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ON THE FUNCTION OF ENDOGENOUS PAIN CONTROLLING SYSTEMS IN HEALTHY SUBJECTS AND PATIENTS WITH PERIPHERAL OR CENTRAL NEUROPATHIC PAIN

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To Anna and Henrik

ABSTRACT

Introduction and aim: Activity in the nociceptive system is modulated by descending inhibitory and facilitatory supraspinal endogenous control. Altered balance between inhibition and facilitation has been suggested to at least partly explain the maintenance of some persistent pain conditions. The aim was to investigate the influence of supraspinal descending endogenous modulation induced by heterotopic noxious conditioning stimulation (HNCS) on spontaneous ongoing pain and dynamic mechanical allodynia in painful peripheral neuropathy (study II) and in central post stroke pain (CPSP) (study III). In addition, the influence of ongoing pain and HNCS on pain sensitivity in a pain-free area was assessed and compared to age- and sex-matched healthy controls (study II and III). To prepare for a proper study design in these studies potential side and time dependant alterations in pain sensitivity were examined in healthy volunteers (study I). The possible involvement of spinal dorsal horn $5HT_3$-receptors in spontaneous ongoing pain and dynamic mechanical allodynia in patients with peripheral neuropathy, an hypothesis derived from animal model research, was examined following intravenous infusion of the $5HT_3$-antagonist ondansetron (study IV).

Methods: Ischemia-induced HNCS was used to activate endogenous pain controlling systems. In patients, the intensity of spontaneous ongoing- and brush-evoked pain was assessed. A semi-quantitative brushing technique was employed, combined with a computerized visual analogue scale (VAS) to monitor the allodynic percept over time and calculating the area under the VAS curve as the total brush-evoked pain intensity. Before, during and following HNCS in study I and II heat- and pressure pain thresholds and sensitivity to suprathreshold heat- and pressure pain were assessed in the pain-free area in patients. For comparison pain sensitivity was assessed also in controls. In healthy volunteers identical assessments were performed bilaterally on the thighs (study I). Before and during 3 hours following an intravenous infusion of ondansetron in study IV the intensity of spontaneous ongoing- and brush-evoked pain was assessed using the described methods.

Results: During unilateral HNCS of the left arm in healthy volunteers no side differences in bilaterally decreased pain sensitivity were found. However, a time factor was demonstrated for reduced suprathreshold pain sensitivity as it was found only on the lastly assessed side without side differences in magnitude. During HNCS in patients with painful peripheral neuropathy the intensity of spontaneous ongoing pain was significantly decreased, whereas brush-evoked pain was unaltered. No significant changes of neither spontaneous- nor brush-evoked pain were found in patients with CPSP. At baseline, significantly increased pressure pain sensitivity in a pain-free area was demonstrated in CPSP patients compared to controls but not in patients with painful peripheral neuropathy. During HNCS higher pressure pain thresholds were demonstrated in patients with painful peripheral neuropathy and CPSP as well as in healthy controls. Intravenous infusion of the $5HT_3$-antagonist ondansetron failed to alter the intensity of spontaneous ongoing- and brush-evoked pain in patients with peripheral neuropathy.

Conclusions: During HNCS the patients with painful peripheral neuropathy reported reduced intensity of the spontaneous pain whereas the intensity of brush-evoked pain was unaltered. No significant alteration of the intensity of spontaneous- or brush-evoked pain were found in CPSP patients indicating lack of modulation from endogenous pain controlling systems on nociceptive activity primarily generated in the stroke affected brain. In CPSP patients the increased pressure pain sensitivity in a remote pain-free area at baseline suggests alterations in corticofugal control of nociceptive sensitivity due to the brain lesion. During HNCS patients with painful peripheral neuropathy and CPSP activated pain modulatory systems interacting with nociceptive input from the spinal level equal to controls. Intravenous infusion of ondansetron did not influence the intensity of brush-evoked- or spontaneous ongoing pain in patients with peripheral neuropathy, indicating lack of involvement of $5HT_3$ receptors in the maintenance of dynamic mechanical allodynia.

Key words: Painful peripheral neuropathy; Central post-stroke pain; Dynamic mechanical allodynia; Endogenous pain modulation; Heterotopic noxious conditioning stimulation; Quantitative sensory testing; Ondansetron.

LIST OF PUBLICATIONS

I. Tuveson B, Leffler AS, Hansson P.
Time dependant differences in pain sensitivity during unilateral ischemic pain provocation in healthy volunteers. Eur J Pain 2006; 10:225-232.

II. Tuveson B, Leffler AS, Hansson P.
Heterotopic noxious conditioning stimulation (HNCS) reduced the intensity of spontaneous pain without altering the intensity of dynamic mechanical allodynia in painful peripheral neuropathy. Eur J Pain 2007; 11:452-462.

III. Tuveson B, Leffler AS, Hansson P.
Influence of heterotopic noxious conditioning stimulation on spontaneous pain and dynamic mechanical allodynia in central post-stroke pain patients.
Submitted.

IV. Tuveson B, Leffler AS, Hansson P.
Ondansetron, a $5HT_3$-antagonist, does not alter dynamic mechanical allodynia or spontaneous ongoing pain in peripheral neuropathy.
Submitted.

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the curve
BP	Blood pressure
CNS	Central nervous system
CPSP	Central post stroke pain
CSF	Cerebrospinal fluid
CT	Computerized tomography
DNIC	Diffuse noxious inhibitory controls
5HT	5-hydroxytryptamine (serotonin)
HNCS	Heterotopic noxious conditioning stimulation
HPT	Heat pain thresholds
HR	Heart rate
LSD	Least significant difference
NMDA	N-methyl-D-aspartate
PAG	Periaqueductal gray
PPT	Pressure pain threshold
QST	Quantitative sensory testing
RVM	Rostral ventromedial medulla
SEM	Standard error of the mean
SHP	Suprathreshold heat pain
SRD	Subnucleus reticularis dorsalis
SPP	Suprathreshold pressure pain
SSRI	Selective serotonin reuptake inhibitor
VAS	Visual analogue scale
WDR	Wide dynamic range

1 INTRODUCTION

Corticofugal (Hagbarth and Kerr, 1954) and bulbo-spinal (Lindblom and Ottosson, 1957; Reynolds, 1969; Mayer et al., 1971) systems have been identified that when activated have the potential to alter nociceptive output from the dorsal horn of the spinal cord. With reference to the concept of pain relief through activation of such systems potential human counterparts have been employed in clinical pain treatment, e.g., electrical stimulation of the thalamus (Hosobuchi et al., 1973), motor cortex (Tsubokawa et al., 1993) and periventricular areas (Boivie and Meyerson, 1982). Such measures have gained widespread clinical use and have demonstrated protruding pain relieving abilities. Subsequent studies have pointed to a great complexity in endogenous pain modulation involving supraspinal areas with, e.g., numerous pain modulatory circuits and involvement of a multitude of neurotransmitters.

Some persistent painful conditions have been considered to at least partly be maintained by dysfunction of endogenous pain controlling systems with altered balance between inhibitory and facilitatory influence such as fibromyalgia (Mense, 1998),peripheral inflammatory conditions and neuropathy (Vanegas and Schaible, 2004). The influence of supraspinally emerging endogenous pain modulation in patients with painful peripheral neuropathy and patients with central post stroke pain (CPSP) has not been extensively studied (De Broucker et al., 1990; Bouhassira et al., 2003; Witting et al., 2003) and subgroups of patients lend themselves to studies of both spontaneous ongoing and stimulus evoked pain. Spontaneous ongoing pain in patients with peripheral neuropathy may reasonably be linked to an increased ectopic neuronal activity (Jensen and Finnerup, 2004), possibly related to aberrant expression of sodium channels (Black et al., 2001). In addition changes in presynaptic calcium channels (Matthews and Dickenson, 2001), involvement of post-synaptic NMDA-receptors (Dickenson et al., 2001) and increased descending facilitation (Porreca et al., 2002) may be involved in the maintenance of such pain states. Peripheral nerve injury often causes sensory deficits but occasionally pain due to a light moving mechanical stimulus which does not normally provoke pain is at hand, i.e., dynamic mechanical allodynia. A crucial role for low threshold A-β afferents as the peripheral substrate in such allodynia has been indicated (Lindblom and Verillo, 1979; Nurmikko et al., 1991; Ochoa and Yarnitsky, 1993). If A-β fibres are the peripheral causative link of dynamic mechanical allodynia in peripheral neuropathy, the converted activity onto the nociceptive system may result in different temporo-spatial activity patterns in the CNS compared to patterns primarily generated by the nociceptive system itself. It may be speculated that such altered activity patterns may be less susceptible to inhibitory endogenous modulation. Descending facilitation from the rostral ventromedial medulla (RVM) has been suggested to result in nerve injury induced mechanical hypersensitivity in rats (Ossipov et al., 2001, Porreca et al., 2002) with a proposed serotonergic link (Suzuki et al., 2004b).

Patients with CPSP complain of continuous ongoing pain and infrequently dynamic mechanical allodynia. The pathophysiological basis for such symptoms/signs in CPSP is not known. Supraspinal endogenous pain modulation in CPSP patients has been studied to a little extent (De Broucker et al., 1990) and no previous study on modulation of spontaneous pain and dynamic mechanical allodynia has been performed.

1.1 ENDOGENOUS MODULATION OF NOCICEPTIVE TRANSMISSION

1.1.1 Spino-bulbo-spinal loops

In 1979 Le Bars and colleagues (Le Bars et al., 1979a,b) put forth the concept of diffuse noxious inhibitory controls (DNIC), i.e., heterotopic conditioning stimulation in the rat inducing widespread inhibitory effects acting plurisegmentally on spinal and trigeminal wide dynamic range (WDR) neurones (Dickenson and Le Bars, 1983; Morton et al., 1987). An involvement of a spino-bulbo-spinal loop was demonstrated and a relay for such a loop was found in subnucleus reticularis dorsalis (SRD) in the caudal medulla (Bouhassira et al., 1992). A widespread bilateral inhibition of neuronal electrical activity from test stimuli without side differences has been demonstrated during conditioning stimulation by immersion of paws, tail and muzzle in hot water (Bouhassira et al., 1990; Bouhassira et al., 1992). Further characterisation of these circuits suggested that nociceptive input from A-δ and C fibres activated this endogenous pain controlling system in a way that was dependant on recruitment of a critical number of spinal neurones (Le Bars, 2002), with ascending projections from the dorsal horn to SRD located in spinoreticular pathways (Le Bars, 2002; Bouhassira et al., 2003). As demonstrated in anterograde and retrograde tracing studies in rats, the projections between the dorsal horn and SRD are found to be preferentially ipsilateral but also contralateral and reciprocal with direct connection between ascending spinal neurones and descending neurones projecting back from SRD (Lima and Almeida, 2002). In an effort to try to mimic supraspinal endogenous pain modulation characteristics found in animal studies, the effect on the nociceptive flexor reflex (RIII) recorded in the ipsilateral biceps femoris muscle has been studied in humans where the sural nerve was electrically stimulated behind the lateral malleolus (Willer et al., 1984). The painful sensation elicited by the electrical stimulation of the sural nerve and the reflex responses were inhibited in a parallel fashion during conditioning painful stimulation (Willer et al., 1984). Later studies have found decreased sensitivity to different painful test stimuli in healthy individuals such as electrical stimulation with conditioning heat provocation (Price and McHaffie, 1988) or heat (Talbot et al., 1987) and CO_2 laser stimulation (Plaghki et al., 1994) during cold pressor test. The pain inhibitory effect in the studies has by some been extrapolated to an effect of DNIC (Willer et al., 1984; Talbot et al., 1987; Price and McHaffie, 1988; Peters et al., 1992; Plaghki et al., 1994) or discussed in terms of the used technique for heterotopic noxious conditioning stimulation (HNCS) only. The latter approach does not presume a specific inhibitory interaction. The neurotransmitter involved in the bulbo-spinal extension of the loop is still unknown (Millan, 2002).

Another spino-bulbo-spinal loop has been identified that comprises the ascending nociceptive pathways, the periaqueductal gray (PAG), the rostral ventromedial medulla (RVM) and finally descending projections from the latter to the dorsal horn of the spinal cord (Fields and Basbaum, 1999). Projections to PAG are also described from the parabrachial nucleus receiving projection neurones from lamina I of the dorsal horn ascending in spino-parabrachial pathways with connections also to amygdala and hypothalamus with subsequent descending projections from the PAG to the RVM (Suzuki and Dickenson, 2005). Increased sensitivity in neuropathic areas of the hindlimb to non-noxious mechanical stimulation induced by spinal nerve ligation in the rat has been demonstrated to be reversed by a lesion of the ipsilateral but not the contralateral spinal dorsolateral funiculus, interpreted as a maintaining role for descending modulation from the RVM of this sign (Ossipov et al., 2000). This notion is also supported by a study demonstrating mechanical hypersensitivity after nerve injury in the rat to be attenuated following microinjection of a local anaesthetic into the PAG

or the RVM (Pertovaara et al., 1996). Involvement of serotonergic mechanisms in modulation of nociceptive activity has been proposed based on findings that intrathecal administration of a non-selective serotonin (5HT)-antagonist (methysergide) prevented both inhibition and facilitation following electrical stimulation of the RVM (Zhuo and Gebhart, 1991) and RVM has been found to be a major source of spinal serotonin (Mason, 2001). In the serotonergic system at least 15 different subtypes of receptors in the peripheral and central nervous system have been found with a possibility for 5HT to exert either antinociceptiv or pronociceptiv effects depending on the receptor involved (Suzuki et al., 2004b). A suggested contribution from both spinal pre- and postsynaptic $5HT_3$-receptors has been put forth in inducing facilitatory influence in the dorsal horn in association with nerve injury (Stewart and Maxwell, 2000; Suzuki et al., 2004b). In addition, intrathecal infusion of the selective $5HT_3$-receptor antagonist ondansetron was demonstrated to reduce neuronal responses to mechanical punctate stimuli following nerve injury in the rat (Suzuki et al., 2004a). Both inhibitory and facilitatory influences with simultaneous action on nociceptive activity have been described in both spino-bulbo-spinal loops via SRD and RVM (Zhuo and Gebhart, 1991; Lima and Almeida, 2002).

1.1.2 Corticofugal modulation

There is increasing evidence for an active role in pain modulation from somatosensory and motor cortices with both inhibitory and facilitatory properties (Millan, 2002) and nociceptive relays at different levels of the neuraxis are under corticofugal control (Villanueva and Fields, 2004). A powerful control of thalamic neuronal activity from the cortex is illustrated by the numerous fibres projecting from the sensory cortex to the thalamus, i.e., 10 times as many as the fibres extending from thalamus to cortex (Deschênes et al., 1998). Cortico-thalamic control has been described to have an exciting effect by reduction of intrinsic inhibition, i.e., disinhibition (Villanueva and Fields, 2004).

1.2 ENDOGENOUS MODULATION OF PAINFUL SYMPTOMS/SIGNS ASSOCIATED WITH PERIPHERAL NEUROPATHY

1.2.1 Modulation by heterotopic noxious conditioning stimulation (HNCS)

Dynamic mechanical allodynia is present in a minority of patients with peripheral neuropathy, and both peripheral and central pathophysiological mechanisms have been discussed to underly the phenomenon (Hansson, 2003). The peripheral mechanisms suggested are peripheral sensitization (Fields et al., 1998), efaptic transmission between large myelinated and nociceptive fibres due to altered insulation after injury (Amir and Devor, 1992) and activation of silent nociceptors (Schmidt et al., 1995; Fields et al., 1998). Proposed central mechanisms are loss of A-β mediated inhibition causing disturbed balance between inhibition and facilitation (Laird and Bennett, 1992), descending facilitation of nociceptive activity (Ossipov et al., 2001), central sensitization by opening of previous existing but silent synapses between A-β afferents and nociceptive specific neurons in the dorsal horn (Cook et al., 1987; Torebjörk et al., 1992) and sprouting of mechanoreceptive fibres in the dorsal horn (Woolf et al., 1992).

The endogenous modulation of spontaneous ongoing pain and dynamic mechanical or static allodynia has recently been explored in clinical studies using HNCS in patients with peripheral neuropathy and an influence has been demonstrated from conditioning stimulation on some test stimuli but not on others (Witting et al.,

2003; Bouhassira et al., 2003). In the study by Witting et al. (2003) conditioning painful stimulation induced by cold-water immersion significantly reduced the area of brush-evoked pain, but had no influence on the intensity of spontaneous- and brush-evoked pain. Bouhassira et al. (2003) used either the cold-pressor test/tourniquet test within the pain-free area to induce HNCS with the aim to compare the effect on both the spinal nociceptive flexion reflex (RIII) response and intensity ratings of the concomitant painful sensation. Inhibition of both the RIII reflex response and the intensity of the painful test stimulus were obtained by the cold-pressor test/tourniquet test. With regard to possible modulation of spontaneous versus stimulus-evoked pain in peripheral painful neuropathy there might be a different capacity from supraspinally emerging endogenous modulation to act on spontaneous nociceptive activity compared to neural activity underlying dynamic mechanical allodynia with a peripheral substrate in A-β afferent fibres and possible novel temporo-spatial activity patterns converging onto WDR-neurones in the spinal cord dorsal horn. On the other hand, if brush-evoked neural activity is converted to the nociceptive system already in the periphery by efaptic transmission the incoming neural activity pattern is physiological and possibly more easily modulated.

1.2.2 $5HT_3$-receptor involvement in modulation of peripheral neuropathic pain

As serotonergic mechanisms have been indicated to influence mechanical hypersensitivity following nerve injury in animals such mechanisms may be of interest to explore also in patients with painful peripheral neuropathy. Involvement of possible descending endogenous serotonergic modulation on components of neuropathic pain has been investigated in patients by infusion of ondansetron, a $5HT_3$-receptor antagonist, and the intensity of spontaneous ongoing neuropathic pain as well as self reported unspecified allodynia was investigated in a double-blind, placebo-controlled crossover study in individuals with chronic neuropathic pain of mixed etiology (McCleane et al., 2003). Significantly reduced ratings of spontaneous ongoing pain intensity was reported at a time-point two hours after a single intravenous injection of ondansetron but no modulating influence on allodynia was found (McCleane et al., 2003).

