Brain processing of experimental muscle pain and its interrelation with proprioception and muscle fatigue
Positron emission tomography study

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Abstract

Chronic muscle pain is a significant medical and social problem and better understanding of the pathophysiological mechanisms involved is an important requirement for further development of diagnostics, treatment and rehabilitation methods. Experimental imaging studies have investigated functional neuroanatomy of different pain components. However, several aspects of brain mechanisms underlying brain processing of muscle pain remain unclear.

The general goal of the present thesis was to study functional brain anatomy of systems underlying perception of muscle pain, processing of proprioceptive information and maintenance of fatiguing muscle contractions with an emphasize on their possible interrelations.

Four series of experiments were carried out. Using an injection of hypertonic saline (HS) into the m. triceps to induce pain comparable with clinical muscle pain a significant activation of insula and putamen as well as decrease of activity in the temporal and occipital cortex in comparison with control stimulation were revealed. An advanced model of prolonged muscle pain were provided by the infusion of the HS during 20 minutes into m. erector spinae A complex dynamics of brain activity during the habituation to nociceptive stimulation was shown: initial activation of insula changed to decrease of activity in this and several other cortical areas. A conjunction analysis identified activations jointly significant in both experiments (despite localization of HS nociceptive stimulation) in the right insula, occipital and left parietal cortical areas. The study of brain activity in response to different modalities of proprioceptive inputs – passive movements, kinesthetic illusions and muscle vibration showed corresponding different patterns of activation in motor and somatosensory areas and temporal areas. Finally, the study of sustained isometric muscle contractions of various force levels and durations revealed that muscle fatigue is associated with contralateral activation of the motor and somatosensory areas and temporal areas and bilateral activation in the supplementary motor areas and cingular cortex, indicating that increased efforts needed to maintain required force and its eventual breakdown with fatigue might induce activation of additional cortical areas. Analysis of data obtained in all experimental series revealed that insula, secondary somatosensory and auditory areas are activated during both perception of muscle pain and processing of somatosensory afferentation.

In conclusion, this thesis has elucidated brain processing of muscle pain showing distributed, bilateral patterns comprised of activated structures predominantly attributed to the medial pain system and deactivated structures. Furthermore, initial and late phases of tonic muscle pain are associated with different brain reactions, namely initial activation of the insula followed by a significant bilateral decrease of activity at the late stage. Area of brain cortex located near lateral sulcus and comprised of secondary somatosensory cortex, posterior part of the insula and adjacent auditory cortex is engaged in the perception of muscle pain and processing of somatosensory afferentation as well as maintenance of fatiguing muscle contractions.

Keywords: Experimental muscle pain; Hypertonic saline; Kinesthesia; Proprioception; Movement; Vibration; Muscle fatigue; Brain; Imaging; Positron emission tomography; Regional cerebral blood flow.
To my mother Irene, daughter Irene and Nadja

Маме, Ире и Надежде
CONTENTS

ORIGINAL PAPERS ............................................................... 6
ABBREVIATIONS ............................................................... 7
INTRODUCTION ................................................................. 8
PET studies of pain ............................................................ 8
The cerebral pain matrix ...................................................... 9
PET studies of muscle pain ................................................ 10
Cerebral processing of proprioceptive inputs ......................... 11
Central effects of fatiguing muscle loads ............................. 13
Summary of the introduction ............................................. 13
OBJECTIVES ................................................................. 15
Specific aims of the papers ................................................. 15
MATERIAL AND METHODS ................................................. 17
Subjects ............................................................................ 17
PET scanning procedure ................................................... 17
PET data acquisition ......................................................... 17
PET data processing ........................................................ 17
Conjunction analysis ........................................................ 18
Experimental condition ..................................................... 19
Induction of muscle pain (Paper I, II and V) ......................... 19
Control group (Paper II) ..................................................... 19
Proprioceptive stimulation (Paper III) ............................... 20
Muscle fatigue (Paper IV) .................................................. 20
EMG recording (Paper III and IV) ...................................... 21
Visual task (Paper I-V) ....................................................... 21
Conditions of PET studies ................................................ 21
RESULTS .................................................................................................................. 24
DISCUSSION ........................................................................................................... 48
The interpretations of increases and decreases of rCBF .................. 48
Functional neuroanatomy of HS induced muscle pain (Papers I, II and V) .................................................. 49
Increase of activity during muscle pain ......................................................... 49
Decrease of activity during muscle pain ..................................................... 51
Reorganisation of functional neuroanatomic system underlying muscle pain due to habituation to acute muscle pain (Paper II) 53
Limitations of the study design ................................................................. 54
Functional neuroanatomic system underlying processing of proprioceptive information (Paper III) ..................... 55
Brain activations after sustained muscle contractions of different levels and durations (Paper IV) ......................... 56
Common elements of neuroanatomical systems underlying muscle pain and activated due to proprioceptive stimulation and fatiguing muscle contractions ......................................................... 58
CONCLUSIONS .................................................................................................. 60
ACKNOWLEDGMENTS ...................................................................................... 61
REFERENCES ...................................................................................................... 63
ORIGINAL PAPERS

This thesis is based on the following papers, referred to by their Roman numerals in the text:


V. Korotkov A, Lyskov E, Kataeva G, Pakhomov G, Medvedev S. Experimental muscle pain induced by injection of hypertonic saline. Comparative analysis of two PET studies. (Submitted)
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<th>Abbreviation</th>
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<td>Maximal Voluntary Contraction</td>
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<td>Positron Emission Tomography</td>
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INTRODUCTION

There is a growing interest in the subject of musculoskeletal disorders due to the increasing number of patients and the economic strain on society. The dominant symptom that determines the clinical picture is chronic pain, although other symptoms (stiffness, soreness, sensation disturbances, etc) are also common. The overall expenses related to chronic pain in Sweden is around 2% of the gross national product (Norlund et al. 2000). According to the Swedish National Board of Occupational Safety and Health’s statistics on work related injuries, about 70 % of all cases were related to musculoskeletal problems brought on by workload (approximately 12 000 individuals). The incidence of chronic muscle pain among adults in the United States has been estimated to range from 2% to 40% of the general population. In Europe the prevalence of muscle pain comes to 14 -50% in the general population (Buckle et al. 2002). Thus, chronic muscle pain poses serious social problems, and a better understanding of underlying pathophysiological mechanisms is necessary to develop effective prevention, diagnostics, treatment and rehabilitation.

Several pathophysiological models of the processes behind chronic muscle pain (Johansson and Sojka 1991, Lund et al. 1991, Arendt-Nielsen et al. 1996) were developed to explain relationships between muscle metabolism, nociceptive afferentation and motor control in different working conditions including monotonous, long-lasting and fatiguing muscle loading. The models are consistent in considering the importance of interactions between peripheral and central regulatory mechanisms. For example, modulations of sensitivity of nociceptors (peripheral sensitisation) and facilitated central processing of peripheral input seems to be the key elements in development of some forms of chronic muscle pain. However, many aspects of the brain processing of muscle pain and its interrelations with proprioception and muscle fatigue remain to be investigated in experimental studies.

PET studies of pain

Brain imaging studies confirm and further elucidate the concept that pain is a complex sensation involving sensory discriminative (including localization of the stimuli, detection of intensity and quality discrimination), affective-motivational (encompassing emotional reactions, an arousal and selective attention to the painful stimuli) and cognitive-evaluative aspects (including anticipation, attention to the painful stimuli and comparison with past experience) (Melzack and Casey, 1968). Brain imaging methods such as PET or fMRI provide unique opportunities to
record functional activity across the whole brain during experimental pain conditions, as well as for patients suffering from chronic pain.

PET methodology allows the study of numerous parameters of cerebral hemodynamics, metabolism and neurotransmitter systems. Judging by the number of articles published on rCBF, PET is the prevalent method in the study with adequate spatial and temporal resolution to detect activation of certain brain structures during pain perception. Various types of experimental nociceptive stimulation were used in PET studies - noxious heat (Coghill et al. 1999, Peyron et al. 1999) and cold (Craig et al. 1996, Petrovic et al. 2000), electrical (Davis et al. 1997, Svensson et al. 1997) and laser (Debrishire et al. 1997) stimulations, and sub- or intracutaneous injections of algic chemical substances (Hsieh et al. 1996, Porro et al. 1998). Results of these studies show a large number of cortical areas and subcortical structures engaged in the distributed neural system underlying the central processing of pain.

The cerebral pain matrix

The structures most consistently responding to pain include secondary somatosensory area (SII), insula, the thalamus, anterior cingulate cortex (ACC), and the PFC and PC (for review see Talbot et al. 1991, Ingvar 1999, Peyron et al. 2000). Activation of the primary somatosensory area (SI), supplementary motor area (SMA), parietal cortex (PC), temporal and occipital cortex and cerebellum were also described. An interpretation of results is commonly referred to a dichotomy of the lateral and the medial pain systems (Treede et al. 1999). The pattern of pain-related cortical activity varies between studies. That may be due to differences in study designs, characteristics of stimuli (e.g., their type and duration) and analysis methods. It was assumed that the pain intensity itself may be the major source of variability of the results (Price 2000). Coghill and co-workers (1999) found pain-intensity related activations in the cerebellum, putamen, thalamus, insula, ACC, SII, SMA and premotor cortex.

The insula is one of the most frequently activated areas in neuroimaging studies of pain (Treede et al. 2000). Pain-related activation of insula correlates positively with stimuli intensity. It was shown that insula participates in the sensory integrative (including visceral sensory and visceral motor integration) and affective components of pain (Coghill et al. 1994, Casey et al. 1994, Derbyshire et al. 1997). It was suggested that SII/insula might be involved in encoding of thermal discrimination (Craig et al. 1996, Casey et al. 1996).

Meanwhile, the affective-motivational component of pain is often associated with activation of the ACC that in turn can be involved in many aspects of cognition, including attention (Vogt et al. 1996, Rainville et al. 1997). Affective
functions of ACC may be related to the integration of affect, cognition and selection of response (Treede et al. 2000). Craig and colleagues (1996) found that activation in the ACC was associated with the thermal grill illusion and also with noxious heat or cold. Authors concluded that increased activity in the ACC appears to be selectively associated with the perception of thermal pain. Also modulation of pain unpleasantness with hypnotic suggestion was shown to affect the functional activity of the ACC (Rainville et al. 1997).

The role of SI remains controversial – activation of the SI has been found in 4 out of 11 neuroimaging (PET and fMRI) studies. The SI is involved in processing of information about localisation and intensity of noxious stimuli and thus subserve sensory discriminative component of pain (Kanda et al. 2000).

Response of the PFC (considered to participate in the brain processing of emotions, memory and attention) to pain is often attributed to the attentional-cognitive activity induced by the noxious stimulation and planning of behavioural response (Derbyshire et al. 1994, Hsieh et al. 1996).

Activation of the thalamus is associated with nociceptive input from ipsilateral projections of the spinothalamic tract. Treede et al. (1999) in summarizing data from several reports concluded that different groups of thalamic nuclei relay the information from the spinal cord to the lateral nociceptive system (SI and SII) and medial nociceptive system (ACC and Insula).

Functions of the lentiform nucleus were attributed to movements (Golebatch et al. 1991) and planned action (Brooks et al. 1993). Activation of contralateral lentiform nucleus during pain may indicate the preparation for the movement to carry out escape behavior (Derbyshire et al. 1997). Results of comparison of cold and heat noxious stimulation reported by Casey et al. (1996) show common activated areas (ACC, premotor cortex, SII and posterior insula, anterior insula and lentiform nucleus.) but cold pain induced higher activation in these areas. Later research of Davis et al. (1998) also detected similar patterns of cold and heat pain-related activations.

**PET studies of muscle pain**

The central network of muscle pain processing is considerably less explored than those of cutaneous pain. The majority of PET and fMRI studies used primarily cutaneous stimulation. At the same time psychophysical studies suggested that different stimuli activate different receptors, pathways and mechanisms (see for review Graven-Nielsen et al. 2003). The issue of similarities/differences between processing of muscle and skin pain remains blurred. Svensson et al. (1997) did not find statistically significant differences between patterns of brain activations in response to muscle pain (elicited with
intramuscular stimulation) and to cutaneous pain (CO2 laser produced). However, the comparison of noxious cutaneous and intramuscular stimulation indicated stronger activation of the ipsilateral premotor, PFC and SII during cutaneous pain (Svensson et al. 1997). It was concluded that differences between acute skin and muscle pain are mediated by differences in the intensity and temporospatial pattern of neuronal activity within similar sets of forebrain structures. This conclusion is in corroboration with the data obtained by other imaging modalities (Chang et al. 2004). The conclusion about similarity of neuroanatomic systems underlying skin and muscle pain was made by Niddam et al. (2002) also using electrical stimulation of muscles. In a recently published study Kupers et al. (2004) suggested that the cerebral processing of experimental jaw-muscle pain may differ from that of cutaneous pain.

