LONG-TERM DEPRESSION OF NOCICEPTION AND PAIN IN HEALTHY VOLUNTEERS

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vorgelegt von

Diplom-Biologin Silke Rottmann

aus Unna

Berichter: Universitätsprofessor Dr. med. Dr. med. habil. Jens Ellrich

Universitätsprofessor Dr. rer. nat. habil. Hermann Wagner

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List of abbreviations

ACC	anterior cingulate cortex
AC-PC	anterior–posterior commissure
Al-TENS	Acupuncture-like TENS
ANOVA	analysis of variance
BA	Brodman area
BOLD	Blood oxygen level dependent
DGSS	Deutsche Gesellschaft zum Studium des Schmerzes
DM	difference of means
EEG	electroencephalography
e.g.	exempli gratia, for example
EMG	electromyography
EPI	T2*-weighted echo planar imaging (fMRI)
EPSP	excitatory postsynaptic potentials
ExpBi	Experiment with bilateral test stimulatian at the right and left hand dorsum, and LFS at the right hand
ExpUni	Experiment with unilateral test stimulatian at the radial and ulnar side of the hand dorsum, and LFS at the radial side
FA	flip angle (fMRI)
fMRI	functional magnetic resonance imaging
FOV	field of view (fMRI)
GABA	γ-Aminobutyric acid
HFS	high-frequency stimulation
I_0	detection threshold
IASP	International Association for the Study of Pain
i.e.	id est, that is
INS	insula
INS A	anterior insula
INS P	posterior insula
I_P	pain threshold
IPL	inferior parietal lobe
I_S	stimulus intensity
ISI	interstimulus interval
L	left (in fMRI study: contralateral)

LFS	low-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
MFG	medial frontal gyrus
N2	second negative peak of SEP (EEG)
NMDA	N-methyl-D-aspartic acid
n. s.	not significant
P2	second positive peak of SEP (EEG)
Post series	test stimulation after LFS or break in the Control experiments
Pre series	test stimulation before LFS or break in the Control experiments
r	correlation coefficient (Pearson)
R	right (in fMRI study: ipsilateral)
RM	repeated measures
S1	primary somatosensory cortex
S2	secondary somatosensory cortex
sem	standard error of mean
SEP	somatosensory evoked cortical vertex potentials (EEG)
SES	Pain Perception Scale (Schmerzempfindungsskala)
SES-A	affective subclass of SES
SES-S	sensory subclass of SES
SPMs	statistical parametric maps (fMRI)
STG	superior temporal lobule
TE	echo time (fMRI)
TENS	transcutaneous electrical nerve stimulation
TR	repetition time (fMRI)
VRS	Verbal Rating Scale (0 - 100)
VRS-I	VRS of intensity
VRS-U	VRS of unpleasantness
vs.	versus

1. Introduction

“Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” This definition by the International Association for the Study of Pain (IASP, www.iasp-pain.org) summarizes the complex nature of pain in one sentence. An adequate stimulus to elicit pain is (potential) tissue damage implicating the important warning function of pain. But pain can also be experienced as tissue damage without any defect, announcing psychological influence. Acute pain serves as an important caution and protection mechanism of the body. Chronic pain persisting more than six month, long after its usefulness as an alarm signal has passed, is without any sense. It is becoming a disease itself significantly lowering the quality of life. According to a study among 46,000 people across Europe, one European adult out of five (19%) suffers from chronic pain (www.paineurope.com). In Germany 17% of the population are afflicted. Chronic pain inflicts great economy loss to the society, in Germany about 25 billion euros per year (German chapter of the IASP, Deutsche Gesellschaft zum Studium des Schmerzes, www.dgss.org). Therefore, research on chronic pain and therapy options is of great interest.

In the present thesis, a specific form of electrical stimulation, so-called low frequency stimulation (LFS), is examined. It might serve as a non-pharmacological treatment in future chronic pain therapy.

1.1. Pain

Pain experience consists of various components with different extend depending on the kind of pain (Schmidt and Lang, 2007) (Fig. 1.1). The sensory-discriminative component is important for identification of location, duration and intensity of the stimulus. The affective-emotional component deals with unpleasantness of the stimulus. As a reaction, the vegetative autonomous and the motor component lead to reflex answers e.g. higher blood pressure, heart frequency and muscle tension, withdraw or fugue. The cognitive component is responsible for the evaluation of the stimulus by comparing the sensation with former experiences. The result of this cognitive process causes pain expression (psycho-motor component), e.g. mimic and vocalization (Fig. 1.1).

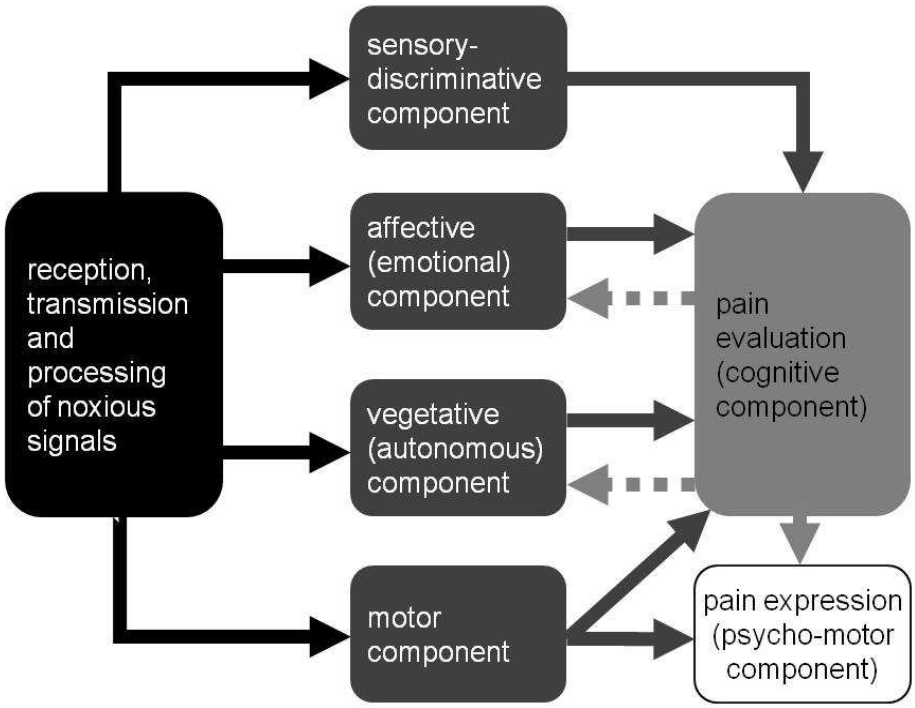


Figure 1.1. Schematic drawing of pain components activated by noxious signals.
Sensory, affective, vegetative and motor components result in pain evaluation and expression. Vice versa, the cognitive component has also influence on the affective and vegetative component (dashed arrows) (Adapted from Schmidt and Lang, 2007).

1.2. Nociception

While pain is a subjective experience resulting from cognitive processing, nociception describes objective processes. Nociception includes not only entrance and transmission but also modulation of noxious stimuli, which takes place at all relay stations. This introduction focuses on cutaneous, spinal nociception.

1.2.1. Peripheral nociceptive system

Nerve fibers that innervate the skin arise from cell bodies in dorsal root ganglia. Based on anatomical and functional criteria, three main groups can be distinguished. Aβ fibers with the largest diameter are myelinated rapidly conducting (30 - 70 m/s) sensory fibers that mostly detect innocuous stimuli, like touch sensation. Thus, they do not belong to the nociceptors that are activated by stimuli causing potential or actual tissue damage. In contrast to this, medium-diameter myelinated Aδ fibers and small-diameter unmyelinated C fibers are activated by noxious stimuli. Most of the nociceptors are polymodal and respond to noxious mechanical, thermal and chemical stimuli, others respond more specialized. The faster Aδ fibers (2 - 33 m/s) mediate

the pinprick-like and well localized “first” pain. The slower C fibers (0.4 - 1.8 m/s) mediate the “second” pain, with a dull and burning sensation (Mackenzie et al., 1975). Electrical stimulation directly excites the free nerve endings. Response to other pain stimuli is mediated by various receptor molecules. For example, heat pain is mainly transduced via vanilloid receptor. Tissue injury results in a local release of various inflammatory agents exciting nociceptive terminals. These factors can also lead to peripheral sensitization resulting in lowered threshold and increased receptive fields. Furthermore silent nociceptors, which are not excitable under normal conditions, can be activated after sensitization. Nociceptors have an efferent function and can release peptides and neurotransmitters (e.g., substance P, calcitonin-gene-related peptide and ATP), which lead to neurogenic inflammation, with vasodilatation and increased vascular permeability. Thus, nociceptors not only mediate but modulate noxious stimuli (Julius and Basbaum, 2001).

1.2.2. Central nociceptive system

Central axons of dorsal root ganglia terminate in the dorsal horn of the spinal cord mainly superficially in laminae I and II or deeper in lamina V and build the first nociceptive synapse. Nociceptive-specific neurons synapse with Aδ and C fibers only. Wide dynamic range neurons also receive input from Aβ fibers, conducting non-noxious mechanical stimuli. Vast majority of primary afferents build excitatory glutamatergic synapses. Excitatory, glutamatergic and inhibitory, GABAergic (γ-Aminobutyric acid) interneurons increase or decrease response of these neurons and thus influences the output of the dorsal horn (D'Mello and Dickenson, 2008).

On spinal and supraspinal level reflex actions are mediated. The nociceptive neurons project to interneurons that are integrated in motoric reflex arcs. Vegetative reflexes are controlled supraspinally by the brainstem in intact organisms, but they can still be determined in modified form after spinalization (Schmidt and Lang, 2007).

Ascending pathways lead from the spinal dorsal horn to the brainstem, thalamus and cortex. According to recent knowledge, no exclusive nociceptive specific tracts or supraspinal pain centers with exclusive nociceptive neurons exist. Main ascending pathway is the spinothalamic tract projecting directly to the thalamus, followed by thalamocortical pathways. Another important pathway is the spinoreticular tract projecting to the reticular formation of medulla and pons, followed by projections to the thalamus (Schmidt and Lang, 2007). There are two different thalamocortical

pathways, that process sensory and affective pain perception. Sensory information is mainly processed via lateral thalamus to primary and secondary somatosensory cortex (S1, S2) and posterior insula. Affective components of pain are processed via medial thalamus to anterior cingulate cortex and anterior insula (Treede et al., 1999). Prefrontal and parietal cortices are involved in cognitive and attentional processes (Kong et al., 2006; Brown et al., 2008). A cortical-limbic pathway projects from S1 and S2 via posterior parietal cortex and insula to amygdala and hippocampus, integrating pain sensation, affect, fear and memory. Other ascending spinal pathways directly access, inter alia, amygdala, hippocampus, hypothalamus and periaqueductal grey, leading to autonomic fear and defensive response (Price, 2002). Main regions involved in descending antinociceptive pathway are periaqueductal grey and raphe nuclei. Direct stimulation of these regions causes analgesia. Inhibiting transmitters are noradrenaline, serotonin, GABA and opioids (Stamford, 1995).

1.3. Synaptic plasticity

1.3.1. Cellular mechanisms of LTP and LTD

The model of bidirectional synaptic plasticity includes long-term potentation (LTP), a long lasting increase of synaptic strength, and its counterpart, long-term depression (LTD), a sustained decrease of synaptic strength. Both phenomena were first investigated in the hippocampus, a brain structure well known to be involved in memory processes (Kandel et al., 2000). Almost 40 years ago, LTP was detected in the dentate area following stimulation with brief high-frequency electrical pulses (HFS) of the perforant path in anaesthetized rabbit (Bliss and Lomo, 1970). After that, a large number of studies on plasticity in the hippocampus were conducted, including LTP and LTD. Prolonged electrical low-frequency stimulation (LFS) was shown to reliably induce LTD. After LFS at the Schaffer collateral projection to area CA1, slope of excitatory postsynaptic potentials (EPSP) in CA1 region decreased for at least one hour (Dudek and Bear, 1992). Furthermore, established LTP showed recovery back to baseline synaptic strength by subsequent electrical LFS in rodents (Barrionuevo et al., 1980). Underlying cellular mechanisms of these long-lasting modifications seem to be important for learning and also “forgetting” processes (Tsumoto, 1993).

LTP and LTD share common properties, HFS and LFS lead to an activation of NMDA (N-methyl-D-aspartic acid) receptors and increase in postsynaptic calcium channels.

For LTP, a high calcium influx preferentially leads to the activation of protein kinases, such as protein kinase C or calcium-calmodulin dependent kinase II, which can subsequently phosphorylate glutamate receptors. Postsynaptically activated nitric oxide could serve as a retrograde messenger, leading to increased transmitter release at presynaptic side. For LTD, a moderate calcium influx leads to preferential activation of protein phosphatases, such as calcium-calmodulin dependent protein phosphatase, which can dephosphorylate inhibitotor 1. Inactivation of inhibitor 1 results in the activation of protein phosphatase 1 and/or 2 and subsequent dephosphorylation of glutamate receptors. At presynaptic side, metabotropic glutamate receptors can lead to a reduction of glutamate release. Retrograde messenger nitric oxide, which is activated postsynaptically, can lead calcium elevation, which is hypothesized to lead to reduction in presynaptic transmitter release (Braunewell and Manahan-Vaughan, 2001; Kemp and Bashir, 2001). Figure 1.2 summarizes the bi-directional model of synaptic strength.

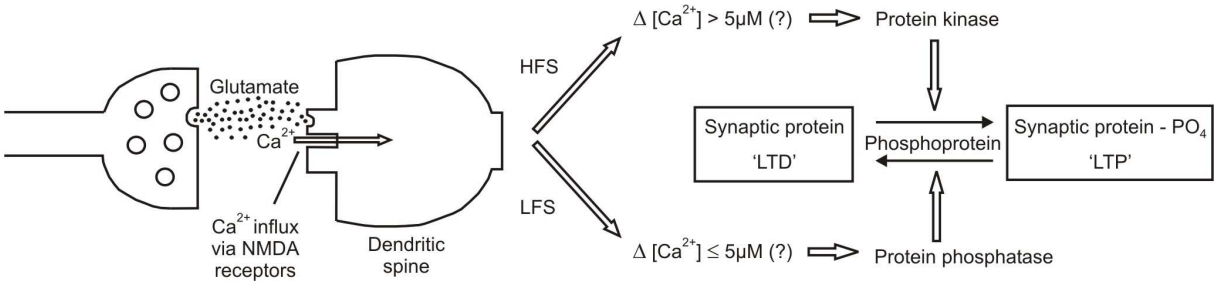


Figure 1.2. Model for the induction of LTP and LTD.

During afferent activity, Ca^{2+} enters dendritic spines through NMDA receptors. During high-frequency stimulation (HFS), Ca^{2+} reaches high levels and preferentially activates a protein kinase. During low-frequency stimulation (LFS), lower Ca^{2+} levels are achieved and this preferentially activates a protein phosphatase. Both the kinases and phosphatases act on a common synaptic phosphoprotein, the phosphorylation state of which controls synaptic strength (Bear and Malenka, 1994).

1.3.2. LTP and LTD in nociceptive system

Both, LTP and LTD, were also examined in the nociceptive system, in the superficial spinal dorsal horn after conditioning stimulation at the attached dorsal root. Repetitive HFS of primary afferents induces LTP in Aδ (Randic et al., 1993) and in C fibers (Liu et al., 1998). Synaptic transmission decreased for more than one hour after noxious

LFS with Aδ fiber intensity. LFS with lower intensity, mainly activating Aβ fibers, only induced transient depression of synaptic transmission for less than 30 minutes (Sandkuhler et al., 1997). Noxious LFS of tongue musculature evoked LTD of craniofacial processing in mice (Ellrich, 2004; Ellrich, 2005). It has been suggested that LTP in nociceptive pathways may be responsible for induction of central sensitization which is assumed to be involved in development of pain memory (Sandkuhler et al., 2000; Ikeda et al., 2003; Ji et al., 2003). Furthermore, LFS to Aδ fibers could reverse HFS induced LTP (depotentiation) and also HFS could not induce LTP once LFS was given (Ikeda et al., 2000). Underlying depotentiation processes are of great interest as they might play an important role in erasing pain memory by noxious LFS. Hence, the current study focuses on LFS as a model of neuromodulation in future analgesic therapy.

1.3.3. Spatial organization of LTD

In vitro studies indicate a sole homosynaptic organization of LTD. In hippocampal slices, LFS of the Schaffer collateral projection to area CA1 induced LTD exclusively at the conditioned pathway. To activate a second converging input, a second stimulating electrode was placed on the opposite (subicular) side of the recording location. This second unconditioned input showed no LTD effect. It was suggested that LTD is input-specific and confined to conditioned synapses (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Kerr and Abraham, 1995). In the same way homosynaptic LTD was induced in the visual cortex of rat and cat (Kirkwood et al., 1993). This homosynaptic effect of LFS has also been shown for the nociceptive system in vitro. LFS of primary afferent Aδ fibers of spinal dorsal roots selectively reduced synaptic transmission in dorsal horn in the conditioned pathway (Chen and Sandkuhler, 2000). These in vitro studies suggest a sole homosynaptic LTD.

1.4. LTD in humans

So far, only a few studies deal with LTD of nociception and pain in humans. Most of them examine trigeminal nociceptive system (Ellrich, 2006). LFS was applied to trigeminal afferents and LTD effect was controlled by evocation of masseter inhibitory reflex (Ellrich and Schorr, 2002) and blink reflex (Schorr and Ellrich, 2002; Yekta et al., 2006), recording of somatosensory evoked cortical potentials (Ellrich and Schorr, 2004) and pain perception rating. In spinal nociceptive system, pain ratings were

investigated (Nilsson et al., 2003; Klein et al., 2004). All studies showed sustained decrease of reflexes, cortical potentials and pain ratings for at least one hour.

Most recent study on LTD in humans investigated the optimum LFS parameter of LTD induction by psychophysical and electrophysiological means (Jung et al., 2009). So far, LFS setting was mostly adopted from experiments performed in rodents under in vitro conditions. In this study, 120 experiments were conducted with varying LFS frequency (0.5, 1, 2 Hz), number of pulses (300, 600, 1200) and intensity (related on pain threshold I_P: $1\times I_P$, $2\times I_P$, $4\times I_P$). Strongest effect on SEP and pain rating was observed after LFS with 1 Hz, 1200 pulses and $4\times I_P$. Furthermore, established LTD after single LFS was amplified by an additional second LFS. Optimum LFS parameter revealed in this study were used in the present thesis.

1.5. Aim of the present thesis

Aim of the present thesis was a further, more detailed investigation of LTD of spinal nociception and pain in healthy humans. Therefore, cutaneous Aδ fibers of the hand dorsum were electrically stimulated and three different aspects of LFS-induced LTD were examined.

(1) Putative homotopy of LTD in human nociception and pain was examined as it was suggested from in-vitro experiments. Cortical potentials and pain perception after LFS were examined in a conditioned and a non-conditioned pathway.

(2) Recent human studies on LTD provided evidence for sustained reduction of global pain perception without any differentiation of various pain qualities. Therefore, sensory and affective pain perceptions were assessed by multidimensional rating scales before, after and during conditioning LFS.

(3) LFS effects on cerebral activation pattern obtained via functional magnetic resonance imaging (fMRI), and pain perception ratings before and after LFS were compared.

Parts of this study were published (Rottmann et al., 2008; Rottmann et al., 2009a; Rottmann et al., 2009b).

2. Methods

The experiments were performed in healthy volunteers, who gave their informed consent prior to their inclusion in the study according to the 1964 Declaration of Helsinki (as amended by the 52nd General Assembly, Edinburgh, Scotland, 2000; http://www.wma.net). The protocol was approved by the local ethics committee. All volunteers had no prior or current skin disease and none of the volunteers were taking analgesic medication. The participants were not informed about the theoretical background of the experiments or possible outcomes.

In the present thesis, electrical stimulation served as pain stimulus. Electrical noxious stimuli (rectangular pulses, 2 ms duration) were applied to hand dorsum via a custom-made concentric electrode. This electrode consists of a small central cathode (diameter: 1 mm) and a large external ring anode (inner diameter: 8 mm; outer diameter: 24 mm). Due to its special geometry, this electrode produces high current density at low current intensities, which leads to preferential activation of cutaneous Aδ fibers (Bromm and Meier, 1984; Kaube et al., 2000; Katsarava et al., 2006). The electrical stimulation was performed with a constant current stimulator (Model DS7A, Digitimer Limited, Hertfordshire, UK).

2.1. Homotopy of LTD

Three experiments and a total of 44 sessions were performed on 30 healthy volunteers (16 females, 14 males) between 21 and 44 years of age. The volunteers were comfortably sitting on a chair with eyes closed.

2.1.1. *Stimulation procedure*

Two concentric electrodes were adjusted at hand dorsum in two different arrangements: unilateral at right hand at radial and ulnar sides (ExpUni) and bilateral at right and left hands at radial sides (ExpBi) (Figs. 2.1A and B). Individual pain threshold (I_P: pricking painful) was determined by applying four series of electrical pulses with decreasing and increasing stimulus intensities using increments of 50 µA according to the method of limits (Gescheider, 1985). Based on threshold detection, the electrical stimulation intensity was adjusted to approximately 5-fold pain threshold, clearly pricking painful and appropriate to elicit reliable evoked potentials.

