Long-Term Depression of Nociception and Pain in Man

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PhD Thesis by

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II. Jung K, Larsen LE, Ellrich J. Heterotopic low-frequency stimulation induces nociceptive LTD within the same central receptive field in man.

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Aalborg, Denmark, February 3rd, 2010

Abbreviations

2PD	Two-point discrimination test
ACC	anterior cingulate cortex
AC-PC	anterior–posterior commissure
Al-TENS	Acupuncture-like TENS
ANOVA	analysis of variance
BA	Brodmann area
cRF	central receptive field
BOLD	Blood oxygen level dependent
DM	difference of means
DNIC	Diffuse Noxious Inhibitory Controls
EEG	electroencephalography
e.g.	exempli gratia, for example
EMG	electromyography
EPSP	excitatory postsynaptic potentials
ExpBack	Experiment with test and conditioning stimulation at the low back
ExpArm	Experiment with test and conditioning stimulation at the forearm
fMRI	functional magnetic resonance imaging
GABA	γ-Aminobutyric acid
HFS	high-frequency stimulation
I_0	detection threshold
IASP	International Association for the Study of Pain
IE	inner electrode
i.e.	id est, that is
INS	insula
I_P	pain threshold
IPL	inferior parietal lobe
I_S	stimulus intensity
ISI	interstimulus interval
LFS	low-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation

ME	master electrode
MFG	medial frontal gyrus
mRF	marginal receptive field
N1	first negative peak of SEP (EEG)
N2	second negative peak of SEP (EEG)
NMDA	N-methyl-D-aspartic acid
n. s.	not significant
OE	outer electrode
oRF	outlying receptive field
P2	second positive peak of SEP (EEG)
PDR	population dendritic response
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)
RF	receptive field
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
SES-A	affective subclass of SES
SES-S	sensory subclass of SES
STG	superior temporal lobule
TENS	transcutaneous electrical nerve stimulation
VRS	Verbal Rating Scale (0 - 100)
VRS-I	VRS of intensity
VRS-U	VRS of unpleasantness

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 months, 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 the Statens Institut for Folkesundhed 19% of the Danish population suffer from chronic pain and are afflicted by chronic pain. Chronic pain is not only the source of a significantly lowered quality of life but also inflicts great economy loss to the society. This thesis focuses on basic research investigating a specific form of electrostimulation, so-called low-frequency stimulation (LFS), as future alternative to pharmacological treatment of chronic pain.

1.1 Pain

Pain experience consists of various components with different extend depending on the kind of pain (Melzack et al. 1968), 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 responses 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 (psychomotor component), e.g. mimic and vocalization.

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.

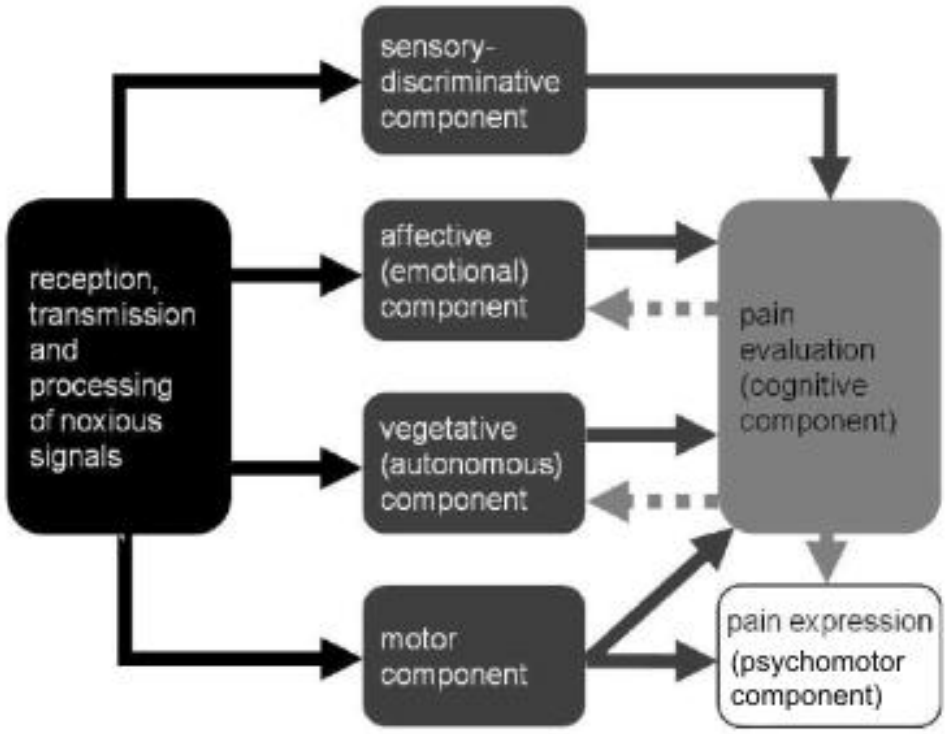

Figure 1.1. Schematic drawing of pain components activated by noxious signals.
Sensory, affective, vegetative and motor components result in pain evaluation and expression. Vice versa, the cognitive component has also influence on the affective and vegetative component (dashed arrows) (adapted from Schmidt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

1.2.1 Peripheral nociceptive system

Nociception begins in the periphery, where the peripheral terminals of sensory fibers (primary afferents) respond to myriad of stimuli and translate this information into the dorsal horn of the spinal cord, where the central ends of these fibers terminate (D'Mello et al. 2008). These 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. Medium-diameter myelinated Aδ fibers and small-diameter unmyelinated C fibers are activated by noxious stimuli (Julius et al. 2001). 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). Activation of nociceptors results in a graded receptor potential, creating noxious signals that reflect the stimulus intensity. Electrical stimulation directly excites afferent fibers. 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 also modulate noxious stimuli (Julius et al. 2001).

