DYNAMICS OF DESCENDING INHIBITION AND NEUROENDOCRINE ANALGESIA

WITH SPECIAL REFERENCE TO THE TRIGEMINAL REGION

Anna Feldreich

Stockholm 2011
Pain is essential for survival, pain may also ruin years of life,

I dedicate this thesis to the patients.
ABSTRACT

In patients with pain, it is of relevance to use clinical assessments that evaluate the pain modulation. In this thesis, the dynamics of descending inhibition or central sensitization and neuroendocrine analgesia were investigated: in healthy volunteers; in patients with chronic closed lock of the temporomandibular joint (TMJ), before and after discectomy; and finally in patients with possible neuropathic pain, (atypical odontalgia). Pain assessment was made with the eleven point numerical rating scale. The pain thresholds were determined to evaluate central sensitization, and cold pressor tests of 2-4 °C were used to provoke descending noxious inhibitory controls. Plasma β-endorphin was determined by radioimmunoassay. The focus of evaluation was on the change induced by provocation or pain relieving surgery.

In study I, we found, that with an exception for the electrical pain threshold over the central maxillary incisor, healthy volunteers increased their electrical and pressure pain thresholds during cold pressor test. There were region and stimuli specific changes between the trigeminal and the spinal region, regarding the change in pain thresholds, but no differences in respect to gender. In study II, we demonstrated that female patients, with chronic closed lock, i.e. limited jaw function and movement-evoked pain from the TMJ, had central sensitization and an elevated neuroendocrine opioid level in plasma. Study III, showed, that in 91% of female patients with chronic closed lock, the movement-evoked pain had disappeared at a median (range) of 8 (6-24) months after TMJ-discectomy. In particular, a clinically substantial reduction in pain intensity was required for a decline in plasma β-endorphin. Central sensitization showed signs to decrease in relation to relief in pain intensity. However, on the whole, central sensitization was not healed. In study IV, we noted that patients with atypical odontalgia had HPA-axis hyperactivity and altered coping together with deficient neuroendocrine opioid release and descending facilitation in the maxillary branch of the trigeminal nerve during cold pressor test.

In conclusion, the outcome measure of pain relieving surgery is feasibly a clinically substantial improvement in maximal pain intensity, here confirmed by a recovery in plasma β-endorphin. Afference from the pulp was absent from descending inhibition in healthy, and possibly promoted by descending facilitation in atypical odontalgia, indicating that the dentoalveolar region is vulnerable to development of chronic pain.
LIST OF PUBLICATIONS

I. Rosén A., Feldreich A., Dabarian N., Ernberg M.
Effect of heterotopic noxious conditioning stimulation on electrical and pressure pain thresholds in two different anatomical regions.
Acta Odontologica Scandinavica, 2008;66:181-188

II. Feldreich A., Ernberg M., Lund B., Rosén A.
Increased β-endorphin levels and generalized decreased pain thresholds in patients with limited jaw opening and movement-evoked pain from the temporomandibular joint.
Journal of Oral and Maxillofacial Surgery, In Press; accepted for publication 2011-09-16.

III. Feldreich A., Ernberg M, Rosén A.
A decrease in plasma β-endorphin marks a clinically substantial decrease in pain intensity after temporomandibular joint discectomy.
Submitted to The Journal of Pain

IV. Feldreich A., Ernberg M, Rosén A.
Atypical odontalgia patients have reduced diffuse noxious inhibitory controls and deficient plasma β-endorphin response to cold pressor test, a prospective case-control study
In manuscript

Study I, reprinted to this thesis in accordance with author rights in the copyright transfer agreement with Informa Health Care

Study II, Reprinted with permission from Elsevier
CONTENTS

1 Introduction .............................................................................................................. 1
  1.1 Pain .................................................................................................................... 1
    1.1.1 Primary types of pain ............................................................... 1
    1.1.2 Acute or chronic pain ................................................................. 2
    1.1.3 Chronic closed lock of the temporomandibular joint .............. 4
    1.1.4 Atypical odontalgia ................................................................. 7
    1.1.5 Central sensitization ............................................................... 9
    1.1.6 Pain processing ................................................................................... 9
    1.1.7 Descending inhibition ............................................................ 12
    1.1.8 Diffuse noxious inhibitory control ........................................... 14
    1.1.9 Descending facilitation .......................................................... 15
  1.2 Activation of the hypothalamic-pituitary-adrenal axis .................. 17
    1.2.1 Cortisol ............................................................................................. 18
    1.2.2 β-endorphin ....................................................................................... 18

2 Aims .......................................................................................................................... 20
  2.1 Specific aims ..................................................................................................... 20
    2.1.1 Study I ............................................................................................. 20
    2.1.2 Study II ............................................................................................. 20
    2.1.3 Study III ............................................................................................. 20
    2.1.4 Study IV ............................................................................................. 20
  2.2 Hypotheses ......................................................................................................... 21
    2.2.1 Study I ............................................................................................. 21
    2.2.2 Study II ............................................................................................. 21
    2.2.3 Study III ............................................................................................. 21
    2.2.4 Study IV ............................................................................................. 21

3 Material and methods ............................................................................................... 22
  3.1 Ethics ................................................................................................................... 22
  3.2 Subjects and study design .................................................................................. 22
    3.2.1 Study I ............................................................................................. 23
    3.2.2 Study II ............................................................................................. 23
    3.2.3 Study III ............................................................................................. 23
    3.2.4 Study IV ............................................................................................. 23
  3.3 Questionnaires .................................................................................................... 24
    3.3.1 General health questionnaire ....................................................... 24
    3.3.2 Coping strategies questionnaire .................................................... 24
  3.4 Experimental procedures ................................................................................... 24
    3.4.1 Venous blood samples ...................................................................... 24
    3.4.2 Assessment of pain ......................................................................... 25
    3.4.3 Threshold assessments ...................................................................... 26
    3.4.4 Cold pressor test ............................................................................. 26
  3.5 Surgical treatment ............................................................................................... 27
  3.6 Experimental protocol ......................................................................................... 28
    3.6.1 Study I ............................................................................................. 28
    3.6.2 Study II ............................................................................................. 28
    3.6.3 Study III ............................................................................................. 28
3.6.4 Study IV .......................................................... 29
3.7 Statistics .......................................................... 30
4 Methodological considerations .................................................. 32
  4.1 Numerical rating scale ............................................. 32
  4.2 Pain pressure threshold .......................................... 32
  4.3 Electrical detection and pain threshold ......................... 32
  4.4 Cold pressor test .................................................. 32
  4.5 Repetitive measurements of pain thresholds ..................... 33
5 Results .................................................................... 34
  5.1 Cold pressor test .................................................... 34
    5.1.1 Healthy volunteers ............................................. 34
    5.1.2 Patients with atypical odontalgia and controls ............ 36
  5.2 Plasma β-endorphins .............................................. 38
    5.2.1 Patients with chronic closed lock and controls .......... 38
    5.2.2 Patients with chronic closed lock after discectomy ...... 39
    5.2.3 Patients with atypical odontalgia and controls ......... 40
  5.3 Pain assessment in TMJ patients .................................. 41
  5.4 Coping strategy questionnaire .................................... 43
6 General Discussion .......................................................... 44
7 Limitation .................................................................. 50
  7.1 Study I ............................................................... 50
  7.2 Study II .............................................................. 50
  7.3 Study III ............................................................ 50
  7.4 Study IV ............................................................ 50
8 Conclusion ................................................................ 52
  8.1 Main conclusion ..................................................... 52
  8.2 Specific conclusions ............................................... 52
    8.2.1 Study I ......................................................... 52
    8.2.2 Study II ......................................................... 52
    8.2.3 Study III ....................................................... 52
    8.2.4 Study IV ....................................................... 53
9 Future perspectives ............................................................ 54
10 Acknowledgements .......................................................... 56
11 References .................................................................. 59
DEFINITIONS

Classification of Chronic Pain from IASP Task Force on Taxonomy, last update 7-14-2011 (Merskey, H. 1994). Reprinted with permission.

Allodynia  Pain due to a stimulus that does not normally provoke pain.

Analgesia  Absence of pain in response to stimulation which would normally be painful.

Hyperalgesia  Increased pain from a stimulus that normally provokes pain.

Neuropathic pain  Pain caused by a lesion or disease of the somatosensory nervous system.

Central neuropathic pain  Pain caused by a lesion or disease of the central somatosensory nervous system. See neuropathic pain note.

Peripheral neuropathic pain  Pain caused by a lesion or disease of the peripheral somatosensory nervous system.

Nociception  The neural process of encoding noxious stimuli.

Nociceptive stimulus  An actually or potentially tissue-damaging event, transduced and encoded by nociceptors.

Nociceptor  A high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli.

Noxious stimulus  A stimulus that is damaging or threatens damage to normal tissues.

Pain threshold  The minimum intensity of a stimulus that is perceived as painful.

Pain tolerance level  The maximum intensity of a pain-producing stimulus that a subject is willing to accept in a given situation.

Sensitization  Increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally sub threshold inputs.

Central sensitization  Increased responsiveness of nociceptive neurons in the central nervous system to their normal or sub threshold afferent input.

Peripheral sensitization  Increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields.
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT</td>
<td>5 hydroxytryptamine, i.e. serotonin</td>
</tr>
<tr>
<td>5HT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5 hydroxytryptamine type 3 receptor, i.e. serotonin receptor type 3</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AO</td>
<td>Atypical odontalgia</td>
</tr>
<tr>
<td>βE</td>
<td>Beta endorphin</td>
</tr>
<tr>
<td>CCDAP</td>
<td>Chronic continuous dentoalveolar pain</td>
</tr>
<tr>
<td>CCL</td>
<td>Chronic closed lock</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene related peptide</td>
</tr>
<tr>
<td>CRF</td>
<td>Corticotrophin releasing factor</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CPT</td>
<td>Cold pressor test</td>
</tr>
<tr>
<td>DNIC</td>
<td>Diffuse noxious inhibitory control</td>
</tr>
<tr>
<td>DRG</td>
<td>Dorsal root ganglion</td>
</tr>
<tr>
<td>EDT</td>
<td>Electrical detection thresholds</td>
</tr>
<tr>
<td>EPT</td>
<td>Electrical pain thresholds</td>
</tr>
<tr>
<td>HNCS</td>
<td>Heterotopic noxious conditioning stimulation</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamus pituitary adrenal</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma aminobutyric acid</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl D-Aspartate</td>
</tr>
<tr>
<td>NMDAR</td>
<td>N-methyl D-Aspartate receptor</td>
</tr>
<tr>
<td>NRS</td>
<td>Numerical rating scale</td>
</tr>
<tr>
<td>PAG</td>
<td>Periaqueductal grey</td>
</tr>
<tr>
<td>PFMC</td>
<td>Prefrontal motor cortex</td>
</tr>
<tr>
<td>PPT</td>
<td>Pressure pain thresholds</td>
</tr>
<tr>
<td>RVM</td>
<td>Rostroventral medulla/Rostro ventrolateral medulla</td>
</tr>
<tr>
<td>SP</td>
<td>Substance P</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TMJ</td>
<td>Temporomandibular joint</td>
</tr>
<tr>
<td>TMJD</td>
<td>Temporomandibular joint disease</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>VGIR</td>
<td>Visually guided irrigation</td>
</tr>
<tr>
<td>WDR</td>
<td>Wide dynamic range</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

1.1 PAIN

The definition of pain is, “An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” (Merskey, H. 1994).

1.1.1 Primary types of pain

The primary types of pain may be divided into nociceptive pain, inflammatory pain, and neuropathic pain (Woolf, C.J. 2004; Zhou, M. 2011, Merskey, H. 1994). The primary afferents are nociceptive specific or polymodal (most). They may be triggered by a specific or by several types of noxious stimuli e.g. of an application of chemical, mechanical or thermal stimuli (Woolf, C.J. 2000). During tissue damage, e.g. during surgery, mechanical overload or infection, the extracellular milieu is changed by tissue- and pathogenic constituents (Thomas, M.V. 2011). Cells from the immune system are activated and recruited to the location of tissue damage where they release inflammatory mediators including cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) (DiPietro, L.A. 1995). During tissue inflammation, many components in the changed microenvironment activate the primary afferents and cause inflammatory pain (Schaible, H.G. 2002). In addition, inflammatory mediators, e.g. prostaglandins and bradykinins, trigger a transformation of the peripheral terminal, into a state of higher sensitivity, i.e. “peripheral sensitization” (Schaible, H.G. 2002; Scholz, J. 2002). The thresholds for the ion channels are lowered, and thus the threshold for transduction and, consequently, the threshold for what normally cause pain (Linley, J.E. 2010; Woolf, C.J. 2000). As a result of this, the activity level in the primary afferent is prone to increase, which in turn results in an “activity dependent” change in gene-expression (Woolf, C.J. 2000). In addition to the level of activity, the neuronal transcription is influenced by extracellular substances such as cytokines released during inflammation (Woolf, C.J. 2000; Scholz, J. 2002). Transcriptional changes may lead to abnormal sensitivity, phenotype switch and eventually morphological changes including denervation (Woolf, C.J. 2000).
If the tissue of a primary afferent is damaged by a lesion, neuropathic pain may develop. Inflammatory pain and neuropathic pain are sharing some characteristics, both may be activated spontaneously, both may be associated with hypersensitivity, reduced threshold and increased afferent response to normal stimuli (Scholz, J. 2002 Woolf, C.J. 2004). During nerve injury the essentials for inflammatory pain and peripheral sensitization may be released. Macrophages releasing proinflammatory cytokines, such as TNF-α, may invade the dorsal root ganglion (Scholz, J. 2007). Damage to the primary afferent may cause changes of the deployment of ion channels and receptors in neighboring afferents (Scholz, J. 2007). Hyperalgesia and spontaneous pain has been suggested to be coupled to such changes. Hyperalgesia and spontaneous pain may also be secondary to morphological changes after nerve injury such as disinhibition i.e. loss of the interneurons releasing gamma amino butyric acid (GABA) in the dorsal-horn and rewiring, i.e. when the primarily innocuous transmitters sprout in the dorsal-horn and connect to nociceptive neurons (Scholz, J. 2002). Neuropathic pain is also referring to changes in pain elicited by organic cause in the central nervous system (CNS), such as pain secondary to stroke or traumatic tissue damage. Central neuropathic pain i.e. pain from a lesion or a disease in the CNS is also functional pain (Woolf, C.J. 2004).