Test stimulation was applied in series of 15 stimuli each with a frequency of 0.125 Hz. Conditioning noxious LFS was applied with a frequency of 1 Hz for 20 minutes, i.e. 1200 pulses, with the same intensity as test stimuli. A recent study of varying LFS parameters revealed the strongest reduction of SEP and pain rating by use of this stimulation protocol (Jung et al., 2009). LFS was always applied to radial side of right hand dorsum. Test stimulation series were alternately applied homotopic to LFS to radial side of right hand dorsum, and heterotopic to LFS to ulnar side of right hand (ExpUni), or to radial side of left hand (ExpBi), respectively (Fig. 2.1). Test stimulus series were repeated every eight minutes before (Pre) and after (Post) LFS. Six alternating Pre series were applied, and after LFS, Post series were continued for one hour. In a Control experiment no LFS was applied but test stimulation was interrupted (test stimulation same as ExpBi) (Fig. 2.1). Ten volunteers participated in ExpUni. Twenty volunteers attended ExpBi, 14 of them took part in Control experiment. They participated in Control and LFS experiment on different days with at least three days in between (Fig. 2.1).

2.1.2. *Recording*

Somatosensory evoked cortical vertex potentials (SEP) were recorded via EEG. Recording electrodes were placed at Cz, Fz and Pz referred to left earlobe (A1) according to the international 10-20-system (bandpass 0.08 to 30 Hz). For artifact control electrooculogram of vertical (bandpass 0.08 to 1000 Hz) and horizontal eye-movements (bandpass 0.08 to 20 Hz), and electromyogram of right masseter muscle (bandpass: 10 to 1000 Hz) were recorded. A ground electrode was fixed on the right forearm (Deuschl and Eisen, 2000). EEG sweeps were recorded from 200 ms before to 600 ms after electrical stimulation.

EEG signals (sampling rate 1000 Hz) were amplified by an EEG bioamplifier VD32 (Schwarzer, Munich, Germany), digitized by a micro CED1401 A/D-Converter (CED, Cambridge, UK), and analyzed by the Signal Software (http://www.ced.co.uk).

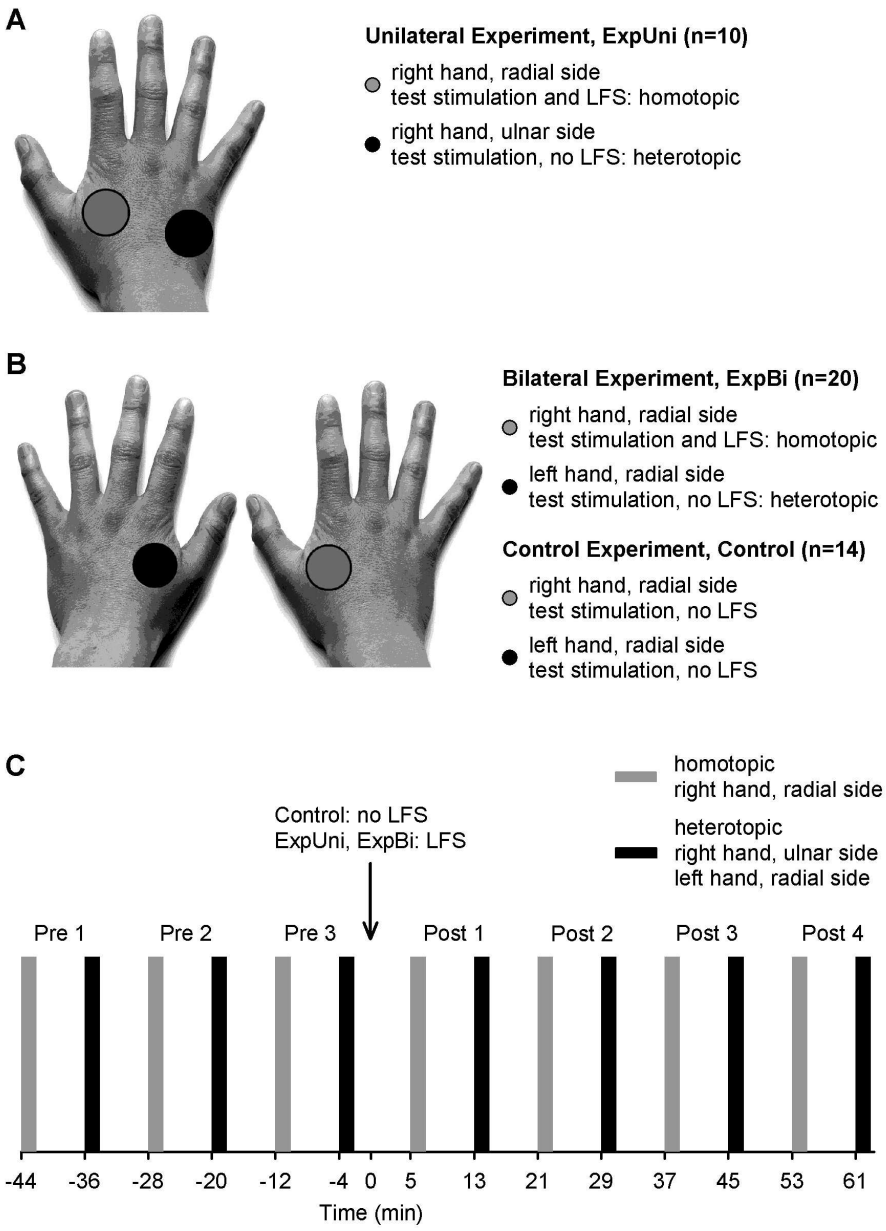


Figure 2.1. Stimulation protocol (Homotopy of LTD).

(A) In unilateral experiment (ExpUni) electrical test stimuli were alternately applied to radial (homotopic) and ulnar (heterotopic) sides of right hand dorsum in 10 volunteers. Electrical low-frequency stimulation (LFS) was applied to radial side of right hand dorsum. (B) In bilateral experiment (ExpBi) electrical test stimuli were alternately applied to radial sides of right (homotopic) and left hand dorsum (heterotopic) in 20 volunteers. LFS was applied to right hand dorsum. In Control experiment electrical test stimuli were alternately applied to radial sides of right and left hand dorsum in 14 volunteers. No LFS was applied. (C) Stimulation protocol. Test stimulation series (15 stimuli per series, 0.125 Hz, 8 min between series) were alternately applied to radial and ulnar sides of right hand dorsum (ExpUni) or to radial sides of right and left hand dorsum (ExpBi, Control). After three Pre test stimulus series each, either LFS (1 Hz, 20 min) was applied to radial side of right hand dorsum (ExpUni, ExpBi) or stimulation was interrupted for 20 min (Control). Afterwards, Post test stimulation series were continued for one hour.

2.1.3. *Pain perception rating*

In addition to this electrophysiological data psychophysical data were collected. An audible signal, 1.5 seconds after each test stimulus, announced volunteers to rate stimulus intensity according to Verbal Rating Scale (VRS: 0=no pain; 100=maximum imaginable painful).

2.1.4. *Data analysis*

Sweeps of test stimulation series were averaged, only sweeps with artifacts were rejected. Latencies of negative peak (N2) and positive peak (P2) of SEP and its amplitude were determined. Subjective pain perception during test stimulation was analyzed. These parameters recorded in all test stimulation series were normalized to baseline by dividing mean value of each series by mean value of three Pre series. Normalized parameters were expressed as percentage changes from baseline. Data were described by arithmetic mean and standard error of mean (sem), by median, 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles (box plot). Statistical analyses within one experiment (time course of different parameters) and between different experiments (Control vs. ExpBi, n=14) at corresponding time points were performed by One Way Repeated Measures ANOVA (F, p value) followed by Holm-Sidak post hoc test (Difference of means=DM, p value) or by Friedman Repeated Measures ANOVA (Chi-square=X^2, p value) and subsequent Student-Newman-Keuls post hoc test (q, p value). Homotopic and heterotopic Post series in ExpUni and ExpBi were compared by Paired t-test (t and p value). Additionally, homotopic and heterotopic Post1 series were separately compared by Paired t-test (ExpUni, ExpBi) and compared to Control experiment by One Way Repeated Measures ANOVA followed by Holm-Sidak post hoc test (Control vs. ExpBi, n=14). The same applied to Post4 series, only LFS experiment and Control experiment were compared by Friedman Repeated Measures ANOVA and subsequent Student-Newman-Keuls post hoc test. The level of significance was set to $p<0.05$. The SigmaStat® software 3.1 (SPSS Inc., Chicago, Illinois, USA) was applied.

2.2. LFS effect on sensory and affective pain components

Two experiments including 40 sessions were performed on 20 healthy volunteers (10 females, 10 males) between 22 and 31 years of age. All participants were native German-speaking students or trainees. Volunteers were comfortably sitting on a chair with eyes closed.

2.2.1. *Electrical stimulation*

Concentric electrode was adjusted at the radial side of the left hand dorsum. Individual thresholds for detection (I_0) and pain (I_P: marginally pricking painful) were determined by applying four series of electrical pulses with decreasing and increasing stimulus intensities using increments of 50 µA according to the method of limits (Gescheider, 1985). Based on threshold detection, the electrical stimulation was adjusted to stimulus intensity with 4fold I_P corresponding to a clearly pricking painful sensation. Both, test stimulation and conditioning noxious LFS were applied via the same electrode. LFS was applied with a frequency of 1 Hz for 20 minutes, i.e. 1200 pulses, with the same intensity as test stimulation (Jung et al., 2009). Test stimulation was applied in series of 15 stimuli each with a frequency of 0.125 Hz (Fig. 2.2A). Test stimulation series were repeated every eight minutes. In order to familiarize volunteers with the experimental procedure the very first test stimulation series was excluded from the analysis (Pre 0). Three test stimulation series before (Pre) and three series after (Post) conditioning LFS were performed (Fig. 2.2A). In the Control experiment, no conditioning LFS was applied but test stimulation was interrupted for 20 minutes. All 20 volunteers participated in Control and LFS experiments on different days with at least three days in between. The order of Control and LFS experiments was balanced.

2.2.2. *Pain perception rating*

Subjective pain perception was obtained by two different pain measurements. Volunteers were asked to rate pain perception according to Verbal Rating Scale (VRS) and to distinguish between pain intensity (VRS-I: 0=not intensive; 100=maximum imaginable intensive) and pain unpleasantness (VRS-U: 0=not unpleasant; 100=maximum imaginable unpleasant).

Additionally, the Pain Perception Scale (Schmerzempfindungsskala, SES) (Geissner, 1995) was applied. The SES is a part of the German pain questionnaire designed by

the German Chapter of the International Association for the Study of Pain (Deutsche Gesellschaft zum Studium des Schmerzes, www.dgss.org). It is an approved tool to determine sensory and affective pain qualities. In this study an enlarged SES questionnaire was used including nine additional sensory items (Türp and Marinello, 2002). Volunteers were asked to judge 19 sensory (SES-S) and 14 affective items (SES-A) on a scale ranging from 0 to 3 (0=not appropriate; 1=largely appropriate; 2=somewhat appropriate; 3=fully appropriate).

After each test stimulus, volunteers were asked to give VRS-I and VRS-U ratings. At the end of each test stimulation series, they filled in an SES questionnaire (Fig. 2.2A). During conditioning LFS, volunteers gave VRS-I and VRS-U ratings every minute. They answered SES questionnaires 10 and 20 minutes after the start of conditioning LFS (Fig. 2.2B).

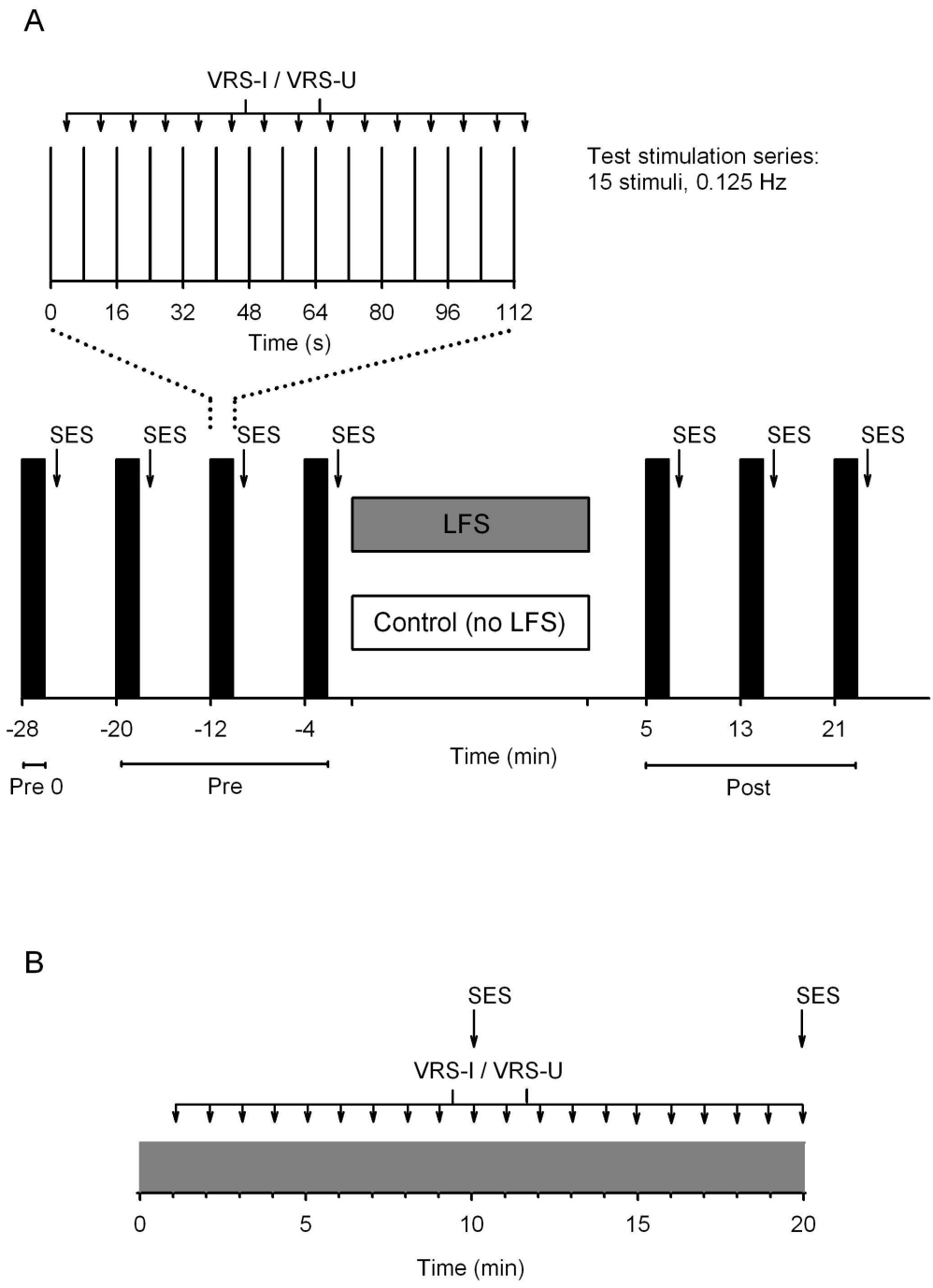


Figure 2.2. Stimulation protocol (LFS effects on sensory and affective pain components). (A) Test stimulation series consisted of 15 test stimuli, applied every 8 seconds. After each test stimulus volunteers were asked to rate pain intensity (I) and unpleasantness (U) on a Verbal Rating Scale (VRS-I, VRS-U). After each test stimulation series volunteers filled in a Pain Perception Scale (SES). Test stimulation series were repeated every eight minutes. First series (Pre 0) was conducted in order to familiarize the volunteers with the rating procedure. Three Pre series and three Post series were analyzed. Between Pre and Post series, either conditioning low-frequency stimulation (LFS) was applied or stimulation was interrupted for 20 minutes (Control). (B) Conditioning LFS was applied for 20 minutes with 1 Hz. Volunteers gave VRS-I and VRS-U rating every minute. They filled in SES after 10 and 20 minutes.

2.2.3. *Data analysis*

Data were described by arithmetic mean and standard error of mean (sem), by median, 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles (box plot). Before statistical analysis, data were transformed into decadic logarithms, as they were not normally distributed. To avoid a loss of zero-values a small constant (0.1) was added to the raw data. Mean values of VRS-I and VRS-U, and SES-S and SES-A, respectively, of Pre and Post condition in the two experiments were compared by Two Way Repeated Measures (RM) ANOVA (F, p value) followed by Fisher LSD post hoc test (Difference of Means=DM, p value). With-in subject factors were time (mean value of Pre and Post series) and experiment (Control vs. LFS experiment). In order to test for possible gender differences, a Three Way Mixed ANOVA with the two with-in subject factors time and experiment and the between subject factor gender was performed. Ratings of LFS experiment were examined by Two Way RM ANOVA. Factor 1 was the time course, comparing test stimulation (Pre and Post series) and conditioning LFS (first 10 minutes of LFS = LFS1, second 10 minutes of LFS = LFS2), factor 2 was the rating dimension (VRS-I vs. VRS-U; SES-S vs. SES-A). Subsequently, Fisher LSD post hoc test was conducted.

In order to sort the SES-S items in meaningful groups of sensory qualities, factor analysis was conducted using ratings of Pre series (Hansen et al., 2007). Factors with Eigenvalue over 1 were retained and rotated using VARIMAX rotation. Rotation maximizes the loading of each variable of one of the extracted factors whilst minimizing the loading on all other factors. Only factor loadings above 0.5 were considered. Cronbach's apha was calculated for each factor, in order to determine the reliability and internal consistency of the groups. LFS effect on each factor was analyzed by Two Way RM ANOVA (Pre vs. Post; Control vs. LFS experiment; Fisher LSD post hoc test). Differences in Pre series, LFS1, LFS2, and Post series were determined for every factor by 1-way RM ANOVA (F, p value) followed by Fisher LSD post hoc test.

The level of significance was set to $p<0.05$. The SigmaStat® software 3.1 and SPSS 16.0 (SPSS Inc., Chicago, IL, USA) were applied.

2.3. LTD of cerebral activation

Two experiments were performed on 17 healthy male volunteers aged between 19 and 28 years. All participants were native German-speaking students or trainees.

2.3.1. *Electrical stimulation*

Concentric electrode was adjusted at the radial side of the right hand dorsum. Individual pain thresholds (I_P) for the first marginally pricking pain were determined by applying four series of electrical pulses with decreasing and increasing stimulus intensities using increments of 50 µA according to the method of limits (Gescheider, 1985). The electrical stimulation was adjusted to a clearly pricking stimulus intensity with 4-fold I_P. Both, test stimulation and conditioning noxious LFS were applied via the same electrode. Individual stimulation intensity was assessed just before the volunteers were placed into the MRI scanner.

2.3.2. *Stimulation protocol*

The fMRI recordings were obtained during four test stimulation series. During each series, 15 test stimuli were applied embedded in a typical box-car design, with three alternating rest and stimulation periods, lasting 15 seconds each. During each stimulation period, 5 stimuli were applied with 0.33 Hz (Fig. 2.3). In a test stimulation series volunteers fixated a white cross on a black screen, presented by MR-compatible LCD goggles (VisuaStim digital, Resonance Technology Inc., Los Angeles, USA). In the LFS experiment, two series were conducted before (Pre) and two after (Post) the conditioning LFS. Interval between first and second Pre series and first and second Post series was 6.5 minutes. LFS was applied with a frequency of 1 Hz for 20 minutes, i.e. 1200 pulses, with an intensity of 4-fold I_P (Jung et al., 2009). A second experiment with the same order of test stimulation series but with 20 minutes no-stimulation time served as a Control (Fig. 2.3). All 17 volunteers participated in both experiments on different days with at least three days in between starting with either Control or LFS experiment in a balanced order.

