Hypocretin deficiency : neuronal loss and functional consequences

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Summary & General Discussion

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In the first part of this thesis the question was examined whether or not hypocretin neurons are lost in neurological disorders in which sleep disturbances similar to those in narcolepsy occur. Furthermore, a screening for auto-antibodies was described, aimed at finding evidence for a putative autoimmune aetiology of human narcolepsy, followed by a report on a placebo-controlled double-blind N=1 trial with intravenous immunoglobulins (IVIg) in one narcoleptic patient.

In the second part of this thesis the consequences of a loss of hypocretin neurons were examined, with a focus on non-sleep-related symptoms of narcolepsy, i.e. obesity (metabolism and autonomic control), vigilance impairment and skin temperature regulation.

Part I: The Hypothalamus and its Hypocretin Neurons

We investigated hypocretin function in other neurodegenerative disorders that are often accompanied by narcolepsy-like sleep disturbances, such as Alzheimer's Disease, Parkinson's Disease and Huntington's Disease. Furthermore, hypocretin functioning was assessed in patients with Prader-Willi syndrome, because of case reports describing cataplexy and sleep disturbances in this syndrome.[1]

Prader-Willi Syndrome.

Prader-Willi Syndrome is characterized by mental retardation, hypogonadism, growth deficiency and most notably by an insatiable hunger. Prader-Willi Syndrome is the most common genetic cause of obesity.[2] Furthermore, patients suffer from excessive daytime sleepiness,[3] and some case reports suggest that a minority of patients experience cataplexy-like attacks.[1] CSF hypocretin values are normal in Prader-Willi Syndrome,[4] although slightly lower than normal values have been reported.[5] To determine whether the hypocretin system is involved, we studied post-mortem hypothalami of eight adult and three infant Prader-Willi Syndrome patients and 11 controls. No difference in the total number of hypocretin-containing neurons was found between Prader-Willi Syndrome patients and controls.

> The number of hypothalamic hypocretin neurons is not abnormally low in Prader-Willi Syndrome. A decline in the number of hypocretin neurons is not the most likely cause of the excessive daytime sleepiness and possible cataplexy reported in this syndrome.
>
> ***Chapter 1***

Future perspectives

Unfortunately no clinical information about sleep disturbances or cataplexy was available for the hypothalami that we studied. The possibility remains that a reduction in the total number of hypocretin neurons can still be found in a few Prader-Willi Syndrome patients with clear-cut cataplexy. Evidence for changes in hypocretin gene expression in Prader-Willi Syndrome has been reported.[6] To further study this possibility, post-mortem material would have to be collected from specific patients with sleep disturbances and clear-cut cataplexy. However, this is unlikely to occur in the near future. Another possibility would be to collect cerebrospinal fluid from these patients, since it can be expected that a loss of hypocretin large enough to result in cataplexy will also be reflected in low CSF levels.

Another explanation for sleep disturbances and cataplexy in Prader-Willi Syndrome might be found in malfunction of the hypocretin receptors. Therefore, we have tried to visualize the hypocretin receptor in post-mortem material from Prader-Willi Syndrome patients, but this proved to be a difficult task due to the low expression of the receptor, specificity problems with the available commercial antibodies and the fixation method of the available Prader-Willi Syndrome and control material. Future experiments should focus on visualizing the hypocretin receptor in frozen tissue using in situ hybridization or more specific antibodies.

Normal Aging

When the control subjects from the Prader-Willi Syndrome study were analyzed together, the total number of hypocretin neurons tended to decline with age.

> The number of hypothalamic hypocretin neurons tends to decline with age in healthy control subjects.
>
> ***Chapter 1***

Future perspectives

The finding that the total number of hypocretin neurons tends to decline with age, raises the interesting question whether this would mean that the hypocretin system would be affected to a greater extent in a brain showing advanced ageing, i.e. Alzheimer's Disease. In addition, it would be of interest to relate the number of hypocretin neurons in controls to the presence of the major histocompatibility complex (MHC) subtype of the immune system that is seen in more then 90% of all narcolepsy with cataplexy patients (HLA, Human Leukocyte Antigen, DQB1*0602). The majority of these patients are hypocretin deficient, which raises the intriguing question whether healthy controls with the same HLA type but without the narcoleptic phenotype, would have a partial loss of hypocretin.

