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# Brain imaging of chronic pain

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ACADEMIC DISSERTATION

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## Abbreviations

ACC	Anterior cingulate cortex
BOLD	Blood oxygen level dependent
CRPS	Complex regional pain syndrome
EEG	Electroencephalography
ECD	Equivalent current dipole
ENMG	Electroneuromyography
fMRI	Functional magnetic resonance imaging
HSV	Herpes simplex virus
IASP	International Association for the Study of Pain
IC	Insular cortex
ISI	Interstimulus interval
LEF	Laser-evoked field
LEP	Laser-evoked potential
MEG	Magnetoencephalography
MI	Primary motor cortex
MRI	Magnetic resonance imaging
MDvc	Ventral caudal part of medial dorsal nucleus
NRS	Numerical rating scale
NS	Nociceptive specific
PFC	Prefrontal cortex
PPC	Posterior parietal cortex
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SQUID	Superconducting quantum interference device
VAS	Visual analog scale
VBM	Voxel-based morphometry
VMpo	Posterior part of ventral medial nucleus
VP	Ventral posterior nucleus
WDR	Wide dynamic range

## **Abstract**

Acute pain serves as a warning sign for protective purposes in the everyday environment and therefore has substantial survival value. Chronic pain, however, lacks survival and adaptive functions, causes a great amount of individual suffering, and consumes the resources of the society through treatment costs and loss of production. The treatment of chronic pain remains challenging because of an inadequate understanding of mechanisms working at different levels of the nervous system in the development, modulation, and maintenance of chronic pain. Especially in chronic pain conditions, in which the aetiology and the pathophysiology are not understood, the treatment may be suboptimal because it can not be targeted to underlying mechanisms.

Noninvasive neuroimaging techniques have contributed to our understanding of brain activity associated with pain. Many previous studies have focused on brain activations to acute experimental pain in healthy individuals, and have consistently demonstrated a widely-distributed network of brain regions that participate in the processing of acute pain. The aim of the present thesis was to employ noninvasive brain imaging methods to better understand the brain mechanisms in patients with chronic pain.

In Study I, we used magnetoencephalography (MEG) to measure cortical responses to painful laser stimulation in healthy individuals for optimization of the stimulus parameters for patient studies. In Studies II and III, we monitored with MEG the cortical processing of touch and acute pain in patients with complex regional pain syndrome (CRPS). We found persisting plastic changes in hand representation area of the primary somatosensory (SI) cortex and attenuated responses in the posterior parietal cortex to both tactile and painful laser stimulation. The primary motor cortex reactivity to acute pain was reduced in patients, and the reactivity correlated with the grip strength and correlated inversely with the amount of ongoing spontaneous pain in the painful hand, suggesting tight coupling between central motor dysfunction and chronic pain in CRPS. In Studies IV and V, we used MEG and functional magnetic resonance imaging (fMRI) to investigate patients

who suffered from recurrent herpes simplex virus infections and from chronic widespread pain in one side of the body. With MEG, we found plastic changes in the SI cortex, suggesting that different types of chronic pain may be associated with similar cortical reorganization. With fMRI, we found functional and morphological changes in the central pain circuitry, suggesting central contribution for the pain.

The results show that chronic pain is associated with morphological and functional changes in the brain. Objective measurement of such changes with functional imaging may aid in the diagnosis and therapy of chronic pain conditions.



## List of publications

This thesis is based on the following publications, which will be referred to by roman numerals.

**I** Raji TT, **Vartiainen NV**, Jousmäki V, Hari R: Effects of interstimulus interval on cortical responses to painful laser stimulation. *J Clin Neurophysiol* 2003, 20: 73–79.

**II** **Vartiainen NV**, Kirveskari E, Forss N: Central processing of tactile and nociceptive stimuli in complex regional pain syndrome. *Clin Neurophysiol* 2008, 119: 2380–2388.

**III** Kirveskari E, **Vartiainen NV**, Gockel M, Forss N: Motor cortex dysfunction in complex regional pain syndrome. *Clin Neurophysiol*, under revision.

**IV** **Vartiainen NV**, Kirveskari E, Kallio-Laine K, Kalso E, Forss, N: Cortical reorganization in primary somatosensory cortex in patients with unilateral chronic pain. *J Pain* 2009, 10: 854–859.

**V** **Vartiainen NV**, Kallio-Laine K, Hlushchuk Y, Kirveskari E, Seppänen M, Autti H, Jousmäki V, Forss N, Kalso E, Hari R: Changes in brain function and morphology in patients with recurring herpes simplex virus infections and chronic pain. *Pain* 2009, 144: 200–208.

Publication I was previously used in the thesis of Tuukka Raji in 2005.



## 1. Introduction

The survival of an organism depends essentially on its ability to perceive pain. Individuals born without the ability to feel pain are vulnerable to life-threatening injuries from the beginning of their lives, because severe damage, such as cuts, fractures, and burns may go unnoticed (Nagasako et al., 2003). In healthy individuals, acute pain is a warning sign that protects against tissue damage in everyday environments. After injury, a decrease of pain threshold and increased responsiveness in the injured area work as motivators that facilitate protective behaviour, until the tissue heals. For example, ongoing pain and tenderness from a sprained ankle motivates for reduced weight bearing and protection, and thereby enhances healing and survival. This hypersensitivity is an expression of temporary and adaptive neural plasticity.

Chronic pain, on the other hand, is maladaptive and reflects pathological changes in the function of the nervous system. It serves no useful purpose from an evolutionary perspective, does not adapt the organism to the environment, and does not lead to performance that enhances survival. Chronic pain causes a considerable negative impact on the individual and society. In 2003, the socioeconomic estimated costs of chronic pain were e.g. in the 9-million-inhabitant Sweden about 8.9 billion € (SEK 87.5 billion). Care-related costs accounted for 0.8 billion € whereas indirect costs due to loss of production attributed to sick leave accounted for 8.1 billion € (Lundberg, 2006).

Treatment of chronic pain remains difficult, and this stems partially from the insufficient understanding of neural mechanisms in the development and maintenance of chronic pain. Even in the perception of acute pain, nociceptive signals produced by harmful stimuli are modulated by complex anti- and pronociceptive activities at all levels of the neuraxis. This modulation is driven also by affective and cognitive factors, such as the state of mind, expectations, previous experiences, mood, attention, and context. In chronic pain, the complexity of such modulation is probably even more profound.

When the aetiology and the pathophysiology of chronic pain are unknown, no mechanism-based treatment is available. Furthermore, unknown aetiology increases the psychological stress, uncertainty, and suffering in the patients, as well as confusion in the physicians trying to treat them. Distinct neural mechanisms may be underlying some chronic pain conditions and therefore better knowledge of these mechanisms could help to develop more rational therapy for chronic pain. However, objective measurement of neural activity related to the complex and highly subjective experience of pain is challenging.

Noninvasive brain imaging has emerged as a tool for the investigation of neural function in healthy individuals and in neurological and psychiatric diseases. Acute pain processing in healthy individuals has been extensively studied with these methods, and there is now increasing enthusiasm for studying neural activity in patients with chronic pain conditions.

The present thesis aims to increase knowledge of pain processing in the central nervous system in patient groups suffering from chronic pain with an unclear origin. In patients suffering from complex regional pain syndrome (CRPS), we used magnetoencephalography (MEG) to follow the cortical processing of tactile and nociceptive stimuli. In patients suffering from recurrent herpes simplex virus (HSV) infections and from chronic pain, we used MEG to study cortical plasticity and functional magnetic resonance imaging (fMRI) to study the central processing of touch and pain to reveal possible central nervous system alterations in these patients.

## **2. Background**

### ***2.1 Anatomy and physiology of pain pathways***

Damaging stimuli activate the peripheral free nerve endings, the nociceptors. The noxious signals from the periphery are transmitted to the central nervous system by the pain pathways, a process referred to as nociception. The experience of pain is normally generated on the basis of noxious input but it is also strongly modulated by affective and cognitive aspects. The subjective experience of pain may exist even in the absence of noxious input, but typically these two are highly correlated. The anatomy and the physiology of the pain pathways are presented below, based on recent reviews (Bromm and Lorenz, 1998; Treede et al., 1999; Schnitzler and Ploner, 2000; Millan, 2002; Craig, 2003; Vogt, 2005; Dostrowsky and Craig, 2006).

#### **2.1.1 Nociceptors and peripheral pathways**

Nociceptors are peripheral free nerve endings, which transduce noxious or potentially noxious stimuli, such as mechanical, thermal and chemical stimuli to action potentials that are sent to the central nervous system along the nociceptive nerve fibres. Nociceptors are widespread in the peripheral tissues, including skin, muscle, joints, and viscera. The noxious signals are transmitted via thinly myelinated A $\delta$ -fibres and unmyelinated C-fibres. The density of the C-fibres in the human skin is assumed higher than that of A $\delta$ -fibres (Bragard et al., 1996). The conduction velocity of A $\delta$ -fibres is around 5–30 m/s and they signal sharp pain from heat and from sharp mechanical stimuli (Konietzny et al., 1981), whereas the conduction velocity of C-fibres is around 0.5–2 m/s and they signal burning pain from heat stimuli and pain from intense pressure (Ochoa and Torebjörk, 1989). Both fibre types signal pain from chemical stimuli. A $\delta$ -fibres are further divided to type I, which are

found in the hairy and glabrous skin, and type II, which are found in hairy skin only. Most of the C-nociceptors respond to several types of noxious stimuli and are therefore termed polymodal nociceptors (Perl, 1996).

About half of the  $A\delta$ -fibres and one third of C-fibres are silent nociceptors, which have very high mechanical thresholds (Meyer et al., 1991). The silent C-fibres are different from the conventional polymodal nociceptors, and they often become responsive to mechanical stimuli in inflamed skin and may therefore be the underlying mechanism for primary mechanical hyperalgesia (Schmidt et al., 1995). The cell bodies of the nociceptive fibres are in the dorsal root ganglia, and the nociceptive fibres terminate in the dorsal horn of the spinal cord or in the trigeminal ganglia in the head area.

### **2.1.2 Dorsal horn and spinal cord**

In the dorsal horn of the spinal cord, the first-order nociceptive neurons synapse with projection neurons that ascend towards more central structures (see Figure 1). The peripheral  $A\delta$ -fibres terminate in the most superficial layer of the dorsal horn, the lamina I, which receives the major input of the all nociceptive fibres, and in the lamina V. The peripheral C-fibres terminate in lamina I and send polysynaptic input to lamina V neurons. Large-diameter myelinated  $A\beta$ -fibres, transmitting mechanoreceptive and proprioceptive input also terminate at the lamina V neurons. Therefore the superficial lamina I receives more specific nociceptive input, whereas the lamina V input represents integration of all afferent input.

In the lamina I of dorsal horn, two nociceptive cell types of the spinothalamic tract can be distinguished: nociceptive specific (NS) cells, which receive predominantly  $A\delta$ -fibre input, and polymodal nociceptive (sensitive for heat, pinch, cold), which receive mainly C-fibre input. In

addition to nociceptive cell types, lamina I also contains non-nociceptive cells that receive modality-specific small-diameter cell input related to e.g. thermoreception and itch (Craig and Andrew, 2002).

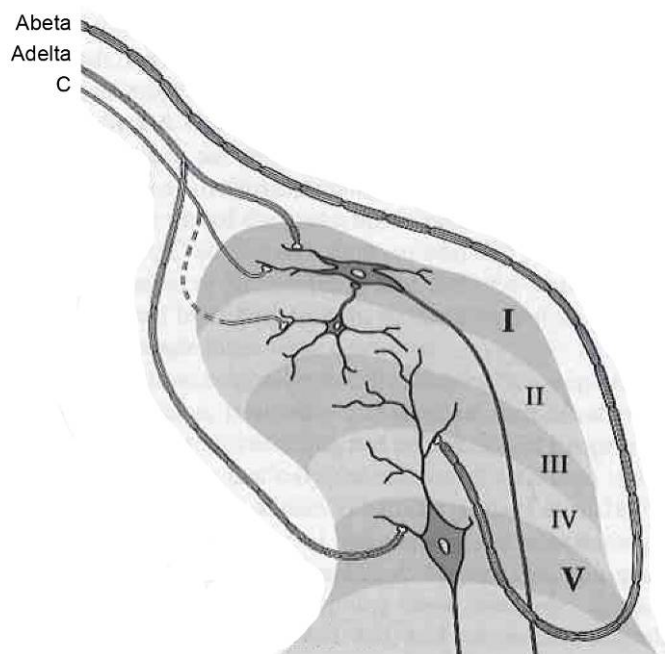


Figure 1. Dorsal horn of spinal cord and nociceptive afferent fibres terminating to projection neurons in laminae I–V. Adapted from Craig (2003) with permission.