1.2.2 Central nociceptive system

The spinal cord is the first relay in the pain pathways from the periphery to the brain. The dorsal horn of the spinal cord is the major receiving zone for primary afferent axons that transmit information from sensory receptors in the skin, viscera, joints and muscle of the trunk and limbs to the nervous 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 (WDR) neurons also receive input from Aβ fibers, conducting non-noxious mechanical stimuli. Primary afferents release a variety of chemical mediators, but all appear to use glutamate as their principal neurotransmitter, and on entering the dorsal horn they form excitatory synapses with the secondary neurons (Todd et al. 2005). Excitatory, glutamatergic and inhibitory, GABA-ergic (γ-Aminobutyric acid) interneurons increase or decrease response of these neurons and thus influences the output of the dorsal horn (D'Mello et al. 2008).

On spinal and supraspinal level reflex actions are mediated. The nociceptive neurons project to interneurons that are integrated in motor 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 (Fig.1.2). According to recent knowledge, no exclusive nociceptive specific tracts or supraspinal pain centers with exclusive nociceptive neurons exist. The main ascending pathway is the spinothalamic tract projecting

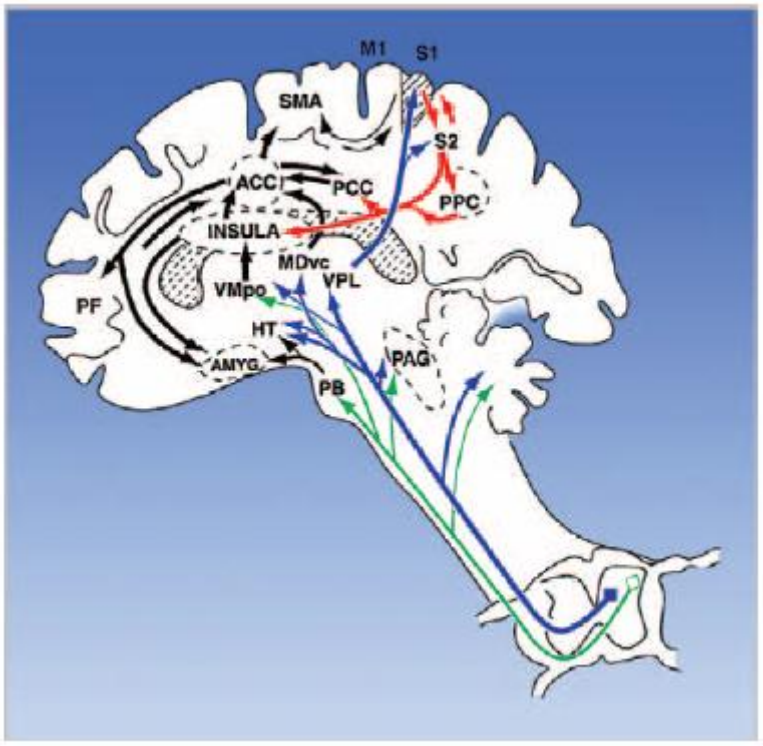

Figure 1.2. Ascending pathways, subcortical structures, and cerebral cortical structures involved in processing pain. PAG, periaqueductal grey; PB, parabrachial nucleus of the dorsolateral pons; Vmpo, ventromedial part of the posterior nuclear complex; MDvc, ventrocaudal part of the medial dorsal nucleus;VPL, ventroposterior lateral nucleus; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; HY, hypothalamus; S1 and S2, first and second somatosensory cortical areas; PPC, posterior parietal complex; SMA, supplementary motor area; AMYG, amygdala; PF, prefrontal cortex (Price 2002).

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 et al. 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 (ACC) 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 (PAG), leading to autonomic fear and defensive response (Price 2002).

Main regions involved in descending antinociceptive pathway are PAG and raphe nuclei. Direct stimulation of these regions causes analgesia. Inhibiting transmitters are noradrenaline, serotonin, GABA and opioids (Stamford 1995).

When proposing the "Gate control theory", Melzack and Wall (1965) suggested that inhibitory interneurons located in the superficial part of the dorsal horn played a crucial role in controlling incoming sensory information before it was transmitted to the brain through ascending pathways (Melzack et al. 1965). This theory thus explains how stimuli that only activate non-nociceptive nerves can inhibit pain. Pain seems to be lessened when the injured area is rubbed because activation of non-nociceptive fibers inhibits the firing of nociceptive fibers in the laminae. In transcutaneous electrical stimulation (TENS), this mechanism can be used where nociceptive fibers are selectively stimulated with electrodes and thereby decrease pain (Chesterton et al. 2002).

1.3 Synaptic plasticity

1.3.1 Cellular mechanisms of LTP and LTD

The model of bidirectional synaptic plasticity includes long-term potentiation (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 (Bliss et al. 1970;Dudek et al. 1992). 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 et al. 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 et al. 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 (Xia et al. 2005). Postsynaptically activated nitric

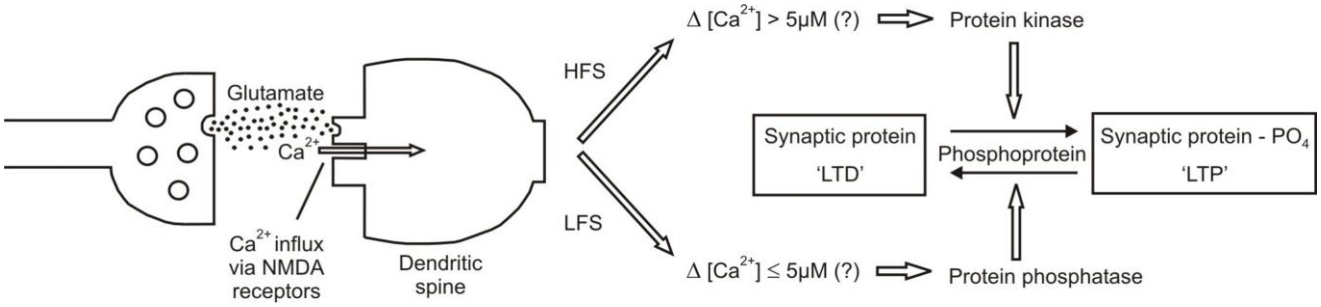

Figure 1.3.1. Model for the induction of LTP and LTD.

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 et al. 1994).

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 inhibitor 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 result in calcium elevation, which is hypothesized to lead to a reduction in presynaptic transmitter release (Braunewell et al. 2001).

