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Recent insights into the pathophysiology of narcolepsy type 1

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ABSTRACT

Narcolepsy type 1 (NT1) is a sleep-wake disorder in which people typically experience excessive daytime sleepiness, cataplexy and other sleep-wake disturbances impairing daily life activities. NT1 symptoms are due to hypocretin deficiency. The cause for the observed hypocretin deficiency remains unclear, even though the most likely hypothesis is that this is due to an auto-immune process. The search for autoantibodies and autoreactive T-cells has not yet produced conclusive evidence for or against the auto-immune hypothesis. Other mechanisms, such as reduced corticotrophin-releasing hormone production in the paraventricular nucleus have recently been suggested. There is no reversive treatment, and the therapeutic approach is symptomatic. Early diagnosis and appropriate NT1 treatment is essential, especially in children to prevent impaired cognitive, emotional and social development. Hypocretin receptor agonists have been designed to replace the attenuated hypocretin signalling. Pre-clinical and clinical trials have shown encouraging initial results. A better understanding of NT1 pathophysiology may contribute to faster diagnosis or treatments, which may cure or prevent it.

1. Introduction

Narcolepsy type 1 (NT1) is one of the central disorders of hypersomnolence and results from hypocretin (Hcrt, also known as orexin) deficiency in the brain. It affects about 20–50 per 100,000 individuals in Europe [1,2]. The core symptom of central disorders of hypersomnolence is excessive daytime sleepiness (EDS). This is often described as the inability to stay awake and includes difficulties with sustained attention, resulting in performance deficits during daily life activities [3]. A hallmark for NT1 is the presence of cataplexy, which is defined as more than one episode of generally brief (<2 min), usually bilaterally symmetrical, sudden loss of muscle tone with retained consciousness [4]. Episodes are often triggered by strong emotions, especially during laughter. Cataplexy can be partial or generalized [5,6]. Other typical NT1 phenomena are weight gain, disturbed nocturnal sleep, hypnagogic (during the onset of sleep) or hypnopompic (during awakening) hallucinations or sleep paralysis, which is the inability to move physically during this sleep-wake transitions [5,6]. Along with these core symptoms, people with NT1 may also experience vivid dreams or cognitive deficits like impairment of memory, alertness and sustained attention. People with NT1 may have difficulties to start and finish tasks, recall and follow multi-step directions. Fatigue, a complaint qualitatively different from sleepiness, is also frequently encountered. Depression and anxiety are frequent comorbidities of NT1. The symptomatology in NT1 is diverse, but not everyone experiences all symptoms [5–8].

The first symptoms occur typically during adolescence and generally include EDS. Cataplexy often develops within the first year after EDS onset but may start a few years later. The occurrence of cataplexy before the development of EDS is rare [9]. Nevertheless, cataplexy in childhood narcolepsy occurs often close to disease onset and may be the most outstanding presenting symptom in this group [10]. Most people with NT1 have seen multiple therapists and physicians. They may have had other diagnoses, such as chronic fatigue syndrome or attention-deficit/hyperactivity disorder (ADHD), before an NT1 diagnosis. This may explain why Europe's mean diagnostic delay is 9.7, and the median is 5.3 years. The median diagnostic delays differ per country from 1.6 to 10 years [9]. This diagnostic delay is a significant burden for the individual, leading to delayed treatment, lost quality-adjusted life years (QALYs), and reduced daily functioning in multiple domains such

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Abbreviations

ADHD	Attention-deficit/hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APC	Antigen presenting cell
BF	Basal forebrain
CNO	Clozapine-N-oxide
CNS	Central nervous system
CRH	Corticotrophin-releasing hormone
CSF	Cerebrospinal fluid
DTA	Diphtheria toxin
DWI	Diffusion-weighted imaging
EDS	Excessive daytime sleepiness
DOX	Doxycycline
DRN	Dorsal raphe nucleus
GABA	γ-aminobutyric acid
HA	Hemagglutinin
Hcrt	Hypocretin
HcrtR	Hypocretin receptor
HDC	Histidine-decarboxylase
HLA	Human leukocyte antigen
KO	Knockout
LC	Locus coeruleus
LH	Lateral hypothalamus
MCH	Melanin-concentrating hormone
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MS	Multiple sclerosis
MSLT	Multiple sleep latency test
NA	Neuraminidase
NARP	Neuronal activity-regulated pentraxin
NP	Nucleoprotein
(N)REM	(Non-)rapid eye movement
NS1	Non-structural 1
NT1	Narcolepsy type 1
NT2	Narcolepsy type 2
OX	Orexin (hypocretin)
PVN	Paraventricular nucleus
QALYs	Quality-adjusted life years
QRFP	Pyroglutamylated RFamide peptide
RFX4	Regulatory factor X4
SOREMPs	Sleep onset rapid eye movement periods
SXB	Sodium oxybate
TBI	Traumatic brain injuries
TMN	Tuberomammillary nucleus
TRIB2	Tribbles homolog 2
tTA	Tetracycline transactivator
VLPO	Ventrolateral preoptic area
VTA	Ventral tegmental area

as education, work, care-giving, and social situations. People often encounter stigma or social misapprehension [11]. A diagnosis of NT1 requires the presence of EDS and the demonstration of either 1) Hcrt deficiency in the cerebrospinal fluid (CSF; <110 pg/ml, when adjusted using Stanford standard samples), or 2) the presence of cataplexy with a mean sleep onset latency of <8 min in the daytime napping test, multiple sleep latency test (MSLT), in combination with ≥2 sleep-onset rapid eye movement sleep periods (SOREMPs) during the MSLT and/or preceding nocturnal polysomnography [4,6].

NT1 is currently not curable, but non-pharmacological and pharmacological symptomatic treatment options are available. Non-pharmacological options include regular bedtimes, scheduled daytime napping, patient education, psychotherapy and, although currently not evidence-based, a diet low on carbohydrates and alcohol [5,12]. Pharmacological treatment options are purely symptomatic. Various wake-promoting agents are effective against EDS and include methylphenidate, dexamphetamine, modafinil/armodafinil, pitolisant and solriamfetol. These compounds act on dopamine, histamine, noradrenaline and other systems [5,11–13]. Commonly used compounds for treating cataplexy are sodium oxybate (SXB) or antidepressants like low-dose clomipramine or venlafaxine [12,13]. SXB is effective against all major narcolepsy symptoms, even though its exact mode of action remains largely unknown. Pitolisant is also effective against cataplectic attacks, but its effect is usually weaker than SXB. Based on individuals' symptoms, different combinations of SXB, wake-promoting agents and antidepressants may be used. The currently available treatments often have adverse side effects, which are essential considerations in finding the optimal treatment regimen [13].