Lamina V is composed mostly of wide dynamic range (WDR) cells. They have large receptive fields and receive input both from tactile and nociceptive afferents. As a population, their activity represents the integration of all afferent input to the dorsal horn.

The spinothalamic tract that projects from the spinal cord to the thalamus is most closely associated with pain and temperature, and it has been known for decades that lesions of this tract results in the loss of these sensations. Almost half of the spinothalamic tract cells are from lamina I and one quarter from laminae IV–VI. Some cells are located in deeper laminae and in the ventral horn. The ascending axons cross the midline at a level near the cell bodies and continue in the spinothalamic tract in anterior and lateral segments. The anterior part contains mainly lamina V neurons, and the lateral part contains mainly lamina I neurons.

Recently, monitoring pain-related neural activity in the dorsal horn of the spinal cord has been accomplished noninvasively using fMRI (Lilja et al., 2006).

### **2.1.3 Ascending nociceptive projections to thalamus and brainstem**

Nociceptive information is transmitted from the spinal cord to the brain through several ascending pathways, the main pathway being the spinothalamic tract. The main projection sites of the spinothalamic tract in the thalamus are the ventral posterior (VP) nuclei, the posterior part of the ventral medial nucleus (VMpo), the ventral caudal part of the medial dorsal nucleus (MDcv), the ventral lateral nucleus, the central lateral nucleus, and the parafascicular nucleus (Dostrowsky and Craig, 2006). The nociceptive specific lamina I pathways project to the VP nuclei, VMpo, and MDvc. Lamina V pathways project to VP nuclei, ventral lateral nucleus, and central lateral nucleus (see Figure 2). The lateral and medial VP nuclei are the main somatosensory nuclei of the thalamus and receive—in addition to nociceptive input—mechanoreceptive and proprioceptive input through the dorsal column medial lemniscus pathways. Using microelectrodes, activity of nociceptive neurons in the human VP nucleus has been recorded, and microstimulation of this area has been shown to evoke pain (Lenz and Dougherty, 1997). In addition, lesion of this structure may lead to central pain (Montes et al., 2005). However, most of the nociceptive information of the spinothalamic tract is conveyed to the cortex through other nuclei (such as VMpo), as the cortical laser-evoked responses were only moderately (33%) attenuated in a patient with a VP lesion (Montes et al., 2005). Interestingly, in patients with central post-stroke pain, incidence of pain evoked by microstimulation of VP nucleus was increased (Davis et al., 1996).

The important functions of the thalamus, in addition to processing noxious information, include processing and relaying both sensory and motor information. Thalamus also takes part in cognitive functions, such as language and memory, and it regulates arousal. Thalamic nuclei project



to one or few cortical areas, which in turn send back information to different thalamic nuclei, forming thalamo-cortico-thalamic circuits (Herrero et al., 2002).

In addition to thalamic targets, nociceptive information is conveyed to numerous homeostatic sites in the brainstem. Catecholaminergic cell groups in the brainstem receive input from lamina I cells (Westlund and Craig, 1996). These cell groups include regions in the ventrolateral medulla, the nucleus of solitary tract, the locus coeruleus, and regions in the dorsolateral pons. They integrate cardiorespiratory and homeostatic function and have pain-modulatory functions. For example, the cell groups in the ventrolateral medulla and in the dorsolateral pons are involved in descending pro- and antinociceptive modulation.



Figure 2. Ascending tracts of lamina I cells (left) and lamina IV–V cells (right) in macaque monkey. MDvc = ventral caudal part of medial dorsal nucleus, VPI = ventroposterior inferior nucleus, VPL = ventroposterior lateral nucleus, VMpo = posterior part of ventral medial nucleus, CL = central lateral nucleus, SI = primary somatosensory cortex, SII = secondary somatosensory cortex, STT = spinothalamic tract. Adapted from Craig (2006) with permission from Elsevier.

Parabrachial nucleus receives spinal input mainly from lamina I cells, and it is connected with the reticular formation of the brainstem (Craig, 1995). Parabrachial nucleus projects to hypothalamus, amygdala, and thalamus, and is thought to integrate nociceptive activity with the homeostatic and autonomic activity.

The periaqueductal gray has a dual role in aversive behaviour and in endogenous analgesia. It receives input primarily from lamina I cells. The closely situated pretectal nuclei have been proposed to have a role in endogenous analgesia, because stimulation of this area produces analgesia (Rees and Roberts, 1993).

The reticular formation receives input through the spinoreticular tract, which consists of neurons in laminae VII and VIII (Kvetter et al., 1982). Many of these neurons respond to stimuli presented to either side of the body and they could therefore contribute to the diffuse nature of many pain conditions.

Traditionally, the nociceptive system has been divided to lateral and medial systems. The lateral pain system has been assumed to serve the sensory-discriminatory aspect of pain processing, whereas the medial system is involved in the cognitive-evaluative, motor, and emotional components of pain. The cortical components of the lateral pain system include the primary and secondary somatosensory (SI and SII) cortices, and the posterior insula, and the corresponding regions of medial system include the anterior cingulate cortex (ACC) and prefrontal cortex (PFC). This division probably oversimplifies the actual picture as separate dimensions of the pain experience probably are not represented by single brain regions.

## 2.1.4 Central structures

### Insula

Anterograde tracing of thalamocortical projections in primates indicates that thalamic nucleus VMpo projects to the posterior insular cortex (IC) (Craig, 2002). Accordingly, electrical stimulation of the posterior IC elicited pain in patients who were undergoing evaluation for the surgical treatment of epilepsy (Ostrowsky et al., 2002; Mazzola et al., 2006). The posterior IC and the closely situated SII cortex are the only cortical regions where electrical stimulation has been shown to elicit pain (Mazzola et al., 2006). Intracranial recordings showed that the posterior IC did not activate to non-painful stimuli but encoded stimulus intensity at painful levels (Frot et al., 2007). The role of posterior IC in the basic sensory aspects of pain is further supported by the sensitivity to the laterality of the stimulation and the somatotopic arrangement of body parts in the posterior IC (Brooks et al., 2002; Bingel et al., 2003; Brooks et al., 2005).

In human brain imaging studies of pain, IC is one of the most consistently activated cortical areas (Peyron et al., 2000; Treede et al., 2000; Apkarian et al., 2005) and it has been thought to be involved in the affective dimension of pain. This is in line with insular damage causing an inadequate emotional response to pain (Berthier et al., 1988). Typically in imaging studies, the activation is spatially extensive, spanning from anterior to posterior IC. Functional segregation of the subregions of the insula has recently been suggested (Schweinhardt et al., 2006a). Clinical pain in patients, with its greater affective component, was associated with activity in the rostral anterior IC, whereas the intensity of experimental (or allodynic) pain was coded by caudal anterior IC (Schweinhardt et al., 2006a).

Insula appears to be a major integration site for interceptive information from the body (Craig, 2002). As the IC also has extensive anatomical connections to limbic structures, it is well suited associate sensory information with emotional responses (Mesulam and Mufson, 1982).

## **Cingulate cortex**

The cingulate cortex belongs to the phylogenetically old limbic system. Nociceptive neurons have been demonstrated in the rabbit ACC; these neurons had large receptive fields and responded to noxious stimulation anywhere in the body (Sikes and Vogt, 1992). Nociceptive neurons have been shown also in the human ACC (Hutchison et al., 1999). Thalamic projections to the Brodmann's area 24 have been demonstrated from thalamic midline, intralaminar (central lateral and parafascicular), and MDvc nuclei (Vogt, 2005). The ACC is one of the most commonly activated areas in brain imaging studies of pain processing (Peyron et al., 2000; Apkarian et al., 2005), and it has been assumed to mediate affective responses to noxious stimuli. The blood-flow based imaging methods show consistent activation in the ACC, but the exact activation areas inside the CC are variable. Activity of the dorsal ACC correlated with the subjective unpleasantness of pain during hypnosis, even while the stimulation energy was the same, and therefore the ACC has been suggested to encode the affective dimension of pain (Rainville et al., 1997). The ACC activity has also been shown to correlate with stimulus energy of experimental pain (Coghill et al., 1999). Büchel et al. (2002) showed several pain-related activation areas in the ACC; the perigenual and posterior ACC coded stimulus intensity and pain, whereas the anterior portion of the ACC showed signal changes related to working memory and attention. Attention-related activation in the anterior ACC has also been observed in single-cell recordings (Davis et al., 2000). Moreover, the ACC has been implicated in motor planning and response selection, attentional orienting, and cognitive control.

Recent subdivision of the CC on cytoarchitectural basis suggests a four-region model including ACC, midcingulate cortex (MCC), posterior cingulate cortex (PCC), and retrosplenial cortex (Vogt, 2005). In this model, the ACC is implicated in emotional and autonomic processing, the MCC is

involved in response selection, and PCC in visuospatial orientation. The role for the posterior MCC in attentional orienting and motor withdrawal is supported by recent intracranial recordings that showed early (120 ms) responses from this area in response to acute laser pain (Frot et al., 2008).

### **Somatosensory cortices**

Single-cell recordings in awake monkeys have demonstrated SI cortex neurons responding in an intensity-correlated manner to noxious stimuli (Kenshalo et al., 1988), suggesting that the nociceptive SI neurons encode the sensory-discriminative component of pain. Accordingly, the loss of discriminative pain sensation (without loss of pain affect) was attributed to a large lesion involving both SI and SII (Ploner et al., 1999a). SI receives input from thalamic lateral and medial VP nuclei, and the SII receives input from inferior VP nucleus. The thalamic nucleus VMpo, which receives almost exclusively spinothalamic lamina I input, has been suggested to project to area 3a in the central sulcus.

The role of the SI cortex in response to acute selectively noxious stimulation has been debated: about half of the studies in a meta-analysis showed no SI activation while the other half did (Peyron et al., 2000). This discrepancy has been explained with different stimulation methods and possible different excitatory and inhibitory effects of pain on SI neurons, which could result in different hemodynamic responses (Peyron et al., 2000; Schnitzler and Ploner, 2000). Pain-related cerebral blood flow increases were somatotopically organized in the SI region (Andersson et al., 1997). According to optical imaging data, area 3a is activated in response to heat pain, probably via thalamic nucleus VMpo (Tommerdahl et al., 1996). In fMRI studies that used nociceptive-specific thulium-laser stimuli, SI has been shown to be involved in coding the side and the intensity of the stimulation (Bornhövd et al., 2002; Bingel et al., 2003). Further, somatotopic representations to such stimuli were demonstrated in SI and in SII (Bingel et al., 2004). Using MEG, several groups have

localized activations in response to painful laser stimulation to SI (Kanda et al., 2000; Ploner et al., 2000; Timmermann et al., 2001), whereas others have localized these activations significantly medial and posterior to area SI, possibly to posterior parietal cortex (PPC) (Forss et al., 2004). It is probable that both the SI and the PPC are activated in response to noxious stimulation (Nakata et al., 2008).

The SII cortices are the first cortical areas activated in response to noxious stimulation in electroencephalography (EEG) and MEG studies (Treede et al., 2000). Intracranial recordings and MEG recordings have demonstrated that the SII cortex encodes stimulus intensity from innocuous to painful level, but shows ceiling effect for higher intensity (Timmermann et al., 2001; Chen et al., 2006; Frot et al., 2007). It was therefore suggested that the SII is involved in recognizing the noxious nature of stimuli. The SII cortex is bilaterally activated also in response to innocuous tactile stimuli and is assumed to be involved in object recognition and integration of sensory input from the two body halves (Simões et al., 2001). The human SII cortex is divided into four cytoarchitectonic areas. A meta-analysis indicated that pain-related activations were clustered in one subarea, whereas activations related to non-painful somatosensory stimulation were found slightly more anteriorly (Eickhoff et al., 2006a; Eickhoff et al., 2006b). Selective SI and SII lesions caused impaired ability to localize painful stimuli and to recognize the stimuli as painful, suggesting that the somatosensory cortices are involved in the sensory-discriminative processing of pain (Ploner et al., 1999a). This is supported by earlier findings of tactile and nociceptive perception deficits when tumour was pressing parietal operculum and elevated pain thresholds in patients with lesions involving the parietal operculum (Greenspan and Winfield, 1992; Greenspan et al., 1999).

### **2.1.5 Descending and modulatory projections**

The pain pathways are under the influence of top-down regulation from hierarchically higher levels. Many supraspinal regions give rise to pathways that monosynaptically descend to the dorsal horn of the spinal cord and may have either inhibitory or facilitatory effects. These include the hypothalamus, parabrachial nucleus, nucleus of the solitary tract, rostral ventral medulla, and periaqueductal grey (Millan, 2002). Pharmacological and electrical stimulation of these areas in animal studies has been shown to modulate nociception, either by direct or indirect spinal projections (Willis and Westlund, 1997; Jasmin et al., 2003). The activity of the descending pathways from rostral ventral medulla is primarily modified by the periaqueductal grey and other regions.