1.1.2 Acute or chronic pain

1.1.2.1 Acute pain

Acute pain is a warning signal to protect from further injury; the pain disappears when normal healing occurs.

Pain contributes to survival in close connection to learning and memory. Studies of conditioning has shown that the more painful a stimuli is the less repetitions it takes to receive an aversive emotional association to a neutral stimulus, presented together with the painful stimulus (Apkarian, A.V. 2008; Schafe, G.E. 2001)

1.1.2.2 Chronic pain

The term chronic pain is used when the pain persists after the normal healing process has been determined. The time point for onset of chronic pain in clinical praxis, including post surgical pain, is debated and the range of 2-6 months is in use (Shipton, E.A. 2011; Benoliel, R. 2010).
The biological mechanisms for developing chronic pain have been studied in animal models. Genetic background may be one of the causes behind different vulnerability to chronic pain (Flores, C.M. 2001; Nitzan-Luques, A. 2011). In particular, genotype selective phenotypic changes in the primary afferent, such as the up regulation of calcitonin gene-related peptide (CGRP) in low threshold mechano fibers, have been found in rodents with hereditary vulnerability to spontaneous pain (Nitzan-Luques, A. 2011). De Felice and colleagues recently showed that after spinal nerve ligation (SNL), the rats subjective to allodynia was relieved from pain after lidocaine microinjection in RVM (De Felice, M. 2011). In contrast, the rats not subjective to allodynia after SNL received allodynia after RVM microinjection (De Felice, M. 2011). This finding suggests that the ON and OFF cells in RVM are important in the development of chronic pain.

Chen-Yu Chiang and Barry Sessle have in two reviews summarized the time window for the, “signal transduction translational/transcriptional”, phase leading to phenotypic changes. In chronic inflammatory pain models, the changes are summarized to peak within 1-7 days and last for 14-21 days and changes in chronic neuropathic pain models, where microglia and astrocytes are involved, peak within 3 days to 3 weeks and is suggested to last for months (Sessle, B.J. 2011; Chiang, C.Y. 2011). An example of the time-line for glial invasion comes from a model for chronic pain, evoked by ligation of the infraorbital nerve (Xu, M. 2008). After partial nerve injury, microglia is activated at day one, and astroglia peaks at day eight, in the superficial lamina of the caudal trigeminal nucleus, while the allodynia in this model lasts three weeks (Xu, M. 2008). After complete nerve transection, the macrophages invading the dorsal root ganglion are still persistent after three months. In a study of mandibular sagittal split osteotomy with intraoperative neurophysiologic measurement, 95% of the nerves were affected. Postoperatively, 1 out of 19 patients had pain after two weeks, while after one year, 2 out of 19 patients suffered from pain, and then the pain was spread (Teerijoki-Oksa, T. 2011).

To understand the mechanisms behind chronic pain in patients studies, investigation of genotypes prevalent in diagnosis of chronic pain, functional magnetic resonance imaging, (fMRI) of affective reactions to pain and stress, and diffuse noxious inhibitory controls (DNIC) have been used (Valdes, A.M. 2011; Zhou, Z. 2008; Mickey, B.J.)
Chronic pain has been associated with grey matter changes in the CNS that may last in at least 12 months after pain declined (Ruscheweyh, R. 2011). Pain and stress is activating the HPA-axis. The HPA-axis, with its key hormone corticotroph releasing factor (CRF), has been associated with a range of chronic diseases such as fibromyalgia, inflammatory bowel disease and temporomandibular disorders (Stohler, C.S. 2001; McEwen, B.S 2010; Hubbard, C.S. 2011). fMRI has revealed that under threat of pain, patients with chronic pain in the form of inflammatory bowel disease have an increased CRF dependent activation of hypothalamus and locus coeruleus in close contact with the amygdala (Hubbard, C.S. 2011). In patients with fibromyalgia and orofacial pain, the level of anxiety is reported to correlate to pain intensity (Ernberg 2000). Several studies suggests that the cortical and sub cortical circuitries that change in chronic pain, basala ganglions, amygdala and prefrontal motor cortex are closely related to emotional learning and memory (Apkarian, A.V. 2008). The chronic anxiety and disability of pain, can lead to maladaptive changes in life-style and coping. Further the development of chronic pain has been associated with major depression (Madland, G. 2001).

1.1.3 Chronic closed lock of the temporomandibular joint

In temporomandibular joint diseases, different classification systems are present, and there is a debate on whether clinical examination or imaging should be the base for treatment and research, especially when surgical intervention is a treatment option (Holmlund, A. 2007; Ribeiro-Rotta, R.F. 2011; Limchaichana, N. 2006).

Chronic closed lock (CCL) of the temporomandibular joint (TMJ) is a clinical diagnosis of limited jaw opening, vertically and/or horizontally, with movement-evoked pain, i.e. internal derangement (Holmlund, A. 2007). The onset may be sudden, or may develop from a state of painful clicking (Holmlund, A. 2007). For reference, the diagnosis corresponds to disc displacement without reduction according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). It also may include clinical signs of osteoarthritis or arthralgia, i.e. palpatory pain in combination with pain at rest or movement-evoked pain, with or without crepitations, according to the RDC/TMD (Dworkin, S.F. 2010; Dworkin, S.F. 1992).
Figure 1. In detail, the temporomandibular joint elevated from the human cranium.

The disc (d.) lies in the auricular fossa between the condyle (c.) and the auricular tubercle. The posterior attachment (p.a.) holds the disc in place while the pterygoideus lateralis (p.l.) attached to the condyle is involved in jaw movement. The auriculo temporal nerve (a.t.n.) innervates the joint.

The etiology behind CCL is not defined and the pathogenesis leading to impaired function is unresolved (Holmlund, A. 2007). Previous trauma, functional overloading, hormonal factors, aging, hyper mobility and systemic diseases may contribute to the internal derangement (Tanaka, E. 2008).

Findings in tissue and synovial fluid suggest that the degree of inflammation correlates to the severity of the disease, also in terms of pain and treatment outcome. In patients with painful clicking, the inflammation in the posterior attachment has by tissue based immunohistochemistry been characterized as mild, CD45RO+, with predominant expression of IL1-α and β. The number of macrophages is increased in generalized joint disease (Kardel, R. 2003).
Specific arthroscopic findings for biopsy verified synovitis of the TMJ, is capillary hyperemia and synovial hyperplasia (Gynther, G.W. 1994). Narrowing and obliteration of vascular lumen in the capsule and the posterior attachment of the disc is more common in painful TMJ, than in asymptomatic joints, and in the endothelial cells of patients with internal derangement, the level of IL-1β, correlated to VAS (Pereira, F.J. 1996; Suzuki, T. 1999).

In two investigations of CCL from Hamada et al, the presence of IL-8, and IL-6 in synovial fluid seem to predict a more severe inflammation, as patients with worse clinical outcome after visually guided TMJ irrigation (VGIR), more frequently had detectable IL-8 in synovial fluid, while the opposite was true for IL-10 (Hamada, Y. 2006; Hamada, Y. 2008). Further, a high degree of joint effusion predicts poor clinical outcome after VGIR (Nakaoka, K. 2009).

Radiographic examinations of patients with TMJ dysfunction and pain have shown a displaced disc in up to 82% of the patients (Tasaki, M.M. 1996; Larheim, T.A. 2005). In a previous study, comparing patients with pain from the TMJ and frequent locking, crepitation, and/or clicking with healthy controls, the incidence of disc displacement was in 72% in the patient group and 35% in the control group (Larheim, T.A. 2001a). The most specific and sensitive signs of TMJ dysfunction on magnetic resonance imaging (MRI) reported, however present in only 23% of the patients, are unilateral marked joint effusion, i.e. increased T2 signal consistent with fluid, or condyle marrow edema and sclerosis (Larheim, T.A. 2001b). The most specific disc disorder is complete anterior disc displacement, present in 16% of the dysfunctional joints, compared to 2% of the healthy control’s (Larheim, T.A. 2001a).

The auriculotemporal nerve of the mandible branch of the trigeminal nerve, is in close connection to the capsule or the condylar neck, and innervates the joint capsule (Loughner, B.A. 1990; Fernandes, P.R. 2003). The nerve passes through the infratemporal fossa and entrapment of the nerve secondary to volume changes may occur (Loughner, B.A. 1990). Especially medial disc displacement has been suggested as the mechanism behind the neurological symptoms associated with internal derangement of the TMJ (Schmidt, B.L. 1998). During internal derangement of the TMJ, the risk for mechanical damage to the joint structures and the nerve is limited by
involvement of brainstem muscle reflexes, limiting the jaw movement. A study of patients presenting with acute closed lock, reported that an intraarticular (IA) injection of lidocain and cortisol, increased the limited jaw opening for at least one week afterwards (Samiee, A. 2011).

The proposed therapeutical approach to CCL is nonsurgical treatment at first, thereafter arthroscopy with lysis and lavage, and lastly discectomy, if the patient does not want to wait for the up to 40% chance of possible relief of symptoms after 2.5 years (Holmlund, A. 2007). In longitudinal follow-up studies the success of open TMJ discectomy has been reported with a success rate of 73% after sixth months and 83-85% after one year (McKenna, S.J. 2001; Holmlund, A. 2007; Eriksson, L. 2001)

1.1.4 Atypical odontalgia

Atypical odontalgia (AO) or chronic continuous dentoalveolar pain (CCDAP) is an orofacial chronic pain condition (Greene, C.S. 2011). The pain is severe and usually continuous, and it is discussed that AO may be a neuropathic pain condition (Baad-Hansen, L. 2008; Melis, M. 2003; Greene, C.S. 2011; List, T. 2007; Baad-Hansen, L. 2006). Recent research has suggested that afferens from teeth partly is supplied by low-thresholds-mechano neurons, “algoneurons”, whose transmission, relay in the superficial lamina of the trigeminal nucleus, and ultimately evokes pain (Fried, K. 2011). It is possible that endodontic therapy or surgical extraction of a tooth may cause nerve damage but in the majority of cases healing occurs. In a small amount of the patients, pain may persist or arise with latency. It has been suggested that AO appears after root canal treatment or tooth extraction, as 3-6% of all root canal treatments results in persistent pain (Baad-Hansen, L. 2008; Melis, M. 2003).
Figure 2. A schematic drawing of the human cranium, with the caudal trigeminal nucleus (V.c.), ganglion gasseri (g.) and the trigeminal nerve, which is the fifth cranial nerve with both sensory and motor functions. The nerve is divided into three parts: n.othalmicus, n.maxillaris (II.) and n.mandibularis (III). The schematic figure show extracted teeth (e.), in the upper right jaw and an area of possible nerve injury near the apex (a.).

Possible peripheral neuropathic explanations to AO may be: the build-up of a neuroma, or inflammatory changes involving the trigeminal root ganglion (Greene, C.S. 2011; Baron, R. 2006; Scholz, J. 2002). Further, neuronal or neuroimmune changes, in the medullary dorsal horn, and in the circuits for pain modulation may be involved, including neuroplastical changes inducing referred pain (Greene, C.S. 2011; Heinricher, M.M. 2009). This can cause tooth ache like pain in other teeth, therefore, it is crucial to point out the importance of avoiding inappropriate irreversible dental treatments (Greene, C.S. 2011). Early diagnosis and treatment is not at least of significance, while secondary psychiatric disease may develop (Clark, G.T. 2006).
1.1.5 Central sensitization

The changes of central sensitization originally referred to changes in the trigeminal nucleus and the spinal dorsal horn. Central sensitization is the term for an afferent input with a response, amplitude and duration larger than normal (Woolf, C.J. 2011). In the clinic, central sensitization may be referred to: reduced pain thresholds, hyperalgesia, overt response, and longer duration of pain after stimuli (Woolf, C.J. 2011). On cellular level, the activity level of nociceptive input triggers the sensitization and increases the synaptic transmission from the first order nociceptors to the second order neurons (Woolf, C.J. 2004). The direct impulse is driven by glutamate, while the joint presence of Substance-P (SP) and N-Methyl-D-Aspartate (NMDA) induces prolonged hyper excitability on the dorsal horn level (Mjellem-Joly, N. 1992). Central sensitization evoked during tissue damage, i.e. surgery can be reduced by, and NMDA block (Stubhaug, A. 1997). In the case of frequent or persisting nociception, transcriptional changes take place and the gene machinery of the cells is eventually changed (Woolf, C.J. 2004).