A better understanding of the pathophysiology of NT1 may contribute to developing better treatment options. More knowledge and awareness may lead to more accurate and faster diagnosis. Here, we focus on recent insights into NT1 pathophysiology, discussing structural and functional changes, immune system involvement, genetic findings and future perspectives for the pathophysiology and treatment options.

2. Neuronal pathways in NT1

2.1. The discovery of hypocretin

In the 1970s, inherited cataplexy in dogs [14] led to a breakthrough in understanding the pathogenesis of narcolepsy. It was found to be an autosomal recessively inherited disorder in Dobermann Pinscher dogs, but due to limited genomic resources, no pathogenic gene was found at that time [15]. Over 20 years later, the mutation of the Hcrt receptor 2 (*HCRTR2*) gene was found to cause narcolepsy in dogs [16]. In 1998, two research groups almost simultaneously reported two Hcrt neuropeptides [17,18], Hcrt type I (Hcrt-1 or orexin-A) and Hcrt type II (Hcrt-2 or orexin-B), both derived from the same hypothalamic-specific precursor protein named prepro-Hcrt. Activation of Hcrt-producing neurons has wake-promoting effects [19,20], while silencing these neurons induces slow-wave-sleep [21]. Two Hcrt receptors have been identified: Hcrt receptor 1 (HcrtR1 or OX1R) and Hcrt receptor 2 (HcrtR1 or OX2R). HcrtR1 binds Hcrt-1 with high affinity, while HcrtR2 is a non-selective receptor for both Hcrt-1 and -2 [17]. Both receptors are classified as G-protein coupled receptors, and by analysis of their mRNA, they displayed a widespread distribution in the rat brain [22]. The HcrtR2 is present in all vertebrate genomes while the HcrtR1 is only found in mammals. Therefore, the HcrtR2 is considered the ancestral form of the two receptors, with HcrtR1 having evolved from HcrtR2 through gene duplication events during the early stages of mammalian evolution [23].

Soon after, two reports suggested the loss of Hcrt-producing neurons in the brain tissue of people with narcolepsy. Immunohistochemistry demonstrated that the peptides encoded by the mRNAs selectively are expressed in cell bodies in the lateral and medial hypothalamic regions [24]. *In situ* hybridization experiments of peri-hypothalamic tissue and peptide radioimmunoassays showed that the number of Hcrt neurons in people with NT1 was reduced by 85 %–95 % [25,26]. Hcrt-1 levels in CSF were also undetectable in most cases [27], suggesting that the lack of Hcrt is the cause of NT1.

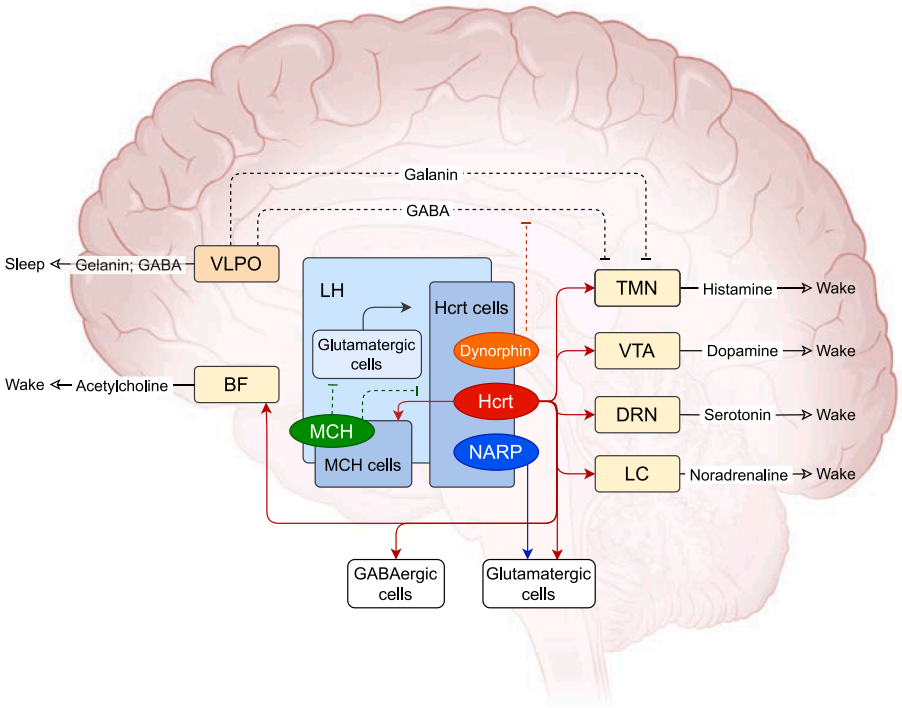

Fig. 1. Schematic visualisation of signaling pathways in sleep and wake. LH = lateral hypothalamus, Hcrt = hypocretin, NARP = neuronal activity-regulated pentraxin, MCH = melanin-concentrating hormone, VLPO = ventrolateral preoptic area, TMN = tuberomammilary nucleus, VTA = ventrotegmetental area, DRN = dorsal raphe nucleus, LC = locus coeruleus, BF = basal forebrain, arrow = stimulation, dotted line = inhibition.

2.2. Hypocretin neuronal circuits

Hcrt has neuromodulatory effects on γ-aminobutyric acid (GABA)- and glutamate-mediated neurotransmission in medial and lateral hypothalamic (LH) neurons [28,29]. As a crucial excitatory neurotransmitter, Hcrt plays a significant role in controlling sleep and wake. Dysfunction of the Hcrt system leads to the entire clinical picture of NT1 [30]. In addition to regulating food intake [17,31], the Hcrt system is also related to neuroendocrine function [28,32], energy regulation [31], cardiovascular [33] and gastrointestinal regulation [34,35], water balance [36] and reward mechanisms [37].

