SOMATOSENSORY EVOKED FIELDS AFTER EXERCISE INDUCED PAIN

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ABSTRACT

Saloranta, Harri. 2017. Somatosensory evoked fields after exercise induced pain. Faculty of sport and health sciences, University of Jyväskylä, Master's thesis, 28 pp,, 2 appendices.

The amount of research utilizing magnetoencephalography (MEG) has increased within the last few decades because of the development and better availability of equipment. With MEG, different areas of brain functionality can be studied in a variety of tasks and pathologies. Somatosensory evoked fields (SEF) are magnetic fields generated by tens of thousands of neurons firing in the primary somatosensory cortex. The purpose of this master's thesis is to study the modulation of SEFs after exercise induced pain.

The study was carried out in Jyväskylä Centre for Interdisciplinary Brain Research (CIBR). The study subjects (N=18, male 10, female 8) were healthy and suitable for MEG measurements. Data was gathered in December 2015 and January 2016 in collaboration with another Master's student. An international article will be published utilizing the data of the present measurement protocol. The study subjects' SEFs were measured from the median nerve of the right arm before and after a 2 minute fatiguing static gripping task.

Two components were analyzed from the SEFs. The first component (N20) can be seen roughly 20 ms after stimulus onset. The second analyzed component (P40-60) was a later response to the stimulus, appearing at 40-60 ms after stimulus onset. The static gripping task had no statistically significant effect on the peak amplitudes of the N20 component (p=0,529) or the P40-60 component (p=0,160). The change in the ratio of N20 and P40-60 components was also analyzed, but no statistically significant difference was found (p=0,169).

With this study protocol, exercise induced pain does not have a statistically significant effect to somatosensory processing in the primary somatosensory cortex. Pain after fatiguing muscle activity seems not to be inhibitory or excitatory to somatosensory processing. Future studies in the subject area should have a larger study group and further develop the study protocol.

Keywords: Magnetoencephalography, pain, physical activity, somatosensory evoked field

ABBREVIATIONS

CNS	Central nervous system
BESA	Brain electric source analysis
ECD	Equivalent current dipole
EEG	Electroencephalography
MEG	Magnetoencephalography
PNS	Peripheral nervous system
PPC	Posterior parietal cortex
SEF	Somatosensory evoked field
SEP	Somatosensory evoked potential
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SQUID	Superconducting quantum interference device
VAS	Visual analogue scale
VPL	Ventral posterior lateral nucleus of thalamus

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INTRODUCTION

1. ANATOMY AND PHYSIOLOGY OF THE SOMATOSENSORY SYSTEM

1.1 Overview of the human nervous system

The human nervous system is divided to the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and the spinal cord, which are central parts of the nervous system. All the remaining parts of the nervous system compose the peripheral nervous system (PNS). These parts include the cranial nerves, spinal nerves, autonomic nerves and ganglia. The PNS consists of afferent and efferent neurons. The afferent neurons are sensory neurons that send impulses to the CNS from receptors. The CNS sends impulses distally via efferent neurons to effectors (Anthony & Thibodeau 1983).

The nervous system can also be divided into the somatic nervous system and the autonomic nervous system. This division is based on the type of effectors that are supplied. The somatic nervous system supplies the skeletal muscles, and it consists of the brain, spinal cord, cranial nerves and spinal nerves. The autonomic nervous system supplies autonomic effectors, including smooth muscle, cardiac muscle and glandular epithelial tissue (Anthony & Thibodeau 1983).

1.2 Overview of the somatosensory system

Sensory information from the extremities is sent via spinal nerves and their different parts. There are 31 pairs of spinal nerves connected to the spinal cord (Anthony & Thibodeau 1983). The somatosensory system is comprised of two major components. These include a subsystem for the detection of different types of mechanical stimuli, including light touch, vibration, pressure and cutaneous tension (the dorsal column-medial lemniscus pathway), and a subsystem for the detection of painful stimuli and temperature (the spinothalamic pathway) (Purves et al 2001). These subsystems give humans the ability to identify different shapes and textures, to detect potentially harmful factors, and assess the internal and external forces affecting the body (Purves et al 2001).

There's a wide variety of specialized sensory receptors in the cutaneous and subcutaneous tissues. According to their function, they can be divided into three groups: mechanoreceptors, nociceptors and thermoceptors. The receptors near the body surface can be further divided into free and encapsulated types. Nociceptors and thermoceptors have free nerve endings; the unmyelinated terminal branches of these neurons spread into the upper regions of the dermis and epidermis. Although their wide range of variety, all somatic sensory receptors work basically the same way. When a stimulus is applied to the skin, the deformation or other change (e.g. temperature) affects the nerve endings. This in turn affects the ionic permeability of the receptor's membrane. As a result, a depolarizing current is generated in the nerve ending, thus producing a receptor potential triggering action potentials. This process is called sensory transduction; a mechanical stimulus is converted into an electrical signal (Purves et al 2001).

1.3 Somatosensory pathways

Impulses in the somatosensory pathways typically travel through three sensory neurons. The first sensory neurons are located in the peripheral nervous system and they send impulses to the central nervous system (Anthony & Thibodeau 1983). The cell bodies of the first sensory neurons are located in the dorsal root ganglion associated with each segmental spinal nerve. Dorsal root ganglion cells are also known as first-order neurons, as they are the initiators of the sensory process (Purves et al 2001). If the receptors of these neurons are in areas supplied by spinal nerves, their dendrites are located in a spinal nerve and their axons terminate in gray matter of the spinal cord or brain stem. If the receptors of the first sensory neurons are located in areas supplied by the cranial nerves, their dendrites lie in a cranial nerve. The bodies of these cells lie in a cranial nerve ganglion and their axons terminate in the gray matter of the brain stem. (Anthony & Thibodeau 1983). The ganglion cells of the first sensory neurons have long peripheral axons that end in the somatic receptors, and shorter central axons that reach the dorsolateral region of the spinal cord (Purves et al 2001).

The second sensory neurons (second-order neurons) lie in the spinal cord or brain stem and ascend to the thalamus. The dendrites and cell bodies of these neurons are located in the

spinal cord or brain stem grey matter. Their axons ascend up the cord through the brain stem to the thalamus, where they synapse with the third sensory neuron dendrites or cell bodies. The third sensory neurons (third-order neurons) arise from the thalamus to the postcentral gyrus of the parietal lobe in the somatosensory area. Thalamocortical tracts consist of bundles of the third sensory neurons. These neurons extend through the internal capsule to the cerebral cortex (Anthony & Thibodeau 1983; Purves et al 2001).

The sensory pathways to the cerebral cortex are mostly crossed pathways. Each side of the brain is thus receiving information from the opposite side of the body. Usually the second sensory neuron crosses over to the opposite side at some level in its ascent to the thalamus (Anthony & Thibodeau 1983). The medial lemniscal system comprises of the tracts that together form the posterior white columns of the cord. These tracts are called the fasciculi cuneatus and gracilis. The medial lemniscus, a flat band of white fibers extending through the medulla, pons and midbrain, is also included in the medial lemniscal system (Anthony & Thibodeau 1983).

The spinothalamic pathway fibers are axons of the second sensory neurons. The spinothalamic pathway transfers information about pain and temperature. The medial lemniscus pathway fibers are axons of the second sensory neurons, like the spinothalamic pathway fibers (Anthony & Thibodeau 1983; Purves et al 2001). Their cell bodies are located in the medulla, where they decussate, and then extend upward to the thalamus, where they terminate. The fibers of the dorsal column-medial lemniscus system mediate tactile discrimination and proprioception (Purves et al. 2001). Tactile discrimination includes awareness of an object's size, shape and texture (stereognosis), the object's precise location, two-point discrimination, weight discrimination and sense of vibrations. Proprioception is the sense of movement and position of body parts (Anthony & Thibodeau 1983).

