Fatigue after Traumatic Brain Injury
Exploring Novel Methods for Diagnosis and Treatment

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To my dear family: Ida, Isak and Noel
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Abstract

Background

Traumatic brain injury (TBI) is one of the most common causes of disability and mortality. While some patients recover quickly, especially at the mild side of the injury severity continuum, many will experience symptoms for years to come. In this chronic phase, patients report a wide array of symptoms, where fatigue is one the most common. This fatigue makes huge impact in several areas of these patients’ lives. Despite the prevalence of fatigue after TBI, the underlying mechanisms are unclear. Further, there are no standardized way for assessment and diagnosis, and there are no treatments with satisfying empirical support. The aim of this thesis was to examine the effects of the novel compound OSU6162 on fatigue in patients with TBI, and to explore functional and structural brain imaging correlates of fatigue after TBI.

Methods

Studies I and III were based on a placebo-controlled, double-blinded clinical trial examining the effects of the monoaminergic stabilizer OSU6162 on fatigue in patients in the chronic phase of traumatic brain injury. In study I, self-assessment scales of fatigue and neuropsychological tests were used as outcomes, while functional magnetic resonance imaging (fMRI) blood-oxygen-level dependent (BOLD) signal was the primary outcome in study III. Studies II and IV used cross-sectional designs, comparing patients with TBI with age- and gender matched healthy controls. Study II examined whether fMRI BOLD signal could be used to detect and diagnose fatigue in patients with TBI, and study IV whether white matter hyperintensities (WMH) contribute to lower cognitive functioning and presence of fatigue after TBI.

Results

Study I revealed no effects of OSU6162 during 28 days of treatment at maximum doses of 15 mg twice daily on measures of fatigue or any other outcome. The results from study II indicated that fatigue after TBI is linked to alterations in striato-thalamic-cortical loops, and suggested that fMRI could be a promising technique to use in the diagnosis of fatigue after TBI. In study III the results revealed effects of treatment in the right occipitotemporal and orbitofrontal cortex. In these areas, the BOLD response was normalized in the OSU6162 group as compared to healthy controls, while the placebo group showed a steady low activity in these areas. The regional effects were located outside the network shown to be linked to fatigue in study II, which might explain why there were no
effects on fatigue after treatment with OSU6162 in study I. Study IV showed that WMH lesions increased with increased TBI severity, but the presence and extent of lesions did not explain lower neuropsychological functioning or fatigue in subjects with previous TBI.

**Conclusions**

In summary, although no effects on fatigue after treatment with OSU6162 were seen, the results provide support to the theory that fatigue after TBI is linked to alterations in striato-thalamic-cortical loops, and on how fatigue after TBI could be assessed or diagnosed using fMRI. Structural damage within white matter was however not related to fatigue.
Abbreviations

BOLD; Blood-oxygen-level dependent
CBT; Cognitive Behavioral Therapy
CT; Computed Tomography
D-KEFS; Delis-Kaplan Executive Functions System
DAI; Diffuse Axonal Injury
ECG; Electrocardiography
fMRI; Functional Magnetic Resonance Imaging
FSS; Fatigue Severity Scale
GCS; Glasgow Coma Scale
HAD; Hospital Anxiety and Depression Scale
IVA; Integrated Visual and Auditory Continuous Performance Test
MFS; Mental Fatigue Scale
MNI; Montreal Neurological Institute
MRI; Magnetic Resonance Imaging
mSDMT; modified Symbol Digit Modalities Test
OSU6162; (−)OSU6162
PASAT; Paced Auditory Serial Addition Test
RAVLT; Rey Auditory Verbal Learning Test
RHIFUQ; Rivermead Head Injury Follow Up Questionnaire
RLS85; Reaction Level Scale
RPQ; Rivermead Post Concussion Symptoms Questionnaire
sMRI; Structural Magnetic Resonance Imaging
TBI; Traumatic Brain Injury
TE; Echo time
TR; Repetition time
WAIS-IV; Wechsler Adult Intelligence Scale, 4th edition
WMS-III; Wechsler Memory Scale, 3rd edition
Original papers


Enkel sammanfattning på svenska

Traumatisk skallskada (engelska traumatic brain injury; TBI) är en av de vanligaste anledningarna till funktions- och förmågebegränsningar hos människor, framför allt bland yngre personer. Många patienter, särskilt de med lätta skallskador, återhämtar sig fort, men vissa får besvär som blir långvariga eller bestående. Ett av de vanligaste symtomen är mental trötthet. Trots att det är så vanligt med trötthet efter skallskada, och trots att det påverkar dessa patienters dagliga liv i mycket stor omfattning, finns det idag inga standardiserade metoder för att mäta eller behandla detta symtom. I denna avhandling undersöks därför nya sätt att behandla och mäta/diagnosticera mental trötthet efter TBI.

I studie I undersökte effekterna av ett nytt sorts läkemedel, den dopamininstabiliserande substansen OSU6162, på trötthet efter skallskada. Resultaten påvisade inga effekter av OSU6162 på tröttheten, som i denna studie undersöktes med självskattningsskalor och neuropsykokologiska tester.

I studie II undersökte huruvida funktionell magnetresonanstomografi (fMRI) kunde användas för att undersöka och diagnosticera trötthet hos patienter med TBI. Resultaten visade att tröttheten efter TBI verkar vara beroende på rubningar i kommunikationen mellan hjärnregionerna striatum, thalamus och frontala cortex, samt att fMRI skulle kunna vara en möjlig teknik att använda för att diagnosticera denna trötthet.

Studie III undersökte huruvida OSU6162 påverkade hjärnaktivitet mätt med fMRI. Här påvisades att OSU6162 hade möjliga behandlingseffekter främst i regionerna occipitotemporala cortex och orbitofrontala cortex. Här verkade hjärnaktiviteten normaliseras för de som behandlades med OSU6162, men inte för de som behandlades med placebo, när jämförelser gjordes med friska försökspersoner. Att effekterna sågs just i dessa regioner kan förklara varför det inte syntes några effekter på trötthet i studie I, eftersom dessa ligger utanför de regioner som visade sig kritiska för trötthet i studie II.

I studie IV undersökte om skador i hjärnans vita substans kan förklara den lägre kognitiva funktionen och tröttheten efter TBI. Resultaten visade att skador i den vita substansen var mycket vanligare hos patienter med TBI än hos friska försökspersoner, och att en högre skadegrad innebar större skador i den vita substansen. Däremot kunde inte fler skador i vitsubstansen förklara den lägre prestationen på olika neuropsykokologiska test eller ökad trötthet hos patienter med TBI.
Sammanfattningsvis påvisar resultaten att OSU6162 inte har effekter på trötthet efter TBI vid denna dos och längd på behandling. För att mäta och diagnosticera trötthet efter TBI kan fMRI vara en lovande teknik. Det krävs dock mer forskning både vad gäller behandling och mätning/diagnosticering av detta för många så handikappande symtom.
Preface

For the last decade, which corresponds to the absolute majority of my clinical career as a psychologist, I have worked in the rehabilitation of patients with traumatic brain injuries at the Neurorehabilitation Clinic at the Northern University Hospital in Umeå. In this work, I have come to appreciate the complicated area of neurorehabilitation, and all aspects of the patient that need to be attended to. For me, this means that rehabilitation medicine is a field of many professionals, and the entire team consisting of physicians, physiotherapists, occupational therapists, social workers, nurses and others, is essential in this complex work.

As psychologists in neurorehabilitation, we take great pride in our ability to assess and detect subtle cognitive impairments in our patients using neuropsychological tests. Impairments that may be hard to understand by patients and their surroundings, but still enough to affect them in their everyday life, their social relationships and their ability to take part in leisure activities or work. This, together with the emotional consequences that often follow after a TBI, makes it a truly inspiring field to work in, a field where I as a psychologist really can make a difference in peoples’ lives.

Over the years, I have learned that some of these cognitive and emotional consequences are more or less common in this patient group. However, the absolute most common symptom that my patients with TBI complain about is fatigue. Even though I believe that I can capture this fatigue in some of my patients using neuropsychological tests, this is far from true for most of them. In a time where objective measures of symptoms are becoming more important, this is truly frustrating for me, and sometimes devastating for patients. Further, we have no way of treating this kind of fatigue in patients, even though the impact of fatigue on patients’ everyday life can be lessened by patients learning the importance of balance between activity and rest. These problems in my everyday clinic is what brought me into research on fatigue after TBI, because we certainly need to find new ways for treatment and diagnosis of this symptom that results in severe disability in so many people.
Introduction

Traumatic Brain Injury

Trauma is one of the most common causes of injury to the human brain, with more than 10 million people suffering a traumatic brain injury (TBI) globally each year.\(^1\) Common reports of the incidence of TBI have been around 200-300/100 000,\(^2\) but the actual number of TBI may be as high as 600/100 000, since many mild TBIs are never treated at hospitals.\(^3\) The fact that TBIs are so common, and that many patients do not seek medical attention and not become part of the official statistics, have prompted several authors to use the term “silent epidemic” when describing TBI.\(^4\)\(^-\)\(^6\) Although the majority of TBIs is not fatal, TBI is the primary cause of death in adolescents and young adults.\(^7\)

A TBI occurs when a mechanical force, direct or indirect, is applied to the brain. This causes an acceleration followed by a deceleration in the brain within the skull, which can cause both focal and diffuse damage. Focal damage includes subarachnoid and intraparenchymal haemorrhages, subdural and epidural hematoma, cerebral contusions, and later edema. These are all possible to detect with a computed tomography (CT) scan,\(^8\) normally the first radiological exam performed on patients suspected to have suffered a TBI.\(^9\) Diffuse damage include microvascular damage and diffuse axonal injury (DAI). In DAI, shearing forces causes a tearing between white and grey matter tissues, which causes axonal lesions in the subcortical white matter. These damages are not detectable on a CT scan, and are sometimes difficult to adequately describe after standard magnetic resonance imaging (MRI).\(^10\) Focal and diffuse damages often coexist, especially in severe cases of TBI, affecting both morbidity and mortality.\(^11\) Mild traumatic brain injuries might not always result in structural damage to the brain that is detectable on standard radiological examinations such as CT or MRI. Instead, the acute clinical symptoms, such as loss of consciousness, dizziness, or confusion, may be attributed to functional disturbances rather than structural damage in the brain.\(^12\)

Severity of brain injury is determined on characteristics at the time point of injury. The most commonly used assessment scale is the Glasgow Coma scale (GCS\(^13\)). This scale examines responses in the patient regarding eye-opening, motor and verbal responses. The patient gets a point in each of these categories for their best response, and receives a total score of 3-15. A total of 3-8 corresponds to a severe TBI, 9-12 to a moderate TBI, and 13-15 to a mild TBI.\(^14\) In Sweden, it has been more common to use the Reaction Level Scale (RLS85\(^15\)), which has shown high consistency with the GCS.\(^16\) However, when an initial assessment using some of these scales have not been performed, other
characteristics at the time of injury have been used to indicate severity. These include length of loss of consciousness, alteration of mental state, posttraumatic amnesia, or findings on structural neuroimaging.\textsuperscript{17} Although there is a relationship between TBI severity and outcome, it is important to note that for the individual patient, the TBI severity does not always correspond to later impairments.\textsuperscript{18} This is especially true for patients at the mild side of the continuum.\textsuperscript{19,20} Thus, it has been proposed that in the later stages of TBI, current cognitive functioning, and not TBI severity, should be used as the basis for planning treatment and rehabilitation.\textsuperscript{21}

