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DYNAMIC MECHANICAL ALLODYNIA IN PERIPHERAL NEUROPATHIC PAIN: PSYCHOPHYSICAL OBSERVATIONS

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To my dad

ABSTRACT

Introduction and aim: Pain due to a light moving mechanical stimulus, dynamic mechanical allodynia, is a protruding symptom/sign in subgroups of patients with peripheral neuropathic pain and frequently as troublesome as spontaneous ongoing pain. The objective of this thesis was to survey psychophysical details of dynamic mechanical allodynia using a novel semi-quantitative method. In addition, the psychophysical characteristics of dynamic mechanical allodynia in the secondary hyperalgesic zone after an intradermal injection of capsaicin were probed with regard to similarities and differences of that phenomenon compared to such allodynia in peripheral neuropathic pain.

Methods: Using a semi-quantitative method brush-evoked allodynia was induced in the innervation territory of the lesioned nervous structure in patients by lightly stroking different distances of the skin 2 or 4 times with brushes of different widths or while varying stroking velocity or brushing force. In study III the patients were also examined in the area outside the flare after an intradermal capsaicin injection in the corresponding contralateral site to the area of painful neuropathy, i.e., in the secondary hyperalgesic area. Age- and sex-matched controls injected with identical amounts of capsaicin were examined in a corresponding area. In all studies the intensity and duration of brush-evoked allodynia was recorded using a computerized visual analogue scale. The total brush-evoked pain intensity, including painful aftersensation was calculated as the area under the curve. Following each stimulus, the subjects selected pain descriptors from a validated instrument. In study II the repeatability of brush-evoked allodynia was examined within and between days in patients with peripheral neuropathic pain.

Results: Significantly increased total brush-evoked pain intensity was demonstrated with increased brushing length and number of strokes, higher brushing force and lower stroking velocity but not while altering brush width. Lack of influence of brush width was further underlined by the finding that brushing of equivalent skin areas resulted in higher total evoked pain intensity if brushing the skin with a thin brush over a longer distance than a thick brush over a shorter distance. A “very good” repeatability of brush-evoked allodynia within and between days was reported using this semi-quantitative method. In patients similarities were found in the relationship between brush-evoked allodynia and temporo-spatial stimulus parameters comparing the capsaicin-induced secondary hyperalgesic area with the area of painful neuropathy. Only 3/9 controls (compared to 8/9 patients) reported brush-evoked pain after capsaicin injection. In all studies the frequency of preferred sensory-discriminative and affective pain descriptors for the brush-evoked pain indicated some similarities, in particular the choice of affective pain descriptors such as ‘annoying’ and ‘troublesome’.

Conclusions: Our findings demonstrated dynamic mechanical allodynia to be a partially graded phenomenon in peripheral neuropathic pain conditions since stimulus parameters such as increased brushing length, increased number of strokes, lower stroking velocity and increased brushing force significantly increased the total brush-evoked pain intensity. However, alterations of the brush width within a limited range did not significantly change the total brush-evoked pain intensity. In addition, dynamic mechanical allodynia in the capsaicin-induced secondary hyperalgesic zone in patients seemingly well reflected perceptual details of such allodynia in the neuropathic condition. In healthy controls, only one-third developed brush-evoked allodynia in the potential secondary hyperalgesic area. Such a low hit frequency calls into question the value of the capsaicin model when aiming at studying dynamic mechanical allodynia. Taken together, these results substantiate the usefulness of this semi-quantitative assessment method in studies on dynamic mechanical allodynia, including longitudinal treatment studies.

Keywords: Dynamic mechanical allodynia; Brush-evoked allodynia; Brush-evoked pain; Neuropathic pain; Pain descriptors; Repeatability; Human pain model; Capsaicin; Psychophysical observations

LIST OF PUBLICATIONS

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Is dynamic mechanical allodynia in the secondary hyperalgesic area in the capsaicin model perceptually similar to the same phenomenon in painful neuropathy?
Submitted.

IV. Samuelsson M, Leffler AS, Johansson B, Hansson, P.
The influence of brushing force and stroking velocity on dynamic mechanical allodynia in patients with peripheral neuropathy.
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CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the curve
CPT	Cold pain threshold
CT	Cold perception threshold
dma	Dynamic mechanical allodynia
HPT	Heat pain threshold
IASP	International Association for the Study of Pain
ICC	Intra-class correlation coefficient
GEE	Generalized correlation coefficient
LED	Light-emitting-diode
LTT	Light touch perception threshold
mN	Millinewton
POM	Pain-O-Meter
SCS	Spinal cord stimulation
SEM	Standard error of the mean
SSRI	Selective serotonin reuptake inhibitor
QST	Quantitative sensory testing
VAS	Visual analogue scale
WT	Warm perception threshold
µg	Microgram
µl	Microlitre

1 INTRODUCTION

Neuropathic pain has been defined by the IASP (International Association for the Study of Pain) as 'pain initiated or caused by a primary lesion or dysfunction in the nervous system' (Merskey and Bogduk, 1994). Recently, a group of authors proposed the following redefinition; 'pain arising as a direct consequence of a lesion or disease affecting the somatosensory system' (Treede et al., 2008).

The prevalence of chronic neuropathic pain is not known in detail. Using blunt and imprecise measures the prevalence has roughly been estimated to 1 - 8 % (Bowsher, 1991; Torrance et al., 2006). The variable outcomes call for more meticulous studies on the matter.

Peripheral neuropathic pain has traditionally been classified either based on the underlying etiology or the anatomical distribution (Hansson, 2003; Jensen et al., 2001; Woolf and Mannion, 1999). A mechanism-based classification of pain, including neuropathic pain has been proposed (Hansson and Kinnman, 1996; Woolf et al., 1998) with the rational to link underlying pathophysiological mechanisms of a painful condition with symptoms and signs. Potential difficulties when trying to implement such a strategy are that one mechanism may give rise to multiple symptoms and signs and one symptom or sign may be caused by different mechanisms (Hansson, 2003; Woolf and Mannion, 1999). At present, while awaiting a detailed mechanism-based classification the traditional classification system should be applied (Hansson, 2003). From a diagnostic work-up point of view it was recently proposed to introduce a grading system of neuropathic pain using three grades; 'definite', 'probable' and 'possible', reflecting different degrees of certainty of the diagnosis (Rasmussen et al., 2004; Treede et al., 2008).

Peripheral neuropathic pain may be expressed by the presence of spontaneous and/or stimulus-evoked pain (Cruccu et al., 2004; Woolf and Mannion, 1999). The spontaneous pain may be continuous (ongoing) with variable intensity or, in a few albeit important conditions, paroxysmal. In subgroups of patients, stimulus-evoked pain such as allodynia or hyperalgesia is present, the former being the most troublesome from the suffering perspective. Most commonly, allodynia is caused by mechanical and thermal stimuli and sometimes such pain may persist after cessation of stimulation, i.e., aftersensation (Gottrup et al., 2003; Lindblom, 1994).

Sensory abnormalities accompanying neuropathic pain may consist of complex combinations of negative and positive signs (Jensen et al., 2001; Leffler and Hansson, 2008; Woolf and Mannion, 1999) and may be subdivided into quantitative (e.g., hypo- or hyperalgesia), qualitative (e.g., allodynia, dysesthesia), spatial (e.g., dyslocalization) and temporal (e.g., abnormal aftersensation) aberrations (Hansson, 1994; Hansson and Kinnman, 1996).

The term *allodynia (allo* (other) and *dynia* (a suffix meaning pain)), was first introduced by Noordenbos and defined as 'pain due to a non-noxious stimulus to normal skin' (IASP Subcommittee on Taxonomy, 1979). In 1992, allodynia was suggested to include 'pain evoked by stimuli activating non-nociceptive afferents' (Hansson and Lindblom, 1992). In 1994, IASP introduced allodynia as 'pain due to a stimulus which does not normally provoke pain' (Merskey and Bogduk, 1994), a definition adopted in this thesis for dynamic mechanical allodynia. Recently, a proposal for redefinition of allodynia has been presented; 'pain in response to a non-nociceptive stimulus' (Loeser and Treede, 2008) and dynamic mechanical allodynia

is suggested to be the only established example of allodynia. This suggestion is obviously an echo of what was put forth earlier by Hansson and Lindblom (Hansson and Lindblom, 1992). Hyperalgesia, which in the terminology from 1994 was defined by IASP as 'an increased response to a stimulus which is normally painful' (Merskey and Bogduk, 1994) is now proposed to be altered to 'increased pain sensitivity' with allodynia as a subgroup (Loeser and Treede, 2008).

Mechanical allodynia has been proposed to be divided into a static (light sustained pressure) or a dynamic (light moving object) subtype (Ochoa and Yarnitsky, 1993). Mechanical allodynia is not unique to neuropathic pain states, but may also be present in nociceptive pain conditions such as after surgery or burn injury. In such instances one can identify primary and secondary hyperalgesic areas. The former is an area with reddening immediately adjacent to injured tissue and the latter is found outside of the flare in undamaged tissue (LaMotte et al., 1991). In the primary hyperalgesic area there is increased sensitivity to both thermal and mechanical stimuli but in the secondary hyperalgesic area this holds true only for mechanical stimuli (LaMotte et al., 1991; Simone et al., 1989).

1.1 DYNAMIC MECHANICAL ALLODYNIA

Pain due to a light moving mechanical stimulus, dynamic mechanical allodynia, is a protruding symptom/sign in subgroups of patients with peripheral neuropathic pain, frequently as troublesome as spontaneous ongoing pain. The prevalence of dynamic mechanical allodynia in different diagnostic entities of peripheral neuropathic pain has not been studied in detail. From limited studies aiming at other issues a fair prevalence estimate, consistent with clinical empiricism, seem to be 20-50% (e.g., Gottrup et al., 2000; Leffler and Hansson, 2008; Martin et al., 2003; Otto et al., 2003; Rasmussen et al., 2004). It interferes extensively with activity of daily living as well as sleep quality (Hensing et al., 2007; Smith and Sang, 2002). Over time, the intensity of dynamic mechanical allodynia may vary, sometimes light stroking of the affected skin may be extremely painful with withdrawal of the affected limb in conjunction with vocalisation and sometimes the perception may instead have the characteristics of dysesthesia. In addition, a patient with a neuropathic skin area may report a mixture of pain and dysesthesia to touch stimuli. Dynamic mechanical allodynia may be extremely localised in some patients with peripheral neuropathic pain, i.e., at the site of a neuroma, but more commonly is widespread in the distribution of the cutaneous innervation territory of the damaged nerve or nerve root (Hansson, 2003).

Treatment studies of neuropathic pain have mainly focused on monitoring spontaneous ongoing pain. In a recent review, disparate effects of a variety of pharmacotherapies on dynamic mechanical allodynia in neuropathic pain were reported (Granot et al., 2007). The imprecise methodologies of stimulus evoked pain used in the included studies, not tested for repeatability, precludes firm conclusions about the efficacy of the different treatment strategies.

1.1.1 Pathophysiology of dynamic mechanical allodynia

Results from studies in neuropathic pain patients (Gracely et al., 1992; Koltzenburg et al., 1994; Ochoa and Yarnitsky, 1993) have clearly pointed to the importance of non-nociceptive mechanoreceptive afferents as the peripheral substrate of dynamic mechanical allodynia, a concept adopted in this thesis based on the clinical phenomenology of included patients with peripheral neuropathic pain, i.e., all devoid

of obvious signs of peripheral sensitization/neurogenic inflammation. When performing experimental compression-ischemia nerve blocks of A-beta fibres, dynamic mechanical allodynia was abolished (Campbell et al., 1988; Ochoa and Yarnitsky, 1993) but was unaffected by local anaesthetic block of A-delta- and C-fibres in patients with peripheral neuropathic pain (Campbell et al., 1988; Nurmikko et al., 1991).

Numerous possible pathophysiological scenarios, alone or in combination, must be taken into account when trying to explain the underlying pathophysiology of the phenomenon (Hansson, 2003; Woolf and Mannion, 1999):

- Peripheral sensitization of A-delta- and C-fibres. However, peripheral sensitization is probably an uncommon feature across diagnostic entities of peripheral neuropathic pain, with the exception of patients with postherpetic neuralgia (Fields et al., 1998).

- Ephaptic transmission or crosstalk between A-beta fibres and nociceptive fibres due to altered insulation between adjacent axons after injury may contribute to dynamic mechanical allodynia (Amir and Devor, 1992). However, reaction time measurements in patients suggested a conduction velocity in the range of A-beta fibres, speaking in favour of large myelinated afferents only to be activated in the periphery (Campbell et al., 1988; Lindblom and Verrillo, 1979).

- Altered balance in the dorsal horn of the spinal cord between facilitatory and inhibitory influences. This could be due to neuropathy-induced loss of A-beta fibres and hence inhibition mediated by such fibres or excitotoxicity influence on inhibitory interneurons leading to cell death (Laird and Bennett, 1992).

- Central sensitization, i.e., the non-nociceptive mechanoreceptive large fibre system gaining access to the nociceptive system in the dorsal horn of the spinal cord (Cook et al., 1987; Fields et al., 1998; Torebjork et al., 1992).

- Descending facilitation of dorsal horn nociceptive neurons from brainstem areas (Ossipov et al., 2001) via a spino-bulbo-spinal loop with serotonergic excitatory influence (Suzuki et al., 2002).

- Sprouting of mechanoreceptive fibres from deeper layers of the dorsal horn to more superficial layers where synaptic couplings to nociceptive neurons may take place (Woolf et al., 1992; Woolf et al., 1995). Others have not been able to demonstrate extensive sprouting but rather only limited branching to lamina II (Bao et al., 2002).

Other afferents than low threshold A-beta mechanoreceptive fibres may be implicated in dynamic mechanical allodynia. In animal studies nociceptive A-beta fibres with low mechanical- and high heat thresholds have been identified (Cain et al., 2001; Djouhri and Lawson, 2004). Also, the existence of A-delta low-threshold mechanoreceptors has been reported in humans (Adriaensen et al., 1983). In addition, in primates the mechanical threshold for C-fibre nociceptors has been reported to be as low as 5 mN (Slugg et al., 2000) and finally, low-threshold mechanoreceptive C-fibres have been identified in human skin that are involved in light touch sensation (McGlone et al., 2007; Vallbo et al., 1993).

1.1.2 Assessment of dynamic mechanical allodynia

In clinical treatment studies, the variety of testing procedures and equipment used to evoke dynamic mechanical allodynia has been extensive including, e.g., a cotton wisp (Leung et al., 2001), cotton wool (Kvarnstrom et al., 2003; Meier et al., 2003), a brush (Attal et al., 2004; Lynch et al., 2005; Wallace et al., 2002) and an electrical toothbrush (Jorum et al., 2003). There is obviously no consensus on how to assess dynamic mechanical allodynia quantitatively with reliable methodology. In order to improve the treatment of dynamic mechanical allodynia it is pivotal to unravel psychophysical details of the allodynic percept and to develop techniques to monitor the experience with a high enough resolution.

1.1.3 The relationship between spontaneous ongoing pain and dynamic mechanical allodynia

The intensity of dynamic mechanical allodynia has been reported to be positively correlated with the spontaneous ongoing pain in patients with pain related to peripheral traumatic nerve injury (Koltzenburg et al., 1994) and in patients with post-herpetic neuralgia (Rowbotham and Fields, 1996). It has been proposed that activity in primary afferent nociceptors, maintaining the spontaneous ongoing pain, causes central abnormalities responsible for induction of dynamic mechanical allodynia (Koltzenburg et al., 1994). The relationship between spontaneous ongoing pain and dynamic mechanical allodynia is a confounding factor while designing treatment studies aiming at relieving such allodynia and the possible influence of spontaneous pain has to be taken into account.

1.1.4 Sensory-discriminative and affective pain descriptors

Certain symptoms such as burning, shooting and shock-like pains have been suggested to be characteristics of spontaneous ongoing neuropathic pain (Boureau et al., 1990; Jensen and Baron, 2003). However, no specific pain descriptors for neuropathic pain were identified when assessing pain patients with and without neuropathic pain (Rasmussen et al., 2004). To our knowledge there has been no study reporting on descriptors for dynamic mechanical allodynia specifically. In addition to surveying the intensity and duration of dynamic mechanical allodynia in patients with peripheral neuropathic pain, multidimensional aspects of the painful experience could be reflected by also having patients using sensory-discriminative and affective descriptors to further detail the psychophysics of the percept.

1.2 A HUMAN EXPERIMENTAL PAIN MODEL WITH CAPSAICIN

In order to develop valid experimental human pain models, i.e., models potentially reflecting mechanisms underlying certain expressions of clinical pain conditions, similarities and discrepancies of symptoms/signs must first and foremost be evaluated comparing the two. Nevertheless, in a situation where symptoms/signs appear to be similar, a potential pitfall with surrogate models would still be that pathophysiological mechanisms in clinical conditions and experimental models might differ, i.e., one symptom/sign may be expressed by a variety of mechanisms. Symptoms and signs caused by intradermally injected capsaicin have been suggested to reflect aspects of the clinical phenomenology of neuropathic pain (Gottrup et al., 2003; Schmelz et al., 2000), e.g., dynamic mechanical allodynia (Baumgartner et al., 2002; Gottrup et al., 2004; Witting et al., 2001; Witting et al., 1998; Witting et al.,

2000). Capsaicin is the algesic ingredient in chilli pepper (LaMotte et al., 1991; Simone et al., 1998) and after injection evokes ongoing pain as a result of vanilloid type 1-receptor activation (Caterina et al., 1997; Schmelz et al., 2000), a receptor found on mechano-heat-insensitive C-fibres (Schmelz et al., 2000; Schmidt et al., 1995). In the area immediately surrounding the injection, capsaicin causes peripheral sensitization (Ali et al., 1996; Hughes et al., 2002), spreading flare (Lewis, 1936) and allodynia to mechanical and thermal stimuli (LaMotte et al., 1991). In the seemingly unaltered tissue outside the flare (Kinnman et al., 1997), classically labelled the secondary hyperalgesic area, allodynia to mechanical stimuli such as brushing is believed to be initiated and maintained by central sensitization and mediated in the periphery by A-beta mechanoreceptive afferents (Torebjork et al., 1992).

In this thesis, psychophysical aspects of dynamic mechanical allodynia were explored using a semi-quantitative assessment method. The purpose was to survey relationships between different stimulus parameters and the painful experience in patients with peripheral neuropathy. Ultimately, such information could be used to suggest standardized methodology for the assessment of dynamic mechanical allodynia. In addition, to evaluate the potential usefulness of the capsaicin model as an experimental model of dynamic mechanical allodynia, the thesis work aimed at comparing perceptual details between clinical dynamic mechanical allodynia and the same phenomenon in the secondary hyperalgesic area in the capsaicin model.