When the first-order axons enter the spinal cord, they bifurcate into ascending and descending branches. These in turn send collateral branches to several spinal segments. Some collateral branches penetrate the dorsal horn of the cord and synapse on neurons that mediate segmental reflexes, e.g. the "knee-jerk" reflex. The majority of the incoming axons ascend ipsilaterally

through the dorsal columns of the cord to the medulla where they terminate by contacting the second-order neurons in the gracile and cuneate nuclei. These nuclei are together referred to as the dorsal column nuclei. The gracile tract, located medially in the dorsal columns, conveys
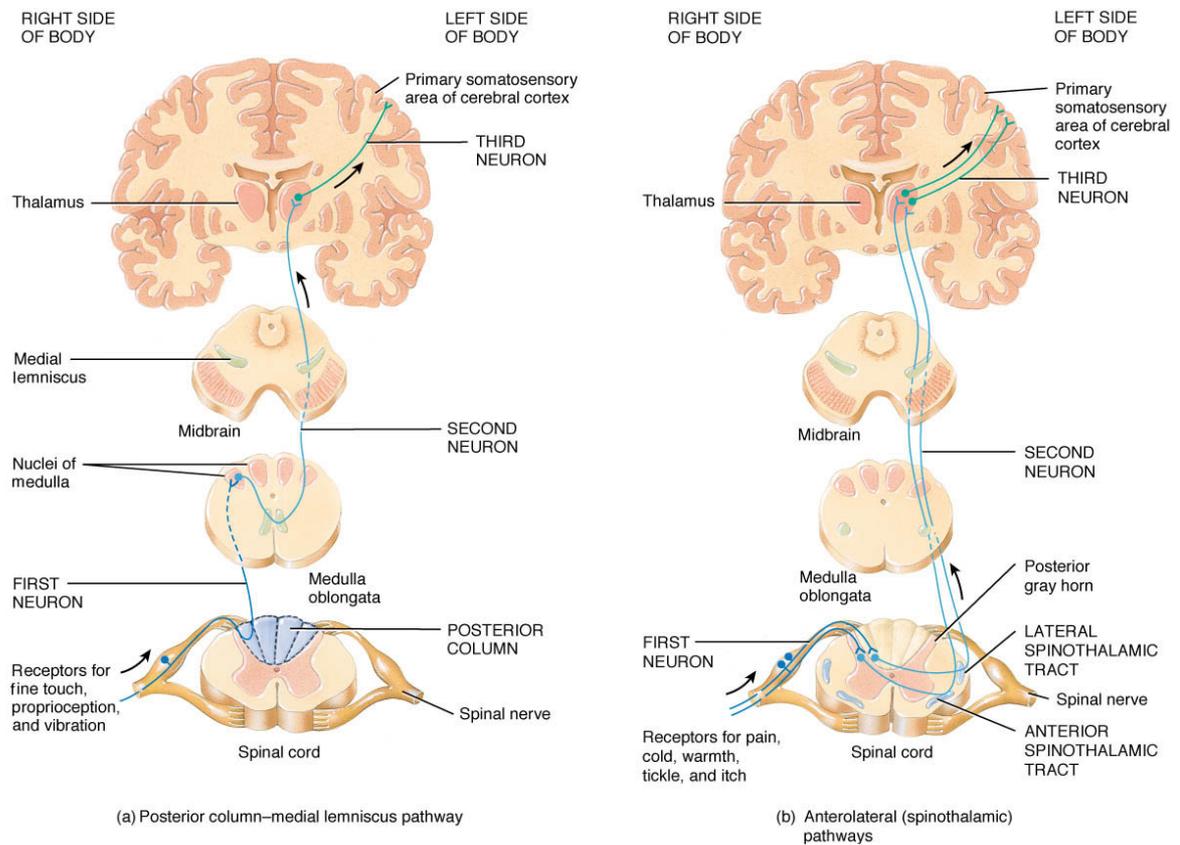
Figure 1. Somatosensory pathways

information from the lower limbs, and the cuneate tract, located laterally in the dorsal columns, conveys information from the upper limbs, trunk and neck (Figure 1). At the upper thorax level, the dorsal columns make up more than a third of the cross-sectional area of the spinal cord (Purves et al. 2001).

The axons of the second-order neurons ascend to the somatic sensory portion of the thalamus. In the dorsal portion of the lower brainstem, the axons from dorsal column nuclei form the internal arcuate tract. The axons of this tract cross the midline and form another dorsoventrally elongated tract, the medial lemniscus. The medial lemniscus axons reach the ventral posterior lateral (VPL) nucleus of the thalamus, where they synapse with the third-order neurons of the dorsal column-medial lemniscus system (Purves et al. 2001).

1.4 Somatosensory information from the upper extremity

The median, radial and ulnar nerves ascending from the upper extremity connect to the brachial plexus, which then connects to the spinal cord. These nerves contain fibers from multiple spinal nerves and they branch out distally innervating the hand and most of the arm. Spinal nerves contain both sensory dendrites and motor axons and are thus called mixed nerves. The median nerve supplies a part of the muscles in the front of the arm and the hand. The median nerve provides sensory information from the palmar surface of the thumb, index and middle fingers (Anthony & Thibodeau 1983).

1.5 The somatosensory cortex
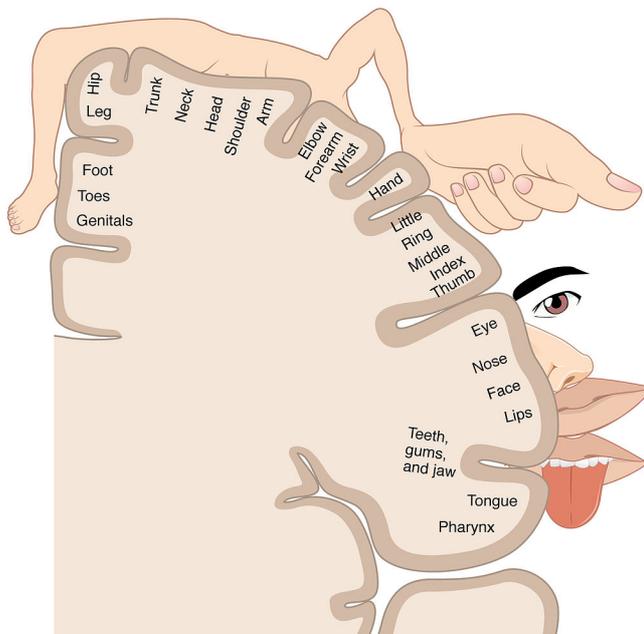
Figure 2. Cortical homunculus

The sensory impulses conducted up the spinothalamic pathway and the medial lemniscal pathway reach the cerebral cortex first in the postcentral gyrus, also known as the somatosensory cortex (Anthony & Thibodeau 1983). The somatosensory cortex is located in the parietal lobe, and is comprised of four regions, known as Brodmann's areas 3a, 3b, 1 and 2. All four areas are involved in tactile information processing, even though 3b is known as the primary somatosensory cortex (SI). Experiments on non-human primates have shown that neurons in area 3b and 1 respond primarily to cutaneous stimuli, and neurons in area 3a respond to stimulation of proprioceptors. The neurons of area 2 process both tactile and proprioceptive stimuli. Each of these areas contain a separate and complete representation of the body. According to these somatotopic maps, the illustration of the "homunculus" has been presented. In the homunculus, different body parts are disproportionate, with large hands and face (Figure 2). These anomalies represent the need for larger processing capability for manipulation, facial expressions and speaking in humans (Purves et al. 2001).

The functional properties of the different areas of the somatosensory cortex are distinct. For example, the neuronal receptive fields in area 3b are relatively simple compared to areas 1 and 2. Area 3b processes generally stimulation of a single finger, while areas 1 and 2 respond to stimulation of multiple fingers. Also the direction of skin stimulation and different shapes of objects affects the area in which the response is elicited (Purves et al. 2001).