1.3.2 Modulation of synaptic transmission: LTP and LTD in the 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. 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 a sustained decrease of excitatory postsynaptic potential (EPSP) amplitude for at least three hours after noxious LFS of the 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. LFS with lower intensity, mainly activating Aβ fibers, only induced transient depression of synaptic transmission for less than 30 minutes (Sandkuhler et al. 1997).

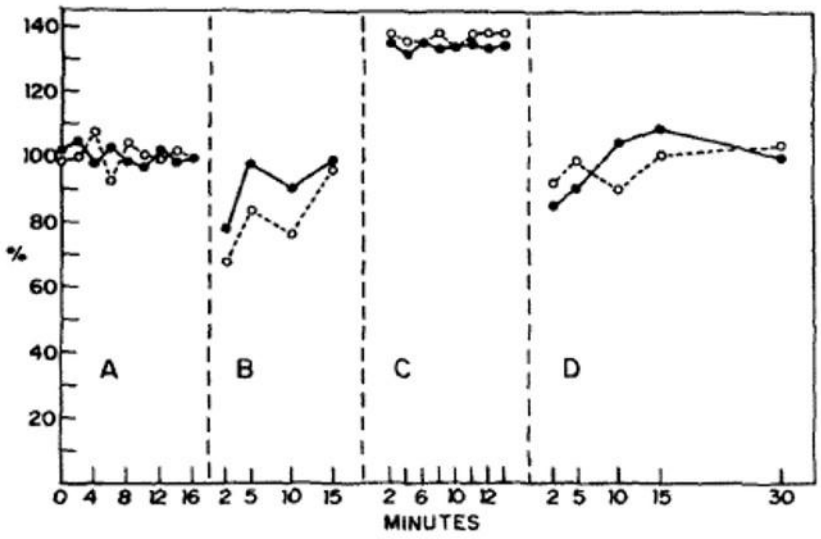

Figure 1.3.2. Effects of LFS and HFS on the slope (open circles) and amplitude (filled circles) of the population dendritic response (PDR) evoked by stimulation of the SCP in one rat.
The experiment consists of four successive periods: (A) control, (B) sample PDRs following the first LFS, (C) Sample PDRs following HFS, (b) sample PDRs following second LFS. Each point represents an average of four responses expressed as a percentage of the mean value for all control (i.e. pro-repetitive stimulation) responses (Barrionuevo et al. 1980).

Noxious LFS of tongue musculature evoked LTD of craniofacial processing in mice (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 2000;Ikeda et al. 2003;Ji et al. 2003).

Furthermore, LFS to Aδ fibers could reverse HFS induced LTP (depotentiation) (Fig.1.3.2) 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 Central sensitization

Central sensitization refers to the increased synaptic efficacy established in somatosensory neurons in the dorsal horn of the spinal cord following intense peripheral noxious stimuli, tissue injury or nerve damage. This heightened synaptic transmission leads to a reduction in pain threshold, an amplification of pain

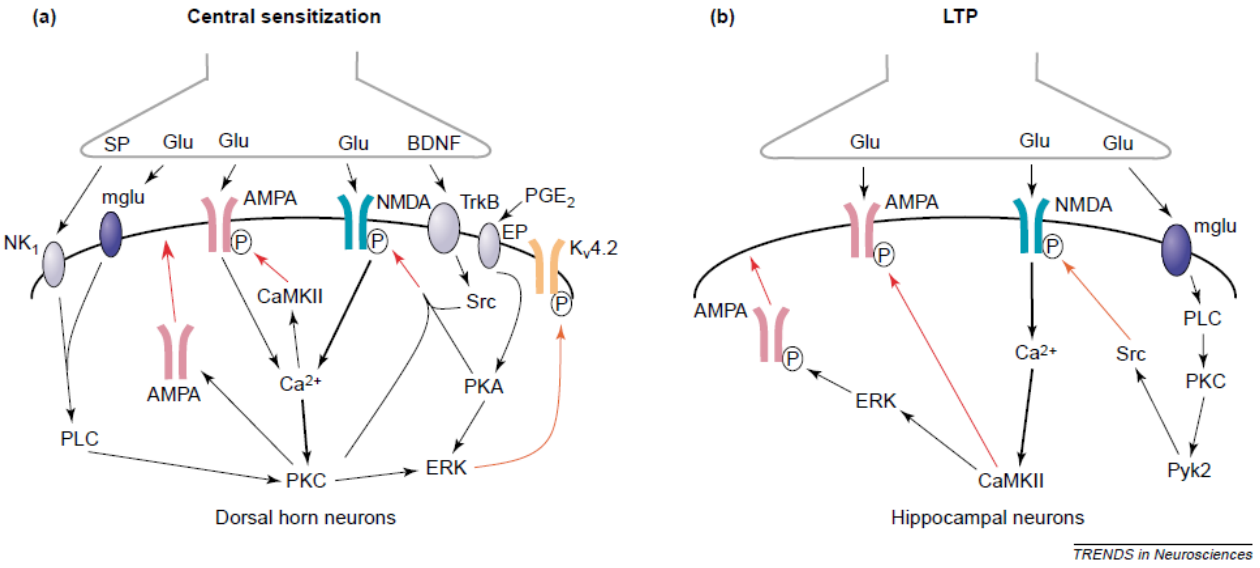

Fig. 1.3.3. Induction of central sensitization and early-phase LTP (Ji et al. 2003)

responses and a spread of pain sensitivity to non-injured areas. Modulation involves activation of intracellular signaling cascades leading to facilitated excitatory synaptic responses and depressed inhibition, thereby amplifying responses to noxious and innocuous inputs. The changes may be restricted to the activated synapse (homosynaptic) or spread to adjacent synapses (heterosynaptic).