Hcrt neurons project to many areas within the central nervous system [24,30]. The Hcrt peptides promote wakefulness arousal by the activation of noradrenergic neurons in the locus coeruleus (LC) [38], dopaminergic neurons in the ventrotegmental area (VTA) [39], serotonergic neurons in the dorsal raphe nucleus (DRN) [40,41], cholinergic neurons in the basal forebrain (BF) [42,43] and histaminergic neurons in the tuberomammillary nucleus (TMN). During sleep, the TMN neurons are inhibited by GABAergic and galaninergic projections from the ventrolateral preoptic area (VLPO) [44,45]. Hcrt-producing neurons express, besides Hcrt, also dynorphin and neuronal activity-regulated pentraxin (NARP) [46]. Dynorphin inhibits the GABAergic innervation into the TMN, indirectly enhancing wakefulness [44] (see Fig. 1). NARP stimulates the clustering of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [47] receptor and regulates glutamate's responsiveness as the target neurotransmitter for AMPA receptors [48]. Hcrt neurons also express NARP in the LC and DRN [49]. The role of NARP within the Hcrt circuit is not entirely clear. Still, the co-expression of NARP and Hcrt neuropeptides may enhance the excitatory effects of glutamate on the target neurons [30,49]. Compared to healthy individuals, reduced levels of dynorphin and NARP have been observed in post-mortem brain tissue of people with NT1. The reduction can be explained by the loss of Hcrt cells since NARP and dynorphin where mainly reduced in regions where it was co-expressed with Hcrt (e.g. the LH) and not in areas where no co-localization with Hcrt was seen (e.g. the PVN) [46].

Like Hcrt neurons, melanin-concentrating hormone (MCH) neurons are located in the LH and project to many of the same loci as Hcrt neurons [50]. MCH neurons have the opposite effect as Hcrt neurons. The MCH and Hcrt cell groups are not co-expressed but interact [51,52]. Hcrt generally excites hypothalamic neurons, including MCH cells [51], whereas MCH has inhibitory effects. MCH inhibits Hcrt neurons directly and indirectly via presynaptic glutamatergic transmission onto Hcrt neurons, serving as a negative feedback regulator of the Hcrt system [50,53] (see Fig. 1).

2.3. Genetics in narcolepsy

Genetic research in people with narcolepsy emerged in 1984 when people with narcolepsy in Japan were found to carry the human leukocyte antigen [54] class II subtype DR2 [55]. HLA class I molecules present antigens to $CD8^+$ T cells and are present in all cells in the body. In contrast, HLA class II molecules are only present on dedicated antigen-presenting cells, such as dendritic cells and macrophages, and present antigens to $CD4^+$ T cells. Genetics research that followed eventually led to the discovery of the HLA class II subtype DQB1*06:02, which forms a haplotype with HLA-DQA1*01:02, the most frequent subtype in narcolepsy [56]. Up to 95 % of people with narcolepsy carry this specific haplotype, compared to a frequency of 20–30 % in the general population. More recent studies report an almost perfect association with nearly 100 % of people with NT1 expressing the HLA-DQB1*06:02 allele [57,58]. HLA-DQB1*06:02 homozygosity confers an even higher risk for developing NT1 [59]. Therefore, HLA-DQB1*06:02 is a necessary genetic factor but is insufficient to produce NT1. Other positive and negative associations between HLA alleles and NT1 are reported, albeit none as strong as the association with HLA-DQB1*06:02. The frequency of HLA-DQB1*03:01 was found to be increased, whereas HLA-DQB1*02:01 (HLA-DQ2),

Table 1
Rodent models of narcolepsy.

Model	Pathophysiology	Phenotype	reference
Hcrt-KO (mouse)	Loss of *Hcrt* precursor gene	SOREMPs; cataplexy; mild sleep fragmentation; increased REM sleep; decreased wake during the dark phase	[72]
Hcrt receptor KO (mouse)	Constitutively absent one or both Hcrt receptors	Mild sleep fragmentation; HcrtR2-KO mice with infrequent cataplexy	[73]
Chemical lesion (rat)	Hcrt-2 conjugated saporin eliminates Hcrt (and neighboring) neurons in the LH	SOREMPs; increased REM sleep, increased NREM sleep, increased total sleep time.	[75]
RNA interference (rat)	Transient inhibition of the gene expression of *HCRT* or its receptors	SOREMPs; cataplexy; increase in fragmented REM sleep	[76]
Hcrt/ataxin-3 transgenic animals (mouse/rat)	Progressive postnatal ablation of Hcrt neurons	SOREMPs; cataplexy; sleep fragmentation; increased REM sleep; decreased wake during the dark phase; obesity	[77,78]
OX-tTA;TetO-DTA (mouse)	*HCRT* expression is dependent on DOX in the diet.	SOREMPs; robust cataplexy; sleep fragmentation; decreased arousal during the dark phase; obesity	[79,80]
Optogenetic model (mouse)	Use of laser light to manipulate Hcrt activity through photosensitive ion channels	Stimulation of Hcrt leads to waking. Inhibition of Hcrt induces deep sleep.	[20,21]
MCH-Cre::OX-KO (mouse)	Ablation of Hcrt neurons and specific activation of MCH neurons by CNO injections	SOREMPs; cataplexy	[81]
OX-HA (mouse)	Expresses HA as a neo-self-antigen in Hcrt neurons	Cataplexy; sleep attack	[82]

Tabel 1. Rodent models of narcolepsy. Abbreviations. Hcrt = hypocretin, OX = orexin (hypocretin), KO = knockout, LH = lateral hypothalamus, REM = rapid eye movement, SOREMPs = sleep onset REM periods, DOX = doxycycline, MCH = melanin-concentrating hormone, CNO = clozapine-N-oxide, DTA = diphtheria toxin, HA = hemagglutinin.

HLA-DQB1*05:01, HLA-DQB1*06:01, HLA-DQB1*06:03 and HLA-DQB1*06:09 were decreased in people with NT1 as compared with healthy controls [57,58,60]. Associations with HLA-DR, HLA-A, HLA-B and HLA-C have also been described [61,62].