2 AIMS OF THE THESIS

The objective of this thesis was to survey psychophysical details of dynamic mechanical allodynia in patients with peripheral neuropathy using a novel semi-quantitative method. In addition, psychophysical characteristics of dynamic mechanical allodynia in the secondary hyperalgesic zone of the human experimental pain model with intradermal injection of capsaicin were probed with regard to similarities and differences of that phenomenon compared to such allodynia in peripheral neuropathic pain.

2.1 SPECIFIC AIMS

Study I

- *To examine the relationship between temporo-spatial stimulus parameters (brushing length, brush width and number of strokes) and brush-evoked pain intensity as well as duration*
- *To examine similarities and discrepancies in the selection of sensory-discriminative and affective descriptors of the painful experience*

Study II

- *To examine the repeatability of brush-evoked allodynia as well as spontaneous ongoing pain within and between days in a short (days) and long (month) term perspective*
- *To examine the relationship between the intensity of spontaneous ongoing pain and the total brush-evoked pain intensity*

Study III

- *To examine psychophysical similarities and discrepancies of the relationship between temporo-spatial stimulus parameters and brush-evoked pain intensity and duration in patients with painful peripheral neuropathy compared to the outcome in the secondary hyperalgesic area in capsaicin-treated skin in patients and in healthy subjects*
- *To examine similarities and discrepancies in the selection of sensory-discriminative and affective descriptors of the painful experience in the area of neuropathy and in the area of secondary hyperalgesia*

Study IV

- *To examine brushing force and stroking velocity and their relationship to brush-evoked pain intensity and duration*
- *To examine similarities and discrepancies in the selection of sensory-discriminative and affective descriptors of the painful experience*

3 MATERIAL AND METHODS

3.1 SUBJECTS

All participating patients were outpatients recruited from the Pain Center, Department of Neurosurgery, Karolinska University Hospital Solna or Pain Unit, Department of Anaestehesia and Intensive Care, Danderyd Hospital, Sweden. Three patients with peripheral neuropathy participated in all studies, 2 patients participated in 3 studies and 6 patients participated in 2 studies. Control subjects participated in study III only. In accordance with the Helsinki declaration, the local ethical committee of the Karolinska University Hospital Solna approved the studies and all subjects gave their informed consent to participation (written informed consent in study II, III and IV).

3.1.1 Study I

3.1.1.1 Patients

Twenty patients participated, eleven females and nine males, with an average age of 43 years (range 27 - 60), suffering from brush-evoked pain, i.e., dynamic mechanical allodynia due to long-term peripheral neuropathy with or without spontaneous ongoing pain. In addition to a diagnosis of neuropathy, with or without spontaneous ongoing pain, a painful sensation evoked by lightly stroking the skin with a soft brush in part of or in the entire innervation territory of the lesioned nervous structure was a prerequisite. On the study day, if applicable, the patients were allowed to continue prescribed medications with stable doses. If the patient was using a spinal cord stimulator, they were requested to switch it off well in advance to eliminate the pain relieving effect of the stimulator. Special care was taken to control this and not to include patients reporting a stimulus-evoked unpleasant sensation only, i.e., dysesthesia. Further exclusion criteria were cardiovascular, other neurological or dermatological diseases or painful conditions localised to the musculoskeletal system. Two male patients were excluded due to inability in understanding the test procedure. Demographic data are shown in Table 1.

Table 1. Demographic data, pain duration, treatment and spontaneous ongoing pain intensity before and during the assessments (VAS translated to 0-100 mm) of 18 patients with dynamic mechanical allodynia due to peripheral neuropathy

Patient gender	**Age (years)**	**Nerves involved**	**Pain duration (years)**	**Treatment: medication and/ or spinal cord stimulation (SCS)**	**Spontaneous ongoing pain intensity before assessments (mm)**	**Spontaneous ongoing pain intensity during assessments x 3 (mm)**
1 F	60	R Ulnar nerve Status post cut injury	2	-	25	21, 22, 39
2 F	51	Scar pain status post carpal tunnel surgery	11	Acetaminophene	88	67, 94, 87
3 M	53	R Brachial plexus Status post tumor, surgery x 2 and radiation	8	Gabapentin SCS	80	82, 84, 92
4 F	36	L Supraclavicular nerve Status post surgery, thenotomy sternocleidomastoid muscle	1.5	Opiod	29	16, 16, 14
5 M	33	L Cervical plexus Status post stab injury	10	SCS	41	42, 51, 49
6 F	50	L Intercostobrachial nerve Status post surgery, mammary cancer	1.5	Amitriptyline	0*	0*
7 M	30	L Sural nerve Scar pain status post surgery, ligamentoplasty	10	-	77	77, 66, 86
8 F	53	R Saphenous nerve Status post surgery, varecous veins	9	-	17	26, 57, 51
9 F	45	R Digital nerve, dig II hand Status post ganglion surgery	6	SCS	88	91, 95, 100
10 F	53	L Brachial plexus Compression/tension injury	2	Opiod SCS	83	81, 87, 96
11 F	32	R Brachial plexus Status post surgery, tumor resection	3	Gabapentin Muscle-relaxant	63	61, 66, 64
12 M	28	L Medial cutaneous nerve of the forearm Status post gun wound	7	SSRI SCS	13	13, 16, 14
13 F	49	Scar pain status post disc surgery L5 – S1	6	Acetaminophene Opiod	68	58, 68, 66
14 F	51	R Saphenous nerve Status post pressure injury	1	Gabapentin	100	97, 100, 96
15 M	27	L Infraorbital nerve Status post fracture and surgery	2	-	0*	0*
16 F	41	L L5 Rhizopathy Status post cyst compression injury	9	Amitriptyline	44	57, 75, 92
17 M	39	L Digital nerve, dig II hand Status post compression injury	5	SCS	68	64, 71, 78
18 M	33	R Femoral nerve Status post stab injury	11.5	Opiod Gabapentin SCS	43	34, 59, 39

F, female; M, male; L, left; R, right. *Two patients reported no spontaneous ongoing pain.

3.1.2 Study II

3.1.2.1 Patients

Ten patients, five females and five males, with an average age of 38, 8 years (range 25 - 52), suffering from dynamic mechanical allodynia (a painful sensation evoked by lightly stroking the skin with a soft brush in part of or in the entire innervation territory of the lesioned nervous structure) and spontaneous ongoing pain due to long-term peripheral neuropathy (range 2-13 years) participated. Patients reporting a stimulus-evoked unpleasant sensation only, i.e. dysesthesia were not considered for inclusion. A power analysis of the standard deviation from an earlier study where this semi-quantitative method for assessment of brush-evoked allodynia was used, guided the number of patients included (Samuelsson et al., 2005). During the study, if applicable, the patients were allowed to continue prescribed medications with stable doses (i.e. gabapentin (n = 1), antidepressants (n = 2)) but were not allowed to start other pain relieving treatments or changing doses of the medications. Patients using a spinal cord stimulator (n = 5) were requested to switch it off well in advance of the examination to eliminate the pain relieving effect of the stimulator. Exclusion criteria were cardiovascular, other neurological or dermatological diseases or painful conditions localised to the musculoskeletal system. One patient was excluded due to not fulfilling the criteria of brush-evoked pain returning to baseline within the maximum sample time of 3 min. Demographic data are shown in Table 2.

Table 2. Demographic data, pain duration and ongoing treatment of 9 patients with spontaneous ongoing pain and dynamic mechanical allodynia due to peripheral neuropathy

Patient gender	**Age (years)**	**Nerves/roots involved**	**Pain duration (years)**	**Treatment: medication and/ or spinal cord stimulation (SCS)**
1 F	52	R Saphenous nerve, status post pressure injury	2	Antidepressant SCS
2 F	42	L L5 root, status post cyst compression injury	10	-
3 M	31	L Sural nerve, Scar pain, status post surgery, ligamentoplasty	12	-
4 M	34	L Cervical plexus, status post stab injury	12	SCS
5 F	25	R Scar pain, status post carpal tunnel surgery	3	-
6 M	29	L Medial cutaneous nerve of the forearm, status post gun wound	8	Antidepressant SCS
7 M	34	R Femoral nerve, status post stab injury	13	Gabapentin SCS
8 M	40	L Digital nerve, dig II hand, status post compression injury	6	SCS
9 F	49	L L5 - S1 roots, status post disc surgery	4	-

F, female; M, male; L, left; R, right.

3.1.3 Study III

3.1.3.1 Patients

Nine patients participated, 5 females and 4 males, with an average age of 41 years (range 28 - 55), suffering from dynamic mechanical allodynia (a painful sensation evoked by lightly stroking the skin with a soft brush) and spontaneous ongoing pain due to long-term (range 1-15 years) peripheral neuropathy in the upper or lower extremity. Special care was taken not to include patients reporting a stimulus-evoked unpleasant sensation only, i.e., dysesthesia. A power analysis from an earlier study where the semi-quantitative method for assessment of brush-evoked allodynia was used, guided the number of patients included (Samuelsson et al., 2005). On the study day, if applicable, the patients were allowed to continue prescribed medications with stable doses. Four patients had no medication and none of the patients used a spinal cord stimulator. Further exclusion criteria were a history of hypertension, cardiovascular-, other neurological- or dermatological diseases or painful conditions localised to the musculoskeletal system. Demographic data is shown in Table 3.

Table 3. Demographic data, pain duration, ongoing treatment and dose of injected capsaicin in mikrog (µg) in 9 patients with spontaneous ongoing pain and dynamic mechanical allodynia due to peripheral neuropathy

Patient gender	**Age (years)**	**Nerves/roots involved**	**Pain duration (years)**	**Treatment: medication**	**Dose of injected capsaicin Microg (µg)**
1 M	34	L sural nerve, scar pain status post surgery, ligamentoplasty	15	-	120
2 M	31	R anteriormedial branches of femoral nerve, status post stripping surgery, varicose veins	4	-	120
3 F	45	L L5 radiculopathy, status post cystic compression injury	13	-	60
4 F	55	R saphenous nerve, status post pressure injury	5	Acetaminophen + codeine, mianserin	60
5 F	28	R scar pain status post carpal tunnel surgery	6	Morphine	120
6 F	54	L superficial peroneal nerve, status post fracture and surgery	1	Amitriptyline, tramadol	60
7 F	34	L lateral cutaneous femoral nerve, status post laparoscopic surgery	9	-	120
8 M	44	L superficial peroneal nerve, status post compression and fasciotomy	7	Pregabalin	120
9 M	42	R ulnar nerve, status post amputation dig V	6	Tramadol, acetaminophen	120

F, female; M, male; L, left; R, right.

3.1.3.2 Controls

Nine healthy and habitually pain-free, age- (± 5 years) and sex-matched volunteers with an average age of 42 years (range 26-59) were recruited. No medication was taken on a regular basis. All controls had heart rate between 60 and 84 beats/min and all but one had resting blood pressure < 140/90 mm Hg (1 control had 140/100 mm Hg, which was normalised at follow-up).

3.1.4 Study IV

3.1.4.1 Patients

Seventeen patients participated, 11 females and 6 males, with an average age of 45 years (range 29 - 62), suffering from dynamic mechanical allodynia (a painful sensation evoked by lightly stroking the skin with a soft brush) due to long-term (range 1-16 years) peripheral neuropathy with or without spontaneous ongoing pain. Special care was taken not to include patients reporting a stimulus-evoked unpleasant sensation only, i.e., dysesthesia. A power analysis from an earlier study where the same semi-quantitative method for assessment of brush-evoked allodynia was used, guided the number of patients included (Samuelsson et al., 2005). On the study day, if applicable, the patients were allowed to continue prescribed medications with stable doses. Eight patients had no medication. If a spinal cord stimulator (n=3) was used, the patients were requested to switch it off at least 12 hours prior to testing. Additional exclusion criteria were cardiovascular-, other neurological- or dermatological diseases. One patient was excluded due to not fulfilling the criteria of brush-evoked pain returning to baseline within the maximum sample time of 3 min. Demographic data is shown in Table 4.

Table 4. Demographic data, pain duration, ongoing treatment, spontaneous ongoing pain intensity before the assessments (VAS translated to 0-100 mm) in 16 patients with dynamic mechanical allodynia due to peripheral neuropathy

Patient gender	Age (years)	Nerves/roots involved	Pain duration (years)	Treatment: medication and/ or spinal cord stimulation (SCS)	Spontaneous ongoing pain intensity before assessments (mm)
1 F	29	R scar pain status post carpal tunnel surgery	7	-	42
2 F	46	L L5 radiculopathy, status post cystic compression injury	14	-	62
3 M	35	L sural nerve, scar pain status post surgery, ligamentoplasty	16	-	66
4 F	56	R saphenous nerve, status post pressure injury	6	Acetaminophen + codeine	20
5 M	45	L superficial peroneal nerve, status post compression and fasciotomy	8	Duloxetine	34
6 M	43	R ulnar nerve, status post amputation dig V	8	-	25
7 F	52	R C8-Th1 radiculopathy, status post neck trauma	8	Pregabalin, Duloxetine	20
8 M	29	L intercostal nerve, status post thoracotomy	3	-	55
9 F	41	L supraclavicular nerve status post thenotomy sternocleidomastoid muscle	7	SR morphine, SCS	0*
10 F	43	L transverse cutaneous nerve of neck, status post stab injury	4	Pregabalin	34
11 F	61	L sural nerve, status post fracture and surgery	2	Pregabalin, Tramadol Capsaicin cream	29
12 F	62	R scar pain, status post carpal tunnel surgery	6	Pregabalin	66
13 M	38	L cervical plexus, status post stab injury	16	SCS	44
14 M	41	L digital nerve dig IV of hand, status post work related injury	1	-	0*
15 F	49	R saphenus nerve, status post hip joint surgery	16	Pregabalin, Gabapentin	50
16 F	42	L digital nerve dig II of hand, status post stab injury and surgery	2	SCS	84

F, female; M, male; L, left; R, right. *Two patients reported no spontaneous ongoing pain.

3.2 METHODS

3.2.1 General procedure

All assessments (author M.S. study I – IV, AS.L. study I) and injections (P.H. study III) were performed by the same investigators. The subjects were carefully familiarised with the different methods to be used before the start of the experiment. During assessments, the subjects were comfortably seated in a chair or lying on a bed.

3.2.1.1 Study 1

To guide sensibility testing the patients were asked to indicate the area of spontaneous ongoing pain and dynamic mechanical allodynia, respectively, on a whole body pain drawing. If present, the spontaneous ongoing pain intensity was rated on a 100 mm visual analogue scale (VAS) before and three times during the experiment (after testing of perception thresholds to touch and temperature as well as following the entire experimental session). The left extreme end of the VAS indicated 'no pain' and the right end 'worst imaginable pain'. To guide quantitative sensory testing (QST) a test area within the region of maximum dynamic mechanical allodynia and in the contralateral homologous area were marked with a pen. The area of dynamic mechanical allodynia was carefully titrated by lightly brushing (Brush-05, SENSELab^{TM}, Somedic Sales AB, Sweden) from the unaffected skin towards an area where the normally non-painful mechanical stimulus was perceived as painful to establish the neuroanatomical border of the phenomenon. Following QST, with a specific brush fitted to an electronic force transducer (SENSEBox Force transducer, Somedic Sales AB, Sweden) pain was induced by lightly stroking the skin, while the patient used a computerized VAS device (SENSEBox VAS, Somedic Sales AB, Sweden) to continuously rate the intensity and duration of the painful sensation. After each stimulus the patients were asked to select descriptors for the sensory-discriminative and affective components of the pain experience, respectively, from a validated instrument, the Pain-O-Meter® (Gaston-Johansson, 1996).

3.2.1.2 Study 2

To guide the assessments of dynamic mechanical allodynia, the patients were asked to indicate the area of spontaneous ongoing pain and dynamic mechanical allodynia, respectively, on a whole body pain drawing. The area of dynamic mechanical allodynia was then titrated by lightly brushing from the unaffected skin towards an area where the normally non-painful mechanical stimulus was perceived as painful. A test area within the region of maximum dynamic mechanical allodynia was marked with a pen. The spontaneous ongoing pain intensity was rated on a VAS before each assessment of brush-evoked allodynia. With a specific brush fitted to an electronic force transducer (SENSEBox Force transducer, Somedic Sales AB, Sweden) pain was induced by lightly stroking the skin (Samuelsson et al., 2005), while the patient used a computerized VAS device (SENSEBox VAS, Somedic Sales AB, Sweden) to continuously rate the intensity and duration of the painful sensation.

3.2.1.2.1 Repeated measures design

The patients participating in the study were examined 4 days during one month, i.e. at day 1, 3, 28 and 30. On each study day the stimulus was repeated 4 times (a, b, c, d) with an inter-stimulus interval of 10 min (after the brush-evoked pain intensity had returned to baseline) (Fig. 1). In total, dynamic mechanical allodynia was assessed 16 times over the 30 days. The assessments were performed under the same conditions, i.e., same place and time of the day. To be able to identify the position of the previous testing the test area was photographed following day 3 to be used as a guide for day 28. The marked area was still visible between day 1 and 3 as well as between day 28 and 30.

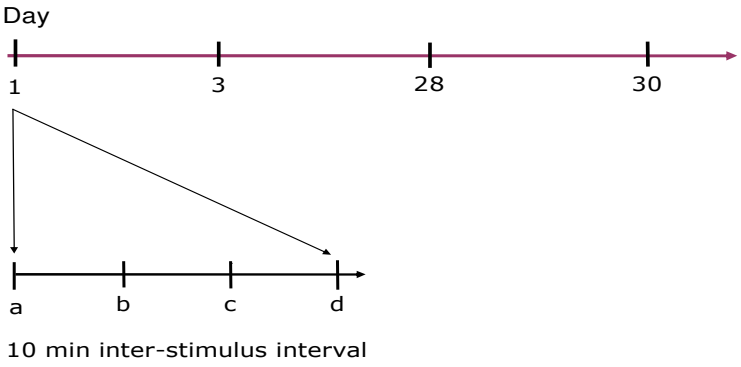

Fig. 1. Each patient was examined 4 days during one month, i.e. at day 1, 3, 28 and 30. On each study day the stimulus was repeated 4 times (a, b, c, d), with an inter-stimulus interval of 10 min.