New imaging methods have contributed to our understanding of these modulatory mechanisms in humans and have shown that forebrain structures such as rostral ACC and PFC are important in pain modulation (Bingel et al., 2007). Rostral ACC covaried with brainstem activity during both placebo and opioid analgesia, and during distraction task (Petrovic et al., 2002; Valet et al., 2004), suggesting that the connectivity of these areas is important for pain modulation. Further, placebo analgesia was associated with PFC activity during anticipation of pain (Wager et al., 2004). This might have also clinical implications, as in Alzheimer patients, the placebo component of analgesic treatment was reduced, indicating the importance of frontal cortex function in the modulation of pain (Benedetti et al., 2006). The interplay between these cortical and brainstem areas related to pain modulation may be important factor in the development of various chronic pain conditions.

### **2.1.6 Pain and the central motor system**

The effect of pain on motor functions is evident in everyday life, as pain impairs and limits motor performance. Studies in healthy subjects have shown that thermal pain activates the primary motor cortex (MI) (Casey et al., 1996; Gelnar et al., 1999). The tight coupling between the central

motor system and pain is further supported by the observation that MI stimulation alleviates chronic pain (Tsubokawa et al., 1991; Garcia-Larrea and Peyron, 2007).

One way to monitor the functional state of the MI is to measure the 20-Hz component of spontaneous oscillatory activity, which is thought to originate mainly in the MI on the basis of intracranial and MEG recordings (Jasper, 1949; Salmelin and Hari, 1994; Salenius and Hari, 2003). Suppression of the 20-Hz rhythm is assumed to reflect excitation and the subsequent rebound is thought to reflect inhibition of the MI. A prior study with healthy subjects showed that the motor cortex 20-Hz rhythm is modulated by painful laser stimuli, possibly for preparation of voluntary movements (Raij et al., 2004). Further, in CRPS patients who had chronic pain in their upper extremity, reactivity of the 20-Hz motor cortex rhythm to tactile stimuli was decreased indicating modified inhibition of the motor cortex (Juottonen et al., 2002).

## **2.2 Chronic pain**

The International Association for the Study of Pain (IASP) defines chronic pain as “*pain without apparent biological value that has persisted beyond the normal tissue healing time*” (usually more than three months). This definition is based on the duration of the pain, and it does not depend on the pain aetiology. Chronic pain can be nociceptive, neuropathic, or neither (i.e., pain occurs without known somatic background). Neuropathic pain was originally defined by IASP as “*pain initiated or caused by primary lesion or dysfunction in the nervous system*” (Merskey, 1994), and recently by an expert group as “*pain arising as a direct consequence of a lesion or disease affecting the somatosensory system*” (Treede et al., 2008). Neuropathic pain can arise from damage to the nerve pathways at any point from the terminals of the peripheral nerves to the cortical neurons in the brain. It is anatomically classified as central (originating from damage in brain or in spinal cord) or peripheral (originating from damage in peripheral nerves). Examples of peripheral neuropathic pain



are posttraumatic pain after mechanical nerve lesion, painful diabetic neuropathy, and postherpetic neuralgia. Central pain is most commonly caused by stroke, spinal cord injury, and multiple sclerosis (Andersen et al., 1995). The term neurogenic pain is defined by IASP as “*pain initiated or caused by a primary lesion, dysfunction, or transitory perturbation in the peripheral or central nervous system*” (Merskey, 1994), and it refers to neuropathic pain with full clinical recovery.

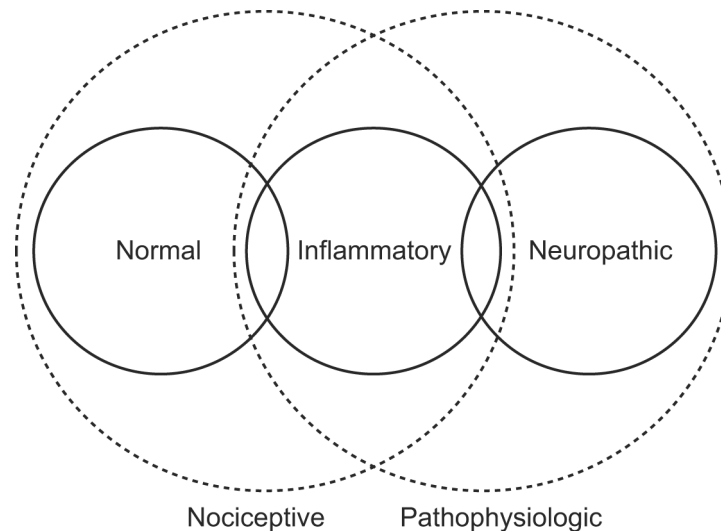


Figure 3. Pain terms. Nociceptive pain can be considered to include normal pain and inflammatory pain (with peripheral and possibly central sensitization). Alternatively, inflammatory pain can be grouped with neuropathic pain under pathophysiologic pain, as changes in pain transmitting system, caused by tissue or nerve pathology, are involved in both. Adapted from Devor (2006) with permission from Elsevier.

Nociceptive pain is usually considered as “normal”, physiological pain. For example when a toe is accidentally hit with a corner of a door, the painful experience matches the mechanical noxious stimulus. Typically minor injuries, such as sprains, burns, and infections, lead to inflammatory reaction, local release of inflammatory mediators, and sensitization of peripheral nociceptors. The resulting symptoms, ongoing pain and hyperalgesia, are adaptive as long as they disappear as the injury heals; temporary hyperresponsiveness enhances survival by protecting the injured body part. Even these physiological forms of pain may induce neuroplastic changes in the central nervous

system leading to hyperalgesia and allodynia (Treede et al., 2008). Neuropathic pain, on the other hand, is a sign of a pathophysiological process in the nervous system (see Figure 3).

Clinical chronic pain conditions may be a combination of nociceptive, inflammatory, and neuropathic pain. The underlying disease, the mechanism of the pain, and the resulting clinical symptoms form a complex relationship. If disease-modifying treatment is available, it should be applied at the first place (such as joint replacement surgery in osteoarthritis). The treatment of the symptoms should be based on the mechanism of pain, and therefore understanding the mechanism of the pain is important (Scholz and Woolf, 2002).

### ***2.3 Complex regional pain syndrome***

The first description of CRPS probably originates from about 150 years ago, when Sir Weir Mitchell reported on “Gunshot wounds and other injuries of peripheral nerves” during the American Civil War (Mitchell et al., 1864). After that, the terms “reflex sympathetic dystrophy” and “causalgia” have been used to describe this syndrome. The clinical characteristics of CRPS include sensory, autonomic, and motor symptoms. Sensory symptoms include spontaneous pain, allodynia, and hyperalgesia, but sensory impairment may also occur. Autonomic nervous system symptoms consist of abnormalities in the skin temperature, colour, and sweating. Common examples of motor symptoms are weakness, dystonia, tremor, and clumsiness especially in fine motor skills. In later stages, clinically apparent signs in the affected limb are atrophy and osteoporosis. The disease is typically triggered by an initiating event, such as minor injury or fracture of a limb. CRPS is divided into two types: type II with nerve lesion and type I without apparent nerve lesion. The developing pain is usually found in the extremity, it is disproportionate to the initiating event, and the pain is not restricted to the area of a single peripheral nerve. The initial diagnostic criteria for CRPS were published by Stanton-Hicks and colleagues (1995). As motor dysfunction has been shown to be

common symptom in CRPS (Veldman et al., 1993; Harden et al., 1999; Birklein et al., 2000), a subsequent revision of the diagnostic criteria has been proposed to include motor symptoms (Harden et al., 2007). The annual incidence is 26 new cases per 100000, and the condition is more common in women than in men (de Mos et al., 2008). The majority of the patients recover spontaneously, but sometimes CRPS may be very resistant to treatment and has led even to amputations (Lausten-Thomsen and Laursen, 2005).

CRPS has been under intensive research, but it still remains unclear what is the pathogenesis behind CRPS, and why it develops after minor trauma in some patients, but not in others (Birklein and Handwerker, 2001; Jänig and Baron, 2003). Possible explanations for the pathogenesis of CRPS include neurogenic inflammation, endothelial dysfunction, abnormal sympatho-afferent coupling, and small-fibre neuropathy combined with ectopic firing of remaining fibres (Oaklander and Fields, 2009). Recent brain imaging studies have shown cortical reorganization in the central sensory and motor systems of CRPS patients (Juottonen et al., 2002; Maihöfner et al., 2007). Such plastic changes have been shown to correlate with the clinical symptoms, such as intensity of pain and tactile discrimination, and with the spread of the pain from its original location (Maihöfner et al., 2003; Forss et al., 2005a; Pleger et al., 2006). Interestingly, decreased pain intensity due to rehabilitation was accompanied by normalization of plastic changes and tactile discrimination ability (Pleger et al., 2005). As the underlying pathophysiology of this condition is still unknown and the treatment options remain limited, functional brain imaging studies could help to understand the possible pathophysiological mechanism and help to develop tailored treatment.

## ***2.4 Herpes simplex virus infections and chronic pain***

HSVs are common pathogens. In the general population, 16% of adults have antibodies against HSV-2 and 70% against HSV-1 (Arvaja et al., 1999). The primary targets of the virus are the sensory

neurons, where the virus can remain latent. During reactivation of the virus, the most common affected areas are the skin and mucosal membranes (Whitley and Roizman, 2001). More severe complications include encephalitis and meningitis. Interesting characteristics of the virus include its ability to invade neural cells and its preference to certain brain areas (Davis and Johnson, 1979).

HSV infections are known to cause transient neuralgia in the trigeminal, cervical, and sacral areas, and also chronic neuropathic pain has been described in the sacral (Haanpää and Paavonen, 2004) and in trigeminal (Gonzales, 1992) dermatomes.

Recently, Kallio-Laine and colleagues described a novel patient group who were suffering from recurrent HSV infections due to subtle immunological abnormalities and from chronic spontaneous pain in one side of the body (Kallio-Laine et al., 2008). The authors suggested that the patients were suffering from central neuropathic pain caused by infections and latent HSV in the brain.

## ***2.5 Brain imaging in acute and chronic pain***

Brain activity associated with pain has been studied in human subjects with multiple brain imaging methods, which either directly or indirectly reflect neural activity. With EEG, MEG, positron emission tomography (PET), and fMRI, a widely distributed network of brain areas has been shown to be activated during pain. The majority of studies have focused on brain activity in healthy individuals elicited by stimulation of peripheral nociceptors with various experimental noxious stimuli, and have demonstrated activations most commonly in SI, SII, ACC, IC, PFC, thalamus, and cerebellum. The different brain areas are thought to reflect the different dimensions of the pain experience, as measured by their activation strengths or correlated activity with other brain regions. To date, possible existence of region devoted solely on processing of pain remains to be demonstrated. In contrast, a highly distributed network of brain regions appears to be involved in the

generation of the pain percept. In addition, intracranial recordings and microneurographic recordings from peripheral nerves have increased our understanding concerning the relationship between neural activity and subjective perception of pain.

The brain activation patterns in response to identical experimental painful stimuli have been compared between patients suffering from various pain conditions and control subjects. Early PET studies showed reduced responses to thermal pain in the ACC of (small groups of) patients suffering from postsurgical pain and from rheumatoid arthritis, but responses were increased in atypical face pain patients (Derbyshire et al., 1994; Jones and Derbyshire, 1997; Derbyshire et al., 1999). However, in larger groups of patients suffering from non-specific low back pain, the responses did not differ from those of control subjects (Derbyshire et al., 2002). The finding was later replicated with fMRI, suggesting that acute pain processing in many chronic pain conditions is not different from that of the healthy controls (Baliki et al., 2006). A recent meta-analysis suggested that prefrontal activation was observed more often in patients with clinical pain than in healthy individuals receiving experimental pain (Apkarian et al., 2005). In this study, the clinical pain referred to constant pain experienced by the patient, or to abnormal evoked sensations, such as hyperalgesia or allodynia. However, brain imaging studies of acute experimental pain in chronic pain patients remains inconclusive, probably because the studied patient populations have varied a lot in pain distribution, history, etiology, and related psychological factors (Apkarian et al., 2005; Kupers and Kehlet, 2006).

In a similar way, laser-evoked potentials (LEPs) have been used to study many patient groups suffering from various pain conditions of peripheral, central, and unclear origin (Kakigi et al., 1991; Treede et al., 1991; Gibson et al., 1994; Casey et al., 1996; Hansen et al., 1996; Lorenz et al., 1996; Truini et al., 2003). The conclusion based on several studies was that suppression of LEPs indicates damage of the spinothalamic tract and supports the diagnosis of neuropathic pain (Cruccu et al., 2004).

