The handle http://hdl.handle.net/1887/49010 holds various files of this Leiden University dissertation.

**Author:** Heide, A. van der  
**Title:** Unravelling narcolepsy : from pathophysiology to measuring treatment effects  
**Issue Date:** 2017-05-24
Astrid van der Heide

UNRAVELLING NARCOLEPSY
FROM PATHOPHYSIOLOGY TO MEASURING TREATMENT EFFECTS
Unravelling narcolepsy

From pathophysiology to measuring treatment effects

Astrid van der Heide
The research described in this thesis was performed at the Department of Neurology and Clinical Neurophysiology of the Leiden University Medical Center, Leiden, the Netherlands.

© 2017 Astrid van der Heide
All rights reserved. No part of this book may be produced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying or otherwise, without the prior permission of the author, or, when appropriate, of the publishers of the publications.
Unravelling narcolepsy
From pathophysiology to measuring treatment effects

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen woensdag 24 mei 2017
klokke 11.15 uur

doors

Astrid van der Heide

goingen te Leeuwarden
in 1984
Promotor

Prof. dr. J.G. van Dijk

Copromotor

Dr. G.J. Lammers

Leden promotiecommissie

Prof. dr. F.H.J. Claas

Dr. S. Overeem (Radboudumc, Nijmegen en Technische Universiteit Eindhoven)

Prof. dr. R.Q. Hintzen (Erasmus MC, Rotterdam)

Prof. dr. M.J. Jager
## CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1</td>
<td>General introduction</td>
<td>7</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>HLA dosage effect in narcolepsy with cataplexy</td>
<td>37</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Immunohistochemical screening for antibodies in recent onset</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>type 1 narcolepsy and after H1N1 vaccination</td>
<td></td>
</tr>
<tr>
<td>Chapter 4</td>
<td>The effects of sodium oxybate on core body and skin</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>temperature regulation in narcolepsy</td>
<td></td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Core body and skin temperature in type 1 narcolepsy in daily life:</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>effects of sodium oxybate and prediction of sleep attacks</td>
<td></td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Comparing treatment effect measurements in narcolepsy: the</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Sustained Attention to Response Task, Epworth Sleepiness Scale</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and Maintenance of Wakefulness Test</td>
<td></td>
</tr>
<tr>
<td>Chapter 7</td>
<td>Summary, conclusions and future perspectives</td>
<td>127</td>
</tr>
<tr>
<td>Chapter 8</td>
<td>Samenvatting, conclusies en toekomstperspectieven</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>About the author</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Curriculum vitae</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>List of publications</td>
<td>151</td>
</tr>
</tbody>
</table>
CHAPTER 1

GENERAL INTRODUCTION

Astrid van der Heide
Gert Jan Lammers

Based on:
INTRODUCTION

Narcolepsy is a disorder of the regulation of sleep and wakefulness, resulting in a variety of symptoms such as excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations, sleep paralysis and disturbed nocturnal sleep. According to the current classification of sleep disorders,\(^1\) narcolepsy can be divided into narcolepsy type 1 and type 2 (previously: narcolepsy with and without cataplexy). Narcolepsy type 1 is considered to be a homogeneous disease entity, a ‘morbus sui generis’, of which the pathophysiological hallmark is a disturbed hypocretin transmission. Narcolepsy type 2 may in contrast be a heterogeneous group of disorders characterised by EDS in combination with abnormal expressions of REM sleep on polysomnography (PSG). Whether the PSG findings in question are at all specific is debatable; there are indications that chronic sleep deprivation in otherwise healthy individuals may be enough to cause similar PSG abnormalities. Most cases of narcolepsy type 2 do not develop cataplexy later on, so type 2 is not simply an early stage of narcolepsy type 1.

This thesis focuses on narcolepsy type 1, or, from now on, ‘narcolepsy’ for short. ‘Narcolepsy type 2’ will be discussed in the sections on pathophysiology and differential diagnosis.

EPIDEMIOLOGY

Narcolepsy is relatively rare, with an estimated prevalence of 25–50 per 100,000 and an estimated incidence of 0.74 per 100,000 person years.\(^2,3\) There usually is a latency of about 10 years between the occurrence of the first symptoms, which emphasises a trend towards late detection, and which may also suggest that detection fails. However, this latency tends to become shorter.\(^4\)

Men and women seem to be affected at equal rates,\(^5\) although one paper reported a higher incidence in men.\(^2\) The disease may start at any age, but most often during adolescence. There is a small second peak in the age at onset at around 35 years of age.\(^6\) Life expectancy of narcolepsy patients does not differ from that of the general population.
CLINICAL FEATURES

EDS

EDS is the leading symptom of narcolepsy. It is invariably present in all patients and usually is the first symptom. It typically develops over weeks to months, but may start over a shorter period of time. EDS is relentlessly present, every day. It is characterised both by an inability to stay awake and, in the majority of patients, by an almost continuous feeling of sleepiness dependent upon the level of activity. Monotonous activities such as watching television, reading, attending a meeting or being a passenger in a car, may all increase the feeling of sleepiness and greatly increase the chance of unintentionally falling asleep, i.e. a ‘sleep attack’. Conversely, intense physical or mental activity decreases sleepiness and prevents sleep attacks. In more severe cases sleep attacks may also occur when patients are relatively active, such as during dinner, while walking or even when riding a bicycle. Sleep attacks tend to last less than 20 minutes and have a temporary refreshing effect. Their frequency varies from one to over ten attacks per day, depending on the severity of the narcolepsy and the circumstances. EDS causes restrictions in daily activity and social embarrassment due to patients falling asleep at inopportune moments. It may also have profound secondary effects, in the form of an increased risk of traffic or job-related accidents.

EDS is typically accompanied by a pronounced difficulty to concentrate and to sustain attention for any length of time, leading to an impaired performance. These attention problems may cause more problems with interpersonal relationships than the actual sleep attacks: people who seem awake but uninterested and not performing as intended apparently receive less compassion than those who are asleep under the wrong circumstances.

Cataplexy

Cataplexy is characterised by a sudden bilateral loss of muscle tone, with preserved consciousness, elicited by emotions. All striated muscles can be involved, with as notable exceptions the external eye muscles and muscles involved in respiration. Cataplexy may be complete or partial. Complete cataplexy involves complete loss of activity of all muscle groups (Figure 1.1). Complete attacks may cause falls. It takes several seconds for a complete attack to build up, so most patients learn to take countermeasures, such as sitting down. Cataplexy most often takes the partial form, in which control over the knees, face and neck
may be lost. Partial attacks may be so subtle that they are only recognised by experienced observers. Occasionally not even patients themselves are aware of subtle attacks. Patients notice partial attacks as their knees ‘giving way’ or sagging of the head or jaw. Muscle twitches and jerky movements may be part of the attack. Over time, most patients learn tricks to prevent or abort attacks, such as ‘trying to think of something else’ or ‘pressing against a firm support surface’.

Cataplexy is most often provoked by an emotional trigger, or its anticipation. Rather than the actual emotion its anticipation may trigger an attack. Common examples are that patients cannot tell the punch line of a joke and cannot score a goal during a sports match. Mirth is the most frequently involved emotion, which usually involves laughing out loud; mere smiling does not usually trigger an attack. Another common trigger is an unexpected meeting with an acquaintance. A range of other triggers can provoke cataplexy. The same emotion need not trigger an attack in all circumstances: experience suggests that there has to be an additional influence for the emotion to evoke cataplexy. A certain mind-set seems to be involved, but its precise characteristics are difficult to define. A degree of relaxation or feeling at ease may be a cataplexy prerequisite, as uncomfortable or stressful situations such as medical consultations usually prevent their occurrence.

The frequency of cataplexy attacks varies from dozens a day to less than once a month. Most last seconds to half a minute, and only sometimes up to two minutes or longer. Partial attacks tend to be shorter at less than 10 seconds. Occasionally it is difficult to establish the duration of an individual attack, especially when the trigger remains present for minutes. In such a situation repeated attacks may occur, giving the impression of a very long lasting single attack. The frequency, severity, and how well patients cope with attacks determine the impact on the quality of life. Patients may learn to avoid situations in which cataplexy
may occur, so they stop laughing out loud, stop visiting comedy shows or even avoid social contact in general.

Although cataplexy is the only truly specific feature of narcolepsy, it is rarely its first symptom: this occurs in fewer than 10% of cases. Usually cataplexy appears shortly after EDS, but it may also only appear months to years afterwards. These patients are likely to be mistaken to have narcolepsy without cataplexy, until the eventual development of cataplexy shows this to have been the wrong diagnosis.

Hypnagogic hallucinations

Hypnagogic hallucinations (HH) are very vivid dreamlike experiences occurring during the transition from wake to sleep. Originally the term ‘hypnopompic hallucinations’ was restricted to mean an occurrence during awakening, but ‘hypnagogic’ is now commonly used for the transition in either direction. The content of the hallucinations varies, but in general they are extremely unpleasant and frightening. In 85% of the hallucinations multiple senses are simultaneously involved: visual, auditory and tactile. In contrast to dreams, the hallucinations are typically ‘pasted’ over the actual environment, so the hallucination seems to occur in the bedroom of the patient. Examples are the presence of someone in the bedroom, or of undergoing surgery without anaesthesia while lying in their own bed. The hallucinations often appear so realistic that patients have difficulty telling them apart from real events after waking up, requiring refutation by others. Narcolepsy patients usually recognise that the content of the hallucination is not real, which helps to distinguish them from hallucinations in a general psychiatric context. Their occurrence during the wake-sleep transition also facilitates the diagnosis. The presence of HH in a patient with narcolepsy should not suggest a psychotic disorder, as these do not occur more often in narcolepsy than in the general population.

HH are not specific for narcolepsy with cataplexy, since they are also present in the general population and in other sleep disorders. However, the prevalence, and probably also the frequency, are higher in narcolepsy.

Sleep paralysis

‘Sleep paralysis’ concerns the inability to move voluntarily when falling asleep or while awakening, while being subjectively awake and conscious. The paralysis may be so complete
that patients cannot raise as much as a little finger. Attacks last up to several minutes, and have similarities with both cataplexy and HH: their timing in the sleep-wake cycle resembles that of HH, whereas the nature and extent of the paralysis resemble complete attacks of cataplexy. Sleep paralysis may occur simultaneously with HH.

Sleep paralysis can occur as an isolated symptom and is therefore not specific for narcolepsy.\textsuperscript{11}

Disturbed nocturnal sleep

Sleep latency in narcolepsy is typically very short: patients usually fall asleep as soon as they lie down and their heads touch their pillow. They have difficulty staying asleep, however, reflected in frequent awakening. Most awakenings are brief but some last more than one hour. The total duration of nocturnal sleep is largely the same as before patients developed narcolepsy,\textsuperscript{13} but in a minority of cases nocturnal sleep time increases temporarily or structurally. Remarkably, there is no clear correlation between the severity of EDS and the extent of nocturnal sleep disruption.

Associated symptoms

The features mentioned until now are considered the classical core symptoms of narcolepsy, which disregards several other features that are also frequently present. These may be related to the inability to sustain attention. One is automatic behaviour, meaning that patients perform semi-purposeful acts but without conscious control. Examples are that someone may continue to write in a state of drowsiness, resulting in illegible writing, or that patients may drive by car following a well-known route, without later knowing how and why they did so. Memory complaints occur frequently.

A final symptom that cannot be directly related to impaired sustained attention is obesity. In a Dutch study about 30\% of patients had a BMI of at least 30 kg/m\textsuperscript{2}, a substantially higher proportion than the 12.5\% which holds for the Dutch population.\textsuperscript{14} Obesity may be explained in part by decreased activity or increased caloric intake, but, since it typically occurs in narcolepsy type 1 patients, and not in patients with a similar sleep-wake phenotype without hypocretin deficiency, may be a direct consequence of hypocretin deficiency.
Co-morbidity

Although EDS in narcolepsy can be accompanied by a feeling of having an energy shortage or fatigue, fatigue is qualitatively different from EDS, and it is important to differentiate between the two. Fatigue is a feature of numerous conditions, not necessarily sleep disorders.

Sleep apnoea and parasomnias are frequently present. One study reported the presence of obstructive sleep apnoea in 25% of the patients with narcolepsy type 1 and type 2. Periodic limb movements in sleep (PLMS) have been described in up to two thirds of the subjects with narcolepsy type 1. How much PLMS contributes to impaired quality of sleep and to EDS is not known; it may be not very relevant. REM sleep behaviour disorder (RBD) occurs more often in narcolepsy than in the general population, affecting 12–36% of the patients.

Since depression by itself may cause sleep problems, EDS as well as a pronounced lack of drive and of energy, it can be difficult to diagnose depression as a separate co-morbid disorder in patients with narcolepsy. Nevertheless, 5–30% of the patients are reported to fulfil criteria for depression, more than the general population.

DIAGNOSIS

Narcolepsy type 1 is diagnosed according to the criteria of the International Classification of Sleep Disorders (ICSD-3) (Table 1.1). EDS must be present, evaluated through careful

Table 1.1  ICSD-3 diagnostic criteria of narcolepsy type 1

<table>
<thead>
<tr>
<th>Criteria A and B must be met:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
</tr>
<tr>
<td>B.</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
</tbody>
</table>

1 In young children, narcolepsy may sometimes present as excessively long night sleep or as resumption of previously discontinued daytime napping.
2 If narcolepsy type I is strongly suspected clinically but the MSLT criteria of B1 are not met, a possible strategy is to repeat the MSLT.
history taking, supplemented or with a decreased hypocretin-1 level in cerebrospinal fluid (CSF) or with the presence of cataplexy in combination with specific findings during a multiple sleep latency test (MSLT) and polysomnography (PSG).

The currently available commercial assessment kit for the hypocretin-1 radioimmunoassay (RIA) has a large inter-assay variation, so reference samples must always be included. Many centres use reference samples from Stanford and convert their values to the Stanford values. For these labs, hypocretin1 levels below 110 pg/ml are diagnostic for narcolepsy.

In atypical cases, such as patients with familial narcolepsy or those who do not carry the human leukocyte antigen (HLA)-allele DQB1*06:02, hypocretin-1 levels are less often low; forming the majority of the patients who have normal levels.

**DIFFERENTIAL DIAGNOSIS**

If a patient presents with the features of narcolepsy type 1, the only remaining relevant question is whether the patient has the idiopathic form or ‘narcolepsy type 1 due to a medical condition’. The latter is primarily found with central nervous system (CNS) disorders, including autoimmune or paraneoplastic disorders associated with anti-Ma2 or anti-aquaporin-4 antibodies, and tumours or other lesions of the hypothalamus.

In the absence of cataplexy diagnosing narcolepsy is more difficult, and depends on whether patients meet the CSF and/or PSG criteria (Table 1.1 and 1.2). Unfortunately, similar clinical and MSLT findings have been described to occur following chronic sleep deprivation. In such cases a low or undetectable hypocretin-1 level can prove the presence of narcolepsy, but a low hypocretin-1 concentration concerns only about 10% of the narcolepsy subjects without cataplexy. In HLA-negative subjects an even lower percentage is found. In case of doubt patients should be advised to follow a regular sleep wake rhythm with enough time in bed to guarantee sufficient nocturnal sleep. Pharmacological treatment should be considered only when complaints remain after having followed such a regime.

From a clinical point of view differentiating narcolepsy and idiopathic hypersomnina (IH) may be difficult, particularly the variant of IH without a long sleep time. In these cases the presence of SOREMPs during the MSLT will help: IH patients do have decreased sleep onset latency, but have less than 2 SOREMPs. Moreover, hypocretin-1 levels are always normal in IH. Table 1.3 summarises the differential diagnoses of EDS and cataplexy.
Table 1.2 ICSD-3 diagnostic criteria of narcolepsy type 2

Criteria A–E must be met:

A. The patient has daily periods of irrepressible need to sleep or daytime lapses into sleep occurring for at least three months.

B. A mean sleep latency of ≤ 8 minutes and two or more sleep onset REM periods (SOREMPs) are found on a MSLT performed according to standard techniques. A SOREM (within 15 minutes of sleep onset) on the preceding nocturnal polysomnogram may replace one of the SOREMPs on the MSLT.

C. Cataplexy is absent.¹

D. Either CSF hypocretin-1 concentration has not been measured or CSF hypocretin-1 concentration measured by immunoreactivity is either > 110 pg/mL or > 1/3 of mean values obtained in normal subjects with the same standardized assay.²

E. The hypersomnolence and/or MSLT findings are not better explained by other causes such as insufficient sleep, obstructive sleep apnoea, delayed sleep phase disorder, or the effect of medication or substances or their withdrawal.

¹ If cataplexy develops later, then the disorder should be reclassified as narcolepsy type 1.
² If the CSF Hcrt-1 concentration is tested at a later stage and found to be either ≤ 110 pg/mL or < 1/3 of mean values obtained in normal subjects with the same assay, then the disorder should be reclassified as narcolepsy type 1.

PATHOPHYSIOLOGY

The hypocretin (orexin) system

The hypocretin system includes two peptides, hypocretin 1 and 2, and two receptors, receptor 1 and 2. The peptides were independently discovered by two groups,²²,²³ explaining why ‘orexin-A and -B’ are synonymous with hypocretin-1 and -2 respectively. Both hypocretin peptides are cleaved from a common precursor (preprohypocretin) and have a different receptor affinity profile. Hypocretin-1 has equal affinity for both receptors, while hypocretin-2 preferentially binds to the hypocretin receptor-2.

Hypocretins are produced by a small number of neurons located in the dorsolateral hypothalamus, centred round the fornix and adjacent areas. Although the hypocretin-producing neurons lie in a small area, their axons project throughout the whole neuraxis, with exception of the cerebellum (Figure 1.2).²⁴-²⁷

The discovery that monogenetic forms of narcolepsy in dogs were caused by mutations in the hypocretin receptor-2 gene, and the report that hypocretin knockout mice develop narcolepsy led to new insights into the pathophysiology of human narcolepsy.²⁸,²⁹ Hypocretin deficiency...
Table 1.3  Differential diagnosis for EDS and cataplexy

<table>
<thead>
<tr>
<th>Excessive daytime sleepiness</th>
<th>Cataplexy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behaviourally induced insufficient sleep syndrome</td>
<td>Isolated cataplexy</td>
</tr>
<tr>
<td>Sleep apnoea</td>
<td>Niemann Pick disease</td>
</tr>
<tr>
<td>Periodic Limb Movement Disorder</td>
<td>Prader-Willi syndrome</td>
</tr>
<tr>
<td>Idiopathic hypersomnia</td>
<td>Norrie disease</td>
</tr>
<tr>
<td>Kleine-Levin syndrome</td>
<td>Secondary to diencephalic tumours</td>
</tr>
<tr>
<td>Drug intoxication/withdrawal</td>
<td>Familial cataplexy</td>
</tr>
<tr>
<td>Circadian rhythm disorders</td>
<td>Coffin Lowry syndrome</td>
</tr>
<tr>
<td>Thalamic infarction</td>
<td>Non-cataplectic attacks</td>
</tr>
<tr>
<td>Metabolic encephalopathy</td>
<td>Syncope</td>
</tr>
<tr>
<td>Depression</td>
<td>Startle syndromes</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Drop attacks</td>
</tr>
<tr>
<td>Malingering</td>
<td>Atonic/gelastic seizures</td>
</tr>
<tr>
<td></td>
<td>Psychogenic</td>
</tr>
<tr>
<td></td>
<td>Malingering</td>
</tr>
</tbody>
</table>

Figure 1.2  Projections of hypocretin.
Hypocretin neurons, located in the lateral hypothalamus, innervate all nuclei of the AAS and the entire cerebral cortex.
Abbreviations: GABA: γ-aminobutyric acid; Gal: galanin; TMN: tuberomammillary nuclei; His: histamine; LH: lateral hypothalamus; Hcrt: hypocretin; vPAG: ventral periaqueductal grey matter; DA: dopamine; Raphe: dorsal and median raphe nuclei; 5-HT: serotonin; LDT: laterodorsal tegmental nuclei; PPT: pedunculopontine tegmental nuclei; ACh: acetylcholine; LC: locus coeruleus; NA: noradrenaline.
General introduction

1. turned out to be the hallmark of human narcolepsy with cataplexy. The first study pointing in this direction was a blinded controlled study in which hypocretin-1 concentrations were measured in the CSF. Hypocretin-1 was undetectable in the majority of patients, in contrast to stable concentrations far exceeding the detection limit that were found in control subjects. Follow-up studies in large numbers of patients confirmed this finding. Attempts to measure hypocretin-2 in CSF have failed, probably because this substance very unstable in the CSF. Since both hypocretin 1 and 2 are derived from the common precursor preprohypocretin, it seems probable that hypocretin 2 is low or absent in narcolepsy with cataplexy as well. This assumption is supported by post mortem brain studies indicating an almost complete selective loss of hypocretin cells in the hypothalamus of patients who suffered from narcolepsy with cataplexy. It is currently presumed that narcoleptic signs and symptoms start once the majority of hypocretin-producing neurons cells have disappeared. The number of degenerated cells may determine symptom severity and the occurrence of cataplexy.

‘Sleep switch’

To understand the pathophysiology of narcolepsy it is essential to understand current concepts of the regulation of sleep and wakefulness.

The main nuclei for the promotion of wakefulness and sleep are located in the hypothalamus and the reticular formation of the mesencephalon and pons, concerning the dorsal and median raphe nuclei (Raphe), the locus coeruleus (LC), the ventral periaqueductal grey matter (vPAG) and the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) (Figure 1.3). Besides projections to the thalamic intralaminar nuclei and the hypothalamus, these nuclei project diffusely to the cortex of the entire hemisphere. Together with the tuberomammillary nuclei (TMN), located in the hypothalamus, these nuclei and their projections are called the ascending arousal system (AAS). The ascending arousal system plays a crucial role in the regulation of wakefulness and comprises two pathways:

1. **Cholinergic branch**: the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT), project to the thalamic reticular nucleus via thalamic relay neurons and activate the cerebral cortex.

2. **Monoaminergic branch**: the locus coeruleus (LC), dorsal and median raphe nuclei (DR), tuberomammillary nuclei (TMN) and ventral periaqueductal grey matter (vPAG) project diffusely to the cortex of the entire hemisphere.
During wakefulness, both the cholinergic and monoaminergic branches are active, and their activity is augmented by the hypocretin neurons from the lateral hypothalamus.

The AAS is influenced by many other systems, among them the ventrolateral preoptic nucleus (VLPO), located in the hypothalamus (Figure 1.4). Activity of the VLPO facilitates sleep. The phase of the diurnal rhythm of the biological clock and the duration of previous wake are major determinants of the activity of the VPLO. During NREM-sleep wake-promoting cholinergic and monoaminergic nuclei are all inhibited by the VLPO. During REM-sleep only the monoaminergic nuclei are inhibited, whereas the cholinergic nuclei are even more active than during wakefulness.

The VLPO inhibits the monoaminergic nuclei, which in turn inhibit the VLPO, resulting in reciprocal inhibition. Such a circuit resembles a ‘flip-flop switch’ (Figure 1.5), a term used
by electrical engineers. By analogy, the circuitry regulating sleep and wakefulness is called the ‘sleep-switch’.34,35

The mutual inhibition in a flip–flop circuit results in either one state or the other, preventing intermediate states. In such a system transitions from one state to the other are abrupt and complete. The ‘sleep-switch’ thus allows only sleep and wake states. Avoiding transitional states may have an evolutionary advantage: sleeping animals are vulnerable; it is necessary for an animal to be able to awaken quickly so it can flee or defend itself. Conversely, it is common experience that one can fall asleep over just a few seconds or minutes. By itself such a circuit allows minor influences to cause abrupt and frequent switches. To maintain wake and sleep for protracted periods hence requires stabilising factors. Hypocretin is considered to act as the stabiliser during wakefulness, when it prevents switching into sleep by reinforcing the arousal systems (Figure 1.2). A breakdown of the hypocretin system means that frequent switches are not prevented. This results in frequent and unwanted transitions
between wake and sleep, and impaired sustained attention. However, up to now it is not clear how hypocretin also stabilises sleep.

**State boundary control**

All signs and symptoms of narcolepsy can be explained by the so-called ‘loss of state boundary control’. The ‘states’ in this concept are the various sleep-wake stages, and the ‘loss of boundary control’ results in two qualitatively different phenomena. The first is that no sleep or wake state can be maintained for a normal length of time: when awake, patients fall asleep easily, and when asleep, they awaken easily. The second is that the various phenomena that normally occur together in a certain sleep or wake stage now

---

**Figure 1.5** The ‘sleep-switch’.
The sleep-switch describes a feedback loop with a self-reinforcing firing pattern resulting in two possible states: sleep and wake. (A) The reciprocal inhibition of the ventrolateral preoptic nucleus (VLPO) and the monoaminergic nuclei (MN). It prevents the occurrence of intermediate states: if there is a transition, it is abrupt and complete. (B) A disadvantage of the sleep-switch is its instability; minor disturbances may lead to abrupt switches. During wakefulness the VLPO is inhibited by the monoaminergic nuclei and hypocretin (Hcrt) reinforces the arousal systems, stabilising the switch in the waking state.
occur out of context. Cataplexy and sleep paralysis are both regarded as the atonia that physiologically occurs during REM-sleep, but now also occurring during wakefulness. HH are considered to be intrusions of dream imagery into the waking state.