The associations with HLA alleles and the presence of NT1 slightly differ between ethnic groups. For example, the HLA-DQB1*06:02 and HLA-DRB1*15 alleles are to a similar level associated with NT1 in Caucasian Americans and Japanese, whereas in African Americans the HLA-DQB1*06:02 allele is more strongly associated than HLA-DRB1*15 [63]. African Americans with narcolepsy are more likely positive for HLA-DQB1*06:02 compared to other ethnic groups. Additionally, African Americans also reported earlier symptom onset and more EDS. Hypocretin deficiency is more prevalent among African Americans, whereas cataplesy is less prevalent compared to Asian, Caucasian or Latino Americans. However, the polysomnography and MSLT measurements do not show differences between ethnic groups [64].

The emergence of genome-wide association studies facilitated the identification of other genetic risk factors for NT1, many of them involved in the immune system's antigen-presenting pathway. T-cell receptor alpha and beta are consistently reported in several studies from different parts of the world [65,66], but also genes such as *CCR1*, *CD207*, *CTSH*, *IFNAR1*, *P2RY11*, *PRF1*, *TNFSF4*, *ZFAND2A* and *ZNF365* surface as a genetic risk factor in multiple studies [66–69]. *CTP1B* stands out in these studies and is primarily involved in mitochondrial transport and energy metabolism without a clear role in immunity [70].

In addition to identifying risk factors for narcolepsy, other applications of genetics are being explored. A recent study was the first to sequence the RNA of Hcrt-producing neurons in late embryonic mice, assessing the transcription of genes distinguishing Hcrt-producing neurons from adjacent MCH-producing neurons [71]. With this approach, it may be possible to identify genes similar to *HCRT*, which are transcribed only in the Hcrt-producing neurons and could lead to understanding their functioning better.

2.4. Rodent models of narcolepsy

Several rodent models have been developed to investigate narcolepsy and the functioning of Hcrt. We discuss the models that are most often used or hold the most promise for future experiments (see Table 1) rather than cover all rodent models.

The only known single-gene model is the **prepro-Hcrt knockout (KO)** mouse [72], the first available rodent narcolepsy model. The non-rapid eye movement (NREM) sleep of Hcrt-KO mice is more dispersed, and rapid eye movement [68] sleep increases during the dark period. REM sleep latency also decreases [72]. **Hcrt receptor KO** models were also created, in which HcrtR2-KO mice showed sleep fragmentation and cataplexy-like attacks [73], and its symptoms are more pronounced than those in HcrtR1-KO mice [74]. This implies that, compared with HcrtR1, HcrtR2 seems to play a more critical role in the pathophysiology of NT1. Hcrt receptor agonists are considered a novel therapy for NT1 [73]. These agonists might replace the shortage of Hcrt signalling by activating the Hcrt receptors.

Neurotoxins can also eliminate Hcrt neurons to some extent. For example, Hcrt-2 conjugated to the ribosome-inactivating toxic protein saporin eliminated up to 90 % of Hcrt neurons but also caused significant loss of neighboring neuronal cells such as MCH neurons, which hampers the usefulness of such models [75]. Transgene or **RNA interference technology** could solve this problem: rat exhibit increased REM and frequent cataplexy after antisense/RNA interference against *HCRT* or its receptors [76], suggesting that a long-term knockdown mouse model might shortly be feasible.

The **Hcrt/ataxin-3** rodent model is born with Hcrt, but, like human narcolepsy, the gradual and specific loss of Hcrt-producing neurons occurs later in life. These transgenic rodents express the *ataxin-3* gene leading to the accumulation of the cytotoxic poly-Q-ataxin-3 gene product controlled by the *HCRT* promotor [77]. Hcrt/ataxin-3 rodents show degeneration of Hcrt-1 in the CSF from the third week of age [77] and develop typical NT1 symptoms like cataplexy, SOREMPs and reduced waking [78].

The development of the **OX-tTA;TetO-diphtheria toxin (DTA)** mouse model was a significant breakthrough [79]. This technique allows control of Hcrt expression by adding or removing doxycycline (DOX) from the diet of Hcrt-tetracycline transactivator (tTA) transgenic mice [80]. Following the removal of dietary DOX, Hcrt-producing neurons undergo apoptosis, and mice exhibit a robust cataplexy phenotype of narcolepsy, including severe sleep fragmentation, SOREMPs, decreased wakefulness during the dark phase, pronounced cataplexy and obesity.

Optogenetic techniques can also control Hcrt gene expression in freely moving mice. The method uses light-sensitive ion channels in the Hcrt neurons that manipulate neuronal activity by delivering laser light to these cells [20,21]. Optogenetic stimulation of Hcrt neurons increases wakefulness in these mice [20], while inhibition induces deep sleep [21].

The **MCH-Cre::OX-KO** mouse model with Hcrt-KO mice expressing Cre recombinase in the MCH neurons was developed in 2018. This

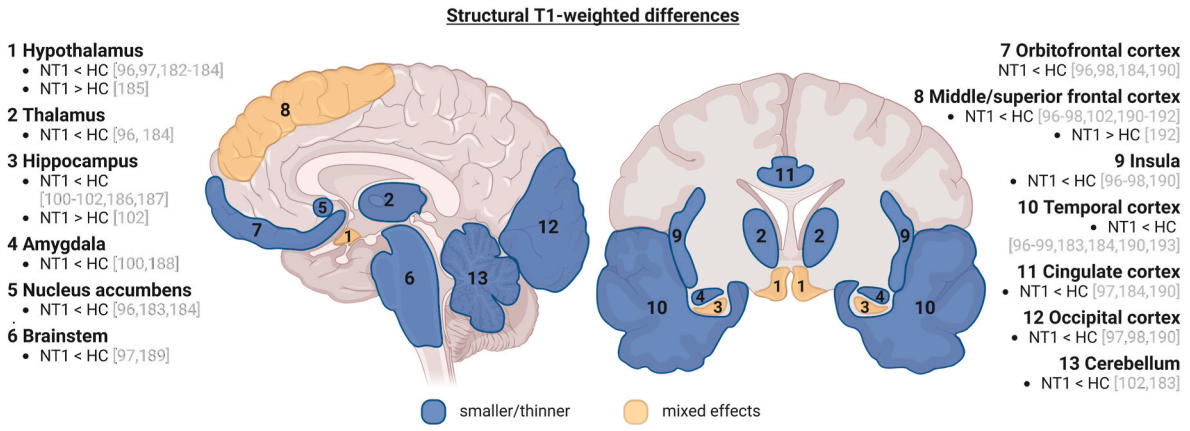

Fig. 2. Structural neuroimaging differences per brain region. Structural T1-weighted magnetic resonance imaging studies investigating narcolepsy type 1. Reported differences could reflect any T1-weighted structural outcome measure, such as volume, cortical thickness and/or surface area. The numbers between brackets correspond to the reference numbers of the studies. HC = healthy controls, NT1, narcolepsy type 1. Figure was created with BioRender.com.