Parkinson's Disease

Although Parkinson's Disease (PD) is primarily characterized by motor symptoms such as tremor and rigidity, sleep disturbances occur often, and include excessive daytime sleepiness, fragmented nocturnal sleep and rapid eye movement (REM)-sleep behavior disorder.[7,8] The combination of these symptoms suggests an overlapping etiology with narcolepsy.[9] Hypocretin levels in CSF were reported to be normal in Parkinson's Disease when samples were obtained using a spinal tap, [10-12] but another study reported low or even absent levels in ventricular CSF.[13] To assess hypocretin function in Parkinson's Disease we determined the total number of hypocretin containing neurons in nine PD patients and nine controls. Hypocretin levels were also determined in post-mortem ventricular CSF of these subjects. Furthermore, cortical brain tissue hypocretin levels were determined in nine PD patients and 16 controls. We found that the hypocretin system was affected in PD. The hypocretin concentration in the cortex was almost 40% lower in PD patients than in controls. Ventricular CSF levels were lower by almost 25%. The total number of hypocretin neurons was about one half of that of controls. In rodents, a reduction in the number of hypocretin neurons of 60-70% results in REM sleep disturbances, which suggests that the cell loss in PD can explain at least part of the sleep disturbances commonly seen in this disorder.

> The hypocretin system is affected in Parkinson's Disease. The total number of hypocretin neurons is 50% lower, the hypocretin concentration in the cortex is 40% lower and the concentration in ventricular CSF is 25% lower in Parkinson's Disease compared to controls. This could at least partly explain the sleep disturbances commonly seen in this disease.
>
> ***Chapter 2***

Future perspectives

The functional relevance of a loss of hypocretin neurons in Parkinson's Disease still needs to be studied. This could involve studying post-mortem hypothalami of Parkinson's Disease patients, combined with a thorough documentation of their sleep disturbances in the last few years of their lives. This would be a difficult task to accomplish. Regrettably, sleep problems, such as excessive daytime sleepiness and REM sleep behavior disorder, are not objectively documented in the clinical histories of the currently available post-mortem material in a way that permits systematic research. Another possibility for future research in patients with Parkinson's Disease could be to perform a treatment trial with narcolepsy medication, for instance gamma hydroxybutyrate or hypocretin-agonists when available for human use. Another option would be administration of the now available hypocretin antagonists to healthy controls to assess the effects of a partial loss of hypocretin neurotransmission.

Huntington's Disease

Huntington's Disease is a neurodegenerative genetic trinucleotide repeat disorder with a dominant mode of inheritance characterized by abnormal dance-like body movements (chorea) and personality changes. Furthermore, patients suffer from severe weight loss, sleep disturbances and autonomic dysfunction, which could partly be due to alterations in hypocretin signalling.[14] Although spinal CSF hypocretin levels were normal in human patients,[15-18] the density of hypocretin neurons was reported to be decreased in two mouse models of the disease.[19] In order to validate and extend these data in Huntington's Disease patients, we counted the total number of hypocretin neurons in 8 HD patients and 8 controls. Hypocretin levels were also measured in post-mortem ventricular CSF of these subjects. Furthermore, cortical brain tissue hypocretin levels were determined in 19 HD patients and 16 controls. Both the total number of hypocretin neurons and the hypocretin concentration in the cortex were 30% lower in HD patients. However, ventricular CSF hypocretin levels were similar to controls. This reduction in hypocretin signalling is in contrast with the strong reduction seen in the R6/2 mouse model of the disease and the contribution to the clinical symptoms of HD patients remains to be investigated.