Most patients with moderate or severe TBI experience long-term or persistent symptoms,\textsuperscript{22,23} but the recovery prognosis in patients with mild TBI is more complicated. While a majority of patients suffering from mild TBI will recover within 3 to 6 months,\textsuperscript{24,25} some will experience persisting and disabling symptoms for years to come.\textsuperscript{26,27} This “miserable minority”\textsuperscript{28} seems to show higher injury-related stress, but also a worse premorbid emotional and physical health than those with better recovery.\textsuperscript{29} Although there is a clear association between symptom severity in the first days or weeks after mild TBI, some may develop persistent symptoms in a later stage.\textsuperscript{30}

The consequences reported by patients with TBI include a wide range of symptoms, of both physical, cognitive and emotional nature. These symptoms can be present in the acute, subacute, or chronic phase of the TBI. Physical symptoms include headaches, dizziness, and sensory disturbances including, but not limited to, light and noise sensitivity.\textsuperscript{31} Common cognitive symptoms after TBI are deficits in attention and mental speed processing,\textsuperscript{32-34} long-term memory,\textsuperscript{32} working memory,\textsuperscript{33} and executive functions such as cognitive flexibility and inhibition.\textsuperscript{34} Patients with TBI also display higher rate of emotional difficulties compared to the general population,\textsuperscript{35} especially depression, anxiety, and increased symptoms of stress.\textsuperscript{36}

**Fatigue after traumatic brain injury - characterization and definition**

Many patients in the chronic phase of TBI suffer from one or several of the symptoms described above, but one of the absolutely most common symptoms in this patient group is fatigue. Although studies vary in the prevalence of fatigue after TBI, around 70\% of patients in the chronic phase of TBI report increased fatigue,\textsuperscript{32,37,38} which by far exceeds the prevalence of fatigue in the general population, often reported to be around 10-20\%.\textsuperscript{39,40} Most improvement (i.e. reductions) in fatigue takes place during the first year after injury, with minimal improvements in the following years for those with persisting symptoms.\textsuperscript{41} Fatigue has been shown to persist both five\textsuperscript{38} and ten\textsuperscript{42} years in these patients,
but probably persists even longer as a chronic symptom. Fatigue is also common in several other disorders affecting the brain, such as stroke,43 Parkinson’s disease,44 and multiple sclerosis.45 Fatigue is therefore not an illness or a diagnostic entity in itself; instead it is a consequence of disturbances in the brain.46

The fatigue that patients experience after suffering a TBI is difficult to describe, since it is subjective in nature,47 and has not been given a clear definition. It is possibly best described by the patients themselves, in Cantor et al47 (p. 876):

- “Anytime I have to focus . . . I’m fatigued.”
- “It’s like you disconnect – I can’t lift my arms, I just can’t – I’m not here so I have to go to sleep.”
- “Like sometimes I feel that I need to be alone, I have to be alone, yes, no more stimulation.”
- “Responding to noise and light and people, in a restaurant, I know, I have to sit on the end of the table and face the wall. Otherwise, I know that I have to leave the restaurant.”
- “Your brain gets full, so it is both a response but also what you can do... it’s inaccuracy in things that you used to be able to do.”
- “You lose what you were talking about, and you can’t hear what anybody else is saying either, and you are trying to grab that thought. It is totally exhausting and fatiguing and it changes your life. It just makes me want to cry thinking about it just because it drives you so easily, you just want to go lay down, you don’t want to participate anymore, you quit and a major part of that fatigue is that it changes how you react with everybody . . . but you lose it and its disconnecting on all levels, then you are frustrated with yourself.”
- “The thing about fatigue is that we all think of fatigue as going to sleep and being oh so tired. I would welcome, welcome feeling tired . . . mostly I wake up, head hurts, I’m not rested, those kinds of things”

This kind of fatigue is difficult to understand, both for the patients themselves and for people around them. Since many of the patients have no visible symptoms of the TBI, it is often hard to accept both for themselves and others that they have to live with “this extreme exhaustion which may appear suddenly, and without previous warning during mental activity, /.../ especially /.../ as the fatigue may appear even after seemingly trivial mental activities” (p. 49146). Around 30% of TBI patients with fatigue are regarded as lazy by their family.48 Together with an often very long mental recovery time,49 it is easy to understand that fatigue affect these patients’ lives deeply. Fatigue in the chronic phase of TBI has indeed been shown to be related to overall disability and the ability to take part in
activities, impaired cognitive function, emotional disturbances, and decreased employment status. Actually, fatigue has been shown to uniquely contribute to disability status after TBI, even when adjusting for injury severity, depression, and impairments in executive functions. In addition, many patients with fatigue after TBI also suffer from sleep wake-disturbances. Fatigue might be an underlying stressor that is a precipitating factor behind insomnia after TBI, but other accompanying factors, such as anxiety or depression, might also be important underlying mechanisms. Having trouble with sleep will of course increase the impact of fatigue in the patients’ everyday life.

It is not only in the everyday life of patients that fatigue is difficult to understand. Researchers have not been able to agree upon a clear scientific definition of fatigue after TBI. Some use what Chaudhuri and Behan call central fatigue, defined as “the failure to initiate and/or sustain attentional tasks (‘mental fatigue’) and physical activities (‘physical fatigue’) requiring self-motivation (as opposed to external stimulation)” (p. 35). This is different from peripheral fatigue, which is not related to mental abilities, but more strictly to neuromuscular output. Others have specified this further by pointing out the causes and defined fatigue as: “The awareness of a decreased capacity for physical and/or mental activity due to an imbalance in the availability, utilization, and/or restoration of resources needed to perform activity.” (p. 46). However, there is not a direct relationship between fatigue after TBI and performance on either cognitive or physical tasks. Thus, Kluger et al, in an attempt to propose a unified taxonomy for fatigue in patients with different kinds of neurological illnesses, distinguished between self-experienced fatigue and fatigability, where the latter is directly related to decreased performance on a given task.

In this thesis, the definition of fatigue is based on Chaudhuri and Behan’s term central fatigue, but with the Kluger et al distinction between fatigue and fatigability kept in mind. The definition of fatigue in this thesis is therefore: A failure to initiate and/or sustain attentional tasks or physical activities, or a self-experienced increase in fatigue that is not directly related to deterioration in performance in attentional tasks or physical activities.

**Mechanisms behind fatigue after traumatic brain injury**

Despite the prevalence of fatigue after TBI, and the impact it has on patients’ lives, the underlying mechanisms remain unclear. However, there are a few theories on the neurobiological basis of TBI-related fatigue. On the cellular level, it has been proposed that TBI causes a low-grade neuroinflammation affecting astrocytes, the supporting cells within the brain. This inflammation affects the possibility of the astrocytes to clear the extracellular space from glutamate, and higher
glutamate levels are suggested to cause unspecific neuronal signaling and thus lack of energy.

On a systems level, Chaudhuri and Behan\textsuperscript{57,58} specified the importance of dysfunction of the non-motor functions of the basal ganglia for fatigue in neurological disorders. According to their theory, disruptions within striato-thalamic-cortical loops may be of either structural, metabolic or neurochemical etiology, thus explaining why fatigue is common in so many different neurological disorders. Due to the importance of dopamine and serotonin within these loops, several studies have examined the potential impact of these neurotransmitters on fatigue. Evidence of relationships between increased levels of serotonin and fatigue have been found in patients with Parkinson’s disease,\textsuperscript{63} multiple sclerosis,\textsuperscript{64} and chronic fatigue syndrome.\textsuperscript{65} Others have stressed the relationship between the dopamine system and fatigue,\textsuperscript{66,67} and even termed the “dopamine imbalance hypothesis” of fatigue in neurological disorders.\textsuperscript{68} This hypothesis states that since either too high or too low levels of the neuromodulator dopamine in the central nervous system is associated with lower cognition, there is a need for balance in dopaminergic neurotransmission for optimal cognitive functioning. Although the “dopamine imbalance hypothesis” has been studied to some extent regarding fatigue in neurological disorders, more well-designed studies are needed to confirm this hypothesis.\textsuperscript{68}

Many of the above-mentioned studies on the neuroanatomical basis of fatigue, both original studies and reviews, mentions that one single explanatory mechanism for such a multidimensional concept as fatigue is unlikely. Others theories include psychosocial,\textsuperscript{69} cognitive,\textsuperscript{70} and genetic\textsuperscript{71} explanations of fatigue after TBI. Even though striato-thalamic-cortical loops seem to play an important part in fatigue after TBI, other factors might be of importance as well.

**Assessment of fatigue after traumatic brain injury**

Assessing fatigue after traumatic brain injury is not a straightforward task. Considering the differences between fatigue and fatigability as described above, there is a need to examine fatigue both regarding self-experience and the effects of fatigue on work output. Since fatigue after TBI mainly regards mental fatigue, this means studying cognitive performance.

There are over 30 self-assessment scales of fatigue,\textsuperscript{72} but no “gold standard” exists for measuring fatigue in patients with TBI.\textsuperscript{47} Many of the scales, such as the Fatigue Severity Scale (FSS\textsuperscript{73}) and the Mental Fatigue Scale (MFS\textsuperscript{49}), have been found to have acceptable psychometric properties. These scales, and most other scales focusing on fatigue, captures what Genova et al\textsuperscript{74} call “trait fatigue”. This means fatigue in everyday life, which is attempted to be assessed by questions
such as “Fatigue causes frequent problems for me” (FSS) or “If you have to take a break, how long do you need to recover after you have worked ‘until you drop’ or are no longer able to concentrate on what you are doing?” (MFS). “Trait fatigue” should according to Genova et al.\(^7^4\) be distinguished from “state fatigue” which is a more transient condition, more able to fluctuate due to internal or external stimuli (such as rest or task occupancy). This kind of fatigue has to my knowledge only been assessed using simple ratings of fatigue on scales from 0-10 or with Visual Analog Scales (VAS). One major problem with using self-assessment is the subjective nature of these scales, in particular when examining patient groups where self-awareness deficits might be common, such as patients with TBI.\(^7^5\) Indeed, it has been recommended that interpretation of self-assessment of fatigue should be made with caution in patients with TBI, due to self-awareness deficits.\(^7^5\)

Thus, to obtain a more objective measure of fatigue, several studies have used neuropsychological tests. Many studies have indeed demonstrated relationships between fatigue after TBI and neuropsychological measures, mainly attention and information processing speed.\(^5^2,7^0,7^5-8^3\) When examining fatigue in patients with TBI, a certain focus on these functions is therefore important.\(^5^6\) Other functions, such as working memory and executive functioning,\(^8^2\) have also been shown to be related to fatigue after TBI. However, these results have not been thoroughly replicated, and the understanding of the relationship between cognitive performance and fatigue is still not fully understood.\(^4^7,6^0\) This might be due to two reasons: 1. The difference between fatigue and fatigability as described by Kluger et al.\(^6^1\) meaning that measuring fatigue subjectively (i.e. with self-assessment scales) and fatigability objectively (i.e. with neuropsychological tests) means that there are two different, although possibly related, constructs that are being measured; 2. The coping hypothesis,\(^8^4\) which postulates that patients with TBI need to compensate for slower mental processing speed and attentional deficits by a constant extra (neuro-) physiological effort, which causes fatigue. Consequently, although patients with TBI that suffer from fatigue may perform as well as healthy controls on neuropsychological tests, the extra physiological effort needed to do this makes them fatigued. Thus, studying only level of performance, and/or only self-experienced fatigue, may not be sufficient when examining fatigue according to the coping hypothesis. Although some have used strictly physiological measures\(^5^2\) when examining fatigue and cognitive functions in TBI, using functional neuroimaging might be one way of directly examining what is going on in the fatigued brain in individuals with a TBI.
Assessing fatigue after traumatic brain injury using functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) uses the fact that neurons signaling need oxygen. Oxygenated and deoxygenated hemoglobin have different magnetic properties. In fMRI, the blood-oxygen-level dependent (BOLD) signal provides information about changes between oxygenated and deoxygenated hemoglobin during performance of a task as compared to a baseline condition. This information given by the BOLD signal can be interpreted as a measure of neural activity, although indirect.