Another approach in studying chronic pain has been to measure brain activations associated with hyperalgesia and allodynia, because these features are common in various chronic pain conditions, especially in neuropathic pain. Measurement of specific cerebral signatures in different pain mechanisms would be useful for diagnostic purposes. Robust brain activations in key pain processing areas such as IC and ACC were shown in response to mechanical allodynia (Schweinhardt et al., 2006a). Similar changes were observed also in experimental hyperalgesia and allodynia models in healthy individuals (Maihöfner et al., 2004b; Zambreanu et al., 2005). However, in other patient groups, mechanical allodynia elicited no IC or ACC activations (Ducreux et al., 2006; Witting et al., 2006). The contradictory results might reflect the varying degrees of spinothalamic tract and lemniscal tract lesions in different patient groups, as well as the varying degree of ongoing background pain in individual patients (Schweinhardt et al., 2006b).

One of the earliest approaches to study chronic pain was to compare the regional cerebral blood flow (rCBF) between patients suffering from ongoing pain and healthy individuals. A decrease of rCBF in the contralateral thalamus was observed in chronic pain conditions including painful mononeuropathy, cancer pain, and posttraumatic pain (Di Piero et al., 1991; Hsieh et al., 1995; Iadarola et al., 1995), suggesting thalamic hypoperfusion in presence of ongoing pain.

More recent studies have examined brain activations with fMRI in patients with ongoing pain but without external painful stimulation. In patients with chronic back pain, the sustained component of the ongoing pain was associated with activity of the medial PFC, whereas the increasing component of pain was associated with activity in IC and other regions involved in acute pain (Baliki et al., 2006). Chronic ongoing pain has also been shown to disrupt the default mode network (Baliki et al., 2008; Cauda et al., 2009). Studying the spontaneous brain activity appears fruitful line of research; we (Malinen S, Vartiainen N, Hlushchuk Y, Koskinen M, Ramkumar P, Forss N, Kalso E, Hari R, unpublished data) have recently found higher-frequency oscillations and altered connectivity of IC in patients with ongoing pain.

## **2.6 Brain imaging methods**

The studies in this thesis were conducted with two complementary brain imaging methods, MEG and fMRI. These two noninvasive techniques open a window to the living human brain for observation of the electric activity on a millisecond timescale (Hämäläinen et al., 1993; Hari and Forss, 1999) and hemodynamic activity with spatial accuracy of millimetre scale.

### **2.6.1 Magnetoencephalography and electroencephalography**

Neural electric activity in the brain produces weak magnetic fields that can be measured outside the head with sophisticated MEG instruments. The synaptic activation in the apical dendrites of the pyramidal cells in the cortex results in postsynaptic intracellular currents. These currents are extremely small, but as they last tens of milliseconds and therefore summate temporally, and occur simultaneously in tens of thousands of neurons, the resulting magnetic fields are strong enough to be detected outside the head with MEG (Hari, 1990; Murakami and Okada, 2006).

Radially oriented currents in a spherical conductor do not produce measurable magnetic field outside the sphere, because the magnetic fields associated with radial intracellular currents and the simultaneous volume currents cancel out each other. In contrast, magnetic fields associated with currents that are tangential to the surface of the spherical conductor are detectable outside the scalp. About two thirds of the cortex of the human brain is located inside the fissures, and the pyramidal cells are oriented perpendicular to the surface of the cortex. Therefore the cortical sulci are well positioned for their activation to be detected with MEG.

Because the magnetic fields generated by the brain currents are small compared to the static magnetic field of the earth (around eight orders of magnitude smaller) it is best to perform MEG

measurements inside a magnetically shielded room. The extreme sensitivity of the MEG method is based on Superconducting Quantum Interference Device (SQUID) sensors that are immersed in liquid helium at a temperature of  $-269\text{ }^{\circ}\text{C}$  to maintain them in superconducting state. Our MEG device has triple sensor elements, each containing two orthogonal planar gradiometers and one magnetometer (Vectorview<sup>TM</sup>, Neuromag Ltd., Helsinki, Finland). Pickup coils of the sensors convert the magnetic flux into electric current in a way that depends from the configuration of the coil. The planar gradiometers are figure-of-eight shaped coils that give maximum signal over the area with the strongest magnetic field gradient, whereas the magnetometers are loop-shaped coils that give the maximum signal over the maxima or minima of the magnetic field. They are more sensitive to deep sources than are the gradiometers, but the trade-off is increased sensitivity to external noise (Hari, 1990).

EEG measures electric potentials between scalp electrodes, but primarily reflects the same underlying neuronal activity that is measured with MEG. Whereas the tissues surrounding the brain, the meninges, the skull, and the skin are transparent to the magnetic fields, the electric potentials are smeared by the different conductivities of these tissues (Hämäläinen et al., 1993). However, EEG is more sensitive than MEG to very deep and radially oriented currents (Hari, 2005). Simultaneous MEG and EEG acquisition may be beneficial, because the information acquired with the methods complement each other.

Cortical responses to external stimuli measured with MEG are typically analyzed by averaging single-trial responses to improve the signal-to-noise ratio (SNR). The activity that is not time-locked to the stimulus is regarded as noise, the level of which reduces as the number of averaged responses increases. In the analysis of averaged responses, a visual search across gradiometer channels obtains the first guess of time windows and activated areas, because the planar gradiometers show the maximum deflections above the current sources. Most commonly used source model is a point-like current dipole (equivalent current dipole, ECD) that represents the neural activity in the brain



underlying the measured MEG signals. Least-squares fit is employed to find an ECD that explains the magnetic field signals from selected area. A result of the fit is a three dimensional location and direction of current dipole. The head is typically modelled as a conductor sphere, which adequately models the brain and other intracranial tissue. The realistically-shaped conductor model does not bring much improvement when compared with the sphere conductor, except in the frontal areas (Tarkiainen et al., 2003). The inverse problem—solving the electrical activity of the brain underlying the measured magnetic field distribution—has no unique solution, but prior knowledge of anatomy and the physiology of the brain as well as the knowledge about the origin of the MEG signal are exploited to constrain the amount of solutions.

To explain the measured signals as completely as possible, additional ECDs can be introduced, resulting in a multidipole model combining several ECDs. The quality of the model can be evaluated by comparing the explanation predicted by the model and the actual measured signals. Under optimal conditions, the localization accuracy of the ECDs is in the order of millimetres.

The magnetic fields of the brain were first detected with a SQUID magnetometer in 1972 (Cohen, 1972). After the introduction of whole-scalp magnetometers (Ahonen et al., 1992), MEG has been widely applied in studies of human brain function in healthy and diseased brains: the sensory and motor systems, spontaneous oscillatory activity, language processing, action observation, neurological conditions such as stroke, epilepsy, and presurgical patients have been studied with MEG (Hari, 1990; Del Gratta et al., 1999; Hari et al., 2000; Kakigi et al., 2000; Salenius and Hari, 2003; Hari, 2005; Mäkelä et al., 2006; Salmelin and Kujala, 2006; Shibasaki et al., 2007).

Pain-related magnetic fields were recorded for the first time in 1983 when the SII cortex was shown to be activated by noxious dental stimulation (Hari et al., 1983). Later, both nasal CO<sub>2</sub> stimulation (Huttunen et al., 1986; Hari et al., 1997) and CO<sub>2</sub> laser has been used for painful stimulation in MEG (Kakigi et al., 1995; Watanabe et al., 1998; Kanda et al., 2000). Recently, a thulium laser that specifically stimulates the myelinated A $\delta$ - and unmyelinated C-fibres has been

used to study the cortical pain processing in healthy individuals (Ploner et al., 1999b; Ploner et al., 2000; Nakata et al., 2004; Raij et al., 2004; Forss et al., 2005b).

## **2.6.2 Functional magnetic resonance imaging**

fMRI is a noninvasive method for mapping brain activity and is based on the phenomenon of nuclear magnetic resonance (NMR). Due to their intrinsic properties, hydrogen nuclei in an external magnetic field precess at a frequency that is proportional to the strength of the magnetic field. By varying the external magnetic field in a systematic manner together with using radiofrequency coils to emit electromagnetic energy and to detect the resulting NMR signal, it is possible to generate three-dimensional images of the structures of human body.

The most commonly used method among fMRI techniques is the measurement of blood oxygen level dependent (BOLD) signal. This signal is based on the differences of the magnetic properties of oxygenated and deoxygenated haemoglobin (Ogawa et al., 1990). As the neurons are active, the relative amount of oxygenated blood increases and the associated BOLD signal changes can be detected (Ogawa et al., 1990). It has been shown that local field potential amplitude, which reflects the postsynaptic activity, is coupled with the BOLD signal, and therefore it is assumed that the BOLD signal reflects synaptic activity (Logothetis et al., 2001). The main advantage of the fMRI when compared to MEG is the high spatial resolution, in order of millimetres, in both cortical and subcortical areas of the brain. However, due to the sluggishness of the hemodynamic response (around 3–6 secs), the temporal resolution of the fMRI is in the orders of seconds, rather than in milliseconds as with MEG (Aguirre et al., 1998).

The end product of a typical fMRI measurement is a large amount of data consisting of three-dimensional arrays of voxels obtained at consecutive time points, which are then subject to several preprocessing steps. The data are corrected for movements by defining parameters for translation and

rotation in three dimensions. The spatial smoothing increases the SNR, decreases the interindividual variance, and helps to meet the statistical assumption by making the noise more normally distributed. Normalization of the data to a common template volume is done by applying linear and nonlinear transformations.

In the statistical analysis, the study protocol forms the basis of a general linear model (Friston et al., 1995). This model is subsequently convolved with the hemodynamic response function to account for the delay between the neuronal activation and the hemodynamic response. The time course of the signal is fitted, voxel by voxel, to the model. This results in parameter estimates and error estimates for each modelled condition in each voxel. Statistical parametric maps are calculated by statistically comparing the parameter estimates of different conditions (e.g. pain vs. warm).

Typically, fMRI studies concentrate on task- or stimulus-related activity. The analysis of spontaneous activity has recently gained popularity and this kind of approach, together with data-driven analysis methods, such as independent component analysis (ICA), may have potential in the study of clinical neurological and psychiatric conditions (Fox and Raichle, 2007).

In addition to studying the brain function, magnetic resonance imaging methods can be used to computationally evaluate the brain morphology with a method called voxel based morphometry (VBM) (Ashburner and Friston, 2000). VBM has been used to compare local gray matter concentration in various disorders, such as in schizophrenia and in chronic pain (Apkarian et al., 2004; Glahn et al., 2008).

### **3. Aims of the study**

The aim of this thesis was to employ noninvasive brain imaging to better understand the brain mechanisms involved in chronic pain. In two patient groups who were suffering from chronic pain with unclear origin, we investigated the brain activation patterns related to touch and pain and compared these activation patterns to those of healthy individuals. The specific aims were the following:

1. To define optimum interstimulus interval (ISI) to obtain best SNR for laser-evoked cortical responses in a fixed measurement time (Study I).
2. To investigate the cortical processing of touch and acute pain in CRPS patients to better understand the pathophysiological mechanisms of hypersensitivity in these patients (Study II).
3. To study the reactivity of the primary motor cortex to acute pain in CRPS patients to find signs of possible motor cortex dysfunction (Study III).
4. To explore whether patients suffering from recurrent HSV infections and from chronic pain of unclear aetiology show chronic-pain-related cortical reorganization (Study IV).
5. To find evidence for central contribution of pain in patients with recurrent HSV infections and chronic pain by investigating their hemodynamic responses to touch and acute pain (Study V).

## 4. Materials and methods

### 4.1 Subjects

Study	N of patients and healthy subjects	Stimuli	Imaging method
I	9 healthy	Laser	MEG and EEG
II	8 CRPS, 9 healthy	Laser, tactile	MEG
III	8 CRPS, 8 healthy	Laser	MEG
IV	8 HSV, 9 healthy	Tactile	MEG
V	8 HSV, 11 healthy	Thermode	fMRI

Table 1. Number of subjects, stimulation type, and brain imaging method in Studies I–V.

#### 4.1.1 Healthy subjects

Nine healthy subjects (8 right-handed, 7 men, 2 women, ages 19–37 years, mean 27 years) participated in Study I. Due to excessive eye movements, the data from one subject were excluded. Nine healthy subjects (8 right-handed, 1 ambidextrous, ages 28–57 years, mean 46 years) participated both in Study II and IV, and eight of them in Study III. Eleven healthy subjects (10 right-handed, 1 ambidextrous, 6 men, 5 women, ages 25–46 years, mean 30 years) participated in Study V.

