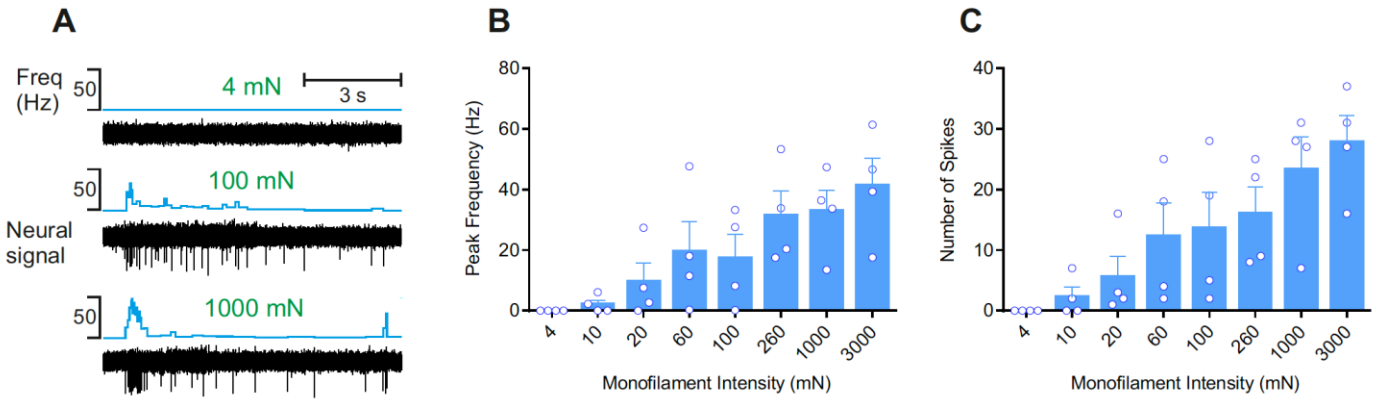

Fig. S4.2.
Response properties of C-HTMRs to graded mechanical stimuli (A) Spike activity of a C-HTMR during monofilament stimulation at three different forces. Compared to an A-HTMR (see Fig. 4.1), the C-HTMR displayed a lower firing rate, and a weaker sustained response during noxious mechanical stimulation. Freq: frequency. (B) Peak discharge rates of C-HTMRs for monofilament stimulation. The data show individual and average (± SEM) responses of C-HTMRs to monofilament stimulation at eight different indentation forces (two units, four trial-sets). (C) Number of spikes produced by monofilament stimulation in C-HTMRs. Individual and average (± SEM) responses of C-HTMRs to monofilament stimulation at eight different indentation forces (two units, four trialsets).

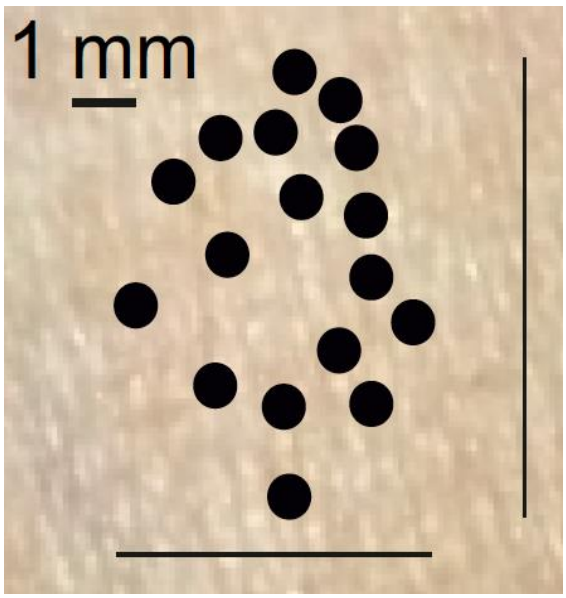

Fig. S4.3. The pattern of receptive field spots, mapped with a Semmes-Weinstein monofilament, is shown for an A-HTMRs. Receptive field spots were redrawn on photographic images so they can be easily seen. The horizontal lines represent the medial-lateral dimension, and the vertical lines represent the proximal-distal dimension.

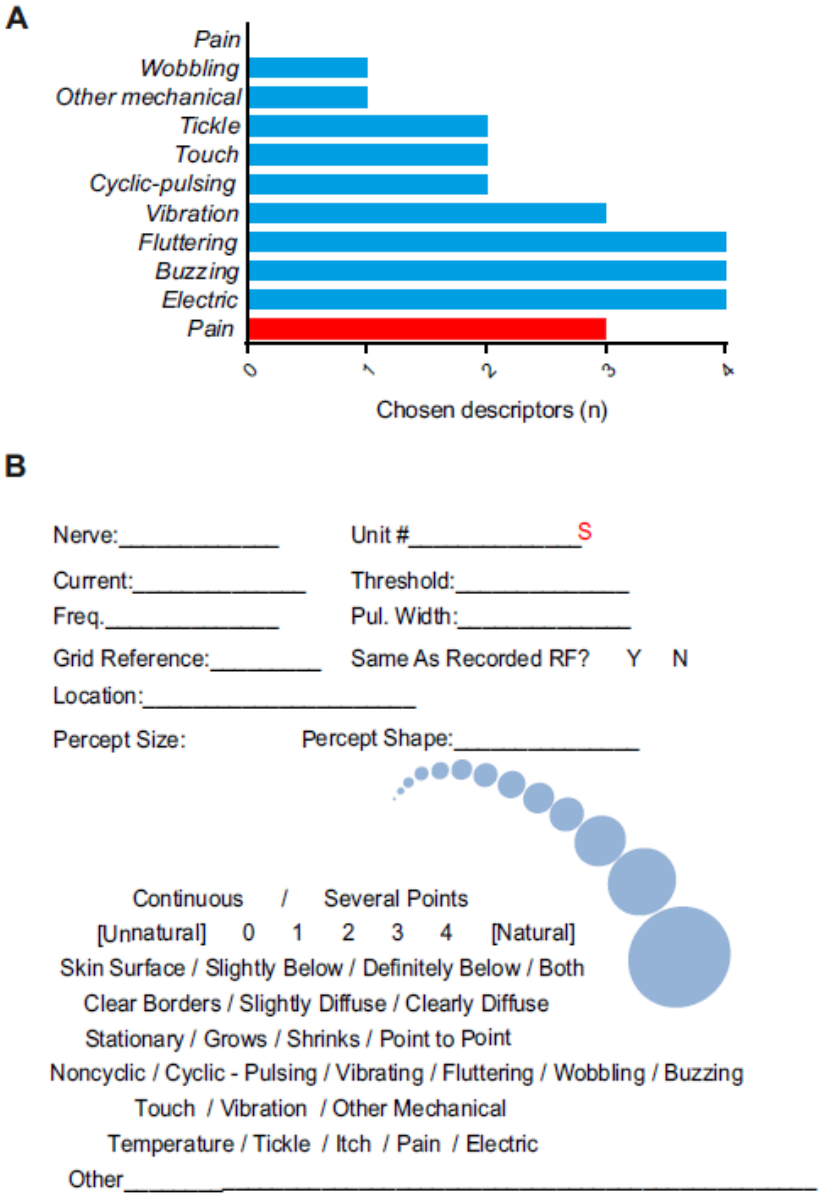

Fig. S4.4.

Intra-neural microstimulation of single Aβ field-LTMRs and Aβ HTMRs produced different sensations, Related to Figures 1 and 2 (A) Activation of single Aβ field-LTMRs (nine units) produced non-painful mechanical sensations – typical of other Aβ LTMR types. Fluttering, buzzing, and electric were the most frequently chosen descriptors for field units. Activation of single Aβ HTMRs (three units) produced painful sensations having a sharp/pinprick quality. (B) Questionnaire for sensory qualities. Activation of single units by intra-neural microstimulation produced conscious percepts that were captured by administering this questionnaire (modified from previous studies (Ochoa and Torebjörk, 1983; Vallbo *et al.*, 1984; Macefield, Gandevia and Burke, 1990)).

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5 PAPER II: RARE HUMAN NERVE GROWTH FACTOR-B MUTATION REVEALS RELATIONSHIP BETWEEN C-AFFERENT DENSITY AND ACUTE PAIN EVALUATION

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5.1 ABSTRACT

The rare nerve growth factor-β (NGFB) mutation R221W causes a selective loss of thinly myelinated fibres and especially unmyelinated C-fibres. Carriers of this mutation show altered pain sensation. A subset presents with arthropathic symptoms, with the homozygous most severely affected. The aim of the present study was to investigate the relationship between peripheral afferent loss and pain evaluation by performing a quantification of small-fibre density in the cornea of the carriers, relating density to pain evaluation measures. In vivo corneal confocal microscopy (CCM) was used to quantify C-fibre loss in the cornea of 19 R221W mutation carriers (3 homozygous) and 19 age-matched healthy control subjects. Pain evaluation data via the Situational Pain Questionnaire (SPQ) and the severity of neuropathy based on the Neuropathy Disability Score (NDS) were assessed. Homozygotes, heterozygotes, and control groups differed significantly in corneal C-nerve fibre density, with the homozygotes showing a significant afferent reduction. Importantly, peripheral C-fibre loss correlated negatively with pain evaluation, as revealed by SPQ scores. This study is the first to investigate the contribution of small-fibre density to the perceptual evaluation of pain. It demonstrates that the lower the peripheral small-fibre density, the lower the degree of reported pain intensity, indicating a functional relationship between small-fibre density and higher-level pain experience.

5.2 INTRODUCTION

The Nerve Growth Factor -β (NGFB) mutation R221W is rare, with a population of 66 living carriers (of whom 3 are homozygous) in the Norrbotten region in northern Sweden, arising from a founder effect as far back as the 17th century (Minde *et al.*, 2004; Minde *et al.*, 2009). Despite its rarity, the R221W mutation offers vital clinical and preclinical insight for pain syndromes by revealing relationships between small-fibre density and pain perception. The R221W mutation (Capsoni *et al.*, 2011; Einarsdottir *et al.*, 2004) selectively affects the density of thin-diameter sensory afferents (Crowley *et al.*, 1994; Larsson *et al.*, 2009; Minde *et al.*, 2004) with severe loss of unmyelinated C-fibres and comparatively moderate loss of thinly myelinated Aδ-fibres (Crowley (Crowley *et al.*, 1994; Larsson *et al.*, 2009; Minde *et al.*, 2004; Minde, 2006). It does not affect large myelinated Aβ-fibres (Minde *et al.*, 2004), which are typically negative for the primary NGF receptor TrkA in the cell bodies (Patapoutian and Reichardt, 2001). Rat cell line models suggest that this missense point mutation affects the cleavage of pro-NGF, limiting the availability of functional mature NGF in the extracellular space (Carvalho *et al.*, 2011; Larsson *et al.*, 2009). The R221W mutation carriers' reduced nociceptive afferent density may thus be a consequence of insufficient trophic support from NGF during development, with possible additional effects of altered regulatory signaling in NGF-mediated nociceptive and inflammatory pathways in adulthood.

The three known homozygous carriers are severely affected, presenting with debilitating and progressive degrees of painless fractures, joint deformation, Charcot arthropathies, bone necrosis, and osteochondritis, resulting in limited mobility (Minde *et al.*, 2004; Minde, 2006; Minde *et al.*, 2009; Minde *et al.*, 2006). These clinical sequelae in homozygotes follow an autosomal recessive heritability pattern. Based on this clinical presentation of painless fractures without anhidrosis or mental retardation, these patients have been classified as having hereditary somatosensory and autonomic neuropathy type V (HSAN-V; (Minde, 2006; Minde *et al.*, 2004). Yet, of the 63 currently identified heterozygous carriers, most do not present with pain- or inflammation-related deficits and have been identified only through pedigree and genetic screening. A proportion of these (19) are vulnerable to carpal tunnel syndrome (Hellgren T, Svensson O, Minde J, unpublished data; (Minde *et al.*, 2009)).

This clinical variability may be underpinned by a uniquely broad range of peripheral unmyelinated afferent density in the R221W carrier population as a whole, thus providing an important window on the role of C-afferent density in pain perception. A previous study (Minde *et al.*, 2004) has quantified small-diameter fibre density in a small sample (3 homozygous and 3 heterozygous carriers) by using electron microscopy of sural nerve biopsies. However, to more completely characterize the relationship between small-fibre reduction and clinical status and pain evaluation, we performed a systematic characterization of fibre peripheral density in a larger sample of carriers.

In the present study, we used corneal confocal microscopy (CCM), a clinical ophthalmic test for the assessment of small-fibre structure. This non-invasive method is increasingly used to quantify C-fibre loss in neuropathy secondary to diabetes and other aetiologies (Quattrini *et al.*, 2007; Tavakoli *et al.*, 2009; Tavakoli *et al.*, 2010; Tavakoli *et al.*, 2012; Gasparotti *et al.*, 2017). To further investigate the contribution of C-fibre density to clinical pain assessment scores and subjective pain evaluation, we also administered the Neuropathy Disability Score (NDS; (Young *et al.*, 1993)) and the Situational Pain Questionnaire (SPQ; Clark and Yang 1983).

5.3 METHODS

5.3.1 Participants

Nineteen individuals (11 males; 3 homozygous carriers; mean age 50 ± 4.9 yr, mean ± SE) with a mutation of the NGFB gene were investigated in this study. Nineteen healthy age-matched controls for the CCM investigation and 12 healthy age-matched controls for the SPQ questionnaire were included in the analysis. Ethical approval was obtained by the ethics board of Gothenburg University. Participants gave informed consent in accordance with the Declaration of Helsinki and were compensated at 200 Swedish crowns per hour. The control subjects for the CCM investigation were recruited and assessed for a clinical study at the University of Manchester and are part of a normative data set for corneal nerve images (Tavakoli *et al.*, 2015). The CCM data were collected in 2 days at the Department of Orthopedics, Gällivare Hospital, Sweden. The homozygous individuals (2 males born in 1990 and 1968, 1 female born in 1983) do not share parents and are only distantly related. Each individual presents with painless fractures, osteochondritis, bone necrosis, and neuropathic joint destruction. Compared with the homozygous carriers, the symptomatic heterozygote carriers in this sample have later onset of symptoms (20–70 yr) and less severe clinical signs. Although not suffering from painless fractures, they can manifest Charcot arthropathies at single or multiple joints, particularly in the lower extremities. Cognitive functions and basic reflexes are normal in carriers (Einarsdottir *et al.*, 2004; Minde *et al.*, 2004; Minde *et al.*, 2006; Minde *et al.*, 2009). A list of the most relevant clinical features of R221W carriers is provided in Table 5.1.

Table 5.1.

Clinical features of the NGFB carriers

Mutation	Gender	DoB	Age of Onset, yr	Charcot Joint	Fractures	NDS	MNSI	CTS
Ho	F	1983	7	Knee, ankle, hip	Yes	7	4.5	No
Ho	M	1990	4	Knee, ankle, spine	Yes	3	4.5	No
Ho	M	1948	7	Knee, ankle, spine, elbow	Yes	5	3	No
He	M	1996		No	No	0		No
He	M	1992		No	No	0	0	No
He	M	1989		No	No	0	1	
He	M	1985		No	No	0	0	No
He	M	1981		No	No	0	0	No
He	F	1967		No	No	0	2	Yes
He	F	1962	15	Hip	No	1		
He	F	1960		No	No	2	2	Yes
He	M	1958		No	No	1	1	Yes
He	F	1952	20	Knee	No	2	0	No
He	M	1951	20	Knee	No	0	1	Yes
He	F	1946		Gonarthrosis	No	1	2	No
He	F	1942		No	No	5	3	Yes
He	F	1936		Gonarthrosis	no	2	0	No
He	M	1934		No	No	5	1.5	Yes
He	M	1933	50	Knee	No	3		Yes

NGFB, nerve growth factor-β; DoB, date of birth; NDS, Neuropathy Disability Score; MNSI, Michigan Neuropathy Screening Instrument; CTS, carpal tunnel syndrome; Ho, homozgyous; He, heterozygous. MNSI scores were collected in a previous investigation (Larsson *et al.*, 2009). CTS scores were collected in a separate study (Hellgren T, Svensson O, Minde J, unpublished data).

5.3.2 Corneal confocal microscopy

Carriers and healthy subjects underwent corneal assessment with the Heidelberg retina tomograph (HRT III, Rostock cornea module) in vivo corneal confocal microscopy, based on an established protocol (Tavakoli and Malik, 2011). The subjects' eyes were anesthetized using a drop of oxybuprocaine hydrochloride (0.4%; Bausch & Lomb), and Viscotears (carbomer polyacrylic acid, 2 mg/g; Alcon) was applied on the front of the eye for lubrication. For each subject, a sterile, disposable Perspex cap (TomoCap, Heidelberg Engineering) was placed over the objective lens, and a drop of Viscotears gel was placed on the tip of the lens. The subject was instructed to fixate on a target with the eye not being examined. Several scans of the entire depth of the cornea were recorded by turning the fine focus of the objective lens backward and forward for 2 min using the section mode, which enables manual acquisition and storage of single images of all corneal layers. This provides *en face* two-dimensional images with a lateral resolution of 2 mm/pixel and a final image size of 400 × 400 pixels of the sub-basal nerve plexus of the cornea from each subject. Six images per subject from the centre of the cornea were selected and examined in a masked and randomized fashion and subsequently analysed using purpose-written, proprietary software (CCMetrics; M. A. Dabbah, Imaging Science, University of Manchester). C-fibres are mainly located in the subbasal plexus between the Bowman's layer and the corneal basal epithelium, whereas Aδ-fibres are located in the stromal layer (Guthoff *et al.*, 2005; Müller *et al.*, 1997). Each main fibre is constituted of a bundle of unmyelinated axons (Al-Aqaba *et al.*, 2010). Three corneal nerve parameters were quantified from the subbasal plexus: 1) corneal nerve fibre density (CNFD), the total number of main fibres per square millimeter of corneal tissue (Al-Aqaba et al. 2010); 2) corneal nerve branch density (CNBD), the number of branches emanating from all main fibres trunks per square millimeter of corneal tissue; and 3) corneal nerve fibre length (CNFL), the total length of all main fibres and branches (mm/mm2) within the area of corneal tissue. The presence and density of Langerhans cells (LCs) in the same images from Bowman's layer were also assessed (Tavakoli *et al.*, 2011). In addition, the presence of Aδ-fibres was also assessed via the examination of images from the stromal layer, although in this study an exact quantification was not performed due to current methodological limitations (Patel and McGhee, 2009). Stromal rather than subbasal nerves appear more robust in surviving post-mortem change; therefore, most of our knowledge from corneal A δ-fibres is from in vitro studies (Al-Aqaba *et al.*, 2010).

5.3.3 Corneal sensitivity

Corneal sensitivity was quantified using a non-contact corneal aesthesiometer (NCCA; Glasgow Caledonian University), which uses a puff of air through a 0.5-mm-diameter bore, lasting 0.9 s, and exerts a force expressed in the millibar range. The stimulus jet is positioned 1 cm from the eye, and the air jet is aligned to the centre of the cornea. The subject feels and acknowledges a sensation on the cornea, which is most commonly described as being “cold” or as a “'breeze.” With the use of a staircase method, each subject is presented with a supramaximal stimulus followed by stimuli of reduced strength until the subject does not feel the air jet anymore. The whole process is repeated three times to derive a threshold. The coefficient of variation for NCCA was 5.6%. Both eyes were tested, and the results are the average of both eye assessments.

5.3.4 Neuropathy Disability Score

The NDS is a widely used tool that assesses neuropathy severity and is based on clinical neurological examination findings. It includes evaluation of vibration, pin prick, and temperature perception as well as the presence or absence of ankle reflexes to establish the severity of neuropathy: NDS 0–2, no neuropathy; NDS 3–5, mild neuropathy; NDS 6–8, moderate neuropathy; and NDS 9–10, severe neuropathy.

5.3.5 Situational Pain Questionnaire

A Swedish translation of the original 30-item SPQ was administered to the subjects (Clark and Yang 1983). The SPQ was originally developed by Clark and Yang (1983). It is used to evaluate how individuals estimate their own pain sensitivity and the pain sensitivity of others. It has previously been used in the context of higher-level pain evaluation in congenital insensitivity to pain (Danziger et al., 2006). The SPQ consists of a series of situations describing low-pain ("I have been bitten by a mosquito") or high-pain events ("The dentist drills in one of my teeth without anaesthesia"). Subjects estimate the amount of pain they might feel in those situations on a Likert scale from 1 ("non-noticeable") to 10 ("worst possible pain"). The SPQ addresses the ability of the subjects to discriminate hypothetical painful from nonpainful situations as well as the amount of pain they think they would experience in them. The interpretation of the SPQ is based on a model of signal detection theory that allows the quantification of the ability to discriminate between the two categories of events and the amount of pain perceived via two scores: P(A) and B (Danziger et al., 2006; Green and Swets 1966; Wickens 2001). P(A) captures the degree of discrimination between high-pain and low-pain situations, whereas the B score reflects the degree to which situations are considered as painful. A high B score reflects an underestimate of pain, whereas a low score reflects a good estimate of pain (for more details, see Danziger et al., 2006).

5.3.6 Data analysis

Statistical comparisons between homozygous carriers, heterozygous carriers, and controls were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL; version 20) using the nonparametric independent-samples Kruskal-Wallis and Mann-Whitney tests. Of all the CCM parameters investigated, particular focus was put on the nerve fibre density (CNFD). Correlation between CNFD, NDS, and SPQ results was investigated with a Spearman's correlation test.

5.4 RESULTS

5.4.1 NGFB carriers show a reduced density of C-fibres

The R221W mutation carriers showed a significant decrease in C-fibre density (CNFD) compared with healthy individuals [$\chi 2(2) = 16.2$, $P = 0.0003$, with a mean rank score of 2 for the homozygous carriers, 15.16 for the heterozygous carriers, and 25.92 for the controls; Fig. 4.1]. For normative values, see also Tavakoli et al. (2015). The CNFD scores varied greatly between the carriers with the lowest value of 0 in two of the homozygous and the maximum value of 40 in one of the male heterozygous, although the nonparametric Levene's test showed no significant difference in the homogeneity of variance ($P = 0.1$). In addition, nerve branch density and nerve fibre length were also significantly reduced in the carriers (see Table 4.2). In seven of the carriers, no Aδ-fibres were observed, although an exact quantification in this case was not methodologically possible. Langerhans cell presence and density of cells was significantly higher in the carriers compared with the control subjects ($P < 0.001$). No difference in corneal nerve parameters between males and females ($P > 0.1$) was observed. CNFD correlated positively with CNBD ($rs = 0.62$, $P < 0.01$) and CNFL ($rs = 0.86$, $P < 0.01$). Corneal sensitivity correlated significantly with CNFD ($rs = -0.66$, $P < 0.01$) and CNFL ($rs = -0.52$, $P < 0.059$).

5.4.2 Nerve fibre density correlates with NDS

The maximum disability score is 10, which would indicate complete loss of sensation to all sensory modalities and absent reflexes. The carriers show a significant negative correlation between peripheral C-fibres density to NDS ($rs = -0.67$, $P < 0.01$; Fig. 5.2). Although the NDS test is not specific for fibre type, these findings suggest a relationship between peripheral density and severity of the neuropathy: the lower the density of small fibres, the higher the severity of the neuropathy.

5.4.3 Nerve fibre density correlates with estimation of pain

The SPQ looks at the ability to discriminate painful vs. innocuous situations via the P(A) score and at the intensity of the imagined pain via the B score. Median scores for P(A) = 0.91 [interquartile range (IQR) = 0.7–0.9] and B = 6.50 (IQR = 5.1–7.2) were observed in the carriers. More specifically, the homozygous carriers (n = 3) scored P(A) = 0.74 (IQR = 0.7–0.8) and B = 7.67 (IQR = 6.7–8.7), whereas the heterozygous carriers (n = 16) scored P(A) = 0.91 (IQR = 0.8–0.9) and B = 6.25 (IQR = 5–7.1). Scores from control subjects (n = 12) were also considered for comparisons: P(A) = 0.92 (IQR = 0.91–0.97) and B = 5 (IQR = 4.8–6.1). The homozygous carriers scored a significantly higher B value than controls ($U = 4$, $P = 0.04$), indicating a lower degree of pain imagined in highly painful situations, whereas a trend for significance was seen between the heterozygous and the controls ($U = 66$, $P = 0.053$). Regarding the P(A) score, no significant difference was found between the three groups ($P = 0.05$) and no correlation was observed between the CNFD score and the P(A) value ($P > 0.05$), indicating that the ability to discriminate between imaginary painful and innocuous situations was intact and did not correlate to the density of C-fibres at the periphery. However, a significant negative correlation between nerve fibre density and B score ($rs = -0.59$, $P < 0.01$; Fig. 5.3) was observed in the carriers, indicating that, on average, the lower the degree of pain imagined, the greater the loss of small fibres measured in the cornea.