GENETIC ASPECTS

As a rule, narcolepsy is a sporadic disease, with only 1–4% of cases having a familial pattern. In most families, an autosomal dominant mode of inheritance has been demonstrated. Except for one unusual case, no mutation in genes encoding for the two hypocretin receptors or preprohypocretin have been identified. The one exception suffered from severe cataplexy with a very early onset at the age of 6 months.

Twin studies have been performed to assess the genetic contribution to the susceptibility for narcolepsy. Concordance for narcolepsy was found in only 25–31% of monozygotic twins (for references see 38), illustrating the contribution of environmental factors. Prevalence studies in sporadic cases demonstrated a 1–2% risk for a first-degree relative of a patient with narcolepsy to develop narcolepsy. This risk is 10–40 times higher when compared to the general population. Besides a higher risk for narcolepsy there is a higher risk for narcolepsy-like complaints that do not fulfil the ICSD criteria for narcolepsy.

HLA association

Narcolepsy has the strongest known association with a specific human leukocyte antigen (HLA)-allele. Narcolepsy is tightly associated with HLA-DQB1*06:02,41-44 which is in linkage disequilibrium with HLA-DQA1*01:02. Worldwide about 85–95% of the narcolepsy with cataplexy patients carry this haplotype, compared to 12–38% of the general population.45 For non-familial cases and those with typical cataplexy the association may even exceed 98%.46 Carrying this haplotype is therefore thought to represent an almost necessary risk factor for the development of narcolepsy, although its mere presence is not sufficient to cause narcolepsy. HLA studies in narcolepsy patients heterozygous for HLA-DQB1*06:02-DQA1*01:02, revealed a role of several accompanying HLA-haplotypes (i.e. located in trans with DQB1*06:02-DQA1*01:02). Heterozygosity with DQB1*03:01, DQA1*06, DQA1*03:03, DRB1*04, DRB1*08, DRB1*11 or DRB1*12 turned out to increase the risk of developing narcolepsy, whereas heterozygosity of DQB1*06:02 with DQB1*06:01, DQB1*06:03, DQB1*05:01 or DQA1*01 (non DQA1*01:02) turned out to decrease the
Furthermore, a two to four times increased risk of developing narcolepsy is reported in Caucasians homozygous for DQB1*06:02.

**IS NARCOLEPSY AN AUTOIMMUNE DISEASE?**

An autoimmune aetiology for narcolepsy has been hypothesised for decades. The aetiology of autoimmune diseases is multifactorial, principally encompassing genetic and environmental factors. As said, the most important genetic factor in narcolepsy is HLA-DQB1*06:02. Other genetic studies demonstrated several additional genetic factors in narcolepsy: polymorphisms in the T-cell receptor alpha locus (TCRα), Cathepsin H (CTSH), Tumor Necrosis Factor (ligand) Superfamily member 4 (TNFSF4, also called OX40L), the purinergic receptor P2RY11 and the DNA methyltransferase DNMT1. These genetic factors suggest T-cell involvement in narcolepsy, supporting the autoimmune hypothesis. It is however unlikely that the immune response is directed against hypocretin or preprohypocretin itself, since there is no evidence for the presence of specific autoantibodies against either peptide. In 2010, three independent groups reported elevated levels of antibodies against a protein that is produced in hypocretin neurons, Tribbles homolog 2 (Trb2). Since Trb2 is not specific for hypocretin-producing neurons, it is unlikely that these antibodies directly injure the hypocretin neurons. These antibodies may arise following earlier damage to hypocretin-producing neurons.

Possible environmental factors involved in autoimmunity are infections. An increased incidence is seen after infections with Streptococcus pyogenes and influenza type A virus, in particular H1N1. Concerning H1N1, a role of vaccination was presumed, but not confirmed. Furthermore, an increased onset was reported in the months following winter related infections in the Chinese population. Like several other autoimmune diseases, narcolepsy starts most often during adolescence, with a small second peak in the age at onset around 35 years of age.

**TREATMENT**

Prevention and cure are the dual ideal results of dealing with any disorder. Unfortunately, narcolepsy can neither be prevented nor cured. Hypocretin substitution might be expected to reduce the symptom burden but it does not, probably because it does not easily cross
the blood-brain barrier. Studies focusing on alternative application routes, such as the nasal route have not shown encouraging results. An alternative causal therapy might be found in hypocretin agonists, but this approach has so far not resulted in a practical treatment either.

Symptomatic treatment remains and can luckily lead to profound improvement. Two treatment modalities have proven to be effective: behavioural modification and pharmacological therapy. As a rule, both are needed to achieve success.

**Behavioural modification**

Patients should be advised to live a regular life, go to bed at the same hour each night as much as possible, and get up at the same time each morning. Scheduled daytime naps may temporarily alleviate and prevent daytime sleepiness, and a short nap just before certain activities demanding a high degree of attention may facilitate the proper completion. The optimal frequency, duration and timing of these naps has to be established on an individual basis.71

Because narcoleptic patients are probably more sensitive to the sleep-inducing properties of carbohydrates, they should not eat large carbohydrate-rich meals.72 For similar reasons alcohol consumption should preferably be avoided.

In general, it is very important that patients learn to accept the diagnosis and its consequences. This highly facilitates the implementation of the behavioural modifications and decreases the burden of the disease. A supportive social environment (e.g., family members, friends, employer, colleagues, patient group organizations and support groups) is also valuable. Despite these behavioural measures, the majority of patients will remain to have residual complaints, requiring adjuvant pharmacological treatment.

**Pharmacological treatment**

A variety of substances are used to treat of narcolepsy, which observation indicates that there is not one drug that works for all patients. As most drugs predominantly act on either excessive daytime sleepiness (EDS) or cataplexy, combinations are often needed to control both symptoms. The only available drug that may improve all major symptoms of narcolepsy is Sodium Oxybate (SXB). Nevertheless, combinations of SXB with, for example, stimulants may have a synergetic effect for the amelioration of EDS, and may therefore be preferred over monotherapy with SXB.
What should be kept in mind when making a choice for a certain drug or combinations of drugs in an individual patient, and how to evaluate its efficacy?

- The expectation and goal of a treatment are of major importance in the judgment of the efficacy. Sleepiness will never be completely alleviated in any patient, whereas cataplexy may completely disappear in some. Long term improvement of disturbed nocturnal sleep is only reached with SXB. Patients must be made aware of this, and this knowledge must guide physicians in trying new drugs or combinations of drugs and in deciding on the right balance between efficacy and side effects.

- Ideally, drug efficacy should be assessed with a generally accepted, objective test to quantify the severity and the individual impact of a symptom. Unfortunately, there is no such test for narcolepsy as a whole, nor for its constituent symptoms. Sleepiness can be assessed with a variety of subjective and objective tests, but none of them is generally accepted as a valid indicator of daytime functioning. In fact, it is uncertain if either the impaired concentration while awake or sleeping during daytime is the more invalidating symptom. In case of the first, vigilance tests are more appropriate than sleep tests.73 Nocturnal sleep, cataplexy, hypnagogic hallucinations and sleep paralysis all present similar assessment problems. Cataplexy cannot be quantified in a simple manner, as its severity depends on many features: frequency, duration, the number of muscles involved, as well as behavioural consequences, such as avoidance of situations in which attacks may occur.

- In the absence of objective tests, history taking is the main instrument to evaluate efficacy and the occurrence of side effects.

- The interpretation of pharmacological trials is hampered by the lack of well-designed studies of older drugs, and a shortage of studies comparing different substances. Moreover, strict inclusion and exclusion criteria prevent the results of large trials to be applicable in all patients.

- Individual differences in efficacy, side effects and tolerability appear large. Knowledge about efficacy of a drug as assessed in groups is therefore of relative importance for individuals.
- Pharmacokinetic aspects, i.e., short and fast acting versus slow and long acting ones may be more important than the expected efficacy.

**Treatment of EDS**

Stimulants are the mainstay of the treatment of EDS.\(^{74,75}\) Useful drugs include dextroamphetamine (5–60 mg/day), methylphenidate (10–60 mg/day), and mazindol (1–6 mg/day). Side effects and the development of drug tolerance are major drawbacks of stimulants. The most important side effects include irritability, agitation, headache and peripheral sympathetic stimulation. These are usually dose-related. Although addiction does not seem to be a problem in narcoleptics,\(^{76}\) some patients tend to increase their dosage because they prefer high alertness. Tolerance develops in about a third of the patients.\(^{76,77}\) Mazindol has been withdrawn in most countries due to observed uncommon, but severe, side effects in related drugs that suppress appetite, in particular fenfluramines. The side effects were pulmonary hypertension and valvular regurgitation.\(^{78}\) As some patients respond better to mazindol than to any other drug it may be considered, provided treatment is closely monitored.

Modafinil (100–400 mg/day) is usually grouped with the stimulants, but is chemically unrelated to amphetamine. The efficacy is probably equal to that of the stimulants, although direct comparisons are lacking. The clinical impression is that all the described side-effects of stimulants, and also tolerance, may occur during treatment with modafinil, but, in general, less frequent and less severe. More specific side effects of modafinil are headache and nausea; however, they usually disappear after 2–3 weeks of treatment. Armodafinil is the \(r\)-enantiomer of modafinil and has shown to be effective in narcolepsy patients. There are no studies that compare its efficacy with modafinil. The drug is available in the USA but not in most European countries.\(^{74}\)

Long-acting agents (modafinil, dexamphetamine, methylphenidate controlled release) are generally better tolerated than the short acting (methylphenidate). The quick and short acting ones can be used to good effect when ‘targeted’ at social events or difficult periods during the day. For this reason, combinations of stimulants may be tailored to the circumstances. Unfortunately, there are no studies assessing the advantages or disadvantages of combinations of stimulants.

Studies with Sodium Oxybate (SXB), the sodium salt of gamma-hydroxybutyric acid, have shown that it is effective in reducing EDS. The usual starting dose is 2.25 grams twice a
night. The dose must be gradually increased, keeping in mind that the optimal daytime effects are reached after weeks. A relevant improvement of EDS is in most patients achieved with higher dosages (6–9 grams/night). The effect on EDS of higher doses is similar to that of modafinil, and side effects are, if present, usually mild. The combination of both therapies is even more effective. The most frequent side effect is nausea, and the most disabling are enuresis and sleepwalking. Lowering the dose may solve these problems. Weight loss may occur.

Follow-up studies provided no evidence for the development of tolerance. Abrupt cessation does not induce rebound cataplexy. However, long-term clinical experience shows that a substantial proportion of patients may develop tolerance for the sleep-promoting effects, although efficacy for the other symptoms remains.

SXB should not be used in conjunction with other sedatives or alcohol. If patients have consumed alcohol in the evening, they should omit one or both doses afterwards. In patients with co-morbid OSAS, treatment should be closely monitored, since SXB may worsen OSAS. Co-treatment with CPAP may be indicated.

Unfortunately, there is concern for misuse. Although potential threats related to misuse may result in hesitation in patients to take, and in physicians to prescribe the substance, it is important to realize that when the drug is properly used, it is safe, and bears no risk for dependence.

Caffeine may alleviate sleepiness, but only weak: the alerting effect of six cups of strong coffee is comparable with that of 5 mg of dexamphetamine. Selegiline and brofaromine may alleviate EDS as well.

Treatment of REM sleep dissociation phenomena

Most studies concerning the treatment of the REM dissociation phenomena focused on cataplexy. Amelioration of cataplexy is generally associated with improvement of hypnagogic hallucinations and sleep paralysis. SXB and tricyclic antidepressants are the most effective treatments. The different tricyclic antidepressants all inhibit the re-uptake of norepinephrine and serotonin and are potent REM sleep inhibitors. The most commonly used ones are imipramine (10–100 mg/day), and clomipramine (10–150 mg/day). Very low doses, such as 20 mg, may sometimes be remarkably effective. Most authors consider clomipramine to
be the treatment of choice. Some patients even experience improvement of EDS when treated with clomipramine. Tolerance may occur. As with stimulants, side effects, and to a lesser extent tolerance, form a major drawback. Side effects are largely due to anticholinergic effects; the most frequently reported ones are a dry mouth, increased sweating, sexual dysfunction (impotence, delayed orgasm, erection and ejaculation dysfunction), weight gain, tachycardia, constipation, blurred vision, and urinary retention. These are severe enough to lead to dose reductions or stopping its use. However, in some patients very low doses may be very effective without causing significant side effects. Tricyclic antidepressants should never be stopped abruptly because of the risk of severe aggravation of cataplexy, which may even lead to a status cataplecticus.

Many alternative antidepressants have been studied, especially selective serotonin re-uptake inhibitors, and more selective noradrenergic reuptake inhibitors such as fluoxetine, zimelidine, viloxazine, femoxetine, fluvoxamine and paroxetine in a relative higher dosage than the tricyclics. All these substances appear to have anti-cataplectic properties and less (disabling) side effects compared to the tricyclics. These substances seem to act mainly via less selective desmethyl metabolites, which are potent adrenergic uptake inhibitors.

During recent years, venlafaxine and atomoxetine have become very popular in the treatment of cataplexy, although there are no randomized placebo-controlled studies. Atomoxetine, however, has occasionally been shown to be effective when the others failed.

SXB is the best-studied drug and is a very potent inhibitor of cataplexy. It has never been compared to an antidepressant, so it is difficult to know whether it is really more effective in this regard. However, the relatively mild side effect profile makes it a more favourable drug, even independent of the beneficial effect of SXB on the other symptoms.

Another alternative less well studied and probably less potent is mazindol. This may, just like SXB, have a combined impact on sleepiness as well as on REM dissociation phenomena.

Several drugs may theoretically be expected to aggravate cataplexy, but the only one for which this is reliably documented is prazosin, an alpha-1 antagonist used to treat hypertension.
Treatment of the disturbed nocturnal sleep

Disturbed nocturnal sleep can be a major complaint of patients. Unfortunately, treatment options are limited, as SXB is the only drug with a proven long-term effect on nocturnal sleep.88 Short-term beneficial effects of benzodiazepines have been described.89 Although nocturnal sleep may (temporarily) be improved with benzodiazepines, improvement of EDS is not the rule.

Treatment of associated symptoms/disorders

Obesity is an associated symptom, to be treated in the same manner as holds for any obese person.

Fatigue or lack of energy may occasionally improve during treatment with stimulants or SXB. There is no other therapy with a proven effect for this complaint.

Treatment of a sleep apnoea does usually not improve EDS; understandably, compliance with CPAP may be limited. Whether apnoea in narcolepsy is in fact a valid indication for specific apnoea treatment is controversial.15 Treatment with SXB may facilitate the acceptance of CPAP treatment. However, since SXB may worsen the course of sleep apnoea it is important in these cases that patients are compliant.81

Treatment of periodic limb movements must be considered if there is co-existent RLS, otherwise only in very severe cases.

Treatment for RBD is rarely indicated, in those cases clonazepam and melatonin can be considered.

Recommendations for the initiation of pharmacological treatment

Pharmacological treatment is supplementary to behavioural advice and should be tailored individually. The recommendations given below should therefore only be considered as a guide to initiate pharmacotherapy.

For patients who predominantly suffer from EDS, modafinil is a good first choice. If EDS is relatively mild or mostly situation based, methylphenidate as ‘on demand’ treatment may be a good alternative. If modafinil monotherapy is not sufficient to reach a satisfactory situation,
combination therapy with SXB or methylphenidate can be considered. Women in the child bearing age who use low dose ethinyloestradiol (30 μg) contraceptives should be advised to switch to a compound with a higher ethinyloestradiol content before the start of modafinil.

Patients with a full-blown symptomatology, or who predominantly suffer from cataplexy and/or disturbed nocturnal sleep are good candidates for first line SXB treatment. In case of co-morbid OSAS, the therapy must be closely monitored and the combination of CPAP and SXB may be considered. If residual EDS complaints remain present, addition of modafinil or methylphenidate may be indicated. If cataplexy is not completely controlled, a very low dose (10 mg) of clomipramine can be added.

FUTURE PHARMACOLOGICAL TREATMENTS

Symptomatic therapies

- A recent study demonstrated promising effects of treatment with pitolisant, an inverse agonist of the histamine H3 receptor, on EDS.90

Immune-based therapy

- Intravenous-immunoglobulins (IVIg) hold promise. These therapies are given close to disease onset and are supposed to modulate the presumed, but not proven, autoimmune process leading to the hypocretin deficiency. A beneficial effect in particular on cataplexy has been claimed.91 Note however that studies were small and not blinded, that possible spontaneous severity fluctuations may have influenced outcome, and that the placebo effect may be large.92

Potential hypocretin-based therapies

- Hypocretin agonists: very attractive from a theoretical point of view. None are as yet available.

- Cell transplantation might potentially provide a cure.93 However, at present the techniques need to be improved and there is the potential problem of an immune reaction to the graft in view of the autoimmune hypothesis of narcolepsy.

- Gene therapy is promising in mice but has potentially dangerous side effects.94
AIMS OF THIS THESIS

This thesis explores several aspects of narcolepsy, varying from pathophysiological to treatment aspects. The first two chapters focus on the autoimmune hypothesis: chapter 2 attempts to shed some light on the role of the HLA-DQ dimer DQ0602 in the aetiology of narcolepsy. To do so, we compared HLA-DQ alleles located in trans with HLA-DQB1*06:02-DQA1*01:02 in Dutch narcoleptic subjects with those of control subjects. Chapter 3 presents the results of a search for antibodies directed against hypocretin-producing neurons. Serum of narcolepsy type 1 patients obtained close to disease onset are screened for antibodies using immunohistochemistry.

The next chapters provide more insight in the temperature regulation in patients with narcolepsy. Chapter 4 describes differences in temperature between narcolepsy type 1 patients and control subjects in a laboratory setting. In particular these concern the effects of sodium oxybate on core body and skin temperature in relation to its effects on sleep. Chapter 5 demonstrates the relation between sleep attacks and changes in skin temperature in everyday life, and further explores temperature regulation and sleep in narcolepsy type 1. Chapter 6 involves the validation of the Sustained Attention to Response Task (SART) to measure treatment efficacy in narcolepsy. Chapter 7 and 8 comprise a summary and discussion of the results of this thesis, and also contains suggestions for further research, respectively in English and Dutch.

REFERENCES


64. Lim ASP, Scammell TE. The trouble with Tribbles: do antibodies against TRIB2 cause narcolepsy? Sleep 2010;33:857–858.


CHAPTER 2
HLA DOSAGE EFFECT IN NARCOLEPSY WITH CATAPLEXY

Astrid van der Heide
Willem Verduijn
Geert W. Haasnoot
Jos J.M. Drabbels
Gert Jan Lammers
Frans H.J. Claas

Immunogenetics 2015;67(1):1–6
Chapter 2

ABSTRACT

Narcolepsy with cataplexy is a sleep disorder caused by the loss of hypocretin producing neurons in the hypothalamus. It is tightly associated with a specific HLA allele: HLA-DQB1*06:02. Based on this, an autoimmune process has been hypothesised. A functional HLA-DQ molecule consists of a DQα and a DQβ chain. HLA-DQB1*06:02 (DQβ) has a strong preference for binding to HLA-DQA1*01:02 (DQα), and together they form the functional DQ0602 dimer. A dosage effect would be expected if the HLA-DQ0602 dimer itself is directly involved in the aetiology. An increased expression of the HLA-DQ0602 dimer is expected in individuals homozygous for HLA-DQB1*06:02-DQA1*01:02, but is also hypothesised in individuals heterozygous for HLA-DQB1*06:02 and homozygous for HLA-DQA1*01:02. To study the impact of the expression of the HLA-DQ0602 dimer on narcolepsy susceptibility, 248 Dutch narcolepsy patients and 1272 Dutch control subjects, all of them positive for DQB1*06:02 (heterozygous and homozygous), were HLA-genotyped with attention not only to DQB1 but also to DQA1*01:02.

DQB1*06:02-DQA1*01:02 homozygosity was significantly more often seen in patients compared to controls (OR 2.29) confirming previous observations. More importantly, a significantly higher prevalence of homozygosity for DQA1*01:02 was found in HLA- DQB1*06:02 heterozygous patients compared to controls (OR=2.37, p<0.001). The latter finding clearly supports a direct role of the HLA-DQ molecule in the development of disease.
INTRODUCTION

Narcolepsy is a disorder of the regulation of sleep and wakefulness, resulting in a variety of symptoms such as excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations, sleep paralysis and disturbed nocturnal sleep.\textsuperscript{1,2} According to the current classification of sleep disorders, narcolepsy can be divided into narcolepsy with and without cataplexy.\textsuperscript{3}

Narcolepsy with cataplexy is caused by a hypocretin-1 (orexin-A) deficiency,\textsuperscript{4} which is thought to be the consequence of a selective loss of hypocretin producing neurons in the hypothalamus.\textsuperscript{5,6} However, the mechanism behind this loss of hypocretin producing neurons has not been elucidated yet. Several findings point into the direction of an autoimmune cause, although no direct evidence for a role of the immune system has been found yet.

By far the strongest argument for an autoimmune aetiology in narcolepsy is its tight HLA (human leukocyte antigen) association: narcolepsy with cataplexy is the disease with the strongest association with a specific HLA-allele, i.e. HLA-DQB1*06:02.\textsuperscript{7} A functional HLA-DQ molecule originates from the binding of an \( \alpha \) chain (DQA1) with a \( \beta \) chain (DQB1) (Figure 2.1). Not all DQ\( \alpha \) and DQ\( \beta \) chains are able to bind to each other (i.e. dimerise). The genes encoding for chains that have affinity to dimerise are often in linkage disequilibrium. Narcolepsy is tightly associated with HLA-DQB1*06:02,\textsuperscript{8-11} which is in linkage disequilibrium with HLA-DQA1*01:02. The opposite is not per se true: HLA-DQA1*01:02 prefers to bind to HLA-DQB1*06:02, but also to the DQ\( \beta \) chains DQB1*05:02, DQB1*06:04 and DQB1*06:09.\textsuperscript{12} The DQ\( \alpha \) and DQ\( \beta \) chain encoded for by this DQB1*06:02-DQA1*01:02 haplotype together form the functional HLA-DQ dimer DQ0602. Worldwide about 85–95\% of the narcolepsy with cataplexy patients carry this haplotype, compared to 12–38\% of the general population.\textsuperscript{13} For non-familial cases and those with typical cataplexy the association may even exceed the 98\%.\textsuperscript{14} Therefore, carrying this haplotype is thought to represent an almost necessary risk factor for the development of narcolepsy, although its mere presence is not sufficient to cause narcolepsy. Results of studies in which exons near the HLA-DQ genes have been sequenced support a direct role for HLA-DQB1*06:02 itself.\textsuperscript{8,14,15} When the HLA-DQ dimer itself is indeed involved in the aetiology, a dosage effect would be expected, meaning that an increased expression of the DQ0602 dimer is associated with a higher susceptibility to the development of narcolepsy. This is expected in individuals homozygous for HLA-DQB1*06:02-DQA1*01:02, but also in individuals heterozygous for HLA-DQB1*06:02 and homozygous for HLA-DQA1*01:02. Similar dosage effects have been described for HLA-DQ molecules associated with diabetes\textsuperscript{16} and celiac disease.\textsuperscript{17,18} If a gene in close linkage with
HLA-DQB1*06:02 is responsible for the increased risk, a dosage effect is only expected in individuals homozygous for HLA-DQB1*06:02, but not in individuals heterozygous for HLA-DQB1*06:02 and homozygous for HLA-DQA1*01:02.

Previous HLA studies in narcolepsy patients heterozygous for HLA-DQB1*06:02-DQA1*01:02, revealed a role of several accompanying HLA-haplotypes (i.e. located in trans with DQB1*06:02-DQA1*01:02). Heterozygosity with DQB1*03:01, DQA1*06, DQA1*03:03, DRB1*04, DRB1*08, DRB1*11 or DRB1*12 turned out to increase the risk of developing narcolepsy,\(^{19,20}\) whereas heterozygosity of DQB1*06:02 with DQB1*06:01,
HLA dosage effect in narcolepsy with cataplexy

DQB1*06:03, DQB1*05:01 or DQA1*01 (non DQA1*01:02) turned out to decrease the risk.\textsuperscript{11,20,21} Furthermore, a two to four times increased risk of developing narcolepsy is reported in Caucasians homozygous for DQB1*06:02.\textsuperscript{14,22}

To further investigate the role of the DQ0602 dimer in developing narcolepsy, we explored the role of HLA-DQB1*06:02-DQA1*01:02 in combination with the haplotype located in trans with it, with special attention to DQA1*01:02. Increased homozygosity for DQA1*01:02 in patients heterozygous for DQB1*06:02 would support a direct role of the HLA-DQ molecule in the development of narcolepsy.

METHODS

Subjects

248 patients and 1272 control subjects, all positive for DQB1*06:02-DQA1*01:02 (heterozygous and homozygous), were included in this study. All patients were recruited from the sleep clinic of the department of Neurology, Leiden University Medical Centre. They all suffered from narcolepsy with clear-cut cataplexy according to the ICSD-3 criteria.\textsuperscript{3} Taking the probable different aetiology of familial narcolepsy into account, identified familial cases were excluded. The DQB1*06:02- DQA1*01:02 positive controls were taken from a panel of randomly selected, healthy unrelated Dutch individuals.\textsuperscript{23} All patients and controls were of European ancestry.