model allows studying MCH activation within a Hcrt-KO model. Due to the expression of the Cre enzymes, MCH neurons can be activated by clozapine-N-oxide (CNO). MCH activation by CNO injections increases REM sleep in Hcrt-KO mice and mice with normal *HCRT* expression. The MCH activation in Hcrt-KO mice also results in short-latency REM sleep and cataplexy. These REM manifestations where only moderate in Hcrt-KO mice with saline injections and further reduced when an MCH receptor antagonist blocked the MCH activation [81].

The **OX-HA** mouse model was developed to assess immunological mechanisms in NT1. Hemagglutinin (HA) is one of the proteins that form the influenza virus. In the OX-HA model, HA is expressed in Hcrt neurons so that HA-specific immune cells can target it, leading to an NT1 phenotype, including cataplexy and sleep attacks [82].

The rodent models can explain most symptoms, as seen in human narcolepsy but does not, for instance, explain why cataplexy in humans often develops later than other sleep-wake disturbances.

2.5. Histology of other cell types

Current narcolepsy (mouse) models focus primarily on losing Hcrt neuronal signalling [83]. Other neurons, neurotransmitters and circuits may also contribute to the pathophysiology of the disease, for example, MCH neurons. MCH neurons are mainly distributed in the LH [84], and regulate NREM and REM sleep [85]. Studies have shown that MCH neurons do not initiate and maintain cataplexy in Hcrt-KO mice [86]. One of the hypotheses is that the reciprocal expression of GABA receptors on MCH and Hcrt-producing neurons may play a role in the homeostatic regulation of their respective activity patterns [87]. Another hypothesis is that the loss of Hcrt signalling favours MCH neuron activation, thereby facilitating the increased REM sleep propensity found in narcolepsy [50,83]. The exact role of the interaction between Hcrt and MCH neurons in sleep/wake regulation remains to be fully elucidated.

Other neurons of interest are histaminergic neurons. A post-mortem study of brain tissue from people with NT1 showed that the number of histamine neurons was significantly increased in NT1. This was found by labelling histidine-decarboxylase (HDC), which synthesizes histamine [88]. Similarly, HcrtR-KO mice showed a 53 % increase in histaminergic neurons in the TMN. Hcrt/ataxin-3 transgenic mice shown an intermediate rise of 28 % [89]. It was believed that the increase in histamine neurons may compensate for Hcrt neuron loss [90]. Nevertheless, the histamine increase was not confirmed by a recent study with Hcrt-KO and OX-HA mice [91]. Those mice did not show a change in the number of HDC neurons or HDC mRNA expression levels compared to control mice. Also, no correlation between the number of Hcrt neurons and HDC neurons was found [91]. Moreover, people with NT1 have no altered histamine levels in their CSF [92] and the CSF histamine remains stable over years [93]. Narcoleptic Dobermann dogs showed a reduction in histamine levels in the thalamus and cortex [94]. Further studies are therefore required to identify the role of histamine in NT1 pathophysiology.

Recent research shows an 88 % reduction in corticotropin-releasing hormone (CRH)-positive neurons in the hypothalamus's paraventricular nucleus (PVN) and significantly fewer CRH-positive fibres in the median eminence. CRH-neurons in the LC and thalamus and other PVN neuronal populations were spared, such as vasopressin, oxytocin, and tyrosine hydroxylase-expressing neurons [95]. CRH regulates humoral and behavioural adaptation to stress as an essential hormone in the hypothalamic-pituitary-adrenal axis. It correlated positively with sleep impairment. Monosynaptic innervations are observed from the CRH neurons in the PVN to the Hcrt neurons in the LH [96]. The underlying mechanism behind the lack of CRH neurons is unknown, but it provides novel targets for diagnostics and therapeutic interventions.

3. In-vivo neuroimaging studies

While histology and genetics aim to provide insight into structural differences on a cellular level between people with NT1 and controls, neuroimaging aims to disentangle functional and macroscopic structural alterations. Neuroimaging, and magnetic resonance imaging (MRI) in particular, has widely been implemented to study brain composition and functioning of people with NT1 [97]. These studies shed light on the macroscopic pathophysiology and consequences of having NT1.

Volumetric analyses have led several studies to observe reduced hypothalamus volumes in NT1 [98,99], but these findings have not been consistently replicated [100,101]. Other structural findings in NT1 include smaller frontotemporal regions of the cortex [98–101] and hippocampus [102–104], which may be related to the attention deficits and subjective memory complaints [105] people with NT1 often experience. The hippocampal volume loss has also been shown to correlate with disease duration [103]. Inconsistent differences within the limbic system in NT1 have been associated to disturbed emotional processing and cataplexy and mainly included reduced volumes of the medial prefrontal cortex, anterior cingulate cortex and amygdala [102,104] (see Fig. 2). One longitudinal study with five years of follow-up focussed on the relationship between disease duration and brain structure. The study revealed increased cortical thinning over time concerning disease progression in NT1 compared to healthy sleepers. The extent of these differences was related to decreased sleep efficiency, increased sleep latency and increased arousal over time, and age at disease onset [106]. Diffusion-weighted imaging [107] studies [108,109] investigate neural connectivity and have reported almost brain-wide impaired connectivity

in people with NT1. The pattern of the affected brain networks is in line with the normally widespread projection pattern of Hcrt-1 [110]. It could partially explain the variety and complexity of symptoms in NT1 (including sleep-wake, emotional and autonomic dysregulation, and cognitive impairment).