> The number of hypocretin containing neurons is 30% reduced in Huntington's Disease. This is in contrast with the strong reduction seen in the R6/2 mouse model of the disease. The contribution to the symptoms of HD patients remains to be investigated.
>
> ***Chapter 3***

Future perspectives

As in Parkinson's Disease, the contribution of a loss of hypocretin neurons to the sleep disturbances seen in Huntington's Disease needs to be studied. Again, sleep problems in Huntington's Disease patients are neither well described nor objectively documented. As such, the currently available post-mortem material does not permit systematic research. As in Parkinson's Disease, narcolepsy medication may improve sleep symptoms in Huntington's Disease.

Narcolepsy: Screening for Autoantibodies

In narcolepsy there is a severe decrease (>95%) of hypocretin containing neurons in the lateral hypothalamus,[20] leading to a general absence of hypocretin in the cortex[21] and in CSF.[22] It is not known how these neurons disappear. The most popular hypothesis concerns an autoimmune process that selectively targets hypocretin neurons. However, no direct evidence for this putative autoimmune process has so far been found. We screened the CSF of 54 patients and the serum of 76 patients and 63 controls for the presence of autoantibodies directed against neurons in the lateral hypothalamus. Detectable autoantibodies were present in only two patients, but also in two controls. Therefore, as shown by immunostaining, humoral immune mechanisms appear not to

play a major role in the pathogenesis of narcolepsy, at least not in the clinically overt stage of the disease.

> As shown by immunostaining, no direct evidence has been found to support the prevailing theory that humoral immune mechanisms (autoantibodies) play a role in the selective loss of hypocretin containing neurons in narcolepsy.
>
> ***Chapter 4***

Future Perspectives

Since the screening was performed on formalin fixed paraffin embedded tissue, a future screening on frozen hypothalamic tissue, although technically challenging, might be advisable. At this stage, it is still a mystery why hypocretin disappears. Lacking evidence for an autoimmune hypothesis, it is important to keep an open mind regarding the cause of narcolepsy. T cells are a fundamental part of many auto-immune diseases, and cellular immune mechanisms should also be examined. In type 1 diabetes mellitus, islet-cell antibodies are abundant around disease onset, but 5 to 10 years later, titers are much lower. Because the symptoms of narcolepsy usually stabilize within the first few years, it is likely that any auto-immune process settles down over time. It is thus advisable to study serum and CSF very soon after disease onset, presumably when the inflammation is ongoing.

Narcolepsy: Trial with Intravenous Immunoglobulins

In line with the prevailing autoimmune theory to explain the pathogenesis of narcolepsy, treatment with high-dose prednisone after acute manifestation of hypocretin deficiency has been tried in an 8-year old boy.[23] This was not effective. However, two open-label studies suggested that treatment with intravenous immunoglobulins (IVIg) shortly after disease onset may dramatically reduce the frequency and severity of cataplexy.[24,25] We performed a double-blind N=1 study in a 55 year old female narcolepsy patient who was suffering from typical narcolepsy with severe cataplexy for 7 years. Open label treatment with IVIg resulted in what appeared to be a dramatic success. However, this striking effect disappeared during the subsequent double-blind placebo-controlled n=1 trial, in which there was no difference between placebo and IVIg treatment. Nevertheless, the placebo effect was impressive. The patient reported fewer cataplectic attacks after the first drug administration of the study, which concerned the placebo. Our findings stress the need for strict adherence to common methodological standards involving blinding and the use of a placebo for future trials.

> During a double-blind placebo-controlled n=1 trial there was no difference between placebo and IVIg treatment. The placebo effect was impressive.
>
> ***Chapter 5***

Future Perspectives

Regarding the effect of IVIg on cataplexy, a double-blind placebo controlled study is needed, in which well-documented baseline measurements are mandatory to control for placebo effects.