HLA genotyping

HLA-DRB and HLA-DQB1 typing was performed with a reversed approach of the PCR-sequence-specific oligonucleotide probe technique described elsewhere.\textsuperscript{24} The typing results of some samples with a rare DRB1-DQB1-DQA1 association were confirmed by PCR-SBT, using the SBT Excellerator HLA-DRB and -DQB kit (Genome Products, Utrecht, The Netherlands). Subsequently, all DQB1*06:02 positive patients and controls were selected to examine their dosage of DQA1*01:02. The absence, heterozygosity or homozygosity for DQA1*01:02 was determined with a sybergreen based rt-PCR, using three different DQA1*SSP primer mixes. On every PCR run the specificity of these primer mixes was checked by adding two controls, one control was homozygous for DQA1*01:02 and the other was negative for DQA1*01:02 (homozygous for DQA1*01:03). Furthermore, to confirm the rt-
PCR results, a routinely used DQA1 PCR-SSP technique was applied on 15 samples and no discrepancy with the rt-PCR results was observed.

**Statistical analysis**

To evaluate statistical significance two-sided Fisher’s exact test was performed. The p values were corrected for multiple comparisons according to the Šidák method. Odds ratios and corresponding 95% confidence intervals (CI) were calculated according to the Woolf Haldane test.

A too large control group could lead to statistically significant differences that are clinically irrelevant. Therefore, corrected p values are standardized (ps) to a sample size of 900 following the method of Good. This sample size is derived by: the total number of patients plus three times the number of patients as maximum allowed size for the control group, and rounded up.

**RESULTS**

**Analysis of HLA-DQB1*06:02-DQA1*01:02 homozygosity**

The relative and absolute distribution of DQB1*06:02-DQA1*01:02 in trans (DQB1*06:02-DQA1*01:02 homozygosity) in DQB1*06:02-DQA1*01:02 positive patients and controls are given in Table 2.1. Homozygosity for HLA-DQB1*06:02-DQA1*01:02 was significantly more prevalent in patients (OR=2.42).

**HLA-DQB1 alleles in trans with HLA-DQB1*06:02**

In patients and controls, the absolute and relative distributions of HLA-DQB1 alleles located in trans with HLA-DQB1*06:02 were calculated and are demonstrated in Table 2.2. DQB1*03:01/03:04 and DQB1*05:02 were significantly more prevalent on the other haplotype compared to controls (OR 1.99 and 3.25, respectively). On the contrary, DQB1*02 and DQB1*06:03 were significantly less prevalent (OR 0.53 and 0.21, respectively). These alleles may be seen to be protective in developing narcolepsy when present in combination with DQB1*06:02, in particular DQB1*06:03.
HLA dosage effect in narcolepsy with cataplexy

The frequency of HLA-DQA1*01:02 located in trans with HLA-DQB1*06:02-DQA1*01:02 was compared between narcolepsy patients and controls (Table 2.3). In heterozygous DQB1*06:02 patients homozygosity for DQA1*01:02, was significantly increased compared to heterozygous controls (OR 2.37). Analysis of the frequency of the specific HLA-DQB1 alleles in trans with HLA-DQB1*06:02 in HLA-DQA1*01:02 homozygous revealed no differences between patients and controls (Table 2.4).
The aim of this study was to obtain more insight into the direct role of the HLA-DQ dimer DQ0602 in the aetiology of narcolepsy. In order to further explore the role of the DQ0602 dimer we studied the HLA-DQ alleles located in trans with HLA-DQB1*06:02-DQA1*01:02, with special attention to DQA1*01:02 homozygosity, in Dutch narcoleptic subjects compared with control subjects.

In concordance with previous studies, homozygosity for DQB1*06:02-DQA1*01:02 was more frequently seen in our patients than in controls.\(^{14,22}\) Homozygosity for DQA1*01:02-DQB1*06:02 leads to the maximum number of expressed predisposing dimers supporting the existence of a dosage effect in the aetiology.

The higher prevalence of homozygosity for DQA1*01:02 in DQB1*06:02 heterozygous narcolepsy patients compared to controls is in line with a direct role of the DQ0602 dimer in the aetiology of narcolepsy. This can be explained in the following way; a functional HLA-DQ molecule is formed by binding of a DQα with a DQβ chain. Every person carries two DQα chains and two DQβ chains. Theoretically, all these chains form functional HLA-DQ molecules

### Table 2.3 Increased homozygosity for HLA-DQA1*01:02 in DQB1*06:02 heterozygous narcolepsy patients and controls

<table>
<thead>
<tr>
<th>DQA1 cis / trans</th>
<th>Patients n (%)</th>
<th>Controls n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
<th>ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>01:02/01:02</td>
<td>36 (17.1)</td>
<td>95 (8.0)</td>
<td>2.366</td>
<td>1.565–3.578</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Presence of trans-HLA-DQA1*01:02 in patients and controls carrying HLA-DQB1*06:02-DQA1*01:02 on the cis haplotype. Ps = p value corrected for standard sample size.\(^{28}\) One of the DQA1*01:02 trans was derived an unusual haplotype with DRB1*15:01, DQB1*06:03 (in a Caucasian patient).

### Table 2.4 Analysis of HLA-DQB1 alleles in trans with HLA-DQB1*06:02 in HLA-DQA1*01:02 homozygous narcolepsy patients and controls

<table>
<thead>
<tr>
<th>DQB1</th>
<th>Patients n (%)</th>
<th>Controls n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
<th>pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>05:02</td>
<td>11 (31)</td>
<td>20 (21)</td>
<td>1.661</td>
<td>0.710–3.887</td>
<td>0.259</td>
<td>0.698</td>
</tr>
<tr>
<td>06:03</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>8.070</td>
<td>0.321–202.751</td>
<td>0.275</td>
<td>0.723</td>
</tr>
<tr>
<td>06:04</td>
<td>21 (58)</td>
<td>64 (67)</td>
<td>0.677</td>
<td>0.310–1.477</td>
<td>0.413</td>
<td>0.881</td>
</tr>
<tr>
<td>06:09</td>
<td>3 (8)</td>
<td>11 (12)</td>
<td>0.768</td>
<td>0.217–2.711</td>
<td>0.757</td>
<td>0.997</td>
</tr>
</tbody>
</table>

HLA*DQB1 alleles present on the other chromosome in narcolepsy patients (n=36) and controls (n=95) heterozygous for HLA-DQB1*06:02 and homozygous for HLA-DQA1*01:02. Pc = p value corrected for multiple testing.\(^{25}\)
by dimerisation. In principle, the \( DQ\alpha \) and \( DQ\beta \) chains of both chromosomes (cis and trans) can dimerise, theoretically leading to four different HLA-DQ molecules in heterozygous individuals. However, \( DQ\alpha \) and \( DQ\beta \) chains differ in affinity for each other and only a portion of the theoretically possible dimers will actually be formed. The affinity of \( DQB1*06:02 \) and \( DQA1*01:02 \) for each other is high. In case of homozygosity for \( DQA1*01:02-DQB1*06:02 \), all expressed DQ molecules are \( DQA1*01:02-DQB1*06:02 \). In individuals heterozygous for both the \( DQ\alpha \) and the \( DQ\beta \) chain, a maximum of four different DQ molecules can be expressed, of which only one is the predisposing one. When a person is homozygous for \( DQA1*01:02 \) and heterozygous for \( DQB1*06:02 \), only two different DQ molecules can be formed, which subsequently results in a higher number of predisposing dimers compared to fully heterozygous individuals. In contrast to the present study, a recent Chinese study did not report a higher prevalence of homozygosity for \( DQA1*01:02 \) in \( DQB1*06:02 \) heterozygous narcolepsy patients (OR=1.15, not significant).\(^{11}\) Nevertheless, the prevalence of homozygous \( DQA1*01:02 \) heterozygous \( DQB1*06:02 \) patients was reported to fall between the prevalence of \( DQA1*01:02-DQB1*06:02 \) heterozygous patients and that of \( DQA1*01:02-DQB1*06:02 \) homozygous patients, suggesting both amount and ratio of \( DQA1*01:02/DQB1*06:02 \) may be important. Remarkably, Tafti et al.\(^{14}\) recently reported a protective effect of \( DQB1*06:09 \), which is in linkage disequilibrium with \( DQA1*01:02 \). This finding is in contradiction with our hypothesis, as a predisposing effect would be expected. Although the numbers are small, neither a predisposing effect nor a protective effect of this allele was found in the present study.

In fully heterozygous patients the known predisposing and protective effects of several alleles in trans cannot be explained by this mechanism. In these patients, different hypotheses have been described. One of those is the \( DQA1-DQB1 \) allelic competition model, in which the trans \( DQ\beta \) molecule competes with \( DQB1*06:02 \) for dimerization with the \( DQA1*01:02 \) molecule. If cross dimerisation is possible, the number of disease predisposing HLA-DQ0602 dimers on the cell surface may decrease, resulting in a lowered risk of developing narcolepsy. The currently identified and previously reported decreased prevalence of \( DQB1*06:03 \) and \( DQB1*06:01 \)\(^{11,20,21}\) is in line with this allelic competition model, as both these alleles are not in linkage disequilibrium with \( DQA1*01:02 \), but are able to dimerise with \( DQA1*01:02 \), and therefore compete with \( DQB1*06:02 \). However, the currently and previously reported predisposing effect of \( DQB1*03:01 \)\(^{11,20,21}\) and currently reported protective effect of \( DQB1*02 \), cannot be explained by one of the above described hypotheses. The mechanism behind the predisposing effect of \( DQB1*03:01 \) can possibly
be found in a theory similar to the DQA1-DQB1 allelic competition model based on cross dimerisation. Such a mechanism is also suggested to play an important role in the development of type 1 diabetes.\textsuperscript{29} DQB1*03:01 is in linkage disequilibrium with DQA1*03. DQB1*03:01 is not able to cross dimerise with DQA1*01:02 in vitro,\textsuperscript{12} but DQB1*06:02 is able to do so with DQA1*03. Subsequently, all DQA1*01:02 chains will exclusively dimerise with DQB1*06:02, leading to a maximal expression of the predisposing dimer DQ0602, explaining the increased susceptibility for the disease. Nevertheless, the observed protective effect of DQB1*02 in trans with DQB1*06:02 in our population does not fit into one of the described hypotheses; DQB1*02 is in linkage-disequilibrium with DQA1*02 and DQA1*05, but these alleles are not able to form stable heterodimers with the DQα and DQβ chains of the predisposing DQ0602 dimer.\textsuperscript{12} Subsequently, this would not influence the availability of the predisposing dimer.

Since it is not possible to explain all protective and predisposing effects of the described alleles by one or multiple of the mechanisms described in the previous section, the explanation may lie in another direction. A possible mechanism explaining the protective or predisposing effect of a certain heterodimer, could be found in a different affinity for the epitopes relevant for the development of narcolepsy.\textsuperscript{30} Heterodimers may compete to bind a particular epitope and when a protective heterodimer has a higher affinity for the epitope, this will negatively affect binding of this peptide to the predisposing heterodimer, resulting in a reduced risk of developing narcolepsy. The opposite might be true for a predisposing heterodimer.

The current genetic study provides additional evidence for a direct role of the HLA-DQ0602 molecule in the aetiology of the disease. The direct role of the HLA-DQB1*06:02-DQA1*01:02 dimer fits perfectly in the autoimmune hypothesis in narcolepsy. The function of HLA-class II molecules like the DQ0602 dimer is to present peptides derived from foreign proteins to the immune system in order to elicit a T cell mediated immune response. Sometimes, T cells reactive with foreign peptides may cross react with self-structures leading to destruction of autologous cells and autoimmunity.

In conclusion, the present study shows a significantly higher prevalence of homozygosity for DQA1*01:02 in HLA-DQB1*06:02 heterozygous patients compared to controls and clearly supports a direct role of the DQB1*06:02-DQA1*01:02 dimer molecule in the development of disease.
REFERENCES


CHAPTER 3
IMMUNOHISTOCHEMICAL SCREENING FOR ANTIBODIES IN RECENT ONSET TYPE 1 NARCOLEPSY AND AFTER H1N1 VACCINATION

Astrid van der Heide
Ingrid M. Hegeman-Kleinn
Els Peeters
Gert Jan Lammers
Rolf Fronczek

Chapter 3

52

ABSTRACT

Narcolepsy type 1 patients typically have undetectable hypocretin-1 levels in the cerebrospinal fluid (CSF), as a result of a selective loss of the hypocretin containing neurons in the hypothalamus. An autoimmune attack targeting hypothalamic hypocretin (orexin) neurons is hypothesised. So far, no direct evidence for an autoimmune attack was found. One of the major limitations of previous studies was that none included patients close to disease onset. We screened serum of 21 narcolepsy type 1 patients close to disease onset (median 11 months), including 8 H1N1 vaccinated patients, for antibodies against hypocretin neurons using immunohistochemistry. No autoantibodies against hypocretin neurons could be detected.
INTRODUCTION

Narcolepsy type 1 is a disorder of the regulation of sleep and wakefulness. Almost all narcolepsy type 1 patients have undetectable hypocretin-1 (orexin A) levels in the CSF.1,2

Hypocretin (orexin) is a neuropeptide, produced by neurons located in the lateral and posterior hypothalamus, most abundant in the perifornical region.3-8 Post-mortem studies in narcolepsy demonstrated a selective loss of the hypocretin containing neurons in the hypothalamus,9-11 explaining the undetectable CSF hypocretin-1 levels. The selectivity of this loss was supported by the fact that intermingling melanin-concentrating hormone neurons appeared to be unaffected.9,10 Although the mechanism behind this specific loss of hypocretin producing neurons has not been elucidated yet, an autoimmune attack targeting hypothalamic neurons that produce hypocretin is hypothesised.

This autoimmune hypothesis is mainly based on the tight association of narcolepsy with HLA-DQB1*06.02,12,13 and is supported by the identification of additional genetic factors that suggest T-cell involvement in narcolepsy: polymorphisms in the T-cell receptor alpha and beta locus (TCRa and TCRβ), Cathepsin H (CTSH), Tumor Necrosis Factor (ligand) Superfamily member 4 (TNFSF4, also called OX40L), the purinergic receptor P2RY11, and the ZNF365 and IL10RB-IFNAR1 loci.14-17 Moreover, an increased incidence of narcolepsy was reported after infections with Streptococcus pyogenes, influenza type A virus, H1N1, and H1N1 vaccination.18-22

In search for evidence for an autoimmune attack, several research groups (including our own) attempted to find autoantibodies against hypocretin neurons. Unfortunately, up to now none of them was convincingly successful.23-26 However, in 2010, three independent groups reported elevated levels of antibodies against a protein that is produced in hypocretin neurons, Tribbles homolog 2 (TRB2).27-29 Since TRB2 is not specific for hypocretin neurons, it is unlikely that these antibodies directly injure the hypocretin neurons. However, these antibodies may be a consequence of hypocretin neuronal damage or may be indirectly involved in the attack of hypocretin neurons.30 In a recent study, sera and CSF from subjects suffering from narcolepsy and other sleep related disorders yielded three immunohistochemical staining patterns in the rat brains. However, none of them concerned hypocretin neurons and the patterns were not specific for narcolepsy.31

All these previous immunohistochemical studies were performed with serum or CSF derived from narcolepsy patients with a relatively long disease duration, or from patients with
unknown disease duration. Due to the delay after clinical onset in those studies, it might be possible that antibody levels already had decreased and no autoimmune response could be found anymore. To further investigate this aspect, we screened serum of narcolepsy type 1 patients close to disease onset, including H1N1 vaccinated patients, for antibodies against hypocretin neurons using immunohistochemistry.

MATERIALS AND METHODS

Subjects

We included 21 narcolepsy type 1 patients. Patients were recruited from the sleep clinic of the department of Neurology, Leiden University Medical Centre, Medisch Centrum Haaglanden, University Medical Centre Groningen and Erasmus MC University Medical Centre, The Netherlands. All patients suffered from narcolepsy type 1 according the International Classification of Sleep Disorders (ICSD-3).32

In addition, sera of 2 subjects without any medical condition were used as controls. Both were used in a previous study in which one of them demonstrated a consistent staining of hypothalamic neurons, and one no staining.23 Serum of a narcolepsy patient who showed consistent staining of hypothalamic neurons in this same previous study was used as third control.

Brain tissue

Immunohistochemistry was performed on sections of encoded human hypothalamus and corpora mammillaria, obtained from the department of pathology of the Leiden University Medical Centre (3 subjects who died of non-neurological disease, age 63 (post-mortem delay [PMD] 1.7 hours), age 37 (PMD 5 hours) and age 48 (PMD 19 hours) respectively). Hypothalami were freshly dissected, fixed in buffered formalin for 60–70 days, paraffin embedded and serially sectioned at 6 μm. In the study we used the sections from the expected hypocretin area, from the level where the fornix touches the paraventricular nucleus to the level where the fornix reaches the corpora mammillaria.
Screening immunohistochemistry

After deparaffinisation and rehydration, endogenous peroxidase activity was blocked in methanol-0.3% H$_2$O$_2$ for 20 minutes. After washes in TBS, sections were incubated for 1h at room temperature (room temperature) with the serum at a 1:400 dilution in supermix (0.05M Tris, 0.15M NaCl, 0.25% gelatin, 0.5% Triton X-100, pH7.6), and overnight at 4°C. The next day, after washes in TBS, sections were labelled with Goat anti-human/biotin in Supermix, dilution 1:4000, 1h at RT, followed by Avidin-Biotin Complex (Vectastain ABC-Elite Kit, Vector Lab, USA) in supermix for 30 minutes at RT. 3,3’-Diaminobenzidine (DAB) - Cobalt Chloride was used to visualise the staining.

Per subject sections of 7 different hypothalamic areas were stained. To identify the area of interest, of all these 7 areas, one section was stained with anti-orexin A/hypocretin-1 antibody (cat. no H-003-30, lot. no 01169-4, Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). Slides incubated only with supermix served as negative control.

All sections were scored separately for staining by two blinded researchers (AvdH and RF).

RESULTS

Subjects (Table 3.1)

21 patients (13 males, 8 females) with an average age of 12 years (range 4–48 years) were included. All, but one patient, were close to disease onset, i.e. within three years after the onset of narcolepsy symptoms, with 33% of patients within 6 months of disease onset. The median duration of symptoms was 11 months (range 2–48 months). All patients suffered clear-cut cataplexy. 20 patients were HLA-typed and were positive for HLA-DQB1*06:02. In 17 patients hypocretin-1 was measured, all were hypocretin-1 deficient. The other four patients were expected to be hypocretin deficient as they suffered clear-cut cataplexy, had a non-familial type of narcolepsy, and were positive for HLA-DQB1*06:02.$^{33}$ 8 patients received a H1N1 vaccination prior to the onset of narcolepsy symptoms.
Table 3.1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Disease duration (months)</th>
<th>H1N1 vaccination</th>
<th>HLA-DQB1*0602</th>
<th>CSF Hypocretin-1 (pg/mL)</th>
<th>Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>M</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>F</td>
<td>2</td>
<td>Unknown</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>M</td>
<td>4</td>
<td>-</td>
<td>N.D.</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>M</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>M</td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>F</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>F</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>F</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>F</td>
<td>10</td>
<td>-</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>F</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>17</td>
<td>M</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>M</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>M</td>
<td>13</td>
<td>N.A.</td>
<td>+</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>17</td>
<td>M</td>
<td>16</td>
<td>N.A.</td>
<td>+</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>17</td>
<td>M</td>
<td>18</td>
<td>N.A.</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>M</td>
<td>18</td>
<td>+</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>F</td>
<td>27</td>
<td>N.A.</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>48</td>
<td>M</td>
<td>27</td>
<td>N.A.</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>16</td>
<td>M</td>
<td>27</td>
<td>N.A.</td>
<td>+</td>
<td>N.D.</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>M</td>
<td>36</td>
<td>N.A.</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>F</td>
<td>48</td>
<td>N.A.</td>
<td>+</td>
<td>≤110</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive patient

| 1       | 38          | M   | 180                       | N.A.             | +             | ≤110                   | +       |

Patient characteristics of the 21 included patients and 1 positive patient from the previous study.23

1 M: male, F: female.

2 N.A.: Not applicable, i.e. patients with disease onset prior to the H1N1 vaccination campaign.

3 N.D.: Not determined.

Hypocretin staining

Hypocretin-1 positive neurons were found in all hypothalamic sections used (see Figure 3.1A for a representative section). Hypocretin-1 positive cell bodies were mainly located in the perifornical area of the lateral hypothalamus, as expected.23

Screening immunohistochemistry

None of the 21 tested patient sera demonstrated consistent staining of neurons in the lateral hypothalamus or corpora mammillaria, i.e. no antibodies were detected, in particular not
in the patients closest to disease onset and in patients who received a vaccination against H1N1. The previous finding of consistent staining of hypothalamic neurons in one control subject and one narcolepsy subject, was confirmed in the present study. The absence of staining in the previously described negative control was also confirmed. See Figure 3.1 for representative hypothalamic sections stained with the sera.

Figure 3.1  Representative examples of staining. Sections from the lateral hypothalamus stained with anti-hypocretin-1 (A), positive serum of the previously described narcolepsy with cataplexy patient (B), positive serum of the previously described control subject (C), and negative serum of 1 narcolepsy with cataplexy patient (D). Scale bars: 100 µm. Abbreviations: F = fornix.

DISCUSSION

The aim of the present study was to find evidence for the autoimmune hypothesis in the aetiology of narcolepsy. To this end, serum of narcolepsy type 1 patients obtained close to disease onset was screened for antibodies against hypocretin neurons using immunohistochemistry. To our knowledge, this is the first study that included patients this close to disease onset.
To overcome the major limitation of these studies, i.e. the relatively long disease duration of the included patients, all patients included in the present study were close to disease onset (median 11 months). Despite this, no autoantibodies against hypocretin neurons could be detected.

The inability to detect autoantibodies in the current study does not contradict the autoimmune hypothesis; it even does not implicate an absence of autoantibodies at any time in the development of the disease. Narcolepsy with cataplexy is associated with loss of at least 90% of hypocretin containing neurons.\textsuperscript{10} It is currently presumed that narcoleptic symptoms start to occur when the vast majority of the hypocretin containing neurons have disappeared.\textsuperscript{34} According to this hypothesis, the actual cell loss – i.e. the immune attack – precedes the appearance of narcoleptic symptoms. Therefore, the time window for detection of an autoimmune process might have passed by the time the patient is diagnosed. Screening patients as short after disease onset as we did, might be too late as well. Moreover, the usage of paraffin fixed hypothalamic sections could have masked the antibody epitopes. Furthermore, narcolepsy is a disorder that seems to be confined to the central nervous system, autoantibodies produced in the brain probably hardly pass the blood-brain barrier. Subsequently, the concentration of autoantibodies might be very low in serum of narcolepsy patients. Theoretically, screening CSF could solve this problem, as the concentration of autoantibodies might be higher in CSF compared to serum. However, so far no hypocretin specific autoantibodies or signs of inflammation have been detected in CSF of narcolepsy patients at all.\textsuperscript{24-26,31,35,36} Moreover, when both serum and CSF were used for immunohistochemistry, CSF was used in a much higher concentration, while the staining pattern was similar, much weaker or even absent with CSF.\textsuperscript{23-26,31} Meanwhile, it is questionable whether narcolepsy is an antibody-mediated disease. Based on the genetic findings, narcolepsy probably is a T-cell mediated autoimmune disease,\textsuperscript{37} and T-cell immunity is not necessarily associated with antibodies.

In conclusion, the present study demonstrates no evidence for autoantibodies in serum of narcolepsy patients close to disease onset and patients after H1N1 vaccination. This finding does not contradict the autoimmune hypothesis, nor implicates an absence of autoantibodies at any time in the development of the disease.
REFERENCES


CHAPTER 4
THE EFFECTS OF SODIUM OXYBATE ON CORE BODY AND SKIN TEMPERATURE REGULATION IN NARCOLEPSY

Astrid van der Heide
Claire E.H.M. Donjacour
Hanno Pijl
Robert H.A.M. Reijntjes
Sebastiaan Overeem
Gert Jan Lammers
Eus J.W. Van Someren
Rolf Fronczek

Journal of Sleep Research 2015;24(5):566–575
Patients suffering from narcolepsy type 1 show altered skin temperatures, resembling the profile that is related to sleep onset in healthy controls. The aim of the present study is to investigate the effects of sodium oxybate, a widely-used drug to treat narcolepsy, on the 24-hour profiles of temperature and sleep-wakefulness in narcolepsy patients and controls. Eight hypocretin-deficient male narcolepsy type 1 patients and eight healthy matched controls underwent twice temperature measurement of core body and proximal and distal skin, and the sleep-wake state for 24 hours. After the baseline assessment, 2 x 3 grams of sodium oxybate was administered for five nights, immediately followed by the second assessment. At baseline, daytime core body temperature and proximal skin temperature were significantly lower in narcolepsy patients (core: 36.8±0.05°C vs. 37.0±0.05°C, F=8.31, p=0.01; proximal: 33.4±0.26°C vs. 34.3±0.26°C, F=5.66, p=0.03). In patients, sodium oxybate administration increased proximal skin temperature during the day (F=6.46, p=0.04) to a level similar as in controls, but did not affect core body temperature, distal temperature or distal-proximal temperature gradient (DPG). Sodium oxybate administration normalised the predictive value of distal skin temperature and DPG for the onset of daytime naps (p<0.01). In conclusion, sodium oxybate administration resulted in a partial normalisation of the skin temperature profile, by increasing daytime proximal skin temperature and by strengthening the known relationship between skin temperature and daytime sleep propensity. These changes seem to be related to the clinical improvement induced by SXB treatment. A causal relation is not proven.
INTRODUCTION

Narcolepsy with cataplexy (narcolepsy type 1) is a sleep disorder characterised by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis and impaired maintenance of nocturnal sleep. A decreased level of hypocretin-1 (orexin-A) in the CSF is the hallmark of the disease and is considered to explain all narcolepsy symptoms.