1.3 ENDOGENOUS PAIN MODULATION OF SYMPTOMS/SIGNS ASSOCIATED WITH CENTRAL POST STROKE PAIN (CPSP)

CPSP is a consequence of stroke in 8 % of the patients during the first year following the stroke (Andersen et al., 1995). At a superficial glance a similar clinical phenomenology is expressed in patients with dynamic mechanical allodynia of peripheral and central neuropathic origin, despite different lesion levels. CPSP has been described to occur following lesions anywhere along the spino-thalamo-cortical pathway from the brainstem to the cerebral cortex (Boivie, 2006) but the pathophysiological background of ongoing spontaneous pain and dynamic mechanical allodynia in such patients is currently poorly understood (Jones and Watson, 2007). Broadly, three hypothetical phenomena have been proposed to underlie abnormal neural activity resulting in CPSP, i.e., disinhibition, sensitization and plasticity and more than one phenomenon may be involved in an individual patient (Craig, 2007).

The technique of HNCS has previously been used in patients with CPSP. During electrically induced HNCS in patients with brainstem infarcts (Wallenberg's syndrome) no inhibition of the spinal nociceptive flexion reflex could be demonstrated (De Broucker et al., 1990), indicating a pivotal brainstem involvement to alter reflex

excitability. In patients with CPSP due to supratentorial stroke the pathophysiology of spontaneous ongoing pain and dynamic mechanical allodynia is reasonably to be found above the brainstem level and should not be modified by spino-bulbo-spinal loops. However, spino-bulbo-cerebral (Lima and Almeida, 2002) and corticofugal (Villanueva and Fields, 2004) projections interfering with pain perception could not be ruled out.

1.4 ENDOGENOUS MODULATION OF NOCICEPTIVE ACTIVITY FROM AN UNAFFECTED PAIN-FREE AREA IN HEALTHY SUBJECTS AND PATIENTS WITH PAINFUL PERIPHERAL NEUROPATHIC PAIN AND CPSP

HNCS has been used to study endogenous pain modulation in healthy subjects (Pertovaara et al., 1982; Plaghki et al., 1994) and in patients with painful clinical conditions such as trapezius myalgia (Leffler et al., 2002a) and rheumatoid arthritis (Leffler et al., 2002b) with induced inhibition of pain perception during various experimental noxious conditioning stimuli. In contrast, lack of altered pain perception during HNCS has been demonstrated in other long-term clinical pain conditions such as temporo-mandibular disorders (Maixner et al., 1995), fibromyalgia (Kosek and Hansson, 1997) and coxarthrosis before but not following successful arthroplastic surgery (Kosek and Ordeberg, 2000). Pain sensitivity at baseline in the pain-free area has been used for comparison with controls, with the possibility to detect endogenous modulation already initiated by the painful condition (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000; Leffler et al., 2002a,b). In humans the net effect only on pain sensitivity can be studied.

2 AIMS OF THE THESIS

The aim was to investigate the influence of supraspinal descending endogenous pain modulation on spontaneous ongoing pain and dynamic mechanical allodynia using heterotopic noxious conditioning stimulation (HNCS) in painful peripheral neuropathy (study II) and in central post stroke pain (CPSP) (study III). In addition, the influence of HNCS and ongoing pain on pain sensitivity in a pain-free area was assessed and compared to age- and sex-matched healthy controls (study II and III). To prepare for a proper study design in these studies potential side and time dependant alterations in pain sensitivity were examined in healthy volunteers (study I). The possible involvement of spinal dorsal horn $5HT_3$-receptors in spontaneous ongoing pain and dynamic mechanical allodynia in patients with peripheral neuropathy, a hypothesis derived from animal model research, was examined following intravenous infusion of the $5HT_3$-antagonist ondansetron (study IV).

2.1 SPECIFIC AIMS

Study I

- *To examine if side and/or time differences in pain thresholds and suprathreshold pain sensitivity for pressure and heat, respectively, could be detected on both thighs in healthy volunteers during unilateral HNCS of the arm.*

Study II

- *To examine if HNCS influences the intensity of spontaneous ongoing neuropathic and brush-evoked pain in patients with peripheral neuropathy.*
- *To examine if HNCS influences pain sensitivity in a pain-free area.*
- *To examine if spontaneous ongoing neuropathic pain influences pain sensitivity in the same pain-free area.*

Study III

- *To examine the effect of HNCS on the intensity of spontaneous ongoing neuropathic and brush-evoked pain in patients with central post stroke pain (CPSP).*
- *To examine if the spontaneous ongoing neuropathic pain influenced the pain sensitivity in a remote pain-free area and to examine the effect of HNCS on pain sensitivity in the same pain-free area.*

Study IV

- *To examine if the intensity of brush-evoked and spontaneous ongoing neuropathic pain in patients with peripheral neuropathy was influenced by an intravenous infusion of ondansetron or placebo (saline).*

3 MATERIALS AND METHODS

3.1.1 Subjects

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

3.1.2 Study I

3.1.2.1 Subjects

Eighteen healthy and habitually pain-free volunteers, ten females and eight males, with an average age of 36 years (range 20 – 54) participated. Seventeen right-handed and one left-handed subject were included. No medication was taken on a regular basis. All participating subjects had resting blood pressure (BP) < 140/90 mm Hg and heart rate (HR) between 50 and 90 beats/min assessed with a digital blood pressure monitor (UA –767 A & D Instruments LTD, Oxford, England).

3.1.3 Study II

Normal resting BP and HR, i.e., BP < 140/90 mm Hg and HR between 50 and 90 beats/min were prerequisites assessed with a digital blood pressure monitor (UA –767 A & D Instruments LTD, Oxford, England).

3.1.3.1 Patients

Fifteen patients, 8 females and 7 males, with an average age of 42 years (range 25 - 60), suffering from long-term peripheral neuropathy (range 1-12 years) participated. Power analysis of standard deviations from earlier studies of our research group using quantitative sensory testing (Samuelsson et al., 2005) and ischemic pain provocation in healthy individuals guided the number of patients included (Leffler et al., 2002a; Tuveson et al., 2006). In addition to a diagnosis of neuropathy, spontaneous ongoing neuropathic pain in conjunction with a clearly 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, i.e., dynamic mechanical allodynia, was a prerequisite. Special care was taken not to include patients reporting a stimulus-evoked unpleasant sensation only, i.e., dysesthesia.

On the study day, if applicable, the patients were allowed to continue prescribed medications with stable doses (i.e., amitriptyline (n=1), gabapentin (n=2), SSRI (n=2)), but were asked to refrain from using additional analgesics (i.e., acetaminophen (n=3), codeine (n=1), tramadol (n=2) and dextropropoxiphene (n=1)) during the 24 hours prior to testing. Six patients had no medication at all. Patients on strong opioid medication were excluded. If the patient was using a spinal cord stimulator (n=6), they were requested to switch it off at least 12 hours in advance to eliminate the pain relieving effect. Exclusion criteria were cardiovascular, other neurological or dermatological diseases or painful conditions localised to the musculoskeletal system.

Table 1. Demographic data, pain duration and the intensity of spontaneous ongoing neuropathic pain before the assessments (VAS translated to 0-100 mm) in 15 patients with dynamic mechanical allodynia due to peripheral neuropathy.

Patient gender	age (years)	Nerves involved	Pain duration (years)	Baseline intensity of spontaneous ongoing neuropathic pain (mm)
1 M	39	L digital nerve, dig II hand, status post compression injury	5	70
2 M	33	R femoral nerve, status post stab injury	12	12
3 F	51	R scar pain, status post carpal tunnel surgery	11	68
4 M	30	L sural nerve, scar pain status post surgery, ligamentoplasty	11	50
5 F	51	R saphenous nerve, status post pressure injury	1	91
6 M	28	L medial cutaneous nerve of the forearm, status post gun wound	7	30
7 F	45	R digital nerve, dig II hand, status post ganglion surgery	6	88
8 F	60	R ulnar nerve, status post cut injury	3	31
9 M	33	L cervical plexus, status post stab injury	11	42
10 F	25	R median nerve, scar pain status post carpal tunnel surgery	2	53
11 F	38	L peroneal nerve, status post compression and fasciotomy	5	52
12 F	42	L L5 rhizopathy, status post cyst compression injury	10	72
13 M	59	R sciatic nerve, status post pelvic fracture	5	33
14 M	55	R branch of femoral nerve, status post knee arthroplasty	3	82
15 F	48	L S1 rhizopathy, status post disc surgery	4	43

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

3.1.3.2 Controls

Fifteen healthy and habitually pain-free, age- and sex-matched volunteers with an average age of 42 years (range 25-59) participated. No medication was taken on a regular basis.

3.1.4 Study III

3.1.4.1 Patients

Ten patients, 4 females and 6 males, with a mean age of 60 years (range 38 – 74), suffering from central post stroke pain (CPSP) for an average of 5.5 years (range 3 months – 12 years) participated. Power analysis of standard deviations from quantitative sensory testing data in previous studies by our group investigating dynamic mechanical allodynia in patients with peripheral neuropathy (Samuelsson et al., 2005) and the effect of ischemia-induced HNCS in healthy individuals guided the number of patients included (Tuveson et al., 2006). In addition to a diagnosis of stroke, spontaneous ongoing pain due to the brain lesion and a clearly painful sensation evoked by lightly stroking the skin with a soft brush were obligatory. Prior to inclusion a supratentorial cerebral lesion, at the left side in seven and at the right side in three patients, had been verified with computerized tomography (CT) (Table 2). All patients in the present study had a history of cerebral haemorrhage/infarct with

an extrathalamic location in 6 patients and with at least a partial thalamic involvement in 4 patients. Five patients had a lesion extending to more than one anatomical location. In 7 patients the haemorrhage engaged the posterior internal capsule and in 5 patients there was an involvement of the striatum. Special care was taken not to include patients reporting a brush-evoked unpleasant sensation, i.e., dysesthesia or patients with static mechanical allodynia only. To crudely assess the cognitive status the patients performed the mini mental test (Folstein, 1975). The average result from the test was 29.5 points (range 28 – 30) out of a maximum of 30 points, and hence no patient was excluded due to mental shortcomings. Interviews and performances of bimanual tasks, included in the mini mental test, did not reveal any neglect problems in the patients included.

Table 2. Demographic data, locations of lesions, pain duration, ongoing medication and the intensity of spontaneous ongoing neuropathic pain (VAS translated to 0-100 mm) before the assessments in 10 patients with dynamic mechanical allodynia due to central post-stroke pain.

Patient (gender)	**Age (years)**	**Location of brain lesion (computerized tomography)**	**Pain duration (years)**	**Baseline intensity of spontaneous ongoing neuropathic pain (mm)**	**Medication**
1 M	70	Left dorsal putamen/posterior internal capsule haemorrhage	12	80	pregabalin, tramadol
2 M	60	Left posterior internal capsule infarct/haemorrhage	4	85	acetaminophen
3 F	70	Right dorsolateral thalamus/posterior internal capsule haemorrhage	3	34	amitriptyline
4 M	67	Left dorsal basal ganglia/posterior internal capsule haemorrhage	8	62	clonazepam, tramadol
5 F	68	Left caudate nucleus, posterior internal capsule and lateral thalamus haemorrhage	11	87	amitriptyline, gabapentin
6 F	56	Left thalamus haemorrhage	2	50	gabapentin, citalopram
7 M	55	Right putamen haemorrhage	0.5	51	pregabalin
8 M	74	Left thalamus/posterior internal capsule haemorrhage	7	68	-
9 M	44	Right posterior internal capsule haemorrhage	2	78	-
10 F	38	Right dorsal putamen haemorrhage	0.3	72	amitriptyline, pregabalin

F, female; M, male.

Six patients were on antihypertensive medication, all with normalized blood pressure. Two patients were on oral treatment for type II diabetes. No symptoms/signs of peripheral polyneuropathy could be found in the patients with type II diabetes during the interview or during clinical bedside sensory examination. Only one patient had overt motor impairment from the stroke (paretic arm). On the study day patients were allowed to continue prescribed medications with stable doses (Table 2). Exclusion criteria were cardiovascular symptoms, other neurological or dermatological diseases or painful conditions localised to the musculoskeletal system. The preparatory diagnostic assessments in patients were performed a few days or some hours prior to the experimental procedure. In addition, all subjects were carefully familiarized with the different methods to be used before the start of the experiment.

3.1.4.2 Controls

Ten healthy and habitually pain-free, age- and sex-matched volunteers with a mean age of 59 years (range 38-72) participated. One volunteer was treated for hypertension with normalized blood pressure.

3.1.5 Study IV

3.1.5.1 Patients

Fifteen patients, 10 females and 5 males, with a mean age of 45 years (range 28 - 61), suffering from long-term peripheral neuropathy for a mean of 5 years (range 0.4 - 15) participated. Power analysis of standard deviations for total brush-evoked pain obtained from earlier studies of our research group using the semi-quantitative brushing technique in patients with painful peripheral neuropathy (Samuelsson et al., 2005) guided the number of patients included.

Table 3. Demographic data, pain duration and the intensity of spontaneous ongoing neuropathic pain before the assessments (VAS translated to 0-100 mm) in 15 patients with dynamic mechanical allodynia due to peripheral neuropathy.

Patient (gender)	**age (years)**	**Location of peripheral nerve injury**	**Pain duration (years)**	**Baseline intensity of spontaneous ongoing neuropathic pain (mm) at the first and second study occasion**	**Treatment: pain medication or spinal cord stimulation (SCS)**
1 F	28	R median nerve, scar pain status post carpal tunnel surgery	5	29, 47	acetaminophen + codeine
2 F	53	L peroneal nerve, status post fracture and surgery	0.9	5, 20	amitriptyline
3 F	45	L L5 radiculopathy, status post cystic compression injury	13	77, 66	-
4 F	54	R saphenous nerve, status post pressure injury	4	38, 39	pregabalin
5 M	42	L digital nerve, dig II of hand, status post compression injury	8	54, 64	SCS
6 M	42	R ulnar nerve, status post amputation dig V	6	42, 20	pregabalin
7 F	52	L S1 radiculopathy, status post disc surgery	7	56, 16	-
8 M	36	L cervical plexus, status post stab injury	14	47, 35	SCS
9 F	42	L cervical tranverse cutaneous nerve, status post stab injury	3	36, 51	tramadol
10 M	28	L intercostal nerve, status post surgery	2	22, 41	-
11 M	44	L peroneal nerve, status post compression and fasciotomy	7	40, 55	-
12 F	44	L peroneal nerve, status post surgery of Mb Morton	0.6	49, 65	amitriptyline, tramadol, gabapentin
13 F	41	L superficial radial nerve, status post stab injury and surgery	1.5	86, 71	acetaminophen + codeine
14 F	60	L sural nerve, status post fracture and surgery	0.3	14, 4	pregabalin, tramadol, cpapsaicin cream
15 F	61	R scar pain status post carpal tunnel surgery	5	65, 44	pregabalin

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

In addition to a diagnosis of neuropathy, spontaneous ongoing neuropathic pain in conjunction with a clearly 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, i.e., dynamic mechanical allodynia, were inclusion prerequisites. Special care was taken not to include patients reporting a stimulus-evoked unpleasant sensation only, i.e., dysesthesia. On the study day patients were allowed to continue prescribed treatment with stable doses (antiepileptics, antidepressants and tramadol, n=7). The patients were carefully informed not to change pain medication between the two study occasions. The patient group without medication comprised eight patients including two patients using short-acting analgesics on demand (i.e. combination of acetaminophen and codeine). The latter two patients were asked to refrain from medication during 24 hours prior to testing. Patients on strong opioid medication were excluded. If a spinal cord stimulator (n=2) 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.

In a tolerability and safety study with a high dose of intravenous ondansetron (32 mg) a statistically significant increase in corrected QT (QTc) interval was described as acute, transient and asymptomatic (Benedict et al., 1996). In addition, in a recent study the risk of pro-arrythmia from ondansetron has been reported to be very low (Charbit et al., 2005). A diagnostic ECG was performed before the start of the experiment, analysed and controlled for normal value of corrected QT- interval and in addition the ECG was monitored continuously during the procedure. All patients had heart rate above 55/min and a normal ECG with a QTc interval corrected for heart rate below 450 ms in men and 470 ms in women, adhering to criteria from the Committee for Proprietary Medicinal Products (London, United Kingdom). One patient was on antihypertensive medication with normalized blood pressure and one used oral treatment for type II diabetes since 2 years. No symptoms/signs of polyneuropathy could be found in the patient with type II diabetes.

3.2 METHODS

3.2.1 General procedure

All tests and sensibility assessments were performed by the same investigators (authors B.T. Study I – IV) and A-S. L. (Study I – III)). All subjects were examined in a relaxed supine position and carefully familiarised with the different methods to be used.

Table 4. Summary of experimental methods.

Method/study	**I**	**II**	**III**	**IV**
HNCS of the left upper arm	x			
HNCS of the upper/lower extremity ipsilateral to the area of brush-evoked pain		x		
HNCS of the upper/lower extremity contralateral to the area of brush-evoked pain			x	
PPT and SPP in a pain-free area	x	x	x	
HPT and SHP in a pain-free area	x	x	x	
Total brush-evoked pain intensity		x	x	x
Intensity of spontaneous ongoing pain (VAS)		x	x	x

Conditioning pain stimulation used in study I - III was performed using a modified submaximal effort tourniquet (Woolf, 1979; Pertovaara et al., 1982). An air-filled

rubber ring was used to drain the arm or leg of venous blood. A cuff, 11 cm wide for the arm and 13 cm for the leg, was placed just proximal to the cubital fossa or around the middle of the thigh and inflated to 240 mm Hg. During inflation with Stille Electronic Tourniquet System® (Stille Surgical AB, Stockholm, Sweden) the subject was instructed to dorsiflex the ankle or wrist joint, the latter with a weight (1 kg for women and 2 kg for men). The effort was stopped following a maximum of 45 repetitions or as soon as the subjects complained of intense pain in the arm or leg, rated as 7 or more out of 10 on a category-ratio-10 scale (Borg, 1982). The tourniquet-induced pain intensity in the arm or leg was rated by the subject using a 100 mm visual analogue scale (VAS). The left extreme end of the VAS indicated 'no pain' and the right end 'worst imaginable pain'. To guide subsequent pressure- and heat pain sensibility testing two separate round spots and two areas of 12.5 cm^2 were marked on the skin overlying the middle anterior part of the thigh (midway between the groin and the basis of patella) (study I – III) or the upper arm (midway between the acromion and the olecranon) (study II – III) in a pain-free area contralateral to the side of peripheral nerve injury or contralateral to the area of allodynia following stroke. Quantitative sensory testing (QST) (Hansson and Lindblom, 1992) was performed in the indicated areas at the thigh or the upper arm at the start of the experiment (baseline values), during HNCS and was repeated after a resting period of 30 min following the HNCS-procedure in study I and II. To guide assessments of brush-evoked pain in the patients with nerve injury (study II – IV), they were asked to indicate the area of ongoing pain and dynamic mechanical allodynia, respectively on a whole body pain drawing. The site of brush-evoked pain was determined by lightly brushing from the unaffected skin towards an area where the normally non-painful mechanical stimulus was perceived as painful using Brush-05 (SENSELab™, Somedic Sales AB, Hörby, Sweden).