Among several experimental techniques used to induce human muscle pain, i.m. injection of HS has gained wide acceptance (Arendt-Nielsen et al. 1999). It has been shown that i.m. injections of HS induce pain by activation of nociceptors, in contrast to electrical stimulation which acts on a much broader spectrum of receptors or afferents (Graven-Nielsen et al. 1997). Injection of HS into muscles induces pain, compatible to the pain accompanying musculoskeletal disorders (Kellgren, 1938). This model has several advantages: the pain produced share many of the features with clinical muscle pain, it has no known side effects and the intensity and the location of the pain can be easily controlled. Until now the HS-model was rarely used in PET studies of pain (Zubietta et al. 2002, Kupers 2004). An advanced method maintaining a rather long (25-30 min and even longer) nociceptive stimulation by means of computer controlled infusion of HS, provides a unique opportunity to assess not only initial but lasting stages of pain.

PET studies of chronic pain are difficult due to the need of comparing data of the same subjects in painful versus pain free conditions. In most studies cerebral effects of other clinical forms such as neuropathic pain, cluster headache etc. Single PET studies on patients with musculoskeletal disorders showed reduced brain responses in PFC and ACC to heat noxious stimulation in comparison with pain-free controls (Derbyshire et al. 1997, Derbyshire et al. 1994). PET study on patients with fibromyalgia showed lower rCBF for patients than controls in the left frontal, temporal, parietal and occipital corticies, whereas retrosplenial cortex was bilaterally higher activated, reflecting according authors increased attention towards subnoxious somatosensory signaling (Wik et al. 2003).

Cerebral processing of proprioceptive inputs

Central processing of proprioceptive afferentation was studied with neuroimaging methods during vibrotactile stimulation (Fox et al. 1987, Meyer et al. 1991, Davis et al. 1998, Naito et al. 1999). A vibration of 110-130 Hz was often
used to map the areas in the brain associated with this kind of peripheral afferent input and the set of structures revealed consistently included SI, SII, thalamus and insula.

A study of central representation of vibration-induced illusion of movement was reported recently (Naito et al. 1999). In this study the rCBF was measured with PET in nine subjects when their left m. biceps tendon was vibrated at 10 Hz (low), at 70 or 80 Hz (illusion), or at 220 or 240 Hz (high) and in a rest condition. When illusion was contrasted with low and high vibration, the contralateral SMA, caudal cingulate motor area, dorsal premotor cortex, and MI were significantly activated, while none of these areas were activated in the contrast of (low + illusion + high) versus rest. This demonstrated that the effects of illusion and vibration were associated with different sets of cortical fields. The activity in motor areas was associated with the kinaesthetic illusions. This was in contrast to the hypothesis that somatosensory areas should be primarily involved in kinaesthetic illusions. Up to now research has not been undertaken to disclose differences in brain systems underlying passive movement and vibration induced illusion of movement.

There is a limited number of studies comparing brain processing of nociceptive and proprioceptive input. During painful stimulation contralateral activation in SI and SII, ACC, anterior insula, the SMA of the frontal cortex, and thalamus was found; whereas vibrotactile stimulation produced activation in contralateral SI, and bilaterally in SII and posterior insula (Coghill et al. 1994). In that study a direct comparison of pain and vibrotactile stimulation revealed that both stimuli produced activation in similar regions of SI and SII, regions long thought to be involved in basic somatosensory processing. In contrast, painful stimuli were significantly more effective in activating the anterior insula, a region heavily linked with both somatosensory and limbic systems. The fact that pain-related activation was found more widely dispersed across both cortical and thalamic regions than for vibrotactile-related activation may reflect the complex nature of pain, involving discriminative, affective, autonomic, and motoric components. A comparison of cortical representation of thermal painful, vibrotactile and motor stimulation was performed by Gelnar et al. (1999). The authors concluded that the network underlying pain perception shares components with the networks underlying perception of touch perception and motor execution. However, the thermal pain perception network also has components that are unique to this perception - SII, insula, and posterior cingulate cortex. An interaction between proprioceptive and nociceptive systems during experimentally induced muscle pain was found in some other studies (Capra et al. 2000). The authors emphasised the importance of further studies on central integration of nociceptive and proprioceptive information.
Central effects of fatiguing muscle loads

Muscle fatigue is a process of acute impairment of performance that includes both an increase in the perceived effort necessary to exert a desired force and an eventual inability to produce that force. It is known that prolonged and sustained muscle actions could be limited not only by the muscle performance “per se” but also by failure of CNS structures (for review see Gandevia 2001). One may speculate that in certain working conditions the peripheral apparatus, i.e. from the spinal cord to the muscle, is capable of generating additional force while central structures become insufficient/sub-optimal in driving them. Taylor and colleagues (2000) suggested that muscle fatigue development could not be fully explained by events at the cortical motoneuron level, but rather reflects changes in voluntary drive “upstream” of motor cortical output (see also Todd et al. 2003). Furthermore, it has been shown that these central changes involve not only excitatory cortical circuitry but also inhibitory networks, and that both changes outlast the contraction for several min (Ljubisavljevic et al. 1996; Gandevia 2001; Taylor et al. 2000). All this indicates complex central processes during the development of fatigue, which are still largely unknown.

A number of imaging studies have investigated the cortical activation during the exertion of various force levels, employing different paradigms. The fMRI technique (e.g. Ludman et al. 1996; Thickbroom et al. 1998; Dai et al. 2001) has been used to study force -related activity of cortical areas during isometric contraction. Also, there have been a few PET studies carried out during sustained isometric contractions (Dettmers et al. 1995; 1996). Consistent findings were that the activated cortical areas were the MI and SI, the SMA, premotor, PFC, PPC, the cerebellum and the cingular cortices. A high correlation between rCBF in these areas and the degree of muscle activation were found (e.g. Dettmers et al. 1995). However, since these studies did not include fatigue situations, they did not yield consistent findings and conclusions about cortical areas involved in the process of fatigue that originates due to sustained muscle contraction.

Summary of the introduction

The cerebral pain matrix as well as brain processing of proprioception have been intensively investigated. However, several important aspects of this problem remain unexplored. Most brain imaging studies of pain have employed cutaneous stimulation. On the other hand, there are prominent differences in psychophysical characteristics and underlying peripheral mechanisms between muscle and cutaneous pain that may lead to distinctions in brain processing of these two pain modalities.

An application of acute or short lasting repeated pain however impedes the ability to follow gradual changes of brain activity during different stages of pain.
i.e., initial, sub-acute, residual, etc. A model providing lasting pain during experimental session would successfully fill the gap between data interpretation of imaging studies of experimental pain and data obtained in clinical examinations in patients with different forms of chronic muscle pain.

There are only a few studies focusing on brain reactivity to proprioceptive afferentation and/or muscle contractions and their functional and anatomical relations with pain-related structures. It is important to elucidate this since muscle pain is associated with alterations of proprioceptive functions and motor control.

Thus, investigations on cerebral processing of nociceptive muscle afferentation and its relations with structures engaged in analysis of other interoceptive inputs from muscles are important for enhancing the knowledge about central mechanisms of the musculoskeletal disorders.
OBJECTIVES

The general goal of the present thesis was to study functional brain anatomy of systems underlying perception of muscle pain, processing of proprioceptive information and maintenance of fatiguing muscle contractions with a special emphasize on their possible interrelations.

Intramuscular injection of 5% HS was used to elicit brain structures that participate in the processing of tonic muscle pain in its initial and late stages. The application of passive movement of the forearm and vibration of tendons was used to detect brain structures involved in processing of proprioceptive information. Fatiguing and non fatiguing isometric muscle contractions were used to detect brain structures involved in the maintenance of fatiguing muscle contractions. Additional analyses of obtained data were used to detect common elements of the neuroanatomical systems being investigated.

Specific aims of the papers

Paper I

The aim of the study was to assess the character and intensity of the brain rCBF changes in response to intramuscular (m. triceps) injections of HS.

Paper II

The aim of the study was to investigate changes in rCBF in response to experimental muscle pain induced by 20 min infusion of HS into the m. erector spinae with separated analysis of PET data at the initial and late phases of pain processing.

Paper III

The aim of the study was to detect brain structures involved in the perception of passive movement, vibration induced illusion of movement and vibration without eliciting the illusion of movement.

Paper IV

The aim of the study was to investigate changes in rCBF associated with fatiguing and not fatiguing sustained isometric muscle contractions of submaximal force levels and of different durations.
Paper V

The aim of the study was to identify common areas of brain responses to HS-induced muscle pain irrespective of the target muscle and method of HS-injection, which were used in paper I and paper II.
MATERIAL AND METHODS

Subjects
The group of subjects consisted of 59 male healthy volunteers that had no history of neurological and psychiatric diseases. This group was subdivided into 16 subjects (age 24.3 ± 7.7) in paper I, 19 subjects (age 26.7 ± 7.0) in paper II, 12 subjects (age 23.5 ± 7.0) in paper III and 12 subjects in paper IV (24.1 ± 6.4).

Their physical and mental conditions were assessed by clinical examination and interview prior to the study. Blood samples were taken to exclude latent forms of anaemia. Written informed consent (including the clear description of experimental goals, protocol and potential risk of the study) was obtained from all subjects prior to their inclusion in the study. Experiments were approved by the local Ethical Committee of the Institute of the Human Brain of Russian Academy of Sciences and were in accordance with the Declaration of Helsinki.

PET scanning procedure
During scanning subjects laid on the couch of the tomograph with their head fixed in a headholder by individual thermoplastic mask in order to avoid interscan replacement. The subject’s head was positioned in the PET scanner parallel to the orbitomeatal line. The study was performed in a dark, quiet room. The eyes of subjects were open, and the ears unplugged. The intravenous catheter for injection of H$_2^{15}$O was inserted in the right antecubital vein prior to the study.

PET data acquisition
All PET scans were done using a Scanditronix PC2048-15B tomograph (15 parallel slices with in-plane spatial resolution of 6.5 mm in the center of the field of view and inter-slice distance of 6.5 mm). A 10-min transmission scan was performed with a $^{68}$Ge rotating pin source for attenuation correction prior to the first emission scan. The distribution of rCBF was measured during 60-sec PET scan using the bolus H$_2^{15}$O methodology without arterial blood sampling. (Fox & Mintun, 1989). The image reconstruction was done using a 7 mm Hanning filter.

PET data processing
The data were analysed with SPM 99 software (Friston et al. 1995). Following the realignment of images from each subject to correct for any changes
in head position between scans, the images were transformed into a standard anatomical space used in SPM 99. In order to increase the signal-to-noise ratio and to accommodate normal variability in functional and gyral anatomy, the images were smoothed with a Gaussian filter of 12x12x12-mm width. The resulting activity data were normalised for differences in global flow by scaling to a global mean of 50 ml/dl/min (McIntosh et al. 1996).

Pixel by pixel comparison was performed to reveal significant differences in rCBF between conditions. Results are displayed as statistical parametric maps of t-statistic (SPMt) showing the areas if significant increases in rCBF (activations) were found. The significance threshold for the resulting SPM was set at p<0.05 and corrected. If no statistically significant activations were detected with this threshold, then the threshold was set at p<0.001 uncorrected and activations were considered significant at p<0.001 at cluster level and cluster sizes >200.

To elucidate possible different functional roles of the activated areas found in the analysis of contrasts, an additional analysis was performed. The SPM analysis using F-statistics (effects of interest) at p<0.0005 corrected was used, revealing the differences between brain areas in different conditions, and scattergrams “fitted responses averaged on each cluster” – “conditions” were build for each cluster (see details in Radovanovic et al. 2002). One of the important steps in SPM analysis is the estimation of the general linear model parameters for every voxel. After parameters are estimated for given voxel, SPM allows to split value observed in voxel for every scan into 3 parts: one which may be attributed to the parameters of interest (conditions effects in our case), a second which may be attributed to the parameters of no interest (subjects effects) and a third considered as a noise. The first part is known in SPM terms as “fitted” model response, and the sum of the first and the third as “adjusted” model response for the voxel under consideration. Averaging the fitted response for all clusters’ voxels then enables comparison of the values of activation in particular clusters by plotting the scattergrams of relative values of activations under all experimental conditions.