Skin and core body temperature play an important role in sleep and wake regulation. Wake is associated with a relatively low skin temperature and a relatively high core body temperature, while sleep is associated with a higher skin temperature and a lower core body temperature. Sleep onset is preceded by a decline in core body temperature and an increase in skin temperature. The decrease in core body temperature is mediated through increased skin perfusion, which consequently leads to the increase in skin temperature, and facilitates cooling of the body. These changes are facilitated in part by the postural change from an upright to a supine position that commonly occurs during sleep.

Previous studies demonstrated an altered diurnal profile of skin temperature in narcolepsy. Compared with controls, patients with narcolepsy show an increased distal skin temperature and a decreased proximal skin temperature in the waking state. This pattern may be considered as characteristic of lowered vigilance or even ‘sleep promoting’, since it is also seen in controls immediately before sleep onset. Indeed, temperature manipulation studies in narcoleptic patients counteracting these changes have shown to improve nocturnal sleep and excessive daytime sleepiness. All together, these findings suggest a relationship between hypocretin function, temperature and sleep regulation.

Gammahydroxybutyrate (GHB) is a hypnotic used to improve nocturnal sleep and EDS in narcolepsy. GHB has a wide range of effects, but the exact mechanisms are still unclear. Altered thermoregulation is one of the effects described in animal studies and human case reports. Rodent studies demonstrate a slight increase in core body temperature after administration of a low dose of GHB (5–10 mg/kg) and a clear decrease in core body temperature in higher doses (<500 mg/kg). Several studies describe hypothermia in humans with GHB intoxication.

Sodium oxybate (SXB) is the sodium salt of GHB and is registered for the treatment of narcolepsy. Its effects are comparable to the effects of GHB. Given the impact of GHB on temperature regulation, the altered pattern of skin temperature in narcolepsy and the positive effects of SXB on sleep in narcolepsy patients, it may be hypothesized that the
treatment effect of SXB may in part be mediated by its possible restorative effect on temperature regulation. The aim of the present study is to investigate the effect of SXB on core body and skin temperature in relation to its effects on sleep. Therefore, we continuously measured sleep, core body temperature and skin temperature for 24 hours in narcolepsy patients and controls, before and after five days of SXB administration during a constant routine protocol.

METHODS

Subjects

Eight male narcolepsy patients (18–65 years of age) were included after informed consent. They all fulfilled the criteria for narcolepsy type 1 according to the International Classification of Sleep Disorders-3 (ICDS-3),1,19 suffered clear-cut cataplexy and were hypocretin-1 deficient. Two patients were drug naive, one patient was tapered from antidepressants ≥ 2 weeks prior to the study, and 2 patients had prior history with SXB; however, no subject took SXB within 20 days of study initiation. The other patients did not take any medication for at least several months prior to beginning the study. Eight healthy male controls, free of any neurologic, endocrine or psychiatric disease, were individually matched for age and body mass index (BMI). Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the Leiden University Medical Centre.

Study design (Figure 4.1)

The results of this study originate from an extensive, constant routine protocol that was described previously.2,20-22

All subjects stayed overnight in the hospital and underwent a baseline 24h temperature measurement and polysomnography. During this measurement subjects remained (semi) supine except for bathroom visits. Lights were switched off at 23:00h and switched on at 7:30h. Subjects were allowed to take daytime naps whenever they wanted. At 8:30h, 13:00h and 18:00h a standardised cold meal was served and during the whole day water and tea (caffeine free) were available. Following the baseline study, subjects ingested SXB for five consecutive days, the first and the 5th day in the hospital. A second 24h temperature measurement and polysomnography was performed on the 5th day of SXB use.
Medication protocol

To monitor possible side effects, the first SXB administration was done in the hospital. Since food reduces the bioavailability of the drug, patients were not allowed to eat for at least 2.5 hours prior to drug administration. Subjects received 3 grams SXB at 23:00h and 3:00h. When no adverse-effects were experienced, subjects were allowed to continue the study and used this dosage of SXB for the next 4 nights. The 5th night the subjects spent in the hospital again for the second measurement. Whether subjects were responders or not was not explicitly determined.

Temperature measurement

During the baseline measurement and 5th night of SXB use, subjects stayed overnight in the hospital and a 24h temperature measurement was performed.

Core body temperature was measured with a wireless monitoring system: an ingestible and biocompatible capsule with Vitalsense monitor (Mini Mitter Company Inc., A Respironics, Inc. Company Bend, Oregon, USA).3,5,23
Skin temperature was measured wirelessly using Thermochron iButtons (type DS1921H-F50; Maxim Integrated products, Inc., Sunnyvale, CA, USA). Skin temperature was measured at 8 locations: bilateral infraclavicular area, both hands, abdomen (1 cm above the umbilicus), left midthigh (musculus rectus femoris) and both feet. Distal skin temperature was obtained from the temperatures at the thenar area at the palmar side of both hands and medial metatarsal area at the plantar sides of both feet. Proximal skin temperature contained the infraclavicular, the thigh and abdominal temperature. Additionally, the distal-proximal temperature gradient (distal minus proximal skin temperature, DPG) was calculated.

Both core body temperature and skin temperature were sampled once per minute with a temperature resolution of 0.125°C.

Sleep analysis

Polysomnographic sleep recordings were performed with a portable, Embletta X100 recorder (Embla Broomfield, CO, USA) and scored by an experienced sleep technician according to the American Academy of Sleep Medicine criteria.

Daytime naps were defined as naps if they fulfilled the following criteria: (1) a period of any sleep stage (I, II, III or REM) during the ‘lights on’ period (between 7:30h and 23:00h), (2) for at least two consecutive minutes, (3) all subjects were awake at least 10 min prior to the nap.

Data analysis and statistics

To compare sleep characteristics between patients and controls unpaired t-tests were used. Paired t-tests were used to analyse sleep characteristics before and during SXB administration. Analysis of differences for the number of daytime naps between patients and controls was performed with the Mann Whitney U test and the Related-Samples Wilcoxon Signed Rank test because of small group size and skewed distribution.

To evaluate group differences, group by time of day differences and administration by time of day effects on temperature, the mean temperature of each episode of 30 minutes was calculated. With these data Generalized Linear Model for repeated measures with Huynh-Feldt corrections were run using IBM SPSS 20 (Illinois) with between factor narcolepsy and within factors SXB and time of day. This analysis was performed on the 24-hours data, and separately for daytime (7:30h–23:00h) and night time (23:00h–7:00h). Posthoc t-tests were
used to evaluate the times of day where narcolepsy or SXB related differences reached significance.

To evaluate the effect of temperature on nap probability in patients at baseline and during SXB administration, mixed effects analysis (R version 3.1.1) was performed. For all analysis, the outcome variable was sleep onset, which was binomially coded for every 30 seconds epoch as wake = 0 and sleep onset = 1 (further sleep-epochs were excluded from analysis). As fixed effects, the different temperatures (proximal, distal, core body and DPG), intervention and time (without interaction term) were entered into the model. As random effects, we had intercepts for subjects. For each of the temperatures, this analysis was performed with three different regressors. The first regressor evaluated was the temperature during the 30 seconds prior to the first sleep epoch. The second and third regressor rather evaluated the predictive value of monotonic changes in temperature prior to sleep onset. To this end, the second regressor was the difference between the temperature immediately prior to the 30-seconds epoch and the temperature 5 minutes before. The third regressor was the difference between the temperature immediately prior to the 30-seconds epoch and the temperature 15 minutes before. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

RESULTS

Subjects

Eight patients (mean age 38.0±4.7 years) and eight controls (mean age 37.9±4.1 years) were included after informed consent. Mean BMI was 28.1±1.6 kg/m² for patients and 27.4±1.4 kg/m² for controls.

Sleep characteristics are given in Table 4.1. During the day, patients were significantly less awake compared to controls (p=0.004). SXB administration resulted in significantly less stage I/II sleep during the day (p=0.049), and a trend towards more wake (p=0.052) was seen. SXB intake demonstrated a significantly higher percentage slow wave sleep during the night in patients (p=0.014) and in controls (p=0.045). SXB administration did not result in change in the prevalence of sleep onset REM periods neither during daytime, nor during night time sleep onset.
<table>
<thead>
<tr>
<th></th>
<th>Patients (N=8)</th>
<th>Controls (N=8)</th>
<th>Patients vs. controls (baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>SXB</td>
<td>Baseline</td>
</tr>
<tr>
<td>Wake day (%)</td>
<td>80.4±4.1</td>
<td>84.9±3.3</td>
<td>0.052</td>
</tr>
<tr>
<td>Wake night (%)</td>
<td>25.8±5.7</td>
<td>19.2±4.3</td>
<td>0.064</td>
</tr>
<tr>
<td>Stage I/II day (%)</td>
<td>14.7±2.9</td>
<td>11.2±2.6</td>
<td>0.049*</td>
</tr>
<tr>
<td>Stage I/II night (%)</td>
<td>55.1±2.5</td>
<td>53.4±3.7</td>
<td>0.497</td>
</tr>
<tr>
<td>SWS day (%)</td>
<td>2.1±0.6</td>
<td>2.7±1.1</td>
<td>0.526</td>
</tr>
<tr>
<td>SWS night (%)</td>
<td>6.5±1.9</td>
<td>16.5±3.0</td>
<td>0.014*</td>
</tr>
<tr>
<td>REM day (%)</td>
<td>4.3±1.7</td>
<td>1.2±0.5</td>
<td>0.050</td>
</tr>
<tr>
<td>REM night (%)</td>
<td>12.6±3.0</td>
<td>10.8±2.1</td>
<td>0.309</td>
</tr>
<tr>
<td>Sleep time day (min.)</td>
<td>254.1±64.4</td>
<td>140.9±30.8</td>
<td>0.117</td>
</tr>
<tr>
<td>Sleep time night (min.)</td>
<td>378.4±29.2</td>
<td>411.9±22.0</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Percentages of sleep stages during the 24 hours of study, before and during SXB administration. Data are shown as mean ± SEM.
Daytime napping occurred in all patients at baseline and during SXB administration, and varied from 3 to 16 naps per patient at baseline and from 3 to 11 naps per patient during administration. At baseline three controls took 2 or 3 daytime naps per person, while during SXB administration five controls took one nap. Both at baseline and during SXB administration, patients had significantly more daytime naps than controls (baseline number of naps for patients and controls, respectively: N=57 and N=8, p<0.01; number of naps during SXB administration for patients and controls, respectively: N=46 and N=5, p<0.01). No significant improvement in the number of daytime naps was seen in controls (p=0.334) or in patients (p=0.248) during SXB administration.

**Temperature in narcolepsy patients vs. controls at baseline**

Temperature profiles are shown in Figure 4.2 and the results of statistical analysis in Table 4.2. Patients had a significantly lower core body temperature. Proximal skin temperature showed a trend to be lower in patients (F=4.13, df=1, p=0.06), while in distal skin temperature and in distal-proximal temperature gradient (DPG) no significant differences were found. Analysis of the effect of group by time of day showed a nearly significant effect of narcolepsy by time of day for proximal skin temperature (F=2.24, df=5.49, p=0.05).

Post-hoc tests indicated a significantly (p<0.05) lower core body temperature in narcolepsy between 16:30 and midnight (00:00) and between 10:00 and 12:00 the next morning. The same was found in proximal skin temperature between 15:30 and 23:00 and between 11:00 and 12:00 the next morning.

**Table 4.2** Results of analysis of temperatures of controls vs. patients at baseline

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>1</td>
<td>4.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>1</td>
<td>0.17</td>
<td>0.69</td>
</tr>
<tr>
<td>DPG</td>
<td>1</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>1</td>
<td>6.46</td>
<td>0.02*</td>
</tr>
<tr>
<td><strong>Group by time of day effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>5.49</td>
<td>2.24</td>
<td>0.05</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>7.46</td>
<td>1.25</td>
<td>0.28</td>
</tr>
<tr>
<td>DPG</td>
<td>8.91</td>
<td>1.75</td>
<td>0.09</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>4.83</td>
<td>1.96</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* p<0.05.
Separate analysis of daytime and night time temperatures demonstrated a significantly lower proximal skin temperature ($F=5.66$, $df=1$, $p=0.03$) and core body temperature ($F=8.31$, $df=1$, $p=0.01$) in patients during daytime. Furthermore, a significant effect of group by time of day was seen for core body temperature during daytime ($F=2.82$, $df=7.11$, $p=0.01$) and for distal skin temperature during night time ($F=4.34$, $df=2$, $p=0.02$).

**Temperature in narcolepsy patients: baseline vs. SXB (Table 4.3, Figure 4.3)**

In patients, a significant main effect of SXB on proximal skin temperature ($F=6.41$, $df=1$, $p=0.04$) as well as a nearly significant SXB by time of day effect ($F=2.22$, $df=4.80$, $p=0.08$) was seen. Additional separate daytime and night time analysis demonstrated that proximal skin temperature was higher during the day in the SXB condition ($F=6.46$, $df=1$, $p=0.04$), but no difference was found during night time ($F=0.08$, $df=1$, $p=0.79$). In post-hoc tests significance ($p<0.05$) was reached from 15:00 to 16:00, from 18:00 to 19:30, and from 8:00 to 10:30 to 12:00 the next morning.

For core body temperature, distal skin temperature and DPG, no main significant effect was found for SXB administration.

Summarising, SXB administration in patients increased proximal skin temperature at several time points during daytime. There was no effect on core body temperature, distal skin temperature and DPG.

**Table 4.3 Results of analysis of temperatures at baseline vs during SXB administration**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td><strong>Administration effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>1</td>
<td>0.48</td>
</tr>
<tr>
<td>DPG</td>
<td>1</td>
<td>1.41</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Administration by time of day effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>5.06</td>
<td>0.61</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>6.48</td>
<td>0.37</td>
</tr>
<tr>
<td>DPG</td>
<td>6.74</td>
<td>0.33</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>5.74</td>
<td>1.65</td>
</tr>
</tbody>
</table>

* $p<0.05$. 
Effects of sodium oxybate on temperature

In controls, no significant effect of SXB or SXB by time of day was found on core body temperature, skin temperatures and DPG.

The predictive value of temperature changes on the onset of daytime naps (Table 4.4)

Since daytime napping was rare in controls, only daytime naps in patients were analysed. Mixed effects analysis of sleep onset in patients at baseline revealed predictive effects of change in proximal skin temperature during the 5 minutes prior to sleep onset, distal skin
During SXB administration the same effects were seen for distal skin temperature and DPG, supplemented with a predictive value of proximal skin temperature, distal skin temperature and DPG during the epoch prior to falling asleep. No predictive value of core temperature was seen for daytime sleep onset, nor at baseline, neither during SXB administration.

Figure 4.3 Mean ± SEM temperature profiles patients at baseline and during SXB administration. (A) distal skin temperature in patients at baseline and during SXB administration, (B) proximal skin temperature in patients at baseline and during SXB administration, (C) distal-proximal temperature gradient (DPG) in patients at baseline and during SXB administration (D) core body temperature in narcolepsy patients at baseline and during SXB administration. The grey area indicates the lights off period and the striped area the period during which the temperature significantly differed according the post-hoc tests (* p<0.05).
The aim of this study was to investigate the effects of SXB on core body and skin temperature in relation to its effects on sleep in patients suffering from narcolepsy type 1. This is the first study in which both core body and skin temperature were measured in combination with continuous sleep registration in narcolepsy. At baseline, patients had significantly lower daytime core body and proximal skin temperatures compared to controls. In patients, SXB increased nocturnal slow wave sleep (SWS), normalised proximal skin temperature, and strengthened the relationship between changes in skin temperature and subsequent daytime sleep onset.

**Table 4.4 Effect of temperature on daytime nap probability**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>P-value</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td><strong>Proximal skin temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.0</td>
<td>0.1</td>
<td>0.588</td>
<td>0.1</td>
<td>0.1</td>
<td>0.013*</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>3.7</td>
<td>1.4</td>
<td>0.006**</td>
<td>0.9</td>
<td>1.4</td>
<td>0.518</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>-1.5</td>
<td>0.8</td>
<td>0.064</td>
<td>0.1</td>
<td>0.8</td>
<td>0.948</td>
</tr>
<tr>
<td><strong>Distal skin temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.1</td>
<td>0.1</td>
<td>0.479</td>
<td>0.4</td>
<td>0.1</td>
<td>0.002**</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>-0.3</td>
<td>0.6</td>
<td>0.571</td>
<td>1.2</td>
<td>0.7</td>
<td>0.110</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.8</td>
<td>0.4</td>
<td>0.029*</td>
<td>1.4</td>
<td>0.4</td>
<td>0.002**</td>
</tr>
<tr>
<td><strong>DPG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.1</td>
<td>0.1</td>
<td>0.578</td>
<td>0.3</td>
<td>0.1</td>
<td>0.020*</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>-0.2</td>
<td>0.6</td>
<td>0.744</td>
<td>1.0</td>
<td>0.7</td>
<td>0.143</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.8</td>
<td>0.4</td>
<td>0.027*</td>
<td>1.2</td>
<td>0.4</td>
<td>0.005**</td>
</tr>
<tr>
<td><strong>Core body temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.0</td>
<td>0.0</td>
<td>0.405</td>
<td>0.0</td>
<td>0.0</td>
<td>0.925</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>-0.1</td>
<td>2.2</td>
<td>0.957</td>
<td>-0.7</td>
<td>2.6</td>
<td>0.787</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.6</td>
<td>1.3</td>
<td>0.666</td>
<td>-1.6</td>
<td>1.6</td>
<td>0.326</td>
</tr>
</tbody>
</table>

Results of linear mixed effects analysis for patients at baseline and patients during SXB administration (nights were excluded), indicating effects of temperature fluctuations as regressor for fluctuations in lapse probability. Analysis was performed for proximal skin temperature, distal skin temperature, distal-proximal temperature gradient (DPG) or core body temperature at the moments: difference between the temperature during the 30-seconds epoch prior to sleep onset and 15 minutes prior to sleep onset, difference between the temperature during the 30-seconds epoch prior to sleep onset and 5 minutes prior to sleep onset or the absolute temperature during the 30-seconds epoch prior to sleep onset. * p<0.05; ** p<0.01.

**DISCUSSION**

The aim of this study was to investigate the effects of SXB on core body and skin temperature in relation to its effects on sleep in patients suffering from narcolepsy type 1. This is the first study in which both core body and skin temperature were measured in combination with continuous sleep registration in narcolepsy. At baseline, patients had significantly lower daytime core body and proximal skin temperatures compared to controls. In patients, SXB increased nocturnal slow wave sleep (SWS), normalised proximal skin temperature, and strengthened the relationship between changes in skin temperature and subsequent daytime sleep onset.
An altered thermoregulatory profile in narcolepsy

In the present study, core body temperature and proximal skin temperature were lower in narcolepsy, mainly caused by significant differences during daytime. No significant differences in distal skin temperature were found, although the nocturnal time course of distal skin temperature significantly differed between patients and controls.

The finding of a decreased daytime proximal skin temperature in narcolepsy patients compared to controls was previously demonstrated as well. In contrast to our current findings, this previous work also reported a higher distal skin temperature. The combination of the increased distal skin temperature and the decreased proximal skin temperature in that study resulted in a higher distal-proximal temperature gradient (DPG). Comparison of the present study with the previous one indicates that the absence of a higher distal skin temperature in patients, and subsequently the absence of a higher DPG, is mainly due to a higher distal skin temperature in controls in the current study. Since a higher distal skin temperature can be a direct consequence of a supine position, maintaining this position throughout our study can be the explanation of the higher distal skin temperature found in controls. Consecutively, these results indicate that narcolepsy patients are likely to attain, even in an upright or sitting position, the high distal skin temperature that healthy controls reach only when remaining in a supine position.

We found a lower core body temperature during the day in patients. In healthy controls, a lower core body temperature is associated with sleep, and would theoretically result in a lower ability to maintain wakefulness. In the past, core body temperature has been more extensively studied than skin temperature. Unfortunately, previous studies in narcolepsy are not conclusive at this point; results vary from an elevated core body temperature to a lowered core body temperature. Manipulation studies demonstrate a minimal effect of manipulation of core body temperature on sleep propensity, however, a high core body temperature is associated with higher vigilance.

SXB normalises temperature profiles in narcolepsy

In patients, SXB administration significantly increased daytime proximal skin temperature, reaching levels comparable with healthy controls. In controls, proximal skin warming resulted in decreased sleep onset latency. In narcolepsy, however, despite the previously described lowered daytime proximal skin temperature, no beneficial effect of daytime proximal skin
warming was found.\textsuperscript{10} These findings do not point to changes in temperature as the primary mechanism through which SXB reduces the amount of daytime sleep attacks.\textsuperscript{33} Warming up the skin by direct manipulation may represent a different physiological mechanism compared with the intrinsic skin warming resulting from SXB administration.

However, two other mechanisms might explain the positive effects of these temperature changes as a consequence of SXB administration. First, SXB induces more consolidation of sleep, i.e. probably lowers the known increased sleep stage shift index.\textsuperscript{34} Second, although for a short time, in high dosages SXB is reported to increase the sympathetic response,\textsuperscript{35,36} while a chronically decreased sympathetic distal vasoconstrictor tone was hypothesised to be causal to the previously found increased DPG and the subsequently increased sleep propensity.\textsuperscript{9}

In healthy subjects, sleep onset is preceded by a decline in core body temperature and an increase in distal skin temperature.\textsuperscript{7} In healthy controls, an increased DPG is associated with a lower vigilance\textsuperscript{12,13} and an accelerated sleep onset.\textsuperscript{3,4} In narcolepsy, a shorter sleep onset latency was found to be associated with an increase of proximal and distal skin temperatures and, to a lesser extent, an increase of the DPG.\textsuperscript{9} However, none of these studies concerned spontaneous daytime napping in narcolepsy patients. Analysis of spontaneous naps in (semi) supine position in the present study revealed a predictive value for proximal skin temperature, distal skin temperature and DPG in narcolepsy patients at baseline and during SXB administration. This relationship between an increase of skin temperature and subsequent sleep onset is known to exist in controls, exists in narcolepsy patients as well, and is even more clear after SXB administration.

**Does altered thermoregulation play a role in SXB’s effects on sleep?**

An increase in nocturnal SWS, previously reported to be one of the principal effects of SXB on sleep,\textsuperscript{33,37-39} was confirmed in this study. If this is mediated by an altered temperature regulation is questionable, since there were no nocturnal temperature effects seen during SXB intake in this study. The relatively high percentage of wake during the night in controls is probably due to the laboratory settings.
Study limitations

Since body position directly affects skin temperature, the major limitation of this study is
the setting in which patients were in (semi) supine position for 24 hours. This body position
differs from the situation in normal daily life, and the setting in previous studies. Moreover,
the clinical effects of SXB on nocturnal sleep can already be experienced with the dose we
have used in the first night of its use, but it usually takes several weeks and a higher dose
to obtain optimal clinical improvement, and significant improvement of cataplexy and EDS.
Subsequently, it is presumable that there are some important long-term effects, particularly
during daytime that may have been missed in this study. Furthermore, the present study
included a relatively small number of patients (of whom two were drug naive and the others
discontinued treatment), and only male subjects, while men and women are equally affected
with narcolepsy. This might have led to an underestimation of the actual effects of SXB and
limits the generalisation of the results.

Conclusion

In conclusion, during a constant routine protocol a decreased daytime core body and
proximal skin temperature were observed in narcolepsy patients compared to controls.
Administration of SXB improved the sleep wake pattern, and partially normalised the
temperature profiles in narcolepsy patients. Furthermore, SXB strengthened the relationship
between skin temperature and subsequent sleep onset – that is known to exist in controls – in
patients. To further explore the role of SXB in temperature regulation and sleep in narcolepsy,
studies with patients and controls of both sexes have to be performed in normal daily life.

REFERENCES

37.
5. van Someren EJ. More than a marker: interaction between the circadian regulation of temperature


CHAPTER 5
CORE BODY AND SKIN TEMPERATURE IN TYPE 1 NARCOLEPSY IN DAILY LIFE:
effects of sodium oxybate and prediction of sleep attacks

Astrid van der Heide
Esther Werth
Claire E.H.M. Donjacour
Robert H.A.M. Reijntjes
Gert Jan Lammers
Eus J.W. Van Someren
Christian R. Baumann
Rolf Fronczek

Sleep 2016;39(11):1941–9
Chapter 5

ABSTRACT

Study objectives: Previous laboratory studies in narcolepsy patients showed altered core body and skin temperatures, which are hypothesised to be related to a disturbed sleep wake regulation. In this ambulatory study we assessed temperature profiles in normal daily life, and whether sleep attacks are heralded by changes in skin temperature. Furthermore, the effects of three months of treatment with sodium oxybate (SXB) were investigated.