#### 4.1.2 Chronic pain patients

Eight CRPS patients (all right-handed females; ages 26–57 years, mean 46 years) participated in Studies II and III. The patients were recruited from Orton Hospital, and a neurologist and a

physiatrist had performed clinical examinations of the patients. Because the patients had no evidence of peripheral nerve lesions, the diagnosis of CRPS type 1 was set according to the criteria by IASP (Stanton-Hicks et al., 1995). All patients suffered from continuous spontaneous upper-limb pain that had lasted for 1–9 years. The pain was best described as burning, stabbing, or tearing, and was rated 5–8 on the Visual Analogue Scale (VAS; from 0 to 10). Brush allodynia and fluctuating edema were present in the affected hands in all patients and vasomotor or sudomotor dysfunction in five patients. All patients had decreased grip strength and clumsiness in fine motor skills of the painful hand. To exclude lesions in the peripheral nerves and in the brain, electroneuromyography (ENMG) and magnetic resonance imaging (MRI) had been performed.

Eight patients (all right-handed, seven females) suffering from recurrent HSV infections and chronic spontaneous unilateral pain participated in Studies IV and V. At the time of the MEG-recordings of Study IV, the ages of the patients were 40–51 years (mean 46 years). The fMRI-measurements took place on average one year later. The patients had spontaneous widespread pain on one side of the body, and the long-term average pain at the time of the MEG-recordings was rated 1–9 on numerical rating scale (NRS, from 0 to 10). The pain had started 3–20 years before the recordings and its intensity fluctuated over time from absent to severe. In three patients, the pain comprised the entire one side of the body, and in five patients, the pain covered at least three of the following areas: the face, trunk, arm, and leg. The patients were seropositive for HSV-1 or HSV-2, and had either active labial or genital herpes (3–12 recurrences per year), or had had recurrent HSV-2 induced meningitis episodes. The patients were recruited from the Pain Clinic of the Helsinki University Central Hospital, and they were a subgroup of the 17 patients described by Kallio-Laine et al. (2008). In six out of eight patients, the pain emerged first in a small area but spread later to wide regions in the ipsilateral body. In five out of eight patients, spontaneous pain worsened during HSV reactivation.

## **4.2 Stimuli**

### **4.2.1 Tactile stimuli**

Tactile stimuli were delivered to the fingertips with pneumatic diaphragms (Mertens and Lütkenhöner, 2000). In Studies II and IV, thumbs and little fingers of both hands were stimulated with 1-s ISI in one session, to evaluate the extent of the hand representation area in the SI cortex, and the index fingers of both hands were stimulated with 3-s ISI in another session, to evaluate the amplitude and latency of the SI response. In Study V, index, middle, and ring fingers were stimulated with a varying stimulus onset asynchrony of 200–600 ms in 20-s blocks. The tactile stimulation blocks alternated with equally long rest periods.

### **4.2.2 Thulium-laser stimuli**

Experimental acute pain was produced in Studies I, II, and III with thulium laser stimulator (Tm:YAG, Baasel Lasertech, Starnberg, Germany). Studies II and III were based on the same recordings. Short laser pulses (duration 1 ms, wavelength 2000 nm) were conducted with an optic fibre inside to the magnetically shielded room. An assisting person manually delivered the laser beam to the dorsum of the hand. The diameter of the circular laser light spot on the skin was about 5 mm, resulting in a stimulated area of about 20 mm<sup>2</sup>. The laser stimulation elicited a fast pricking pain, often accompanied by a slower burning sensation. The stimulation site was changed between successive pulses within a skin area of about 5 cm<sup>2</sup> to avoid adaptation and burns of the skin. In Study II, the individual pain thresholds before the MEG recordings were individually determined by increasing the stimulus energy until subjects reported pain (VAS = 1). This procedure was repeated three times and the mean stimulus energy obtained was set as the pain threshold. During the measurement, the stimulus energy of 1.4–1.5 times the pain threshold was administered (740 mJ in

control subjects, 520 mJ in patients). Already at this intensity level, the patients experienced severe pain and therefore higher intensity level stimulation was impossible. Additional energy of 1.1 times the pain threshold (540 mJ) was used in control subjects for control purposes. After the stimulation sessions, the subjects evaluated the mean pain intensity.

### **4.2.3 Thermode stimuli**

In Study V, thermal pain was produced by two Peltier elements (TSA II, Medoc, Israel). The elements (16 mm x 16 mm) were placed on the hand dorsa. The stimulus temperature increased at 10 °C/s and decreased at 8 °C/s. Prior to experiment, we tested the appropriate stimulation temperature by increasing the stimulus temperature in a step-by-step manner to highest level tolerated by the subject. During the experiment, 10-s thermal stimuli, either painful heat (mean  $\pm$  SEM  $47.4 \pm 0.2$  °C in control subjects,  $46.6 \pm 0.7$  °C in patients) or innocuous warmth (42 °C), were pseudorandomly delivered to each hand with 30-s rest periods (22 °C) in between.

## **4.3 Magnetoencephalographic and electroencephalographic recordings**

The MEG and EEG recordings were carried out in the magnetically shielded room in the Brain Research Unit of the Low Temperature Laboratory, Helsinki University of Technology. The cortical responses were recorded with a 306-channel neuromagnetometer (Vectorview<sup>TM</sup>, Neuromag Ltd., Helsinki, Finland). To align the MEG and the MRI coordinate systems, four indicator coils were placed on the scalp and their positions with respect to the anatomical landmarks were measured with a three-dimensional digitizer. The head position in relation to the sensors was determined by measuring the magnetic signals produced by currents led into the indicator coils. During recordings, the subject was sitting with the head supported against the helmet-shaped sensor



array of the magnetometer. The signals were band-pass filtered through 0.03–200 Hz and digitized at 600 Hz. The analysis period was from –200 to 500 ms in Studies I, II, and IV, and from –1000 to 3500 ms in Study III. Altogether 110–120 tactile responses, and 40–50 laser-evoked responses were averaged for each stimulation site. Simultaneously recorded electro-oculograms (EOG) were monitored for rejection of MEG epochs that coincided with excessive eye movements or blinks.

Laser-evoked fields in Studies I and II, and somatosensory evoked fields in Study II were modelled by multiple current dipoles (see 2.6.1). In Study IV, the somatosensory evoked fields measured over the SI cortex were modelled with single dipoles. In Study III, the temporal spectral evolution (TSE) of the level of the motor-cortex 20-Hz rhythm was monitored by first filtering the oscillatory activity through 15–25 Hz, then rectifying, and finally averaging it time-locked to laser stimuli (Salmelin and Hari, 1994; Silén et al., 2000). The channel showing maximum reactivity was chosen, and the suppression, rebound and reactivity of the rhythm were calculated for each subject.

#### ***4.4 Functional magnetic resonance imaging measurements***

The fMRI measurements in Study V were conducted at the Advanced Magnetic Imaging Centre, Helsinki University of Technology with 3.0 T Signa EXCITE scanner (General Electric, Milwaukee, WI, USA). A gradient-echo echo-planar imaging sequence with the following parameters was used: repetition time (TR) 2000 ms, echo time (TE) 32 ms, flip angle (FA) 75°, field of view (FOV) 20 cm, and matrix size 64 x 64. Imaging of the whole brain required 31–33 oblique slices, each 4 mm thick, with no spacing in between. Parameters for the T1-weighted anatomical images, obtained with 3D inversion-pulse prepared spoiled-gradient-recalled (SPGR) acquisition, were the following: TR 9.0–9.2, TE 1.9–2.0, inversion time 300 ms, FA 15°, matrix size 256 x 256, FOV 24 or 26 cm, and slice thickness 1 mm.

The fMRI data were preprocessed with BrainVoyager QX (Brain Innovation, Maastricht, The Netherlands). VBM analysis was conducted with the SPM2 software package (Wellcome Department of Imaging Neuroscience, London, UK, <http://www.fil.ion.ucl.ac.uk/spm>) and Matlab 6.51 (Mathworks, Natick, MA).

## 5. Experiments

### 5.1 *Optimising interstimulus interval for cortical laser-evoked responses*

Laser-evoked responses have been widely used in the basic research of cortical pain processing and in clinical applications such as in the assessment of the nociceptive pathway function (Bromm and Lorenz, 1998; Spiegel et al., 2000; Cruccu et al., 2004).

For successful use in clinical settings, the SNR of the responses should be maximized by optimizing the stimulus parameters. Increasing the number of averaged responses enhances the SNR as the (stationary) noise decreases in proportion to the square root of the number of averaged responses. In a fixed measurement time, the number of responses can be increased by shortening the ISI. On the other hand, the responses have certain recovery cycles; the amplitude of the response decreases with shortening ISI, whereas it increases with longer ISI to a certain saturation point (Wikström et al., 1996). Further, long-latency responses are known to be sensitive to changes of attention (Garcia-Larrea et al., 1997). Therefore, extensively long measurement session may decrease attention and vigilance and affect the amplitude of the responses.

Although laser-evoked responses are widely in use, their recovery cycles had not been previously systematically studied. The aim of this study was to measure the recovery cycles of LEFs and LEPs to obtain the optimal ISI for best SNR in fixed measurement time.

#### 5.1.1 Results

The laser pulses were described as pricking pain, followed by weaker burning pain. The subjects rated the intensity of the pain significantly lower at the 4-s than at 0.5-s ISI ( $3.6 \pm 0.4$  vs.  $4.4 \pm 0.6$ ,  $p = 0.01$ ; mean  $\pm$  SEM, 0–10 scale), and the lowest intensity rating was obtained with the longest ISI of 16 sec ( $3.1 \pm 0.5$ ).

The laser pulses elicited bilateral activations in all subjects in the SII regions at 150–205 ms and contralateral activation in seven out of eight subjects in the SI region at 160–195 ms. The simultaneously registered LEPs from the derivation Cz–mastoid comprised a surface-negative peak at 190–230 ms and a subsequent surface-positive peak at 310–330 ms.

The MEG responses showed good replicability during the measurement session, but the EEG peak-to-peak potentials decreased 25–54% ( $p < 0.05$ ) from the beginning to the end of the session.

Increasing the ISI from 0.5 s to 4 s strongly increased the amplitudes of all responses, but further increase had little additional effect. On the basis of the curve fitting, an exponential curve with time constant of 3.5 s best explained the measured recovery cycle of responses. As shown by Ahlfors et al. (1993), the optimal ISI for the best SNR obtained in a fixed measurement time is 1.26 x time constant. Therefore, the optimal ISI for pain-related magnetic and electric responses is around 4–5 s.

### **5.1.2 Discussion**

The results show that the amplitudes of the LEFs and LEPs increase with increasing ISI, and start to saturate after 4-s ISI. Based on the recovery cycles of LEFs and LEPs, the optimum ISI for recording laser-evoked responses in a fixed measurement time is around 4–5 s. Our findings agree with and complement the findings of other groups who have shown amplitude increase of pain-related responses with increasing ISI (Jacobson et al., 1985; Truini et al., 2004).

## **5.2 Responses to tactile and noxious stimulation are altered in complex regional pain syndrome**

Patients with CRPS commonly suffer from hyperalgesia and allodynia to external stimulation, in addition to chronic ongoing pain. Prior brain imaging studies in CRPS patients have demonstrated increased cortical activations in SI, SII, IC, and ACC during mechanical hyperalgesia and cortical reorganization of the hand representation area in the SI cortex contralateral to the affected limb (Juottonen et al., 2002; Maihöfner et al., 2003; Pleger et al., 2004; Maihöfner et al., 2005). The amount of reorganizational changes has been shown to correlate with the severity of ongoing pain and with the tactile sensitivity. In CRPS patients in whom the symptoms are alleviated, spontaneously or by therapy, the reorganization appears to be reversible (Maihöfner et al., 2004a; Pleger et al., 2005), but it remains unknown whether the cortical reorganization persists when CRPS becomes chronic. We investigated the cortical processing of touch and acute pain in CRPS patients with MEG, to better understand the central mechanisms of hyperesthesia, and to find out whether the plastic changes in the SI cortex are permanent in chronic CRPS patients.

### **5.2.2 Results**

In the patients, the pain thresholds to laser pain were lower than in the control subjects, not only for the painful ( $p = 0.003$ ) but also for the healthy hand ( $p = 0.02$ ) (see Figure 4). The laser stimuli of similar energy (520–540 mJ) were rated more painful by the patients than control subjects, both in the painful ( $7.5 \pm 0.7$  vs.  $2.3 \pm 0.3$ ,  $p = 0.005$ ) and healthy hands ( $5.4 \pm 1.0$  vs.  $2.3 \pm 0.3$ ,  $p = 0.05$ ).

