Unravelling narcolepsy : from pathophysiology to measuring treatment effects

Heide, A. van der

CHAPTER 1
GENERAL INTRODUCTION

Astrid van der Heide
Gert Jan Lammers

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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 it 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.[7] 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

Figure 1.1 Photographs showing a complete cataplectic attack. Note that it takes several seconds for the attack to develop fully.

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'.[8]

Cataplexy is most often provoked by an emotional trigger, or its anticipation.8 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.[8] 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.[9] 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.[10] 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.[10]

HH are not specific for narcolepsy with cataplexy, since they are also present in the general population and in other sleep disorders.[11] However, the prevalence, and probably also the frequency, are higher in narcolepsy.[12]

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.[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,[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^2, a substantially higher proportion than the 12.5% which holds for the Dutch population.[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.[15] Periodic limb movements in sleep (PLMS) have been described in up to two thirds of the subjects with narcolepsy type 1.[16] 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.[17]

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.[18]

DIAGNOSIS

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

Table 1.1 ICSD-3 diagnostic criteria of narcolepsy type 1

Criteria A and B 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.[1]
B. The presence of one or both of the following: 1. Cataplexy *and* a mean sleep latency of ≤ 8 minutes and two or more sleep onset REM periods (SOREMPs) on an MSLT performed according to standard techniques. A SOREMP (within 15 minutes of sleep onset) on the preceding nocturnal polysomnogram may replace one of the SOREMPs on the MSLT.[2] 2. 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.

[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.[19] 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.[20] 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 hypersomnia (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.[21] 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 SOREMP (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

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

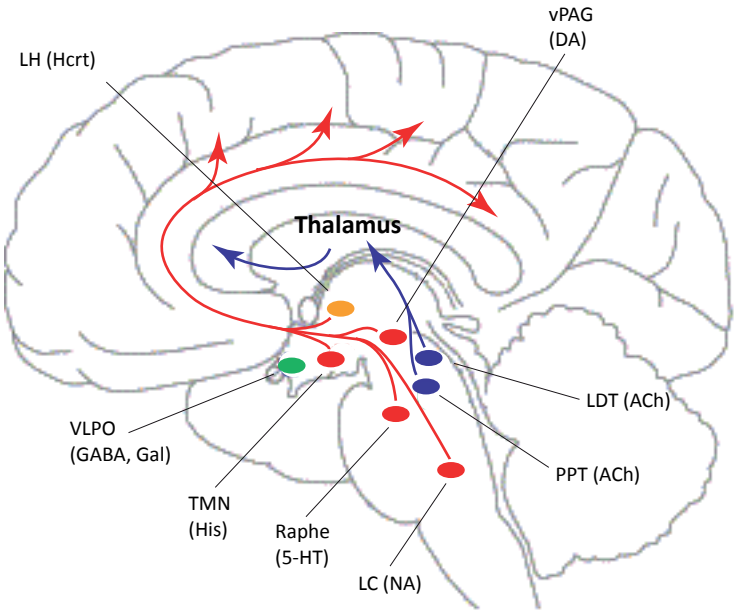

Figure 1.2 Projections of hypocretin.
Hypocretin neurons, located in the lateral hypothalamus, innervate all nuclei of the AAS and the entire cerebral cortex.
Abbreviations: GABA: γ-aminobutyric acid; Gal: galanin; TMN: tuberomammillary nuclei; His: histamine; LH: lateral hypothalamus; Hcrt: hypocretin; 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.

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.[30] 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.[31] 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.[32,33]

'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.