Part II: When Hypocretin Neurons are Absent: Narcolepsy

Vigilance

Excessive daytime sleepiness (EDS) is considered to be the main complaint in narcolepsy.[26] However, this focus on inadvertently falling asleep may have led to undervaluation of the perhaps most serious complaint: impaired performance in the waking state.[27] This realisation suggested that tests aiming to measure vigilance might be useful in narcolepsy. The Sustained Attention to Response Task (SART) appeared to be a good candidate.[28] This test takes only a short time to perform and is easy to administer, which make it useful in a clinical setting. To explore the role of the SART in quantifying vigilance as an essential aspect of the severity of narcolepsy, we compared the SART with 2 instruments commonly used to measure sleepiness: the MSLT [29] and the Epworth Sleepiness Scale (ESS).[30] We found that the SART, measuring attention, was abnormal as often as the MSLT, measuring sleepiness. Still, the two tests measure different aspects of the disease, as SART and MSLT results showed no correlation with each other or with the Epworth Sleepiness Scale. The range of the MSLT latency was considerably larger in controls than in patients, while the reverse applied to an even stronger degree for the range of the SART error scores.

> Difficulty in remaining vigilant during the day may be the most serious concern in narcolepsy, since it impairs performance. The Sustained Attention to Response Task quantifies this neglected symptom and is valid, easy to administer and takes little time to perform.
>
> ***Chapter 7***

Future Perspectives

Vigilance impairment is an important aspect of narcolepsy that deserves more attention. The fact that the range of SART results in narcoleptic patients is large, may be advantageous, in that it may offer a better resolution to quantify vigilance as a severity indicator of narcolepsy, which may be of use in measuring treatment effects. However, before the SART can be used in treatment trials and other studies, its sensitivity to treatment effects has to be studied in more detail. This could involve administration of the SART on multiple timepoints during several testing days as a long-term measure for vigilance before and after treatment in narcolepsy and possibly other sleep disorders.

Obesity

Obesity is a consistent feature of narcolepsy.[31] The identification of hypocretin deficiency as the cause of human narcolepsy with cataplexy and the potential role of hypocretin peptides in metabolic control has sparked interest in the pathophysiology of obesity in narcolepsy. Obviously, eating too much or moving too little are straightforward explanations. In contrast to such expectations narcoleptic subjects in fact consumed less food than healthy controls,[32] while there were no signs pointing to a reduced amount of physical activity.[33] Therefore, the link between hypocretin deficiency and obesity must be less straightforward than assumed. We studied basal metabolic rate and variation in blood pressure and heart rate in hypocretin-deficient narcoleptic subjects and healthy controls, hypothesizing that sympathetic tone might be diminished and/or that basal metabolic rate would be reduced in narcoleptic subjects. We did not find a reduced basal metabolic rate in narcoleptic subjects. However, we did find a higher variability in heart rate and blood pressure, which could point to a changed sympathetic tone. The role of this latter finding in the pathophysiology of obesity in narcolepsy remains to be elucidated.

> Using indirect calorimetry no reduced basal metabolic rate in hypocretin deficient narcoleptic humans can be detected. However, a higher variability in heart rate and blood pressure could point to a reduced sympathetic tone. The role of this latter finding in the pathophysiology of obesity in narcolepsy remains to be elucidated.
>
> ***Chapter 8***

Future Perspectives

We did not find a change in basal metabolic rate in human patients. A normal basal metabolic rate has also been observed in hypocretin knock-out rodents (C. Sinton, personal communication). However, it was found that energy expenditure is reduced in the ataxin-3 mouse model of narcolepsy, with profound sleep/wake fragmentation as the leading cause.[34] Indirect calorimetry in the fasted, rested state could be too insensitive to detect subtle changes in basal metabolic rate. It is thus of interest to study metabolism using more sensitive methods. A study using a 24-hour metabolic chamber in combination with doubly labelled water should provide the most reliable information about metabolism in narcoleptic patients. Furthermore, research might address autonomic regulation in narcolepsy. However, this is not an easy task, as the sympathetic and parasympathetic efferent command streams to relevant target organs cannot be measured directly. Concepts such as sympathetic tone and sympathovagal balance are frequently used, but difficult to validate. The finding of an increased variation in heart rate and blood pressure should be replicated.