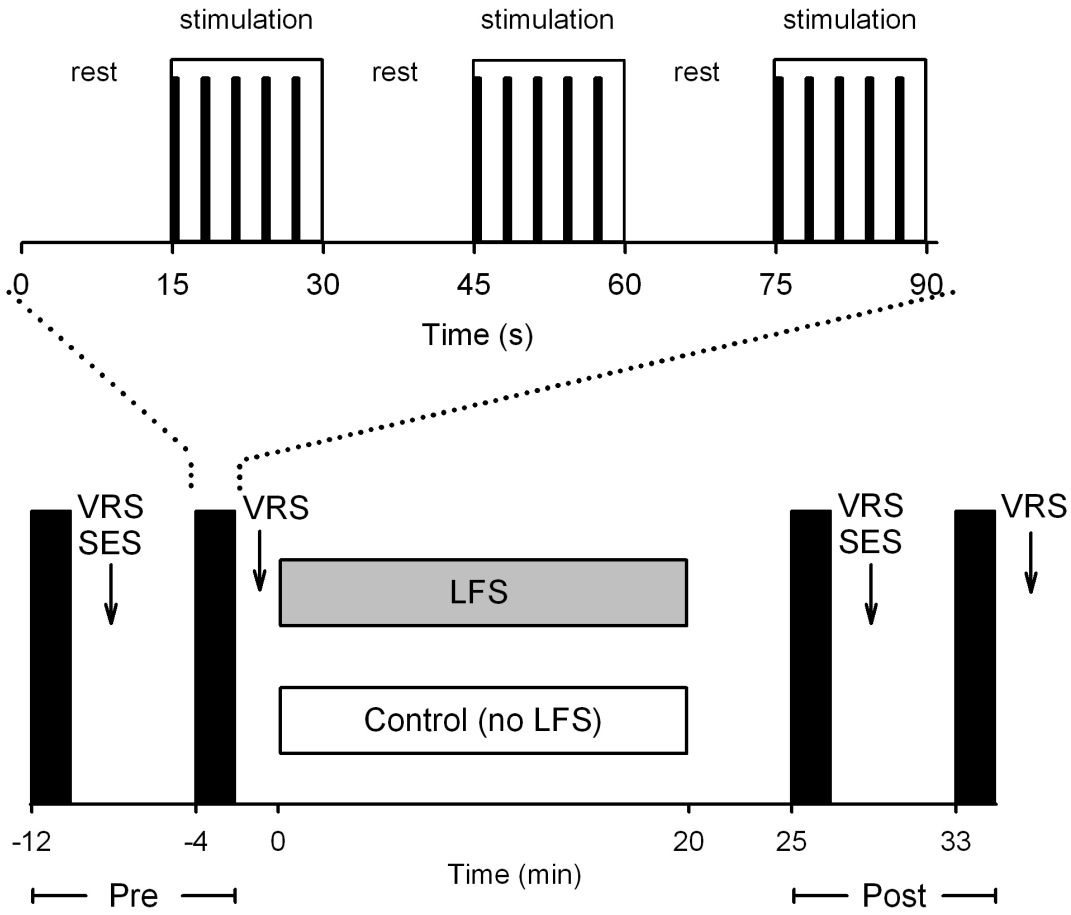


Figure 2.3. Stimulation protocol (LTD of cerebral activation).

The fMRI recordings were conducted during 4 test stimulation series. During each series, three alternating rest and stimulation periods were conducted. After 15 seconds rest period, stimulation period occurred with application of 5 test stimuli for 15 seconds (0.33 Hz). Overall 15 test stimuli were applied during one series. Two series were performed before (Pre) and two series after (Post) conditioning LFS (1Hz, 1200 pulses, 20 minutes) or an interruption in the Control experiment. Interval between first and second Pre series and first and second Post series was 6.5 minutes. Volunteers rated pain perception according to Verbal Rating Scale (VRS) after each series and filled in a Pain Perception Scale (SES) after first Pre and first Post series.

2.3.3. *Pain perception rating*

Subjective pain perception was obtained by two assessments. After each series, volunteers were asked to rate pain perception according to a Verbal Rating Scale (VRS: 0=not painful; 100=maximum imaginable painful).

Furthermore, the Pain Perception Scale (SES: 0=not appropriate to 3=fully appropriate) was applied (confer chapter 2.2.2). The SES was provided between first and second Pre series and between first and second Post series (Fig. 2.3). SES items were displayed on LCD goggles (VisuaStim XGA, Resonance Technology Inc.,

Los Angeles, USA). Ratings were delivered via an audio system (Commander XG, Resonance Technologies Inc., Los Angeles, USA). During LFS, volunteers were asked to rate their pain perception according to VRS every five minutes and to answer SES afterwards. Rating during LFS was conducted to assure that the volunteers paid attention to the stimulation, results were not analyzed.

Data were described by arithmetic mean and standard error of mean (sem), by median, 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles (box plot). Before statistical analysis, data were transformed into decadic logarithms, as they were not normally distributed. To avoid a loss of zero-values a small constant (0.1) was added to the raw data. Mean values of VRS, SES-S and SES-A, respectively, of Pre and Post condition in the two experiments were compared by Two Way repeated measures (RM) ANOVA (F, p value) followed by Fisher LSD post hoc test (Difference of Means=DM, p value). With-in subject factors were time (mean value of Pre and Post series) and experiment (Control vs. LFS experiment). The level of significance was set to $p<0.05$. The SigmaStat® software 3.1 (Systat Software Inc., http://www.systat.com) was applied.

2.3.4. *Image acquisition*

All measurements were conducted at the University Hospital of RWTH Aachen University using a whole body Philips 3 T MRI “Modell Achieva” (Philips Medical Systems, Nederland B.V.) with a standard head coil and foam padding to restrict movements. After orienting the axial slices in the anterior–posterior commissure (AC–PC), plane functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a repetition time (TR) of 2300 ms, an echo time (TE) of 30 ms and a flip angle (FA) of 90 degrees. During one scan, 42 volumes were collected, consisting of 33 contiguous slices (3.75×3.75 mm^2 in-plane resolution; 3.5 mm slice thickness; 0.5 mm gap) measured interleaved with whole brain coverage. A 64x64 matrix with a field of view (FOV) of 240 mm was used. Each fMRI scan started with five dummy scans that were not recorded for data analysis to allow tissue to reach steady state magnetization. Image artifacts were avoided by stretching the stimulation cables as much as possible and by using non-magnetic brazen electrodes.

2.3.5. *Image processing and statistical analyses*

Image processing and statistical analysis were performed using SPM2 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/). All functional images were realigned to the first image and spatially normalized into the anatomical space of the MNI brain template (Montreal Neurological Institute). The voxel sizes of the normalized images were 4 mm isotropically. Spatial smoothing was performed using a Gaussian filter of 8×8×8 mm^3 in order to enhance signal-to-noise ratio. The effect of the electrical stimulation on regional blood oxygenation level-dependent (BOLD) responses was estimated according to the general linear model (Friston et al., 1995). Statistical parametric maps (SPMs) with t-statistics for each voxel were generated using the hemodynamic response function. Single subject t-contrasts were computed for stimulation periods compared to rest periods in order to examine brain activation under Pre and Post stimulation in LFS and Control experiment. The two series before (Pre) and the two series after (Post) LFS or after the break in Control experiment, respectively, were considered as one condition. Contrast images were entered into a second level statistical analysis to test for effects on a between subject basis. This approach corresponds to a random effects analysis, which extends the scope of inference to the population from which the subjects were initially recruited.

2.3.5.1. Group Analyses

Group analyses were conducted considering brain activations of all 17 volunteers without regard to the ratings. Simple contrasts, comparing stimulation periods with rest periods (Pre LFS; Post LFS; Pre Control; Post Control) were calculated with One Sample t-tests. Paired t-tests were used to examine differences in simple contrasts in one experiment (Pre LFS - Post LFS; Post LFS - Pre LFS; Pre Control - Post Control; Post Control - Pre Control) and between LFS and Control experiment (Pre LFS - Pre Control; Pre Control - Pre LFS; Post LFS - Post Control; Post Control - Post LFS). Significance threshold was set to $p<0.001$ uncorrected for all contrasts. In order to establish an appropriate voxel contiguity Monte-Carlo simulation of the brain volume was conducted (Slotnick et al., 2003). This correction has the advantage of higher sensitivity, while still correcting for multiple comparisons across the whole brain volume. Assuming an individual voxel type I error of $p<0.001$, a cluster extent of 7 contiguous resampled voxels was indicated as necessary to correct for multiple voxel comparisons across the whole brain at $p<0.05$ (based on 10,000 iterations). All

complex contrasts were inclusively masked by the minuend with p<0.05 uncorrected. For example, in the Pre - Post contrast only regions with activation under Pre condition were considered. Deactivation under Post condition that are probably artifacts from blood flow away from this region to the region of high neuronal activity could otherwise lead to a seemingly significant positive effect in the Pre - Post contrast. This mask is a pure graphical operation without the use of statistics. Finally, coordinates of activation were transformed from MNI to Talairach space (Talairach and Tournoux, 1988) using the Matlab function mni2tal.m implemented by Matthew Brett (Brett et al., 2002).

2.3.5.2. Correlation with pain rating

Simple regression analyses between changes in cortical activation and rating after LFS in contrast to Pre LFS were conducted. Differences in VRS, SES-S and SES-A rating (mean rating Pre LFS - mean rating Post LFS) were tested against Pre LFS - Post LFS contrast to examine putative correlation between pain relief and decreased brain activity after LFS. In order to determine correlation between pain relief and increased pain activity after LFS, simple regression analyses were conducted using the Post LFS - Pre LFS contrast. For the simple regression analyses, Monte-Carlo simulation of the brain volume was conducted (Slotnick et al., 2003). Assuming an individual voxel type I error of p<0.01, a cluster extent of 12 contiguous resampled voxels was indicated as necessary to correct for multiple voxel comparisons across the whole brain at p<0.05 (based on 10,000 iterations). Finally, coordinates of activation were transformed from MNI to Talairach space (Talairach and Tournoux, 1988) using the Matlab function mni2tal.m implemented by Matthew Brett (Brett et al., 2002).

Brain coordinates assessed by simple regression analyses (SPM2) were further examined by use of the statistical program SigmaStat 3.1 (Systat Software, Inc.). Pearson Correlation was calculated in order to measure the association between change in brain activity and pain rating. The correlation coefficient (r) ranging between –1 and 1 indicated the relationship between the two variables. Scatter plots were prepared to visualize the results.

3. Results

3.1. Homotopy of LTD

In all 44 sessions I_P, SEP and pain perception ratings were recorded. Stimulus intensity of about 5-fold I_P elicited a definite pinprick-like painful sensation and stable SEP. Absolute values under Pre baseline conditions are summarized in table 3.1. There were no significant differences at the two electrode positions within the different experiments.

Table 3.1. Absolute values under baseline condition.

Parameter	Ulnar right	Radial right
ExpUni (n=10)		
I_P (mA)	0.8±0.1	0.7±0.1
I_S (mA)	6.1±0.7	5.0±0.7
N2 latency (ms)	140.1±3.3	140.1±2.1
P2 latency (ms)	221.8±11.1	218.7±9.0
SEP amplitude (µV)	37.1±4.0	34.7±3.5
VRS Rating	40.0±4.9	38.5±4.7
	Radial left	Radial right
ExpBi (n=20)		
I_P (mA)	0.8±0.1	0.9±0.1
I_S (mA)	3.2±0.3	3.5±0.3
N2 latency (ms)	146.6±2.3	149.2±2.8
P2 latency (ms)	232.9±5.8	234.2±5.9
SEP amplitude (µV)	28.3±1.9	30.5±2.2
VRS Rating	29.3±3.4	27.7±3.3
Control (n=14)		
I_P (mA)	1.0±0.1	1.0±0.1
I_S (mA)	3.6±0.4	3.6±0.3
N2 latency (ms)	148.6±4.2	147.6±3.8
P2 latency (ms)	226.6±6.6	222.6±5.8
SEP amplitude (µV)	27.6±2.5	29.0±2.7
VRS Rating	35.3±4.2	31.6±3.8

Pain threshold (I_P), Stimulus intensity (I_S), N2 and P2 latency, SEP amplitude and VRS rating were determined in every session. There were no differences within the experiments under baseline condition. Data are presented as mean±sem.

3.1.1. *LFS within one hand (ExpUni)*

Ten volunteers were tested in order to examine homotopic nature of LFS within one hand (ExpUni). Considering time course, SEP amplitude elicited by homotopic test stimulation significantly decreased after LFS compared to baseline (-34.6±5.4%, $F=15.5$, $p<0.001$). Post hoc test showed significant differences of SEP amplitude between all Pre series on one hand and all Post series on the other hand ($p<0.001$). SEP amplitude elicited by heterotopic test stimulation decreased after LFS as well (-22.7±2.7%, $F=7.6$, $p<0.001$). There was no difference in SEP amplitude between last Pre series (Pre3) before LFS and first Post series (Post1) after LFS. All other comparisons between Pre and Post series revealed a difference ($p<0.05$) (Figs. 3.1A and B). Mean amplitude changes were significantly stronger under homotopic test stimulation compared to heterotopic test stimulation ($t=-3.1$, $p<0.05$) (Fig. 3.1B). Analyzing Post1 and Post4 separately indicated a difference between homotopic and heterotopic test stimulation in both series (Post1: $t=-3.0$, $p<0.05$; Post4: $t=-3.0$, $p<0.05$). N2 and P2 latencies did not change after LFS. Homotopic pain perception rating under radial test stimulation significantly decreased after LFS (-44.1±8.6%, $F=20.7$, $p<0.001$). Post hoc test revealed significant differences of rating between all Pre series on the one hand and all Post series on the other hand ($p<0.001$). By contrast, heterotopic pain perception rating remained constant. Comparison of homotopic and heterotopic pain perception under Post stimulation indicated a significant difference ($t=-5.2$, $p<0.001$) (Fig. 3.1C).

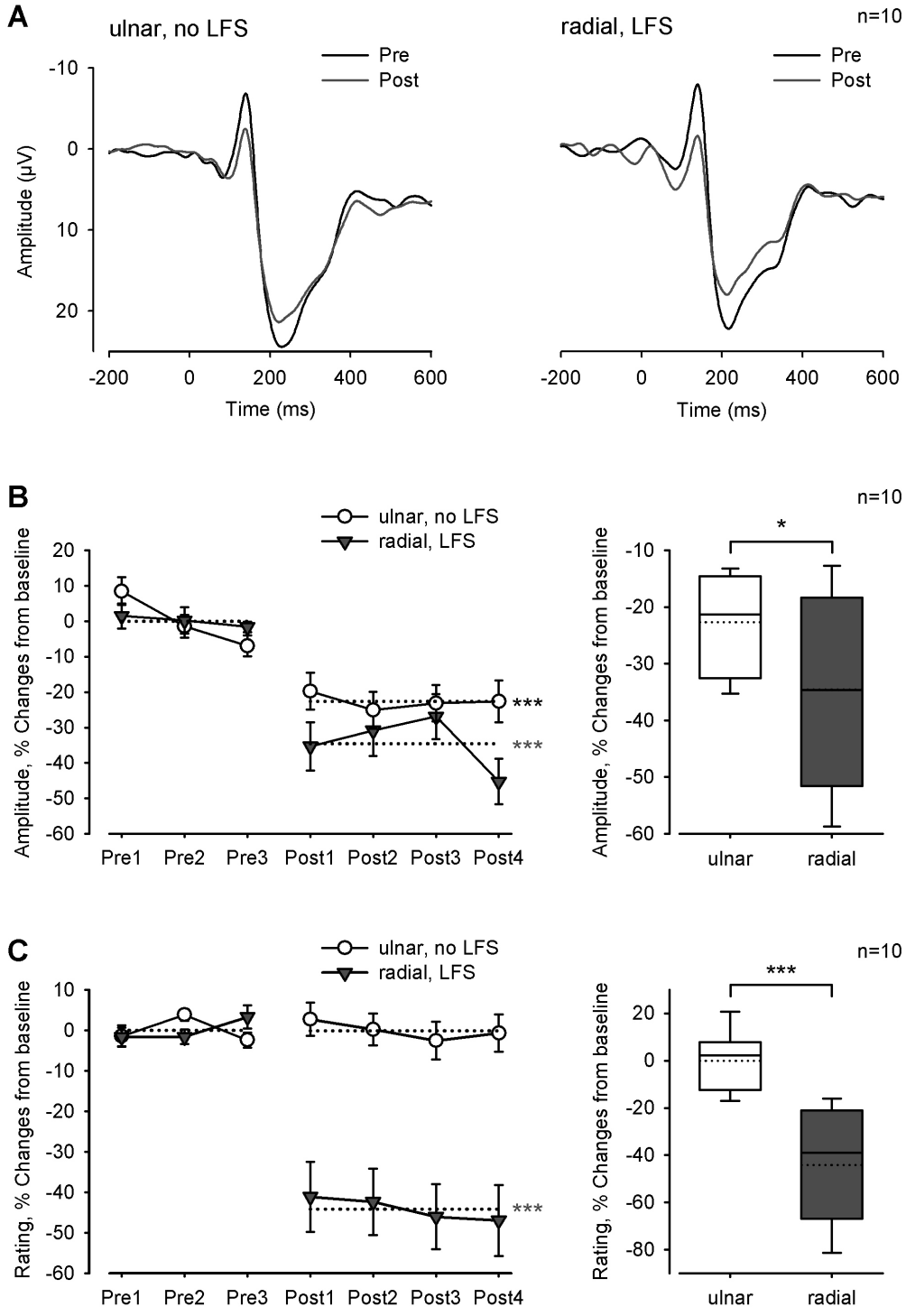


Figure 3.1. Unilateral experiment (ExpUni) in 10 volunteers.

Effects of radial LFS on SEP amplitude (A, B) and pain rating (C) under homotopic radial and heterotopic ulnar test stimulation. (A) Grand mean average of SEP under test stimulation before (Pre, black) and after LFS (Post, gray). (B, C) Time courses of percentage changes from baseline (mean±sem) of SEP amplitude (B) and rating (C) are presented. Box plots summarize changes after LFS. Both amplitude and rating under homotopic test stimulation indicated a significantly stronger reduction than under heterotopic test stimulation. Asterisks mark significant changes as analyzed by (time course) One Way Repeated Measure ANOVA and (boxplot) Paired t-test (* $p<0.05$, *** $p<0.001$).

3.1.2. *LFS at two hands (ExpBi)*

Twenty volunteers were tested to investigate homotopic nature of LFS at two hands (ExpBi). Comparison of Pre and Post stimulation in time course revealed a significant decrease of SEP amplitude (homotopic, right: -33.6±3.8%, $F=23.6$, $p<0.001$; heterotopic, left: -16.0±3.0%, $F=8.1$, $p<0.001$). Post hoc test of SEP amplitude during homotopic test stimulation revealed differences between all Pre series on the one hand and all Post series on the other hand ($p<0.001$). Under heterotopic test stimulation there were no differences between Pre3 vs. Post1 and Pre2 vs. Post1 and Post2, other comparisons between Pre and Post series showed significant differences ($p<0.05$) (Figs. 3.2A and B). SEP reduction under homotopic test stimulation was significantly stronger than under heterotopic test stimulation ($t=-3.7$, $p<0.01$) (Fig. 3.2B). Separate analyses of Post1 and Post4 revealed a difference between homotopic and heterotopic test stimulation in both series (Post1: $t=-4.0$, $p<0.001$; Post4: $t=-3.0$, $p<0.01$). N2 and P2 latencies remained constant during experiment. Homotopic pain perception rating significantly decreased after LFS (-29.1±4.4%, $F=13.1$, $p<0.001$). Post hoc test indicated significant differences of rating between all Pre series on the one hand and all Post series on the other hand ($p<0.05$), except comparison of Pre1 and Post2. Heterotopic pain perception rating remained stable. The reduction of homotopic pain perception rating was significantly different from heterotopic pain rating ($t=-6.0$, $p<0.001$) (Fig. 3.2C).

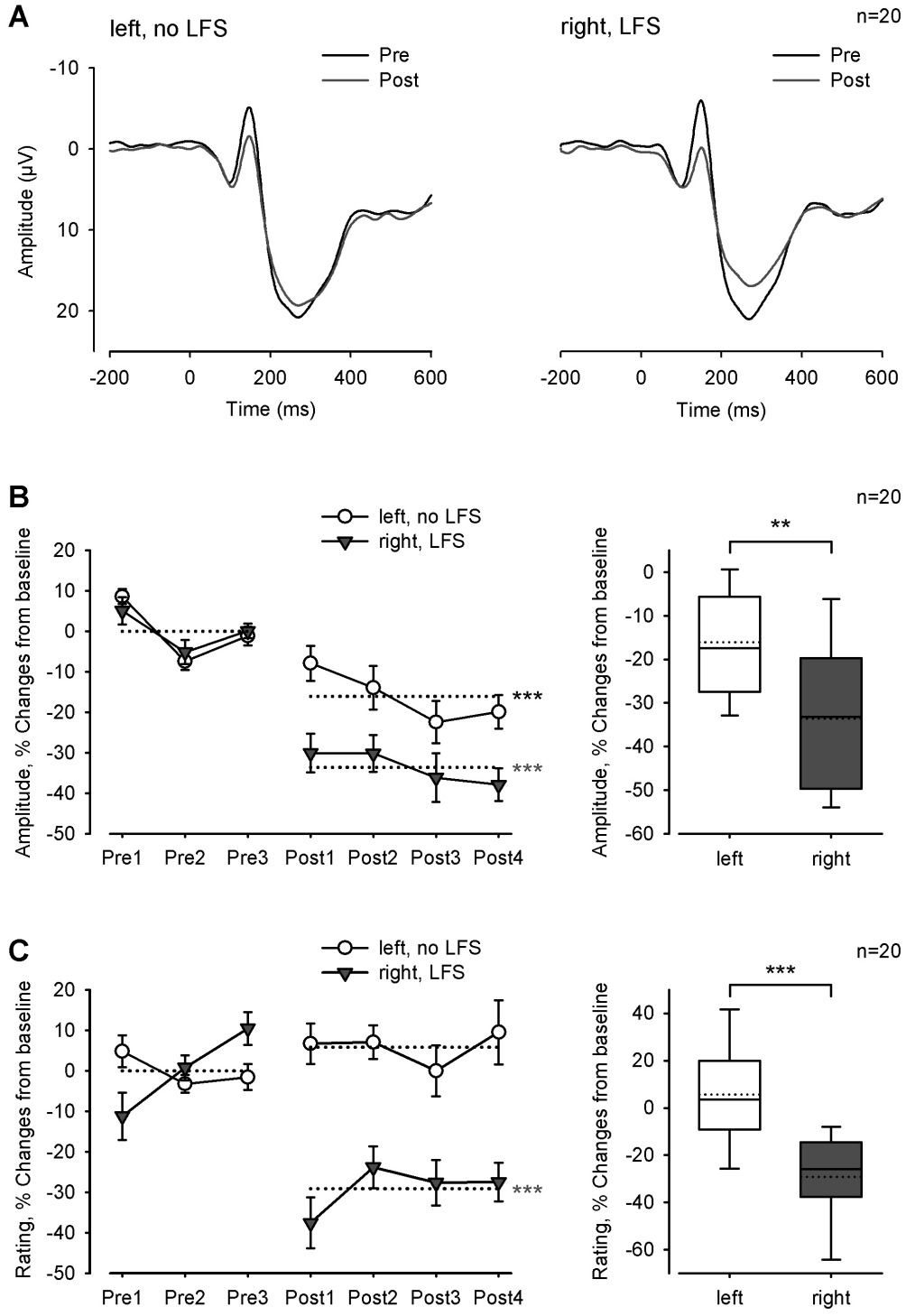


Figure 3.2. Bilateral experiment (ExpBi) in 20 volunteers.