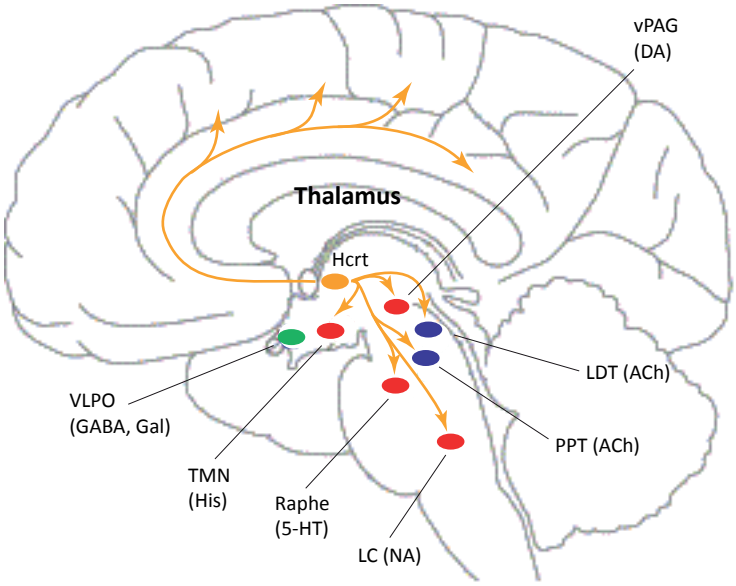

Figure 1.3 Ascending arousal system (AAS).
The AAS comprises two pathways: (1) *Monoaminergic branch (red)*; the locus coeruleus (LC), dorsal and median raphe nuclei (Raphe), tuberomammillary nuclei (TMN) and ventral periaqueductal grey matter (vPAG) project diffusely to the cortex of the entire hemisphere. This pathway receives also contribution from peptidergic neurons in the lateral hypothalamus (LH) containing hypocretin (Hcrt). (2) *Cholinergic branch (blue)*; the pedunculopontine and laterodorsal tegmental nuclei (PPT, LDT), project to the thalamic reticular nucleus via thalamic relay neurons and activate the cerebral cortex. Abbreviations: VLPO: ventrolateral preoptic nucleus; GABA: γ-aminobutyric acid; Gal: galanin; His: histamine; DA: dopamine; 5-HT: serotonin; ACh: acetylcholine; NA: noradrenaline.

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

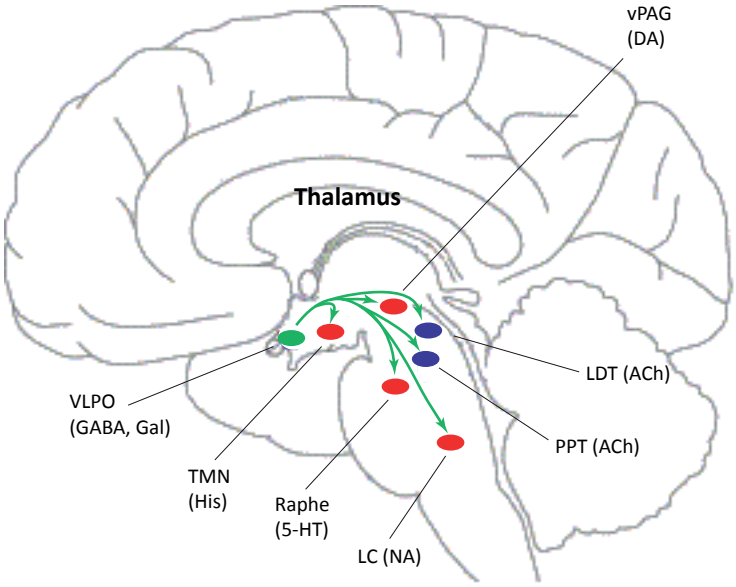

Figure 1.4 Projections of the ventrolateral preoptic nucleus (VLPO).
The VLPO has projections to the nuclei of both the monoaminergic branch (red) and the cholinergic branch (blue).
Abbreviations: GABA: γ-aminobutyric acid; Gal: galanin; TMN: tuberomammillary nuclei; His: histamine; vPAG: ventral periaqueductal 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.

