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## **Hypocretin deficiency : neuronal loss and functional consequences**

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# General Introduction and Scope of the Thesis

**INTRODUCTION**

# General Introduction and Scope of the Thesis

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## The dual discovery of hypocretin

The hypocretins were discovered in 1998 nearly simultaneously by two different groups. One group named these newly found peptides *hypocretins* because of their *hypothalamic* origin and a weak sequence homology to the *incretin* hormone family.<sup>1</sup> Only six weeks later, another group named the same peptides *orexins*, because intracerebroventricular injection of these neurotransmitters stimulated food intake in rats (ορεξιη = appetite).<sup>21</sup>

From the precursor molecule preprohypocretin two peptides are produced: hypocretin-1 and -2.<sup>1</sup> Hypocretin 1 is 33 amino acids in length, with an N-terminal pyroglutamyl residue and an amidated C-terminal. Four cysteine residues in the peptide form two sets of intrachain disulfide bonds. Hypocretin-2 is a 28 amino acid peptide with an amidated C-terminal (Figure 0.1).<sup>2</sup> There are two types of hypocretin receptors. Both are 7-transmembrane G-protein coupled receptors encoded by 7 exons. The hypocretin receptor 1 has a preferential affinity for hypocretin-1, whereas hypocretin receptor 2 binds both hypocretins with equal affinity.<sup>2</sup>

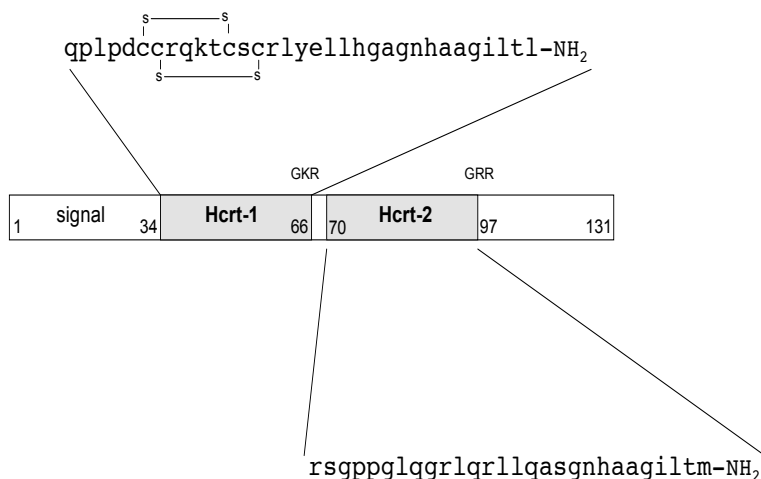
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## Anatomy of the hypocretin system

Hypocretin is produced by neurons in a subregion of the hypothalamus (see Box 1), the dorsolateral hypothalamus (see Box 2), centered around the fornix and adjacent areas. In rats, estimates of the number of hypocretin containing neurons range from 1,000 to 4,000, depending on the antiserum and/or estimation method.<sup>3</sup> In the human brain, this number was estimated at 15,000-20,000 using in situ hybridization<sup>3</sup> and 50,000-80,000 using immunocytochemistry.<sup>4</sup> The cell bodies of hypocretin producing neurons all lie together in a rather small area, but this does not hold at all for their projections, which are found throughout the brain.<sup>5,6</sup> In accordance with this finding, hypocretin receptors are also found throughout the brain.

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*1 Currently, 'orexin' is used more by basic researchers studying animal models and metabolism, while 'hypocretin' is used more by clinical sleep specialists. In this thesis 'hypocretin' will be used, since this is the name given by the group that was the first to describe these peptides. Furthermore, in the OMIM and MGD genetic databases, the term hypocretin is used.*



**Figure 0.1** Diagram of preprohypocretin

GKR and GRR depict dibasic residues, that are potential cleavage sites for prohormone convertases. The derived amino acid sequence for hypocretin-1 and hypocretin-2 are shown as well. The C-terminal end of both peptides are amidated. Note the two intrachain disulfide bridges in hypocretin-1.