3.2.1.1 Study I

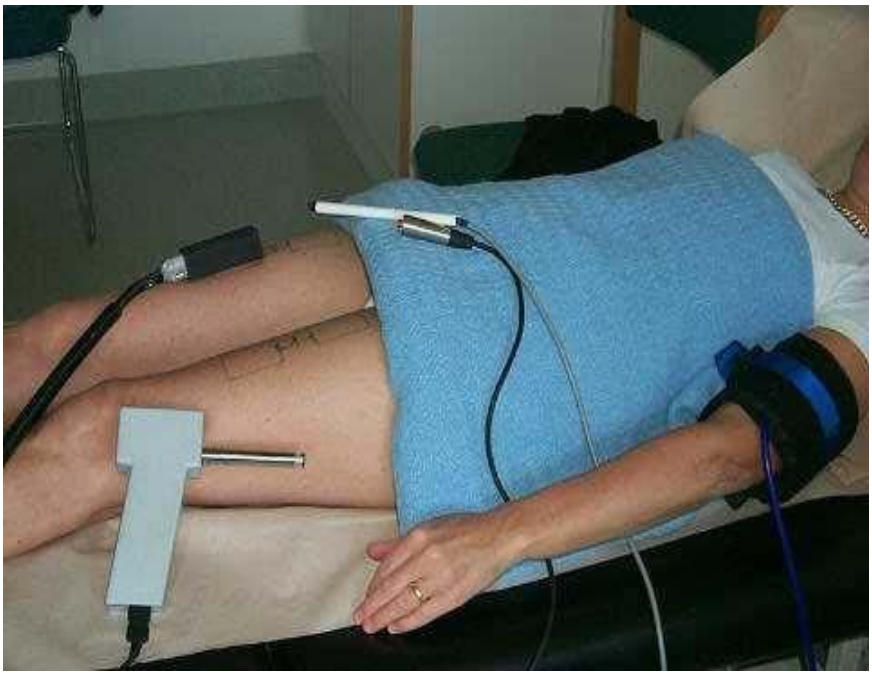

Figure 1. Experimental set up for study I with blood pressure cuff on the left arm to induce ischemic pain, an algometer for assessments of pressure pain and a thermode for assessments of heat pain. Both the algometer and the thermode of the thermotest were connected to hand hold buttons and the subjects were instructed to press the button when the pressure or the heat turned into a painful sensation.

HNCS was induced by ischemia in the left arm and quantitative sensory testing was performed bilaterally in the indicated areas on the thighs at the start of the experiment (baseline values), during HNCS and repeated after a resting period of 30 min following the HNCS-procedure. The session time line is illustrated in figure 2 and the experimental set up in figure 1. Pain intensity, BP and HR were assessed at regular intervals during the HNCS-procedure, i.e., before the start of QST and following QST on the first and secondly assessed thigh, the latter just before cuff deflation. During the resting period the pain intensity in the left arm was rated 1 min following cuff deflation and repeated after 5, 10, 15 and 30 min in conjunction with assessments of BP and HR. The HNCS-procedure was completed in approximately 10 minutes.

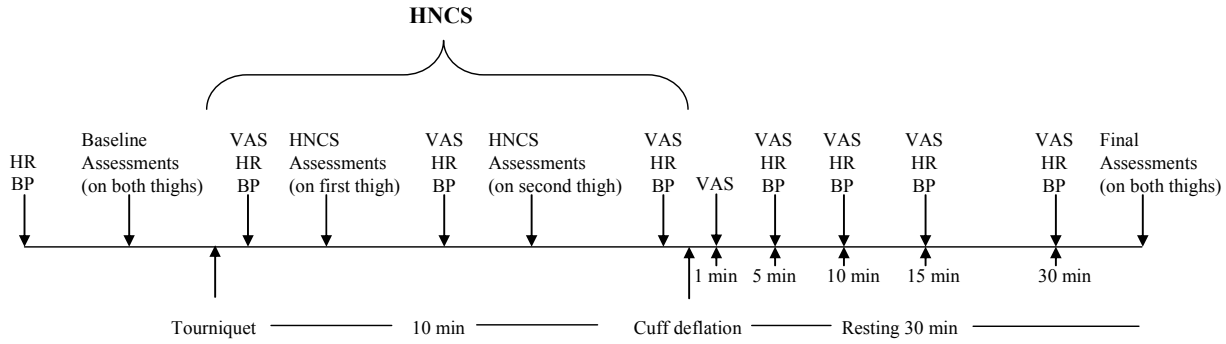

Figure 2. Timeline for the experimental session before, during and following heterotopic noxious conditioning stimulation (HNCS). Pain intensity (VAS), heart rate (HR) and blood pressure (BP) were repeatedly measured during and after the pain provocation.

3.2.1.2 Study II

HNCS was induced in the ipsilateral arm in patients with unilateral peripheral neuropathy in the lower extremity and vice versa. Quantitative sensory testing was performed in the indicated areas at the upper arm or the thigh contralateral to the nerve injury at the start of the experiment (baseline values), during HNCS and repeated after a resting period of 30 min following the HNCS-procedure, the latter as a screening for protracted inhibition. The session time line is illustrated in figure 3. HNCS-induced pain intensity, BP and HR were assessed at regular intervals during the HNCS-procedure, i.e., before the start of QST and following QST, the latter just before cuff deflation. During the resting period the pain intensity in the provoked arm or the leg was rated 1 min following cuff deflation and repeated after 5, 10 and 30 min in conjunction with assessments of BP and HR. The HNCS-procedure was completed with a mean of 9 min (range 7-13 min) with the same duration for the patients and their matched controls.

Patients: In patients the influence of HNCS on spontaneous ongoing neuropathic pain and brush-evoked allodynia was examined. The intensity of brush-evoked allodynia was assessed before and during HNCS as well as following the resting period of 30 min. Spontaneous ongoing neuropathic pain was rated before the assessments of brush-evoked allodynia as well as at 1, 5 and 10 min during the resting period. Due to care taken to possible time dependent alterations in pain sensitivity (Tuveson et al., 2006) from the HNCS-procedure during the three different assessment periods, ratings of

spontaneous ongoing neuropathic pain intensity and assessments of dynamic mechanical allodynia were performed before QST for half of the patients and following QST for the other half. The matched controls were examined in the same order as their patients and care was taken to maintain the same duration of the ischemic pain provocation in the controls.

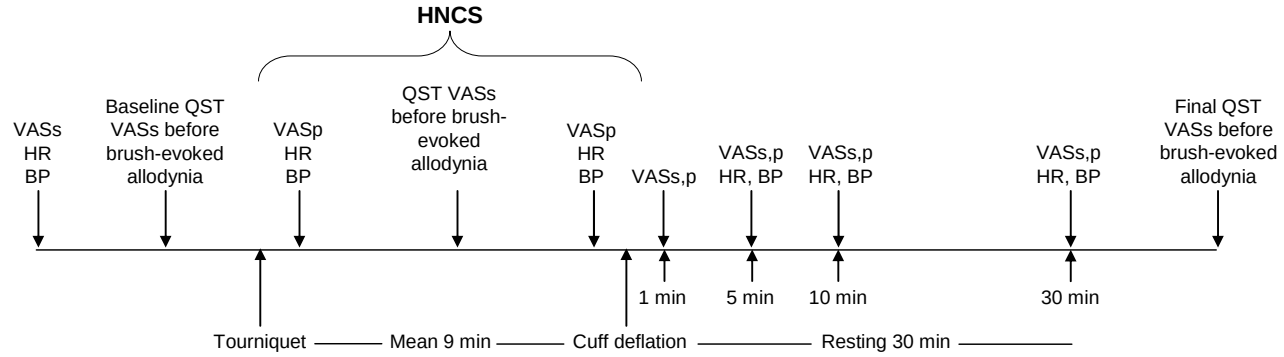

Figure 3. Timeline for the experimental session before, during and following heterotopic noxious conditioning stimulation (HNCS). Visual analogue scale pain intensity ratings of the spontaneous ongoing neuropathic pain (VASs), and the provoked pain intensity (VASp); BP, blood pressure; HR, heart rate. Assessment of spontaneous ongoing neuropathic pain intensity and of dynamic mechanical allodynia were performed before QST for half of the patients and following QST for the other half and the controls were examined in the same manner.

3.2.1.3 Study III

HNCS was induced by ischemia in the contralateral lower extremity in patients suffering from allodynia in the upper extremity and vice versa. Quantitative sensory testing was performed in the indicated areas at the thigh or the upper arm at the start of the experiment (baseline values) as well as during HNCS. The session time line is illustrated in figure 4. HNCS-induced pain intensity, blood pressure and heart rate were assessed at regular intervals during the HNCS-procedure, i.e., before the start of QST and following QST, the latter just before cuff deflation. During the resting period the pain intensity in the provoked arm or leg was rated 1 min following cuff deflation and repeated after 5 and 15 min in conjunction with assessments of BP and HR. The HNCS-procedure was completed with a mean of 7.3 min (range 6 – 11 min) and with the same duration for the patients and their controls.

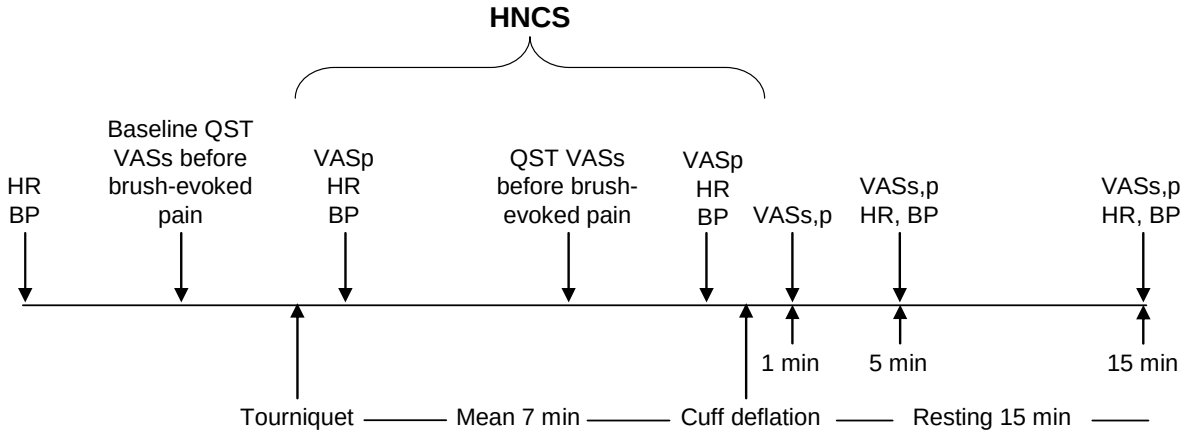

Figure 4. Experimental session timeline including before, during and following heterotopic noxious conditioning stimulation (HNCS). Visual analogue scale pain ratings of the spontaneous ongoing neuropathic pain (VASs); provoked pain intensity (VASp); blood pressure (BP); heart rate (HR).

Patients: In patients the influence of HNCS on ongoing central post-stroke pain and dynamic mechanical allodynia was examined as well as the pain sensitivity in a pain-free area contralateral to the side of ongoing pain. The area of maximum dynamic mechanical allodynia was found in the volar region of the lower arm in seven patients and in the dorsal region of the foot in two patients. In these patients a 60 mm long test area within the region of maximum dynamic mechanical allodynia was marked with a pen. Due to painful withdrawals from the brushing procedure within the area of maximum dynamic mechanical allodynia a less painful area located in the region of lateral upper arm was used in one patient. The intensity of brush-evoked pain was assessed before and during HNCS. Due to care taken to possible time dependent alterations in pain sensitivity from the HNCS-procedure (Tuveson et al., 2006) ratings of ongoing- and brush-evoked pain were performed at the end of the HNCS period. The matched controls were examined in the same order as the patients and care was taken to maintain the same duration of the ischemic pain provocation in the controls.

3.2.1.4 Study IV

The area of dynamic mechanical allodynia in patients with peripheral neuropathy was determined as described in study II and III. ECG was monitored continuously during the whole procdure. The spontaneous pain intensity was rated by the subject using a 100 mm visual analogue scale (VAS). The left extreme end of the VAS indicated 'no pain' and the right end 'worst imaginable pain'.

The study was randomised, double-blind and placebo-controlled with a crossover design. The patients were randomly allocated to be given odansetron or saline at two different sessions separated by at least 3 days (intra-session median=10 days, range 3 – 50 days). An intravenous catheter was inserted in fossa cubitis in all but one patient, where the catheter was inserted in the dorsum of the hand. The catheter was never inserted ipsilateral to a nerve injury in the arm. The intravenous infusion was prepared by a nurse who did not participate in the rest of the experiment. Ondansetron 8 mg (4 ml) added to 96 ml saline or pure 100 ml saline was given as an intravenous infusion during 10 min using a volume controlled infusion pump IVAC Signature (Alaris Medical Systems, San Diego, USA). Stroking in the area of dynamic mechanical allodynia was performed before and immediately following the intravenous infusion and was then repeated every 15 minutes during 3 hours, i.e., at 14 different time-points. Assessment of blood pressure (BP) and heart rate (HR) was performed at the start of the experiment and immediately following the intravenous infusion. HR was then repeatedly assessed at every 15 min and BP at every 30 min during a period of 3 hours. The estimated plasma halflife of ondansetron is 3.0 - 3.5 hours (Blackwell and Harding, 1989). Any side effects were actively asked for intermittently during the observation periods and before the second session. Care was taken to encourage the patients to get in contact with the investigator (author B.T.) to report additional side effects following the sessions.

3.2.2 Intensity ratings of spontaneous ongoing neuropathic and brush-evoked pain

The spontaneous ongoing pain intensity was rated using a VAS (0-100 mm) before each assessment of brush-evoked pain (Study II, III och IV), i.e., before (Study II and III) and during HNCS as well as following HNCS at 1, 5, 10 and 30 min (Study II) or at 1, 5 and 15 min (Study III) during the resting period. In study IV the spontaneous pain

intensity was rated before and immediately following the intravenous infusions as well as every 15 min during 3 hours (i.e., altogether at 14 different time-points).

3.2.2.1 Assessment of brush-evoked pain (Study II, III and IV)

Brush-evoked pain was induced by lightly stroking 60 mm of the neuropathic skin 4 times using a brush with a width of 8 mm, connected to a modified electronic von Frey analyzer (Somedic Sales AB, Hörby, Sweden). The patients were carefully instructed to rate the intensity of brush-evoked pain separately from the ongoing pain. The brush strokes were always performed in the same direction (proximal to distal), with the examiner keeping a fairly constant brushing 'force' (4 – 25 g) and velocity (20 mm/s). Brushing force was monitored on-line on the computer screen and if the force exceeded 25 g or was below 4 g the stroke was disregarded and a new attempt was made. Using a computerized VAS, preset to record values exceeding 2 mm and stopped when values were below 2 mm, the subjects continuously rated the intensity of the brush-evoked pain. Measurements were stored in a data base that enabled recordings of seconds to onset of brush-evoked pain (> 2 mm VAS), the maximum value of the total brush-evoked pain intensity as well as when pain intensity had returned to baseline, all as a basis for calculating the total brush-evoked pain intensity as the integrated value of the graph over time, i.e., the area under the curve (Samuelsson et al., 2005). Good repeatability has been demonstrated using this technique (Samuelsson et al., 2007).

3.2.3 Quantitative sensory testing

QST was performed using the method of limits (Hansson et al., 1988; Jensen et al., 1986).

3.2.3.1 Assessment of pressure pain threshold and sensitivity to suprathreshold pressure pain (Study I, II and III)

Pressure pain sensitivity was assessed using a pressure algometer (Somedic Sales AB, Sweden), the reliability of which has been reported previously (Jensen et al., 1986; Kosek et al., 1993). Pressure algometry has been reported to mainly reflect pressure pain sensitivity of deeper tissues (Kosek et al., 1999). The two adjacent pen marked spots on the thigh or the upper arm were used for assessment of the pressure pain threshold (PPT) and the sensitivity to suprathreshold pressure pain (SPP), respectively. The perception level to pressure pain was assessed three times, with an intended pressure rate of 50 kPa/s and an inter-stimulus interval of 10 s. The mean of the last two perception levels was calculated as the PPT. The sensitivity to SPP was assessed once and with the same pressure rate by asking the subjects to push the button when they would rate the pressure pain intensity as 4 out of 10 on a category-ratio-10 scale (Borg, 1982). The probability of harming the underlying tissue was regarded to be low at this intensity.

3.2.3.2 Assessment of heat pain threshold and sensitivity to suprathreshold heat pain (Study I, II and III)

Testing of heat pain sensitivity was carried out using a commercially available Peltier element based thermode (MSA Thermotest™, Somedic Sales AB, Hörby, Sweden) with an area of 12.5 cm^2. All measurements started from skin temperature, assessed with an infrared skin temperature analyser (Tempett®, Somedic Sales AB, Hörby, Sweden). The perception level to heat pain was assessed three times with a stimulus

rate of 2 °C/s and an inter-stimulus interval of 10 s. The average of the last two pain levels was calculated as the heat pain threshold (HPT). The sensitivity to suprathreshold heat pain (SHP) was assessed once and with the same stimulus rate by asking the subjects to push the button when they would rate the heat pain intensity as 7 out of 10 on the category-ratio-10 scale. The stimulus never exceeded the cut off temperature of 50 ºC, thereby minimising the risk of skin damage.

3.3 STATISTICS

Table 5. Statistical methods used for analysis of assessed parameters in study I – IV.

Statistical method/ assessed parameter	Spontaneous pain	Brush-evoked pain	Provoked pain	PPT	SPP	HPT	SHP	BP	HR
One-way ANOVA		II							
Two-way ANOVA				I, II, III	I, II, III	I,II, III	I, III	I, II, III	I, II, III
Three-way ANOVA		IV		I	I	I	I	IV	IV
Four-way ANOVA		IV							
Mixed procedure	IV					II			
Friedman´s ANOVA	II, III, IV	III, IV	I, II, III				II		
Mann- Whitney U test	II	II		I, II	I, II	II	I,II		
Page test	III		III						
Sign test		III, IV	III				III		
Spearmann rank order correlation	III	III							

3.3.1 Study I

A normal distribution was found for PPT, SPP and HPT. The distribution for SHP was skewed and to meet the requirements for an adequate ANOVA the data have been transformed to a log scale (-ln(51-x)) (Kirk, 1995). The data were analysed using a three-way ANOVA with repeated measures on three factors. The factors were group with the two levels ipsilateral (left;1st assessed side)/ contralateral (right;2nd assessed side) and contralateral (right;1st)/ ipsilateral (left;2nd), side with the two levels ipsilateral as well as contralateral and time with the three levels before, during and following HNCS. In case of significant three-way interaction, two-way interactions were examined. In order to study possible side and/or time dependent differences during the HNCS-procedure the data were analysed separately for the first and the lastly assessed side. If the F-ratio for the time factor was significant from the simple model, the Fisher's LSD test was performed to make pairwise comparisons among the mean values, provided that the sphericity assumptions were met. Otherwise planned comparisons were performed between the means. When significantly decreased sensitivity was found, comparing baseline and outcomes during HNCS, Mann-Whitney U test was used for an analysis between sides of the magnitude of the altered PPT, SPP and SHP, respectively, separately for the primarily and secondly assessed sides. Due to care taken to possible time dependent effects of HNCS during the three different assessment periods, the measurements on the first assessed side were performed on the contralateral thigh in 10 subjects and on the ipsilateral thigh in 8 subjects.