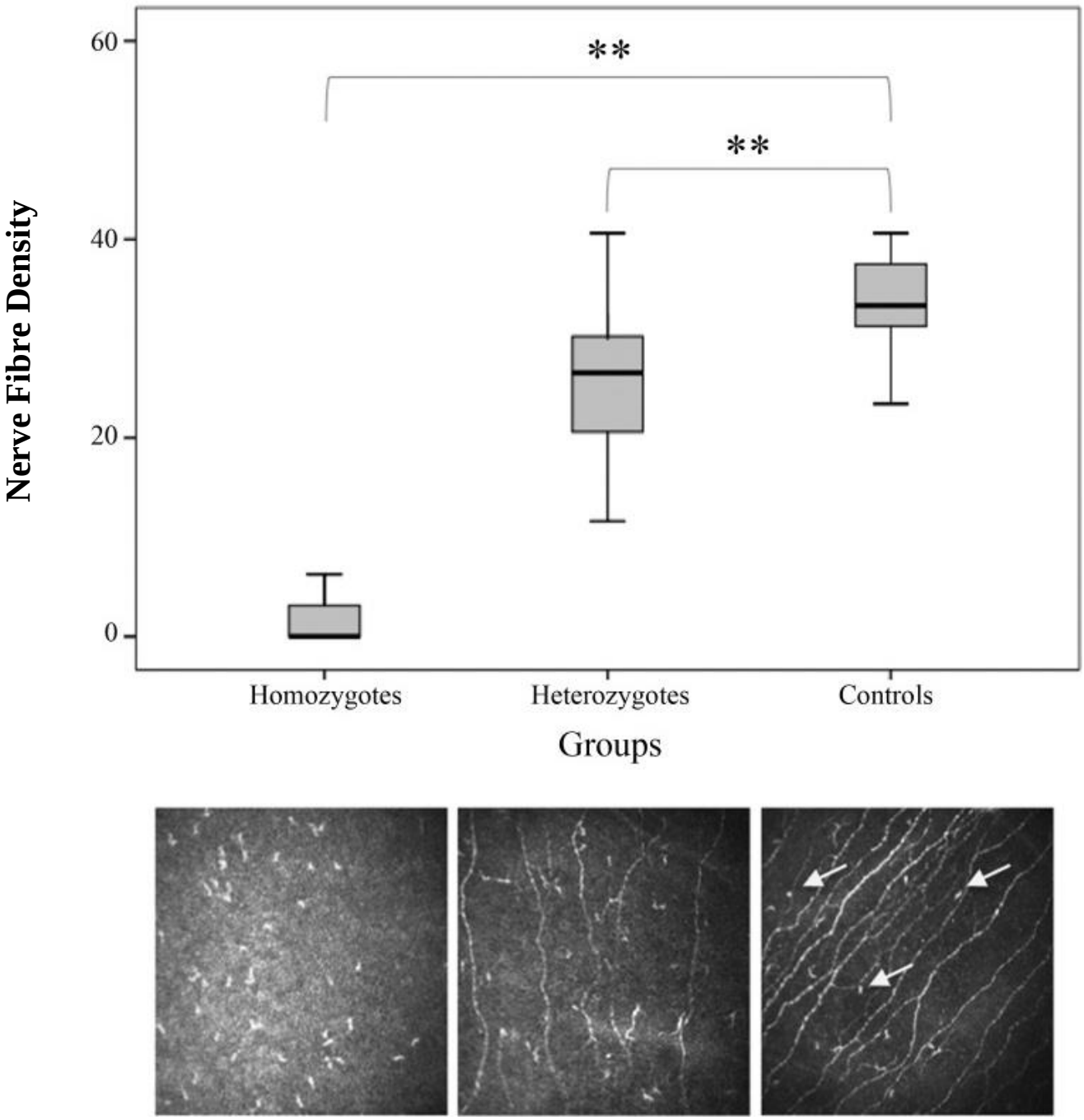

Fig. 5.1.

Box plot showing CNFD values (top) and CCM images (bottom) for homozygous (left), heterozygous (centre), and control (right) subjects. NGFB carriers show a significant reduction of C-afferent density in the cornea. **P < 0.01. Arrows indicate Langerhans cells.

Table 5.2.

Corneal sensitivity and corneal nerve morphological parameters in NGFB carriers and controls

				***P* Value**		
Parameter	**Homozygous Carriers**	**Heterozygous Carriers**	**Controls**	**Homozygous vs. Heterozygous**	**Control vs. Homozygous**	**Control vs. Heterozygous**
NCCA-R, mbar	5.55 (1.54–12.50)	1.30 (0.68–4.85)	0.56 (0.12–0.88)	0.073	0.006	<0.001
CNFD, fibres/mm²	0 (0–6.25)	26.56 (11.61–40.62)	33.33 (23.44–40.62)	0.007	0.006	0.002
CNBD, nerve branches/mm²	0 (0–0)	10.68 (2.08–23.96)	58.33 (15–183.33)	0.007	0.006	<0.001
CNFL, mm/mm²	0 (0–1.42)	12.22 (3.87–16.05)	23.73 (13.94–32.58)	0.007	0.006	<0.001
LCs, %presence	100	93.7	20			
LC density, cells/mm²	158.13 ± 83.46	93.09 ± 24.83	14.1 ± 4.13	0.342	0.0001	0.0001
Stromal Aδ-fibres, %presence	0	56	100			

Data are medians with interquartile range (IQR) in parentheses, percentages, or means ± SE for parameters in homozygous carriers (*n* = 3), heterozygous carriers (*n* = 16), and healthy controls (*n* = 19). NCCA-R, non-contact corneal aesthesiometer-right side; CNFD, nerve fibre density; CNBD, nerve branch density; CNFL, nerve fibre length; LC, Langerhans cells.

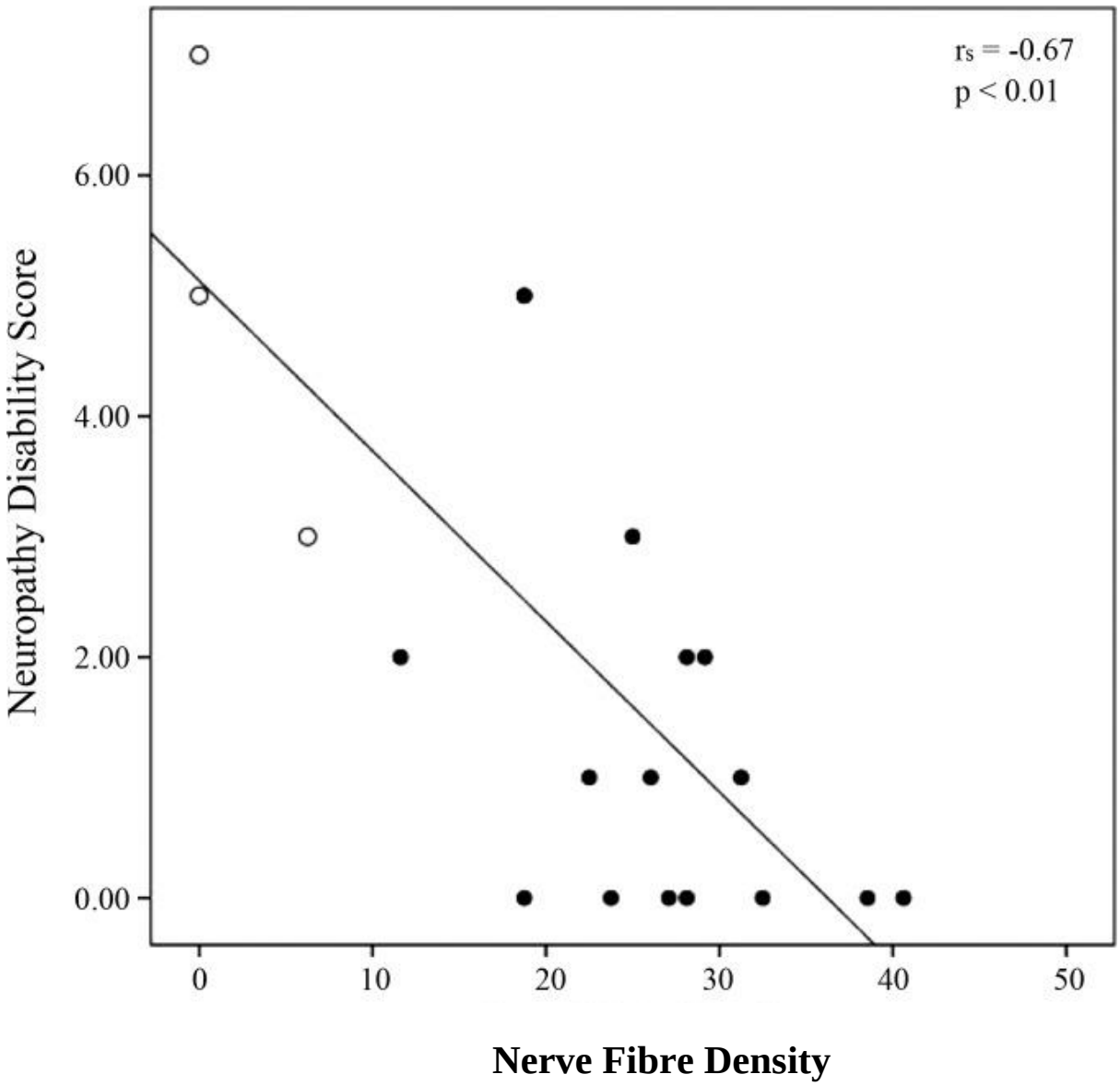

Fig. 5.2.

Correlation between CNFD and NDS scores. The carriers show a significant negative correlation between C-fibre density and the severity of neuropathic symptoms (rs = −0.67, P < 0.01). Open circles represent homozygous carriers' scores.

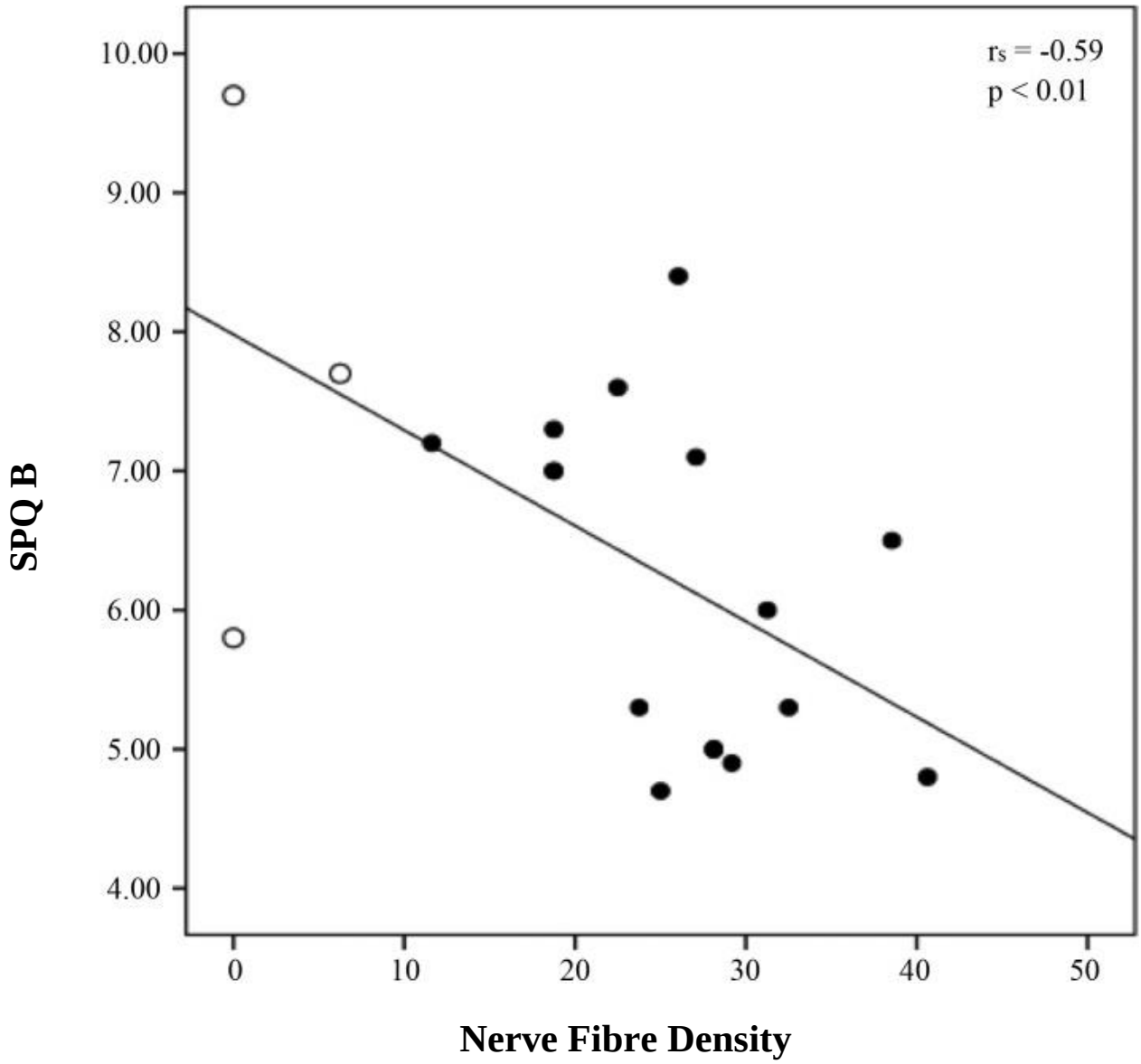

Fig. 5.3.

Correlation between CNFD and SPQB scores. The degree of pain imagined is negatively correlated to the average loss of C-fibres in the carriers (rs = −0.59, P < 0.01). Open circles represent homozygous carriers' scores.

5.5 DISCUSSION

These findings indicate that C-afferent nerve fibre density affects acute pain evaluation in carriers of the R221W mutation. Individual variability of carriers' CNFD is related to variability on clinical and subjective measures of acute pain (NDS and SPQ). This is the first study to show a direct relationship between peripheral C-fibre afferent density, assessed using CCM, and the evaluation of acute pain. The cornea is the most highly innervated tissue in the human body. It contains myelinated Aδ-fibres, which are large-diameter (6 µm), straight nerves that respond primarily to mechanical stimuli, and unmyelinated C-fibres, which are small-diameter (2–4 µm), beaded nerves that respond to thermal and chemical stimuli (Müller *et al.*, 1997). The nerves penetrate the cornea radially, and ∼70–80 nerve trunks, which contain 900-1,200 myelinated and unmyelinated axons, enter the midstroma (Müller *et al.*, 1997). The myelinated nerve bundles lose their myelin sheaths and perineurium early after entering the stroma, and all the nerve bundles lose their Schwann cell sheath before penetrating Bowman's layer to enter the epithelium, allowing the cornea to remain transparent (Tavakoli and Malik, 2011).

The homozygous carriers showed a marked reduction of small fibres compared with both heterozygous carriers and healthy controls, with almost a complete lack of C-afferents in the cornea. The heterozygous group was more variable, with individual values ranging from low CNFD approaching that of the homozygote group to high CNFD approaching that of healthy controls. This suggests that in homozygotes the consequences of the R221W mutation on thin-diameter sensory afferents are severe and definite. In contrast, the consequences are milder on average, yet more variable, in the heterozygote group.

The density of corneal nerve fibres (CNFD) was negatively correlated with NDS scores of neuropathy severity in the carrier group, indicating that the lower the CNFD, the higher the severity of symptoms. The SPQ questionnaire provided a self-reported measure of pain intensity estimates in the context of potential painful situations. The groups did not significantly differ on the ability to distinguish written descriptions of painful from nonpainful situations [SPQ P(A)]. However, differences in pain intensity estimates for painful situations (SPQ B) were observed, especially in the homozygotes whose ratings were significantly lower than the controls. This might indicate a general pain underestimation bias among carriers as a group, which is especially pronounced among the homozygotes. In the whole carrier group, this underestimation bias also correlated with small-fibre CNFD: the lower the amount of fibres in the periphery, the lower the pain intensity estimates.

The R221W carriers reduced small-fibres density was previously assessed through sural biopsies in six carriers (Minde et al., 2009). Although crucial in characterizing the peripheral physiology of the R221W mutation, this assessment was limited in scope, partly due to the invasive nature of nerve biopsy. Furthermore, the biopsy data were skewed to a more severely affected sample, consisting of three homozygotes and three heterozygotes. CCM allowed us to noninvasively assess more detailed morphological properties of small-fibre epithelial C-afferents in a broader sample and to take variability into account, especially among heterozygote carriers.

The haplotype of the R221W mutation is on chromosome 1p11.2-p13.2, restricted to the 8.3-Mb region, flanked by the single nucleotide polymorphism markers rs2490334 and rs2275607. The mutation involves a basic arginine (CGG) to a nonpolar tryptophan (TGG) substitution at position 221 in the NGFB amino acid sequence. Rat cell line models suggest that this missense point mutation affects the cleavage of pro-NGF and that the resulting intracellular accumulation of pro-NGF limits the availability of mature NGF in the extracellular space (Carvalho et al., 2011, Larsson et al., 2009).

NGF plays a role not only in trophic support and cell differentiation in development but also in pain and inflammation in adulthood (Capsoni *et al.*, 2011; Lewin and Moshourab, 2004). The R221W mutation carriers' phenotype may thus be a consequence of lack of trophic support during development, resulting in reduced nociceptive afferent density. It may also involve altered regulatory signaling in nociceptive

pathways during pain and injury. NGF binding to the TrkA receptor was unchanged in rat cell lines and transgenic mouse models of the mutation (Capsoni et al., 2011, Larsson et al., 2009). However, in the transgenic mouse model (R100W), the mutated form of NGF exhibited a reduced ability to activate downstream TrkA-dependent signaling pathways, in particular PLC-γ, suggesting that the carriers' phenotype might be explained by altered nociceptive regulatory actions (Capsoni et al., 2011, Larsson et al., 2009).

In the human carriers, however, the wide phenotypic variance and the sharp differences in clinical status between heterozygote and homozygote carriers suggest a gene dosage or codominance effect. Heterozygote carriers do not manifest severe pain indifference, inflammation, or arthropathy, although as a group they show subtle effects that are related to their degree of epithelial C-afferent innervation, and some individuals develop arthropathies with increasing age (Minde et al., 2009). In this study, corneal fibre loss was regarded as an index of the general reduction in nerve fibres that was observed in the sural nerve biopsy samples (Minde et al., 2004). It is reasonable to expect that such a general, congenital reduction of nociceptive fibres would result in atypical functional physiology of the nerve pathways, including downstream signaling in the relevant subcortical nuclei, as well as the relevant cortical projections. We thus predicted that the R221W mutation would not only affect nociceptive processing at the level of sensory perception but also bias pain processing at the level of experience, memory, and behavior. The results from the SPQ show that carriers are biased to underestimate the painfulness of hypothetical situations, despite being able to accurately differentiate painful from nonpainful situations. This indicates that the reduced CNFD associated with the R221W mutation might bias pain processing at the central, and probably cortical, level. This central-level bias may also influence carriers' behavioral responses to pain; for example, underestimation of acute pain could imply underreaction to pain-relevant stimuli.

The extreme inflammation and injury in homozygote carriers' load-bearing joints may reflect the cumulative effects of even subtle deficits in the ability to produce appropriate and timely behavioral responses to nociceptive signals. Radin et al. (1990) termed this “microklutziness.” In this scenario, arthropathic symptoms are the result of wear and tear on the joints as a consequence of poor behavioral adjustments to pain and discomfort, creating negative feedback loops of inflammation and further injury. We observed a high incidence of Langerhans cells in the carriers, a type of epithelial dendritic cell (Seré *et al.*, 2012). These antigen-presenting dendritic cells are released as part of the immune response to injury and lodge throughout the body. This may bear some relationship to the carriers' history of pain events. However, Langerhans density did not correlate with surgical history or any of the measures collected in this study.

CCM allows the collection of several parameters, of which we focused only on the CNFD since evidence from previous studies had shown reduced small fibres' density in the carriers (Minde et al., 2004). Future studies should provide further insights on whether other CCM parameters might correlate to components of pain perception.

This study demonstrates that corneal confocal microscopy can be used as a non-invasive technique to assess relationships between afferent density and morphology and clinical or preclinical presentation in pain syndromes or in neuropathic conditions with consequences for patient pain. It reveals a relationship between CNFD and clinical, subjective, and behavioral pain outcomes. These findings have wider implications for understanding the contribution of peripheral afferent populations to pain-related pathways across levels of the nervous system and the nociceptive mechanisms involved in acute, superficial pain evaluation.

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6 PAPER III: THE ENIGMATIC C-TACTILE AFFERENT: THE MISSING SPINOTHALAMIC LINK

Andrew Marshall, Manohar Sharma, Kate Marley and Francis McGlone

6.1 ABSTRACT

Growing neurobiological evidence indicates that tactile sensation has an affective-emotional dimension. Human hairy skin is innervated by unmyelinated, low-threshold mechanoreceptive fibres, C-Tactile afferents, that are tuned to slow, gentle caress-like stroking. C-Tactile afferents, the putative peripheral neurobiological substrate for affective touch, are proposed to form a distinct coding channel projecting to emotional cortical systems but, in contradistinction to the Aβ dorsal column/medial-lemniscal discriminative touch stream, less or not at all toward classical somatosensory areas. A prediction of this dual pathways model of somatosensation is that C-Tactile afferents ascend alongside other C-fibre modalities in the spinothalamic tract. Assessments of affective touch were performed in 19 patients before and after undergoing anterolateral cordotomy for unilateral cancer related pain. Cordotomy induced clear-cut contralateral deficits in the canonical spinothalamic modalities of temperature, pain and itch. However, the benchmark measures of affective touch, including the characteristic inverted U-shaped stimulus-response curve that binds psychophysical ratings to the characteristic velocity dependence of C-Tactile firing, were unaltered. The findings indicate that perceptual judgments about touch pleasantness do not depend on the integrity of the spinothalamic tract. Fibres ascending the dorsal columns provide sufficient information to conserve judgements about touch pleasantness after spinothalamic tract ablation. These findings diverge from those seen in HSAN-V and HSAN-III suggesting that evaluation of touch pleasantness including the characteristic tuning to slow gentle stroking critically depend on the co-processing of Aβ and CT inputs during normal neurodevelopment.

6.2 INTRODUCTION

The canonical view of the sense of touch having only discriminative and haptic functions, transmitted purely by large myelinated Aβ afferents, has been challenged by the discovery of a system of cutaneous low threshold mechanosensitive C-fibres. These so-called C-tactile (CT) afferents have slow conduction velocities (~1 m s^{-1}) which, along with other neurophysiological properties such as fatigue to repeated stimulation, makes them poorly suited for tactile discrimination (Vallbo, Olausson and Wessberg, 1999; Olausson *et al.*, 2010; McGlone, Wessberg and Olausson, 2014).

A dominant theory of the functional role of CT afferents, the Social Touch Hypothesis, proposes that they form a specific coding channel that carries information pertinent to pleasurable/rewarding aspects of gentle touch (Morrison, Löken and Olausson, 2010; McGlone, Wessberg and Olausson, 2014) that have emotional, affiliative and social relevance. This hypothesis was primarily built on the characteristic response properties of CT afferents: microneurographic recordings demonstrate that they respond preferentially to gentle caress-like touch (Nordin, 1990; Vallbo, Olausson and Wessberg, 1999). Indeed, a defining characteristic is that their firing rates are strikingly dependent on the force and velocity of a soft brushing stimulus, spiking most vigorously to velocities of 1-10 cm s^{-1} and significantly less so for faster and slower stimuli (Loken *et al.*, 2009; Ackerley *et al.*, 2014a). Remarkably, this inverted U-shaped neural stimulus-response function shows strong positive correlation with psychophysical ratings of touch pleasantness (Essick, James and McGlone, 1999; Loken *et al.*, 2009; Essick *et al.*, 2010).

A prediction of the Social Touch Hypothesis is the existence of distinct pathways for projection and processing of cutaneous CT inputs, and that these are at least partially segregated anatomically from discriminative touch pathways (Morrison, Löken and Olausson, 2010; McGlone, Wessberg and Olausson, 2014). In support of this premise, soft stroking touch of hairy skin activates dorsal posterior insular cortex but not primary sensory cortex in individuals lacking Aβ low threshold mechanoreceptor afferent fibres (Olausson *et al.*, 2002). Stroking with velocities optimal to CT afferents induces higher blood-oxygen-level-dependent response that with sub-optimal speeds (Morrison, Bjornsdotter and Olausson, 2011). Conversely, the same tactile stimulus in patients with Hereditary Sensory and Autonomic Neuropathy type V (HSAN-V), who show reduced C-fibre innervation density, presumably affecting CT afferents, is not only perceived as less pleasant than in controls but is also associated with flattening of the characteristic inverted U-shaped velocity dependence and reduced activation of dorsal posterior insula cortex (Morrison *et al.*, 2011). Together these imply not only that primary cortical targets for Aβ and CT afferents are distinct but also that CT afferents are placed within a wider interoceptive system including bodily sensations such as pain, temperature and itch that serve a function more interoceptive than exteroceptive (Craig, 2002; McGlone, Wessberg and Olausson, 2014), and one that is primarily protective.

The major somatosensory input into dorsal posterior insular cortex in primates is from posterior ventral medial nucleus of thalamus (Craig *et al.*, 1994; Craig and Zhang, 2006). Spinal inputs to this relay derive almost exclusively from dorsal horn lamina I via the spinothalamic tract (Craig and Zhang, 2006).
A deal of anatomical and physiological evidence suggests that the affective touch stream, similar to projections from other small diameter inputs ascends, in the spinothalamic tract. The central terminals of unmyelinated C-LTMR afferents, the animal equivalent of CT afferent, arborise in laminae II/IIIi of the spinal cord dorsal horn (Light and Perl, 1979; Sugiura, 1996; Li *et al.*, 2011; Abraira and Ginty, 2013). Lamina II cells activated by C-LTMRs arborise in lamina I (Lu and Perl, 2005; Maxwell *et al.*, 2007; Lu *et al.*, 2013) where they can contact projection neurons via an excitatory interneuronal relay (Lu *et al.*, 2013). Furthermore, a sub-population of L1 projection neurons in rats, in keeping with activation by C-LTMR inputs, fire in response to slow velocity gentle stroking (Andrew, 2010). However, this population are 'wide dynamic range' neurons, and also fire, indeed more vigorously so, in response to cold and noxious mechanical stimulation. A C-LTMR specific pathway has yet to be found.

Chronic intractable unilateral cancer pain below the dermatomal level C4 can be treated by lesioning the contralateral anterolateral funiculus, which contains the spinothalamic tract, at the C1/C2 cervical level (Harsh and Viswanathan, 2013). Pain relief is typically associated with marked deficits in noxious and innocuous temperature and noxious mechanical sensation, classical spinothalamic tract modalities, contralateral to the lesion (Lahuerta *et al.*, 1994; Bain, Hugel and Sharma, 2013; Harsh and Viswanathan, 2013). Subjective alterations in tactile sensation following cordotomy, that might reflect altered affective touch processing (Lahuerta *et al.*, 1994), have been described but this pre-dates the discovery of CT afferents in humans. To investigate the hypothesis that the human spinothalamic tract relays ascending information derived from CT inputs and is necessary for the perception of affective tactile sensation, pleasant touch perception was quantified using benchmark affective touch psychophysics in patients before and after anterolateral cordotomy. Subjective responses to CT and non-CT targeted brushing stimuli as well as descriptor ratings for emotional and discriminative aspects of touch were assessed ipsilateral and contralateral to lesioning. It was predicted that cordotomy would result in both a reduction of subjective pleasantness to gentle dynamic touch and a flattening of the inverted-U velocity tuning contralateral to lesioning whilst leaving discriminative touch capacity intact.