Design: Two-centre observational study.

Subjects: 25 narcolepsy patients and 15 healthy controls.

Interventions: Core body, proximal and distal skin temperatures, and sleep-wake state were measured simultaneously for 24 hours in ambulatory patients. This procedure was repeated in 16 narcolepsy patients after at least three months of stable treatment with SXB.

Measurements and results: Increases in distal skin temperature and distal-to-proximal temperature gradient (DPG) strongly predicted daytime sleep attacks (p<0.001). As compared to controls, patients had a higher proximal and distal skin temperature in the morning, and a lower distal skin temperature during the night (all p<0.05). Furthermore, they had a higher core body temperature during the first part of the night (p<0.05), which SXB decreased (F=4.99, df=1, p=0.03) to a level similar to controls. SXB did not affect skin temperature.

Conclusions: This ambulatory study demonstrates that daytime sleep attacks were preceded by clear changes in distal skin temperature and DPG. Furthermore, changes in core body and skin temperature in narcolepsy, previously only studied in laboratory settings, were partially confirmed. Treatment with SXB resulted in a normalisation of the core body temperature profile. Future studies should explore whether predictive temperature changes can be used to signal or even prevent sleep attacks.
INTRODUCTION

Core body and skin temperature are closely linked to sleep and alertness. In healthy controls, wake is associated with a relatively high core body temperature and a relatively low skin temperature. The opposite pattern, i.e. a decreased core body temperature and an increased skin temperature, is seen during sleep. Transitions from wake to sleep and vice versa are characterised by changes in these temperatures. Sleep onset is preceded by a decline in core body temperature and an increase in skin temperature. The decrease in core body temperature is mediated through increased skin perfusion, which consequently leads to the increase in skin temperature, facilitating cooling of the body. A relatively high distal skin temperature compared to proximal skin temperature, i.e. a high distal-to-proximal temperature gradient (DPG), was demonstrated to promote sleep onset. When a ‘sleepy state of core body and skin temperature’ is seen during wake, this is typically associated with lowered vigilance. This may probably be called ‘sleep promoting’, since it is also seen immediately before sleep onset.

Narcolepsy type 1 (narcolepsy with cataplexy) is a disorder of the regulation of sleep and wakefulness. Besides the pathognomonic symptom cataplexy, narcolepsy patients suffer from excessive daytime sleepiness (EDS), hypnagogic hallucinations, sleep paralysis and disturbed nocturnal sleep. In studies concerning temperature measurements in narcolepsy patients, an altered diurnal temperature profile has been demonstrated in both core body and skin temperature. Narcolepsy patients are reported to have a relatively high daytime distal skin temperature, a lowered proximal skin temperature, and subsequently a higher DPG during daytime. Furthermore, a lower nocturnal distal skin temperature was seen in narcolepsy. Studies regarding core body temperature in narcolepsy were not conclusive. Results vary from an elevated core body temperature to a lowered core body temperature.

The hypothesised relationship between an altered temperature pattern and disturbed sleep wake regulation in narcolepsy was supported by temperature manipulation studies. These studies demonstrated that cooling the distal skin resulted in a better ability to maintain wakefulness, and increasing core body temperature improved vigilance during the day. Furthermore, counteracting the altered nocturnal skin temperature pattern by subtle skin temperature manipulations during the night, improved nocturnal sleep.

All these previous studies regarding skin temperature and most of the studies concerning core body temperature were performed in a laboratory setting, but whether laboratory
findings are representative for actual daily life has not been assessed. The current study was performed to overcome this limitation and to further explore the interaction between temperature regulation and sleep in narcolepsy in normal daily life. We aimed first to assess whether in an ambulatory setting, sleep attacks are heralded by changes in skin temperature. Our second aim was to further explore core body and skin temperature profiles and sleep in narcolepsy type 1 in daily life, before and after stable monotherapy with sodium oxybate (SXB) a widely-used drug to improve nocturnal sleep, cataplexy and EDS in narcolepsy.\textsuperscript{17} We expected to confirm the previously found daytime increased distal and lowered proximal skin temperature and consequently an increased DPG. Furthermore, we measured 24h core body temperature patterns during day and night, these patterns have not been studied in detail before.

Patients and controls underwent 24h ambulatory core body and skin temperature measurement together with ambulatory polysomnography. Additionally, the patients who were treated successfully with SXB monotherapy underwent the same measurements after three months stable treatment with SXB.

METHODS

Subjects

Twenty-five narcolepsy patients (16 male; mean BMI 27.6) were recruited from the narcolepsy outpatient clinics of Leiden University Medical Centre, The Netherlands (N=14) and University Hospital Zurich, Switzerland (N=11) between 2007 and 2012. They all fulfilled the criteria for narcolepsy type 1 according to the International Classification of Sleep Disorders-3 (ICDS-3).\textsuperscript{18} Of 25 patients, 23 underwent a lumbar puncture which revealed an undetectable hypocretin-1 in all these patients.

Patients were all naïve to SXB treatment, and were scheduled to start with SXB. The decision for treatment with SXB was part of their therapeutic plan; i.e. no patients were put on SXB treatment for the purpose of participation in this study. 6 patients used narcoleptic medication other than SXB, which was stopped at least 14 days prior to the start of the study.

Fifteen healthy controls (9 male; The Netherlands (N=9); Switzerland (N=6); mean BMI 25.2), free of any neurologic or psychiatric disease were recruited using notices in local newspapers. Controls were matched for age and gender.
Exclusion criteria for both patients and controls were cognitive impairment due to neurological disorders other than sleep-wake disorders, the use of hypnotics or sleep-wake active drugs other than SXB, and age below 18 or above 70 years.

The protocol was approved by the medical ethical committees of both institutions and written informed consent was obtained from all subjects prior to the study.

**Study design**

At baseline, all subjects underwent an ambulatory baseline 24h temperature measurement and polysomnography simultaneously. Following the baseline study, twenty-three patients were treated with SXB by their treating physician. They received the usual therapeutic dose of SXB (4.5–9.0 g/day). Due to side effects and/or environmental factors, seven patients discontinued SXB within three months. Subsequently, after three months stable single-drug treatment a second 24h temperature measurement and polysomnography was performed in sixteen patients. Controls followed the procedure only at baseline.

**Temperature measurement**

The temperature measurement methods have previously been described.11 A wireless monitoring system was used for core body temperature measurement: an ingestible and biocompatible capsule with Vitalsense monitor (Mini Mitter Company Inc., A Respironics, Inc. Company Bend, Oregon, USA).19

Thermochron iButtons (type DS1921H-F50; Maxim Integrated products, Inc., Sunnyvale, CA, USA) were used for wireless skin temperature measurement.20 Skin temperature was measured at 7 locations: left infraclavicular area, both hands, abdomen (1 cm above the umbilicus), left midthigh (musculus rectus femoris) and both feet. Distal skin temperature contained the temperatures at medial metatarsal area at the plantar sides of both feet and the thenar area at the palmar side of both hands.21 Proximal skin temperature was obtained from the infraclavicular, the thigh and abdominal temperature. To calculate the DPG, proximal skin temperature was subtracted from the distal skin temperature.

Both core body temperature and skin temperature were sampled once per minute with a resolution of 0.125°C.
Sleep recording and analysis

Polysomnographic sleep recording was performed in all subjects during day and night for 24 hours with a portable Embletta X100 (Leiden) or a portable Embla A10 (Zürich) recorder (Embla Broomfield, CO, USA). Sleep recordings were scored according to the American Academy of Sleep Medicine criteria by an experienced sleep technician.22

Additionally, daytime sleep attacks were classified based on the following criteria: (1) a period of any sleep stage (I, II, III or REM) during daytime, (2) for at least two consecutive minutes, (3) no sleep was registered at least 10 minutes prior to the sleep attack.

Data analysis and statistics

Because of skewed distribution, the Mann Whitney U test was used to compare sleep characteristics and number of daytime naps between patients and controls at baseline, and the Related-Samples Wilcoxon Signed Rank test was used to analyse sleep characteristics and daytime naps before and during SXB treatment in patients.

For subsequent analyses on the 24-hour profiles, the mean temperature of each episode of 30 minutes was calculated. Group differences, group by time of day differences and treatment by time of day effects on temperature were analysed with Generalized Linear Model for repeated measures with Huynh-Feldt corrections (IBM SPSS 20, Inc., Chicago, IL, USA) with between factor narcolepsy, within factors SXB and time of day and covariate geographical site (Leiden or Zürich). This analysis was separately performed with the real clock time data and with the data anchored for nocturnal bed times (with the actual bedtime coded as time-point 0). Furthermore, analysis was run on the 24-hour data, and separately for daytime and night time. Where narcolepsy or SXB related differences reached significance, posthoc t-tests were used to evaluate differences in the time of day.

Mixed effects logistic regression analysis (R version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria) was performed to evaluate the effect of temperature on the onset of spontaneous daytime sleep attacks in patients at baseline. Since these temperatures were measured once per minute, and sleep scoring was performed in 30-second epochs, the temperatures were interpolated into 30-second values. Nocturnal temperatures were excluded. For all analysis, the outcome variable was sleep onset, which was binomially coded for every 30-second epoch as wake = 0 and sleep onset = 1 (further sleep-epochs were
excluded from analysis. The different temperatures (proximal, distal and DPG) and time of day were entered into the model as fixed effects. Intercepts for subjects were defined as random effects. To evaluate different epoch durations prior to sleep onset, three analyses were performed, each with a different regressor representing the temperature profile in an epoch prior to sleep onset. The first analysis evaluated the last temperature value during the epoch prior to sleep onset. The second and third regressor evaluated the predictive value of monotonic changes in temperature prior to sleep onset. To this end, the second regressor evaluated changes immediately preceding a sleep bout, quantified as the difference between the last temperature readout immediately prior to the 30-seconds epoch and the temperature 5 minutes before. The third regressor evaluated slower changes, which were quantified as the difference between the last temperature readout immediately prior to the 30-seconds epoch and the temperature 15 minutes before. These three analyses were repeated for each of the temperatures (proximal, distal and DPG). P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

RESULTS

Subjects

Twenty-five patients (mean age 34.8±13.4 years) and fifteen controls (mean age 33.9±14.0 years) were included. Sixteen patients were available for the second study part. The average of their scheduled treatment dosages was 5.8±2.2 g SXB/night.

Sleep

Sleep characteristics are given in Table 5.1. Due to technical problems, polysomnographic registration failed in one patient, and two controls did not give permission for polysomnographic registration.

During the day, patients were significantly less awake compared to controls (p<0.001). Subsequently, all sleep stages were more frequently seen during the day in patients compared to controls. Patients were more awake during the night, but spent overall more time asleep during 24 hours. The distribution of sleep stages during the night was similar in patients and controls. In patients, treatment with SXB resulted in an increase of slow
### Table 5.1 Sleep variables before and after SXB administration

<table>
<thead>
<tr>
<th></th>
<th>Patients (N=24)</th>
<th>Controls (N=13)</th>
<th>p-value</th>
<th>Patients (N=16)</th>
<th>SXB</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake total (%)</td>
<td>59.1±7.7</td>
<td>64.2±6.3</td>
<td>0.033*</td>
<td>57.3±7.8</td>
<td>59.3±7.4</td>
<td>0.214</td>
</tr>
<tr>
<td>Wake day (%)</td>
<td>90.1±5.5</td>
<td>99.4±1.9</td>
<td>&lt;0.001**</td>
<td>90.1±4.7</td>
<td>90.9±7.2</td>
<td>0.070</td>
</tr>
<tr>
<td>Wake night (%)</td>
<td>12.4±10.7</td>
<td>5.8±7.3</td>
<td>0.009*</td>
<td>12.3±10.2</td>
<td>10.0±5.8</td>
<td>0.278</td>
</tr>
<tr>
<td>Stage I total (%)</td>
<td>8.7±3.5</td>
<td>4.8±2.3</td>
<td>0.001**</td>
<td>9.4±3.9</td>
<td>8.1±3.5</td>
<td>0.179</td>
</tr>
<tr>
<td>Stage I day (%)</td>
<td>3.2±2.0</td>
<td>0.2±0.5</td>
<td>&lt;0.001**</td>
<td>3.7±2.1</td>
<td>3.3±2.5</td>
<td>0.532</td>
</tr>
<tr>
<td>Stage I night (%)</td>
<td>16.8±6.5</td>
<td>12.5±5.8</td>
<td>0.052</td>
<td>16.5±6.3</td>
<td>16.0±6.9</td>
<td>0.679</td>
</tr>
<tr>
<td>Stage II total (%)</td>
<td>17.0±4.3</td>
<td>18.6±3.3</td>
<td>0.301</td>
<td>17.9±4.2</td>
<td>16.8±4.9</td>
<td>0.211</td>
</tr>
<tr>
<td>Stage II day (%)</td>
<td>3.4±2.5</td>
<td>0.5±1.4</td>
<td>&lt;0.001**</td>
<td>3.6±2.3</td>
<td>3.9±3.7</td>
<td>0.737</td>
</tr>
<tr>
<td>Stage II night (%)</td>
<td>37.5±8.4</td>
<td>49.3±8.0</td>
<td>0.001**</td>
<td>37.8±8.3</td>
<td>37.8±8.8</td>
<td>0.877</td>
</tr>
<tr>
<td>SWS total (%)</td>
<td>5.2±3.1</td>
<td>3.2±2.6</td>
<td>0.054</td>
<td>4.9±2.6</td>
<td>6.4±3.8</td>
<td>0.070</td>
</tr>
<tr>
<td>SWS day (%)</td>
<td>1.2±2.0</td>
<td>0.0±0.0</td>
<td>&lt;0.001**</td>
<td>0.9±1.6</td>
<td>5.7±18.8</td>
<td>0.646</td>
</tr>
<tr>
<td>SWS night (%)</td>
<td>11.6±7.2</td>
<td>8.4±6.6</td>
<td>0.192</td>
<td>10.9±6.5</td>
<td>14.8±10.3</td>
<td>0.023*</td>
</tr>
<tr>
<td>REM total (%)</td>
<td>10.0±3.3</td>
<td>9.2±3.0</td>
<td>0.524</td>
<td>10.5±3.5</td>
<td>9.5±4.6</td>
<td>0.118</td>
</tr>
<tr>
<td>REM day (%)</td>
<td>2.1±2.0</td>
<td>0.1±0.2</td>
<td>&lt;0.001**</td>
<td>1.8±1.4</td>
<td>1.6±2.9</td>
<td>0.140</td>
</tr>
<tr>
<td>REM night (%)</td>
<td>21.6±5.5</td>
<td>24.0±5.2</td>
<td>0.127</td>
<td>22.5±6.1</td>
<td>20.8±8.3</td>
<td>0.469</td>
</tr>
<tr>
<td>Sleep time total (min.)</td>
<td>586.8±111.1</td>
<td>514.2±89.8</td>
<td>0.034*</td>
<td>613.0±112.4</td>
<td>571.5±89.6</td>
<td>0.059</td>
</tr>
<tr>
<td>Sleep time day (min.)</td>
<td>86.1±52.4</td>
<td>5.8±17.1</td>
<td>&lt;0.001*</td>
<td>83.7±43.7</td>
<td>77.9±61.3</td>
<td>0.079</td>
</tr>
<tr>
<td>Sleep time night (min.)</td>
<td>500.6±127.0</td>
<td>509.1±88.5</td>
<td>0.750</td>
<td>529.3±134.6</td>
<td>496.9±83.6</td>
<td>0.301</td>
</tr>
</tbody>
</table>

Percentages of sleep stages during the 24 hours of study, before and during SXB treatment. Night is defined by the time in bed during night time. Data are shown as mean ± SD. * p<0.05; ** p<0.01.
wave sleep during the night (p=0.023), and in a trend towards more time spent awake during the day.

Daytime napping occurred in all patients at baseline and in 13 out of 16 patients during SXB treatment, varying from 1 to 7 naps per patient at baseline and from 0 to 9 naps per patient during treatment. 3 controls had 1 daytime nap each; the other controls did not show any daytime sleep. At baseline, patients had significantly more daytime naps than controls (baseline mean number of naps for patients and controls, respectively 2.79±0.318 and 0.23±0.122, p<0.001). Significant improvement in the number of daytime naps was seen in patients during SXB treatment (baseline mean number of naps for patients at baseline and during treatment with SXB, respectively 2.79±0.318 and 1.88±0.539, p=0.037).

**Temperature in narcolepsy: patients vs. controls (Table 5.2, Figure 5.1)**

Temperature profiles are shown in Figure 5.1 and the results of statistical analysis in Table 5.2. No differences were seen between patients and controls by analysis over the full 24 hours. Analysis of the effect of group by time of day demonstrated a significant effect of narcolepsy by time of day for proximal skin temperature and a trend for core body temperature (respectively, F=2.93, df=10.23, p<0.01 and F=1.92, df=7.32, p=0.07).

Separate analysis of daytime and night time temperatures demonstrated a trend towards a higher core body temperature in patients during the entire night (F=3.50, df=1, p=0.07). Furthermore, a significant effect of group by time of day was seen for proximal skin temperature during the day and for both proximal and distal skin temperature during

| Table 5.2 Results of analysis of temperatures of controls vs. patients at baseline |
|--------------------------------|------|------|------|
|                                 | df   | F    | p-value |
| **Group effect**               |      |      |        |
| Proximal skin temperature      | 1    | 0.13 | 0.72   |
| Distal skin temperature        | 1    | 0.02 | 0.90   |
| DPG                            | 1    | 0.04 | 0.85   |
| Core body temperature          | 1    | 0.58 | 0.45   |
| **Group by time of day effect**|      |      |        |
| Proximal skin temperature      | 10.23| 2.93 | <0.01**|
| Distal skin temperature        | 6.08 | 2.55 | 0.02*  |
| DPG                            | 5.61 | 1.19 | 0.31   |
| Core body temperature          | 7.32 | 1.92 | 0.07   |

* p<0.05; ** p<0.01.
Post-hoc tests indicated a significantly (p<0.05) higher proximal skin temperature in patients between 7:30h and 11:30h, a significantly higher core body temperature in patients between 0:00h and 3:30h, a significantly lower distal skin temperature in patients between 1:00h–4:00h and 5:00h–5:30h, and a significantly higher distal skin temperature in patients between 7:30h–9:30h and 10:30h–11:00h.

A similar analysis was performed anchored to the bedtimes instead of actual clock times. This did not change the findings. This analysis is not presented here but can be found in Supplementary Figures S5.1 and S5.2 and Supplementary Tables S5.1 and S5.2.
In conclusion, as compared to controls, patients had a higher proximal and distal skin temperature in the morning; and a lower distal skin and a higher core body temperature during the night.

**Temperature in narcolepsy patients: baseline vs. SXB (Table 5.3, Figure 5.2)**

No significant differences in temperature were seen at baseline compared to during treatment with SXB. Analysis of treatment by time of day revealed a significant effect on proximal skin temperature and nearly on core body temperature (respectively $F=2.14$, $df=7.73$, $p=0.03$; $F=1.90$, $df=6.94$, $p=0.07$). Furthermore, in post-hoc tests core body temperature was lower after SXB treatment from 5:00h to 9:30h.

![Figure 5.2](image_url) **Figure 5.2** Mean ± SEM temperature profiles of patients at baseline and during SXB administration. (A) distal skin temperature in patients at baseline (red) and during SXB administration (blue), (B) proximal skin temperature in patients at baseline and during SXB administration, (C) distal-proximal temperature gradient (DPG) in patients at baseline and during SXB administration (D) core body temperature in narcolepsy patients at baseline and during SXB administration. The grey area indicates the period during which the temperature significantly differed according the post-hoc tests (* $p<0.05$).
Additional separate daytime and night time analysis demonstrated that core body temperature was lower during the night during SXB treatment ($F=4.99$, $df=1$, $p=0.03$), and analysis of the effect of treatment by time of day demonstrated a significant effect for proximal skin temperature during daytime ($F=3.45$, $df=7.71$, $p<0.01$).

Summarising, treatment with SXB in narcolepsy lowered nocturnal core body temperature to a level seen in controls. Indeed, no significant difference in nocturnal core body temperature was found anymore for patients during treatment with SXB compared to controls ($F=0.0$, $df=1$, $p=0.94$).

The predictive value of temperature changes on the onset of daytime naps (Table 5.4, Figure 5.3)

Since daytime napping was rare in controls, only daytime naps in patients were analysed. Mixed effects logistic regression analysis in patients at baseline revealed that the probability of sleep onset increased with a higher distal skin temperature (Odds Ratio (OR) and 95% confidence interval (CI) $1.7 \ [1.3–2.1] / ^\circ C/30$ seconds, $p<0.001$) and DPG (OR $1.9 \ [CI 1.5–2.4]$, $p<0.001$) during the previous epoch. Furthermore, the probability of sleep onset increased the steeper the slope of increasing distal skin temperature and DPG over the previous 5 minutes (respectively OR $7.8 \ [CI 3.5–17.3]$, $p<0.001$, and OR $6.1 \ [CI 3.0–12.4]$, $p<0.001$) and 15 minutes (respectively OR $2.5 \ [CI 1.6–3.8]$, $p<0.001$, and OR $2.2 \ [CI 1.5–3.3]$, $p<0.001$) prior to sleep onset prior to falling asleep was found. No predictive value of proximal skin

<table>
<thead>
<tr>
<th>Table 5.3 Results of analysis of temperatures at baseline vs during SXB treatment in patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment effect</strong></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
</tr>
<tr>
<td>Distal skin temperature</td>
</tr>
<tr>
<td>DPG</td>
</tr>
<tr>
<td>Core body temperature</td>
</tr>
<tr>
<td><strong>Treatment by time of day effect</strong></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
</tr>
<tr>
<td>Distal skin temperature</td>
</tr>
<tr>
<td>DPG</td>
</tr>
<tr>
<td>Core body temperature</td>
</tr>
</tbody>
</table>

* $p<0.05$; ** $p<0.01$. 
Table 5.4  Effect of temperature on daytime nap probability

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal skin temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>-0.29</td>
<td>0.24</td>
<td>0.7</td>
<td>0.5–1.2</td>
<td>0.225</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>0.98</td>
<td>0.74</td>
<td>2.7</td>
<td>0.6–11.5</td>
<td>0.186</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.09</td>
<td>0.37</td>
<td>1.1</td>
<td>0.5–2.3</td>
<td>0.803</td>
</tr>
<tr>
<td><strong>Distal skin temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.50</td>
<td>0.12</td>
<td>1.7</td>
<td>1.3–2.1</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>2.06</td>
<td>0.40</td>
<td>7.8</td>
<td>3.5–17.3</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.90</td>
<td>0.22</td>
<td>2.5</td>
<td>1.6–3.8</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><strong>DPG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.64</td>
<td>0.13</td>
<td>1.9</td>
<td>1.5–2.4</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>1.81</td>
<td>0.36</td>
<td>6.1</td>
<td>3.0–12.4</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.80</td>
<td>0.20</td>
<td>2.2</td>
<td>1.5–3.3</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Results of mixed effects logistic regression analysis for patients at baseline (nights were excluded), indicating effects of temperature fluctuations as regressor for fluctuations in sleep attack probability. Analysis was performed for proximal skin temperature, distal skin temperature or distal-proximal temperature gradient (DPG) at the moments: difference between the temperature during the 30-seconds epoch prior to sleep onset and 15 minutes prior to sleep onset, difference between the temperature during the 30-seconds epoch prior to sleep onset and 5 minutes prior to sleep onset or the absolute temperature during the 30-seconds epoch prior to sleep onset. * p<0.05; ** p<0.01.

temperature in daytime sleep onset was seen. During treatment with SXB, similar effects were found, although less strong (Supplementary Table S5.3).

Core body temperature was excluded from this analysis; due to technical problems, too many core body temperature measurements were missing to perform this analysis reliably. In patients at baseline 11% of the measurements were missing, during treatment with SXB 3% and in controls 21%.

**DISCUSSION**

We aimed to assess whether in everyday life, sleep attacks are heralded by changes in skin temperature, and to further explore temperature regulation and sleep in narcolepsy type 1.

Interestingly, changes in distal skin temperature and DPG strongly predicted the onset of daytime sleep attacks. Compared to controls, patients had a higher core body temperature during the first part of the night and a different time course of distal skin, proximal skin and core body temperature. Treatment with SXB resulted in normalisation (i.e. lowering) of core
body temperature during the night, an increased amount of slow wave sleep during the night and a reduced number of daytime naps.

**Distal skin temperature and DPG changes are predictive for sleep onset**

An exciting finding in the present study is the predictive value of distal skin temperature and DPG in daily life daytime sleep attacks, 30 seconds, 5 minutes, 15 minutes and before sleep onset. In particular, the increase of distal skin temperature and DPG in the five minutes prior to sleep onset are strongly related to the onset of sleep attacks. These findings may be evaluated for possible use as a warning of sleep attack risk in narcolepsy patients. It is tempting to imagine the development of a temperature-driven alarm or even cooling devices, to warn narcolepsy patients or even prevent them from falling asleep when not appropriate.