Skin Temperature

In healthy subjects there is a relation between skin temperature and sleep. When the temperature of the distal skin (hands and feet) increases relative to the temperature

of the proximal skin, the process of falling asleep is facilitated.[35] This increase in the temperature of the hands and feet results from increased blood flow in the skin of the extremities and is, among other factors, controlled by the hypothalamic circadian clock, as is sleep.[36] Because narcolepsy is characterized by hypothalamic alterations, we studied skin temperature in narcoleptic patients. We found that the distal skin temperature was higher in narcoleptic subjects compared to healthy controls throughout the day in the waking state, while the proximal skin temperature was lower. The increase in the gradient between the distal and the proximal skin temperature (the distal-to-proximal gradient, DPG) was related to a shorter subsequent sleep latency. Once asleep, narcoleptics maintained their elevated distal skin temperature and DPG, whereas proximal skin temperature increased to reach normal levels. This dramatic alteration of daytime skin temperature control in narcolepsy suggests that hypocretin deficiency in narcolepsy affects skin temperature regulation, which in turn may affect sleep and vigilance.

The temperature of the distal skin (hands and feet) is higher in narcoleptic subjects in the waking state, while the temperature of the proximal skin is lower. These alterations are related to a shorter subsequent sleep-onset latency. Hypocretin deficiency affects skin temperature regulation.

Chapter 9

Our next goal was to investigate a contribution of skin temperature regulation disturbances to impairments in the ability to maintain vigilance and wakefulness, two major complaints of patients with narcolepsy. The Psychomotor Vigilance Task [37] and the Maintenance of Wakefulness Test [38] were repeatedly assessed, while skin and core body temperature were manipulated using a thermosuit and hot or cold food and drinks.[39] Compared to core cooling, core warming improved the time-on-task decline in Psychomotor Vigilance Task response speed by 25%. Slightly increasing core body temperature, -which was relatively low in the narcolepsy patients-, towards a more normal level, thus improves vigilance. As compared to distal skin warming, distal skin cooling increased the time that the patients were able to maintain wakefulness by 24%. Cooling the hands and feet and warming the proximal skin thus decreases daytime sleepiness in narcolepsy. Core body and skin temperatures causally affect vigilance and sleepiness in narcolepsy. This may have future therapeutic consequences.

In narcoleptic subjects vigilance can be improved by slightly increasing core body temperature towards normal levels, while sleepiness can be decreased by cooling the hands and feet and warming the proximal skin. Although these effects were moderate, this may have future therapeutic consequences.

Chapter 10

Apart from sleepiness and decreased vigilance, disturbed night time sleep is another core symptom of narcolepsy that can severely affect quality of life.[40] Nocturnal polysomnography shows a fragmentation of the normal sleep pattern and frequent arousals.[40,41] To investigate a causal contribution of temperature alterations to the

disturbed sleep in narcolepsy, we manipulated proximal and distal skin temperature during nocturnal polysomnography. Throughout the night, skin temperature was manipulated to slowly cycle within a range normally observed during sleep. The sleep-inducing combination of proximal skin warming and distal skin cooling led to a 160% increase in the duration of slow wave sleep, a 50% increase in REM-sleep and a 68% decrease in wakefulness, compared to the wakefulness-inducing combination of proximal skin cooling and distal skin warming (note, that due to the protocol used, temperature manipulations can only be compared to one another, but not to a 'thermoneutral' situation). These effects are similar in magnitude to the effects of the currently used hypnotic sodium oxybate (gamma hydroxybutyrate).[42] Skin temperature manipulations under controlled conditions thus ameliorated the typical nocturnal sleep problems of narcoleptic patients - i.e. they led to increased slow wave sleep and decreased wakefulness–, making their sleep more comparable to that of healthy persons. These results indicate that skin temperature control could have clinical relevance in the management of disturbed nocturnal sleep in narcolepsy.

In narcoleptic subjects night time sleep can be improved by subtle skin manipulations (distal skin cooling and proximal skin warming) that counteract the temperature alterations found earlier, making their sleep more comparable to that of healthy control subjects. Skin temperature control could have clinical relevance in the management of disturbed nocturnal sleep in narcolepsy.