Several studies have used fMRI to investigate cognitive and neurophysiological abnormalities after TBI, mainly within working memory paradigms. Two of the first studies in the field were conducted in the late 1990s by McAllister et al,85,86 with results showing mainly increased activations during working memory tasks within the frontal lobes in patients with TBI as compared to healthy controls. Thereafter, numerous studies have shown both regional hyper- and hypoactivation in patients with TBI when performing mental speed, attention, working memory, and long-term memory tasks (for a review, see Mayer87). In a meta-analysis, Bryer et al88 showed that this discrepancy in results may be task-dependent, with hyperactivation in patients being more consistently observed in continuous or high load tasks, and hypoactivation in discrete or low load tasks. Bryer et al88 interpreted hypoactivation as a result of patients with TBI being less engaged by the task, or that there is heterogeneity within the TBI sample in engagement of the task. Others have concluded that hypoactivation is due to failure in neural recruitment after injuries.89 Hyperactivation has been explained as a normal response to increased load due to cognitive difficulties and neural inefficiency,90 inhibitory difficulties,91 neural compensation,85,86 and effects of brain reorganization.92

Some fMRI studies with patients suffering from TBI have interpreted alterations in the BOLD signal as indicative of extra mental effort or fatigue.90,93-95 However, at the beginning of this decade, only one fMRI study had explicitly examined fatigue in patients with TBI. Kohl and colleagues96 used a sustained attention and processing speed task, the modified Symbol-Digit Modalities test (mSDMT), in an attempt to find neural correlates of mental fatigue. While there were no differences in task performance, patients with TBI showed increased activation in several regions over time during a 21-minute task. These regions included the superior parietal cortex, the middle frontal gyrus, the basal ganglia (caudate), and the anterior cingulate. Healthy controls showed decreased activity over time in the same regions. These results were in line with previous research from the same research group investigating fatigue in patients with multiple sclerosis,97 With a theoretical basis in the coping hypothesis,84 the authors interpreted increased activations over time as indications of fatigue. Increased activity within the basal
ganglia was of specific interest in view of the theory that patients with neurological disorders and fatigue show dysfunction within striato-thalamic-cortical loops.\textsuperscript{57,63} Unfortunately, neither Kohl et al\textsuperscript{96} nor Deluca et al\textsuperscript{97} examined fatigue in patients in any other way, such as with subjective self-report measures.

During the recent years, a few other studies have investigated fatigue after TBI using fMRI. One study showed that fatigue after TBI was related to increased activation in caudate nucleus during a working memory task, but to decreased activation during the mSDMT processing speed task mentioned above.\textsuperscript{98} At rest, the human brain at normally shows neural signaling within organized networks,\textsuperscript{99} often termed the default mode network.\textsuperscript{100} fMRI studies of resting state connectivity have shown that disruptions in the connectivity between thalamus and middle frontal cortex at rest are related to fatigue in patients with TBI.\textsuperscript{101,102} Recent arterial spin labeling studies have found similar alterations to be related to fatigue after TBI.\textsuperscript{103,104} In addition, several studies have examined fatigue in other neurological patient groups and shown that fatigue is related to alterations in striato-thalamic-cortical loops using both task\textsuperscript{105-108} and rest fMRI.\textsuperscript{109,110}

According to the results from the studies summarized above, fMRI seems to offer a possibility to detect fatigue in patients with TBI or other neurological disorders. Still, functional MRI is rarely used in clinical practice in patients with TBI, while structural magnetic resonance imaging (sMRI) is very commonly used. sMRI uses different sequences to acquire high resolution images of the brain. These sequences have different properties to detect special features of abnormalities in the brain. The T1-weighted sequence return high resolution images with high grey-white matter contrast. The T2-weighted sequence can accurately establish presence of gliosis or edema within the brain, and characterize location or boundaries of cerebrospinal fluid. The gradient echo sequence is particularly sensitive in detecting hemosiderin depositions (residual byproducts after a bleeding). The fluid attenuated inversion recovery (FLAIR) is specifically sensitive in detecting pathologies within white matter. Abnormalities in white matter will show up as bright areas on the FLAIR image, and is thus called white matter hyperintensities (WMH). Studies on sMRI have revealed relationships between fatigue and structural brain lesions within striato-thalamo-cortical loops both in TBI\textsuperscript{111} and other patient groups.\textsuperscript{112-114} Fatigability, but not self-assessed fatigue, was in a recent study\textsuperscript{115} related to total lesion volume in white matter, indicating that a decrease in connectivity could be an important factor.

In summary, there seems to be some evidence that fatigue after TBI, and fatigue in other neurological disorders, is related to disturbances in striato-thalamic-cortical loops. However, the underlying mechanisms behind these alterations are not fully understood, neither exactly how these alterations relate to fatigue. Thus, additional studies using functional and structural imaging of the brain to examine
these alterations and to detect or diagnose fatigue in these patients are needed. Further, these previous studies give no clear indication how fatigue should be treated.

**Treatment of fatigue after traumatic brain injury**

Due to the difficulties in the reliable assessment of fatigue after TBI, together with an incomplete understanding of the underlying mechanisms, finding treatments for this disabling symptom have been difficult. All systematic reviews on treatments of fatigue after TBI during the last five years conclude that there are no treatments to be recommended, neither pharmacological nor behavioral, due to a lack of conclusive scientific evidence.\(^{116-120}\) Treating other symptoms and disorders (e.g. depression, anxiety, pain, sleep-wake disturbances) that might increase the fatigue, and finding a balance between rest and activity, is today the best recommendation for patients with fatigue after TBI.\(^{56,121}\) However, there are several promising treatments that should be studied further.

**Behavioral interventions of fatigue after traumatic brain injury**

There are few studies that have examined the effects of different behavioral interventions after TBI and used fatigue as primary outcome. However, several studies have examined fatigue as secondary outcome of the intervention. At least six studies have examined the effects of cognitive behavioral therapy (CBT) in patients with TBI and used fatigue as secondary outcomes. Three case studies examined the effects of CBT on insomnia.\(^{122-124}\) All of these showed indications of effects on fatigue, but the effects were generally weak, and difficult to interpret due to the study designs. Other CBT studies for social anxiety,\(^{125}\) post-concussion symptoms,\(^{126}\) and depression after TBI,\(^{127}\) also revealed effects on fatigue, although this was not the primary target. Johansson and colleagues performed two studies\(^{128,129}\) examining the effects of Mindfulness-based stress reduction aimed at reducing fatigue after stroke or TBI. They found effects on fatigue of both person-to-person, and internet delivered mindfulness, possibly with the latter being superior. These promising results of CBT inspired Nguyen and colleagues\(^{130}\) to design an adapted form of CBT treatment, specifically aimed at sleep disturbances and fatigue after TBI. Their initial pilot study of this therapy programme showed effects both on sleep quality, depression and fatigue. In a similar fashion, Raina et al\(^{131}\) developed an internet-based manualized CBT intervention combining psychoeducation and problem-solving therapy targeting fatigue management after TBI. With small to medium effect sizes on several fatigue measures, the authors found effects of the intervention in a small TBI sample. It should be made clear that there is a need for replication of the results from all of these studies, especially including larger samples and using more robust study designs, before any definitive conclusions can be drawn.
There have also been some studies on different physical exercise regimes after TBI that have included fatigue as secondary outcome. While some studies have not found any effects on mental fatigue,\textsuperscript{132-136} others have found effects on both physical fatigability and mental fatigue.\textsuperscript{137,138} Several other interventions such as working memory training,\textsuperscript{139} electroencephalographic biofeedback,\textsuperscript{140} light therapy,\textsuperscript{141} and cranial electrotherapy stimulation\textsuperscript{142} have indicated effects on fatigue, but these results have not been replicated.

**Pharmacological interventions of fatigue after traumatic brain injury**

There have been a number of studies that have tested different pharmacological interventions with fatigue as primary target in patients with TBI. These can broadly be divided on basis of the kind of drug: Modafinil, Methylphenidate, other interventions, and OSU6162.

Of three randomized, double-blinded, placebo-controlled trials of modafinil,\textsuperscript{143-145} all found effects on sleep quality or daytime sleepiness, but only one found effects on fatigue in patients with TBI.\textsuperscript{145} Thus, treating sleep effectively does not seem to be enough to alleviate fatigue in these patients.

One randomized, double-blinded placebo-controlled study of methylphenidate found effects both on fatigue and fatigability in patients with TBI.\textsuperscript{146} Two studies\textsuperscript{147,148} of methylphenidate that used randomized, unblinded cross-over designs found significant decreases in mental fatigue and increases in information processing speed after four weeks of treatment. These positive effects were dose-dependent, as patients with higher (or normal) doses of methylphenidate (60 mg/day) showed more improvement than those with low doses (15 mg/day). The improvements were better than for patients receiving no treatment. The same research group also showed long-term effects of treatment with methylphenidate on both mental fatigue and several cognitive functions using a pre-post assessment observational design.\textsuperscript{149}

Additional studies have evaluated other pharmacological interventions such as donepezil,\textsuperscript{150} piracetam,\textsuperscript{151} and recombinant human growth hormone,\textsuperscript{152} and included fatigue as secondary outcome. These studies have had mixed results, and often suffered from low sample size and flawed study designs, such as unblinded pre-post designs.

In summary, there have been a number of both behavioral and pharmacological interventions evaluated for treatment of fatigue after traumatic brain injury, but due to mixed results, possibly reflecting poor study design quality and small samples sizes, there are still no treatments with reasonable scientific support
available for this disabling symptom. Thus, there is a need for more trials examining the effects of different forms of treatment, both previously tried and novel forms.

\(-\)-OSU6162

A novel chemical compound is the OSU6162. OSU6162 has been classified as a monoaminergic stabilizer, which stems from the fact that the direction of response to treatment with OSU6162 is dependent on dopaminergic tone.\(^{153}\) It has been shown to affect both motor systems\(^{154}\) and cognition\(^{153}\) in rodents, displaying these stabilizing properties. OSU6162 is well tolerated in healthy human subjects, and causes only minor side effects at high doses, including small elevations of heart rate and serum prolactin levels.\(^{155}\) The compound has shown affinity to mainly D2/D3 receptors,\(^{156,157}\) binding to the allosteric site for stimulation and dopamine release, and to the orthosteric site for inhibition.\(^{158}\) It has also shown a possible affinity to serotonergic 5-HT2A receptors,\(^{157,159}\) thus potentially affecting both dopaminergic and serotonergic systems. Since both these systems, and the homeostasis within them, have been shown to influence fatigue after TBI,\(^ {68,160}\) there have been a few studies examining whether OSU6162 can alleviate fatigue in neurological patient groups.