Consequently, the measurements on the secondly assessed side were performed on the contralateral thigh in 8 subjects and on the ipsilateral thigh in 10 subjects.
The data from assessments of BP and HR as well as VAS ratings during pain provocation were analysed using a two-way ANOVA with repeated measures on two factors. The within factors for time were before, during with three time points (before the first QST and following QST on the first and second thigh, respectively) as well as following the conditioning stimulation during the resting period with four time points (after 5, 10, 15 and 30 minutes). If the F-ratio for the time factor was significant, planned comparisons were performed between the mean values. The p-values were then corrected according to the Bonferroni procedure. Statistical significance was accepted at $p \leq 0.05$.

3.3.2 Study II

3.3.2.1 Assessments of pressure and heat pain thresholds as well as sensitivity to suprathreshold pressure

Data were analysed with a two-way ANOVA with repeated measures of two factors. The within factors were group (patient and control) and time (before, during and following HNCS). Differences between levels of the within factor time were evaluated by the Fisher LSD test if the sphericity condition was tenable otherwise specific contrasts were applied. In case of a significant interaction between the factors, simple effects were examined, i.e., effects of one factor holding the other factor fixed. Due to a skewed distribution for the patients, HPT was analysed by mixed procedure and univariate planned comparisons, whereas only mixed procedure was used for normally distributed HPT in controls.

3.3.2.2 Assessments of sensitivity to suprathreshold heat pain and provoked pain intensity ratings in the arm or the leg during and following HNCS

SHP was analysed by Friedman's ANOVA due to a ceiling effect and skewed distribution. The analysis was followed by multiple comparisons between groups (patients and controls) and between three time-points (before, during and following HNCS) for the sensitivity to SHP as well as six time-points for the provoked pain intensity ratings (see timeline figure 3).

3.3.2.3 Pain intensity ratings of spontaneous ongoing neuropathic pain

Data were analysed by Friedman's ANOVA followed by multiple comparisons between the eight time-points (see timeline figure 3).

3.3.2.4 Assessments of brush-evoked allodynia

The data for the total evoked pain intensity have been log-transformed due to skewed distribution in order to meet the requirements for an adequate ANOVA and were analysed with one-way repeated measures ANOVA. The within factor was time (before, during and following HNCS). Differences between levels of the within factor time were evaluated by the Fisher LSD test if the sphericity condition was tenable, otherwise specific contrasts were applied. To investigate the influence of brush-strokes performed before or following QST a between factor, order, was included in the ANOVA model.

3.3.2.5 Assessments of systolic and diastolic blood pressure as well as heart rate

Data were analysed with a two-way ANOVA with repeated measures of two factors. The within factors were group (patient and control) and time with six time-points, i.e., at baseline, during the HNCS-procedure, i.e., before and following QST and as well as 5, 10 and 30 min following the HNCS-procedure during the resting period. Differences between levels of the within factor time were evaluated by specific contrasts. In case of a significant interaction between the factors simple effects were examined, i.e., effects of one factor holding the other factor fixed.
Mann-Whitney U test was used for simple comparison between means of psychophysical parameters (PPT, SPP, HPT, SHP and dynamic mechanical allodynia) and intensity of spontaneous pain between patients with and without medication.
Statistical significance was accepted at $p \leq 0.05$.

3.3.3 Study III

3.3.3.1 Pain intensity ratings of spontaneous ongoing neuropathic pain and assessments of brush-evoked pain and their relationship

The ongoing pain intensity was normally distributed and analysed using Friedman´s ANOVA by ranks, followed by the Page Test and multiple comparisons between time points based on ranks. The Page Test tests the hypothesis that the measures are the same versus the alternative that the measures are ordered in a specific sequence. The p-values were adjusted according to the Bonferroni procedure due to multiple comparisons.

The total brush-evoked pain intensity had a skewed distribution and was analysed using non-parametric statistics, i.e., Sign Test and Friedman's ANOVA.

Spearman rank order correlation was used to analyse the relationship between spontaneous ongoing- and brush-evoked pain before and during HNCS.

3.3.3.2 Assessments of pressure- and heat pain thresholds as well as sensitivity to suprathreshold pressure and heat

Data was analysed with a two-way ANOVA with repeated measures on two factors. The within factors were group (patient and control) and time (before and during HNCS). The data for PPT and SPP has been log-transformed due to a skewed distribution in order to meet the requirements for an adequate ANOVA. Due to a ceiling effect of the variable SHP a nonparametric statistic approach, the Sign Test, was used to evaluate the group and time effects.

3.3.3.3 Ratings of provoked pain in the arm or the leg during HNCS

Within patients and controls Friedman´s ANOVA by ranks was used followed by the Page Test and multiple comparisons between time points based on ranks. The Sign Test was used to compare patients and controls at different time points. The p-values were then adjusted according to the Bonferroni procedure.

3.3.3.4 Assessments of systolic and diastolic blood pressure as well as heart rate

Data was analysed with a two-way ANOVA with repeated measures on two factors. The within factors were group (patient and control) and time (at baseline, during the HNCS-procedure, i.e., before and following QST as well as during the resting period at 5 and 15 min following the HNCS-procedure). When factor time was significant, multiple comparisons between time points were performed. The p-values were then adjusted according to the Bonferroni procedure.
Statistical significance was accepted at $p \leq 0.05$.

3.3.4 Study IV

3.3.4.1 Assessments of the total brush-evoked pain intensity, the maximum value of the total brush-evoked pain intensity and the duration as well as the frequency of painful aftersensation

The total brush-evoked pain intensity had a skewed distribution and has been square root-transformed in order to meet the requirements for an adequate ANOVA. A three-way ANOVA with repeated measures on two factors was used to analyse the total brush-evoked pain intensity. The between factor was treatment sequence, i.e., ondansetron/saline or saline/ondansetron, and the within-groups factors were treatment (ondansetron or saline) and time with 14 time points (before and 13 time points following the infusion i.e. every 15 min). In addition, a four-way ANOVA was used to analyse the influence from the between factor with or without pain medication. The maximum value of the total brush-evoked pain intensity and the duration of the aftersensation were analysed by Friedman´s ANOVA. The Sign test was used to calculate the frequency of aftersensation.

3.3.4.2 Pain intensity ratings of spontaneous ongoing pain

The spontaneous ongoing pain intensity was analysed by Friedman's ANOVA. Due to inability of Friedman's ANOVA to analyse more than one factor at a time analysis was also performed with parametric statistics with mixed procedure to analyse the interaction of the sequence and ongoing pain medication.

3.3.4.3 Assessments of heart rate as well as systolic and diastolic blood pressure

Data was analysed using a three-way ANOVA with repeated measures on two factors. The between-group factor was treatment sequence (ondansetron/saline or saline/ondansetron) and the within-group factors were treatment (ondansetron or saline) and time (before and 13 time points following the infusion for the heart rate and 7 time points for the blood pressure). In case of a significant interaction between the factors simple effects were examined, i.e., effects of one factor holding the other factor fixed.

4 RESULTS

4.1 STUDY I

4.1.1 Quantitative sensory testing

4.1.1.1 Pressure pain threshold (PPT; kPa) (Figure 5)

There was no significant three-way interaction between the three factors group, side and time (Table 6). In the two-way repeated measures ANOVA without the factor group there was no significant interaction between side and time (Table 7). The PPT was significantly lower at the left thigh compared to the right during the whole experiment and there was a significant change over time. Compared to baseline, analysed by planned comparison, the pressure pain threshold increased significantly during the HNCS-procedure ($F(1,17) = 16.82$, $p<0.001$) and returned to baseline following the HNCS-procedure on both sides alike ($F(1,17) = 23.81$, $p<0.001$) (Figure 5). No significant difference in magnitude of the altered PPT during the HNCS-procedure was seen between sides for the first or the lastly assessed side.

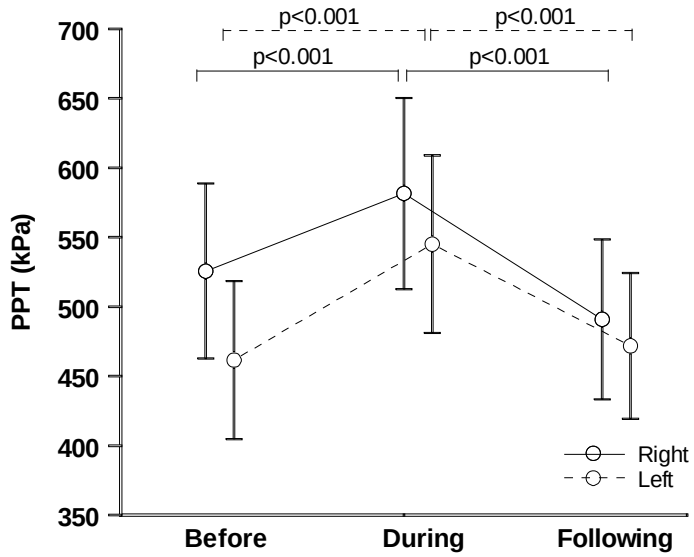

Figure 5. Results, absolute values, from assessment of the pressure pain threshold (kPa), on the right and left thigh in 18 healthy subjects, before, during and following HNCS of the left forearm. Mean values and 95 % confidence intervals are presented. Significant differences in the ANOVA are indicated by p-values.

Table 6.

Results of three-way ANOVA

	group/side (right/left)	time/side	group/time	group/side/time
PPT	NS	NS	NS	NS
SPP	NS	NS	NS	$F(2,32) = 3.33$, $p<0.05$
HPT	NS	NS	NS	NS
SHP	NS	NS	NS	$F(2,32) = 3.91$, $p<0.03$

The p-values are presented. NS, statistically non-significant. PPT, pressure pain threshold; SPP, suprathreshold pressure pain sensitivity; HPT, heat pain threshold; SHP, suprathreshold heat pain sensitivity.

Table 7.

Results of two-way ANOVA without factor group

	side (right/left)	time	side/time
PPT	F(1,17) =5.22, p<0.04	F(2,34) =14.48, p<0.001	NS
SPP	F(1,17) =8.53, p<0.01	F(2,34) =4.40, p<0.02	NS
HPT	NS	NS	NS
SHP	NS	F(2,34) =4.81, p<0.02	NS

The p-values are presented. NS, statistically non-significant. PPT, pressure pain threshold; SPP, suprathreshold pressure pain sensitivity; HPT, heat pain threshold; SHP, suprathreshold heat pain sensitivity.

4.1.1.2 Suprathreshold pressure pain (SPP; kPa) (Figure 6)

There was a significant three-way interaction between the three factors group, side and time, indicating different time courses for the first and secondly assessed side (Table 6). The two-way repeated measures ANOVA without factor group showed significantly lower SPP on the left compared to the right thigh during the whole experiment and there was a significant change over time (Table 7).

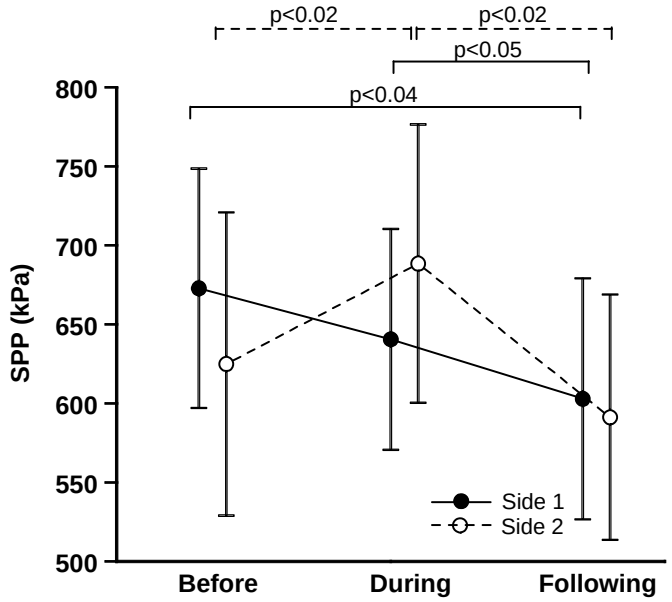

Figure 6. Results, absolute values, from assessment of the sensitivity to suprathreshold pressure pain (kPa), the pressure at which the subjects reported pain intensity as 4 out of 10 on the category ratio 10 scale. The results are presented for the primarily and the lastly assessed thigh in 18 healthy subjects, before, during and following HNCS of the left forearm. Mean values and 95 % confidence intervals are presented. Significant differences in the ANOVA are indicated by p-values.

When separately analysing the first and secondly assessed side a significant two-way interaction was found between group and time (Table 8). On the first assessed side planned comparison demonstrated a significantly increased sensitivity to SPP was seen following the HNCS procedure compared to baseline (F(1,17) =5.42, p< 0.04) and during HNCS (F(1,17) =4.46, p<0.05), respectively (Figure 6). At the lastly assessed

side, compared to baseline, the sensitivity to SPP decreased significantly during the HNCS ($F(1,17) = 6.60$, $p<0.02$) and returned to baseline following the HNCS-procedure ($F(1,17) = 7.90$, $p<0.02$) (Figure 6). No significant difference in the magnitude of the decrease in sensitivity to SPP was seen between sides for the lastly assessed side (n =8 on the contralateral side and n =10 on the ipsilateral side, respectively).

Table 8.

Results of two-way repeated measures ANOVA without factor side

	group	time	group/time
PPT	NS	$F(2,34) = 14.48$, $p<0.001$	NS
SPP	NS	$F(2,34) = 4.40$, $p<0.02$	$F(2,34) = 3.41$, $p<0.05$
HPT	NS	NS	NS
SHP	NS	$F(2,34) = 4.81$, $p<0.02$	$F(2,34) = 4.28$, $p<0.03$

The p-values are presented. NS, statistically non-significant. PPT, pressure pain threshold; SPP, suprathreshold pressure pain sensitivity; HPT, heat pain threshold; SHP, suprathreshold heat pain sensitivity.

4.1.1.3 Heat pain threshold (HPT; ºC)

No significant three- or two-way interaction was seen for HPT and the HNCS-procedure did not change HPT, neither for the first nor the lastly assessed thigh (Table 6 - 8).

4.1.1.4 Suprathreshold heat pain (SHP; ºC) (Figure 7)

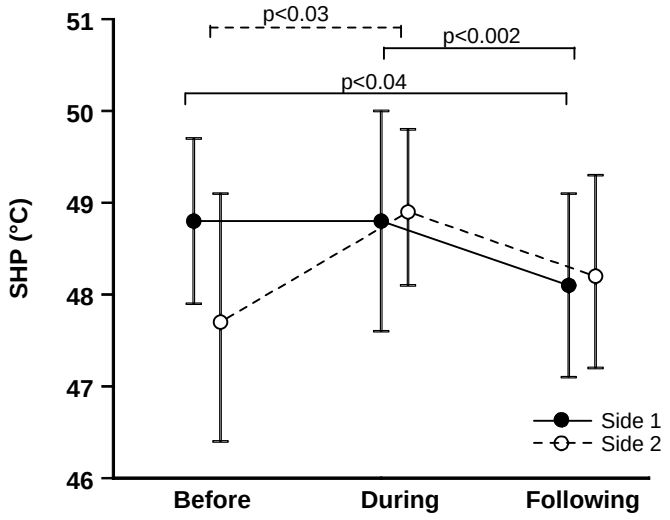

Figure 7. Results, absolute values, from assessment of the sensitivity to suprathreshold heat pain (ºC), the temperature at which the subjects reported pain intensity as 7 out of 10 on the category ratio10 scale; cut off temperature of 50 ºC. The results are presented for the primarily and the lastly assessed thigh in 18 healthy subjects, before, during and following HNCS of the left forearm. Mean values and 95 % confidence intervals are presented. Significant differences in the ANOVA are indicated by p-values.

During assessment of SHP several subjects reached the cut off temperature of 50 ºC without signalling SHP as 7 out of 10 on the category-ratio-10 scale, and in such cases 50 ºC was used in the statistical analysis. There was a significant three-way interaction between the three factors group, side and time, indicating different time courses (Table

6). In the two-way ANOVA without factor group there was a significant change over time (Table 7). When separately analysing the first and secondly assessed sides, there was a significant two-way interaction between side and time (Table 8). On the first assessed thigh analysed by planned comparison, significantly increased sensitivity to SHP was seen following the HNCS-procedure compared to baseline ($F(1,17) = 5.03$, $p<0.04$) and during HNCS ($F(1,17) = 14.68$, $p<0.002$), respectively (Figure 7). At the secondly assessed thigh compared to baseline, the sensitivity to SHP decreased significantly during HNCS ($F(1,17) = 6.15$, $p<0.03$) and returned almost to baseline following the HNCS-procedure ($F(1,17) = 3.26$, $p<0.09$). No significant difference in the magnitude of the decrease in sensitivity to SHP was seen between sides for the secondly assessed thigh (n=8 on the contralateral side and n=10 on the ipsilateral side, respectively).

4.1.2 Number of lifts and pain intensity ratings in the left forearm during and following the HNCS-procedure

Median number of weight lifts were 43 (25^{th} and 75^{th} percentiles; 31 and 45) until the pain intensity in the left forearm was rated as 7 on the category-ratio-10 scale. There was a tendency to higher VAS-scores in the left forearm before the first QST during HNCS for the subjects starting assessments on the contralateral thigh ($p=0.05$). No significant difference was seen between the two groups during the remaining HNCS-procedure. The induced forearm pain intensity decreased significantly one minute following cuff deflation ($p<0.001$) and after ten minutes no subjects reported pain in the left forearm.