From the primary somatosensory cortex, somatosensory information is further distributed to higher-order cortical fields and subcortical structures. One of these cortical structures is called the secondary somatosensory cortex (SII) and is adjacent to the SI. The SII receives convergent projections from the SI and sends projections to limbic structures, i.e. the amygdala and hippocampus. The latter pathway is theorized to have an important role in tactile learning and memory. Motor cortical areas in the frontal lobe also receive tactile information from the anterior parietal cortex and provide feedback to several cortical somatosensory regions (Purves et al. 2001).

1.6 The association cortices

The association cortices comprise of the major part of the cerebral surface in the human brain. These cortices function to process the complex relationship between the arrival of input in the primary sensory cortices and behavior generation. The overall functions of the association cortices are referred to as cognition, which includes the ability to monitor external stimuli or internal motivation, to identify the importance of the stimuli, and to plan appropriate responses to them. The neural processes of the cortices are immensely complex, and they receive and integrate information from multiple sources, and they also influence a variety of cortical and subcortical targets. The association cortices receive projections from the primary and secondary sensory and motor cortices, the thalamus and the brainstem. Information from these cortices is sent to the hippocampus, the basal ganglia, the cerebellum, the thalamus and other association cortices. The parietal association cortex is highly important for attending to stimuli in the external and internal environment and the temporal association cortex is important for identifying the nature of the stimuli. The frontal association cortex has an especially significant role in planning proper behavior in response to the stimuli (Purves et al. 2001).

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The primary and secondary somatosensory and motor cortices account for only about a fifth of the cerebral cortex. A large part of the remaining cortex attends to complex stimuli and their properties. These more integrative functions include identifying the relevant features of the stimuli and related objects, and planning suitable strategies in response. The association cortices in the parietal, temporal and frontal lobes are responsible for these functions. The association cortex in the occipital lobe mainly processes visual information (Purves et al. 2001).

A large portion of the cortex is neocortex, which has six cellular layers, or laminae. The layers have distinct cell populations, varying in size, shape, density, input and output. Although there is an overall uniformity, based on the laminar features about 50 subdivisions have been identified in the cerebral cortex. The subdivisions are referred to as cytoarchitectonic areas. Knowledge on the varying functions of the cytoarchitectonic areas is mainly based on studies of patients who have damage in one or more of these areas. Electrophysiological mapping in laboratory animals and neurosurgical patients have also added to this knowledge. Different brain regions identified on histological grounds have also been found to be distinct functionally, and by the physiological response properties of their constituent cells. In addition, these regions can be distinguished by their local and long-distance connection patterns (Purves et al. 2001).

There are variations among cytoarchitectonic areas, but all cortical regions have some common properties: 1) Each cortical layer has a primary input source and output target, radial and lateral connections. 2) Similarly functioning cells tend to be organized in radially aligned groups spanning through all cortical layers, and they receive information from segregated radial and columnar bands. 3) Horizontally aligned axons in the cortex that link functionally similar cell groups extend widely from interneurons within specific cortical layers (Purves et al. 2001).

The connections of the association cortices are noticeably different from primary and secondary sensory and motor cortices, especially their inputs and outputs. For example, there are three thalamic nuclei that do not relay primary sensory or motor information but provide subcortical input to the association cortices. These are the pulvinar nuclei, which project to

the parietal association cortex, the lateral posterior nuclei projecting to the temporal association cortex, and the medial dorsal nuclei projecting to the frontal association cortex. The input to these nuclei comes from other association cortices. The information that the association cortices receive through the thalamus has already been processed in the primary sensory and motor areas of the cortex, and is fed back to the association regions (Purves et al. 2001).

The association cortices also receive direct projections from other parts of the cortex. These are referred to as corticocortical connections, which make up the majority of the input to the association cortices. Ipsilateral corticocortical connections to the association cortices originate from primary and secondary sensory and motor cortices and other association cortices of the same hemisphere. Interhemispheric connections through the corpus callosum and anterior commissure are received from corresponding and noncorresponding association cortices of the opposite hemisphere. In addition, the association cortices have innervation from the dopaminergic, noradrenergic and serotonergic nuclei in the brainstem reticular formation, as well as cholinergic nuclei of the brainstem and basal forebrain. Information from these sources is projected to different layers of the cortex and affects the individual's mental status continuum ranging from the state of deep sleep to high alertness (Purves et al. 2001).

2. MAGNETOENCEPHALOGRAPHY

2.1 Introduction to magnetoencephalography

Magnetoencephalography (MEG) is a neuroimaging technique, in which weak magnetic fields generated by cerebral currents are detected outside of the head with an array of sensors. In MEG, an array of superconducting sensors is used to detect magnetic fields. The device is set in a magnetically shielded room to ensure minimal magnetic noise from unwanted sources. The MEG system is used to map the magnetic field pattern as reliably as possible and to use this pattern to calculate the sources of the magnetic fields, i.e. the equivalent current dipole (ECD). MEG, and also EEG (electroencephalography) are superior in their time resolution capacity compared to other neuroimaging techniques, for example functional MRI. There are also certain limitations to MEG. There is no uniform solution to the problem of localizing the

source of neuronal signals of the brain. Computational models are used to calculate the location of the sources as accurately as possible. Accepted difference of localization between replications is 4-5 mm (Hari & Forss 1999, Hansen et al. 2010).

Magnetoencephalography signals are recorded outside the scalp surface, the technique is completely non-invasive and it does not have any effect on the brain's electrophysiological function. The signals are generated from tens of thousands of neurons firing synchronously in the cerebral cortex and the activity is mainly comprised of postsynaptic currents in apical dendrites. Although MEG and EEG are unrivalled in their time resolution capabilities, they have the problem of source localization. In MEG/EEG, this is referred to as the inverse problem (Lopes da Silva 2010).

2.2 The inverse problem in magnetoencephalography

An inverse problem is the opposite of a forward problem. A forward problem starts with the causes and calculates the results, whereas an inverse problem starts with the results and then, using appropriate models, calculates the causes. There are theoretically an infinite number of possible solutions in finding the source of any electromagnetic field, and knowledge of the context in which the observations were made is crucial in localizing the source as accurately as possible. In MEG source localization, source models are used, which take into account the geometry of the head, conductivity of tissues and sensor locations. The localization of the sources of MEG signals is partly dependent on these models and the assumptions correspondent to them, and thus has always some degree of uncertainty. The solutions to the inverse problem in MEG are based on research in source localization in EEG, finding the origins of signals in electrocardiography (ECG) and magnetocardiography (MCG) (Baillet 2010).

2.3 Special considerations to magnetoencephalography

The folding of the brain cortex allows the recording of MEG signals. The gyrus and sulcus formations have neuronal populations that are oriented in different ways. At the top of the gyri the apical dendrites are perpendicular to the overlying skull and in the walls of the sulci the dendrites are oriented parallel to the skull. MEG can record magnetic fields generated by

the currents in the tangentially oriented dendrites as these dendrites generate magnetic fields outside the skull. The currents in the radially oriented dendrites generate magnetic fields inside the skull. These currents can be recorded with EEG. However, there are many inbetween orientations in the cortex, currents which are recorded in varying degrees and strengths (Lopes da Silva 2010).

With MEG, it is difficult to detect dipoles that are generated in the deeper parts of the brain, as the magnetic fields rapidly decrease as the distance between the source and the MEG sensors increase. Another disadvantage is the difficulty of detecting multiple dipoles. In order to solve this problem, special algorithms are needed. It is also impossible for MEG to detect activity in the white matter of the brain (Kakigi et al. 2000).