NMDA receptors in the pain modulating system, including the rostro ventral medulla/Rostro ventrolateral medulla (RVM), and NMDA receptor subtype NRB2 in the forebrain has been associated with neuroplasticity following pain and inflammation in supraspinal structures (Terayama, R. 2000; Miki, K. 2002; Wei, F. 2001). In addition to the hyper excitability of the synapses, this neuroplasticity includes many of the processes of central sensitization on the spinal level, such as gene expression and phenotype switch e.g. with changes in receptor distribution (Ji, G. 2007; Miki, K. 2002; Wei, F. 2001).

1.1.6 Pain processing

When the nociceptors have been trigged, the impulses project through the dorsal root ganglion into the dorsal horn of the spinal cord. A total of 85% of the most superficially neurons of the dorsal horn in lamina I are either high threshold neurons or sensitive to noxious cold, to larger extent the rest are wide dynamic range (WDR), with convergent A-δ and c-fiber input (Suzuki, R. 2005). After reception of a noxious high threshold, or originally cold stimuli, these neurons project through the spino-parabrachial tract to the periaqueductal grey (PAG), the hypothalamus, and amygdala, centers involved in affective and autonomic response and/or descending control of nociceptive input (Suzuki, R. 2005).
Figure 3.
Schematic drawing of ascending and descending pathways involved in pain and pain modulation (Fig. 3).

Ascending pathways are depicted in red, connections between the parabrachial nucleus and the midbrain PAG-RVM system in yellow and descending pathways in blue. The levels of illustration include from bottom up:

A. The spinal level with the dorsal horn, where ascending pathways origin from the primary afferent. The spino-parabrachial pathways origin in superficial laminas, predominantly lamina I, and conveys noxious stimuli via the parabrachial nucleus (P) to the midbrain PAG-RVM system, and further to hypothalamus (H) and amygdala (A), involved in emotional-affective and autonomic response involved in threat, pain and stress (Gauriau, C. 2002; Hubbard, C.S. 2011; Rasmussen, N.A. 2009). From the dorsal horn the spino-thalamic tract is decussating to the contralateral ventral part from where it is conveying sensory discriminative and thermal input to the thalamus for further distribution, including the somatosensory cortex (Gauriau, C 2002).

B. The medulla with the medullary dorsal horn including and the RVM. In the medullary dorsal horn, the trigeiminal caudal nucleus is receiving sensory input from the trigeiminal nerve. The superficial lamina, predominantly lamina I is projecting predominantly to the ipsilateral parabrachial area and project further to the amygdala and the hypothalamus (Ding, Y.Q. 1995; Bester, H. 1995; Suzuki, R. 2005). The RVM is a part of the midbrain PAG-RVM system and has descending monoaminergic projections that are involved in the regulation of the transmission in the dorsal horn (Heinricher, M.M. 2009; Giordano, J. 2004).

C. Pons with the parabrachial nucleus and the noradrenergic locus coeruleus. The parabrachial area conveys information from two of three proposed major main pathways (Gauriau, C. 2002). Focus is laid on the spino-parabrachial circuits projecting to the hypothalamus (H), the amygdala (A) and the midbrain PAG-RVM system. This circuit is suggested to be involved in the fight and flight reaction, emotional-aggressive behaviors, fear-evoked-avoidance learning, anxiety and pain-modulation (Gauriau, C. 2002). The spino-(Trigemino) parabrachiohypothalamic pathway is suggested to be involved in neuroendocrine adaptations to noxious afference (Bester, H. 1995). The deep lamina projections via the parabrachial internal sub nucleus to the caudal reticular nuclei and medial thalamus for further distribution back to nociceptive lamina and to the striato cortical prefrontal compartment is not shown, this system is suggested to be involved in attentional, motivational and motor responses to pain (Gauriau, C. 2002).

D. The periaductal area with the periaqeductal grey matter (PAG). PAG organized in columns and included in the PAG-RVM system, where PAG receives input from the prefrontal motor cortex (PFMC), the anterior cingulated cortex (ACC), hypothalamus (H), amygdala (A) and the parabrachial circuit (Heinricher, M.M. 2009; Gauriau, C. 2002; Bandler, R. 1994). The ventrolateral columns receive nociceptive input from viscera and deep muscles and is identified to be involved in the flight reaction, with hypotension, bradycardia, passive emotional coping strategies and long-lasting opioid mediated analgesia (Gauriau, C. 2002; Bandler, R. 1994). The lateral PAG receives superficial, cutaneous nociceptive input and is involved in the fight reaction, with hypertonia and tachycardia, and mediates, “short lasting”, non opioid mediated analgesia (Gauriau, C. 2002; Bandler, R. 1994). The midbrain PAG-RVM system is a major supraspinal center for pain modulation and descending control

E. Prefrontal motor cortex, the anterior cingulate cortex (ACC), the thalamus, the amygdala (A) and the hypothalamus (H). Hypothalamus (H), amygdala (A) and thalamus are targets of the major nociceptive pathways the spino-thalamic-tract, spino-reticular pathway and the spino-parabrachial pathway (Gauriau, C. 2002; Suzuki, R. 2005). From the prefrontal-motor-cortex (PMFC), the anterior cingulated cortex (ACC), the hypothalamus (H) and the amygdala (A) input is given to the midbrain PAG-RVM system involved in pain modulation, emotional and autonomic response to pain (Heinricher, M.M. 2009).
The discriminative parts of a sensory input, including pain and temperature signals from the whole body except from the orofacial region, travel along the lateral spinothalamic tract targeting the ventral posterolateral nucleus (VPL) of the thalamus. From the thalamus the information is transmitted to parts of the brain dealing with affective and discriminative parts of the sensation (Gauriau, C. 2002). The sensory input from the orofacial area transmits via the trigeminal nerve which has three divisions; the ophthalmic nerve, the maxillary nerve and the mandibular nerve.

The nerve endings from trigeminal ganglion (semilunar ganglion) transmit to the trigeminal sensory nuclei: the sub-nucleus oralis, the sub nucleus interpolaris and the sub nucleus caudalis. The information is then transmitted to trigemino-parabrachial tract and the lateral trigemino-thalamic tract, eventually reaching primarily the PAG, the hypothalamus, the amygdala, and the VPL, respectively.

By fMRI both emotional – arousal activated circuits and cortical areas have been identified, for certain patterns of pain and concomitant stimuli. The anterior cingulated cortex seems to be important in pain and affect (Rainville, P. 1997). Isolated, burning C-fiber stimulation seems to especially activate circuits involving the dorsolateral prefrontal cortex (Veldhuijzen, D.S. 2009). Finally, an increased pattern of communication between locus coeruloes and hypothalamus with amygdala in IBS patients, activated by threat of pain, seems to be CRF dependent (Hubbard, C.S. 2011).

### 1.1.7 Descending inhibition

Normally, ascending neurons are under tonic inhibition by descending controls. The descending control projects to a number of neuron-types, with a range of transmitter substances (Millan, M.J. 2002). Forty years ago Reynolds and colleagues discovered that electrical stimulation of the PAG produced analgesia enough to perform abdominal surgery in rats (Reynolds, D.V. 1969). Likewise, stimulation of the periventricular gray (PVG) of the hypothalamus inhibits dorsal horn neuronal nociceptive input (Carstens, E. 1995). Further, lidocain injections into the RVM and PAG induces reversible tactile allodynia and deep brain stimulation of the PVG and the PAG are effective in treating severe chronic nociceptive pain (Pertovaara, A. 2000; Nguyen, J.P. 2011). The PAG and the RVM with the nucleus raphe magnus (NRM)
plays a pivotal role in the descending inhibition of pain, where the projection from PAG to RVM produces monoaminergic release on spinal level (Aimone, L.D. 1986). Early in the 1980-ties, the control neurons with converging C-fiber and A-δ input WDR was identified function (Dickenson, A.H. 1980).

Figure 4. Descending noxious inhibitory control (DNIC). Primary afferents project to the dorsal horn of the medulla and the caudal trigeminal nucleus where the first order synapse is situated. Ascending pathways stimuli is relaying in superficial and deep lamina, and relay on levels above (pink line ascending). When heterotopic noxious conditioning stimulation (HNCS) is applied descending control is activated by the RVM through the dorsolateral funiculus (DLF) (blue arrow). In the case of serotonergic transmission from the RVM, interneurons (green) are suggested to be activated and impose descending control via opioid and GABA (Giordano, J. 2004, Giordano J, 2001; Hao, J.-X. 1992). In the middle of the picture, a WDR neuron with convergent c-fiber (purple arrow) and A-δ input (pink arrow) is inhibited by GABA, and in the lateral part of the picture, a synapse inhibited by endogenous opioids are depicted. WDRs are the primary target for DNIC and are present in both superficial and deep lamina (Dickenson, A.H. 1980, Suzuki, R. 2005) Nociceptive afferens may be reduced in both ipsilateral and decussating projections (red lines) (Baron, R. 2006, Lapirot, O. 2009).

Descending inhibition, is thought to be the analgesic mechanism of systemic opioid administration, and may also be activated by morphine stimulation in PAG, which induces subsequent monoaminergic descending control (Kuraishi, Y. 1983). Further in experimental models of chronic pain, inflammation induced by carrageenan or nerve lesion in rat, increases the tissue levels of dynorphin B (DynB) and met-enkephalin-Arg-Phe (MEAP) in the PAG (Rosen, A. 2000). In addition, peripheral injections of
morphine or a 5HT-re uptake blocker increase the tissue levels and release of SP to the PAG (Rosen, A. 1995; Rosen, A. 2004). Furthermore, SP or Noceceptin/orphanin FQ injections in the PAG increase the antinociceptive thresholds to heat and loading in rats (Rosen, A. 2004; Bytner, B. 2001). The descending projection from the midbrain PAG-RVM system has been suggested to exercise its effect on the spinal level, by an inhibition of the afferent transmission through interneurons releasing opioids and/or GABA after 5HT stimulation (Giordano, J. 2004). The RVM is receiving input from PAG and additional supraspinal centers, as well as the parabrachial area. Within the RVM a structure of ON- and OFF- cells were identified by Fields and colleagues 1983. The OFF-cell activity is dependent on opioid agonism or antagonism, and may be electrically activated just below the threshold for analgesia (Fields, H.L. 1983). The opioid agonism is increasing the threshold for inhibition of the OFF-cell, an inhibition that correlates with the tail-flick secondary to thermal stimuli (Fields, H.L. 1983). In contrast, when the tail flick occurs, the on-cell are activated (Fields, H.L. 1983). These on-cells have been destroyed in animal models of nerve injury and are suggested to induce the descending facilitation necessary for maintaining hypersensitivity, while OFF-cells have been harder to study in models of chronic pain (Porreca, F. 2002). Opiate agonism on the level of the dorsal horn inhibits excitation of nociceptors by inhibiting release of SP, while GABA is suggested to block WDR neurons postsynaptically (Jessell, T.M. 1977; Hao, J.X. 1992).

### 1.1.8 Diffuse noxious inhibitory control

Diffuse noxious inhibitory control (DNIC) involves ascending and descending pathways (Le Bars, D. 1979; Le Bars, D. 1979; Pertovaara, A. 2000; van Wijk, G. 2010). When the nociceptors have been trigged by noxious stimuli, the afferents transmit the information to supraspinal structures through ascending pathways. From supraspinal circuits, including the midbrain PAG-RVM system, the descending pathways originate and travel down to the spinal cord and to the trigeminal nucleus (Dickenson, A.H. 1980; Heinricher, M.M. 2009). The DNIC response is activated by nociceptive stimulation applied outside the excitatory receptor fields. DNIC effects have generally been found at heterotopic areas or extra segmental, why the term heterotopic noxious conditioning stimuli (HNCS), also has been used. However, recent research has found pain inhibition even when homotopic stimuli are applied (Pud, D. 2005).
The WDR neurons have been shown to play a pivotal role in the DNIC response. Most of the WDR neurons are found in lamina V (Le Bars, D. 2002). They may be triggered by noxious and innocuous stimuli (Le Bars, D. 2002). There are different ways to trigger the DNIC response in experimental settings. The type and duration of the stimuli applied determines the level of inhibition (Oono, Y. 2008). The most used conditioning stimuli are thermal, i.e. cold or heat, or ischemic (Baad-Hansen, L. 2005; Kosek, E. 2000). The test stimuli that have been used are for example heat, electrical, pressure or chemical. This has resulted in studies with a range of different methodological approaches. Pud et al have proposed a more homogenous methodological approach, because it is difficult to compare the results from one study to another (Pud, D. 2009).