4.1.3 Blood pressure (BP) and heart rate (HR)

Compared with baseline, the systolic and diastolic BP increased significantly during the HNCS-procedure (systolic: before QST ($F(1,17) = 24.05$, $p<0.001$), between QST on the primarily and secondly assessed thigh ($F(1,17) = 18.54$, $p<0.002$) and before cuff deflation ($F(1,17) = 25.23$, $p<0.001$) and diastolic: before QST ($F(1,17) = 24.67$, $p<0.001$), between QST ($F(1,17) = 35.54$, $p<0.001$) and before cuff deflation ($F(1,17) = 21.16$, $p<0.001$)) and returned to baseline 5 min following cuff deflation. The HR increased significantly during the HNCS-procedure compared to baseline (before QST ($F(1,17) = 10.79$, $p<0.02$) as well as before cuff deflation ($F(1,17) = 7.12$, $p<0.05$)). Compared to baseline, the HR had decreased significantly 15 min following the cuff deflation ($F(1,17) = 10.53$, $p<0.02$).

4.2 STUDY II

The main finding of the present study was the disparate influence of HNCS on ongoing pain and brush-evoked allodynia. Eight patients (six with no medication and two with acetaminophen/ codeine medication on demand, however not taken in at least 48 hours) were compared with seven patients on medication with CNS modulatory mechanisms of action (gabapentin, amitriptyline, tramadol and SSRI). No significant differences were found between the two groups before or during HNCS for all included psychophysical parameters or spontaneous pain intensity (data not shown).

4.2.1 The influence of spontaneous ongoing neuropathic pain on pain sensitivity in a remote pain-free area

At baseline, no significant difference in pain sensitivity was found between patients and controls for pressure- and heat pain thresholds as well as for the sensitivity to suprathreshold pressure- and heat pain.

4.2.2 The influence of HNCS on clinical pain and experimental pain components

There was no interaction between the three factors group, time and order (before or following QST) during the whole experiment.

4.2.2.1 The influence of HNCS on spontaneous ongoing neuropathic pain intensity (mm) (Figure 8)

A significant change over time (Friedman's ANOVA $p<0.001$) was found. Compared to baseline (mean 54 ± 5.9 (SEM) mm), the spontaneous ongoing neuropathic pain intensity decreased significantly during the HNCS-procedure (mean 43 ± 6.5 mm; $p<0.05$), with no significant difference following HNCS.

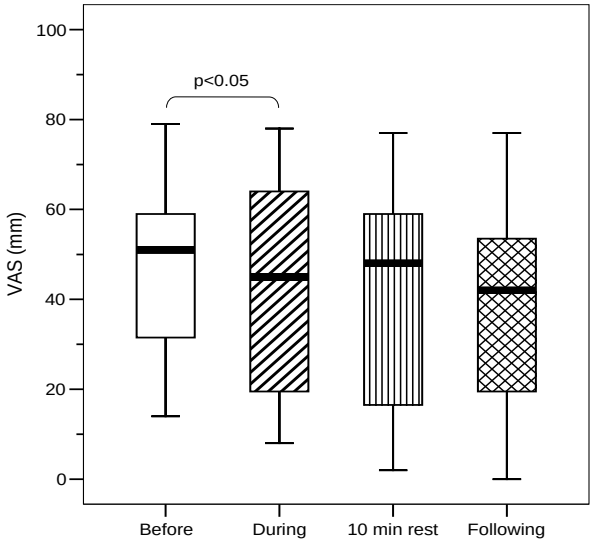

Figure 8. Results, absolute values, from VAS ratings of the intensity of spontaneous ongoing neuropathic pain in 15 patients with peripheral neuropathy and dynamic mechanical allodynia before, during as well as at 10 and 30 min following heterotopic noxious conditioning stimulation (HNCS). Median values of the intensity of spontaneous ongoing neuropathic pain are presented within the 25 – 75 interquartile box, non-outlier maxima and minima (whiskers). Significant differences from pair wise comparison between time-points are indicated by p-values.

4.2.2.2 The influence of HNCS on total brush-evoked pain intensity (Figure 9)

There was a significant change over time for the total brush-evoked pain intensity ($F(2,28) =4.4$, $p<0.05$). This was due to significantly higher total brush-evoked pain intensity following the HNCS-procedure compared to during conditioning pain stimulation ($p<0.01$). However, comparing baseline with during HNCS, no significant change of the total brush-evoked pain intensity was found.

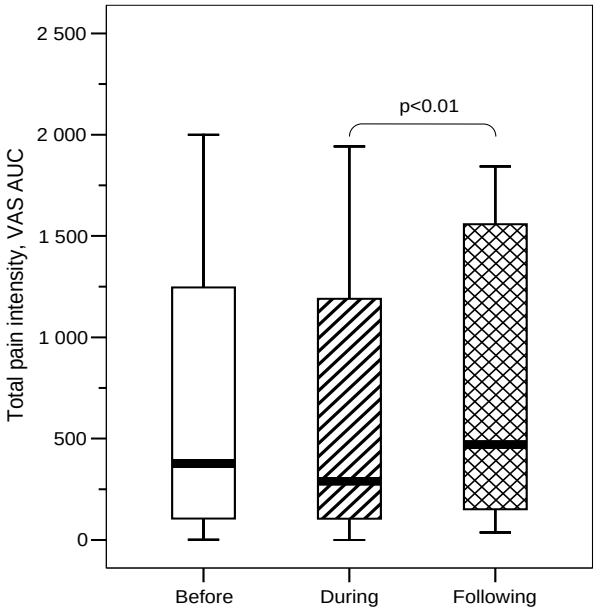

Figure 9. Results, absolute values, from VAS ratings of the total brush-evoked pain intensity in 15 patients with peripheral neuropathy and dynamic mechanical allodynia before, during and following heterotopic noxious conditioning stimulation (HNCS). Median values of the total brush-evoked pain intensity (area under the curve (AUC)) are presented within the 25 – 75 interquartile box, non-outlier maxima and minima (whiskers). Significant difference from post hoc LSD test is indicated by *p*-value.

4.2.2.3 Tourniquet-induced pain in the arm or leg

The patients performed fewer movement efforts than the controls before 7 on the category-ratio-10 pain scale were reached (wrist; patients median 20 (25 % (Q25) and 75 % quartiles (Q75); 7 - 33), controls median 38 (Q25-Q75; 23-45), ankle; patients median 0 (Q25-Q75; 0-23), controls median 5 (Q25-Q75; 0-41)).
There was no significant difference in the tourniquet-induced pain intensity between patients and controls during or 30 min following conditioning pain stimulation. The provoked pain intensity decreased significantly 5 min following cuff deflation in patients and controls alike ($p<0.001$ and $p<0.001$, respectively). Following the resting period, one patient but no controls reported pain from the tourniquet procedure (arm 21 mm).

4.2.2.4 The influence of HNCS on pain sensitivity in a pain-free area

Pressure pain threshold (PPT; kPa) (Figure 10)

There was no significant difference between patients and controls in PPT during the whole experiment. A significant change over time was found (Table 9). Compared with baseline, the sensitivity to pressure pain decreased significantly during HNCS ($F(1,14) = 11.27$, $p<0.01$) and returned to baseline following conditioning stimulation ($F(1,14) = 32.17$, $p<0.001$) in patients and controls alike.

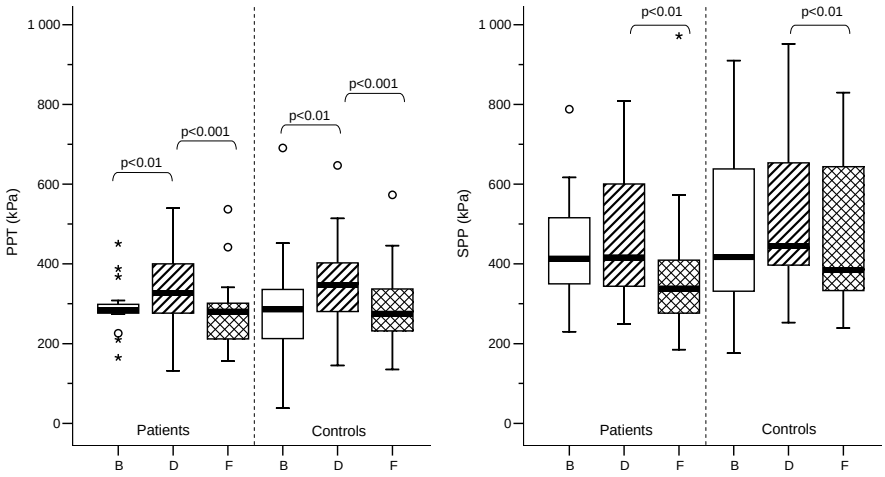

Figure 10. Results, absolute values, from quantitative pain testing at the thigh or the upper arm in 15 patients with peripheral neuropathy and dynamic mechanical allodynia and their age and sex-matched controls before (B), during (D) and following (F) heterotopic noxious conditioning stimulation (HNCS). Median values are presented within the 25 – 75 interquartile box, non-outlier maxima and minima (whiskers), circles denote outliers and asterisks extremes. PPT, pressure pain threshold; SPP, suprathreshold pressure pain. Significant differences from planned comparison of PPT and post hoc LSD test for SPP are indicated by p-values.

Suprathreshold pressure pain (SPP; kPa) (Figure 10)

There was no significant difference between patients and controls in the sensitivity to SPP during the whole experiment. A significant change over time was found (Table 9). Compared with during HNCS, the sensitivity to suprathreshold pressure pain was significantly increased following conditioning stimulation in patients and controls alike ($p<0.01$).

Table 9

Results of the two-way repeated measures ANOVA

	Group	Time	Group/Time
PPT	*NS*	$F(2,28) = 12.44$, $p<0.001$	*NS*
SPP	*NS*	$F(2,28) = 4.06$, $p<0.03$	*NS*
HPT	$F(1,14) = 7.21$, $p<0.02$	*NS*	$F(2,28) = 4.93$, $p<0.03$
Systolic BP	*NS*	$F(5,70) = 25.13$, $p<0.001$	$F(5,70) = 5.75$, $p<0.006$
Diastolic BP	*NS*	$F(5,70) = 30.28$, $p<0.001$	$F(5,70) = 2.54$, $p<0.04$
HR	$F(1,14) = 20.93$, $p<0.001$	$F(5,70) = 10.78$, $p<0.001$	*NS*

p-values are presented. NS, statistically non-significant. PPT, pressure pain threshold; SPP, suprathreshold pressure pain sensitivity; HPT, heat pain threshold; BP, blood pressure; HR, heart rate.

Heat pain threshold (HPT; ºC) (Figure 11)

There was a significant interaction between the factors group and time (Table 9). Compared with during HNCS, the sensitivity to heat pain in patients was significantly

increased following conditioning stimulation ($p<0.01$). Compared with baseline, the sensitivity to heat pain in controls was significantly decreased during and following the HNCS-procedure ($p<0.01$ and $p<0.05$, respectively).

Suprathreshold heat pain (SHP; ºC) (Figure 11)

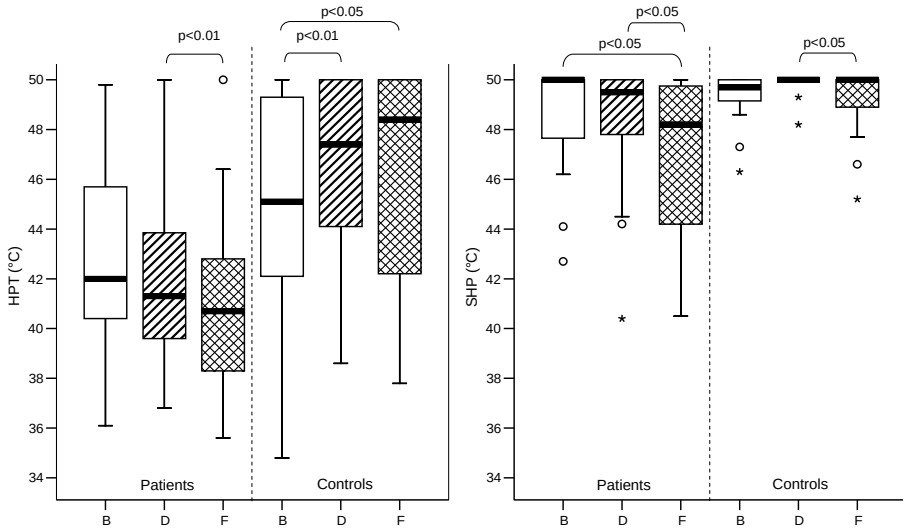

Figure 11. Results, absolute values, from quantitative pain testing at the thigh or the upper arm in 15 patients with peripheral neuropathy and dynamic mechanical allodynia and their age and sex-matched controls before (B), during (D) and following (F) heterotopic noxious conditioning stimulation (HNCS). Median values are presented within the 25 – 75 interquartile box, non-outlier maxima and minima (whiskers), circles denote outliers and asterisks extremes. HPT, heat pain threshold; SHP, suprathreshold heat pain. Significant differences from univariate planned comparison and mixed procedure (HPT) and multiple comparisons (SHP) are indicated by p-values.

During assessment of SHP several subjects reached the cut off temperature of 50 ºC without signalling SHP as 7 out of 10 on the category-ratio-10 scale. In such cases 50 ºC was used in the statistical analysis. There was a significant change over time for patients ($p<0.01$) and controls ($p<0.05$), analysed with Friedman's ANOVA. Compared with during HNCS, the sensitivity to SHP was significantly increased following conditioning pain stimulation in controls ($p<0.05$) and in patients ($p<0.05$), respectively, and in patients also comparing following HNCS with baseline ($p<0.05$).

4.2.2.5 Autonomic responses (blood pressure (BP; mm Hg) and heart rate (HR; beats/min))

No significant difference was seen between patients and controls in systolic or diastolic BP at baseline. During the HNCS-procedure the systolic and diastolic BP increased in both groups alike and a significant interaction was found between the two factors group and time for both systolic and diastolic BP (Table 9). In controls, but not in patients, the systolic and diastolic BP returned to baseline 5 min following cuff deflation (Table 10). Significantly higher HR was seen in patients compared with controls at baseline and during the whole experiment (Table 9). Compared to baseline, significantly higher HR was seen during the HNCS-procedure, whereas significantly lower HR was seen in both groups alike during the resting period at 5 and 10 min following HNCS (Table10).

Table 10

Results of contrast estimates compared to baseline and absolute values (mean (±SEM))

	Baseline	HNCS before cuff deflation	5 min rest	10 min rest	30 min rest
Systolic BP patients	120 (3.6)	137 (5.7) $p< 0.001$	126 (3.6) $p< 0.05$	128 (4.0) $p< 0.004$	125 (3.2) $p< 0.05$
Systolic BP controls	122 (3.1)	133 (5.3) $p< 0.02$	120 (3.8) NS	118 (3.3) NS	121 (3.5) NS
Diastolic BP patients	75 (2.2)	87 (3.9) $p< 0.001$	78 (2.8) $p< 0.04$	79 (2.9) $p< 0.004$	81 (2.9) $p< 0.003$
Diastolic BP controls	73 (2.1)	83 (2.6) $p< 0.001$	73 (3.2) NS	73 (2.8) NS	74 (2.6) NS
HR patients	69 (2.2)	73 (2.5) $p< 0.03$	68 (2.6) $p< 0.02$	67 (2.3) $p< 0.005$	69 (2.9) NS
HR controls	60 (2.2)	62 (1.9) $p< 0.03$	57 (1.9) $p< 0.02$	57 (2.0) $p< 0.005$	57 (1.9) NS

p-values are presented. NS, statistically non-significant. BP, blood pressure (mm Hg); HR, heart rate (beats/min). As no significant interaction was found for HR the effect of time is generalized to influence both groups alike.

4.3 STUDY III

All patients reported spontaneous ongoing- as well as brush-evoked pain during all assessments of the total brush-evoked pain intensity. The median value of VAS ratings of the ongoing pain at baseline was 70 mm (mean 67, range 34 – 87).

The main finding of the present study was that there was no influence of HNCS on the ongoing- or total brush-evoked pain intensity. In the pain-free area significantly increased pain sensitivity to threshold- and suprathreshold pressure pain could be found in patients compared to controls at baseline, whereas no significant differences could be demonstrated during HNCS between patients and their controls.

4.3.1 The influence of HNCS on spontaneous ongoing pain (mm) and total brush-evoked pain intensity (AUC; area under the curve) (Table 11)

Table 11

Results from assessments of spontaneous ongoing neuropathic pain intensity (mm), total brush-evoked pain intensity (area under the curve = AUC) and the provoked pain intensity in the leg or arm (absolute mean and range values) during the ischemia-induced HNCS procedure.

	Baseline	HNCS			Resting time		
		Cuff inflation	Before/at QST	Cuff deflation	1 min	5 min	15 min
Spontaneuos ongoing pain intensity (mm)	67 (34 -87)		63 (29 – 86)		67 (34 – 100)	58 (32 -85)	62 (36 – 87)
Total brush-evoked pain intensity (AUC)	618 (150- 2694)		309 (18 -783)				
Provoked pain intensity in patients (mm)		83 (66 - 100)		73 (38 - 97)	21 (0 - 60)	8 (0 – 40)*	2 (0 – 12)**
Provoked pain intensity in controls (mm)		67 (58 – 76)		58 (38 – 84)	20 (0 – 48)	5 (0 – 13)*	0 (0 – 1)**

No significant difference was found for the intensity of spontaneous ongoing pain or the total brush-evoked pain intensity comparing assessments at baseline and during HNCS. The decrease of the provoked pain intensity was significant at 5 ($P<0.001$)* and 15 min ($P<0.001$)** during the resting period compared to cuff inflation in patients and controls alike.

There was no significant change over time for the spontaneous ongoing pain intensity or the total brush-evoked pain intensity when comparing baseline with the pain during HNCS. However, the mean value of total brush-evoked pain intensity was reduced by half during HNCS (with an unaltered median value) ($P<0.06$), and 6 out of 10 patients rated a reduction of approximately 50 % or more of the total brush-evoked pain intensity during the HNCS procedure.

4.3.1.1 The relationship between the intensity of spontaneous ongoing pain and total brush-evoked pain intensity (Figure 12)

At baseline a significant inverse correlation was demonstrated between the intensity of spontaneous ongoing neuropathic pain and the total brush-evoked pain intensity ($P<0.05$, $r_s= -0.7$), whereas no significant correlation was found during the pain provocation procedure.

