Imaging Chronic Pain and Inflammation

Positron Emission Tomography Studies of Whiplash Associated Disorder

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Abstract

This thesis is on chronic neck pain after a rear impact car injury, so called whiplash associated disorder (WAD). Three empirical studies using positron emission tomography (PET) with different radioligands have been performed.

The first study evaluated resting state regional cerebral blood flow (rCBF) in WAD patients and healthy controls, by use of oxygen-15 labeled water. Patients had heightened resting rCBF bilaterally in the posterior parahippocampal and the posterior cingulate gyri, in the right thalamus and in the right medial prefrontal gyrus. Attenuated tempo-occipital blood flow was also observed in the patient group as compared to healthy controls. Alterations in rCBF were related to patients’ neck disability ratings. Study I suggests an involvement of the posterior cingulate, the parahippocampal and the medial prefrontal gyri in WAD. This altered resting state neural activity may be linked to an increased self-relevant evaluation of pain and stress.

The second study evaluated central expression of the neurokinin-1 (NK1) receptor in WAD patients and healthy controls. Using a carbon-11 labeled specific NK1 antagonist, the receptor availability was measured. Patients displayed lowered NK1 receptor availability in the insula, anterior cingulate, frontal lobe, hippocampus, amygdala and in the periaqueductal gray matter, consistent with results from animal models of chronic pain. NK1 receptor availability was most reduced in the ventromedial orbitofrontal cortex, where attenuations were linearly related to patients fear and avoidance of movement.

Thirdly, carbon-11 labeled D-deprenyl was used to investigate the presence of locally inflamed soft tissue in the cervical neck in WAD patients. Although the retention mechanism of [11C]D-deprenyl is not known, the results suggest that WAD patients have chronic inflammatory processes in the neck, most commonly in the adipose tissue at the spinal nerve process of the second vertebra.

In summary, this thesis provides evidence for altered central blood flow and receptor characteristics in WAD patients. Further, WAD patients may also have signs of persistent peripheral tissue damage. Both central and peripheral pain mechanisms have been demonstrated and visualized in patients with whiplash associated disorder.

Keywords: Whiplash Associated Disorder, PET, brain, chronic pain, rCBF, neurokinin-1, Substance P, inflammation, D-deprenyl, fear of movement, kinesiophobia,

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The thesis is based on the following publications, which are referred to in the text by their Roman numerals:


## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Populärvetenskaplig sammanfattning på svenska</td>
<td>9</td>
</tr>
<tr>
<td>Introduction</td>
<td>11</td>
</tr>
<tr>
<td>Pain, chronic pain and whiplash</td>
<td>11</td>
</tr>
<tr>
<td>Tissue injury and inflammation</td>
<td>16</td>
</tr>
<tr>
<td>Central mechanisms of pain processing</td>
<td>17</td>
</tr>
<tr>
<td>Substance P and the neurokinin-1 receptor</td>
<td>18</td>
</tr>
<tr>
<td>Pain processing in the brain</td>
<td>20</td>
</tr>
<tr>
<td>Subcortical nuclei</td>
<td>20</td>
</tr>
<tr>
<td>Cortical pain processing</td>
<td>20</td>
</tr>
<tr>
<td>Pain behaviors</td>
<td>22</td>
</tr>
<tr>
<td>Chronic versus acute pain</td>
<td>24</td>
</tr>
<tr>
<td>Brain plasticity and neuronal loss in chronic pain</td>
<td>24</td>
</tr>
<tr>
<td>Functional neuroimaging of whiplash-associated disorder</td>
<td>25</td>
</tr>
<tr>
<td>Brain damage in WAD?</td>
<td>27</td>
</tr>
<tr>
<td>Aims and hypothesis of the thesis</td>
<td>28</td>
</tr>
<tr>
<td>Study I</td>
<td>28</td>
</tr>
<tr>
<td>Study II</td>
<td>28</td>
</tr>
<tr>
<td>Study III</td>
<td>29</td>
</tr>
<tr>
<td>Methods</td>
<td>30</td>
</tr>
<tr>
<td>Positron emission tomography</td>
<td>30</td>
</tr>
<tr>
<td>The principles of PET</td>
<td>30</td>
</tr>
<tr>
<td>Analysis of PET data</td>
<td>33</td>
</tr>
<tr>
<td>Statistical methods in neuroimaging</td>
<td>33</td>
</tr>
<tr>
<td>PET data analysis of the neck</td>
<td>35</td>
</tr>
<tr>
<td>PET scanner specifications</td>
<td>35</td>
</tr>
<tr>
<td>Pain and disability measurement</td>
<td>36</td>
</tr>
<tr>
<td>Visual Analogue Scale</td>
<td>36</td>
</tr>
<tr>
<td>Neck Disability Index</td>
<td>36</td>
</tr>
<tr>
<td>Tampa scale for kinesiophobia</td>
<td>36</td>
</tr>
<tr>
<td>Spielberger state anxiety inventory-state portion</td>
<td>37</td>
</tr>
<tr>
<td>Screening Questionaire for Post Traumatic Stress Disorder</td>
<td>37</td>
</tr>
<tr>
<td>Whiplash related disorder questionnaire</td>
<td>37</td>
</tr>
</tbody>
</table>
The empirical studies ................................................................. 38
  Participants and behavioral ratings ............................................. 38

Study I. Regional cerebral blood .................................................. 41
  Results ......................................................................................... 41
  Discussion ..................................................................................... 44
Study I. Additional analyses ....................................................... 45
  Relation to Kinesiophobia ............................................................ 45
  Individual differences in rCBF ..................................................... 46

Study II. Nerokinin-1 receptors .................................................... 48
  Results ......................................................................................... 48
  Discussion ..................................................................................... 52

Study III. Chronic inflammation ................................................ 54
  [11C]D-deprenyl ................................................................. 54
    Quantifying PET tracer uptake in neck regions ....................... 54
    Test- retest reliability ............................................................... 56
  Results ......................................................................................... 57
  Discussion ..................................................................................... 62

General discussion ...................................................................... 64
  Clinical implications ................................................................. 64
  Cause or consequence .............................................................. 65
  Future directions ........................................................................ 66
    Bringing the studies together: Multiple tracer analyses .......... 66
    Sprained ankles as a model for tissue inflammation ............... 70
  Closing comments ..................................................................... 71

Acknowledgments ....................................................................... 72

References .................................................................................. 74

Appendix with anatomical illustrations ......................................... 88
Abbreviations

ACC  anterior cingulate cortex
AUC  area under curve
BA   Brodmann area
CBT  cognitive behavioral therapy
CSF  cerebrospinal fluid
CT   computed tomography
ECD  ethyl cyteinate dimer
FDG  fluorodeoxyglucose
fMRI functional magnetic resonance imaging
HMPAO hexamethylpropyleneamineoxime
MRI  magnetic resonance imaging
NDI  neck disability index
NK1  neurokinin-1
PAG  periaqueductal gray matter
PB   parabrachial nucleus
PCC  posterior cingulate cortex
PET  positron emission tomography
PFC  prefrontal cortex
PPC  posterior parietal cortex
PTSD post traumatic stress disorder
rCBF regional cerebral blood flow
ROI  region of interest
RVM  rostral ventromedial medulla
S1   primary somatosensory cortex
S2   secondary somatosensory cortex
SP   substance P
SPM  statistical parametrical mapping
SPECT single photon emission computed tomography
STAI-s Spielberger state trait anxiety inventory, state portion
SUV  standardized uptake value
TAC  time activity curve
TSK  Tampa scale for kinesiophobia
VAS  visual analogue scale
vmOFC ventral medial orbitofrontal cortex
WAD  whiplash-associated disorder
Smärta i nacken efter en trafikolycka där man har blivit påkört bakifrån brukar betecknas som pisksnärtsskada eller "whiplash". De symptom som kan uppträda är mångfacetterade. Utöver smärta och stelhet i nackmuskulaturen förekommer bland annat domningar i armar och händer, huvudvärk och yrsel. Skadan har god prognos och majoriteten av alla drabbade tillfrisknar inom några veckor. En del får dock bestående besvär med bland annat långvarig smärta och begränsad rörlighet i nacken. Det är en påtaglig brist på metoder för diagnos efter whiplashskada. Någon skada syns sällan på röntgen eller magnetkamerabilder. Vidare är det känt att relativt lite om hur långvarig smärta tilltar sig till uttryck i hjärnans aktivitet. Man vet heller inte om det finns tecken på kvarstående inflammation i nacken hos de patienter som inte tillfrisknar.

Med hjälp av positronemissionstomografi (PET) kan man studera biokemiska processer i kroppen. Tekniken innebär att ett radioaktivt spårämne injiceras i kroppen för att märka in exempel blodflöde, receptorer för signalsubstanser, eller inflammationsprocesser. När det radioaktiva ämnet sönderfaller uppstår gammastrålning som kan registreras och avbildas med hjälp av en PET kamera. På så vis kan man få ett mått på till exempel hjärnans blodflöde, eller var i kroppen ett spårämne ansamlas.

Den här avhandlingen baserar sig på tre PET studier, där olika aspekter av kronisk smärta hos whiplashpatienter har undersöks och jämförts med frika, smärtfria kontrollpersoner.

I den första studien undersöktes hjärnans blodflöde i vila hos patienter och friska. Blodflödet i hjärnan är kopplat till hjärnans nervaktivitet. Det visade sig att patienterna hade en förhöjd hjärnaktivitet i delar av bakre cingulum - en hjärnstruktur som är inblandad i smärtupplevelse, minnen och kroppsuppfattning. Patienterna hade även sänkt aktivitet i området mellan tinningloben och nackloben, och ju mer nackbesvär patienterna hade, desto lägre var aktiviteten i detta område. Det tyder på att kronisk smärta förändrar hjärnans viloaktivitet i vissa specifika områden.

I studie två undersöktes mängden neurokinin 1(NK1)-receptorer i hjärnan. NK1-receptorer aktiveras av en signalsubstans som heter Substans P, som är inblandad i smärta och rädsla. NK1-receptorer har tidigare mest studerats i försöksdjur. Mängden receptorer minskar i hjärnan på djuret om det utsätts...
för smärta. Studie två visar, för första gången, att mängden NK1-receptorer är förändrad även hos människor med kronisk smärta. Mängden tillgängliga receptorer var betydligt lägre hos whiplashpatienter i flera olika hjärnstrukturer som är inblandade i smärtupplevelse. Framför allt fanns det färre receptorer i den undre ytan av främre hjärnbarken. De patienter som var mest rörelserädda och undvek att utsätta sig för smärta, hade minst antal tillgängliga NK1-receptorer i detta område. Det förefaller som att whiplashpatienter har en förändring i sitt smärtsystem, där den långvariga smärtan kan ha minskat antalet NK1-receptorer. Alternativt så är mängden kroppsegen substans P förhöjd i dessa delar av hjärnan, vilket gör receptorerna mindre tillgängliga. Att undvika att röra på sig, av rädsla för att det gör ont, är en viktig aspekt av långvarig smärta. Frågan är om man undviker att röra på sig för att man har ont, eller om man har ont för att man undviker att röra på sig. Det kan vi i nuläget inte svara på, men NK1-receptorer kan vara en viktig pusselbit i svaret.

I studie tre användes en PET spårsubstans som indikerar inflammation. Med vanlig röntgen eller magnetkamera kan man oftast inte se någon skada i Nacken på whiplashpatienter. I studie tre observerades att ungefär hälften av de undersökta patienterna hade ett förhöjt upptag av spårsubstansen i områden kring nackrosetten. Upptaget var framför allt förlagt till den fettvävnad som omger de djupliggande musklerna vid andra nackkotan. Detta kan tyda på att många whiplashpatienter har en pågående kronisk inflammation i Nacken, viket inte kunnat visualiseras tidigare.

Sammantaget så har whiplashpatienter förändrad hjärnaktivitet i vila, färre lediga NK1-receptorer och även tecken på kronisk inflammation i Nacken. Dessa resultat tyder inte bara på att whiplash kan ge långvarig inflammation i Nacken, utan också på att det sker förändringar i hjärnan vid långvarig smärta. Det visar att det sker ett intrikat samspel mellan kropp och smärtupplevelse. Förhoppningsvis kommer de här resultaten att leda till en ökad förståelse för whiplashsyndromet och för patienter med kronisk smärta.
Introduction

This thesis is on brain mechanisms and inflammatory processes in patients with chronic neck pain after a rear impact car accident, so called whiplash-associated disorder, WAD. Using the nuclear imaging technique positron emission tomography (PET), three empirical studies of patients with chronic WAD and healthy controls have been carried out. The general aim was to investigate; (I) if patients have alterations in brain activity during rest, (II) if patients have alterations in neurokinin-1 receptor expression in the brain, and (III) if patients show signs of chronic inflammatory processes in the soft tissue in the neck. How alterations in the above three systems are related to the negative emotions associated with pain is also explored.

First a general background on pain and WAD is outlined. Peripheral and central mechanisms are described, from injury to pain perception, modulation and chronification. In this section, some references concerning general pain mechanisms have been omitted. Instead, the reader is referred to several excellent reviews of the field.1-7

Secondly, the existing literature on neuroimaging of WAD is reviewed. Thereafter, the methods, results and interpretations of the empirical works in this thesis are summarized. Finally this thesis provides a preliminary attempt to integrate the results of the three studies, and future lines of research are discussed.

Pain, chronic pain and whiplash

Pain can be defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”.8 This definition highlights the multifaceted role of pain, both as a discriminative perception, an autonomic drive, a reflexive motor stimulus and an affective motivation.9

From an evolutionary perspective, the experience of pain is a vital protective mechanism. Acute pain serves as a warning that demands attention and action, and prioritizes escape. After the initial escape reaction, pain and sensitization of damaged tissue serves to promote recuperative actions such as immobilization and quiescence, usually promoting healing. With time and proper care, tissue usually heals, and pain diminishes. But sometimes tissue does not heal properly and pain persists. Further, not all human pain syn-
dromes can be traced to identifiable tissue damage. Chronic pain, especially in the absence of damaged tissue, has no ecological value and has been described as “an unfortunate evolutionary free rider”.\textsuperscript{10} From a neurobiological perspective, the development of chronic pain may rely on a combination of pain sensitization and activity dependent neuronal plasticity. Pain sensitization is the process through which an injured body part becomes more sensitive to pain. This encourages us to immobilize the injured area, thus promoting healing. Activity dependent neuronal plasticity is the process through which the human brain adapts to experience. When we experience something repeatedly over time, our capability to process the experience can be enhanced. Both these processes have an adaptive value, but in combination, they may lead to the maladaptive development of persistent pain and hyperalgesia (an increased response to a painful stimulus) or allodynia (a painful response to a normally non-painful stimulus).\textsuperscript{11}

From a psychological perspective, the etiology of chronic pain is intimately related to the emotional reaction to pain. Pain-related fear and avoidance appears to be an essential feature of the development of a chronic problem for a substantial number of patients with musculoskeletal pain.\textsuperscript{12, 13} A “fear-avoidance” model of pain, developed by Lethem and colleagues\textsuperscript{14}, proposes that individuals can respond with either confrontation or avoidance to the experience of pain (figure 1). Acceptance and confrontation of pain leads the individual to resume an increasing range of physical activity as the damaged tissue heals, thus minimizing maladaptive behavioral schemes such as fear of movement and reinjury, avoidance, anxiety and depression. By contrast, pain avoidance through immobilization provides short term negative reinforcement, but may hinder proper healing and diminish muscle strength. Further, adopting an avoidant strategy promotes exaggerated pain perception and may lead to a maladaptive downward spiral with pain related anxiety and depression, which can promote the development of chronic pain.
A common misconception is that psychological models exclude the possibility of physiological reasons for chronic pain. On the contrary, in their original publication, Lethem and colleagues stress that “the majority, if not all, patients with chronic pain have physical injury or some other definable organic lesion at the outset of their history of pain”. Therefore, the fear-avoidance model can be applied to patients with either identifiable tissue damage, healed tissue, no tissue damage, or no evidence of tissue damage.
One such chronic pain syndrome, where there is little direct evidence of tissue damage, is neck pain after a rear impact car accident. This type of injury is commonly called “whiplash”. The presence of debilitating pain, restricted movement and other symptoms at six months or more after a such an accident is called late whiplash syndrome (LWS). In addition to neck pain, patients sometimes experience more widespread pain, tingling in the arms, dizziness, tinnitus, fatigue, visual changes, memory and concentration deficits, depression and sleeping difficulties. This complex set of symptoms is named whiplash-associated disorder, WAD.