6.3 METHODS

6.3.1 Participants

Twenty patients were recruited in accordance with the Health Research Authority National Research Ethics Service (study reference 14/NW/1247). All patients were admitted to the Walton Centre for Neurology and Neurosurgery, Liverpool, UK and suffered from intractable unilateral cancer related pain below the cervical level C4 with an expected lifespan of less than 12 months. The patients' demographic and clinical details are shown in Table 6.1. The majority of patients carried a diagnosis of pleural mesothelioma with treatment resistant chest wall pain. No patient had symptoms or signs of neurological impairment in the region of sensory testing. All patients were medicated with regular and *pro re nata* opioids as well as a variety of non-opioid analgesia. The most common descriptor terms on the McGill Pain Questionnaire (Melzack, 1987) for the cancer related pain were aching, shooting and stabbing (fig. 6.1). The median and range for numeric rating scale of average 4 hours pain, maximum pain in the past 4 hours and current pain were 76 (20-90), 98 (79-100) and 50 (10-81) respectively. A large number (13/19) of patients had previously received chemotherapy with potential peripheral neurotoxicity although no patient described ongoing symptoms potentially attributable to this.

Opioid treatment (Martel *et al.*, 1995; Case *et al.*, 2016a), chronic pain (Case *et al.*, 2016a) and chemotherapy induced neurotoxicity (Geber *et al.*, 2013; Krøigård *et al.*, 2014) could all in principle impact on sensory testing. However, pre-procedural thermal and thermal pain detection thresholds were normal in the area of sensory testing and there was no pre-procedural evidence of an alteration in either the sensory discriminative or affective aspects of touch (see below). Furthermore, since the study paradigm compared lesioned versus non-lesioned sides and pre-versus post-lesion states one would expect a right-left or pre-post difference in measures of affective or discriminative touch to be detected even if there was an underlying subtle (drug or pain induced) baseline 'abnormality' in the function or processing of CT afferents or a generalised procedural effect.

The antero-lateral cordotomy was performed at the C1/C2 level contralateral to the cancer related pain. The procedure was performed with sedation and local anesthesia. Following dural puncture with a 20G spinal needle the cordotomy electrode was advanced into the antero-lateral quadrant of the spinal cord. Positioning in the spinothalamic tract was verified by eliciting cold, heat or other painful sensations, encompassing the region of cancer related pain, using 50Hz electrical stimulation through the cordotomy electrode. Motor twitch threshold using 10Hz stimulation was also performed to assess for proximity to the corticospinal tract. Adjustments of the electrode were made to maximise location with the spinothalamic tract and minimise proximity to motor pathways. The spinothalamic tract was disrupted using a radiofrequency current which produces a heat induced lesion. This was performed in steps, typically starting at 65°C for 25-30s, with a maximum temperature of 85°C. Lesioning of the spinothalamic tract was confirmed in the operating theatre by demonstrating a contralateral loss of temperature sensation. Operative details for all cases are shown in Table 6.1.

	Age	M/F	Oncological diagnosis and treatment	Chemotherapy	Radiotherapy	Surgery	Pain location (Approximate dermatomal distribution)	Pain NRS (average 24 hours / maximum /current)	Post-Lesion Pain NRS	Medication	Operative medication	Thermocoagulation	Intra-operative sensation
1	53	F	Carcinoma of breast, bone metastases	No	Yes	Yes	Left hip and thigh (L2-L3)	72 / 100 / 36	5	Naproxen, Amitryptiline, Oxycodone	Midazolam 2mg Fentanyl 75 mcg	1 x 25s 75°C	Heat left trunk, upper and lower limb
2	70	M	Mesothelioma, chest wall invasion	No	No	Yes	Right thoracic (T4-T10)	77 / 100 / 61	5	Ibuprofen, Gabapentin, Fentalyl patch	Midazolam 2mg Fentanyl 110 mcg	2 x 25s 75°C, 1x25s 90°C	Burning / stinging right arm and chest
3	54	F	Carcinoma of lung, chest wall invasion	No	Yes	No	Right thoracic and axilla (T2-T4)	90 / 100 / 81	0	Paracetamol, Pregabalin, Lidocaine patch, Lorazepam, Oxycodone, Ketamine	Propofol, Fentanyl 80 mcg	1 x 25s 75°C, 2 x 25s 80°C	Burning right arm, hand, abdomen, chest
4	68	M	Mesothelioma, chest wall invasion	No	No	Yes	Right thoracic (T3-T11)	90 / 94 / 58	0	Paracetamol, Naproxen, Gabapentin, Morphine	Propofol, Fentanyl 110mcg	1x25s 75°C, 2x25s 80°C	Cold sharp freezing blast right upper limb
5	67	M	Mesothelioma, chest wall invasion	Yes - pemetrexed and carboplatin	No	Yes	Right thoracic (T3-T8)	88 / 93 / 52	10	Gabapentin, Oxycodone	Propofol, Fentanyl 120mcg	1 x 25s 70°C, 2 x 25s 78°C, 1 x 25s 84°C	Heat right hand, shoulder and upper trunk
6	60	M	Mesothelioma, chest wall invasion	Yes - pemetrexed and cisplatin	No	Yes	Right thoracic (T3-T8)	81 / 95 / 71	0	Amitriptyline, Oxycodone	Propofol, Fentanyl 140mcg	1x25s 74 °C, 1 x 25s 80°C	Sharp and cold freezing blast right upper limb
7	73	M	Mesothelioma, chest wall invasion	No	No	Yes	Left thoracic (T3-T10)	81 / 100 / 57	30	Gabapentin, Methadone, Oxycodone	Propofol,Midazolam 2mg, Fentanyl 140mcg	1 x 25s 70°C, 1 x 75°C, 2 x 25s 80°C	Heat and sharp pain right trunk, upper and lower limb
8	56	M	Mesothelioma, chest wall invasion	Yes - pemetrexed and cisplatin	No	Yes	Right thoracic (T3-T9)	77 / 94 / 48	0	Pregabalin, Lidocaine Patch, Tramadol, Oxycodone	Propofol, Remifentanyl	2 x 25s 75°C, 1 x 25s 80 °C	Heat right upper limb and upper trunk
9	56	M	Non-small cell lung carcinoma, vertebral and right hip metastases	No		Yes	Right lower limb (L2-L5)	79 /98 /45	0	Ibuprofen, Dexamethazone, Gabapentin, Lorazepam, Oxycodone, Fentanyl IR	Midazolam 2mg, Fentanyl 75 mcg	1 x 25s 75°C, 2 x 25s 80°C, 1 x 25s 85°C	Burning / stinging right upper limb and chest
10	59	M	Mesothelioma, chest wall invasion	Yes - pemetrexed and carboplatin	No	Yes	Left thoracic (T2-T8)	51 / 100 /38	0	Paracetamol, Pregabalin, Morphine, Fentanyl patch	Propofol, Midazolam 2mg, fentanyl 80mcg	1 x 25s 75°C, 1 x 25s 80°C, 2 x 25s 85°C	Heat sensation left upper limb and chest

Table 6.1. Demographic and clinical data of patients undergoing anterolateral cordotomy

11	65	M	Carcinoma of Rectum, pelvic metastases	Yes – folinic acid, fluorouracil and irinotecan	No	Yes	Left lower limb (S1-S2)	20 / 88 /48	15	Paracetamol, Pregabalin, Oxycodone	Propofol, Fentanyl 80 mcg	1 x 25s 70 °C, 2 x 25s 80 °C	Heat left trunk, upper and lower limb
12	59	F	Non-small cell lung carcinoma, chest wall invasion	Yes – cisplatin and docetaxel	Yes	Yes	Left thoracic (T5-T10)	65 / 79 / 70	0	Gabapentin, Oxycodone	Propofol, Remifentanyl	2 x 25s 75°C, 1 x 25s 85°C	Heat left upper limb
13	71	M	Mesothelioma, chest wall invasion	Yes - pemetrexed and carboplatin	Yes	Yes	left thoracic (T3-T10)	50 /80 /70	0	Amitrypyiline, Oxycodone	Propofol, Remifentanyl	1 x 25s 75°C, 1 x 25s 80°C	Heat / buring left upper limb and trunk
14	62	M	Mesothelioma, chest wall invasion	Yes - pemetrexed and carboplatin	No	Yes	left thoracic (T2-T8)	65 / 100 / 20	5	Ibuprofen, Amitryptiline, Morphine	Propofol, Remifentanyl	1 x 25s 75°C, 1 x 25s 80°C, 3 x 25s 85°C	Heat left shoulder / upper limb / chest followed by pleasant sensation centred on area of allodynia aroud left nipple
15	51	F	Carcinoma of Rectum, pelvic metastases	Yes – folinic acid, fluorouracil and irinotecan	No	Yes	Right lower limb (L5)	55 / 100 / 40	0	Dexamethazone, Oxycodone	Propofol, Fentanyl 80mcg	1 x 25s 75 °C, 1 x 25s 80 °C, 2 x 25s 85 °C	Heat right trunk, upper and lower limb
16	47	F	Carcinoma of Cervix, pelvic metatsases	Yes – fluorouracil and cisplatin	Yes	No	Left lower limb (L1-L4)	75 / 100 / 65	20	Naproxen, Dexamethazone, Gabapentin, Diazepam, Oxycodone	Propofol, Remifentanyl	2 x 75°C, 2 x 80°C, 1 x 85°C	Heat left trunk, upper and lower limb
17	51	F	Carcinoma of Lung, bone metastases and chest wall invasion	Yes – carboplatin and docetaxel	Yes	No	Left thoracic (T2-T6)	90 / 100 / 65	0	Etoricoxib, Amitryptiline, Pregabalin, Morphine	Propofol, Fentanyl	2 x 75°C, 1 x 80°C, 1 x 85°C	Burning left upper limb and upper trunk
18	63	M	Mesothelioma, chest wall invasion	Yes – pemetrexed, cisplatin and bevacizumab	No	Yes	Right thoracic (T3-T6)	50 / 80 / 45	0	Paracetamol, Naproxen, Gabapentin, Oxycodone	Propofol, Remifentanyl	2 x 75°C, 1 x 80°C, 1 x 85°C	Heat right chest wall and right arm
19	55	F	Carcinoma of Lung, bone metastases and chest wall invasion	Yes – carboplatin and docetaxel	Yes	No	Right thoracic and axilla (T2-T4)	45 / 90 / 10	0	Paracetamol, Dexamethazone, Gabapentin, Oxycodone	Propofol, Fentanyl	2 x 25s 75°C, 1 x 25s 85°C	Heavy hot sensation right upper limb and upper trunk

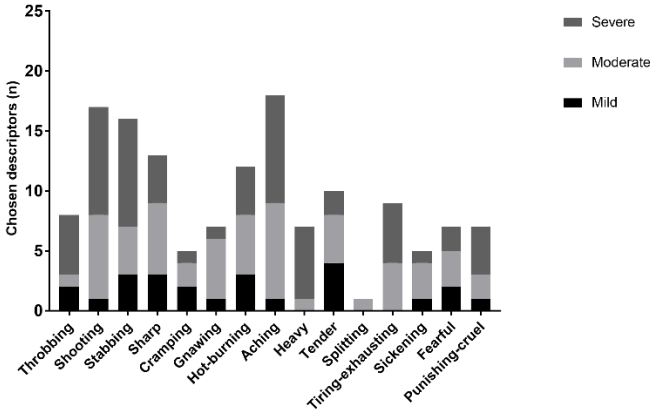

Fig. 6.1
Short form McGill Pain Questionnaire showing descriptors for the clinical pain prior to anterolateral cordotomy.

6.3.2 Experimental design

All patients underwent pre-procedure testing, either on the morning of or day before the cordotomy. Post-cordotomy testing was undertaken at least four hours following the procedure to allow for recovery from operative sedation. All post-cordotomy assessments were performed within 72 hours of the procedure. Pre-procedure and post-procedure testing typically lasted approximately 90 minutes.

Pleasant touch. Assessment of gentle dynamic touch was made using stimuli delivered to the dorsal aspect of the right and left forearm using a 70mm goat's hair artist brush. Patients were prevented from seeing the tested extremity throughout the experiment. Stimuli were given in a proximal to distal direction. Stimuli were delivered manually over a 10cm distance marked on the forearm at velocities of 0.3, 3 and 30 cm s^{-1}, chosen to reflect C-tactile optimal (3 cm s^{-1}) and sub-optimal (0.3 and 30 cm s^{-1}) stimuli. All stimuli were performed by AGM who had been trained to deliver the stimuli at the required velocities. A computerized visual meter was used during training and testing sessions. Six stimuli at each velocity were given on each side in a computer-generated pseudorandom order. An inter-stimulus interval of at least 10s was allowed to prevent fatigue in CT afferent fibres. The order of testing with respect to right and left was randomized. After each stroke patients rated both the pleasantness and intensity of the stimulation using a 20cm paper visual analogue scale (VAS). Anchor points for touch intensity were no sensation (0) and very intense (10). For pleasantness anchor points were 'unpleasant' (−10) and 'pleasant' (10) with 0 representing a neutral stimulus.

Tactile Acuity and Graphesthesia. Mechanical detection thresholds on the dorsal aspect of the forearm were determined using standardized set of von Frey monofilaments (Optihair2- Set Nervtest, Germany), which bend at forces between 0.25 and 512mN (Frey 1896) according to the 'method of limits' (Rolke *et al.*, 2006a). Two-point discrimination (TPD) on the dorsal forearm was determined using mechanical sliding calipers with a precision of 1mm. Assessment commenced with 1mm between the two points and was gradually increased until the subject discerned two points. Five ascending and descending assessments, centred around the subject's TPD threshold, were conducted. The geometric mean of the obtained values was calculated for the threshold. Assessment of graphesthesia was used as a test of dorsal column function (Bender, Stacy and Cohen, 1982). The numbers 3, 4 and 5 were drawn, approximately 6cm in top-bottom dimension on the dorsolateral forearm using the blunt end of a Neurotip (Owe Mumford Ltd, UK). Initially testing was performed with the eyes open to ensure that the task was understood. Each number was presented three times in a pseudorandom order with eyes closed. The order of testing with respect to right and left was randomized.

Thermal threshold testing. Assessment of cold sensation detection threshold (CDT), warm sensation detection threshold (WDT), cold pain threshold (CPT) and heat pain threshold (HPT) was made using the method of limits with the MEDOC TSA II (Medoc, Ramat Yishai, Israel) on the dorsal aspect of the right and

left forearm. The thermode had a surface area of 9.0cm2 and baseline temperature of 32°C. Thresholds were obtained using ramped stimuli of 1°C/s, the patient terminating the ramp with a button press. The mean of three consecutive temperature thresholds was calculated. The order of testing with respect to right and left was randomized.

Pinprick testing. Assessment of pinprick sensation on the right and left dorsolateral forearm was made using a Neurotip (Owe Mumford Ltd, UK).

Itch. Assessment of itch sensation was made using cowhage. Cowhage spicules contain the pruritogen mucunain (Reddy *et al.*, 2008; Davidson and Giesler, 2010) and on contact with the skin induce a histamine independent itch via activation of proteinase-activated receptors-2 and -4 (Reddy *et al.*, 2008; Davidson and Giesler, 2010). Recordings in primates have shown that cutaneous application of cowhage activates ascending spinothalamic projection neurons (Davidson *et al.*, 2012). Approximately 20 cowhage spicules were collected onto a cotton bud and rubbed directly on a $1cm^2$ skin site for 20 seconds. Spicules were then removed with a strip of lightly-adhesive paper tape (Micropore, 3M, USA) immediately after application. Assessments were made post-cordotomy only, on the right and left side. Patients were asked to rate the intensity of itch on a numeric rating scale (0-100). If no perception of itch was elicited cowhage application was repeated up to a maximum of three times before the sensation was judged to be absent.

***Questionnaires*.** The Touch Perception Task (TPT) was administered to evaluate the qualitative aspects of discriminative and emotional touch of different materials delivered to the dorsal aspect of the right and left forearm. The TPT was developed as a validated descriptive scale for touch perception (Guest *et al.*, 2011). The full TPT consists of 26 sensory and 14 emotional descriptors that provide information about differing aspects of touch in relation to specific tactile stimulations. For the purpose of the current study a shortened form of the TPT consisting of 28 descriptors was administered omitting seven sensory (firm, gritty, jagged, lumpy, rubbery, sticky and vibrating) and five emotional (sexy, thrilling, enjoyable, soothing and relaxing) descriptors (Table 6.2).

Stimuli were administered using a manual tactile stimulator that delivers a force-controlled stimulus at a pressure of 0.22N. To this either sandpaper (grade: P120; see (Verrillo, Bolanowski and McGlone, 1999)) for classification of the sandpaper) or artificial fur (soft 10 mm long hairs) were attached with an application dimension of 80 × 50 mm. Artificial fur and sandpaper have been used previously (see (Ackerley *et al.*, 2014b)) to provide extremes of tactile stimuli. The manual tactile stimulator was moved over the skin at 3 $cm\ s^{-1}$ over a 10cm distance in a proximal to distal direction. The order of testing with respect to right and left as well as type of material was randomized.

Sensory Descriptors	Emotional Descriptors
Bumpy	Arousing
Burning	Calming
Cold	Comfortable
Damp	Desirable
Dry	Discomfort
Fluffy	Exciting
Fuzzy	Irritating
Greasy	Pleasurable
Hairy	Sensual
Hard	
Hot	
Prickly	
Rough	
Sharp	
Slippery	
Smooth	
Soft	
Warm	
Wet	

Table 6.2. List of sensory and emotional descriptors used in the Touch Perception Task.

6.3.3 Data analysis

Statistical analyses were carried out with SPSS (version 23; IBM, Armonk, NY), Excel 2010 (MicrosoftTM) and Graphpad Prism (version 7.04; GraphPad Software, La Jolla, CA).

Rating data for pleasantness and intensity were averaged for each participant and each velocity and these average values were used in the reported analysis of variance (ANOVA) and regression analyses. For each condition regression analysis was performed both on average pleasantness rating scores and on each participant's raw data to extract negative quadratic term and intercept values on a group and individual basis.

Since both the populations studied were substantially older than in previous studies and that an abbreviated version of the TPT was used in the current study, a further factor analysis using information obtained in the pre-cordotomy state and healthy control participants was performed to reduce the number of variables into fewer numbers of factors. Given that the aim was to separate sensory and emotional aspects of touch scores from sensory and emotional descriptors were entered in separate factor analyses to yield sensory and emotional factors respectively. The approach was similar to that used in previous studies (Guest *et al.*, 2011; Ackerley *et al.*, 2014b).

The suitability of principal components analysis was assessed prior to analysis. Inspection of the correlation matrix showed that all variables had at least one correlation coefficient greater than 0.3. The overall Kaiser-Meyer-Olkin (KMO) measure for sensory and emotional descriptors was 0.8 and 0.87 respectively. Individual KMO measures for emotional descriptors were all > 0.75 and for sensory descriptors >0.5. Bartlett's test of sphericity was statistically significant ($p < .0005$), indicating the likelihood that the descriptors cluster into factors. To enable better differentiation between the factors independent orthogonal (Varimax) rotation and related oblique (Promax) rotation were tested (Russell, 2002). Oblique rotation for both sensory and emotional analyses was used because significant correlations were found between factors. The Promax procedure after an initial orthogonal Varimax rotation initially, allows correlations between factors to improve the factor fit, resulting in factors that are more realistic (Fabrigar et al., 1999; Russell, 2002; Schmitt, 2011). To establish how many factors to retain the eigenvalue-one criterion, percentage of variance explained (>5%), inspection of scree plots and finally the interpretability criterion which considers the concept of "simple structure" and whether the final solution makes sense.

From the 17 sensory descriptors principle component analysis revealed four components, termed ‘texture’, ‘pile’, ‘slip’ and ‘heat’, that had eigenvalues greater than one and which explained 39.5%, 14.0%, 11.6% and 8.1% of the total variance, respectively. Visual inspection of the scree plot indicated that four components should be retained. In addition, a four-component solution met the interpretability criterion. As such, four components were extracted. The descriptor ‘cold’ did not load highly on any of the factors. Three factors, termed ‘positive’, ‘arousal’ and ‘negative’ which explained 65.6%, 14.8% and 8.1% of the total variance were extracted from the emotional descriptor terms. These findings are broadly consistent with previous investigation (Guest *et al.*, 2011; Ackerley *et al.*, 2014b).

The extracted factors were composed of significantly contributing descriptors (loadings of >0.3; Field, 2009). Factor scores were determined using the weightings of significant descriptor contributions. Since oblique rotation was used both pattern and structure matrices were generated; the former containing information relating to how much a descriptor predicts that factor (i.e. regression coefficients) and that latter how much a descriptor relates to each factor (i.e. correlation coefficients). Factor loadings (regression and correlation coefficients) for significantly contributing descriptors are presented in order of magnitude along with the variance and covariance incorporated in each factor are shown in Tables 6.3 and 6.4. A factor weight matrix using information from the structure and pattern matrices was then used to compute factor scores. Overall factor scores for each sensory and emotional factor were subsequently being used to explore differences following cordotomy.

Repeated measures ANOVA was used to explore significant differences in pleasantness and intensity rating data, intercept and quadratic terms as well as mechanical detection and two-point discrimination thresholds. All models had factors of time (pre- and post-cordotomy) and side (pain-affected and control). A third factor of either velocity (0.3, 3 and 30 cm s^{-1}) or material (fur and sandpaper) were used when appropriate. Model assumptions were checked by plotting model residuals in combination with verification that the Shapiro-Wilk test of normality for model residuals was not significant. Data were checked for sphericity (i.e., equality in variance in the different levels of the factor) using Mauchly's test. Data were logarithm transformed when appropriate (Shapiro-Wilk's test of normality $p < .05$). In the case of outliers, assessed as a value greater than 3 box-lengths from the edge of the box, analyses were repeated after removal. All analyses were robust to outlier removal. Significant interaction effects were followed up using simple main effects and pairwise comparisons with Sidak’s correction (denoted in the text as P_s). F approximations to Pillai’s trace are reported.

Wilcoxon signed rank test was used to explore pre- and post-cordotomy as well as pain affected versus control side differences in non-parametric distributed data. Spearman rank correlation and where appropriate Pearson r correlation were used to explore the relationship between the pre- to post-cordotomy change in touch ratings, quadratic and intercept terms as well as thermal thresholds. Relevant variables were then entered in a linear regression model (detailed below). Statistical significances were sought at the $p < 0.05$ level.

Factor Name	**Factor 1: Texture / Roughness**				**Factor 2: Pile**				**Factor 3: Heat**				**Factor 4: Slip**			
Variance	**39.4%**				**14.0%**				**10.1%**				**8.4%**			
Output	**Regression**		**Correlation**		**Regression**		**Correlation**		**Regression**		**Correlation**		**Regression**		**Correlation**	
Descriptor and Loading	Bumpy	0.92	Hard	0.86	Warm	0.91	Fluffy	0.86	Hot	0.93	Hot	0.95	Slippery	0.86	Slippery	0.86
	Dry	0.82	Rough	0.85	Hairy	0.85	Soft	0.80	Burning	0.88	Burning	0.86	Greasy	0.86	Greasy	0.86
	Sharp	0.81	Sharp	0.84	Fuzzy	0.78	Smooth	0.79			Prickly	0.49				
	Hard	0.79	Dry	0.76	Smooth	0.70	Hairy	0.76			Sharp	0.45				
	Prickly	0.66	Prickly	0.76	Fluffy	0.65	Fuzzy	0.76								
	Rough	0.64	Bumpy	0.64	Soft	0.53	Warm	0.71								
	Warm	0.41	Smooth	-0.53			Hard	-0.51								
	Fluffy	-0.42	Fluffy	-0.73			Rough	-0.71								
	Soft	-0.54	Soft	-0.79												

Table 6.3. Sensory descriptors factor analysis

Three significant factors were found in the emotional descriptors data (those contributing >5% of the variance; detailed in the Methods) and named Texture, Pile, Heat and Slip. The descriptors and their significant loadings (>0.3) are shown for both the regression (pattern matrix) and the correlation (structure matrix) factor analysis output.

Factor Name	**Factor 1: Positive Affect**				**Factor 2: Arousal**				**Factor 3: Negative Affect**			
Variance	**65.6%**				**14.8%**				**8.1%**			
Output	**Regression**		**Correlation**		**Regression**		**Correlation**		**Regression**		**Correlation**	
Descriptor and Loading	Calming	1.01	Comfortable	0.97	Arousing	0.94	Arousing	0.90	Discomfort	0.98	Discomfort	0.95
	Comfortable	0.98	Pleasurable	0.97	Exciting	0.92	Sensual	0.89	Irritating	0.87	Irritating	0.93
	Pleasurable	0.97	Calming	0.95	Sensual	0.73	Exciting	0.89			Calming	-0.53
	Desirable	0.79	Desireble	0.95			Desirable	0.72			Comfortable	-0.57
			Sensual	0.72			Comfortable	0.61			Desirable	-0.58
			Arousing	0.54			Pleasurable	0.60			Pleasurable	-0.60
			Exciting	0.53			Calming	0.59				
			Discomfort	-0.51								
			Irritating	-0.61								

Table 6.4. Emotional descriptors factor analysis

Three significant factors were found in the emotional descriptors data (those contributing >5% of the variance; detailed in the Methods) and named Positive Affect, Arousal and Negative Affect. The descriptors and their significant loadings (>0.3) are shown for both the regression (pattern matrix) and the correlation (structure matrix) factor analysis output.