Anatomical identification of activations was made on the basis of the Talairach and Tournoux brain atlas (Talairach and Tournoux, 1988) using MNI space utility and Talairach space utility (http://www.ihb.spb.ru/~pet_lab).

**Conjunction analysis**

To identify common areas of brain responses to HS induced muscle pain, irrespective of the target muscle and means of HS injection as well as to muscle pain and passive movement, vibrotactile stimulation and fatiguing isometric contraction conjunction analyses were performed. This kind of PET data processing allows only detecting of overlapping in patterns of activations in order
to find common elements of neuroanatomical systems, but not to determine exact mechanisms of their interaction.

**Experimental conditions**

**Induction of muscle pain (Paper I, II and V)**

An experimental model for muscle pain with i.m. injection of HS was used to elicit brain structures involved in the brain processing of muscle pain. In study I, 2 ml of HS was injected into the left m. triceps (in the middle of lateral head) using a single bolus injection. In study II in order to allow continuous infusion, prior to the study a catheter (size 27-29G) was inserted into the right m. erector spinae at the level of L3 spinous process. A LabView program running on a computer controlled an infusion pump (Harvard Apparatus). The pump was connected to the catheter via a plastic extension tube. The HS was injected according to a feed forward compensation algorithm (Zedka, 1999), and the infusion rate was gradually increased from 300 to 500 µl/min to provide pain a longer time (tonic pain). The total amount of HS injected was approximately 10 ml.

The subjects rated their pain on an 11-points numerical rating scale (Breivik et al. 2000) once every min, and immediately before and after each scan. The subjects were novel to the technique of i.m. injection of HS. State and trait anxiety was assessed before and after the experiments with Spilberger State Trait Anxiety Inventory to estimate their levels in general and possible changes after experiments.

**Control group (Paper II)**

During a prolonged experiment rCBF might be affected by factors other than those directly involved in pain processing. Since these possible effects may be of a non-monotonous character, it was necessary to investigate a control group undergoing a similar protocol that included performance of the visual task, but not experiencing pain. The control group, 15 healthy volunteers (right-handed males, mean age 25.2±3.4 year), was selected from a previous experimental series (Tervaniemi et al. 2000). Subjects performed distracted cognitive task (detection of grammatical gender of words) with simultaneous binaural acoustical stimulation that consisted of standard and deviant tones. The first four consecutive scans of this experiment were analyzed in the same way as in the experimental pain group (i.e. with the same statistical thresholds and contrasted pairs). Since characteristics of the audio-stimulation (lateralisation and the order of the standard and deviant stimuli) were counterbalanced between scans, they could not contribute to the certain patterns of the rCBF responses. On the other hand, it was important that visual presentation of the task (position, brightness, contrast of video display,
exposure duration, timing) had the same characteristics as in the present study. Therefore we assume that brain activity associated with visually presented distraction task, as well as possible non-specific time effects due to non-randomized consequence of scans, were comparable in both groups.

**Proprioceptive stimulation (Paper III)**

Subjects laid supine on the couch of the tomograph with the head fixed in the headholder and the ears plugged. The left forearm was positioned and fixed in the specially developed arm holder to which a torque motor is attached. Passive movements were performed horizontally with a frequency of 15 flexions and 15 extensions per min around 25° in both directions starting from 140° to avoid extreme positions of the joint. Two vibrators were attached to the arm holder in such a way that they could easily be connected to the m. biceps and m. triceps tendons.

During the training, frequency and amplitude for the strongest movement illusions as well as that of vibration not exerting movement illusions were determined - the frequency was changed from 10 to 100 Hz with 10 Hz steps to find the optimal frequency that would induce the illusion that was comparable with an actual arm movement (*ILLUSION* frequency). Then the frequency was dampened with 10 Hz steps until the movement illusion disappeared, and then decreased by 10 Hz more (*NO ILLUSION* frequency). Switching between m. triceps and m. biceps were performed every 2 sec to attain the timing properties of passive movement.

**Muscle fatigue (Paper IV)**

Subjects lay supine on the couch of the PET tomograph, with their head fixed in the headholder with individually molded thermoplastic mask. To prevent movements during efforts to maintain contractions, the support for the subjects’ left elbow was fixed to the couch. The forearm was positioned upright, with a 90° elbow angle, to hold the handle of the dynamometer. The handle was adjusted for comfortable gripping. The wrist joint was fixed by a plastic holder to the handle in order to stabilize the forearm and minimize the activation of wrist flexor muscles. The construction permitted subjects to develop and maintain isometric voluntary contractions engaging primarily their m. biceps, through flexion of the forearm in the vertical plane. A dynamometer was connected to the scale, which the subject could view and thus adjust and maintain the required level of contraction force. Before the PET session, the magnitude of MVC was measured. The required force level, in each of the conditions, was then defined as a percentage of developed MVC. Before the start of the session, the subjects were requested to exert and keep the required force level for a short period of time in order to get familiar with the
whole experimental procedure. During the scans, subjects were instructed to relax completely and to perform a visual task.

After the end of the contractions, subjects were instructed to relax completely during the scan. In order to start the PET recording as soon as possible after the contractions to avoid prolonged recovery time and to avoid the confounding effects of repetitive MVC contractions on rCBF muscle fatigue was not quantified, by repeating the post-contraction MVC measurement. However, in order to estimate the level of fatigue after different conditions in our experiments we chose a similar cohort of six, age and sex matched subjects (age 24.8 ± 8.2; mean ± SD), and repeated all three experimental conditions (CO, 30 and 50). Without subsequent PET scan, and measured the post-condition MVC after the end of contractions. There were no differences in the level of post-condition MVC after CO and 30 conditions, as compared with pre-condition level. After 50 condition all subjects were unable to reach pre-contraction MVC levels, thus indicating force-generating capacity failure once the endurance point was surpassed. Therefore, 50 condition was considered as the “fatigue” condition.

EMG recording (Paper III and IV)

EMG activity was recorded with surface electrodes (Blue Sensor type Q-10-A, Medicotest, Denmark) over the m. biceps and brachioradialis muscles. Signals were amplified (x 300), filtered (101000 Hz) and sampled -2000 Hz/channel (Polyneurograph DK 86, St. Petersburg, Russia), for later off-line analysis (Spike 4 software, Cambridge Electronic Design, Cambridge, UK). Changes in EMG activity were estimated by calculating the root mean square (RMS) level of the rectified EMG signal in 10 s intervals.

Visual task (Paper I-V)

In order to minimize the state of arousal and standardize attention during all scans across studies I-IV, the subjects performed a simple task. They had been asked to calculate one of three visual stimuli presented on a computer screen placed approximately two meters in front of their eyes. Either black circles presented in the left or right half of a white screen, or two circles on both sides of the screen were used. The presentation of stimuli began 20 s before the injection of \( H_2^{15}O \) and finished after the end of each scan.

Conditions of PET studies

Paper I

The following conditions were used: A. Rest 1 - subjects were instructed to relax completely and refrain from moving. B. Needle – a syringe needle was
inserted into the m. triceps of the left arm 2 min before the scan. C. Rest 2- subjects were instructed to relax completely and refrain from moving. D. Pain- 2 ml of HS were injected into the m. triceps of the left forearm 2 min before the scan. The conditions were not randomised (one scan per condition).

**Paper II**

The following conditions were used: A. Baseline (BL) - subjects were instructed to relax completely and refrain from any movements. After this scan the pump was started, and infusion of HS began. B. Early pain (EP) - scan was performed 4 min after the start of infusion. C. Late pain (LP) - scan was performed 21 min after the start of infusion. After that, the infusion was stopped and 20 min after the fourth scan (D) was performed: Post pain (PP) - subjects were instructed to relax completely and refrain from any movements. The conditions were not randomised (one scan per condition).

**Paper III**

The following conditions were used: A. Rest (RE) - subjects were instructed to relax completely and refrain from any movement; the arm holder was not moving, vibrators - not attached to the skin and switched off. B. Vibration not elicited illusion of movement (VN): arm holder was in the fixed position; VN-frequency vibration was started 20 sec before the injection of the radioactivity. C. Vibration induced illusion of movement (VI): arm holder was in the fixed position; VI-frequency vibration was started 20 sec before the injection; D. Passive movement (PM): passive flexion and extension of the left elbow was started 60 sec before the injection and will be continued to the end of the scan; vibrators - not attached to the tendons and switched off. The conditions were counterbalanced (two scans per condition).

**Paper IV**

The following conditions were used: A: Rest condition (RE)- No contraction; the subjects were instructed to relax completely and perform only the visual task during the scan. B. Short contraction condition (CO) -Contraction at 30% of MVC was applied for 60 s; starting 70 s before the injection of H215O and ending 10 s before the scan. C. Prolonged contraction (30) - Contraction at 30% of MVC was applied for 120 s; starting 130 s before the injection of H215O and ending 10 s before the scan. D. Contraction at 50% of MVC condition (50) - was applied for 120 s; starting 130 s before the injection of H215O and ending 10 s before the scan. The conditions were counterbalanced (two scans per condition).
Paper V

The following conditions from paper I and II were used for conjunction analysis: A (from paper I). Rest 2- subjects were instructed to relax completely and refrain from moving. B (from paper I). Pain- 2 ml of HS were injected into the m. triceps of the left forearm 2 min before the scan. C (from paper II). Baseline (BL) - subjects were instructed to relax completely and refrain from any movements. After this scan the pump was started, and infusion of HS began. D (from paper II). Early pain (EP) - scan was performed 4 min after the start of infusion of HS.
RESULTS

Paper I

Following injection of HS in the m. triceps all subjects perceived localized, cramp-like, aching pain. The averaged pain intensity rating for all 16 subjects was 3.5 and 0.25 during Pain and Needle condition, respectively (Fig. 1).

Figure 1. Pain intensity ratings of 16 subjects after injection of 5 % HS into the left m. triceps (circles) and insertion of a needle without HS injection (squares). The light vertical bar indicates the period of PET scans – Pain and needle respectively.

To detect brain areas involved in processing of HS-induced muscle pain, six pairs of contrasts were analysed: Pain vs. Needle, Needle vs. Pain, Pain vs. Rest2, Rest2 vs. Pain, Needle vs. Rest1 and Rest1 vs. Needle. A comparison of Pain and Needle conditions revealed an rCBF increase in the right insula and putamen, and a decrease in occipital and temporal cortex of the right and left hemispheres (BA 17, 18, 19 and 37) (in the right hemisphere extending to the cerebellum) and in the left frontal cortex (BA 9, 10, 46) (Table 1 and Fig. 2).
Table 1. Significant increases in regional brain activity in response to HS injection into the left m. triceps (cluster based analysis).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>$p_{corrected}$ for the cluster level</th>
<th>Coordinates of local maxima (x y z)</th>
<th>$p_{corrected}$ for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain-Rest</td>
<td>1. Right: Insula, Superior Temporal gyrus (BA 22)</td>
<td>617</td>
<td>0.000</td>
<td>52 4 -2</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td>1. Right: Cerebellum, Lingual gyrus (BA 17, 18), Inferior Occipital gyrus (BA 17, 18), Fusiform gyrus (BA 17, 18), Cuneus (BA 17)</td>
<td>2201</td>
<td>0.000</td>
<td>36 -74 -16</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>2. Left: Medial Frontal Gyrus (BA 9, 46), Inferior Frontal gyrus (BA 44)</td>
<td>718</td>
<td>0.000</td>
<td>-36 24 32</td>
<td>0.328</td>
</tr>
<tr>
<td>Rest – Pain</td>
<td>3. Left: Inferior Parietal Lobulus (BA 40)</td>
<td>408</td>
<td>0.002</td>
<td>-48 -44 4</td>
<td>0.351</td>
</tr>
<tr>
<td>Needle-Rest</td>
<td>1. Left: Inferior, Medial and Superior frontal gyri (BA 10 and 46)</td>
<td>324</td>
<td>0.006</td>
<td>-26 64 -4</td>
<td>0.520</td>
</tr>
<tr>
<td>Pain-Needle</td>
<td>Right: Insula, Putamen</td>
<td>348</td>
<td>0.004</td>
<td>22 -6 -6</td>
<td>0.117</td>
</tr>
<tr>
<td>Needle-Pain</td>
<td>1. Right: Cerebellum, Fusiform Gyrus (BA 18, 19), Inferior Temporal Gyrus (BA 37)</td>
<td>1472</td>
<td>0.000</td>
<td>34 -76 -18</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>2. Left: Medial Temporal gyrus (BA 39), Angular gyri (BA 39), Inferior parietal Lobulus (BA 40)</td>
<td>543</td>
<td>0.000</td>
<td>-50 -40 50</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>3. Right: Cuneus (BA 17, 18, 19), Medial Temporal gyrus (BA 37), medial Occipital gyrus (BA 18, 19)</td>
<td>1589</td>
<td>0.000</td>
<td>46 -76 20</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>4. Left: Medial Frontal gyrus (BA 9, 10, 46)</td>
<td>517</td>
<td>0.000</td>
<td>-38 38 24</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>5. Left: Inferior Temporal gyrus (BA 37), medial Occipital gyrus (BA 19)</td>
<td>256</td>
<td>0.016</td>
<td>-56 -66 -4</td>
<td>0.145</td>
</tr>
</tbody>
</table>

For cluster analysis uncorrected threshold $p<0.001$ was used and clusters were considered as significant if corrected $p<0.05$ at cluster level and cluster size $>200$ voxels.