Most excitatory input to pain pathway neurons is subthreshold, and increased gain results in recruitment of these inputs to the output of the neurons, causing them to fire to normally ineffective inputs (Woolf et al. 1990). These changes constitute central sensitization and are responsible for pain produced by low-threshold afferent inputs and the spread of hypersensitivity to regions beyond injured tissue (Kilo et al. 1994;Ali et al. 1996). Analysis of the molecular mechanisms underlying the generation and maintenance of central sensitization and LTP indicates that both mechanisms share distinct similarities (Fig.1.3.3). Central sensitization involves activation of ligand-gated ion channels (NMDA, AMPA and/or kainate receptors), G-protein coupled metabotropic receptors, the substance-P receptor neurokinin-1 (NK1) and metabotropic glutamate (mglu) receptors, and tyrosine kinase receptors (trkB and Eph). Two major mechanisms appear to contribute to the resultant increased synaptic efficacy: alterations in ion channel and/or receptor activity owing to posttranslational processing, and trafficking of receptors to the membrane. Activation of several protein kinases leads to the phosphorylation of ionotropic glutamate receptors, increasing synaptic efficacy by altering channel open-time, increasing bursting, removing the Mg^{2+} channel blockade, and promoting trafficking of receptors to the synaptic membrane.

1.3.4 LTD in humans

Only a few studies deal with LTD of nociception and pain in humans. Most of them examine the trigeminal nociceptive system (Ellrich 2006). LFS was applied to trigeminal afferents and LTD effect was controlled by evocation of masseter inhibitory reflex (Ellrich et al. 2002) and blink reflex (Yekta et al. 2006;Aymanns et al. 2009), recording of somatosensory evoked cortical potentials (SEPs) (Ellrich et al. 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.

So far, LFS setting was mostly adopted from experiments performed in rodents under in vitro conditions. Animal experimental studies under in vitro conditions suggest LFS with at least 900 pulses and a frequency of 1 Hz in order to induce stable LTD of synaptic strength. Hence, information regarding optimum stimulation parameters for spinal LTD in man is still missing but represents a prerequisite in order to induce maximum LTD effect.

1.3.5 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 et al. 1992;Mulkey et al. 1992;Kerr et al. 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 et al. 2000). These in vitro studies suggest a sole homosynaptic LTD. Recent studies in human volunteers support the assumption of homotopic organization. Application of LFS to right hand dorsum solely depressed rating on right hand but had no effect on left hand rating. Stimulation of radial side of right hand dorsum exclusively decreased perception on radial but not on ulnar side of the same hand (Rottmann et al. 2008). Experiments performed bilaterally on the forehead showed a decrease of pain perception solely after ipsilateral but not after contralateral LFS (Yekta et al. 2006). Furthermore trigeminal pain perception was solely inhibited by homotopic LFS after unilateral application of LFS to all three sensory branches of the trigeminal nerve (Aymanns et al. 2009).

1.3.6 Sensory and affective components in LTD

A previous human study demonstrated a sustained LTD of sensory and affective components of pain induced by LFS (Rottmann et al. 2010a). Pain ratings concerning test stimulation and LFS were obtained by multidimensional assessment including Verbal rating scale of perceived stimulus intensity (VRS-I) and unpleasantness (VRS-U) and pain perception scale with sensory (SES-S) and affective items (SES-A). Application of 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 was shown to correlate with the affective component of pain (Rainville et al. 1999). The quality of pain revealed by the SES questionnaire gave 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). 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 after LTD induction.

1.3.7 Central mechanisms involved in LTD

LTD of pain-related cerebral activation was recently demonstrated in a functional magnetic resonance study (fMRI) study (Rottmann et al. 2010b). A significant activity decrease after LFS application was found in bilateral S1 and S2, ACC, ipsilateral insula, inferior parietal lobe (IPL), and superior temporal gyrus (STG). A positive correlation between pain relief and increased brain activation after LFS indicated an involvement of endogenous pain control mechanisms in LTD. This insight is a big step forward in clarifying involved cerebral mechanisms. However, as fMRI has excellent spatial but relatively low temporal resolution, it can hardly address the involvement of different temporal stages in LTD processing. As fMRI is based on blood flow changes, it does not reflect the electrical activity in neurons. Therefore, it is not the most suitable method for addressing the question of when during painful stimulation the different brain areas become active and hence, in what processing step each area is involved. In order to determine the electrical activity of the brain, dipole source analysis of evoked potentials recorded by electroencephalogram (EEG) were examined in study III.

1.4 Aim of the present thesis

Due to the putative role of LTD in chronic pain therapy it is essential to obtain further extensive information about possible mechanisms involved in LTD induction. Thus, the aim of the present thesis was a further, more detailed investigation of LTD of spinal nociception and pain in healthy humans. In order to evaluate these LTD mechanisms cutaneous Aδ fibers of the hand dorsum were electrically stimulated and three different aspects of LFS-induced LTD were examined.

1) The optimum stimulation paradigm for inducing LTD in human nociception and pain was investigated as it was suggested from in-vitro experiments. Cortical potentials and pain perception before and after LFS were assessed. (Study I)
2) Spatial organization of LTD in human pain perception was examined as it was suggested from in-vitro experiments. Pain perception before and after LFS application was examined in a conditioned and a non-conditioned pathway. (Study II)
3) LFS effects on cerebral activation pattern obtained via multi-channel EEG, and pain perception ratings before and after LFS were compared. (Study III)

Clarifying possible mechanisms that contribute to LTD induction will be a further step to elucidate the precise mechanisms involved in LTD induction for qualifying this approach for neuromodulatory treatment of pain.

Parts of this study were presented at conferences (Jung et al. 2009;Jung et al. 2010;Larsen et al. 2010).

2 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. This chapter provides a discussion of the applied methods.

2.1 Electrical stimulation

2.1.1 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 (Fig.2.1) which reaches superficial skin layer, where nociceptive free nerve endings are located (Novotny et al. 1988). Experiments on human blink reflex provide further evidence for preferential 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 et al. 2006;Peddireddy et al. 2006;Di Clemente et al. 2007). Furthermore, SEPs 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 et al. 1984b). Another reliable pain model is the intracutaneous stimulus.