1.1.9 Descending facilitation

Several mechanisms of descending pronociceptive and antinociceptive stimulation involving a wide range of supraspinal structures has been described (Millan, M.J. 2002). A range of receptors have been reported to be involved in pro- as well as antinociceptive mechanisms (Millan, M.J. 2002). The RVM has been identified as a center for descending control, where the term descending facilitation has been used for the admittance of discriminatory fast A-δ sensory information, while the c-fiber nociceptive WDR neurons are inhibited. A wider use of the term descending facilitation may involve sensory afferent signaling that is enhanced within the CNS; this may result in decreased pain thresholds secondary to HNCS. The dynamics of the RVM are changed during injury and inflammation (Heinricher, M.M. 2009). Of great importance is to elucidate which mechanisms that are dominating the clinical picture. If tissue-damage with resulting inflammatory response is or has been present, this has to be taken in consideration, as neuroimmune mechanisms are suggested to modulate the transmission both at spinal and supraspinal levels. Descending facilitation in long-lasting pain, resulting in an increased response to innocuous as well as noxious stimuli has been proved by several studies (Pertovaara, A. 2000; Guo, W. 2007; Wei, F. 2008; Villa, G. 2010). In an experimental animal model of allodynia after chronic constriction injury to the infraorbital nerve, the following changes occur in the RVM: Glia is activated after the injury, and stays activated for at least two weeks. Further, the presence of IL-1β and TNF-α increased, and the NMDA-receptor subunit NR1 is up regulated (Wei, F. 2008). The Authors were able to decrease the alldynia by inhibiting glia, and by inhibiting IL1-β and TNF-α, both NR-1 phosphorylation and hyperalgesia
decreased (Wei, F. 2008). This glial activation secondary to a nerve injury within the trigeminal area has the potential to lead to increased excitability in the midbrain PAG-RVM system. Further, peripheral inflammation in the orofacial area induced by complete Freund’s adjuvant (CFA) intraarticular injection into the TMJ, or in the masseter, as well as ligation of the infraorbital nerve or the inferior alveolar nerve, activates microglia cells and astrocytes in superficial lamina of the trigeminal nucleus (Villa, G. 2010; Guo, W. 2007; Xu, M. 2008). In addition, immunohistochemistry studies in a rat models of chronic pain after either sciatic nerve ligation or CFA induced peripheral inflammation, using intrathecal 5HT3 antibodies has revealed a neuronal-glial-neuronal interaction possible resulting in descending facilitation. It is suggested that spinal activation of microglia through fractalkine expressing 5HT3 receptor positive neurons, increases synaptic transmission through a two step mechanism, where first the microglia by production of IL-18 triggers the astrocytes to IL-1β, and secondly the IL-1β increases the activity of NMDA receptors in the afferent synapse (Gu, M. 2011). To summarize, there are emerging evidence from animal models, that peripheral inflammation or nerve injury, seems to activate glia both in the medullary dorsal horn and in the RVM, which leads to hyper excitability both in the PAG-RVM system and on the level dorsal horn transmission possibly leading to a switch from descending inhibition to descending facilitation.
Figure 5. Descending facilitation explained by neuron-glial-neuronal interaction.

In experimental animal models, it was recently suggested that a switch to descending facilitation could be secondary to neuronal-glial-neuronal interactions. Descending facilitation involves, in the middle of the picture, activation of glia by a 5HT$_3$ (5HT$_R$) activated neuron (green) which releases the ligand fractalkine CX3CL1 and thus attracting microglia (pink) with the receptor CX3CXR. The microglia in turn is activating the astrocyte (blue) by release of IL-18. The astrocyte is modulating synaptic transmission by production of IL-$\beta$ and activation of NMDAR (Gu, M. 2011) The synaptic transmission is modulated and ascends via ipsilateral and decussating pathways (pink, purple and red arrows). For comparison and further legends, please see fig 3.

1.2 ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

When the pain signal reaches the brain, a new mission develops, namely to stop what is provoking the pain, and the focus in the brain changes. When the human is in stress, as when being in a situation of acute pain, a fight and flight reaction may develop. The reaction is characterized by a decreased parasympaticus tonus and an increased sympaticus tonus. Hypothalamus plays a pivotal role in the pain and stress response and is the starting point for the hypothalamus pituitary adrenal (HPA)-axis. Nociceptive input and adrenergic input to the hypothalamus discharge CRF. CRF is the peptide initiating the HPA-axis, further CRF-receptors are widespread in the brain, where they plays an important role in modulating the emotional-affective response to pain (Ji, G.
Below the hypothalamus lies the pituitary gland that is closely connected with the hypothalamus by the pituitary portal system. Hormones from the anterior pituitary gland are released into the blood by signals from the hypothalamus. The pituitary gland is also under control from the brain by the infundibular stalk. Chronic stress has been shown to induce hypercortisolemia able to dislocate the hippocampus command of the hypothalamus, causing a HPA-axis sensitization (Southwick, S.M. 2005). For example, different types of experimental models of stress in rats, increases the plasma levels of corticosteron and tissue levels of SP in the PAG (Rosen, A. 1992; Brodin, E).

1.2.1 Cortisol
Cortisol is produced in the zona fasciculata of the adrenal gland cortex secondary to adrenocorticotrophic hormone (ACTH) stimulation. Cortisol has a negative feedback on the pituitary release of ACTH and β-endorphin (βE) and has a diurnal rhythm, with the highest levels during the morning. In blood cortisol the total amount is divided into pools, the majority bound to corticosteroid binding globulin, and minority as free cortisol, considered active. Cortisol has widespread effects on the homeostasis, including effects on the immune system, metabolism, pain and mood. In 50% of patients with major depression a state of sensitization or dysregulation of the HPA-axis has been seen, together with persistent hypercortisolemia (Southwick, S.M. 2005).

1.2.2 β-endorphin
βE is an endogenous opioid with strong analgesic effects released into plasma by the anterior lobe of the pituitary gland (Hargreaves, K.M. 1983; Hartwig, A.C. 1991). In the pituitary gland CRF stimulates and regulate the large precursor hormone, pro-opiomelanocortin (POMC) production and splicing into βE, and other POMC-derivates such as ACTH. These peptides are released into the blood stream. Of the βE in the intermediate lobe, 96% is acetylated, and viewed as inactive as an analgesic. In addition to the pituitary, POMC is produced in the arcuate nucleus of the hypothalamus. A mediobasal lesion of the hypothalamus decreases the βE level in plasma and CSF, while a hypofysectomy decreases the level of βE only in plasma. This indicates an “up-stream” position of the hypothalamus in the distribution of βE in relation to the pituitary (Barna, I. 1992; Baysefer, A. 1999). The axons of POMC cell are lining the wall of the third ventricle, the midbrain, PAG and locus coeruleus. The extent of the role of intraventricular βE in the antinociception obtained with electrical
stimulation in PAG is not known (Reynolds, D.V. 1969, Basbaum A.I. 1984). The main role of \( \beta E \) is suggested to be to bind to \( \mu \)-receptors on nociceptors (Kageyama, K. 2011; Mechlin, B. 2007; Hargreaves, K.M. 1990; Hartwig, A.C. 1991; Rasmussen, NA. 2009).

HPA-axis activation and plasma \( \beta E \) are argued to be involved in analgesia secondary to prolonged pain and stress (20 min) (Basbaum, A.I. 1984). Further, three minutes of stress and pain to the forepaw in rodents produces a descending inhibition that is opioid dependent and is inhibited by RVM lesion, probably partly mediated by opioid and SP stimulation in PAG (Basbaum, A.I. 1984). Acute stress evoked by swim stress induced analgesia, induces descending inhibition, which may be both opioid and none opioid dependent, regarding on stimuli. Four degree cold water stress induces a non-opioid NMDA dependent analgesia, while a less painful stress, (warmer water, > 15 C\(^\circ\)), reveals an opioid involvement (Marek, P. 1992; De Felipe, C. 1998). Visceral pain and deep-muscle pain activates the ventrolateral column of PAG, an area where opioid microinjections may induce analgesia (Bandler, R. 1994; Gauriau, C. 2002). In contrast, pain from superficial structures such as the skin, activates the lateral PAG, an area related to hypertension and tachycardia, and results in a short lasting analgesia (Bandler, R. 1994; Gauriau, C. 2002). In the dorsal and the lateral column of PAG, the binding to the GABA receptor is modulated by estrogen in female, but not in male rats (Schwartz-Giblin, S. 1995). Females have been reported to have a pain decrease during HNCS, and non-opioid mediated descending inhibition originating from the lateral column of PAG, may be one mechanism behind this.
2 AIMS

The general aim for this thesis was to investigate the dynamics of descending inhibition and neuroendocrine analgesia in patients with chronic pain in the trigeminal area and healthy controls.

2.1 SPECIFIC AIMS

2.1.1 Study I
The key question was to examine the reaction to cold pressor test on pain thresholds in the trigeminal versus the spinal region, and to find out whether the response to cold pressor test differs between gender and by type of test stimuli.

2.1.2 Study II
The main aim was to investigate, if the level of plasma βE is altered, and if central sensitization is present, in patients with chronic closed lock of the temporomandibular joint.

2.1.3 Study III
The main aim of this study was to examine the long term response after temporomandibular joint discectomy in patients with chronic closed lock, regarding chronic pain relief, and the relation between chronic pain relief and potential changes in central sensitization and presence of neuroendocrine opioid in plasma.

2.1.4 Study IV
The aim of this study was to assess if patients with atypical odontalgia have changes in the dynamics of descending controls and neuroendocrine analgesia, and whether these patients have an altered pattern of coping.
2.2 HYPOTHESES

2.2.1 Study I
Pain modulation is less efficient in the orofacial region than in the spinal region. Endogenous pain modulation is more susceptible to pain evoked by pressure stimulation than by pain evoked by electrical stimulation. Pain modulation is less efficient in females than it is in males.

2.2.2 Study II
Patients with chronic closed lock (CCL) have increased plasma βE levels and decreased pain thresholds.

2.2.3 Study III
At follow-up of patients with chronic closed lock more than six months after TMJ-discectomy, the following mechanism is hypothesized to win through: “If the pain-intensity decreases, the plasma βE will decrease and the pain-thresholds will increase.”

2.2.4 Study IV
Patients with atypical odontalgia will have disturbed descending controls, a sensitized HPA-axis with a deficient neuroendocrine opioid response and altered coping.
3 MATERIAL AND METHODS

3.1 ETHICS

The methods and selection of subjects were approved by the regional ethical review board in Stockholm. All studies followed the principles for medical research according to the guidelines of the Declaration of Helsinki. All research persons in this thesis were over 18 years of age and gave their verbal and written consent to participate. Students at present undertaking courses at the Division of Oral and Maxillofacial Surgery were not eligible as controls.

3.2 SUBJECTS AND STUDY DESIGN

Table 1 gives an overview of the study design used.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Variable</th>
<th>Instruments, provocations and interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Prospective, EST</td>
<td>EST(^{1}), EPTs(^{2}), PPTs(^{3}),</td>
<td>Algometer, PainMatcher®, Pulp-tester, NRS,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pain-tolerance, pain-intensity(^{6})</td>
<td>CPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unpleasantness(^{7}), DNIC</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Prospective, EDT(^{1})</td>
<td>EDT(^{1}), EPTs(^{2}), PPTs(^{3}),</td>
<td>Algometer, PainMatcher®, NRS</td>
</tr>
<tr>
<td></td>
<td>Case/Control</td>
<td>pain-intensity(^{4}), p-βE</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Prospective, longitudinal</td>
<td>EDT(^{1}), EPTs(^{2}), PPTs(^{3}),</td>
<td>Algometer, PainMatcher®, NRS, Discectomy</td>
</tr>
<tr>
<td></td>
<td>follow-up</td>
<td>pain-intensity(^{6}), p-βE</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Prospective, EDT(^{1})</td>
<td>EDT(^{1}), EPTs(^{2}), PPTs(^{3}),</td>
<td>Algometer, PainMatcher®, Pulp-tester, NRS,</td>
</tr>
<tr>
<td></td>
<td>Case/Control, CPT</td>
<td>pain-intensity(^{6}), unpleasantness(^{7}),</td>
<td>CPT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Cortisol, p-βE DNIC</td>
<td></td>
</tr>
</tbody>
</table>

CPT=cold pressor test, DNIC=Diffuse noxious inhibitory controls, DF=Descending facilitation, EST=Electrical sensory threshold, EDT=Electrical detection threshold, EPT=Electrical pain threshold, PPT=Pressure pain threshold. \(^{1}\) EST/EDT measured over finger. \(^{2}\) EPTs, over the index finger and over the central maxillary incisor. \(^{3}\) PPTs over the index finger and over the masseter muscle. \(^{4}\) Pain intensity at rest- and evoked by movement from the TMJ. \(^{5}\) Pain intensity at rest- and evoked by movement from the TMJ as well as pain-elsewhere and maximal pain intensity. \(^{6}\) Pain intensity during CPT measured by NRS and PainMatcher®. \(^{7}\) Unpleasantness during CPT measured by NRS and PainMatcher®.
3.2.1 Study I
This is a prospective study investigating DNIC between and within genders in 15 males and 15 females with a mean (SD) age of 25.1 (4.4) years. Inclusion criteria: no pain (NRS=0) and healthy.

3.2.2 Study II
This prospective case/control study compared eighteen, consecutive female patients who were undergoing TMJ discectomy with eighteen age-matched female healthy controls. Inclusion criteria for patients were movement-evoked pain from the TMJ, and limited jaw functions, i.e. CCL, corresponding to clinical signs of a diagnosis of disc displacement without reduction, according to the research diagnostic criteria for temporomandibular disorders (TMDs). Inclusion criteria for healthy controls were, no pain i.e. (NRS=0). Exclusion criteria were generalized joint diseases. The patients had a median age of 42.5 years (range, 19 to 72 years). The median age of the control group was 41.5 years (range, 23 to 63 years).

3.2.3 Study III
This is a prospective follow-up of eleven female patients with a diagnosis of CCL who had undergone discectomy at the division of oral and maxillofacial surgery at least 6 months earlier. The inclusion criteria for the patients were: CCL. Patients with generalized joint disease, e.g. rheumatoid arthritis, were excluded. The median age of the patients was median 49 years, range (18-72), and the pain duration was 2.5 years, range (1-8). The postoperative assessment took place 6-24 months after TMJ discectomy.