One study used a non-randomized, crossover design to test the tolerability and efficacy of OSU6162 in 15 patients with Huntington’s disease.\(^ {161}\) The results revealed effects on one item (‘vitality’) of the Short-Form 36 Questionnaire, but no other treatment effects were seen, including different cognitive tests and self-assessment scales. In a recent randomized double-blind placebo-controlled study of OSU6162 in 62 patients with Chronic Fatigue Syndrome, there were no effects when examining the entire group, but in a subsample of patients, with concomitant pharmacological treatment for depression, OSU6162 did have effects on fatigue.\(^ {162}\) Furthermore, in an open-label single-arm study, OSU6162 showed effects on mental fatigue and depression in 30 patients with multiple sclerosis.\(^ {163}\)

Only one study has examined the effects of OSU6162 on fatigue in patients with TBI. In a randomized crossover, double-blind and placebo-controlled pilot study, Johansson et al\(^ {164}\) included 6 patients with TBI and 6 patients with stroke, all suffering from fatigue. These patients were treated with OSU6162 or placebo in increasing doses from 15 to 45 mg twice daily over four weeks. Patients receiving OSU6162 showed improvements on both self-assessment of mental fatigue, and trends towards improvements on neuropsychological tests of attention and processing speed. This latter study was the inspiration for the clinical trial of OSU6162 (Studies I and III) that forms the basis of this thesis.
Thesis Rationale and Aims

Due to the prevalence of fatigue in patients with persisting symptoms after TBI, and the limited understanding of mechanisms behind fatigue after TBI and how it should be assessed and treated, the aims of this thesis were:

1. To evaluate the effects of monoaminergic stabilizer OSU6162 on mental fatigue, as assessed by self-assessment scales and neuropsychological tests, in patients in the chronic phase of TBI (Study I).

2. To evaluate whether fMRI BOLD signal could be used to detect and diagnose fatigue in patients in the chronic phase of TBI (study II).

3. To examine the effects of OSU6162 on fMRI BOLD signal in patients in the chronic phase of TBI (Study III).

4. To examine whether white matter hyperintensities (WMH) in patients in the chronic phase of TBI may influence cognitive function and fatigue (Study IV).
Materials and Methods

Study Designs
The studies in the thesis were approved by the Regional Ethical Review Board in Umeå (dnr 2011-346-31M, with supplement dnr 2015-87-32M), and the Swedish Medical Products Agency. The studies were performed in accordance with the principles stated in the World Medical Association’s Declaration of Helsinki. The clinical randomized trials (Study I and III) were conducted according to the study protocol (EudraCT clinical trial registration no. 2011-004990-10).

Studies I and III were randomized double-blinded placebo-controlled clinical trials of the effects of OSU6162. In study I, the outcome was measured with self-assessment scales and neuropsychological tests. In study III, outcome was measured using fMRI.

Studies II and IV were cross-sectional studies analyzing differences in fMRI-data (Study II) and structural MRI-data (Study IV) in patients with different severities of TBI and healthy controls.

Participants and Inclusion Procedure
All patients with TBI were the same for Studies I-IV, and were thus included through the same inclusion process. The patients were recruited using registers over all 490 patients with ICD-10 diagnostic codes S06.x (intracranial injury), F07.2 (postconcussional syndrome), or T90.5 (sequelae of intracranial injury) that had visited the Neurorehabilitation Clinic in Umeå during 2006 to 2013. The inclusion criteria were:

1. Suffered a Traumatic brain injury > 12 months ago.
2. Age 18 to 65.
3. Moderate disability or better recovery (i.e. > 5) on the Extended Glasgow Outcome Scale. This means that patients must be able to take care of themselves in their home and be able to travel and go grocery shopping alone. Still they can have impairments in their ability to work or take part in leisure activities.
4. Self-experienced fatigue, defined as a score > 36 on the Fatigue Severity Scale.

Exclusion criteria were:

1. Other psychiatric or neurological diseases such as severe depression and anxiety, bipolar disease, obsessive-compulsive disorder, psychosis and addiction to alcohol or drugs.
2. Sick sinus syndrome; resting heart rate below 50 beats per minute; congestive heart failure classified as functional Class III or IV by the New York Heart Association; myocardial infarction within six months of randomization; a prolonged corrected QT at screen or pretreatment (defined as a corrected QT interval of > 450 milliseconds for males or > 470 milliseconds for females); other clinically significant heart conditions which would negatively impact on the patient completing the study.

3. Clinically significant liver disease and/or an elevation in either total bilirubin, alkaline phosphatase, lactate dehydrogenase or serum glutamic-oxaloacetic transaminase of > 2 times the laboratory reference.

4. Clinically significant renal disease and/or an elevation in serum creatinine of > 1.5 times the laboratory reference.

5. Presence of active neoplastic disease.

6. Electroconvulsive therapy within the last 90 days.

7. Surgical or medical conditions, which, in the judgment of the principal clinical investigator, might have interfered with the absorption, distribution, metabolism or excretion of the drug.

8. Drug treatments capable of inducing hepatic enzyme metabolism (e.g., barbiturates, rifampicin, carbamazepine, phenytoin, primidone) within the previous 30 days of enrollment in this study (or 5 half-lives of inducing agent, whichever longer).

9. Personal or immediate family medical history of seizures.


11. Unstable therapies were not allowed, but stable therapies were. A stable therapy was defined as having started at least 6 months before the study and continued to be unchanged during the study period. Examples of such medications are anti-depressant therapy such as citalopram (highest allowed dose 40 mg/daily), mirtazapine (highest allowed dose 45 mg daily) or sertraline (highest allowed dose 100 mg/daily). Other stable therapies with hypnotics and anxiolytics were also allowed if given at doses recommended by the manufacturers. Analgesics such as nonsteroidal anti-inflammatory drugs, acetyl salicylic acid and paracetamol were permitted as well as stable anti-hypertensive therapy.

12. Pregnancy

13. Women of childbearing age not on contraceptives.

14. Abnormal laboratory parameters: such as hemoglobin, white blood cells count, electrolytes, thyroid-stimulating hormone, thyroxine, vitamin B12 or folic acid above the laboratory references level.

15. Severe dementia.

16. Incapability of giving informed consent.
After reading of medical records, 278 patients were excluded due to fulfilling one or more of the exclusion criteria, or residing outside the county of Västerbotten. 212 patients received an initial phone call, at the time of which 129 patients were excluded, mainly due to not experiencing fatigue. After another 13 patients who initially agreed to participate declined participation before baseline, a total of 70 patients performed all baseline assessments, excluding MRI/fMRI examination. Three of these patients were excluded on basis of the exclusion criteria, and one was excluded due to not suffering from fatigue. Another two patients dropped out after the baseline examination. Thus, a total of 64 patients were randomized to receive either OSU6162 ($n = 33$) or placebo ($n = 31$). See figure 1 for a summary of the inclusion process (also, see Figure 1 in Study I for a more detailed flow chart on reasons for exclusion). TBI severity was based on physician’s diagnosis based on self-reported or medical journal confirmed loss of consciousness in accordance with VA/DoD Clinical Practice Guideline for the Management of Concussion-Mild Traumatic Brain Injury. 17

To investigate differences in functional and structural brain imaging between patients with TBI and people with no known brain injury, healthy controls were recruited for studies II-IV. These healthy controls were friends and families of patients that volunteered to participate. The inclusion and exclusion criteria were the same as for patients, but healthy controls were also excluded if they had suffered a concussion or more severe TBI. A total of 57 healthy controls reported interest, but to have groups with approximately equal size, only 30 healthy controls were included. These were chosen from the initial 57 to match the patient groups as closely as possible on age, gender, and education. Three of these healthy controls were excluded after performing all examinations, two due to scoring above cut-offs on self-assessment scales for clinical conditions, and one due to incidental findings during MRI/fMRI examination. Thus, a total of 27 healthy controls were included in the analyses in studies II-IV.

**Procedure**

At the beginning of the study, all patients received written and oral information regarding the study from the principal investigator and head physician, and all signed informed consent. Patients then went through medical examinations, electrocardiography (ECG), vital signs assessment and blood samples collection. After this, they completed self-assessment scales and went through
neuropsychological testing. If no reason for exclusion appeared during this first visit, the patient returned one week later for the MRI/fMRI examination, randomization to OSU6162 or placebo, and to start treatment. The treatment duration was 28 days, and the treatment group received OSU6162 in increasing dosage, starting with 5 mg twice daily during week one, 10 mg twice daily during week two, and 15 mg twice daily during weeks 3 and 4. To ensure the double blinding, the placebo group received the placebo in a similar pseudo-increasing dosage. All patients visited the clinic once weekly during the entire treatment period to meet with the principal investigator, go through medical examinations, vital signs assessment and to complete self-assessment scales. These weekly examinations were performed primarily for safety, and was not included in the outcome analyses in any study. On the last day of treatment (day 28), all patients returned and performed all examinations again, starting with the MRI/fMRI session, followed by medical assessments, self-assessment scales and neuropsychological testing. For illustration of the examination and treatment procedure, see figure 2.

Figure 2. Flow chart for the examination and treatment procedure for patients included in the clinical trial. Superscript Roman numerals indicate in which studies the examinations have been used as outcomes.

Healthy controls went through all examinations at one time point only, and performed all tests on the same day. After receiving written and oral information regarding the study and signing informed consent, they first went through MRI/fMRI examination, had a 30-minute break, went through neuropsychological testing and ended the day completing self-assessment scales.
Instruments

Self-Assessment Scales
To capture “trait fatigue”, two self-assessment scales of fatigue were used: The Fatigue Severity Scale (FSS\textsuperscript{73}) and the Mental Fatigue Scale (MFS\textsuperscript{49}). Both scales focus on fatigue, but while the former is more focused on physical symptoms of fatigue and consequences in everyday life, the latter focuses on mental or cognitive fatigue and includes consequences on emotional functioning. The FSS is a 9 item Likert scale, with responses on each question ranging from 1 to 7. Mean scores of 4 or above (or a total of > 36) have been used to indicate severe fatigue,\textsuperscript{73} but later studies indicate that the cutoff should be set higher to avoid overdiagnosing.\textsuperscript{40} The scale shows high internal consistency and validity for fatigue in patients with TBI.\textsuperscript{81,168,169} Although less well studied than the FSS, the MFS also shows high internal consistency,\textsuperscript{49} and has been validated towards fatigue in patients with TBI.\textsuperscript{170} This scale consists of 15 questions rated on a scale from 0 to 3. Questions incorporate both emotional, cognitive and sensory aspects of fatigue. A cut-off of 10.5 have been suggested to indicate problems with mental fatigue.\textsuperscript{170}

The participants also completed the Hospital Anxiety and Depression Scale (HAD\textsuperscript{171}), a common measure of depression and anxiety in patients with somatic disorders, including TBI.\textsuperscript{172} Lastly, all participants completed the Rivermead Post Concussion Symptoms Questionnaire (RPQ\textsuperscript{173}) and the Rivermead Head Injury Follow Up Questionnaire (RHIFUQ\textsuperscript{174}). These scales capture common symptoms after concussions and more severe head injuries (RPQ), and changes from before to after the injury in daily activities, such as the ability to participate in conversations or to perform leisure or work-related activities (RHIFUQ).

Neuropsychological tests
All participants underwent an extensive neuropsychological test battery. The tests were given in the following order:

- Rey Auditory Verbal Learning Test (RAVLT\textsuperscript{175}) This is a test of verbal learning and memory. It consists of five trials of learning, one distraction list, short term recall and long-term recall. The long-term recall part of this test was performed after the Trail Making Test below.
- Coding from Wechsler Adult Intelligence Scale, 4\textsuperscript{th} edition (WAIS-IV\textsuperscript{176}) measures processing speed.
- Digit Span from Wechsler Memory Scale, 3\textsuperscript{rd} edition (WMS-III\textsuperscript{177}) is a test of auditory attention and working memory.
- Trail Making Test from Delis-Kaplan Executive Functions System (D-KEFS\textsuperscript{178}) consists of four trials of visual scanning, number sequence,
letter sequence, and number-letter switching, and measures visual search ability, attention, and the executive function switching.