6.4 RESULTS

6.4.1 Anterolateral Cordotomy caused profound effects on clinical pain, evoked pain, temperature and itch

In all patients' clinical pain was either abolished or dramatically reduced following cordotomy (Table 6.1). Similarly, a demonstrable deficit in pinprick sensation was evident in the forearm on the pain affected side after lesioning. Prior to cordotomy cold, warm as well as cold and heat pain thresholds were equivalent on pain affected and control sides. Also, temperature detection and thermal pain thresholds did not differ significantly from those seen in age-matched healthy control participants (data not shown). Lesioning of the anterolateral funiculus resulted in profound impairments in temperature detection and thermal pain thresholds (Table 6.5 and fig. 6.2). Following cordotomy cowhage reliably caused a sensation of itch within two applications (mean itch rating (0-100) = 35.6 +/- 11.6) on the control side but failed to induce itch in three applications on the pain affected side.

	Pain Affected		**Control**	
	Pre-cordotomy	**Post-cordotomy**	**Pre-cordotomy**	**Post-cordotomy**
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
CDT	30.1 (29.5-30.8)	2.0 (0.0-12.0) ****	30.2 (29.8-30.5)	30.0 (29.4-30.2)
WDT	34.8 (34.3-35.3)	46.1 (39.8-48.1) ****	34.9 (34.5-35.1)	35.2 (34.8-35.5)
CPT	5.0 (1.4-12.0)	0.0 (0.0-1.0) ****	6.2 (3.1-10.5)	6.2 (1.2-10.8)
HPT	46.2 (44.8-47.8)	50.0 (49.1-50.0) ****	45.4 (43.9-46.9)	46.1 (44.6-46.8)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
MDT	3.35 (0.80)	3.53 (0.86)	3.28 (0.83)	3.43 (0.86)
TPD	30.4 (5.6)	30.6 (5.9)	30.2 (5.3)	30.7 (6.3)

Table 6.5. Summary of thermal threshold and discriminative touch sensation testing in the pre-cordotomy and post-cordotomy states.

Significant differences (Related-Samples Wilcoxon Signed Rank Test) between the pre-cordotomy and post-cordotomy states are marked with asterisks and show ****$p < 0.0005$. Abbreviations: CDT, Cold Detection Threshold; WDT, Warm Detection Threshold; CPT, Cold Pain Threshold; HPT, Heat Pain Threshold; MDT, Mechanical Detection Threshold; TPD, Two-Point Discrimination; IQR, Interquartile Range.

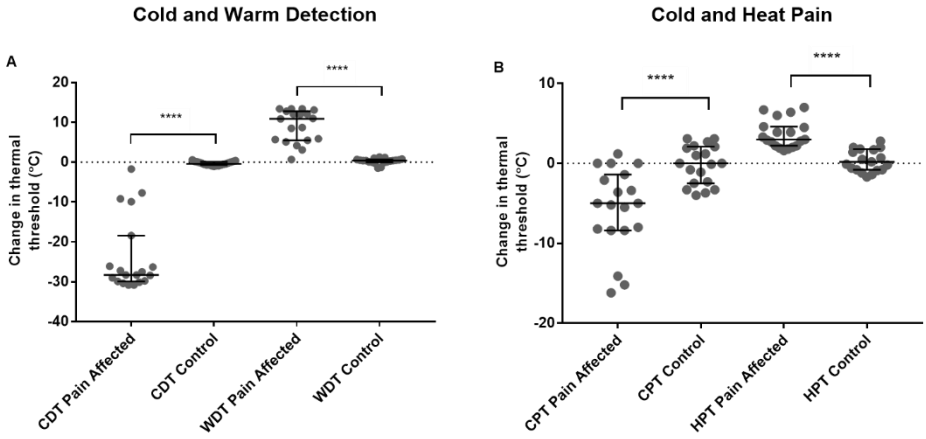

Fig. 6.2.

Dot plot showing changes in pre-cordotomy to post-cordotomy thermal detection (A) and pain (B) thresholds. Data are presented as median and interquartile range. Significant differences (Related-Samples Wilcoxon Signed Rank Test) between the pain affected and control sides are marked with asterisks and show ****$p < 0.0005$. Abbreviations: CDT, Cold Detection Threshold; WDT, Warm Detection Threshold CPT, Cold Pain Threshold; WPT, Warm Pain Threshold.

6.4.2 Discriminative touch is unaltered by anterolateral cordotomy

Mechanical detection thresholds, two-point discrimination as well as graphesthesia showed no deficit and were equivalent on control and pain affected sides prior to cordotomy. Lesioning of the anterolateral tract produced no significant ipsilateral or contralateral change in these measures of touch discrimination (Table 6.5).

6.4.3 Perceived touch pleasantness is not affected by cordotomy

Pleasantness ratings for stroking touch were almost always positive across all velocities. In keeping with previous published results (Essick, James and McGlone, 1999; Essick *et al.*, 2010; Loken *et al.*, 2009; McGlone, Wessberg and Olausson, 2014) group ratings for touch pleasantness in patients on control and pain affected sides as well as in healthy control participants showed a pattern consistent with an inverted U-shape (fig 6.3). No effect of anterolateral tract lesioning was seen on ratings for touch pleasantness (Table 6.6 and fig 6.3).

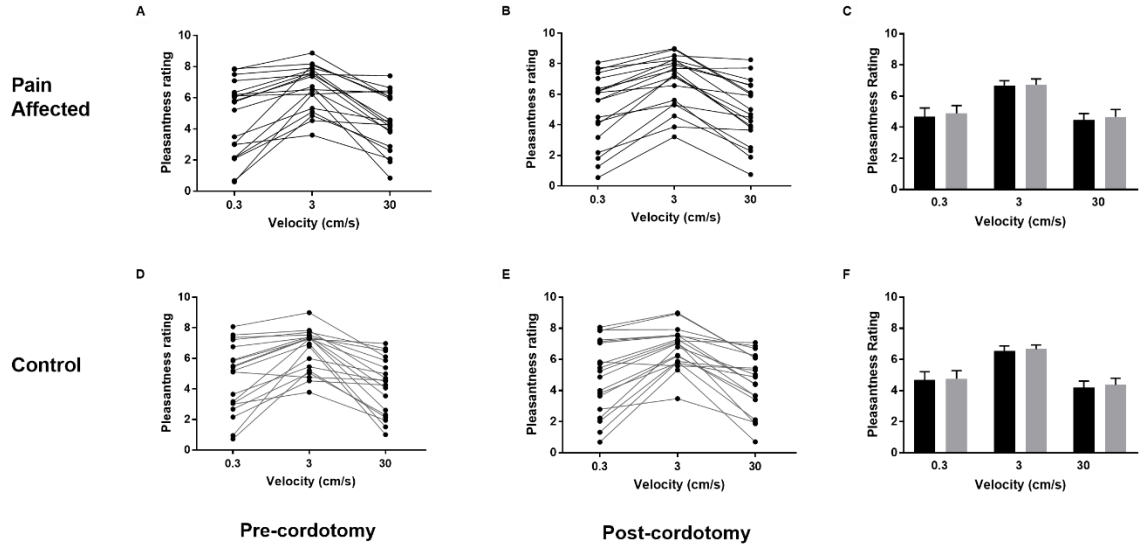

Fig 6.3.

Raw and group data for VAS pleasantness ratings for stroking touch at C-Tactile optimal and sub-optimal velocities. (A) Raw data for the pain affected side pre-cordotomy (B) Raw data for the pain affected side post-cordotomy (C) Group data for the pain affected side pre-cordotomy (black) and post-cordotomy (grey) (D) Raw data for the control side pre-cordotomy (B) Raw data for the control side post-cordotomy (C) Group data for the control side pre-cordotomy (black) and post-cordotomy (grey). Group data are presented as mean + standard error mean.

To further explore the effect of velocity on the pattern of pleasantness ratings following cordotomy, two additional analyses were performed for each of the four conditions (pre- and post-, control and pain affected). Firstly, a measure of the preference for C-tactile targeted touch, the C-tactile preference index (CTPI) was calculated as:

$$CTPI = \frac{1/2\ \left((VAS\ rating\ 3cms/s - VAS\ rating\ 0.3cm/s) + (VAS\ rating\ 3cm/s - VAS\ rating\ 3cm/s)\right)}{1/3\ (VAS\ rating\ 0.3\ cm/s + VAS\ rating\ 3cm/s + VAS\ rating\ 30cm/s)}$$

Where VAS ratings represent pleasantness rating at the appropriate velocity. The CTPI was not affected by cordotomy (Table 6.6 and fig. 6.4).

Secondly, regression analysis was performed on group to assess the shape of rating curves. Using logarithm-transformed values for the independent variable, 'velocity', rating data were entered into the regression model as both linear and quadratic terms. Analysis was performed on both a group level, using average rating scores, and individually, using all individual rating scores, to extract negative quadratic term and intercept values (Morrison *et al.*, 2011). These values describe the two key components of typical pleasantness ratings to gentle dynamic touch in healthy individuals: the degree of the inverted U-shape provides a measure of the velocity-dependent preference for CT- targeted touch, whereas, the intercept value reflects overall perceived touch pleasantness across all velocities. Quadratic terms that are more negative represent a greater preference to CT-targeted velocities when compared to fast and very slow touch. Intercept values that are higher reflect higher pleasantness ratings encompassing all velocities. For group data in all four conditions the negative quadratic term provided a better fit for average rating scores than the linear term ($p<0.0005$) (fig. 6.4). Furthermore, when quadratic terms from individual participants were plotted against intercept values, no separation was seen across the four conditions (fig. 6.4). Indeed, no significant difference in rating patterns across conditions was found when quadratic term and intercept values were entered separately into a repeated measures ANOVA with independent variables side (control and pain affected) and time (pre- and post-cordotomy) (Table 6.5). Finally, there was no significant difference in the coefficient of variance for pleasantness ratings across conditions.

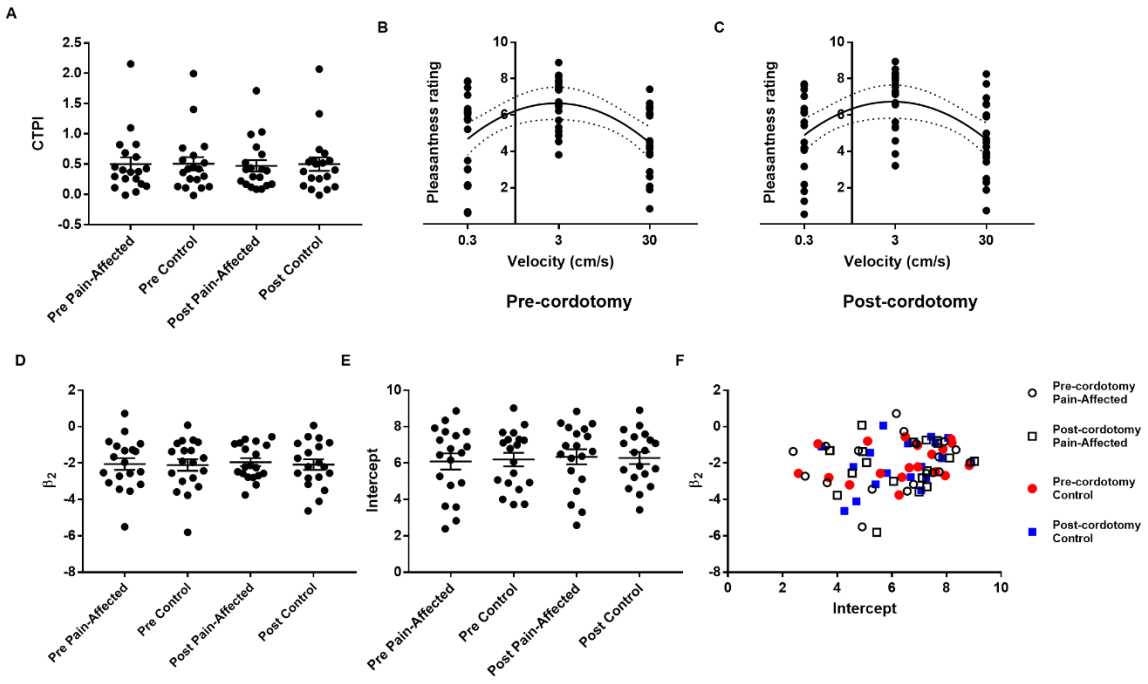

Fig. 6.4.

Measures of preference for C-Tactile targetted touch and overall touch pleasantness are unaffected by anterolateral cordotomy. (A) Dot plots of the CTPI for pre-cordotomy and post-cordotomy states for both the pain-affected and control side. Dot plots of the mean individual ratings for touch pleasantness on the pain-affected side both pre-cordotomy (B) and post-cordotomy (C) are shown. The lines of best fit with 95% confidence intervals are shown. β_2 and intercept values for the pre-cordotomy and post-cordotomy states are (-2.07, 6.22) and (-1.95, 6.34) respectively. Dot plots of individual values for β_2 and intercept pre-cordotomy and post-cordotomy states for both the pain-affected and control side are shown in (D) and (E). Scatterplot of β_2 and intercept values from regression analysis of individual pleasantness rating tuning curves (F). There is no clear separation of datapoints for the pre-cordotomy pain-affected (white circles), post-cordotomy pain-affected (white squares), pre-cordotomy control (red circles) or post cordotomy control (red squares) states. Data in A, D and E are presented with mean +/- SEM. Abbreviations; CTPI, C-Tactile Preference Index; SEM, standard error of mean.

Source of Variation	*df*	Pleasantness		Coefficient of Variation Pleasantness		Intensity		CTPI		Quadratic Term		Intercept	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Time	1	0.810	.380	0.641	.434	13.889	**.002**	0.000	.995	0.226	.640	0.687	.418
Side	1	1.073	.314	0.039	.845	14.516	**.001**	0.011	.919	0.537	.473	0.021	.888
Velocity	2	24.727	**<.0005**	9.083	**.001**	119.819	**<.0005**	N/A	N/A	N/A	N/A	N/A	N/A
Time x Side	1	0.029	.867	0.417	.526	20.043	**<.0005**	0.057	.814	0.189	.669	0.399	.536
Time x Velocity	2	0.212	.810	0.197	.822	1.195	.286	N/A	N/A	N/A	N/A	N/A	N/A
Side x Velocity	2	1.450	.248	1.068	.354	4.197	0.023	N/A	N/A	N/A	N/A	N/A	N/A
Time x Side x Velocity	2	0.441	.647	3.918	**.029**	0.598	.555	N/A	N/A	N/A	N/A	N/A	N/A

Table 6.5. Summary of three-way repeated measure ANOVA for the effects of velocity, side (control versus pain affected) and time (pre-cordotomy versus post-cordotomy) on pleasantness ratings, co-efficient of variation of pleasantness ratings, CTPI, intensity ratings, negative quadratic term and intercept. Analysis was log10 transformed for CTPI and coefficient of variation.

6.4.4 The perceived intensity of gentle stroking touch is reduced by anterolateral tract lesioning

Ratings for the intensity of stroking stimuli were significantly affected by stroking velocity, side (pain affected versus control) and time (pre- versus post-cordotomy) and all of their two-way interactions, but not three-way interactions (Table 6.5). Intensity ratings increased with increasing velocity in all four conditions as well as in healthy control participants (fig 6.5) and were significantly lower in the pain-affected side post-cordotomy when compared to both the pre-cordotomy state and control side post-cordotomy. The percentage change in touch intensity from the pre-cordotomy to post-cordotomy states on the pain affected and control states was significantly affected by side (pain-affected versus control) (two-way repeated measures ANOVA $F(1, 18) = 24.410$ $p < .0005$) (fig 6.5 (C)). The percentage difference in touch intensity between the pain-affected and control sides in the pre-cordotomy and post-cordotomy was significantly affected by time (pre-cordotomy versus post-cordotomy) (two-way repeated measures ANOVA $F(1, 18) = 23.399$ $p < .0005$) (fig 6.5 (D)).

6.4.5 Relationship between touch intensity and pleasantness

It was observed that patients in whom, following cordotomy, had lower ratings for touch intensity tended concomitantly to have higher ratings for touch pleasantness. Indeed, on the pain affected side, absolute and percentage change in overall touch intensity (defined as sum average ratings at 0.3. 3 and 30 cm s^{-1}) showed a significant negative correlation with change in overall touch liking (defined as sum of average ratings at 0.3. 3 and 30 cm s^{-1}) and the change in intercept values obtained on regression analysis of individual pleasantness ratings (fig. 6.5). Linear regression was used to determine the proportion of variation in change of pleasantness ratings (intercept value) explained by the change in intensity.

The change in overall touch intensity significantly predicted the change in perceived touch pleasantness, $F(1,17) = 9.602$, $p = 0.007$ (fig. 6.5) with change in touch intensity accounting for 38.2% of the variation in overall touch pleasantness (intercept value) with an adjusted $R^2 = 0.346$. There was no significant correlation between a change in touch intensity and change in indices of preference to CT targeted touch (CTPI or regression quadratic term) (fig. 6.5).

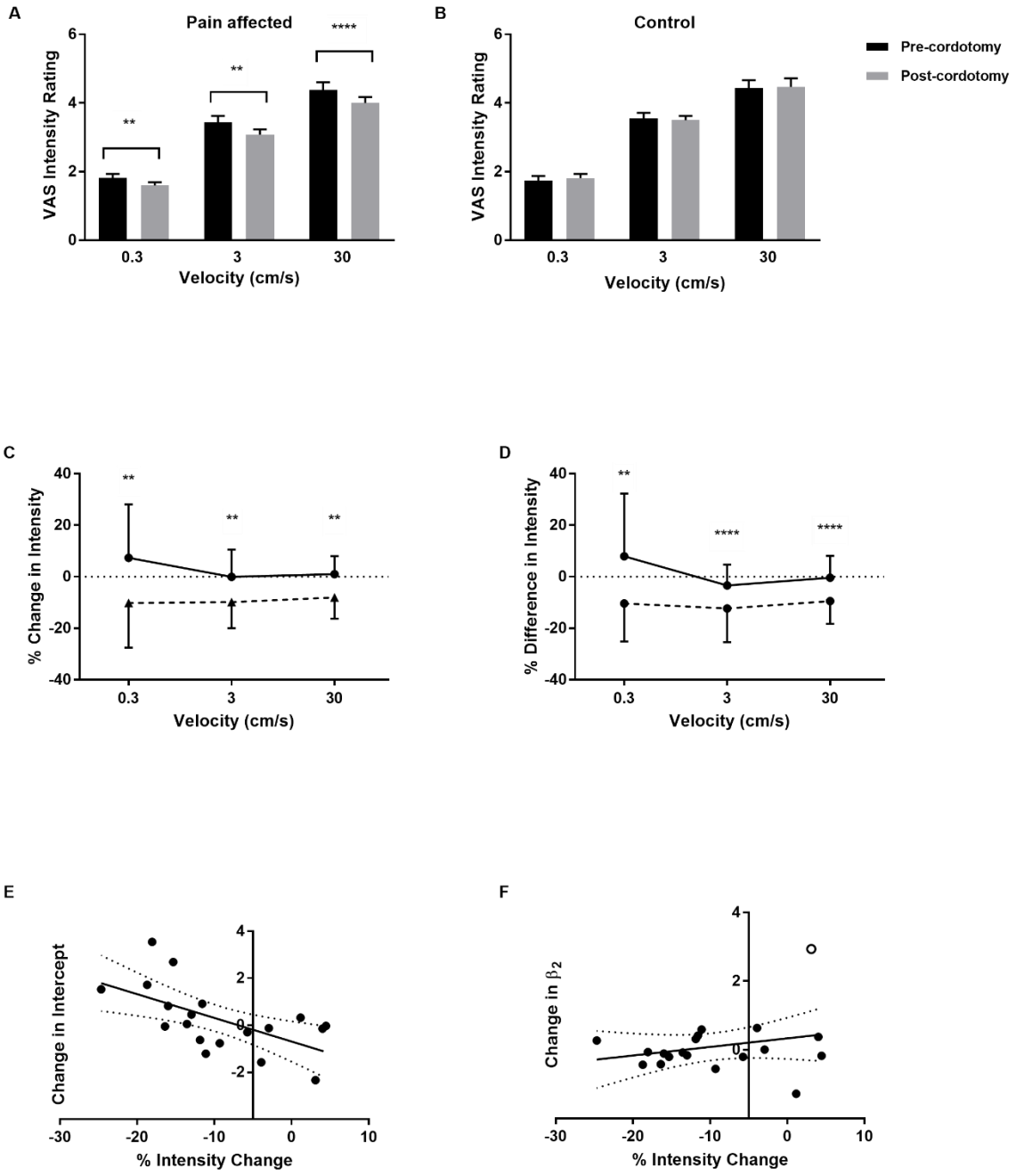

Fig. 6.5.

Ratings for the intensity of touch on hairy skin are reduced by anterolateral cordotomy. Pre-cordotomy and post-cordotomy group data (mean + SEM) for the pain-affected (A) and control (B) sides are shown. The intensity of touch is significantly reduced by cordotomy across all velocities on the pain-affected side. No significant changes are seen on the control side. The percentage change in touch intensity from the pre-cordotomy to post-cordotomy states on the pain affected (dashed line, mean - SEM) and control (solid line, mean + SEM) states is shown in (C). The percentage difference in touch intensity between the pain-affected and control sides in the pre-cordotomy (solid line, mean + SEM) and post-cordotomy (dashed line, mean - SEM) are shown in (D). A scatter plot showing the relationship between the percentage change in overall touch intensity (all velocities) and the change in intercept (a marker of overall touch pleasantness rather than CT

specific touch) between the pre-cordotomy and post-cordotomy states on the pain affected side is shown in (E). Patients showing the greatest reduction in touch intensity showed a shift towards higher overall touch pleasantness ratings. The line of best fit and 95% confidence intervals are shown. The change in overall touch intensity significantly predicted the change in perceived touch pleasantness, $F(1,17) = 9.602$, $p = .007$. A similar plot is showing the relationship with change in touch intensity and the β_2 value is shown in (F). In contrast to overall touch pleasantness there was no evidence of a significant relationship between change in touch intensity and change in CT specific touch. Significant differences are marked with asterisks and show **$P_s < .01$, *** $P_s < .001$, **** $P_s < .0005$. Abbreviation; SEM, standard error of mean.

6.4.6 Touch Perception Task

Inspection of radar plots depicting average scores in for each sensory and emotional descriptor in the four conditions (figs. 6.5 and 6.6) suggest that whilst ratings for a pleasant material, fur, are not substantially altered by anterolateral tract lesioning there are clear differences following cordotomy for stimulation with sandpaper. Specifically, there is a shift from high ratings for negative descriptors such as discomfort and irritating to higher ratings for more positive terms as well as evidence of lower ratings for descriptor terms relating to stimulus texture/roughness.

This was confirmed using overall factor scores (Table 6.6 and fig 6.7). Of note, there was a highly significant reduction in negative and increase in positive emotional terms as well as reduction in texture/roughness and increase in smoothness/pile sensory terms following cordotomy for stimulation with sandpaper. No significant effect on sensory or emotional factors were seen following cordotomy for stimulation with fur. These data were ostensibly the same using factor weightings derived from previous work (Guest *et al.*, 2011; Ackerley *et al.*, 2014b) (data not shown).

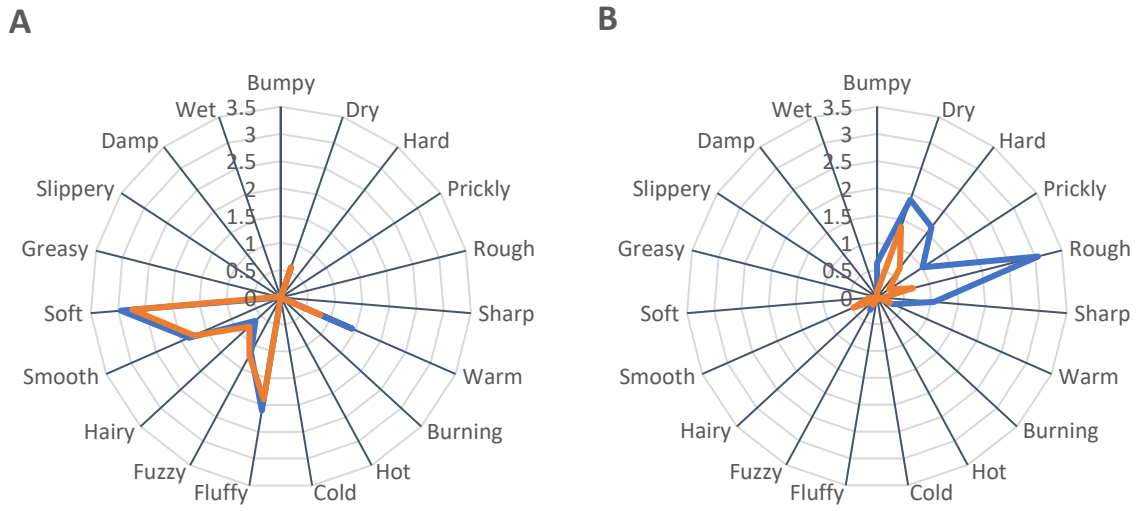

Fig. 6.6.

Radar plot of ratings for sensory descriptor terms in the pre-cordotomy and post-cordotomy states on the pain affected side. (A) The mean of individual rating for sensory descriptors for stroking with fur in both the pre-cordotomy (blue line) and post-cordotomy (orange line) state on the pain affected side are shown. Note that the lines are almost superimposed. (B) In contrast descriptor ratings for sandpaper are clearly altered by spinothalamic tract lesioning with markedly lower mean ratings for dry, hard, prickly, rough and sharp post-cordotomy (orange line). Radar plots for descriptor ratings to stimulation with fur and sandpaper on the control side (not shown) were superimposable for respective pre-cordotomy and post-cordotomy states as well as for the equivalent material in the pre-cordotomy state on the pain affected side.