---

**Figure 5.3** Temperature prior to daytime sleep onset in patients at baseline. Mean ± SEM temperatures in patients at baseline from 30 minutes prior to sleep onset until 15 minutes after sleep onset. (A) Distal skin temperature (B) Proximal skin temperature (C) Distal-proximal temperature gradient (DPG). The horizontal arrows indicate the temperature change 15 and 5 minutes prior to sleep onset, the vertical arrow indicates the temperature 30 seconds prior to sleep onset, **p<0.01.**
Previous temperature manipulation studies in healthy young and elderly subjects, insomniacs and narcolepsy patients showed that manipulation of skin temperature can influence the ability to maintain wakefulness, daytime vigilance, sleep onset latency and sleep depth. In all subjects, distal skin warming decreased sleep onset latency and enhanced sleep depth. In narcolepsy patients, distal skin cooling increased the ability to maintain wakefulness. In elderly and insomniacs, proximal skin warming markedly slowed the response speed in vigilance tasks.

Altered thermoregulatory profile in narcolepsy

Patients had a higher core body temperature during the first part of the night, which is in line with the only other ambulatory core body temperature study that has been performed in narcolepsy. All other previous studies concerning core body temperature were performed in a laboratory setting, and showed a large variation in methods and results. As high core body temperature is associated with higher vigilance or wakefulness, a nightly higher core body temperature might be related to the disturbed nocturnal sleep in narcolepsy.

In previous laboratory studies, daytime proximal skin temperature was repeatedly described to be lower in patients than in controls, a finding that could not be replicated in the current study. Conversely, we even found a higher proximal skin temperature in the morning in patients. However, none of these previous studies included ambulatory patients. These previous studies were respectively performed when subjects stayed (semi) supine during the measurements, and during a Multiple Sleep Latency Test (MSLT). Since body position directly influences skin and core body temperature, this might be an explanation for the differences between previous findings and ours. As a consequence of the absence of a lowered proximal skin temperature, no higher distal-proximal temperature gradient (DPG) was found in patients. Furthermore, the time course of both distal and proximal skin temperature is similar (Figure 5.1), so significant differences in DPG are not expected.

The different time course of distal skin temperature in patients, being higher in the morning and lower during night time, does correspond with the findings in the previous studies. As described in the introduction section, a high distal skin temperature during the day is associated with lowered vigilance and increased sleep propensity, and low distal skin temperature is expected to correspond with an increased maintenance of wakefulness. Subsequently, a lowered nocturnal distal skin temperature might be related to a disturbed nocturnal sleep.
The effects of SXB on temperature profiles in narcolepsy

Patients had an increased nocturnal core body temperature compared to controls prior to treatment, while during treatment with SXB, core body temperature reached levels similar to the levels seen in controls. This normalisation of nocturnal core body temperature might be associated with the known improvement of nocturnal sleep in patients during SXB treatment.\(^2\) However, in our previous study no treatment effects on core body temperature were seen at all.\(^1\) In that study, patients were only treated with SXB for 5 consecutive days, probably a too short period to detect these changes in core body temperature. Furthermore, methodology differed in that patients stayed (semi) supine throughout the whole study. This difference in methodology might as well be an explanation for the absence of an increase, i.e. normalisation, of daytime proximal skin temperature in patients after treatment with SXB described in our previous study.\(^1\) Nevertheless, based on manipulation studies,\(^16,25,28\) an increase of proximal skin temperature lowers vigilance and increases sleep onset. Therefore, this previously found increased daytime proximal skin temperature would be in contradiction with the known effects of SXB, i.e. a reduction of daytime sleep attacks.

Limitations

Since previous studies were performed in laboratory settings, the results are not representative for daily life. For this reason, we performed our study ambulatory, which is a benefit in measuring and predicting daytime sleep attacks, however, due to being ambulatory, all circumstances differed between the subjects. Subjects were allowed to follow their usual habits, therefore all bedtimes differed, food and drinks varied as well as their temperatures. Furthermore, subjects went outside whenever they wanted while the climate differed season by season, and due to technical problems core body temperature measurements were partially incomplete in a few subjects. These differences make the comparison of temperature profiles more difficult, however, we already performed these measurements in a laboratory setting.\(^2\) Moreover, no information about body position, which influences temperature, was available. All these differences probably influenced core body and skin temperatures and its analysis. Moreover, due to several clinical and/or personal reasons, not all included patients started or continued treatment with SXB for a period of at least three months, making the post-treatment group smaller.
Conclusions

Daytime sleep attacks were preceded by clear changes in skin temperature. An increase in distal skin temperature and DPG during the fifteen minutes, 30 seconds, and even more during the five minutes prior to daytime sleep onset, was highly significantly associated with the occurrence of sleep attacks. It is intriguing to speculate whether these findings in the future might possibly lead to methods to warn narcolepsy patients or prevent falling asleep when not appropriate.

In an ambulatory setting, core body temperature was higher and distal skin temperature lower during the night in narcolepsy patients compared to controls. These changes might be associated with the known disturbed nocturnal sleep in narcolepsy. Furthermore, the previously described higher daytime distal skin temperature was also seen in the present study, although only in the morning. This finding might be related to excessive daytime sleepiness in narcolepsy. Treatment with SXB improved nocturnal sleep and normalised the core body temperature profile.

ACKNOWLEDGEMENTS

We would like to thank J.G. van Dijk, S. Overeem, C. Bassetti and R. Khatami for their involvement in study design; M. Bach, J. Meier, S. Weber and J.G. van Vliet – de Regt for their help in acquiring the data.

REFERENCES


25. Raymann RJ, van Someren EJ. Time-on-task impairment of psychomotor vigilance is affected by mild skin warming and changes with aging and insomnia. Sleep 2007;30:96–103.


28. Raymann RJEM, Van Someren EJW. Diminished capability to recognize the optimal temperature for sleep initiation may contribute to poor sleep in elderly people. Sleep 2008;31:1301–1309.

### SUPPLEMENTARY TABLES

**Supplementary Table S5.1**  Results of analysis of temperatures of controls vs. patients at baseline based on nocturnal sleep times

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>1</td>
<td>0.09</td>
<td>0.77</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>1</td>
<td>0.02</td>
<td>0.88</td>
</tr>
<tr>
<td>DPG</td>
<td>1</td>
<td>0.00</td>
<td>0.98</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>1</td>
<td>0.12</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Group by time of day effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>11.20</td>
<td>2.28</td>
<td>0.01*</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>5.95</td>
<td>2.46</td>
<td>0.03*</td>
</tr>
<tr>
<td>DPG</td>
<td>5.18</td>
<td>0.96</td>
<td>0.45</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>8.76</td>
<td>3.43</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01.

**Supplementary Table S5.2**  Results of analysis of temperatures at baseline vs during SXB treatment in patients based on nocturnal sleep times

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>1</td>
<td>0.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>1</td>
<td>0.61</td>
<td>0.44</td>
</tr>
<tr>
<td>DPG</td>
<td>1</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>1</td>
<td>1.55</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Treatment by time of day effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal skin temperature</td>
<td>8.72</td>
<td>1.30</td>
<td>0.24</td>
</tr>
<tr>
<td>Distal skin temperature</td>
<td>6.05</td>
<td>0.63</td>
<td>0.71</td>
</tr>
<tr>
<td>DPG</td>
<td>5.75</td>
<td>0.93</td>
<td>0.47</td>
</tr>
<tr>
<td>Core body temperature</td>
<td>8.49</td>
<td>2.75</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01.
### Supplementary Table S5.3  Effect of temperature on daytime nap probability

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal skin temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.28</td>
<td>0.70</td>
<td>1.3</td>
<td>0.3–5.2</td>
<td>0.692</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>1.83</td>
<td>1.25</td>
<td>6.2</td>
<td>0.5–72.6</td>
<td>0.142</td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.47</td>
<td>0.40</td>
<td>1.6</td>
<td>0.7–3.5</td>
<td>0.241</td>
</tr>
<tr>
<td><strong>Distal skin temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.59</td>
<td>0.29</td>
<td>1.8</td>
<td>1.0–3.2</td>
<td>0.042*</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>0.64</td>
<td>0.59</td>
<td>1.9</td>
<td>0.6–6.0</td>
<td>0.277</td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.68</td>
<td>0.20</td>
<td>2.0</td>
<td>1.3–2.9</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><strong>DPG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change 15 minutes prior to sleep onset</td>
<td>0.59</td>
<td>0.29</td>
<td>1.8</td>
<td>1.0–3.2</td>
<td>0.040*</td>
</tr>
<tr>
<td>Change 5 minutes prior to sleep onset</td>
<td>0.93</td>
<td>0.57</td>
<td>2.5</td>
<td>0.8–7.8</td>
<td>0.104</td>
</tr>
<tr>
<td>At 30 seconds prior to sleep onset</td>
<td>0.65</td>
<td>0.17</td>
<td>1.9</td>
<td>1.4–2.7</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Results of mixed effects logistic regression analysis for patients during treatment with SXB (nights were excluded), indicating effects of temperature fluctuations as regressor for fluctuations in sleep attack probability. Analysis was performed for proximal skin temperature, distal skin temperature or distal-proximal temperature gradient (DPG) at the moments: difference between the temperature during the 30-seconds epoch prior to sleep onset and 15 minutes prior to sleep onset, difference between the temperature during the 30-seconds epoch prior to sleep onset and 5 minutes prior to sleep onset, the absolute temperature during the 30-seconds epoch prior to sleep onset. * p<0.05; ** p<0.01.
Supplementary Figure S5.1  Mean ± SEM temperature profiles of patients vs. controls. Temperature curves anchored on bedtimes instead of real clock times. (A) distal skin temperature in patients (red) and controls (green) at baseline, (B) proximal skin temperature in patients and controls at baseline, (C) distal-proximal temperature gradient (DPG) in patients and controls at baseline (D) core body temperature in narcolepsy patients and controls at baseline. The grey area indicates the period during which the temperature significantly differed according the post-hoc tests (* p<0.05).
Supplementary Figure S5.2  Mean ± SEM temperature profiles of patients at baseline and during SXB administration.
Temperature curves anchored on bedtimes instead of real clock times. (A) distal skin temperature in patients at baseline (red) and during SXB administration (blue), (B) proximal skin temperature in patients at baseline and during SXB administration, (C) distal-proximal temperature gradient (DPG) in patients at baseline and during SXB administration, (D) core body temperature in narcolepsy patients at baseline and during SXB administration. The grey area indicates the period during which the temperature significantly differed according the post-hoc tests (* p<0.05).
CHAPTER 6
COMPARING TREATMENT EFFECT MEASUREMENTS IN NARCOLEPSY:
THE SUSTAINED ATTENTION TO RESPONSE TASK, EPWORTH SLEEPINESS SCALE AND MAINTENANCE OF WAKEFULNESS TEST

Astrid van der Heide
Mojca K.M. van Schie
Gert Jan Lammers
Yves Dauvilliers
Isabelle Arnulf
Geert Mayer
Claudio L. Bassetti
Claire-Li Ding
Philippe Lehert
J. Gert van Dijk

Sleep 2015;38(7):1051–8
ABSTRACT

**Study objectives:** To validate the Sustained Attention to Response Task (SART) as a treatment effect measure in narcolepsy, and to compare the SART with the Maintenance of Wakefulness Test (MWT) and the Epworth Sleepiness Scale (ESS).

**Design:** Validation of treatment effect measurements within a randomised controlled trial (RCT).

**Patients:** 95 patients with narcolepsy with or without cataplexy.

**Interventions:** The RCT comprised a double-blind, parallel-group, multi-centre trial comparing the effects of 8-week treatments with pitolisant (BF2.649), modafinil or placebo (NCT01067222). MWT, ESS and SART were administered at baseline and after an 8-week treatment period. The severity of excessive daytime sleepiness and cataplexy was also assessed using the Clinical Global Impression scale (CGI-C).

**Measurements and results:** The SART, MWT and ESS all had good reliability, obtained for the SART and MWT using two to three sessions in one day. The ability to distinguish responders from non-responders, classified using the CGI-C score, was high for all measures, with a high performance for the SART ($r=0.61$) and the ESS ($r=0.54$).

**Conclusions:** The SART is a valid and easy to administer measure to assess treatment effects in narcolepsy, enhanced by combining it with the ESS.
INTRODUCTION

While narcolepsy has an undisputed profound impact on daily life, quantifying how it impairs daily life is difficult. The severity of narcolepsy is currently assessed using measures of the ability to stay awake in boring conditions, such as the Maintenance of Wakefulness Test (MWT), or measures of subjective sleepiness, for which the Epworth Sleepiness Scale (ESS) is often used. However, sleepiness and sleep propensity are not the only aspects of the burden of narcolepsy. An aspect that is gradually more recognised is the quality of the awake state, for which the ability to sustain attention is an important requisite. The Sustained Attention to Response Task (SART), designed to assess this function, has previously been used in narcolepsy and has shown clear potential to quantify the impairment in function during wake in narcolepsy.

The SART is a go/no-go task in which the no-go target appears unpredictably and rarely, and in which both accuracy and response speed, quantified as reaction time (RT), are important. The SART was developed to investigate lapses of sustained attention in individuals with neurological impairment, and proved to be a useful tool to investigate sustained attention in a number of other clinical conditions, including sleep disorders.

To date, the validation of the SART as a tool to measure sustained attention in sleep disordered patients is based on a comparison of SART results between patients with narcolepsy and healthy controls. The SART discriminated well between these groups, i.e. it demonstrated good construct validity. Between-subjects variability in SART performance was higher in the narcolepsy group than in the control group. No correlations were found between SART performance and subjective sleepiness (ESS) or between SART performance and the average sleep onset latency during multiple sleep latency tests (MSLT), i.e. the SART showed discriminant validity with these measures of sleepiness/sleep propensity.

As the SART quantifies the impairment of the waking condition in narcolepsy, it should also be a useful tool to measure treatment effects in narcolepsy. Hence, the objective of this study was to validate the SART as a measurement of treatment in narcolepsy, and to compare it with the MWT and ESS, two tests frequently used in treatment-effect studies in hypersomnias that, however, have never explicitly been validated for their capability to measure treatment effects in narcolepsy. As the initial studies of the SART in sleep disorders have neither assessed the reliability of the test, nor the statistical properties of its outcome measures (i.e. descriptive statistics, statistical distribution of the data), these characteristics were also investigated in this study and compared to those of the ESS and MWT.
METHODS

Subjects

The analysis was conducted on data originating from a double-blind, parallel-group, multi-centre trial comparing the effects of eight-week treatment with the experimental drug BF2.649 (pitolisant) to effects of the proven effective drug modafinil and to placebo in narcolepsy (NCT01067222). Inclusion criteria were the presence of narcolepsy with or without cataplexy diagnosed according to the International Classification of Sleep Disorders (ICSD)-2 criteria and a score of ≥14 on the Epworth Sleepiness Scale (ESS) during the baseline period.

The trial was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by central and local ethics committees and written informed consent was obtained from all subjects prior to the study. The results of this study were published separately.

Design

Eligible patients started with a baseline period of seven days in which they were not allowed to take psychostimulants, medication with sedating properties, tricyclic antidepressants, psychoactive agents, or medication interacting with modafinil. Patients were allowed to take their anticataplectic drugs (sodium oxybate and nontricyclic antidepressants). The baseline period was completed by an inclusion visit. Patients continuing to meet the inclusion criteria were randomly assigned to one of three equally sized treatment groups for a total duration of eight weeks, with possible titration after two weeks and, if necessary, also after three weeks. A control visit took place after seven weeks, and an endpoint visit took place after the eight-week treatment period.

The SART and the MWT were performed at the inclusion visit and the endpoint visit (or the last on-study visit). A SART session was administered prior to each of four MWT sessions, starting at 10:00 hrs and at two-hour intervals thereafter. Patients were requested to take their morning treatment and to have a light breakfast before 08:00 hrs, arriving at the trial centre around 09:00 hrs. Patients took trial medication and had lunch immediately after the second MWT session. Patients were to refrain from stimulating beverages such as coffee or tea during these visits.
Sustained Attention to Response Task

The SART involved withholding key presses to 1 in 9 target stimuli during a 4-minute 19-second period. A number from 1 to 9 was shown 225 times in white on a black computer screen in a quasi-random way, while patients were seated on a chair in front of a computer screen. The font size was randomly chosen from 26, 28, 36, or 72 points. Each number was presented for 250 milliseconds, followed by a blank screen for 900 milliseconds. Subjects had to respond to the appearance of each number by pressing a button, except when the number was a 3. Subjects had to press a button before the next number appeared and were instructed to give equal importance to accuracy and speed in performing the task.6,12

The primary outcome measure of the SART is the total number of errors, consisting of, firstly, key presses when no key should be pressed (i.e. after a ‘3’, a so-called ‘no-go trial’) and, secondly, absent presses when a key should have been pressed (i.e. after anything but a ‘3’, the so-called ‘go trials’). Errors on no-go trials, with a maximum count of 25, are called commission errors. Errors on go trials, omission errors, have a theoretical maximum count of 200. The sum of commission and omission errors, the total error count, was also analysed.

Maintenance of Wakefulness Test

The MWT consisted of four 40-minute sessions in a quiet and dimly lit room. Subjects were instructed to stay awake while comfortably seated in a semi-supine position. Movements or vocalisations were not allowed. The session was terminated either when sleep-onset occurred, defined as either three consecutive 30-second epochs of stage 1 sleep or a single 30-second epoch of any other sleep stage, or after 40 minutes of being awake.13 The mean of the four sleep-onset latencies was considered the primary outcome measure of the MWT.

Epworth Sleepiness Scale

The ESS was administered twice at baseline (at the start of the baseline period and at the inclusion visit) and twice after treatment (at the control visit and the endpoint visit). The two early and the two late measurements were treated as separate sessions in order to assess reliability, i.e. they were not averaged. The four sessions were also separately used in the analysis of treatment efficacy.
Clinical Global Impression

The severity of EDS and of cataplexy was assessed by the local investigator using the Clinical Global Impression of Severity (CGI-S), a 6-point scale, at both baseline visits. Their average value was used for analysis. Any changes in severity of EDS and of cataplexy were measured by the investigator using the Clinical Global Impression of Change (CGI-C) at each follow-up visit. Ratings of this 7-point scale were averaged for the control and endpoint visit to create the final CGI-C score. CGI-S and CGI-C were rated based on a clinical interview before the administration of other scales or tests. The CGI-S and CGI-C scores were linearly transformed into a range from 0 to 4 to enhance comparability, with low values indicating higher severity in the CGI-S and more worsening in the CGI-C.

Statistical analysis

The statistical analyses were carried out with R statistical package (R, version 2.12.2). Unless specified otherwise, we conducted two-sided tests with a significance level of 0.05.

Descriptive statistics

Normality of SART, MWT and ESS outcome measures was assessed by descriptive statistics, parameters of asymmetry and kurtosis, box plots, and the Kolmogorov-Smirnov (KS) test for normality. In case of non-normality, this was repeated for the log transformation of the respective outcome measures. Floor and ceiling effects and homoscedasticity (homogeneity of variance) were tested in subgroups based on age range and gender.

Reliability

A test is considered reliable when within-patient variability is low; no significant change in the test value should occur during a period in which no change is expected, and the value should respond when such a change in condition occurs. We calculated the reliability of each outcome measure with a linear mixed model (see Appendix 6.1).

Reliability is high when within-patient variability is low compared to the variability of the studied outcome measure. To express this comparison as a number the intra-class correlation coefficient (ICC) of reliability was used. The ICC was estimated from our model as follows: the within-patient variability (squared) was divided by total variability, which is the within-patient variability (squared) plus the variability of the studied outcome measure (squared).
The optimal value of the ICC is 1, meaning there is no within-patient variability, and all variability is explained by variability of the studied outcome parameter. An ICC > 0.8 is accepted as indicating good reliability.\textsuperscript{16,17}

When one measurement or test session proves to have an insufficiently high reliability, this reliability can be increased by repeating the test.\textsuperscript{18} As the SART and MWT were each performed four times on a test day, the ICCs resulting from the first 2 to all 4 sessions were calculated using the Spearman-Brown expression for stepped-up reliability.\textsuperscript{19}

**Sensitivity**

As we aimed to investigate the validity of the SART in the context of narcolepsy, the CGI-C was considered the most appropriate standard to compare SART results with, as it reflects clinically pertinent changes in a patients' condition, assessed in a manner reflecting normal medical practice in a patient-physician interview. We calculated the sensitivity of each outcome measure for treatment efficacy by dividing subjects into responders and non-responders. Such a classification provides two groups that are supposed to differ in the true level of the constructs, but that are quite homogeneous within each group. The best dichotomy between the categories was found through assessing the linearity of the scale (Logit model between CGI-C and first factor from a confirmatory factor analysis), corroborated with a Rasch Analysis. On this basis, a responder was defined as being ‘much’ or ‘very much’ improved on the CGI-C, and all other results were classified as non-responders. This strategy is commonly used in various studies.\textsuperscript{20-23} Analysis of covariance (ANCOVA) was used to compare outcome measures between responders and non-responders, corrected for baseline values, age, and sex.

The difference in the mean outcome measure between responders and non-responders was divided by its standard deviation (called ‘residual standard deviation’) to calculate the so-called Cohen’s Effect Size (ES) or standardised mean difference. An ES > 0.5 is considered clinically relevant. If baseline and final values of the same outcome measures are correlated, the residual standard deviation is reduced, leading to a higher ES. A corrected effect size taking into account this correlation was calculated by multiplying the ES by the square root of the coefficient of correlation between the baseline and final values. The effect size was also measured using a linear mixed model, in which the interaction between treatment effect and time provided a more accurate measure of the effect size.
Finally, associations between CGI-C, MWT, ESS, and SART were investigated using factor analysis to demonstrate the contribution of each outcome measure to the CGI-C score.\textsuperscript{24,25}

**Missing values**

Reliability and sensitivity were estimated on the available data set. The trial from which our data originated was considered a pivotal Phase III trial. As such, missing data were rare, with no missing data at baseline and less than 7% at final time. These data were not imputed, but directly handled by the mixed model.\textsuperscript{26}

For sensitivity purposes, we repeated our analyses in imputing missing data by using Last Observed Carried out Forward techniques (LOCF), Baseline Carried Forward (BCF) and multiple imputation. We calculated the relative error between the values of the three techniques $Q_i$ with our suggested method $Q$ in calculating $E=100 \times \frac{|Q_i-Q|}{Q}$. These values were 0.8%, 1.3%, and 1.7% respectively. We therefore concluded that imputation of missing data did not change the results.

**RESULTS**

**Subjects and data characteristics**

Patient characteristics are summarised in Table 6.1. None of the SART accuracy measures was normally distributed (KS, $p<0.001$). After logarithmic transformation, the commission errors and the total number of errors became normally distributed (KS, $p=0.14$). No suitable transformation was found that resulted in a normal distribution for omission errors (KS, $p<0.001$). The ESS showed a slightly platycurtic normal distribution (KS, $p>0.55$). A ceiling effect was observed for the MWT, caused by the maximum score of 40 minutes; this made the nature of the distribution difficult to define with precision. A log-normal distribution was suspected (KS, $p=0.23$) and was therefore used in further analysis. As observed more often after log transformations, between-category heteroscedasticity was found for log-MWT.

**Reliability**

Table 6.2 presents within-patient variability and variability of the studied measure (i.e. the various SART error counts, ESS, and MWT) as modelled. With the aid of these estimates the ICC was calculated. The ICC was highest for the ESS at 0.83; ICC for log-SART total error
Comparing treatment effect measurements in narcolepsy

Table 6.1 Patient characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Responders (N=51)</th>
<th>Non-responders (N=44)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>0.737</td>
</tr>
<tr>
<td>Sex (males (%))</td>
<td>38.24±14.08</td>
<td>39.25±15.36</td>
<td>0.971</td>
</tr>
<tr>
<td>Baseline ESS</td>
<td>18.70±2.79</td>
<td>18.13±2.39</td>
<td>0.291</td>
</tr>
<tr>
<td>Baseline CGI-S</td>
<td>1.63±1.04</td>
<td>0.93±0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline SART total errors</td>
<td>15.65±13.69</td>
<td>11.62±7.21</td>
<td>0.079</td>
</tr>
<tr>
<td>Baseline MWT sleep latency (min.)</td>
<td>10.6±8.9</td>
<td>13.2±10.6</td>
<td>0.196</td>
</tr>
<tr>
<td>Endpoint ESS</td>
<td>9.76±6.56</td>
<td>15.02±4.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endpoint CGI-C</td>
<td>3.26±0.83</td>
<td>0.91±0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endpoint SART total errors</td>
<td>8.77±7.03</td>
<td>11.48±8.91</td>
<td>0.145</td>
</tr>
<tr>
<td>Endpoint MWT sleep latency (min.)</td>
<td>23.6±14.6</td>
<td>12.4±11.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ESS: Epworth Sleepiness Scale; CGI-S: Clinical Global Impression of Severity; CGI-C: Clinical Global Impression of Change; SART: Sustained Attention to Response Task; MWT: Maintenance of Wakefulness Test; log: log-transformed; min: minutes; SD: standard deviation.