Four grades of WAD have been defined as follows:

- Grade I: injuries involving complaints of neck pain, stiffness or tenderness, but no physical signs.
- Grade II: neck complaints accompanied by decreased range of motion and point tenderness (musculoskeletal signs).
- Grade III: neck complaints accompanied by neurologic signs such as decreased or absent deep tendon reflexes, weakness and/or sensory deficits.
- Grade IV: injuries in which neck complaints are accompanied by fracture or dislocation.
- Other symptoms such as dizziness, tinnitus, headache, memory loss, dysphagia, and temporomandibular joint pain can be present in all grades.

Neck pain after traffic collisions is common, around 300 per 100,000 inhabitants visit the hospital emergency department each year. In the majority of cases, the injury is resolved within a few months. Nonetheless, pain in the neck is the most common symptom after traffic injuries. In Sweden, neck pain accounts for a majority of the sick leave and disability pension caused by traffic injuries. In the United States, the annual cost for WAD related sick leave and litigation has been estimated to between $13 and $29 billion and to £2.6 billion in the United Kingdom (1990 prices).

Although the name ‘whiplash’ is suggestive of an accident process, the injury mechanisms behind WAD are not clear. Studies using either cadavers or biomechanical modelling suggest that a rear impact car accident causes the cervical spine to bend in an initial S-shape. This is thought to occur because the lower cervical spine is thrust forward by the car seat, while the head, due to its inert mass, remains level. This may cause a shearing force on the cervical spinal column, which may lead to abnormal stretching of the lower cervical segments, strains of the facet joint capsular tissue or surface, and strains on the cervical muscles.

The authenticity of WAD is often questioned. One survey found that a substantial number of physicians regarded prolonged symptoms to be of psychogenic origin or a result of pending litigation. There is some support for this view, in that the type of legislative system and compensation for pain
and suffering is associated with the incidence and prognosis of whiplash injury.\textsuperscript{25, 26} Further, there are large cultural differences in the prevalence of WAD, and also in the expectations of pain and chronicity among victims.\textsuperscript{27}

The scientific field of WAD is riddled with controversies, and several publications suffer from poor methodological standards. One recent review found 54\% of the 1203 studies reviewed to be of insufficient quality.\textsuperscript{28} Further, published studies are often commented, debated and criticized by advocates of the opposing opinion.

At the heart of the controversies around WAD is the lack of an objective diagnosis. There is scientifically acceptable evidence that WAD patients have less mobility and range of motion in the cervical spine compared with controls, but with considerable overlap between groups.\textsuperscript{29} WAD patients also have less neck muscle strength and endurance than healthy controls. Magnetic resonance imaging (MRI) studies have revealed a higher degree of fatty infiltration in the cervical muscles in chronic WAD patients.\textsuperscript{30, 31} However, it is not clear whether these muscular deficits are the cause or consequence of long term pain. Structural MRI studies of the cervical spine to identify pathology have relatively low reliability and a high degree of false positives. For example, one study found disk degeneration in almost 60\% of asymptomatic individuals above 40 years old.\textsuperscript{32} In another study, MRI within 2 days of the whiplash neck-sprain injury could not detect pathology connected to the injury nor predict symptom development or outcome.\textsuperscript{33}

Some MRI studies have demonstrated significantly more high-grade lesions in craniovertebral ligaments and membranes in WAD patients compared to noninjured subjects.\textsuperscript{34} However, a recent study indicates that alterations in the alar ligaments is almost as common in healthy controls (40\% of the cases) as in WAD patients (49\% of the cases).\textsuperscript{35} Thus, the clinical relevance of MRI imaging in acute and chronic WAD is not clear.

This lack of peripheral findings has led to the hypothesis that chronic pain after whiplash trauma has centrally mediated components. The fear avoidance model of chronic pain predicts that stressful life events, personal pain history, avoidant coping strategies and personality traits such as neuroticism are risk factors for the development of chronic pain.\textsuperscript{14} This idea has some support, but the scientific evidence is inconclusive.\textsuperscript{36} Initial pain intensity and initial neck pain related disability after injury have moderate predictive value in determining long term disabilities.\textsuperscript{37} After controlling for pain intensity, fear of movement and pain catastrophizing are also predictive of disability and depression after a whiplash injury.\textsuperscript{12} There is some support for an association between lower self efficacy and greater post traumatic stress and the development of chronic pain.\textsuperscript{38-41} However, the “big five” personality traits (openness, conscientiousness, extraversion, agreeableness, and neuroticism)\textsuperscript{42}, general psychological distress, wellbeing, social support, life control and psychosocial work factors have not sufficiently been shown to be associated with the development of chronic pain.\textsuperscript{36}
In summary, WAD is a multifaceted phenomenon with high societal costs, unclear etiology, few diagnostic options, and, most importantly, individual suffering for the afflicted subjects. In order to understand WAD, we must study it from several different perspectives. The biological underpinnings of tissue damage and the body’s protective inflammatory response to trauma needs to be related to perceptual, emotional and behavioral mechanisms. Further, the transition from acute injury to chronic illness may depend on a range of biological, psychological and also sociological factors. This thesis is aimed at providing a bio-psychological perspective of how WAD is manifested, both in the brain and in the neck, with the overall aim to search for new biomarkers that can elucidate chronic pain.

Tissue injury and inflammation

Around the year 25 A.D. Aulus Cornelius Celsus described the body’s acute reaction following a traumatic event such as a microscopic tear of a ligament or muscle. His original wording: “Notae vero inflammationis sunt quatuor: rubor et tumor cum calore et dolore” (True signs of inflammation are four: redness and swelling with heat and pain) still holds. *Functio laesa* or ‘loss of function’ is commonly quoted as a fifth sign. Tissue healing can be divided into three phases: the inflammatory, proliferative, and remodeling phases. The inflammatory phase, with the above five signs, generally occurs during the first 72 hours after injury.

The nerve cell endings that initiate the sensation of pain are called nociceptors (from *nocere*, “to hurt”). Nociceptors are activated by a variety of stimuli such as pressure, temperature and chemicals. When stimuli intensity surpasses the neurons threshold, an action potential is sent by the neuron. Damaged tissue releases a wide range of substances, the “inflammatory soup”, which makes the nociceptors more sensitive to stimuli. In addition, electrical signaling in the nociceptive neurons causes them to release neurotransmitters and peptides, including substance P, which further contributes to the inflammatory response. The function of this complex response is to protect the damaged area by making it hyperalgesic, or over-sensitive to stimuli. Further, the inflammatory response promotes healing and guards against infection by means of increased blood flow and accumulation of white blood cells. The white blood cells, or leukocytes, adhere or stick to the damaged area and begin repairing it. About two or three days after the tissue damage, fibroblasts begin to accumulate in the damaged region, marking the onset of the proliferative phase even before the inflammatory phase has subsided. The main function of fibroblasts is to rebuild the structural integrity of the wounded tissue by secreting precursors to the extracellular matrix. This phase is characterized by the production and simultaneous degeneration of the fibrous protein collagen. Collagen is generated, and degenerated in a
non-orderly way to quickly rebuild strength in the injured region. This process can last for up to six weeks, depending on the extent and severity of the tissue injury. The damaged area is still hyperalgesic, but peripheral and central sensitization (see below) typically declines as the tissue heals. In this phase, we begin carefully and gradually using our damaged body part.

In the final maturation and remodeling phase, which can last up to 12 months, the production and degeneration of collagen has reached equilibrium, and the activity of the fibroblasts and macrophages diminishes. The originally disorganized collagen fibers are remodeled and aligned to further increase strength and function, and scar tissue is eventually replaced by normal, fully healed tissue. In this process, the threshold for pain usually returns to pre-injury levels. Immobilization may hinder the proper remodeling of collagen and can increase stiffness and long term disabilities. Therefore, patients are instructed to move and use the injured limbs early in the proliferation and remodeling phase, as this promotes healing and rehabilitation.

Central mechanisms of pain processing

Sensory and nociceptive neurons are usually divided based on the speed their axons conduct the action potential. Sensory neurons that are responsible for the perception of touch have fast, myelinated axons. Specialized relatively fast-conducting Aδ fibers convey sharp initial pain, where as slower, unmyelinated C-fibers convey duller, longer lasting second pain. These neurons responsible for pain originate with other sensory neurons in the dorsal root ganglia, and the axons enter the spinal cord via the dorsal roots. Aδ fibers synapse in lamina 1 and 5, and C fibers in lamina 1 and 2, to second order neurons. The axons of the second order neurons ascend to higher centers in the brainstem and thalamus through a pathway called the anterolateral system (see figure 2).

In addition to the peripheral sensitization in the inflammatory process, the excitability of neurons may also increase in the dorsal horn of the spinal chord, a process called central sensitization. The substance P (SP) receptor neurokinin-1 (NK1) modulates this process. Activity from nociceptive afferents, which previously were below the pain threshold, may become sufficient to generate neuronal signaling in the dorsal horn, thereby contributing to increased pain sensitivity. The result is that a normally innocuous stimulus, such as movement or light touch, becomes painful.

The neurons in the anterolateral system project to several nuclei in the brain stem and further up in the thalamus, amygdala, and cortical regions. In these regions, the afferent information is processed and modulated, and, importantly, there are descending projections to the dorsal horns, allowing for modulation of the pain signaling at all levels of the system. The cortical
network for pain processing, modulation and integration with emotion and cognition is called the pain matrix. The main components of this network, with a special emphasis on the SP/NK1 system, are briefly described below.

Substance P and the neurokinin-1 receptor

Substance P (SP) is a small peptide that functions as a neurotransmitter and a neuromodulator. It was discovered in 1931 by Ulf von Euler and John Gaddum.\textsuperscript{44} SP is considered to be an evolutionary old neurotransmitter, as it is present both in mammals, reptiles, fish and some invertebrates.\textsuperscript{45, 46} Although initially described for its vasodilating effects and its involvement peripherally in inflammatory responses and in C-fiber signaling of pain, there is abundant evidence that SP is also involved in central modulation of pain and anxiety. Both SP and its primary binding site, the neurokinin-1 receptor (NK1), are widely distributed throughout the brain and body. In the central nervous system, SP and NK1 receptors are largely co-located with serotonin, and with high concentrations in areas known to be involved in emotional processing, such as the amygdala, the hippocampus, the hypothalamus, the periaqueductal gray, and the frontal cortex. Also, NK1 receptors are particularly abundant in the putamen and caudate nucleus.

It seems that SP may play a role particularly when the nervous system is stressed, challenged or afflicted by disease.\textsuperscript{47} The NK1 receptor system is involved in inflammation and pain signaling, but also in emotional processes such as anxiety and depression. As pain patients may have both inflammation and affective disorders, the role of NK1 receptors in chronic pain syndromes is particularly interesting. Animal experiments indicate that selective NK1 receptor antagonists might be effective in the treatment of pain and anxiety, but to much disappointment, NK1 antagonists in various human pain and anxiety conditions have thus far mostly failed to show clinical efficacy.\textsuperscript{48}
Figure 2. The pain circuitry in the human brain. Red and blue arrows are afferent projections, and green efferent projections. RVM rostral ventromedial medulla, PB parabrachial nucleus, PAG periaqueductal gray matter, Tha thalamus, Amy amygdala, vmOFC ventromedial orbitofrontal cortex, PFC prefrontal cortex, ACC anterior cingulate cortex, PCC posterior cingulate cortex, PPC posterior parietal cortex, S1 primary somatosensory cortex. Adapted from Price, 2000, and Mello & Dickerson, 2008.
Pain processing in the brain

Subcortical nuclei

The ascending neurons in the spinal cord, many superficially in lamina I, project to the thalamus, the periaqueductal gray and to the parabrachial area. A large proportion of these neurons express the NK1 receptor.⁶

The rostral ventromedial medulla (RVM) has many descending pathways to the spinal dorsal horn and has been implicated in both inhibition and facilitation of pain. Recently, it was demonstrated that activation of NK1 receptors in the RVM contributes to hyperalgesia.⁴⁹

The parabrachial nucleus (PB) has direct connections to the central nucleus of the amygdala, and may relay neuronal signaling to the insula. It has a role in the homeostatic regulation and the autonomic response to pain. It also projects NK1 containing neurons downwards to the RVM.

The periaqueductal gray matter (PAG) is involved in the fight-flight response and automatic behavioral coping strategies to pain. It has long been thought of as one of the key region for endogenous pain regulation.⁵⁰ It has reciprocal projections both to the RVM and to the thalamus and the amygdala, and receives input from the anterior cingulate (ACC), the insula and the ventral medial orbitofrontal cortex (vmOFC).⁵¹ In contrast to the role of NK1 receptors in the RVM, activation of the PAG area either by SP⁵² or other selective NK1 agonists⁵³ can be antinociceptive. Further, endogenous SP released in the PAG acts as a neuromodulator of nociceptive information through interaction with the opioid system.⁵⁴-⁵⁶

The amygdala complex is generally though of as a set of ‘danger detection nuclei’, and much research has focused on its role in classical conditioning and in affective disorders. In pain processing, it is thought to attach emotional significance to painful stimuli, and it can both enhance and reduce pain, depending on the emotional context.⁵⁷ Animal studies indicate that SP injections into the basolateral amygdala enhances passive pain avoidance.⁵⁸

The thalamus consists of several nuclei that receive nociceptive signalling from the different lamina of the anterolateral system. Through the ventral posterior lateral thalamic nucleolus, sensory and discriminative aspects of pain are relayed to the primary and secondary somatosensory cortex. Through the medial thalamic nuclei, projections reach the insular and anterior cingulate cortex.