The number of patients to reached 90% and 95% significance were calculated to 10 and 11 respectively, based on an interim power estimation.

3.2.4 Study IV
The patients were referred to the department of Dental Medicine division of Oral Maxillofacial Surgery, Karolinska Institutet, Stockholm, as a part of their multidisciplinary investigation of their orofacial pain. They were screened for suitability for this prospective case/control study. The inclusion criteria used for AO were: 1) persistent pain localized to the region of an extracted tooth with pain duration of > 6 months, 2) The patients pain could not be explained by clinical, radiographical or
laboratory examinations 3) >18 years of age. Exclusion criteria: trigeminal neuralgia (TN). The controls were matched to the AO patients according to age and gender. The inclusion criteria were: pain-free, good general health and >18 years of age. Exclusion criterion from analysis was: any pain revealed in the general health questionnaire, no present sign of clinical signs of systemic disease e.g. hypertonia. The controls (21) underwent blood pressure control, blood sampling, general health questionnaire and CPT. AO was confirmed in 9 patients, while age-match and absence of present pain and systemic disease e.g. hypertonia was confirmed in 14 controls. The research persons withdrawing from the CPT were excluded from further analysis (n=4), while one was excluded from analysis secondary to prolonged blood sampling (>45 min). AO patients (n=9), and valid controls (n=9), passing the CPT without withdrawal, were included for analysis on the dynamics of their neuroendocrine analgesia and their descending controls. The median (range) age of the AO patients (6 female, 3 male), was 53 (32-68) years, compared to 47 (30-63) years, for the healthy controls (7 female, 2 male).

3.3 QUESTIONNARIES

3.3.1 General health questionnaire
General health and present medications were recorded by questionnaire for study I-IV.

3.3.2 Coping strategies questionnaire
To investigate the coping strategies of AO-patients, the Swedish version of the Coping strategies questionnaire (CSQ) was used. The 27 items validated to have high internal consistency was used together with the two coping effectiveness items, remaining in a 48 question order, in similarity to the validation study (Robinson, M.E. 1997). The CSQ was investigated item-wise and based on the validated subclasses, where, for example, distancing replaced reinterpretation.

3.4 EXPERIMENTAL PROCEDURES

3.4.1 Venous blood samples
Blood samples were collected between 8.45 am and 9.00 am. The participants were fasting and were not allowed to smoke within 4 hours prior to sampling.
To avoid discrepancies related to diurnal variances in stress hormones, the samples were acquired between 8:45 to 9:00 a.m. The participants were instructed to be non-smoking and fasting from 4:00 a.m. Immediately after sampling, the vials were put on ice, and were transported without delay to the laboratory, where they were centrifuged at 2,700 revolutions/min at 4°C for 10 minutes. Thereafter the samples were snap frozen and kept in -70 °C until analysis. An antibody against the N-terminal of the peptide (R-776212, Eurodiagnostica, Malmö, Sweden), was allowed for analysis by radioimmunoassay (RIA). The reactivity of the antibody was 100% against βE, and the cross reactivity against α-endorphin was 69%. In addition the cross reactivity against β-lipotropin 61-69 was 67% and against β-lipotropin 61-87, 43%. Furthermore, the cross reactivity towards additional β-lipotropin, leucine-enkephalin, methionine-enkephalin was below 10%. The RIA was carried out in a certified laboratory (Neurochemical Laboratory, Mölndal Hospital, Sweden).

Total serum cortisol was analysed by the certified laboratory (Karolinska University Laboratory) by Electrochemiluminecens immunoassay, “ECLIA” on the Modular E170 analytics immunoassay analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

3.4.2 Assessment of pain

The Visual Analogue Scale (VAS) and the numerical rating scale (NRS) are the most used rating scales for pain assessment in the clinic. However, in chronic pain patients the NRS scale is recommended (Farrar, J.T. 2001). To evaluate the outcome in clinical settings it is important to present the change in pain intensity after treatment. To define the level of change in pain intensity the NRS scale is recommended. In addition, a decrease on the NRS scale of ≥4 points or ≥50% appear to mirror substantial improvements and is associated with patient ratings of “very much improved” (Farrar,J.T. 2001;Dworkin,R.H. 2005). Both the patients and the healthy controls were asked to score their present pain. They were also asked to estimate the pain in the area of pain as well as “pain elsewhere” e.g. back pain, headache (Brandsborg, B. 2009). An eleven point numeric rating scale (NRS), with the end points of 0 (no pain) and 10 (worst imaginable pain).
3.4.3 Threshold assessments

Another method for pain estimation is threshold recordings. The detection threshold is defined as the least level of stimulation that can be detected, and the pain threshold as the least experience of pain which a subject can recognize (Merskey, H. 1994). We measured the detection thresholds by the PainMatcher®.

3.4.3.1 Electrical detection and electrical pain thresholds
The term Electrical sensory threshold (EST), and the term electrical detection threshold (EDT), refers both to the detection threshold as defined above. The term EST was used in study I, while the term EDT was used in study II-IV. In study I-IV, the EDTs and EPTs were measured over the finger with the PainMatcher®, a double-blinded device (Cefar Medical AB, Lund, Sweden). In study I and IV, the EPT over the central maxillary incisor were recorded with an electrical pulp tester (Vitality scanner; Analytic Technology, Redmond, WA, USA; range 0-80 µA, pulse rate intervals 0-99).

3.4.3.2 Pressure pain thresholds
The pressure pain thresholds (PPTs) were measured over the finger and over the masseter muscle (the muscle was at rest during the recordings), with an electronic pressure algometer (Somedic Sales AB, Hörby, Sweden). The algometer consists of a pistol grip connected to a power supply with a pinch handle attached to the algometer. The participants were asked to push a button when the pain threshold was reached. A pressure rate of 30 kPa/s was used for all studies in this thesis (Ayesh, E.E. 2007). The digital screen on top of the Algometer was used to control the pressure rate interval. All participants were carefully instructed and undertook a training session before the experiment.

3.4.4 Cold pressor test
To activate a DNIC (diffuse noxious inhibitory control) response i.e. by immersion of the hand and wrist contra lateral to the test side into a cold water bath (2°C) (Baad-Hansen, L. 2005). Recordings of pain thresholds started 30 s after immersion of the hand. Pain intensity, unpleasantness and tolerance time were recorded. For study I and IV. In study I the temperature of the water was 2-4°C and in study IV, 2°C.
3.5 SURGICAL TREATMENT

Study 3 is a postoperative follow-up of discectomy. The patients followed ordinary preoperative procedures from the Karolinska University Hospital, Huddinge, to decrease the risk for acute perioperative pain. Before they were sent to the operating theatre, they received acetaminophen and oxycodone. The surgery was performed under general anesthesia by diprivan and remifentanil, using routine prophylaxis for postoperative nausea and vomiting with betamethasone, ondansetron and droperidol. Prior to start of surgery, local anesthetic was administrated subcutaneously and intraarticularly to the TMJ. Discectomy was performed as described by Holmlund et al (Holmlund, A.B. 1993a; Holmlund, A.B. 1993b), via a preauricular approach the superior part of the capsule was cut horizontally. After exploration of the upper compartment the dissection was continued to the lateral pole of the condyle. The disc attachment was cut and the underlying compartment was inspected. After attachment of a Kirschner Wire of 1.1 mm to the condyle and the eminence, the joint space was enlarged by retraction. Disc clamps were positioned anteriorly and posteriorly of the disc, and complete discectomy was completed with a scalpel. After removal of the clamps, the cartilage was investigated for sections hindering remodeling. If needed, arthroplasty was executed with a curved bone file. No implants were inserted. No perioperative antibiotics were routinely administrated. Postoperatively the patients stayed over night at the hospital, routine follow-up and discharge was made by the surgeon. The postoperative analgesic treatment consisted of acetaminophen and non-steroidal anti inflammatory drugs (NSAID). Postoperatively the patients followed the normal routines from the Division of Oral and Maxillofacial Surgery.
3.6 EXPERIMENTAL PROTOCOL

3.6.1 Study I
All participants received a research number. For those with even numbers, the right hand was used for the test side and the recordings started in the orofacial region with recording of EDTs over the maxillary incisor followed by measurements of EPTs and then recordings for PPTs over the masseter muscle, while for participants with uneven numbers, the left side was tested and the estimations started over the index finger with PPTs before recordings for EPTs over the finger. After baseline recordings in the orofacial region as well in the spinal region the participants immersed their contra lateral hand in the water bath as long as they could endure, but for a maximum of 5 min, instructed to take up the hand whenever the pain become intolerable. Thirty seconds after start of CPT the pain thresholds were recorded in the orofacial area as well as over the finger. Five and 15 min after end of CPT the EDTs and pain thresholds were again measured in the same manner as at baseline. During the experimental procedure the participants were asked to score the pain intensity and unpleasantness of the painful sensation on the NRS scale.

3.6.2 Study II
Both the patients and the healthy controls were asked to score their present pain level. The patients were asked to score their TMJ pain at rest and at movement. The participants were seated in a supine position during the threshold recordings. The PPTs over both index fingers were measured as well the PPTs over both masseter muscles. The EDTs over both fingers were assessed as well as the EPTs over both fingers. As has been recommended by the Sex, Gender and pain Special Interest Group of the IASP, the study was preformed only in women due to the incidence of a female male ratio of 9:1 at our centre.

3.6.3 Study III
Patients with CCL were examined preoperatively and scheduled for a postoperative control 6-24 moths after discectomy. The postoperative examination followed the same protocol as the preoperative experimental procedure in (study II).
3.6.4 Study IV

The samples were collected from the hand contra lateral to the hand immersed into the cold water bath. Both patients and healthy controls were asked to fill in the CSQ and their present pain intensity was assessed on NRS. The experimental procedure regarding the threshold measurements and the CPT followed the same procedure as for study 1. Blood sampling of βE was repeated 20 minutes after start of CPT.
3.7 STATISTICS

For statistical analyses, Sigma Stat version 3.1 (Systat Software Inc) (study I), Statistica version 9.1 (Stat soft Inc, Tulsa, Oklahoma, USA) (study II) and Statistica version 10 (Stat soft Inc, Tulsa, Oklahoma, USA) (study III-IV) were used. The Kolmogorov-Smirnov (study I) and the Shapiro-Wilks test was used to test for normality (study II). In Study I Student’s unpaired t-test were used to test threshold differences between genders at baseline. Two-way repeated measures ANOVA was used to prove changes in pain thresholds during the experiment. Separately, ANOVA, two level and two ways, were used to test for the impact of stimulus, region, gender, order side and order of test sites, i.e. electrical and mechanical; trigeminal and spinal; male and female; right or left; trigeminal or spinal, on pain thresholds. Time (four levels: baseline, during and 5 min and 15 min after HNCS) was the repeated factor. As post-hoc test, the Holm-Sidak method for multiple comparison procedure was used versus baseline. Differences in pain-tolerance were controlled with the unpaired t-test, while Pearson’s product moment correlation test was used to test correlation between pain-tolerance and changes in pain thresholds. To control for multiple correlation analyses, the p-values were corrected according to Bonferroni. Nonparametric statistics were used in (study II-III); all tests were two-tailed. Descriptive data were presented with median and range (max-min). Dependent variables were studied with Wilcoxon-matched-pair method (study III). Fisher’s exact test was used to compare binary outcomes between independent subgroups, e.g. a substantial decrease in pain intensity or not (study III). The Mann-Whitney U-test was used to compare non-binary differences including categorical i.e. numerical rating scales and Likert scales between the groups (study I-IV) and independent subgroups (study III). The Mann-Whitney U-test was used to compare non-parametric outcomes in continuous variables i.e. detection and pain thresholds and βE levels between groups (study II and IV and between independent subgroups (study III). The Friedman-test was used to study changes in the detection thresholds and pain thresholds before, during and after CPT, the Friedman-ANOVA was confirmed with Wilcoxon-signed-rank test, and only changes confirmed by both methods were considered (study IV). Spearman rank-correlation test (two-tailed) was used to investigate the correlations between pain-intensity and pain thresholds (study I), βE, EDTs, EPTs and PPTs, (study II), pain thresholds, βE and changes in pain intensity (study 3) and cortisol and βE (study IV). In study IV, The CSQ was analyzed item by
item and the subclasses for cognitive coping strategies and behavioral coping strategies were analyzed by comparison of area under the curve; all CSQ analyses were made with Mann-Whitney U-test (study IV). A p-value < 0.10 was considered as a trend and a p-value < 0.05 was considered as significant (study I-IV).
4 METHODOLOGICAL CONSIDERATIONS

4.1 NUMERICAL RATING SCALE
The numerical rating scale (NRS) was used for measuring pain intensity and unpleasantness. The use of an eleven point NRS scale is a widespread for measuring self-reported pain intensity. There are different scales for evaluating pain, i.e. visual analogue scale VAS and NRS, the NRS is recommended for chronic pain. The NRS is preferred by patients, abstract and easy to understand (Dworkin, R.H. 2005). Decreases on the eleven-point NRS-scale after clinical interventions have been evaluated against the perception of clinical improvement by chronic pain patients (Farrar, J.T. 2001). The NRS is an ordinal scale and non-parametric statistics should be used for evaluation.