Effects of right LFS on SEP amplitude (A, B) and pain rating (C) under homotopic right and heterotopic left test stimulation. (A) Grand mean average of SEP amplitude under test stimulation before (Pre, black) and after LFS (Post, gray). (B, C) Time courses of percentage changes from baseline (mean±sem) of SEP amplitude and rating are presented. Box plots summarize changes after LFS. Both amplitude and rating under homotopic test stimulation revealed a significantly stronger decrease than under heterotopic test stimulation. Asterisks mark significant changes as analyzed by (time course) One Way Repeated Measure ANOVA and (boxplot) Paired t-test (** p<0.01, *** p<0.001).

3.1.3. *Control experiment*

Fourteen volunteers were examined in a Control experiment. In this experiment no LFS was applied, but test stimulation was interrupted for 25 minutes. Electrode location was the same as in experiment ExpBi (Fig. 2.1B). Regarding time course Post SEP amplitude decreased (right: -14.4±3.8%, $F=4.4$, $p<0.001$; left: -5.8±6.7%, $X^2=13.3$, $p<0.05$). SEP amplitude under right test stimulation revealed significant differences in Pre1 vs. Post2, Post3 and Post4, Pre2 vs. Post3 and Post4, Pre3 vs. Post4 ($p<0.05$). There was no difference between Pre3 and Post1. Under left hand test stimulation difference only occurred in Pre3 vs. Post3 ($p<0.05$). N2 and P2 latencies remained constant. Pain perception rating did not change during the whole experiment (Fig. 3.3).

3.1.4. *ExpBi versus Control experiment*

Results from the fourteen volunteers who participated in both LFS experiment (ExpBi) and Control experiment were compared, in order to differentiate between LFS effects and habituation effects. Comparison of mean SEP reduction in Post series in these two experiments demonstrated a significant difference ($X^2=13.8$, $p<0.01$). Post hoc test revealed that SEP amplitude under homotopic test stimulation at right hand dorsum decreased significantly stronger compared to other conditions (LFS, heterotopic, left: $q=5.7$; Control, right: $q=4.3$; Control, left: $q=5.2$; all conditions: $p<0.05$). SEP amplitude under heterotopic test stimulation after LFS did not differ from Control experiment (Fig. 3.3A). Examining Post1 series separately indicated the strongest decrease in SEP amplitude under homotopic test stimulation ($F=5.3$, $p<0.05$; LFS, heterotopic, left: $DM=19.8$, $p<0.05$; Control, right: $DM=22.3$, $p<0.01$; Control, left: $DM=22.5$, $p<0.01$). Same is true for Post4 series ($X^2=8.1$, $p<0.05$; LFS, heterotopic, left: $q=4.5$; Control, right: $q=3.5$; Control, left: $q=3.9$; all conditions: $p<0.05$). Homotopic pain perception at right hand dorsum significantly decreased after LFS compared to other conditions ($F=8.0$, $p<0.001$; LFS, heterotopic, left: $DM=34$, $p<0.001$; Control, right: $DM=33.2$, $p<0.001$; Control, left: $DM=24.9$, $p<0.01$). Heterotopic pain perception in LFS experiment and pain perception in Control experiment remained constant during the whole session (Fig. 3.3B).

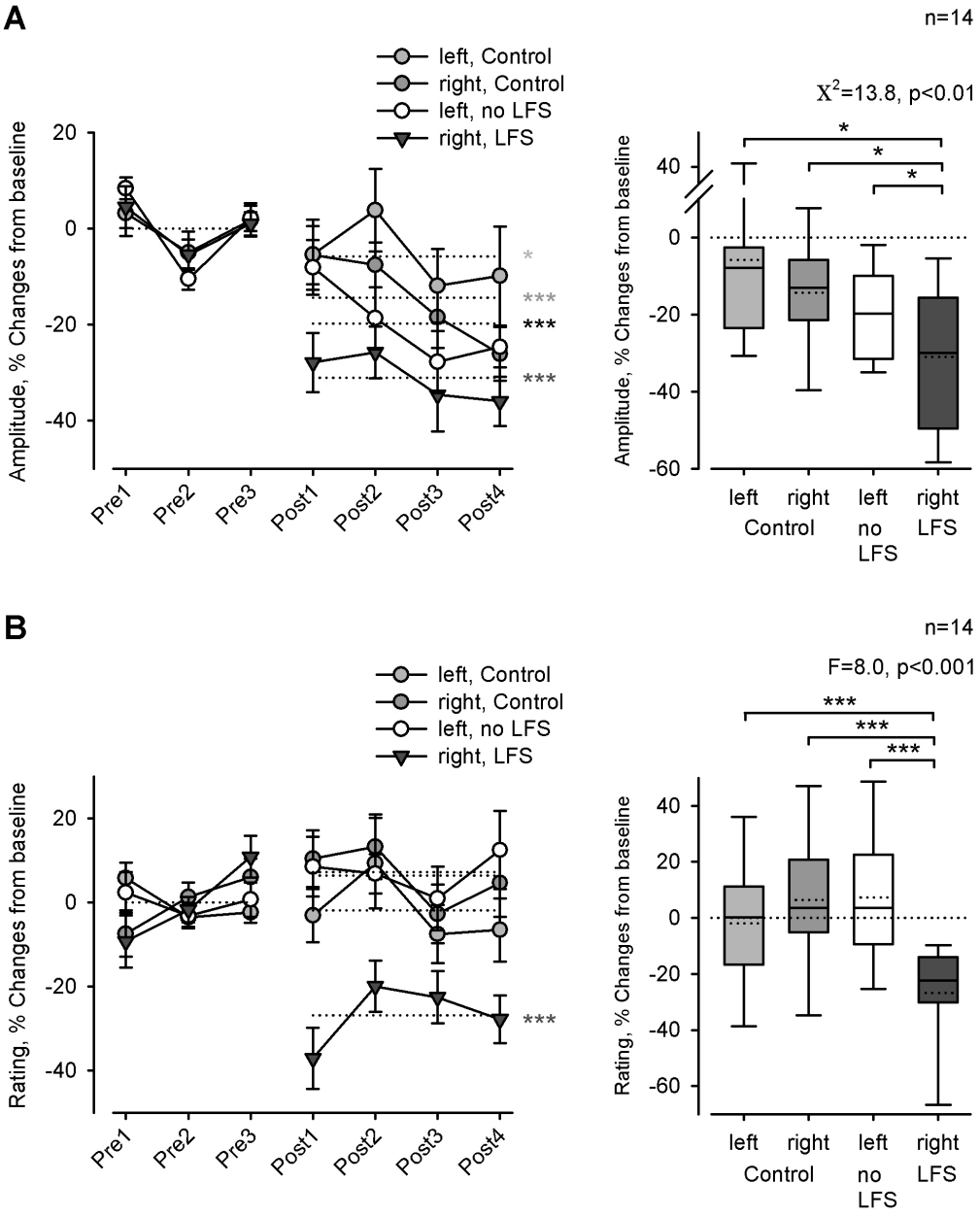


Figure 3.3. Comparison between LFS and Control experiments (ExpBi vs. Control; n=14). Effects of right LFS on SEP amplitude (A) and pain rating (B) under homotopic right and heterotopic left test stimulation compared to Control experiment without conditioning stimulation. Time courses of percentage changes from baseline (mean±sem) of SEP amplitude and rating are presented. Box plots summarize changes after LFS and no stimulation period. Changes in both amplitude and rating revealed a significant effect of LFS under homotopic test stimulation as compared to effects under heterotopic test stimulation and effects in Control experiment. Left, Time course: Asterisks mark significant changes as analyzed by One Way Repeated Measure ANOVA. Right, Boxplot: P values correspond to Friedman Repeated Measures ANOVA followed by Student-Newman-Keuls test (A) and to One Way Repeated Measures ANOVA followed by Holm-Sidak tests (B). Number of asterisks corresponds to level of significance (* p<0.05, *** p<0.001).

3.2. LFS effect on sensory and affective pain components

In all 40 sessions sensory thresholds and pain perception ratings were recorded. The mean test stimulus intensity was 2.5±0.16 mA (mean±sem), corresponding to 9.1-fold I_0 (0.3±0.02 mA) and 4.0-fold I_P (0.6±0.04 mA). This stimulus intensity elicited a painful pinprick sensation. Electrical thresholds under Pre condition in LFS experiment (I_0: 0.3±0.02 mA; I_P: 0.6±0.06 mA) and Control experiments (I_0: 0.3±0.03 mA; I_P: 0.6±0.06 mA) did not differ (Paired t-test).

3.2.1. *VRS rating*

Comparing Control and LFS experiment, VRS-I showed significant changes over time (Two Way RM ANOVA: $F=22.2$, $p<0.001$) with significant interaction between time and experimental condition ($F=13.9$, $p<0.001$) (Fig. 3.4A). VRS-U revealed changes due to experimental condition (Two Way RM ANOVA: time $F=25.6$, $p<0.001$; experiment $F=17.2$, $p<0.001$; interaction between time and experiment $F=13.2$, $p<0.01$) (Fig. 3.4A). Gender as in-between factor showed no interaction in VRS ratings.

In the time course of the LFS experiment, considering test stimulation (Pre, Post) and conditioning LFS (first 10 minutes: LFS1 and last 10 minutes: LFS2; Fig. 3.4B), VRS-I and VRS-U significantly changed (Two Way RM ANOVA, Tab. 3.2). VRS-I and VRS-U solely differed under Post condition, pointing to a stronger decrease of VRS-U after conditioning LFS.

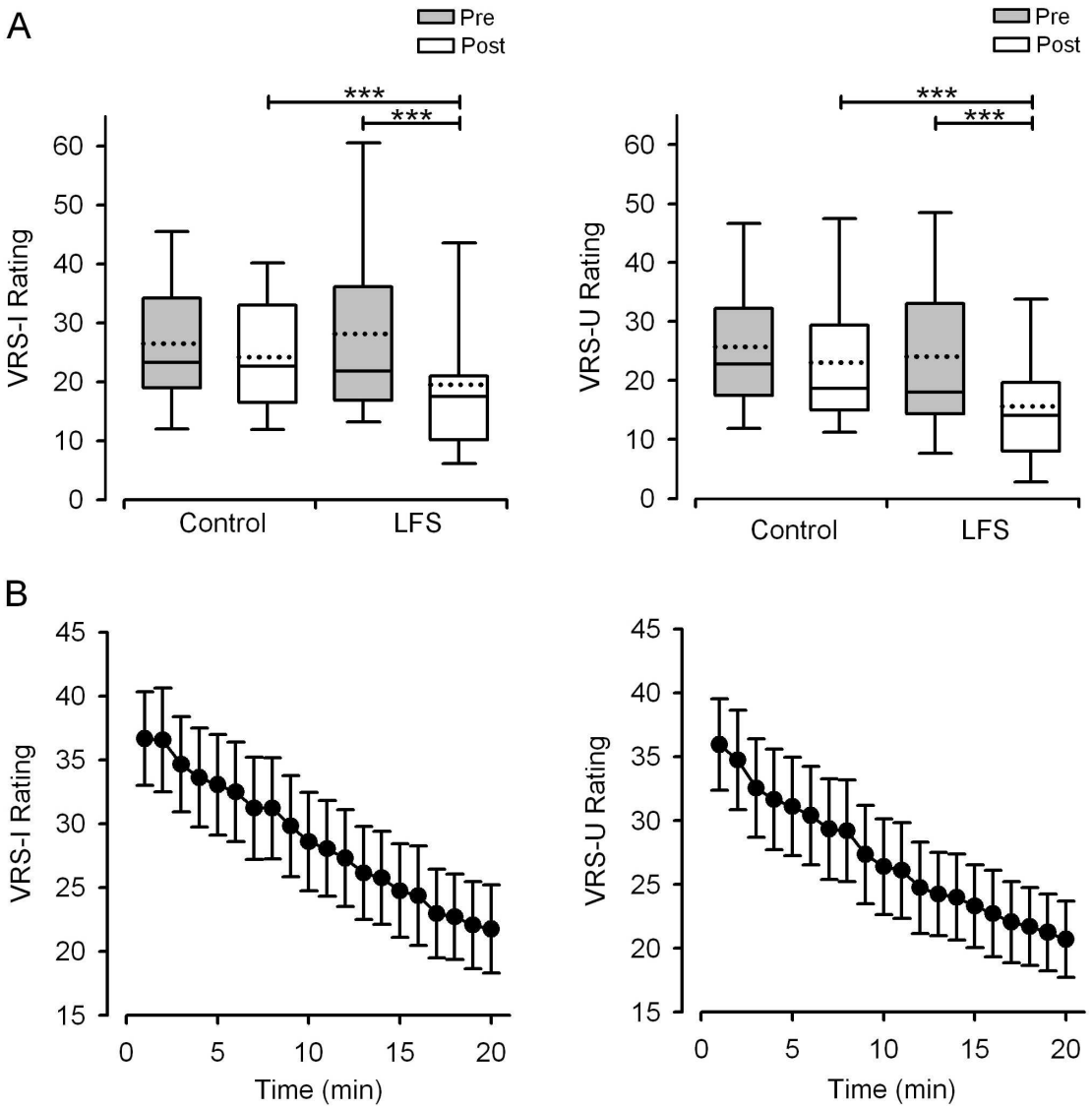


Figure 3.4. VRS rating during test stimulation (A) and during conditioning LFS (B) in 20 healthy volunteers.

(A) Effects of conditioning LFS on intensity VRS-I rating (left) and unpleasantness VRS-U rating (right) compared to Control experiment without conditioning stimulation. Boxplots of averaged data of Pre and Post series are presented. VRS-I and VRS-U ratings decreased after conditioning LFS, but not under Control conditions. (B) Time courses (mean±sem) of VRS-I (left) and VRS-U (right). Both VRS-I and VRS-U ratings continually decreased during conditioning LFS. Asterisks mark significant changes as analyzed by Fisher LSD post hoc tests after Two Way RM ANOVA (*** $p<0.001$).

Table 3.2. Intensity VRS-I and unpleasantness VRS-U ratings in the time course of the LFS experiment (n=20).

VRS	Pre	LFS1	LFS2	Post
VRS-I	28.1±3.7	32.9±3.8	24.6±3.5	19.5±3.0
Pre vs.		n. s. (DM=0.08)	n. s. (DM=0.09)	p<0.001 (DM=0.19)
LFS1 vs.			p<0.001 (DM=0.17)	p<0.001 (DM=0.27)
LFS2 vs.				n. s. (DM=0.09)
VRS-U	24.0±3.2	30.9±3.7	23.1±3.2	15.6±2.3
Pre vs.		p<0.05 (DM=0.12)	n. s. (DM=0.06)	p<0.001 (DM=0.23)
LFS1 vs.			p<0.001 (DM=0.17)	p<0.001 (DM=0.35)
LFS2 vs.				p<0.001 (DM=0.18)
VRS-I vs. VRS-U	n. s. (DM=0.08)	n. s. (DM=0.04)	n. s. (DM=0.04)	p<0.01 (DM=0.13)

VRS-I and VRS-U (mean±sem) in test stimulation (Pre, Post) and conditioning LFS (first 10 minutes: LFS1, last 10 minutes: LFS2) were compared by Two Way RM ANOVA. Factors were time course (Pre, LFS1, LFS2, Post) and rating dimension (VRS-I, VRS-U). Time course ($F=13.7$, $p<0.001$) and interaction with rating dimension ($F=6.2$, $p<0.001$) revealed significance. Results from Fisher LSD post hoc test are presented (DM=difference of means, p value, n. s.: not significant).

3.2.2. *SES rating*

The profile of SES rating under Pre test stimulation revealed high rating of sensory items (Fig. 3.5A). The prevailing sensation stinging (2.0±0.1) was rated twice as high as the subsequent highest items pulling (0.9±0.1), sharp (1.0±0.2) and shooting (0.9±0.2). In contrast to sensory items mean ratings of all affective items were lower than 0.5.

Examining Control and LFS experiment, SES-S showed significant changes over time (Two Way RM ANOVA: time $F=15.6$, $p<0.001$) with a significant interaction between time and experimental condition ($F=4.5$, $p<0.05$) (Fig. 3.5B). SES-A significantly changed under the two experimental conditions (Two Way RM ANOVA: time $F=14.6$, $p<0.001$; experimental condition $F=69.0$, $p<0.001$; interaction between time and experiment $F=51.1$, $p<0.001$) (Fig. 3.5B). Gender as in-between factor showed no interaction in SES rating.

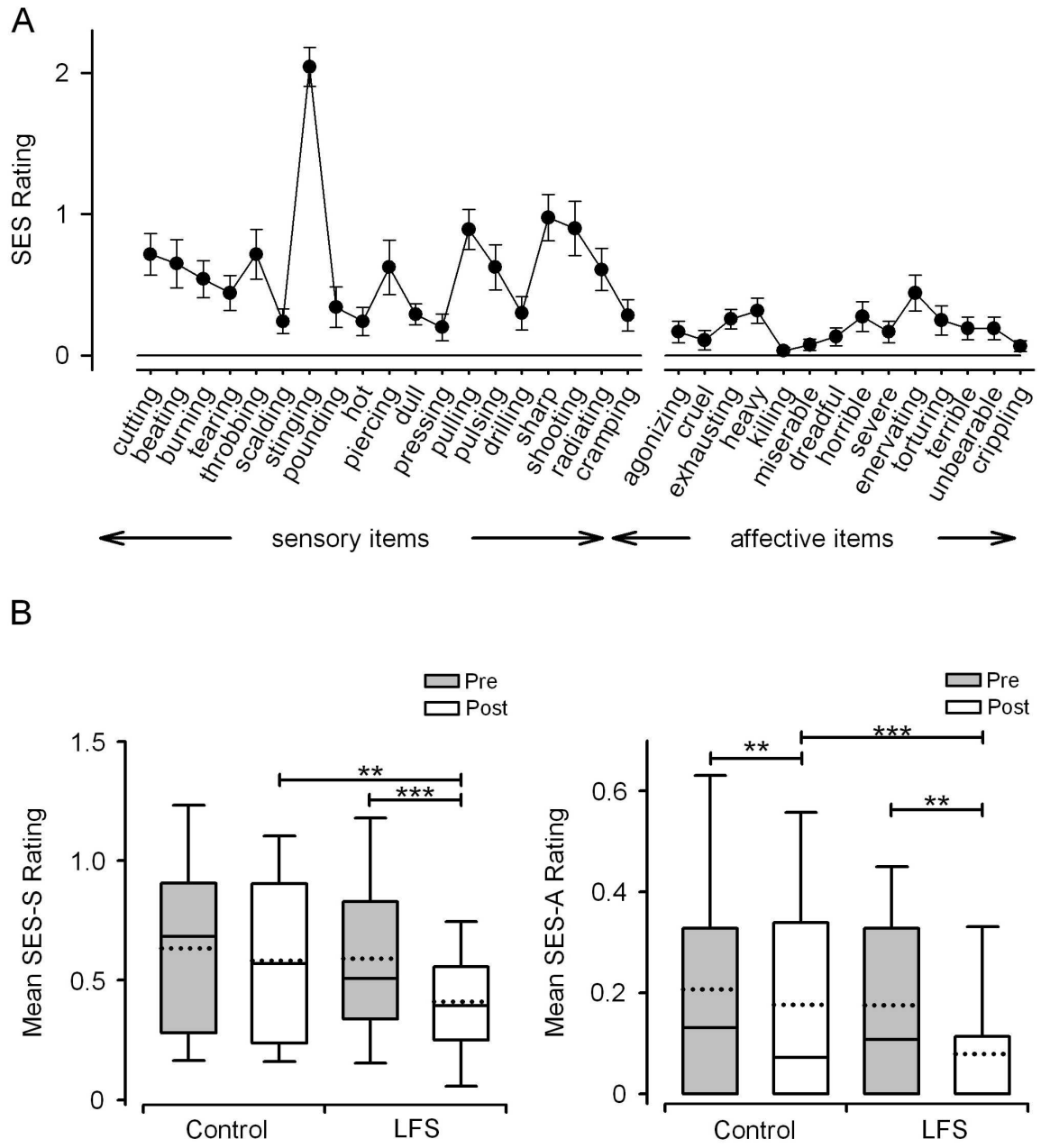


Figure 3.5. SES rating during test stimulation in 20 healthy volunteers.