Using functional neuroimaging methods, multiple studies have reported decreased resting-state activity within the default mode network as the main resting network [111,112], which also seemed less connected to networks that are normally active during cognitive tasks (salience, dorsal attention and executive network) [113]. The combination of deactivation of the default mode network and upregulation of the cognitive networks is normally essential to perform well in cognitive tasks. The decreased coupling of these networks in narcolepsy suggests a dysregulation of mental resources in favour of staying awake over actual task performance [114], a phenomenon that another functional MRI study during cognitive tasks also suggested [115]. Limbic network activity has been thoroughly assessed in emotional processing and cataplexy attacks [116–118]. These studies have reported mixed increased and decreased hypothalamus activity in combination with mainly enhanced amygdala and reward system activity. Loss of hypothalamic control over the mesolimbic reward system in NT1 during emotional stimuli seems responsible for triggering cataplexy attacks. Functional neuroimaging data in narcolepsy should take into account the key confounding factor of sleepiness and drowsiness [119]. Some of the mentioned studies did not take this into account, adding more uncertainty to their results.

The observed neuroimaging differences in NT1 align with its clinical presentation and microscopic pathology. The extensive differences between people NT1 and healthy individuals that have been reported in especially diffusion-weighted imaging studies are surprising, given the presence of just 100,000–160,000 Hcrt-1 neurons in healthy individuals [110].

4. Autoimmunity in NT1

The discovery of the strong association of narcolepsy with HLA subtypes and the subsequent identification of the loss of Hcrt in the brain of people with narcolepsy led to the auto-immune hypothesis. This hypothesis assumes the existence of an auto-immune process in the brain that leads to the destruction of Hcrt-producing neurons in the hypothalamus. The emergence of a new H1N1 pandemic virus renewed interest in this hypothesis.

4.1. H1N1 pandemic

An increased incidence of NT1 was observed in various European countries during the pandemic and after the widespread vaccination campaign against influenza A(H1N1)pdm09 (hereafter referred to as H1N1) viral pandemic during the winter of 2009–2010. The Pandemrix® (GlaxoSmithKline Biologicals, Wavre, Belgium) vaccine containing AS03 as an adjuvant was widely used in Europe [120]. The increase in new narcolepsy cases was first observed in Sweden and Finland [121,122]. In 2010, a 17-fold rise in incidence was observed in Finnish children compared to 2002–2009. All children had received the Pandemrix® vaccine within the last 242 days before symptom onset. Most of these new narcolepsy cases developed cataplexy rapidly after EDS onset [121]. The same pattern was seen in Norway. A 10-fold increase in new narcolepsy cases was observed in vaccinated children compared to unvaccinated children. Most children developed symptoms within the first six months after vaccination with Pandemrix® [123]. France, Ireland, and Czech Republic also had an increase in child narcolepsy associated with the Pandemrix® vaccination, albeit not as substantial as in Scandinavia [124–126]. European studies estimated the relative risk for children and adolescents to develop NT1 after Pandemrix® vaccination between 1.5 and 25.0, and between 1.1 and 18.8 for adults [127]. A large international study could not identify the increased incidence in Spain, the Netherlands and the United Kingdom [128]. Non- or other-adjuvanted vaccines used in other countries showed no association with the development of narcolepsy [120].

Nevertheless, a seasonal increase in narcolepsy onset was observed in China six months after the peak of the H1N1 infections during the winter of 2010. A 3-fold increased incidence was observed, but the majority was not vaccinated against H1N1 [129]. At least two known cases developed narcolepsy symptoms shortly after an H1N1 infection [129]. The incidence peak in the United States was lower than in Europe or China, but a 1.6-fold increased incidence of narcolepsy was also observed in 2010. The new narcolepsy cases were associated with the H1N1 infection rates eight months earlier [130]. A possible relationship between H1N1 vaccinations other than Pandemrix® and narcolepsy onset has not been reported. Studies on other vaccinations showed that a vaccine against tick-borne encephalitis have also been suggested as potential risk factor for the development of NT1 [131].

4.2. Other triggers

The discovery that immune system-related triggers such as Pandemrix® vaccination and H1N1 infection are associated with NT1 development, in combination with the H1N1 virus not circulating for decades before its reintroduction in 2009–2010, has sparked interest into the possibility of other triggers also causing narcolepsy. Multiple other triggers have been described, but the direct causal evidence and understanding of underlying pathophysiological mechanisms remain limited. Reported triggers have mainly been associated with the rapid onset of narcolepsy symptomatology. Mainly streptococcal infections have been associated with the rapid onset of narcolepsy in multiple studies [132,133]. A post-streptococcal immune process can cause Sydenham chorea. Three patients with the diagnosis of both Sydenham chorea and NT1 within a few months were investigated in a case series. Coincidence of simultaneous disease development is unlikely but independent development due to a common immune trigger is possible [134]. Nevertheless, an association between streptococcal infection and narcolepsy has not been observed in a large Chinese study population [135]. Fluctuating narcolepsy incidence rates have been reported in several European countries in the past decade with a child-specific peak in 2013 [126]. This has been suggested to be linked to fluctuations in the severity and dominance of flu strains in prior flu seasons, but direct correlational studies on narcolepsy incidence rates and flu season characteristics have not yet been performed.

It remains unclear why immunological events rapidly trigger the onset of narcolepsy symptoms in some (typically within weeks to months). In contrast, in others that experienced the same trigger, narcolepsy symptoms take many years to arise. In delayed onset cases, it could be that the initial stimulus induced a slow-paced auto-immune process that remained asymptomatic at first or that additional triggers, closer to narcolepsy symptom onset, were needed to trigger onset. A first trigger could not have been strong enough or occurred under different environmental circumstances and did not directly trigger symptomatic narcolepsy. This idea aligns with the multiple-hit model [5] in which people with a specific genetic predisposition must experience multiple environmental triggers to develop narcolepsy. Except for the descriptive case reports and a study with narcoleptic mice [136] fitting the multiple hit hypothesis, there so far is limited pathophysiological evidence that supports this theory. Extended evidence for the multiple-hit model is already obtained within other fields, for example, for developing various cancer types [137,138].