2.4 Instrumentation

The cerebral magnetic fields are very weak, and a highly sensitive magnetic field detector is needed. The only sensor sensitive enough for MEG recording is the SQUID, Superconducting Quantum Interference Device. When a superconducting ring is placed in a magnetic field, the field induces a shielding current around the ring. The current is dependent on the applied field. This shielding current provides an indirect measure of the magnetic field. Conventional current measurement cannot be used to measure the current in the ring, since the continuous superconducting loop would be destroyed in the process. The ring is broken by a thin layer of electrical insulator, through which the electron pairs can still tunnel. These "Josephson junctions", or "weak links" allow for an interference, which then transfers to a measurable physical quantity, a resistance across the SQUID (Lopes da Silva 2010).

3. SOMATOSENSORY EVOKED FIELDS

3.1 Introduction to somatosensory evoked fields

Somatosensory evoked fields (SEF) are magnetic fields generated by electrical currents in the brain. These fields can be recorded with MEG and the equivalent current dipole (ECD) locations can be calculated based on the evoked fields. In addition to somatosensory evoked fields, also auditory, visual, language and motor evoked and movement-related magnetic fields can be recorded (Burgess et al. 2011). The somatosensory system in humans can be stimulated peripherally by mechanical stimuli, by heating and cooling, or with short electrical pulses, which activate the nerve directly (Parkkonen 2010). Using mechanical stimuli is essential when stimulating sites close to the MEG sensors, for example lips or tongue, to get clear MEG data without stimulus artifacts (Burgess et al. 2011).

Frequently used sites of electrical stimulation in SEF examination include the median nerve and tibial nerve. Other mixed nerves can also be used (e.g. ulnar, posterior tibial, femoral, sural (sensory) nerves) (Burgess et al. 2011). Electric pulses generated through cutaneous electrodes are typically very brief, 100-200 µs. A typical current at the motor threshold when stimulating the median nerve is 5-10 mA (Parkkonen 2010). A pulsating stimulus is used often with a frequency of 5 Hz. Roughly 100-300 stimuli are required to acquire an adequate number of acceptable repetitions and the responses are averaged on-line. The final averages are done off-line from the raw data. The purpose of the averaging is to acquire a reliable response with minimal interference from unwanted sources. Indications for the recording of SEFs include localization of primary SI or more specific areas of SI (e.g. presurgical procedure, scientific research) (Burgess et al. 2011).

3.2 Generation of SEFs

Somatosensory evoked fields (SEF) are generated by the electrical potentials in the somatosensory cortices in response to peripheral nerve or cutaneous stimulation (Berger & Blum 2007). Slice recordings have indicated, that brain tissue volumes of less than 10mm^3 can generate a clear signal for MEG to pick up at typical measurement distance of MEG devices (Hari & Forss 1999). Basically, when SEFs are recorded in magnetoencephalography,

the MEG system records the fields generated by somatosensory evoked potentials (SEP). SEPs can be directly measured by electroencephalography (EEG), although the signal is changed by the medium between the measuring electrodes and the nerves generating the potential. This phenomena is called volume conduction. In MEG, this problem is not present. SEFs are easy to produce and they can be used to assess the functional integrity of the ascending somatosensory pathways (Berger & Blum 2007).

It is theoretically possible to use any sensory or mixed nerve stimulation sites in recording SEFs, but the sites that are mostly used are the median and posterior tibial nerves. These nerves are easy to stimulate and there is wide existing data of their use in SEF-recordings. The median nerve has contributions from the medial and lateral parts of the brachial plexus and it's fibers span from C5 to T1 roots. Stimulation of sensory receptors in the skin initiates activation of peripheral sensory nerves, which extend through the brachial plexus to the dorsal root ganglia. These bipolar neurons transmit this physiological activation centrally through the appropriate spinal root and into the spinal cord. From here, fibers project through the brainstem tegmentum to the contralateral VPL nucleus of thalamus. The contralateral VPL nucleus of thalamus has widespread connections to the contralateral (to the site of stimulation) somatosensory cortex in the parietal lobe (Berger & Blum 2007).

3.1 Activation of the somatosensory cortical network

According to Vanni et al (1996), cortical sources of SEFs in humans are in agreement of the somatotopical arrangement in the somatosensory cortex. In their research, Vanni et al used individual MRI data of seven study subjects in source location of median and ulnar nerve SEFs. Two distinct sources were identified after median and ulnar nerve stimulation in Brodmann area 3b in the contralateral SI. The first source (M20) peaked at 21-22ms with a current of opposite direction at 32ms. The second source (M40) was located 7mm medial compared to the first source and had two peaks, 25ms and a more prominent peak at 42ms. For median nerve stimulation, the source for M20 was 7mm more lateral compared to the ulnar nerve stimulation. For M40 the organization was less clear.

In their review article of somatosensory evoked magnetic fields, Kakigi et al. (2000) summarize research in the field of study. A complete homunculus was established by

Nakamura et al (1998) with SEF recordings (Figure 3), demonstrating the efficacy and accuracy of MEG. Considering upper limb and median nerve stimulation, the researchers noted that they found the typical components, N20m-P30m-N40m-P60m-N90m and their counterparts. Using
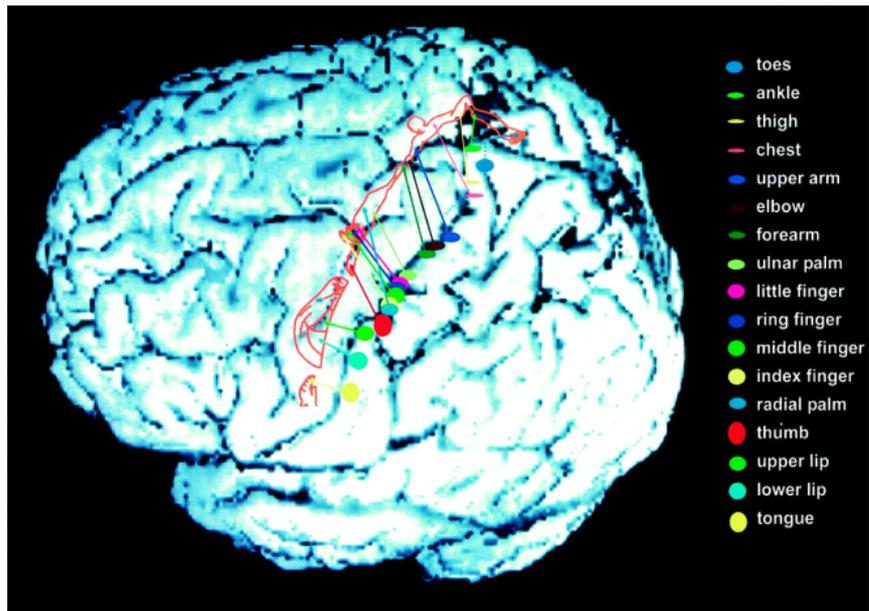
Figure 3. Detailed somatosensory receptive map according to a MEG study by Nakamura et al (1998)

unilateral middle finger stimulation, there were components found to be located in the SII bilaterally at 80-100ms. This finding suggests that in humans, SII has a bilateral function. The researchers have also studied activity in the posterior parietal cortex (PPC) in response to upper limb stimulation. Using a five dipole model with brain electric source analysis (BESA) system the ECDs of the middle-latency SEF were identified in the SI contralaterally and in the SII and PPC bilaterally.

Mauguière et al (1997) studied the somatosensory cortical network in the human brain by recording SEFs after median nerve stimulation. In their study source modelling was combined with magnetic resonance imaging (MRI). The results suggest that there's six identifiable sources on the cortex: (1) the posterior bank of the rolandic fissure (area SI), the upper bank of the sylvian fissure (parietal opercular area SII) and the banks of the intraparietal fissure contralateral to stimulation, (2) in the SII area ipsilateral to stimulation and (3) in the mid-frontal or inferior frontal gyri on both sides. All of these source areas were simultaneously active at 70-140 ms after stimulus onset, the SI source being the only one active at already 20-60ms.