Coordinates of the local maxima were defined according to the brain atlas of Talairach & Tournoux (1988); maxima were considered as significant if corrected $p<0.05$ at voxel level.
Figure 2. Selected contrasts of the study I. The panels show three standard orthogonal views of the “glass” brain. The stereotactic space is that defined in the SPM software (MNI space), SPMt threshold is at $P<0.001$ uncorrected and cluster size $>200$ voxels. The left side of image is the left side of the brain. See table 1 for exact anatomical localization of the selected clusters.
Paper II

All subjects reported subjective experiences of localised, cramp-like, aching pain during i.m. infusion of HS into the right m. erector spinae. A graph of the averaged pain ratings is shown in Fig. 3.

**Figure 3.** Pain responses of the nineteen subjects during the course of the experiment (Mean ±SD). Vertical bars indicate time of PET scans.

The difference between average pain ratings during early and late pain was significant (p=0.012, paired t-test). Before the PET scan the mean trait anxiety score was 41±10.3 (Mean ± SD) and after it was -39±8.8 revealing a statistically significant decrease during the study (p=0.02, paired t-test). The state anxiety score was 37±6.4 before the study and 35±6.8 after. The decrease of state anxiety was not significant (p=0.28, paired t-test). To detect brain areas with changes in rCBF in response to HS-induced low back pain and to track changes in the functional state of those structures, 12 contrasts were analysed (see Fig. 4 and Table 2 for detailed outline of results).

**Changes in rCBF as compared to Baseline condition**

During the *Early Pain* condition there were three clusters with reduced rCBF and one with increased rCBF as compared to the *Baseline* condition (see
Fig. 4). Among these three clusters with reduced blood flow, two large ones located in the occipital, temporal cortex and PC involving BA 18, 19, 37 and BA 19, 21, 22, 37,

Table 2. Significant increases in regional brain activity in response to HS infusion into the right m. erector spinae (cluster based analysis).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>( p_{\text{corrected}} ) for the cluster level</th>
<th>Coordinates ( b ) of local maxima (x y z)</th>
<th>( p_{\text{corrected}} ) for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pain – Baseline</td>
<td>1. Right. Insula, Superior Temporal Gyrus (BA 22, 38)</td>
<td>387</td>
<td>0.002</td>
<td>42 –4 –8</td>
<td>0.039</td>
</tr>
<tr>
<td>Baseline – Early pain</td>
<td>1. Left. Fusiform Gyrus (BA 37, 19); Middle Occipital Gyrus (BA 18, 19); Inferior Temporal Gyrus (BA 37); Inferior Occipital Gyrus (BA 18); Lingual Gyrus (BA 18, 19); 2. Right. Fusiform Gyrus (BA 37); Inferior Temporal Gyrus (BA 37); Middle Temporal Gyrus (BA 21, 39); Middle Occipital Gyrus (BA 19); Superior Temporal Gyrus (BA 22); Inferior Parietal Lobule (BA 40)</td>
<td>2529</td>
<td>0.000</td>
<td>-36 –66 –8</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>3. Left. Superior Temporal Gyrus (BA 22); Inferior Parietal Lobule (BA 40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early pain – Late pain</td>
<td>1. Left. Insula, Superior Temporal Gyrus (BA 38); Inferior Frontal Gyrus (BA 47)</td>
<td>413</td>
<td>0.001</td>
<td>-42 14 –10</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>2. Right. Insula, Superior Temporal Gyrus (BA 22, 38)</td>
<td>590</td>
<td>0.000</td>
<td>42 0 –10</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>3. Right. Cingulate Gyrus (BA 24, 32)</td>
<td>651</td>
<td>0.000</td>
<td>6 16 32</td>
<td>0.137</td>
</tr>
<tr>
<td>Late pain - Early pain</td>
<td>1. Right. Inferior Occipital Gyrus (BA 18)</td>
<td>290</td>
<td>0.008</td>
<td>36 –96 –12</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>2. Left. Inferior Occipital Gyrus (BA 18); Middle Occipital Gyrus (BA 19)</td>
<td>301</td>
<td>0.007</td>
<td>-30 –88 –4</td>
<td>0.064</td>
</tr>
</tbody>
</table>
Table 2 (continued).

<table>
<thead>
<tr>
<th>Baseline - Late pain</th>
<th>Coordinates</th>
<th>Z-score</th>
<th>Cluster Size</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Right Cingulate Gyrus (BA 32,24); Medial Frontal Gyrus (BA 6,8,9)</td>
<td>813</td>
<td>0.000</td>
<td>12 24 32</td>
<td>0.048</td>
</tr>
<tr>
<td>2. Right Superior Temporal Gyrus (BA 22); Inferior Parietal Lobule (BA 40);</td>
<td>907</td>
<td>0.000</td>
<td>56 -42 18</td>
<td>0.048</td>
</tr>
<tr>
<td>3. Right Middle Frontal Gyrus (BA 9,46,10); Superior Frontal Gyrus (BA 9);</td>
<td>355</td>
<td>0.003</td>
<td>34 44 30</td>
<td>0.068</td>
</tr>
<tr>
<td>4. Left Cerebellum; Fusiform Gyrus (BA 37,19); Middle Occipital Gyrus (BA 19); Medial Temporal Gyrus (BA 39)</td>
<td>990</td>
<td>0.000</td>
<td>-32 -64 -6</td>
<td>0.085</td>
</tr>
</tbody>
</table>

*a SPMt threshold is at P<0.001 uncorrected and cluster size >200 voxels. Clusters were considered as significant if corrected p<0.01 at cluster level.

*b The stereotactic space is that defined in SPM software (MNI space).

39 and 40 respectively, and a smaller one located in the left (contralateral) hemisphere involving BA 22 and 40. The cluster with increased blood flow was located in the insula and superior temporal gyrus (BA 22 and 38) on the right (ipsilateral) side.

During the Late Pain condition, there was one large cluster with increased blood flow in the left frontal cortex (BA 8, 9 and 10) and five clusters with reductions in blood flow in comparison to Baseline. One of the clusters with reduced blood flow involved the right cingulate gyrus and frontal cortex (BA 6, 8, 9, 32 and 24); a second cluster involved the cerebellum and areas of temporal and occipital cortices (BA 19, 37 and 39); a third one involved the temporal and posterior PC (BA 22 and 40), a fourth one involved the frontal cortex (BA 9, 10, 46); and finally a fifth one involved the insula/lateral frontal cortex (BA 44, 45).

In the Post Pain condition there was only one cluster with a reduction in blood flow in the temporal and occipital cortex (involving BA 19, 37 and 39).

Changes in rCBF between Early Pain and Late Pain conditions

A decrease in rCBF was found during the Late Pain condition as compared with the Early Pain condition in three clusters; one comprising left insula, Inferior
Frontal (BA 47) and Superior Temporal (BA 47) cortex; the second comprising the right insula and temporal areas (BA 22, 38); and the third comprising the right cingulate gyrus (BA 24, 32). An increase in rCBF was found in the occipital cortex bilaterally (BA 18, the right hemisphere and BA 18; 19 the left hemisphere).

**Figure 4.** Selected contrasts of the study. The panels show three standard orthogonal views of the “glass” brain. The stereotactic space is that defined in the SPM software (MNI space), SPMt threshold is at P<0.001 uncorrected and cluster...
size >200 voxels. The left side of the image is the left side of the brain. See table 2 for exact anatomical localization of the selected clusters.

**Changes in rCBF in the control group**

Clusters of significantly increased rCBF due to non-pain related factors are presented in table 3. Changes in brain activity in left cerebellum and BA 37 were detected in contrast between scan 1-3 (corresponding to Baseline - Late Pain) and in contrast between scans 1-4 (corresponding to contrast Baseline - Post Pain).

Table 3. Significant changes in regional brain activity obtained in the contrasts of control group (cluster based analysis)

<table>
<thead>
<tr>
<th>Corresponding contrasts of pain study</th>
<th>Hemisphere, Areas activated (Brodmann area)</th>
<th>Cluster size (voxels)</th>
<th>( P_{\text{corrected}} ) for the cluster level</th>
<th>Coordinates ( x,y,z ) of local maxima</th>
<th>( P_{\text{corrected}} ) for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline - Late pain</td>
<td>Left, Fusiform Gyrus (BA 37); Cerebellum</td>
<td>544</td>
<td>0.000</td>
<td>-32 -44 -26</td>
<td>0.015</td>
</tr>
<tr>
<td>Postpain – Baseline</td>
<td>1. Right, Precentral Gyrus (BA 6);</td>
<td>374</td>
<td>0.001</td>
<td>+62 -12 +42</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>2. Left, Cingulate Gyrus (BA 23)</td>
<td>295</td>
<td>0.005</td>
<td>-14 -48 +24</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>3. Left, Precentral Gyrus (BA 6);</td>
<td>785</td>
<td>0.000</td>
<td>-42 -14 +34</td>
<td>0.056</td>
</tr>
<tr>
<td>Baseline – Postpain</td>
<td>Left, Fusiform Gyrus (BA 37); Cerebellum</td>
<td>392</td>
<td>0.001</td>
<td>-42 -58 -26</td>
<td>0.416</td>
</tr>
</tbody>
</table>

\( a \) SPMt threshold is (for both studies) at \( P<0.001 \) uncorrected and cluster size >200 voxels. Clusters were considered as significant if corrected \( p<0.01 \) at cluster level. 

\( b \) The stereotactic space is that defined in SPM software (MNI space).

**Paper III**

All 12 subjects reported feelings of flexion-extension movements throughout the trials with a high-frequency vibration stimulation (Vf). Nine subjects experienced strong, steady kinesthetic illusion of movement during the scan, which did not differ from the one elicited before the test, while in three subjects the illusion sensation was slightly weaker towards the end of the scan. None of the subjects experienced kinesthetic illusions during low frequency vibration (VN), passive movements (PM) or the resting (RE) conditions.
Changes in rCBF were located in the right (contralateral to the stimulated arm) hemisphere. Six contrasts were analyzed – PM-RE, VI-RE, VN-RE, PM-VN, PM-VI and VI-VN. Voxel-based analysis revealed significant rCBF changes in five contrasts: PM-RE, VI-RE, VN-RE, PM-VN and PM-VI (Table 4 and Fig. 5). The comparison of passive movement with other conditions (contrasts PM-VI, PM-VN, PM-RE) showed similar localizations of the rCBF increases in the MI (BA 4), SMA (BA 6), and SI (BA 1, 2, 3). In PM-RE and PM-VN contrasts, areas of increased rCBF extended to the SSA (BA 5).