- Paced Auditory Serial Addition Test (PASAT\textsuperscript{179}) measures information processing ability, both quantity and speed.
- Symbol Search from WAIS-IV\textsuperscript{176} measures processing speed.
- Block Span from WMS-III\textsuperscript{177} is a test of visual attention and working memory.
- Color-Word Interference Test from D-KEFS\textsuperscript{178} primarily measures the executive functions of inhibition and switching.
- Verbal Fluency from D-KEFS\textsuperscript{178} is also a test of executive functions, primarily word fluency and switching.
- The computerized Integrated Visual and Auditory Continuous Performance Test (IVA\textsuperscript{180}), which measures sustained attention and response inhibition. This last test was performed after participants had completed the self-assessment scales, and not as a part of the rest of the neuropsychological test regime.

**Structural and functional Magnetic Resonance Imaging**

For studies II and III, fMRI was the main outcome measure, and for study IV, structural MRI imaging was the variable of interest. The same MRI equipment and procedure were used for all patients with TBI and healthy participants on all scanning occasions.

**MRI Imaging procedure**

The scannings were performed on the same 3T Discovery MR 750 General Electric scanner (General Electric Company, Chicago, Illinois, USA, Figure 3).
The fMRI sequence was performed first. In this sequence, BOLD-contrast sensitive T2-weighted single-shot gradient echo-planar imaging sequences were used. These were collected as 37 transaxial slices, each with a slice thickness of 3.4 mm, with 0.5 mm spacing and an Echo time (TE) of 30 ms, Repetition time (TR) of 2000 ms, flip angle of 80°, and field of view of 25 × 25 cm. The fMRI sequence was followed by the anatomical image acquisition. T1-weighted images were collected using a 3D fast spoiled gradient-echo sequence with 176 slices, and a slice thickness of 1 mm; TR = 8.2 ms, TE = 3.2 ms, flip angle = 12° and field of view = 25×25 cm. Fluid attenuated inversion recovery (FLAIR; used in study IV) images were acquired using a 2D T2 FLAIR sequence in 48 slices with 3 mm thickness; TR: 8000 ms, TE: 120 ms, field of view: 24x24 cm.

In the preprocessing stage, all fMRI data were slice-time and motion corrected, followed by coregistration to the T1 image. The T1 images were segmented, and a Dartel template was created for each participant, which was used for normalization in Montreal Neurological Institute (MNI) space. Smoothing were performed using a Gaussian filter with full width half maximum of 8 mm.

All structural images were reviewed by a clinical radiologist to examine the extent of damage in patients with TBI, and to discover possible abnormalities in healthy controls.
fMRI task – The modified Symbol Digit Modalities Test
During fMRI scanning, participants performed a 27-minute fatiguing attention and processing speed task called the modified Symbol Digit Modalities Test (mSDMT), which have been used in several previous studies to assess fatigue in patients with neurological disorders. The task was programmed and administered using E-prime software (version 2.0.10.353, Professional Psychology Software Tools, Inc, Pittsburgh: www.pstnet.com/eprime). In this task, participants viewed a digit-symbol code-key, with digits 1-9 paired with a specific symbol (Figure 4). Below this code-key, one symbol-digit pair appeared. Participants were supposed to click a response button on an MRI-compatible keypad (Current Designs package 932, Current Designs, Philadelphia, Pennsylvania) with their right index finger if the pair matched a pair in the code key, and with their left index finger if it did not. The code-key changed after each trial to avoid learning effects. The trial was shown until a response was given, or until 6 seconds had elapsed. After this, a randomly varying inter-trial-interval passed until the next trial began. These intervals were of 0, 4, 8 or 12 seconds in length, with a fixation cross on the screen during this time. The test consisted of a total of 192 trials, lasting approximately 27 minutes. The participants practiced this task on a laptop before entering the scanner, to make sure that they had understood the task. This practice continued until participants answered at least five consecutive trials correctly.

Figure 4. Description of the modified Symbol Digit Modalities Test as administered to participants in studies II and III. Trial on the left displays a matching trial, and trial on the right displays a non-matching trial. ITI = Inter-trial-interval; s = seconds.

Assessment of “state fatigue” before and after fMRI task
To capture how this task affected the “state fatigue” of participants, they had to rate their fatigue directly before and after performing the task, while lying in the scanner. Participants were asked to rate their fatigue at that precise moment on a scale from 0 to 10, where 0 indicated no fatigue at all, and 10 indicated the worst possible fatigue.
Other examinations

For safety, and for sensitivity analyses in study I, patients with TBI in the clinical trial was examined medically with ECG, vital signs assessment and blood samples were drawn, at baseline and at treatment end. The blood samples were analyzed in regards to hormonal function (prolactin), pancreatic function (serum amylase, glucose), kidney/renal function (uric acid, urea nitrogen, creatinine), liver function (total protein, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline- phosphatase, gamma-glutamyl transferase), serum electrolyte changes (Sodium and Potassium) and cholesterol and folic acid. Further, in study I, plasma concentrations of OSU6162 were evaluated in the treatment group. This was done by using liquid chromatography–tandem mass spectrometry, with a method previously described.\textsuperscript{156}

Statistics

All analyses of statistical data that were not fMRI data, were analyzed using SPSS statistics IBM Corporation, Armonk, New York) versions 22 (studies I and II) or 24 (studies III and IV). The alpha-level was generally set to 0.05 in all analyses, except for fMRI-data. If not otherwise specified, differences between groups were tested using independent t-tests for continuous variables and Chi\textsuperscript{2}-tests for categorical variables. fMRI data were analyzed using SPM 12\textsuperscript{183} or an inhouse batch program called DataZ.

Study I

To examine effects of treatment, differences between pre- and post-treatment assessments were tested in linear regressions with treatment group as explanatory variable. These linear regression models were later performed adjusting for several covariates (gender, age, education, employment status, time since injury, and pathologic MRI/CT findings). Within-group differences from before to after treatment were tested using paired-samples t-tests. This study used an intention-to-treat protocol, where missing observations at the post-treatment assessment were compensated by the last observation carried forward method.

Study II

fMRI

To investigate whether there were any time effects on both fMRI BOLD signal and performance of the fMRI task, the fMRI session was divided into three parts. Part 1 corresponded to the first 64 trials of the task, Part 2 to trials 65-128, and Part 3 to trials 129-192.
To analyze fMRI data, a regression analysis was set up in SPM12, with a relative masking threshold of 0.15 and a high pass filter of 130 seconds. To adjust for serial correlations, an autoregressive model was used. 6 regressors were defined, 3 for experimental trials (Part1, Part2 and Part3) of the task, and 3 for the corresponding inter-trial-intervals (Cross1, Cross2, Cross3), and then convolved with the hemodynamic response. For each individual, two contrasts were set up: One for the entire task (task effect; Part1+Part2+Part3-Cross1-Cross2-Cross3), and one for time effects (Part1-Cross1)-(Part3-Cross3). These two contrasts were then analyzed and compared between healthy controls and patients with TBI using two-sample t-tests. This comparison was done with a whole-brain approach using the family-wise error method (FWE) to control for multiple comparisons, with $p < 0.05$ (cluster size > 10). This analysis was later adjusted for differences in reaction time on the fMRI task and in presence of depressive symptoms. Subsequent analyses for the time effects used an uncorrected $p < 0.005$, in on beforehand hypothesized regions of the basal ganglia (caudate nucleus and putamen) thalamus, cingulate cortex and middle frontal gyrus. The same statistical threshold was used for correlational analyses between fMRI data and self-assessment of fatigue.

**Behavioral data**
Analyses comparing healthy controls and patients with TBI on self-assessment scales, neuropsychological tests and results the fMRI task (mSDMT) used ANCOVAs with education as covariate due to differences in this variable and its known effects on neuropsychological tests. Repeated measures ANOVA were used when examining differences between groups in “state fatigue” from before to after performance of the mSDMT (2x2, group vs time), and for testing differences between groups in reaction time and accuracy on the mSDMT changes during parts 1, 2 and 3 of the task (2x3, group vs time).

**Study III**
In Study III, the same fMRI contrast as in Study II (task effect) was used, one for the pre-treatment and one for the post-treatment examination. To evaluate the effects of treatment on changes in these contrasts from before to after treatment, a 2X2 (group by time) repeated measures ANOVA was used. An initial whole brain FWE-controlled analysis was followed by a whole brain uncorrected analyses at $p < 0.001$ and cluster size > 10. To test differences of contrast values in clusters with treatment effects between patients participating in the trial and healthy controls, independent samples t-tests were used. To examine treatment effects on behavioral data, a 2X2 (group by time) repeated measures ANOVA was used. It is important to note that in this study, in contrast to study I, the last observation carried forward method was not used.
Study IV

All WMH lesions were segmented using the Lesion Segmentation Tool (LST) toolbox version 2.0.15 (www.statistical-modelling.de/lst.html). Within this tool, the Lesion Growth Algorithm segmented the T1 image into grey matter, white matter and cerebrospinal fluid, and then combined this with a coregistered FLAIR image. By doing this, only hyperintense areas within white matter are obtained. This segmentation creates a map over WMH lesions, and the number of such lesions and total volume of such lesions in milliliters are obtained. The threshold (kappa) for the creation of lesions map was set to 0.3, as recommended by the developers. The number and volume of WMH lesions in both patients with TBI and healthy controls were highly skewed. For this reason, non-parametric tests were used in each analysis including WMH lesions as dependent variable. For the same reason, Spearman’s Rank Correlations were used in correlation analyses. Linear regressions were used to investigate relationships between WMH data and neuropsychological measures and fatigue. This was first done by examining differences between patients with TBI and healthy controls on these measures, and subsequently adding WMH lesions and age in these regression models to investigate whether the differences were affected by these adjustments. After these initial regressions had been performed, linear regressions within the TBI group only investigated whether number of WMH lesions or WMH lesion volume predicted performance on neuropsychological tests or self-assessment of fatigue, with or without adjustment of age. Age was used as a covariate in these analyses since WMHs are frequently seen in normal aging.
Results

Participants

Study I
Of the 64 patients included, 33 were randomized to receive treatment with OSU6162, and 31 to receive placebo. There were no differences between groups at baseline regarding age, gender, time since injury, education or employment status. There was, however, a higher degree of pathologic findings on MRI/CT in the control group as compared to the treatment group ($p = 0.017$).

One patient in the placebo group stopped taking medication in the first week of treatment after experiencing severe stomach pain, and this was the only patient not returning for post-treatment assessment. However, all data were carried forward in the intention-to-treat analysis. Two patients, one in the treatment group and one in the placebo group stopped taking the medication during week three after experience side effects, but both these patients returned for post-treatment assessments on day 28 (See flowchart in figure 5).