4. RESEARCH QUESTIONS AND HYPOTHESIS

The research questions for this study are:

1. Are the SEFs elicited by stimulating the median nerve and their location in the current study similar to previous research data in the field?

2. What is the subjective quality and quantity of exercise induced pain?

3. Is there a change in the amplitude or the configuration of the waveform in the SEFs after exercise induced pain?

Hypotheses:

The null hypothesis and alternative hypothesis for this study are: H0 = Exercise induced pain does not have an effect on the amplitude or the configuration of the waveform of SEFs. H1 = Exercise induced pain has an effect on the amplitude or the configuration of the waveform of SEFs.

5. METHODS

5.1 Study subjects

The study subjects (N=18, 10 male, 8 female) (Tables 1. and 2.) were healthy and suitable for MEG-study and right-handed for ease of measurement. They were screened for depression symptoms with the Finnish modification of the short form of the Beck Depression Inventory (RBDI) (All participants with a score of 5 or less included) and they signed an informed consent before participating in the study. Before starting the measurements, the study subjects were seated in the neuromagnetometer seat to check for any subject-dependent noise or disturbance in the MEG-channels. In addition to this study, MEG-measurements for another master's thesis and an international study were made for the same study group. This study was approved by the ethics committee of the University of Jyväskylä. The data was recorded at the Jyväskylä Centre for Interdisciplinary Brain Research in December 2015 and January 2016.

Table 1. Study subjects' characteristics and descriptive statistics for age, height, weight and BMI.

ID	Sex	Age	Height	Weight	BMI
101	M	27	177	70	22,3
102	M	36	185	78	22,8
103	M	32	173	71	23,7
105	M	18	180	68	21
106	M	32	183	68	20,3
107	M	28	176	95	30,7
108	M	30	189	92	25,8
109	M	30	177	94	30
110	M	33	203	93	22,6
111	M	41	188	85	24
201	F	27	170	59	20,4
202	F	40	160	60	23,4
204	F	27	169	63	22,1
205	F	24	177	64	20,4
206	F	36	159	59	23,3
207	F	23	163	63	23,7
208	F	37	173	73	24,4
209	F	26	162	58	22,1

	Range	Mean	Std. Deviation
Age (years)	18-41	30,39	6,099
Height (cm)	159-203	175,78	11,384
Weight (kg)	58-95	72,94	13,251
BMI	10,4	23,50	2,8924

5.2 Instrumentation and devices

The measurements were made with a 306-channel neuromagnetometer (Elekta Neuromag® TRIUX) in a magnetically shielded room. A Polhemus FASTRAK® digital tracker was used for 3D head digitization with 5 head point indicator (HPI) coils attached. The nasion and the left and right pre-auricular points were indicated. In addition, the study subject's head shape

was digitized using the Polhemus FASTRAK® digital tracker by drawing three antero-posterior lines across the top of the head, one line across the forehead and one on the bridge of the nose. Two EMG electrodes were attached for the use of electrooculography (EOG). No heart beat detection was used. A ground cuff was attached to the right forearm and a stimulator was set over the right median nerve. For producing the electrical stimuli, Presentation –software and a Digitimer –stimulator (Digitimer Ltd, model DS7A, Welvyn Garden City, UK) was used. For the static gripping task, a standard hand gripper was used with aluminium foil shielding to prevent magnetic disturbances in the recording room.

5.3 Study protocol

The participant was asked to remove any jewellery and was provided with metal-free clothing if in need. The study subject was seated and the cords from the EOG, ground cuff and HPI-coils were attached to the neuromagnetometer. The stimulus intensity was set to exceed the motor threshold. The stimulus intensity ranged from 3,5 to 9 mA, and the mean value was 5,7 mA. The placement of the median nerve stimulator (Figure 4) was determined to be correct and the stimulus intensity high enough when there was flexion movement in the thumb joints and the subject felt electrical sensation in the thumb and index finger region. The subject was given the instructions according to which the measurement proceeded.

Figure 4. Median nerve stimulator placed on the wrist.

The median nerve stimulation was executed and raw data was recorded with MEG. On-line averaging was also used with the recording software for visualization purposes. 300 stimuli were programmed for the median nerve stimulation with a 5Hz frequency. Thus one set of 300 stimuli lasted for 60 seconds. After measuring the first SEF, the subject was instructed to

begin the static gripping task (Figure 5) via audio connection. A period of 2 minutes was timed for the gripping task, after which a 0 to 10 score (visual analogue scale, VAS) for the pain induced by the exercise was inquired from the subject, along with the quality of pain, according to the Finnish modification McGill pain questionnaire. After this, the second set of 300 stimuli was executed and the data acquisition was started. The subject was instructed to adjust the median nerve stimulator at the beginning of the second set only if the stimulator had shifted from it's position during the static gripping task and no clear thumb movement was seen. The second run of the SEF recording was recorded similarly to the first run. After the second set of 300 stimuli, the subject was again asked the pain score and quality of pain, if there was any residual pain in the thumb and thenar area.

Figure 5. The gripper used in the static gripping task. Gripper's metal part was covered with aluminium foil to prevent disturbances to the MEG device.

5.4 Data acquisition

Elekta's own software was used for data acquisition. A sampling frequency of 1000 Hz was used. Both the MEG and the electro-oculogram (EOG) lowpass frequency was set to 330 Hz and highpass frequency to 0,1 Hz. Data was stored on a server and was secured with a password. During data analysis, data was stored on personal workstations.

5.5 Data pre-processing

For data analysis, Brainstorm –software was used. Before using Brainstorm, the data was filtered with MaxFilter –program (Elekta Neuromag) and saved. The data analysis process was carried out according to Brainstorm –software online tutorials. In the Brainstorm – software, an averaged MRI-based 3D-model of the brain was used, since no individual MRI-

data was obtained. The raw data file was linked to the default anatomy and the digitized head points were used to warp and scale the head shape to match the default anatomy head shape (Figure 6). After this the head model was computed. The model contains the different organic structures of the intra- and extracranial space which slightly affect the magnetic fields. The noise levels were evaluated by estimating the power spectrum of the signals over the recordings. There was no clear or major continuously occurring noise patterns at any specific frequencies, thus no notch filter was
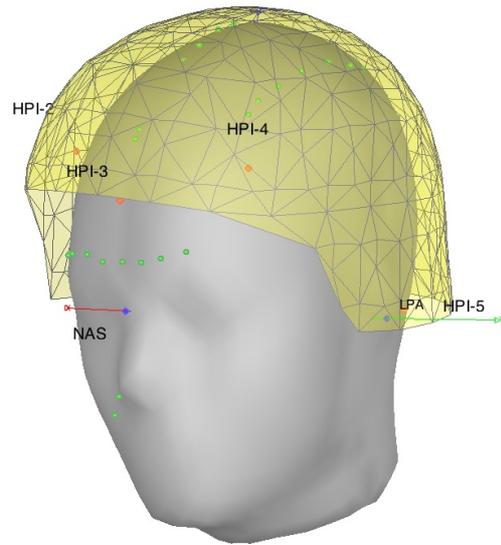
Figure 6. Nasion (NAS), left pre-auricular point (LPA), and HPI-coil locations in Brainstorm -software.

used for removal of noise frequencies (e.g. powerline currents). The eye blinks were observed and detected with the software. The blinks were removed by using the Signal-Space Projection (SSP) approach.