(A) Rating of single items during Pre test stimulation (mean±sem of Control and LFS experiment). (B) Effects of conditioning LFS on sensory SES-S rating (left) and affective SES-A rating (right) compared to Control experiment without conditioning stimulation. Boxplots of averaged data of Pre and Post series are presented. SES-S rating revealed sole reduction after conditioning LFS. SES-A also decreased during Control. Reduction after conditioning LFS was stronger. Asterisks mark significant changes as analyzed by Fisher LSD post hoc tests after Two Way RM ANOVA (*** $p<0.001$, ** $p<0.01$).

Considering the time course of the LFS experiment, SES-S and SES-A revealed significant differences (Two Way RM ANOVA: time course Pre, LFS1, LFS2, Post; rating dimension SES-S, SES-A) as shown in table 3.3.

Table 3.3. Sensory SES-S and affective SES-A rating in the time course of the LFS experiment (n=20).

SES	Pre	LFS1	LFS2	Post
SES-S	0.59±0.08	0.94±0.09	0.75±0.10	0.41±0.05
Pre vs.		p<0.001 (DM=0.20)	n. s. (DM=0.07)	p<0.05 (DM=0.13)
LFS1 vs.			p<0.05 (DM=0.13)	p<0.001 (DM=0.33)
LFS2 vs.				p<0.001 (DM=0.20)
SES-A	0.18±0.05	0.46±0.09	0.35±0.09	0.08±0.03
Pre vs.		p<0.001 (DM=0.32)	p<0.001 (DM=0.18)	p<0.01 (DM=0.14)
LFS1 vs.			p<0.01 (DM=0.14)	p<0.001 (DM=0.47)
LFS2 vs.				p<0.001 (DM=0.33)
SES-S vs. SES-A	p<0.001 (DM=0.47)	p<0.001 (DM=0.35)	p<0.001 (DM=0.36)	p<0.001 (DM=0.48)

SES-S and SES-A (mean±sem) in test stimulation (Pre, Post) and conditioning LFS (first 10 minutes: LFS1 and last 10 minutes: LFS2) were compared by Two Way RM ANOVA. Factors were time course (Pre, LFS1, LFS2, Post) and rating dimension (SES-S, SES-A). Both factors (time course: F=26.4, p<0.001; rating dimension: F=79.4, p<0.001) and the interaction (F=4.3, p<0.01) showed significances. Results from Fisher LSD post hoc test are presented (DM=difference of means, p value, n. s.: not significant).

Factor analysis of sensory SES items in Pre series revealed five factors with Eigenvalue above 1 (Tab. 3.4) explaining 83.9% of variance. Two items, pounding and piercing, had factor loadings above 0.5 after VARIMAX rotation in two factors and were placed in the factor with the higher loading. Factor 1 comprising five items was suggested to describe deep rhythmic pain. Factor 2 consisting of three items was taken as superficial heat pain. Factor 3 contains three items which were interpreted to represent deep constant pain. Factor 4 comprising six items was defined to describe superficial sharp pain. Factor 5 consisted of the item pulling. Item cutting which had the same loading for factor 2 and factor 4 was placed to factor 4. Item cutting rather belongs to a factor describing sharp pain, than to heat pain.

The five factors were examined in Control and LFS experiment regarding putative LFS effect (Two Way RM ANOVA: time (Pre vs. Post), experiment (LFS vs. Control)). Factors 1, 2, and 5 did not show any significance. Factor 3 revealed a decrease of deep constant pain over time ($F=10.6$, $p<0.01$) without interaction. Factor 4 decreased from Pre to Post series ($F=9.5$, $p<0.01$). Interaction between time and experiment was close to level of significance ($F=4.1$, $p=0.056$) with significant reduction of factor 4 after conditioning LFS ($DM=0.17$, $p<0.001$). During Control, factor 4 remained stable.

Considering the LFS experiment, the five factors were examined regarding possible differences in test stimulation (Pre, Post) and conditioning stimulation (LFS1, LFS2) (One Way RM ANOVA, Tab. 3.5).

Table 3.4. Factor analysis of sensory SES-S items in Pre series (n=20).

	Factor 1: Deep Pain/ Rhythm	Factor 2: Superficial Pain/Heat	Factor 3: Deep Pain/ Constant	Factor 4: Superficial Pain/Sharp	Factor 5: Pulling
Eigenvalue	6.2	3.9	2.7	1.8	1.3
% of variance	25.3	17.1	16.7	15.6	9.1
SES-S items	Factor loadings				
beating	***0.94***	-0.02	0.22	-0.01	-0.13
throbbing	***0.92***	-0.11	0.25	-0.08	-0.04
radiating	***0.82***	-0.20	0.06	0.18	-0.13
pulsing	***0.81***	0.25	0.15	0.07	0.22
pounding	***0.73***	0.03	*0.59*	-0.02	0.07
scalding	-0.02	***0.94***	0.17	-0.01	-0.03
hot	0.03	***0.91***	-0.26	0.02	0.16
burning	-0.04	***0.84***	0.28	0.16	-0.18
tearing	0.28	0.06	***0.91***	0.09	0.16
cramping	0.24	0.20	***0.76***	0.09	-0.28
pressing	0.50	0.04	***0.69***	0.01	0.26
drilling	-0.02	0.05	0.13	***0.90***	0.03
sharp	-0.22	0.48	0.08	***0.67***	-0.02
piercing	*0.63*	-0.01	-0.15	***0.66***	0.03
cutting	-0.01	*0.65*	0.11	***0.65 ****	0.11
shooting	0.23	0.02	-0.63	***0.59***	-0.10
stinging	0.35	-0.12	-0.06	***0.53***	0.44
pulling	0.40	0.05	0.43	0.34	**0.65**
dull	0.22	-0.02	-0.01	0.03	-0.88
Cronbach's alpha	0.93	0.87	0.85	0.81	

Factors with Eigenvalue over 1, percentage of variance they explain after VARIMAX Rotation and factor loadings for every SES-S item are presented. Factor loadings above 0.5 were considered (italic). Items were sorted by highest factor loadings (bold). * Item cutting with exactly the same loading for 2 factors was placed in the factor, which fits better regarding Cronbach's alpha and literature.

Table 3.5. Factors of SES-S in the time course of the LFS experiment (n=20).

Factor		ANOVA	Pre	LFS1	LFS2	Post
1	Deep Pain / Rhythym	F=88.3, p<0.001	0.5±0.1	1.1±0.2	1.0±0.2	0.3±0.1
	Pre vs.			p<0.001 (DM=0.37)	p<0.001 (DM=0.31)	n. s. (DM=0.08)
	LFS1 vs.				n. s. (DM=0.07)	p<0.001 (DM=0.45)
	LFS2 vs.					p<0.001 (DM=0.38)
2	Superficial Pain / Heat	F=6.8, p<0.001	0.4±0.1	0.8±0.2	0.7±0.2	0.3±0.1
	Pre vs.			p<0.01 (DM=0.26)	n. s. (DM=0.13)	n. s. (DM=0.11)
	LFS1 vs.				n. s. (DM=0.12)	p<0.001 (DM=0.36)
	LFS2 vs.					p<0.01 (DM=0.24)
3	Deep Pain / Constant	F=8.4, p<0.001	0.3±0.1	0.5±0.1	0.3±0.1	0.1±0.1
	Pre vs.			p<0.01 (DM=0.23)	n. s. (DM=0.04)	p<0.05 (DM=0.18)
	LFS1 vs.				p<0.05 (DM=0.19)	p<0.001 (DM=0.42)
	LFS2 vs.					p<0.01 (DM=0.22)
4	Superficial Pain / Sharp	F=8.4, p<0.001	0.9±0.1	2.0±0.2	0.8±0.2	0.7±0.1
	Pre vs.			p<0.001 (DM=0.37)	p<0.05 (DM=0.17)	p<0.05 (DM=0.16)
	LFS1 vs.				p<0.001 (DM=0.54)	p<0.001 (DM=0.53)
	LFS2 vs.					n. s. (DM=0.01)
5	Pulling	F=1.0, n. s.	0.9±0.2	0.9±0.2	0.9±0.2	0.6±0.1

Factors of sensory components of pain perception (SES-S) (mean±sem) in test stimulation (Pre, Post) and conditioning LFS (first 10 minutes: LFS1 and last 10 minutes: LFS2) were compared by One Way RM ANOVA (F, p value) followed by Fisher LSD post hoc test (DM=difference of means, p value, n. s.: not significant).

3.3. LTD of cerebral activation

The mean test stimulus intensity was 3.21±0.14 mA (mean±sem), corresponding to 4.0-fold I_P (0.80±0.04 mA). This stimulus intensity elicited a definite pinprick like painful sensation. Thresholds (I_P) and stimulus intensities (I_S) in Control (I_P: 0.76±0.05 mA; I_S: 3.04±0.20 mA) and LFS experiment (I_P: 0.84±0.05 mA; I_S: 3.38±0.20 mA) did not differ (Paired t-test).

3.3.1. *Pain perception ratings*

Pain perception ratings indicated significant changes dependent on conditioning LFS. Comparison of VRS in Control and LFS experiment showed significance (Two Way RM ANOVA: time (Pre vs. Post) $F=11.5$, $p<0.01$; experiment (LFS vs. Control) $F=16.8$, $p<0.001$; interaction between time and experiment $F=14.4$, $p<0.01$). VRS was solely reduced after LFS compared to Pre LFS (DM=0.06, $p<0.001$) and Post Control (DM=0.07, $p<0.001$) (Fig. 3.6A).

SES-S reflecting sensory aspects of pain indicated significant interaction between time and experiment (Two Way RM ANOVA, $F=6.4$, $p<0.05$). After LFS, SES-S decreased compared to Pre LFS (DM=0.07, $p<0.05$) and Post Control (DM=0.10, $p<0.01$) (Fig. 3.6B). SES-A, an index of affective pain magnitude, revealed significant changes (Two Way RM ANOVA: time (Pre vs. Post) $F=10.1$, $p<0.01$; experiment (LFS vs. Control) $F=13.4$, $p<0.01$; interaction between time and experiment $F=15.0$, $p<0.001$). SES-A in Post LFS was reduced compared to Pre LFS (DM=0.27, $p<0.001$) and Post Control (DM=0.32, $p<0.001$) (Fig. 3.6B). VRS, SES-S and SES-A showed no differences under Pre condition in Control and LFS experiment. During Control experiment, pain perception ratings remained stable (Fig. 3.6).

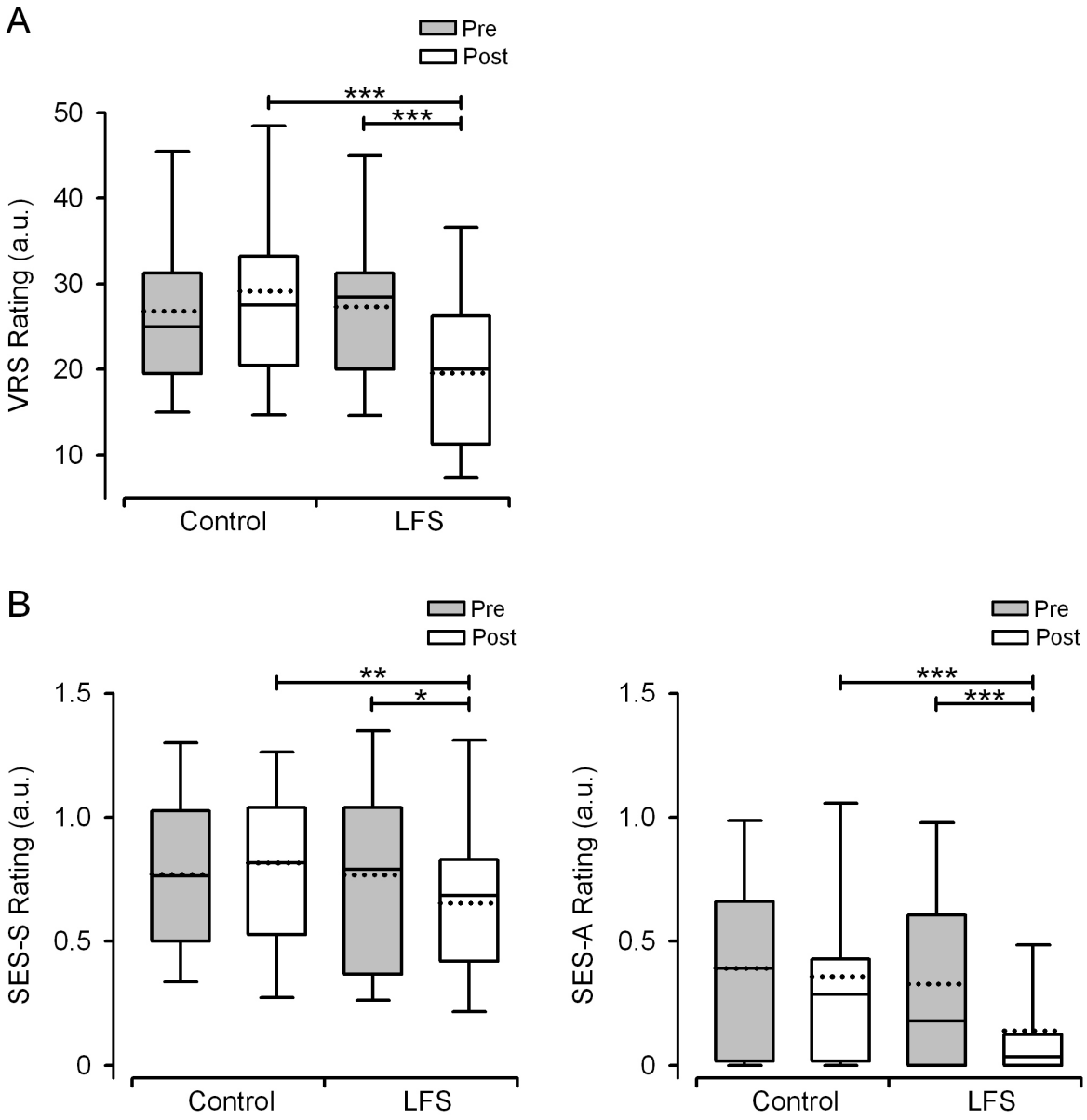


Figure 3.6. Pain perception rating.

Effects of LFS on VRS rating (A) and SES rating (B), subdivided into sensory SES-S rating (left) and affective SES-A rating (right) compared to Control experiment without conditioning stimulation. Boxplots of averaged data of Pre and Post series are presented. VRS, SES-S and SES-A ratings decreased after LFS, but not under Control conditions. Asterisks mark significant changes as analyzed by Fisher LSD post hoc tests after Two Way RM ANOVA (*** $p<0.001$, ** $p<0.01$, * $p<0.05$).

3.3.2. *fMRI data*

3.3.2.1. Group analyses

Comparison of the two experiments revealed no difference under Pre condition (Pre LFS - Pre Control and Pre Control – Pre LFS: Paired t-test, $p<0.05$ corrected, masked incl. minuend, $p<0.05$). Brain activation during Control experiment remained stable under Pre and Post conditions (Pre Control – Post Control and Post Control – Pre Control: Paired t-test, $p<0.05$ corrected, masked incl. minuend, $p<0.05$). These two facts assure that changes during LFS experiment are due to conditioning LFS. Therefore, the focus is on the LFS experiment. Comparison of stimulation periods with rest periods under Pre LFS condition (Pre LFS) revealed that electrical test stimulation led to a significant activation of various areas (Tab. 3.6, Figs. 3.7 and 3.8). Bilateral S1 (Brodmann area (BA) 1, 2, 3), S2 (BA 43), anterior and posterior insula (BA 13) and anterior cingulate cortex (ACC; BA 32) were activated. In parietal lobule, activation was observed ipsilateral inferior (BA 40). In superior frontal lobule, ipsilateral, right BA 10 was activated. Bilateral activation was found in inferior frontal lobule (BA 44, 45, 47) and in superior temporal lobule (STG; BA 22, 38) (Tab. 3.6, Figs. 3.7 and 3.8). Comparison of stimulation periods with rest periods after LFS (Post LFS) indicated sole activation in ipsilateral, right inferior parietal lobe (IPL, BA 40) (Tab. 3.6, Figs. 3.7 and 3.8). Pre LFS - Post LFS contrast (Tab. 3.7, Figs. 3.7 and 3.8) demonstrated significant reduction in brain activation after LFS compared to Pre LFS. Decrease of activation was found in bilateral S1 (BA 1, 2, 3) and S2 (BA 40, 43), bilateral ACC (BA 24, 33) and ipsilateral posterior insula (BA 13). Further reductions after LFS were observed in ipsilateral IPL (BA 40) and in ipsilateral STG (BA 22) (Tab. 3.7, Figs. 3.7 and 3.8). Post LFS - Pre LFS contrast revealed no increased activation after LFS. Comparison of LFS and Control experiments during Post condition, indicated reduced bilateral ACC activity (BA 24) after LFS compared to Post Control (Post Control - Post LFS; Paired t-test, $p<0.05$ corrected, masked incl. minuend, $p<0.05$; x: 0, y: 24, z: 21; z-value: 3.66; voxels: 7). In the Post LFS - Post Control contrast, no activation was observed.

Table 3.6. Significant activation in brain regions during stimulation period compared with rest period before (Pre) and after (Post) LFS.

Brain regions	Talairach coordinates x	y	z	t-value	z-value	voxels
Pre LFS						
R inferior frontal gyrus (BA 47)	**40**	**27**	**-5**	**7.44**	**4.82**	**379**
R superior temporal gyrus (BA 22)	55	11	-4	7.10	4.71	
R postcentral gyrus (BA 3), S1	63	-18	38	5.77	4.18	
R postcentral gyrus (BA 43), S2	63	-15	15	5.71	4.16	
R insula (sub-lobar), INS A	36	16	-1	5.67	4.14	
R inferior parietal lobule (BA 40), IPL	55	-30	31	5.34	4.00	
R insula (sub-lobar), INS	44	-4	4	5.04	3.84	
R postcentral gyrus (BA 2), S1	55	-29	42	4.64	3.64	
L insula (BA 13), INS P	**-44**	**-11**	**4**	**6.80**	**4.60**	**193**
L postcentral gyrus (BA 1, 2, 3), S1	-63	-21	38	6.60	4.55	
L postcentral gyrus (BA 43), S2	-63	-15	19	5.59	4.10	
L superior temporal gyrus (BA 22)	-55	8	0	5.36	4.00	
L inferior frontal gyrus (BA 45)	-59	13	21	4.80	3.72	
L inferior frontal gyrus (BA 44)	-59	12	10	4.16	3.38	
L superior temporal gyrus (BA 38)	**-44**	**11**	**-11**	**5.09**	**3.87**	**26**
R anterior cingulate (BA 32), ACC	**4**	**32**	**24**	**4.75**	**3.70**	**43**
L anterior cingulate (BA 32), ACC	-4	36	24	4.59	3.61	
R superior frontal gyrus (BA 10)	**36**	**59**	**15**	**4.69**	**3.67**	**18**
L inferior frontal gyrus (BA 47)	**-32**	**31**	**-2**	**4.37**	**3.50**	**9**
L insula (BA 13), INS A	-32	19	1	3.98	3.27	
Post LFS						
R inferior parietal lobule (BA 40), IPL	**51**	**-48**	**50**	**4.26**	**3.43**	**10**
R inferior, superior parietal lobule (BA 40, 7)	44	-56	54	4.00	3.28	

One-Sample t-test, $p<0.05$ corrected. Bold: Local maxima with the highest z-value within an activated voxel cluster. L: left contralateral, R: right ipsilateral, BA: Brodmann area, ACC: anterior cingulate cortex, INS: insula (A: anterior, P: posterior), IPL: inferior parietal lobe, S1: primary somatosensory cortex, S2: secondary somatosensory cortex

Table 3.7. Significant differences in brain activation before (Pre) in contrast to after (Post) LFS.