Study II
Of the 64 patients eligible, 60 went through fMRI examination at baseline. One of these misunderstood the task, answered randomly, and was therefore excluded. Two patients were excluded during the initial fMRI data analysis due to signal attrition caused by structural damage in the brain. Thus, a total of 57 patients were analyzed, and compared to the 27 healthy controls as outlined above. There were no differences between groups regarding age and gender, but the control group did have a higher degree of education ($p = 0.026$).
Study III
Of the 64 patients randomized in study I, two patients in each treatment arm did not go through baseline fMRI. One patient in the treatment group and three in the placebo group were excluded during initial fMRI data analysis due to movement that could not be corrected for or signal attrition due to structural brain damage. Further, two patients in the treatment group and one in the placebo group declined fMRI examination at the post-treatment assessment. One patient in the placebo group did not return for post-treatment assessment. Since the last observation carried forward method was not used in this study in the analysis of the effects of OSU6162, 28 patients in the treatment group and 24 patients in the placebo group were included in the analysis (see figure 6). There were no differences between groups at baseline, including no differences in TBI severity. In this study, the 27 healthy controls were included in the sensitivity analyses.

Figure 6. Flow chart for patient randomization and analysis in Study III.

Study IV
60 patients went through the baseline MRI/fMRI scanning for study II. One of these individuals was not examined using the FLAIR sequence, thus 59 patients and the 27 healthy controls were included in the analysis in study IV. As in study II, there was a difference in education ($p = 0.03$), but no differences regarding age or gender between groups.

Key findings
The key findings from studies I-IV are presented below under each headline. For more in-depth information regarding results from each specific study, see the original studies in the appendix.

Study I
The aim in study I was to examine the effects of OSU6162 on fatigue, as measured by self-assessment scales and neuropsychological tests. Both the placebo and the
treatment group showed significant improvements on the primary outcomes, self-assessment scales of fatigue; the Fatigue Severity Scale and the Mental Fatigue Scale (both $p < 0.01$). However, there were no significant differences between groups on changes on these scales (Figure 7), either on initial unadjusted analyses, or in analyses adjusting for the effects of gender, age, education, employment status, time since injury, or presence of pathologic MRI/CT findings.

![Figure 7. Results from primary outcome measures of fatigue, Mental Fatigue Scale and Fatigue Severity Scale, before and after treatment in patients treated with OSU6162 and placebo. Error bars indicate standard error of mean. No significant effects of treatment were found.](image)

In other self-assessment scales and neuropsychological tests there were some within-group improvements. However, there were no differences between groups in changes in any of these scales in both unadjusted and adjusted linear regression analysis, except for one neuropsychological test result (IVA Auditory Stamina), where the fully adjusted model revealed significantly greater improvements for the placebo group ($p = 0.02$).

The adverse events/safety analysis showed that OSU6162 was well tolerated, and there were no differences in number of reported side effects in the placebo vs the treatment group. The side effects were generally mild.

Sensitivity analysis revealed significantly greater changes from before to end of treatment in the treatment group regarding heart rate ($p = 0.009$), folic acid levels ($p = 0.02$), and prolactin levels ($p = 0.03$). There were no differences between groups in other blood test results or physical examinations.

Analysis of plasma concentrations of OSU6162 showed measurable concentration of OSU6162 in all patients in the treatment group (M = 0.14, SD = 0.09, range 0.01-0.32 µM).
Study II
The objective of study II was to investigate whether fMRI could be used to detect and diagnose fatigue in patients with TBI. This was examined by comparing BOLD fMRI data between patients and age- and gender-matched healthy controls, during performance of a 27-minute fatiguing attention task (mSDMT).

Results revealed that both patients and healthy controls experienced increased fatigue while performing the task (both $p < 0.001$), but patients showed a significantly greater increase in fatigue ($p < 0.001$). In the fMRI task, patients showed slower reaction times in the beginning (part 1), middle (part 2) and end (part 3) of the task (all $p < 0.001$). There were no differences between groups in reaction time change, nor in response accuracy during the entire task.

The fMRI data analyses revealed significantly increased BOLD signal during task in healthy controls compared to patients in several regions: the apriori hypothesized bilateral caudate and thalamus (Figure 8 A and B, red contours), but also the bilateral anterior insula, fusiform, calcarine, the left hippocampus, precentral sulcus, and the right supplemental motor area, middle cingulate cortex, frontal middle gyrus, and superior parietal lobule. Due to the differences in reaction time between patients and healthy controls, an analysis adjusting for reaction time differences were performed. The differences in BOLD signal were somewhat attenuated but remained significant (Figure 8A) within the bilateral caudate, thalamus, fusiform, and the left anterior insula. Further, differences in presence of depressive symptoms were adjusted for, which attenuated the BOLD signal differences to a lesser extent (Figure 8B). There were no regions where patients showed significantly increased task-related BOLD signal relative to controls.
Differences in task activation between patients with traumatic brain injury and healthy controls, adjusted for differences in reaction time (A; hot colors) and scores on the Hospital Anxiety and Depression Scale – depression (B; hot colors). Hot colors indicate increased activation in healthy controls during task relative to patients. Red contours show clusters with differences in the unadjusted analysis (p < 0.05, FWE-Corrected, cluster size ≥ 10).

The largest group differences between groups during the entire task were found within the bilateral caudate nucleus. The peak voxel in the left caudate (t = 7.62, p < .001, MNI coordinates –12, 14, 6) was used to test the diagnostic value of the fMRI BOLD signal (Figure 9). With a cutoff of 1.0 in the contrast value, 81% of healthy controls showed higher values (specificity), and 91% of patients showed lower values (sensitivity). This corresponds to a positive predictive value of 91%, and a negative predictive value of 81%.
Figure 9. Contrast values of the left caudate (MNI coordinates $-12, 14, 6$), in healthy controls and patients with traumatic brain injury. The solid line represents the cutoff tested in the sensitivity/specificity analysis.

To examine time effects on the BOLD signal, changes from parts 1 to 3 of the scanning was used. Differences between patients and controls were significant at an uncorrected threshold ($p < 0.005$, cluster-size $\geq 10$) in a few of the a priori hypothesized regions such as the bilateral caudate and the anterior medial thalamus. All patterns of changes were the same, where healthy controls showed higher deactivation over time than patients.

Increases in “state fatigue” from before to after performance of the fMRI task correlated positively to BOLD signal in the bilateral operculum ($rs = 0.44-0.56, p < 0.001$, cluster size $\geq 10$).

**Study III**

Study III examined the effects of OSU6162 on fMRI BOLD signal. As in study I, there were no effects on any self-assessment scales, or in any behavioral data of the fMRI task. An uncorrected analysis ($p < 0.001$, cluster size $\geq 10$) revealed fMRI BOLD signal differences during task between groups in two small clusters, one in the right thalamus, and one in the right para-hippocampal area. In these areas, the treatment group showed higher BOLD signal during task than the placebo group. These differences were not present at the post-treatment examination.

There were significant treatment effects (group by time interactions) on the BOLD signal in the right occipitotemporal cortex, the right brain stem and the
right orbitofrontal cortex. Of special interest were the effects in the occipitotemporal cortex and the orbitofrontal cortex, since they were within the task network, indicating relevance to the task. In the regions with treatment effects, there were no differences between groups at baseline. At the post-treatment examination, the placebo group showed no differences from before to after treatment, but the OSU6162 group showed increases in BOLD signal during task from before to after treatment. This increase in the treatment group meant that the BOLD signal levels after treatment were similar to that of healthy controls (Figure 10).

Figure 10. Regions (Hot colors) with significant treatment effects of OSU6162 on BOLD signal (uncorrected p < 0.001, cluster size ≥10), in the right occipitotemporal cortex (A), and the right orbitofrontal cortex (B).

**Study IV**

Study IV investigated the prevalence of WMH lesions in patients with TBI with different severities, in relation to healthy controls. Further, the relationships between TBI-related WMH lesions and several neuropsychological measures and fatigue were examined.
Healthy controls had lower degree of both number of WMH lesions ($p = 0.032$) and total lesion volume ($p = 0.025$) than patients with TBI (Figure 11). Comparing healthy controls with TBI patients with different severities revealed that healthy controls differed significantly from both the moderate ($p = 0.049$ for number of WMH lesions and $p = 0.032$ for lesion volume) and severe ($p = 0.009$ for number of WMH lesions and $p = 0.005$ for lesion volume) TBI groups, but not from the mild TBI group.

Figure 11. Mean number of white matter hyperintensity (WMH) lesions and total WMH lesion volume in healthy controls and patients with traumatic brain injury (TBI). The bars on the left represent all healthy controls and all TBI patients, while the bars to the right represent TBI patients divided after TBI severity (Mild, Moderate, Severe). Error bars indicate standard error of mean.

The mild TBI group was significantly different from the severe TBI group both regarding number of WMH lesions ($p = 0.017$) and total lesion volume ($p = 0.006$). There was a trend towards differences between the mild TBI group and the moderate TBI group regarding WMH lesion volume ($p = 0.079$), but there were no significant differences regarding number of WMH lesions. No significant
differences were found between the moderate and severe TBI groups regarding WMH lesions (all $ps > 0.1$), possibly due to low power.

Linear regression analyses showed significant differences between healthy controls and patients with TBI in most measures of neuropsychological functioning and fatigue, but adding WMH lesions and age to the statistical models did not affect these differences. In the fully adjusted models, increased WMH lesion volume was related to worse performance on the RAVLT – Delayed Recall ($p = 0.047$) and PASAT ($p = 0.037$). Increased number of WMH lesions was however related to lower self-assessment of fatigue on the MFS ($p = 0.013$) in the fully adjusted model (Table 1).

Table 1. Results from linear regressions comparing healthy controls and patients with TBI

<table>
<thead>
<tr>
<th>Lesion Volume</th>
<th>Lesion Volume and Age</th>
<th>Nr of Lesions</th>
<th>Nr of Lesions and Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue Severity Scale</td>
<td>23.94*</td>
<td>24.13*</td>
<td>24.14*</td>
</tr>
</tbody>
</table>

Note: Values indicate the unstandardized coefficient (difference) between healthy controls and patients with TBI. Symbols denote significant contribution of that variable to the regression. *Healthy controls vs TBI ($p < 0.001$); ^WMH lesion ($p < 0.05$); 'Age ($p < 0.05$).

The relationship between number of WMH lesions and self-assessment of fatigue on the MFS was the same in the TBI group only, when adjusting for age ($p = 0.026$; Table 2). No other relationships between WMH lesions and measures of neuropsychological functioning or fatigue were seen in the TBI group.

Table 2. Results from linear regressions within the TBI group only

<table>
<thead>
<tr>
<th>Lesion Volume</th>
<th>Lesion Volume and Age</th>
<th>Nr of Lesions</th>
<th>Nr of Lesions and Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue Severity Scale</td>
<td>-0.18</td>
<td>-0.18</td>
<td>-0.16</td>
</tr>
<tr>
<td>Mental Fatigue Scale</td>
<td>-0.11</td>
<td>-0.15</td>
<td>-0.17</td>
</tr>
</tbody>
</table>

Note: Values indicate the unstandardized coefficient. Symbols denote significant contribution of that variable to the regression. ^WMH lesion ($p < 0.05$).
Discussion

Fatigue is a one of the most common symptoms in the chronic phase of TBI. Still there are no valid ways of objectively measuring this fatigue, nor are there any evidence-based treatments. Thus, this thesis includes four studies aimed at expanding the research base regarding both assessment and treatment of fatigue in the chronic phase of TBI. Study II and IV investigated functional and structural neuroimaging correlates to fatigue in an attempt to find ways of assessing and diagnosing fatigue in patients with TBI. Studies I and III examined the effects of novel compound OSU6162 on this disabling symptom in the same patients.