Chapter 11

Future Perspectives

The studies into regulation and manipulation of temperature in narcolepsy were performed in a laboratory setting under strictly controlled circumstances. The extent to which such fairly subtle effects also occur in daily life is as yet unknown, suggesting the need for monitoring under normal circumstances in patients' homes. An intriguing consequence of monitoring thermoregulatory profiles could be the prediction of sleep

Table 1. Overview Part 1

	Hypocretin System			
	In Vivo	Post Mortem		
Disorder	Spinal CSF	Ventricular CSF	Tissue Level	Hcrt-1 IR Cell Number
Narcolepsy	↓↓↓↓	↓↓↓↓	↓↓↓↓	↓↓↓↓ (-95%)
Prader-Willi Syndrome	=	=	=	=
Huntington's Disease	=	=	↓ (-30%)	↓ (-30%)
Parkinson's Disease	=	↓ (-25%)	↓ (-40%)	↓↓ (-50%)
Alzheimer's Disease	?	?	?	?
Normal Aging	=	?	?	=/↓?

CSF, Cerebrospinal Fluid; IR, immuno-reactie; hcrt-1, hypocretin 1.

attacks. Furthermore, the effects of manipulation of skin temperature on night time sleep are promising. These effects were obtained using slowly cycling manipulation patterns. The most optimal pattern found (proximal skin warming and distal skin cooling) might then be applied during multiple nights and compared to other patterns to confirm its beneficial effect in the long run.

General conclusions

Neuronal Loss and Narcoleptic Symptoms

As stated in the first part of this thesis, a loss of hypocretin containing neurons is not limited to narcolepsy, but also occurs in other disorders. The speed of the process that targets hypocretin containing neurons in narcolepsy is unknown, but the process is highly selective and complete, in contrast to the situation in Parkinson's and Huntington's Disease, where the hypocretin cell loss is not complete and where various neuronal populations are at risk. We know that other cell types are reduced to a lesser extent (for example the melanin-concentrating hormone neurons in Huntington's Disease in chapter 3), but maybe other cell types to an even greater extent (for example dopamine neurons in Parkinson's Disease) than the hypocretin containing neurons.

A complicating issue is the fact that it is difficult to distinguish a loss of neurons from a loss of a cell marker, such as hypocretin-1. Strictly speaking, a decreased number of neurons that express hypocretin-1 does not mean that there is actually a loss of these neurons. Neurons that used to express hypocretin-1 could still be present and functionally active. In fact, some researchers hypothesize that hypocretin neurons are not actually lost in narcolepsy, but just stop making hypocretin.[43] The fact that the expression of dynorphin and neuronal activity-regulated pentraxin (NARP), which are normally co-expressed by the majority of hypocretin neurons,[44,45] is also lost in narcoleptic hypothalami, does still not prove that the neurons are really gone. These cells may produce fewer peptides on a global level. However, despite this unanswered question, the functional consequences of a 'real' loss of neurons on the one hand or a loss of 'only' the marker on the other hand, are similar. But if the neurons that formerly produced hypocretin are still alive and can be turned intro active hypocretin neurons again, this could potentially be a way to cure narcolepsy.

The link between the complete narcoleptic phenotype and a complete loss of hypocretin has been well established. Hypocretin is undetectable in the spinal CSF of narcoleptic patients with cataplexy[22] and hypocretin knockout rodent models show the complete narcoleptic phenotype.[46] However, the exact relationship between loss of hypocretin containing neurons and the occurrence of clinical symptoms remains unknown. At this moment, it is impossible to quantify hypocretin neurons in vivo and as such the only clues originate from post-mortem research.

Some clues to solve this issue originate from research into disorders other than narcolepsy. In brains from patients who had been suffering from Parkinson's Disease for some years, the degree of cell loss in the substantia nigra pars compacta turned out to be at least 75%.[47,48] However, the question still remains how many dopamine neurons have to

be lost before clinical symptoms will start to appear. The only disorders in which in vivo evidence for the relationship between cell loss and function is available, are disorders of the motor neuron. In Amyotrophic Lateral Sclerosis (ALS) the number of remaining motor units can be estimated using an electrophysiological calculation method (Motor Unit Number Estimation, MUNE). It has been shown that subjects can lose up to 25% of their motor units without a reduction of muscle strength, meaning that function can be maintained through compensatory processes. [49]