3.2.1.3 Study 3

The patients were first examined in the area of painful neuropathy and subsequently in the corresponding contralateral site, i.e., in the potential secondary hyperalgesic area outside the flare after an intradermal injection of capsaicin. Aged and gender matched controls were examined in a corresponding area after an intradermal injection of capsaicin.

3.2.1.3.1 Patients

To guide subsequent assessments of dynamic mechanical allodynia, the patients were asked to indicate the area of spontaneous ongoing pain and dynamic mechanical allodynia, respectively, on a whole body pain drawing. The area of allodynia was then titrated by lightly brushing (Brush-05, SENSELab^{TM}, Somedic Sales AB, Sweden) from the unaffected skin towards an area where the normally non-painful mechanical stimulus was perceived as painful. The spontaneous ongoing pain intensity was rated on a VAS before assessment of brush-evoked allodynia. With a specific brush fitted to an electronic force transducer (SENSEBox Force transducer, Somedic Sales AB, Sweden) pain was induced by lightly stroking the skin (Samuelsson et al., 2005), while the patient used a computerized VAS device (SENSEBox VAS, Somedic Sales AB, Sweden) to continuously rate the intensity and duration of the painful sensation. After each stimulus, sensory and affective pain descriptors were selected from a validated instrument, the Pain-O-Meter® (Gaston-Johansson, 1996).

Following assessments in the area of painful neuropathy capsaicin was injected intradermally in the corresponding contralateral area. The capsaicin-induced ongoing pain intensity was rated on a VAS 1 min following the injection, before the assessment of brush-evoked allodynia. Within a few minutes an area of flare developed around the injection site. Repeated testing with a brush was performed continuously from the unaffected skin towards the injection site to allow for brush-evoked allodynia to develop outside the flare. If so, a test area for subsequent semi-quantitative examination was marked well outside the area of flare. The same testing

procedure of brush-evoked allodynia was then applied in the capsaicin treated skin as in the area of painful neuropathy.

3.2.1.3.2 Controls

Aged and gender matched controls were injected intradermally with capsaicin in a corresponding area to the injection site of capsaicin in patients and assessed following the same protocol as used for the patients.

3.2.1.4 Study 4

To guide subsequent assessments of dynamic mechanical allodynia, the patients were asked to indicate the area of spontaneous ongoing pain and dynamic mechanical allodynia, respectively, on a whole body pain drawing. If present, the spontaneous ongoing pain intensity was rated on a VAS before assessment of brush-evoked allodynia. The area of allodynia was then titrated by lightly brushing (Brush-05, SENSELab^{TM}, Somedic Sales AB, Sweden) from the unaffected skin toward an area where the normally non-painful mechanical stimulus was perceived as painful. With a specific brush fitted to an electronic force transducer (SENSEBox Force transducer, Somedic Sales AB, Sweden) pain was induced by lightly stroking the skin (Samuelsson et al., 2005), while the patient used a computerized VAS device (SENSEBox VAS, Somedic Sales AB, Sweden) to continuously rate the intensity and duration of the painful sensation. After each stimulus the patients were asked to select descriptors for the sensory-discriminative and affective components of the pain experience, respectively, from a validated instrument, the Pain-O-Meter® (Gaston-Johansson, 1996).

3.2.2 Quantitative sensory testing (study I)

To outline the profile of neuropathy, all patients in study I were examined using conventional QST measures.

3.2.2.1 Assessment of tactile perception threshold

Low threshold mechanoreceptive function (perception threshold to light touch) was assessed using von Frey-like filaments made of optical glass (Optihair von Frey Filaments, MARSTOCK nervtest, Dr Fruhstorfer, Marburg, Germany) (Fruhstorfer et al., 2001) according to the method of limits (Weinstein, 1962) as previously described (Leffler et al., 2000).

3.2.2.2 Assessment of thermal perception threshold

Quantitative testing of thermal non-noxious and noxious sensibility was carried out employing a threshold tracking unidirectional stimulation technique and the methods of limits (Hansson et al., 1988), using a Peltier element based contact thermode with a size of 18 x 18 mm (MSA Thermotest®, Somedic Sales AB, Hörby, Sweden). The method has been thoroughly described elsewhere (Leffler et al., 2000).

3.2.3 Assessment of spontaneous ongoing pain (Study I – IV)

If present in study I, the spontaneous ongoing pain intensity was rated on a VAS before and three times during the experiment, after testing of perception thresholds to touch and temperature as well as following the entire experimental session. The left extreme end of the VAS indicated ‘no pain’ and the right end ‘worst imaginable

pain'. In study II the intensity of the spontaneous ongoing pain was rated on a VAS before each assessment of brush-evoked allodynia.

The spontaneous ongoing pain intensity in the area of neuropathy was in study III rated on a VAS before assessment of brush-evoked allodynia and the capsaicin-induced ongoing pain intensity was rated on a VAS 1 min following the injection. If present in study IV, the spontaneous ongoing pain intensity was rated on a VAS before assessment of brush-evoked allodynia.

3.2.4 Assessment of brush-evoked pain (Study I – IV)

In study I, II and IV, a 60 mm long and 20 mm wide test area within the area of maximum dynamic mechanical allodynia in patients with painful peripheral neuropathy was marked with a pen. In study III, a 20 mm long and 20 mm wide test area within the area of maximum dynamic mechanical allodynia in patients with painful peripheral neuropathy and when present, in the secondary hyperalgesic area in capsaicin treated skin in patients and their controls was marked with a pen. Brush-evoked allodynia was induced by lightly stroking the test area using brushing stimuli with varying characteristics, i.e., brushing length, width of the brush, number of strokes, stroking velocity and brushing force. The brushes were fitted to an electronic force transducer (SENSEBox Force transducer, Somedic Sales AB, Sweden) (Fig. 2). A database application was used where all stimulus parameters (brushing length, width of the brush, number of strokes, brushing pressure and stroke velocity) were recorded.

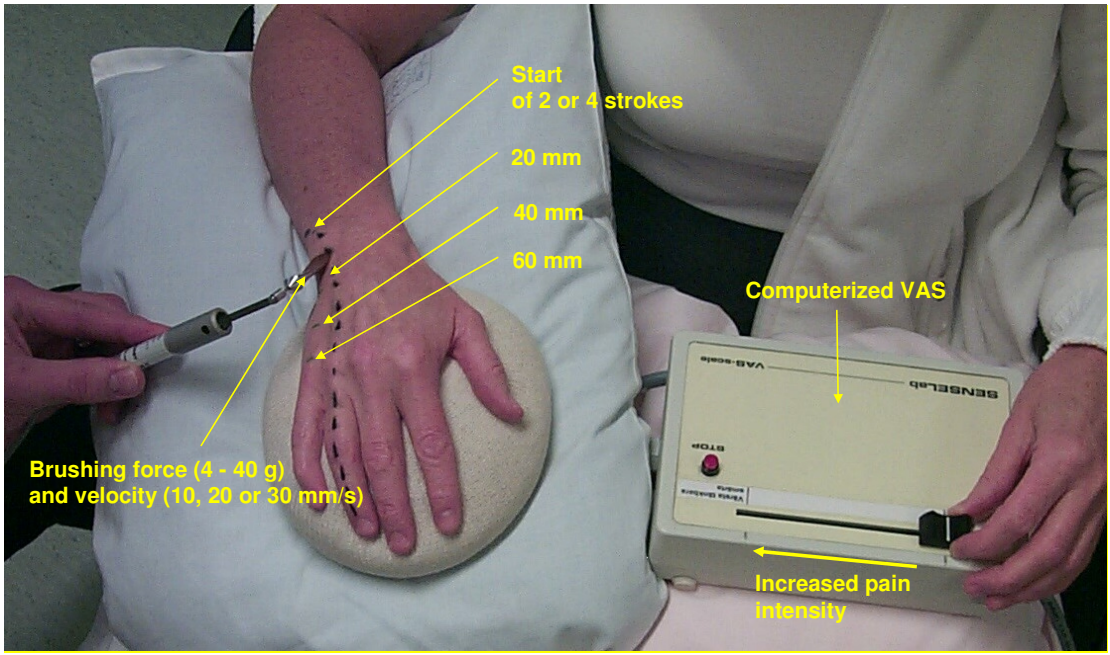

Fig. 2. Assessment of dynamic mechanical allodynia using brushing stimuli with varying characteristics.

In study I, II and III, the examiner (author M.S.) kept a fairly constant brushing force of 4 – 25 g (4 - 20 g, study I) and a stroking velocity of approximately 20 mm/s. Brushing force was monitored on-line on the computer screen and if exceeding 25 g (20 g, study I) or if below 4 g, the stroke was disregarded and a new attempt was made. In study IV, nominal values of brushing forces and stroking velocities were carefully practiced by the examiner (author M.S.). The brush, fitted to the electronic force transducer handle, provided visual feedback of the brushing force through five light-emitting diodes (LEDs) of different colours located in the handle. The centre LED indicated that the applied force was within $\pm$ 2.5 g of the nominal force. The two LEDs on each side of the centre LED indicated a further deviation of 2.5 or 5 g, respectively, from the nominal force. The database application recorded the variability of brushing force and stroking velocity from the nominal values, monitored on-line on the computer screen (Table 5).

Before each study session the brushing device was balanced, holding the brush in the air approximating the stroking position and activating the automatic balance function. The brush strokes were always performed in the same direction, i.e., proximal to distal. To avoid minor movement related influences on the handheld sensor the database recording start was set to be triggered as the brushing force exceeded 4 g. The stimuli were delivered manually with an inter-stimulus interval of 1-4 minutes depending on differences in duration of aftersensation of brush-evoked allodynia and time consumption for the selection procedure of pain descriptors.

Using a computerized VAS, preset to record values exceeding 2 mm and stopped when values were below 2 mm, the subjects continuously rated the development of the intensity of brush-evoked allodynia. Measurements were stored in a database that enabled recordings of seconds to onset of brush-evoked pain (> 2 mm VAS), maximum brush-evoked pain intensity, as well as when the brush-evoked pain intensity had returned to baseline, calculating the total brush-evoked pain intensity as the integrated value of the graph over time, i.e., the area under the curve. In the present studies, aftersensation was defined as the time from cessation of each stimulus plus an additional 5 s to enable the subjects to fully estimate their brush-evoked pain intensity and until the brush-evoked pain intensity had returned to baseline. The subjects were carefully instructed to rate the intensity of brush-evoked allodynia separately from spontaneous ongoing pain.

Table 5. Nominal force and velocity values, applied force and velocity values calculated as mean ± SEM and range of applied force and velocity during the 18 stimuli in 16 patients with dynamic mechanical allodynia due to peripheral neuropathy (study IV)

Brushing stimulus	**Nominal force**	**Applied force, mean ± SEM**	**Applied force, range**	**Nominal velocity**	**Applied velocity, mean ± SEM**	**Applied velocity, range**
1	10	12.3 ± 0.50	8.9 – 15.9	10	10.7 ± 0.26	9.5 – 13.0
2	10	10.6 ± 0.39	8.0 – 13.2	10	11.5 ± 0.38	9.2 – 13.0
3	10	12.3 ± 0.39	8.8 – 14.8	20	19.9 ± 0.58	16.2 – 26.1
4	10	11.7 ± 0.40	7.8 – 14.1	20	19.6 ± 0.61	17.1 – 26.1
5	10	11.1 ± 0.57	7.0 – 15.7	30	27.4 ± 0.83	20.7 – 35.3
6	10	11.4 ± 0.82	7.0 – 19.2	30	27.9 ± 0.80	23.1 – 33.3
7	20	20.6 ± 0.30	18.2 – 22.7	10	11.1 ± 0.35	9.7 – 14.6
8	20	19.6 ± 0.43	14.6 – 22.4	10	11.2 ± 0.23	9.8 – 13.6
9	20	20.4 ± 0.52	18.1 – 26.5	20	19.1 ± 0.35	15.8 – 21.4
10	20	20.0 ± 0.54	16.7 – 24.5	20	18.5 ± 0.49	14.6 – 21.4
11	20	21.2 ± 0.53	17.9 – 24.9	30	26.9 ± 0.61	23.1 – 31.6
12	20	21.1 ± 0.79	16.9 – 28.3	30	26.5 ± 0.62	22.1 – 31.6
13	40	38.7 ± 0.71	33.1 – 44.2	10	11.0 ± 0.26	9.7 – 13.0
14	40	38.3 ± 0.63	33.6 – 43.4	10	10.8 ± 0.26	8.8 – 13.6
15	40	37.7 ± 1.31	32.0 – 50.9	20	18.7 ± 0.59	14.3 – 24.0
16	40	37.1 ± 1.13	27.5 – 44.9	20	18.4 ± 0.53	13.3 – 21.4
17	40	38.8 ± 0.90	31.1 – 44.9	30	26.9 ± 0.49	22.2 – 30.0
18	40	41.3 ± 1.15	34.3 – 48.9	30	27.2 ± 0.52	23.1 – 30.0

3.2.4.1 Study I

Brush-evoked allodynia was induced by lightly stroking different lengths (20, 40 and 60 mm) in the test area with one of three brushes of different widths (4, 8 and 16 mm) 2 or 4 times. A standardized protocol based on 18 stimuli defining the sequence of brushing length, width of the brush and number of strokes was used. Each three stimulus parameter combination was introduced ones, i.e., 18 stimuli altogether. To eliminate possible sequence dependency of stimuli the sequences were altered, starting with the 4 mm brush and finishing with the 16 mm brush in half of the subjects and vice versa for the other half. The protocol was completed in approximately 40 minutes.

3.2.4.2 Study II

With fixed stimulus parameters and identical assessment conditions, brush-evoked allodynia was induced by lightly stroking the skin 60 mm with 4 consecutive strokes using an 8 mm wide brush 4 times at each study day.

3.2.4.3 Study III

Brush-evoked allodynia was induced by lightly stroking different lengths (10 or 20 mm) in the test area with one of two brushes of different widths (4 or 16 mm) 2 or 4 times. A standardized protocol based on 16 brush stimuli (i.e., 8 stimuli in the area of painful neuropathy and 8 stimuli in the capsaicin-induced secondary hyperalgesic area, respectively) in the patients and on 8 brush stimuli in the controls was used.

3.2.4.4 Study IV

Each assessment of brush-evoked allodynia was induced by lightly stroking the skin for the full length of 60 mm, with 2 consecutive strokes using a 16 mm wide brush, providing the best practical prerequisites for a reproducible brushing stimulus. A standardized protocol based on 9 different stimulus combinations (18 stimuli) was used, composed from each 3 levels of stroking velocity and brushing force. Stroking velocities were 10, 20 or 30 mm/s, used in combination with brushing forces of 10, 20 or 40 g.

3.2.5 Assessment of sensory-discriminative and affective pain descriptors (Study I, III and IV)

A self-administered pain assessment tool, the Pain-O-Meter® (POM), has been developed for the purpose of improving assessment of multidimensional aspects of clinical acute and chronic pain (Gaston-Johansson, 1996). The POM is a hand-held plastic device, comprising two methods for pain assessment, a VAS for assessment of pain intensity and two separate lists of pain descriptors. The Swedish version used in the present studies consisted of 12 sensory-discriminative and 11 affective words. Following each stimulus of the protocol, the patients were asked to choose as many sensory-discriminative and affective descriptors as needed to adequately describe the pain experience. The Swedish sensory-discriminative and affective words used in the present studies are translated into English according to recent publications of Swedish studies using the POM (Hofgren et al., 1994) and a Swedish-English dictionary. The descriptors have been demonstrated to discriminate differences in pain intensity but were in these studies used only to describe the experience of pain without a hierarchic intensity rating. Test-retest reliability and concurrent as well as construct validity of the POM has been demonstrated in studies on American patients with acute or chronic pain, i.e., labour pain, rheumatoid arthritis and postoperative pain (Gaston-Johansson, 1996).

3.2.6 Experimental pain model - intradermal injection of capsaicin (Study III)

A solution of capsaicin (6 mg/ml) and polysorbat 80 diluted in isotonic saline prepared as described previously (Gazerani et al., 2005) was injected intradermally (Fig. 3). Guided by how the injection-induced pain intensity was tolerated in the patients, either 120 µg (20 µl) or 60 µg (10 µl) capsaicin was injected (Table 3). The matched controls were given identical amounts of capsaicin as the corresponding patient. The injections were performed manually with the use of a 0.3 ml plastic

syringe and a 30 gauge needle. The rational for injecting the patients with intradermal capsaicin contralateral to the area of painful neuropathy was to secure an intra-individual evaluation of psychophysical parameters comparing the clinical with the experimental situation.

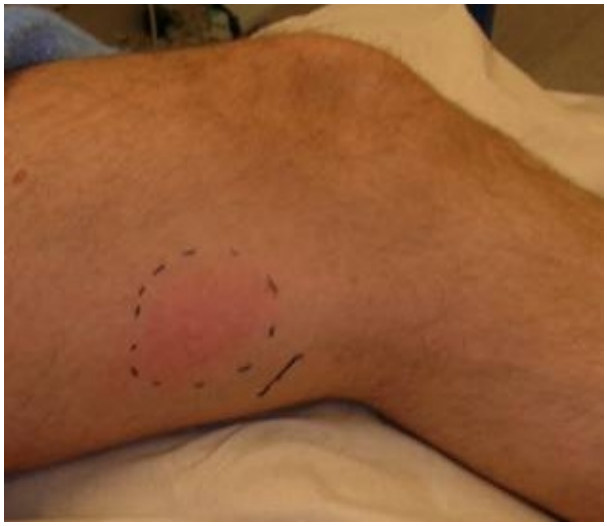

Fig. 3. Primary (flare) and secondary hyperalgesic area (outside the hatched circle) after intradermal injection of capsaicin.

3.3 STATISTICS

Table 6. Statistical methods used for analysis of assessed parameters in study I – IV

Statistical method/ assessed parameter	**Spontaneous Pain**	**Spontaneous pain/ brush-evoked pain; relation**	**Brush-evoked pain; total pain intensity**	**Brush-evoked pain; aftersensation**	**Brush-evoked pain; max VAS/ aftersensation**
One-way ANOVA	II		II		
Two-way ANOVA			IV		
Three-way ANOVA			I		
Four-way ANOVA			III		
Five-way ANOVA			III		
GENMOD procedure				I, III, IV	
Spearman rank order correlation		II			I, III
Intra-class correlation coefficient, $ICC_{2,1}$	II		II		
Mann-Whitney U-test			I	I	
Sign test	III			III	

3.3.1 Study I

The data from assessments of the total pain intensity during brush-evoked allodynia were analyzed using a three-way ANOVA with repeated measures on three factors (Kirk, 1995). The factors were, 'brushing length' with three levels (20 mm, 40 mm and 60 mm), 'brush width' with three levels (4 mm, 8 mm and 16 mm) and 'number of strokes' with two levels (2 and 4 times). In case of a significant main effect, the LSD post-hoc test was performed to make all pair wise comparisons among means.