## Function of the hypocretin system

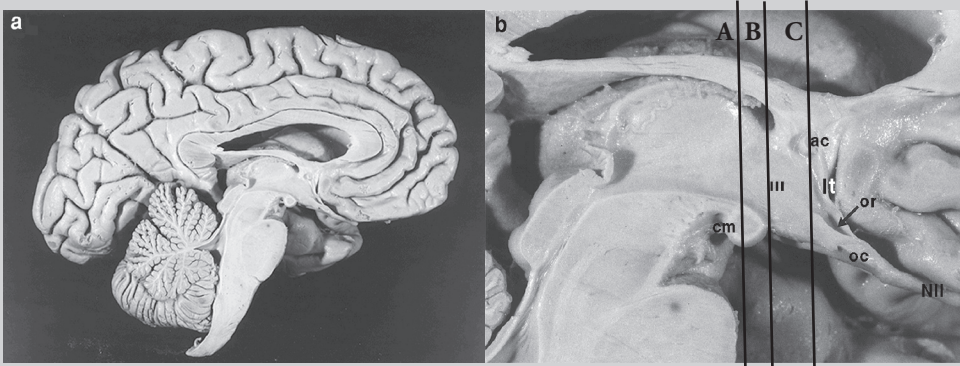
When hypocretins were first discovered they were thought to be mainly involved in the regulation of food intake. Local injection of hypocretin-1 in several hypothalamic areas, such as the dorsomedial nucleus, induced feeding behaviour,<sup>7</sup> while administration of hypocretin-1 antibodies suppressed feeding in rats.<sup>8</sup>

Hypocretin administration does not however alter total 24 hour food consumption and neither does prolonged administration affect body weight in rats.<sup>9</sup> Furthermore, the appetite-inducing activity of hypocretin is much less compared with for example that of the most well-known appetite inducing peptide Neuropeptide Y (NPY) and sometimes even absent.<sup>10</sup> These findings suggest that the major function of hypocretin must be another than the regulation of food intake.

The prevailing view that the main function of the hypocretin system regulates food intake underwent a change following the discovery that dogs (Dobermans and Labradors) suffering from an autosomal recessive inheritable form of the sleep disorder narcolepsy (see Box 3) have a mutation in the type 2 receptor for hypocretin. This prompted the view that hypocretins are crucial for the regulation of sleep. As said, hypocretin neurons project widely throughout the brain, but closer scrutiny revealed a notable concentration in wake stimulating areas.<sup>5</sup> Soon further evidence for the role of hypocretin in regulating sleep and activity/arousal was found. In a number of animal studies central administration of hypocretin-1 resulted in general hyperactivity together with stereotypical motor activities, such as burrowing and grooming.<sup>10-12</sup> Both hypocretins

## Box 1: The Human Hypothalamus

The human hypothalamus represents only a very small portion of the adult human brain: with 4 cm<sup>3</sup> it amounts to only 0.3% of the adult brain. It is nevertheless extraordinarily complex, containing as it does many different cell groups with different structural and molecular organizations that are critically involved in a great many physiological, endocrine and behavioral processes.<sup>29</sup> Among these are the regulation of food intake, autonomic tone, the sleep-wake cycle and temperature.



**Figure 0.2** Medial surface of the human brain (a: overview), (b: detail with the hypothalamus)  
ac = anterior commissure, NII = optic nerve, lt = lamina terminalis, oc = optic chiasm, or = optic recess, III = third ventricle, cm = corpus mamillare.

The exact location of the boundaries of the hypothalamus itself is quite arbitrary.<sup>30,31</sup> Moreover, the various cell types within the hypothalamus do not respect the anatomical boundaries of the different nuclei. However, the borders of the hypothalamus are generally considered to lie as follows. *Rostrally*, the border is the lamina terminalis, and *caudally* it is the plane through the posterior fissure and the posterior edge of the mamillary body (Figure 0.2). It should be noted, however, that the nucleus basalis of Meynert (that does not belong to the hypothalamus *sensu stricto*) extends even more caudally than the mammillary bodies (Figure 0.2). The *ventral* border of the hypothalamus includes the floor of the third ventricle that blends into the infundibulum of the neurohypophysis. The exact location of the *lateral* boundaries is less clear, i.e. the striatum/nucleus accumbens, amygdala, the

increase blood pressure and heart rate in rats when injected intracerebroventricularly.<sup>13,14</sup> Moreover, hypocretin-1 and -2 increased the firing rate of the histaminergic neurons, which play a prominent role in arousal.<sup>15</sup>