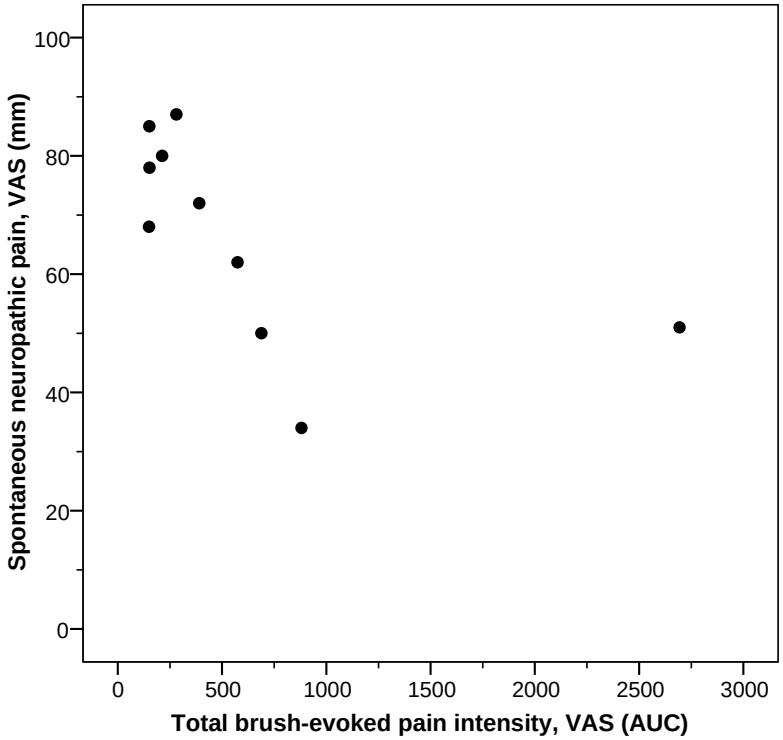

Figure 12. The correlation between the intensity of spontaneous central post-stroke pain and total brush-evoked pain intensity at baseline in ten patients with central post-stroke pain ($r_s=-0.7$, $P< 0.025$). AUC; area under the curve.

4.3.2 The influence of spontaneous ongoing pain and HNCS on pain sensitivity in a remote pain-free area

There was no interaction between the two factors group and time.

4.3.2.1 The influence of spontaneous ongoing pain on pain sensitivity in a pain-free area (Figure 13)

Sensitivity to threshold pressure pain (PPT; kPa), suprathreshold pressure pain (SPP; kPa), threshold heat pain (HPT; ºC) and suprathreshold heat pain (SHP; ºC)

At baseline significantly lower pressure pain thresholds were found in patients compared to controls (P<0.05) in conjunction with a significantly increased sensitivity to suprathreshold pressure pain (P=0.05). There was no difference between patients and controls in the sensitivity to heat pain or suprathreshold heat pain.

4.3.2.2 The influence of HNCS on pain sensitivity in a pain-free area (Table 12, Figure 13).

Sensitivity to threshold pressure pain (PPT; kPa), suprathreshold pressure pain (SPP; kPa), threshold heat pain (HPT; ºC) and suprathreshold heat pain (SHP; ºC)

There was a significant change over time for patients and controls alike (P<0.001). Compared to baseline, significantly higher pressure pain thresholds were found during HNCS in patients and controls alike. There was no significant change over time in the sensitivity to suprathreshold pressure pain in patients or controls. However, comparing baseline with assessments during HNCS a significantly 15 % reduced sensitivity to suprathreshold pressure pain was found in controls only (P<0.05, 95% confidence interval 1.04–1.27).

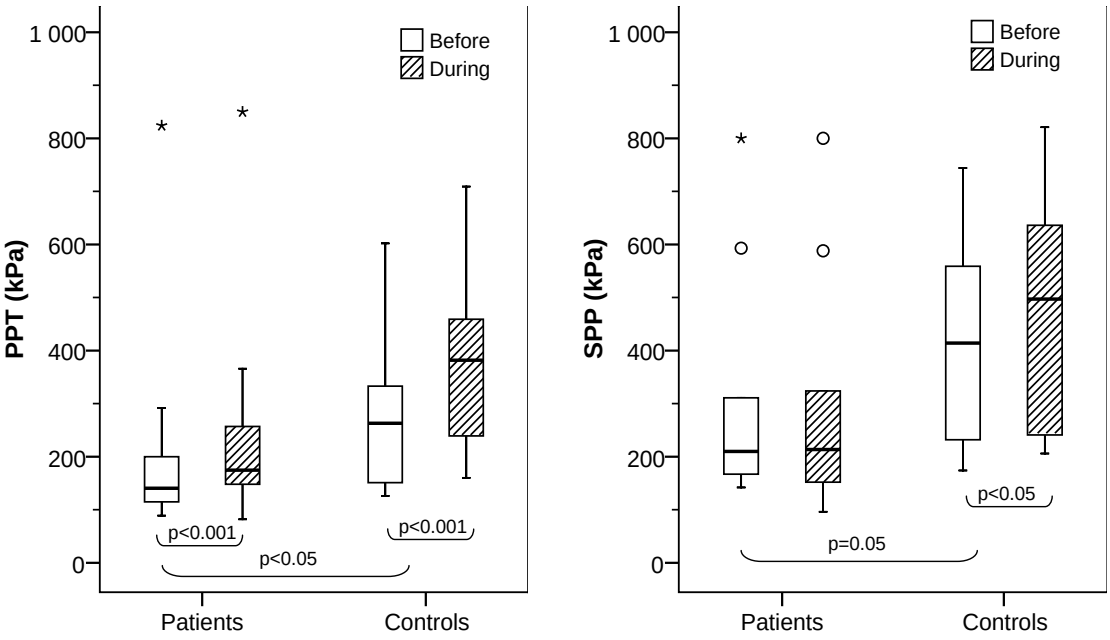

Figure 13. Results, absolute values, from quantitative pain testing at the pain-free arm or leg in 10 patients with central post-stroke pain and brush-evoked pain and their age- and sex-matched controls before and during heterotopic noxious conditioning stimulation (HNCS). Median values (-) are presented within the 25 – 75 interquartile boxes, whiskers denote non-outlier maxima and minima, circles denote outliers and asterisks extremes. Pressure pain threshold (PPT); sensitivity to suprathreshold pressure pain (SPP). Significant differences from the ANOVA for PPT and SPP are indicated by P-values.

No significant change over time was found for heat pain thresholds or sensitivity to suprathreshold heat pain in patients or controls.

Table 12

Results of the two-way repeated measures ANOVA

	Group	Time	Group/Time
PPT	*F*(1,9)=5.78,*P*< 0.05	*F*(1,9)=75.42,*P*<0.001	NS
SPP	*F*(1,9)=4.9,*P*=0.05	NS	NS
HPT	*NS*	NS	NS
Systolic BP	*NS*	*F*(4,36)=20.28,*P*<0.001	NS
Diastolic BP	*NS*	*F*(4,36)=11,40,*P*<0.001	NS
HR	*NS*	*F*(4,36)=4.58,*P*<0.01	NS

P-values are presented. NS=statistically non-significant. PPT=pressure pain threshold; SPP=suprathreshold pressure pain sensitivity; HPT=heat pain threshold; BP=blood pressure; HR=heart rate.

4.3.3 Provoked pain in the upper or lower extremity (Table 11)

Following cuff inflation to 240 mm Hg the patients performed none and the controls 3 movement efforts before at least 7 was rated on the category-ratio-10 scale in the provoked leg. The two patients with provoked pain in the arm performed 6 more movement efforts than the controls (median 40 compared to 34).

There was no significant difference in the tourniquet-induced pain intensity between patients and controls during HNCS or at 1 and 5 min following conditioning stimulation. Compared to during the HNCS procedure, the provoked pain intensity decreased significantly at 5 min (P<0.001) and 15 min (P<0.001) following HNCS in patients and controls alike.

4.3.4 Autonomic responses (blood pressure (BP; mm Hg) and heart rate (HR; beats/min)) (Table 12 and 13)

At baseline there were no significant differences in systolic or diastolic BP between patients and controls. No interaction between the two factors group and time was found, whereas a significant change over time was demonstrated for the systolic (P<0.001) and diastolic (P<0.001) BP in patients and controls alike. Compared to baseline, a significant BP increase was seen during HNCS in both groups alike (P<0.001). Five min following cuff deflation the BP had returned to baseline in both patients and controls.

There was no interaction between the two factors group and time for HR, whereas a significant change over time was found (P<0.01). Compared to baseline, no significant change in HR was seen during the HNCS-procedure, whereas significantly

lower HR was found in both groups alike during the resting period at 5 min (P<0.01) and 10 min (P<0.05).

Table 13

Results from assessments of blood pressure and heart rate at baseline, during HNCS and during the resting period.

Absolute values (mean (±SEM))

	Baseline	During HNCS after cuff inflation	5 min rest	15 min rest
Systolic BP patients	136 (5.0)	153 (8.3) *P*<0.001	137 (4.2) NS	135 (3.7) NS
Systolic BP controls	126 (5.5)	139 (6.4) *P*<0.001	130 (4.6) NS	123 (4.6) NS
Diastolic BP patients	81 (3.8)	89 (3.8) *P*<0.001	84 (3.7) NS	85 (3.5) NS
Diastolic BP controls	75 (2.6)	85 (3.3) *P*<0.001	78 (3.3) NS	75 (3.0) NS
HR patients	76 (2.7)	78 (3.1) NS	73 (2.4) *P*<0.01	73 (2.7) *P*<0.05
HR controls	71 (1.9)	72 (3.0) NS	66 (1.9) *P*<0.01	66 (1.9) *P*<0.05

P-values are presented. NS=statistically non-significant. BP=blood pressure (mm Hg); HR=heart rate (beats/min). Since no significant interaction was found the effect of time is assumed to influence both groups alike.

Table 14. Comparison of the results indicated by arrows from the present study on CPSP patients and a previous study in patients with peripheral neuropathy and spontaneous ongoing neuropathic pain as well as dynamic mechanical allodynia (Tuveson et al., 2007). Identical methodology with ischemia induced HNCS was used in both studies.

	Before HNCS		During HNCS	
	Peripheral neuropathy	CPSP	Peripheral neuropathy	CPSP
PPT	↔	↓ *P*< 0.04	↑ *P*< 0.005	↑ *P*< 0.001
SPP	↔	↓ *P*= 0.05	↔	↔
HPT	↔	↔	↔	↔
SHP	↔	↔	↔	↔
Spontaneous pain			↓ *P*< 0.02	↔
Brush-evoked pain			↔	↔

P-values are presented. PPT, pressure pain threshold; SPP, suprathreshold pressure pain sensitivity; HPT, heat pain threshold; SHP, suprathreshold heat pain sensitivity.

4.4 STUDY IV

4.4.1 Total brush-evoked pain intensity (AUC; area under the curve), maximum value of the total brush-evoked pain intensity (mm) and the duration (s) as well as the frequency of aftersensation (Figure 14)

There was no interaction between the factors treatment sequence (i.e., ondansetron/saline or saline/ondansetron), time and the factor with or without ongoing pain medication during the whole experiment. No significant change in the total brush-evoked pain intensity was found over time following infusion of neither ondansetron nor saline. In addition, no significant alteration in the duration and frequency of aftersensation could be demonstrated during the whole experiment following infusion of neither ondansetron nor saline.

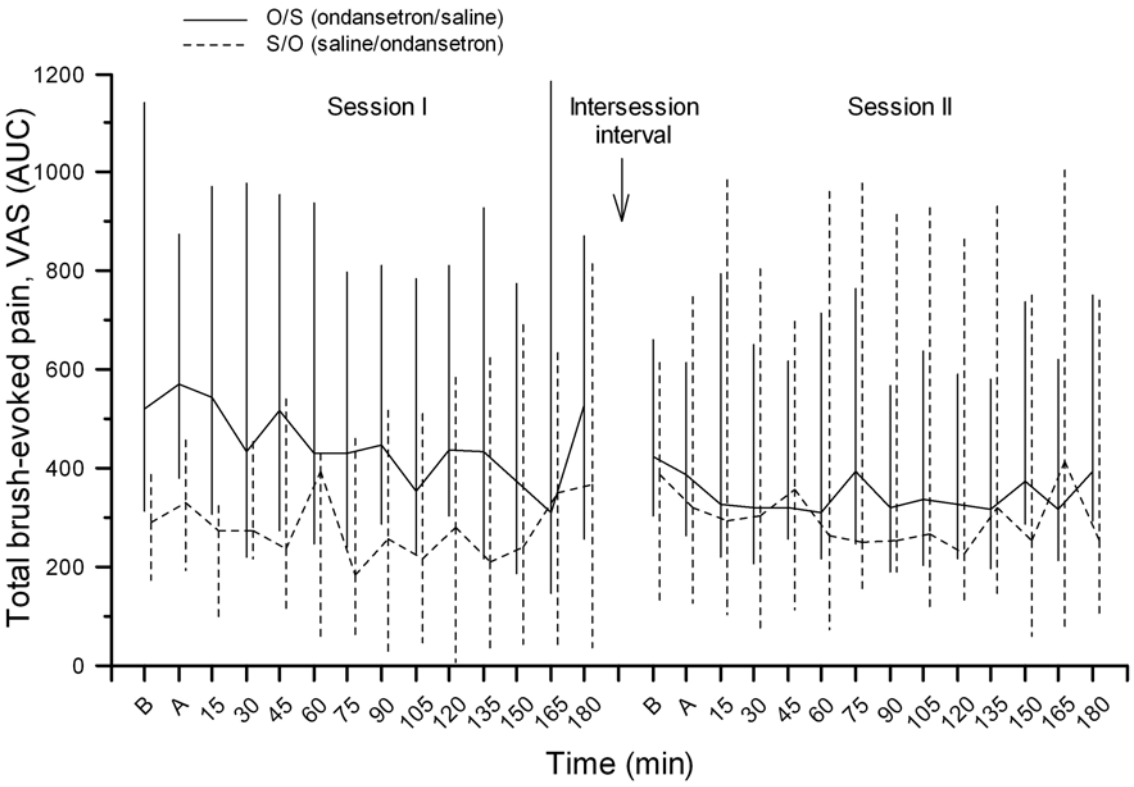

Figure 14. Results, absolute values, from VAS ratings of the total brush-evoked pain intensity in 15 patients with peripheral neuropathy and dynamic mechanical allodynia before (B) infusion of ondansetron/saline and immediately after (A) as well as every 15 min during 3 hours for treatment sequence ondansetron/saline (continuous line) and saline/ondansetron (broken line). The horizontal lines present the median values for the intensity of brush-evoked pain (VAS – area under the curve) and whiskers the 25 – 75 interquartile.

4.4.2 Intensity of spontaneous ongoing pain (VAS; mm) (Figure 15)

The intensity of ongoing pain was not significantly altered following infusion of neither ondansetron nor saline. To control for possible influence from the factors with or without ongoing pain medication and treatment sequence the data was analysed with a three and four-way ANOVA, respectively. No significant interaction was found between the factors pain medication and treatment sequence. There was a significant difference in ongoing pain intensity between patients with and without pain medication ($P<0.05$) calculated using least squares means (variance). The patients with ongoing pain medication rated the pain intensity with a range of 16 – 38 mm, compared to 34 - 54 mm for the patients without pain medication.

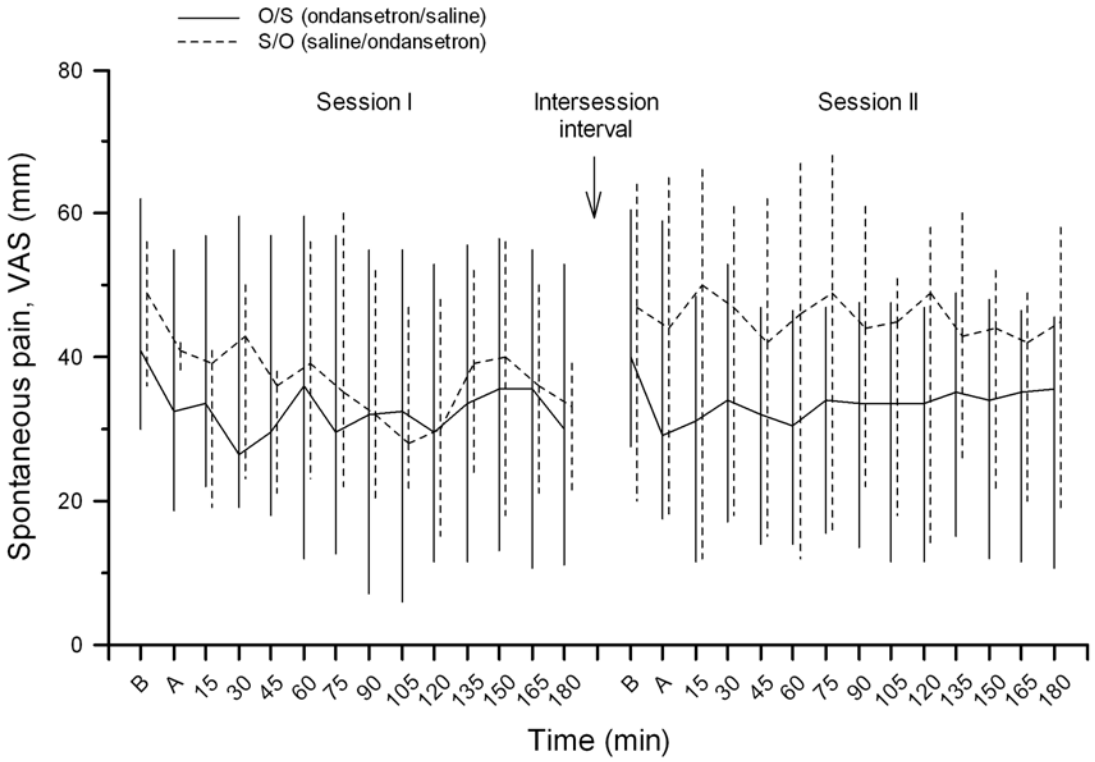

Figure 15. Results, absolute values, from VAS ratings of the intensity of spontaneous ongoing neuropathic pain in 15 patients with peripheral neuropathy and dynamic mechanical allodynia before (B) infusion of ondansetron/saline and immediately after (A) as well as every 15 min during 3 hours for treatment sequence ondansetron/saline (continuous line) and saline/ondansetron (broken line). The horizontal lines present the median values for the intensity of spontaneous ongoing neuropathic pain (VAS; mm) and whiskers the 25 – 75 interquartile.

4.4.3 Power analysis

A power analysis of the total brush-evoked pain intensity and the spontaneous ongoing pain intensity was performed following completion of the result analysis with an estimated clinically relevant reduction of both parameters of 30 % (Farrar et al., 2001). A power analysis of differences in standard deviations demonstrated that 80 % power

would be obtained from assessments of the total brush-evoked pain intensity in a sample size of 16 patients and from ratings of the spontaneous ongoing pain intensity in a sample size of 8 patients.