The duration of aftersensation (s) was categorised into four categories as the variable had a skewed distribution with many outcomes equal to zero. The data were analysed using analysis of variance (ANOVA) for repeated measures for ordinal responses (Procedure GENMOD in SAS®) (Stokes et al., 2000). The model was set up with the within factors 'brushing length' (20 mm, 40 mm and 60 mm), 'brush width' (4 mm, 8 mm and 16 mm) and 'number of strokes' (2 and 4 times). The estimates from the models were odds ratios and 95% confidence intervals.

The relationship between the duration of the painful aftersensation and the maximum rated pain intensity during assessments of brush-evoked allodynia was analysed with Spearman rank order correlation.

For post hoc analysis, Mann-Whitney U-test was used for comparison of the total brush-evoked pain intensity and the duration of the aftersensation between two subgroups of patients, i.e., patients with and without signs of heat allodynia.

Statistical significance was accepted at $P \leq 0.05$.

3.3.2 Study II

For analysis of the variation between repeated measurements of the spontaneous ongoing pain as well as of the total brush-evoked pain intensity within and between days one-way repeated measures analysis of variance (ANOVA) was used. Intra-class correlation coefficient ($ICC_{2,1}$) was then calculated from the variance estimates obtained from the ANOVA and interpreted according to established grading criteria: < 0.20 poor, 0.21 – 0.40 fair, 0.41 – 0.60 moderate, 0.61 – 0.80 good and 0.81 – 1.00 very good (Altman, 1991; Shrout and Fleiss, 1979).

The relationship between the intensity of spontaneous ongoing pain and the total brush-evoked pain intensity was calculated using Spearman rank order correlation coefficient (Siegel and Castellan, 1988).

3.3.3 Study III

Data from assessments of the total pain intensity during brush-evoked pain in patients were analyzed using a four-way analysis of variance (ANOVA) with repeated measures on four factors (Kirk, 1995). The factors were, 'side' with two levels (area of painful neuropathy and of capsaicin-induced secondary hyperalgesia), 'brushing length' with two levels (10 mm and 20 mm), 'brush width' with two levels (4 mm and 16 mm) and 'number of strokes' with two levels (2 and 4 times). In addition, a five-way ANOVA with repeated measures on the aforementioned factors and a between-groups factor 'dose of injected capsaicin' (120 µg or 60 µg) was used. In the statistical analysis a non-painful stimulus was included and calculated as a zero value.

The data from assessments of the duration of aftersensation of brush-evoked pain in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in patients was analyzed using the Sign test.

Furthermore, the aftersensation was categorised into two categories as the variable had a skewed distribution with many outcomes equal to zero. The categories were 'aftersensation' or 'no aftersensation'. The data were analysed by a generalized estimating equations (GEE) model with the GENMOD procedure in SAS® (Stokes et al., 2000). The GEE strategy is a useful approach for repeated measurements analysis of ordered categorical- and binominal outcomes in a longitudinal study. The model was set up with the within factors 'side' (area of painful neuropathy and the capsaicin-induced secondary hyperalgesic area), 'brushing length' (10 mm and 20 mm), 'brush width' (4 mm and 16 mm) and 'number of strokes' (2 and 4 times). The estimates from the models were odds ratios and 95% confidence intervals.

The relationship between the duration of the painful aftersensation and the maximum rated pain intensity during assessments of brush-evoked pain was analysed with Spearman rank order correlation.

Sign test was used to analyze data from assessments of spontaneous ongoing pain 1 min after the intradermal injection of capsaicin in patients and their matched controls.

Statistical significance was accepted at $P \leq 0.05$.

3.3.4 Study IV

Data from assessments of the total pain intensity during brush-evoked allodynia were analyzed using a two-way analysis of variance (ANOVA) with repeated measures on two factors (Kirk, 1995). The factors were, 'brushing force' with three levels (10, 20 and 40 g), and 'stroking velocity' with three levels (10, 20 and 30 mm/s). In the statistical analysis a non-painful stimulus was included and calculated as a zero value. In case of a significant main effect, post-hoc test with pair wise T-tests was performed.

The aftersensation was categorised into two categories as the variable had a skewed distribution with many outcomes equal to zero. The categories were 'aftersensation' or 'no aftersensation'. The data were analysed by a generalized estimating equations (GEE) model with the GENMOD procedure in SAS® (Stokes et al., 2000). The model was set up with the within factors 'brushing force' (10, 20 and 40 g) and 'stroking velocity' (10, 20 and 30 mm/s). The estimates from the models were odds ratios and 95% confidence intervals.

Statistical significance was accepted at $P \leq 0.05$.

4 RESULTS

4.1 STUDY I

4.1.1 Quantitative sensory testing

All patients demonstrated sensory abnormalities within the proper innervation territory of the damaged peripheral nervous structure at both bedside examination and QST (Table 7). There was no difference in skin temperature between the dynamic mechanical allodynia site and the corresponding homologous site (data not shown). Nine patients reported lower heat pain threshold in the area of dynamic mechanical allodynia compared to the homologous contralateral site with a temperature difference ranging from 1.7 – 12.9 ºC. Ten patients reported a higher cold pain threshold in the area of dynamic mechanical allodynia compared to the homologous contralateral site with a temperature difference ranging from 0.8 – 21.6 ºC. Five patients reported both types of alterations.

Table 7. Results from quantitative sensory testing of non-nociceptive and nociceptive perception thresholds (absolute values) in the area of dynamic mechanical allodynia (dma) and in the corresponding contralateral (cont lat) area in 18 patients with dma due to peripheral neuropathy

Patient Sex	Δ CT (ºC) dma	Δ CT (ºC) cont lat	Δ WT (ºC) dma	Δ WT (ºC) cont lat	HPT (ºC) dma	HPT (ºC) cont lat	CPT (ºC) dma	CPT (ºC) cont lat	LTT (g) dma	LTT (g) cont lat
1 F	5.5	0.8	13.0	5.5	48.1	47.0	11.9	16.3	0.34	0.03
2 F	1.2	1.9	2.1	2.0	39.0	46.9	27.8	10.0	0.34	0.03
3 M	0.9	2.6	3.1	15.8	37.1	50.0	26.9	10.0	0.34	0.34
4 F	8.1	3.5	no warmth	3.6	50.0	40.3	10.0	10.0	2.1	0.34
5 M	23.1	20.2	no warmth	14.3	50.0	49.5	10.0	10.0	3.58	0.34
6 F	6.4	1.7	9.7	4.2	49.1	40.2	10.0	10.0	2.1	0.18
7 M	4.7	8.8	9.1	5.5	49.3	45.8	23.1	10.0	2.1	0.34
8 F	8.0	3.6	11.0	14.3	33.8	44.6	16.3	23.8	2.1	0.34
9 F	1.1	1.5	4.4	2.4	50.0	43.0	31.6	10.0	0.03	0.03
10 F	1.8	1.8	7.7	3.4	43.7	48.6	24.7	10.0	0.03	0.03
11 F	6.6	2.2	6.5	4.2	49.8	44.4	14.4	10.0	3.58	0.34
12 M	1.8	1.0	3.9	2.6	50.0	50.0	25.8	10.0	0.64	0.34
13 F	1.3	1.3	2.3	2.6	36.8	39.0	25.4	27.1	0.64	0.34
14 F	2.0	3.4	14.3	11.3	45.8	50.0	24.8	24.0	8.5	2.1
15 M	2.2	1.0	2.6	2.6	41.0	47.3	21.8	10.0	0.03	0.03
16 F	3.0	2.9	16.2	13.5	50.0	49.4	10.0	10.6	2.1	0.34
17 M	1.1	1.6	2.6	1.8	36.4	49.2	27.9	24.0	0.03	0.03
18 M	15.4	1.5	7.8	5.8	48.0	49.7	10.0	10.0	3.58	2.1

CT, cold perception threshold; WT, warm perception threshold; Δ °C, the average temperature difference from baseline (skin temperature); HPT, heat pain threshold; CPT, cold pain threshold; LTT, light touch perception threshold.

4.1.2 The relationship between total brush-evoked pain intensity and temporo-spatial stimulus parameters

Sixteen patients reported brush-evoked allodynia during the 18 stimuli. One patient reported brush-evoked allodynia during 4 and one patient during 15 out of 18 stimuli. In the three-way ANOVA there was no significant interaction between the three factors brushing length, brush width and number of strokes. In the post hoc analysis, there was no significant difference in total brush-evoked pain intensity between the two subgroups of patients, with and without signs of lowered heat pain threshold in the neuropathic area with dynamic mechanical allodynia (data not shown).

4.1.2.1 Brushing lengths (20, 40 or 60 mm)

Significantly higher total brush-evoked pain intensity was demonstrated with increased brushing length (F (2, 34) = 29.09, $P < 0.001$). The total brush-evoked pain intensity induced by brushing 20 mm was significantly separated from 40 and 60 mm ($P < 0.001$ and $P < 0.001$, respectively), respectively and 40 mm was significantly separated from 60 mm ($P < 0.01$) (Fig. 4).

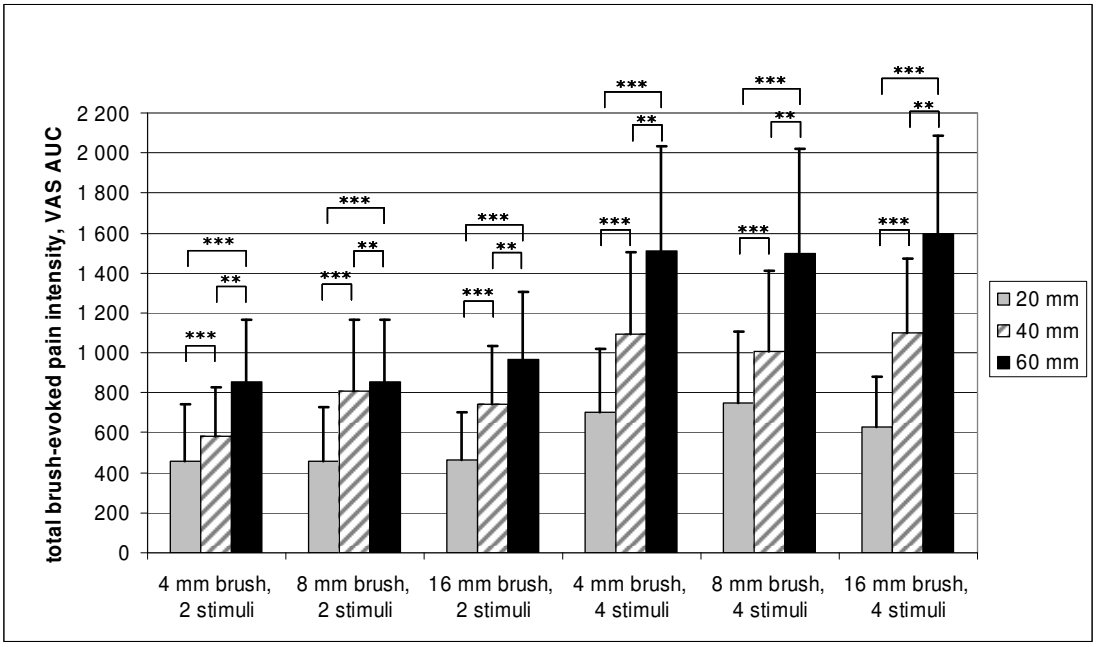

Fig. 4. The relationship between brushing lengths (20, 40 or 60 mm) and brush-evoked pain following 2 or 4 strokes using brushes of various width (4, 8 or 16 mm) in 18 patients with dynamic mechanical allodynia due to peripheral neuropathy. Mean VAS ratings of total brush-evoked pain intensity ± SEM (area under the curve (AUC)) is presented. In the three-way ANOVA significant differences are indicated by P-values in the figure (** $P < 0.01$, *** $P < 0.001$).

4.1.2.2 Number of strokes (2 or 4)

Significantly higher total brush-evoked pain intensity was found for 4 strokes compared with 2 strokes (F (1, 17) = 37.52, $P < 0.001$) (Fig. 5).

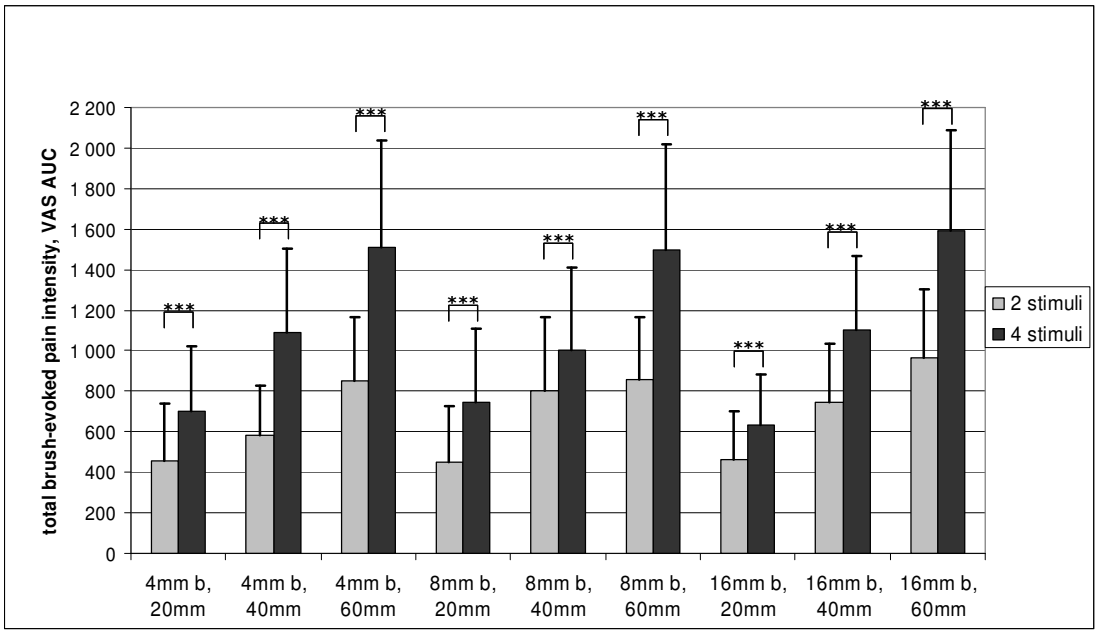

Fig. 5. The relationship between number of strokes (2 or 4) and brush-evoked pain following brushing of different lengths (20, 40 or 60 mm) using brushes of various width (4, 8 or 16 mm) in 18 patients with dynamic mechanical allodynia due to peripheral neuropathy. Mean VAS ratings of total brush-evoked pain intensity $\pm$ SEM (area under the curve (AUC)) is presented. In the three-way ANOVA significant differences are indicated by *P*-values in the figure (*** $P < 0.001$).

4.1.2.3 Brush widths (4, 8 or 16 mm)

Altering brush width did not significantly affect the total brush-evoked pain intensity.

4.1.2.4 The relationship between brushing area (i.e., brush length and width) and total brush-evoked pain intensity

Activation of two equivalent areas of 160 mm², i.e., brushing the skin 2 or 4 times using a thin brush (4 mm) over a longer distance (40 mm) resulted in higher total brush-evoked pain intensity than using a wider brush (8 mm) over a shorter distance (20 mm). In addition, activation of two equivalent areas of 320 mm², i.e., brushing the skin 2 or 4 times using a narrow brush (8 mm) over a longer distance (40 mm) resulted in higher total brush-evoked pain intensity than using a thick brush (16 mm) over a shorter distance (20 mm) (Fig. 6).

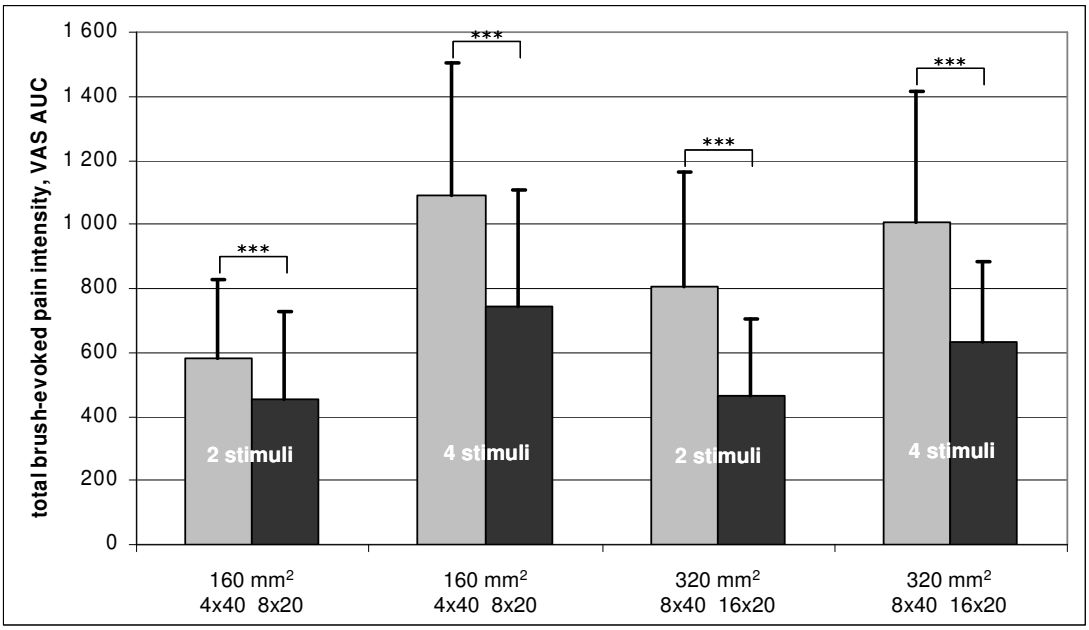

Fig. 6. The relationship between brushing area and brush-evoked pain in 18 patients suffering from dynamic mechanical allodynia due to peripheral neuropathy. For details see text. Mean VAS ratings of total brush-evoked pain intensity ± SEM (area under the curve (AUC)) is presented. Significant differences are indicated by *P*-values in the figure (*** $P < 0.001$).

4.1.3 The relationship between the duration of painful aftersensation (s) and temporo-spatial stimulus parameters

Aftersensation was reported during 12-18 stimuli by 13 patients, during 7-11 stimuli by 2 patients and during 1-6 stimuli by 2 patients. One patient reported no aftersensation. Two typical curves of brush-evoked pain development obtained from one patient with and one without aftersensation are depicted in Fig. 7.