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

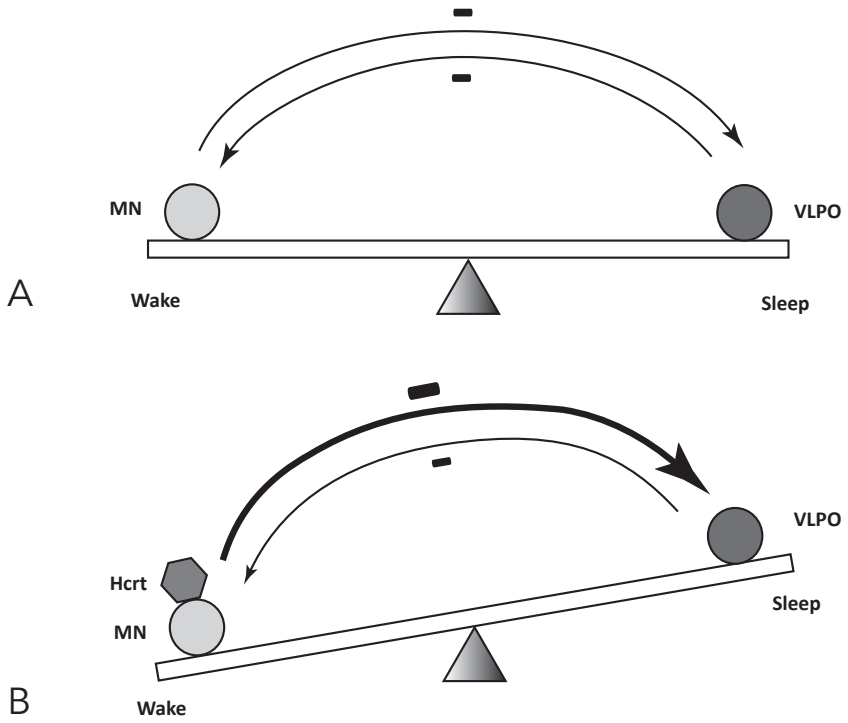

Figure 1.5 The 'sleep-switch'.
The sleep-switch describes a feedback loop with a self-reinforcing firing pattern resulting in two possible states: sleep and wake. (A) The reciprocal inhibition of the ventrolateral preoptic nucleus (VLPO) and the monoaminergic nuclei (MN). It prevents the occurrence of 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.

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'.[36] 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

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,[37] 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.[39] 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.[40] 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,[47,48] 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

risk.[44,48,49] Furthermore, a two to four times increased risk of developing narcolepsy is reported in Caucasians homozygous for *DQB1*06:02*.[46,50]

IS NARCOLEPSY AN AUTOIMMUNE DISEASE?

An autoimmune aetiology for narcolepsy has been hypothesised for decades.[51] The aetiology of autoimmune diseases is multifactorial, principally encompassing genetic and environmental factors.[52] 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*.[53-56] 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.[57-60] In 2010, three independent groups reported elevated levels of antibodies against a protein that is produced in hypocretin neurons, Tribbles homolog 2 (Trb2).[61-63] 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.[64]

Possible environmental factors involved in autoimmunity are infections.[65] An increased incidence is seen after infections with Streptococcus pyogenes and influenza type A virus, in particular H1N1.[66-68] Concerning H1N1, a role of vaccination was presumed, but not confirmed.[69,70] Furthermore, an increased onset was reported in the months following winter related infections in the Chinese population.[68] 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.[6]

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 s 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.[79] 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.[80]

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.[81]

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.[82]

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.[76,77] Selegiline and brofaromine may alleviate EDS as well.[75]

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).[5,74,75] Very low doses, such as 20 mg, may sometimes be remarkably effective. Most authors consider clomipramine to

be the treatment of choice.[83] 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, femoxitine, fluvoxamine and paroxetine in a relative higher dosage than the tricyclics.[5,75,84] 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.[85]

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.[86]

SXB is the best-studied drug and is a very potent inhibitor of cataplexy.[87] 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.

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