Assessing and diagnosing fatigue after TBI using functional and structural MRI

Study II showed major differences in functional brain activity, as measured by BOLD fMRI, between patients with TBI suffering from fatigue and healthy controls, during a fatigue-inducing attention task. These brain activity differences were present although there were no differences in accuracy between patients and controls on the behavioral task. When the differences in BOLD signal were adjusted with differences in reaction time and presence of depressive symptoms, the BOLD signal differences were only mildly attenuated. Thus, the differences, mainly located within striato-thalamic-cortical loops, is a clear indication that fatigue after TBI is dependent on adequate functioning within these loops, confirming results from other fMRI studies in both TBI and other neurological disorders.

Novel data from study II indicates that fMRI might be a promising technique in diagnosing fatigue in patients with TBI. Using the voxel with the most profound differences between patients and healthy controls, and testing it for sensitivity and specificity, we correctly identified 81% of controls (specificity), and 91% of patients (sensitivity). This corresponds to the same values regarding negative and positive predictive values, respectively. That is, a clinician with a patient showing lower contrast values than the cutoff value, could be 91% sure that this patient has suffered a TBI with following experience of fatigue. Or on the other side, if the value is higher than the cutoff, the clinician can be 81% sure that this patient is not a patient with TBI suffering from fatigue. However, this is only based on data from study II, and more studies are needed before fMRI can be used clinically to diagnose fatigue after TBI.

Finding instruments that can objectively and correctly diagnose fatigue in patients with TBI is crucial. Many patients struggle to prove their deficits to
insurance companies and to take part of support from welfare systems, and establishing if a patient suffers from fatigue after a TBI is often difficult. As noticed above, the results should be replicated in future studies. Such studies could change the task to possibly make the differences in BOLD signal even larger. This might be done by adjusting task duration or difficulty as this might affect neural recruitment in patients with TBI. Since the results from study II indicate that the differences were largest in the beginning of the task, making the task longer is not likely of interest. Instead, altering the task difficulty might prove more worthwhile. One way of doing this is changing the task from being a symbol-digit task to a symbol-symbol task. This means that participants are not helped by the order in the code key, but instead must use visual search in a more extensive sense. Since rapid visual search is often affected in patients with TBI, this might be a suitable alteration of the task.

Furthermore, the analysis of changes in BOLD signal over time showed that controls deactivated more over time than patients in regions such as the caudate and thalamus. This was seen even though there were no group differences in changes in reaction time or accuracy over time. The observed deactivation in controls, despite no changes in performance, confirms results from several studies, and has been interpreted as practice effects, enhanced efficacy in processing, and habituation. The more constant level of activation in TBI patients during the task, might be a source contributing to the self-experienced fatigue in this patient group. This fatigue was seen in the self-assessments done directly before and after the fMRI task, where patients showed significantly higher fatigue due to the task than healthy controls. These results are in line with the coping hypothesis of fatigue, which states that fatigued patients may perform on the same behavioral level as healthy controls, but this causes higher (neuro-) physiological activity, or as in the case of this study, an inability to disengage over time, and therefore the higher level of fatigue. Thus, further research should evaluate whether an inability to learn to process information more effectively, or lack of habituation, may be underlying mechanisms of fatigue in patients with TBI.

One other finding from study II that are in line with the coping hypothesis, were the positive correlations between increases in “state fatigue” and BOLD signal in the bilateral operculum. Operculum has previously been related to fatigue in multiple sclerosis. The novel results from study II might indicate that an increased neurophysiological effort in these regions is directly related to increased subjective fatigue in TBI as well.

Neither controls nor patients with TBI revealed deterioration in performance (reaction time or correctness) during the entire 27-minute task. This means that this task cannot capture central fatigue, where one important aspect, according
to Chaudhuri and Behan,\textsuperscript{57,58} is difficulties to sustain attentional performance over time. However, patients with TBI (as well as healthy controls) did increase their subjective fatigue while performing this task, but patients became significantly more fatigued than controls. Hence, the task was fatiguing according to the Kluger et al\textsuperscript{61} definition, where fatigue is the individual perception of weariness, exhaustion, or increasing sense of effort that does not correspond to expected output. This should be contrasted with performance fatigability, which is defined as changes in performance over time, and thus not captured by the mSDMT. This difference between subjective fatigue and performance fatigability is always important to keep in mind when examining fatigue in patients with TBI. Both have been difficult to assess in a more objective way, especially the subjective perception of fatigue, but maybe the results of study II are an indication of this being possible.

Study IV showed that structural white matter hyperintensity lesions were more common in patients with TBI than in age- and gender-matched healthy controls. As expected according to results from previous studies,\textsuperscript{191-193} both number of WMH lesions and total WMH lesion volume increased with injury severity. Further, a relationship between number of WMH lesions and fatigue were seen. Surprisingly this relationship was negative, indicating that a higher number of WMH lesions was related to less self-assessed fatigue. This relationship was however weak, and might be an outcome of multiple comparisons. It is reasonable to assume that the increased lesion load does not protect the patient from fatigue per se. However, within the group of TBI patients, the increased lesion load might be related to other factors such as difficulties with self-awareness, or other impairments, that possibly make patients pursue a less activity-full daily life, which in turn causes less fatigue. Further, WMH lesion load was related to TBI severity, and several studies have shown that self-assessed fatigue is not related to TBI severity.\textsuperscript{81,169,194} There are only a few studies that have shown relationships between structural MRI lesions and fatigue after TBI.\textsuperscript{111,115} As suggested above, functional measures, such as fMRI used in Study II, may thus be more suitable to detect fatigue in patients with TBI. Still, more research both regarding structural and functional correlates of fatigue after TBI are needed before any definitive conclusions can be drawn.

The treatment of fatigue after TBI using OSU6162

The clinical trials (Studies I and III) revealed that OSU6162 does not seem to have effects on fatigue in this patient group after 4 weeks of treatment at a dosage of 15 mg twice daily. Fatigue was in this case measured with self-assessment scales, neuropsychological tests, and BOLD fMRI. How come there were no effects of OSU6162 in these studies, while other studies\textsuperscript{161-164} have found promising effects of OSU6162 alleviating fatigue both in TBI and other neurological disorders?
Most importantly, studies I and III are the largest, most rigorously controlled studies that have been performed to investigate the effects of OSU6162 in patients with TBI. The only previous study that included patients with TBI,\textsuperscript{164} only had six TBI patients. Further, the randomized double-blind placebo-controlled design with parallel groups holds some advantages over the randomized placebo-controlled crossover-studies performed.\textsuperscript{161,164} This is specifically in regard of carry-over effects in the crossover designs, that might affect outcome, especially when there is a considerable placebo effect. The only other randomized double-blind placebo-controlled study with parallel design of approximately the same size that has been performed, studied the effects of OSU6162 on fatigue in patients with chronic fatigue syndrome.\textsuperscript{162} Although no effects were seen when comparing all patients included, this study showed effects on fatigue when looking at a subsample of patients with antidepressant medication. Further, there were also relationships between plasma levels of OSU6162 and improvements in fatigue and depression. These subgroup analyzes were however not specified on beforehand, limiting the conclusions that can be drawn from them. Still, there might be treatment effects of OSU6162 in patients with chronic fatigue syndrome, but not in patients with TBI, possibly due to different mechanisms underlying fatigue in these conditions. One other important factor to explain that other studies showed effects on fatigue is that all these studies used doubled or tripled dosage of OSU6162 compared to studies I and III. Studies I and III showed that OSU6162 was safe to use, and caused only minor side effects. There were no differences in side effects reported in the treatment group in comparison to the placebo group. This is not surprising since preclinical studies have showed tolerability of up to 90\textsuperscript{156} and 150\textsuperscript{155} mg of OSU6162 per day, and the maximum dosage in studies I and III was only 30 mg daily. However, tolerability was for the first time established in patients with TBI. If low dosage was the reason for lack of effects on fatigue, there seems to exist a reasonable possibility of increasing the dosage in future studies.

Sensitivity analysis in study I revealed that the group treated with OSU6162 showed increased heart rate and prolactin levels, previously known effects of OSU6162.\textsuperscript{155,156} Further, all patients in the treatment group had measurable levels of OSU6162 concentrations in plasma. Thus, problems with compliance does not seem to be able to explain the lack of effects on fatigue. There was also a significant treatment effect on levels of folic acid, that has not been seen in any previous studies. This might simply be an outcome of the multiple comparisons problem, but future studies should investigate folic acid in participants treated with OSU162, to examine whether there is a true treatment effect.

Both the OSU6162 and the placebo group did however show improvements on most self-assessment scales and neuropsychological tests. The improvements on neuropsychological tests might mainly be attributed to test-retest or practice
effects, common phenomena in both healthy participants and patients with TBI. The improvements in self-assessment scales could be due to placebo effects. There are of course other explanations, such as observer (Hawthorne) effects or observer-expectancy effects that might have driven these improvements. Nonetheless, the improvements in the placebo group might have concealed true effects of OSU6162, which could not be seen in the results. Still, these within-group improvements show the importance of using a control group, blinded for treatment assignment, when examining treatment effects on self-assessed fatigue och neuropsychological tests in patients with TBI.

Study III revealed effects of OSU6162 on BOLD fMRI signal, as compared to placebo. However, these effects were seen in regions within the occipitotemporal cortex and the orbitofrontal cortex. Scanning the fMRI literature on fatigue in neurological disorders gives few indications that these areas are connected to fatigue. Study II in this thesis (discussed above) did not show any relationships between these areas and fatigue. Two other fMRI studies of fatigue in TBI have found fatigue-related BOLD signal changes in the right superior parietal cortex and fusiform, that are close to areas showing treatment effects in the present study. Another study using the same task as study II, but examining fatigue in multiple sclerosis found fatigue-related altered BOLD signal in the orbitofrontal cortex. However, several other studies have not found any relationships between areas affected by treatment in study III and fatigue in neurological disorders. It is important to note that all of these studies showed relevance of alterations in the basal ganglia, and specifically the striatum, to fatigue in neurological disorders, despite using different tasks. Another recent study found that stimulating the fronto-striatal network might alleviate on-task fatigue in patients with multiple sclerosis. These results indicate that treatments should, at least to some extent, affect functional activity within the basal ganglia to have effect on fatigue in neurological disorders. Thus, the lack of effect on functional brain activity in the striatum in study III, might explain why OSU6162 had no effect on fatigue in study I.

There were no effects of treatment on behavioral output in the fMRI task, even though the treatment effects on BOLD signal changes were within the cerebral task network. This implies that the fMRI technique possibly is more sensitive in detecting subtle treatment differences than behavioral measures, and thus fMRI might be helpful in the evaluation of clinical trials.

The baseline assessments suggested a successful randomization, since there were few differences between the placebo and the treatments groups before start of treatment. In study I there was one difference regarding the presence of pathological MRI/CT findings. Adjusting for this did not alter the main findings. In study III, there were differences in BOLD signal, where the treatment group
showed higher BOLD signal in the right thalamus and the para-hippocampal area. Especially the baseline differences in the right thalamus might have been of interest, since it was within the striato-thalamic-cortical network shown to be important for fatigue in study II. However, the differences were rather small, and only found in uncorrected analyses. Further, after treatment, there were no differences between groups within this area, and the changes from before to after treatment were not significantly different between groups. This shows that there were no treatment effects present in this area.

Studies I and III are not the first studies that have shown lack of treatment effect on fatigue after TBI. Although some studies have found promising results, there are no pharmacological or behavioral treatments with satisfying scientific evidence to be recommended for treatment of fatigue after TBI. Why is it so difficult to find effective treatments of this disabling symptom that so many patients are suffering from? One explanation is that treatment of fatigue after TBI with different compounds has not been thoroughly tested. Actually, the first clinical trials aimed specifically at fatigue after TBI, dates back only about ten years. Thus, research needs more time to find effective treatments.