Brain regions	Talairach coordinates x	y	z	t-value	z-value	voxels
Pre LFS - Post LFS						
R postcentral gyrus (BA 2, 3) S1	**59**	**-18**	**30**	**5.74**	**4.17**	**127**
R postcentral gyrus (BA 43) S2	59	-15	19	5.71	4.16	
R inferior parietal lobule (BA 40) IPL	51	-26	27	4.84	3.75	
R insula (sub-lobar), INS P	44	-15	19	4.38	3.50	
R superior temporal gyrus (BA 22)	67	-38	13	3.88	3.21	
L postcentral gyrus (BA 1, 2, 3) S1	**-63**	**-21**	**38**	**4.95**	**3.80**	**39**
L postcentral gyrus (BA 40, 43) S2	**-63**	**-19**	**12**	**4.69**	**3.66**	**18**
L, R cingulate gyrus (BA 24)	**0**	**-14**	**38**	**4.23**	**3.42**	**12**
L, R anterior cingulate (BA 33, 24) ACC	**0**	**13**	**21**	**4.00**	**3.28**	**8**

Paired t-test, $p<0.05$ corrected, masked incl. by the minuend, $p<0.05$. Bold: Local maxima with the highest z-value within an activated voxel cluster. L: left contralateral, R: right ipsilateral, BA: Brodmann area, ACC: anterior cingulate cortex, INS: insula (P: posterior), IPL: inferior parietal lobe, S1: primary somatosensory cortex, S2: secondary somatosensory cortex

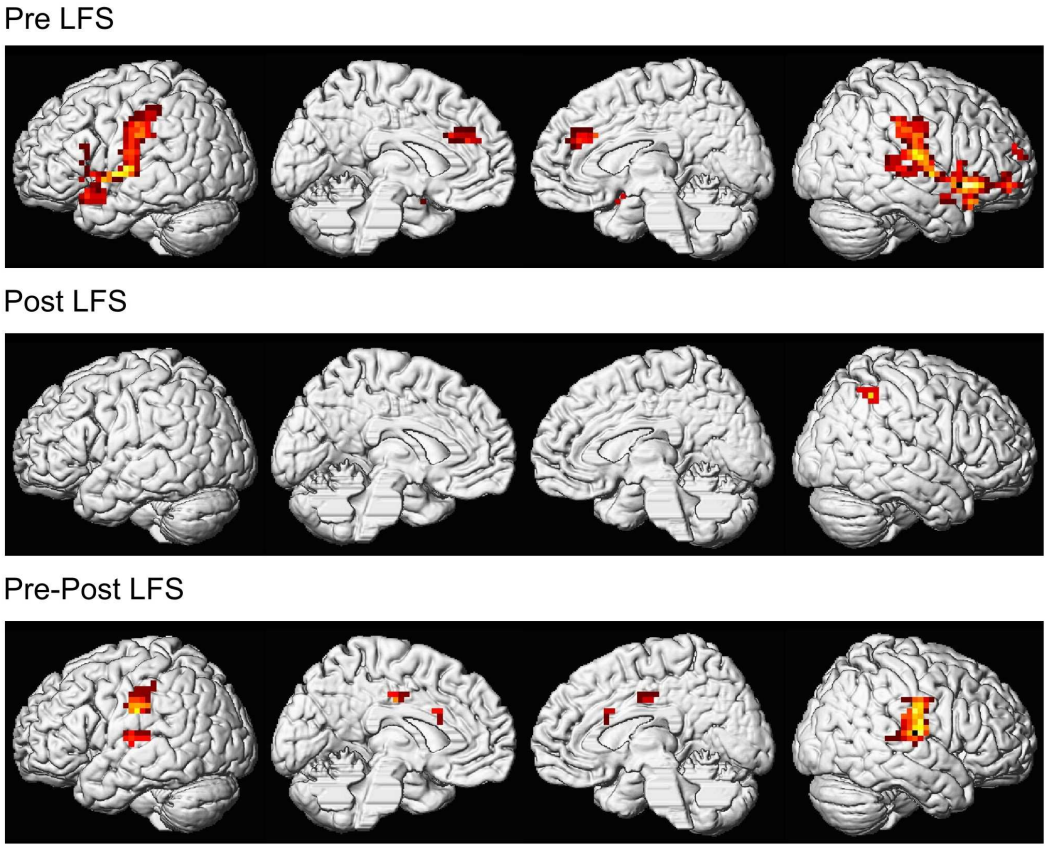


Figure 3.7. Significant brain activation in LFS experiment (whole brain).

Activation during stimulation period was compared with rest period before (Pre LFS) and after LFS (Post LFS) by One Sample t-test, $p<0.05$ corrected. Pre LFS - Post LFS contrast (Paired t-test, $p<0.05$ corrected, inclusively masked by the minuend $p<0.05$) demonstrated significant reduction after LFS.

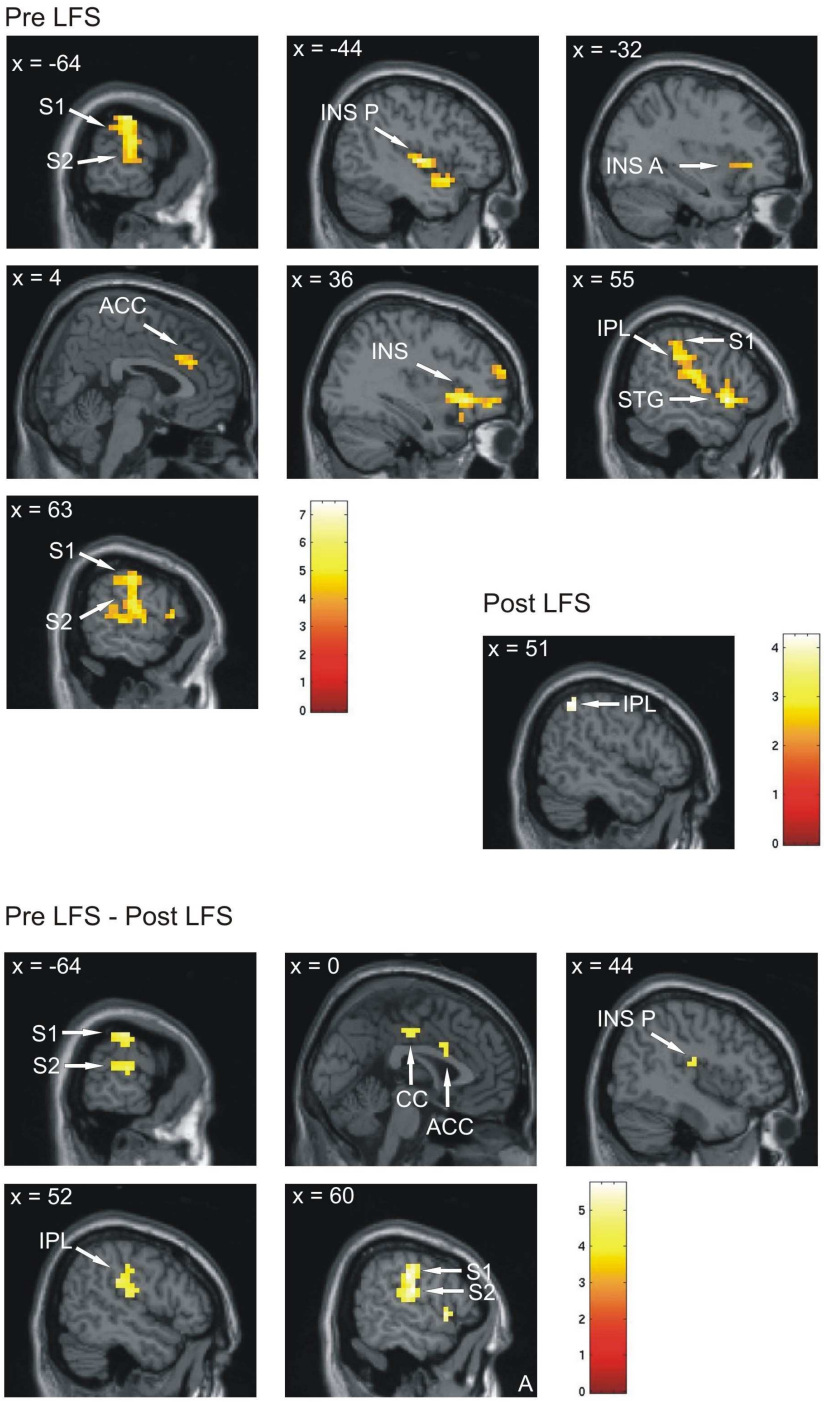


Figure 3.8. Significant brain activation in LFS experiment (sagittal sections).
Activations of specific areas before (Pre LFS) and after LFS (Post LFS) (stimulation period - rest period; One Sample t-test, $p<0.05$ corrected) and Pre LFS - Post LFS contrast (Paired t-test, $p<0.05$ corrected, inclusively masked by the minuend $p<0.05$) are presented by use of sagittal (x) sections. Before LFS, various pain-related areas were activated. After LFS, only right IPL was activated. Contrast Pre LFS - Post LFS revealed decrease in pain-related areas after LFS. ACC: anterior cingulate gyrus (BA 24, 32, 33), CC: cingulate gyrus (BA 24), INS A: anterior insula, INS P: posterior insula (BA 13), IPL: inferior parietal lobule (BA 40), S1: primary somatosensory cortex, S2: secondary somatosensory cortex, STG: superior temporal gyrus (BA 22)

3.3.2.2. Correlation with pain rating

Simple regression analyses revealed no correlation between changes in VRS rating and brain activity after LFS. No positive correlation was detected between the decrease in SES rating and the decrease in brain activation after LFS (Pre LFS - Post LFS). However, the decrease in SES rating was correlated with increased brain activation after LFS (Post LFS - Pre LFS contrast) (Tab. 3.8, Fig. 3.9). Decrease in SES-S rating was correlated with increased activation after LFS bilateral in cingulate gyrus (BA 24, 32) including the rostral part of ACC. Activation of ACC was also observed under test stimulation before LFS (Pre LFS contrast) and activation decreased after LFS (Pre LFS – Post LFS contrast). The group analyses revealed an activation in a more medial part of the ACC. Correlation between brain activation and decreased SES-S rating was observed in the ipsilateral medial frontal gyrus (BA 9). This area was not activated before LFS. Decrease in SES-A rating was correlated with increased activation in bilateral ACC (BA 32) after LFS. Rise of activity was observed in the rostral part of ACC, comparable to the increased activity in rostral ACC correlated with decreased SES-S rating. Contralateral striatum, consisting of caudatum and putamen, showed increased activity with increased pain relief. There was no activation in the striatum under test stimulation before LFS. Ipsilateral anterior insula (BA 13) correlated with SES-A. Before LFS, bilateral anterior and posterior parts of insula were activated by test stimulation. The Pre LFS – Post LFS contrast revealed decreased activation in ipsilateral posterior insula after LFS. Increments of activity correlated with decrease of SES-A rating were detected in the ipsilateral frontal gyrus, medial (BA 11) and inferior (BA 47), in STG (BA 38), and in IPL (BA 40). Medial frontal gyrus was not activated under test stimulation before LFS. Activation in bilateral STG was observed before LFS. The Pre LFS – Post LFS contrast revealed decreased activation in ipsilateral STG after LFS. Decreased activation was more lateral, caudal and dorsal than the increased activation correlated with decreased SES-A rating. Group analyses revealed ipsilateral IPL activation before and after LFS and a significant reduction in this area after LFS. Activation before LFS was more rostral and ventral than after LFS. Reduction of IPL activity in Pre LFS - Post LFS contrast was more lateral, rostral and ventral than the increased activation in IPL correlated with pain relief. Simple regression analyses showed contralateral activations in the parietal lobe, in the precuneus (BA 19) and

angular gyrus (BA 39). Both areas were not activated by test stimulation before LFS (Tab. 3.8, Fig. 3.9). Pearson Correlation revealed significant, positive correlation between pain relief and increased brain activity after LFS (Fig. 3.9).

Table 3.8. Simple regression between Post LFS - Pre LFS contrast and sensory SES-S rating and affective SES-A rating, respectively.

	Talairach coordinates					
Brain regions	x	y	z	t-value	z-value	voxels
SES-S						
R anterior cingulate (BA 24) ACC	**4**	**28**	**10**	**3.54**	**2.97**	**19**
R anterior cingulate (BA 32) ACC	12	36	13	2.77	2.45	
R medial frontal gyrus (BA 9)	**4**	**33**	**32**	**3.24**	**2.77**	**43**
L cingulate gyrus (BA 32)	-4	25	32	2.93	2.57	
SES-A						
R anterior cingulate (BA 32)	**4**	**31**	**-8**	**5.32**	**3.93**	**66**
L caudate, caudate head	-16	19	-1	3.02	2.63	
L lentiform nucleus, putamen	-16	8	-4	2.79	2.46	
R medial frontal gyrus (BA 11)	**44**	**46**	**-9**	**3.97**	**3.23**	**20**
R inferior frontal gyrus (BA 47)	48	42	-12	3.41	2.89	
R inferior parietal lobe (BA 40)	**44**	**-49**	**36**	**3.60**	**3.05**	**13**
R insula (BA 13) INS A	**40**	**19**	**-4**	**3.57**	**2.99**	**27**
R superior temporal gyrus (BA 38)	44	15	-14	2.90	2.54	
L anterior cingulate (BA 32)	**-8**	**36**	**24**	**3.56**	**2.98**	**25**
L precuneus (BA 19)	**-40**	**-72**	**40**	**3.05**	**2.65**	**23**
L angular gyrus (BA 39)	-44	-64	36	2.97	2.59	

Magnitude of pain relief after LFS was correlated with increased brain activation after LFS.
Simple Regression analyses, $p<0.05$ corrected. Bold: Local maxima with the highest z-value within an activated voxel cluster. L: left contralateral, R: right ipsilateral, BA: Brodmann area, ACC: anterior cingulate cortex, INS: insula (A: anterior)

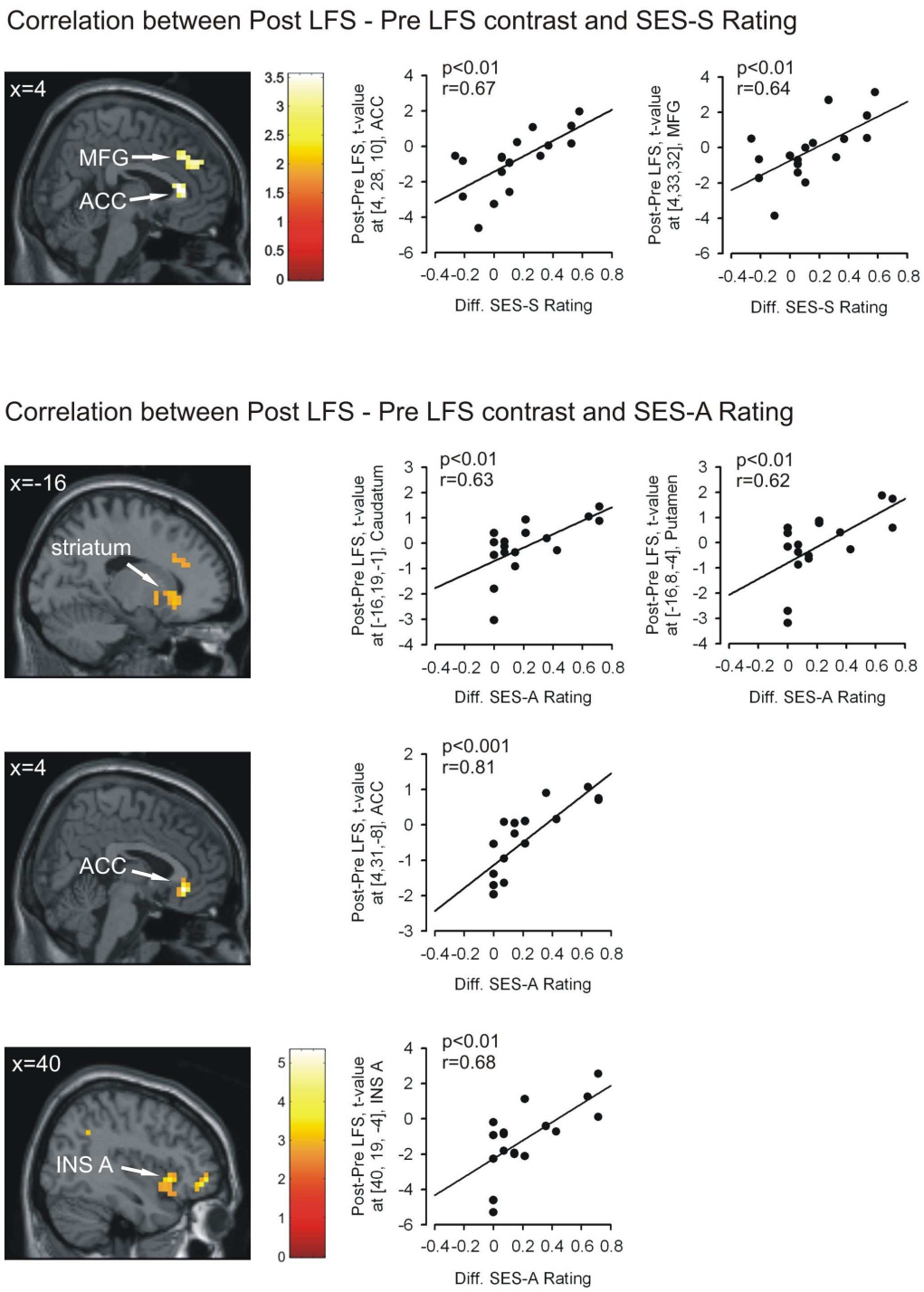


Figure 3.9. Simple regression analyses.

Simple regression between Post LFS - Pre LFS contrast and sensory SES-S rating and affective SES-A rating, respectively ($p<0.05$ corrected). Left: Specific areas are presented by sagittal sections. Magnitude of pain relief after LFS was correlated with increased brain activation after LFS. Right: Scatter plots show correlation between the difference in pain rating (Pre LFS – Post LFS) and the intensity (t-value) of brain activation after LFS (Post LFS – Pre LFS contrast). Pearson correlation test was conducted, p value and r value are presented. ACC: anterior cingulate gyrus (BA 24, 32), INS A: anterior insula (BA 13), MFG: medial frontal gyrus (BA 9), striatum (caudatum and putamen)

4. Discussion

The present thesis indicated prolonged decrease of nociception and pain after LFS for at least one hour. LFS induced a homotopic Aδ fiber mediated LTD, that was expressed by decreased SEP, sensory and affective pain perception rating and pain-related cerebral activation. Reduction of nociceptive processing might be due to peripheral effects on the first nociceptive synapse as it is suggested from in vitro studies. Furthermore an involvement of endogenous descending pain pathways is suggested as the magnitude of pain relief was correlated with increased brain activation after LFS.

The first part of the thesis demonstrated a pure homotopic effect of LFS. SEP amplitude and pain rating under homotopic test stimulation decreased after LFS. The decrease of SEP amplitude under heterotopic test stimulation was of the same amount as the decrease in Control experiment. Immediately after LFS, SEP amplitude under homotopic test stimulation significantly reduced. In contrast to this, SEP amplitude under heterotopic test stimulation remained unchanged directly after LFS. In Control experiment, SEP amplitude in first series after no stimulation period remained constant. The restriction to conditioned pathway and the magnitude of decrease fit well to the results of in vitro studies. Pure homotopic LTD was reported on the electrical conditioned pathway in different brain regions including hippocampus (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Kerr and Abraham, 1995), visual cortex (Kirkwood et al., 1993), perirhinal cortex (Cho et al., 2000) and amygdala (Wang and Gean, 1999). In the nociceptive system, LFS with Aδ fiber exciting intensity induces a homosynaptic LTD between Aδ fibers and second order neurons in the superficial spinal dorsal horn in rat slice preparation (Chen and Sandkuhler, 2000).

The second part of the thesis indicated sustained LTD of sensory and affective components of pain induced by LFS. Conditioning LFS resulted in a persistent decrease of VRS-I and VRS-U, and SES-S and SES-A ratings as compared to Pre LFS series and Control series. Sensory and affective components of pain are processed in two different pathways (Treede et al., 1999). However, the sensory component of pain correlates with the affective component of pain (Rainville et al., 1999). Hypnotic suggestions to modulate sensory component of pain produced parallel changes in pain intensity and unpleasantness. Suggestions directed at

affective component produced specific changes in unpleasantness, but not in pain intensity (Rainville et al., 1999). There are two possible explanations for reduced pain perception after LFS in the present thesis. Sensory and affective components of pain might decrease independently or the reduction in sensory pain component might cause the decline in affective pain component.
Furthermore quality of pain revealed by SES questionnaire gives an explanation about the involved nerve fibers. Stimulation of Aδ fibers elicits a pinprick-like painful sensation, whereas stimulation of C fibers evokes burning sensation (Mackenzie et al., 1975). SES item “stinging” was the prevailing sensation under test stimulation, suggesting preferentially Aδ fiber stimulation. The different pain sensations originated from factor analyses of 19 sensory SES items were similar to the subclasses proposed for the original SES questionnaire (Geissner, 1995). Besides the known subclasses superficial sharp pain, heat pain and deep rhythmic pain, the additional subclass describing deep constant pain was yielded in the present thesis. Perception of deep rhythmic pain decreased over time. Deep constant pain and superficial heat pain were not affected. The factor representing superficial sharp pain was the only factor that showed a decrease after LFS compared to Pre stimulation and Control, pointing to a reduced impact of Aδ fiber input on central nervous system pain processing.

The fMRI data of the third part of the thesis demonstrated that electrical test simulation activates brain areas involved in sensory, affective and cognitive pain processing. Group analyses revealed no difference between Control and LFS experiment under Pre condition. After LFS, brain activity was significantly reduced. Brain activation remained unchanged in the Control experiment. These results were in line with the reduction in the pain rating, pointing to LTD of pain-related cerebral activation induced by noxious LFS pointing to an effect at the first nociceptive synapse (Sandkuhler et el., 1997).
Simple regression between brain activation and pain rating revealed that peripheral effects at the first nociceptive synapse are not the only explanation for the LFS effect. Decrease in pain rating was not correlated with decrease of brain activation after LFS. Therefore, reduced brain activity after LFS resulted in a general decrease of pain perception, but had no direct influence on the magnitude of pain relief. In contrast to this, increased brain activation after LFS seemed to be important for the strength of rating reduction. In volunteers with a greater extent of pain relief, brain

activity under Post condition was increased in brain areas involved in endogenous pain control.