After the initial data preprocessing procedures the responses were averaged with the Brainstorm –software (off-line averaging). Both runs of the SEF-recordings in the study protocol were averaged similarly and the same amount of responses were used. If the subject had adjusted the stimulator location during the second run, only the suitable responses collected after the adjustment were used in the averages and the same amount of responses was averaged from the first run. 13 of the 18 study subjects needed to adjust the stimulator, and the average number of responses for offline averaging was 248 (min 150, max 300 responses).
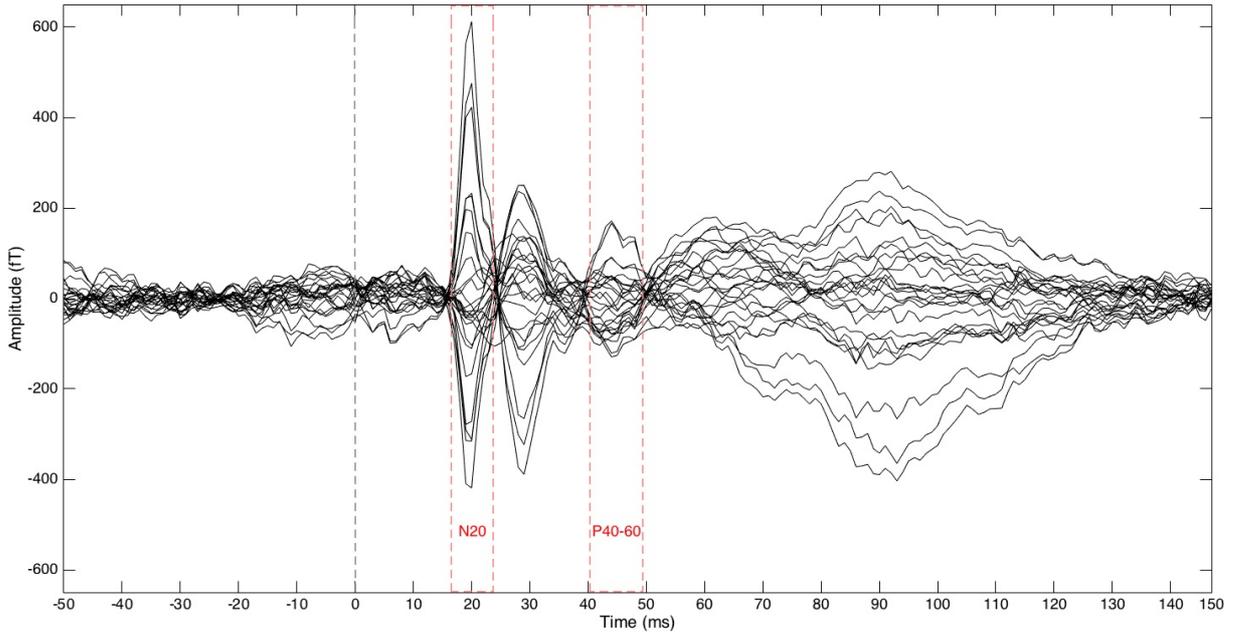
Figure 7. SEF waveforms of single study subject, from the left parietal gradiometers. The analyzed components outlined (red) in image.

5.6 Data analysis

The components to be analyzed were the first visible component in the SEF, typically seen at 20ms after stimulus onset, and a component appearing at 40-60ms after stimulus onset, which depicts later neural data processing (Figure 7). The source for these components is in the primary somatosensory cortex (SI). The peak amplitudes of the components were acquired by using "scouts" in Brainstorm. The user can place the scout on a selected point on the cortex and determine the size for the scout. The
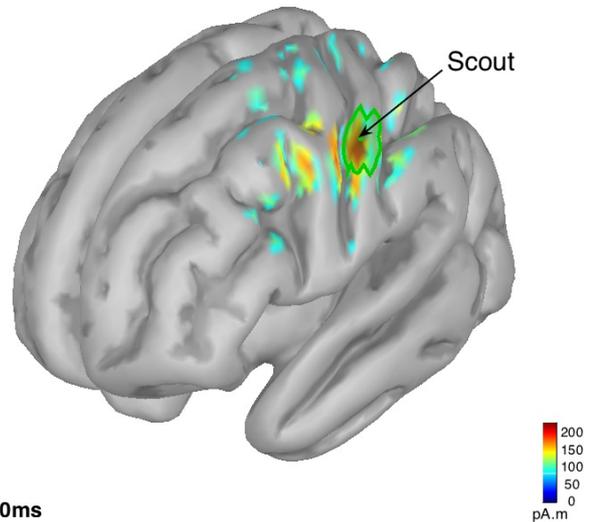
Figure 8. Placement of a scout on a focal point of activity.

size of the scout used was 40 vertices, which is a common size used according to the Brainstorm − software online manuals. The scout is placed on a focal point of activity at the

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peak of the component (Figure 8). After this, the amplitudes (Table 3) can be acquired from the MEG-signals "underneath" the scout for analysis.

Table 3. Peak amplitudes for N20 and P40-60-components, difference pre and post exercise and time point. Amplitudes are in picoamperes (pAm).

|	N20 pre	N20 post	Difference	Time (ms)	P40-60 pre	P40-60 post	Difference	Time (ms)
ID101	39,3	51	11,7	21	58,2	39,2	-19	48
ID102	66,1	70,8	4,7	26/25	22,7	26,8	4,1	47/46
ID103	117	89,7	-27,3	20	28,7	4,6	-24,1	45
ID105	65,7	58,3	-7,4	22	84,1	96,8	12,7	58
ID106	59,4	49,5	-9,9	21/20	63,5	62,8	-0,7	44
ID107	48,1	49,7	1,6	19	79,9	75,9	-4	45
ID108	46,9	42	-4,9	23	23,3	30,3	7	53
ID109	37,6	42,5	4,9	19	31,8	31,5	-0,3	60
ID110	55,4	64,7	9,3	21	22	18,6	-3,4	40/42
ID111	36,7	26,3	-10,4	30	29,3	32,3	3	60/58
ID201	31,5	33,8	2,3	18/19	82,2	69,9	-12,3	54
ID202	32	38,8	6,8	19	46,9	42,4	-4,5	57/60
ID204	45,6	85,7	40,1	20	73,6	57,9	-15,7	60
ID205	29,7	23,2	-6,5	20	40,9	23	-17,9	40
ID206	76,6	68,4	-8,2	19	23	33,7	10,7	49
ID207	43,1	43,4	0,3	18	39,6	41,8	2,2	53
ID208	39	42,7	3,7	21	27	29,6	2,6	51
ID209	52,2	59,1	6,9	19ms	59	53,3	-5,7	40

Table 4. Component mean peak latency and range.

	N20	P40-60
Component mean peak latency	20,7ms	50,3ms
Range	18-30ms	40-60ms

5.6. Statistical analysis

Statistical analysis of the amplitudes of the N20- and P40-60 components was made with IBM SPSS statistics –software. The amplitudes of the components both pre- and post-exercise (static gripping task) were input. The data was analyzed with the paired samples t-test.

Since there appeared to be some non-uniform variation in the N20-component between the two runs of SEFs (see table 4: amplitude difference in the N20-component), it was suspected that the change in the position of the median nerve stimulator had affected the intensity of the stimuli and subsequently the amplitude of the SEFs. Because of this, in order to "standardize" the P40-60 component to the possible change of the intensity of the stimulus, the P40-60-component amplitudes were divided with the N20-component amplitudes both pre and post exercise (Table 5). After this, the resulting ratios pre and post exercise were statistically analyzed.

Table 5. P40-60 to N20 ratio pre and post exercise.