4.2 PAIN PRESSURE THRESHOLD
The PPT is validated and is reliable both between different operators and between different measurement times in the same operator-research person setting; however, the result is dependent on site of measurement (Ohrbach, R. 1989a; Ohrbach, R. 1989b). At least a mean of two measurements are suggested (Ohrbach, R. 1989a; Ohrbach, R. 1989b). In this thesis we used a mean of two or three measurements since this have been shown to be more reliable than single measurements (Isselee, H. 1997; Ohrbach, R. 1989a)

4.3 ELECTRICAL DETECTION AND PAIN THRESHOLD
The PainMatcher® is a valuable tool for measuring detection and pain thresholds (Lund, I. 2005). However, the tool may both act as a matcher of pain and to measure the pain threshold (Alstergren, P. 2003).This dual use may induce an instructor or operator induced error. It offers a blinded measurement, reducing operator bias since it may be used by the patient herself.

4.4 COLD PRESSOR TEST
A range different conditioning stimuli been used to produce DNIC, e.g. cold and heat, ischemic, and muscle pain induced by glutamate or saline injections (van Wijk, G. 2010). It has been shown in animal swim-stress-model, that a stimulation degree of (4-15ºC), allows for activation of a non-opioid dependent DNIC (Marek, P. 1992; De Felipe, C. 1998).
In study I, all healthy volunteers initiating the CPT of 2-4° C was included for analysis. The CPT was both used as an HNCS and a measure of pain tolerance, the HNCS did not differ between gender and was mean (SD) 196 (63) s, with a range between 64-300 s, (n=30). However, with regards to findings in animal models, times in the lower part of this range might allow only for activation of a non-opioid dependent DNIC.

In study IV we had a strict threshold for CPT as we only included patients that endured having the patients as long as it took for the pain measurements (approx 4 min 30 sec. to 5 min). The research persons were thoroughly informed that they were free to elevate their hand from the ice-bath at any time. Research persons withdrawing their hand was excluded from the subsequent analysis. Previously Bullinger et al have shown that cold pressor test with “ice water” for 60 seconds did not produce any changes in healthy volunteers (Bullinger, M. 1984). Inclusion to analysis only of research persons enduring CPT is in line with other investigators (Arendt-Nielsen 2008). In another study, 17% of the research persons were unwilling to hold their hand in 10° C, why the withdrawal of 25% is not overtly strange. However, this level of withdrawal signals that the level of pain endured by the research persons was considerable, and the NRS rating of pain and unpleasantness were median both median 6 and 7, for healthy controls and patients respectively.

4.5 REPETITIVE MEASUREMENTS OF PAIN THRESHOLDS

It may be argued that the use of the median of three measurements is more appropriate than the use of mean. The median would provide a more correct measure of three repetitive measurements, than the mean, since normality is hard to argue. Furthermore a non-parametric approach is favorable since a perceived sensation is measured. The use of medians, instead of means allows also for less data handling errors, since the calculation of a median of three is more easy and quicker than the calculation of a mean.
5 RESULTS

5.1 COLD PRESSOR TEST

5.1.1 Healthy volunteers

There were baseline significant differences between genders, regarding the EPT over the incisor (p=0.014), between males with a mean (SD) of 29.8 (8.9) V, and females, 22.3 (6.5) V, and PPT over the finger (p=0.003), where males reached mean (SD) 555 (135) kPa, and females 395 (136) kPa. Region and stimuli specific changes are shown in figure 6.

![Figure 6](image)

**Figure 6.** The mean (SD) relative changes to baseline (100%) of electrical (EPT) and pressure pain thresholds (PPT) in the finger and orofacial region during and 5 and 15 min after heterotopic noxious conditioning stimulation (HNCS) in 15 healthy female subjects and 15 age-matched male subjects. (A) EPT in the finger, (B) EPT in the orofacial region (incisor), (C) PPT in the finger, and (D) PPT in the orofacial region (masseter muscle). EPT and PPT in the finger and PPT in the orofacial region increased significantly during HNCS for the entire group compared to baseline (p<0.001). EPT in the finger was also significantly increased compared to baseline 5 and 15 min after HNCS (p=0.008 and p=0.038, respectively), as was PPT in the masseter muscle (p<0.001 for both time-points). The EPTs over the central maxillary incisor remained unchanged during the while experiment (B).

There were no significant differences between males and females regarding DNIC response. However, there were differences between regions and stimuli types in the
same region, for details see figure 7. The pain tolerance for CPT of 2-4°C, and thus the HNCS did not differ between gender and was mean (SD) 196 (63) s, with a range between 64-300 s, (n=30).

**Figure 7.** Bars denoting the mean (SD) changes relative to baseline (100%) of pressure pain thresholds (PPT) and electrical (EPT) and over the finger and in the trigeminal region, over the masseter muscle and over the central maxillary incisor respectively in 30 healthy subjects. The recordings of pain thresholds were made during-, and 5 and 15 min after heterotopic noxious conditioning stimulation by cold pressor test. *Significant difference between regions; #significant difference between stimulus type in the same region (Holm-Sidak method; p<0.05)
5.1.2 Patients with atypical odontalgia and controls

In AO patients the EPTs over the central maxillary incisor decreased (p=0.012) during CPT, while healthy controls remained unchanged. The difference between the groups were significant, and was the only difference found when comparing differences in changes from baseline between the AO and controls (Figure 8). The healthy controls had low electrical pain thresholds in the dentoalveolar region compared to AO before and after CPT, but not during CPT. Only dynamics in pain thresholds significant by Friedmann Anova were further analysed. In AO, The EPTs and PPTs over the finger were unchanged during, 5 min and 15 min after the termination of the CPT, while the PPT over the master muscle increased from baseline. The healthy controls increased in PPT over both the masseter and PPT and EPT over the finger, for details please see table 2.

Figure 8. Bars (median, whiskers denoting 10:e and 90:e percentile) showing the change in percent in pain thresholds induced during 2 degree Celsius water cold pressor test (CPT) in nine patients with atypical odontalgia (AO) and in nine healthy controls. * Significant change in the electrical pain threshold over the maxillary incisor, comparing baseline and CPT, Mann Whitney U-test (two-tailed), (U=11.5, Z=2.10, p=0.028). Bars not filled represents AO patients, dashed bars represents healthy controls.
Table 2 Median (min-max) electrical detection thresholds (EDTs), electrical pain thresholds (EPTs) and pressure pain thresholds (PPT) in patients with atypical odontalgia (n=9), and healthy volunteers controls (n=9), at baseline, during cold pressor test (CPT), as well as 5 and 15 min thereafter.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>During CPT</th>
<th>5 min after</th>
<th>15 min after</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incisor (V)</td>
<td>19 (11-41)</td>
<td>-</td>
<td>26 (11-48)</td>
<td>23 (11-48)</td>
</tr>
<tr>
<td>Finger (0-99 au)</td>
<td>4 (3-6)</td>
<td>-</td>
<td>5 (3-7)#</td>
<td>5 (3-8)</td>
</tr>
<tr>
<td>EPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incisor (V)*</td>
<td>28 (17-99)</td>
<td>23 (19-48)γ</td>
<td>35 (23-99)</td>
<td>30 (24-99)</td>
</tr>
<tr>
<td>Finger (0-99 au)</td>
<td>9 (4-9)</td>
<td>8 (5-9)</td>
<td>11 (3-99)</td>
<td>10 (4-99)</td>
</tr>
<tr>
<td>PPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masseter (kPa)*</td>
<td>260 (0-800)</td>
<td>417 (1-800)#</td>
<td>303 (1-800)#</td>
<td>373 (1-800)#</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incisor (V)</td>
<td>20 (11-32)</td>
<td>-</td>
<td>19 (7-34)</td>
<td>15 (10-37)</td>
</tr>
<tr>
<td>Finger (0-99 au)*</td>
<td>4 (3-6)</td>
<td>-</td>
<td>5 (3-7)#</td>
<td>4 (3-7)#</td>
</tr>
<tr>
<td>EPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incisor (V)</td>
<td>21 (15-36)τ</td>
<td>24 (13-35)</td>
<td>21 (19-50)τ</td>
<td>21 (18-37)τ</td>
</tr>
<tr>
<td>Finger (0-99 au)**</td>
<td>9 (5-16)</td>
<td>13 (6-21)#</td>
<td>12 (6-19)#</td>
<td>12 (5-19)#</td>
</tr>
<tr>
<td>PPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masseter (kPa)**</td>
<td>339 (253-441)</td>
<td>467 (262-672)#</td>
<td>448 (260-497)</td>
<td>405 (283-591)</td>
</tr>
<tr>
<td>Finger (kPa)**</td>
<td>723 (617-938)</td>
<td>983 (793-1213)#</td>
<td>922 (559-981)</td>
<td>777 (472-978)</td>
</tr>
</tbody>
</table>

V=volt, au=arbitrary units, ** Significant difference induced by CPT, Friedmann ANOVA (p<0.01). * Significant difference induced by CPT, Friedmann ANOVA (p<0.05). # Significant increase induced by CPT compared to baseline (p<0.05). ## Significant increase induced by CPT compared to baseline (p<0.01). γ Significant decrease induced by CPT compared to baseline (p<0.05). τ Significant difference between the groups (p<0.05).

There were no significant difference between patients and controls regarding the pain intensity and unpleasantness scored on the NRS scale and PainMatcher® during CPT.
5.2 PLASMA $\beta$-ENDORPHINS

5.2.1 Patients with chronic closed lock and controls
At the preoperative assessment, female patients with CCL had significantly higher plasma $\beta$E levels, compared to age matched healthy female controls (Figure 9).

**Figure 9.** Box plot (median 25% and 75% percentiles as well as min and max values showing the plasma $\beta$E in 18 female patients with chronic closed lock (CCL) of the temporomandibular joint compared to age and sex matched controls. The plasma $\beta$E levels in the patients were significantly higher ($p=0.013$) compared to the controls.
5.2.2 Patients with chronic closed lock after discectomy

Figure 10. Box plot showing changes of plasma β-endorphin (βE) (pmol/L) measured preoperatively and median (range) 8 (6-24) months after discectomy in patients with (n = 8) or without (n = 3) a substantial decrease of pain intensity (≥50%) measured on an eleven point NRS scale, (Mann Whitney U-test; p=0.024). The square is denoting the median and the box are limited by the 25% and 75% percentiles while the whiskers are showing min and max values.

The plasma level of βE significantly decreased (p=0.032), to median (range), 12 (9-13) pmol/L, from the preoperative level of 14 (9-29) pmol/L. A clinically substantial decrease in maximal pain intensity, i.e. a 50% decrease in NRS or more, was necessary for a decrease in plasma βE (p=0.003), i.e. all the patients, 7/7, that decreased in plasma βE had a substantial decrease in maximal pain intensity, vs. none of the patients that did not reach this level of pain relief, 0/4. The relation between a clinically substantial decrease and plasma βE is shown in figure 10. The plasma βE level decreased with time (months) after surgery (r_s=-0.62, p=.041, n =11).
5.2.3 Patients with atypical odontalgia and controls

The BE response was significantly higher in healthy controls vs. AO (p=0.014), (Figure 11).

* Denoting an increase in healthy controls, CPT stimulated a significant increase in plasma βE (p=0.021), this increase was absent in patients with atypical odontalgia (p=0.5).
5.3 PAIN ASSESSMENT IN TMJ PATIENTS

The PPTs over the most painful masseter muscle was significantly lower in patients compared to female controls. In addition, the patients also showed significantly lower PPTs over the fingers. This findings show that patients with CCL have central sensitization which is lowering the threshold for pain, for details, please see table 3.

Table 3. Electrical detection threshold (EDT) and pain thresholds (EPT; au), as well as, pressure pain thresholds (PPT kPa), in 19 female patients with limited jaw function and movement evoked pain of the temporomandibular joint and 18 female age-matched controls

<table>
<thead>
<tr>
<th>Threshold and site</th>
<th>Side</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTs finger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>4 (2-8)</td>
<td>4 (2-6)</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>4 (3-7)</td>
<td>3 (2-6)</td>
<td></td>
</tr>
<tr>
<td>EPTs finger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>8 (3-19)</td>
<td>11 (5-25)</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>8 (4-30)</td>
<td>10 (5-17)</td>
<td></td>
</tr>
<tr>
<td>PPTs finger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>*493 (197-1584)</td>
<td>825 (585-1283)</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>*566 (173-1304)</td>
<td>829 (624-1232)</td>
<td></td>
</tr>
<tr>
<td>PPTs masseter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>*331 (167-501)</td>
<td>424 (243-1157)</td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>369 (176-755)</td>
<td>397 (253-941)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median (range). The EDTs and EPTs were recorded over the fingers, while the PPTs were recorded bilaterally over the masseter muscle. *Significant difference compared to the healthy subjects (p < 0.05).

At the follow-up, median 8 months, range (6-24), the pain intensity had significantly decreased, both pain at rest and movement-evoked pain from the TMJ, for details see table 4. In contrast, there were no significant changes of EDTs, EPTs or PPTs comparing the preoperative and postoperative levels.
In specific, the preoperative EPTs over the fingers correlated to the preoperative pain intensity at rest (ipsilateral: $r_s = -0.70, p=0.016, n =11$, and contra lateral: $r_s=-0.61, n=11, p=0.044$). The change in ipsilateral EPT correlated to the decrease in movement-evoked pain, and the decrease in maximal pain-intensity, both ($r_s= 0.71, p= 0.014 n =11$). Finally, a decrease of maximal pain intensity correlated inversely to PPT over the contra lateral finger ($r_s=- 0.68 p= 0.020, n=11$).