The distance between the thumb (D1) and the little finger (D5) representations in SI was smaller for the painful compared with the healthy hand (mean  $\pm$  SEM;  $6 \pm 2$  vs.  $10 \pm 2$  mm,  $p = 0.02$ ). The mean SI response amplitude to tactile stimulation was 33% stronger ( $p = 0.05$ ) for the

painful than the healthy hand whereas the SII responses did not differ between the hands. The PPC responses to tactile stimulation were found in only three patients, whereas they were observed in all control subjects.

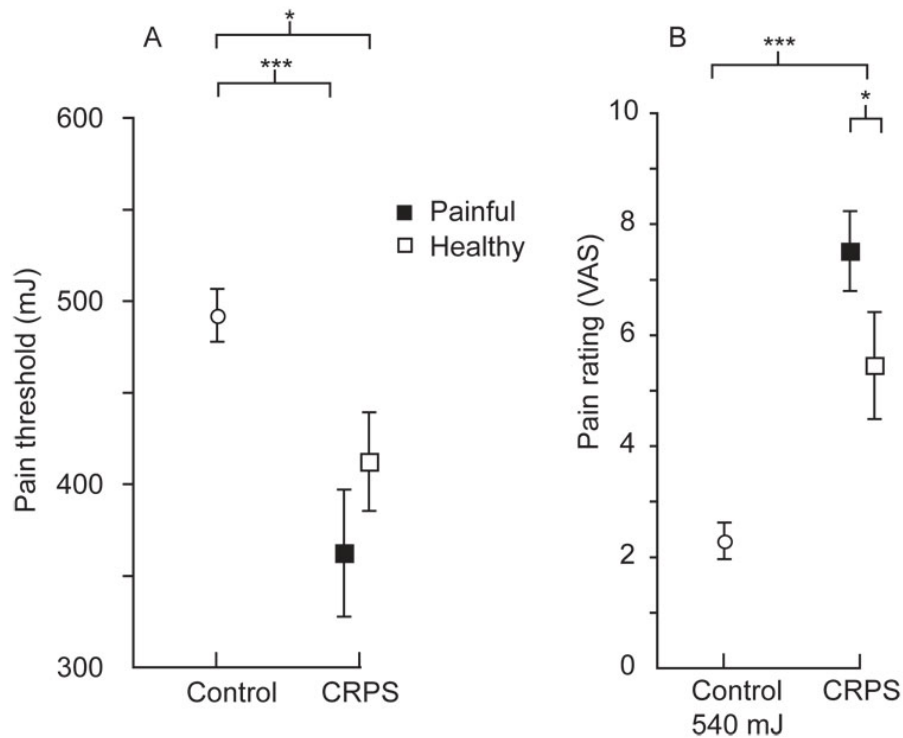


Figure 4. Pain thresholds (A) and ratings (B) in control subjects (circles) and in CRPS patients (squares). Black/white squares correspond to painful/healthy hands. \* $p < 0.05$ , \*\*\* $p < 0.005$ .

Figure 5 shows that to painful laser stimulation with similar energy (520–540 mJ), the mean SII cortex activation strength was 12–15 nAm in CRPS patients, and 5–10 nAm in control subjects, but this difference was not statistically significant. The PPC was similarly activated in control subjects and in patients. When the stimulation energies of 1.4–1.5 times the pain threshold were compared (520 mJ in CRPS vs. 740 mJ in control subjects), the SII activation strengths did not differ significantly, but the PPC activation was stronger in control subjects than in patients (controls  $13.8 \pm 3.5$  nAm vs. patients healthy hand  $4.0 \pm 2.0$ ,  $p = 0.03$ ; patients painful hand  $4.5 \pm 3.7$ ,  $p = 0.09$ ; PPC source was observed in seven control subjects, but only in two CRPS patients to painful

and three to healthy hand stimulation). The PPC activation correlated with the pain rating in the control subjects ( $r = 0.60$ ,  $p = 0.01$ ), but such correlation was not observed in the patients.

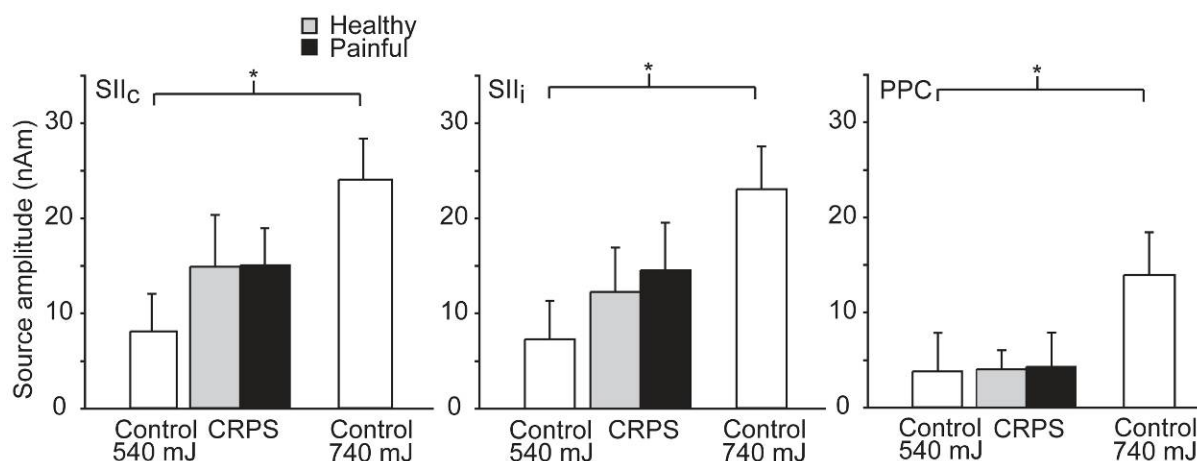


Figure 5. Source strengths to painful laser stimulation in contra- and ipsilateral somatosensory cortices (SIIc and SIIi) and in posterior parietal cortex (PPC). In control subjects, sources were stronger to 740 mJ than to 540 mJ stimulation ( $*p < 0.05$ ). Black/grey = painful/healthy hand.

### 5.2.3 Discussion

Earlier studies have demonstrated that the cortical reorganization in SI in CRPS patients reversed back to normal, together with the clinical improvement of the symptoms (Maihöfner et al., 2004a; Pleger et al., 2005). The present results show that the D1–D5 distance remains reduced in chronic CRPS patients.

The lowered pain threshold and the elevated perceived pain intensity, not only in the painful but also in the healthy hand, indicate a generally altered perception of pain, rather than hypersensitivity restricted to only one side of body. This result agrees with a previous study which showed that the pain thresholds also in the unaffected limb of CRPS patients tend to be lower than in the control subjects (Kemler et al., 2000).

With similar stimulation energy (520–540 mJ), SII cortices were clearly activated in control subjects and in patients, suggesting that the nociceptive pathways in CRPS patients transmit

noxious impulses from the peripheral A $\delta$ -fibres normally at least to the SII cortex. Because the SII activation was not statistically significantly enhanced in patients compared with control subjects, although the patients experienced more severe pain, it is possible that some other pain-processing area such as ACC or IC, could contribute to the hyperesthesia to pain.

The patients' decreased PPC activation to laser stimulation could be explained by the lower stimulation energy. However, the almost absent PPC activations to tactile stimulation in the patients may indicate malfunction of PPC. This is supported by recent study in CRPS patients, which demonstrated slowed grasping movements of the hand in a kinematic analysis, suggesting disturbed integration of visual and proprioceptive in PPC. In the same study, the motor performance in the patients correlated with PPC activity (Maihöfner et al., 2007).

### ***5.3 Motor-cortex reactivity to noxious stimulation is decreased in complex regional pain syndrome***

The diagnostic criteria of CRPS have traditionally concentrated on the sensory, autonomic, and trophic features (Stanton-Hicks et al., 1995). Because motor dysfunction is common in CRPS (Veldman et al., 1993; Birklein et al., 2000), revised diagnostic criteria for CRPS have been proposed to include also motor symptoms (Harden et al., 2007). Brain imaging studies in CRPS have demonstrated bilateral disinhibition of the motor cortex and adaptive changes of the central motor circuits (Schwenkreis et al., 2003; Maihöfner et al., 2007). We studied the motor cortex reactivity in response to acute painful stimuli, to clarify the role of possible motor cortex dysfunction and its relationship with clinical symptoms in CRPS patients.



### 5.3.1 Results

Spectrum of the spontaneous oscillatory activity recorded over the sensorimotor region in both hemispheres during rest with eyes open showed spectral peaks at around 10 and 20 Hz in both patients and control subjects. The mean ( $\pm$  SEM) peak frequency in both groups was  $18.8 \pm 0.5$  Hz, and the amplitudes of these peaks did not differ between the control subjects ( $10 \pm 2$  fT/cm and  $11 \pm 2$  fT/cm for right and left hemisphere) and patients ( $10 \pm 2$  fT/cm and  $11 \pm 2$  fT/cm for hemispheres contralateral to painful and healthy hand).

The painful laser stimuli elicited clear changes in the level of the 20-Hz rhythm measured over the contralateral MI region (see Figure 6). In the control subjects, the strength of the reactivity (peak-to-peak amplitude measured between suppression and rebound peaks) did not differ between the 740 mJ and 540 mJ energy stimulation, but the suppression peaked earlier to 740 mJ than to 540 mJ stimulation ( $432 \pm 61$  ms *vs.*  $757 \pm 82$  ms  $p = 0.005$ ). In the CRPS patients, 20-Hz rhythm reactivity in the hemisphere contralateral to the affected hand was attenuated compared with the high and low intensity stimulation in control subjects ( $4.1 \pm 1.5$  fT/cm *vs.*  $13.9 \pm 3.9$  fT/cm;  $p = 0.04$ , high;  $4.1 \pm 1.5$  fT/cm *vs.*  $10.2 \pm 3.0$  fT/cm;  $p = 0.03$ , low). The peak latencies of suppression and rebound did not differ significantly between the groups. In the patients, the level of reactivity correlated inversely with the intensity of spontaneous pain in the affected hand ( $r = -0.95$ ,  $p = 0.0002$ ). Furthermore, the level of reactivity correlated with the grip strength in the affected hand ( $r = 0.61$ ,  $p = 0.05$ ).

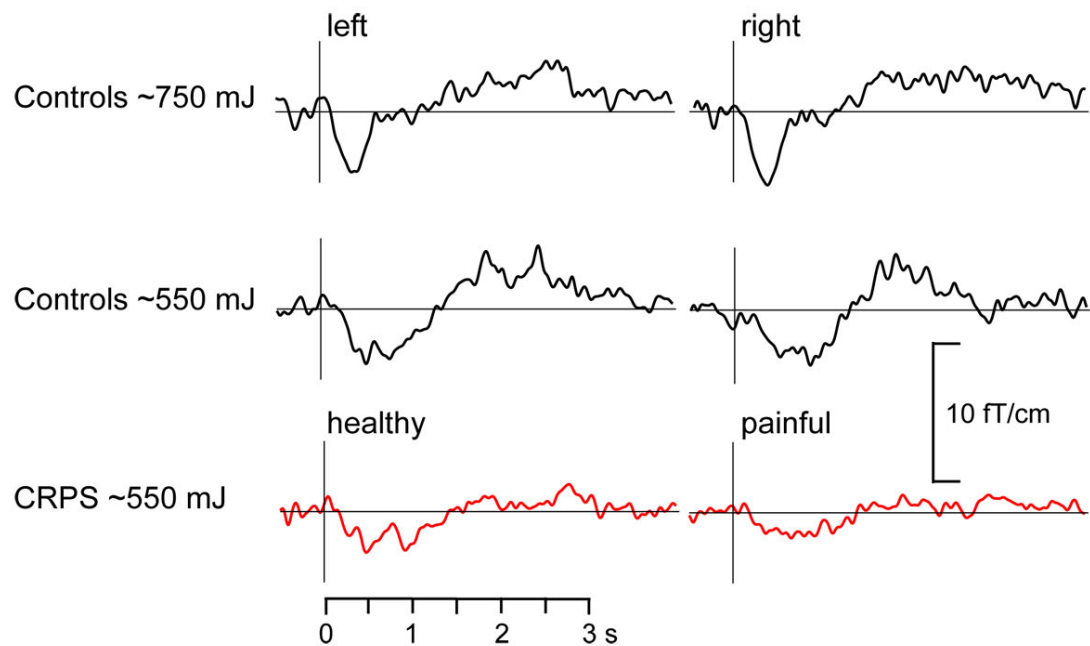


Figure 6. Grand averages of level of motor-cortex 20-Hz rhythm time-locked to painful laser stimulation in control subjects and in patients. Control subjects were stimulated with two energy levels (740 mJ and 540 mJ) and patients were stimulated with one energy level (520 mJ).