	Pre	post
ID101	1,48	0,77
ID102	0,34	0,38
ID103	0,25	0,05
ID105	1,28	1,66
ID106	1,07	1,27
ID107	1,66	1,53
ID108	0,50	0,72
ID109	0,85	0,74
ID110	0,40	0,29
ID111	0,80	1,23
ID201	2,61	2,07
ID202	1,47	1,09
ID204	1,61	0,68
ID205	1,38	0,99
ID206	0,30	0,49
ID207	0,92	0,96
ID208	0,69	0,69
ID209	1,13	0,90

6. RESULTS

The SEFs recorded with MEG in this study are similar to previous data in the field (Figure 7), answering research question 1. The activity was localized in the posterior wall of the central sulcus, in Brodmann's area 3b of the SI (Figure 7). The second research question concerned the quality and quantity of pain. The subjective quality and quantity of exercise induced pain, measured with the McGill pain questionnaire and the visual analogue scale (VAS), varied greatly among the study subjects. The mean for VAS after the static gripping task was 4,6 and the range was 2-8. The mean for VAS after the second run of SEF was 0,4 and the range was 0-3,5. The VAS values diminished among all study subjects. Only 3 of the 18 study subjects reported some residual pain or sensations after the second run of SEF. As for the quality of pain, the study subjects reported 12 different descriptions for the quality of pain, the most common being "väsyttävä/väsynyt" (tiring).

Table 6. Quality and quantity of pain

After static gripping task		After second run of SEF	
VAS	Quality of pain	VAS	Quality of pain
6	väsyttävä	1	-
6	viiltävä	0	-
4	jomottava	0	-
6	pakottava	0	-
4	pistävä	0	-
7	särky	0	-
4	väsynyt	0	-
3	puuduttava	0	-
5	väsyttävä	0	-
4	pistävä	0	-
6	poltteleva, viiltävä	3,5	jäykkä, jomottava
4	polttava	0	-
8	jäykkä	0	-
2	tasainen	0	-
3	-	0	-
4	paineen tunne	0	-
2	painava	0	-
5	väsyttävä	2	kihelmöivä

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The rest of the results, considering research question 3, were drawn from the statistical analysis of the peak amplitudes of the two analyzed components of the SEFs, N20 and P40-60. The amplitude values were analyzed with the paired samples t-test. The statistical analysis showed that there was no statistically significant difference between the pre and post exercise conditions across the study group in the analyzed components (Table 7). For the first component, N20, the p-value was 0,529 and for the second component, P40-60, the p-value was 0,160. For the ratio of these two components pre and post exercise the p-value was 0,169, the level of significance being 0,05.

Table 7. Paired samples t-test

	Mean	Std. Deviation	Std. Error mean	95% CI lower	95% CI upper	p-value*
N20	-2,17778	14,36878	3,38675	-9,3232	4,96765	0,529
P40-60	3,62778	10,47736	2,46954	-1,58249	8,83805	0,160
N20 - P40-60 ratio	0,12389	0,36556	0,08616	-0,05790	0,30568	0,169

*Level of significance <0,05

7. DISCUSSION

The study protocol was executed successfully in the included study population. All of the study subjects completed the static gripping task according to instruction and the desired exercise induced pain was established. All of the study subjects reported pain immediately after the gripping task. There was some feeling of pain (VAS 1-3,5) in three study subjects after the second set of stimuli (duration 60 seconds), among the rest of the subjects the pain diminished and disappeared during the second set of stimuli. An average amount of 248 responses were averaged offline among the study subjects (min 150, max 300). 13 out of 18 study subjects needed to adjust the stimulator placement in the beginning of the second set of stimuli, since the stimulator placement had changed during the static gripping task and no movement in the thumb was seen. This meant that the stimuli did not target the median nerve and the motor threshold was not reached. After the adjustment an adequate number of responses considering reliable results from averaging was acquired from all study subjects.

The study was carried out according to ethical guidelines in scientific research. The study subjects were informed of the study protocol and agreed to participating in the study. Screening of suitability for MEG study was made according to common practice in the field of study. The study subjects were aware of their right to stop their participation at any time. The study protocol included other measurements that were not a part of this study, for example a cold water immersion for the hand. This measurement can be painful. The study subjects were screened for depression with the Beck Depression Inventory (RBDI) with this measurement in mind. Participating in a MEG study can also be harmful if the study subject has anxiety or a fear of confined spaces, because the MEG device is located in a shielded room and the study subject's freedom of movement is somewhat restricted while seated in the device. All participants completed the measurements without any unexpected discomfort. Their anonymity was secured in storing and handling the data.

The SEFs recorded in this study were similar to previous study in the field. A typical waveform was seen with components similar to earlier studies. The location of activity both pre and post exercise was in the SI, Brodmann's area 3b (posterior wall of the central sulcus), also according to previous literature. The location of activity was unchanged between the two runs of SEFs.

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With this study setting there was no significant difference in the analyzed component amplitudes of the SEFs. The changes in the SEFs were non-uniform among the study subjects; in some study subjects there was an increase in the amplitude of the components and in some there was a decrease. Exercise induced pain in the present design doesn't seem to have an effect to somatosensory processing, i.e. it does not alter the central inhibition or excitability in response to electrical stimulation of the median nerve.

The study setting may have contributed to the non-uniformity of the changes in the component peak amplitudes. Firstly, the change in the placement of the stimulator might have affected the amplitude, even though the stimulator was re-adjusted. Secondly, the pain induced by the static gripping task may have diminished significantly during the first half of the second set of stimuli. Thus, after picking the responses to be averaged (e.g. responses 120 to 300 out of all 300) the effect of the pain may have not been recorded optimally. If a different type of stimulator was used, for example an air puff stimulator, the results may have been more consistent. There is limitations for usage of different stimulators in this study setting (static gripping task), and the electric stimulator was best suitable. Thirdly, no head position tracking was used in this study protocol.

The head position was recorded only at the start of recording each data file. In this study this means that the head position was recorded in the beginning of the first and second run of SEF. Since the magnetic fields generated in the cerebral cortex are very weak, they diminish quite rapidly when distance between the source and the MEG sensor cap increases. Any head movement or for example slouching slightly while seated in the MEG device may affect the results of the recordings. Ou et al. (2007) pointed out that changes in the relative head position can contribute to the variation between two different data sets. This would also be the case if no head position tracking was used and there was a change in the head position while obtaining data. In this study, especially the participants who had to adjust the median nerve stimulator, may have unintentionally moved their head in the second run of SEF. The head tends to turn towards the point where the eyes are fixed, in this case, the right wrist. After the adjustments the participants were again asked to look straight forward, but there still might be a change to the initial head position.

The hypothesized change in the component amplitudes or the waveform of SEF would be a sign of central excitation or inhibition of somatosensory processing in the presence of pain in muscle tissue after exercise. This study setting aimed to find out the immediate change of somatosensory processing after exercise induced pain. Nociceptive information is relayed from the peripheral parts of the body up the spinal cord to the thalamus via the spinothalamic tract. Nociceptive pathways terminate in the thalamus, and from there nociceptive information is relayed to various cortical and subcortical regions, including the hypothalamus, basal ganglia, amygdala, periaqueductal grey and regions of cerebral cortex (Garland 2012). In this study setting, the presence of pain was hypothesized to have an effect on the SEFs, due to SIs role in somatosensory and nociceptive processing. This would suggest ascending central modulation of pain.

The brain's function in receiving nociceptive information is not passive, but the brain instead regulates transmission of sensory information by influencing the spinal dorsal horn through descending projections from the brainstem nuclei, more accurately the periaqueductal grey and the rostral ventromedial medulla. It is proposed that there is gating of the perception of noxious stimuli in the substantia gelatinosa of the dorsal horn. Afferent signals are integrated with downstream modulation from the brain (Garland 2012, Schaible 2006). This proposed theory would suggest that there may be a mechanism through which the SEFs could also be modified in the presence of pain. In the current study, when the second set of stimuli is executed and pain in the thenar area is present, there could be some gating of the afferent somatosensory information in the substantia gelatinosa of the dorsal horn. Thus there would also be a change in the SEFs generated in the SI.