Concentric electrode

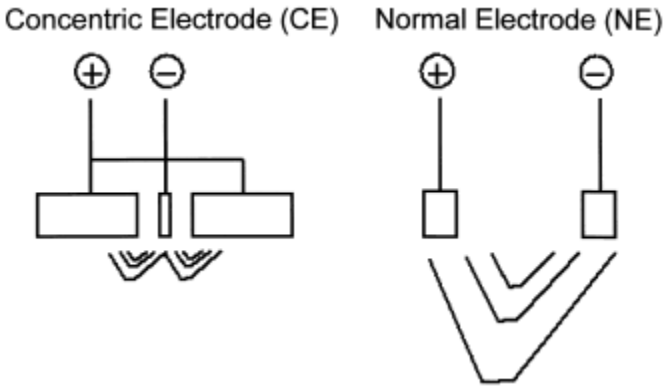

Figure 2.1: New concentric electrode (CE) and the normal electrode (NE).
Electric fields are intimated (Kaube et al. 2000).

It induces pain sensation and cerebral potentials very similar to the concentric electrode (Bromm et al. 1984a). Both pain models result in low pain thresholds (I_P) 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). A recent human study revealed evidence for preferential Aδ fiber stimulation (Rottmann et al. 2010a). Psychophysical data revealed 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.

2.1.2 Multiarray electrode

A special designed multiarray electrode was developed in order to examine the LTD effect on different receptive field (RF) areas. The advantageous design of the concentric electrode characterized by a large anode with a small central cathode was adopted. This development led to a multiarray electrode consisting of 6 rows with 4 pin electrodes each, resulting in 24 pin electrodes. A large surface electrode that was placed on the outer forearm opposed to the multiarray electrode served as anode. In order to operate all 24 pin electrodes separately each row was energized by a particular stimulator that powered 4 pin electrodes. This

new electrode design and the RF distribution on the forearm allowed a distinct stimulation of nociceptive afferents located in the central, marginal or outlying RF area.

2.2 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 et al. 2002;Ellrich et al. 2004;Yekta et al. 2006;Ellrich 2006) and spinal nociceptive system (Klein et al. 2004). Decrease in VRS rating was coupled with decrease in brainstem 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 et al. 1968).

Therefore a previously performed study investigated the impact of noxious LFS on the sensory and affective aspects of pain perception by multidimensional rating scales (Rottmann et al. 2010a). Volunteers were asked to rate electrical 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 (Tuerp et al. 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). This questionnaire gives not only ratings for 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. In addition to the superficial sharp and heat pain and the deep rhythmic pain, another subclass describing deep constant pain was revealed. The results 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.

2.3 Two-point discrimination test (2PD)

2PD is a well-established property of the sensory systems in order to discriminate and separate mechanical and noxious inputs (Dellon et al. 1987). It represents the ability to identify separate regions of perception evoked from simultaneously stimulating two discrete regions of the body. This test measures the minimum distance (two-point threshold) at which two stimuli are perceived as separate. This threshold varies for different body regions and describes the spatial resolution of a specific skin region (Weinstein 1968).

According to the sensory homunculus developed by Penfield and Jasper (Penfield et al. 1954), the forearm and low back were chosen for this study as RFs in these areas are relatively large adding up to 40 mm (Weinstein 1968). Thus, these large RFs allow a fixation of electrodes apart from each other within and outside the same RF. A commercially available compass-type instrument, commonly used in clinical investigation and specifically designed for this purpose, was used in the assessment of 2PD in the low-back and forearm region. Testing was performed according to a standardized protocol as previous studies emphasized the importance of detailed description of test performance, with special reference to the pressure applied and the testing protocol (Lundborg et al. 2004).

Previous human studies investigating spatial organization of LTD examined the hand and face region. Due to the small size of RFs on the hand dorsum and face region, respectively (Rottmann et al. 2008;Aymanns et al. 2009) it was hardly possible to selectively stimulate skin area that is innervated by terminals of only one primary sensory neuron. When multiple primary sensory neurons converge on a single secondary sensory neuron, their individual RFs merge into a single, large secondary RF, referred to as central RF. Defining central RFs via 2PD illustrates a possibility to converge animal and human experimental setups in order to gain more insight into spatial mechanisms of LTD in man. Application of 2PD represents an important tool for bridging results obtained from animal experiments with human studies.

2.4 Somatosensory evoked potentials (SEP)

Recording of SEP amplitude via EEG is a valid method to investigate nociceptive processing. SEPs are reproducible and constant on different days (Bromm et al. 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 et al. 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 et al. 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.

2.5 Dipole source modeling

Understanding of the cortical components of cerebral pain processing has been advanced by the use of modern brain imaging techniques (Iannetti et al. 2005). Such studies have shown that pain experience involves a widely distributed network of activity across the S1 and S2, insular cortex, ACC and prefrontal cortices (Apkarian et al. 2005;Tracey et al. 2007).

During the last years, methods based on blood flow changes, like fMRI, gained in importance as they present an efficient tool for visualizing activated brain areas after painful stimulation. However, activation and deactivation of multiple areas in the brain takes place shortly after stimulation, and many of these processes are common for other sensory events not related specifically to pain. Such complexity causes major difficulties in the interpretation of evoked pain response when slowly changing alterations in blood flow are used for analyzing (Davis 2003). In contrast to these methods, EEG-based experiments detect the neuronal activity with a very high temporal resolution (Valeriani et al. 2001). One study of this thesis takes advantage of the high temporal resolution of EEG recordings in order to allow a determination of different temporal pain components that are involved in LTD.

A 64-channel model was chosen for this study as this number of electrodes was previously shown to result in reliable EEG source imaging. A simulation study investigated the influence of channel numbers on source estimation using nine different electrode configurations between 25 and 181 channels (Lantz et al. 2003). The influence of the number of electrodes on source localization precision was shown to be not linear. The precision increased from 25 to around 100 electrodes and then reached a plateau. An almost perfect localization was already achieved with 64 electrodes, supporting the chosen electrode set-up in this study.

3 Discussion of study I-III

Collected data of this thesis indicated a prolonged decrease of nociception and pain after LFS for at least one hour in healthy volunteers. Present results imply an enhancement of LTD effects in man by optimizing stimulation parameters. LFS application induced a heterotopic Aδ fiber mediated LTD within the same RF, that was expressed by a decreased pain perception rating. Reduction of nociceptive processing might be due to peripheral effects on the first nociceptive synapse as it is suggested from in vitro studies. However, a performed dipole source analysis showed a decreased activity for the affective pain component and demonstrated a posterior shift of the ACC dipole generator after LTD induction.