Cortical pain processing

Cortical pain processing can be conceptualized as two interacting systems; the lateral and the medial system. This division is based on the projections from the medial and lateral thalamic nuclei to the cortex, and provides a convenient (but a bit simplistic) view of cortical pain processing. The lateral
system, involving the primary and secondary somatosensory cortex, mediates the sensory and discriminatory aspects of pain: the location and quality of stimulation. The medial system, consisting of the anterior cingulate and prefrontal cortical regions, process the affective motivational aspects of pain: intensity, unpleasantness, fear and anxiety. In between these systems is the insula, which receives projections from both the medial and lateral thalamic nuclei, and can be thought of as a region where sensory and affective pain processes are integrated.

In addition to interpreting signalling from the periphery, there is extensive descending modulation and control of pain. The relationship between reported pain intensity and the peripheral injury that evokes it depends on several factors such as the level of arousal, anxiety, depression, attention and expectation. A classic example of this is that soldiers wounded in battle may experience little pain despite extensive injuries.59

**Somatosensory cortex**

The primary somatosensory cortex (S1) is positioned behind the central sulcus, and consists of Brodmann areas 1, 2 and 3. Input from body regions is represented in distinct regions in the cortex, and it encodes the location of both painful and non-painful stimulation.

The secondary somatosensory cortex (S2), as the name suggests, receives its input from S1, and processes higher order somatosensory functions. S1 and S2 are anatomically interconnected with posterior parietal cortices where the sense of touch and pain is integrated with auditory and visual modalities, and perhaps memory functions. Further, pathways from S1 and S2 project to the insula and ultimately to limbic and frontal regions, see figure 2.

**Insula**

The insula receives input from the ventral and medial thalamic nuclei, and is thought to play a central role in interoception, or the sense of the physiological condition of one’s body.60 Its caudal region is reliably activated in neuroimaging studies of experimental pain,1 and a recent metaanalysis suggests that clinical pain is processed in more rostral parts of the insula, an area where aversive and anxiogenic stimuli are also processed.61 Recent animal studies have demonstrated that prolonged pain reduces NK1 receptor mRNA expression in the rat insula.62, 63

**Cingulate cortex**

The cingulate gyrus stretches around the corpus callosum in the midline of the brain. Brodmann divided it into an anterior and posterior region, but recent definitions suggest four major portions, with subregions, based on its functional role.5 These regions their subdivisions are the anterior cingulate cortex (ACC; subgenual; pregenual), the midcingulate cortex (MCC; ante-
rior; posterior), the posterior cingulate cortex (PCC; dorsal; ventral), and the retrosplenial cortex (RSC).

The subgenual ACC is an emotion regulating region that is activated in sadness, whereas pregenual ACC has been described as encoding the suffering part of pain and noxious visceral pain. The MCC is activated by cutaneous pain, and its anterior regions are involved in fear-avoidance behaviours. Posterior regions of MCC are involved in orienting the body in response to sensory stimuli, including pain. The PCC region is less well understood, but it has been described as an emotional pre-processor, where the self-relevance of emotional stimuli is assessed. The RSC is also a poorly understood region, but it has been suggested that it is involved in the interaction between emotion and episodic memory.

A recent autopsy study revealed no major alterations in NK1 receptor density in the cingulate cortex in subjects with mood disorders, schizophrenia or depression compared to controls. However, depressed patients had alterations in NK1 density distribution in the different cortical lamina.

Prefrontal regions
The prefrontal cortex (PFC) has extensive reciprocal connections to the sensory and motor systems, as well as the subcortical structures described above. This allows for complex top down modulation that may both inhibit and enhance peripheral pain signals, as well as the subserving brain structures. Further, prefrontal regions are thought to be responsible for complex behavioural and cognitive strategies such as avoidance, pain catastrophizing, and coping strategies. Prefrontal regions also contribute to elaborations on the significance of pain, such as perceived interference with one’s life, difficulties enduring pain over time and implications for one’s future well being and life in general. The investigation of how the brain regulates these more complex aspects of the pain experience is an emerging field in pain research. A few studies of particular relevant to this thesis are described below.

Pain behaviors
Anticipation and expectation
The threat of pain is in itself a highly aversive experience, and anticipation of pain can cause mood changes that exacerbate the suffering of pain. In experimental models, when painful stimulation is signaled by a cue, the expectation period between the cue and the pain causes an increase in activity in the pain matrix. However, there is evidence that anticipation of pain specifically activated the anterior medial frontal cortex, the anterior insula and the posterior cerebellum, and the middle and posterior cingulate gyrus. When the cue preceding pain indicates that the pain will be of higher intensity, subjects report higher pain, even if the
stimulus is of lower intensity,\textsuperscript{69} an effect that seems dependent on the ACC and the medial prefrontal cortex.\textsuperscript{70}

**Fear of pain and pain catastrophizing**

In accordance with the fear avoidance model of chronic pain, individuals who are more afraid of pain are at risk of developing long term pain. The neural correlates of pain-related fear and anxiety are just beginning to be mapped in the brain. A study in healthy controls found a positive correlation between anxiety sensitivity and medial prefrontal activation to painful thermal stimulation. Further, there was a positive correlation between fear of pain and activity in the right lateral orbitofrontal cortex as well as with activity in the posterior cingulate gyrus.\textsuperscript{71} In contrast to this, chronic back pain patients with exaggerated pain illness behavior displayed less activity in the PCC in response to unpleasant tactile stimulation. One possible explanation for these discrepant results is that chronic pain patients have an altered resting state brain activity. If posterior cingulate blood flow is elevated already at baseline, no increase will be seen at stimulation. A recent fMRI study of chronic back pain patients supports this hypothesis.\textsuperscript{72} Pain patients exhibited less deactivation compared to normal subjects mainly in the medial prefrontal cortex, the amygdala and in the PCC. These new results are in line with those of study I in this thesis.

**Control over pain**

The degree to which pain is perceived as controllable affects tolerance, motivation and the ability to cope with pain. Perceived control can be experimentally induced by allowing participants to stop a painful stimulation. Using fMRI, perceived controllability was linked to decreased activity in the ACC, the insula and S2.\textsuperscript{73} In a later study, controllable, versus uncontrollable pain resulted in higher activity in the right anterior ventrolateral prefrontal cortex.\textsuperscript{74} Further, prefrontal activity has been related to the way individuals cope with pain.\textsuperscript{75}

**NK1 receptor expression in the prefrontal cortex**

The NK1 receptor is expressed in the frontal cortices, and a recent study indicated age and gender differences in receptor density.\textsuperscript{76} However, the functional role of the NK1 receptor in the human PFC is not known. NK1 receptor expression has been studied in a post-mortem study of major depression, where NK1 receptor expression was reduced in the rostral orbitofrontal cortex in patients.\textsuperscript{77}
Chronic versus acute pain

Most of our knowledge of the pain system in the brain has been derived from experimental studies of healthy individuals, stimulated with acute pain. Neuronal signaling is usually studied by measuring pain induced alterations in the brains regional cerebral blood flow (rCBF) or blood oxygen level dependent (BOLD) signal. Neuroimaging of chronic pain states is more challenging. First and foremost, chronic pain is enduring and unyielding, making experimental manipulations difficult. One strategy has been to use identical paradigms in patient cohorts as in healthy subjects, but the interpretation of these results is unclear, as differences may reflect altered pain processing in the patient group, or differences in attention to the experimental stimuli.\(^1\) Acute pain stimulation may redirect attention from the chronic condition, and thus temporarily “alleviate” the experience of chronic pain, similar to biting ones fist in order to cope with something more painful. A more direct experimental approach is to provoke the chronic pain, either pharmacologically or mechanically, and measure cerebral activations to the provocation. Such studies have revealed, in general, that chronic pain, as compared to acute, is more often associated with prefrontal brain regions critical for cognitive/emotional assessments.\(^1\) A third strategy, employed in study I in this thesis, is to measure neuronal activity in the resting state. The rational behind this is that the brain has extensive neuronal processing also in the absence of external stimuli.\(^78\) As patients experience pain also at rest, this will influence the resting state neuronal activity.\(^72, 79, 80\)

Brain plasticity and neuronal loss in chronic pain

Neuronal plasticity refers to the changes that occur in the organization of the nervous system as a result of experience. Long term pain and stress can cause reorganization and local morphologic alterations of the brain. High resolution structural MRI studies have revealed lower gray matter density and reduced cortical thickness in a range of chronic pain conditions.\(^81\) Attenuations are seen in the cingulate, orbitofrontal and insular cortices, and, with less consistent evidence, in the brainstem and thalamus. There is limited knowledge of the specific neurotransmitter systems mediating these functional and structural alterations in human patients. In animal models of pain and stress, hippocampal atrophy caused by excessive glucocorticoids is a well established finding.\(^82\) This has also been observed in post traumatic stress patients.\(^83\) Of particular of interest in this thesis, NK1 receptor mRNA expression is reduced in the hippocampus and the insula in animal models of pain and stress.\(^62, 63, 84\)

To date, only one structural neuroimaging study of WAD has been published.\(^85\) The study, encompassing 21 chronic (5-16 months) WAD patients
and 18 healthy controls, did unfortunately not investigate local changes of gray matter density. Instead, only the global ratio between ventricle volume and total brain volume was used as the outcome measure. No difference was seen between patients and controls.

Functional neuroimaging of whiplash-associated disorder

To date, there are 12 functional neuroimaging studies of WAD patients, which are briefly reviewed below.

The first neuroimaging study of whiplash patients was a single photon emission tomography (SPECT) study on 28 WAD patients, eight low back pain patients and 15 normal controls. A unilateral and bilateral parieto-occipital hypoperfusion was seen in 24 out of 28 WAD patients, in one of eight low back pain patients, and in no healthy control. The SPECT images were evaluated qualitatively by two independent blinded readers. Several further studies by the same group have reported similar results, with parieto-occipital hypoperfusion in around 75% of WAD patients. Altogether, at least 100 patients were evaluated with the SPECT blood flow marker $^{99m}$Tc-hexamethylpropyleneamineoxime (HMPAO). Further, 200 patients were evaluated with another blood flow tracer, $^{99m}$Tc-L,L-ethyl cyteinate dimer (ECD). This large patient cohort was compared qualitatively and quantitatively to 15 HMPAO and 20 ECD healthy controls, and to eight patients with acute low back pain. In addition, results from ten WAD patients with $^{18}$F-fluorodeoxyglucose (FDG) PET were reported. This WAD patient group was compared to 20 healthy controls. The FDG study revealed bilaterally lowered glucose metabolism in the posterior parietal occipital region, evaluated with a region of interest (ROI) approach. The authors speculate that the parieto-occipital hypoperfusion is caused by vasoactive peptides released by pain sensitive afferents in the upper cervical spine, but do not exclude the possibility of hypometabolism in parieto-occipital regions as a consequence of diffuse axonal lesions due to acceleration forces. Further, they speculate that parieto-occipital perfusion deficits could be the substrate of cognitive disturbances in chronic whiplash patients. Unfortunately, it is not clear if the publications above included unique or similar patient cohorts.

Other neuroimaging studies have reported conflicting results. Bicik and coworkers investigated 13 WAD patients with HMPAO SPECT, FDG PET and MRI. Patients were compared to a database with FDG images of 16 melanoma patients. Statistical parametrical mapping (SPM) of the FDG images revealed decreased metabolism in the putamen, frontal cortex and lateral anterior temporal cortex. No alterations were seen in the parieto-temporo-
occipital area. FDG hypometabolism in the frontal cortex was correlated with the Beck Depression Index score. Relatively reduced FDG and HMPAO uptake was correlated with cortical thickness. In individual cases, the reliability to detect hypometabolism was relatively low. The authors conclude that FDG PET or HMAO SPECT should not be used as a routine diagnostic tool in WAD. However, the use of melanoma patients as a control material has been questioned. In a later study by the same group, 21 WAD patients were investigated with HMAO SPECT, FDG PET and 

\[^{15}O\]water PET. Working memory and divided attention assessments were performed, but no control group was evaluated. No significant correlations were found between regional perfusion or metabolism and scores of working memory or divided attention. The authors suggested that working memory and divided attention deficits in WAD are not signs of brain damage.

In a study published in 2002, 20 WAD patients were evaluated with neuropsychological tests, MRI, HMPAO SPECT and P300 event related potential electroencephalography (EEG). No healthy controls were imaged with SPECT, but EEG was performed in 15 patients and nine healthy controls. No structural brain damage was observed on the MRI’s, but diminished perfusion was observed in the temporal lobe (8/20 patients), occipital lobe (3/20) and frontal lobe (2/20), and two patients showed asymmetric perfusion in the basal ganglia. Eight patients had abnormal P300 ERP latency or amplitude. However, these abnormalities showed no correlation to neuropsychological test scores.

In a recent SPECT study, Sundström and colleagues reported no significant differences between 27 whiplash patients and 15 healthy controls. Further, they observed greater perfusion alterations in nontraumatic chronic neck pain patients (n = 18) than in WAD patients compared to healthy controls. However, there was a sub-significant perfusion reduction in the right temporal lobe and the left temporo-parietal region in the WAD patients.

To date, only one functional magnetic resonance imaging (fMRI) study on WAD has been published. Five WAD patients, five asymptomatic, fully recovered subjects with whiplash trauma, and seven healthy controls were included. The study paradigm was a visual motion stimulation task, and WAD patients displayed a significant reduction in their ability to perceive coherent visual motion. WAD patients also displayed significantly lower BOLD response in the middle temporal/V5 visual cortex region compared to healthy controls and asymptomatic whiplash patients.

Taken together, there is a reasonable amount of evidence suggesting perfusion reductions in the temporo-occipito-parietal transition zone. The interpretation of these findings is still unclear, as this region is not usually involved in pain processing. Further, results should be interpreted with caution, as rather liberal significance thresholds were used in several studies, and neuroimaging methods and statistical analysis procedures have developed substantially over the last ten year period.
Brain damage in WAD?

In addition to pain and stiffness, WAD patients often report cognitive disturbances such as impaired memory, poor concentration and mental fatigue and slowness. Sleep impairment, sensory sensitivity, visual disturbances and vertigo is also common. By one year after the injury only a small fraction of patients still have symptoms, but cognitive symptoms can persist. As these symptoms are similar to mild post-concussion symptoms, whiplash has sometimes been claimed to cause mild traumatic brain injury or diffuse axonal injury.

Primate experiments with acceleration injuries have revealed that diffuse axonal lesions may result from pure acceleration trauma, without head impact. Injured animals were all rendered unconscious for at least 15 minutes after the trauma, and acceleration forces were much higher than what is common in motor vehicle accidents. Animals that were exposed to milder acceleration violence regained consciousness promptly, and did not have evidence of axonal injury.

The results from SPECT and PET studies of whiplash have sometimes been interpreted as evidence of mild traumatic brain injury or diffuse axonal lesions. This interpretation is controversial, as brain perfusion is highly variable, and both blood flow alterations and cognitive symptoms may be accounted for by the persistence of pain and its sequels. While it can not be fully excluded, it remains unlikely that whiplash trauma without head impact and loss of consciousness causes brain injury.