Attenuated cerebral activity after LFS in well-known pain-related brain areas might be due to LTD effects at the first nociceptive synapse as described in in-vitro studies (Sandkuhler et al., 1997). Consequently, a decrease of synaptic strength would cause inhibition of activity in all subsequent areas of the central nervous system. However, alternatively LFS may activate areas of the endogenous pain control system resulting in a decreased activity of pain-related areas as seen in the group analyses. The decrease of activity was not correlated with the amount of pain relief in the statistical analyses. The assumed activation of brain areas involved in endogenous pain control could not be demonstrated in the group analyses as it was below the significance threshold. However, the regression analyses revealed a correlation between the activation of brain areas and the amount of pain relief. This result suggests an involvement of the endogenous pain control system in LTD of pain processing.

General observations and possible spinal and supraspinal mechanisms of LFS-induced LTD will be discussed in detail.

4.1. General observations during test stimulation and LFS

Test stimulation via concentric electrode resulted in reliable SEP, sensory and affective pain perception and pain-related brain activation. SEP and global pain ratings obtained in the first part of the thesis were comparable to SEP recorded after intracutaneous electrical stimulation (Bromm and Meier, 1984) and pain ratings evoked by concentric electrode stimulation in previous LTD studies (Schorr and Ellrich, 2002; Ellrich and Schorr, 2002; Ellrich and Schorr, 2004; Yekta et al., 2006, Jung et al., 2009).

Differentiation into sensory and affective pain perception in the second part of the thesis revealed that electrical test stimulation evoked both, the sensory and the affective component of pain, with a preponderance of sensory pain perception. Under experimental conditions with limited stimulus duration and the possibility to withdraw at any time point, the affective component was expected to be less pronounced. Furthermore, the affective component is less marked for phasic pain (e.g. pain elicited by electrical stimulation) than for tonic pain (Chen and Treede, 1985; Rainville et al., 1992). SES item “stinging” was the prevailing sensation under test

stimulation, suggesting preferentially Aδ fiber stimulation.

The fMRI examination in the third part of the thesis revealed activation of various pain-related brain areas, including S1, S2, insula, ACC, STG, IPL and prefrontal cortex, evoked by test stimulation. S1 and S2 are important for sensory pain processing, including localization and discrimination of stimulus intensity (Bornhovd et al., 2002; Kulkarni et al., 2005). Many brain-imaging studies report only contralateral S1 activation (e.g. Christmann et al., 2007). Currently, there is a debate on the involvement of ipsilateral S1 during painful stimulation in healthy volunteers. Electrical median nerve stimulation activated S1 not only on the contralateral but also on the ipsilateral side (Nihashi et al., 2005). Furthermore, somatotopic organization was shown for contralateral and ipsilateral S1 after laser stimulation at hand and foot (Bingel et al., 2004). Both working groups suggested that the ipsilateral S1 might be activated via the corpus callosum from contralateral S1. Insula is involved in sensory and affective pain processing. Activation of the posterior insula indicated sensory processes while activation in anterior insula pointed to affective processes (Carlsson et al., 2006). ACC (BA 24, 32) is the most frequently reported area in pain-related imaging studies (Peyron et al., 2000). It is a multi-integrative structure involved in affective, cognitive and attentional processes and motor response planning (Peyron et al., 2000). Activation in STG (BA 22, 38) may be explained by salience of painful stimuli, which is processed via fronto-parietal-cingulate network, including temporo-parietal junction (BA 22, 39, 40), frontal operculum and ACC (Downar et al., 2003). Activity in right IPL has been associated with spatial attention to painful stimuli (Kulkarni et al., 2005). Prefrontal activation (BA 10, 44, 45, 47) was interpreted as a consequence of attention, cognitive evaluation, and planning of motor behavior in response to pain (Baron et al., 1999).

Pain perception ratings were obtained during LFS. Similar to test stimulation, sensory pain ratings were more pronounced than the affective ratings. Global, affective and sensory ratings were higher during LFS than during test stimulation. This was probably due to temporal summation caused by the higher frequency of LFS. Temporal summation was shown for the concentric electrode used in the present thesis (Katsarava et al., 2006). Pain ratings decreased during LFS, reaching a saturation level at the end of stimulation. Decrease of global, sensory and affective pain rating in the time course of LFS might be explained by habituation to repeated stimuli (Milne et al., 1991). An fMRI study showed reduced activation in brain areas

related to sensory (S2) and affective (anterior insula) components of pain following habituation (Bingel et al., 2007).

After LFS, SEP amplitude, pain ratings and brain activation were significantly reduced indicating sustained LTD. Putative LTD mechanisms are discussed below.

4.2. LFS effects at the first nociceptive synapse

It is interesting to discuss the results revealed from the previous study of our working group regarding the optimum LFS parameter (Jung et al., 2009). This study determined the optimum stimulation paradigm, regarding frequency, number of pulses, intensity and repetition of LFS application. Strongest LTD effect induced by 1 Hz is consistent with frequency optimum in in vitro (Dudek and Bear, 1992; Nakano et al., 2004) and in vivo studies (Manahan-Vaughan, 2000). Animal studies reported a switch from LTD induction with low 1 Hz frequencies to LTP induction with higher 10 Hz frequencies, no effect was reported after 3 Hz stimulation (Wang and Wagner, 1999). The idea of applying frequencies above 2 Hz in order to obtain a frequency-response curve with a greater spectrum (Mayford et al., 1995; van Dam et al., 2004) could not be realized as higher frequency stimulations under identical experimental conditions were not acceptable to participants due to strong pain.
Frequency dependent switch from LTD to LTP is thought to be dependent on postsynaptic intracellular calcium level (Artola and Singer, 1993). LFS leads to a moderate increase of Ca^{2+} preferentially activating protein phosphatases necessary for LTD, while HFS leads to a high elevation of Ca^{2+} preferentially activating protein kinases necessary for LTP (Bear and Malenka, 1994).
LFS with 1200 pulses showed the maximum decrease of SEP amplitude, indicating that a prolonged conditioning duration is necessary for the induction of LTD. Several previous studies investigated the correlation between number of pulses and LTD induction in animals, indicating that LFS with at least 900 pulses is essential to induce sustained depression of synaptic strength (Dudek and Bear, 1993; Manahan-Vaughan, 2000). Not only the above mentioned frequency dependent amplitude but also the duration of postsynaptic Ca^{2+} elevation seems to be important for the induction LTD (Mizuno et al., 2001). There is evidence that Ca^{2+}/Calmodulin dependent phosphatase plays a role in the induction of LTD and needs a prolonged rise in Ca^{2+} at a moderate level to be activated (Yasuda et al., 2003; Xia and Storm, 2005).

There is rare information about the most effective stimulation frequency and number of pulses in man, though our findings are in agreement with human studies suggesting 1 Hz stimulation and 1000 pulses (Klein et al., 2004) to 1200 pulses (Schorr and Ellrich, 2002; Ellrich and Schorr, 2002; Ellrich and Schorr, 2004; Yekta et al., 2006) as appropriate parameter.

Varying stimulation intensity indicated LTD after LFS with an intensity of $2\times I_P$ and $4\times I_P$ and not after LFS with an intensity of $1\times I_P$ (Jung et al., 2009). Electrical stimulation via concentric electrode preferentially activates nociceptive fibers (see below, 4.7.1. Concentric electrode) resulting in sustained LTD even after LFS with low intensity. Differences in stimulation intensities described in various reports are mostly due to different kinds of electrodes or stimulation sites. Certain studies reported application of intensities ranging between 2.3 and $2.5\times I_P$ on the mental nerve area and forehead, respectively, for investigating masseter inhibitory and blink reflex (Schorr and Ellrich, 2002; Ellrich and Schorr, 2004). In addition, LTD in humans was induced by punctuate electrodes with intensities of $10\times I_0$ and $20\times I_0$ corresponding to LFS intensities in present study (Klein et al., 2004).

The first part of this thesis revealed the homotopic nature of LFS-induced LTD. The restriction to conditioned pathway was shown in in vitro studies in the nociceptive system. LFS with Aδ fiber exciting intensity induces a homosynaptic LTD between Aδ fibers and second order neurons in the superficial spinal dorsal horn in rat slice preparation (Chen and Sandkuhler, 2000). This points to an effect at the first nociceptive synapse. Furthermore, after induction of LTD, EPSP amplitude, dendritic spine size, and the number of pre- and postsynaptic structures decreased in hippocampal slices (Zhou et al., 2004; Shinoda et al., 2005).

The second part of the thesis indicated sustained LTD of sensory and affective components of pain induced by LFS. Analysis of pain quality offers information on the activated fiber type. Stimulation of Aδ fibers elicits a pinprick-like painful sensation, whereas stimulation of C fibers evokes burning sensation (Mackenzie et al., 1975). SES item “stinging” was the prevailing sensation under test stimulation, suggesting preferentially Aδ fiber stimulation. The factor representing superficial sharp pain was the only factor that showed a decrease after LFS, pointing to a reduced impact of Aδ fiber input on central nervous system pain processing. The factor representing superficial heat pain did not change after LFS, suggesting no involvement of C fibers.

Preferential activation of Aδ fibers was achieved by the concentric design of the electrode used in this study (see below, 4.7.1. Concentric electrode). Results from this part of the thesis not only support the activation preference of the concentric electrode, they also show an Aδ fiber mediated LTD.

The fMRI data from the third part of the thesis demonstrated strong decrease of pain-related brain activation after LFS. While before LFS various pain-related areas were activated (see above, 4.1. General observations during test stimulation and LFS), after LFS, solely right IPL was activated. Activation after LFS was more posterior and superior than activation before LFS. As activity in right IPL has been associated with spatial attention to painful stimuli (Kulkarni et al., 2005), activation after LFS may be due to the request to focus on each stimulus and rate pain perception after each session. In contrast to Pre LFS, activity in IPL was reduced after LFS. This decrease cannot simply be explained by attenuated attention during the time course of experiment, as there was no change in Control experiments. Another study, examining acute muscle pain, indicated activation in right IPL during both painful and non-painful activation with stronger activation during painful stimulation (Niddam et al., 2002). This leads to the suggestion that reduced IPL activity in the present study is directly linked to decreased pain perception after LFS. Contrast between Pre and Post LFS further revealed significant reduction in S1, S2, insula, ACC and STG, indicating a sustained decrease in sensory, affective and cognitive pain processing after LFS.

Taken together these results suggest LFS effects at the first nociceptive synapse in the spinal cord. Homotopic, Aδ fiber mediated LTD, dependent on the frequency and duration of LFS to obtain the necessary intracellular rise of Ca^{2+}, leads to a decrease of SEP amplitude, sensory and affective pain perception and cerebral activity involved in sensory, affective and cognitive pain processing.

4.3. Supraspinal mechanisms of LTD

The homotopy of LFS in spinal nociceptive system shown in the first part of the study suggested effects at the first nociceptive synapse. In contrast to this, another study in trigeminal nociceptive system investigating the human blink reflex revealed heterosynaptic LTD. Pain perception was only reduced after ipsilateral LFS, whereas blink reflex was reduced bilaterally (Yekta et al., 2006). Bilateral reduction of blink reflex was probably due to bilateral projections of supraorbital nerve afferents onto

spinal trigeminal nuclei, suggesting an involvement in LFS effects beyond the first nociceptive synapse.

Animal studies demonstrated an involvement of the endogenous pain control system in LTD. Conditioning stimulation of the sciatic nerve, which induced LTD in rats with intact descending pathways, led to LTP in spinalized rats (Liu et al. 1998). Various neurotransmitters, e.g. opioid, dopamine and serotonin, influenced LFS-induced LTD. Exogenously applied and endogenously released opioids can act to facilitate LTD of the Schaffer collateral input to CA1 pyramidal neurons (Wagner et al., 2001). LTD in spinal dorsal horn was blocked by μ-opioid receptor antagonist (Zhong and Randic, 1996). There are some evidences for an influence of dopamine on LTD. D2-like receptor activation prevented LTD, and D2-like receptor blockade amplified LTD of orofacial sensorimotor processing in anesthetized mice (Ellrich, 2005). Administration of serotonin increased the incidence of primary afferent-evoked LTD in rat deep dorsal horn neurons (Garraway and Hochman, 2001).

The depression of sensory and affective rating observed in the second part of the thesis can at least partly be explained by endogenously released neurotransmitters. The μ-opioid receptors played a central role in the regulation of sensory and affective components of pain (Zubieta et al., 2001). Fentanyl, a μ-opioid receptor agonist, produced nearly equal reductions in sensory and affective response to experimental pain (Price et al., 1986). Treatment with tropisetron, a 5-HT3 receptor antagonist, resulted in reduced activation in sensory and affective pain related brain areas in fibromyalgia patients (Koeppe et al., 2004). D2-receptor-mediated neurotransmission was positively associated with ratings of sensory and affective qualities of pain (Scott et al., 2006). These studies suggest an involvement of descending systems, modulating sensory and affective components of pain, in LTD effects after LFS.

The third part of the thesis directly provides evidence for supraspinal effects of LFS. Magnitude of pain relief after LFS was correlated with increased activation in ACC, anterior insula, caudatum and putamen, frontal, temporal and parietal cortex. These brain areas are suggested to take part in the endogenous nociceptive descending control that is mediated by the above mentioned neurotransmitters, opioid, dopamine and serotonin. Electrical stimulation of ACC decreased the response to noxious stimuli in dorsal horn neurons in rats (Senapati et al., 2005). Authors hypothesized that ACC suppressed noxious input at spinal level via descending inhibitory system.

Furthermore the ACC, especially the rostral part, was implicated in opioid analgesia (Casey et al., 2000). The anterior insula further contains dopamine receptors that modulate long-term nociception in rat (Coffeen et al., 2008). The striatum, consisting of caudate nucleus and putamen, and striatal dopamine receptors were involved in pain regulation (Hagelberg et al., 2004b). Furthermore, opioids affected the dopaminergic system. Opioid-dopamine interactions were demonstrated in frontal and temporal cortical regions in healthy human (Hagelberg et al., 2004a). Another human study investigated the effect of serotonin on pain modulation. Intensity of cold pressure pain was inversely correlated with serotonin receptor binding potential in multiple cortical areas, including the insula, prefrontal and cingulate cortices (Martikainen et al., 2007).

Correlation between brain activation after LFS and pain relief in the present thesis led to the suggestion that LFS affected the endogenous descending system resulting in a stronger depression of pain.

4.4. Other mechanisms than LTD

The above mentioned decrease in pain perception ratings and cerebral activation was solely observed after LFS. There was no change after the break in Control experiments. These results led to the suggestion that the reduction is purely due to LFS-induced LTD. SEP amplitude also decreased in Control experiments and under heterotopic test stimulation after LFS, but reduction after homotopic LFS was significantly stronger. Furthermore, SEP amplitude under homotopic test stimulation was significantly reduced directly after LFS, as measured in the first Post series. In contrast to this, SEP amplitude under heterotopic test stimulation and in Control experiment remained unchanged in the first Post series compared to the last Pre series. Significant stronger decrease after homotopic LFS was still evident after one hour, as determined in the last Post series. The decline of SEP after no stimulation period and after heterotopic LFS was likely due to habituation. Progressive decrease in SEP amplitude during repetitive electrical stimulation is defined as habituation (Condes-Lara et al., 1981). Application of electrical stimuli with a constant interstimulus interval as in the present thesis leads to a stronger effect. As shown in a previous study examining habituation after electrical stimulation, there were no changes in recruitment of primary afferents, so this phenomenon is not due to transmission fatigue (Milne et al., 1991). As in the present study cutaneous afferents

were stimulated this decrease cannot be explained by receptor adaptation. Other mechanisms like diffuse noxious inhibitory control can be excluded. Otherwise in the first part of the thesis also the unconditioned heterotopic side had been affected (Le Bars et al., 1979). Taken together, there is strong evidence that LFS induces LTD of nociception and pain.

4.5. Clinical advantage

Repetitive HFS of primary afferents induces LTP in Aδ (Randic et al., 1993) and in C fibers (Liu et al., 1998) in vitro and in vivo. LFS with Aδ fiber intensity induces de-novo LTD in rat spinal dorsal horn in vitro (Sandkuhler et al., 1997) and in vivo (Liu et al., 1998). Furthermore, LFS to Aδ fibers could reverse HFS-induced LTP (depotentiation) and HFS could not induce LTP once LFS was given (Ikeda et al., 2000). LTP may be an underlying mechanism of afferent induced hyperalgesia, as it can not only be evoked by electrical stimulation but also by natural stimulation of heat-, mechano- or chemosensitive nociceptors in the skin or by acute nerve injury (Sandkuhler and Liu, 1998). LTP and injury-induced hyperalgesia share signal transduction pathways, time course and pharmacological profile, which makes LTP at Aδ and C fiber synapses an attractive cellular model of hyperalgesia, central sensitization and chronification of pain (Sandkuhler et al., 2000). As LFS is able to de-potentiate established LTP, induce de-novo LTD and prevent further LTP, it might be used as a neuromodulary treatment of pain. Therefore it is of great interest to investigate the mechanisms of LFS-induced LTD in humans.

4.6. Comparison with transcutaneous nerve stimulation (TENS)

So far, clinically used treatment of chronic pain is transcutaneous electrical nerve stimulation (TENS). It is used for more than 30 years, but there are only a few valid investigations on the efficacy of TENS (Chesterton et al., 2003; Ainsworth et al., 2006). Two forms of TENS with different stimulation parameters exist. “Acupuncture-like TENS” (Al-TENS) was applied with 4 Hz and with “to tolerance” intensity whereas “conventional TENS” was performed with 110 Hz and “strong but comfortable” stimulation intensity (Chesterton et al., 2002). Al-TENS intensity “to tolerance” was defined as very strong and uncomfortable. Even though it can be assumed that Al-TENS induces painful sensations, the intensity of Al-TENS can not be directly compared to stimulation intensity in the present study due to missing pain quantification in the literature. Conventional TENS with high frequency and low

intensity caused hypoalgesic effect only during stimulation. Al-TENS with high intensity reduced pain perception for 20 minutes after stimulation. Al-TENS was applied for 30 minutes. In contrast to this, the present thesis showed sustained LTD of nociception and pain for at least one hour already after 10 minutes after LFS. The different electrode designs play an important role for the varying effect durations. In the present thesis a concentric electrode was used that preferentially activates Aδ fibers. In contrast to the specific activation of these nociceptive afferents, TENS electrodes activate the whole A fiber spectrum without any preference (large diameter electrodes). Conventional TENS recruits only Aα/β fibers and hypoalgesic effect is probably mediated by inhibitory GABAergic interneurons as proposed within gate control theory (Melzack and Wall, 1965). The longer-lasting hypoalgesia after tolerable painful TENS additionally requires recruitments of Aδ fibers, but it is limited to a short period. In the present thesis Aδ fibers are preferentially stimulated resulting in a long-lasting decrease of nociception and pain for at least one hour and therefore matching the criteria of long-term depression (Braunewell and Manahan-Vaughan, 2001).

4.7. Discussion of methods

LTD in human was investigated by use of psychophysical, electrophysiological and brain imaging methods. Applied methods were important to gain more insight into the effect of LFS and may be a step forward to future therapy of chronic pain. However, more experiments are necessary to examine the precise mechanisms of LTD and to develop an optimal treatment protocol. This chapter provides a discussion of the applied methods and methods that are recommended for future studies.

4.7.1. Concentric electrode

In this thesis electrical stimuli evoked by a concentric electrode served as pain stimulus. This electrode consists of a small central cathode and a large ring anode. Due to the concentric design, low current intensities produce high current field density which reaches superficial skin layer, where the nociceptive free nerve endings are located (Novotny and Gommert-Novotny, 1988). Experiments on human blink reflex provide further evidence for preferentially Aδ fiber activation. Electrical stimulation with conventional surface electrode at the forehead elicits R1 and R2 response. Electrical stimulation via concentric electrode could not elicit R1 response (Kaube et al., 2000). This early component could be elicited by innocuous

mechanical stimuli, mediated via Aβ fibers, but not by noxious stimuli activating selectively Aδ fibers, as demonstrated by use of laser stimulation (Ellrich et al., 1997). The R2 response, elicited by innocuous and noxious stimuli, could be evoked by use of standard and concentric electrodes. After blockade of Aδ and C fibers by cutaneous anesthesia, R2 was slightly depressed after standard stimulation, but almost abolished after stimulation with the concentric electrode (Kaube et al., 2000). This nociceptive specific blink reflex evoked by concentric electrode has been validated in various studies (Koh and Drummond, 2006; Peddireddy et al., 2006; Di Clemente et al., 2007). Furthermore, SEP could no longer be elicited via concentric electrode after blockade of Aδ and C fibers by cutaneous anesthesia. Mean conduction velocity estimated after electrical stimulation with concentric electrode was in Aδ fiber range with 11.6±5.1 m/s (Katsarava et al., 2006). This is similar to conduction velocities measured with electrical stimulation via needle electrode and laser stimulation, which is known to selectively activate Aδ fibers (Inui et al., 2002). SEP latencies in the present thesis coincide with that after laser stimulation (Spiegel et al., 2000; Truini et al., 2005; Ristic et al., 2008) considering a nociceptor activation time of about 40 ms (Bromm and Treede, 1984). Another reliable pain model is the intracutaneous stimulus. It induces pain sensation and cerebral potentials very similar to the concentric electrode (Bromm and Meier, 1984). Both pain models result in low pain thresholds as compared to conventional surface electrodes and elicit a definite, well localized sharp sensation that is typical for Aδ fiber mediated pain.