The changes in the thenar muscle's chemical cellular environment does not affect the SEFs from the periphery. This means that the stimulus information travels unchanged by muscle fatigue from the periphery at least to the spinal chord, where descending projections from the medulla may have a gating action on the stimuli (Taylor et al. 2016). Instead, central inhibition or excitation may be present due to the somatosensory and nociceptive information received by the SI. There is a change in the neurochemistry of the SI in the presence of exercise induced pain and muscle fatigue. When the stimulus signal arrives to the SI from the periphery, the altered neurochemical environment and synapse activity causes a different neural activity, which can be seen in the SEFs.

Peripheral fatigue occurs at or distal to the neuromuscular junction. However, also neural drive determines the degree to which extent muscle fibers contract. The processes within the CNS can limit the neural drive to the muscles; this phenomenon is called central fatigue (Taylor et al. 2016). In the current study setting, exercise induced pain, or muscle fatigue, is hypothesized to alter the somatosensory processing in the SI. This would mean that the somatosensory information received from the upper extremity and/or it's processing would appear modulated in the SEFs. This would further on have an effect in the processes in the motor cortex and efferent signals, i.e. neural drive to the muscles, from the CNS. According to Taylor et al. (2016), it is probable that all the neurons in the brain are affected by both excitatory and inhibitory signals. The neurotransmitters serotonin, dopamine and noradrenaline have a major role in signal transduction between the neurons in the brain, and the changes in the their concentrations have been linked to the central fatigue –hypothesis. Considering this study setting, these neurotransmitters could be the factors behind the hypothesized changes in the SEFs.

Physical activity and pain and their different aspects seem to be interrelated. Pain perception can be different depending on physical activity levels. According to Law & Sluka (2017), experience of pain is modulated by physical activity levels; higher levels of physical activity are associated with greater conditioned pain modulation. The researchers also note that the development of chronic pain is affected by the level of physical activity. Those with sedentary lifestyles have a higher incidence of chronic pain conditions. These findings could mean that the current study's subject's physical activity levels would affect the changes in the SEFs. A more active study subject could have a different modulation in the recorded SEF after exercise induced pain compared to subject who is more sedentary. Further on, it could be hypothesized that the processing of somatosensory information would differ depending on the subject's physical activity levels, which would have an effect on later processing in the cortices and efferent signals to the extremities.

Exercise induced pain may affect early somatosensory processing, but there may also be a change in the processing pathway with a longer latency. Klingner et al. (2016) propose in their study of somatosensory processing that information from the extremities is conveyed via the thalamus to the SI and SII in parallel. The processing then switches to serial processing.

There are neurons in the SII that receive direct information from the thalamus but also process input from the SI. Considering the current study, this could imply that it would be necessary to analyze both SI and SII sources independently, while also look into a longer somatosensory processing window.

This study aimed to show a modulation in the study subjects' SEFs after exercise induced pain. With this study protocol, there was mixed results and no conclusion to the study question at hand could be drawn. Future approaches aiming to study the research question at hand should develop the study protocol further. A more natural stimulus and a different muscle or muscle group could be used. In addition to SI activity, other areas of the brain could also be studied.

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APPENDICES

Appendix 1.
MOOD QUESTIONNAIRE (RBDI)   Date: _____   Subject ID: _____
Finnish modification of the short form of the Beck Depression Inventory

In this questionnaire we would like to ask you about your mood. *Please circle the number before the alternative that best describes how you are feeling at present.*

1. How are you feeling?
 	
    1 I am feeling quite optimistic and good
    2 I do not feel sad
    3 I feel sad or blue
    4 I am blue or sad all the time and I can't snap out of it
    5 I am so sad or unhappy that I can't stand it

2. How do you see your future?
 	
    1 I am full of hope about my future
    2 I am not particularly pessimistic or discouraged about the future
    3 I feel discouraged about the future
    4 I feel I have nothing to look forward to
    5 I feel that the future is hopeless and that things cannot improve

3. How would you describe your life?
 	
    1 my life has been generally successful
    2 I do not feel like a failure
    3 I feel I have failed more than the average person
    4 as I look back on my life, all I can see is a lot of failures
    5 I feel I am a complete failure as a person

4. How satisfied or dissatisfied are you with your life?
 	
    1 I am quite satisfied with my life
    2 I am not particularly dissatisfied
    3 I don't enjoy things the way I used to
    4 I don't get satisfaction out of anything anymore
    5 I am dissatisfied with everything

5. How do you feel about yourself?
 	
    1 I feel quite good about myself
    2 I don't feel particularly guilty
    3 I feel bad or unworthy a good part of the time
    4 I feel quite guilty
    5 I feel as though I am very bad or worthless

6. Are you disappointed in yourself?
 	
    1 I am happy with myself and with what I have achieved
    2 I don't feel disappointed in myself
    3 I am disappointed in myself
    4 I am disgusted with myself
    5 I hate myself

7. Do you have thought of harming yourself?   1 I have never thought about suicide

2	I don't have any thoughts of harming myself
3	I feel I would be better off dead
4	I have definite plans about committing suicide
5	I would kill myself if I had the chance

8. How do you feel about meeting new people?

1	I enjoy meeting people and talking with them
2	I have not lost interest in other people
3	I am less interested in other people than I used to be
4	I have lost most of my interest in other people and have little feeling for them
5	I have lost all my interest in other people and don't care about them at all

9. What are your feelings about making decisions?

1	making decisions is easy for me
2	I make decisions about as well as ever
3	I try to put off making decisions
4	I have great difficulty in making decisions
5	I can't makea ny decisions at all anymore

10. How do you feel about your appearance?

1	I am quite happy with my appearance
2	I don't feel that I look any worse than I used to
3	I am worried that I am looking old and unattractive
4	I feel that there are permanent changes in my appearance and they make me look unattractive
5	I feel that I am ugly or repulsive-looking

11. Do you have problems with sleep?

1	I don't have any problems with sleeping
2	I can sleep as well as usual
3	I wake up more tired in the morning than I used to
4	I suffer from sleeplessness
5	I suffer from sleeplessness, difficulties in getting to sleep or too early awakening

12. Do you ever feel tired or exhausted?

1	I almost never feel tired
2	I don't get any more tired than usual
3	I get tired more easily than I used to
4	I get tired from doing anything
5	I get too tired to do anything

13. How is your appetite?

1	my appetite is very good
2	my appetite is no worse than usual
3	my appetite is not as good as it used to be
4	my appetite is much worse now
5	I have no appetite at all anymore

14. Are you tense or distressed?

1	I have good control over my feelings and do not become tense or distressed easily
2	I do not feel tense or distressed
3	I become distressed quite easily
4	I become anxious, tense or distressed very easily
5	I feel anxious or tense all the time as if I had lost my nerves

Appendix 2.
McGill pain questionnaire

What Does Your Pain Feel Like?

Some of the words below describe your present pain. Circle ONLY those words that best describe it. Leave out any category that is not suitable. Use only a single word in each appropriate category—the one that applies best.

1	2	3	4
Flickering	Jumping	Pricking	Sharp
Quivering	Flashing	Boring	Cutting
Pulsing	Shooting	Drilling	Lacerating
Throbbing		Stabbing	
Beating		Lancinating	
Pounding			

5	6	7	8
Pinching	Tugging	Hot	Tingling
Pressing	Pulling	Burning	Itchy
Gnawing	Wrenching	Scalding	Smarting
Cramping		Searing	Stinging
Crushing			

9	10	11	12
Dull	Tender	Tiring	Sickening
Sore	Taut	Exhausting	Suffocating
Hurting	Rasping		
Aching	Splitting		
Heavy			

13	14	15	16
Fearful	Punishing	Wretched	Annoying
Frightful	Grueling	Blinding	Troublesome
Terrifying	Cruel		Miserable
	Vicious		Intense
	Killing		Unbearable

17	18	19	20
Spreading	Tight	Cool	Nagging
Radiating	Numb	Cold	Nauseating
Penetrating	Drawing	Freezing	Agonizing
Piercing	Squeezing		Dreadful
	Tearing		Torturing