Table 4. Significant increases in regional brain activity in response to different types of proprioceptive stimulation: passive movements, kinaesthetic illusion, vibration (voxel based analysis).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>( p_{\text{corrected}} ) for the cluster level</th>
<th>Coordinates ( ^b ) of local maxima (x y z)</th>
<th>( p_{\text{corrected}} ) for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM-RE</td>
<td>1. Right G.Postcentralis (BA 1,2,3) SI, G.Precentralis (BA 4) MI, G.Frontalis medialis (BA 6) SMA, Lobulus paracentralis (BA 5) SSA, 2. Right Lobulus parietalis inferior (BA 40) SII, G.Temporalis superior (BA 22, 42) AA, G.Temporalis transversi (BA 41) AI</td>
<td>1350</td>
<td>0,000</td>
<td>+12 -18 +54</td>
<td>0,000</td>
</tr>
<tr>
<td>VI-RE</td>
<td>1. Right G.Temporalis superior (BA 22, 42) AA, G.Temporalis transversi (BA 41) AI, Lobulus parietalis inferior (BA 40) SII</td>
<td>782</td>
<td>0,000</td>
<td>+52 -30 +22</td>
<td>0,000</td>
</tr>
<tr>
<td>VN-RE</td>
<td>1. Right Lobulus parietalis inferior (BA 40) SII</td>
<td>6</td>
<td>0,020</td>
<td>+46 -26 +22</td>
<td>0,033</td>
</tr>
<tr>
<td>PM-VI</td>
<td>1. Right G.Postcentralis (BA 1,2,3) SI, G.Precentralis (BA 4) MI 2. Right G.Frontalis medialis (BA 6) SMA</td>
<td>424</td>
<td>0,000</td>
<td>+44 -28 +58</td>
<td>0,000</td>
</tr>
<tr>
<td>PM-VN</td>
<td>1. Right G.Postcentralis (BA 1,2,3) SI, G.Precentralis (BA 4) MI, G.Frontalis medialis (BA 6) SMA, Lobulus paracentralis (BA 5) SSA</td>
<td>1787</td>
<td>0,000</td>
<td>+14 -16 +52</td>
<td>0,000</td>
</tr>
</tbody>
</table>

\(^a\) SPMt were thresholded at \( p<0.05 \) corrected

\(^b\) The stereotactic space is that defined in SPM software (MNI space).
In addition, in the **PM-RE** contrast, increases in rCBF were found in the SII (BA 40); the auditory cortex, primary, AI (BA 41); and the associative, AA (BA 22, 42). Vibration with illusion of movement (**VI**) contrasted with RE (contrast **VI-RE**) demonstrated increases in rCBF in SII (BA 40) and the AI (BA 41) and AA (BA 22, 42). In **VN-RE** contrast, an increase in rCBF was found in SII (BA 40) only.

**Figure 5.** Selected contrasts of the study. The panels show three standard orthogonal views of the “glass” brain. The stereotactic space is that defined in the SPM software (MNI space), SPMt threshold is at P<0.05 corrected. The left side of image is the left side of the brain. See table 4 for exact anatomical localization of the selected clusters.
As the voxel-based analysis revealed no supra-threshold clusters in \textit{VI-VN} contrast, the cluster-based analysis was applied for this comparison. In \textit{VI-VN} contrast, clusters of rCBF increases were found in the MI (BA 4); and the SMA (BA 6) (Table 4a and Fig. 6).

\textbf{Table 4a. Significant\textsuperscript{a} increases in regional brain activity obtained in cluster based analysis of \textit{VI-VN} contrasts.}

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>\textit{p}\textsubscript{corrected} for the cluster level</th>
<th>Coordinates \textit{b} of local maxima (x y z)</th>
<th>\textit{p}\textsubscript{corrected} for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI-VN</td>
<td>1. Right G.Precentralis (BA 4) MI, G.Frontalis medialis (BA 6) SMA</td>
<td>439</td>
<td>0.002</td>
<td>+14 –12 +54</td>
<td>0.099</td>
</tr>
</tbody>
</table>

\textsuperscript{a} SPM\textit{t} were thresholded at \textit{p}<0.001 uncorrected and cluster size >200 voxels; clusters with corrected \textit{p}<0.05 at cluster level were considered as significant.

\textsuperscript{b} The stereotactic space is that defined in SPM software (MNI space).

\textbf{Figure 6.} Contrast VI-VN of the study. The panels show three standard orthogonal views of the “glass” brain. The stereotactic space is that defined in the SPM software (MNI space), SPM\textit{t} threshold is at \textit{P}<0.001 uncorrected and cluster size >200 voxels. The left side of image is the left side of the brain. See table 4a for exact anatomical localization of the cluster.
F-statistic analysis (effect of interest) revealed three different clusters. These clusters are equivalent to those appearing in contrasts. See Table 5 for a detailed description of each particular cluster. Scattergrams ("fitted responses averaged on each cluster" – "conditions"), showing relative values of activation under different conditions, are given in Fig. 7. In these scattergrams two different patterns can be seen. In the first two diagrams (A and B), related respectively to the first two clusters located in SMA, SSA, SI and MI areas, a similar activation between RE and VN conditions was found. The third diagram (C), related to the cluster located in SII, AA and AI areas, shows a small difference in activation between PM and VI conditions.

Table 5. Significant\(^a\) differences between all conditions used in the study

<table>
<thead>
<tr>
<th>Localization of activated area (Brodmann area)</th>
<th>Cluster size</th>
<th>Coordinates of primary local maxima (in mm)</th>
<th>P corrected for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. G Frontalis medialis (BA 6) SMA&lt;br&gt;Loebulus paracentralis (BA 5) SSA</td>
<td>227</td>
<td>+12 –14 +52</td>
<td>0.000</td>
</tr>
<tr>
<td>2. G Postcentralis (BA 1,2) SI&lt;br&gt;G Precentralis (BA 4) MI</td>
<td>106</td>
<td>+32 –32 +56</td>
<td>0.000</td>
</tr>
<tr>
<td>3. Lobulus parietalis inferior (BA 40) SII&lt;br&gt;G Temporalis superior (BA 42) AA&lt;br&gt;G Temporalis transversi (BA 41) AI</td>
<td>124</td>
<td>+54 –26 +16</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\(^a\) SPMF were thresholded at \(p<0.0005\) (corrected);

\(^b\) The stereotactic space is that defined in SPM software (MNI space).
Figure 7. Scattergrams of “fitted responses averaged on each cluster” – “conditions” (see “Materials and methods” section for detailed description). Scattergram A represents cluster 1 in Table 5 – SMA and SSA; B represents cluster 2 of the same table – SI and MI areas; and C represents cluster 3 in Table 5 – SII, AA and AI areas

**EMG activity**

EMG records did not show additional activation in the majority of trials. However, occasionally low-level EMG activity was noticed. That was presented in the m. triceps and followed the passive movement. In two subjects, EMG activity was also noticed during the VI test. One could not exclude that this occasional low-level muscle activation might have caused cortical activation in the VI condition. However, when those two subjects were excluded from the analysis and the data recalculated, no differences in brain activation pattern were revealed. Therefore, the overall analysis included all 12 subjects.

**Paper IV**

Two min of the contraction at 50% of MVC effectively provided distinctive muscle fatigue. All twelve subjects were unable to maintain the required level of force, reaching only between 30 and 40% of the previously recorded MVC level instead. Also, all subjects reported that they were fatigued at the end of these
conditions, this confirming our expectations that the 50% MVC condition could be considered as fatiguing.

Six PET contrasts were analyzed – 50-RE, 50-CO, 50-30, 30-RE, 30-CO and CO-RE. Voxel-based analysis revealed significant rCBF changes in four contrasts: 50-RE, 50-CO, 50-30 and CO-RE (Table 6 and Fig. 8).

### Table 6. Significant increases in regional brain activity obtained in voxel based analysis of 50-RE, 50-CO, 50-30 and CO-RE contrasts.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>( p_{\text{corrected}} ) for the cluster level</th>
<th>Coordinates of local maxima (x y z)</th>
<th>( p_{\text{corrected}} ) for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-RE</td>
<td>1. Right: G.Postcentralis (BA 1,2,3) SI, G.Precentralis (BA 4) MI, Precuneus (BA 7), Lobulus parietalis inferior (BA 40) SII, Insula, G.Temporalis superior (BA 22) AA, G.Temporalis transversi (BA 41) AI, G.Temporalis medius (BA 21), Right and Left: G.Frontalis medialis (BA 6) SMA, Lobulus paracentralis (BA 5, 7) SSA, GCinguli (BA 24, 31)</td>
<td>4800</td>
<td>0.000</td>
<td>( +28 -36 +58 )</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2. Right: Lobulus parietalis inferior (BA 40), G.Temporalis superior (BA 42, 22) AA,</td>
<td>148</td>
<td>0.000</td>
<td>(+68 :-36 +28)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>3. Left: G.Temporalis superior (BA 22) AA,</td>
<td>57</td>
<td>0.001</td>
<td>(-48 :-16 -2)</td>
<td>0.010</td>
</tr>
<tr>
<td>50-CO</td>
<td>1. Right: Insula, G.Temporalis superior (BA 22) AA, G.Temporalis transversi (BA 41) AI</td>
<td>611</td>
<td>0.000</td>
<td>(+42 -24 +8)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2. Right and Left: GCinguli (BA 24, 32, 33)</td>
<td>141</td>
<td>0.000</td>
<td>(+2 +20 +26)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>3. Right: Lobulus parietalis inferior (BA 40), G.Supramarginalis (BA 40)</td>
<td>57</td>
<td>0.001</td>
<td>(+68 -34 +34)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

table is continued on the next page
Table 6 continued.

| 4. Right and Left: G.Frontalis medialis (BA 6) SMA, G.Cinguli (BA 24, 31) | 1842 | 0.000 | 0 –10 +46 | 0.000 |
| 5. Right: G.Postcentralis (BA 1,2,3) SI, G.Precentralis (BA 4) MI, Lobulus parietalis inferior (BA 40) | 550 | 0.000 | +36 –32 +56 | 0.000 |
| 50-30 | 1. Right: Lobulus paracentralis (BA 5) SSA, R: G.Cinguli (BA 32), Right and Left: G.Frontalis medialis (BA 6) SMA, G.Cinguli (BA 24, 31) | 1277 | 0.000 | +2 –6 +42 | 0.000 |
| 2. Right: G.Postcentralis (BA 3) SI, G.Precentralis (BA 4) MI, | 269 | 0.000 | +32 –32 +58 | 0.005 |

a SPMr were thresholded at p<0.05 corrected. Only clusters of volume > 10 voxels are given.

b The stereotactic space is that defined in SPM software (MNI space).

The comparison of the 50 condition with other conditions (contrasts 50-RE, 50-30, and 50-CO) showed similar bilateral localization of rCBF increases in the SMA (BA 6), and g. cinguli (BA 24, 31). Contralateral activation was only found in a portion of the SI (BA 3) area and the MI (BA 4) area.

In addition, portions of the SI (BA 1, 2), the SII (BA 40), and of BA 40 not included in SII, the AA (BA 22) and the AI (BA 41) areas were activated only in the right (contralateral) hemisphere, when comparing the 50 condition with conditions without fatigue, i.e. RE and CO. The right insula was also activated only in contrasts 50-RE and 50-CO (see table 6 for the detailed description of each particular cluster). Contralateral activation only was also seen in the CO-RE contrast, and included the g. lingualis (BA 18), the cuneus (BA 17) and the g. cinguli (BA 23, 30).
Differences between the RE condition and the 30 condition were significant only at the cluster level. The areas activated were the g. lingualis (BA 18, 19) and the cuneus (BA 17). Also, significant differences were found in the SI (BA 2, 3) and the parietal lobulus (BA 40). All activations were contralateral (see table 6a and Fig. 9). Contrast 30-CO did not reveal statistically significant differences, both at voxel and cluster level.
Table 6a. Significant increases in regional brain activity obtained in 30-RE contrasts. (cluster based analysis).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>p_corrected for the cluster level</th>
<th>Coordinates b of local maxima (x y z)</th>
<th>p_corrected for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-RE</td>
<td>1. Right: G.Lingualis (BA 18, 19), Right and Left: Cuneus (BA 17)</td>
<td>1313</td>
<td>0.000</td>
<td>-4 -84 +14</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>2. Right: G.Postcentralis (BA 2,3) SI, Lobulus parietalis inferior (BA 40)</td>
<td>548</td>
<td>0.000</td>
<td>+34 -38 +50</td>
<td>0.218</td>
</tr>
</tbody>
</table>

a SPMt were thresholded at p<0.001 uncorrected; clusters with corrected p<0.001 at cluster level were considered as significant. Only clusters of volume > 200 voxels are given.

b The stereotactic space is that defined in SPM software (MNI space).