For an empirical evaluation and discussion of the validity of individual brain scans as signs of pathology, see section “Individual differences in rCBF” below.
Aims and hypothesis of the thesis

In order to better understand and treat illness, we need adequate descriptions of the biological processes that are altered, and knowledge of how those alterations are related to the patients’ behaviors and experiences of illness. The overall aim of this thesis is to improve our understanding of whiplash-associated disorder, a debilitating, common syndrome for which there are few diagnostic biomarkers. Little is known about how WAD influences neuronal processing in the brain. Even less is known of how neurokinin-1 receptors are affected in this chronic pain condition. A further question is if there is evidence of persistent inflammation in the cervical neck in WAD patients.

By means of positron emission tomography, these three issues have been partially addressed, potentially giving us a better understanding of the disorder.

Study I
This was a PET study performed with $[^{15}O]$water to measure resting state rCBF in WAD patients and healthy controls.

Based on functional neuroimaging studies of chronic pain, we hypothesized elevated rCBF within the medial and lateral pain matrix in WAD patients as compared to healthy controls. Attenuated parieto-occipital rCBF in WAD patients was also hypothesized, based on previous neuroimaging studies of WAD. An additional aim was to relate rCBF alterations within the WAD patient group to clinical symptoms.

Study II
This was a PET study performed with the carbon-11 labeled NK1 antagonist $[^{11}C]$GR205171 to investigate NK1 receptor availability in the brain.

Based on functional and structural neuroimaging studies of chronic pain patients, and on animal studies of NK1 receptor alterations caused by pain and stress, we hypothesised that WAD patients, as compared to controls, would have altered NK1 receptor availability in the medial pain system, amygdala, hippocampus and in the periaqueductal gray matter. The functional significance of NK1 receptor availability was explored by correlating NK1 levels to patients’ ratings of pain, anxiety, neck disabilities and kinesiophobia.
Study III

This was a PET study to explore the use of $[^{11}\text{C}]\text{D-deprenyl}$ as a marker for chronic inflammation in neck regions.

Given that $[^{11}\text{C}]\text{D-deprenyl}$ accumulates in tissue with ongoing inflammation, we hypothesized the presence of locally elevated $[^{11}\text{C}]\text{D-deprenyl}$ in chronic WAD patients compared to healthy, pain free controls.
Methods

Positron emission tomography

All three studies in this thesis used PET to visualize and/or quantify neuronal processes, neuroreceptor distribution and indications of chronic inflammatory processes.

A GE Discovery whole body PET-CT scanner, photo courtesy of GE Healthcare, Uppsala Imanet.

The principles of PET

Unstable, short lived, positron emitting radionuclides are produced using a cyclotron, and then incorporated into biologically active molecules, such as water or more complex molecules, called radiotracers. The most commonly used radionuclides are $^{11}$C, $^{13}$N, $^{15}$O and $^{18}$F, which have a very short half life, approximately 20, 10, 2 and 110 minutes respectively. Due to the short
half lives of most radionuclides, the radiotracers are often produced using a cyclotron and radiochemistry laboratory that is in close proximity to the PET imaging facility. A very large number of organic molecules can be labeled, especially with $^{11}$C, offering the opportunity to investigate specific biological processes \textit{in vivo}. The main clinical use of PET is in diagnosis of cancer. A common use of PET in neuroscience is to investigate biodistribution of neurotransmitters and their receptors.

The radiotracer is injected intravenously in the arm of the subject, where it can indicate blood flow (in the case of $[^{15}\text{O}]$water), accumulate in tissue or circulate through the vascular system, cross the blood brain barrier and bind to specific receptors in the brain. The unstable radionuclide decays and emits positrons. After travelling a few millimeters, the positron meets its antimatter counterpart, an electron. The meeting results in the annihilation of both, and the simultaneous emission of two high energy photons in opposite directions. The photons are detected by a ring of detectors in the scanner. As the photons are emitted simultaneously, the location of the annihilation event can be computed. The photon detections are stored in a computer with respect to position in space and time. After data acquisition of tens of thousands of annihilation events, the distribution of the positron emitting isotopes in the brain or body can be calculated.
PET imaging generates large amounts of data, usually presented as a series of slices through the brain or body, combined into a three dimensional map of voxels. PET imaging can result in either static images (reflecting one time point) or dynamic imaging sequences (reflecting several points in time). Dynamic imaging facilitates kinetic analysis of the radiotracer, to investigate specific binding and interaction processes with the target receptors.

The distribution of the radiotracer depends on the blood flow in the body, blood brain barrier transport, trapping of the molecule in tissue and binding to the target molecules. An illustration of how a radioligand may accumulate in tissue over time is provided in figure 4.
Figure 4. Schematic illustration of how a radiotracer may behave in tissue. Blood flow ligands such as \([^{15}\text{O}]\text{water}\) display a rapid influx and efflux, whereas a pharmaceutically active radioligand may accumulate or bind irreversibly to tissue, thereby gaining higher radioactive signal over time.

**Analysis of PET data**

Statistical methods in neuroimaging

As PET imaging generates large amounts of data, statistics in neuroimaging is a field of research in its own right. In this thesis, statistical parametric mapping methods are used in study I and II to enable between-group comparisons of radioligand distribution in patients and healthy controls. Further, correlation analyses within the patient group are also performed to explore links between brain alterations and behavior. In order to do this, the PET images first need to go through some preprocessing steps.

Human brains differ in size and shape, and to make a statistical analysis on several subjects, each subject’s brain needs to have the same spatial representation. Therefore, the three-dimensional image of every participant’s brain is warped into a common standardized brain shape. This way, the coordinates of say the amygdala in one subject will correspond to the same
coordinates in all the other subjects. The PET data are represented as voxels (little boxes, usually 2×2×2 mm) that build up a 3 dimensional volume. Every voxel has an assigned position and value. Thus, a voxel value representing the blood flow or receptor density in one subject may be compared statistically to the other subjects, in essence by performing a t-test on the voxel value.

But before the statistical analysis, images are smoothed, so that voxel values are averaged with their neighbors, typically using a Gaussian filter. This is done to cope with functional anatomical variability that is not accounted for by normalizing, and to improve the signal to noise ratio of the images.

In a standard image analysis, a statistical model is fitted to the data, to give model parameters. The model parameters are used to look for an effect, such as the difference between patients and healthy controls. To do this, a statistic is calculated for each voxel in the brain. The statistical analysis may be a simple t-test between groups, regressions or ANOVAs. The general linear model is used to perform these analyses for each voxel value in the brain, and nuisance variables that may confound the analysis can be entered into the model to be accounted for.

For example, in study I, the difference between healthy subjects and patients in rCBF is modeled as a t-test performed on every voxel value in the brain. The resulting t-value for each t-test is visualized as a map of t-values across the brain.

Now, what should be considered a significant difference? Performing a statistical test on every voxel value runs the risk of generating false positives, and some correction method is necessary to evaluate what is a true difference. Bonferroni correction, i.e. dividing the statistical threshold by the number of comparisons performed, disregards the spatial proximity of voxels. If several neighboring voxels reach significance, the probability that they represent a true difference increases. This is modeled using the theory of Gaussian fields. For an in depth description of this, the reader is referred to the works of Karl Friston and colleagues.100

One way to efficiently reduce the problem of multiple comparisons is to define a priori regions of interest (ROIs). This way, if we have reason to suspect that a particular brain structure is altered, only the voxels within that structure are investigated. There are still multiple comparisons performed, but much fewer than if the whole brain is analyzed.

In Study I and II the image preprocessing and subsequent statistical parametrical mapping was performed using the Matlab based neuroimaging software SPM2 (www.fil.ion.ucl.ac.uk/spm). Brain locations are described by their names and as (x, y, z) coordinates in the standard Talairach space.101

In Study I, the statistical detection threshold was set at p<0.001 uncorrected, equivalent of alterations exceeding 3.09 standard deviations. This threshold is commonly adapted in neuroimaging studies, but results, especially small unilateral alterations, should be interpreted with caution.
In Study II, *a priori* hypothesis allowed for directed searches in pre-defined ROIs. This decreases the number of comparisons made, thus giving more power to the analyses. Further, exploratory whole brain analyses were performed. The statistical threshold for ROI analyses and exploratory analyses was set at $p<0.05$, corrected for multiple comparisons. This threshold is more conservative than the ($p<0.001$ uncorrected) threshold.

**PET data analysis of the neck**

In study III, PET data were not analyzed in a voxel vise manner, as no standardized space exists for the cervical neck. Instead, the amount of trapped radioligand was measured by extracting the area under time activity curve (AUC) in a series of ROIs. Differences between ROI AUC values were analyzed using students t-test. See section “Study III: Quantifying $[^{11}C]$D-deprenyl uptake in the neck” for further details.

**PET scanner specifications**

In study I, II and III, an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, USA) was used. The scanner enables acquisition of 63 contiguous planes of data with a distance of 2.46 mm resulting in a total axial field of view of 15.5 cm. A 10 min transmission scan was performed using three retractable Germanium-68 rotating line sources. Thereafter, the patients received an intravenous bolus injection of the radiotracer, and data collection commenced immediately after injection. The emission scans were reconstructed with a filter back projection using a 4 mm Hanning filter, resulting in a spatial resolution of about 5 mm in the field of view.

In study III, three WAD patients and the control group were investigated on a GE Discovery ST PET-CT scanner (General Electric Medical Systems, Milwaukee, WI), which has 24 rings of bismuth germinate crystal detectors. This scanner enabled the acquisition of 47 contiguous planes of data with a distance of 3.27 mm, resulting in a total axial field of view of 15.7 cm. The PET-CT investigation was initiated with an attenuation CT scan (140kV; auto mA 10-80 mA; pitch 1.75; speed 35) for attenuation correction of the PET emission data. Following this scan the radioligand was injected as described above. PET data was reconstructed using the OSEM (Ordered Subset Expectation Maximization) algorithm with 2 iterations and 32 subsets and with a Gaussian post-processing filter 5.14 mm in width.

All PET data were corrected for photon attenuation, decay, scattered radiation, random coincidences and physical decay of the radioligand. In study III, three WAD patients were first investigated in the ECAT HR+ scanner, and one month later re-investigated in the PET-CT scanner to obtain a test-retest measure for the different scanners and sessions.
Pain and disability measurement

Self-reporting is considered to be the standard for pain measurement in humans. From the subjective report of pain, there is usually no way to distinguish between the experiences due to tissue damage and to psychological factors. Therefore, both pain intensity and pain related behavior and affects are measured through questionnaires. The pain and disability measures used in this thesis are briefly described below:

Visual Analogue Scale

The VAS is a simple and robust measurement, usually designed as a 100 millimeter line, with descriptions of pain intensity at each end, where the patient will mark her current experience of pain intensity, as exemplified below:

No Pain × Worst possible pain

The pain rating is a number between 0 and 100, 30 in the case above. Although the assessment is dependent on what the subject considered the worst possible pain, experimental studies have shown the VAS rating scale to be valid for measurement of, and comparison between, chronic and acute pain.¹⁰²

Neck Disability Index

The NDI is a 10 item scaled questionnaire aimed at assessing disability due to neck pain. The NDI consists of 10 items; pain intensity, personal care, lifting, sleeping, driving, recreation, headaches, concentration, reading and work. The activity limitation for each item is rated from 0 (no activity limitations) to 5 (major activity limitations) and summed up to a total disability score, ranging from 0 to 50. The questionnaire has been validated in English and Swedish populations, and is considered a reliable instrument to measure disability due to neck pain.¹⁰³,¹⁰⁴

Tampa scale for kinesiophobia

Kinesiophobia, defined as excessive, irrational, and debilitating fear of physical movement and activity resulting from a feeling of vulnerability to painful injury or reinjury, was measured with the Tampa scale for kinesiophobia, TSK.¹⁰⁵ The questionnaire consists of 17 items, where the respondents are asked to indicate their level of agreement to each of the 17 statements on a 4 point response scale, with scoring alternatives from “strongly disagree” to “strongly agree”. Total scores range from 17 to 68. Psychomet-
ric evaluations of the TSK have deemed it valid and reliable. Kinesiophobia was not measured in the control group, as the TSK is aimed at measuring injury related behaviour and cognitions.

Spielberger state anxiety inventory-state portion
The STAI-s is a 20 item scale assessing transient levels of anxiety. The scale ranges from 20 to 80, and it is well validated and widely used.

Screening Questionnaire for Post Traumatic Stress Disorder
The SQ-PTSD questionnaire was developed by Frans and colleagues and is based on the diagnostic criteria for PTSD according to the diagnostic and statistical manual of mental disorder, DSM-IV. The questionnaire also assesses to what degree a trauma caused enduring emotional distress. Validity, reliability and specificity of the SQ-PTSD are satisfactory.

Whiplash related disorder questionnaire
The questionnaire consists of WAD specific items such as accident description, sick leave status, pain drawings, health prior to whiplash injury, symptoms immediately after injury, and current symptom ratings. It is not a rating scale, but rather a general descriptive tool.
The empirical studies

Participants and behavioral ratings

In total, 22 WAD patients and two control groups consisting of 18 and 8 healthy pain free subjects respectively were investigated. Of the 22 patients, 21 were investigated with \(^{15}\text{O}\)water, 18 with \(^{11}\text{C}\)GR205171, and 22 with \(^{11}\text{C}\)D-deprenyl, see table 1.

Patients were recruited from hospital registries, and controls through advertisements. Initial screening was done by telephone interview. Exclusion criteria for patients were loss of consciousness at the accident, neurological symptoms, WAD due to other accidents than by car, and pain not related to WAD. Exclusion criteria for both WAD patients and healthy subjects were current depression or other psychiatric disorder, organic brain disorder, somatic disease, left-handedness, substance abuse and pregnancy. All participants refrained from analgesics and anti-inflammatory drugs one to three days prior to scanning, from tobacco, alcohol and caffeine 12 h before, and from food 3 h before PET investigations.

Approvals were obtained from the regional ethical review board in Uppsala, Sweden and the local radiation ethics committee. Written informed consent was obtained from all participants.

Patients and controls did not differ with respect to age, but in study I and II, the patient group contained more females (80%) than the control group, which was sex balanced (50%). In study III, the control group consisted women only.

Patients were all diagnosed as WAD grade II, and had pain between 6 and 24 months post trauma. The average time since injury was 15 (±7) months. Patients had significantly higher VAS pain ratings, NDI ratings and SQ-PTSD scores. In study I, patients did not differ from controls with respect to STAI-s, but in study II, controls rated their anxiety lower than patients, leading to a significant difference. VAS pain intensity measurements and STAI-s ratings were obtained from all participants directly after each PET investigation, whereas NDI, TSK and SQ-PTSD ratings were collected by mail in questionnaires. For details, see table 1 and 2.
Table 1. PET investigations and ratings in patients and control groups, • data available, × data missing, - data not collected. See text for abbreviations.