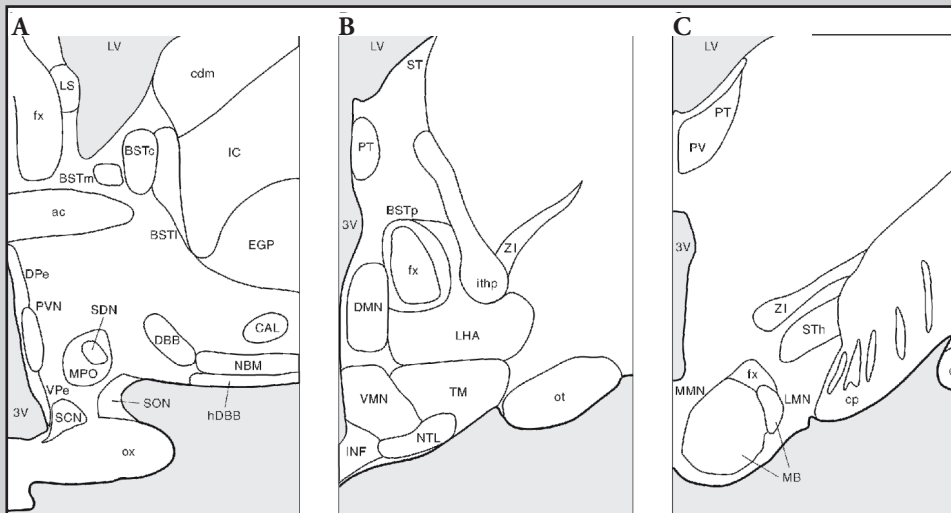
### Lack of hypocretin: Narcolepsy

Shortly after these animal discoveries **narcolepsy** in man (see Box 3) was also shown to be due to a malfunction of the hypocretin system. In healthy persons hypocretin

(Box 1 continued)

posterior limb of the internal capsule and basis pedunculi and, more caudodorsally, the lateral border of the subthalamic nucleus.<sup>31,32</sup>

Most authors distinguish three hypothalamic regions (Figure 0.3),<sup>31</sup> (A) the chiasmatic or preoptic region, (B) the cone-shaped tuberal region (which surrounds the infundibular recess and extends to the neurohypophysis) and (C) the posterior or mammillary region, which is dominated by the mammillary bodies that abut the midbrain tegmentum.<sup>32</sup>

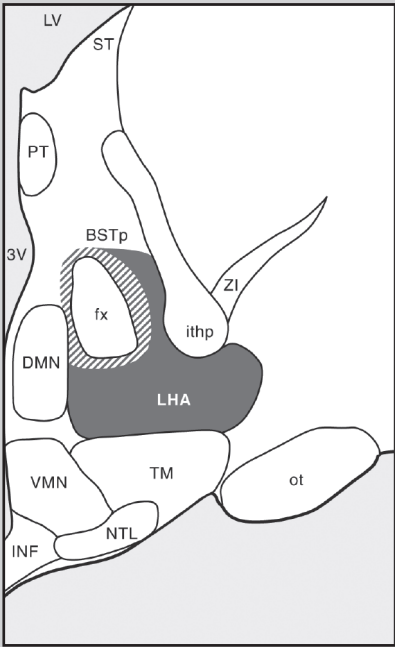


**Figure 0.3** Nuclei of the human hypothalamus in three representative coronal cuts

Abbreviations: Ox: optic chiasma, NBM: nucleus basalis of Meynert, hDBB: horizontal limb of the diagonal band of Broca, SDN: sexually dimorphic nucleus of the preoptic area, SCN: suprachiasmatic nucleus, BST: bed nucleus of the stria terminalis, (c = centralis; m = medialis; l = lateralis; p = posterior); PVN: paraventricular nucleus, SON: supraoptic nucleus, DPe: periventricular nucleus dorsal zone, VPe: periventricular nucleus ventral zone, fx: fornix, 3V: third ventricle, ac: anterior commissure, VMN: ventromedial hypothalamic nucleus, INF: infundibular nucleus, OT: optic tract, MB: mamillary body i.e. MMN: medial mamillary nucleus + LMN: lateromamillary nucleus, cp: cerebral peduncle. (Adapted from Fernández-Guasti et al., 2000; Fig. 2.)