4.4.4 Autonomic responses (blood pressure (BP; mm Hg) and heart rate (HR; beats/min)) (Table 15)

There were no interactions in HR between the factors treatment, time and treatment sequence and no significant differences in HR between patients with or without ongoing pain medication. No significant change in HR was found over time following infusion of neither ondansetron nor saline. The three-way ANOVA showed a significant change over time for both systolic ($P<0.01$) and diastolic BP ($P<0.001$) following infusion of either ondansetron or saline (Table 2), with an initial decrease during 30 – 90 min following the infusion and a return to baseline during the later phase. Comparing baseline values, a significant difference in BP was demonstrated between the two treatment-groups with lower BP before the infusion of ondansetron compared to saline (systolic $P<0.05$; diastolic $P<0.05$). In addition, there was a significant interaction between treatment, time and treatment sequence (systolic $P<0.05$; diastolic $P<0.05$). These interactions were caused by minute differences in absolute values of the systolic and diastolic BP with no reasonable influence on brush-evoked- or ongoing pain intensity. No interaction was found for the factor 'with or without ongoing pain medication' for either the systolic or diastolic BP.

Table 15

Results of the three-way ANOVA with repeated measures on two factors, time (8 time points) and treatment (ondansetron or saline)

Between groups factor is sequence (ondansetron/saline and saline/ondansetron)

	Treatment	Time	Treatment/sequence	Time/sequence	Treatment/time	Treatment/time/sequence
Systolic BP	F(1,13)= 5.6, P<0.05	F(7,91)=3.53, P<0.01	NS	NS	NS	F(7,91)=2.35, P<0.05
Diastolic BP	F(1,13)=5.05, P<0.05	F(7,91)=7.32, P<0.001	F(1,13)=8.93, P<0.05	NS	F(7,91)=2.38, P<0.05	F(7,91)=2.46, P<0.05

P-values are presented. NS=statistically non-significant. BP=blood pressure.

4.4.5 Side effects

Tiredness was the most frequently reported side effect experienced by 3 patients following infusion of ondansetron and by 4 patients following infusion of saline. Two patients reported headache and three patients reported dizziness, nausea or light-headedness following saline infusion.

5 DISCUSSION

5.1 ISCHEMIA-INDUCED HNCS IN HEALTHY SUBJECTS (STUDY I)

5.1.1 Side and time dependant influence of HNCS on pain sensivity in healthy subjects

During HNCS the pressure pain threshold increased significantly on both sides alike and returned to baseline 30 minutes following pain provocation. No significant difference in the magnitude of the altered pressure pain threshold was seen between sides for the first or the lastly assessed side. A time factor was demonstrated for alterations in sensitivity to SPP an SHP during HNCS with decreased sensitivity on the lastly assessed side only, on both sides alike, and without magnitude differences between sides. Suprathreshold stimuli were used in the present study due to outcome of previous studies (Leffler et al., 2002a,b). For PPT and SPP higher values were found on the right side for the seventeen right-handed subjects and the one left-handed subject. A laterality difference for pressure pain with higher baseline values on the dominant right side has previously been described (Brennum et al., 1989; Petersen et al., 1992; Jensen et al., 1992; Kosek et al., 1993). The increase of the pressure pain thresholds during HNCS in the present study with no side and time differences is in accordance with previous findings in healthy individuals (Kosek and Hansson, 1997; Kosek and Ordeberg 2000; Leffler et al., 2002a,b), possibly explained by activation of DNIC-related mechanisms (Willer et al., 1984; Talbot et al., 1987; Price and McHaffie, 1988). While HNCS has been shown to alter PPT, inconsistent effects of HNCS on heat pain sensitivity have been described in healthy individuals. HNCS has been demonstrated to elevate HPT in some studies using the cold pressor test (Talbot et al., 1987; Plaghki et al., 1994) but not in studies using the tourniquet for pain provocation (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000; Leffler et al, 2002 a), the latter in accordance with the results of the present study. However, a time dependant, delayed alteration was seen for both SPP and SHP indicating a difference in modulation susceptibility between suprathreshold stimuli compared to threshold stimuli, i.e., the pressure pain threshold in the present study. Regarding SPP and SHP decreased sensitivity were observed only on the lastly assessed side. On the primarily assessed sides there was an increased sensitivity to SPP and SHP 30 minutes following the HNCS. This may be due to peripheral sensitization caused by the repeated testing procedures (Bessou and Perl, 1969; Fitzgerald and Lynn, 1977) or centrally mediated descending facilitation (Pertovaara, 1998). A similar response with increased thermal pain (following temporal summation of test stimuli near heat pain tolerance) during the early phase of testing was recently described using conditioning cold immersion (Edwards et al., 2003). Compared with pain provoked by heat or cold, ischemic noxious conditioning stimulation can be used over a longer period of time and thus creates a possibility to study more protracted time dependant differences. During HNCS using ischemic pain no sex differences in presumed inhibition have been demonstrated (France and Suchowiecki, 1999).

A positive correlation has been described between the conditioning-induced pain and the perceived intensity of the noxious test stimuli in humans (Willer et al., 1984; Talbot et al., 1987). During the HNCS procedure a strong tendency ($p= 0.05$) to higher pain intensity was found in the left arm before the start of QST for those subjects starting the assessments on the right thigh. No differences in pain intensity of the left arm were found during the rest of the conditioning stimulation period and could

therefore not explain the time dependant differences in SPP and SHP during HNCS found in the present study. In accordance with previous findings no laterality differences were seen in the present study for HPT (Taylor et al., 1993) and SHP (Long, 1994; Vierck et al., 2001). The findings of laterality differences for PPT and SPP but not for HPT and SHP support the suggestion that these differences may be related to deep rather than cutaneous pain sensibility (Taylor et al., 1993; Sarlani et al., 2003).

5.1.2 HNCS-induced autonomic responses in healthy subjects

Previous studies using conditioning ischemic pain stimulation (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000) and the present study demonstrated increased systolic and diastolic BP during HNCS. Analgesia induced by baroreceptor activation (Dworkin et al., 1994) and pain threshold elevation during increased systolic BP (Pertovaara et al., 1984) have been reported and could have affected the currently demonstrated increase in PPT and the decreased sensitivity to SPP and SHP, respectively, during pain provocation on the lastly assessed side. A moderate statistically significant increase in HR of 7.3 % (5 beats/min), which was found in the present study, had also previously been described (Kosek and Hansson, 1997). During HNCS with the submaximal effort tourniquet no increase of adrenocorticotropin was found by Pertovaara et al. (1982) as opposed to exercise-induced sensory threshold increase with an increase of HR of more than 50 % compared with baseline (Kemppainen et al., 1985). Due to the aforementioned moderate increase in HR in the present study, stress-induced analgesia is not considered to be a relevant basis for the sensory alterations found during conditioning stimulation. In addition, presumed inhibition during HNCS has been described to be attention insensitive (Talbot et al., 1987; De Broucker et al., 1990; Kakigi, 1994; Edwards et al., 2003).

5.2 THE INFLUENCE OF ISCHEMIA-INDUCED HNCS ON PAIN PARAMETERS IN PATIENTS WITH PAINFUL PERIPHERAL NEUROPATHY AND CPSP (STUDY II AND III)

5.2.1 The influence of HNCS on the intensity of spontaneous ongoing pain in patients with peripheral neuropathy

In contrast to findings in a recent study by Witting et al. (2003), using heterotopic cold-water immersion, significantly and time independent reduced intensity of spontaneous ongoing neuropathic pain was found during the ischemic HNCS. Compared to the study by Witting et al. (2003) all patients in the present study reported spontaneous ongoing neuropathic pain with comparatively higher intensity (mean 54 mm compared to 22 mm), which may have influenced the result. In addition, a sub-analysis of the Danish patients with spontaneous ongoing pain intensity of more than VAS 20 mm did not change the result (Witting et al., 2003). Studies in non-neuropathic pain states using ischemia-induced HNCS have demonstrated decreased spontaneous ongoing pain during conditioning painful stimulation in patients with osteoarthritis (Kosek and Ordeberg, 2000) and acute orofacial pain due to pulpitis (Sigurdsson and Maixner, 1994), conditions with a clear pathophysiological nociceptive background to the pain. However, unaltered spontaneous pain intensity during ischemia-induced HNCS was seen in patients with pain of unknown etiology, i.e., chronic pain due to temporomandibular disorder (Maixner et al., 1995) and fibromyalgia (Kosek and Hansson, 1997).

5.2.2 The influence of HNCS on the intensity of spontaneous ongoing pain in central post stroke pain (CPSP) patients

HNCS has been used to activate endogenous pain modulatory systems. The consequence of HNCS induced in areas with input to the spinal cord is triggered by activity in spinoreticular pathways (Bouhassira et al., 1993), with preferential ipsilateral but also contralateral projections from the dorsal horn to subnucleus reticularis dorsalis (SRD) in the caudal medulla as demonstrated in anterograde and retrograde tracing studies in rats (Lima and Almeida, 2002). Relevant to the current study, projections from SRD to the thalamus have also been demonstrated to be bilateral with contralateral dominance and mainly connected to the medial thalamic nuclei and to a smaller extent to the ventroposteriorlateral nucleus (Leite-Almeida et al., 2006). Thalamo-cortical pathways are also under powerful corticofugal control illustrated by cortico-thalamic projections having 10 times the number of fibres compared to the former (Deschenes et al., 1998). In the context of ascending activity triggered by HNCS no side dependant difference was found in healthy subjects regarding the ability of HNCS to alter pain sensitivity in an area distant to the conditioning stimulation (Tuveson et al., 2006). In this study ischemia was induced in the unaffected side ipsilateral to the brain lesion. The possible impact of laterality on spontaneous- and/or brush-evoked pain is unknown and was not aimed at being resolved.

During HNCS in the leg or arm ipsilateral to the brain lesion no significant alteration of the intensity of ongoing pain was found. This points to the inability of endogenous pain controlling systems activated by HNCS to alter pain generated as a direct consequence of a brain lesion, i.e. lack of influence on pain pathways of the brain from corticofugal- and spino-bulbo-cerebral pain controlling systems (Villanueva and Fields, 2004).

In patients with peripheral neuropathy the ongoing pain intensity was unaltered using conditioning cold-water immersion (Witting et al., 2003) but reduced during ischemia-induced HNCS (Tuveson et al., 2007). Separating the ascending pain network in a medial and lateral system has been proposed (Albe-Fessard et al., 1985) where the lateral system is supposed to process the sensory-discriminative and the medial system the affective-motivational component of the pain experience (Treede et al., 1999). An imaging study indicated activation of preferentially the medial pain system by ongoing peripheral neuropathic pain (Hsieh et al., 1995). We have earlier put forth arguments favouring the possibility that the medial pain system is more prone to influence from HNCS than the lateral system (Tuveson et al., 2007). This suggestion was derived from our previous findings in patients with peripheral neuropathy where ongoing spontaneous pain was significantly reduced as opposed to the intensity of brush-evoked pain (Tuveson et al., 2007). In this study, with unaffected spontaneous pain during HNCS we are unable to pinpoint if the brain lesion preferentially affected the lateral or medial pain system and the literature does not give any guidance on the cortical activation pattern in CPSP. The lack of effect of HNCS could alternatively be explained by a loss of crucial connections within endogenous pain controlling systems caused by the brain lesion.

5.2.3 The influence of HNCS on the intensity of brush-evoked pain in patients with painful peripheral neuropathy

The novel semi-quantitative technique used in the present study to assess brush-evoked allodynia offers detailed insights into several dimensions of brush-evoked allodynia (Samuelsson et al., 2005). The total intensity of brush-evoked allodynia was not significantly changed during the HNCS-procedure in the present study, supporting results from the study by Witting et al. (2003). Compared to activity in the nociceptive system generating spontaneous ongoing neuropathic pain it may be hypothesised that the afferent volley giving rise to dynamic mechanical allodynia is more intense and therefore more difficult to overcome by conditioning stimulation. Data from experiments in patients with peripheral neuropathic pain indicate a crucial role for low threshold A-beta afferents in the generation of hypersensitivity to light mechanical stimuli (Lindblom and Verillo, 1979; Nurmikko et al., 1991; Ochoa and Yarnitsky, 1993). Assuming that large myelinated fibres are the peripheral link in the generation of dynamic mechanical allodynia in the present study, their mode of converting activity onto the nociceptive system may result in temporo-spatial activity patterns that are more resistant to interference than pattern primarily generated in the nociceptive system itself.

Another argument favouring the idea of the spontaneous ongoing pain percept being more prone to modulation from HNCS could be adopted from the outcome of earlier positron emission tomography data indicating a preferential activation of the medial pain system in ongoing neuropathic pain (Hsieh et al., 1995) and the lateral system when experiencing dynamic mechanical allodynia (Petrovic et al., 1999). Since only ongoing pain was modified one may argue that the interaction is likely not to take place on the spinal level but rather at supraspinal sites where the ascending pathways separate into a medial and lateral system (Albe-Fessard et al., 1985; Willis and Westlund, 1997). Hence, endogenous pain modulation set up by compression/ischemia preferably seems to interact with the medial pain system, thus influencing spontaneous pain intensity but not brush-evoked pain.

The Danish group found a decreased area of brush-evoked allodynia during cold-water immersion (Witting et al., 2003), a parameter not examined here. A number of studies have previously demonstrated shrinkage of the allodynic area as result of a variety of interventions (Marchettini et al., 1992; Belfrage et al., 1995) and this parameter seems to be more susceptible to manipulation than the pain intensity of dynamic mechanical allodynia. The clinical significance, from the perspective of the quality of life of the patient, of a smaller area of allodynia versus reduced pain intensity in the remaining area with dynamic mechanical allodynia must be scientifically evaluated in forthcoming clinical studies.

Several previous studies have demonstrated a significant positive correlation between the ongoing spontaneous pain intensity and the intensity of dynamic mechanical allodynia (Koltzenburg et al., 1994; Rowbotham and Fields, 1996). It is therefore interesting to note that a statistically significant reduction, albeit numerically not extensive, of the intensity of spontaneous ongoing pain was not paralleled by a reduction in the intensity of dynamic mechanical allodynia in this study. This finding indicates a complex relationship between the two painful percepts.

Serious criticism has been raised regarding the clinical relevance of behavioural testing procedures presumed to reflect sensory hypersensitivity in animal models of peripheral neuropathy, e.g., von Frey filament poking of the neuropathic paw (Hansson, 2003). This offsets a valid discussion of our data taking into account also results from animal studies.

5.2.4 The influence of HNCS on the intensity of brush-evoked pain in CPSP patients

On a group level unaltered intensity of brush-evoked pain was demonstrated, although 6 out of 10 subjects reported a reduction of approximately 50 % during HNCS. Unaltered brush-evoked pain intensity has been reported during both ischemia- and cold-induced HNCS in patients with peripheral neuropathy (Tuveson et al., 2007; Witting et al., 2003). A novel communication between non-nociceptive and nociceptive pathways, explaining the initiation and maintenance of brush-evoked pain following stroke, is likely to be found in the brain. Details of such an interaction are unknown. During functional imaging of patients with CPSP after brainstem infarct, a normally non-painful object (frozen water in a flat plastic container) now perceived as painful, was demonstrated to activate areas of both the medial and lateral pain system (Peyron et al., 1998). If our previously suggested (Tuveson et al., 2007) preferential medial pain system interaction from HNCS has a bearing on our results, the lack of a group level effect on brush-evoked pain by HNCS may be explained by the mixed activation of the medial and lateral pain system during the allodynic percept in CPSP patients (Peyron et al., 1998).

In the present study a significant inverse relationship was found at baseline between the intensity of spontaneous- and brush-evoked pain. This is at variance with study outcomes in peripheral neuropathy where a significant positive relationship was demonstrated (Koltzenburg et al., 1994; Rowbotham and Fields, 1996; Samuelsson et al., 2005). The inverse correlation was lost during HNCS, a finding most reasonably related to the fact that spontaneous ongoing pain was unaltered but 6 out of 10 patients experienced about a 50 % reduction of brush-evoked pain.

5.2.5 The influence of HNCS on pain sensitivity in a remote pain-free area in patients with painful peripheral neuropathy

The outcome during HNCS of psychophysical assessments using natural stimuli in a remote pain-free area has previously not been studied in neuropathic pain patients. The assessments were performed repeatedly at the same skin area but at different sites for threshold and suprathreshold measurements. This precaution was taken across modalities since significant differences have been found for PPT between adjacent muscles in the same body region (Kosek et al., 1993).

During HNCS a higher PPT was demonstrated in the remote pain-free area in patients and controls alike, indicating that patients with peripheral neuropathy have normal inhibition of the pressure pain sensitivity at threshold level in a pain-free area.

In human studies the outcome of heat pain perception during HNCS is variable, with elevated HPT in healthy individuals in some studies (Talbot et al., 1987; Plaghki et al., 1994) but not in other studies (Kosek and Ordeberg, 2000; Leffler et al., 2002a). In the healthy controls similar alterations were observed to pressure- and heat pain during HNCS. However, a modality specific difference was found for the patients with decreased sensitivity to pressure pain but not to heat pain in the remote pain-free area during HNCS. A plausible explanation for this is currently unattainable.

Increased sensitivity was found following HNCS in patients and controls alike for suprathreshold perception of both pressure- and heat pain. This may be a result of peripheral sensitisation due to the repeated testing procedure (Bessou and Perl, 1969; Fitzgerald and Lynn, 1977) or centrally mediated descending net facilitation

(Pertovaara, 1998) and has previously been demonstrated for suprathreshold pressure- and heat stimuli in healthy volunteers (Tuveson et al., 2006).

5.2.6 The influence of HNCS on pain sensitivity in a remote pain-free area in CPSP patients

Increased PPT during HNCS indicates normal function of endogenous pain modulation in areas unaffected by the stroke and has previously been demonstrated in a remote pain-free area in healthy subjects (Tuveson et al., 2006), patients with rheumatoid arthritis (Leffler et al., 2002b), trapezius myalgia (Leffler et al., 2002a) and painful peripheral neuropathy (Tuveson et al., 2007). In three patients with sensory loss due to thalamic stroke electrically induced HNCS in the hand on the lesioned side resulted in inhibition of the nociceptive RIII-reflex following electrical stimulation of the contralateral sural nerve (De Broucker et al., 1990). Comparable results are available from healthy subjects (Willer et al., 1984). Taken together, the outcome of our study indicates normal function of spino-bulbo-spinal endogenous modulation of nociceptive input from pressure stimulation. No change in HPT or suprathreshold heat pain sensitivity was demonstrated. In healthy individuals (Plaghki et al., 1994; Talbot et al., 1987) and patients with non-neuropathic painful conditions (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000; Leffler et al., 2002a; Leffler et al., 2002b) HPT alterations during HNCS are variable, with elevated HPT in some studies (Leffler et al., 2002b; Plaghki et al., 1994; Talbot et al., 1987) but not in others (Kosek and Hansson, 1997; Kosek and Ordeberg, 2000; Leffler et al., 2002a).