In the three-way ANOVA, there was no significant interaction between the three factors brushing length, number of strokes and brush width. In the post hoc analysis, there was no significant difference in duration of aftersensation between the two subgroups of patients, with and without signs of lowered heat pain threshold in the neuropathic area with dynamic mechanical allodynia (data not shown).

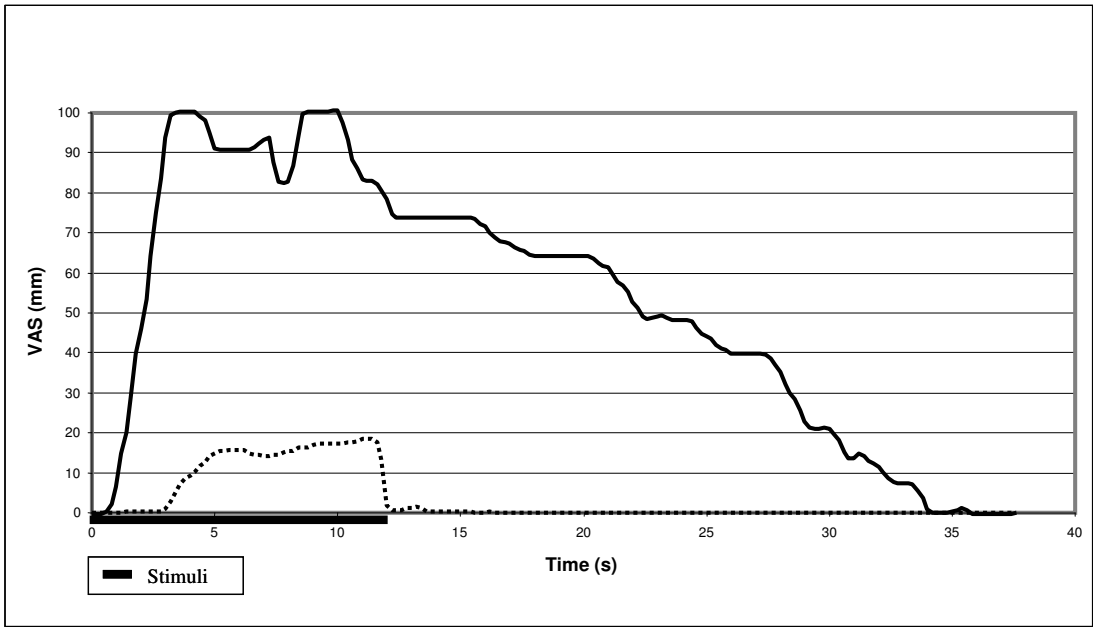

Fig. 7. Temporal profile of brush-evoked pain intensity, in two patients with peripheral neuropathy and dynamic mechanical allodynia, one with and one without aftersensation, induced by brushing the skin two 60 mm strokes using a brush with a width of 4 mm.

4.1.3.1 Brushing lengths (20, 40 or 60 mm)

Significantly increased duration of aftersensation was demonstrated with increased brushing length ($P < 0.008$). The ratio for a shorter aftersensation was 2.5 times higher following a brushing length of 20 mm compared with 60 mm ($P < 0.001$) and 1.7 times higher following a brushing length of 40 mm compared with 60 mm ($P < 0.001$). There was no significant difference in painful aftersensation following a brushing length of 20 mm compared with 40 mm.

4.1.3.2 Number of strokes (2 or 4) and brush widths (4, 8 or 16 mm)

Number of strokes and brush width did not significantly affect the duration of the aftersensation.

4.1.4 The relationship between the duration of painful aftersensation (s) and the maximum pain intensity (mm)

A significant positive correlation was demonstrated between the duration of painful aftersensation and the maximum brush-evoked pain intensity for the various temporo-spatial stimulus parameters in the protocol, with the exception of stroking twice a length of 20 mm using a brush with a width of 4 mm (r_s = 0.55-0.85, $P < 0.02$).

4.1.5 Sensory-discriminate and affective pain descriptors

The most commonly used sensory-discriminative descriptors during brush-evoked allodynia were pricking (24%), burning (16%), and sore (11%) and for the affective descriptors annoying (30%) and troublesome (28%) (Table 8).

Table 8. Results, absolute values and relative frequencies of sensory-discriminative and affective pain descriptors from 18 patients with dynamic mechanical allodynia due to peripheral neuropathy using the Swedish version of the Pain-O-Meter

Sensory-discriminative words	**Absolute values, relative frequencies (%)**	**Affective words**	**Absolute values, relative frequencies (%)**
Cutting	52 (9)	Annoying	155 (30)
Dull	40 (7)	Terrifying	2 (0)
Pricking	140 (24)	Troublesome	145 (28)
Squeezing	3 (1)	Suffocating	13 (3)
Cramping	16 (3)	Killing	14 (3)
Tearing	36 (6)	Intolerable	30 (6)
Aching	51 (9)	Fearful	37 (7)
Smarting	16 (3)	Tiring	27 (5)
Burning	95 (16)	Nagging	17 (3)
Sore	66 (11)	Unbearable	43 (8)
Gnawing	19 (3)	Torturing	35 (7)
Pressing	48 (8)		
Sum	582 (100)		518 (100)

4.2 STUDY II

The two first sessions were performed on day 1 and 3 in all patients. The third session was performed on day 27 in one patient and on day 29 in two patients. The fourth session was performed in three patients on day 28, 31 and 38, respectively. In all but two patients, all assessments were made at the same time of the day. In the remaining two patients the time of examination deviated between 3 and 7 hours from schedule.

All patients reported spontaneous ongoing pain as well as brush-evoked allodynia during all 16 assessments. The mean VAS-ratings and range of the spontaneous ongoing pain intensity and the maximum intensity of the brush-evoked allodynia are presented in Table 9.

In the one-way ANOVA there was no significant difference in the total brush-evoked pain intensity or the spontaneous ongoing pain intensity within or between days.

Table 9. Mean VAS-ratings and range of the spontaneous ongoing pain intensity rated before each assessment of brush-evoked allodynia and the maximum intensity of the brush-evoked allodynia for the 16 assessments, respectively, in 9 patients with peripheral neuropathy (VAS translated to 0-100 mm)

Patient	**Spontaneous ongoing pain intensity Mean (mm)**	**Spontaneous ongoing pain intensity Range (mm)**	**Maximum intensity of brush-evoked allodynia Mean (mm)**	**Maximum intensity of brush-evoked allodynia Range (mm)**
1	34.9	24 - 48	26.4	9.6 – 39.9
2	56.7	44 - 86	71.0	50.4 – 92.6
3	49.9	37 - 59	30.9	14.5 – 40.9
4	76.9	65 - 89	15.8	6.5 – 27.5
5	42.6	27 - 55	53.0	41.4 – 75.8
6	16.1	12 - 20	12.9	8.9 – 17.5
7	29.8	20 - 39	38.2	27.4 – 48.1
8	80.8	74 - 92	96.9	68.1- 100
9	15.1	10 - 20	8.4	2.3 – 15.1

4.2.1 Repeatability of total brush-evoked pain intensity within days

The repeatability of the total brush-evoked pain intensity for the four consecutive assessments (a, b, c, d) within day 1, 3, 28 and 30 was “very good” ($ICC_{2,1}$ = 0.89, 0.93, 0.93 and 0.95, respectively). The individual assessments of the total brush-evoked pain intensity within days are presented in Fig. 8.

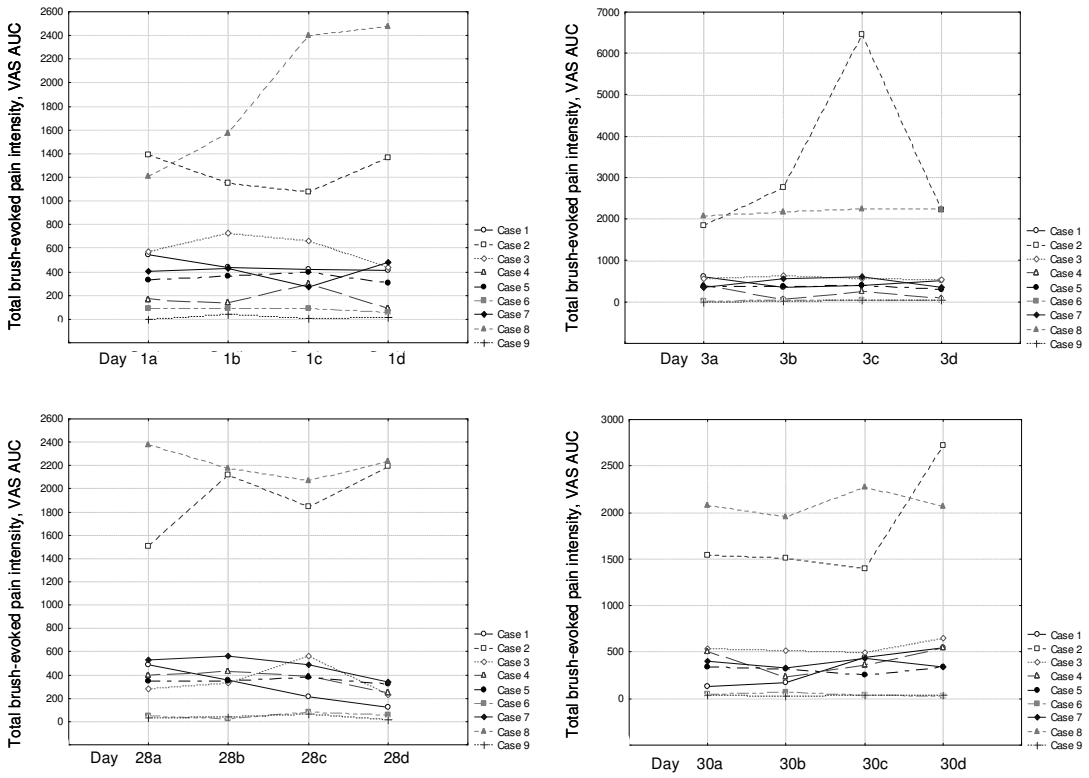

Fig. 8. The total brush-evoked pain intensity during four assessments (a, b, c, d) with an inter-stimulus interval of 10 min within day 1, 3, 28 and 30 in 9 patients with peripheral neuropathy. AUC; area under the curve.

4.2.2 Repeatability of spontaneous ongoing pain intensity within days

The repeatability of the spontaneous ongoing pain intensity for the four consecutive assessments (a, b, c, d) within day 1, 3, 28 and 30 was "very good" ($ICC_{2,1}$ = 0.92, 0.91, 0.96 and 0.93, respectively).

4.2.3 Repeatability of total brush-evoked pain intensity between days

The repeatability of the total brush-evoked pain intensity between all days was "very good" ($ICC_{2,1}$ = 0.86 - 0.92) (Table 10). The individual assessments of the total brush-evoked pain intensity between days are presented in Fig. 9. The short term repeated assessments of the total brush-evoked pain intensity (i.e. between day 1 and 3; between day 28 and 30) ($ICC_{2,1}$ = 0.84 - 0.97) as well as the long term repeated assessments (i.e. between day 1 and 28; between day 3 and 30) ($ICC_{2,1}$ = 0.77 - 0.94) were "very good" (Table 10).

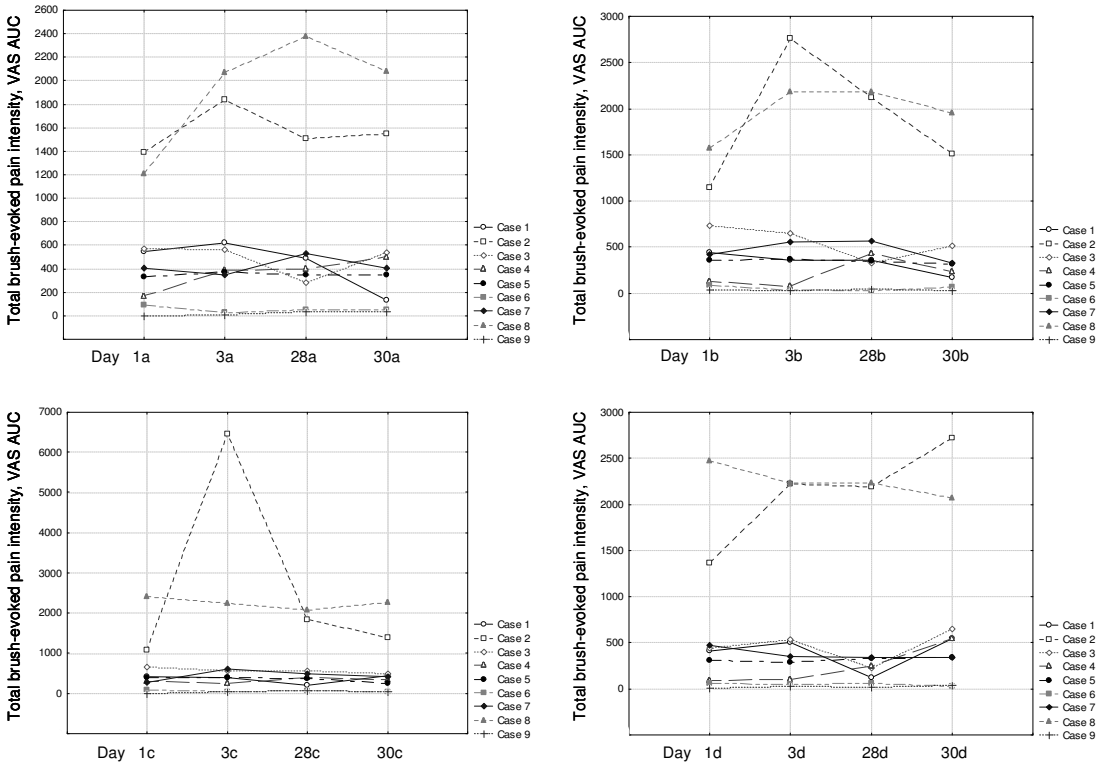

Fig. 9. The first (a), second (b), third (c) and fourth (d) assessment of total brush-evoked pain intensity at day 1, 3, 28 and 30 in 9 patients with peripheral neuropathy. AUC; area under the curve.

Table 10. The repeatability of the spontaneous ongoing pain intensity and the total brush-evoked pain intensity between all days (i.e. day 1a, 3a, 28a and 30a; 1b, 3b, 28b and 30b; 1c, 3c, 28c and 30c; 1d, 3d, 28d and 30d) as well as short- and long-term repeated assessments analysed with intra-class correlation coefficient ($ICC_{2,1}$) in 9 patients with peripheral neuropathy

	Assessment a ICC2,1	**Assessment b ICC2,1**	**Assessment c ICC2,1**	**Assessment d ICC2,1**
Day 1, 3, 28, 30	*0.93*	*0.87*	*0.96*	*0.95*
Between all days	0.86	0.92	0.88	0.91
Day 1 - 3	*0.93*	*0.79*	*0.95*	*0.97*
Short term	0.93	0.94	0.84	0.97
Day 28 - 30	*0.97*	*0.98*	*0.97*	*0.98*
Short term	0.93	0.92	0.95	0.87
Day 1 – 28	*0.91*	*0.92*	*0.92*	*0.94*
Long term	0.77	0.87	0.80	0.93
Day 3 – 30	*0.93*	*0.84*	*0.98*	*0.93*
Long term	0.88	0.92	0.94	0.92

The first number in each pair (in italics) represents the spontaneous ongoing pain and the second number the brush-evoked pain.

4.2.4 Repeatability of spontaneous ongoing pain intensity between days

The repeatability of the spontaneous ongoing pain intensity between all days was "very good" ($ICC_{2,1}$ = 0.87 - 0.96) (Table 10). The short term repeated assessments of the spontaneous ongoing pain intensity (i.e. between day 1 and 3; between day 28 and 30) ($ICC_{2,1}$ = 0.79 - 0.98) as well as the long term repeated assessments (i.e. between day 1 and 28; between day 3 and 30) ($ICC_{2,1}$ = 0.84 - 0.98) were "very good" (Table 10).

4.2.5 Relationship between intensity of spontaneous ongoing pain and total brush-evoked pain intensity

A significant positive correlation was demonstrated between the spontaneous ongoing pain intensity and the total brush-evoked pain intensity for 10 out of the 16 assessments (r_s = 0.56 - 0.88, $P < 0.042$) (Table 11). Such a correlation was also found between the mean intensity of spontaneous ongoing pain and the mean of total brush-evoked pain intensity for all 16 assessments collectively ($r_s = 0.68$, $P < 0.042$) (Fig. 10).

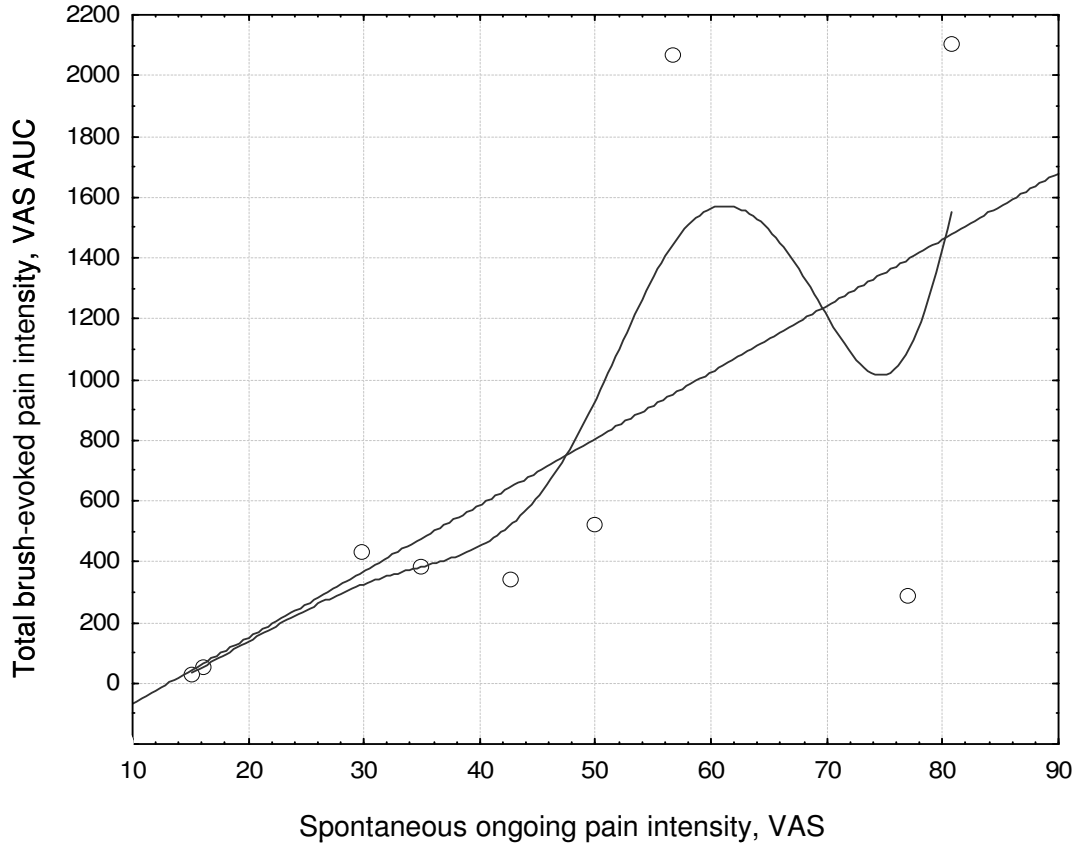

Fig. 10. The correlation between the mean intensity of spontaneous ongoing pain and the mean of total brush-evoked pain intensity for all 16 assessments collectively in 9 patients with peripheral neuropathy. AUC; area under the curve. The correlation is presented with two curves, one distance weighted and one linear.