Figure 9. Contrast 30-RE of the study. The panels show three standard orthogonal views of the “glass” brain. The stereotactic space is that defined in the SPM software (MNI space), SPMt threshold is at P<0.001 uncorrected and cluster size >200 voxels. The left side of image is the left side of the brain. See table 6a for exact anatomical localization of the selected clusters.
Analysis with “effect of interest” revealed three clusters. The localization of these clusters was similar to those appearing in contrasts (see Table 7 for the detailed description of each particular cluster).

Table 7. Significant\(^a\) differences between all conditions used in the study

<table>
<thead>
<tr>
<th>Localisation of activated areas (Brodmann area)</th>
<th>Cluster size</th>
<th>Coordinates (^b) of primary local maxima (in mm)</th>
<th>(P) corrected for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Right and Left: G. Frontalis medialis (BA 6) SMA, G. Cinguli (BA 24, 31), Right Lobulus paracentralis (BA 5) SAA,</td>
<td>1020</td>
<td>+8, –12, +50</td>
<td>0.000</td>
</tr>
<tr>
<td>2. Right: G. Postcentralis (BA 1, 2, 3) SI, G. Precentralis (BA 4) MI, Lobulus parietalis inferior (BA 40)</td>
<td>559</td>
<td>+36 –34 +58</td>
<td>0.000</td>
</tr>
<tr>
<td>3. Right: G. Temporalis superior (BA 22)AA, G. Temporalis transversi (BA 41) AI, Insula</td>
<td>18</td>
<td>+42 –24 +4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\(^a\) SPMF were thresholded at \(p<0.0005\) (corrected);\n\(^b\) The stereotactic space is that defined in SPM software (MNI space).

A scattergram of fitted responses averaged on each cluster versus conditions is given in Fig. 10. In this scattergram, the level of rCBF gradually increases from the \(RE\) condition to the \(CO\), 30 and 50 conditions for all three clusters.
Figure 10. Scattergram of fitted responses averaged on each cluster versus conditions (see the “Methods” section for a more detailed description). Filled circles correspond to cluster 1 in Table 6: SMA, SSA and g. cinguli (BA 24, 30); empty circles correspond to cluster 2 in Table 7: SI, MI and BA 40; filled squares correspond to cluster 3 in Table 7: AI, AA and insula.

EMG activity

During contractions the RMS amplitude of the EMG increased in both muscles gradually reaching its maximal level towards the end of the contraction, thereby indicating recruitment of additional motor units over time in both muscles. However, when we compared normalized RMS values of the EMG of both muscles at the end of each condition, we found that while RMS of m. brachioradialis amounted to 52% of the m. biceps in CO condition, it reached 40% in 30 condition and 35% in 50 condition. This indicates that while activation of both muscles increased, the activation of m. brachioradialis did not increase to the same extent as m. biceps. Thus, the prolonged fatiguing 50% MVC contraction was maintained...
predominantly at the expense of additional m. biceps activation. Although, the relative contribution of the co-activation of hand and wrist flexor muscles to PET signal could not be avoided, in a fatigue condition it was probably minor compared to m. biceps, as estimated by changes in EMG signal.

**Paper V. Results of the conjunction analysis of data obtained in Study I and II**

Coordinates and sizes of significant clusters in contrasts Pain-Baseline2 (Study 1) and Early pain-Baseline (Study 2) and their mirror contrasts selected for conjunction analysis, are presented in Tables 8a and 8b respectively.

<table>
<thead>
<tr>
<th>Table 8a. Significant activation obtained for selected contrasts in Study I.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contrast</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Pain-Rest 1.</td>
</tr>
<tr>
<td>Pain-Rest 2.</td>
</tr>
<tr>
<td>Rest – Pain 1.</td>
</tr>
<tr>
<td>Rest – Pain 2.</td>
</tr>
</tbody>
</table>

*For cluster analysis, an uncorrected threshold $p<0.001$ was used, and clusters were taken into consideration if corrected $p<0.01$ and voxel size $>200$.

*Coordinates of the local maxima are given in stereotactic space that defined in SPM software (MNI space).*
Table 8b. Significant activation obtained for selected contrasts in Study II.

<table>
<thead>
<tr>
<th>Contrast (Study)</th>
<th>Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>( p_{\text{corrected}} ) for the cluster level</th>
<th>Coordinates ( b ) of local maxima ((x \ y \ z))</th>
<th>( p_{\text{corrected}} ) for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pain – Rest</td>
<td>Right. Insula, Superior Temporal Gyrus (BA 22, 38)</td>
<td>387</td>
<td>0.002</td>
<td>+42 -4 -8</td>
<td>0.039</td>
</tr>
<tr>
<td>Rest – Early pain</td>
<td>1. Right. Fusiform Gyrus (BA 37); Inferior Temporal Gyrus (BA 37); Medial Temporal Gyrus (BA 21, 39); Medial Occipital Gyrus (BA 19); Superior Temporal Gyrus (BA 22); Inferior Parietal Lobulus (BA 40)</td>
<td>2295</td>
<td>0.000</td>
<td>+46 -56 -12</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2. Left. Superior Temporal Gyrus (BA 22); Inferior Parietal Lobulus (BA 40)</td>
<td>275</td>
<td>0.010</td>
<td>-46 -42 +30</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>3. Left. Fusiform Gyrus (BA 37, 19); Medial Occipital Gyrus (BA 18, 19); Inferior Temporal Gyrus (BA 37); Inferior Occipital Gyrus (BA 18); Lingual Gyrus (BA 18, 19);</td>
<td>2529</td>
<td>0.000</td>
<td>-36 -66 -8</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\( a \) For cluster analysis, an uncorrected threshold \( p<0.001 \) was used, and clusters were taken into consideration if corrected \( p<0.01 \) and voxel size >200.

\( b \) Coordinates of the local maxima are given in stereotactic space that defined in SPM software (MNI space).

The results of conjunction analysis are presented in Table 9 and Fig. 11, giving coordinates and sizes of the three areas that showed statistically significant changes in rCBF irrespective of features of the experimental series.

Data show a cluster of increase of rCBF during pain located in the right insula and two clusters of decrease of rCBF located in the right inferior temporal gyrus (BA 37) and in left inferior parietal lobule (BA 40).
Table 9. Clusters with significantly\textsuperscript{a} increased activity during both HS injection into the m. triceps and HS infusions into m. erector spinae. Results of the conjunction analysis.

<table>
<thead>
<tr>
<th>Hemisphere, Areas activated \textsuperscript{a} (Brodmann area)</th>
<th>Cluster size (voxels)</th>
<th>Coordinates\textsuperscript{b} of local maxima (x y z)</th>
<th>( P ) corrected for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase of rCBF Right. Insula</td>
<td>191</td>
<td>+42 -2 -6</td>
<td>0.000</td>
</tr>
<tr>
<td>Decrease of rCBF 1. Right. Inferior Temporal gyrus (BA 37)</td>
<td>226</td>
<td>+46 -64 -12</td>
<td>0.000</td>
</tr>
<tr>
<td>2. Left. Posterior Parietal lobule, (BA 40)</td>
<td>68</td>
<td>-40 -46 +48</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\textsuperscript{a} SPM\textsubscript{t} were thresholded at \( p<0.05 \) corrected. Only clusters that have analogue in results of study 1 and 2 are presented.

\textsuperscript{b} Coordinates of the local maxima are given in stereotactic space that defined in SPM

Figure 11. Parameter estimates and their standard errors built for local maximum of cluster detected in conjunction analysis in right insula (upper panel) and in right Inferior Temporal gyrus (BA 37) and left Posterior Parietal lobule, (BA 40) (lower panel). X axis - conditions from Study 1 (RE1, RE2-rest, NL-needle, P1-pain) and Study 2 (BL - Baseline, EP - Early Pain, LP - Late pain, PP - Postpain). Y axis – size of effect.
Results of Conjunction analysis of data obtained in Study I and III, and in Study I and IV

Conjunction analysis of data obtained in study I and III as well as in study I and IV are presented in Tables 10 and 11 (see also Fig. 12) respectively revealed a cluster located near the lateral sulcus of the right (contralateral to side of stimulation) hemisphere and comprised of SII, the posterior part of insula and the AI and AA (BA 21, 42 respectively).

Table 10. Clusters with significantly\(^a\) increased activity during both HS injection into the m. triceps and different types of proprioceptive stimulation of triceps-biceps muscle group. Results of the conjunction analysis.

<table>
<thead>
<tr>
<th>Contrasts included into the conjunction analysis. Localisation of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>Coordinates(^b) of primary local maxima (in mm)</th>
<th>(P) corrected for the voxel level</th>
</tr>
</thead>
</table>
| Pain-Rest (I) and PM-Rest (III)  
1. Right: Insula, G.Temporalis superior (BA 22) AA, G.Temporalis transversi (BA 41) AI, Lobulus parietalis inferior (BA 40) SII | 335 | +44 -28 +20 | 0.000 |
| Pain-Rest (I) and VI-RE (III)  
1. Right: Insula, G.Temporalis transversi (BA 41) AI, Lobulus parietalis inferior (BA 40) SII | 225 | +44 -28 +20 | 0.000 |
| Pain-Rest (I) and VN-RE (III)  
1. Right: Insula, G.Temporalis superior (BA 22) AA, | 23 | +72 -36 +28 | 0.007 |

\(^a\)SPM\(t\) were thresholded at \(p<0.05\) corrected  
\(^b\) Coordinates of the local maxima are given in stereotactic space that defined in SPM
Table 11. Clusters with significantly\textsuperscript{a} increased activity during both HS injection into the m. triceps and after fatiguing muscle contraction. Results of the conjunction analysis

<table>
<thead>
<tr>
<th>Contrasts included into the conjunction analysis. Location of activated areas, (Brodmann areas)</th>
<th>Cluster size (voxels)</th>
<th>Coordinates\textsuperscript{b} of primary local maxima (in mm)</th>
<th>( P ) corrected for the voxel level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain-Rest (Study I) and 50-Rest (Study IV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Right: Insula, G.Temporalis superior (BA 22) AA, G.Temporalis transversi (BA 41) AI, Lobulus parietalis inferior (BA 40) SIH</td>
<td>554</td>
<td>+40 -20 +4</td>
<td>0.000</td>
</tr>
<tr>
<td>2. Left: Cingulate gyrus (BA 24)</td>
<td>9</td>
<td>-8 -8 +42</td>
<td>0.018</td>
</tr>
</tbody>
</table>

\textsuperscript{a}SPM\textsubscript{t} were thresholded at \( p<0.05 \) corrected

\textsuperscript{b} Coordinates of the local maxima are given in stereotactic space that defined in SPM

\textbf{Figure 12.} Cluster with significantly increased activity during both HS injection into the left m. triceps and passive movement of the left forearm. The panels show three standard orthogonal views of the “glass” brain. The stereotactic space is that defined in the SPM software (MNI space), SPM\textsubscript{t} threshold is at \( p<0.05 \) corrected. The left side of image is the left side of the brain. See table 10 for exact anatomical localization of the selected clusters
DISCUSSION

Experimental imaging studies have investigated neuro-anatomy of different pain components in the medial and lateral pain systems in the brain. However, these studies mainly employed cutaneous stimulation. On the other hand, interrelations between sensor-discriminative, emotional-affective and cognitive-evaluative components of muscle and skin pain are different, that might lead to a distinction in brain processing of these two pain modalities. Therefore, application of nociceptive stimulation specific for muscle is highly relevant. Application of acute or short lasting repeated pain stimuli are typical for imaging experiments, because possibility of repetitive trials and their randomisation. However, short application of painful stimulus does not allow the following of changes of brain activity during different stages of pain – initial, sub-acute, residual etc, whereas long lasting pain stimuli may provide valuable information about this dynamics. Finally, since muscle pain modulates motor functions and co-ordination, and in turn, motor activity has an influence on the perception of muscle pain, comparative analysis of different experiments could delineate the functional role of brain structures involved in these processes.

The interpretations of increases and decreases of rCBF

Our studies on the effects of experimental muscle pain induced by i.m. injection of HS revealed both increases and decreases of rCBF during muscle pain.