Traumatic brain injuries (TBI) have also been described to cause Hcrt deficiency and sleep-wake disturbances in 72 % of people with TBI [107]. Interestingly, EDS was most often reported in the absence of cataplexy, and the sleep-wake disturbances and Hcrt deficiency tended to be transient. TBI is rarely a trigger for NT1 development. The EDS symptoms generally disappeared within six months, and Hcrt-1 levels normalized in most subjects [107]. Other rare causes and triggers of

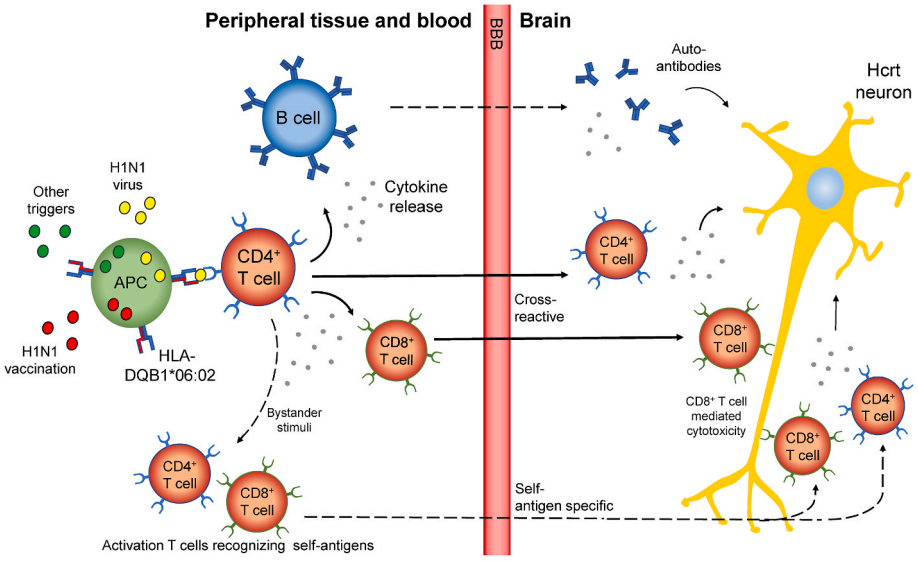

Fig. 3. The autoimmune hypothesis. Hcrt = hypocretin, APC = antigen-presenting cell, BBB = blood brain barrier.

(secondary) narcolepsy have been described in case reports and series, including Guillain-Barré syndrome, Wernicke's encephalopathy, enteroviral gastroenteritis and hypothalamic tumoral and multiple sclerosis (MS) lesions [139–143]. MS lesions in the hypothalamus are identified in people with EDS but without cataplexy and Hcrt deficiency. MS and narcolepsy can also coexist in a second phenotype where patients have no lesions in the hypothalamus and do have cataplexy and low/undetectable CSF Hcrt levels [144]. NT1 has also been described as a secondary disease in association with paraneoplastic encephalitis. Here it is believed that an autoimmune response against tumor antigens cross-reacts with antigens in 'healthy' hypocretin neurons resulting in tissue damage and NT1 symptoms [145–147].

4.3. Humoral immune response: autoantibodies

Genetic and epidemiological findings point towards an auto-immune aspect in the pathogenesis of narcolepsy (see Fig. 3). This resulted in the search for autoantibodies or T-cell reactivity associated with narcolepsy.

As most known auto-immune processes of the central nervous system, such as different types of auto-immune encephalitis, are mediated by autoantibodies, a logical first step would be to search for autoantibodies. The search for autoantibodies directed towards prepro-Hcrt, Hcrt-1, Hcrt-2, Hcrt neurons or -receptors yielded no explanatory results [148–152]. No antibodies against prepro-Hcrt, Hcrt-1, Hcrt-2 or HcrtR2 were detected in the sera of 31 people with Pandemrix®-related NT1 [149]. Another study found no signs of Hcrt-specific antibodies in the sera of people with NT1, even though all participants tested HLA-positive and samples were drawn close to symptom onset [150]. Autoantibodies produced in the brain may not pass the blood-brain barrier and are undetectable in the blood. Stainings for antibodies against Hcrt-related peptides in the CSF or Hcrt neurons in the LH of people with NT1 were also primarily negative [152,153]. Sera or brain tissue stained positive for antibodies against Hcrt neurons or receptors in a few cases, but this was also seen in controls [151,152]. The positive stainings in people with NT1 were not associated with HLA status, cataplexy, or sleep disturbances [151]. Overall, the evidence for Hcrt-specific autoantibodies is poor and leads to investigating other immune mechanisms that may explain Hcrt deficiency in NT1 development.

In 2010, multiple studies detected elevated levels of the anti-Tribbles homolog 2 (TRIB2) autoantibody in people with NT1 compared to controls and those with other hypersomnolence disorders or inflammatory neurological disorders. This antibody was strongly associated with cataplexy and the highest titers was observed within the first 2–3 years after symptom onset [154]. *Anti*-TRIB2 was undetected in people with narcolepsy; low titers were also seen in some controls. TRIB2 is not specific for Hcrt-producing neurons, making it unlikely that this antibody is the (only) direct cause of Hcrt-producing neuronal destruction. Later studies could not confirm any involvement of TRIB2 in the pathophysiology of narcolepsy [149,155,156].

An international study with recent onset NT1 found elevated antibody markers of post-streptococcal infection [133]. Other autoantibodies have been observed in sera of people with NT1, like prostaglandin receptor D2 [54] and neurexin-1 [157]. However, these studies showed that these observations were not specific for people with NT1, were not replicable or were only seen in a minority of patients [148].

Due to the H1N1 pandemic, autoantibodies related to H1N1 were investigated in relation to the development of narcolepsy. No evidence was found for a higher infection rate in people with NT1 compared to controls [47,158,159]. H1N1 hemagglutinin (HA) is the most crucial epitope of the influenza virus and shows structural homology with hypocretin [47,160]. Antibody levels and affinity directed toward HA turned out to be similar between people with NT1 and controls [47]. The H1N1 non-structural 1 (NS1) protein is produced during an actual H1N1 infection, meaning the Pandemrix® vaccine does not contain this recombinant protein. Similar levels of NS1 recombinant were detected between people with NT1 and controls [158,159].