Table 11. The correlation between the spontaneous ongoing pain intensity and the total brush-evoked pain intensity for the 16 assessments was analysed with Spearman rank order correlation in 9 patients with peripheral neuropathy

Assessments	Spearman (r_s)	p-level
Day 1 a	0.78	0.013
Day 1 b	0.70	0.036
Day 1 c	0.73	0.026
Day 1 d	0.68	0.042
Day 3 a	0.70	0.036
Day 3 b	0.57	0.112
Day 3 c	0.57	0.112
Day 3 d	0.56	0.116
Day 28 a	0.73	0.025
Day 28 b	0.58	0.104
Day 28 c	0.68	0.042
Day 28 d	0.60	0.088
Day 30 a	0.88	0.002
Day 30 b	0.71	0.032
Day 30 c	0.73	0.025
Day 30 d	0.65	0.058

The Spearman rank order correlation coefficient was significant for values $P < 0.05$.

4.3 STUDY III

4.3.1 The relationship between the total brush-evoked pain intensity (AUC; area under the curve) and temporo-spatial stimulus parameters in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in patients

All patients reported brush-evoked pain in their area of painful neuropathy during all stimuli. Six out of 9 patients reported brush-evoked pain in an area outside the flare during all stimuli and 2 patients during 6 and 7 out of 8 stimuli, respectively. The area of secondary hyperalgesia developed in the 8 patients with a latency of 7-22 min and lasted up to 38 min (range 17 – 38 min). Data from the total brush-evoked pain intensity during different stimulus combinations in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in patients is presented in Fig. 11.

In the four- and five-way ANOVA there was no significant interaction between the factors side, brushing length, brush width, number of strokes and dose of injected capsaicin.

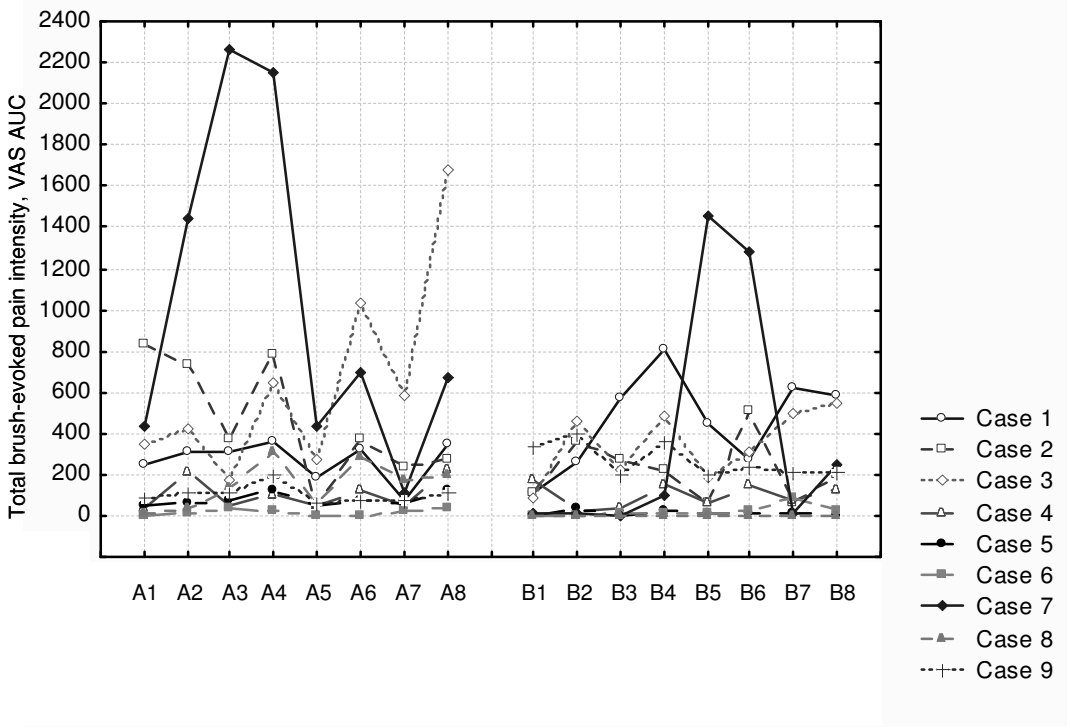

Fig. 11. Total brush-evoked pain intensity at different stimulus combinations in the area of painful neuropathy (A) and in the capsaicin-induced secondary hyperalgesic area (B) in 9 patients. The different stimulus combinations were **1**; 4 mm brush, 2 strokes, 10 mm brushing length. **2**; 4 mm brush, 4 strokes, 10 mm brushing length. **3**; 4 mm brush, 2 strokes, 20 mm brushing length. **4**; 4 mm brush, 4 strokes, 20 mm brushing length. **5**; 16 mm brush, 2 strokes, 10 mm brushing length. **6**; 16 mm brush, 4 strokes, 10 mm brushing length. **7**; 16 mm brush, 2 strokes, 20 mm brushing length and **8**; 16 mm brush, 4 strokes, 20 mm brushing length. Data is presented as total brush-evoked pain intensity, AUC (area under the curve).

4.3.1.1 Side (area of painful neuropathy and capsaicin-induced secondary hyperalgesic area)

There was no significant difference between sides regarding the relationship between the total brush-evoked pain intensity and the various temporo-spatial stimulus parameters (brushing length, brush width and number of strokes).

4.3.1.2 Number of strokes (2 or 4)

Significantly higher total brush-evoked pain intensity was demonstrated for 4 compared to 2 strokes in both sides (F (1, 8) = 20.61, $P < 0.01$) (Fig. 12).

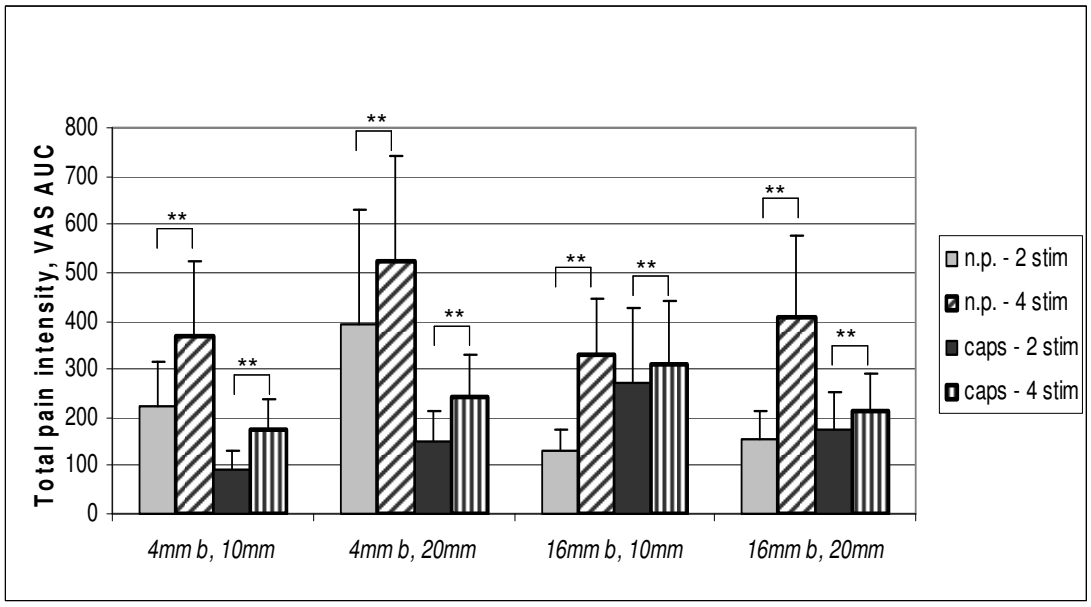

Fig. 12. The relationship between number of strokes and the total brush-evoked pain intensity following brushing of different lengths using brushes of various widths in neuropathic (n.p.) and capsaicin induced pain (caps) in patients; n=9. Mean VAS ratings of total brush-evoked pain intensity ± SEM (area under the curve (AUC)) is presented. In the four-way ANOVA significant differences are indicated by *P*-values in the figure (** $P < 0.01$). b; brush widths (4 or 16 mm). mm; brushing lengths (10 or 20 mm). stim; number of strokes (2 or 4).

4.3.1.3 Brushing lengths (10 or 20 mm)

Altering brushing length did not significantly affect the total brush-evoked pain intensity in any side.

4.3.1.4 Brush widths (4 or 16 mm)

Altering brush widths did not significantly affect the total brush-evoked pain intensity in any side.

4.3.1.5 Dose of injected capsaicin (120 µg or 60 µg)

Six patients were injected with 120 µg and 3 patients with 60 µg capsaicin. Regardless of injected dose there was no significant difference in the relationship between the total brush-evoked pain intensity and the various temporo-spatial stimulus parameters.

4.3.2 The relationship between the total brush-evoked pain intensity (AUC; area under the curve) and temporo-spatial stimulus parameters in the capsaicin-induced secondary hyperalgesic area in patients and their controls

Three out of 9 controls reported brush-evoked pain in an area outside the flare during 4, 5 and 6 out of 8 stimuli, respectively. The area of secondary hyperalgesia developed in the 3 controls with a latency of 7-11 min and lasted up to 22 min (range 19–22 min). Data from the total brush-evoked pain intensity during different stimulus

combinations in the capsaicin-induced secondary hyperalgesic area in patients and controls is presented in Fig. 13. No statistical analysis was done due to the few controls reporting an area of secondary hyperalgesia.

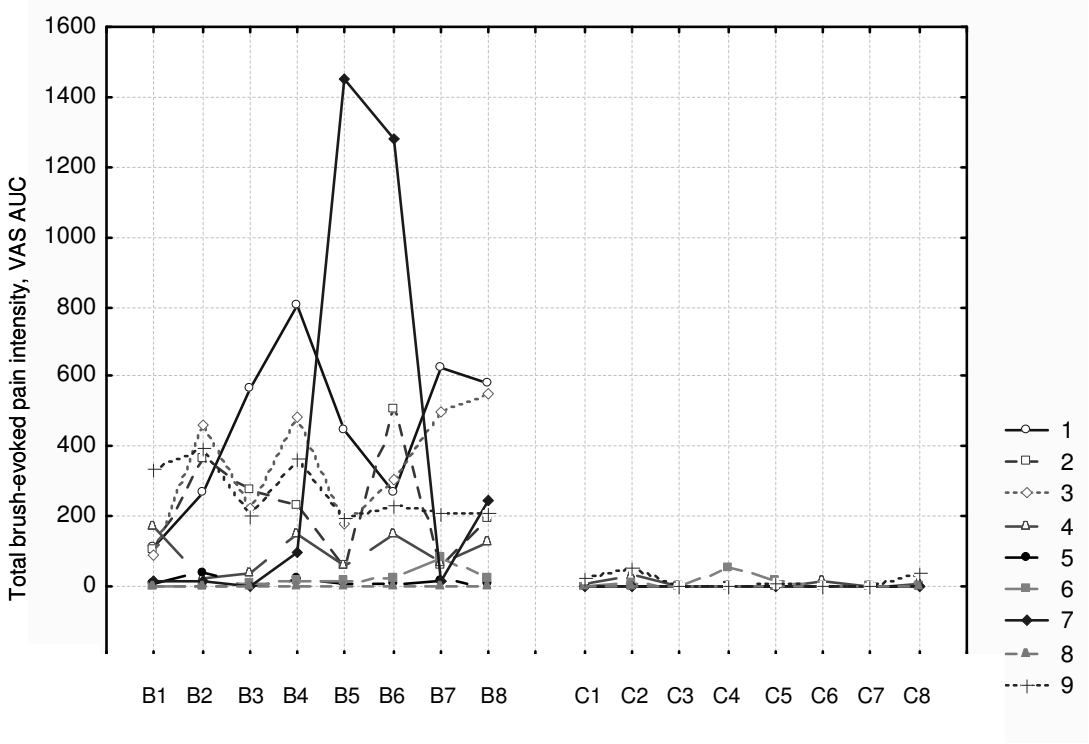

Fig. 13. Total brush-evoked pain intensity at different stimulus combinations in the capsaicin-induced secondary hyperalgesic area in 9 patients (B) and their matched controls (C). The different stimulus combinations were **1**; 4 mm brush, 2 strokes, 10 mm brushing length. **2**; 4 mm brush, 4 strokes, 10 mm brushing length. **3**; 4 mm brush, 2 strokes, 20 mm brushing length. **4**; 4 mm brush, 4 strokes, 20 mm brushing length. **5**; 16 mm brush, 2 strokes, 10 mm brushing length. **6**; 16 mm brush, 4 strokes, 10 mm brushing length. **7**; 16 mm brush, 2 strokes, 20 mm brushing length and **8**; 16 mm brush, 4 strokes, 20 mm brushing length. Data is presented as total brush-evoked pain intensity, AUC (area under the curve).

4.3.3 The relationship between the frequency and duration of painful aftersensation (s) after brushing stimuli and the different temporo-spatial stimulus parameters in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in patients

Aftersensation was reported during 5-8 stimuli in the area of painful neuropathy by 6 patients and in the capsaicin-induced secondary hyperalgesic area by 2 patients. During 1-4 stimuli 2 patients reported aftersensation in the area of painful neuropathy and 6 patients in the capsaicin-induced secondary hyperalgesic area. Lack of aftersensation was reported by 1 patient in the area of painful neuropathy.

The odds ratio for any aftersensation following a brushing length of 10 mm was 5.4 times higher in the area of painful neuropathy compared to the capsaicin-induced

secondary hyperalgesic area ($P < 0.05$). The odds ratio for any aftersensation in the area of painful neuropathy was 3 times higher following a brushing length of 10 mm compared to 20 mm ($P < 0.01$).

Significantly longer duration of aftersensation was demonstrated in the area of painful neuropathy compared to the capsaicin-induced secondary hyperalgesic area when brushing 2 or 4 strokes with a 4 mm brush for 10 mm, respectively ($P < 0.05$) (Fig. 14).

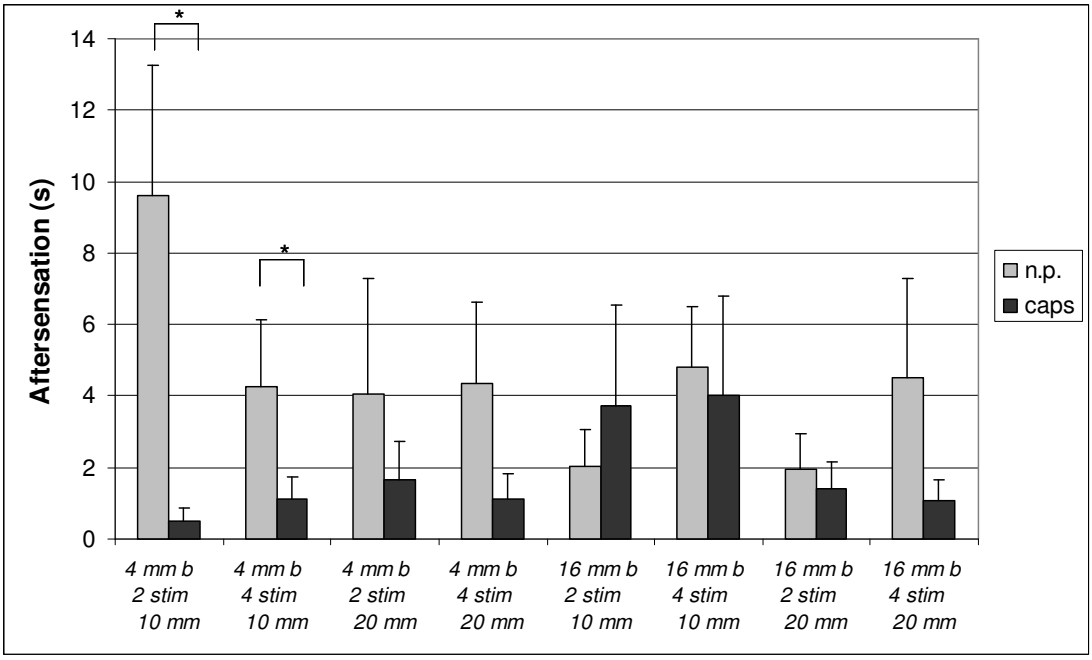

Fig. 14. Significantly longer duration of aftersensation (s) was demonstrated in the area of painful neuropathy (n.p.) compared to the capsaicin-induced secondary hyperalgesic area (caps) in patients; n=9 when brushing with a 4 mm brush, 10 mm brushing length using 2 or 4 strokes, respectively (* $P < 0.05$). Mean values of aftersensation (s) $\pm$ SEM is presented. b; brush widths (4 or 16 mm). stim; number of strokes (2 or 4). mm; brushing lengths (10 or 20 mm).

4.3.4 The relationship between duration of painful aftersensation (s) after brushing stimuli and maximum brush-evoked pain intensity (mm) in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in patients

A non-significant correlation was demonstrated between the duration of the painful aftersensation and the maximum brush-evoked pain intensity for the various temporo-spatial stimulus parameters in the area of painful neuropathy (r_s = 0.02-0.56) and in the capsaicin-induced secondary hyperalgesic area (r_s = 0.18-0.65).