Study I

This study investigated the optimum stimulation paradigm for inducing LTD and demonstrated a stimulation protocol with 1 Hz, 1200 pulses and 4-fold pain threshold (I_P) as most effective for inducing strong LTD effect at the spinal level in man. It was also shown that established LTD after single LFS could be amplified by an additional second LFS.

LFS with 0.5, 1, and 2 Hz induced significant reduction of SEP and pain ratings as compared to Control group. Obtained results demonstrated that the effect on SEP amplitude after 1 Hz LFS preponderated that of 2 Hz stimulation. These results are consistent with previous work investigating the frequency optimum in animal experiments (Dudek et al. 1992;Nakano et al. 2004) showing that 1 Hz stimulation induced the strongest LTD effect (Study I, Fig.3). There is evidence for a shift from LTD to LTP induction above a certain frequency threshold in rodents leading to a frequency-response curve. These reports showed almost no consistent effect on baseline responses with a stimulation frequency about 3 Hz whereas 10 Hz resulted in a potentiation of synaptic transmission (Wang et al. 1999). However, the idea of applying frequencies above 2 Hz in order to obtain a frequency-response curve with a greater spectrum could not be realized as higher frequency stimulations under identical experimental conditions were not acceptable to participants due to strong pain. Investigating the influence of applied number of pulses showed an SEP suppression augmentation with increasing number of pulses revealing 1200 pulses as the most effective parameter (Study I, Fig.4). Thus, a minimum number of pulses might be necessary to induce stable 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 et al. 1993;Manahan-Vaughan 2000). A key aspect for LTD induction seems to be the intracellular Ca^{2+} concentration, as LTD can be blocked by applying Ca antagonists (Sandkuhler et al. 1997). An explanation

for the necessity of this long-lasting stimulation with at least 900 pulses to induce LTD in rodents seems to be the activation time of Calcineurin. 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 et al. 2005). LFS with intensities 2-fold I_P and 4-fold I_P evoked sustained depression of SEP and pain perception in comparison to Control and 1-fold I_P LFS (Study I, Fig.5). The results illustrated that the concentric electrode allows sustained depression of nociceptive transmission already with low stimulus intensities as there was no significant difference between 2-fold I_P and 4-fold I_P LFS. The special design of this electrode produces a higher current field density that is responsible for depolarizing nociceptive fibers in superficial skin layers at lower intensities than conventional electrodes (Kaube et al. 2000). A further decrease of amplitude and pain perception was shown by applying an additional LFS clearly separated from preceding conditioning stimulation (Study I, Fig.6). This amplification is in keeping with previous animal studies (Dudek et al. 1992;Sandkuhler et al. 1997) showing this enhancement of LTD after two separate LFS in rats. Consequently, a stimulation paradigm (1 Hz, 1200 pulses and 4-fold I_P) with repetitive episodes of LFS is assumed as optimal in order to achieve the strongest analgesic effect.

Study II

The second study of this thesis examined the spatial organization of LTD. In order to allow more precise conclusions about the size of skin area that is involved in LTD, two different designs of electrodes were applied on the low back area and the forearm. A sustained pain reduction after LFS application to different RFs on the forearm and low back was observed. The idea of focusing on the specific involvement of different RF areas in human LTD induction was the unique feature of this study. For the first time it was shown that the most efficient LTD effect was obtained in the central and marginal area of the RF compared to the less affected outlying area.

The study was divided into two parts defined as ExpBack and ExpArm according to the site of electrical stimulation (Study II, Fig.3+4). In ExpBack, pain perception was exclusively inhibited after LFS application within the same RF but did not change after LFS application to a distinct dermatome (Study II, Fig.5). These results are congruent with data from in vitro studies that showed pure homotopic LTD of the electrically conditioned pathway in various brain regions including hippocampus (Dudek et al. 1992;Mulkey et al. 1992;Kerr et al. 1995), visual cortex (Kirkwood et al. 1993), and amygdale (Wang et al. 1999). Literature suggests that LTD is input specific and confined to the stimulated synapses. Electrical LFS of one afferent input to a postsynaptic neuron selectively evoked LTD of the same synapse (Chen et al. 2000). However,

differences in the experimental setup between the present study and the described in vitro literature need to be discussed. In animal studies LFS with Aδ fiber intensity was applied to primary afferent fibers of the dorsal root. Intracellular recordings from rat dorsal horn neurons showed homosynaptic LTD at the synapse between Aδ fibers and second order neurons in the superficial spinal dorsal root (Chen et al. 2000). Even though the results from this study are consistent with that data, restrictions of experimental setups in human experiments should be taken into consideration. The described in vitro experiments focused on effects on the first nociceptive synapse whereas this study provides information about involved RFs of higher-order neurons. Many axons from primary neurons converge onto a single second-order sensory neuron in the dorsal horn. This information is conveyed via third order neurons to the cerebral cortex where sensory perception occurs. Consequently, RFs of second and higher-order sensory neurons are larger and more complex than those of receptor neurons as they receive convergent input from many hundreds of receptors, each with a slightly different but overlapping primary RF. Thus, present data gave rather evidence about central RF involvement in LTD induction than information about possible homosynaptic mechanisms.

Results of ExpBack are in agreement with recently published studies in human volunteers investigating spatial organization of LTD. Application of LFS to right hand dorsum solely depressed rating on right hand but had no effect on left hand rating. Stimulation of radial side of right hand dorsum exclusively decreased perception on radial but not on ulnar side of the same hand (Rottmann et al. 2008).

The presented observations from ExpArm further supported the hypothesis that LTD is restricted to the RF where LFS is applied. Data indicate that noxious LFS is able to induce LTD not only at the exact stimulation site but also within a certain area of the same central RF (Study II, Fig.6). The applied optimized multiarray electrode design showed the strongest LTD effect in the marginal and central RF area compared to the outlying region. These findings play an important role when introducing this electrostimulation for clinical use. By developing an electrode array that is able to stimulate a wide skin area, this non-pharmacological treatment can induce a broad analgesic effect corresponding to the dimension of the involved central RFs.