Table 6.2 Variability and intra-class coefficient of correlation of SART, MWT and ESS

<table>
<thead>
<tr>
<th></th>
<th>Within-patient variability</th>
<th>Variability measure</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SART commission errors (log)</td>
<td>0.14</td>
<td>0.23</td>
<td>0.71</td>
</tr>
<tr>
<td>SART omission errors (log)</td>
<td>0.30</td>
<td>0.34</td>
<td>0.56</td>
</tr>
<tr>
<td>SART total errors (log)</td>
<td>0.20</td>
<td>0.28</td>
<td>0.65</td>
</tr>
<tr>
<td>MWT sleep latency in min. (log)</td>
<td>0.26</td>
<td>0.47</td>
<td>0.76</td>
</tr>
<tr>
<td>ESS</td>
<td>1.09</td>
<td>2.45</td>
<td>0.85</td>
</tr>
<tr>
<td>SART commission errors</td>
<td>2.85</td>
<td>4.39</td>
<td>0.70</td>
</tr>
<tr>
<td>SART omission errors</td>
<td>8.28</td>
<td>7.97</td>
<td>0.48</td>
</tr>
<tr>
<td>SART total errors</td>
<td>8.67</td>
<td>10.50</td>
<td>0.59</td>
</tr>
<tr>
<td>MWT sleep latency in min.</td>
<td>7.44</td>
<td>13.48</td>
<td>0.77</td>
</tr>
</tbody>
</table>

ICC: intra-class coefficient of correlation, calculated as follows: within-patient variability (squared) divided by the total variability, which is the within-patient variability (squared) plus the variability of the studied outcome measure (squared). The last four rows illustrate that non-log-transformed SART and MWT have a lower ICC compared to their log-transformed match.

count was 0.65, and for log-MWT it was 0.76. The influence of replication is presented in Table 6.3; repeating the test improved the reliability for the MWT to 0.87 for the first two tests and to 0.82 for the first two log-transformed SART commission error counts.
Sensitivity

SART, ESS and MWT results differed significantly between the responder group and the non-responder group with lower SART and ESS scores and higher MWT sleep latencies for responders (Table 6.4). The corrected ES was ≥ 0.5 for all outcome measures except for the SART omission error count (Table 6.5). The highest effect size was seen for the ESS.

Using these results, we calculated which sample size would be needed to perform a treatment-effect study based on common assumptions (i.e. \( \alpha = 0.05 \), \( 1 - \beta = 0.9 \), two-sided

### Table 6.3  Influence of the number of sessions on the intra-class coefficient of correlation

<table>
<thead>
<tr>
<th></th>
<th>ICC</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SART commission errors (log)</td>
<td>0.70</td>
<td>0.82</td>
</tr>
<tr>
<td>SART omission errors (log)</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>SART total errors (log)</td>
<td>0.65</td>
<td>0.79</td>
</tr>
<tr>
<td>MWT sleep latency in min. (log)</td>
<td>0.76</td>
<td>0.87</td>
</tr>
<tr>
<td>ESS</td>
<td>0.83</td>
<td>0.91</td>
</tr>
</tbody>
</table>

ICC: intra-class coefficient of correlation. An ICC > 0.80 is regarded as good reliability. The ICC resulting from the first 2 to all 4 sessions was calculated using the Spearman-Brown expression for stepped-up reliability. In bold the minimum number of sessions necessary to provide good reliability (ICC>0.80).

### Table 6.4  ANCOVA non-responders versus responders corrected for age and sex

<table>
<thead>
<tr>
<th></th>
<th>Delta</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SART commission errors (log)</td>
<td>0.13</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SART omission errors (log)</td>
<td>0.17</td>
<td>0.31</td>
<td>0.007</td>
</tr>
<tr>
<td>SART total errors (log)</td>
<td>0.21</td>
<td>0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MWT sleep latency in min. (log)</td>
<td>-0.33</td>
<td>0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESS score</td>
<td>6.90</td>
<td>5.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SART commission errors</td>
<td>1.83</td>
<td>3.08</td>
<td>0.007</td>
</tr>
<tr>
<td>SART omission errors</td>
<td>5.30</td>
<td>7.77</td>
<td>0.002</td>
</tr>
<tr>
<td>SART total errors</td>
<td>7.29</td>
<td>8.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MWT sleep latency in min.</td>
<td>-13.00</td>
<td>11.50</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Delta is calculated by subtraction of the values of the parameters of the responders from the non-responders.
Comparing treatment effect measurements in narcolepsy

To do so, we performed an analysis of covariance, in which we corrected for the baseline values, age and sex. As the results show (Table 6.6), using the log-transformed SART commission error count or the total error count allows studies to be designed with lower numbers of subjects than holds if non-transformed outcome parameters are used.

**Table 6.5  Cohen’s effect size**

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Coefficient of correlation</th>
<th>Effect size</th>
<th>Corr. effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>SART commission errors (log)</td>
<td>0.81</td>
<td>0.81</td>
<td>0.68</td>
</tr>
<tr>
<td>SART omission errors (log)</td>
<td>0.64</td>
<td>0.55</td>
<td>0.41</td>
</tr>
<tr>
<td>SART total errors (log)</td>
<td>0.76</td>
<td>1.05</td>
<td>0.85</td>
</tr>
<tr>
<td>MWT sleep latency in min. (log)</td>
<td>0.63</td>
<td>1.01</td>
<td>0.88</td>
</tr>
<tr>
<td>ESS</td>
<td>0.34</td>
<td>1.33</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Cohen’s effect size (ES) was calculated by dividing the difference in the mean outcome measure between responders and non-responders by its standard deviation. The corrected effect size was calculated by multiplying the ES by the square root of the coefficient of correlation between the baseline and final values. An ES > 0.50 is regarded as good.

**Table 6.6  Necessitated sample sizes**

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SART commission errors (log)</td>
<td>16</td>
<td>14</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>SART omission errors (log)</td>
<td>74</td>
<td>58</td>
<td>53</td>
<td>50</td>
</tr>
<tr>
<td>SART total errors (log)</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>MWT sleep latency in min. (log)</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>ESS</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

log: log-transformed. N1 until N4 is the sample size necessitated in case of 1/2/3/4 tests or sessions in standard conditions (α=0.05, 1−β=0.9, two-sided test), calculated by an analysis of covariance of the values of the outcome measure, corrected for the baseline values, age and sex.
Comparison of the MWT, ESS and SART

Figure 6.1 shows the results of the factor analysis in which the CGI-C noted at the final patient visit was compared to the mean change from baseline (or relative variation) of the SART, ESS and MWT. There was a significant correlation between CGI-C and all outcome measures with the highest correlation for delta log-SART total error count ($r=0.606$) and the ESS ($r=0.535$).

Figure 6.1  Factor analysis of CGI-C, SART, ESS, and MWT.
(A) Factor analysis of the delta scores of the MWT (log-transformed), ESS, SART (log-transformed total error count) and CGI-C. (B) Factor analysis of the delta scores of SART outcome measures (all log transformed), and CGI-C.

The direction of the arrows represents the degree of correlation between the various measures. When arrows point in the exact same direction, they are perfectly positively correlated. Arrows pointing in opposite directions, with an angle between them of 180° indicate a perfect inverse, i.e. negative correlation. Arrows at right angles to one another reflect that the two measures are completely independent. The dashed line represents the 180° opposite of the CGI score.

Figure A shows that the ESS and SART are more parallel to the CGI than the MWT, and the angle between them suggests that they capture different aspects.

DISCUSSION

This study demonstrated that the SART is a useful tool to measure treatment efficacy in narcolepsy. Of the various SART outcome measures, the log-transformed total error count proved most sensitive to treatment effects, as established by the CGI-C. The log transformed commission error count proved the most reliable across sessions performed on the same day; a good reliability of >0.8 was already reached after performing the SART twice. Performing the SART three times allowed the log-transformed total error count to exceed this threshold as well.
Reliability of the SART, ESS and MWT

Tests are considered reliable when no significant changes in their outcome measures are observed in periods when no change is expected, and when such changes do occur when there is a change in condition. We used the intra-class correlation coefficient to compare reliability of the SART, the ESS and the MWT. As the ESS needed to be administered only once to reach a high level of reliability, the ESS proved the most reliable test. Note that repeated administration of the ESS differed from repeated administration of SART and MWT: the ESS was repeated with an interval of one week, while SART and MWT sessions were repeated on the same day. However, we did not consider this a limitation of high importance, as we aimed at comparing the reliability of SART, MWT and ESS in their usual schedule of administration. The ESS measures experienced sleepiness over the past week(s) or month(s), and is therefore not administered several times per day.

The SART can achieve the same level of reliability as the ESS, but to do so it needed to be administered twice when the log-transformed commission error count was used, and three times when the log-transformed total error count was used.

The MWT reached a similar level of reliability after two sessions, regardless of log transformation. The distribution of the MWT exhibits a ceiling effect meaning that using it for statistical analysis is complex and should be treated with caution.

These results suggest that SART and MWT can measure treatment effects reliably using only the first two or three sessions on one day instead of the four sessions that are conventionally used, given the fact that they are performed at the same time of day as in this study. More than three sessions probably do not relevantly further explain variability and using four tests will be accompanied by higher costs and longer duration. Those who wish to investigate a time of day effect on treatment results might wish to use four or even more tests, but should realise that time of day (morning vs afternoon) did not affect SART performance in a recent study.27

Sensitivity of the SART, ESS and MWT

Note that a high reliability of a test does not necessarily mean that it also reflects clinical improvement well. We investigated the latter aspect, sensitivity, for which we used the CGI-C as a gold standard. The ESS, SART and MWT all showed high sensitivity, with highest
sensitivity for the ESS. The highest effect size of SART was found for the log-transformed total error count. We also found that the change in clinical condition from baseline to endpoint (CGI-C) was significantly correlated with the changes (delta scores) of all three tests. In fact, of the three studied measurements, the change in a SART parameter (log-transformed total errors count) reflected the change in clinical condition most closely, followed by the ESS.

Which aspect of improvement do the various tests reflect?

The SART, ESS and MWT need not reflect the same aspects of the burden of narcolepsy. In fact, in previous studies the SART error count was not related to the ESS, which reflects perceived sleepiness, and the MSLT, which reflects the propensity to fall asleep quickly.4,5 In these same studies, ESS and MSLT results were correlated. We attempted to unravel the correlation between our outcome measures through factor analysis (Figure 6.1). The arrows of the ESS and MWT roughly point in the same direction, which means that changes in MWT and ESS during the study largely reflect the same aspect of the narcolepsy burden. Of these two measures, the treatment response as expressed in the delta ESS score is the better representative of the investigator’s impression of treatment response, as the angle between CGI-C and delta ESS is smaller than between CGI-C and MWT. The delta scores of the ESS and SART explain the CGI-C score quite well (i.e. lie close to the 180º opposite of the CGI-C arrow) in a similar magnitude. Interestingly, the delta scores of the ESS and SART form a large angle (close to 90º) among themselves, indicating that they indeed explain different aspects of the CGI-C score. The factor analysis thus shows that the investigator’s impression is both based on sustained attention and the ability to stay awake.

The optimal test battery to measure treatment response in narcolepsy

Measures of sleep propensity (MWT) or perceived sleepiness (ESS) on the one hand, and sustained attention on the other hand (SART), are complementary. This study indicates that a combination of the SART with either the MWT or ESS comprises the most suitable combination of the three investigated tests to measure treatment response in narcolepsy. As the MWT and the ESS in part seem to explain the same variability, the question rises which of these tests is best suited to measure treatment effects. The MWT and the SART measure more distinct phenomena than the ESS and SART, as the angle between delta-MWT and delta-SART is closer to 90º in the factor analysis. Another argument in favour of the MWT is that it is easier for a patient to manipulate an ESS result for whatever reason than an MWT.
result. Then again, there are a number of arguments against the MWT. It can be manipulated by reducing previous amount of sleep; it is not uniformly carried out: some use 20-minute sessions, others 40-minute ones; some use four, others five sessions; the definition of sleep onset also varies. However, these disadvantages could be overcome by using the protocol recommended in the AASM manual. Furthermore, the MWT is performed in an artificial setting that need not represent daily life, and, finally, it is time-consuming. Compared to the MWT the ESS is inexpensive, has a high degree of internal consistency and can easily be re-rated for follow-up studies. While these arguments were already known the present study adds new ones in favour of the ESS over the MWT: it had the highest reliability of all three tests and was more sensitive to treatment efficacy than the MWT.

An interesting characteristic of the SART has to do with the balance between a subjective and objective assessment. An ‘objective’ test reflects a quantitative test measurement rather than a patient’s opinion of disease severity. The SART (and MWT) as objective tests offer the advantage of immunity to manipulation in one direction: it is possible to perform the test worse than one’s conditions allows, but not better. However, ‘subjective’ assessment by patients often forms the primary reason to alter treatment in patient care. The SART has the advantage of objectivity as well as a close relation to subjective changes in severity, reflected in the CGI-C.

We conclude that a single ESS accompanied by two to three SART sessions, depending on the chosen SART outcome parameter, provides a good method to evaluate treatment effects in narcolepsy. This battery comprises two key aspects of narcolepsy, perceived sleepiness and sustained attention, and is easy and cheap to administer.

**Detailing SART analysis**

Which SART parameter should be used? The factor analysis revealed only minor differences among the various outcome measures of the SART (Figure 6.1b), indicating that they represent the same part of the CGI-C. The highest effect size was found for the total error count. This needs three SART sessions, compared to two for the commission error count. The latter parameter also had a better distribution. The omission error count did not perform as well in terms of distribution and reliability. Still, the total error count did perform well, and, as it contains the omission error count as well, counting omission errors may have a role. The relative importance of omission, commission and total error counts can differ
between disorders.\textsuperscript{28} We accordingly advise to use the total error count as the primary SART outcome measure.

Reaction time can also be used as a SART parameter, but measuring RT accurately requires special equipment, whereas measuring error counts can be done with standard personal computers. In the present multicentre study, RTs were not measured.

A different test to measure sustained attention is the Psychomotor Vigilance Task (PVT), which has been used and validated in sleep deprivation studies.\textsuperscript{29-31} PVT results in narcoleptics differed from those of healthy controls.\textsuperscript{32} The PVT is sensitive to treatment efficacy in obstructive sleep apnoea syndrome,\textsuperscript{33} but its role in assessing treatment efficacy in narcolepsy would require an assessment similar to the present study, which is currently not available.

**Study limitations**

Our study is limited to the three measurements of treatment effect that we evaluated, so there is no way for us to tell whether any of the other possible parameters to measure disturbed sleep and its consequences would be useful to measure treatment efficacy.

Our results are based on data from a study designed to evaluate effects of pitolisant. This means that patients were not selected to represent a typical spectrum of severity of narcolepsy. However, the selection was not limitative, the statistical analysis was prepared before the analysis of the drug trial, and the analysis was conducted independent of the main and secondary endpoints of the trial.

**Conclusion**

In conclusion, this study shows that the SART, in particular the commission errors and the total error score, is a valid measure to detect treatment effects. A combination of the SART and ESS includes a comprehensive evaluation of treatment effects in narcolepsy since the ESS represents a subjective estimate of how sleepy patients feel, while the SART is objective in nature. Together they share the advantages of not requiring much time or money, and they correlate well with the clinical global assessment of patient improvement.
REFERENCES


APPENDIX 6.1

Reliability

We calculated the reliability of each outcome measure as the ratio of its observed variability divided by the variability of the true value of the construct that was measured. As this true value cannot be measured directly, it was estimated from our data by means of a linear mixed model. We defined the following linear mixed model to compare the reliability of SART accuracy measures, MWT sleep latency and ESS score:

\[ Y(i) = K + Time \times [1+N(0, \sigma_e)] + age + sex + \sigma T \]

In this model, we assumed that the value of the outcome measure (Y) depended on a constant value (K), the variability of the studied outcome measure (\(\sigma T\)), some random variability expressed as the interaction of within-patient variability (\(\sigma e\)) with time, and the effects of age and sex. The model contained a random factor for the short time interval in which the value of the outcome measure was not expected to vary within each subject.

Sensitivity

Effect size was also measured using a linear mixed model assuming that the value of the outcome measure depended on a constant value, time, being a responder or not, the interaction of the latter two, age, and sex:

\[ Y() = K + Time + Responder + Time \times Responder + age + sex \]
CHAPTER 7
SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES
SUMMARY AND CONCLUSIONS

The first part of this thesis concerned an overview of the pathophysiology, symptoms and treatment of narcolepsy type 1. The second part elaborated some pathophysiological aspects, focusing on the autoimmune hypothesis of narcolepsy. The third part focused on alterations of temperature regulation and on measuring treatment effects of symptomatic treatment on sustained attention, i.e. vigilance.

Chapter 1 presented an overview of the clinical features, diagnostic criteria, pathophysiology and current treatment options. Narcolepsy is a disorder of the regulation of sleep and wakefulness, with as its major features excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations, sleep paralysis and disturbed nocturnal sleep. The diagnosis is reached using the criteria of the International Classification of Sleep Disorders (ICSD-3), including a clinical assessment, polysomnographical studies (multiple sleep latency test (MSLT)) and/or hypocretin-1 measurement in the cerebrospinal fluid (CSF). The level of the neuropeptide hypocretin-1 in the CSF is so low as to be undetectable in almost all patients with narcolepsy with cataplexy patients. This deficiency is thought to be the cause of the typical narcolepsy symptoms. Post mortem studies demonstrated a selective loss of hypocretin-containing neurons in the hypothalamus. Although the mechanism behind this specific loss of hypocretin-producing neurons has not yet been elucidated, an autoimmune attack has been proposed targeting hypothalamic neurons that produce hypocretin/orexin. Narcolepsy can at present be prevented nor cured, leaving symptomatic treatment at the sole option; luckily, this may lead to a substantial improvement. Treatment includes behavioural modification and pharmacological treatment with sodium oxybate (SXB), psychostimulants and/or antidepressants.

HLA in narcolepsy

An autoimmune aetiology of narcolepsy has been hypothesised for decades. It is mainly based on the tight association of narcolepsy with HLA-DQB1*06:02. In fact, this association is the strongest known for any disease. Worldwide, 85–95% of patients suffering from narcolepsy with cataplexy carry this haplotype, compared to 12–38% of the general population. For non-familial cases and those with typical cataplexy the rate may even exceed 98% of cases. HLA-DQB1*06:02 itself seems to be a risk factor for the development of narcolepsy, but another gene in close linkage with it could also be responsible for the
increased risk. If the \textit{HLA-DQB1*06:02-DQA1*01:02} (\textit{HLA-DQ0602}) dimer would itself be involved in the aetiologia of narcolepsy, a dosage effect would be expected: an increased expression of the \textit{HLA-DQ0602} dimer \textit{should} be associated with a higher susceptibility to the development of narcolepsy. This would be the case in individuals homozygous for \textit{HLA-DQB1*06:02-DQA1*01:02}, but also in individuals heterozygous for \textit{HLA-DQB1*06:02-DQA1*01:02} and homozygous for \textit{HLA-DQA1*01:02}.

In \textbf{Chapter 2} we investigated the \textit{HLA-DQ} alleles located in \textit{trans} with \textit{HLA-DQB1*06:02-DQA1*01:02}. As expected, homozygosity for \textit{DQB1*06:02-DQA1*01:02} was more frequently seen in our patients than in controls. We indeed found a higher prevalence of homozygosity for \textit{HLA-DQA1*01:02} in \textit{HLA-DQB1*06:02-DQA1*01:02} heterozygous narcolepsy patients. Both these findings support a direct role of the \textit{HLA-DQB1*06:02-DQA1*01:02} dimer molecule in the development of narcolepsy.

This direct role of the \textit{HLA-DQ0602} dimer fits perfectly well with the autoimmune hypothesis in narcolepsy: the function of HLA-class II molecules like the \textit{HLA-DQ0602} dimer is to present peptides derived from foreign proteins to the immune system in order to elicit a T cell mediated immune response. Sometimes, T cells reactive with foreign peptides may cross react with self-structures leading to destruction of autologous cells and autoimmunity.

\textbf{Narcolepsy and auto-immunity}

In a search for evidence for the autoimmune hypothesis we screened the serum of narcolepsy patients for antibodies against hypocretin-producing neurons using immunohistochemical methods (\textbf{Chapter 3}). Similar studies in the past have not been successful.\textsuperscript{8-11} These previous studies were performed with serum or CSF derived from narcolepsy patients with either a relatively long or unknown disease duration. Current thinking holds that narcoleptic symptoms start when the vast majority of the hypocretin-producing neurons have been destroyed. As an immune attack is only active as long as there are target cells to attack, the auto-immune activity will be active only before or at the first appearance of narcoleptic symptoms. Afterwards auto-immune activity would stop. The major limitation of the previous studies might therefore be the relatively long disease duration of the included patients. To overcome this, we screened serum of 21 narcolepsy type 1 patients close to disease onset, including H1N1 vaccinated patients, for antibodies against hypocretin producing neurons using immunohistochemistry. Unfortunately, no autoantibodies against hypocretin neurons
could be detected. This finding does not contradict the autoimmune hypothesis, nor does it imply an absence of autoantibodies at any time in the development of the disease.

**Altered temperature regulation in narcolepsy**

Skin and core body temperature play important roles in sleep and wake regulation. The waking state is associated with a relatively low skin temperature and a relatively high core body temperature, while sleep is associated with the opposite pattern. Sleep onset is preceded by a decrease in core body temperature and an increase in skin temperature. The decrease in core body temperature is mediated through increased skin perfusion, which consequently leads to the increase in skin temperature, facilitating cooling of the body.

In narcolepsy, an altered diurnal profile of skin temperature has been demonstrated, suggesting a relationship between hypocretin function, temperature and sleep regulation. Sodium oxybate (SXB) is a drug that is registered for the treatment of narcolepsy. Given the altered pattern of skin temperature in narcolepsy and the positive effects of SXB on sleep in narcolepsy patients, it was hypothesised that the effect of SXB may in part be mediated by its possible restorative effect on temperature regulation.

In Chapter 4 we studied the differences in core body and skin temperature between narcolepsy patients and controls, as well as the effects of SXB on body temperature in relation to its effects on sleep. Eight male narcolepsy patients and eight healthy male controls, matched for age and body mass index, underwent a 24-hour temperature measurement and polysomnography. During the experiment subjects stayed in the hospital. They remained supine or semi supine except for bathroom visits, received standardised cold meals at fixed times, and were allowed to take daytime naps. Following the baseline study, subjects ingested SXB for five consecutive days. A second 24-hour temperature measurement and a polysomnographic study were performed on the 5th day of SXB use.

At baseline, core body temperature and proximal skin temperature were lower in narcolepsy, mainly caused by significant differences during daytime. In contrast to previous studies, no significant difference in distal skin temperature was found. This was thought to be mainly due to a higher distal skin temperature in controls, since a higher distal skin temperature can be a direct consequence of a supine position. In patients, SXB administration resulted in a partial normalisation of the skin temperature profile, by increasing daytime proximal skin
temperature to levels comparable with healthy controls and by strengthening the known relationship between skin temperature and daytime sleep propensity.

Following this hospital-based strictly controlled study we performed a similar study in ambulant patients and controls (Chapter 5). To do so, 25 narcolepsy patients and 15 healthy controls underwent an ambulatory baseline 24-hour temperature measurement and polysomnographic test. This procedure was repeated in 16 narcolepsy patients after at least three months of stable treatment with SXB. The aim of the study was to further explore temperature regulation and sleep in narcolepsy type 1; we chose an ambulatory setting to study daily life as much as possible, and to find out whether spontaneous sleep attacks were heralded by changes in skin temperature.

At baseline, patients had a higher core body temperature than controls during the first part of the night, a higher proximal and distal skin temperature in the morning, and lower distal skin temperature during night time. As sleep is associated with a low core body temperature and high distal skin temperature, these findings could be related to disturbed nocturnal sleep in narcolepsy. Treatment with SXB resulted in a decrease of core body temperature, reaching levels similar to the levels seen in controls. This normalisation of nocturnal core body temperature might be associated with the known improvement of nocturnal sleep in patients during SXB treatment.\textsuperscript{21} Furthermore, SXB reduced the amount of daytime sleep and number of daytime naps. A remarkable finding was that daytime sleep attacks were preceded by clear changes in temperature: increase of distal skin temperature and distal-to-proximal temperature gradient (DPG) during the fifteen minutes, 30 seconds, and even more during the five minutes prior to daytime sleep onset, were highly significantly associated with occurrence of these sleep attacks.