One other important factor that may contribute to lack of treatment effects for fatigue after TBI, is that the underlying mechanisms are still poorly understood. Several hypotheses on the etiology of fatigue after TBI have been suggested. Neuroanatomical explanations include disruptions within the basal ganglia and its connectivity, dopaminergic imbalance, and astrocytes having difficulties to clear the extracellular space from glutamate. While study II strengthens the theory of the importance of striato-thalamic-cortical networks in fatigue, there may be other explanations for fatigue in these patients. In addition to the neuroanatomical explanations of fatigue mentioned above, other aspects such as genetics, cognition, affective symptoms (e.g. stress, depression, and anxiety), and social support could be significant contributors to fatigue in patients with TBI. As discussed above, several studies have also indicated that fatigue after TBI is not related to injury severity. As noted, Study IV added to this by showing that the only relationship between fatigue and WMH, was negative, meaning that a higher number of WMH lesions (in other words, larger structural damage) was actually related to lower self-assessed fatigue. Strictly examining or focusing on the severity of the TBI or extent of structural damage in the brain does not seem to be suitable in the case of fatigue after TBI. In accordance with this, most authors suggest that both the examination of mechanisms behind fatigue and the treatment thereof should embrace a multidimensional view on fatigue. Thus, it could be of interest for future studies to combine interventions in a multimodal fashion, which might yield better effects on fatigue after TBI. A biopsychosocial model of fatigue after
stroke has been proposed, possibly the same would be beneficial for the understanding, treatment and rehabilitation of fatigue after TBI.

**Methodological considerations**

Whenever a clinical trial is faced with negative results, the question of power must be addressed. Indeed, the number of participants in studies I and III was relatively small, and when looking at effect sizes from the primary outcome FSS, the OSU6162 group did show larger improvements. The effect size difference was however only 0.12 and non-significant. The power calculation for the clinical trial was based on one previous study of OSU6162 aimed at fatigue in patients with TBI and stroke. This suggested that 30 patients in each treatment arm would be enough to detect treatment effects (with a significance level 0.05 and power of 80%). Increasing the number of patients would have increased power, and thus the possibility to detect effects of treatment. An alternative could have been to use a cross-over design with each patient receiving both OSU6162 and placebo and serving as their own control could have been performed. This would have increased power using the same number of patients, but there might have been problems with carry-over effects. Further, even though there might have been a statistically significant effect of treatment with a larger sample or other designs, it is not clear whether there would be a clinically significant effect.

Results from other self-assessment scales and neuropsychological tests were quite random, where the treatment group improved more in some scales, and the placebo group improved more in others. Actually, the only significant treatment effect found was favoring the placebo group. This effect was however rather weak, and could be random due to multiple comparisons.

The possibility of type I errors due to multiple comparisons must always be considered in research. In study I this did not seem to be a problem, since no effects of treatments were seen, more than already known effects (such as increased heart rate and prolactin levels). However, in studies II and III, where the main outcome were fMRI data, the risk of rejecting the null hypothesis, although it is true, is always a factor due to the several comparisons that are being made. The main results in study II were thus adjusted using the family-wise error method, giving higher confidence to the results presented and the interpretation thereof. The results from the time-dependent analysis in study II, and the treatment effects in study III, was not corrected for multiple comparisons. Although a standard correction method should be used when interpreting fMRI data, uncorrected data should also be presented as long as this lack of correction can be defended. Since there have been very few fMRI studies examining fatigue after TBI, and no previous study examining the effects of OSU6162 on fMRI data, studies II and III should be regarded exploratory studies. Thus, we
chose to minimize the risk of type II errors, that is to miss possible differences or treatment effects that could be of interest for future studies. The same was true for study IV, where both number and volume of WMH lesions were correlated to several neuropsychological outcome measures. Therefore, it should be stressed that some of the findings in studies II-IV did not survive correction for multiple comparisons and should be regarded as tentative until confirmed by future research.

One major aspect to be considered in all four studies is how fatigue was examined and measured. There is no “gold standard” for how to measure fatigue after TBI. The self-assessment scales and neuropsychological tests used as outcomes have satisfying psychometric properties, but are not thoroughly validated towards fatigue after TBI, which no scales are. As always when studying this complex symptom, there is a possibility that fatigue was not correctly captured by the instruments.

One major methodological issue is whether fMRI is a suitable technique for examining and diagnosing fatigue. That is, are we detecting fatigue in the brain or simply observing the association of fatigue in the brain? It has often been said that fMRI is strictly correlational. Indeed, BOLD fMRI does not directly measure neural activity, but instead the hemodynamic changes, which is a strong predictor of neuronal activity. This means that, for example, the results from study II showing differences in neural activity within striato-thalamic-cortical loops, actually shows differences in hemodynamic changes between patients with TBI and healthy controls within these loops. Still the differences are present, performance of this task during fMRI scanning revealed these differences in hemodynamic changes while increasing fatigue in participants. Although the entire chain of causality cannot be fully explained, the conclusion that fatigue is related to the changes within striato-thalamic-cortical loops is still valid.

There are other ways to strengthen this assumption that the differences within striato-thalamic-cortical loops was really due to fatigue, and not any other aspects of solely having suffered a TBI. Patients with TBI that are not experiencing fatigue but do have similar cognitive impairments and emotional disturbances as the patients in the study, could have been included. This could also have added extra valuable information to study IV. Further, other variables often associated with fatigue in TBI, such as pain, stress, and sleep-wake disturbances could have been examined, and possibly adjusted for in many analyses.

One especially important factor in all studies is the TBI sample. The sample was quite heterogenous when it comes to TBI severity and time since injury. All patients were suffering from fatigue, but differed in other symptoms caused by the TBI. This is indeed a problem in all TBI research, since each individual TBI is
unique and sustained in highly individualized circumstances, and thus causes different patterns of damage to the brain, even within the same injury severity. Because of this, finding effective treatments and accurate diagnostic markers can be troublesome within this group of patients, and the generalization also becomes somewhat limited. In the case of the TBI sample in the current thesis, all had moderate or better recovery according to the Extended Glasgow Outcome Scale, making generalization to patients with less favorable outcome after TBI limited. In addition, many of the exclusion criteria, such as history of seizures and other neurological or psychiatric disorders are quite common after TBI, further limiting generalizability to the general TBI population.

The assessment of TBI severity in this thesis, and especially in study IV, might be questioned. The most common tool for assessment of TBI severity has been the Glasgow Coma scale (GCS). Since it has been more common to use the Reaction Level Scale (RLS85) in Sweden, the absolute majority of patients did not have a GCS score from time of injury. Many patients did not have an RLS85 score either, especially the majority of patients with mild TBI. Thus, self-reported or medically journal confirmed length of loss of consciousness was used to indicate severity. Although this can be a valid measure of TBI severity, aspects such as sedation or pharmacological treatments in the acute phase affects the accuracy in moderate to severe cases. It is also recommended that multiple aspects, including loss of consciousness but also alteration of mental state, posttraumatic amnesia, or findings on structural neuroimaging are used when assessing TBI severity. In lack of data from many of these variables, the choice was made to use loss of consciousness as indicator of TBI severity, since this would be easiest for participants to self-report. Of course there are sources of error in reporting length of loss of consciousness as well, including recall bias.

A similar limitation concerns the inclusion of healthy controls, and specifically how lack of concussion or more severe TBIs in this group was confirmed. This was based single-handedly on self-report, which might also be influenced by recall bias, or possibly that these healthy controls concealed having sustained a concussion or having other disorders included in the exclusion criteria due to a wish to participate in the study. Indeed, two healthy controls showed significant clinical conditions that were reasons for exclusion at the examination day, that was not revealed during initial inclusion. These two participants were excluded from further analyses.

Scientific contributions and future considerations

There is no doubt that fatigue poses a significant problem for people with TBI and that there are very limited treatment options showing efficacy. Therefore, intervention studies in this area are important. To find out that OSU6162 at this
dosage and treatment duration does not show any effects on fatigue was of course disappointing. Still, the results give some valuable information to the research field. The negative results in itself are clear evidence that OSU6162 at this dosage does not give effects on fatigue in this patient group, and thus alterations of treatment in future studies may be needed. Since OSU6162 was well tolerated in this patient group, to investigate a higher dose of OSU6162 in this patient group with the outcome of fatigue would be of interest. To increase treatment duration would also be of interest.

Clinical trials with negative results are far more likely not to be submitted for publication or being published than trials with positive findings. With the increasing attention to the problem of publication bias, publishing a rigorously done negative study is a way of helping research to avoid this bias, by showing other researchers that it is possible to publish clinical trials showing no therapeutic effect of the treatment studied.

Study II is currently, to my knowledge, the largest study examining fatigue in patients with TBI using fMRI. The results clearly demonstrate that fatigue after TBI is related to alterations in striato-thalamic-cortical loops, adding to previous fMRI research on fatigue after other neurological disorders, but for the first time shown in a larger study with TBI patients. However, future studies must examine the underlying mechanisms of these alterations, be it imbalance in the dopamine system or any other explanation. This is crucial to both increase the understanding of fatigue after TBI, and to find effective treatments.

Study II also revealed an impressing diagnostic accuracy of the fMRI technique, an accuracy that to my knowledge has not been seen in any other study. This is a promising result for the field of research on fatigue after TBI, and for patients suffering from fatigue: It might be possible to objectively establish who suffers from this disabling symptom and who does not. Still, the findings must of course be evaluated in future studies.

Study III used a novel method of investigating whether a treatment method of fatigue would reveal effects on fMRI BOLD signal. In accordance with the results from Study II and several other fMRI studies on fatigue in neurological disorders, using fMRI as a main outcome seemed reasonable. The treatment effects on the fMRI BOLD signal in the occipitotemporal and the orbitofrontal cortex helped explain the lack of treatment effects in Study I, but also gave information about some other possible indications for OSU6162. Altered BOLD signal in the orbitofrontal cortex have been related to addiction-related disorders, and the results from study III might thus explain the promising effects of OSU6162 on alcohol addiction treatment. This shows that using fMRI may be of value when
examining treatment effects (or lack thereof), but might also give suggestions for other possible treatment indications.

Study IV showed that both number of white matter hyperintensities and total WMH lesion volume increased with TBI injury severity. Although this has been shown in previous studies, this was for the first time demonstrated using the fully automated Lesion Segmentation Tool. Further, TBI-related WMH lesions were not associated to any neuropsychological measures, but a negative relationship between self-assessed fatigue and number of WMH lesions were found. Such a relationship has never been found before. Although this should be interpreted cautiously, it is a clear indication that increased structural damage in the brain, in this case WMH lesions, is not directly related to fatigue. Thus, using other measures, for example functional neuroimaging as in Study II, might be more adequate to capture fatigue in patients with TBI. This is an interesting future research area, to investigate if functional MRI should be used clinically instead of structural MRI when examining fatigue (and possibly also other sequelae) after TBI.

**Conclusions**

In summary, this thesis contributes to the research field regarding fatigue in patients in the chronic phase of TBI, both when it comes to assessment and treatment. Fatigue seems to be dependent on alterations in striato-thalamic-cortical loops, and OSU6162 only affected functional brain activity outside these loops, which might be an important explanation of lack of treatment effects on fatigue. This field does however still require much more research to help researchers develop more valid ways of assessment and treatment, to aid clinicians in the rehabilitation of their patients, and, most importantly, to help patients with TBI suffering from fatigue and their close ones to understand what this fatigue is and to help them alleviate it.
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