4.3.5 The intensity of spontaneous ongoing pain in the capsaicin-induced secondary hyperalgesic area in patients and their controls

All subjects reported spontaneous ongoing pain after an intradermal injection of capsaicin. Significantly higher pain intensity was demonstrated in the capsaicin-induced secondary hyperalgesic area in patients compared to their controls ($P < 0.01$) (Table 12).

Table 12. Spontaneous ongoing pain intensity in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in 9 patients and their controls, respectively (VAS translated to 0-100 mm)

Patient/Controls	**Spontaneous ongoing pain due to neuropathy (VAS)**	**Capsaicin-induced ongoing pain in patients (VAS)**	**Capsaicin-induced ongoing pain in controls (VAS)**
1	82	94	42
2	61	88	22
3	42	100	39
4	36	92	51
5	29	51	30
6	24	100	29
7	77	100	28
8	28	48	16
9	37	97	45

4.3.6 Choice of sensory-discriminative and affective pain descriptors to characterize brush-evoked pain in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in patients and their controls

The most commonly used sensory-discriminative descriptors during brush-evoked pain in the area of painful neuropathy were smarting (15%) and burning (14%) and for the affective descriptors troublesome (36%) and annoying (28%). The most commonly used sensory-discriminative descriptors during brush-evoked pain in the capsaicin-induced secondary hyperalgesic area in patients were smarting (30%) and burning (23%) and for the affective descriptors troublesome (48%) and annoying (41%) The most commonly used sensory-discriminative descriptors (n=3) during brush-evoked pain in the capsaicin-induced secondary hyperalgesic area in controls were smarting (56%) and burning (13%) and for the affective descriptors (n=2) annoying (90%) (Table 13).

Table 13. Absolute values and relative frequencies of selected sensory-discriminative and affective pain descriptors in the area of painful neuropathy and in the capsaicin-induced secondary hyperalgesic area in 9 patients and their controls, respectively using the Swedish version of the Pain-O-Meter

Sensory-discriminative words	Absolute values and relative frequencies (%) neuropathy	capsaicin patients	capsaicin controls	Affective words	Absolute values and relative frequencies (%) neuropathy	capsaicin patients	capsaicin controls
Cutting	19 (11)	4 (3)	1(4,5)	Annoying	27 (28)	22 (41)	9 (90)
Dull	10 (6)	7 (6)	3 (13)	Terrifying	0 (0)	0 (0)	0 (0)
Pricking	22 (13)	16 (13)	1 (4,5)	Troublesome	34 (36)	26 (48)	0 (0)
Squeezing	3 (2)	0 (0)	0 (0)	Suffocating	1 (1)	0 (0)	0 (0)
Cramping	5 (3)	0 (0)	0 (0)	Killing	0 (0)	0 (0)	0 (0)
Tearing	0 (0)	0 (0)	0 (0)	Intolerable	1 (1)	0 (0)	0 (0)
Aching	19 (12)	11 (9)	1 (4,5)	Fearful	12 (13)	2 (4)	0 (0)
Smarting	24 (15)	36 (30)	13 (56)	Tiring	1 (1)	1 (2)	0 (0)
Burning	23 (14)	28 (23)	3 (13)	Nagging	0 (0)	0 (0)	0 (0)
Sore	22 (13)	15 (13)	0 (0)	Unbearable	11 (12)	3 (5)	0 (0)
Gnawing	1 (0)	0 (0)	0 (0)	Torturing	8 (8)	0 (0)	1 (10)
Pressing	18 (11)	4 (3)	1(4,5)				
Sum	166 (100)	121 (100)	23 (100)		95 (100)	54 (100)	10 (100)

4.4 STUDY IV

Fourteen out of 16 patients reported spontaneous ongoing pain before assessment of brush-evoked allodynia.

4.4.1 The relationship between the total brush-evoked pain intensity (AUC; area under the curve) and the various stimulus parameters

Fifteen out of 16 patients reported brush-evoked allodynia during all stimuli and one during 8 out of 9 stimulus combinations.

In the two-way ANOVA there was no significant interaction between the factors brushing force and stroking velocity.

4.4.1.1 Stroking velocity (10, 20 or 30 mm/s)

Significantly higher total brush-evoked pain intensity was demonstrated with a lower stroking velocity (F (2, 30) = 43.09, $P < 0.001$). In the post hoc analysis, the total brush-evoked pain intensity induced by a stroking velocity of 10 mm/s was significantly different from a stroking velocity of 20 or 30 mm/s ($P < 0.001$ and $P < 0.001$), respectively, and 20 mm/s was significantly different from 30 mm/s ($P < 0.01$) (Fig. 15).

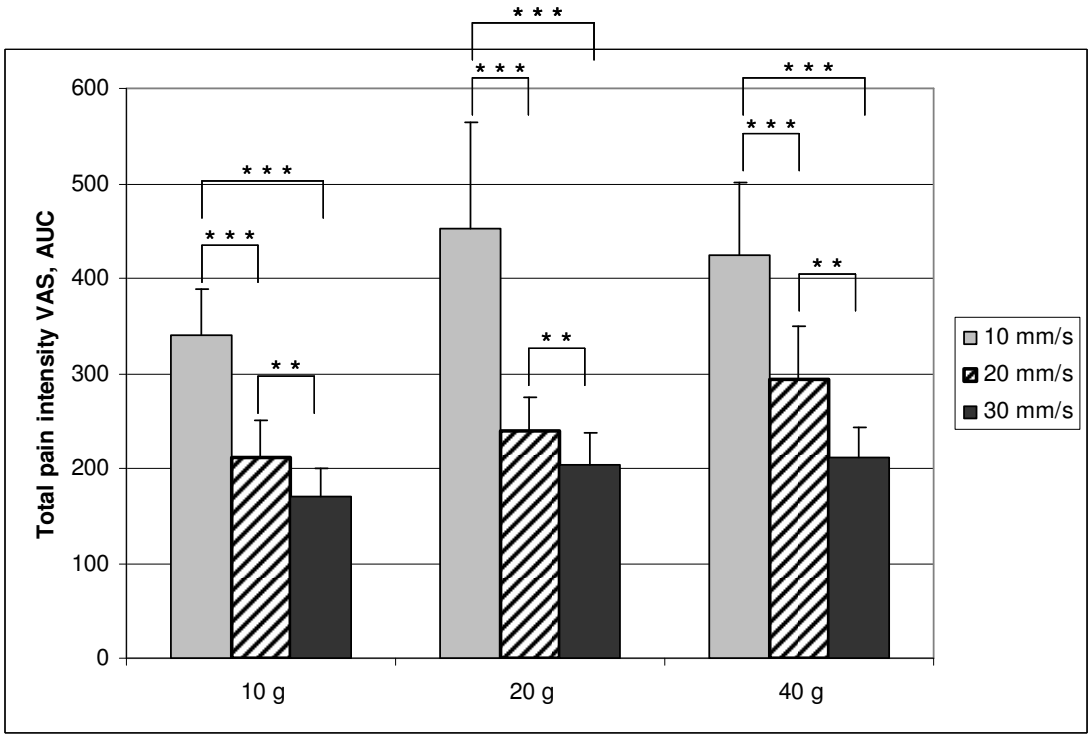

Fig. 15. The relationship between stroking velocity (10, 20 or 30 mm/s) and the total brush-evoked pain intensity following brushing with different forces (10, 20 or 40 g) in 16 patients with dynamic mechanical allodynia due to peripheral neuropathy. Mean VAS ratings of total brush-evoked pain intensity ± SEM (area under the curve (AUC)) is presented. In the two-way ANOVA significant differences are indicated by *P*-values in the figure (** $P < 0.01$, *** $P < 0.001$).

4.4.1.2 Brushing force (10, 20 or 40 g)

Significantly higher total brush-evoked pain intensity was demonstrated with a higher brushing force (F (2, 30) = 3.97, $P < 0.05$). In the post hoc analysis, the total brush-evoked pain intensity induced by a brushing force of 10 g was significantly different from a brushing force of 40 g ($P < 0.05$) (Fig. 16). There was no significant difference in the total brush-evoked pain intensity induced by 10 g compared to 20 g or the latter compared to 40 g, respectively.

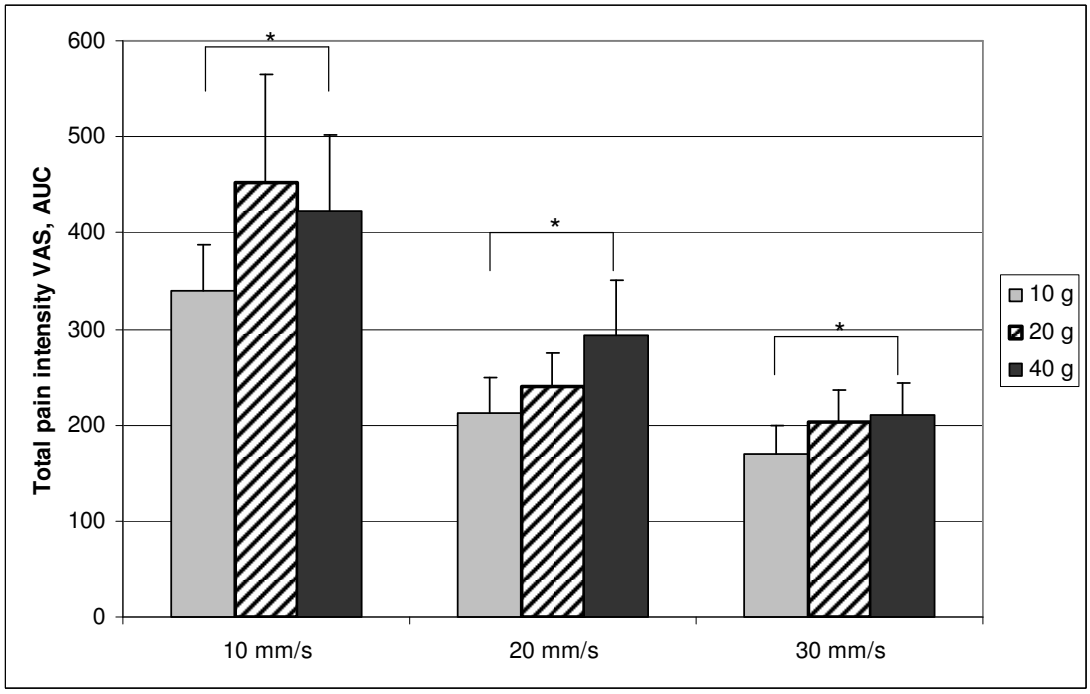

Fig. 16. The relationship between brushing force (10, 20 or 40 g) and the total brush-evoked pain intensity following brushing with different stroking velocities (10, 20 or 30 mm/s) in 16 patients with dynamic mechanical allodynia due to peripheral neuropathy. Mean VAS ratings of total brush-evoked pain intensity ± SEM (area under the curve (AUC)) is presented. In the two-way ANOVA significant differences are indicated by *P*-values in the figure (* $P < 0.05$).

4.4.2 The frequency of painful aftersensation after brushing with various stimulus parameters

Aftersensation was reported during 8 stimuli by 1 patient, during 4-6 stimuli by 2 patients and during 1-3 stimuli by 5 patients. Lack of aftersensation was reported by 8 patients.

In the GEE model there was no significant interaction between the two factors brushing force or stroking velocity. Alterations in brushing force or stroking velocity did not significantly change the frequency of aftersensation.

4.4.3 Choice of sensory-discriminative and affective pain descriptors to characterize brush-evoked pain

The most commonly used sensory-discriminative descriptors during brush-evoked allodynia were sore (14%), aching (13%) and pressing (11%) and for the affective descriptors troublesome (34%) and annoying (27%) (Table 14).

Table 14. Absolute values and relative frequencies of selected sensory-discriminative and affective pain descriptors from 16 patients with dynamic mechanical allodynia due to peripheral neuropathy using the Swedish version of the Pain-O-Meter

Sensory-discriminative words	**Absolute values and relative frequencies (%)**	**Affective words**	**Absolute values and relative frequencies (%)**
Cutting	45 (11)	Annoying	87 (27)
Dull	24 (6)	Terrifying	5 (2)
Pricking	43 (11)	Troublesome	109 (34)
Squeezing	29 (7)	Suffocating	14 (4)
Cramping	21 (5)	Killing	8 (2)
Tearing	5 (1)	Intolerable	20 (6)
Aching	55 (13)	Fearful	13 (4)
Smarting	41 (10)	Tiring	23 (7)
Burning	36 (9)	Nagging	17 (5)
Sore	56 (14)	Unbearable	10 (3)
Gnawing	7 (2)	Torturing	18 (6)
Pressing	47 (11)		
Sum	409 (100)		324 (100)

5 DISCUSSION

5.1 PRESUMED PATHOPHYSIOLOGY OF DYNAMIC MECHANICAL ALLODYNIA (STUDY I – IV)

Patients in this thesis where included based on their similar clinical phenomenology of dynamic mechanical allodynia at bedside examination. Allodynia was defined by the IASP (Merskey and Bogduk, 1994) solely based on the clinical presentation that a normally non-painful stimulus is perceived as painful, possible underlying pathophysiological mechanisms were not taken into account. Therefore, both peripheral and central mechanisms need to be considered (Hansson, 2003). For the following reasons we believe that the majority of patients in our study group were devoid of significant peripheral sensitization of mechanonociceptors as a basis for dynamic mechanical allodynia:

- No patient had overt signs of neurogenic inflammation/peripheral sensitization, i.e., reddening, swelling or temperature increase in the skin area affected by neuropathy compared to the contralateral homologous area.

- Allodynia to thermal and mechanical stimuli are common signs in patients with peripheral sensitization. In study I, only 9 out of the 18 patients reported lowered heat pain threshold in the neuropathic area with dynamic mechanical allodynia compared to the homologous contralateral site. The lack of correlation between these phenomena does not favour peripheral sensitization as a common denominator underlying the pathophysiology of dynamic mechanical allodynia in the majority of the study patients. Increased heat pain sensitivity may be due to either peripheral or central mechanisms, or a combination thereof and we cannot, of course, rule out the possibility of peripheral processes contributing to the lowered heat pain threshold in some of the patients. In addition, the post-hoc test of patients with and without a lowered heat pain threshold did not reveal any differences in total brush-evoked pain intensity or duration of aftersensation.

Based on these suggestions, it seems reasonable to assume that the dynamic mechanical allodynia at least in the majority of the study patients was due to peripheral activation of mechanoreceptive A-beta afferents with subsequent conversion of their message to the nociceptive system in the periphery (ephapses) and/or in the central nervous system. Current knowledge suggests that both fast and slowly adapting mechanoreceptive afferents may be recruited by a light moving non-painful mechanical stimulus (Johnson, 2001; Lundstrom, 2002).

Importantly, caution should be exercised when trying to fit our psychophysical results into a conceptual pathophysiological context since other afferents than low threshold A-beta mechanoreceptive fibres may be implicated in dynamic mechanical allodynia such as nociceptive A-beta fibres (Cain et al., 2001; Djouhri and Lawson, 2004), A-delta low-threshold mechanoreceptors (Adriaensen et al., 1983), C-fibre nociceptors with low mechanical threshold (Slugg et al., 2000) and low-threshold mechanoreceptive C-fibres (McGlone et al., 2007; Vallbo et al., 1993). The four abovementioned nociceptors/mechanoreceptors all have activation thresholds below the brushing forces used in the thesis studies and their involvement in dynamic mechanical allodynia could therefore not be out ruled.

5.2 ASSESSMENT OF DYNAMIC MECHANICAL ALLODYNIA

5.2.1 The relationship between dynamic mechanical allodynia and stimuli with varying characteristics (study I, II and IV)

In study I increased brushing length and number of strokes significantly increased total brush-evoked pain intensity, which was not the case while increasing brush width. This finding points to dynamic mechanical allodynia being a graded phenomenon when varying certain stimulus parameters but not others. The lack of influence of brush width on total brush-evoked pain intensity is further underlined by the finding that activation of equivalent areas, either 160 mm² or 320 mm², resulted in higher total brush-evoked pain intensity if brushing the skin with a thin brush over a longer distance than using a thick brush over a shorter distance. Data from experiments on patients with neuropathic pain indicate a crucial role for low threshold A-beta fibres in the generation of hypersensitivity to light mechanical stimuli (Campbell et al., 1988; Lindblom and Verrillo, 1979; Nurmikko et al., 1991; Ochoa and Yarnitsky, 1993; Price et al., 1989). If we assume that large myelinated fibres are the peripheral link in the generation of dynamic mechanical allodynia in this study, the ceiling effect regarding induced pain intensity within the studied range of brush widths may reflect, if CNS mechanisms are implied, that central temporo-spatial summation from low-threshold mechanoreceptive afferents onto the nociceptive system is satiable and hence requires activity only in a restricted number of simultaneously activated fibres to become saturated. Such a scenario hints the possible contribution of dynamic receptor activation and deactivation (on and off) of rapidly adapting cutaneous mechanoreceptors to the phenomenon of dynamic mechanical allodynia in this study. Since a group of patients with, e. g., inflammatory based tactile allodynia was not included we are unable to suggest if activity set up in sensitized nociceptive afferents by a brushing stimulus would result in different response patterns to the variable stimulus parameters. Also, the mechanism converting activity in the mechanoreceptive system to the nociceptive system may influence response characteristics, e. g., ephaptic transmission in the periphery as a possible means of intermodality interaction may demonstrate specific characteristics.

One of the main outcomes of study IV was the significant impact of stroking velocity on dynamic mechanical allodynia with higher total brush-evoked pain intensity linked to a lower stroking velocity across a stroking distance of 60 mm. The patients included had no signs of inflammation, supporting our position that brush-evoked allodynia in such patients likely is mediated in the periphery by low-threshold A-beta mechanoreceptive afferents, as discussed previously. Whatever the level of conversion of such activity onto the nociceptive system, be it peripheral or central, the amount of peripheral inflow from low-threshold mechanoreceptors should have a bearing on the intensity and duration of dynamic mechanical allodynia. Interestingly, in human volunteers the number of stimulus-evoked action potentials and the duration of stimulus-evoked activity in low-threshold mechanoreceptors recorded during microneurography have been demonstrated to increase with lower brushing velocity (Edin et al., 1995; Essick and Edin, 1995). As the stroking velocities in those studies were comparable with the ones used in this study, these findings in human volunteers seem relevant to our results.