could be detected in the cerebrospinal fluid, but in narcoleptic patients the amount was so low that its presence could not be detected.<sup>16</sup> Further research showed that the lack of hypocretin was caused by a specific loss of hypocretin containing neurons.<sup>3</sup> At present it is not clear how the amount of cell loss translates to disease severity. How these cells are lost is also at yet unknown. There is only one report about a genetic mutation causing the narcolepsy phenotype showing an autosomal dominant mode of inheritance.<sup>17</sup> The most popular hypothesis concerns an autoimmune process that selectively targets hypocretin neurons, but no direct proof for such a process has yet been found.<sup>18</sup> The strongest argument for this hypothesis is the fact that almost all

## Box 2: The Lateral Hypothalamus



The area lateral of the preoptic nucleus and the paraventricular nucleus (PVN) does not belong to a well circumscribed nucleus and is called the lateral hypothalamic area (LHA) or lateral hypothalamic zone (figure 0.4). The relatively sparse neurons in this zone, which include the hypocretin producing neurons, are interspersed around the fibres of the fornix.<sup>31</sup> Large, darkly staining neurons are found scattered through the lateral hypothalamic area. These cells merge with the tuberomammillary nucleus neurons. The cells of the lateral hypothalamic area project on other hypothalamic areas, on the cerebral cortex, brainstem and spinal cord.<sup>31</sup> The LHA is involved in the regulation of food intake and body weight, together with the infundibular nucleus, paraventricular nucleus (PVN), dorsomedial nucleus (DMN), and the ventromedial hypothalamic nucleus (VMN).<sup>32</sup>

**Figure 0.4** | Schematic representation of the nuclei of the human hypothalamus.  
The lateral hypothalamus is indicated in grey.

narcolepsy with cataplexy patients share the same major histocompatibility complex (MHC) subtype of immune system (HLA, Human Leukocyte Antigen, DQB1\*0602).<sup>19</sup>

### *The hypocretin system in other disorders*

Abnormalities of sleep resembling those seen in narcolepsy, inspired an interest in hypocretin function in neurodegenerative disorders, such as **Alzheimer's Disease**, **Parkinson's Disease** and **Huntington's Disease**. Furthermore, intriguing reports about sleep disturbances and even a state resembling cataplexy in the **Prader-Willi Syndrome** have led to an interest in hypocretin functioning in this genetic disorder that affects the hypothalamus.<sup>20</sup> There are not many tools to assess hypocretin functioning. Electrophysiological tests, imaging techniques, blood- and even CSF measurements provided inconclusive results.<sup>21-23</sup> Therefore, we decided to study post-mortem brain material from these disorders.

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### **Involvement of hypocretin in narcoleptic symptoms other than sleep**

Although the link between hypocretin deficiency and the sleep-related symptoms of narcolepsy has been well established, there are other consequences of a lack of hypocretin that have to be studied in more detail. Most importantly, it is still unknown how a

lack of hypocretin results in the emotion-triggered cataplectic attacks that characterize narcolepsy (see box 3). In fact, the relationship between hypocretin deficiency and cataplexy is stronger than that with excessive daytime sleepiness. Virtually all patients suffering from cataplexy are hypocretin-deficient, but narcoleptic patients that do not have cataplexy often still have detectable amounts of hypocretin in their CSF.<sup>24</sup>

#### *Metabolism*

Hypocretin-deficient narcoleptic patients are more obese than healthy controls and subjects suffering from idiopathic hypersomnia, a disorder resembling narcolepsy in that subjects also suffer from EDS, but in whom there is no hypocretin deficiency.<sup>25</sup> Obesity in narcolepsy thus seems to be related in some way to hypocretin deficiency. Involvement of the hypocretin system in metabolism is also indicated by effects of hypocretin administration and hypocretin antagonists administration on food intake, as well as by anatomical connections between the hypocretin system and the hypothalamic circuitry responsible for the regulation of metabolism.

#### *Thermoregulation*

Relationships between skin temperature and sleep have been discovered in the 1930's but were largely neglected afterwards until recently.<sup>26</sup> Core body temperature is higher during the day than during the night. In contrast, skin temperature follows the opposite pattern, i.e., it is higher during the night and lower during the day.<sup>27</sup> The core body temperature rhythm is intrinsically linked to that of sleep and wakefulness. Warmer hands and feet promote the onset of sleep, while active manipulation of the skin temperature affects sleepiness.<sup>27</sup> Thermoregulation and sleep/wake regulation are both major functions of the hypothalamus and as such hypocretin deficiency may be involved in the regulation of temperature.