Whether the moderate loss of hypocretin containing neurons in Parkinson's Disease and Huntington's Disease will lead to clinical symptoms remains an intriguing question. In rodents a loss of 60-70% of hypocretin neurons results in REM-sleep disturbances. This would imply that the more than 50% loss we found in Parkinson's Disease (reflected in lower ventricular CSF levels) could at least partially explain the sleep disturbances (excessive daytime sleepiness, REM-sleep Behavior Disorder) commonly seen in this disorder. The loss of hypocretin containing neurons in Huntington's Disease is less severe (with normal ventricular CSF levels) and is thus less likely to result in clinical symptoms.

The exact contribution of a loss of hypocretin neurons to sleep disturbances still needs to be studied. This should involve studying post-mortem hypothalami of patients, whose sleep disturbances were well documented during the last few years of their lives. Furthermore, we found an indication for a decrease in hypocretin cell number with age in the controls of our study looking into hypocretin functioning in Prader-Willi Syndrome. This raises the interesting question whether this would mean that the hypocretin system would be affected to an even greater extent in a brain showing advanced ageing, i.e. Alzheimer's Disease. Interestingly, preliminary observations pointing to changes in the hypocretin system in Alzheimer's Disease were indeed mentioned in an abstract by Harper et al.[50] Sleep disturbances and autonomic disorders are frequently present in Alzheimer's Disease.[51] A loss of hypocretin neurons could partly explain these phenomena. We are, therefore, currently examining the hypocretin system in normal ageing and in Alzheimer's Disease. In addition, as mentioned before, it would be of interest to relate the number of hypocretin neurons in controls to the presence of the HLA DQB1*0602 subtype of the immune system that is seen in more then 90% of all narcolepsy with cataplexy patients.

Body Temperature and Narcolepsy

In narcolepsy a relatively high skin temperature of the hands and feet compared to the temperature of the proximal skin is related to a shorter sleep latency. By manipulating skin and core body temperature we were able to influence sleepiness, vigilance and night time sleep. Note that the beneficial manipulations were all in a direction that counteracted the narcoleptic alterations in temperature that we found earlier. This indicates that the hypothalamic circuitry involved in the coupling between temperature and sleep are basically intact in narcolepsy and that manipulation of core body and skin temperature can causally affect sleep and vigilance in narcolepsy.

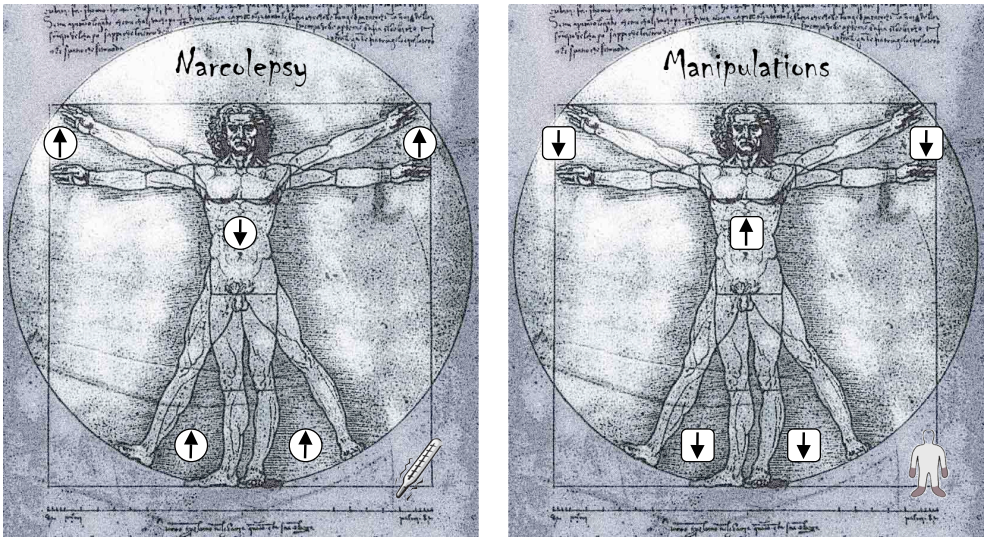

Figure 12.1 | **Overview Temperature Studies**
Scheme indicating the alterations in skin temperature control we found in narcolepsy (left, circles) and the manipulations of skin and core body temperature that had beneficial effects on vigilance, sleepiness and night time sleep (right, squares). Note that the beneficial manipulations (right) are all in the opposite direction compared to the alterations found (left).