<table>
<thead>
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<th>Subject</th>
<th>PET HR+ scanner</th>
<th>PET-CT scanner</th>
<th>Subjective ratings</th>
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<td>Patient 1</td>
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<td>Patient 2</td>
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<td>Patient 3</td>
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<td>Patient 4</td>
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<td>Patient 5</td>
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<td>Patient 6</td>
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<td>Patient 7</td>
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<td>Patient 9</td>
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<td>Patient 18</td>
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<td>Patient 19</td>
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<tr>
<td>Patient 20</td>
<td>•</td>
<td>-</td>
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<tr>
<td>Patient 21</td>
<td>•</td>
<td>-</td>
<td>•</td>
</tr>
<tr>
<td>Patient 22</td>
<td>•</td>
<td>-</td>
<td>•</td>
</tr>
</tbody>
</table>

n = 21 18 22 3 22 20 16 22 20

Control group A 18 18 - - 18 - - 18 18
Control group B - - - 8 8 8 - 8 8
Table 2. Demographics and behavioral state and trait measures. Mean values ±standard deviation. Significant differences are in **bold**.

<table>
<thead>
<tr>
<th></th>
<th>WAD</th>
<th>Control A</th>
<th>Control B</th>
<th>WAD vs C A</th>
<th>WAD vs C B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male</td>
<td>18/4</td>
<td>9/9</td>
<td>0/8</td>
<td><strong>p = 0.04</strong></td>
<td><strong>p = 0.6</strong></td>
</tr>
<tr>
<td>Age</td>
<td>37±11</td>
<td>35±9</td>
<td>33±9</td>
<td><strong>p = 0.64</strong></td>
<td><strong>p = 0.63</strong></td>
</tr>
<tr>
<td>VAS Pain</td>
<td>35±19</td>
<td>0.6±2</td>
<td>4±7</td>
<td><strong>p &lt; 0.001</strong></td>
<td><strong>p &lt; 0.001</strong></td>
</tr>
<tr>
<td>NDI</td>
<td>18±5</td>
<td>-</td>
<td>2±1</td>
<td><strong>p &lt; 0.001</strong></td>
<td><strong>p &lt; 0.001</strong></td>
</tr>
<tr>
<td>SQ-PTSD</td>
<td>6±5</td>
<td>2±3</td>
<td>1±1</td>
<td><strong>p = 0.01</strong></td>
<td><strong>p = 0.02</strong></td>
</tr>
<tr>
<td>STAI-s</td>
<td>31±7</td>
<td>27±8</td>
<td>30±5</td>
<td><strong>p = 0.16</strong></td>
<td><strong>p = 0.83</strong></td>
</tr>
</tbody>
</table>

VAS = Visual Analog Scale, NDI = neck disability index, SQ-PTSD = Post Traumatic Stress Disorder, STAI-s = Spielberger state trait anxiety inventory, state portion. a Refers to study I, in study II controls rated their anxiety slightly lower leading to a significant difference between patients and controls in STAI-s ratings at **p = 0.016**.

When describing symptoms, patients reported moderate to severe neck pain (89%), shoulder pain (61%), headache (61%), upper back pain (39%) and lower back pain (28%). Further, patients reported moderate to severe restricted head movement (61%), arm numbness (44%), concentration deficits (39%), vertigo (33%), memory deficits (28%), visual disturbances (22%), tinnitus (17%), jaw pain (17%), nausea (11%), and leg numbness (6%).
Study I. Regional cerebral blood

$[^{15}\text{O}]$water was used in to measure resting state rCBF in 21 WAD patients and 18 healthy controls. There is a relatively linear relationship between local field potentials (or neuronal signaling) and rCBF. By labeling water with $^{15}\text{O}$, an isotope with a half life of ~ 2 minutes, the rCBF can be measured with PET, thus providing an indirect index for neuronal signaling.

Based on functional neuroimaging studies of chronic pain, we hypothesized elevated rCBF within the medial and lateral pain matrix in WAD patients as compared to healthy controls. Attenuated parieto-occipital rCBF in WAD patients was also hypothesized, based on previous neuroimaging studies of WAD. An additional exploratory aim was to relate rCBF alterations within the WAD patient group to clinical symptoms.

Results

WAD patients, as compared to controls, had elevated rCBF bilaterally in the posterior cingulate gyrus (Brodmann Area (BA) 29) and the parahippocampal gyrus (BA 30) as compared to controls. Further, rCBF was elevated in the left lingual gyrus (BA 19), the tail of the right caudate nucleus, the right thalamus, the right inferior temporal gyrus (BA 37), the anterior lobe of the right cerebellum, the right middle frontal gyrus (BA 11), the left precentral gyrus (BA 4), and the right postcentral gyrus (BA 2).

Lower rCBF in the WAD group as compared to healthy controls was observed bilaterally in the tempo-occipital transition zone encompassing BA 39 and 19. No significant differences were observed in the insular or anterior cingulate cortices, see figure 5 and table 3.

The rCBF of the cluster found in the right middle frontal gyrus was correlated to NDI ($r = 0.53$, $p = 0.020$). Further, NDI was negatively correlated with the lowered rCBF of the right temporo-occipital transition zone ($r = 0.53$, $p = 0.021$). VAS pain was correlated to the elevated activity in right precentral gyrus ($r = 0.52$, $p = 0.017$). However, these correlations did not survive Bonferroni correction for multiple comparisons.
Figure 5. A statistical parametrical map revealing bilaterally elevated resting rCBF in the posterior cingulate and the parahippocampal gyri in patients with whiplash-associated disorder compared to healthy pain-free controls. The functional images are superimposed on a template MRI image. The hair cross indicates the voxel with peak rCBF difference, at Talairach coordinates (x = -14, y = -44, z = 1). The color bar indicates T-levels.
Table 3. Differences in rCBF between WAD patients and healthy controls. P-values uncorrected for multiple comparisons.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Peak x,y,z</th>
<th>Peak location / (enclosed grey matter)</th>
<th>voxels</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAD&gt;Controls</td>
<td>-14, -44, 11</td>
<td><em>Left posterior corpus callosum</em></td>
<td>264</td>
<td>5.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(-16, -45, 2)</td>
<td>(L. parahippocampal gyrus, BA 30)</td>
<td></td>
<td>(4.48)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>(-18, -47, 2)</td>
<td>(L. lingual gyrus, BA 19)</td>
<td></td>
<td>(4.36)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>(-12, -44, 8)</td>
<td>(L. posterior cingulate, BA 29)</td>
<td></td>
<td>(4.25)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>18, -42, 11</td>
<td><em>Right posterior corpus callosum</em></td>
<td>115</td>
<td>4.21</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>(18, -42, 9)</td>
<td>(R. parahippocampal gyrus, BA 30)</td>
<td></td>
<td>(4.03)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>(20, -38, 11)</td>
<td>(R. caudate tail)</td>
<td></td>
<td>(4.02)</td>
<td>(&lt;0.001)</td>
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<tr>
<td></td>
<td>(20, -36, 11)</td>
<td>(R. posterior thalamus, pulvinar)</td>
<td></td>
<td>(3.86)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>(16, -40, 11)</td>
<td>(R. posterior cingulate, BA 29)</td>
<td></td>
<td>(3.83)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>67, -55, -9</td>
<td><em>R. inferior temporal gyrus, BA 37</em></td>
<td>10</td>
<td>3.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>16, -19, 5</td>
<td><em>R. thalamus</em></td>
<td>24</td>
<td>3.64</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>12, -55, -19</td>
<td><em>R. cerebellum, anterior lobe, dentate</em></td>
<td>31</td>
<td>3.57</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>44, 42, -19</td>
<td><em>R. middle frontal gyrus, BA 11</em></td>
<td>6</td>
<td>3.46</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>-28, -15, 47</td>
<td><em>L. precentral gyrus, BA4</em></td>
<td>6</td>
<td>3.43</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>36, -23, 38</td>
<td><em>R. postcentral gyrus, BA2</em></td>
<td>2</td>
<td>3.39</td>
<td>0.001</td>
</tr>
<tr>
<td>WAD&lt;Controls</td>
<td>53, -65, 20</td>
<td><em>R. middle temporal gyrus, BA 39</em></td>
<td>20</td>
<td>3.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(55, -63, 16)</td>
<td>(R. middle occipital gyrus, BA19)</td>
<td></td>
<td>(3.37)</td>
<td>(0.001)</td>
</tr>
<tr>
<td></td>
<td>(-44, -79, 22)</td>
<td>(L. middle occipital gyrus, BA 19)</td>
<td>6</td>
<td>3.51</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(-44, -78, 24)</td>
<td>(L. middle temporal gyrus, BA 39)</td>
<td></td>
<td>(3.35)</td>
<td>(0.001)</td>
</tr>
</tbody>
</table>

Clusters are indicated in italic font. Peak voxel coordinates are in the Talairach space. As clusters cover more than one functional area, grey matter Brodmann Areas (BA) peaks enclosed by clusters are indicated in parenthesis.
Discussion

Elevated resting state rCBF in the pain matrix, and attenuated rCBF in the parieto-occipital cortical regions in WAD patients as compared to controls was hypothesized.

WAD patients did have rCBF attenuations bilaterally in the temporo-parieto-occipital cortex, and this finding has now been replicated in at least 4 studies, including the present. Temporo-parieto-occipital regions are not usually considered as part of the pain matrix, but attenuations in this region have been observed in experimental models of pain. This was interpreted as a shift of activity away from brain areas not involved in the neuronal processing of pain. In the present study, attenuations were negatively correlated with neck disability ratings but not with VAS pain ratings. Further studies are needed to elucidate whether these decreases reflect an epiphenomenon, or have a functional role in the symptomatology of WAD.

One could speculate that these attenuations of blood flow caudally have a role in the visual disturbances often reported by WAD patients.

The posterior cingulate (PCC) and parahippocampal gyrus had the most pronounced elevations in rCBF. Enhanced activity in the posterior cingulate gyrus has been reported in several imaging studies of acute experimental muscular pain and also in clinical pain syndromes. Pain processing in the PCC is thought to depend on emotional aspects and self relevance of pain, rather than nociceptive input per se. The parahippocampal gyrus has been found to be hyperactive in patients with somatoform pain disorder in response to noxious heat, and also in healthy subjects when heightened pain sensitivity was induced by capsaicin or anxiety. Thus, the present results may be indicative of elevated pain sensitivity or aberrant emotional pain processing.

An alternate, but not necessarily contradictory, interpretation of the heightened posterior cingulate activity in the WAD group is that it reflects stress symptoms. There are many overlapping clinical and epidemiological features between WAD and PTSD. Although no formal diagnostic interview was performed in the present study, the patients reported significantly more PTSD-like symptoms than did healthy controls. The PCC and retrosplenial cortex are involved in relating autobiographical memory to the present emotional state, and the PCC has been implicated in several neuroimaging studies of PTSD. Thus, the elevated resting state rCBF in the posterior cingulate and the parahippocampal gyrus may perhaps be attributed to elevated self referential (pain) processing in WAD.

The elevated rCBF in the right middle frontal gyrus in WAD patients may reflect higher order pain and/or stress relevant cognitions, such as pain catastrophizing. This interpretation is strengthened by the observed correlation between elevated medial prefrontal blood flow and neck disability ratings.
The areas with elevated rCBF in Study I largely coincide with areas recently shown to have lower cortical thickness in fibromyalgia patients.\textsuperscript{136} Areas of elevated rCBF may reflect a compensatory mechanism for regional atrophy.\textsuperscript{137} However, as MRI images were not obtained of the present population, this remains a speculation.

The areas with elevated rCBF also partly correspond to the proposed “default network” described in healthy controls during rest.\textsuperscript{78} When healthy subjects are resting without external stimuli, the medial prefrontal gyrus, the posterior cingulate gyrus and the lateral parietal cortex have elevated and correlated activity. These areas, dubbed the ‘default mode network’, work in concert in a task negative manner, usually displaying rCBF decreases to external stimuli.\textsuperscript{138} In the present data, the patients default mode network seems disrupted at rest, and we suggested that imaging the default mode network in chronic pain patients may be fruitful. Indeed, two recent studies demonstrate similar alterations in the default mode network in chronic pain patients\textsuperscript{72}, and modulation of the network by acupuncture.\textsuperscript{80}

In conclusion, Study I replicated previous studies indicating attenuated temporo-parieto-occipital blood flow in WAD patients. Further, elevated resting state blood flow in the posterior cingulate, parahippocampal, and medial prefrontal areas was demonstrated. It could be speculated that the altered resting state activity is associated with increased self-relevant evaluation of pain and stress in WAD patients as compared to healthy individuals.

**Study I. Additional analyses**

**Relation to Kinesiophobia**

In addition to the analyses in study I, correlation analyses between regional cerebral blood flow and kinesiophobia ratings were performed. For this purpose, the mean rCBF in the clusters with altered rCBF was extracted, and mean values were correlated to TSK ratings. In order to reduce the risk of false positives, only the four largest clusters were investigated, that is the right and left PCC/parahippocapal region with elevated rCBF, and the right and left temporo-occipital clusters with attenuated rCBF.

Kinesiophobia ratings correlated positively with activity in the right PCC ($r = 0.517$, $p = 0.04$) and negatively with activity in right temporo-occipital region ($r = -0.515$, $p = 0.041$). Although these correlations are modest, and do not survive Bonferroni correction, they indicate that the altered rCBF may be related to fear of movement.

In addition, kinesiophobia ratings were correlated with rCBF throughout the brain using SPM2 in an exploratory analysis within the patient group. Positive correlations between kinesiophobia ratings and blood flow were found in the globus pallidus ($Z = 3.62$, $r = 0.79$, $p<0.001$) (figure 6), in the
left middle temporal gyrus ($Z = 3.49, r = 0.77, p<0.001$), and in the sub-
genual ACC ($Z = 3.40, r = 0.76, p<0.001$). The p-values are not corrected for multiple comparisons.

The globus pallidus exerts major inhibitory influence on the thalamus and subserving motor pathways, and has been described as a limbic-somatic motor interface for the planning and inhibition of movement. There are direct projections from the amygdala to the globus pallidus, making it a functionally and anatomically interesting region for fear of movement. Also ACC has previously been linked to fear of movement and affective modulation of pain perception.

Although these analyses are explorative, they indicate that fear of movement may be a core component in the patients pathology, that readily maps on to neuronal alterations. These observed relationships could serve the basis for new, hypothesis driven, investigations of fear of movement.

Figure 6. The relation between rCBF in the Globus Pallidus and patients ratings of kinesiophobia. The Person correlation $= 0.79$, $p<0.001$, gray lines indicate the 95% confidence interval.

Individual differences in rCBF

Previous studies have reported perfusion attenuations in individual subjects, and suggested that these attenuations may be indicative of minimal traumatic brain injury. However, such an interpretation is highly controversial.
In the present patient cohort, no subjects lost consciousness at the time of trauma, which is usually a prerequisite for traumatic brain injuries. Thus, we did not interpret the results as signs of brain injury. However, whether or not individual rCBF PET scans can be interpreted this way deserves an empirical evaluation.

In order to evaluate the validity and reliability of single subject imaging, a statistical single subject analysis was performed. The rCBF image of each WAD patient was compared to the 18 control subjects in a general linear model in SPM2. To evaluate the specificity of this analysis, each control subject was compared to the remaining 17 control subjects in a similar fashion. The significance level was set at p<0.05, corrected for multiple comparisons.

Three of the 21 WAD patients had significant alterations in rCBF (both attenuated and elevated) compared to the control group. None of these three patients reported cognitive disturbances. In the healthy group, 3 of the 18 controls also had significant alterations in rCBF (attenuations and elevations).