Study III

Information regarding central mechanisms involved in LTD induction and maintenance are still rare. Therefore, the third study of this thesis investigated changes in activation pattern of pain-related brain areas after LFS application. SEPs were recorded with 64-channels that allowed a dipole source modeling in order to obtain information about involved brain generators. In addition to a strong reduction of pain perception (Study III, Fig.2), dipole modeling indicated a significant decrease and a posterior shift of the source

generator in the ACC after LFS application (Study III, Fig.5, Table 1). No changes were observed under Control condition. These results indicate possible neuroplastic changes within the involved pain pathways evoked by LTD induction.

A recently performed fMRI study (Rottmann et al. 2010b) demonstrated a positive correlation between pain relief and increased brain activation after LFS, indicating an involvement of endogenous pain control mechanisms in LTD. Thus, described fMRI results supported the hypothesis on central mechanism involvement in LTD induction. Averaged EEG sweeps showed reproducible positive and negative deflections for both experimental conditions. The identified components were consistent with previous studies on pain responses. Two components were defined as pain-relevant (Bromm et al. 1982a) since they correlated significantly and exclusively with the subjects' reported pain and could be specifically attenuated by analgesic drugs (Scharein et al. 1998). These two pain-relevant components are part of the late N2-P2 complex. Consequently, differences of N2-P2 amplitude can serve as an objective estimate for the induced degree of experimental pain.

A significant reduction of N2 and P2 amplitude after LFS application was accompanied by a strong decrease of pain perception in this study. Thus, a decrease of pain rating as a subjective measurement could be confirmed by an objective vertex potential analysis.

Dipole source analysis revealed a reproducible generator for N1 that was localized in the contralateral S2 area for Pre LFS and Pre Control. Dipole parameter like position and magnitude for N1 did not change during LFS and Control experiment. Recording of N1 component over the scalp has handy implications in both clinical and experimental practices, since the N1 was shown to reflect relatively early cortical processing which is less susceptible to cognitive modulation than the subsequent vertex potentials (Legrain et al. 2002;Garcia-Larrea et al. 2003). The observed stable N1 dipole magnitude and position after LTD induction indicated constant nociceptive transmission reaching cortical areas that did not change even though pain perception decreased. This finding provides an indication that the measured pain perception was likely coupled to the affective pain component whereas the sensory part played a secondary role.

Source modeling of the location of generators of scalp SEPs revealed that the best fit was obtained by including a deep anterior midline source corresponding to the cingulate gyrus. Several lines of evidence support a role of the ACC in pain processing (Vogt et al. 1993). A decrease of dipole magnitude was exclusively found in the P2 component which represents the affective pathway generated in the cingulate cortex. The sensory component (N1) did not show a reduced activity in the somatosensory region after LFS.

This led to the suggestion that LTD was primarily caused by a strong reduction of the affective pain component whereas the sensory pathway was less affected.

The findings of P2 magnitude and location changes indicate that pain reduction after LFS led to changes in cortical projections of the nociceptive system. Studies investigating spatiotemporal aspects of brain activity in chronic tension-type headache (TTH) patients support this assumption (Buchgreitz et al. 2008). The authors showed a reduction of P200 dipole strength only in healthy controls after electrical stimulation whereas no difference was found in TTH patients. These results give evidence for a possible abnormal supraspinal response that may be explained by impaired inhibition of nociceptive input in chronic pain patients. The demonstrated reduction of N2 and P2 amplitude and magnitude, and the posterior shift of P2 may be related to central neuroplastic changes within the involved pain pathways evoked by LTD induction. However, whether this relates to peripheral mechanisms, caused by the recruitment of a lower number of nociceptive fibers, or by central changes, such as hypoexcitability, or activation of endogenous inhibitory control mechanisms, cannot be determined from the SEP characteristics alone.

3.1 Supraspinal mechanisms of LTD

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 et al. 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 et al. 2001).

A previous study from our group provided evidence for supraspinal effects of LFS (Rottmann et al. 2010b). 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 was shown to decrease 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 of the described fMRI study led to the suggestion that LFS affected the endogenous descending system resulting in a stronger depression of pain.

3.2 Inhibiting mechanisms apart from LTD

A decrease of pain perception and SEP amplitude could also be observed under Control condition. However, the reduction in pain and SEPs was significantly stronger after LFS application compared to Control experiment. Furthermore, it seems important to point out that SEP amplitude and pain rating were significantly reduced directly after LFS application, as measured in the first Post series. In contrast to this, SEP amplitude and pain perception in Control experiment remained unchanged in the first Post series compared to the last Pre series. Under Control condition, a consistent decline of these two parameters was observed after the first Post series. However, there was a significant stronger decrease after LFS after one hour, as determined in the last Post series compared to Control.

The decline of SEP after no stimulation 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 (DNIC) can be excluded as this phenomenon was shown to affect also the unconditioned heterotopic side (Lebars et al. 1979). In contrast, the second study clearly provided evidence for a LTD effect strictly related to the stimulated area skin. Therefore, it seems very unlikely that the depression of pain and nociception that was observed during these

studies was caused by DNIC. Taken together, there is strong evidence that LFS induces LTD of nociception and pain.

3.3 Clinical advantage

Repetitive HFS of primary afferents induces LTP in Aδ (Randic et al. 1993) and in C fiber synapses (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 et al. 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 depotentiate established LTP, induce de-novo LTD and prevent further LTP, it might be used as a neuromodulatory treatment of pain. Therefore it is of great interest to investigate the mechanisms of LFS-induced LTD in humans.

3.4 Comparison with transcutaneous electrical 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) is applied with 4 Hz and with “to tolerance” intensity whereas “conventional TENS” is performed with 110 Hz and “strong but comfortable” stimulation intensity (Chesterton et al. 2002). Al-TENS intensity “to tolerance” is defined as very strong and uncomfortable. Even though it can be assumed that Al-TENS induces painful sensations, the intensity of Al-TENS cannot 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 Aβ fibers and the hypoalgesic effect is probably mediated by inhibitory GABAergic interneurons as proposed within the gate control theory (Melzack et al. 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 LTD of nociception and pain for at least one hour and therefore matching the criteria of LTD (Braunewell et al. 2001).