There was also a substantial decrease in maximal pain intensity in 8 out of 11 patients. The decrease in maximal pain intensity correlated significantly with the decrease of plasma $\beta$E (Figure 12).

![Figure 12](image)

Figure 12. Scatter plot, showing the correlation between change of plasma $\beta$-endorphin ($\beta$E) level (pmol/L) and change of maximal pain intensity (NRS 0-10) > 6 months after TMJ discectomy in patients with chronic closed lock (Spearman rank correlation; $r_s=-0.63, p=0.038, n=11$).
5.4 COPING STRATEGY QUESTIONNAIRE

The AO patients had a tendency towards a higher score to the factor “catastrophizing” and a decreased score for the factor distancing. The scores for the individual items, significantly different between AO and controls are depicted in table 5.

<table>
<thead>
<tr>
<th>Items/ Questions (Likert scale range)</th>
<th>Patients’ score median (range)</th>
<th>Controls’ score median (range)</th>
<th>Mann Whitney U-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>When I am in pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I feel like life is not worth living (0-6)*</td>
<td>1 (0-4)</td>
<td>0 (0-0)</td>
<td>0.036*</td>
</tr>
<tr>
<td>I relax (0-6)</td>
<td>3 (0-6)</td>
<td>5 (4-6)</td>
<td>0.036*</td>
</tr>
<tr>
<td>I ignore the pain (0-6)</td>
<td>2 (0-6)</td>
<td>5 (3-6)</td>
<td>0.018*</td>
</tr>
<tr>
<td>I know I will be able to handle the pain no matter what (0-6)</td>
<td>3 (0-5)</td>
<td>4.5 (2-6)</td>
<td>0.036*</td>
</tr>
<tr>
<td>I worry that the pain never will disappear (0-6)</td>
<td>2 (0-5)</td>
<td>0 (0-3)</td>
<td>0.026*</td>
</tr>
<tr>
<td>I pretend it is not me who is in pain (0-6)</td>
<td>0 (0-3)</td>
<td>2.5 (1-4)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Effectiveness rating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How great is your control over your pain an ordinary day (0-6)</td>
<td>3 (0-6)</td>
<td>5.5 (4-6)</td>
<td>0.013*</td>
</tr>
</tbody>
</table>
6 GENERAL DISCUSSION

In this thesis, the dynamics of descending inhibition or central sensitization and neuroendocrine analgesia were investigated; in healthy volunteers, during painful internal derangement from the temporomandibular joint, following pain-relieving discectomy, and finally in patients with possible orofacial neuropathic pain.

Before discectomy, female CCL patients showed signs of central sensitization, with significantly lower PPTs over the most painful masseter muscle and over the index fingers, compared to healthy age-matched controls. In addition, the βE levels were elevated. Central sensitization has been pointed out as an important factor in osteoarthritis of the knee (Arendt-Nielsen, L. 2010). Previous studies have shown that patients with TMD have lower pain thresholds in the area of pain, as well as generally (Svensson, P. 2001; Maixner; W. 1995; Ayesh, E.E. 2007). At follow up, median (range) 8 (6-24) months after surgery, the pain pressure thresholds in these female CCL patients did not increase in the group as a whole. Instead, after pain-reliving discectomy, the increase in PPT in CCL patients was related to the magnitude of decrease in pain intensity. The restoration of βE levels, in turn, were dependent both on time since surgery (months) and a on a clinically substantial decrease in pain-intensity.

In this study we measured a blood sample, and pain thresholds at regions distant from the TMJ. When analyzing these outcome measures, not immediately related to the TMJ, but most probably also effected by the overall homeostasis, we were paying attention to differences in the maximal pain intensity present. In this measure we included not only pain at rest from the TMJ, and movement-evoked pain from the TMJ, but also pain-elsewhere, this to get an overall measure of the pain of the patient. In animal studies it has been shown that when central sensitization is developed, changes in the receptor fields due to prolonged nociceptive inputs is present (Grubb, B.D. 1993). Furthermore, central sensitization is lowering the threshold for pain as modification of the first order synapse occurs, and the descending control is modulated (Grubb, B.D. 1993; Pertovaara, A. 2000; Woolf, C.J. 2004). To counteract the increased hyperexcitability, the descending control of deep lamina nociceptive specific neurons and WDR neurons is increased, and in response to concomitant stress, pain and inflammation, βE is released (Grubb, B.D. 1993). Pain and inflammation activates
NMDA NR1 receptor in arcuate nucleus of the hypothalamus (Peng, J.M. 2011) 
NMDA is activated both on spinal and supraspinal level as a part of the sensitization secondary to tissue damage, and in hypothalamus the NMDA receptor activation is increasing (ACTH) adrenocorticotrophic hormone release (Farah, J.M., Jr 1991; Bach, F.W. 1995). In particular, during CCL inflammation, IL-1β is present in the TMJ endothelia, in amounts correlating to VAS (Suzuki, R. 2005). IL-1β activates hypothalamic CRF, the initial peptide of the HPA-axis and a trigger for down-stream βE release (Kageyama, K. 2009; Melik Parsadaniantz, S. 1994). Further, βE is released by adrenergic stimulation; both acting direct on the pituitary gland and subsequently to noradrenergic input from the locus coeruleus, stimulating the CRF release in the hypothalamus during the fight-and flight reaction (Ishac, E.J. 1987; Ziegler, D.R. 1999). In line with several models of stress and pain, the increased preoperative βE level in CCL patients may be secondary to an activation of the HPA-axis by inflammatory pain, modulated by peripheral and central sensitization. However, the level of stress in the situation of preoperative planning cannot be ruled out as a factor influencing the increased preoperative βE level.

At the follow up after surgery all of the patients were pain free over the TMJ at rest, while the jaw movements were pain free in 10 out of 11 patients. It has been recommended to report not only a significant improvement of pain intensity, but also if chronic pain patients reached a substantial (clinically relevant) improvement in the pain intensity (Farrar, J.T. 2001). This is characterized by a decrease of ≥50% or ≥4 points on the NRS scale which is associated with ratings of “very much improved” by the patients (Farrar, J.T. 2001; Dworkin, R.H. 2005). The association between βE recovery and this important level of clinical improvement indicate that the nerve-system and the HPA-axis may readapt from changes induced by chronic pain and disability when the patient is in a pain free state. Further, this indicates that the of the HPA-axis may normalize, at the same time as the afferent input to the hypothalamus decreases. This suggests further that the perception of a clinically relevant improvement in pain is mirrored in the neuroendocrine response, suggesting a psychoneuroendocrinological coupling between the emotional perception of pain and the plasma βE level. It seems like both plasma βE and PPT depend on pain intensity, although the plasma βE is more likely to recover after pain relieving surgery. The NRS have been criticized, but the present findings, suggest that the long-time postoperative change in pain-intensity,
measured by an eleven point NRS-scale, is related to the changes in neuroendocrine opioid release and possibly also to changes in central sensitization.

The reports concerning gender differences in DNIC are diverging. Several reports have found no gender difference in the DNIC response while other studies have shown gender differences (Arendt-Nielsen, L. 2008; Goodin, B.R. 2009, Lautenbacher, S. 2008; Pud, D. 2005; Baad-Hansen, L. 2005). In healthy controls, in our setting, there was no difference between gender regarding the DNIC effect which is in line with about half of the published studies on sex differences in DNIC response (van Wijk, G. 2010). However, although healthy volunteers did not show any gender differences regarding the DNIC response, there were region and stimuli specific changes. In contrast to AO, all pain thresholds in healthy controls and volunteers except the EPTs over the central maxillary incisor increased during DNIC. In healthy controls, Fujii et al have found that ischemic pain trigger the DNIC response over the tooth, but the effect is depending on the effect, intensity and quality of the stimuli (Fujii, K. 2006). In none of our studies we could prove a substantial DNIC effect over the tooth at pain threshold level. The different findings may be explained by the use of different methods for heterotopic noxious conditioning stimuli and by the suprathreshold level used by Fuji et al. to evaluate DNIC. While DNIC was predicted to be maximal during CPT, analgesia elicited by βE was expected in the later measurements, at 5 min and 15 min. The plasma βE increased significantly in healthy controls after CPT, also compared to AO. The healthy controls had in addition significant increases in electrical detection thresholds and electrical pain thresholds over the finger at 15 minutes, these changes were absent in AO. However, the increase in thresholds at 15 minutes was not significant when comparing the two groups, and no opioid antagonist were supplied, why a possible neuroendocrine analgetic effect could not be supported.

To our knowledge, this is the first study examining patients with AO by DNIC and plasma βE. The healthy controls increased their plasma βE after cold pressor test. This is in line with (al'Absi, M. 2004) who showed that this response and the ACTH elevation secondary to CPT were gender non-specific, and could not be blocked with naloxone. In contrast, patients with AO did not respond with an βE elevation after CPT, which compared to healthy controls may be regarded as an absence of a dynamic neuroendocrine analgesic response to CPT. In particular; AO had significantly higher
plasma cortisol levels than healthy controls. Further, in healthy controls there was a trend to a correlation between plasma cortisol and plasma βE, which was absent in AO patients.

Baad Hansen has previously suggested that the chronic pain in AO-patients with pain from a tooth, or from an area where a tooth has been removed, is not due to peripheral sensitization. The argumentation is that as the response to capsaicin induced pain was present on both the painful and the non painful side, a central mechanism to the increased pain was involved (Baad-Hansen, L. 2006). The AO-patients in this thesis, all had pain from an area where a tooth, or teeth had been removed. Compared to healthy controls, the AO had higher EPTs in the dentoalveolar region at baseline, and at 5 min and 15 min. In contrast, the EPT over the central maxillary incisor in the patients with AO were reduced during CPT, whereas they were unchanged in healthy controls, with a significant difference between groups. The reduced DNIC is a sign of a change in the pain modulation. In a previous study Arendt-Nielsen and colleagues found that a reduced DNIC response was present when two experimental stimuli were applied simultaneously (Arendt-Nielsen, L. 2008). Patients with AO often report more than one pain area and often the pain is spread to other regions, in some patients the pain are crossing the midline. If DNIC is influenced by how many pain areas the patient report in the body at a given time, the state of central sensitization might be prolonged by an impaired DNIC response (Arendt-Nielsen, L. 2008; van Wijk, G. 2010; Yarnitsky, D. 2008). This is in agreement with Lautenbacher and Rollman, who showed that the electrical pain thresholds were decreased during thermal stimulation in patients with fibromyalgia (Lautenbacher, S. 1997). Further, Kosek and colleagues found that the PPT in fibromyalgia patients was decreased during DNIC performed by a sub maximal effort tourniquet test (Kosek, E. 1997). In another study, patients with atypical trigeminal neuralgia had less descending inhibition compared to patients with classical trigeminal neuralgia and healthy controls (Leonard, G. 2009). Further, a shift towards pain facilitation in the descending pain modulating system has been proposed to partly explain reduced PPTs over the tibialis anterior following fatiguing muscle contraction in fibromyalgia patients (Ge, H.Y. 2011). The reduced EPT of AO in the dentoalveolar area, may be explained in similar terms, by the presence of pain facilitation. Astrocytes have been suggested to be involved, both when the pain crosses the midline, and recently in descending facilitation (Obata, H. 2010; Gu, M. 2011, Obata, H. 2010). By diverse means, e.g. by tooth pulp injury, by nerve ligation injury in the trigeminal area,
by experimental inflammation, i.e. CFA injection into the TMJ, or into the masseter muscle, microglia is up-regulated in the trigeminal caudal nucleus (Canzobre, M.C. 2010; Villa, G. 2010; Xu, M. 2008; Guo, W. 2007). In particular, after third molar surgery, measured by bilateral pain thresholds, the hyperalgesia spreads across the midline and may be present during at least a week after tooth extraction (Emberg, M. 2007). A nerve injury model of the infraorbital nerve have further shown that glial activation occurs not only in the trigeminal nucleus, but also in the RVM, where NMDAR is up regulated (Wei, F. 2008). Activation of RVM would normally lead to increased descending control, a 5HT dependent activation of interneuron and subsequent suppression of dorsal horn neurons has been suggested (Giordano, J. 2004). However, Gu and colleagues recently suggested, after studies in both nerve ligation model and peripheral inflammatory model, that electrical stimulation of RVM may result in descending facilitation mediated by a neuronal-glial-neuronal signaling at the spinal level (Gu, M. 2011). Thus, hypothetically, in light of these findings, our results of a decrease EPT over the central maxillary incisor during CPT can be explained by pain facilitation mediated by RVM hyperexcitation and neuronal-glia-neuronal facilitation of noxious transmission in the caudal trigeminal sub nucleus. In this model of explanation, neuroimmune changes involving glia at both in the midbrain PAG-RVM system, and at medullary dorsal horn level (caudal trigeminal sub nucleus) is part of the mechanism leading to descending facilitation. In the neuronal-glia-neuronal model, IL-1β produced by astrocytes is presented as the substance that up regulate NMDAR (Gu, M. 2011). IL-1β is also increasing the permeability of the blood brain barrier (Blamire, A.M. 2000). The vast majority (96%), of the βE suggested to be released from the pituitary secondary to inflammatory pain is acetylated (Basbaum, A-I. 1984). Interestingly, in a neuroinflammatory model, βE decreases the evoked potential mediated by 5HT (Hansson, E. 2008). This suggests that plasma βE participates in a neuroinflammatory feedback loop during acute inflammatory pain. βE is allowed passage to the area of IL-1β production by a permeable blood brain barrier, and well there they may inhibit the astrocyte and decrease the pronociceptive neuroinflammatory activity. The above suggestion of a neuroinflammatory feedback loop find partly support in studies of direct or acute changes, the situation is more uncharted in the chronic state.
The βE has been shown to decrease in parallel the severity of rheumatoid arthritis, and a correlation both with inflammation, pain and more advanced medication is possible (Elbeialy, A. 1997). Chronic morphine load and inflammation has been suggested to increase descending facilitation of tail stimulation in a rat model where micro injections of lidocain in RVM recovered DNIC induced by thermal conditioning in a heat ramp (Okada-Ogawa, A. 2009). The interpretation of the findings from the present study discussed so far may result in the hypothesis that; the presence of pain facilitation in AO, together with an HPA-axis sensitization, and a supposedly chronically activated neuroendocrine opioid system, is a sign of a globally sensitized pain modulating system.