5.2.7 The influence of HNCS on autonomic responses in patients with painful peripheral neuropathy

Increased systolic and diastolic BP during the HNCS procedure were demonstrated in the present study as well as in previous studies using conditioning ischemic pain provocation (Kosek and Hansson, 1997; Tuveson et al., 2006). Inhibition of pain perception induced by baroreceptor activation has been demonstrated (Dworkin et al., 1994) and also pain threshold elevation during increased systolic BP (Ghione, 1996). Furthermore, a significant positive correlation has been demonstrated between cardiovascular reactivity and endogenous pain inhibition (Edwards et al., 2004). Therefore, the increased BP and higher HR in the present study may have contributed to higher PPT and decreased sensitivity to SHP as found in patients and controls alike. During the resting period following the HNCS-procedure, persisting systolic and diastolic blood pressure elevation was found in the patients, despite the increased heat pain sensitivity in the remote pain-free area.

5.2.8 The influence of HNCS on autonomic responses in CPSP patients

Increased systolic and diastolic BP during HNCS was demonstrated here, corroborating results from previous studies using conditioning ischemia (Kosek and Hansson, 1997; Tuveson et al., 2006; Tuveson et al., 2007). Inhibition of pain perception due to baroreceptor activation has been demonstrated (Dworkin et al., 1994) as well as perception- and pain threshold elevation during increased systolic BP (Ghione, 1996) and antihypertensive treatment did not change the hypoesthesia (Ghione et al., 1988). Despite treated hypertension in 6 out of 10 CPSP patients, pressure pain sensitivity was significantly lowered at baseline, the opposite compared to the anticipated influence from hypertension.

5.3 THE INFLUENCE OF SPONTANEOUS ONGOING NEUROPATHIC PAIN ON PAIN SENSITIVITY IN PATIENTS WITH PAINFUL PERIPHERAL NEUROPATHY AND CENTRAL POST STROKE PAIN (STUDY II AND III)

5.3.1 The influence of spontaneous ongoing neuropathic pain on pain sensitivity in a remote pain-free area in patients with painful peripheral neuropathy

Unaltered pain sensitivity in the pain-free area at baseline does not support a net activation of widespread inhibitory/excitatory central nervous system mechanisms by the spontaneous ongoing neuropathic pain. Inability of clinical pain to activate widespread endogenous pain modulation has been described previously in various pain states, e.g., in pain experienced from musculoskeletal structures (Leffler et al., 2003), chronic back pain, acute postoperative pain (Peters et al., 1992) and in a recent study investigating patients with pain due to peripheral neuropathy (Bouhassira et al., 2003). It has been suggested that the ability of spontaneous ongoing pain to activate endogenous pain modulation could be pain intensity dependent (Peters et al., 1992; Leffler et al., 2003). Although the spontaneous ongoing neuropathic pain intensity was rather high in the present study (mean VAS 54 mm at baseline) perception of natural psychophysical painful stimuli were unaltered in the remote pain-free area, adding to earlier data on electrical test-stimuli in patients reporting even higher pain intensity (mean VAS 66.6 mm in patients with spontaneous ongoing pain) (Bouhassira et al., 2003).

5.3.2 The influence of spontaneous ongoing neuropathic pain on pain sensitivity in a remote pain-free area in CPSP patients

Compared to healthy controls significantly decreased PPT and increased sensitivity to SPP at baseline in a remote pain-free area ipsilateral to the brain lesion indicates disruption of corticofugal control of nociceptive input by the brain injury. Either increased facilitation or decreased inhibition, or a combination, may be at hand. In this study the pain sensitivity at baseline was compared to healthy controls on a group level. In accordance with findings in the present study unaltered HPTs were also found in CPSP patients comparing the affected and contralateral side with normative data (Greenspan et al., 2004).

Activation of endogenous pain modulation by ongoing pain has been suggested to be pain intensity dependent in two different non-neuropathic pain states (Leffler et al., 2003; Peters et al., 1992). The high intensity of spontaneous pain at baseline in the present study was comparable with studies using HNCS in peripheral neuropathy (Bouhassira et al., 2003; Tuveson et al., 2007), with no demonstrated influence at baseline on the nociceptive R III-reflex (Bouhassira et al., 2003) and unaltered perception threshold and suprathreshold sensitivity to pressure- and heat pain in the pain-free area (Tuveson et al., 2007), arguing against the pain intensity to be a crucial factor.

5.4 THE INFLUENCE OF THE $5HT_3$-ANTAGONIST ONDANSETRON ON THE INTENSITY OF BRUSH-EVOKED PAIN AND ONGOING PAIN IN PATIENTS WITH PAINFUL PERIPHERAL NEUROPATHY (STUDY IV)

5.4.1 The influence on the intensity of brush-evoked pain

Recent work on animal models of neuropathy has indicated a role for spinal $5HT_3$-receptors in descending facilitatory influence on mechanical sensitivity from brainstem areas such as the rostral ventromedial medulla (RVM) (Oatway et al., 2004; Suzuki et al., 2004a; Zeitz et al., 2002). Behavioural signs of mechanical hypersensitivity have indirectly been shown to correlate with electrophysiological alterations of dorsal horn neurons (Suzuki et al., 2004a) and such hypersensitivity was reversed by a lesion of the ipsilateral but not the contralateral dorsolateral funiculus supporting a role for descending modulation from the rostral ventromedial medulla (Ossipov et al., 2000). The suggested $5HT_3$-link in the dorsal horn of the spinal cord has indicated a possible role for intrathecally administered ondansetron in the treatment of mechanical hypersensitivity following nerve injury (Suzuki and Dickenson, 2005; Suzuki et al., 2004a). In this context, it deserves to be mentioned that a study comparing knock-out mice lacking the $5HT_3$-receptor with wild type mice, where withdrawal thresholds to mechanical and thermal stimuli were similarly lowered following partial nerve injury, reported that hypersensitivity after nerve injury is not dependant on activation of $5HT_3$-receptors (Zeitz et al., 2002).

The negative outcome on brush-evoked pain reported here, using a recently developed semi-quantitative technique (Samuelsson et al., 2005; Samuelsson et al., 2007), from the use of intravenous ondansetron in patients with peripheral neuropathic pain, does not support a role for a $5HT_3$-link in the maintenance of such allodynia. In a previous human study the effect of a single intravenous injection of 8 mg of ondansetron was investigated in patients with neuropathic pain of mixed etiology. Such a diagnosis was based on the presence of more than ‘3 cardinal symptoms’, i.e., burning pain, paresthesia/dysesthesia, shooting/lancinating pain, numbness or allodynia (McCleane et al., 2003). In that study the intensity of an unspecified allodynia was self reported by 17 out of 23 patients on an 11-point Likert scale (no clinical examination) and was unaltered by ondansetron infusion. The present study, using a more high resolution technique to assess brush-evoked pain, arrived at the same conclusion.

5.4.2 The influence on the intensity of spontaneous ongoing pain

No animal studies using $5HT_3$-antagonists have focused on the difficult task of assessing spontaneous pain behaviour. In a previous clinical study a significant effect on pain intensity compared to placebo was reported only at the 2 hour time-point during a 48-hour follow-up after intravenous injection of ondansetron (McCleane et al., 2003). Here, we report unaltered intensity of spontaneous ongoing pain during a 3 hour follow-up. A dose of 8 mg of ondansetron was used in both studies. The baseline mean pain intensity was slightly higher in the study by McCleane et al. (2003), about 58 mm compared to 44 mm in the present study, which may influence the outcome since a higher baseline pain intensity has been suggested to more likely result in a positive outcome of a trial in neuropathic pain (Katz et al., 2008). Also, in the present

study the placebo response was protruding which has been shown to reduce the ability to detect benefits of efficacious treatments (Katz et al., 2008).

5.5 METHODOLOGICAL SHORTCOMINGS

5.5.1 Study I and II

Pain inhibition during HNCS has been described to be attention insensitive (Talbot et al., 1987; De Broucker et al., 1990; Edwards et al., 2003). In support of this, powerful inhibition of the RIII-reflex by noxious but not painful electrical stimulation was obtained when applied to the lesioned hand of patients with a unilateral thalamic infarction (De Broucker et al., 1990). Also, a post-stimulatory inhibitory effect following HNCS has been found in both humans (De Broucker et al., 1990; Sigurdsson and Maixner, 1994) and animals (Bouhassira et al., 1990), arguing against distraction as the only cause for reduced pain sensitivity.

5.5.2 Study III

Normally distributed data for spontaneous pain intensity has a higher statistical power than skewed distribution of data for brush-evoked pain intensity. Marked reduced brush-evoked pain intensity in 6 out of 10 patients, with a lack of significance on a group level could indicate a type II error and the study was performed in a comparatively small group of CPSP patients due to difficulties in recruiting patients fulfilling preset criteria. Still, the number of included patients was, as mentioned in the materials and methods section, based on analysis of data from studies on patients with peripheral neuropathy (Samuelsson et al., 2005) and healthy individuals (Tuveson et al., 2006).

Pain inhibition during HNCS in humans has been suggested to be attention insensitive (De Broucker et al., 1990; Edwards et al., 2003; Talbot et al., 1987). In a recent publication (Tuveson et al., 2007) we have argued against diminished attention to be a prominent part of the manifestations of HNCS.

5.5.3 Study IV

The interpretation of the combined behavioural and electrophysiological techniques used in animal studies investigating the effect of ondansetron (Suzuki et al., 2004a; Suzuki et al., 2005) may not translate to brush-evoked pain in humans (Hansson, 2003; Vierck et al., 2008). More specifically, von Frey filament poking in neuropathic animals may not necessarily activate the same sensory channels as brushing in patients with allodynia to such a provocation.

A major difference and a potential confounding factor between animal and human studies is the administration route of ondansetron, i.e., intrathecally in the animal studies (Suzuki et al., 2004a; Suzuki et al., 2005) and intravenously in this study and the one by McCleane et al. (2003). In uninjured rats, the concentration of ondansetron in brain tissue was probed 30 minutes following an intravenous infusion and the cerebral- and plasma concentrations were comparable, indicating a good penetration of the blood brain barrier (Marchi et al., 2006). We found no human study addressing the concentration of ondansetron in cerebrospinal fluid (CSF) after intravenous administration. Only one study investigated the concentration of ondansetron in CSF following 5 oral doses given to six patients and the result indicated a low capacity of the drug in penetrating the blood brain barrier (Simpson et al., 1992).

The described side effects of ondansetron as headache, tiredness and also seizure and visual affection indicate CNS penetration of the drug (according to summary of product characteristics). A bell-shaped dose-response curve for the action of ondansetron has been described in several clinical studies aiming at treatment of anxiety, migraine and reduction of alcohol consumption with an effect of a low but not of a higher dose (Wolf, 2000). The uncertainty of optimal dosage of ondansetron in pain states is a complicating factor and prompted us to use identical dosing as in the study by McCleane et al. (2003).

The power analysis performed following completion of the study and related to the differences of standard deviations indicated that a sufficient number of subjects were included to obtain reliable results for both brush-evoked and spontaneous ongoing pain.

6 THESIS SUMMARY

Study I

No side differences in pain sensitivity were demonstrated on the thighs during unilateral HNCS of the left upper arm. However, a time factor was found for alterations in sensitivity to suprathreshold pressure- and heat pain without side differences in magnitude. Only at the lastly assessed side the sensitivity to suprathreshold pressure- and heat pain decreased significantly compared to baseline during the HNCS procedure and returned to baseline following the pain provocation. The results have implications for future design of HNCS-related experimental and clinical studies.

Study II

In patients with painful peripheral neuropathy HNCS altered differentially spontaneous- and brush-evoked pain. During the HNCS procedure the intensity of spontaneous ongoing neuropathic pain was significantly decreased, whereas the total brush-evoked pain intensity was unaltered. At baseline, in a remote pain-free area the pain sensitivity was unaltered indicating lack of activation of endogenous modulatory mechanisms from the spontaneous ongoing activity in the pain system. During conditioning pain stimulation higher pressure pain thresholds were demonstrated in the pain-free area in patients and controls alike. Surveying endogenous pain modulating systems and their interplay with different aspects of clinical pain conditions may in the future facilitate development of therapeutic strategies augmenting mechanisms involved in such systems.

Study III

No significant alterations of ongoing- or brush-evoked pain were found during HNCS in CPSP patients. This indicates a lack of modulation from endogenous pain controlling systems on nociceptive activity primarily generated in the stroke-affected brain. The increased pressure pain sensitivity in a remote pain-free area at baseline suggests alterations in corticofugal control of nociceptive sensitivity due to the brain lesion. Nociceptive input from the spinal level in patients during HNCS was modulated equal to controls, indicating normal function of spino-bulbo-spinal modulatory systems.

Study IV

Intravenous infusion of ondansetron did not influence the intensity of brush-evoked- or spontaneous ongoing pain in patients with peripheral neuropathy, indicating the lack of involvement of 5HT3 receptors in a previously proposed spino-bulbo-spinal loop with descending facilitation acting on spinal mechanisms related to the maintenance of dynamic mechanical allodynia.

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8 SAMMANFATTNING PÅ SVENSKA

Bakgrund och syfte: Inflödet av smärtsignaler påverkas på ryggmärgsnivå av nedåtstigande hämmande och förstärkande kroppsegen kontroll utgående från centralnervösa centra ovan ryggmärgen. Förändrad balans mellan hämning och förstärkning har delvis ansetts kunna förklara vissa långdragna smärttillstånd. Syftet var att undersöka påverkan från centralnervöst utlöst kroppsegen smärtmodulation på intensiteten av spontansmärta och smärtsam beröringsutlöst överkänslighet orsakad av perifer nervskada samt nervsmärta efter stroke. Den kroppsegna smärtkontrollen aktiverades med hjälp av smärtprovokation inom ett avlägset smärtfritt område. Dessutom undersöktes påverkan från den pågående spontansmärtan respektive smärtprovokationen på smärtkänsligheten i ett smärtfritt område vilken jämfördes med ålders- och könsmatchade friska kontroller (studie II och III). För att skapa ett adekvat protokoll för undersökning av patienter studerades först möjligheten av sidoskillnader eller förändring över tid i smärtkänsligheten hos friska försökspersoner under en smärtprovokation (studie I). I djurförsök har man rapporterat att en speciell receptor för signalsubstansen serotonin i ryggmärgens bakhorn, dvs. $5HT_3$-receptorn, skulle kunna ha betydelse för utveckling av beröringsutlöst smärta efter nervskada. Den eventuella betydelsen av receptorn för utveckling av spontansmärta samt smärtsam beröringskänslighet undersöktes efter intravenös tillförsel av läkemedlet ondansetron, en substans som blockerar $5HT_3$-receptorn (studie IV).

Metod: Smärtprovokation för att aktivera den kroppsegna smärtmodulationen utfördes i studie I, II och III genom att en blodtrycksmanschett pumpades upp i arm eller ben, vilket ger smärta orsakad av manschettens åtstramning och syrebrist i vävnaden. En förstärkning av smärtan kunde vid behov ske med muskelarbete. Intensiteten på spontansmärtan skattades hos patienterna med visuell analogskala (VAS) före, under och efter smärtprovokation respektive intravenös infusion av ondansetron. För undersökning av smärtsam beröringskänslighet användes en borste kopplad till en dator för registrering av tryck och hastighet under hudstrykningen med samtidig registrering av patientens smärtupplevelse via en datoriserad visuell analogskala. Detta gav möjlighet att registrera arean under kurvan som ett mått på den totala smärtupplevelsen över tid under hudstrykningen med borsten. Före, under och i studie I och II även efter smärtprovokationen bestämdes smärttrösklar för tryck och värme samt övertrösklig känslighet för tryck- och värmesmärta i ett smärtfritt område. För jämförelse bestämdes smärtkänsligheten hos friska matchade kontroller på motsvarande sätt. I studien med friska försökspersoner jämfördes smärtkänsligheten mellan de båda benen (studie I). Före och under 3 timmar efter intravenös tillförsel av läkemedlet ondansetron i studie IV undersöktes intensiteten av spontansmärtan och den smärtsamma beröringskänsligheten på patienter med perifer nervskada med de beskrivna metoderna.

Resultat: Under ensidig smärtprovokation av vänster arm på friska försökspersoner registrerades inga sidoskillnader i den minskade smärtkänsligheten mätt på båda benen. Däremot påvisades en reducerad övertrösklig smärtkänslighet utan sidoskillnad av förändringens storlek bara på den sist undersökta sidan. Hos patienter med perifer nervskada reducerades intensiteten hos spontansmärtan signifikant under smärtprovokationen i motsats till intensiteten på beröringsutlöst smärta som förblev oförändrad. Smärtprovokationen framkallade inga signifikanta förändringar av intensiteten på spontansmärtan eller den beröringsutlösta smärtan hos patienter med smärta efter stroke. I utgångsläget noterades signifikant ökad smärtkänslighet i ett

smärtfritt område hos patienter med smärta efter stroke i motsats till opåverkad smärtkänslighet hos patienter med perifer nervskada jämfört med kontroller. Under smärtprovokation påvisades högre trösklar för trycksmärta hos patienter med perifer nervskada och smärta efter stroke liksom för friska kontroller. Intravenös tillförsel av ondansetron påverkade inte intensiteten på spontan- eller beröringsutlöst smärta hos patienter med perifer nervskada.

Slutsatser: Under smärtprovokationen skattade patienterna med perifer nervskada en minskad intensitet på spontansmärtan medan den beröringsutlösta smärtans intensitet var opåverkad. Ingen signifikant förändring av intensiteten på spontansmärtan eller den beröringsutlösta smärtan påvisades hos patienter med neuropatisk smärta efter stroke, vilket talar för avsaknad av inverkan från kroppsegen smärtmodulation på smärtuppkomst primärt orsakad av hjärnskada efter stroke. Den påvisade ökade känsligheten för trycksmärta i smärtfritt område vid utgångsläget för patienter med smärta efter stroke talar för förändrad nedåtstigande smärtkontroll från hjärnbarken orsakad av hjärnskadan. Under smärtprovokationen aktiverade patienter med perifer nervskada och smärta efter stroke den kroppsegna smärtkontrollen som påverkar inflödet av smärtsignaler på ryggmärgsnivå på samma sätt som hos friska kontroller, vilket visar på normalt fungerande kroppsegen smärtkontroll. Intravenös tillförsel av 8 mg ondansetron påverkade inte intensiteten av spontan- eller beröringsutlöst smärta hos patienter med perifer nervskada. Detta talar för bristande betydelse av $5HT_3$-receptorn för underhållet av beröringsutlöst smärta.

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