These results are most likely not signs of morbidity. Rather, we interpret them as a methodological consequence due to the high interindividual variability in cerebral blood flow. Further, as neither patients nor controls with significantly altered rCBF had cognitive deficits, it seems that cerebral blood flow varies considerably on the individual level, without necessarily leading to behavioural consequences. Thus, we conclude that reports of hypoperfusion in individual subjects may have low specificity, and interpreting hypoperfusion as evidence of brain injury can be seriously questioned, also from a methodological perspective.
Central neurokinin-1 receptor availability was measured in vivo with the carbon-11 labeled NK1 receptor antagonists GR205171, a compound which has a very high affinity for the NK1 receptor. For an in depth description of synthesis, pharmacokinetics and binding properties of GR205171, see Bergström and colleagues. The uptake and binding of [11C]GR205171 was measured over 60 minutes in multiple time frames. The binding of [11C]GR205171 to NK1 receptors was modeled using the Patlak graphical method. When tracer kinetics are irreversible, this method produces a slope value that represents the net accumulation rate of [11C]GR205171 from plasma to the irreversible brain compartment, which is proportional to NK1 receptor density. Although the endogenous substance P (SP) competition with [11C]GR205171 is low, it is not negligible. Therefore, NK1 slope values are interpreted as a measure of receptor availability (NK1 receptor density minus occupancy with endogenous SP).

The parametrical Patlak slope value images were coregistered with [15O]water rCBF images to improve spatial resolution, and normalized to the MNI space using SPM2. The difference between patients and controls was analyzed with a between group ANCOVA, using gender and age as nuisance variables to dissociate potential differences between patients and healthy controls from gender and age effects.

Based on functional and structural neuroimaging studies of chronic pain patients, and on animal studies of NK1 receptor alterations caused by pain and stress, we hypothesised that patients would have decreased NK1 receptor availability in the medial pain system, amygdala, and hippocampus as well as in the periaqueductal gray matter. The functional significance of NK1 receptor availability was explored by correlating NK1 levels to patients’ ratings of pain, anxiety, neck disabilities and kinesiophobia.

Results

WAD patients (n = 18) had widespread reductions of NK1 receptor availability in the medial pain system. The whole brain analysis demonstrated significantly (p = 0.012, corrected) lower NK1 receptor availability in the right ventromedial orbitofrontal cortex (vmOFC) in patients compared to controls (figure 7). The ROI based analysis further revealed that patients had...
significantly (p<0.05, corrected) lower NK1 receptor availability in the left superior frontal gyrus, right anterior cingulate, right and left insula, left hippocampus, left amygdala and in the PAG, see table 4. The thalamus and the primary somatosensory cortex displayed no significant alterations. No region with higher receptor availability in the patient group was observed, even at liberal significance thresholds.

NK1 receptor availability in the brain region with the most pronounced attenuation, the vmOFC, was negatively correlated to TSK ratings of kinesiophobia (n = 12, Z = 3.19, r = -0.808, p = 0.009 corrected), see figure 8, but not to the duration of patients pain, VAS pain, STAI-s, or NDI ratings.

**Figure 7.** Lower neurokinin-1 receptor availability in the ventromedial orbitofrontal cortex in chronic WAD patients as compared to healthy pain free controls. The statistical image, displayed at p<0.001, indicating differences in [11C]GR205171 slopes, is overlaid on a MRI template. The scale indicates T values. Crosshair is located at the peak difference, Talairach coordinates (x = 10, y = 34, z = -17).
Figure 8. Scatter plot of NK1 receptor availability in the vmOFC and patients ratings of kinesiophobia. NK1-Patlak slope values are from the voxel at (x = 12, y = 40, z = -17). The Pearson correlation was $r = -0.81$, $p = 0.009$ corrected.
Table 4. Areas with lower neurokinin-1 receptor availability in WAD patients compared to controls.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Peak x y z</th>
<th>Region (Brodman area)</th>
<th>Z-value</th>
<th>p value cluster</th>
<th>p value voxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Brain</td>
<td>10, 34, -17</td>
<td>R. vm orbitofrontal cortex (11)</td>
<td>4.89</td>
<td><strong>0.012</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R. Insula</td>
<td>44, -21, -2</td>
<td>R. insula (13)</td>
<td>3.39</td>
<td><strong>0.048</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>38, -42, 19</td>
<td>R. Insula (13)</td>
<td>3.19</td>
<td>0.075</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>40, -35, 4</td>
<td>R. Insula (13)</td>
<td>3.11</td>
<td>0.075</td>
<td>0.001</td>
</tr>
<tr>
<td>L. Insula</td>
<td>-42, 12, 10</td>
<td>L. anterior insula (13)</td>
<td>3.34</td>
<td><strong>0.032</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>-48, -40, 19</td>
<td>L. posterior insula (13)</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-46, 2, 5</td>
<td>L. anterior insula (13)</td>
<td>3.21</td>
<td>0.075</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R. ACC</td>
<td>6, 31, -10</td>
<td>R. ACC (32)</td>
<td>3.42</td>
<td><strong>0.038</strong></td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>14, 37, -2</td>
<td>R. ACC (32)</td>
<td>3.42</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L. ACC</td>
<td>n.s</td>
<td></td>
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</tr>
<tr>
<td>R. Frontal</td>
<td>10, 34, -17</td>
<td>R. vm orbitofrontal cortex (11)*</td>
<td>4.89</td>
<td><strong>0.004</strong></td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>6, -13, 50</td>
<td>R. medial frontal gyrus (6)</td>
<td>4.04</td>
<td>0.087</td>
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<td>59, 30, 11</td>
<td>R. inferior frontal gyrus (46)</td>
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<tr>
<td></td>
<td>20, 33, 46</td>
<td>R. superior frontal gyrus (8)</td>
<td>3.41</td>
<td>0.395</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>46, 0, 33</td>
<td>R. precenral gyrus (6)</td>
<td>3.31</td>
<td>0.332</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>30, 38, -9</td>
<td>R. middle frontal gyrus (11)</td>
<td>3.17</td>
<td>0.589</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L. Frontal</td>
<td>-18, 58, -8</td>
<td>L. superior frontal gyrus (11)</td>
<td>3.82</td>
<td><strong>0.012</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-18, 36, -14</td>
<td>L. middle frontal gyrus (11)</td>
<td>3.65</td>
<td>0.127</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-50, -2, 6</td>
<td>L. precenral gyrus (6)</td>
<td>3.62</td>
<td>0.138</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-50, 50, -9</td>
<td>L. middle frontal gyrus (47)</td>
<td>3.17</td>
<td>0.619</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R. Hippo</td>
<td>n.s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. Hippo</td>
<td>-14, -29, -5</td>
<td>L. posterior hippocampus</td>
<td>3.48</td>
<td><strong>0.038</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R. amygdala</td>
<td>n.s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. amygdala</td>
<td>-20, -1, -17</td>
<td>L. amygdala</td>
<td>2.66</td>
<td><strong>0.039</strong></td>
<td>0.004</td>
</tr>
<tr>
<td>PAG</td>
<td>-4, -38, 13</td>
<td>PAG</td>
<td>2.80</td>
<td><strong>0.044</strong></td>
<td>0.003</td>
</tr>
<tr>
<td>L. thalamus</td>
<td>n.s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. thalamus</td>
<td>n.s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. SI</td>
<td>n.s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. SI</td>
<td>n.s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ROI region of interest, R right, L left, vm ventromedial, ACC anterior cingulate cortex, Frontal, frontal lobe, PAG periaqueductal gray matter, SI primary somatosensory cortex. n.s not significant. *Same region as in whole brain analysis. Peak locations are indicated in Talairach coordinates. Cluster p-values are corrected for multiple comparisons with the FWE method, with significant differences in **bold**, voxel p-values are uncorrected.
Discussion

Significantly lower neurokinin-1 receptor availability in chronic WAD patients was observed throughout the brain regions known to be involved in pain processing. The PAG, as well as the left amygdala and left hippocampus had lower NK1 receptor availability, consistent with animal models of pain and stress. There was also lower availability of NK1 receptors in the insula, anterior cingulate and dorsal regions of the prefrontal cortex, predominantly on the right side, a pattern which largely overlaps with the medial network of pain modulation. Previous studies have mostly used functional neuroimaging measurements of blood flow to image pain processes. The present results extend those findings by highlighting the importance of the NK1 receptor in prefrontal, limbic and brain stem regions.

Of particular interest, receptor availability was lowest in the vmOFC (BA 11), a brain region involved in emotional regulation. Further, the amount of available NK1 receptors in the vmOFC was negatively correlated with patients’ ratings of kinesiophobia, suggesting that the higher fear of movement patients’ display, the less available NK1 receptors they have in this emotion regulating area. As the vmOFC has few direct sensory inputs, but efferent projections to both limbic structures and the PAG, the vmOFC is a likely candidate for forebrain modulation of emotionally motivated behaviour. The vmOFC is involved in the extinction of learned fear, and thinner ventromedial orbitofrontal cortex is associated with less ability to remember that a stimuli is no longer dangerous. Patients with somatoform pain disorder and fibromyalgia have less activity in the vmOFC in response to noxious stimulation, and in healthy controls, prefrontal activation is negatively correlated with pain catastrophizing. Recent fMRI also studies indicate that orbitofrontal regions modulate pain-related fear and anxiety and avoidance of a painful threat. In animal models, SP injections into the basal forebrain or basolateral amygdala enhances passive pain avoidance. Taken together, it is plausible that the vmOFC is involved in orchestrating fear induced pain avoidance, and perhaps the extinction of “pain memories”.

The SP/NK1 system interacts with the opioid system in the descending modulation of pain. Recently a combined [11C]diprenorphine PET and fMRI study found increased blood flow and evidence of endogenous opioid release in the right medial orbitofrontal cortex as a function of acupuncture analgesia. We did not observe altered regional cerebral blood flow in the vmOFC in study I, but this was during rest when patients received no external stimulation. One possibility is that NK1 attenuation in the vmOFC does not influence baseline rCBF, but influences rCBF when central inhibition of pain is executed.

Several lines of evidence suggest that there is gray matter loss in chronic pain syndromes, particularly in the cingulate, insular and orbitofrontal cor-
tex, where we observed attenuated NK1 receptor availability. Animal studies indicate decrements in hippocampal NK1 receptor mRNA expression, and also volume alterations (initially increased edema) in the hippocampus as a consequence of prolonged pain or stress.\textsuperscript{62, 63, 84} Also, in animals, NK1 receptor antagonist treatment inhibit stress induced endocytosis of the NK1 receptor\textsuperscript{152} and stress induced hippocampal atrophy.\textsuperscript{153} Decrements in NK1 receptor expression have also been observed in a post-mortem study of major depression.\textsuperscript{77} In that study, NK1 receptor expression was reduced in the rostral orbitofrontal cortex, a reduction that coincided with thinning of gray matter. Thus, one hypothesis worth exploring is that reductions of gray matter density and volume are indicative of NK1 neuronal loss.

Whether the reduced NK1 availability is a cause or a consequence of chronic pain can not be determined in the present study. If reduced NK1 receptor availability was a function of the duration of pain, a negative correlation would be expected, but this was not observed. However, the animal literature supports the notion that chronic pain and stress reduces NK1 receptor expression, and reductions were seen within a few days in those studies.

These results may also shed some light on the discrepant results of NK1 antagonists in animal and human pain. First, the rationale of treating chronic pain patients with NK1 antagonists is questionable if the NK1 system is already down-regulated. Second, animal pain models typically quantify analgesic effects as alterations in locomotive pain behaviour, whereas human clinical trials typically rely on self-report of pain intensity. But pain behaviour and pain self report are not interchangeable constructs. For example, a recent study on cognitive behavioural therapy focusing on exposure and acceptance strategies in whiplash patients found that pain intensity ratings were not affected, but kinesiophobia, functioning and life satisfaction were much improved in the treatment group.\textsuperscript{154} It is possible that other aspects of the human pain experience, i.e. kinesiophobia, may be better suited to evaluate clinical efficacy of drugs targeting the NK1 system.

In fibromyalgia and post traumatic stress patients, elevated SP levels in the cerebrospinal fluid (CSF) have been reported,\textsuperscript{155, 156} but the opposite results have also been observed.\textsuperscript{157} One study reports elevated SP levels in the blood plasma of WAD patients,\textsuperscript{158} and SP levels in the blood plasma are correlated to CSF levels.\textsuperscript{159} Thus, it is possible that reduced binding of \textsuperscript{[11C]}GR205171 can be explained by locally elevated endogenous SP levels.

To summarize, Study II demonstrated extensive reductions in NK1 availability throughout the medial pain matrix in WAD patients. The NK1 receptors in the ventral medial orbitofrontal cortex seem to be involved in pain related fear and avoidance, a behaviour that may contribute to the persistence of pain.
Study III. Chronic inflammation

$^{[11\text{C}]}\text{D-deprenyl}$

D-deprenyl is the mirror isomer of L-deprenyl (commercial name Selegiline) which is a potent inhibitor of the enzyme monoamine oxidase B (MAO-B). L-deprenyl has been labeled with carbon-11 and successfully used as a radioligand to image the regional distribution of MAO-B in the brain.\textsuperscript{160} D-deprenyl is 20 to 500 times less potent as an MAO-B inhibitor.\textsuperscript{161, 162} Because of its lower affinity, $^{[11\text{C}]}\text{D-deprenyl}$ was originally developed as an inert control radioligand for $^{[11\text{C}]}\text{L-deprenyl}$. It was later observed that $^{[11\text{C}]}\text{D-deprenyl}$ shows elevated uptake in inflamed tissue, but the mechanism of this retention is not known.

Danfors and colleagues used $^{[11\text{C}]}\text{D-deprenyl}$ in a study of patients with rheumatoid arthritis, and high tracer retention was observed in the synovium of the knee joint.\textsuperscript{163} Intraarticular corticosteroid injections resulted in clinical improvement and a significant reduction of radioligand retention, suggesting that $^{[11\text{C}]}\text{D-deprenyl}$ may be used as measure of inflammatory processes.

The purpose of study III was to explore the use of PET to indicate chronic inflammation in WAD. Given that $^{[11\text{C}]}\text{D-deprenyl}$ accumulates in tissue with ongoing inflammation, we investigated the presence of locally elevated $^{[11\text{C}]}\text{D-deprenyl}$ in chronic WAD patients and healthy, pain free controls using PET. Although the study was explorative, we hypothesized locally elevated tracer uptake in cervical soft tissue regions in WAD patients as compared to controls.

Quantifying PET tracer uptake in neck regions

All patients were investigated using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, USA). The control group was investigated using a GE Discovery ST PET-CT scanner (General Electric Medical Systems, Milwaukee, WI). Three subjects were investigated with both the HR+ scanner and the PET CT scanner.

Regions of interest (ROIs) were defined in non glandular regions with elevated uptake, in the regions adjacent to the spineous process of axis, in the spongeous bone of the cervical vertebra 2 through 6, and in muscle tissue without visually evident elevated radioligand uptake. The definitions of the ROIs are summarized in table 5. ROIs were positioned manually in the last
frame of the PET investigation (35-45 minutes post tracer administration), and across subjects, ROIs were of identical size and shape. The positions of the ROIs are illustrated in figure 9 and 10.