Increased neuronal activity leads to increased energy metabolism and augmentation of CBF (Fox et al. 1987). Hence increase of rCBF detected by means of comparison of two conditions mirrors increased level of activity (i.e. activation) of a particular brain area not discerning between processes of excitation and inhibition. A decrease in rCBF, on the other hand, is commonly explained by a depression of synaptic activity, i.e., deactivation (Iacoboni et al 1999). From another point of view such a decrease may indicate suppression of a remote excitatory processes or autoregulatory mechanism for global CBF that does not reflects synaptic activity (Casey 1999).

Thus, changes of rCBF indicate just changes of activity of the certain structure and using task-control protocol only indicates localised reaction to different stimuli.
**Functional neuroanatomy of HS induced muscle pain (Papers I, II and V)**

**Increase of activity during muscle pain**

Increase of activity during muscle pain (Study 1 and 2) was detected in insula, putamen, adjacent temporal cortex (BA 22, 38) and frontal cortex (BA 8, 9, 10). Conjunction analysis of rCBF data pooled from the two studies indicated that statistically significant increase of rCBF in the right insula was observed independently on stimulated muscle.

All areas activation of which was detected in our studies (paper I, II and V) were previously described as elements of neuroanatomical system underlying processing of pain (Apkarian et al 1995, Casey 1999, Peyron et al. 1999, Treede et al.1999, Bromm 2001, Porro et al 2003). However, our data suggested that HS induced muscle pain is associated mainly with activation of structures traditionally attributed to the medial nociceptive system, since thalamo-cortical projections of the lateral system (e.g. SI/SII) did not show statistically significant reactions of rCBF. Apart from methodological limitations due to spatial resolution, this fact could be explained by relatively poor sensory-discriminative component of muscle pain in comparison with skin pain.

Existence of such differences was not statistically proved previously (Svensson et al. 1997, Niddam et al. 2002). Svensson and co-authors (1997) comparing changes of rCBF during painful electrical stimulation of skin and muscle did not detect statistically significant differences while described tendency to higher level of activation of premotor area, SII and PFC during skin pain. Electrical stimulation of muscle with parameters that induces intensive but not painful or moderately painful sensations was used by Niddam et al. (2002). Muscle pain was associated with activation of the SMA, the supramarginal and superior temporal gyri, precuneus, posterior cingula, insula, thalamus and caudate. In comparing these results with the literature the authors made the conclusion about similarity of neuroanatomic systems underlying skin and muscle pain.

Recently Kupers et al. (2004) published data that suggested differences between cerebral processing of muscle and skin pain. This PET study revealed that experimental jaw-muscle pain was associated with activation of the dorsal-posterior insula (bilaterally), ACC and PFC, right posterior PC, brainstem, cavernous sinus and cerebellum while no rCBF changes occurred in SI or SII and thalamus.

In our opinion such disagreement is the result of the particulars of different methods of pain induction used in neuroimaging studies. Electrical stimulation
which is widely used in neuroimaging research of pain, has several incontestable advantages - the level of pain and its duration are easily controlled, the stimulation of different localization is available in frames of one study, and it evokes no “aftereffects”. Nonetheless electrical stimulation bypasses the receptor transduction and depolarises the afferent fibres directly. Thick myelinated (non-nociceptive) afferents are activated at lower stimulus intensities than unmyelinated fibres, therefore, electrical stimuli are not specifically nociceptive. Activation of large diameter afferents could possibly induce activation of cerebral structures with an important role in the sensory-discriminatory aspect of pain.

The specificity of HS induced pain model applied in our studies, as well as in the study of Kupers et al. (2004) is a rather isolated activation of muscle group III afferents in response to injected substances since subjects experienced a dull, strain painfullnes, which was moderate in intensity and reminiscent of deep somatic muscle pain.

Study I showed that experimental muscle pain in the left m. triceps elicited by i.m. injection of HS induces significant increases of rCBF in clusters comprised of the contralateral insula, superior temporal cortex and putamen. Although the Needle condition cannot be considered as a complete ‘placebo’, the Pain vs. Needle and Needle vs. Pain contrasts provide relevant information about brain regions activated by experimental HS-induced muscle pain since it excludes the cutaneous pain component induced by skin stimulation.

Disturbances in motor control are common in acute and chronic muscle pain. It has been shown that HS injections closely mimic clinical musculoskeletal pain both in subjectively perceived quality as well as in its effects on motor performance (Arendt-Nielsen et al. 1996). The cluster of pain-related activation (in the Pain–Needle contrast) included the putamen, an important element of the motor control system (Jueptner et al. 1998). The subconscious control of skeletal muscle tone and the coordination of learned movement patterns is considered the main functional role of the putamen as a part of the extrapyramidal system. However, the role of central structures in the interplay between muscle pain and motor-control derangement remains to be established. The results of this and previous studies dealing with the central processing of pain indicate that there might be an interaction also on central levels. It is important to recognize that the links between experimental muscle pain and changes in motor control may not be the same in clinical conditions with chronic muscle pain.

Changes in rCBF within medial PFC following experimentally induced pain in normal subjects have been shown in several PET studies especially if tonic, or continuos, pain was used as a stimulus (for review, see Peyron et al. 2000). Further, hyperactivity in medial PFC (and insula) in response to painful thermal
stimulation was found in an fMRI study on patients with sympathetically mediated chronic pain (Apkarian et al. 2001). In PET studies, increases of rCBF in medial PFC have been found in a patient with chronic facial pain (Kupers et al, 2000), patients with neuropathic pain (Hsieh et al 1995), and following opioid anaesthesia (Petrovic et al. 2002).

Derbyshire and co-workers (Derbyshire et al 1994) examined patients with atypical facial pain, and found decreases in rCBF in the PFC, and increases of rCBF in the ACC. They hypothesise about reciprocal (possibly inhibitory) connections between PFC and ACC in the processing of pain in patients with this disorder. Our data support the possible existence of reciprocal relationships between the PFC and ACC. In the present study, activation of medial PFC was associated with inhibition of the right insula, right ACC (BA 24,32) and lateral frontal cortex in LP vs. BL contrast. However changes of rCBF obtained in the LP-BL contrast might be related to some uncontrolled factors in our study (e.g. a decrease of anticipation of pain during the course of the study).

Several particulars of the design of our study should be noted. We used visual task to standardize the state of subjects during the PET study. Previously it was shown that distraction of attention from painful stimulation might diminish both subjective ratings of pain and brain response to pain (Peyron et al. 1999, Bantick et al. 2002) and may lead to false negative results (namely lack of activation in SI or thalamus). However, in study of Kupers et al. (2004) activation of primarily medial nociceptive system was revealed without distraction of attention of subjects. Pain intensity registered in the above cited study was higher then in ours.

**Decrease of activity during muscle pain**

Areas of rCBF decrease were detected during HS induced muscle pain both in m.triceps and m.erector spinae (Papers I and II). Conjunction analysis of selected contrasts from two different studies in this thesis identified brain areas equally responding to HS induced muscle pain irrespective of localization of stimulation, i.e. rCBF decrease in the left PC (BA 40) and right occipital (BA 37) cortex.

Engagement of PPC in pain processing is in accord with a number of experimental pain studies (Talbot et al. 1991, Svensson et al. 1997, Peyron et al. 1999) as well as with data obtained on patients with neuropathic pain (Hsieh et al. 1995, Petrovic et al. 1999). In most of those studies increase of activity was observed. The acknowledged participation of PPC in maintenance of attention, working memory, goal oriented processes, suggest that its activation might mediate
cognitive dimension of pain processing associated with encoding of attended stimulus (Peyron et al. 1999). Furthermore, enhanced activation of the PPC is associated with enhanced vigilance and attentiveness to the sensory (e.g. nociceptive) afferentation that accompanied chronic neuropathic pain (Hsieh et al. 1995).

According to our data HS induced muscle pain leads to an opposite effect i.e. decrease of rCBF. This type of reaction in the PC was reported in studies with experimentally induced pain (Vogt et al. 1996) and clinical observation (Rosen et al. 1994), and was interpreted as a possible indication of disengagement of these areas for attention and visually guided processes due to nociceptive stimulation. This unusual reaction characteristic in the PPC might indicate certain peculiarity of cognitive-evaluative component of induced muscle pain. This assumption however should be taken with cautious because of the different opinions on the nature of rCBF decrease (local inhibitory processes or suppression of a remote excitatory processes or autoregulatory mechanism for global CBF).

Decrease of rCBF was also detected in the occipito-temporal cortex (BA 37). The possible role of this area in the pain processing remains unclear. Svensson et al. (1997) noted that one of the common features for skin and muscle pain in their experiments was significant deactivation in the primary and secondary visual cortex, but the relation of this finding to pain was unclear. Two possible mechanisms of rCBF decrease in occipito-temporal cortex can be proposed. First, it might indicate redistribution of blood flow from initially activated visual projection areas, since during all scans including baseline, subjects performed visual task. Second, changes in this area may have pain-independent character reflecting either changes in default mode of brain function (Raichle et al. 2001) or task-independent effect of time (Rajah et al. 1998).

Thus results presented in papers I, II and V indicate that experimental HS induced muscle pain led to bilateral brain response, and the pattern of brain reaction to muscle pain comprised of areas with increased as well as decreased rCBF. Our results showed involvement of multifunctional brain areas engaged in decision making, selective attention and motor control in maintenance of muscle pain perception and suggested involvement primarily medial nociceptive system in maintenance of muscle pain perception.
Reorganisation of functional neuroanatomic system underlying muscle pain due to habituation to acute muscle pain (Paper II)

Results obtained in Paper II showed that at the beginning of HS infusion changes in rCBF indicate an activation of the ipsilateral insula and massive bilateral deactivation in the occipito-temporal cortex. Scans recorded 15 min later on the other hand, indicated a decrease of rCBF in the left insula, inferior frontal (BA 47) and superior temporal (BA 47) cortex, the right insula and temporal areas (BA 22, 38) and right cingulate gyrus (BA 24, 32), and an increase in occipital cortex bilaterally (BA 18 of right hemisphere and BA 18 and 19 of left). Thus, it is apparent that the cerebral response to experimental muscle pain differs in the acute and tonic phases.

The LP scan was performed 15 min after EP, when the subjective pain level gradually decreased despite the continuation of HS injection. Hence, we expected the functional state of the brain areas involved in habituation to tonic muscle pain to be different. Indeed the pattern of rCBF during the LP scan was different from that found during the EP scan. In the contrast EP vs. LP, there was a bilateral increase of rCBF in the occipital cortex and decreases in rCBF in the bilateral insula and ipsilateral ACC. The increase of rCBF in the occipital cortex is possibly related to a functional redistribution of blood flow in the opposite direction as compared to the BL vs. EP contrast following habituation to pain and hence a "refocusing" on the visual task.

The inactivations of the ipsilateral ACC and bilateral insula might be associated with a functional adaptation to pain following a cognitive evaluation of the experimental pain condition. ACC had been shown to encode affective component of pain (Rainville et al 1997) and response selection following a stimulus (Treede et al 2000).

Attenuation of signal during repeated noxious heat stimulation was observed in a study of Becerra et al. (1999). In this study noxious stimulation was organised in four 29 sec sessions. A decrease of level of activation was observed in the last 2 sessions in the frontal gyri, the cingulate gyrus, the insula, the medial temporal gyrus and the precentral gyrus. The issue of a differential pattern of brain activation to initial and lasted experimental pain was put forth in study of Petrovich et al. (2000), however those study implemented comparatively shorter (1-2 min) time slots.
Limitations of the study design

To study the effects of muscle pain induced by i.m. injection of HS we used a non-randomised design in Study I to avoid possible ‘post-pain’ effects (the continuous feeling of heaviness in the muscle injected with HS after the cessation of pain, and the possible sensitisation of peripheral receptors by HS, Mense et al. 1993), and in Study II in accordance with its goal – to study habituation to muscle pain. It has been reported that with scan repetitions certain brain areas could be activated or deactivated because of non-specific time effect due to habituation, learning or tiredness (Paus, 1997; Rajah, 1998). Such task-independent effects may have monotonous or nonmonotonous character of changes through the sequence of scans. In Study I no clusters showed such a character of changes. Only one cluster (BA 19,37) in study II showed a monotonous decrease in rCBF from BL to PP conditions. These changes in rCBF reached statistical significance only in BL vs. PP contrasts. The coordinates of this cluster partly overlapped with a larger cluster in BL vs. LP contrasts. Therefore, conclusions about the engagement of the cluster observed in the contrasts BL vs. LP in pain processing must be made with caution, since possible effects related to habituation, learning or tiredness might be involved. In this context, the activation of the insula found in both studies supports the view that the insula indeed increases its activity because of experimental muscle pain, since this structure has been shown to decrease its activity as a result of the non-specific time-effect.