Activation of Aδ fibers seems to be essential in order to induce LTD of nociception and pain. In vitro experiments in the spinal nociceptive system revealed sustained decrease of EPSP amplitude for at least three hours after noxious LFS at attached dorsal root with Aδ fiber intensity (Sandkuhler et al., 1997). LFS with higher intensities, additional recruiting C fibers, did not lead to a stronger decrease. Thus, C fiber activity is not required for maximal expression of LTD. LFS with lower intensity, activating mainly Aβ fibers, led to a short-term reduction for less than 30 minutes (Sandkuhler et al., 1997). Recent human study revealed LFS effect only after clearly painful LFS intensities with 4-fold pain threshold. After LFS with intensity of 1-fold pain threshold, SEP amplitude and pain ratings did not differ from Control experiment without LFS. Results from the present thesis gave further evidence for preferentially Aδ fiber stimulation. The second part of the thesis showed that SES item “stinging” was the prevailing sensation under test stimulation, suggesting preferentially Aδ fiber

stimulation. Sensation of superficial sharp pain was affected by LFS, suggesting Aδ fiber mediated LTD.

4.7.2. Pain perception rating

In order to examine subjective pain experience volunteers were asked to give pain perception ratings. A simple but very effective way is to ask the volunteers “How strong is your pain on a scale from 0 (no pain) to 100 (maximum imaginable pain)?” This Verbal Rating Scale (VRS) was used in all previous LTD studies in humans and revealed a strong percentage decrease after LFS in trigeminal nociceptive system (Schorr and Ellrich, 2002; Ellrich and Schorr, 2002; Ellrich and Schorr, 2004; Ellrich, 2006; Yekta et al., 2006) and spinal nociceptive system (Klein et al., 2004). Decrease in VRS rating was coupled with decrease in brain stem reflexes and cortical potentials. Therefore, VRS could serve as a ‘gold standard’ with which new measures could be compared.

Asking the volunteers about the magnitude of pain is a good method to determine general LTD effect on pain perception, but it is limited to one dimension. Pain is known to be a multidimensional phenomenon, consisting of different components. Sensory-discriminative components refer to location, duration and intensity of noxious stimuli, while affective-emotional components deal with the unpleasantness evoked by pain. The cognitive component is responsible for the evaluation of the stimulus by comparing the sensation with former experiences (Melzack and Casey, 1968).

In the second part of the thesis, the impact of noxious LFS on sensory and affective aspects of pain perception was obtained by multidimensional rating scales. Volunteers were asked to rate stimulation according to VRS and to distinguish between pain intensity (VRS-I: 0=not intensive; 100=maximum imaginable intensive) and pain unpleasantness (VRS-U: 0=not unpleasant; 100=maximum imaginable unpleasant). VRS-I may account for the sensory component and VRS-U for the affective component of pain. Furthermore, volunteers filled in an enlarged Pain Perception Scale (Schmerzempfindungsskala, SES) (Geissner, 1995) including nine additional sensory items (Türp and Marinello, 2002). They were instructed to judge 19 sensory (SES-S) and 14 affective items (SES-A) on a scale ranging from 0 to 3 (0=not appropriate; 1=somewhat appropriate; 2=largely appropriate; 3=fully appropriate). The SES is part of the German pain questionnaire designed by the

German Chapter of the International Association for the Study of Pain (www.dgss.org) and is a validated tool to determine sensory and affective pain qualities. This questionnaire gives not only ratings for the sensory and affective components of pain it also provides insights into the quality of pain. The sensory items were grouped into different pain sensations by factor analyses based on the ratings obtained in the second part of the thesis. Factors were similar to the subclasses proposed for the original SES questionnaire with 10 sensory SES items (Geissner, 1995). In addition to the superficial sharp and heat pain and the deep rhythmic pain, another subclass describing deep constant pain was revealed. This part of the thesis showed sustained decrease of global, sensory and affective pain perception rating after LFS. Sensation of superficial sharp pain was affected by LFS, suggesting Aδ fiber mediated LTD.

4.7.3. Somatosensory evoked potential (SEP)

Recording of SEP amplitude via EEG is a valid method to investigate nociceptive processing. SEP are reproducible and constant on different days (Bromm and Scharein, 1982b). Component analysis of SEP to mechanical and electrical stimulation revealed different components discriminating between quality and quantity of stimulation. Two components (N150-P260: Amplitudes with negativity at 150 ms and positivity at 260 ms) were detected which distinguished between painful and non-painful stimulation and therefore may be denoted as specific pain-related components (Bromm and Scharein, 1982a). Dental stimulation experiments demonstrated that N175-P260 amplitudes are correlated rather with subjective painfulness than stimulus intensity (Chen et al., 1979). Decrease of pain perception rating after pharmacological treatment is highly correlated with the decrease in late SEP amplitude (Chen and Chapman, 1980; Kochs et al., 1996). In the present thesis, SEP amplitude decreased after LFS compared to Pre LFS baseline and Control without conditioning stimulation, indicating sustained decrease in nociceptive processing.

4.7.4. Functional magnetic resonance imaging (fMRI)

FMRI is a non-invasive brain imaging method measuring blood oxygenation level dependent (BOLD) signal as an indirect marker of changes in brain activity. While oxygenated hemoglobin is diamagnetic and has no influence on MR signal, deoxygenated hemoglobin is paramagnetic and attenuates the MR signal in a

concentration-dependent manner. Activation of brain areas leads to an increase in cerebral blood flow resulting in an oversupply of oxygenated blood. Although the BOLD signal is dependent on the blood flow response that follows neural activity, they are suggested to be linearly related (Logothetis, 2003; Marcar and Loenneker, 2004).

Pain-related brain activation was investigated in imaging studies showing that pain is processed in various cortical structures (Peyron et al., 2000). Sensory aspects of pain are mainly processed in S1, S2 and posterior insula (Alkire et al., 2004) and affective aspects in ACC and anterior insula (Rainville et al., 1997; Carlsson et al., 2006). Prefrontal and parietal cortices are involved in cognitive and attentional processes (Kong et al., 2006; Brown et al., 2008). In the third part of the study, these pain-related brain areas were activated by electrical stimulation. After LFS, activation in all these areas was decreased.

4.7.5. Methods for future investigation of LFS

In the first part of the thesis, the homotopic organization of LTD was shown. In order to investigate LTD and effects of repeated LFS for several days, results from the unconditioned heterotopic side can be used as an endogenous control.
Further investigation of the spatial organization of LTD in the human low back area provides evidence that LFS effect is restricted to one receptive field (Jung et al., 2008). In order to affect a larger area by LFS, the development and optimation of a multielectrode array is necessary. Inducing a more widespread LTD effect will be a prerequisite for introducing this kind of electrical stimulation in chronic pain therapy.

The sensory, affective and cognitive components of pain were examined in the second part of the thesis. It would be interesting to further investigate the vegetative and motoric component (Schmidt and Lang, 2007). Vegetative autonomous response can be determined by measurement of blood pressure, heart frequency, skin conductance and respiration rate. Furthermore, pupil size can be used as an indicator for autonomic response. Painful stimulation reliably elicits pupil dilation, indicating sympathetic activity, which increases with increasing stimulus intensity (Chapman et al., 1999; Hofle et al., 2008). Pupillary light reflex, a light flash induced constriction and redilatation of the pupil, provides insight into the interaction of sympathetic and parasympathetic components (Heller et al., 1990). Combined measurement of pupillary light reflex and cardiovascular functions provides valuable

information about LFS effect on vegetative pain components.
Influence of LFS on the motoric component of pain in the spinal system could be investigated by EMG recording of muscle tension or withdraw reflex. In previous studies, LFS was examined in sensorimotor processing in the trigeminal nociceptive system by use of masseter inhibitory reflex and blink reflex (Schorr and Ellrich, 2002; Ellrich and Schorr, 2002; Yekta et al., 2006). It was suggested, that the reduction of motor response is due to an inhibitory effect on sensory neurons (Ellrich, 2006). But the effect on sensory or motoric part of the reflex arc remains to be determined.

In the third part of the thesis, fMRI recordings were conducted to examine the LFS effect in the brain. FMRI is a good method to determine spatial localization, but the temporal solution is rather poor. Results were restricted to comparisons before and after LFS application. Multi-channel EEG recording that directly measure neuronal activity in real-time (Michel et al., 2004) allows an examination of temporal changes in brain activity during LFS. Therefore, it might provide information about brain areas that are responsible for LTD induction after a certain time period of LFS application.

5. Conclusion

The present thesis investigated LTD in human by use of psychophysical, electrophysiological and brain imaging methods. LFS induced prolonged decrease of nociception and pain for at least one hour. LTD induction was dependent on stimulation parameter. Optimum frequency (1 Hz) and number of pulses (1200) are suggested to be important to induce a moderate prolonged rise in intracellular Ca^{2+} in order to obtain LTD. Low stimulation intensities of $2\times I_P$ and $4\times I_P$ already induced clearly pricking painful sensation and sustained LTD. Due to the concentric design of the electrode low stimulation intensities produce high current density leading to a preferential activation of Aδ fibers. In vitro studies in spinal dorsal horn proofed the necessity of Aδ fiber stimulation in order to induce sustained LTD. Furthermore, analyses of pain quality in the present thesis showed a decrease of superficial sharp pain indicating Aδ fiber mediated LTD.

LTD was solely observed at the place where the LFS was applied, indicating a homotopic effect. Homosynaptic LTD was shown in in vitro studies. After LFS, SEP amplitude, sensory and affective pain perception and pain-related cerebral activation were reduced. These results indicate an effect at the first nociceptive synapse as suggested from in vitro studies. Furthermore, correlation of pain ratings and cerebral activation revealed an increased activity of brain areas involved in endogenous inhibitory pain pathways after LFS with increasing pain relief. Therefore LFS induces mechanisms at the first nociceptive synapse and in supraspinal regions leading to a sustained LTD of nociception and pain.

This thesis was important to gain more insight into the effect of LFS and may be a step forward to future therapy of chronic pain. However, more experiments are necessary to examine the precise mechanisms of LTD and to develop an optimal treatment protocol.

6. Summary

Synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), represents a cellular model of learning and memory. LTP, a long-lasting increase of synaptic strength, can be induced by electrical stimulation with a high frequency. Low-frequency stimulation (LFS) leads to a decrease of synaptic transmission referred to as LTD. Synaptic plasticity was shown in the nociceptive system. LTP is suggested to be involved in central sensitization of pain, leading to a so-called pain memory. As LFS is able to reverse LTP, it might be useful to attenuate or even erase this pain memory. Therefore it is of great interest to investigate LFS-induced LTD in humans, in order to use it in future therapy of chronic pain. So far, most studies were conducted in animals, showing sustained homosynaptic LTD after LFS of spinal afferents. The few studies in humans investigated the influence of LFS on trigeminal reflexes, evoked potentials and general pain perception.

Present thesis is dealing with a detailed investigation of LTD in spinal nociceptive processing in healthy human. It is divided into three parts:
(1) Homotopy of LTD; (2) LFS effects on sensory and affective pain components;
(3) LTD of cerebral activation.
In all parts nociceptive Aδ fibers were electrically stimulated by a concentric electrode. Painful test stimulation series were applied before (Pre) and after (Post) conditioning LFS (1 Hz, 20 min) to the hand dorsum. In Control experiments with the same volunteers no LFS was applied, but stimulation was interrupted. LFS effect was examined by electrophysiological, psychophysical and brain imaging methods.

In the first part of the thesis, putative homotopy of LTD was investigated in 30 volunteers by alternating application of test stimulation series unilateral to radial and ulnar side of right hand dorsum or bilateral to radial side of right and left hand dorsum. Conditioning LFS was applied to radial side of right hand dorsum. Somatosensory evoked cortical potential were recorded and volunteers rated stimulus intensity. After homotopic LFS, amplitude of cortical potential and pain rating significantly decreased. Amplitude reduction after heterotopic LFS did not differ from habituation effects in Control experiment without LFS. Heterotopic pain perception was not affected. This part demonstrated homotopic organization of LTD.

Investigation of LFS effect on sensory and affective pain perception in the second part of the study was performed on 20 healthy volunteers, who were asked to rate

their pain on multidimensional assessment including Verbal Rating Scale of perceived stimulus intensity and unpleasantness and Pain Perception Scale with sensory and affective items. After LFS, pain perception ratings were reduced as compared to Pre series and Control experiments. During LFS, ratings decreased. Factor analysis of sensory items of the Pain Perception Scale revealed sole reduction of superficial sharp pain perception after LFS in contrast to Control experiment. Perception of deep rhythmic pain decreased over time. Deep constant pain and superficial heat pain were not affected. This study showed sustained LTD of sensory and affective components of pain. Reduction of sharp pain points to Aδ fiber mediated LTD.

In the third part of the thesis, LFS effects on cerebral activation were investigated in 17 healthy male volunteers by functional magnetic resonance imaging. Volunteers rated sensory and affective pain perception. Electrical test stimulation activated brain areas involved in sensory (primary and secondary somatosensory cortex, posterior insula) and affective (anterior cingulate cortex, anterior insula) pain processing. After LFS, activity was significantly reduced. There was no change during Control experiments. Sensory and affective pain rating solely decreased after LFS. Pain relief was correlated with increased activity after LFS in rostral part of anterior cingulate cortex, anterior insula, striatum, frontal and temporal cortex. This part revealed LTD of pain-related cerebral activation, involving sensory and affective processes. Increased brain activation after LFS suggested involvement of endogenous pain modulatory systems leading to stronger LTD.

The present thesis indicated sustained reduction of nociception and pain after LFS for at least one hour. Homotopic Aδ fiber mediated LTD was expressed by declined somatosensory evoked potentials, sensory and affective pain perception rating and pain-related cerebral activation. Reduction of nociceptive processing might be due to peripheral effects on the first nociceptive synapse as it is indicated from in vitro studies. The magnitude of pain relief was correlated with increased brain activation after LFS, suggesting an involvement of endogenous pain modulatory pathways.

This thesis was an important step towards the understanding of LTD in humans. Detailed knowledge about LTD is a prerequisite in order to use LFS as therapy in chronic pain patients in future.

7. Zusammenfassung

Synaptische Plastizität, bestehend aus Langzeitpotenzierung (LTP) und Langzeithemmung (LTD), stellt ein zelluläres Modell des Lernens und Gedächtnisses dar. LTP, eine lang anhaltende Steigerung der synaptischen Übertragung, kann durch hochfrequente elektrische Stimulation hervorgerufen werden. Niederfrequenzstimualtion (LFS) führt zu einer Senkung der synaptischen Übertragungsstärke, bekannt als LTD. Synaptische Plastizität wurde im nozizeptiven System gezeigt. Es wird angenommen, dass LTP zu der Bildung eines so genannten Schmerzgedächtnisses führt. Da LFS eine bestehende LTP aufheben kann, könnte sie nützlich sein um das Schmerzgedächtnis zu löschen oder zumindest abzumildern. Die meisten Studien wurden bisher in Tieren durchgeführt. Diese zeigten eine lang anhaltende homosynaptische LTD. Die wenigen Studien in Menschen untersuchten den Einfluss der LFS auf trigeminale Reflexe, evozierte Potentiale und generelle Schmerzempfindung.

Die vorliegende Doktorarbeit zeigt eine detaillierte Untersuchung der LTD im spinalen nozizeptiven System von gesunden Probanden. Dabei wurden nozizeptive Aδ Fasern des Handrückens über eine konzentrische Elektrode stimuliert. Teststimulationsserien wurden vor (Pre) und nach (Post) einer konditionierenden LFS (1 Hz, 20 Min) appliziert. In Kontrollexperimenten mit den gleichen Probanden wurde keine LFS appliziert, sondern eine Pause eingelegt. Die Effekte der LFS wurden anhand von elektrophysiologische, psychophysiologischen und bildgebenden Methoden untersucht.

Der erste Teil der Doktorarbeit befasst sich mit der möglichen Homotopie der LTD. Bei 30 Probanden wurden abwechselnd Teststimulationsserien unilateral auf der radialen und ulnaren Seite der Handfläche, oder bilateral auf der rechten und linken Hand an der radialen Seite appliziert. Dabei wurden somatosensorisch evozierte kortikale Potentiale und ein Schmerzrating aufgenommen. Nach homotoper LFS sanken die Amplitude der kortikalen Potentiale und das Rating signifikant. Die Reduktion der Amplitude bei heterotoper LFS unterschied sich nicht von Habituationseffekten im Kontrollexperiment. Das heterotope Rating blieb unbeeinflusst. Somit wurde die homotope Organisation der LTD gezeigt.

Im zweiten Teil wurde der Einfluss der LFS auf die sensorischen und affektiven Schmerzkomponenten untersucht. Zwanzig Probanden sollten die Intensität und

Unangenehme des Schmerzes beurteilen und eine Schmerzempfindungsskala mit sensorischen und affektiven Items ausfüllen. Nach der LFS sanken die sensorischen und affektiven Empfindungen im Vergleich zu den Pre Serien und zur Kontrolle signifikant ab. Eine Faktorenanalyse zeigte, dass die Empfindung von scharfem Schmerz im Vergleich zur Kontrolle abnahm. Rhythmischer Tiefenschmerz sank über die Zeit ab. Konstanter Tiefenschmerz und Hitzeschmerz waren unbeeinflusst. Das Absinken des scharfen Schmerzes deutet auf eine Aδ Faser vermittelte LTD hin. Diese reduzierte sowohl sensorische als auch affektive Schmerzempfindungen.

Im dritten Teil wurde der LFS Effekt auf die zerebrale Aktivierung an 17 Probanden mittels funktioneller Magnetresonanztomographie gemessen. Sensorisches und affektives Schmerzrating wurde aufgenommen. Elektrische Teststimulation aktivierte Gehirnareale, die an der Verarbeitung der sensorischen (primärer und sekundärer somatosensorischer Kortex, posteriore Insula) und affektiven (anteriorer cingulärer Kortex, anteriore Insula) Schmerzverarbeitung beteiligt sind. Nach der LFS war diese Aktivität signifikant reduziert. Während der Kontrollexperimente gab es keine Veränderungen. Der Schmerznachlass korrelierte mit einer steigenden Aktivität nach der LFS in folgenden Gehirnarealen: rostraler Teil des anterioren cingulären Kortex, anteriore Insula, Striatum, Frontal- und Temporalkortex. Es zeigte sich eine LTD in Gehirnarealen, die an der Verarbeitung von sensorischen und affektiven Schmerzprozessen beteiligt sind. Es wird vermutet, dass die steigende Aktivität nach der LFS mit dem endogenen schmerzmodulierenden System im Zusammenhang steht, das zu einem stärkeren LTD Effekt führt.

Diese Doktorarbeit zeigte eine langanhaltende Reduktion von Nozizeption und Schmerz nach LFS. Die homotope Aδ Faser vermittelte LTD stellte sich durch eine Reduktion der kortikalen Potentiale, der sensorischen und affektiven Schmerzempfindung und der schmerzbezogenen Gehirnaktivität dar. Das Absinken der nozizeptiven Verarbeitung könnte auf Effekte an der ersten nozizeptiven Synapse zurückzuführen sein, wie es in vitro Studien vermuten lassen. Die Korrelation von Schmerznachlass und dem Ansteigen der Gehirnaktivität deutet auf eine Beteiligung des endogenen schmerzmodulierenden Systems hin.

Diese Doktorarbeit war ein wichtiger Schritt zum Verstehen der LTD beim Menschen. Ein detailliertes Wissen über die LTD ist eine Vorraussetzung um LFS zukünftig in der Therapie chronischer Schmerzpatienten einzusetzen.

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Lebenslauf:

Persönliche Daten:

Name:	Rottmann
Vorname:	Silke
Geburtstag:	20. 02.1979
Geburtsort:	Unna
Staatsangehörigkeit:	Deutsch

Qualifikationen:

1998: Abitur, Goethe Gymnasium Dortmund

1998 bis 2004: Biologiestudium, Ruhr Universität Bochum
Abschluss: Diplom am Lehrstuhl für Allgemeine Zoologie und Neurobiologie
„Die Rolle des Colliculus superior bei der Steuerung von zielgerichteten Armbewegungen beim Rhesusaffen (*Macaca mulatta*)“

2005: Beginn der Promotion, Interdisziplinäres Zentrum für klinische Forschung IZKF-Biomat., Medizinische Fakultät der RWTH-Aachen Universität

2008 September bis Dezember: Gastforscher an der Aalborg Universität, Dänemark, Medical Physiology & Experimental Pharmacology Group, Center for Sensory-Motor Interaction SMI, Department of Health Science and Technology, Medical Faculty

ab 2009 Juli: Junior Manager im Bereich International Clinical Quality Assurance, Pierrel Research Europe GmbH, Essen