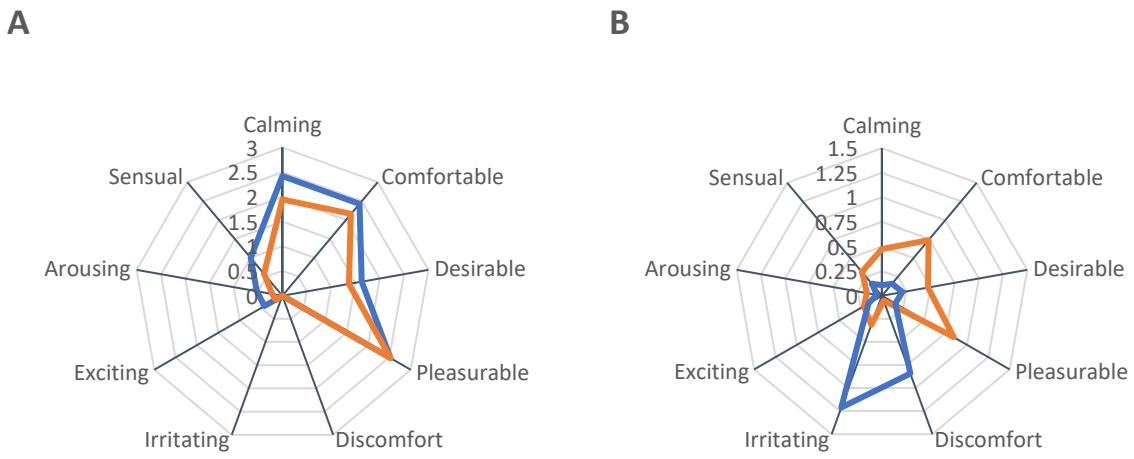

Fig. 6.7.

Radar plot of ratings for emotional descriptor terms in the pre-cordotomy and post-cordotomy states on the pain affected side. (A) The mean of individual rating for emotional descriptors for stroking with fur in both the pre-cordotomy (blue line) and post-cordotomy (orange line) state on the pain affected side are shown. The lines broadly follow the same patterns with, if anything, the post-cordotomy state rated higher for positive factors (B) In contrast descriptor ratings for sandpaper are clearly altered by spinothalamic tract lesioning. There is a clear divergence in the shape of the lines with pre-cordotomy ratings for negative descriptors being higher than positive descriptors and *visa versa* for the post-cordotomy state. Radar plots for descriptor ratings to stimulation with fur and sandpaper on the control side (not shown) showed the same pattern as the pre-cordotomy ratings on the pain affected side. Note that the scale for mean descriptor ratings are different.

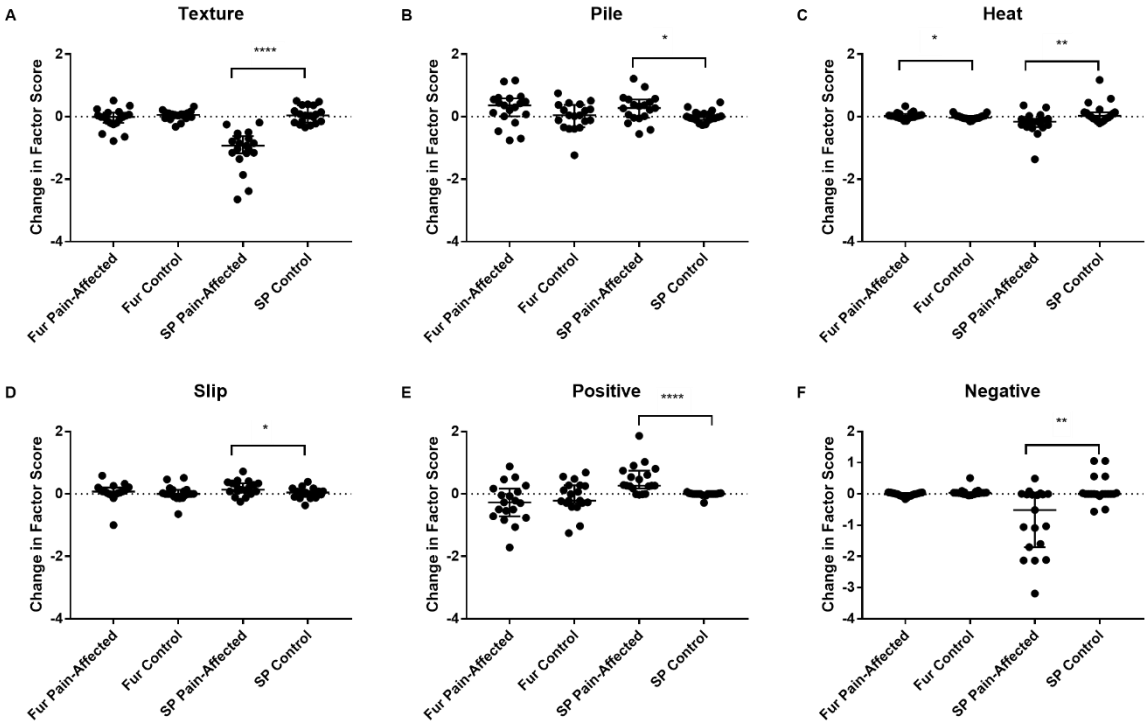

Fig. 6.8.

Descriptor ratings for sensory and emotional terms in the TPT are markedly affected by anterolateral cordotomy for stimulation with sandpaper but not fur. The absolute change in the factor score between the pre-cordotomy and post-cordotomy states for stimulation with fur and sandpaper on the pain-affected and control sides are shown in the dot plots for sensory (A-D) and emotional (E-F) factors. Factor scores for stroking with sandpaper are significantly affected by cordotomy with evidence of a marked reduction in ratings for the texture group (A) and a more modest reduction in ratings for heat terms (C). There are significant increases in ratings for descriptor terms in the pile (B) and slip (D) group. Only heat (C) is significantly altered for stimulation with fur. Emotional factors are markedly affected by cordotomy with highly significant increases and decreases in factor scores for positive (E) and negative (F) terms respectively. These are unaffected for stroking with fur. No significant change in the emotional factor 'arousal' was seen (data not shown). Bars depicting median and interquartile ranges are shown. Significant differences (Related-Samples Wilcoxon Signed Rank Test) between the pain-affected and control sides are marked with asterisks and show *p<0.05, **p<0.01, ***p<0.001, ****p < 0.0005. Abbreviation: SP, sandpaper.

Material / Factor	Pain Affected		Control	
	Pre-cordotomy	Post-cordotomy	Pre-cordotomy	Post-cordotomy
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Sensory				
Fur				
Texture	-0.34 (-0.61 – -0.25)	-0.45 (-0.53 – -0.26)	-0.53 (-0.65 – -0.19)	-0.36 (-0.58 – -0.23)
Pile	1.29 (0.92 – 2.59)	1.83 (1.39 – 2.10)	1.42 (0.93 – 2.42)	1.34 (1.04 – 2.38)
Heat	0.19 (0.14 – 0.27)	0.22 (0.17 – 0.26)	0.24 (0.15 – 0.28)	0.22 (0.16 – 0.27)
Slip	-0.12 (-0.27 – 0.09)	-0.06 (-0.23 – 0.18)	-0.09 (-0.29 – -0.04)	-0.14 (-0.23 – 0.01)
Sandpaper				
Texture	1.50 (0.95 – 2.16)	0.51 (0.29 – 0.71) ****	1.64 (0.84 – 1.97)	1.51 (1.14 – 1.98)
Pile	-0.21 (-0.33 – 0.19)	0.06 (-0.06 – 0.29) *	-0.20 (-0.28 – -0.02)	-0.18 (-0.37 – 0.01)
Heat	0.24 (0.04 – 0.29)	0.01 (-0.04 – 0.06) *	0.23 (0.09 – 0.38)	0.28 (0.13 – 0.41)
Slip	-0.29 (-0.41 – -0.19)	-0.17 (-0.26 – -0.07) **	-0.35 (-0.47 – -0.26)	-0.38 (-0.47 – -0.22)
Emotional				
Fur				
Positive	2.33 (1.80 – 3.15)	2.11 (1.08 – 2.67)	2.32 (1.77 – 2.88)	2.05 (1.36 – 2.89)
Arousal	0.26 (-0.11 – 1.05)	-0.05 (-0.12 – 0.4)	0.42 (-0.06 – 0.75)	0.30 (-0.12 – 0.74)
Negative	0.03 (0.00 – 0.06)	0.02 (0.00 – 0.06)	0.00 (-0.03 – 0.00)	0.02 (0.01 – 0.05)
Sandpaper				
Positive	0.00 (-0.01 – 0.02)	0.27 (0.27 – 0.81) ****	0.00 (-0.02 – 0.01)	0.00 (-0.01 – 0.01)
Arousal	0.01 (0.00 – 0.01)	-0.01 (-0.03 – 0.02)	0.01 (0.00 – 0.01)	0.01 (0.00 – 0.15)
Negative	0.50 (0.00 – 2.11)	0.01 (-0.02 – 1.55) **	0.50 (0.00 – 1.55)	1.06 (0.00 – 2.11)

Table 6.6. Summary of factor scores for sensory and emotional factors in the Touch Perception Task for pain affected and control sides in the pre-cordotomy and post-cordotomy states.

Significant differences (Related-Samples Wilcoxon Signed Rank Test) between the pre-cordotomy and post-cordotomy states are marked with asterisks and show *p<0.05, **p<0.01, ***p<0.001, ****p < 0.0005. Abbreviation: IQR, Interquartile Range.

6.5 DISCUSSION

The recognition that touch has an emotional domain has led to the suggestion that tactile sensation is served by two main processing streams; a 'fast', Aβ mediated, discriminative pathway projecting via the dorsal columns and ventral posterolateral nucleus of the thalamus to the primary somatosensory cortex; and a 'slow', CT mediated, affective touch pathway that has the insula cortex as its primary cortical target (Morrison, Löken and Olausson, 2010; McGlone, Wessberg and Olausson, 2014). Despite a wealth of evidence suggesting that the 'slow' touch stream ascends to interoceptive regions of thalamus and cortex (Olausson *et al.*, 2002; Olausson *et al.*, 2008b; Morrison, Bjornsdotter and Olausson, 2011; Morrison *et al.*, 2011; McGlone *et al.*, 2012; McGlone, Wessberg and Olausson, 2014); and that it does so via the spinothalamic tract (Sugiura, 1996; Lu and Perl, 2005; Maxwell *et al.*, 2007; Andrew, 2010), no deficits in standard affective touch metrics were observed following anterolateral cordotomy. The straightforward implication of these findings is that perceptual judgments about touch pleasantness and, in particular touch pleasantness predicated on the distinctive velocity tuned firing patterns of CT afferents, do not depend on the integrity of the spinothalamic tract.

Clearly this conclusion relies on the assumption that lesioning did not consistently spare a particular region of the spinothalamic tract. The percutaneous cordotomy results in largely confluent lesions in the anterolateral funiculus. Spinal cord destruction is not uniform with significant heterogeneity in lesion extent as well as location (Lahuerta *et al.*, 1994; Vedantam *et al.*, 2017) and lesion extension to the contralateral side may occur. Lesions are usually centred anatomically in the ipsilateral anterolateral cord, the typical location of the spinothalamic tract, and less extensively in ventromedial regions. In cats fibres ascending ventromedially in the anterolateral funiculus form a separate pathway, the ventral spinothalamic tract (Jones *et al.*, 1987). Ventral spinothalamic tract projection neurons respond to innocuous tactile stimulation with large, occasionally bilateral, receptive fields (Meyers and Snow, 1982). However, the same cells also respond to proprioceptive, visceral as well as noxious cutaneous stimulation and have neuron cell bodies located in deep dorsal horn laminae (IV-V and VII-VIII) (Meyers and Snow, 1982) whereas C-LTMR afferents synapse in superficial layers (Light and Perl, 1979; Sugiura, 1996; Li *et al.*, 2011; Abraira and Ginty, 2013). No pathological material or neuroimaging data are available for the current study. However, all patients developed substantial canonical spinothalamic sensory deficits and no patient exhibited demonstrable ipsilateral sensory impairment. Thus, it is improbable over the series of patients studied that a given area of the anterolateral funiculus, including ventromedial regions, remained consistently unlesioned; or that there was regular appreciable lesion extension to the contralateral side. It is reasonable, therefore, to conclude that in the post-cordotomy state patients are largely relying on mechanosensory information ascending in the dorsal column pathway. In this respect there are two not necessarily mutually exclusive, broad mechanistic possibilities

(1) CT-afferent inputs also have a signalling pathway, either direct or indirect, in the dorsal columns.

(2) The perceptual consequences of lesioning the 'slow' touch pathway are so subtle they are not detected by standard psychophysical evaluation.

6.5.1 Unmyelinated low threshold mechanosensory inputs to the dorsal columns

A substantial proportion, up to 25%, of long, directly projecting fibres within the dorsal columns are unmyelinated (Briner *et al.*, 1988; Patterson *et al.*, 1989; Patterson *et al.*, 1990; Garrett *et al.*, 1992; Patterson, Chung and Coggeshall, 1992), many of which these immunostain for Calcitonin Gene Related Peptide (Briner *et al.*, 1988; Patterson *et al.*, 1990). Whilst a proportion may be nociceptive,

and potentially contribute to the pain caused by intense stimulation of the dorsal columns (Nashold and Friedman, 1972), it is uncertain whether they represent the diversity of C-fibres, including CT-afferents. C-LTMR afferents not only connect to Lamina I projection neurons but also input a complex interconnected dorsal horn network that receives terminations also from Aβ and Aδ LTMR afferents (Li *et al.*, 2011; Abraira and Ginty, 2013; Abraira *et al.*, 2017). Descending modulatory pathways also connect to this network (Abraira *et al.*, 2017). The large majority of neurons in this network are, however, intrinsic interneurons (Abraira *et al.*, 2017), the various inhibitory and excitatory sub-classes of which receive and process information from multiple but distinct LTMR inputs. Therefore, there is a vast potential for processing LTMR inputs into perceptually relevant ascending information which, in rodents, ascends the post-synaptic dorsal column pathway (Abraira *et al.*, 2017). This projection, of which the neuronal firing pattern and receptive field characteristics in cats and primates are consistent with relay of processed primary afferent inputs (Whitsel *et al.*, 1972; Nathan, Smith and Cook, 1986; Noble and Riddell, 1988), reflect a pathway by which CT afferents could impart emotionally relevant firing patterns on ascending information. In this respect CT afferents might not necessarily have an exclusive, unitary role in tactile coding, but instead shape ascending dorsal horn projection neuron outputs. Given their slow conduction velocity and subsequent latency difference relative to myelinated LTMR inputs, such a conditioning process would impact on the response to touch applied closely following CT activation rather than the contemporaneous tactile stimulus.

Isolated dorsal column lesions in humans are extremely rare and assessments of the emotional aspects of touch following disruption of this pathway have not yet been performed.

6.5.2 Pleasant touch is dissociated from canonical spinothalamic modalities in individuals with a neurodevelopmentally intact tactile system

The absence of change in affective touch is in marked contrast to the profound effects of anterolateral cordotomy on the perception of temperature, itch and pain. Noxious cutaneous stimuli that activate pruriceptor and nociceptor afferents demand attention so interruption of their principle second order pathway will be very noticeable. However, touch pleasantness is a less assertive and more imprecise perception than pain or itch. When evaluating descriptor terms or rating pleasantness, judgements will depend on other factors including context and, importantly, non-CT LTMR inputs. Following disruption of the putative spinothalamic 'slow' touch pathway patients can still use the velocity, force and texture of the tactile stimulus, properties largely subserved by myelinated afferents (Weber *et al.*, 2013; Pei and Bensmaia, 2014; Lieber *et al.*, 2017), to judge if a CT targeted stimulus feels pleasant. In the current study, patients who rated stroking as less intense post-cordotomy were most likely to maintain pleasantness ratings across all stimulus velocities. This suggests that Aβ LTMR inputs were indeed considered when judging pleasantness. Gentle brushing stimulation of glabrous skin, which lacks CT afferent innervation (Olausson *et al.*, 2010; McGlone, Wessberg and Olausson, 2014), is perceived as pleasant and also shows inverted U-shaped velocity tuning (Löken, Evert and Wessberg, 2011). However, in support of dual discriminative and emotional touch systems and unlike the current findings, descriptor ratings for sensory factors are higher and ratings for positive affect lower for palm compared to hairy skin stimulation (McGlone *et al.*, 2012). Indeed, it has been argued, based on differences in brain activation between glabrous and hairy skin stimulation, that affective touch on the palm is largely attributable to secondary reinforcement (McGlone *et al.*, 2012; McGlone, Wessberg and Olausson, 2014).

It is currently unknown what perception is generated with pure CT afferent stimulation. The only evidence available is from two neuronopathy patients who lack Aβ fibres (Olausson *et al.*, 2002; Olausson *et al.*, 2008a; Olausson *et al.*, 2008b) in whom gentle stroking of hairy skin, despite eliciting

a robust sympathetic skin response, evokes no more than a barely perceptible, poorly localised tactile sensation. This implies both that that the full expression of pleasant touch depends on concomitant Aβ LTMR inputs and that the loss of such a weak sensation, particularly in the presence of a substantial Aβ LTMR volley, might also be, at most, barely perceptible. Certainly, the deficit associated with disruption of an ascending affective touch stream might not be striking enough to alter a psychophysical tuning curve for which individuals have 'learned' to associate an Aβ input evoked by a 3 cm s^{-1} stroking stimulus with the vigorous firing of CT-afferents.

The dissociation of C-fibre deficits seem post-cordotomy contrasts with the encompassing impairments associated with the profound loss of peripheral unmyelinated afferents in HSAN-V. Analogous to the post-spinothalamic tract lesioned state patients with HSAN-V (Morrison *et al.*, 2011) and HSAN-III (Macefield *et al.*, 2014) have impairments in thermoperception. They also have congenital insensitivity to pain. However, in contrast to the post-cordotomy state, patients with HSAN-V and HSAN-III do not show an inverted U-shaped velocity tuning curve for ratings of pleasant touch. Instead velocity tuning appears either flat or linearly increases with touch velocity, suggesting reliance on Aβ fibre inputs. The incongruous findings for affective touch are likely to reflect differences in neurodevelopment. The congenital deficit in small afferent fibres seen in cases of HSAN is very likely to have downstream consequences for affective touch processing in the dorsal horn, sub-cortical regions as well as distributed cortical networks. In HSAN Aβ LTMR activity occurs without the full emotionally salient input from CT afferents. Therefore, unlike neurologically intact individuals, the association or conditioning process that occurs between Aβ LTMR inputs and the velocity tuning of CT-afferents will be underdeveloped. Intriguingly, the divergent findings in cordotomy patients and individuals with HSAN suggest that touch pleasantness and the expression of the characteristic velocity tuned preference for a slow caress might have its roots during development.

6.5.3 Touch and the spinothalamic tract

Previous lesion studies suggest that the anterolateral funiculus transmits information pertinent to touch. Touch sensation remains partially intact after lesions that interrupt dorsal column pathways but spare the anterolateral funiculus (Vierck, 1977; Wall and Noordenbos, 1977; Nathan, Smith and Cook, 1986; Danielsson and Norrsell, 1989). Ostensibly complete deficits occur with additional lesioning of the anterolateral funiculus.

Although there were no effects of cordotomy on metrics of CT afferent function the perception of a gentle brushing stimulus was not left completely unaltered. The *intensity* of the soft brush stimulus to all velocities was reduced after spinothalamic tract lesioning. Therefore, fibres ascending the anterolateral funiculus whether they be output neurons for Aβ, Aδ or C-afferents, contribute to intensity perception. It is unlikely that this change directly relates to a deficit in CT afferent processing since ratings to stroking were reduced to the same extent (~10% pre-cordotomy values) across all velocities. Stroking touch is perceived as more intense with increasing stimulus velocity (Case *et al.*, 2016b; Case *et al.*, 2016a; Case *et al.*, 2017). Since LTMR Aβ firing rates positively correlate with stroking velocity (Loken *et al.*, 2009; McGlone, Wessberg and Olausson, 2014), touch intensity has been used as a proxy of large myelinated function in hairy skin. There is evidence that touch intensity and pleasantness are dissociated at the level of cortical processing (Case *et al.*, 2016b; Case *et al.*, 2017) and a causal role for secondary somatosensory cortex (S2) in tactile intensity perception has been suggested (Case *et al.*, 2017). S2 is also implicated in the coding of pain intensity (Timmermann *et al.*, 2001; Lockwood, Iannetti and Haggard, 2013) .

S2 receives significant spinothalamic input from both lateral spinothalamic projections via ventral posterolateral thalamus (Stevens, London and Apkarian, 1993) and from ventrally located

spinothalamic pathways originating from laminae IV-V and VII-VIII wide dynamic range projection neurons (Meyers and Snow, 1982; Jones *et al.*, 1987; Stevens, Apkarian and Hodge, 1991). Therefore, there exist plausible pathways by which cordotomy can reduce tactile intensity.

The alterations in sensory descriptor ratings in the TPT following cordotomy were unexpected. Significant changes were seen for stimulation with sandpaper (average particle diameter 125 μm) but not fur (Although not formally measured the average diameter of the fake fur would be expected to be significantly less than 100 μm and likely in the region of 50 μm). In glabrous skin tactile perception of texture is thought to be driven by two distinct mechanisms (Hollins and Risner, 2000).) The perception of coarse textures (elements more than 100 μm in size) is mediated largely by SA I afferents (Yoshioka *et al.*, 2001; Manfredi *et al.*, 2014; Weber *et al.*, 2013). Conversely the perception of fine textures is thought to be driven by vibrotactile channels (i.e. Pacinian Meissner corpuscles) (Manfredi *et al.*, 2014). The pattern of post-cordotomy impairment in texture perception would as such be in keeping with a loss of ascending information from SA1 fibres. However, SA1 inputs to the dorsal horn arborize in laminae III/IV and project via the ipsilateral dorsal column pathway (Li *et al.*, 2011; Abraira and Ginty, 2013; Abraira *et al.*, 2017) and would as such be unlikely to have been disrupted by lesioning of the anterolateral funiculus. Interestingly, although not force controlled, the Aβ-HTMR fibres described in PAPER I were strongly excited by a rough brush stimulus. Indeed, this was used during the search procedure for this afferent type. It is therefore possible that HTMR afferents, perhaps of all classes, that project via pathways in the anterolateral funiculus play a role in texture discrimination.

Collectively, these finding suggest that, at least in hairy skin, inputs ascending in the spinothalamic tract contribute to discriminative touch perception.

Whether the cordotomy also disrupts descending pathways that affect sensory processing in the dorsal horn is uncertain. However, it has been shown that sensory inflow through the spinal cord is modulated by corticospinal projections originating from primary and secondary somatosensory cortex. Indeed, interruption of the corticospinal tract, which is adjacent to the spinothalamic pathway, impairs behavioural responses to light touch in rodents (Liu *et al.*, 2018).

6.5.4 Conclusion

Despite a wealth of evidence suggesting that the spinothalamic tract represents the major second order projection pathway for CT afferents no alterations in benchmark metrics affective touch were observed following anterolateral cordotomy. The findings indicate in individuals with a neurodevelopmentally normal somatosensory system that fibres ascending the dorsal columns provide sufficient information to conserve judgements about touch pleasantness. At the level of the spinal tracts it is apparent that the dual pathways model for discriminative and affective touch represents an oversimplification.

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7 PAPER IV: DISSOCIATION OF SPINOTHALAMIC MODALITIES FOLLOWING ANTEROLATERAL CORDOTOMY

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7.1 CASE REPORT

Whether the brain interprets somatosensory inputs that are encoded by particular afferent activity patterns or that transmit via modality specific, potentially anatomically segregated, labelled lines has been long debated (Ma, 2010). According to the labelled line theory of somatosensation specialised receptors in the periphery capture specific attributes of a stimulus which is then conveyed to cortical regions, such as the somatosensory cortex. According to this theory, ascending spinal pathways also carry this sensory information in specific labelled lines.

The spinothalamic tract (STT), which ascends the anterolateral funiculus of the spinal cord, is long-established as the second-order pathway conveying information about innocuous cutaneous temperature and noxious mechanical and thermal stimulation from the dorsal horn of the spinal cord. Indeed, STT lesioning with anterolateral cordotomy is used as a palliative procedure to ameliorate contralateral, intractable cancer-related pain (Tasker, 1990).

We present a case of a 62-year-old man who, following an anterolateral cordotomy, developed dissociated spinothalamic sensory loss implying segregation of ascending thermal and mechanical nociceptive pathways. The case is discussed in relation to both its clinical relevance and the labelled line theory of somatosensation.

Nine years following the initial mesothelioma diagnosis our patient developed chest wall bony destruction and pain affecting the left hemithorax and axilla. The pain was initially successfully controlled with chest wall radiotherapy and opioid analgesia. However, with disease progression, it became refractory to opioids. He was referred to our unit for consideration of palliative percutaneous anterolateral cordotomy. The pain was described as a persistent dull ache with severe paroxysms of sharp, shooting pain typically precipitated by movement. On examination there was a small area of dynamic tactile allodynia around the left nipple (Fig. 7A). Otherwise the neurological examination was normal. His analgesic regime at presentation consisted of morphine slow release 500 mg twice daily, morphine immediate release liquid 100 mg up to five times day, amitryptiline 20 mg at night, paracetamol 4 g/day, and ibuprofen 1.2 g/day.

A right-sided percutaneous cervical cordotomy between first and second cervical vertebrae was performed. The cordotomy electrode was inserted through a 20-gauge spinal needle into anterolateral spinal cord. Sensory stimulation through the cordotomy electrode at 50 Hz, rather than inducing the typical intense and unpleasant heat or cold sensation (Lahuerta *et al.*, 1994), reproducibly evoked an unusual poorly described, although not unpleasant, cutaneous sensation over the left chest wall and upper limb, centred on the area of allodynia. No evoked motor phenomena were observed at 2 Hz stimulation indicating that the corticospinal tract was remote to the electrode tip. Three incremental heat lesions, each lasting 25 seconds, were performed at 75°C, 80°C, and 85°C. The patient described immediate and complete pain relief in his left chest wall. No demonstrable clinical deficits to temperature sensation were elicited in the operating room. It was decided not to progress to any further lesioning. On post-cordotomy review, the patient remained pain-free with no objective evidence of altered thermal perception. Pinprick sensation was lost in the left C4-S1 dermatomes (Fig. 7B). Thermal detection and pain thresholds, performed with a Medoc II TSA (Medoc Ltd., Ramat Yishai, Israel) using the method of limits, demonstrated normal cold and warm detection thresholds in the left forearm (C6), chest wall (T2), and foot dorsum (L5) on both sides. Heat pain thresholds also showed no significant side-to-side asymmetry. Cold pain

thresholds were at floor level bilaterally (Table 7). The patient had complete pain relief sustained at 4 weeks follow-up and indeed had discontinued all opioid and non-opioid analgesia.