Figure 9. Sagittal display of a $[^{11}\text{C}]$D-deprenyl PET image of a healthy control. Regions of interest in (A) the spongyous bone of the vertebra (VERT_2) and in (B) normal muscle tissue (MUSCLE) are indicated.

Figure 10. Horisontal, coronal and sagittal display of C2_LEFT and C2_RIGHT ROIs in a $[^{11}\text{C}]$D-deprenyl PET image.

Table 5. Region of interest (ROI) definitions

<table>
<thead>
<tr>
<th>ROI</th>
<th>Size (mm$^3$)</th>
<th>Shape</th>
<th>Position/definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAK_1</td>
<td>500</td>
<td>Sphere</td>
<td>Non glandular soft tissue with maximum uptake</td>
</tr>
<tr>
<td>PEAK_2, 3</td>
<td>500</td>
<td>Sphere</td>
<td>2$^{nd}$ and 3$^{rd}$ highest non glandular soft tissue uptake</td>
</tr>
<tr>
<td>C2_LEFT</td>
<td>4200</td>
<td>Sphere</td>
<td>Left of the spineous process of C2</td>
</tr>
<tr>
<td>C2_RIGHT</td>
<td>4200</td>
<td>Sphere</td>
<td>Right of the spineous process of C2</td>
</tr>
<tr>
<td>VERT_2-6</td>
<td>500</td>
<td>Sphere</td>
<td>Spongyous bone/bone marrow of C2 through C6</td>
</tr>
<tr>
<td>VERT_M</td>
<td>Mean of VERT_2-6</td>
<td>Prolate spheroid</td>
<td>Muscle region with low uptake</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>6300</td>
<td>Prolate spheroid</td>
<td>Muscle region with low uptake</td>
</tr>
</tbody>
</table>
The standardized uptake value (SUV) is a widely used semi-quantitative PET measure, calculated as a ratio of tissue radioactivity concentration and injected dose at the time of injection divided by body weight.\textsuperscript{164} An image analysis program (Amide Medical Image Data Examiner, version 0.9.1)\textsuperscript{165} generated time activity data for each ROI in each subject, representing levels of radioactivity at different time points expressed as SUV. For each time activity curve, the area under the curve (AUC) (figure 11) was calculated in order to obtain values for comparisons between patients and healthy volunteers.

Test- retest reliability

Three patients were investigates with both the HR+ PET scanner and the Discovery PET-CT scanner. To evaluate between scanner and session test-retest reliability, SUV values for radioligand uptake at all time points were extracted. The Pearson correlation between scanners within subjects was calculated for the five vertebra ROIs \texttt{VERT\_2-6}, and for the three peak ROIs \texttt{PEAK\_1, 2 &3}.
Results

WAD patients had significantly higher $[^{11}\text{C}]\text{D-deprenyl}$ uptake than controls in tissue regions adjacent to spineous process of the second vertebra ($C_{2\_\text{RIGHT}} p = 0.004, C_{2\_\text{LEFT}} p = 0.021$) see table 6, figure 12 and 13. Maximal uptakes were located around the spineous process of axis in most patients, and also at the insertion of rectus capitis posterior major in the occipital bone. From the three patients investigated with combined PET and CT, it became evident that the $[^{11}\text{C}]\text{D-deprenyl}$ uptake was located in the fat tissue surrounding the muscle, see figure 12. In the healthy control group, uptake in the fat between the sternocleidomastoid muscle and the levator scapulae muscle was the most common finding, see table 7, and the appendix for an anatomical overview. The uptake of $[^{11}\text{C}]\text{D-Deprenyl}$ at $C_{2\_\text{RIGHT}}$ and $C_{2\_\text{LEFT}}$ was not correlated to patients pain, neck disability or fear of movement.

The AUC for the tissue with peak uptake, PEAK_1, was elevated in the patient group ($p = 0.071$, n.s). In the ROI of presumably normal the muscle tissue, MUSCLE, AUC values were also elevated in the patient group ($p = 0.11$, n.s.). In the vertebra, VERT_M, AUC values were not markedly different ($p = 0.33$, n.s.). See table 6.

The test-retest reliability between scanners was high, both in the presumably normal tissue of the vertebral column and in PEAK areas. The correlation between the first and the second scan in AUC measures of VERT_2-6 was high ($r = 0.86, p<0.001$) Peak uptakes were in the same anatomical position in scan one and two, and the test-retest correlation was high also for PEAK_1,2 &3 (mean $r = 0.80, p<0.001$).
Figure 12. The left column displays horizontal, coronal and sagittal images of a WAD patient with presumably abnormal $[^{11}\text{C}]$D-deprenyl uptake at the occipital bone and at the right spinoceous process of axis. To the right is a healthy control subject. The color scale indicates standardized uptake values. The PET image is superimposed on a CT image.
Figure 13. Mean SUV time activity curves in C2_RIGHT (diamonds) and C2_LEFT (squares) in WAD patients (red) and controls (blue). Error bars indicate standard errors.

Table 6. Area under curve measures for $[^{11}\text{C}]\text{D-deprenyl}$ retention in regions of interest.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Patients AUC (±SD)</th>
<th>Controls AUC(±SD)</th>
<th>t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEAK_1</td>
<td>71.6 ±29.6</td>
<td>53.9±19.0</td>
<td>0.071</td>
</tr>
<tr>
<td>C2_RIGHT</td>
<td>40.4±15.3</td>
<td>26.0±8.5</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>C2_LEFT</td>
<td>41.3±19.7</td>
<td>27.9±9.8</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>VERT_M</td>
<td>57.1±14.0</td>
<td>51.8±12.2</td>
<td>0.33</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>25.6±6.9</td>
<td>21.0±6.4</td>
<td>0.11</td>
</tr>
</tbody>
</table>
There was an overlap between AUC measures for patients and controls (figure 14). By using the AUC metric for \texttt{C2\_RIGHT} and \texttt{C2\_LEFT}, the sensitivity and specificity of this metric to predict WAD was calculated, see figure 14 & 15. With uptake superseding one standard deviation as a criterion for abnormality, the sensitivity was 63%, with a specificity of 88%. The positive predictive value with this criterion was 93%, and the negative predictive value was 47%. Using a more conservative cut-off value of 2 standard deviations above mean, sensitivity dropped to 50%, but with 100% specificity in this sample. The positive predictive value was then 100%, but with a negative predictive value of 42%.

\textbf{Figure 14.} Individual time activity curve area (AUC) measures (×100) for [\textsuperscript{11}C]D-deprenyl retention in \texttt{C2\_RIGHT} and \texttt{C2\_LEFT}, each subject contributes to two measure points. Solid line indicates mean value for controls and patients, dashed lines indicate one standard deviation.

\textbf{Figure 15.} Sensitivity and specificity calculation matrix.
Table 7. Locations of the most elevated $[^{11}\text{C}]$D-deprenyl uptake and its area under curve of the time activity data in patients and controls. Locations are in non glandular soft tissue. Anatomical locations are approximate and may refer to tissue surrounding the muscle.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Location of PEAK_1</th>
<th>AUC of PEAK_1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 5</td>
<td>Left semispinalis cervicis muscle at C2</td>
<td>147.0</td>
</tr>
<tr>
<td>Patient 1</td>
<td>Left oblicuus capitis inferior muscle</td>
<td>124.0</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Right rectus capitis posterior major</td>
<td>120.0</td>
</tr>
<tr>
<td>Patient 10</td>
<td>Left insertion of rectus capitis posterior major in occipital bone</td>
<td>102.0</td>
</tr>
<tr>
<td>Patient 20a</td>
<td>Left fat between sternocleidomastoid and levator scapulae</td>
<td>95.9</td>
</tr>
<tr>
<td>Patient 12</td>
<td>Left semispinalis cervicis at C2</td>
<td>88.7</td>
</tr>
<tr>
<td>Patient 16</td>
<td>Nuchal ligament at axis</td>
<td>78.4</td>
</tr>
<tr>
<td>Patient 13</td>
<td>Left insertion of rectus capitis posterior major in occipital bone</td>
<td>68.8</td>
</tr>
<tr>
<td>Patient 22a</td>
<td>Right fat between semispinalis capitis and C3</td>
<td>67.6</td>
</tr>
<tr>
<td>Patient 6</td>
<td>Left semispinalis cervicis muscle at C7</td>
<td>66.8</td>
</tr>
<tr>
<td>Patient 14</td>
<td>Left semispinalis cervicis at C2</td>
<td>62.1</td>
</tr>
<tr>
<td>Patient 21a</td>
<td>Right fat at upper insertion of rectus capitis posterior major</td>
<td>59.7</td>
</tr>
<tr>
<td>Patient 8</td>
<td>Left levator scapulae at C4</td>
<td>57.3</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Right oblicuus capitis inferior / semispinalis capitis at C2</td>
<td>56.9</td>
</tr>
<tr>
<td>Patient 7</td>
<td>Right multifidus muscle at C2</td>
<td>56.3</td>
</tr>
<tr>
<td>Patient 18</td>
<td>Right semispinalis at C6</td>
<td>53.3</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Right rectus capitis posterior major</td>
<td>51.5</td>
</tr>
<tr>
<td>Patient 15</td>
<td>Splenius capitis muscle at C5</td>
<td>47.0</td>
</tr>
<tr>
<td>Patient 9</td>
<td>Left sternocleidomastoid muscle at occipital bone</td>
<td>46.2</td>
</tr>
<tr>
<td>Patient 19</td>
<td>Left semispinalis at occipital insertion</td>
<td>45.1</td>
</tr>
<tr>
<td>Patient 11</td>
<td>Right insertion of sternocleidomastoid in occipital bone</td>
<td>44.3</td>
</tr>
<tr>
<td>Patient 17</td>
<td>Right sternocleidomastoid muscle</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>71.6 (±29.6)</td>
</tr>
</tbody>
</table>

| Control 1b | Right fat behind sternocleidomastoid muscle             | 81.8          |
| Control 2b  | Right fat behind rectus capitis muscle                   | 78.3          |
| Control 8b  | Right fat between semispinalis and spinalis capitis at C2 | 66.1          |
| Control 4b  | Left fat behind sternocleidomastoid muscle               | 48.7          |
| Control 7b  | Right fat behind sternocleidomastoid muscle              | 46.8          |
| Control 6b  | Right fat behind sternocleidomastoid muscle              | 41.4          |
| Control 5b  | Right splenius capitis                                  | 35.0          |
| Control 3b  | Right fat behind sternocleidomastoid muscle             | 33.5          |
|            | Mean (±SD)                                              | 53.9 (±19.0)  |

*PET & PET-CT  **PET-CT
Discussion

PET demonstrated elevated uptake of $^{[11}C]D$-deprenyl in cervical soft tissue areas in patients with chronic pain after whiplash injury. In the three patients examined with PET-CT, it became evident that the $^{[11}C]D$-deprenyl uptake is located to the adipose tissue surrounding the deep cervical muscles. This pattern of results may indicate that there is persistent inflammation in deep cervical muscles regions in WAD.

Autopsy$^{166}$ and electromyography$^{167}$ studies suggest an increase in type IIIC muscle fibers in the neck flexor muscles in chronic neck pain patients, and WAD patients have also been demonstrated to have high fatty infiltration in the cervical muscles.$^{30, 31}$ Also, impairments in intramuscular microcirculation have been demonstrated in painful muscles.$^{168}$ These alterations in the microstructure and tissue composition of cervical tissue in WAD patients are some of the possible mechanisms which could lead to accumulation and trapping of $^{[11}C]D$-deprenyl.

Two main hypotheses for $^{[11}C]D$-deprenyl uptake in inflamed tissue have been postulated: the trapping in low pH regions as demonstrated with lysosomotropic agents, or binding to a specific (unknown) molecular structure$^{163}$. The pharmacology of deprenyl is complex and extends beyond MAO-B inhibition.$^{169-171}$ As D-deprenyl is 20 to 500 times less potent as an MAO-B inhibitor than is its mirror isomer L-deprenyl,$^{161, 162}$ the observed uptake is probably independent of MAO-B. The independency from MAO-B is further supported by the observation by Danfors and colleagues that administration of 10 mg L-deprenyl did not block $^{[11}C]D$-deprenyl uptake, but rather increased it slightly.$^{163}$

Interesting characteristics of D-deprenyl are its cytoprotective and antiapoptotic properties,$^{172-174}$ possibly acting through an adaptive increase in superoxide dismutase activity.$^{170, 175}$ This effect is seen independently of MAO-B inhibition, thus making superoxide dismutase a possible candidate for a binding site of $^{[11}C]D$-deprenyl. Also, deprenyl can increase cell-cell adhesion, an important process in the accumulation of leukocytes and regeneration of the extracellular matrix in inflamed tissue.$^{176}$

In cancer studies, when 2-$^{[18}F$]-fluoro-2-deoxy-glucose (FDG) is used to detect tumors, an uptake resembling the current finding is sometimes seen in patients who are cold and freeze during the PET investigation.$^{177, 178}$ This uptake is attributed to sympathetic noradrenergic outflow, leading to elevated metabolism of the brown adipose tissue, i.e. a normal bodily reaction to keep blood and body temperature at homeostasis.

At the time of the study, we did not consider the possibility that $^{[11}C]D$-deprenyl might also be susceptible to temperature driven brown adipose tissue trapping. This is possible, although all subjects were indoors in a centrally heated environment kept at 21°C for at least one hour prior to tracer administration. The prevalence of brown fat FDG uptake is also much lower.
than in the present study. Here we observed elevated uptake in at least 50% of the patients. In cancer patients, one large study identified 17 cases of FDG uptake in brown fat in 638 patients (2.5%) screened for tumors. However, the elevated $[^{11}\text{C}]$D-deprenyl uptake in the patients group could be mediated by pain induced activity in the sympathetic nervous system. Noxious mechanical stimulation increases sympathetic efferent nerve activity in the brown adipose tissue of the neck in rats, and the sympathetic nervous system has been implicated in the initiation and maintenance of several pain syndromes. Specifically, noradrenalin levels and sympathetic nervous system reactivity has been shown to be elevated in WAD patients, especially those with post traumatic stress symptoms. One possibility, in light of the current observations, is an active involvement of (brown) adipose tissue in the inflammatory response to a neck sprain. This may be one starting point of a chronic vicious feed back loop of inflammation, pain, muscle disuse, and altered enervation, involving both peripheral tissue, sympathetic outflow and central modulation of pain.

To conclude, Study III provides, to our knowledge, the first visualization of possible chronic inflammatory mechanisms in WAD patients. We have demonstrated an elevated uptake of $[^{11}\text{C}]$D-deprenyl in local areas of adipose tissue in patients with chronic pain after whiplash. Although there was a wide variation between patients, the fat tissue at the spineous process of axis had particularly high levels of radioligand retention. Patients with WAD may have persistent inflammation in the neck, and PET studies with $[^{11}\text{C}]$D-deprenyl may contribute to the development of a sensitive, specific and reliable biomarker for injury in this large patient population. Further studies are necessary to elucidate the binding mechanism of $[^{11}\text{C}]$D-deprenyl, the possible role of adipose tissue in the inflammatory response, and how $[^{11}\text{C}]$D-deprenyl can be used for prognosis evaluation in WAD.
General discussion

This thesis provides three new insights to the multifaceted problem of chronic pain after a rear impact car accident:

1. Whiplash patients have alterations in resting state regional cerebral blood flow, with attenuations in temporo-occipital regions and elevations in the posterior cingulate and parahippocampal gyrus.