### **Box 3: Narcolepsy**

Narcolepsy is a sleep/wake disorder that affects between 25 and 50 per 100,000 people.<sup>33</sup> It is a severely disabling disorder characterized by an instability of wakefulness and the various sleep stages, meaning that these cannot be maintained for long periods, so frequent unwanted transitions between these states ensue.<sup>34</sup> The classical symptoms of narcolepsy are:<sup>35,36</sup> excessive daytime sleepiness, cataplexy (a sudden, bilateral loss of muscle tone luxated by strong emotional stimuli -such as laughter- with preserved consciousness), hypnagogic hallucinations (very vivid, often frightening dream-like experiences that occur during the transition between wakefulness and sleep) and sleep paralysis (an inability to move during the onset of sleep or upon awakening, while patients are subjectively awake). Other important symptoms are fragmented nocturnal sleep, disturbed vigilance<sup>37</sup> and obesity<sup>25</sup>. Determination of the hypocretin levels in the cerebrospinal fluid (CSF) has become a diagnostic test for narcolepsy with cataplexy.<sup>24,38</sup> Treatment of narcolepsy is currently based on antidepressants working against cataplexy, sleep paralysis and hypnagogic hallucinations. Stimulants, such as modafinil and methylphenidate, are used to treat excessive daytime sleepiness. Gammahydroxybutyrate is a relatively new hypnotic that may improve all symptoms.<sup>39</sup>



### *Autonomic Nervous System*

Fat tissue is densely innervated by both sympathetic and parasympathetic fibers. Metabolism is increased when sympathetic tone is higher.<sup>28</sup> A higher temperature of the distal skin is linked to an increased loss of heat. This can in turn be due to peripheral

## **Scope of the Present Thesis**

In this thesis many of the above-mentioned links of the hypocretin system are investigated.

### *Part I - The Hypothalamus and its Hypocretin Neurons*

The first three chapters deal with the hypothalamic hypocretin system in disorders that are accompanied by narcolepsy-like sleep disturbances, i.e. **Prader-Willi Syndrome** (chapter 1), **Parkinson's Disease** (chapter 2) and **Huntington's Disease** (chapter 3). To determine whether the hypocretin system is affected in these disorders, the total number of hypocretin neurons was determined using quantitative techniques in post-mortem human hypothalami. Furthermore, hypocretin levels in both post-mortem CSF and brain tissue were measured in patients with Parkinson's and Huntington's Disease.

The reason why hypocretin neurons disappear in **narcolepsy** is still a mystery. A putative autoimmune aetiology has been hypothesized, but a screening for **auto-antibodies** and a n=1 trial with **intravenous immunoglobulins** yielded no unequivocal results in favor of this hypothesis (chapters 4 and 5).

### *Part II - When Hypocretin Neurons are Absent: Narcolepsy*

The consequences of hypocretin deficiency in narcoleptic patients are explored, focussing on **vigilance** (chapter 7), **metabolism** and the **autonomic nervous system** (chapter 8) and **skin temperature regulation** (chapters 9-11).

The ability of a specific neuropsychological test to measure **vigilance** as a severity indicator for narcolepsy is explored in chapter 7.

Two possible causes for the obesity commonly seen in narcolepsy are a decreased **basal metabolic rate** and a changed **autonomic tone**, reflected in an abnormal heart rate and blood pressure variability. In chapter 8 both elements are examined in hypocretin-deficient narcoleptic subjects.

To assess the influence of hypocretin deficiency on **skin temperature regulation**, thermoregulatory profiles of the proximal and distal skin of narcoleptic subjects were compared to profiles of healthy controls during a daytime sleep registration in chapter 9. To further study whether changes in skin temperature regulation can causally affect sleep, both core body and skin temperatures were **manipulated** while sleep and vigilance were measured in chapters 10 and 11.

vasodilatation caused by a decrease in sympathetic tone.<sup>26</sup> The regulation of body weight, metabolism and body and skin temperature is influenced by the autonomic nervous system. Since integration of autonomic function with many other bodily functions is situated in the hypothalamus, it is possible that the autonomic nervous system has a role in narcoleptic symptoms.

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