Although the anterolateral cordotomy procedure typically produces contralateral deficits in pain and temperature sensation in the same segmental distribution (Tasker, 1990), dissociations in STT modalities have been described previously in a small number of patients (Bowsher, 2005; Friehs, Schröttner and Pendl, 1995). Bowsher (2005) investigated STT sensory modalities using quantitative methods in a series of patients following anterolateral cordotomy. In some patients, pain relief and loss of mechanical pain to pinprick stimuli was associated with preserved cold and/or warm sensation. A single patient exhibited preservation of heat pain as well as innocuous heat and cold sensation. Evidence of segregation within ascending thermal and mechanical nociceptive pathways is also provided by microstimulation studies of thalamic nuclei during deep brain stimulation surgery in humans (Lenz *et al.*, 1993b), as well as in patients with lesions of the brainstem and thalamus (Bowsher, 2005). Our patient also demonstrated a dissociation in nociceptive mechanical and heat sensation. Although a deal of interaction and processing of diverse modality-specific primary afferent inputs probably occurs in the spinal cord (Ma, 2010), this case further demonstrates that there remains anatomical-functional segregation in broadly labelled lines within the STT.

It has been argued that more ventrally placed STT lesions will preferentially disrupt ascending nociceptive fibres, whilst sparing thermal pathways (Friehs, Schröttner and Pendl, 1995). Indeed more ventral and medial lesions are associated with greater pain relief and loss of mechanical pain sensation (Lahuerta *et al.*, 1994). In the current case neither neuroimaging nor pathological information is available to support a ventromedial lesion. However, a more ventrally placed lesion may be suggested by the unusual non-painful, non-temperature-related cutaneous sensation elicited during 50 Hz high-frequency stimulation, the nature of which suggests activation of ascending non-nociceptive pathways. This potentially reflects activation of the ventral STT. Although poorly described in humans fibres destined for the ventral STT in cats arise in lamina VII and VIII of the dorsal horn and have been shown to respond to both innocuous as well as noxious range mechanical stimuli to deep and superficial tissues from the contralateral body side (Meyers, Kelly and Snow, 1984). It is also possible that the evoked sensations could reflect stimulation of ascending second-order pathways of primary mechanosensitive low threshold A-δ or C-tactile afferent fibres (McGlone, Wessberg and Olausson, 2014).

The findings have potential clinical implications. An appropriately placed lesion may produce a beneficial outcome without sacrifice of temperature, including thermo-noxious pathways. Furthermore, the absence of clear deficits in cold sensation in the operating room does not necessarily imply that additional lesions, with the associated potential risk of motor tract damage, are required. Careful questioning about ongoing pain and assessment of noxious mechanical stimulation are of importance in this respect.

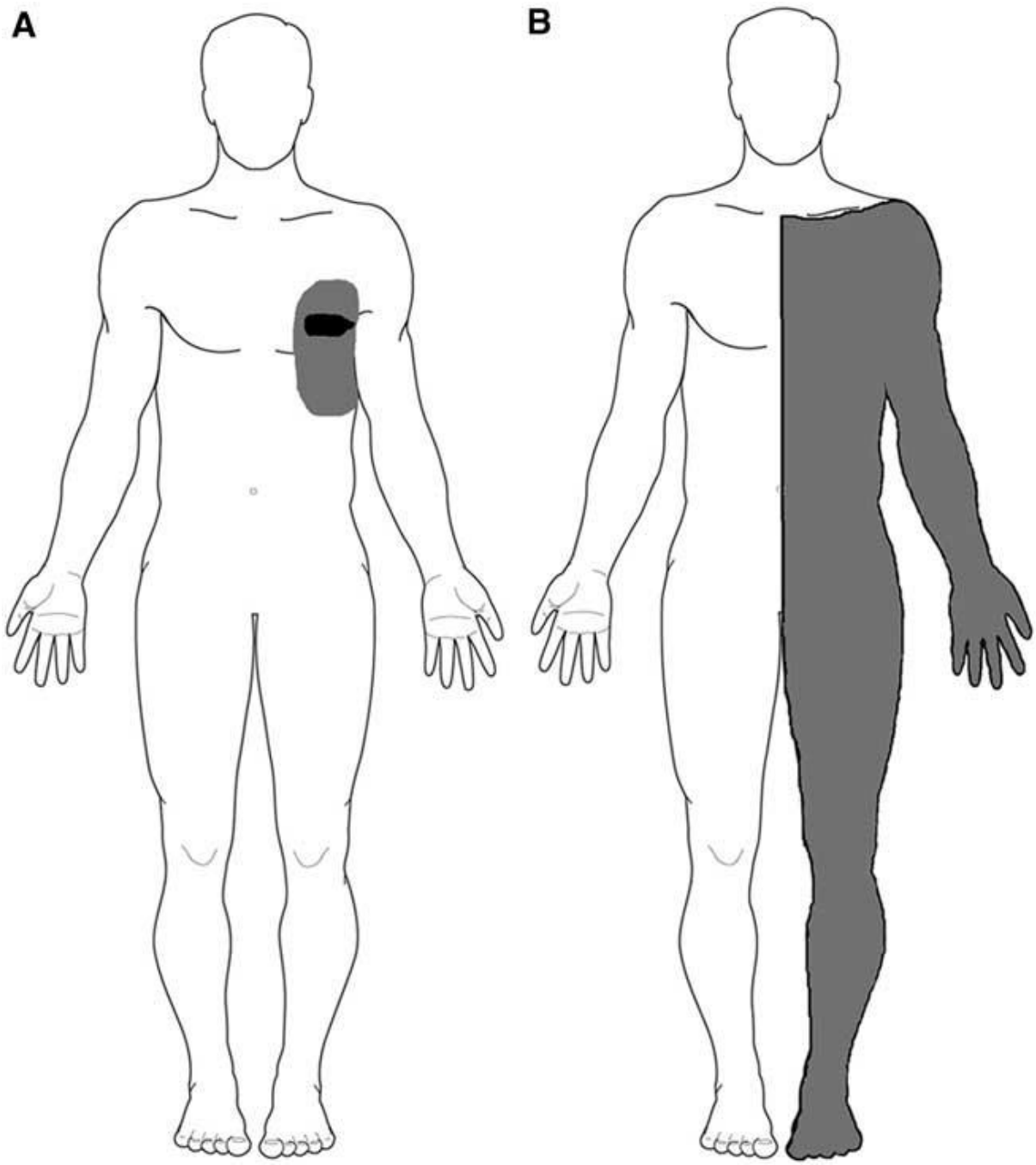

Fig. 7.

(A) Pre-lesion map of the region affected by the cancer-related pain (grey shading) and tactile allodynia (black shading); (B) post-lesion deficit to pinprick (grey shading).

Table 7. Pre- and post-lesion results for thermal detection and thermal pain thresholds

Pre-lesion

Segment tested	Left				Right			
	CDT (°C)	**WDT (°C)**	**CPT (°C)**	**HPT (°C)**	**CDT (°C)**	**WDT (°C)**	**CPT (°C)**	**HPT (°C)**
C6	30.5	36.1	0.5	44.8	30.2	35.2	1.0	46.7

Post-lesion

	Left				Right			
C6	30.1	36.4	0.2	45.6	29.2	36.1	0.7	47.3
L5	28.3	37.8	0	47.1	28.9	37.1	0	46.7
T2	29.8	37.4	1.5	44.9	29.0	37.8	2.1	46.1

CDT=cold detection threshold; WDT=warm detection threshold; CPT=cold pain threshold; HPT=heat pain threshold. The baseline thermode temperature was 32°C. Pre-lesion studies were only performed in the arm (C6).

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8 PAPER V: DISCRIMINATIVE AND AFFECTIVE TOUCH IN HUMAN EXPERIMENTAL TACTILE ALLODYNIA

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8.1 ABSTRACT

Recently, several studies have suggested a role for unmyelinated (C-tactile, CT) low-threshold mechanoreceptive afferents in the allodynic condition. In this psychophysical study we explored the integrity of both Aβ and CT afferent processing following application of the heat capsaicin model of tactile allodynia on the left forearm in healthy subjects (n = 40). We measured tactile direction discrimination (TDD) to target the integrity of Aβ processing (n = 20). The TDD accuracy was significantly lower in the allodynic compared to a control zone. In addition, we measured the perceived pleasantness and pain of brush stroking at CT targetted (slow) and CT sub-optimal (fast) stroking velocities to investigate the integrity of CT processing (n = 20). When comparing touch pleasantness in the allodynic and control zone, there was a significantly larger difference in ratings for CT targetted compared to CT suboptimal stimulation. The results suggest a disturbance in both Aβ-mediated discriminative and CT-mediated affective touch processing in human experimental tactile allodynia. Our findings support the canonical view that tactile allodynia is signalled by Aβ afferents but that CTs seem to contribute by the loss of a pain inhibiting role.

8.2 INTRODUCTION

The canonical view is that tactile allodynia is signalled by fast-conducting, myelinated low-threshold mechanoreceptors (Aβ-LTMRs) gaining access to pain signalling pathways following central sensitization (Woolf, 2011). The critical role of Aβ-LTMRs in signalling tactile allodynia is based on numerous human selective nerve block studies indicating that tactile allodynia is abolished by compression or ischemic block of these afferents (Woolf, 2011), but cf.(Woolf, 2011; Nagi *et al.*, 2011).

However, recent studies show that slowly-conducting, unmyelinated, C-LTMRs may also have a role in the pathophysiology (Nagi *et al.*, 2011; Seal *et al.*, 2009; Liljencrantz *et al.*, 2013; Andrew, 2010). C-LTMRs are considered the animal homologue of human C-tactile (CT) afferents and, under normal conditions, CTs are thought to signal pleasant aspects of light, stroking touch (Loken *et al.*, 2009; Olausson *et al.*, 2002; Morrison, Löken and Olausson, 2010). However, in the heat capsaicin experimental model of tactile allodynia, the sensory input from CT afferents is altered (Liljencrantz *et al.*, 2013). This was demonstrated in two rare patients lacking Aβ afferents who, following application of the model, did not perceive allodynia but a reduced C-touch sensation (Liljencrantz *et al.*, 2013; Treede and Cole, 1993).

The heat capsaicin model of tactile allodynia induces a primary hyperalgesic area, surrounded by a secondary hyperalgesic area (Petersen and Rowbotham, 1999). Generally, within the secondary hyperalgesic area a transient, smaller area of dynamic tactile allodynia develops. Sometimes this area is surrounded by, and incorporated within, a larger area of tactile hypoesthesia (Magerl and Treede, 2004), which is considered to reflect altered central processing of Aβ fibre input.

Tactile direction discrimination (TDD) is a psychophysical measure highly sensitive in detecting Aβ deficits in the clinical setting (Olausson, Wessberg and Kakuda, 2000; Löken *et al.*, 2010). Here, we investigated TDD as an indicator of Aβ processing in the secondary hyperalgesia and control zones.

CTs are highly sensitive mechanoreceptors and show fatigue (i.e. decreased responsiveness to repeated stimulation of the receptive field) (Nordin, 1990). CTs show strong responses to slow (1–10 cm s^{-1}) stroking, but poor response to fast (30 cm s^{-1}) stroking and CT firing correlates with ratings of pleasantness (Loken *et al.*, 2009). On the contrary, Aβ afferents fire with a higher discharge rate to faster brush stroking (Loken *et al.*, 2009). This physiological difference provides a means for preferentially stimulating each afferent type (Loken *et al.*, 2009; Gordon *et al.*, 2013; Bennett *et al.*, 2014; Morrison *et al.*, 2011). Here, our subjects rated touch pleasantness and pain for CT targetted and CT sub-optimal brush stroking as an indicator of CT processing in the secondary hyperalgesia and control zones.

We hypothesized decreased TDD accuracy, as well as reduced touch pleasantness, implying that both types of LTMR processing are affected in tactile allodynia.

8.3 MATERIAL AND METHODS

8.3.1 Participants

The ethical review boards at the University of Gothenburg approved the procedures. The experiments were performed in accordance with the Declaration of Helsinki. Informed, written consent was obtained from 40 healthy subjects (median age 25 years, range 19–43 years, 20 men).

8.3.2 Heat/capsaicin sensitization

A Peltier thermode (3 × 3 cm, Medoc, TSA 2001, Thermosensory Analyzer, Rimat Yishai, Israel) was used to deliver a 5 min 45 °C stimulus to the subject's left forearm dorsum. Then capsaicin cream (Capsina, 0.075%, Hants, UK) was applied to that same, preheated, skin area for 30 min. The subjects reported ongoing pain from the treated skin as numeric ratings (no pain–worst pain imaginable, 0–10). The median pain rating at the end of the heat stimulus was 1.1 (range 0–5.5) and after removal of the capsaicin 1.5 (range 0–5.1) (n = 40). All participants developed a flare.

Following model application, half of the subjects participated in TDD and half in stroking pleasantness and pain testing. All testing was on the long axis of the forearm radial or ulnar to the primary hyperalgesia zone (depending on where the allodynic percept to light cotton swab stroking was most prominent). For comparison, the same stimuli were applied in a control zone, 12 cm proximal or distal to the capsaicin zone. We used a pseudo-randomized block design; subjects were allocated in a balanced design for the site of model application (i.e. proximal or distal forearm), zone where testing commenced (i.e. allodynic or control zone), and all stimulus sequences (although limited to a maximum of 4 consecutive identical stimulations). The areas of punctate hyperalgesia, tactile hypoesthesia and tactile allodynia were quantified after the main test protocols. Subjects were prevented from seeing the tested extremity throughout the experiment. As all testing was completed within 30 min of the model application, rekindling was not required.

8.3.3 Questionnaire

After the testing all subjects completed the Short Form-McGill Pain Questionnaire (SF-MPQ) (Melzack, 1987).

8.3.4 TDD

All stimuli were delivered by the same experimenter using a hand-held stimulator (half cylinder contact surface covered in fine woven fabric, diameter 4 mm × length 15 mm), vertical load 16 g, stimulation velocity 1 cm s^{-1}(Olausson, Wessberg and Kakuda, 2000). Participants were instructed to verbally report the direction (distal or proximal) after each movement. The test started with motion over an 18 mm distance: three consecutive correct responses shortened the distance whereas one incorrect response increased it. The best (i.e. lowest) score obtainable was 18 points (Olausson, Wessberg and Kakuda, 2000; Löken *et al.*, 2010). The paradigm consisted of 32 trials in each zone.

8.3.5 Stroking pleasantness and touch evoked pain

Tactile stimuli were delivered manually (soft goat's hair brush: 0.5 cm wide, 3 cm long) to the allodynic and control zones, respectively (stroking distance 5 cm, approximate application force 0.3 N (Loken *et al.*, 2009; Liljencrantz *et al.*, 2013). Two different stimulation velocities were used for preferential activation; 3 cm s^{-1} for CT and 30 cm s^{-1} for Aβ afferents (Loken *et al.*, 2009; Morrison *et al.*, 2011; Gordon *et al.*, 2013; Bennett *et al.*, 2014),. To control for differences in stimulus duration 10 consecutive strokes were applied at 30 cm s−1 (10 × 30 cm s^{-1}). A single stroke stimulus of 30 cm

s^{-1} was also included. All stimuli were delivered manually by the same experimenter who was trained to apply the strokes with constant force and velocity. Ten stimuli of each type were delivered in each subject, pseudorandomized order. The participants were instructed to rate their subjective perception of each stroking on a computerized visual analogue scale (VAS) with the endpoints unpleasant and pleasant (0–10) (Essick *et al.*, 2010). Similarly, a pain rating for each stroking stimulus was recorded using a VAS with the endpoints no pain and worst pain imaginable (0–10).

8.3.6 Mapping of the secondary zone

In all subjects, the areas of punctate hyperalgesia, hypoesthesia and tactile allodynia surrounding the primary zone were mapped. Skin stimulation was initiated outside the affected skin area and moved towards the primary zone along eight orientations (45°, 90° angles). Punctate hyperalgesia was mapped with a monofilament (calibrated indentation force 0.25 N) and the area measured 1064 mm2 ± 118 (mean ± SEM, n = 40). Tactile hypoesthesia and allodynia were mapped by 0.5 cm strokes with a cotton swab (approximate application force 0.3 N) (Liljencrantz *et al.*, 2013; Loken *et al.*, 2009). Subjects were asked to report hypoesthesia i.e. sudden intensity drop or numbness of the tactile stimulus, area 652 mm2 ± 119 (mean ± SEM, n = 40) and tactile allodynia i.e. unpleasant or painful sensation to light touch, area 391 mm2 ± 72 (mean ± SEM, n = 40).

8.3.7 Statistics

Statistical comparisons were made using SPSS (PASW Statistics for Windows, Version 18.0. Chicago, IL). Significances were sought below $P < 0.05$. Kolmogorov–Smirnov tests of normality were run for all data and parametric or non-parametric statistics were used accordingly.

8.4 RESULTS

8.4.1 Questionnaire

The most common SF-MPQ descriptors selected for stroking in the allodynic zone were “hot-burning” (n = 30), “tender” (n = 22), and “stabbing” (n = 10) (Fig. 8.1). None of the descriptors were applicable in the control zone.

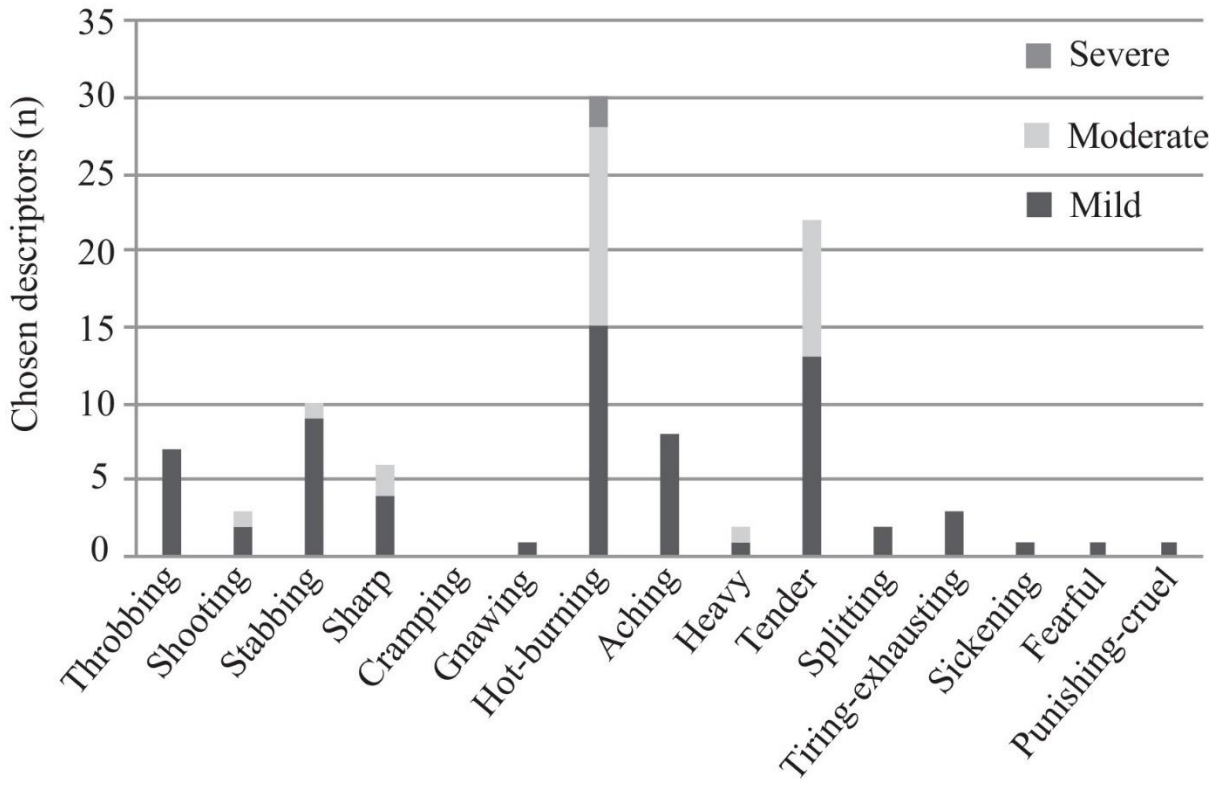

Fig. 8.1.

SF-MPQ of pain descriptors related to stroking stimuli in the allodynic zone, the most commonly chosen descriptors were “hot-burning”, “tender”, and “stabbing”.

8.4.2 TDD

Subjects were significantly less accurate in the allodynic compared to the control zone (Wilcoxon: P = 0.001, allodynic median 21, control median 18, n = 20; Fig. 8.2). There was no significant difference in ongoing pain ratings throughout the testing of the two zones (Wilcoxon: P = 1.000). There was one extreme outlier (Fig. 8.2), but the difference remained significant after exclusion of this data point (P = 0.002). There were no significant correlations between TDD scores and mapped areas of punctate hyperalgesia, hypoesthesia or tactile allodynia (Spearman's rho).

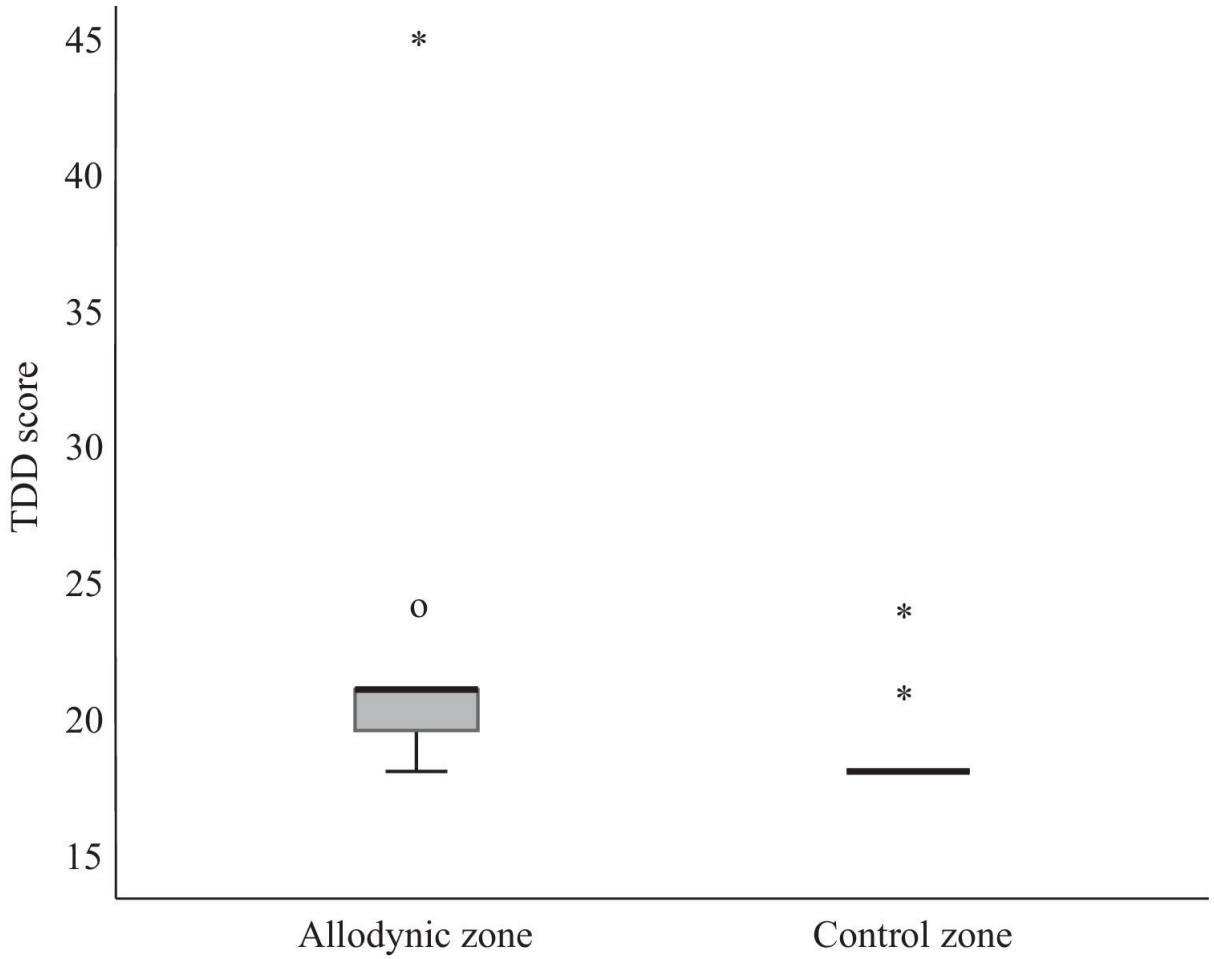

Fig. 8.2.

TDD accuracy was significantly less accurate in the allodynic compared to the control zone. The lowest TDD score obtainable is 18. T-bars extend to 1.5 times the inter-quartile range (IQR). Outliers are represented as circles and extreme outliers as asterisks (defined as values greater than 1.5 times and 3 times the IQR, respectively).

8.4.3 Stroking pleasantness and touch evoked pain

A significant decrease in pleasantness was found when comparing stroking in the allodynic compared to the control zone for 3 cm s^{-1} and for single 30 cm s^{-1} stroking but not for 10 × 30 cm s^{-1} stroking (Fig. 8.3; Table 8.1). The decrease in pleasantness ratings between the allodynic and control zone for stroking at 3 cm s^{-1} was significantly larger than for 10 × 30 cm s^{-1} but not for single 30 cm s^{-1} stroking (Repeated measures ANOVA: $F(2,18) = 6.0$, $P = 0.01$. Post-hoc Bonferroni corrected pairwise comparisons, $P = 0.006$ and $P = 0.18$ respectively; Fig. 8.3). There was no significant difference between the single 30 cm s^{-1} stroking and the 10 × 30 cm s^{-1} condition ($P = 0.22$; Fig. 8.3A).