Of course, these findings were obtained in a laboratory setting under strictly controlled circumstances. The narcoleptic alterations in skin temperature control and the beneficial effects of manipulation thus need to be replicated and confirmed in a different setting: outside the hospital and at home. As described in chapters 10 and 11, one could then even think of practical applications such as chairs or bedding that measure and differentially manipulate proximal and distal skin temperature. At this point, the findings can be summarised as follows: To stay alert, drink your hot coffee, but don't hold it, and hold your ice cream, but don't eat it.

Narcolepsy: Search for the Cause and Therapy

The last few years have seen an enormous increase in knowledge concerning the role of hypocretin in the regulation of sleep. However, more studies are needed to assess the effects of hypocretin deficiency on other important aspects: metabolism, vigilance, autonomic control and temperature regulation in humans.

Regarding narcolepsy, the two most exciting future fields of research are the search for the cause and the search for a new therapy.

Cause

As of yet no evidence for the autoimmune hypothesis has been found. However, immune mechanisms could still be involved. An environmental factor, such as a pathogen, may lead to a B- or T-cell mediated immune reaction and a selective cell

loss in genetically susceptible individuals. Nonetheless, it is important to keep an open mind regarding the cause of narcolepsy. It has been shown that hypocretin neurons are specifically vulnerable to N-methyl-D-aspartate (NMDA) mediated excitotoxicity.[52] One could also hypothesize that a loss of hypocretin is not directly responsible for the narcoleptic symptoms, but just an epiphenomenon of another, unknown disease process in narcolepsy. However, knock-out animal models of the disease show the complete narcoleptic phenotype including cataplexy,[46] which renders it less likely that additional factors are involved. Clues may be found in familial cases of narcolepsy with cataplexy without a loss of hypocretin neurons. However, it is most likely that mutations in those families will affect either the hypocretin signalling pathway or susceptibility to the narcolepsy disease proces. But families with multiple cases are scarce and rarely have more than two affected individuals.[53] Genome-wide studies have reported potential linkage to chromosome 4p13-q21 in eight small multiplexes, Japanese families,[54] and evidence for linkage to chromosome 21q in a large French family.[55]

The existence of narcolepsy without cataplexy, where hypocretin is most often present, shows that the narcoleptic phenotype of excessive daytime sleepiness with sleep-onset REM can occur without hypocretin deficiency. This raises the intruiging question whether *narcolepsy with cataplexy* and *narcolepsy without cataplexy* might be two completely distinct disease entities with different pathophysiological mechanisms. It even may be the case that what we now classify as *narcolepsy without cataplexy* is in fact no single disease entity. Some of these subjects will be narcolepsy patients that are going to develop cataplexy in the future, being most often hypocretin deficient. A few subjects in this group will have various other pathology causing excessive daytime sleepiness and sleep-onset REM. For many subjects, however, their complaints could be due to lifestyle (sleep deprivation). One could even hypothesize these complaints may be due to HLA DQB1*0602 positivity.[56]

Therapy

The most obvious new treatment option for narcolepsy with cataplexy would be administration of hypocretin. However, systemic hypocretin-1 hardly crosses the blood-brain barrier to produce therapeutic effects. So treatment with the peptide itself does not seem to be a good option. The development of hypocretin analogues that do reach the brain will be needed to further explore this therapeutic pathway in humans. Reportedly, pharmaceutical companies are working on a hypocretin agonist. However, it has been almost 8 years since the discovery that hypocretin deficiency is the cause of human narcolepsy with cataplexy. So far, it has been disappointingly silent on this topic.

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