Further in study IV, increasing the brushing force had a significant impact on the total brush-evoked pain intensity, i.e., a force of 40 g compared to 10 g significantly increased the total pain intensity. In the aforementioned studies with forces employed

that were comparable to ours, increased brushing force resulted in an increased number of action potentials and discharge rate in low-threshold A-beta mechanoreceptive afferents (Edin et al., 1995; Essick and Edin, 1995). In addition, increased brushing force may result in an increased recruitment of mechanoreceptive afferents in areas adjacent to the stimulus device (Edin et al., 1995; Essick and Edin, 1995). Again, if assuming that low-threshold mechanoreceptors play a pivotal role in dynamic mechanical allodynia in the studied patient population our findings of increased total pain intensity linked to higher brushing force may be explained by an increased peripheral temporo-spatial inflow in the non-nociceptive mechanoreceptive system before the message is converted onto the nociceptive system.

The main outcome of study III was the within patients findings when comparing the two sides (the area of painful neuropathy and the capsaicin-induced secondary hyperalgesic area), demonstrating similarities regarding the relationship between the total brush-evoked pain intensity and the employed temporo-spatial stimulus parameters. Thus, the experimental pain model, when administrated to patients contralateral to the area of neuropathy, seemingly well reflected perceptual aspects of the dynamic mechanical allodynia in the clinical pain condition. Further, within patients an increased number of strokes significantly increased the total brush-evoked pain intensity, which was not the case while increasing brushing length or brush width. The results on number of strokes and brush width coincide with earlier findings in study I where dynamic mechanical allodynia was reported to be a graded phenomenon. The outcome from altering brushing length is at variance with our previous results demonstrating increased total brush-evoked pain intensity while increasing brushing length (20, 40 and 60 mm) (Samuelsson et al., 2005). In study III, the limited variation of the brushing lengths (10 and 20 mm), necessitated by the limited spread of secondary hyperalgesia in capsaicin-injected areas found in preliminary experiments, may have a bearing on the non-significant difference of the total brush-evoked pain intensity.

5.2.2 Aftersensation of dynamic mechanical allodynia (study I, III and IV)

In study I the results pointed to aftersensation being a common phenomenon. Significantly increased duration of aftersensation was demonstrated with increased brushing length. The finding that number of strokes and brush width did not significantly affect the duration of the aftersensation is interesting. Time (seconds) is the parameter when calculating aftersensation in relation to stimulus parameters. This is obviously different from the parameter “total intensity of brush-evoked pain” used when calculating its relationship to different stimulus parameters. The results may therefore not coincide. We also would like to point out (see statistics) that due to different data distributions (i.e., “for total brush-evoked pain intensity” and “aftersensation”, respectively) different statistical methods have been applied. The finding may also, again, reflect the satiability of the system within the studied stimulus range. The fact that duration of aftersensation was significantly correlated to the maximum intensity of brush-evoked pain in all stimulus combinations but one suggests, in neurophysiological terms, that neuronal hyperexcitability with spontaneous afterdischarge is more pronounced the higher the peak activity (i.e., maximum impulse frequency) in the afferent system.

Our results in study III demonstrated aftersensation to be a more common phenomenon in the area of painful neuropathy than in the capsaicin-induced

secondary hyperalgesic area in patients. In both areas there was no correlation between the duration of painful aftersensation and the maximum brush-evoked pain intensity as earlier demonstrated in study I (Samuelsson et al., 2005). However, caution should be exercised when comparing these results since this study included a comparably limited number of patients, small variations in brushing lengths and few subjects reporting any aftersensation.

In study IV, aftersensation was an infrequent phenomenon in patients with dynamic mechanical allodynia due to peripheral neuropathy which is at variance with our results from study I, where all except one out of 18 patients reported aftersensation although not after all stimuli (Samuelsson et al., 2005).

5.2.3 Repeatability of dynamic mechanical allodynia (study II)

Subgroups of patients with neuropathic pain across different etiological diagnostic entities suffer from dynamic mechanical allodynia, sometimes as troublesome as the ongoing pain. Treatment measures aimed at relieving such pain are needed. A valid and reliable stimulation technique is crucial when introducing dynamic mechanical allodynia as an efficacy parameter in treatment studies. Previous treatment studies (for review see Granot et al., 2007) have used techniques not surveyed in detail and therefore the outcome of such studies must be questioned. The currently employed technique proved to be a promising tool for future treatment studies since, overall, the ICC of the total brush-evoked pain intensity within the same day and between the four days was generally high confirming a “very good” repeatability for all used combinations of measurements.

Our results also demonstrated a “moderate to good” positive correlation between the mean intensity of spontaneous ongoing pain and the mean total brush-evoked pain intensity of all 16 assessments collectively, confirming results arrived at by others using different methods for analysis (Koltzenburg et al., 1994; Rowbotham and Fields, 1996). This relationship between evoked and ongoing pain must be taken into account while designing treatment studies focusing at dynamic mechanical allodynia. It is not known if treatment modalities in peripheral neuropathic pain may uncouple this relationship causing reduction of only one of the two. It may be hypothesised that this is one possible outcome of treatment considering the numerous possible underlying mechanisms of dynamic mechanical allodynia which have been reported on in the animal experimental literature (Hansson, 2003).

5.2.4 Sensory-discriminative and affective pain descriptors (study I, III and IV)

The distribution of sensory and emotional descriptors in study I did not indicate any common denominators. A survey of the two subgroups with and without lowered heat pain threshold in the affected area compared to the contralateral homologous area indicated no obvious differences in preferred descriptors. If, hypothetically, stimulating preferably sensitised nociceptors in the group with lowered heat pain threshold this might have resulted in a different distribution of descriptors compared to the group with unaltered heat pain threshold.

In study III, the preferred sensory-discriminative and affective pain descriptors for the brush-evoked pain did not indicate any differences between the area of painful peripheral neuropathy and the secondary hyperalgesic area in capsaicin treated skin in patients. The results in the area of neuropathy coincided with findings from study I.

The distribution of preferred sensory-discriminative and affective pain descriptors for the brush-evoked pain in study IV indicated some similarities with our earlier findings, in particular the choice of affective pain descriptors such as ‘annoying’ and ‘troublesome’.

5.3 CAPSAICIN-INDUCED DYNAMIC MECHANICAL ALLODYNIA (STUDY III)

Importantly, only 3 of the 9 healthy controls reported brush-evoked pain in an area outside the flare, i.e., the secondary hyperalgesic area after an intradermal injection of capsaicin. This low hit frequency seriously questions the model when aiming at studying dynamic mechanical allodynia in an area devoid of peripheral sensitisation in controls. Although poorly defining the area of secondary hyperalgesia, others have reported a similar low frequency of brush-evoked allodynia using the same methodology (Geber et al., 2007).

The expression of brush-evoked pain in the secondary hyperalgesic area after capsaicin injection in 8 out of 9 patients compared to the few controls may indicate a neuronal preparedness, i.e., spread of central hyperexcitability across dorsal horns of the spinal cord in patients with unilateral painful peripheral neuropathy. Animal studies have suggested commissural connections transferring sensory information onto neurons in the contralateral dorsal horn (Koltzenburg et al., 1999; Petko and Antal, 2000; Sotgiu and Biella, 1998). In addition, after nerve injury an increased bilateral metabolic activity in the dorsal horns of the spinal cord of rats has been reported, which may indicate increased neuronal activity (Mao et al., 1992). Another factor of potential importance could be spinal glia activation. Results from animal studies in rats after partial nerve injury have raised the possibility of spinal glia and proinflammatory cytokines to be involved in contralateral spread of hyperexcitability in the cord and hence contributing to mirror-image mechanical hypersensitivity (Milligan et al., 2003; Spataro et al., 2004). Importantly, mirror-image pain related symptoms and signs, spontaneous and/or stimulus-evoked are extremely rare, if at all existent, in patients with peripheral neuropathic pain.

Since it has been reported that spontaneous ongoing pain after an intradermal capsaicin injection rapidly declines (Kinnman et al., 1997; LaMotte et al., 1992) we assessed such pain intensity already at 1 min after injection to secure activity in the nociceptive system as a basis for development of brush-evoked pain. Significantly higher spontaneous ongoing pain intensity was demonstrated in patients compared to controls. It seems reasonable, again, to implicate central hyperexcitability spreading across dorsal horns of the spinal cord in patients with unilateral peripheral neuropathy.

5.4 METHODOLOGICAL SHORTCOMINGS

5.4.1 Study I

Several methodological considerations deserve to be mentioned. A handheld brush was used, which allows for variability in brushing force. Prior to start of the study a window of allowed variability in brushing force was set at 4-20 g, a non-painful brushing intensity in normal skin, and author M.S. was carefully trained to perform repeatable strokes within this range. The frequency of disregarded attempts was less than once during each session with 18 stimuli. A stroking velocity of about 20 mm/s

was arbitrarily decided on and was carefully practiced but was not monitored with high resolution during the experiments. Recording of subjective brush-evoked pain intensity started when 2 mm on the computerized VAS was reached and stopped when below 2 mm to establish with reasonable certainty that there was a conscious action from the patient. During the 18 stimuli, two patients reported a number of non-painful percepts, which in the statistical analysis of the total brush-evoked pain intensity were recorded as zeros. In addition, patients without aftersensation due to non-painful percepts during brushing or, more commonly because the pain vanished immediately after cessation of stimulation were included in the statistical analysis and recorded as zeros.

5.4.2 Study II

A few methodological aspects need to be considered. In reports on dynamic mechanical allodynia the patients have rated their brush-evoked pain in a number of different ways (Attal et al., 2002; Baumgartner et al., 2002; Finnerup et al., 2002; Koltzenburg et al., 1994; Rowbotham and Fields, 1996; Witting et al., 2003). By the currently employed computerized VAS the patients rated both intensity as well as duration of pain during dynamic mechanical allodynia which provides a more high-resolution assessment of dynamic mechanical allodynia than a global rating of the pain intensity only. The basis for how patients compose different aspects of the percept, including duration, into a single number has never been studied.

The semi-quantitative technique used in this study has some weaknesses, which have been discussed previously (Samuelsson et al., 2005). Here we point out that although variability in pressure and speed of the stimulus is an inherent part of the technique due to the manual handling of the device such shortcomings did not off-set the methodology when challenged from the point of view of repeatability.

5.4.3 Study III

Some methodological considerations deserve to be mentioned. Here we have defined the secondary hyperalgesic area after an intradermal capsaicin injection, i.e., the test area, as the uninjured area outside the visible flare (Kinnman et al., 1997) where brush-evoked pain was present. By defining the test area in this way we, during the brushing, aimed at avoiding activation of sensitised nociceptive afferents and instead activating mechanoreceptive A-beta fibres.

The statistical analysis of the total brush-evoked pain intensity related to the injected dose of capsaicin (60 or 120 µg) in patients showed no dose-dependency, which is at variance with results of others reporting dose-dependent intensity and area of brush-evoked pain (Scanlon et al., 2006; Simone et al., 1989). Interestingly, 2 out of 3 controls that developed brush-evoked pain were injected with a dose of 60 µg capsaicin only. Although the same injection technique was repeated and a bleb indicated the intradermal nature of the injections in all subjects there might have been small deviations in injected dose due to the relatively low resolution of the syringe measures.

Other methodological considerations related to the method of examination with a handheld brush, techniques for recording brushing force and velocity have been detailed previously (Samuelsson et al., 2005).

5.4.4 Study IV

Methodological considerations related to this semi-quantitative method of examination have been detailed previously (Samuelsson et al., 2005; Samuelsson et al., 2007). In addition, variations in applied stroking velocity and brushing force were difficult to fully eliminate due to the use of a handheld brush (regarding applied values, see Table 5). Following completion of the study the power analysis indicated that a sufficient number of patients had been included to be able to obtain reliable results regarding brush-evoked pain with an 80 % power for the stroking velocity parameter. For the brushing force an 80 % power would have necessitated an inclusion of 30 patients (51 % power was obtained with the included patients).

6 THESIS SUMMARY

6.1 STUDY I

This is the first study demonstrating a relationship between brush-evoked pain and some temporo-spatial stimulus parameters during dynamic mechanical allodynia. Increased brushing length and number of strokes significantly increased total brush-evoked pain intensity. In addition, a significantly increased duration of aftersensation was demonstrated with increased brushing length. Further probing of the reliability of the allodynic percept stands out as an important prerequisite before performing treatment studies of this enigmatic symptom/sign of neuropathic pain.

6.2 STUDY II

A very good repeatability of brush-evoked allodynia within and between days has been reported using this semi-quantitative method. We have provided evidence to support this assessment technique as the current method of choice for short- and long-term evaluation of dynamic mechanical allodynia in treatment studies.

6.3 STUDY III

Similarities were found in the relationship between brush-evoked allodynia and temporo-spatial stimulus parameters in the capsaicin-induced secondary hyperalgesic area contralateral to the area of painful neuropathy, reflecting seemingly well the outcome when testing the area of neuropathic pain. In healthy controls, only one-third developed brush-evoked allodynia in the potential secondary hyperalgesic area, which put into question the usefulness of the capsaicin model when aiming at studying dynamic mechanical allodynia outside of the flare. Still, it is possible that the few healthy subjects reporting such allodynia might experience details of the percept that were similar to what was reported by the patients in the area of neuropathy. To collect a reasonable number of such responders, a larger population of individuals needs to be examined.

6.4 STUDY IV

In conclusion, our findings demonstrated a significantly increased total brush-evoked pain intensity following lower stroking velocity and higher brushing force in patients with dynamic mechanical allodynia due to peripheral neuropathy. We now believe that a critical mass of data has been accumulated to substantiate the usefulness of this semi-quantitative assessment method in longitudinal studies on dynamic mechanical allodynia.

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8 SAMMANFATTNING PÅ SVENSKA

Bakgrund och syfte: Enligt International Association for the Study of Pain (IASP) definieras perifer neuropatisk smärta som ’smärta initierad eller orsakad av en primär lesion eller dysfunktion i det perifera nervsystemet’, ett svårbehandlat tillstånd. Hos en undergrupp av patienter med perifer neuropatisk smärta (ca 20-50 %) kan, förutom en ständigt pågående spontansmärta, utvecklas en smärtsam överkänslighet för lätt strykning av huden i det nervskadade området, s.k. dynamisk mekanisk allodyni. Beröringsutlöst smärta är för de drabbade ett påtagligt symtom som påverkar många situationer i det dagliga livet då lätt beröring av t.ex. klädesplagg, duschstrålar och sängkläder inom den överkänsliga kroppsdelen ger upphov till smärta. Kontinuerligt ökar kunskapen kring patofysiologiska mekanismer rörande den perifera neuropatiska smärtans uppkomst och underhåll men fortfarande saknas behandlingsstrategier med hög träffsäkerhet. Syftet med avhandlingsarbetet var att undersöka psykofysiska karaktäristika avseende dynamisk mekanisk allodyni med hjälp av en standardiserad metod. Därutöver var syftet att studera likheter och olikheter mellan beröringsutlöst smärta hos patienter med perifer nervskada och samma fenomen i en ofta använd experimentell smärtmodell, d.v.s. efter injektion i huden av kapsaicin (ingrediens i chilipeppar) då denna modell ofta används som surrogat för att studera smärta efter nervskada.

Metod: Kartläggning av psykofysiska förhållanden kring smärtintensitetsutveckling och dess relation till olika stimuleringsparametrar utgör basen i den användna undersökningsmetodiken. Den beröringsutlösta smärtan i området med neuropatisk smärta hos patienterna framkallades genom att med olika breda penslar lätt stryka huden olika långa sträckor samt genom att variera antalet strykningar, penseltryck och strykningshastighet. I studie III undersöktes patienterna även i ett friskt område på motsatta sidan till nervskadan efter en ytlig (intradermal) hudinjektion av kapsaicin. Området intill den rodnad som uppstod efter injektionen undersöktes med avsikt på förekomst av beröringsutlöst smärta. Ålders- och könsmatchade kontroller undersöktes i motsvarande område efter en liknande injektion av kapsaicin. I alla studier skattade försökspersonerna/patienterna intensitet och varaktighet av den beröringsutlösta smärtan med hjälp av en datorbaserad visuell analog skala (VAS). Som ett mått på den totala smärtintensiteten beräknades arean under VAS kurvan. Efter varje penselstrykning fick försökspersonerna välja ord från ett validerat instrument för att beskriva smärtupplevelsens sensoriska och känslomässiga innehåll. I studie II undersöktes patienterna vid upprepade tillfällen samma dag samt fyra gånger under en månad med avsikt att studera repeterbarheten hos den beröringsutlösta smärtan vid en bestämd hudretning med pensel.

Resultat: Signifikant ökad total beröringsutlöst smärtintensitet kunde påvisas med ökad strykningslängd, ökat antal strykningar, ökat penseltryck och lägre strykningshastighet men inte då penselbredden varierades. När lika stora ytor av huden beströks med pensel kunde en ökad total smärtintensitet påvisas vid användande av en smal pensel över en längre sträcka i jämförelse med en bredare pensel över en kortare sträcka. En mycket hög grad av repeterbarhet av den totala beröringsutlösta smärtintensiteten påvisades med den använda metodiken vid upprepad mätning inom och mellan dagar. Likheter kunde påvisas i relationen mellan den beröringsutlösta smärtan och stimuleringsparametrarna i det nervskadade området och i det kapsaicininducerade smärtområdet, utanför den av kapsaicinet orsakade rodnaden. Endast 3 av 9 kontroller (att jämföra med 8 av 9 patienter) utvecklade dynamisk mekanisk allodyni i ett område utanför rodnaden efter kapsaicininjektionen.

De vanligast använda smärttermerna i studie I, III och IV överensstämde till stora delar, framför allt avseende de affektiva termerna ’irriterande’ och ’besvärlig’.

Slutsatser: Resultaten visar att dynamisk mekanisk allodyni är ett delvis graderat fenomen då stimulering med ökad strykningslängd, ökat antal strykningar, ökat penseltryck och lägre strykningshastighet signifikant ökade den totala beröringsutlösta smärtintensiteten vilket dock inte var fallet då penselbredden varierades. Därutöver påvisades likheter i relationen mellan smärtintensitetsutvecklingen och olika stimulusparametrar vid jämförelse mellan nervskadat och kapsaicininducerat smärtområde hos patienterna. Endast en tredjedel av kontrollerna utvecklade ett område med kapsaicinorsakad beröringsutlöst smärta utanför rodnaden. Den låga frekvensen beröringsutlöst smärta i det sekundärhyperalgetiska området ger anledning att ifrågasätta användbarheten av kapsaicinmodellen vid undersökning av dynamisk mekanisk allodyni hos friska försökspersoner. Sammanfattningsvis kan den utvecklade undersökningsmetodiken anses användbar vid studier av dynamisk mekanisk allodyni, inkluderande longitudinella behandlingsstudier.

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