Tactile stimulation with a soft brush stroke was rated as minimally painful for all touch conditions in the allodynic zone (Fig. 8.3B; Table 8.1). There was no significant difference in touch evoked pain between stimulus types ($P = 0.68$, related samples Friedman's two-way analysis of variance by ranks).

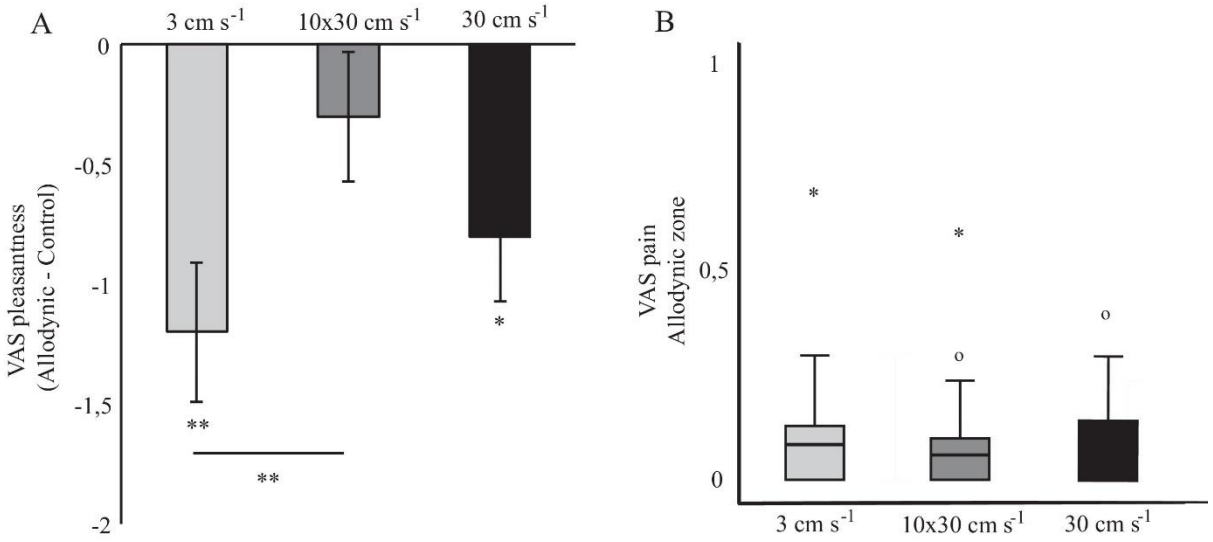

Fig. 8.3.

(A) Psychophysical testing of perceived pleasantness. There was a significant decrease in pleasantness ratings, when comparing brush stroking in the allodynic and the control zone for stroking at 3 cm s^{-1} and single stroking at 30 cm s^{-1} but not for stroking at 10 × 30 cm s^{-1}. The decrease in ratings were significantly greater for the CT targetted (3 cm s−1) than the CT suboptimal (10 × 30 cm s^{-1}) brush stroking. Error bars indicate SEM. (B) Pain ratings for touch in the allodynic zone. There were no significant differences in pain ratings across the three stimulus conditions. Conventions as in Fig. 2.

Table 8.1. Mean pleasantness values compared using Bonferroni corrected one sample t-tests against zero. Median pain values compared using one sample Wilcoxon signed rank test against zero.

Paradigm	**VAS pleasantness**		**Significances testing (allodynic vs control)**	**VAS pain**		**Significance testing (allodynic vs control)**
	Allodynic	**Control**		**Allodynic**	**Control**	
3 cm s^{-1}	6.1	7.4	t = 4.2, P = 0.003	0.09	0	P = 0.001
10 × 30 cm s^{-1}	5.6	5.9	t = 1.0, P = 0.95	0.06	0	P = 0.001
30 cm s^{-1}	5.9	6.6	t = 2.8, P = 0.03	0.04	0	P = 0.003

There was no significant difference in ongoing pain ratings throughout the testing of the two zones (Wilcoxon: P = 0.5). There was a significant correlation between the differences in pleasantness ratings for the two zones and the area of punctate hyperalgesia for 3 cm s^{-1} (Pearson: r = −0.63, P = 0.003) and for 10 × 30 cm s^{-1} (Pearson: r = −0.58, P = 0.007). A correlation was also seen for the difference in pleasantness ratings at 10 × 30 cm s^{-1} and the mapped area of hypoesthesia (Pearson: r = −0.53, P = 0.02). However, this significance was driven by one outlier (>3 SD); when this subject was removed the significance was lost. There were no significant correlations between the differences in pleasantness ratings for the two zones and the area of tactile allodynia (Pearson).

8.4 DISCUSSION

Our findings suggest that there was a disturbance in both Aβ and CT afferent processing; the deficits were suggested from decreased TDD accuracy and reduced stroking pleasantness.

8.4.1 Decreased TDD accuracy in the allodynic zone

TDD testing evaluates Aβ function with high sensitivity and specificity (Löken *et al.*, 2010). Our findings show a consistent and significant decrease in TDD accuracy in the allodynic zone. It seems unlikely that distraction by the capsaicin induced pain could explain the difference in TDD scores since the ongoing pain from the treated skin area was the same after testing in the allodynic and the control zones.

It has previously been shown that following a capsaicin injection, there is numbness and reduced tactile detection in an area surrounding the allodynic zone (Magerl and Treede, 2004). This is explained in terms of pain-induced inhibition of non-nociceptive somatosensory input, i.e. tactile peripheral input is re-routed resulting in cross-talk into nociceptive pathways, leading to the loss of tactile sensitivity (Magerl and Treede, 2004). Physiological alteration of somatosensory processing supporting this inhibition has been demonstrated at the level of the spinal cord (Dougherty, Willis and Lenz, 1998), the thalamus (Brüggemann, Shi and Apkarian, 1998), and the contralateral primary somatosensory cortex (Apkarian *et al.*, 2000). We did not find a significant correlation between the degree of perceived hypoesthesia and TDD (Magerl and Treede, 2004). However, another method for quantifying the area of hypoesthesia (e.g. tactile detection thresholds using monofilaments) may have been more sensitive (Kauppila *et al.*, 1998).

Two-point discrimination (TPD) and other measures of tactile acuity are typically reduced in chronic pain conditions with (and without) allodynia (Hollins, Sigurdsson and Morris, 2001; Moriwaki and Yuge, 1999; Maihöfner *et al.*, 2006; Moseley, 2008; Stanton *et al.*, 2013; Lewis and Schweinhardt, 2012). Chronic pain patients may have a re-organization of their somatosensory cortex and the extent of this re-organization seems related to their pain intensity as well as their reduced tactile acuity (Flor *et al.*, 1995; Flor *et al.*, 1997; Maihöfner *et al.*, 2004; Pleger *et al.*, 2005). Further, as the pain diminishes the tactile acuity increases (Pleger *et al.*, 2005; Maihöfner *et al.*, 2004; Nathan, 1960).

8.4.2 Pain and decreased touch pleasantness in the allodynic zone

Pleasant touch in humans is a construct of many factors; the input from CT afferents, Aβ afferents, homeostatic state as well as contextual factors (Craig, 2002). Recent studies have implicated CTs in the pathophysiology of dynamic tactile allodynia (Nagi *et al.*, 2011; Liljencrantz *et al.*, 2013; Seal *et al.*, 2009). In this study we present further evidence suggesting affected CT processing in experimental allodynia alongside with affected Aβ processing. In the current study, the greatest drop in pleasantness ratings in the area of experimental allodynia was seen for CT targetted brush stroking (3 cm s^{-1}). This indicates an altered processing of CT information (Liljencrantz *et al.*, 2013), but does not indicate that CT afferents drive allodynia (Nagi *et al.*, 2011; Seal *et al.*, 2009). In the allodynic zone, the pleasantness ratings for the CT targetted stroking dropped to that of the Aβ targetted stroking suggesting that the CT processing was suppressed (Liljencrantz *et al.*, 2013). A similar finding has been observed in CT-denervated patients (Morrison *et al.*, 2011). The significant pleasantness drop seen for the single stroking at 30 cm s^{-1} may be explained by there being a slight, yet suboptimal, CT response in the control zone (Loken *et al.*, 2009) that was suppressed in the allodynic zone. However, for the 10 × 30 cm s^{-1} intense stroking the CTs are likely to fire even less due to the repeated stimulation which may fatigue CTs, almost to the point of inexcitability (Nordin,

1990). For the decrease in touch pleasantness a significant correlation was found with the mapped area of punctate hyperalgesia; the area of hyperalgesia may be indicative of the extent of the development of the model.

One explanation for not finding any differences in pain ratings across the stimulus conditions could be that the rating scale used was too crude in its endpoint worst pain imaginable to detect the fine grain differences between the different stroking stimuli. This suggestion is supported by the fact that the pain ratings were indeed very low.

Another explanation (in line with the canonical view), is that tactile evoked pain is solely mediated by Aβ afferents gaining access to pain signaling pathways. This theory is supported by two previous studies showing that Aβ denervated subjects do not develop experimental, tactile evoked pain (Liljencrantz *et al.*, 2013; Treede and Cole, 1993). For CT afferents which are suggested to signal touch pleasantness through the spinothalamic tract under normal touch conditions there might be a gating resulting in a significant decrease in pleasantness perception (Andrew, 2010; Craig, 2002) to prioritize the nociceptive information from the periphery.

8.4.3 Previous work implicating CT afferents in experimental allodynia

Experimental evidence for a role for CTs in allodynia were suggested through a C-LTMR knock-out mouse model, targetted against the vesicular glutamate transporter type 3 (VGLUT3). The knock-out mice showed reduced mechanical hypersensitivity following inflammation and nerve injury (Seal *et al.*, 2009). However, more recent findings suggest that VGLUT3 lineage sensory neurons are divided into two groups depending on their VGLUT3 expression: transient expression neurons seem to be myelinated mechanoreceptors whereas persistent expressers are likely unmyelinated neurons (Lou *et al.*, 2013). Mice with a conditional knock-out of VGLUT3-persistent neurons have largely, but not completely, unaffected acute and chronic mechanical pain thus instead suggesting that VGLUT3-transient neurons may control the mechanical hypersensitivity (Lou *et al.*, 2013).

This finding is more in line with a previous finding from our group. Following the heat/capsaicin model, two sensory neuronopathy patients lacking Aβ afferents did not report tactile allodynia (Liljencrantz *et al.*, 2013) but instead reported their C-touch percept (faint sensation of pleasant touch) to be significantly weaker in the allodynic zone compared to untreated skin. Accordingly, functional magnetic resonance imaging (fMRI) showed that stroking in the allodynic and control zones evoked different responses in the primary cortical receiving area for CTs: the posterior insular cortex. These findings suggest that dynamic tactile allodynia is associated with a reduced CT hedonic touch processing. A similar reduced hedonic touch processing was recently observed in patients with a congenital denervation of CT afferents (Morrison *et al.*, 2011). Restoring normal CT processing may thus be a novel therapeutic strategy against neuropathic pain.

In a recent study this question was pursued using a novel specific marker of C-LTMRs: a chemokine-like secreted protein called TAFA4 which is predominantly co-expressed with VGLUT3 (Delfini *et al.*, 2013). The authors speculate that upon activation C-LTMRs might release TAFA4, and that this protein then acts to prevent mechanical hypersensitivity. Following inflammation and nerve injury TAFA4-null mice show enhanced mechanical and chemical hypersensitivity that was reversed by application of recombinant TAFA4 protein (Delfini *et al.*, 2013) i.e. restoring normal C-LTMR functional signalling.

A previous electrophysiological study in rats suggested that C-LTMR targetted input may inhibit C-nociceptive messages in the dorsal horn (Lu and Perl, 2003). A specific inhibitory pathway was identified between substantia gelatinosa neurons receiving direct peripheral C-LTMR afferent

projections and other substantia gelatinosa cells receiving direct nociceptive input (Lu and Perl, 2003). This unmyelinated circuit represents a potential pathway for innocuous C-LTMR impulses to suppress nociceptive impulses (Lu and Perl, 2003). A disruption of this circuit due to central sensitization may cause a loss of the nociceptive balancing effect of C-LTMRs again supporting the notion that normalizing CT function may be a treatment strategy for tactile allodynia (Seal *et al.*, 2009; Liljencrantz *et al.*, 2013; Andrew, 2010; Delfini *et al.*, 2013; Lou *et al.*, 2013).

In summary, both Aβ and CT processing were affected in the allodynic zone of the heat capsaicin experimental model of human dynamic tactile allodynia. We found no differences in touch evoked pain between CT optimal and suboptimal stimuli suggesting that CTs do not have a critical role in mediating tactile allodynia (Nagi *et al.*, 2011; Seal *et al.*, 2009), supporting the view that Aβ afferents signal this sensation. Instead the contribution of CTs in allodynia seems to be the loss of a pain inhibiting role.

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9 DISCUSSION

This thesis aims to further our understanding of noxious and innocuous range cutaneous mechanosensation in humans.

At the level of the primary afferent, **PAPER I** provides direct evidence for the existence of a hitherto unrecognised population of fast conducting, Aβ range, mechanically sensitive fibres in human skin that have both high mechanical thresholds and the potential to encode in the noxious range. Importantly, evidence is presented from intraneural microstimulation as well as psychophysical studies in patient groups selectively either lacking Aβ or small diameter afferents, showing that these fibres are likely to be relevant to human nociception and pain perception. However, in patients with selective small diameter afferent denervation due to HSAN-V, the *prima facie* presence of Aβ HTMRs does not compensate for their nociceptive deficits - they have 'congenital insensitivity to pain' and, as **PAPER II** demonstrates, alterations in the perceptual evaluation of pain that relate to peripheral C-fibre density.

With respect to the ascending spinal pathways of mechanosensation, **PAPER III** shows that disruption of the canonical 'second order' C-fibre projection, despite causing indisputable deficits in temperature, pain and itch sensation, does not affect benchmark metrics of the slow, CT-afferent, touch pathway. This dissociation of C-fibre deficits contrasts with the 'all-inclusive' impairments associated with the profound loss of peripheral unmyelinated afferents in HSAN-V. Despite the polymodal nature of most peripheral nociceptor afferents, **PAPER IV** indicates that 'second order' projections subserving mechanical pain are anatomically segregated from thermal and thermo-noxious pathways.

Finally, in the setting of touch-induced pain, **PAPER VI** demonstrates that allodynia is associated with alterations in both discriminative and affective touch. This implies that plasticity in the central processing of both CT-afferent and Aβ LTMR inputs can be caused by vigorous nociceptor input to the dorsal horn.

9.1 FAST NOCICEPTORS

Unexpected exposure to a potentially damaging stimulus, be it mechanical, chemical or thermal, as well as causing pain also results the coordinated withdrawal of the affected limb from the source of harm. The neural circuitry underlying this nocifensive behaviour, initially defined by Sir Charles Sherrington in a classic series of experiments (Sherrington, 1910), involves a polysynaptic spinal reflex arc that is driven by activity in cutaneous nociceptor afferents. The facility for **prompt** reflexive withdraw from the source of potential harm has obvious benefits in terms of protection and limiting tissue injury. In this respect to have nociceptor afferents with fast conduction velocities capable of driving nociceptive withdrawal reflexes would be advantageous. It is widely assumed that nociceptors are sensory neurons with thinly myelinated (Aδ) or unmyelinated (C) axons, however, the putative role of thickly myelinated Aβ nociceptors has been largely ignored. Myelinated HTMR afferents with conduction velocities in the Aβ range have been reported in a large number of species (Lawson, 2002a; Djouhri and Lawson, 2004; Treede, Meyer and Campbell, 1998).

A specific aim of this thesis was to determine whether Aβ field LTMRs in humans, as has recently been suggested for their mouse namesake (Bai *et al.*, 2015), have nociceptive properties. In contrast to the mouse equivalent, human Aβ field LTMRs have mechanical thresholds similar to other LTMRs and fail to encode skin indentations in the perceptibly noxious range. However, the findings in **PAPER I** demonstrate that humans are equipped with fast conducting mechanosensitive receptor afferents with properties in accord with the characteristics of a nociceptor - that is (1) high mechanical thresholds and (2) the capacity to encode the force of cutaneous mechanical stimuli in a physiologically relevant noxious range (Burgess and Perl, 1967; Perl, 1968).

The classification of afferents into Aβ and Aδ groups on the basis of conduction velocity is not entirely clear-cut, and necessarily arbitrary. Aδ and Aβ fibres populations have been shown, anatomically and physiologically, to make up two normally distributed populations the tails of which overlap (see Boyd and Davey, The composition of peripheral nerve, 1968). It might therefore be argued that the HTMR afferents with fast conduction velocities described in **PAPER I** represent the tail-end of the Aδ population that impinges into the Aβ range. Aδ fibres are encountered infrequently in human microneurography experiments (Adriaensen *et al.*, 1983), which may relate to the fact that they are small diameter as well as being insulated with myelin. Single unit recordings of HTMR afferents, classified as Aδ fibres, in the human radial nerve were reported to have a range of velocities of 2-33 m s^{-1} (Adriaensen *et al.*, 1983). Since upper limb conduction velocities of myelinated afferents are typically greater than those in the lower limb (Kimura, 2013), direct comparison with the current sample, predominantly recorded from the peroneal nerve, is more difficult. All but two of the HTMR afferents recorded in **PAPER I** had conduction velocities greater than the slowest Aβ LTMR. Furthermore, within this sample the conduction velocity of HTMR afferents was statistically indistinguishable from Aβ LTMRs. Therefore, it seems reasonable to classify these afferents as Aβ nociceptors.

Further investigations are needed to clarify the precise nature of these afferents including a systematic assessment of their response to different forms of stimulation (e.g. thermal and chemical sensitivity as well as other forms of mechanical stimulation (e.g. hair pull and pinch)), axonal properties (e.g. electrical stimulation paradigms assessing activity-dependent slowing and response to burst stimulation) as well as documentation of frequency dependent evoked-sensations during intraneural microstimulation. In-vitro human and animal studies will be required to characterise

their molecular properties as well as anatomical delineation of their cutaneous/subcutaneous endings.

Whilst direct evidence that Aβ HTMR afferent activation in humans (or other species) drives the nociceptive withdrawal reflex is currently lacking, given their short transmission latency they would be well-suited for such a role. Indeed, preferential electrical stimulation of Aβ fibres, provided it is given as a high frequency train, is sufficient to cause pain and elicit a withdrawal reflex (Willer, Boureau and Albe-Fessard, 1978; Willer, Boureau and Albe-Fessard, 1980; Willer and Albe-Fessard, 1983).

As well as a putative role in nocifensive behaviour **PAPER I** provides evidence that Aβ HTMRs also play a role in pain perception. Although systematic investigation of frequency-dependence of evoked sensation was not assessed, INMS of single Aβ HTMRs produced a painful percept with a sharp/pinprick quality. This is consistent with roles in both the perception of 'first' pain as well as in stimulus localisation. Furthermore, in comparison to C HTMRs, Aβ HTMRs display a higher firing rate, a more finely grained receptive field with multiple small high-sensitivity spots and relative insusceptibility to fatigue during sustained noxious mechanical stimulation. These characteristics likely ensure a large, continuous transfer of information to the central nervous system with content rich on stimulus intensity and localization (Adriaensen *et al.*, 1983; Andrew and Greenspan, 1999; Garell, McGillis and Greenspan, 1996; Slugg, Meyer and Campbell, 2000; Arcourt *et al.*, 2017; Ghitani *et al.*, 2017).

The importance of the Aβ system for human pain perception is further suggested by the aberrant pain ratings to noxious punctate stimuli in Aβ deafferented subjects, who have preserved small-fibre functions. Clearly under normal circumstances noxious mechanical skin stimulation will co-activate multiple mechanosensitive afferent types, both low and high threshold. The resulting perceptual and behavioural consequences will depend on the relative strength and spatio-temporal pattern of inputs from these fibre subtypes (Arcourt *et al.*, 2017; Browne *et al.*, 2017). Recent evidence in rodents clearly suggests that Aβ co-activation modulates reflex nociceptive withdrawal and prevents overflow of the motor response (Arcourt *et al.*, 2017). Also, in humans, mechanically evoked pain increases under conditions of Aβ blockade (Andrew and Greenspan, 1999) suggesting that the net effect of Aβ stimulation is anti-nociceptive.

Given that a prime role of A-fibre nociceptors is almost certainly that of an early warning system to prevent injury it is of interest that individuals with HSAN-V, who have congenital insensitivity to pain, have a normal psychophysical profile to graded mechanical skin stimulation. HSAN-V is caused by NGF-beta mutation R221W (Einarsdottir *et al.*, 2004; Minde *et al.*, 2004; Minde *et al.*, 2009; Capsoni *et al.*, 2011) and follows an autosomal recessive pattern of inheritance. Homozygotes are severely affected presenting with debilitating and progressive degrees of painless fractures, joint deformation, Charcot arthropathies, bone necrosis, and osteochondritis, resulting in limited mobility (Minde *et al.*, 2004; Minde, 2006; Minde *et al.*, 2006; Minde *et al.*, 2009). Heterozygotes are less markedly affected and display considerable variability in clinical severity (Minde *et al.*, 2009). Detailed neuropathological investigation of sural nerve biopsies in a small number of R221W carriers, both homozygous and heterozygous, demonstrate a profound reduction in unmyelinated C-fibres and comparatively moderate loss of thinly myelinated Aδ-fibres (Crowley *et al.*, 1994; Larsson *et al.*, 2009; Minde *et al.*, 2004; Minde *et al.*, 2006). A moderate loss of Aβ fibres was seen in one heterozygote but they also suffered with Type 2 diabetes (Minde *et al.*, 2004). The heterozygote carriers studied in PAPER I all had marked deficits in cold sensation detection, suggesting significant Aδ fibre loss (Magerl *et al.*, 2010; Rolke *et al.*, 2006b). This implies that the conserved pain ratings to

noxious punctate stimuli are largely due to activity in mechanosensitive Aβ fibres with the capacity to encode in the noxious range.

The clinical phenotype of HSAN-V is less severe than in patients with congenital insensitivity to pain due to HSAN-IV (Miura *et al.*, 2000; Indo, 2002) and those with loss of function mutations affecting the voltage gated sodium channel NaV1.7 (Goldberg *et al.*, 2007). Carriers of the R221W mutation present predominantly with deep pain insensitivity (Minde *et al.*, 2004; Minde *et al.*, 2006; Minde *et al.*, 2009; Sagafos *et al.*, 2016). Although they develop multiple skeletal complications such as painless fractures and Charcot arthropathies they have relative preservation of superficial mechanical pain (Minde *et al.*, 2009; Sagafos *et al.*, 2016)[3]. For example, one homozygote R221W carrier noted that "**Cuts do not hurt, but pinpricks do**" (Minde *et al.*, 2004) and most, including homozygotes, have preserved pinprick sensation (Minde *et al.*, 2009). The divergence in deficits of deep and superficial pain sensation could relate to differences in tissue innervation. Bone cortex, marrow and periosteum are innervated by thinly myelinated and unmyelinated fibres but have little in way of Aβ supply (Mantyh, 2014). Furthermore, there are significant differences in the populations of C-fibres innervating skin and bone (Mantyh, 2014). Therefore, the deep pain insensitivity and propensity to skeletal complications seen in HSAN-V could be explained by a profound C- and Aδ denervation that, in contrast to cutaneous tissues, is not partially compensated by innervation with Aβ HTMRs.

[3] In contrast to patients with HSAN IV and loss of function mutations in NaV1.7, carriers of the R221W mutation do not present with self-mutilation (e.g. biting or chewing on the tongue) (https://neuromuscular.wustl.edu/time/hsn.htm). The situation with heat pain is less clear-cut. Whilst homozygous as well as heterozygous cases of HSAN-V do not develop painless burns (Minde et al., 2004) they do have abnormal heat pain thresholds (Minde et al., 2004 but see also Sagofas et al., 2016) and appear to have a delayed reaction to noxious heat (Minde et al., 2004).

9.2 C-FIBRE DENERVATION AND HIGHER LEVEL-PAIN PROCESSING

Whilst the existence of a population of unaffected Aβ HTMRs could account for preserved cutaneous noxious-range mechanosensation it clearly does not fully counteract for the deficits in thinly myelinated and unmyelinated afferent innervation. In **PAPER II** the relationship between the level of peripheral C-fibre innervation and clinical deficits as well as subjective measures of acute pain were investigated in carriers of the R221W mutation.

The density of C-fibres in the cornea, assessed using CCM, was used as a proxy of general body C-fibre innervation. This noninvasive method, which has been applied to quantify C-fibre loss in neuropathy secondary to diabetes and other aetiologies (Quattrini *et al.*, 2007; Tavakoli *et al.*, 2009; Tavakoli and Malik, 2011; Tavakoli *et al.*, 2012), allowed a large number of carriers to be studied. As expected, the cornea of R221W carriers showed reduced numbers of C-afferents. There was a clear gene dosage effect with homozygotes having almost a complete loss of C-fibres whereas heterozygote carriers showing a lesser, but variable, degree of corneal denervation. Correspondingly, greater degrees of corneal denervation were associated with greater neuropathy severity. Whilst carriers were able to distinguish between painful and non-painful situations, they significantly underestimated the intensity of imagined painful situations (i.e. they had a higher SPQ-B) compared to healthy controls. Importantly, this underestimation bias also correlated with corneal innervation: the lower the peripheral small fibre innervation - the greater the underestimation of pain intensity.