### 5.3.3 Discussion

Our results demonstrate decreased reactivity of the motor-cortex 20-Hz level rhythm in response to acute laser pain in CRPS patients. The suppression of the 20 Hz level oscillations in healthy individuals has been interpreted to reflect excitation or disinhibition of the motor cortex, and the subsequent rebound has been assumed to reflect inhibition (Salmelin and Hari, 1994; Salenius et al., 1997).

The correlation between decreased reactivity and the amount of ongoing spontaneous pain in the affected hand suggests a causal relationship between the ongoing pain in CRPS and the motor cortex reactivity. TMS studies in CRPS patients have indicated disinhibition or hyperexcitability of the motor cortex, either bilaterally or contralaterally to the painful side (Schwenkreis et al., 2003; Eisenberg et al., 2005). However, we did not find increased reactivity to acute pain as a sign of hyperexcitability, but instead decreased reactivity (consisting of decreased suppression and rebound)

as a sign of defective interplay between excitation and following inhibition. If under normal circumstances the meaning of the suppression and subsequent rebound of the motor cortex originated 20-Hz rhythm is the preparation of voluntary movements, one could speculate that ongoing pain might lead to exhaustion—or overexcitation—of the motor cortex, so that acute pain does not adequately alarm the motor system.

Interestingly, the reactivity decrease in the motor cortex correlated with the decreased grip strength in the affected hand, suggesting that central motor system dysfunction could partly explain the observed motor deficits in CRPS. This is in line with recent fMRI study showing correlation between the motor cortex activity and motor performance in CRPS patients (Maihöfner et al., 2007).

The results indicate a tight relationship between central motor system dysfunction and clinical symptoms such as ongoing pain and weakness in CRPS. Therapy aimed at normalizing the motor function in CRPS might be beneficial also for the pain symptoms.

#### ***5.4 Patients with chronic pain and recurrent herpes simplex virus infections show plastic changes in the primary somatosensory cortex***

Plastic changes in the SI cortex have been demonstrated in patients suffering from various chronic pain conditions, such as phantom limb pain, CRPS, low-back pain, and painful carpal tunnel syndrome (Flor et al., 1995; Flor et al., 1997; Juottonen et al., 2002; Tecchio et al., 2002). Although the plastic changes have been linked primarily with pain, the influence of reduced afferent input due to peripheral nerve damage or due to lack of use of the limb can not be ruled out. We aimed to clarify, whether cortical reorganization in SI cortex is observed in a patient group who suffered from chronic pain in one side of the body (including the upper limb), but were able to use the painful limb in normal manner and had no signs of peripheral nerve damage.

### 5.4.1 Results

The cortical SI responses to tactile stimulation peaked at the same latency and were equally strong in the control subjects and in the patients (latency:  $58 \pm 1$  ms controls vs.  $57 \pm 2$  painful,  $54 \pm 1$  ms healthy, n.s.; strength: 28.9 nAm controls vs. 23.3 nAm painful, 23.1 nAm healthy, n.s.). These parameters did not differ between the healthy and the painful hands in the patients. Figure 7 shows in two representative HSV patients that the D1–D5 distance in the SI cortex was decreased in the hemisphere contralateral to the painful hand compared to the other hemisphere. At group level, the mean ( $\pm$  SEM) D1–D5 distance in SI was shorter to the stimulation of the painful compared to the healthy hand ( $7 \pm 1$  vs.  $13 \pm 2$  mm,  $p = 0.04$ ).

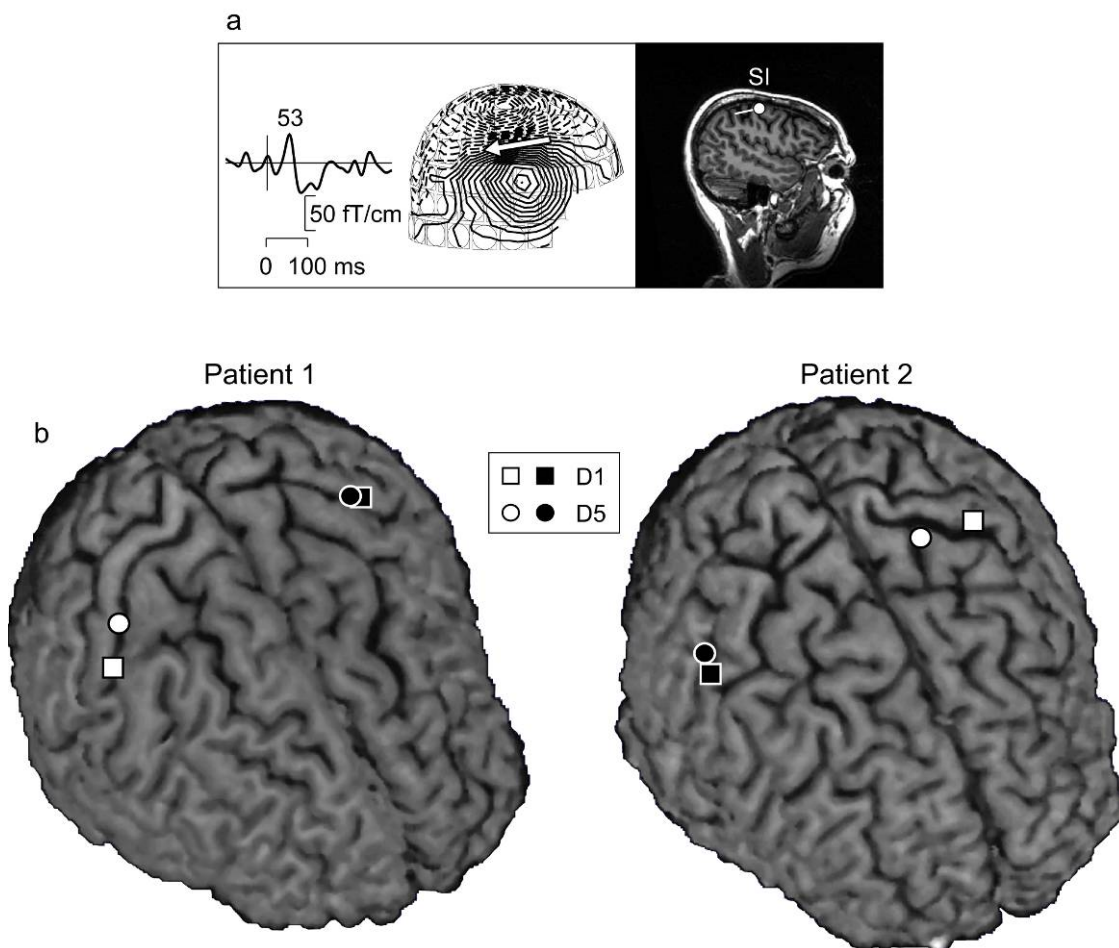


Figure 7. Finger representations in SI in two HSV patients. Black symbols correspond to affected and white symbols to healthy hemisphere.

## 5.4.2 Discussion

The observed decreased D1–D5 distance in SI in HSV patients indicates that cortical plasticity may be a common phenomenon in various chronic pain conditions. Earlier, reorganization has been observed in patients who have reduced afferent input due to nerve trauma or a tendency to immobilize the hand (Flor et al., 1995; Juottonen et al., 2002). Our patients had no indication of peripheral nerve lesions and the pain did not restrict the use of the affected hand. Therefore the influence of reduced proprioceptive input in the development of reorganization is unlikely.

The mechanism of pain-related cortical reorganization is unclear, but it is possible that continuous pain could interfere with the tactile processing and cause cortical changes. It is known that peripheral nerve damage induces extensive neuroplastic changes in the dorsal horn of the spinal cord, and similar changes may occur also at supraspinal level (Woolf and Mannion, 1999).

## ***5.5 Chronic pain in patients with recurrent herpes simplex virus has a central contribution***

Recently an interesting chronic pain patient group was described by Kallio-Laine et al. (2008). The patients suffered from recurrent HSV infections and from unexplained chronic pain widespread in one side of the body. The clinical picture suggested central pain, but direct supporting evidence was lacking. Our aim was to study, with functional and morphological brain imaging, whether the HSV patients have functional or structural abnormalities in the central pain-processing circuits.

### 5.5.1 Results

Subtle sensory abnormalities were found in the patients. Temperature sensitivity in the hand was lower on the painful side, compared with the healthy side (cold difference 1.4 °C,  $p = 0.04$ ,

warmth difference  $2.1\text{ }^{\circ}\text{C}$ ,  $p = 0.06$ ). The tactile sensitivity was lower on the painful side compared with the healthy side ( $p = 0.04$ ).

The perceived intensity of painful thermal stimuli ( $47.4 \pm 0.2\text{ }^{\circ}\text{C}$  in control subjects,  $46.6 \pm 0.7\text{ }^{\circ}\text{C}$  in patients; n.s.) was rated similarly in both groups ( $6.5 \pm 0.5$  in control subjects,  $6.3 \pm 0.6$  in patients).

In the control subjects, a well-known network of pain-processing areas was activated in response to thermal pain, including the IC, SII cortices, ACC, PPC, thalamus, striatum, frontal, and prefrontal cortices. In patients, the activations were statistically significantly weaker in the bilateral IC, in ACC, and in thalamus. Moreover, whereas the insular responses in the contralateral hemisphere were symmetric in control subjects to both left- and right-hand stimuli, in patients the responses to healthy hand stimulation were weaker compared to painful hand stimulation (see Figure 8). The responses to tactile stimulation in SI and SII cortices were similar in both groups. Figure 9 shows that the individual touch-related responses in SI and SII cortices were similar in control subjects and patients, but the insular responses were smaller in patients than in control subjects. VBM demonstrated decreased gray matter density in the frontal and prefrontal areas and in the ACC.

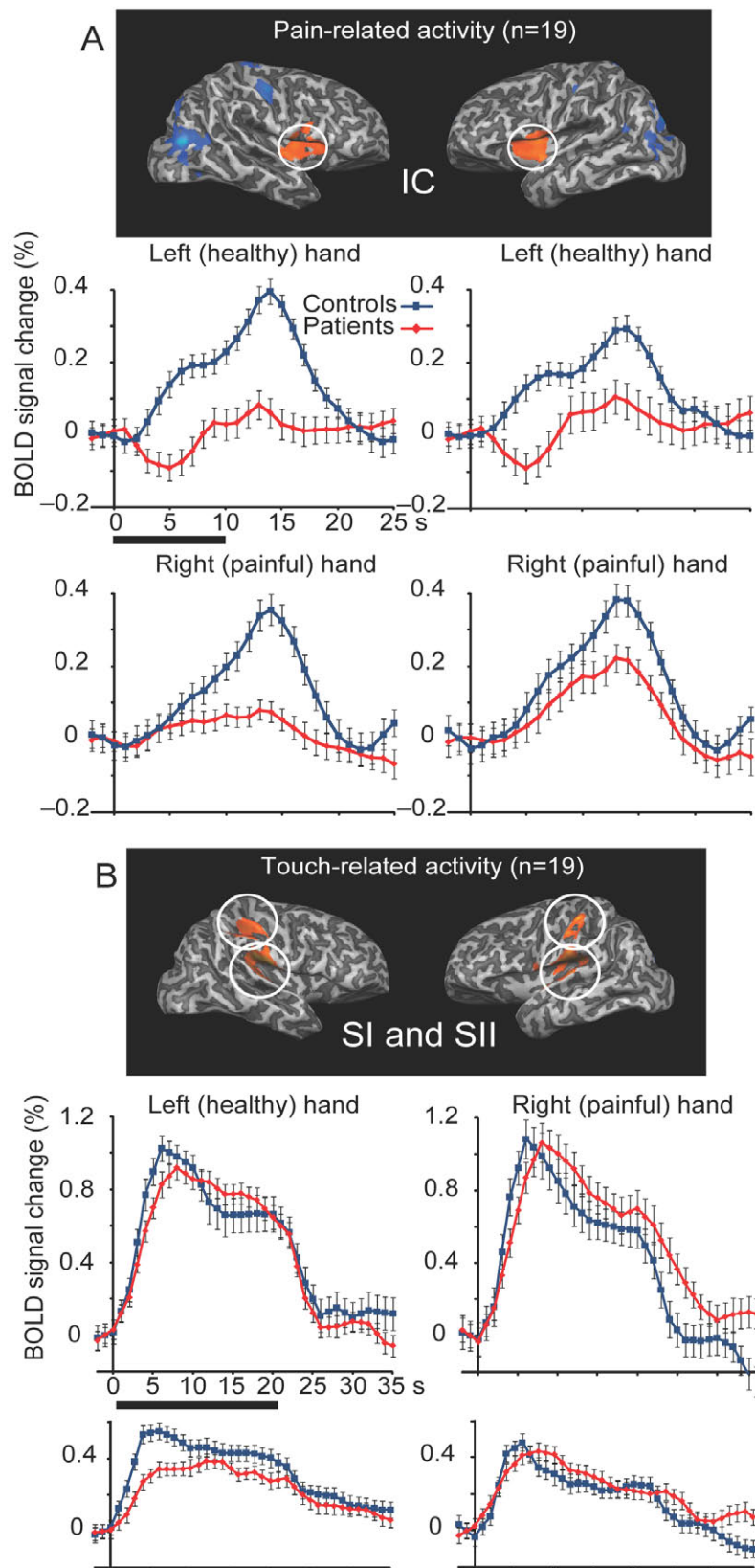


Figure 8. Group level hemodynamic responses to pain (A) and touch (B). Blue traces represent controls and red traces represent patients. Half of patients were flipped along midsagittal axis, normalizing painful side of the body to right.