Measuring treatment effect in narcolepsy: the Sustained Attention to Response Task

Narcolepsy has an undisputed profound impact on daily life. One aspect that is gradually recognised as a severe handicap is the lower quality of the awake state, for which the ability to sustain attention is an important requisite. The Sustained Attention to Response Task (SART), designed to assess this function, has previously been used in narcolepsy,\textsuperscript{22,23} and has shown clear potential to quantify the impairment in function during wake in narcolepsy. The SART is a go/no-go task in which the no-go target appears unpredictably and rarely, and in which both accuracy and response speed, quantified as reaction time (RT), are important.
Chapter 6 described the validation of the SART as a tool to measure treatment effects. The analysis was conducted on data originating from a double-blind, parallel-group, multi-centre trial comparing the effects of eight-week treatment with the experimental drug pitolisant (an inverse agonist of the histamine H3 receptor) to effects of the proven effective drug modafinil and to placebo in narcolepsy. In this study, the severity of EDS and of cataplexy was assessed by the local investigator using the Clinical Global Impression of Severity (CGI-S), and any changes in severity of EDS and of cataplexy were measured using the Clinical Global Impression of Change (CGI-C). The results of both CGI scales were compared with the SART, the Maintenance of Wakefulness Test (MWT) and the Epworth Sleepiness Scale (ESS). Based on the analyses, we concluded that two to three SART sessions accompanied by a single ESS, constitute a good method to evaluate treatment effects in narcolepsy. This battery comprises two key aspects of narcolepsy, perceived sleepiness and sustained attention, and is easy and cheap to administer.

FUTURE PERSPECTIVES

The discovery of hypocretin-1 deficiency in narcolepsy type 1 at the end of the last century, represented a major advance in the understanding of the pathophysiological mechanism behind the development of narcolepsy. In accordance with the tight HLA-DQB1*06:02 association, attention was mainly focused on auto-immunity. Although more and more supporting findings have been published, no direct proof for this hypothesis has yet been found. Up to now, most genetic findings pointed to T-cell involvement. Furthermore, a remarkable increased incidence of narcolepsy was reported after infections, in particular after H1N1 vaccination and infection with H1N1. Our finding of a dosage effect of HLA-DQ0602 in narcolepsy provides additional evidence for the T-cell autoimmune hypothesis.

With this in mind, future studies should focus on the possibility of narcolepsy being a T-cell mediated autoimmune disease. Exploring this possibility will be challenging as the immune attack in narcolepsy is not only presumably transient and of short duration, but also likely to precede the appearance of narcoleptic symptoms: the opportunity to detect an autoimmune process may have passed by the time its need becomes apparent. To complicate matters further the autoimmune response might well be confined to the central nervous system, making it appreciably more difficult to detect than a systemic response is. The latter problem is mainly due to the low number of lymphocytes in CSF. As a result of a low concentration auto reactive T-cells will be difficult to detect, requiring a large amount of
CSF. Nevertheless, future studies should focus on T-cell autoimmunity in CSF of recent onset narcolepsy patients, with special attention to infection with and/or vaccination for H1N1.39

We studied differences in core body and skin temperature between narcolepsy patients and controls in this thesis, first in a clinical and later in an ambulatory setting, more extensively than in previous studies. Narcolepsy patients were already known to have an altered temperature profile compared to controls, which we replicated.

Treatment with SXB resulted in a partial normalisation of this altered temperature pattern, in particular during night time. These changes might well be related to the improvement of nocturnal sleep in narcolepsy during treatment with SXB; however, it is unclear whether temperature changes during SXB use are related to daytime sleep attacks in narcolepsy. A replication of these studies, including more ambulant patients and controls, could yield more insight. Future studies might well focus on the relation between the increase of distal skin temperature and distal-to-proximal temperature gradient (DPG) and the onset of daytime sleep attacks, i.e. to explore whether these temperature changes can reliably predict sleep attacks. If sleep onset can be predicted from these temperature changes, this might lead to methods to warn narcolepsy patients when falling asleep and even prevent them falling asleep when not appropriate. Moreover, manipulation of distal skin temperature might have therapeutic potential as well, this has to be further explored.

Now that the SART has been validated as a tool to measure treatment efficacy, it can be used to measure vigilance in narcolepsy. Doing so will not only be helpful to evaluate treatment effects of existing or new drugs, but may also be implemented in the evaluation of fitness to drive a motor vehicle as well. At present only the Maintenance of Wakefulness Test (MWT) is used for this purpose. The MWT is performed in an artificial setting that does not approximate driving a car and negotiating traffic. Vigilance probably resembles the demands posed by paying attention while driving a motor vehicle more closely than the ability to stay awake in a semi-supine position in a quiet and dimly lit room, which is what the MWT assesses.

REFERENCES


CHAPTER 8
SAMENVATTING, CONCLUSIES EN TOEKOMSTPERSPECTIEVEN
SAMENVATTING EN CONCLUSIES

In het eerste deel van dit manuscript wordt een overzicht gegeven van de pathofysiologie, symptomatologie en behandeling van narcolepsie type 1. Het tweede deel gaat dieper in op de pathofysiologie, en wel in het bijzonder op de auto-immuun hypothese. Het derde en laatste deel verschaf inzicht in de afwijkende temperatuurregulatie bij narcolepsiepatiënten en op het meten van de effecten van symptomatische behandeling op volgehouden aandacht (vigilantie).

Narcolepsie is een stoornis van de slaap-waak regulatie, gekenmerkt door overmatige slaperigheid overdag, kataplexie, hypnagoge hallucinaties, slaapparalyse en een verstoorde nachtslaap. **Hoofdstuk 1** geeft een overzicht van deze klinische symptomen, van de diagnostische criteria, de pathofysiologie en behandelmogelijkheden van narcolepsie type 1. De diagnose wordt gesteld met behulp van de ‘International Classification of Sleep Disorders (ICSD-3)’ en omvat een klinische beoordeling, polysomnografisch onderzoek (‘multiple sleep latency test’ (MSLT)) en/of bepaling van hypocretine-1 (een neuropeptide) in de liquor. Een verlaagd hypocretine-1 gehalte in de liquor wordt bij bijna alle narcolepsiepatiënten met kataplexie gevonden en wordt gezien als de oorzaak van de typische narcolepsiesymptomen. Post-mortem wordt bij narcolepsiepatiënten een selectief verlies van hypocretine-producerende neuronen in de hypothalamus gezien, wat het bijzonder lage gehalte hypocretine-1 in de liquor verklaart. Het mechanisme achter dit specifieke verlies van hypocretine-producerende neuronen is nog niet volledig opgehelderd, maar er wordt gedacht aan een auto-immuunreactie gericht tegen hypocretine-producerende neuronen in de hypothalamus. Het is momenteel niet mogelijk de ziekte te voorkomen of genezen; wat rest is symptomatische behandeling, die een wezenlijke verbetering kan geven. De behandeling omvat gedragsaanpassingen en farmacologische behandeling, met natriumoxybaat (SXB), psychostimulantia en/of antidepressiva.

HLA en narcolepsie

Reeds tientallen jaren wordt gedacht dat narcolepsie een auto-immuunziekte kan zijn. Deze auto-immuunhypothese is vooral gebaseerd op de sterke associatie van narcolepsie met HLA-DQB1*06:02. Narcolepsie met kataplexie heeft van alle ziekten de sterkste associatie met een specifiek HLA-allel. Wereldwijd draagt 85–95% van de patiënten met narcolepsie met kataplexie dit haplotype, terwijl dit in de algemene bevolking slechts 12–38% betreft.
Onder patiënten met een niet-familiaire vorm van narcolepsie en patiënten met typische kataplexie, komt dit percentage zelfs boven de 98%. HLA-DQB1*06:02 lijkt daarom de belangrijkste risicofactor voor het ontwikkelen van narcolepsie te zijn, al zou een aan HLA-DQB1*06:02 nauw gerelateerd gen theoretisch gezien ook verantwoordelijk kunnen zijn voor het verhoogde risico. Als de HLA-DQB1*06:02-DQA1*01:02 (HLA-DQ0602) dimeer daadwerkelijk betrokken is bij het ontstaan van narcolepsie, ligt een dosis-effect voor de hand: een verhoogde expressie van de HLA-DQ0602 zou moeten leiden tot een verhoogde kans op het ontwikkelen van narcolepsie. Dit zou gelden voor personen die homozygoot zijn voor HLA-DQB1*06:02-DQA1*01:02, maar ook voor personen die heterozygoot zijn voor HLA-DQB1*06:02 en homozygoot zijn voor HLA-DQA1*01:02.

In hoofdstuk 2 hebben we onderzoek gedaan naar de allelen die in trans gelokaliseerd zijn met HLA-DQB1*06:02-DQA1*01:02. Zoals verwacht kwam homozygotie voor HLA-DQB1*06:02-DQA1*01:02 vaker voor bij patiënten dan bij controlepersonen. Ook werd er inderdaad een verhoogde prevalentie van HLA-DQA1*01:02 homozygoten gevonden onder patiënten die heterozygoot zijn voor HLA-DQB1*06:02-DQA1*01:02. Beide bevindingen ondersteunen de hypothese dat de HLA-DQB1*06:02-DQA1*01:02-dimeer een directe rol speelt bij de ontwikkeling van narcolepsie.

Een directe rol voor de HLA-DQ0602 dimeer past uitstekend bij de auto-immuunhypothese voor narcolepsie: de functie van een HLA klasse II molecuul als de HLA-DQ0602 dimeer is het presenteren van lichaamsvreemde peptiden aan het immuunsysteem, teneinde een T-cel gemedieerde immuunrespons te genereren. In sommige gevallen vindt er een kruisreactie plaats en genereren de T-cellen, die normaal gesproken op lichaamsvreemde peptiden reageren, een immuunrespons tegen lichaamseigen structuren.

### Narcolepsie en auto-immuniteit

Op zoek naar bewijs voor de auto-immuunhypothese hebben we serum van narcolepsie-patiënten immunohistochemisch onderzocht op antilichamen tegen hypocretine-producerende neuronen (hoofdstuk 3). Vergelijkbare eerdere onderzoeken faalden. Deze voorgaande onderzoeken zijn echter verricht met serum of liquor afkomstig van patiënten met een onbekende of een relatief lange ziekteduur. De huidige gedachtegang is dat de symptomen van narcolepsie zich pas openbaren op het moment dat de overgrote meerderheid van de hypocretine-producerende neuronen is verdwenen. Een auto-
immuunrespons is alleen actief zolang er nog cellen resten waartegen de respons gericht is; daarna stopt de aanval. De auto-immuunrespons bij narcolepsie kan dan ook zeer wel een tijdelijk fenomeen zijn, dat alleen actief is voorafgaand aan of tijdens het eerste optreden van symptomen. Om de kans zo groot mogelijk te maken om antilichamen te vinden, hebben we serum van 21 patiënten met narcolepsie type 1 onderzocht bij wie de klachten recent ontstaan waren. De groep bevatte patiënten die gevaccineerd waren tegen H1N1. Er werden helaas geen antilichamen tegen hypocretine-producerende neuronen gevonden. Deze negatieve bevinding weerspreekt de auto-immunhypothese niet, noch betekent het dat er op geen enkel moment in het ontstaan van de ziekte antilichamen aanwezig zijn.

Veranderde temperatuurregulatie bij narcolepsiepatiënten

De huid- en kernlichaamstemperatuur spelen een belangrijke rol bij de regulatie van slaap en waak.12-14 Waak is geassocieerd met een relatief lage huidtemperatuur en een relatief hoge kerntemperatuur, terwijl slaap geassocieerd is met het omgekeerde patroon. In slaap vallen wordt vooraf gegaan door een daling van de kerntemperatuur en een stijging van de huidtemperatuur. De daling van kerntemperatuur wordt veroorzaakt door een toename van huidperfusie, die tegelijk ook leidt tot een stijging van de huidtemperatuur.15,16 Het lichaam kan hierdoor afkoelen.

Uit eerder onderzoek is gebleken dat narcolepsiepatiënten een ander huidtemperatuurprofiel hebben gedurende de dag, wat een relatie tussen de functie van hypocretine, temperatuur en slaapregulatie suggereert.17-19 Natriumoxybaat (SXB) is een medicijn dat geregistreerd is voor de behandeling van narcolepsie. Gegeven het afwijkende huidtemperatuurprofiel en het positieve effect van SXB op de slaap van narcolepsiepatiënten, zou het kunnen dat het effect van SXB deels bereikt wordt door een herstellend effect van SXB op de temperatuurregulatie.

Hoofdstuk 4 betrof de verschillen tussen kern- en huidtemperatuur bij narcolepsiepatiënten en gezonde controleproefpersonen. Tevens werden de effecten van SXB op de lichaamstemperatuur in relatie tot de effecten op slaap onderzocht. Acht mannelijke narcolepsiepatiënten en acht mannelijke gezonde controlepersonen met óvereenkomstige leeftijd en ‘body mass index’ (BMI), ondergingen een polysomnografie en temperatuurmeting gedurende 24 uur. Gedurende deze tijd verbleven ze in het ziekenhuis in liggende of halfliggende houding (toiletbezoeken uitgezonderd). Ze aten gestandaardiseerde, koude
maaltijden op vaste tijden en mochten dutjes doen. Aansluitend aan de eerste metingen namen de proefpersonen gedurende vijf aaneengesloten dagen SXB. De volgende 24-uurs meting werd verricht op de 5e dag van inname van SXB.

De eerste meting leverde een lagere kerntemperatuur en proximale huidtemperatuur op bij narcolepsiepatiënten. Dit verschil werd vooral veroorzaakt door significante verschillen gedurende de dag. In tegenstelling tot eerdere onderzoeken vonden we geen verschillen in distale huidtemperatuur. Omdat een liggende houding de distale huidtemperatuur verhoogt,20 denken we dat dit ontbrekende verschil mogelijk het gevolg was van een hogere distale huidtemperatuur bij de controlepersonen. Inname van SXB resulteerde bij patiënten in een gedeeltelijke normalisatie van het huidtemperatuurprofiel, ten eerste door verhoging van de proximale huidtemperatuur tot waarden vergelijkbaar met gezonde controleproefpersonen en ten tweede door versterking van het bekende verband tussen huidtemperatuur en de kans om overdag in slaap te vallen.

In aansluiting op dit gestandaardiseerde onderzoek in een laboratoriumomgeving, werd ook een vergelijkbaar onderzoek verricht bij ambulante patiënten en controle proefpersonen (hoofdstuk 5). Daartoe ondergingen 25 narcolepsiepatiënten en 15 gezonde proefpersonen een ambulance 24-uurs temperatuurmeting en een polysomnografie bij aanvang van het onderzoek. Na behandeling met SXB gedurende minimaal drie maanden werden deze metingen bij 16 patiënten nogmaals verricht. Het doel van het onderzoek was om de temperatuurregulatie en slaap bij narcolepsie type 1 patiënten verder te onderzoeken, met een nadruk op de situatie in het dagelijks leven. Tevens konden we bestuderen of spontane dutjes overdag vooraf gegaan worden door veranderingen in huidtemperatuur.

Tijdens de nulmeting bleken patiënten ’s nachts een hogere kerntemperatuur en een lagere distale huidtemperatuur te hebben en ’s ochtends een hogere proximale en distale huidtemperatuur. Aangezien slaap gepaard gaat met een lage kerntemperatuur en een hoge distale huidtemperatuur, zou de gestoorde nachtslaap bij narcolepsiepatiënten verklaard kunnen worden door deze hogere kerntemperatuur en lagere distale huidtemperatuur ’s nachts. Behandeling met SXB resulteerde in een daling van de nachtelijke kerntemperatuur tot een niveau vergelijkbaar met gezonde proefpersonen. Het normaliseren van de nachtelijke kerntemperatuur zou een rol kunnen spelen bij de reeds bekende verbetering van nachtslaap als gevolg van behandeling met SXB.21 Daarnaast werd een afname van de slaapduur en het aantal dutjes overdag gezien tijdens behandeling met SXB. Een opvallende bevinding was dat in slaap vallen overdag werd voorafgegaan door een
duidelijke temperatuursverandering. Vooral een stijging van de distale huidtemperatuur en distale-proximale gradiënt (DPG) gedurende vijf minuten voor het in slaap vallen was heel sterk geassocieerd met het in slaap vallen overdag, maar ook in de periodes 15 minuten voor en 30 seconden voor het in slaap vallen was een significant verband te zien.

Het meten van behandeleffect bij narcolepsie: de ‘Sustained Attention to Response Task’

Zonder twijfel heeft narcolepsie een grote invloed op het dagelijks leven. Een steeds beter erkende factor daarbij is dat patiënten met narcolepsie niet goed wakker zijn, waarbij het vermogen een tijd lang de aandacht ergens bij te houden (vigilantie) belangrijk is. De ‘Sustained Attention to Response Task’ (SART) is ontworpen om dit aspect te meten. De SART is een ‘actie’/’geen actie’ beslissingstaak, waarbij de ‘geen actie’ taak slechts sporadisch en onvoorspelbaar voorkomt; zowel nauwkeurigheid als reactietijd zijn belangrijk zijn. Deze test is eerder gebruikt bij narcolepsiepatiënten en heeft de potentie de verminderde aandacht tijdens het wakker zijn te kwantificeren.22,23

In hoofdstuk 6 wordt de SART gevalideerd als meetinstrument voor behandeleffecten bij narcolepsie. De analyses zijn verricht op gegevens die verkregen zijn gedurende een dubbelblind onderzoek met parallelle groepen in meerdere centra. Gedurende dit onderzoek werden bij narcolepsiepatiënten de effecten van 8 weken behandeling met het experimentele medicijn pitolisant (een antagonist van de histamine H3 receptor) vergeleken met behandeling met het effectieve medicijn modafinil en met een placebo.24

De ernst van overmatige slaperigheid overdag en kataplexie werden beoordeeld door een plaatselijke onderzoeker met behulp van de ‘Clinical Global Impression of Severity (CGI-S)’ en verandering in de ernst van overmatige slaperigheid overdag en kataplexie werd beoordeeld middels de ‘Clinical Global Impression of Change (CGI-C)’.25 Deze schalen werden vergeleken met de SART, de Maintenance of Wakefulness Test (MWT) en de Epworth Slaperigheidsschaal (ESS). Op basis van deze analyses hebben we geconcludeerd dat twee tot drie SART sessies, in combinatie met één afnamemoment van de ESS, een goede methode vormen om behandeleffecten bij narcolepsiepatiënten te meten. Deze testbatterij omvat de twee belangrijkste aspecten van narcolepsie, namelijk slaperigheid en volgehouden aandacht, en is bovendien goedkoop en eenvoudig af te nemen.
Samenvatting, conclusies en toekomstperspectieven

TOEKOMSTPERSPECTIEVEN

De ontdekking van hypocretine-1 deficiëntie bij patiënten met narcolepsie type 1 eind vorige eeuw betekende een grote vooruitgang in het begrip van het pathofysiologische mechanisme achter narcolepsie. Door de sterke associatie met HLA-DQB1*06:02 is de aandacht hierbij vervolgens vooral uitgegaan naar auto-immuniteit. Hoewel er steeds meer ondersteunende bevindingen voor deze hypothese worden gedaan, is er nog geen direct bewijs gevonden. De meeste genetische bevindingen duiden op betrokkenheid van T-cellen. Tevens wordt er een opvallende toename in voorkomen van narcolepsie gezien na infecties, in het bijzonder na infecties met H1N1 en vaccinatie tegen dit virus. Het door ons gevonden dosiseffect van HLA-DQ0602 ondersteunt de T-cel auto-immunhypothese.

Met deze resultaten in het achterhoofd zou toekomstig onderzoek gericht kunnen worden op de mogelijkheid dat narcolepsie inderdaad een door T-cellen tot stand komende auto-immuunziekte is. Het aantonen hiervan zal erg lastig zijn: de immuunreactie is bij narcolepsie waarschijnlijk tijdelijk en kortdurend, en gaat bovendien waarschijnlijk vooraf aan het optreden van symptomen, zodat de periode waarin de immuunreactie vast te stellen is mogelijk al is afgelopen op het moment dat de patiënt de diagnose krijgt. Tevens zou deze immuunreactie beperkt kunnen zijn tot het centraal zenuwstelsel, wat de vaststelling lastiger maakt dan als er een systemische immuunreactie is. Dit laatste probleem wordt veroorzaakt door het feit dat er bij maar weinig patiënten lymfocyten in de liquor aanwezig zijn. Als gevolg van deze lage concentratie lymfocyten is het moeilijk om actieve T-cellen te detecteren en is een vrij grote hoeveelheid liquor voor onderzoek nodig. Desondanks zullen toekomstige onderzoeken zich moeten richten op T-cel auto-immuniteit in de liquor van patiënten die sinds kort klachten van narcolepsie hebben. Daarnaast zou er extra aandacht moeten zijn voor een doorgemaakt infectie met H1N1 of een daartegen gerichte vaccinatie.

Dit proefschrift beschrijft verschillen in kerntemperatuur en huidtemperatuur tussen narcolepsiepatiënten en gezonde personen. Het onderzoek is zowel in een klinische omgeving als in een ambulante omgeving verricht; met name dit laatste aspect was nog nooit zo uitvoerig onderzocht. Het onderzoek bevestigde dat narcolepsiepatiënten een ander temperatuurprofiel hebben dan gezonde proefpersonen.

Behandeling met SXB resulteerde in een gedeeltelijke normalisatie van dit afwijkende temperatuurprofiel, met name gedurende de nacht. Deze veranderingen hebben mogelijk
te maken met de verbetering van de nachtelijke slaap die gezien wordt tijdens behandeling met SXB bij narcolepsiepatiënten. Het is echter niet duidelijk in hoeverre de tijdens behandeling met SXB gevonden temperatuursveranderingen overdag gerelateerd zijn aan het in slaap vallen overdag. Herhaling van onze onderzoeken met meer ambulante patiënten en gezonde personen zou daar meer inzicht in kunnen geven, al is het de vraag in hoeverre dit tot nieuwe klinisch relevante inzichten leidt. Toekomstige onderzoeken kunnen mogelijk beter gericht worden op het verband tussen een stijging van de distale huidtemperatuur en DPG met het ontstaan van slaapaanvallen: kunnen deze temperatuursveranderingen het in slaap vallen betrouwbaar voorspellen? Als in slaap vallen kan worden voorspeld aan de hand van deze temperatuursveranderingen, zou dit kunnen leiden tot methoden om narcolepsiepatiënten te waarschuwen en mogelijk zelfs kunnen helpen met het voorkomen van het in slaap vallen op ongewenste momenten. Daarnaast zou manipulatie van distale huidtemperatuur therapeutische mogelijkheden kunnen bieden, ook dit zal verder uitgezocht moeten worden.

Nu de SART gevalideerd is als meetinstrument om behandel Effecten bij narcolepsie te meten, kan het worden gebruikt om vigilantie (volgehouden aandacht) te meten bij narcolepsiepatiënten. Naast het evalueren van behandel Effecten van bestaande of nieuwe medicijnen, zou deze test ook een rol kunnen spelen bij de beoordeling van iemand geschikt is om een motorvoertuig te besturen. Op dit moment wordt alleen de MWT voor dit doel gebruikt. De MWT wordt echter verricht in een kunstmatige omgeving die geenszins lijkt op het besturen van een auto of deelnemen aan het verkeer. Het meten van vigilantie lijkt dichter te liggen bij de voorwaarde om een motorvoertuig veilig te besturen, namelijk continue alertheid, dan datgene wat de MWT meet, namelijk het vermogen wakker te kunnen blijven in een half liggende houding in een rustige kamer met gedimd licht.

REFERENCES

Samenvatting, conclusies en toekomstperspectieven


Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration,
National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural
Research Programs; 1976.


27. de Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with


29. Faraco J, Lin L, Kornum BR, et al. ImmunoChip study implicates antigen presentation to T cells in


32. Koepsell TD, Longstreth WTJ, Ton TGN. Medical exposures in youth and the frequency of narcolepsy
with cataplexy: a population-based case-control study in genetically predisposed people. J Sleep Res
2010;19:80–86.


34. Han F, Lin L, Warby SC, et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1

35. Wijnans L, Lecomte C, de Vries C, et al. The incidence of narcolepsy in Europe: before, during, and
after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. Vaccine 2013;31:1246–1254.

increase in the incidence of childhood narcolepsy in Finland. PLoS ONE 2012;7:e33536.


38. Schuld A, Uhr M, Pollmächer T. Oligoclonal bands and specific antibody indices in human narcolepsy.

ABOUT
THE AUTHOR
CURRICULUM VITAE

Astrid van der Heide was born on April 22, 1984 in Leeuwarden. She attended secondary school at “Slauerhoff (later named Piter Jelles) college” and graduated in 2002. In the same year she started her study of medicine at the University of Groningen. In 2008, she performed a scientific traineeship at the department of Child Neurology and Clinical Neurophysiology of the University Medical Centre in Utrecht (supervisors Dr. F.E. Jansen and Prof. Dr. A.C. van Huffelen). This traineeship focussed on the identification of the epileptogenic zone in patients with tuberous sclerosis. After obtaining her medical degree in 2009 (cum laude), she became resident (not in training) in neurology at the Rijnland Hospital in Leiderdorp (Dr. J. Haan). In the same year she started her PhD research at Leiden University Medical Centre, under supervision of Prof. Dr. J.G. van Dijk and Dr. G.J. Lammers, which investigations are described in this thesis. Her neurology residency program started in 2011 (Prof. Dr. R.A.C. Roos and Prof. Dr. J.J. van Hilten). Part of her training was at the St. Antonius Hospital in Nieuwegein (Dr. S.C. Tromp) and at the Haga Hospital in The Hague (Dr. S.F.T.M. de Bruijn). In 2016, she was trained in pediatric neurology at the University Medical Centre in Utrecht (Prof. Dr. K.P.J. Braun). She expects to qualify as a (pediatric) neurologist in 2018.
LIST OF PUBLICATIONS


Book chapter