Forecasting whether a noxious event reflects a real threat for tissue damage is crucial for the selection and modification of behavioural responses (Morrison *et al.*, 2013). This process will depend upon the ability to both gauge and predict pain intensity. Judgements about pain, rather than being a simple expression of nociceptor activity, reflect a multifaceted higher-lever experience (Morton, Sandhu and Jones, 2016). This is particularly true when it comes to estimating how much pain is likely to be felt in a particular situation, a process that will rely on expectations of pain resulting from past and learned pain experiences (Morton, Sandhu and Jones, 2016). Whilst individuals with HSAN-V are able to forecast whether a particular situation could be painful, a pervasive tendency to underestimate the degree of pain could account for repeated low-level trauma and subsequent skeletal manifestations seen in R221W carriers (Minde *et al.*, 2006; Minde *et al.*, 2009). The relationship between the central-level bias in pain estimation and peripheral small fibre innervation in HSAN-V described in **PAPER II** also implies that judgements about expected pain intensity, presumably anchored to an individuals' perception of pain, are set by the density of peripheral innervation. Intriguingly, the correlation with unmyelinated innervation density, suggests that it might be primary C-nociceptor rather than high threshold A-fibre afferents (i.e. the primary afferent fibres primarily associated with the emotional rather than sensory-discriminative aspects of pain) that are of prime importance in calibrating the memory for pain intensity.

It is important to recognise that the effects of C-fibre denervation seen in HSAN-V extend beyond pain and that nociceptor afferents should not be considered in isolation (Morrison *et al.*, 2011). Carriers of the R221W mutation perceive gentle stroking touch as less pleasant than healthy control subjects and have alterations in the velocity tuning of psychophysical ratings for pleasantness; findings that are likely attributable to reduced peripheral CT afferent innervation (Morrison *et al.*, 2011). In both healthy controls and carriers' ratings of pleasantness for observed touch closely match those for felt touch. Therefore, just as pain intensity estimation is referenced to personal

experience, higher-level judgements about the pleasantness of touch are also dependent on peripheral innervation (Morrison *et al.*, 2011).

Given the complex interactions between touch and pain (see section 1.3) it is possible that the overall effects of congenital C-fibre denervation on somatosensation partly depend on the balance of CT afferent and C-nociceptor deficits. Such a process would depend on integration of CT afferent, C-nociceptor and indeed other relevant afferent inputs (e.g. Aβ mechanosensitive fibres), which could occur at a number of anatomical levels. In this respect, the congenital deficit in small afferent fibres seen in carriers of the R221W mutation is very likely to have downstream consequences for both nociceptive and hedonic touch processing and the interaction between these modalities at the level of the dorsal horn, sub-cortical regions as well as cerebral cortex. Indeed, evidence of such downstream cortical effects are suggested by preliminary magnetic resonance diffusion imaging data. Using Tract-Based Spatial Statistics in R221W carriers the fractional anisotropy of white matter adjacent to the mid-cingulate cortex was shown to correlate with peripheral C-fibre innervation density (Perini personal communication). The cingulate cortex is a region that both receives spinothalamic input via ventral caudal portion of the medial dorsal nucleus of the thalamus (Craig, 2003) and is consistently activated during pain perception (Vogt, Berger and Derbyshire, 2003; Vogt, 2005; Shackman *et al.*, 2011; Wager *et al.*, 2013). Interestingly, an afferent mid cingulate cortex–posterior insula pathway has recently been identified as being of importance in gating sensitivity to painful stimuli (Tan *et al.*, 2017). Therefore, the inadequate behavioural response to pain in HSAN-V could potentially be related to maldevelopment of the spinothalamic projection to the cingulate and/or less efficient cortico-cortical connectivity between cingulate and posterior insula. Where and how CT-afferents, and indeed other types of LTMR afferent, feed in to these spinal and supra-spinal systems is not currently clear. A critical first step will be to understand the comparative anatomy for nociceptor and CT pathways.

9.3 SLOW TOUCH PATHWAYS

The dual pathways model of touch (Morrison, Löken and Olausson, 2010; McGlone, Wessberg and Olausson, 2014) hypothesises the existence of two main processing streams for inputs resulting from innocuous tactile stimulation - a fast, Aβ mediated, discriminative pathway projecting via the dorsal columns and ventral posterolateral nucleus of the thalamus to the primary somatosensory cortex; and a 'slow', CT mediated, affective touch pathway that has the insula cortex as its primary cortical target. Despite a wealth of evidence suggesting that the 'slow' touch stream ascends to interoceptive regions of thalamus and cortex (Lu and Perl, 2005; Olausson *et al.*, 2002; Seal *et al.*, 2009; Andrew, 2010; Morrison, Bjornsdotter and Olausson, 2011; Lu *et al.*, 2013; McGlone, Wessberg and Olausson, 2014); and that it does so via the spinothalamic tract (Andrew, 2010; Lu *et al.*, 2013; Lu and Perl, 2005), no deficits in standard affective touch metrics were observed following anterolateral cordotomy (**PAPER III**). The straightforward implication of these the findings is that perceptual judgments about touch pleasantness and, in particular touch pleasantness predicated on the distinctive velocity tuned firing patterns of CT afferents, do not depend on the integrity of the spinothalamic tract. Clearly this conclusion relies on the assumption that lesioning did not consistently spare a particular region of the spinothalamic tract or, in distinction to the situation for temperature, pain and itch, that there is significant redundancy in affective touch projections such that judgements can be made on a much-reduced complement of fibres.

The percutaneous cordotomy procedure induces a number of thermally generated lesions in the anterolateral funiculus (Tasker, 1990). Spinal cord destruction is not uniform with significant heterogeneity in terms of lesion extent as well as location (Lahuerta *et al.*, 1994; Vedantam *et al.*, 2017). For example, in 21 patients for whom pathological information was available the cross-sectional area of cord destruction in the transverse plane ranged from 2.5 to 53% following unilateral cordotomy (Lahuerta *et al.*, 1994). Contralateral extension of the lesion, although much less extensive, was not uncommon although this was not usually associated with identifiable sensory deficits. No pathological material or neuroimaging data are available for **PAPERS III & IV**. However, all patients displayed substantial canonical spinothalamic sensory deficits as well as immediate term pain relief. No patient exhibited demonstrable ipsilateral sensory deficits. Therefore, it seems most unlikely that over the 20 patients studied that a given area of the anterolateral funiculus remained consistently unlesioned or that there was regular appreciable lesion extension to the contralateral side.

Therefore, it is reasonable to conclude that in the post-cordotomy state patients are largely relying on mechanosensory information ascending in non-spinothalamic projections, the most likely being the dorsal column pathway. In this respect there are three, not necessarily mutually exclusive, broad mechanistic possibilities:

1) CT-afferent inputs have a signaling pathway in the dorsal columns reliant on the first order unmyelinated projections (Briner *et al.*, 1988; Patterson *et al.*, 1989; Patterson *et al.*, 1990; Patterson, Chung and Coggeshall, 1992; Garrett *et al.*, 1992), the function of which is currently unclear, or post-synaptic pathways containing information processed within dorsal horn LTMR networks (Abraira *et al.*, 2017).

2) CT-afferent inputs, in the course of development or post-developmental tactile experience, have shaped neural responses to, and therefore perceptual interpretations of, myelinated LTMR inputs.

3) The perceptual consequences of lesioning the 'slow' touch pathway are so subtle they are not detected by standard psychophysical evaluation

Perceptual judgements about tactile sensation will be strongly influenced by functional connections that have been shaped by past experience (Gallace and Spence, 2009). In this respect it is useful to compare the deficits following cordotomy with those seen in patients with HSAN. Analogous to the post-spinothalamic tract lesioned state patients with HSAN-V (Minde *et al.*, 2004; Einarsdottir *et al.*, 2004; Minde *et al.*, 2009) and HSAN-III (Macefield *et al.*, 2014; Axelrod, 2004) have significant impairments in thermoperception. They also have congenital insensitivity to pain. However, in contrast to the post-cordotomy state, patients with HSAN-V and HSAN-III do not show an inverted U-shaped velocity tuning curve for ratings of pleasant touch (Morrison *et al.*, 2011; Macefield *et al.*, 2014). Instead velocity tuning appears either flat or linearly increases with touch velocity, suggesting reliance on Aβ fibre inputs.

In contrast to neurologically intact individuals, in whom the central nervous system networks that process tactile information will receive and associate inputs from Aβ and CT-afferents, in individuals with HSAN, these networks will have developed deficient in CT-afferent influence. It is of note that the 'wiring' between these afferent types and the potential for CT afferents to shape Aβ LTMR inputs could occur at any number of anatomical levels, from dorsal horn through to distributed somatosensory cortical networks. For example, in HSAN, CT afferents will not fully impart their velocity tuned firing signature within the dorsal horn networks and this will be reflected in interneuron outputs conveying processed information relevant to innocuous touch perception. However, save for the potential loss of descending pathways, this network will be intact following cordotomy.

When considering higher level perceptual inferences, the brain will take in to account all relevant information. Whilst CT afferents are tuned to the velocity of a tactile stimulus this does not mean they are velocity detectors. Indeed, for reasons described earlier they are poorly suited for such a discriminative role (McGlone, Wessberg and Olausson, 2014). Instead, the speed of a tactile stimulus is likely to be represented by spatiotemporal patterns of activity in neural populations (Pei and Bensmaia, 2014). This discriminative function is likely to be subserved by Aβ LTMR afferent neural pathways (Pei and Bensmaia, 2014). Both neurologically intact and C-fibre denervated individuals will be able to discriminate the velocity of a tactile stimulus. However, only in neurologically intact individuals will there have been an association or conditioning process between velocity discriminating Aβ LTMR inputs and the velocity tuning of CT-afferents. It is currently unknown what perception is generated with pure CT afferent stimulation. The only evidence available is from two neuronopathy patients who lack Aβ fibres (Olausson *et al.*, 2002; Cole *et al.*, 2006; Olausson *et al.*, 2008a). Here, gentle stroking of hairy skin evokes no more than a barely perceptible, poorly localised tactile sensation. The loss of such a weak sensation, particularly in the presence of a substantial Aβ LTMR volley, might also be, at most, barely perceptible. Therefore, even if ascending 'slow' touch pathways were disrupted by cordotomy, patients could use the velocity, force and texture of the tactile stimulus, properties largely subserved by myelinated afferents, to judge if a CT targetted stimulus feels pleasant. Certainly, the deficit might not be striking enough to disrupt a psychophysical tuning curve for which individuals have 'learned' to associate an Aβ input evoked by a 3 cm s^{-1} stroking stimulus with the vigorous firing of CT-afferents. Such an associative process is deficient in individuals with HSAN because their Aβ LTMR activity occurs without the full emotionally salient input from CT afferents.

The findings in this thesis do not provide a definitive answer to the question "what is the second order pathway for CT-afferents?" For the reasons described above the dominant pathway might still be the spinothalamic tract. Future investigations in patients undergoing cordotomy incorporating psychophysiological measures such as heart rate variability, galvanic skin response, facial electromyography or evaluative conditioning paradigms (Pawling *et al.*, 2017b; Pawling *et al.*, 2017a; Triscoli *et al.*, 2017) in addition to ratings of pleasantness, might provide further clues if the perceptual manifestations of a loss of ascending CT drive are subtle. It is of interest in this respect that Aβ deafferented individuals show a robust sympathetic skin response to a gentle stroking tactile stimulus (Olausson *et al.*, 2008a) even though the associated perception of touch is barely present. Structural MRI of the spinal cord post-cordotomy might enable the lesion location and extent to be correlated with psychophysical evaluations. Functional imaging such as fMRI might also detect alterations in patterns of cortical activation following cordotomy, just as differences in activation are seen when stroking hairy and glabrous skin (McGlone *et al.*, 2012). Further studies with a longer follow-up period[4] could assess whether, due to a fading of 'tactile memory' (Gallace and Spence, 2009), see also (Löken, Evert and Wessberg, 2011) for potential 'affective touch memory' relating to the order of stimulation of CT and non-CT innervated skin) deficits develop over time.

Although there were no effects of cordotomy on metrics of CT afferent function the perception of a gentle brushing stimulus was not left completely unaltered. The intensity of the soft brush stimulus to all velocities was reduced after spinothalamic tract lesioning. Spinal cord sectioning in cats and non-human primates, as well as human clinical case studies, have documented that touch sensation remains partially intact, albeit with reduced spatial acuity, after lesions that interrupt the dorsal columns but spare the anterolateral funiculus (Vierck, 1977; Wall and Noordenbos, 1977; Nathan, Smith and Cook, 1986; Danielsson and Norrsell, 1989). More profound, or ostensibly complete, deficits in tactile sensation occur if the anterolateral tracts are also interrupted (Danielsson and Norrsell, 1989). Ill-defined changes in the perception of dynamic touch following cordotomy described as numbness, deadness or reduced sensation have been reported by some patients with otherwise seemingly normal discriminative tactile sensation (Nathan, 1990). These clinical observations parallel the reduction in touch intensity to brushing quantified in this study. Stroking touch is perceived as being more intense with increasing stimulus velocity (Case *et al.*, 2016b; Case *et al.*, 2016a; Case *et al.*, 2017). Aβ LTMR firing rates positively correlate with the velocity of the brushing stimulus (Loken *et al.*, 2009; McGlone, Wessberg and Olausson, 2014) and touch intensity has as such been used as a proxy of large myelinated function. There is evidence that touch intensity and pleasantness can be dissociated both at the level of cortical processing and perception. fMRI BOLD responses in S1 and S2 cortex correlate with perceived touch intensity but show no relationship with touch pleasantness (Case *et al.*, 2016b). Furthermore, inhibitory rTMS over S2 reduces both the S2 BOLD response and touch intensity without affecting pleasantness ratings (Case *et al.*, 2017), suggesting a causal role for S2 in tactile intensity perception. However, S2 is also implicated in the perception of intensity for noxious stimuli (Timmermann *et al.*, 2001; Lockwood, Iannetti and Haggard, 2013). Anatomical tracer studies have demonstrated a significant spinothalamic input to the region via VPI thalamus (Stevens, London and Apkarian, 1993). S2 also receives significant spinothalamic input from ventrally located spinothalamic pathways originating from laminae IV-V and VII-VIII wide dynamic range projection neurons (Meyers and Snow, 1982; Jones *et al.*, 1987; Stevens, Apkarian and Hodge, 1991). Therefore, plausible anatomical pathways exist by which anterolateral cordotomy could reduce touch intensity.

[4] However, in addition to the ethical and logistic implications longer term studies in this patient population, it can also be argued both that the lesion extent will be maximal the closer in time to the procedure (e.g. due to spinal cord oedema) and that a greater time interval would give a greater chance for adaptive plasticity (see Danielsson and Norresell, 1989).

The alterations in sensory descriptor ratings in the TPT following cordotomy were unexpected. Significant changes were seen for stimulation with sandpaper (average particle diameter 125 µm) but not fur (Although not formally measured the average diameter of the fake fur would be expected to be significantly less than 100 µm and likely in the region of 50 µm). In glabrous skin tactile perception of texture is thought to be driven by two distinct mechanisms (Hollins and Risner, 2000).) The perception of coarse textures (elements more than 100 µm in size) is mediated largely by SA I afferents (Yoshioka *et al.*, 2001; Manfredi *et al.*, 2014; Weber *et al.*, 2013). Conversely the perception of fine textures is thought to be driven by vibrotactile channels (i.e. Pacinian Meissner corpuscles) (Manfredi *et al.*, 2014). The pattern of post-cordotomy impairment in texture perception would as such be in keeping with a loss of ascending information from SA1 fibres. However, SA1 inputs to the dorsal horn arborize in laminae III/IV and project via the ipsilateral dorsal column pathway (Li *et al.*, 2011; Abraira and Ginty, 2013; Abraira *et al.*, 2017) and would as such be unlikely to have been disrupted by lesioning of the anterolateral funiculus. Interestingly, although not force controlled, the Aβ-HTMR fibres described in PAPER I were strongly excited by a rough brush stimulus. Indeed, this was used during the search procedure for this afferent type. It is therefore possible that HTMR afferents, perhaps of all classes, that project via pathways in the anterolateral funiculus play a role in texture discrimination.

Collectively, these finding suggest that, at least in hairy skin, inputs ascending in the spinothalamic tract contribute to discriminative touch perception.

Whether the cordotomy also disrupts descending pathways that affect sensory processing in the dorsal horn is uncertain. However, it has been shown that sensory inflow through the spinal cord is modulated by corticospinal projections originating from primary and secondary somatosensory cortex. Indeed, interruption of the corticospinal tract, which is adjacent to the spinothalamic pathway, impairs behavioural responses to light touch in rodents (Liu *et al.*, 2018).

A single patient did display a dissociation between noxious mechanical and noxious and innocuous temperature sensation following cordotomy (**PAPER IV**), thus supporting the premise that there do exist broad anatomically segregated labelled lines within the spinothalamic tract (Craig and Dostrovsky, 2001; Andrew and Craig, 2002a; Craig, 2003; Andrew and Craig, 2001; Ma, 2010). Spinothalamic axons transmitting inputs from innocuous range thermoreceptive afferents ascend in more lateral regions of the spinothalamic tract (Lahuerta *et al.*, 1994; Friehs, Schröttner and Pendl, 1995). A more ventrally or medially placed lesion could spare these fibres. The dissociation between noxious heat and mechanical pain sensation suggests that the two types of lamina I projection neuron relaying nociceptive information are also anatomically segregated. NS cells, which predominantly receive A-fibre nociceptor afferents, signal sharp 'first' pain (Andrew and Craig, 2002b). Conversely HPC cells predominantly receive C-fibre input from polymodal nociceptors (Andrew and Craig, 2002b) and likely signal 'second' burning pain. The pattern of deficits displayed by the patient in **PAPER IV** would be consistent with preferential disruption of ascending NS fibres. Studies in cordotomy patients that, in addition to systematic thermal threshold assessment, also use graded mechanical skin stimulation will provide further insights in this respect. They may also provide clues as to the nature of the ascending pathways for the 'fast' nociceptors described in **PAPER I**.

9.4 MECHANOSENSATION AND PAIN

An overarching aim of somatosensory research is to be able to apply knowledge of the fundamental mechanisms of somatosensation to gain an understanding of, and ultimately treatments for, pathological conditions. Pain and tactile disturbances are intimately linked. In neuropathic pain normally innocuous cutaneous mechanical stimuli can cause disabling pain, a symptom termed tactile allodynia (Rasmussen *et al.*, 2004). It has been widely believed that central sensitisation, for example due to peripheral nerve injury, alters tactile signaling in the dorsal horn such that Aβ LTMR inputs now gain access to ascending nociceptive projections (Woolf, 1993; Campbell and Meyer, 2006). The recent elucidation of spinal circuits relaying inputs from Aβ LTMR to lamina I output cells that are unmasked in pain models support this view (Cheng *et al.*, 2017; Koch, Acton and Goulding, 2018) as does the lack of allodynia associated with Aβ nerve loss or blockade in human pain models (Koltzenburg, Lundberg and Torebjörk, 1992; Torebjörk, Lundberg and LaMotte, 1992; Cervero and Laird, 1996; Landerholm and Hansson, 2011; Liljencrantz *et al.*, 2013).

The role of CT afferents in signaling tactile allodynia remains unclear with contradictory evidence being presented in both animal (Seal *et al.*, 2009; Lou *et al.*, 2013; Delfini *et al.*, 2013) and human literature (Koltzenburg, Lundberg and Torebjörk, 1992; Torebjörk, Lundberg and LaMotte, 1992; Cervero and Laird, 1996; Nagi *et al.*, 2011; Landerholm and Hansson, 2011; Liljencrantz *et al.*, 2013). However, evidence is emerging that under conditions of central sensitisation CT afferents, rather than having a direct role in signaling touch hypersensitivity, play a modulatory role in prioritising nociceptive transmission (Liljencrantz *et al.*, 2013; Liljencrantz and Olausson, 2014). For example, selective activation of CT afferents in Aβ denervated subjects does not elicit allodynia in the heat-capsaicin model. It does, however, cause a reduction in the perceived intensity of touch (Liljencrantz *et al.*, 2013) consistent with reduced hedonic signaling and decreased CT mediated pain inhibitory mechanisms. The findings presented in **PAPER V** further support this hypothesis. Ratings of pleasantness to soft brush stroking were reduced in an area experimental allodynia in healthy subjects. Consistent with a reduction in CT signaling the decrease in ratings were significantly greater for CT targetted than CT suboptimal stroking velocities when stimulus duration was equivalent. In distinction, there were no differences in ratings for touch-evoked pain. In keeping with an alteration in Aβ LTMR processing (Olausson, Wessberg and Kakuda, 2000; Löken *et al.*, 2010) tactile direction discrimination was also less accurate in the allodynic zone. Therefore, there is evidence that both discriminative and affective aspects of touch are affected in this model. As well as developing tactile hypersensitivity a region of hypoesthesia to touch was also seen around the test site. Tactile hypoethesia around regions of clinical (Hollins, Sigurdsson and Morris, 2001; Maihöfner *et al.*, 2006) and experimental pain (Magerl and Treede, 2004; Geber *et al.*, 2008) have been documented previously and has been proposed to be secondary to a re-routing of low threshold somatosensory inputs to nociceptive projections (Magerl and Treede, 2004). Whether this pain-induced inhibition of tactile input occurs predominantly at spinal (Geber *et al.*, 2008; Dougherty, Willis and Lenz, 1998) or supraspinal levels (Brüggemann, Shi and Apkarian, 1998; Apkarian *et al.*, 2000); or whether processing of large or small diameter LTMR afferents is differentially affected remains open to question. Indeed, in **PAPER V** no significant correlation was seen between the area of tactile hypoesthesia and the reduction either in TDD accuracy or pleasantness ratings.

9.5 THERAPEUTIC TARGETS

It is evident that if CT afferent signaling, and thus pain inhibitory effects, are suppressed in allodynia (Liljencrantz *et al.*, 2013; Liljencrantz and Olausson, 2014), **PAPER V**) then interventions which enhance their effects might be used as a novel therapeutic strategy to treat the symptoms of neuropathic pain. Indeed, there is evidence in rodents that would support such a strategy. Pre-ganglionic terminals of C-LTMR afferents release the chemokine TAFA-4 (Delfini *et al.*, 2013; Kambrun *et al.*, 2018). Conditional knock-out of C-LTMRs in mice result in enhanced mechanical hypersensitivity in inflammatory as well as neuropathic pain models. This hypersensitivity is reversed by intrathecal administration of TAFA-4. Therefore, at least in rodents, pharmacological reversal of reduced C-LTMR processing is sufficient to behavioral manifestations of allodynia. One might envisage treating refractory localised neuropathic or inflammatory pain with appropriate dermatomal level intrathecal TAFA-4 agonists.

It may also be feasible to activate CT pathways rostral to the spinal cord dorsal horn, for example with highly focussed spinal cord stimulation. However, the continuing ambiguities surrounding the ascending projection pathways (**PAPER III**) would need to be resolved before a neuromodulatory target can be defined. The study of the neural circuitry underlying allodynia has focused heavily on the dorsal horn. However, there are almost certainly, as yet poorly defined, sites of pain-slow touch integration at supraspinal-levels where aberrant signally could generate tactile hypersensitivity. These too could represent neuromodulatory targets. For example, neurons representing potential targets of CT pathways which specifically respond to slow stroking stimuli have been identified in superficial layers of cortical areas 3b/1 (i.e. primary somatosensory cortex) (McKenna, Light and Whitsel, 1984). Interestingly this region shows strong reciprocal connectivity to a specific sub-region of area 3a, a cortical area recently identified as receiving strong nociceptive input (Vierck *et al.*, 2013; Whitsel *et al.*, 2018). The afferent mid-cingulate to dorsal posterior insula cortical pathway (Tan *et al.*, 2017) detailed above in the discussion of **PAPER II** is also a potential candidate for neuromodulation. The precise role of CTs in nociception and pain perception needs to be defined but the modulation of Aβ nociceptors (**PAPER I**) and their rostral projections might also represent a therapeutic strategy. Lightning/lancinating sensations are not uncommon in patients with neuropathic pain (Rasmussen *et al.*, 2004). These symptoms, which are unlikely reflect firing in primary C-fibre afferents, could potentially be related to ectopic or spontaneous firing predominantly of myelinated HTMRs. Patients with predominantly large fibre neuropathies can also present with pain (Tavakoli *et al.*, 2012). One potential risk of 'silencing' nociceptor fibres to treat pain is the risk of loss of their protective role (which is another reason why enhancing CT-afferent signally might reflect a more attractive strategy). If the prime role for Aβ HTMRs is nocifensive it might be appropriate in some circumstances to consider how to selectively spare rather than silence these fibres. Similarly, selective modulation of highly specific nociceptor projections within the spinothalamic tract would make an appealing option as it could minimise collateral deficits. The finding of anatomical-functional segregation of nociceptive pathways in the spinal cord (**PAPER IV**) might facilitate this approach, particularly if specific fibre tracts could be identified reliably intra-operatively.

10 CONCLUSIONS

PAPER I

It is concluded that human hairy skin is innervated by a population of hitherto undiscovered fast conducting mechanosensitive afferent fibres with high mechanical thresholds. These Aβ HTMRs are relevant for human pain perception and have properties well suited for reflexive nociceptive withdrawal from noxious mechanical stimuli.

PAPER II

It is concluded that carriers of the R221W mutation that causes HSAN-V show reduced C-fibre innervation of the cornea. Lower peripheral C-fibre densities are associated with a greater underestimation about how painful a particular situation will be. Therefore, individual experiences of pain may determine central-level perceptual evaluations of pain.

PAPER III

It is concluded that there is a dissociation between the obvious deficits in canonical spinothalamic modalities and affective touch following anterolateral cordotomy. Perceptual judgments about touch pleasantness do not depend on the integrity of the spinothalamic tract. Fibres ascending the dorsal columns provide sufficient information to conserve judgements about touch pleasantness after spinothalamic tract ablation. These findings diverge from those seen in HSAN-V and HSAN-III suggesting that evaluation of touch pleasantness including the characteristic tuning to slow gentle stroking critically depend on the co-processing of Aβ and CT inputs during normal neurodevelopment.

PAPER IV

It is concluded that there is at least partial anatomical segregation of fibres that have specific functional modalities in the spinothalamic tract. ‘Second order’ projections for mechanical pain are anatomically segregated from thermal and thermo-noxious pathways.

PAPER V

It is concluded that both discriminative and affective aspects of touch are affected in experimental tactile allodynia. This implies that vigorous nociceptive input acutely induces plasticity in LTMR afferent inputs to the dorsal horn of the spinal cord.

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