Unravelling narcolepsy : from pathophysiology to measuring treatment effects

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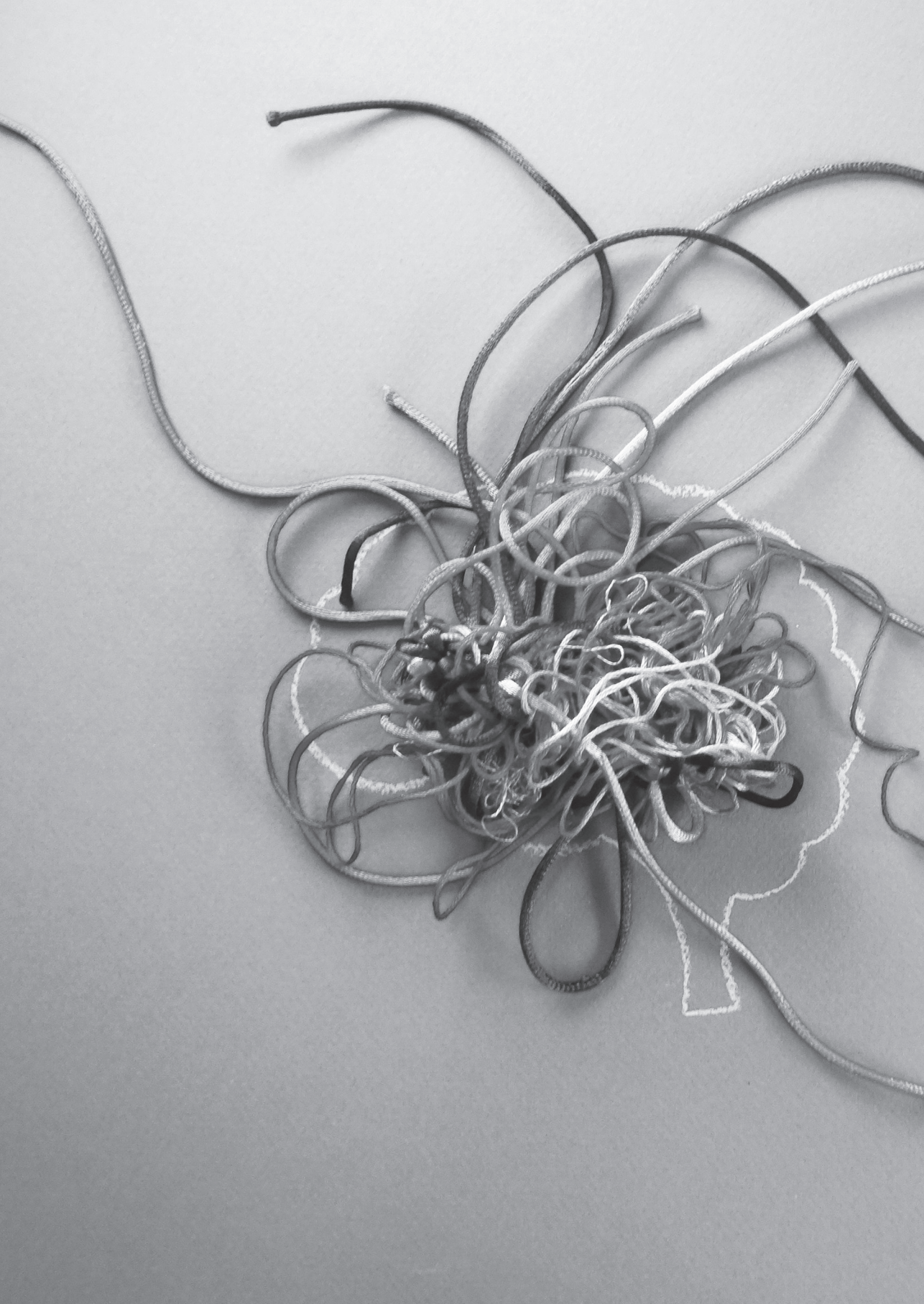

CHAPTER 7

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, focussing 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),[1] 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.[2] Post mortem studies demonstrated a selective loss of hypocretin-containing neurons in the hypothalamus.[3-5] 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*.[6,7] 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.[6] For non-familial cases and those with typical cataplexy the rate may even exceed 98% of cases.[7] *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 *HLA-DQB1*06:02-DQA1*01:02* (*HLA-DQ0602*) dimer would itself be involved in the aetiology of narcolepsy, a dosage effect would be expected: an increased expression of the *HLA-DQ0602* dimer *should* be associated with a higher susceptibility to the development of narcolepsy. This would be the case 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*.

In **Chapter 2** we investigated the *HLA-DQ* alleles located in *trans* with *HLA-DQB1*06:02-DQA1*01:02*. As expected, homozygosity for *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 *HLA-DQA1*01:02* in *HLA-DQB1*06:02-DQA1*01:02* heterozygous narcolepsy patients. Both these findings support a direct role of the *HLA-DQB1*06:02-DQA1*01:02* dimer molecule in the development of narcolepsy.

This direct role of the *HLA-DQ0602* dimer fits perfectly well with the autoimmune hypothesis in narcolepsy: the function of HLA-class II molecules like the *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.

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 (**Chapter 3**). Similar studies in the past have not been successful.[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.[12-14] 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.[15,16]

In narcolepsy, an altered diurnal profile of skin temperature has been demonstrated, suggesting a relationship between hypocretin function, temperature and sleep regulation.[17-19] 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.[20] 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 a 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.[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,[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.[24] 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).[25] 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,[26,27] 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.[28-31] Furthermore, a remarkable increased incidence of narcolepsy was reported after infections, in particular after H1N1 vaccination and infection with H1N1.[32-37] 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.[38] 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.

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