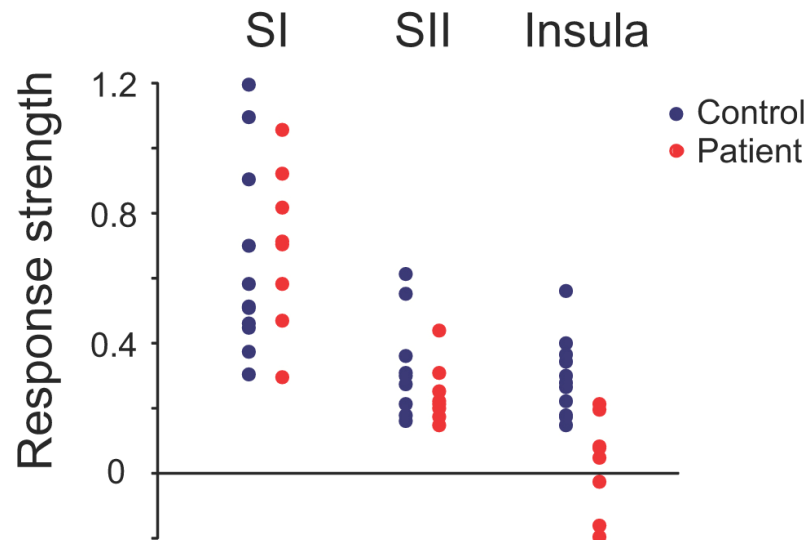


Figure 9. Response strengths of touch-related responses from contralateral primary and secondary somatosensory (SI and SII) cortices and pain-related responses from contralateral insular cortex (IC).

### 5.5.2 Discussion

We found functional and structural changes in the central nervous system in patients suffering from chronic pain and from recurrent HSV infections. The areas that are normally activated to acute experimental pain—the IC, ACC, and thalamus—showed reduced responses to acute painful heat, suggesting altered processing of noxious input in these patients. Morphological changes were observed in the frontal and prefrontal areas, which have been associated with chronic pain in earlier VBM and fMRI studies (Apkarian et al., 2004; Baliki et al., 2006).

In the patients, the hemodynamic responses to healthy hand stimulation were reduced compared with the painful hand. Such a reduction could be explained by functional impairment of the nociceptive pathways. An interesting possibility is that recurrent HSV infections have led to functional and structural changes in the brain, giving rise to central pain. This is supported by the



known neuroinvasiveness of the HSV, and by its tendency to affect especially temporal and frontal areas. The relationship between HSV and central pain should be studied in future.

An alternative option is that functional and structural changes are secondary to chronic pain, as frontal decrease of gray matter has been observed earlier in brain imaging studies in chronic pain patients (Apkarian et al., 2004).

## 6. General discussion

Functional brain imaging methods are powerful tools for studying the central nervous system mechanisms involved in the development and maintenance of chronic pain. Particularly in chronic pain conditions with unknown aetiology and pathophysiology, functional brain imaging may be used to pinpoint neural dysfunction related to, or underlying the pain. Abnormalities found in the central somatosensory and nociceptive processing could facilitate the mechanism-based diagnosis and therapy for chronic pain.

Measurements of brain responses to external stimuli in patients suffering from chronic pain can be challenging. Using the same stimulus energy in hypersensitive patients and in control subjects may be impossible. The mechanism underlying the pain is difficult to conclude on the basis of the symptoms and the aetiology alone, because of complex relationship between disease process, mechanisms of pain, and clinical symptoms. Brain imaging may be beneficial in clarifying these relationships and be a step towards the mechanism-based treatment of chronic pain.

### ***6.1 Primary somatosensory cortex reorganization and chronic pain***

Reorganizational changes in SI have been observed in many chronic pain conditions with a wide range of aetiologies, such as phantom limb pain, neuropathic pain, and CRPS (Flor et al., 1995; Flor et al., 1997; Juottonen et al., 2002; Tecchio et al., 2002). The amount of cortical reorganization has been shown to correlate with the intensity of pain, both in the sensory and motor cortices (Flor et al., 1995; Lotze et al., 2001; Maihöfner et al., 2004a). However, the directionality between the cortical plasticity and pain has not been definitely concluded (Flor, 2008). The pain itself could cause cortical plasticity, as it has been shown that acute pain can modify the cortical maps in healthy subjects (Sörös et al., 2001). On the other hand, the process of maladaptive plasticity could

at least partially cause the chronification of the pain. Our finding of similarly decreased D1–D5 distance in the SI contralateral to the painful limb in both HSV and CRPS patients suggests that cortical reorganization can be induced by various chronic pain conditions.

The mechanism of cortical reorganization is unclear. Cortical reorganization may also reflect changes occurring at thalamic or spinal level, as reorganizational changes in phantom limb patients have been demonstrated also in thalamus (Davis et al., 1998). Increased activity of peripheral nociceptors is known to lead to changes in the synaptic structure of the spinal cord (Woolf and Salter, 2006). One possibility is that the continuous pain in our patients could gate somatosensory input at spinal, supraspinal, or cortical level and lead to SI reorganization.

Because our results indicate a tight relationship between chronic pain and cortical reorganization, therapy aimed at normalizing the reorganizational changes in CRPS and in other chronic pain conditions might be beneficial also for the pain symptoms. Cortical reorganization might be an additional measure of chronic pain, and it could be useful in diagnostics and follow-up in chronic pain conditions.

## ***6.2 Changes in other cortical areas in chronic pain***

LEPs have been widely used in the studies of pain-related cortical processing in healthy subjects and in various neurological patient groups with altered pain perception (Bromm and Lorenz, 1998; Kakigi et al., 2005). The most prominent and the most commonly measured LEP component is the vertex potential N200–P300, which probably is generated predominantly in the ACC (Bromm and Lorenz, 1998; Lenz et al., 1998; Frot et al., 2008). Lesions in the spinothalamic system are associated with attenuated LEPs (Cruccu et al., 2004). We studied the cortical processing of touch and pain in CRPS patients with MEG, because this method is well suited to measure activity from somatosensory cortices (Hari and Forss, 1999).

In our CRPS patients, the SII responses were at least as strong as in the control subjects, when laser stimulation that mainly excites the A $\delta$ -fibres was used, suggesting that the nociceptive pathways conduct impulses from the periphery to the cortical level. Dysfunction of small fibres (A $\delta$ - and C-fibres) has been suggested to be a possible mechanism behind the chronic pain in CRPS (Oaklander and Fields, 2009). Therefore it would be interesting, although technically demanding, to study the C-fibre function in CRPS patients.

The observed attenuated PPC responses to tactile stimulation and the decreased motor cortex reactivity to acute pain might indicate a malfunction of these cortical areas in CRPS. The decrease of the reactivity of the motor cortex correlated with the amount of spontaneous pain, suggesting tight coupling between spontaneous pain and motor cortex dysfunction. It is interesting that the motor cortex reactivity correlated with the grip strength in our patients and that both the motor cortex and PPC activities have been shown to correlate with motor performance in a recent fMRI study (Maihöfner et al., 2007). It is possible that the continuing pain in our CRPS patients could have caused constant excitation state of the motor cortex, so that acute painful stimuli did not cause additional activation. Both the continuous pain as well as the motor cortex malfunction could also contribute to the dysfunction of PPC. Taken together, malfunction of the motor cortex and the PPC—induced by chronic pain—could contribute to the motor symptoms and neglect-like symptoms that are commonly observed in CRPS patients. Therapy aimed at normalizing the function of these areas might be beneficial in CRPS. It might be useful to study whether motor and visuospatial training might lead to alleviation of the CRPS symptoms.

### ***6.3 Chronic pain and recurrent herpes simplex virus infections***

The observed functional and morphological changes in the central pain circuitry, together with the widespread distribution of pain, support the hypothesis for central involvement in the development of pain in patients suffering from recurrent HSV infections.

The reduced hemodynamic responses to pain in ICs, ACC, and in thalamus, together with the normal hemodynamic responses to touch in SI and SII cortices suggest that in these patients, the pain-processing areas are specifically affected. The reduced responses could be a sign of a spinothalamocortical tract lesion that is too subtle to be otherwise detected. In these patients, the cortical responses to acute laser pain in the SII and PPC areas measured with MEG did not differ from those of the healthy control subjects, and the motor cortex reactivity to acute pain was normal (Vartiainen N, unpublished data). This fits well with these patients not having disturbing motor and neglect-like symptoms. This finding also suggests that chronic pain as such does not always lead to motor cortex and PPC dysfunction. On the contrary, the plasticity in SI was a common finding in both patient groups.

It is interesting that in most patients with recurrent HSV infections, there were no clinical signs of central nervous system infection. Two patients had meningitis, and one had suspected encephalitis. The possibility that a recurrent labial or genital virus infection in some susceptible individuals could cause changes in central pain processing circuits and cause central pain, should motivate further studies, especially among patient groups vulnerable to such infections. In susceptible patients, more aggressive treatment could possibly prevent the development of central nervous system changes and chronic pain.

#### **6.4 Future goals**

It is clear that the treatment of chronic pain should be based on the mechanism of pain. Brain imaging methods may be helpful in reaching this goal, as brain activity associated with certain mechanism may be distinct and could thereby be used for classification of pain conditions and for tailored pharmacotherapy and rehabilitation. One possible future approach may be the studying of resting state activity of the brain in chronic pain patients.

Brain imaging has helped us to understand which brain areas are activated when an individual perceives acute pain, and different areas are assumed to play different roles in contributing to the subjective experience of pain. Simultaneously with the experience of pain, complex pro- and antinociceptive modulation takes place along the neuraxis. Better understanding of this modulation by functional imaging may help to understand the development of various chronic pain conditions, and why some individuals may be susceptible to develop chronic pain. As this modulation occurs already at the level of the brain stem and the spinal cord, technical development allowing functional imaging of these regions will be necessary.

As several analgesic drugs are being developed for the treatment of chronic pain, and especially neuropathic pain, functional imaging of central nervous system may be used to facilitate the pharmacological development by quantifying central correlates of pharmacological effects.

## 7. Conclusions

The main results of this thesis work were the following:

The optimum ISI for recording cortical laser-evoked responses with best possible SNR in a restricted time slot is around 5 s.

In chronic CRPS, the plastic changes in the SI cortex appear to be permanent, at least during the three-year follow-up. The about 33% increased SI responses to painful compared to healthy hand stimulation may reflect central sensitization. Lowered pain thresholds and higher perceived pain estimates in both sides of the body suggest general hyperresponsiveness in these patients. Intact SII responses to painful laser stimulation suggest integrity of nociceptive ascending pathways from peripheral A $\delta$ -fibres to the SII cortex. The attenuated PPC responses and the attenuated motor-cortex reactivity might reflect PPC and motor cortex dysfunction that would explain the neglect-like symptoms and the motor weakness in the affected hand. It remains to be demonstrated what causes the observed dysfunction in cortical systems; is it the ongoing pain, or the (still unknown) primary pathophysiological process underlying CRPS?

Similar kind of reorganizational changes in the hand representation area of the SI cortex were found in CRPS patients and in HSV patients, suggesting that chronic pain of various aetiologies is associated with SI plasticity.

The morphological and functional changes in the central pain circuitry found in the patients suffering from recurrent HSV infections and from spontaneous fluctuating pain in one side of the body suggest central nervous system involvement in the development of pain. As the pain was on clinical grounds suspected to be associated with virus infections, it is possible that the observed central changes could be related to the virus and its latency in the brain. It might be clinically relevant to target patients who are susceptible to recurrent HSV infections, with more aggressive treatment, so that development of chronic pain could be avoided.

Chronic pain is a burden that reduces the quality of life and causes economical losses to individuals, companies, and to society as a whole. A better understanding of the pathophysiological processes in the development and maintenance of pain, especially when the origin of chronic pain is unclear, is needed. Suffering related to these conditions could be reduced by prevention and alleviation of symptoms with pharmacotherapy, physiotherapy, and psychological techniques that are target the underlying mechanism. In future, brain imaging could be used to facilitate mechanism-based diagnosis of chronic pain.



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