2. Whiplash patients have extensive reductions in neurokinin-1 receptor availability throughout the medial pain matrix, including the ventromedial orbitofrontal cortex, where the magnitude of NK1 reductions is linearly related to kinesiophobia.

3. Whiplash patients have an indication of chronic inflammatory processes in the soft tissue in the cervical neck.

The results bridge over the Cartesian division between mind and body. Chronic pain, whether associated with actual or potential tissue damage, has profound behavioral and emotional consequences, that in turn have physiological manifestations in the brain. The debate surrounding the origin of chronic pain in WAD patients will no doubt continue, but the present results contribute to a more integrative view, where the mind and body are not seen as separate entities.

Clinical implications

How may the results from this thesis contribute to the clinical management of WAD? A recent review of treatment options in WAD recommends early physical activity in acute WAD.\textsuperscript{183} In chronic WAD, a combination of cognitive behavioral therapy (CBT) with physical therapy interventions and coordination exercise has moderate scientific support. If our interpretations of the results in Study I are correct (an elevated introspective and emotional pain processing in WAD), CBT programs that address pain cognitions and memories may be helpful. Evaluating if CBT can alter neuronal pain processing and resting state brain activity is an interesting future endeavor. A recent report on CBT for irritable bowels syndrome indeed suggest reductions in posterior cingulate resting state activity after therapy.\textsuperscript{184}

Study II implies that the NK1 system could be a treatment target in chronic pain. Unfortunately, treating pain patients with NK1 inhibiting drugs
has been unsuccessful, but, to our knowledge, NK1 antagonists have not been evaluated in WAD patients. Also, if the NK1 system is already down regulated centrally, the rationale for NK1 antagonist treatment can be questioned. Further, Study II suggests the possibility that NK1 receptors are not primarily involved in pain perception, but in pain related avoidance behavior. Thus, pharmacological or behavioral therapy targeting fear of movement may be beneficial for WAD patients.

The results from Study III may be the first step towards a clinical application. [11C]D-deprenyl provides a relatively specific and sensitive marker for WAD, which has not been available before. The incidence of inflammation could potentially be regarded as a dividing factor between WAD with tissue damage and WAD without tissue damage, and the two subtypes may require different treatment strategies. In patients with locally elevated [11C]D-deprenyl, the affected areas may perhaps be targeted directly. Intramuscular injections of corticosteroids or anesthetic agents are used clinically, but have not received strong scientific support. However, by first localizing the locus of the inflammation with [11C]D-deprenyl, such treatment may be more effective. This remains to be evaluated.

**Cause or consequence**

One puzzling clinical question is why some individuals recover from a rear impact neck injury, and some develop long term disabilities. Whether the alterations in the brain and cervical soft tissue are the cause or consequence of chronic pain can not be determined by the present studies. It is possible that, for example, an attenuated NK1 receptor system is a risk factor present in some individuals already before an injury. The same goes for the elevated D-deprenyl uptake. If it is not indicative of inflammation, but rather is indicative of metabolically active brown fat, we must ask why this is more common amongst chronic WAD patients. Metabolically active brown fat has relatively low prevalence in the adult population, around 2-10%, and, if D-deprenyl is not indicative of inflammation, the results from study III would suggest that brown fat in the cervical neck region could be a risk factor for WAD. A third possibility, in light of the recent findings on fat content in cervical muscles, is that not using the deep cervical muscles because of inflammation, pain or kinesiophobia, will increase their fatty infiltration. Elevated [11C]D-deprenyl could perhaps be indicative of this process.

During the initial phase of an injury, avoiding movement enhances the healing process, but, as stated before, fear of movement is also predictive of the development of chronic pain. Is fear of movement the villain in the drama? Or is fear of movement just a logical consequence, as moving an injured body part produces pain, and thereby serving as negative reinforcement for immobilization? Studying rCBF, NK1 receptor availability and D-
deprenyl neck uptake in the acute phase of an injury would shed light on the progression from acute injury to chronic pain. Prospective studies are needed to determine what risk factors (psychological, physiological, or genetic) contribute to chronic pain. But studying pain prospectively is difficult, as we rarely know who will be injured, and thus, a pre-injury baseline measurement can not readily be obtained. Also, in the acute phase, either an equivalent trauma, or an accurate quantification of tissue damage would be necessary in order to compare individuals.

Future directions

Bringing the studies together: Multiple tracer analyses

How do the results of these studies relate to each other? Based on the presence or absence of inflammation in the cervical neck, is it possible to predict rCBF for NK1 receptor availability? Or vice versa? Are rCBF alterations associated with NK1 receptor availability? These questions are beyond the scope of this thesis, but some preliminary analyses and results are none the less outlined below.

![Figure 16. Illustration of a three dimensional coordinate system, where the results from study I, II and III could be mapped out.](image)

Relating rCBF measures to NK1 receptor expression, both in the same brain regions, and in distal parts within the networks of the brain, require the development of multi-tracer PET protocols. This field holds much promise, as blood flow measurements such as $[^{15}O]$water PET and fMRI methods give
no direct evidence of what neurotransmitters are released in the synapses. For example, an increased rCBF or BOLD signal is indicative of increased local field potentials or neuronal signaling, but whether this signaling causes glutamate, GABA, monoamine, or other neurotransmitter release is not known. Further, if local signaling has excitatory or inhibitory effects on other brain regions, and other neurotransmitter release, also remains to be explored.

The NK1 system was altered throughout the medial pain matrix in patients, whereas resting state rCBF was normal in the insula and anterior cingulate, and with only minor increases prefrontally. This may indicate that $[^{11}C]$GR205171 imaging is more sensitive to pathology than blood flow measurements. Further, receptor imaging is intimately related to pharmacology, as the radioligands used often are drug candidates in development. Thus, identifying alterations in receptor systems may be a shortcut towards clinical applications and drug development.

To begin investigating the relationship between rCBF, NK1 receptors and peripheral inflammation, an explorative analysis was performed in the patient group. The mean rCBF in the left and right posterior cingulate/parahippocampal gyrus from study I was extracted using the software package Marsbar. The same was done for study II, by extracting the mean Patlak slope value of the vmOFC cluster with reduced NK1 receptor availability. From study III, the mean AUC measure for C2_RIGHT and C2_LEFT was used. These values, along with neck disability ratings and the Tampa scale for kinesiophobia, were correlated with each other.

The analysis revealed that rCBF of the right PCC was positively correlated to TSK ratings, and negatively to vmOFC NK1 receptor availability (table 8). As noted in study II (with a different method), NK1 receptor availability in vmOFC was negatively correlated to TSK ratings also here. All other correlations were low and non-significant. With Bonferroni corrections for multiple comparisons, only the negative correlation between rCBF in the right PCC and NK1 levels in the vmOFC was significant, $p = 0.012$ corrected.
Table 8. Correlations within the patient group between the major altered metrics from study I, II & III.

<table>
<thead>
<tr>
<th>Metric</th>
<th>rCBF_L.PCC</th>
<th>NK1_vmOFC</th>
<th>C2 ROI</th>
<th>NDI</th>
<th>TSK</th>
</tr>
</thead>
<tbody>
<tr>
<td>rCBF_L.PCC</td>
<td>0.24</td>
<td>-0.12</td>
<td>-0.29</td>
<td>0.23</td>
<td>0.06</td>
</tr>
<tr>
<td>rCBF_R.PCC</td>
<td>-0.70**</td>
<td>0.12</td>
<td>0.41</td>
<td>0.41</td>
<td>0.52*</td>
</tr>
<tr>
<td>NK1_vmOFC</td>
<td>-0.11</td>
<td>-0.24</td>
<td>-0.59*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2 ROI</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDI</td>
<td></td>
<td></td>
<td></td>
<td>0.37</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Interpreting these preliminary results requires caution. It may be that they reflect common behavioral processes, perhaps manifested as an increased introspective emotional evaluation of pain, in combination with an exaggerated fear of movement. Also the correlation between kinesiophobia and rCBF in the Globus Pallidus suggest that tonic inhibition and avoidance of movement is an important aspect of chronic pain. These speculations may form the basis for future hypothesis driven experimental designs. Voxel vise whole brain analyses, for example evaluating where in the brain blood flow and NK1 receptor availability is correlated and anti-correlated, are on the future agenda.

D-deprenyl and regional cerebral blood flow

The indication of inflammation in the cervical neck, from study III, was not correlated to pain, neck disability, fear of movement, altered brain blood flow, or to NK1 receptor availability. The incidence of inflammation could potentially be regarded as a dividing factor between WAD with tissue damage and WAD without tissue damage. Perhaps the first but not the latter patients will respond to systemic or local anti-inflammatory treatment. If WAD patients can be divided into subgroups with different pathologies, the rCBF and NK1 measures may be different in the two subgroups, and the results in study I and II represent the alterations common to both subgroups.

To explore the relationship between D-deprenyl uptake and rCBF, a correlation analysis within the WAD group was performed. The $[^{11}\text{C}]$D-deprenyl AUC from C2 ROI was used as a regressor to rCBF in patients. If $[^{11}\text{C}]$D-deprenyl is an index of peripheral damage, one might expect subjects with high D-deprenyl uptake to have high nociceptive inflow, and vice versa. Thus, the lateral and medial pain matrix would have higher blood flow the higher the D-deprenyl uptake.

The preliminary regression analysis revealed no significant correlations between peripheral $[^{11}\text{C}]$D-deprenyl and central rCBF using stringent statistical criteria. However, at a threshold of $p<0.001$, uncorrected, the activity of
the left amygdala, and the right caudate tail were correlated to $[^{11}C]\text{D-deprenyl}$ uptake. Inflammation has been shown to activate extracellular signal-regulated kinase in the amygdala, a process necessary for long lasting hyperalgesia associated with persistent inflammation. Further, the amygdala is generally thought of as a threat detecting region involved in fear. As the amygdala has projections to sympathetic neurons, and the brown fat is sympathetically enervated, these relationships are worth further exploration.

An intriguing result was a negative correlation between D-deprenyl uptake and rCBF in the left tempo-occipital transitions zone. Patients displayed reduced rCBF as compared to controls in this area, and also, the rCBF in this region was negatively correlated to the D-deprenyl uptake in the neck ($r = -0.72$, $p<0.001$). As originally speculated by Otte and coworkers, it may be that inflammation in the neck tissue causes the release of vasoactive peptides. These peptides, perhaps substance P, could then reduce posterior cortical blood flow via influence on the posterior cerebral artery. Although speculative, this is another relationship that deserves further exploration.

**Neurokinin-1 availability and $[^{11}C]\text{D-deprenyl}$ uptake**

A preliminary correlation analysis between NK1 receptor availability and $[^{11}C]\text{D-deprenyl}$ uptake at the spineous process of axis was performed. The analysis revealed a significant positive correlation ($r = 0.80$, $Z = 4.08$, $p<0.001$ uncorrected, $p = 0.008$ corrected) between $[^{11}C]\text{D-deprenyl}$ uptake and NK1 receptor availability in the right occipital lingual gyrus, BA 18. In the corresponding region in the left hemisphere, there was also a negative correlation, but less striking ($Z = 3.67$, $p<0.001$ uncorrected, $p = 0.22$ corrected). These results are similar to the negative correlation between the parietal/occipital cortex blood flow and inflammation. Mechanisms may be similar, in that the inflammation in the neck affects SP content in the posterior vertebral artery. This SP, or its metabolites, may perhaps pass the blood brain barrier and subsequently influence NK1 receptor availability in the parietal/occipital region. Another important question to explore is whether the NK1 receptor system is up regulated peripherally, and down regulated centrally, as some recent animal studies indicate.

Taken together, these preliminary analyses suggest the potential of using multiple radiotracers to map disorders, and the need for an integrative and multifaceted perspective on brain and body function during disease. Brain blood flow and receptor expression may well be influenced both by behavioral and psychological processes, as well as physiological aspects of inflammation and perhaps also the anatomy of the brains blood supply.
Sprained ankles as a model for tissue inflammation

One major limitation to the interpretation of Study III is that the binding mechanism of $[^{11}C]$D-deprenyl is not known. To explore how $[^{11}C]$D-deprenyl behaves in acutely injured and inflamed tissue, we are currently investigating $[^{11}C]$D-deprenyl uptake in patients with acute ankle sprains. Eight subjects have been investigated at the time of writing, both acutely (~5 days post trauma), and with a 5 week follow-up. All patients had substantial pain, were unable to support their weight on the injured foot, and had edema around the sprained ankle. $[^{11}C]$D-deprenyl uptake was highly elevated in area of the injured tendon, and the location of uptake corresponded well to the patients’ experience of pain. The exact location of the elevated $[^{11}C]$D-deprenyl uptake has not yet been thoroughly analysed, but preliminary analyses suggest that the uptake is concentrated to fat tissue surrounding injured ligaments, much in the same way that we observed in chronic WAD patients. The other, non sprained ankle, serves as a control region, and had no notable tracer uptake. At the 5 week follow up, all patients were walking without difficulty and had little visible edema. Patients still experienced occasional residual pain. The $[^{11}C]$D-deprenyl uptake was however still highly elevated, approximately the same level as acutely. Interestingly, at the 5 week follow-up, $[^{11}C]$D-deprenyl retention had shifted slightly, from the injured tendon towards the bone surface. We suspect that $[^{11}C]$D-deprenyl shows retention to some inflammatory process that is present both acutely and fore some time into the proliferative healing phase. One candidate process is the regeneration of the extracellular matrix after an injury, perhaps the turnover process from myofibroblast to collagen. A 6 month follow up, when patients are fully recovered, is planned.

These results point to the potential of $[^{11}C]$D-deprenyl PET to measure soft tissue injury processes. PET could potentially serve as an objective biomarker in several debilitating pain syndromes, such as lower back pain, fibromyalgia and rheumatoid arthritis. Perhaps also cardiovascular, metabolic and neurodegenerative syndromes with inflammatory components can be imaged, but that is a future field of investigation.
Closing comments

Whiplash-associated disorder is a multifaceted problem that needs to be studied from several different perspectives. This thesis gives evidence of objectively measurable alterations in both the neck and in the brain in WAD patients, something that up to now has been quite scarce in the whiplash field. Hopefully, these results will inspire further investigation, leading to a better understanding of how our mind and our body interact in response to pain and inflammation, and the progression from acute injury to chronic illness. Ultimately, such knowledge will help us find new and improved treatment options for chronic pain disorders.
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Clas, Uppsala, den 20 oktober 2008
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Appendix with anatomical illustrations


Muscles of the neck, lateral view
Superficial muscles of the back
Deep muscles of the back
A doctoral dissertation from the Faculty of Social Sciences, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Social Sciences. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Social Sciences”.)