Additional analysis was performed to detect nonmonotonous rCBF changes related to non-specific time-effect. We analyzed respective contrasts of a consecutive scans in control group of subjects, that performed a similar distracting task, but without exposition to experimental muscle pain (see Paper V for the details). Clusters located in the left cerebellum and BA 37 were found in contrasts corresponding to the contrasts Baseline-Late Pain and Baseline-Post-pain.

As both analyses revealed clusters in the left cerebellum and temporal lobe (BA 37), judgments about possible engagement of these areas in the processing of tonic muscle pain have no experimental support in our study.

Another limitation of the study design is related to possible co-effects of mechano-stimulation due to i.m. infusion, especially taking into account that infusion volume gradually increased during the experiment. Such non-nociceptive mechano-stimulation may contaminate the cerebral activation patterns during the EP and LP phases. In this context application of the isotonic saline with the same increment of infusion rate would be relevant control to differentiate between pain-related reactions and effects of mechanostimulation. One may mention, however, that changes in brain activity due to injection of isotonic saline were significantly weaker in comparison with HS induced pain in other studies (Zubieta et al. 2002;
Chang et al. 2003). The appearance of distinctive painful sensations during the repeated injection of isotonic saline also was highlighted (Chang et al. 2003).

Thus, data presented in paper II show that initial and late phases of tonic experimental muscle pain are associated with different patterns of brain response, namely initial increase of rCBF in the insula followed by a significant bilateral decrease of rCBF at the late stage.

**Functional neuroanatomic system underlying processing of proprioceptive information (Paper III)**

The involvement of MI (BA 4), SMA (BA 6), SI (BA 1, 2, 3), SSA (BA 5), and SII (BA 40) in processing of proprioceptive information as revealed in our study, is in accord with previous literature (Burton et al. 1993, Weiller et al. 1996, Mima et al. 1999, Naito et al. 1999).

An unexpected finding deserving particular attention, especially in context of the current theses was that in some of the conditions (PM and VI contrasted vs RE), the primary auditory cortex (BA 41) and the auditory association cortex (BA 22, 42) in the contralateral temporal lobe were activated. This activation cannot be regarded as a response to noise. First, auditory stimuli activate the auditory cortex bilaterally, while we saw only unilateral activation in the right hemisphere. Second, during the tests, the motor remained switched on during all conditions, including rest, and in this situation subtraction as a step in data processing eliminates all consequences of noise. It appears more likely that it occurred due to interactions between the somatosensory and auditory systems. Evidence for such interactions was previously adduced in several studies (Makeig et al. 1996; Levanen et al. 1998; Jousmäki and Hari 1999; Foxe et al. 2000), where somatosensory stimulation evoked responses not only in SI and SII, but also in the temporal cortex adjacent to the sulcus lateralis, as was the case in our study. Previously, Roland (1982) demonstrated the existence of similar interactions with the $^{133}\text{Xe}$ intracarotid technique. Altogether, these data indicate that the auditory cortex might be involved in processing afferent information and contribute to somatosensory integration processes. In our study, the auditory cortex was activated only in conditions where the sense of movement existed, irrespective of the existence of real movement, i.e., in passive and vibratory illusion conditions. No auditory cortex activation occurred in the $VN$ condition.

Both the perception of passive movements and the perception of illusory movements induced by tendon vibration activated area in the PC and temporal cortex (SII, AA, AI). The comparison of activation intensities under different
functional conditions suggested that SII is involved mainly in stimulus perception
generation and areas SI/MI and SMA in the processing of proprioceptive input.

Thus, analysis of rCBF during passive movements and tendon vibration
elicited and not elicited illusion of movements revealed involvement of primary
and associative auditory cortex in processing of proprioceptive information and
suggested that SII is involved mainly in stimulus perception generation while areas
SI/MI and SMA – mainly in the processing of proprioceptive input.

**Brain activations after sustained muscle contractions of different levels and durations (Paper IV)**

In this study we observed after-effects of muscle contractions on brain
activation by comparing changes in brain activation after sustained contractions of
different levels and durations while the subjects were relaxed. This should enable
the detection of more subtle changes in brain activation encompassing the
complexity of the cortical processes, not being overridden, at the same time, by
motor cortex activation “per se”. However, while it is almost certain that afferent
discharges differed between different conditions, the exact changes in post-
contraction sensory afferent activity in our study cannot be estimated, particularly
when comparing contractions of different intensity, duration and metabolic status
(i.e. fatigue).

Previous studies have shown that muscle fatigue evokes prolonged post-
contraction group III and IV muscle afferent discharges, while their activation was
shown to be dependent on the intensity of muscle contraction and metabolic status
(Decherchi et al. 1998; see also Gandevia 1998). Thus, we can only assume that
stronger and longer contractions in our study were associated with increased group
III and IV muscle afferent inflow. It is not surprising therefore, that contrasting 30-
CO did not reveal statistically significant differences, at both voxel and cluster
levels. Furthermore, comparisons of the conditions CO and 30 to the RE condition,
contrasts CO-RE and 30-RE, show almost identical activation in the respective
brain areas, arguing for the similarity of those conditions concerning brain activity.
As compared to contrasting CO-RE, contrasting 30-RE showed an additional
cluster in the SI area. This difference might be due to the fact that condition 30 had
a contraction time twice as long as condition CO, the longer contraction time
evoking different, probably increased afferent inflow from the contracting muscle.
Some earlier studies suggest that during dynamic hand contraction, neural input
from muscle spindles or metabolically sensitive nerve fibers is not required for
rCBF increase (Williamson et al. 1996). However, the classical findings about
subjects mismatch in the estimation of force during muscle vibration (McCloskey
et al. 1974; Jones and Hunter 1985) suggest that peripheral information from group I muscle spindle afferents plays a role in force estimation. Therefore, we believe that at least some of the changes in brain activation are consequences of increased afferent input from the periphery as post-contraction afferent discharges, from group III and IV afferents (Decherchi et al. 1998; for reviews see Gandevia 1998; Taylor et al. 2000) or from muscle spindle afferents (Gilhodes et al. 1992).

The activation of the contralateral insula during increased effort is in line with earlier findings implicating a role for the insula during exercise (Williamson et al. 1997; Zamrini et al. 1990). The rCBF was significantly increased in the contralateral insula during active exercise without such changes in the ipsilateral insula (Williamson et al. 1997). It was suggested that the insula could be involved in cortical cardiovascular control and regulation of the autonomic activity. This has also been found in earlier animal studies (Oppenheimer et al. 1992). The magnitude of insular activation varied with the intensity of perceived effort and, hence, of central commands (Williamson et al. 1999).

Activation of temporal areas BA 22, 41, 42, which only occurs in the 50 condition, have been described earlier in connection with processing of afferent information and somatosensory integration processes (Szczepaniak and Moller 1993; Makeig et al. 1996; Foxe et al. 2000; Radovanovic et al. 2002). It might have played a similar role in the 50 condition that involves the strongest activations and effort.

Additional analysis revealed three separate clusters: one comprising the MI and SI, the second comprising the SMA and SAA, and the third comprising the insula as well as temporal areas (AA/AI). The activity in all clusters increased proportionally with increasing force level and duration of contraction in different conditions (from CO to 50). At the same time the fatiguing contraction (condition 50) has the same qualitative characteristics as the other two sustained contractions, that induced only additional activation of the same areas.

Thus, our study indicated gradual increase in activation of the insula as well as temporal areas (AA/AI) during more demanding contractions and suggests that this area may have a role in regulating increasing demands (for instance, cardiovascular) under conditions of fatiguing contractions.
Common elements of neuroanatomical systems underlying muscle pain and activated due to proprioceptive stimulation and fatiguing muscle contractions

Comparison of results obtained in studies I, III, and IV, including conjunction analysis of selected contrasts indicate that area, comprised of SII, posterior insula and auditory cortex (BA 21, 42) are involved in the perception of both muscle pain and proprioceptive functions as well as in maintenance of fatiguing muscle contraction.

SII, posterior insula and auditory areas were previously grouped as a somatosensory associative area (Petrovic et al. 2000, Treede et al. 2000). Brooks and co-workers (2002) consider that the reason for such unification is limitations related to the low resolution of PET technique and visualization of activated areas on axial slices. Acceding in part to this statement, however one can see another reason to consider these structures to be united to one functional zone – the similarity of functions maintained.

The presence of nociceptive neurons in the SII (classical somatosensory area) and posterior insula was shown with neuronal recording in monkeys (Robinson and Burton, 1980, Dong et al. 1989). Characteristics of the response of nociceptive neurons in the SII suggested participation of those neurons in the maintenance of learning and attention to painful events and assessment of stimuli quality (Treede et al. 1999, 2000). In a recent MEG study of coding of pain intensity it was shown that SII may subserve recognition of noxious nature and attention toward painful stimuli. (Timmermann et al. 2001).

Overlapping of areas activated during both vibrotactile and nociceptive stimulation was found only in 30% of the activated areas within parietal operculum (Gelnar et al. 1999). According to findings of Greenspan et al. (1999), who compared patterns of lesions in parasilvian cortex with loss of pain sensitivity, SII is essential for normal pain perception. There is an evidence that SII encode spatial aspect of nociceptive information without additional tactile information (Bingel et al. 2004).

The insula is believed to serve a sensory integrative function for pain, taste and other visceral sensations. Recent imaging studies evidenced that the anterior insula is involved mainly in maintenance of pain, whereas the posterior insula involved in maintenance of touch (Davis et al. 1998). Treede with co-workers
(2000) in summarizing electrophysiological and neuroimaging data, concluded that it seems to be considerable overlap in tactile and nociceptive areas in the parietal operculum, however, there is a trend toward a deeper location (closer to the circular sulcus of insula) of the nociceptive area in humans (Treede et al. 2000). Involvement of the clastrium-insula region in tactile-visual crossmodal transfer of information was shown in the study of Hadjikhani and Roland (1998).

Thus, area of brain cortex located near the lateral sulcus and comprised of SII, posterior part of the insula and auditory cortex (BA 41, 22) participate in the maintenance of perception of both muscle pain and movement.
CONCLUSIONS

1. Experimental muscle pain induced by the injection of HS led to a bilateral brain response as measured by rCBF changes. The pattern of brain reaction to experimental muscle pain comprised of areas with increased as well as decreased rCBF.

2. Multifunctional brain areas engaged in decision making, selective attention and motor control were activated during perception of muscle pain.

3. Initial and late phases of tonic experimental muscle pain are associated with significantly different patterns of brain response, namely initial increase of rCBF in insula followed by significant bilateral decrease of rCBF at the late stage.

4. Proprioceptive afferentation due to passive movements or illusions of movements is associated with different patterns of brain activity. Activation in SI/MI and SMA were observed only in response to passive movement whereas both perception of passive movements and perception of illusory movements induced by tendon vibration activated areas in temporal and parietal cortices (SII, AA, and AI).

5. Analysis of rCBF during passive movements and tendon vibration elicited and not elicited illusion of movements revealed involvement of primary and associative auditory cortex in processing of proprioceptive information and suggested that area SII is involved mainly in stimulus perception generation while areas SI/MI and SMA – mainly in the processing of proprioceptive input.

6. A fatiguing contraction in m. biceps was associated with contralateral activation of the MI, the SI, the SII, the AA and AI, the SMA and the cingulate cortex. Gradual increase in activation of the insula as well as temporal areas (AA/AI) during more demanding contractions suggests that this area may have a role in regulating increasing demands (for instance, cardiovascular) under conditions of fatiguing contractions.

7. Analysis of brain reactions to nociceptive and somatosensory stimulation show that area of brain cortex comprised of secondary somatosensory cortex, posterior part of the insula and auditory cortex (BA 41, 22) is participated in the maintenance of perception of both muscle pain and movement as well as maintenance of fatiguing muscle contraction.
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