4 Conclusion and perspectives

Presented studies yielded novel information about the optimum stimulation protocol, spatial organization and possible central mechanisms involved in LTD induction and maintenance. An amplification of LTD effect was obtained by choosing an optimum stimulation paradigm and developing an advanced multi array electrode design. The possible involvement of central mechanisms in LTD induction provides further insight into possible supraspinal mechanisms responsible for the analgesic effect after LFS application.

Due to the ability of electrical LFS to reverse potentiated synaptic transmission back to baseline conditions in hippocampus, application of LFS and LTD in chronic pain might be a mechanism-based treatment in order to attenuate or even erase pain memory. This thesis focused on clarifying basic mechanisms and characteristics of LTD as a future alternative to pharmacological treatment of chronic pain in healthy human volunteers. However, detailed knowledge about LTD in humans is essential for introducing LFS as an approach for future chronic pain treatment. Hence, this work may be a step forward to future therapy of chronic pain. Nevertheless, more experiments are necessary to examine the precise mechanisms of LTD and to develop an optimal treatment protocol.

5 Danish summary

Synaptisk plasticitet, inklusiv long-term potentiation (LTP) og long-term depression (LTD), repræsenterer en cellulær model af indlæring og hukommelse. LTP, en længerevarende forøgelse af synaptisk styrke, kan induceres med højfrekvent elektrisk stimulation. Lavfrekvent stimulation (LFS) fører til en reducering af synaptisk transmission, hvilket refereres til som LTD. Synaptisk plasticitet er blevet påvist i smertesystemet. LTP er blevet indikeret som værende involveret i central sensibilisering af smerte, som fører til en såkaldt smertehukommelse. Eftersom LFS er i stand til at reversere LTP, ville det være nyttigt at svække eller måske slette denne smertehukommelse. Derfor ville det være af stor interesse at undersøge LFS-induceret LTD i mennesker med henblik på brug i fremtidig behandling af kroniske smerter. Indtil videre har de fleste studier været udført på dyr, hvor resultaterne har vist vedvarende LTD efter LFS af afferente spinale nervebaner. De få studier, der er udført på mennesker har undersøgt, hvordan LFS påvirker den trigeminale refleks, evokerede potentialer og generel smerteperception.

Denne afhandling indeholder en detaljeret undersøgelse af LTD i den spinale nociceptive processering hos raske mennesker. Afhandlingen er inddelt i tre dele: (1) Optimering af elektriske stimuleringsparametre; (2) Spatial organisering af LFS; (3) Neuroplastiske forandringer som følge af LTD induktion.

I alle tre dele blev nociptive Aδ fibre elektrisk stimuleret med et koncentrisk elektrode design. Smertefulde testserier blev påført før (Pre) og efter (Post) konditioneret LFS (1 Hz, 20 min) på håndryggen, forarmen eller den nedre rygregion. I kontroleksperimenter med de samme forsøgspersoner blev LFS ikke påført. Effekten af LFS blev undersøgt med hensyn til elektrofysiologisk, psykofysisk og hjernebilleddannelse teknikker.

Formålet med den første del af afhandlingen var, at optimisere stimuleringsprotokollen til at inducere LTD i mennesker. Derfor blev forskellige parametre som frekvens, antal stimuleringer og stimuleringsintensitet undersøgt. LFS blev påført på den højre håndryg. Somatosensoriske evokerede kortikale potentialer blev optaget og forsøgspersonerne vurderede stimuleringsintensiteten. Dette studie demonstrerede et stimuleringsparadigme med 1 Hz, 1200 stimuleringer og 4-gange smertetærsklen, som det mest effektive til at inducere en stærk LTD effekt på spinalt niveau. Studiet viste også, at når en LTD effekt var opnået med et enkelt LFS stimuleringsparadigme, kunne denne effekt forstærkes med et ekstra LFS stimuleringsparadigme.

Den anden del af afhandlingen beskæftigede sig med at undersøge den spatiale organisering af LTD. For at være i stand til at lave mere præcise konklusioner om det hudområde, der er involveret i LTD, blev to forskellige elektrodedesign anvendt på den nedre rygregion og forarmen. Størrelsen af receptive områder (RF) blev registreret og elektroder påsat i den centrale, marginale og et fjerntliggende område af RF. Smerte perception blev kun reduceret efter LFS påførelse indenfor det samme RF og forblev upåvirket når LFS blev

påført et fjerntliggende RF på den nedre rygregion. Det blev påvist, at den mest effektive LTD effekt blev opnået i den centrale og det marginale område af RF og det fjerntliggende område var mindre påvirket. Disse resultater indikerer, at en heterotopisk smertereduktion indenfor det samme RF eksisterer, som også understøttes af tidligere fund i dyr og mennesker.

Information omkring centrale mekanismer, der er involveret i LTD induktion og vedligeholdelse er stadig sparsom. Derfor undersøgte den tredje del af denne afhandling forandringer i smerterelaterede hjerneområder efter påførelse af LFS. Somatosensoriske evokerede kortikale potentialer blev optaget med 64-kanal encefalografi, som muliggør en dipole source modellering, der giver informationer om involverede hjernegeneratorer. Ud over en stærk reducering i smerteperception, indikerede dipole modellering en signifikant reduktion og en flytning af hjernegeneratoren i anterior cingulate cortex til en mere posterior position efter LFS påførelse. Disse resultater indikerer mulige neuroplastiske forandringer indenfor den involverede smertenervebane, der evokeres af LTD påførelse.

De præsenterede studier indeholder ny information omkring den optimale stimuleringsprotokol, spatial organisering og mulige centrale mekanismer, der er involverede i LTD induktion og vedligeholdelse.

Denne afhandling var et vigtigt skridt imod forståelsen af LTD i mennesker, da detaljeret viden om LTD er en forudsætning for at benytte LFS som en fremtidig analgesisk terapimulighed i kroniske smertepatienter.

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