The CSQ showed signs of altered coping strategies and a decreased pain control in patients with AO. Specifically, the CSQ question regarding ambivalence to life during pain was significantly higher for AO patients than for healthy individuals. Furthermore the patients had a tendency to catastrophizing compared to healthy controls. Ambivalence is a sign of hopelessness, and hopelessness has been associated to suicidality, (Beck, A.T. 1974) Longitudinal studies suggest that chronic pain patients are at higher risk for completed suicide than comparable controls (Fishbain, D.A. 1991; Penttinen, J. 1995). Suicide is a preventable cause of death, and one patient planned for CPT was admitted to acute psychiatric care for suicidality. Secondary psychiatric illness, not at least depression is a known co morbidity in AO, and this stresses why psychiatric competence need to be included in the multidisciplinary team in care of these patients (Clark, G.T. 2006; Dickinson, B.D. 2010; Madland, G. 2001) Cognitive-Behavioral-Therapy has improved catastrophizing and depression scores, as well the amount of patients with clinically substantial pain-relief in patients with TMD (Turner, J.A. 2006). AO and TMD share a moderate to severe score for depression, and the same level of pain intensity (Baad-Hansen, L. 2008, Ernberg 2011). In conclusion, considering the altered coping presented in this study, a CBT-intervention may have beneficial effects also in AO.
7 LIMITATION

7.1 STUDY I
No control bath was used. Different devices were used to measure the electrical detection and pain threshold over the tooth compared to over the finger. In addition, measurement was made over different tissues, end-phalanx of index finger and tooth. Pain-tolerance was defined as the time of the hand in the ice-bath, why the provocation stimuli was not set at a distinct level.

7.2 STUDY II
The goal of this study was to include CCL patients. TMD is a heterogenic-group, therefore we chose to investigate CCL patients, to investigate patients with long-term pain. The duration of pain from the TMJ before assessment in this study was 1-17 years, which possibly might have an effect on the stress level, the co morbidity and underlying neurobiological changes.

The level of stress, was not estimated by biological measures exclusive from plasma-βE. Most probably CRF, ACTH and cortisol would have added valuable information.

7.3 STUDY III
In line with the previous study, this study was confined to females in accordance to the recommendations from the special interest group for the study of pain (IASP) (Greenspaan, J. 2007).

This study was initiated while the first study was underway, why this does not constitute a follow-up of study II.

7.4 STUDY IV
This was designed as a prospective case control study of a strict interpretation of the AO diagnosis. However, the patient flow through the study was hindered by a frequent withdrawal from CPT. Further, when registrating the data, it was revealed from the general health questionnaire that a proportion of the control group were in pain, NRS>0, or had chronic pain problems, such as headache and back pain why they were excluded from analysis.
The diagnostic criteria’s of AO are under ongoing discussion. In this study we only included patients with pain from an area with an extracted tooth or teeth. The inclusion criteria for this study are mirroring the proposed diagnostic criteria’s of CCDAP (chronic continuous dentoalveolar pain).

This study includes both male and female participants, while there were no gender differences in study I. However, this is an extrapolation, whether or not the absence of gender differences concerns patients is not known. The recommendations from the special interest group for the study of pain (IASP) should if possible be regarded in this diagnosis as well (Greenspaan, J. 2007).

In summary, it is difficult predicting which patients that will fulfill the diagnosis of AO, while the diagnostic criteria is fulfilled only if there is no other explanation to the pain. In this study, three patients under investigation for possible AO were diagnosed with trigeminal neuralgia. We wanted to study pain, why we chose research persons without any pain. This selection allows for bias and furthermore does imply, that some parts of the pattern here specific for AO, might be present in persons considering themselves of general good health.
8 CONCLUSION

8.1 MAIN CONCLUSION

The outcome measure of pain relieving surgery is feasibly a clinically substantial improvement in maximal pain intensity, here confirmed by a recovery in plasma β-endorphin. Afference from the pulp was absent from descending inhibition in healthy, and promoted by descending facilitation in atypical odontalgia, indicating that the dentoalveolar region is vulnerable to development of chronic pain.

8.2 SPECIFIC CONCLUSIONS

8.2.1 Study I

Except for over the maxillary incisor where no effect could be obtained, healthy controls showed a DNIC effect during cold pressor test. The proportion of change in pain thresholds and test stimuli type during DNIC differed between the orofacial and spinal region regarding pressure and electricity. In this study, there were no gender differences regarding the DNIC response.

8.2.2 Study II

Patients with CCL show signs of central sensitization with decreased pain thresholds in the orofacial area and over both index fingers. These patients also showed increased plasma βE levels. The increased neuroendocrine opioid level in the bloodstream is probably a counteract to the central sensitization, peripheral inflammation and stress.

8.2.3 Study III

In CCL patients, with a median range 8 (6-24) months after discectomy, plasma βE decreased with time. The movement-evoked-pain from the TMJ disappeared in 10 out of 11 patients. Noteworthy, a clinically substantial decrease in maximal pain intensity was needed for a decrease in plasma βE. In addition, increased PPT, a possible sign of decrease in central sensitization correlated to the decrease in maximal pain intensity. On the whole, central sensitization could not be proven relieved.
8.2.4 Study IV

Patients with atypical odontalgia have signs of HPA-axis hyperactivity axis with disturbed neuroendocrine opioid release and altered ability to coping.
9 FUTURE PERSPECTIVES

The first study investigates differences in the spinal and trigeminal region. The results raise the question regarding differences in anatomical regions, if pain threshold measurements would have been streamlined, e.g. pressure measurements on muscles versus muscles in the trigeminal region, i.e. stimuli specific and region specific changes.

Standardized protocols regarding DNIC studies need to be developed to make the comparison of results possible between different studies. Furthermore, standardized protocols are needed not only for research but also for future possibly clinical use.

The function of the DNIC effect with regards to the evolutionary aspect is interesting. For example, it would be interesting to investigate DNIC effects larger muscle groups involved in mobility and breathing as during an experimental fight and flight reaction.

Further the morphological picture of the joint, both radio graphically and intraoperatively in correlation to the pain intensity, central sensitization, the cytokine levels from the joint, in relation to the plasma levels and the neuroendocrine activation measuring both βE, ACTH, cortisol and CRH would be of interest. The most interesting finding would be a peripheral biomarker correlating to the severity of joint degeneration and inflammation.

Predictors for long-term postoperative pain need to be found. The preoperative assessment and the postoperative follow-up are two time-points where information could be gathered to find such predictors. If patients at risk for persistent postoperative pain and disabling central sensitization is found at the preoperative assessment these might need increased support postoperatively if surgery is inevitable.

Maximal pain intensity, pain at rest and movement-evoked pain is found to be clinical measures with a correlation to pain thresholds and neuroendocrine response. The value of these bedside measures as predictors of clinical outcome need to be further evaluated.
The presence of central sensitization in CCL patients, extending to pain facilitation in the AO patients suggests an involvement of cytokine driven changes of astrocyte and microglia origin in the central nerve system. Antidepressants have previously been shown to have positive effects in a subpopulation of chronic pain patients having undertaken total knee replacement secondary to osteoarthritis. In addition to a pharmacological intervention of the serotonin system, anti-inflammatory and immunosuppressive therapy ought to be evaluated in patients with persistent pain.
10 ACKNOWLEDGEMENTS

Many people contributed to this thesis, I specially want to to express my sincere gratitude to all research persons, all patients and healthy volunteers, that participated in these studies

In particularly to,

My supervisor, Associate Professor Annika Rosén, Head of the Division for Oral and Maxillofacial Surgery, for introducing me to the research field of pain. I am sincerely grateful for all science you have taught me during these years. Thanks for keeping your door open, for your enthusiasm, and the discussions about life and science.

My deepest appreciation goes to my co-supervisor Associate Professor Malin Emberg. I heartly thank you for all science you thought me during this thesis. Thanks for sharing your knowledge with me, and for your generous support in many ways.

My external mentor, Med. Dr. Irene Lund for support and discussions and fun work together with the television program about pain for utbildningsradion

I wish to thank people who inspired me to start a research career, Professor Kaj Fried, for the trigeminal nuclea you drew on a whiteboard in Solna when I was a student, Anna Josephson for being the most pedagogic teacher, Bertil Fredholm for giving me an understanding how complex things are.

A special thank goes to Professor Ernst Brodin, for being a role model in teaching. Thank for your ability to understand, and teach research.

To Anders Heimdahl, former Head of the Division of Oral and Maxillofacial Surgery, Thanks for support during the beginning of my career!

I wish to thank Professor, emeritus, Rolf Ekman. For you took the time and answered all my questions about β-endorphin.
A special thank goes to professor, Anders Holmlund for your skills in the field of TMJ in research and surgery.

I greatly thank to my co-author, Bodil Lund for being competent co-author and for being a good friend.

I would like to thank Rita Persson, Karin Coster, Kerstin Bergman and Ann-Sophie Thormann for accurate and for including research persons, and for skillful help in the lab.

A special thanks to Agneta Engström for great support

I wish to thank all present and former staff of the Department of Dental Medicine, Division of Oral and Maxillofacial surgery.

I feel grateful for having so lovely friends: Katarina Gospic, Nussrat Kahlon, Anna Eliasson, Johanna Nielsen, Lisa Jahanfar, Saba Saad, Ilka Kenyeres, Sofia Hidén, Marcus Jonsson, Marcus Sandström Olsson, thanks for having so fantastic personalities. I really enjoy sharing my life with you!

A special thank to Ene Leipalu, for introducing me to Marathon, and to Johanna Nielsen, Anna Eliasson and Caroline Karlsson for running together with me, you rock!

I would like to thank my wonderful family for love and support. You share my happiness in success and support me in the rough times. A special thank to my mother and father. A great thank to, Bengt Feldreich and Anna Lisa Feldreich for encouragement through life and always believing in me. Thanks to Ewert Karlsson and Elisabeth Rimeika, Linnea Rimeika, Mats Larsson and Monica Larsson, Gerd Feldreich, Ann Feldreich, Annelie Feldreich, Sten Feldreich och Tina Feldreich Danielsson, Lotta Rimeika, Alf Rimeika, Dangule Rimeika, Svante Gelinder, Malin Rudberg, Björn Larsson, Andreas Larsson, Johanna Robinson, Jonatan Robinson, Larry Robinson and Irene Rimeika. Thanks, Jonas & Lotta and Tomas & Maja!
I wish to thank my dear children Eleonora Feldreich and Beatrice Feldreich for seeing the beauty in life and sharing your happiness with me—I love you!

A special thank goes to Nicolas Karlsson, for all the moments of joy, for refreshing life, you are the most amazing person person I know— I love you!

The financial support from the Department of Dental Medicine, Karolinska Institutet, Swedish Dental Society and American Dental Society of Sweden is gratefully acknowledged.
11 REFERENCES


Hao JX, Xu XJ, Yu YX, Seiger A, Wiesenfeld-Hallin Z: Baclofen reverses the hypersensitivity of dorsal horn wide dynamic range neurons to mechanical


Hubbard CS, Labus JS, Bueller J, Stains J, Suyenobu B, Dukes GE, Kelleher DL, Tillisch K, Naliboff BD, Mayer EA: Corticotropin-releasing factor receptor 1 antagonist alters regional activation and effective connectivity in an emotional-


Limchaichana N, Petersson A, Rohlin M: The efficacy of magnetic resonance imaging in the diagnosis of degenerative and inflammatory temporomandibular joint


Pud D, Granovsky Y, Yarnitsky D: The methodology of experimentally induced
diffuse noxious inhibitory control (DNIC)-like effect in humans. Pain 144:16-19,
2009.

Pud D, Sprecher E, Yarnitsky D: Homotopic and heterotopic effects of endogenous

Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC: Pain affect encoded in

Rasmussen NA, Farr LA: Beta-endorphin response to an acute pain stimulus. J

Rassnick S, Sved AF, Rabin BS: Locus coeruleus stimulation by corticotropin-
releasing hormone suppresses in vitro cellular immune responses. J Neurosci

Reynolds DV: Surgery in the rat during electrical analgesia induced by focal brain

Ribeiro-Rotta RF, Marques KD, Pacheco MJ, Leles CR: Do computed tomography
and magnetic resonance imaging add to temporomandibular joint disorder treatment?

Robinson ME, Riley JL, 3rd, Myers CD, Sadler IJ, Kvaal SA, Geisser ME, Keefe FJ:
The coping strategies questionnaire: A large sample, item level factor analysis. Clin J

Rosen A, Zhang YX, Lund I, Lundeberg T, Yu LC: Substance P microinjected into
the periaqueductal gray matter induces antinociception and is released following