4.4. Cellular immune response: T-cell response

The search for autoantibodies involved in NT1 rendered no convincing evidence for the involvement of mediators of humoral immunity, T-cell reactivity in NT1 has been investigated.

4.4.1. Cellular immune response in the blood

Hcrt-specific $CD4^+$ T-cells have been detected in the blood of both people with NT1 and narcolepsy type 2 (NT2, another form of narcolepsy with EDS but without Hcrt deficiency and generally without cataplexy) and even some healthy controls [161,162]. Interestingly, one of the people with Hcrt-specific $CD4^+$ T-cells that was included with a diagnosis of NT2, later developed cataplexy which changed the diagnosis to NT1. This underscores that some people with NT2 in time evolve to NT1 [161]. The exact role of the detected $CD4^+$ T-cells is still unclear. The observed autoreactivity was only identified against (amidated fragments of) the Hcrt peptides, not against Hcrt neurons itself [161,

162]. One study found that most autoreactive T-cells recognizing Hcrt were HLA-DR-restricted instead of the expected HLA-DQ restriction [161]. An explanation for this finding could be that the Hcrt-specific autoreactivity represents a secondary phenomenon that occurs after the Hcrt neurons have already decayed. Other studies did not replicate the presence of Hcrt-specific $CD4^+$ T-cells in NT1 [160]. Specific $CD4^+$ T-cell reactivity against regulatory factor X4 (RFX4), which is highly enriched in Hcrt neurons, has been detected in the sera of some people with NT1 but also in a few unaffected siblings [163]. However, unlike $CD8^+$ T-cells, $CD4^+$ T-cells cannot directly destroy Hcrt neurons [82]. $CD8^+$ T-cells recognize HLA type I molecules expressed on neuronal cell surfaces. In contrast, $CD4^+$ T-cells only recognize HLA type II that is expressed by professional antigen-presenting immune system cells. $CD4^+$ T-cells necessitate cognate effector cells to lead to the destruction of the Hcrt neurons. A study screened for $CD8^+$ T-cell autoreactivity towards a pool of NT1-related proteins in the blood of people with NT1 and controls [164]. Among these proteins were prepro-Hcrt, HcrtR2, RFX4 and TRIB2. Enhanced autoreactivity was observed in the people with NT1 compared to HLA-DQB1*06:02-positive controls [164]. Hcrt-reactive cytokine-producing $CD4^+$ and $CD8^+$ T-cells were also increased in the blood of children with NT1 compared to their peers [165]. Cytokines are signalling molecules immune cells produced during an (auto)immune response. Increased cytokine levels in the sera of people with NT1 were reported [166].

4.4.2. Cellular immune response in the CSF

Hcrt-specific T-cells in CSF were identified in one study where two $CD8^+$ T cell clones were found in the CSF of someone with recent NT2 symptom onset [161]. The authors argue that this might represent a case of recent-onset NT2 that is still evolving in NT1 and that the $CD8^+$ T cells represent a process of ongoing, but unfinished destruction of Hcrt neurons. Overall, no differences have been observed in the total frequency of $CD4^+$ and $CD8^+$ T-cells in the CSF between patients and controls [167,168]. Convincing alterations of cytokine levels in the CSF were not detected when comparing people with NT1 to controls or people with other hypersomnias [166,169], while gene expression in CSF cells in NT1 resembles non-inflammatory disease (idiopathic incranial hypertension) more than inflammatory disease (multiple sclerosis) [170]. However, a potential mediating role for cytokines in the auto-immune process of autoantibodies or autoreactive T-cells cannot be ruled out.

4.4.3. Cellular immune response in brain tissue

In 2013, the post-mortem tissue of a person with paraneoplastic NT1 contained hypothalamic $CD8^+$ T-cell infiltration. The type of inflammatory reaction showed that $CD8^+$ T-cells were responsible for inducing tissue damage [147]. This argues that $CD8^+$ T-cell infiltration was associated with a complete loss of hypocretinergic neurons, even though it is unclear whether this mechanism is the same as what happens in sporadic NT1 [164]. In a mouse model, non-specific $CD4^+$ T-cells easily infiltrate the central nervous system (CNS) and cause local inflammation, but they do not cause loss of Hcrt neurons or clinical manifestations of narcolepsy [171]. On the contrary, transferring $CD8^+$ T-cells that recognize Hcrt leads to T-cell infiltration in the hypothalamus and specific destruction of Hcrt neurons [82]. Additionally, in NT1 patients relatively close to disease onset PET imaging showed no increased microglial density compared to controls, rather arguing slightly against a central role for neuroinflammation in the pathophysiology of NT1 [172].

4.5. H1N1 immune response in NT1

The increased NT1 incidence during the H1N1 pandemic suggests that an immune response towards H1N1 might also destroy Hcrt neurons and NT1 development. Despite people with NT1 having a stronger T-cell reactivity towards the H1N1 epitopes HA, neuraminidase (NA) and nucleoprotein (NP) [162,173,174], no evidence was found for cross-reactivity between Hcrt and H1N1-HA or H1N1-NP [160–162,173]. Interestingly, mice infected with the H1N1 virus developed narcoleptic-like symptoms without any B- and T-cells. The hypothalamic area, including Hcrt neurons, was affected in some mice. This suggests that an H1N1 infection by itself could cause sleep-wake dysregulation without the involvement of the adaptive immune system [175].

4.6. Bystander activation

An alternative hypothesis to cross-reactive T-cells, bystander activation might be involved in the destruction of Hcrt neurons. In this scenario, active $CD4^+$ T-cells provide bystander stimuli (cytokines) as a side effect of their response to foreign antigens. These cytokines can stimulate surrounding non-pathogen-specific T-cells, in this case autoreactive T-cells against Hcrt neurons as part of a broader immune response [146,176].

5. Future directions

5.1. Pathophysiology

The symptom combinations that people with NT1 can experience are diverse: not all people have the same expression of symptoms. The intensity or frequency of symptoms can differ between people. Not all symptoms are always present in every individual with NT1. It remains unclear why the phenotype is that different between individuals. People also do not respond equally to the available treatment options, even when experiencing similar symptoms. Future studies should focus on these inter-individual differences between people with NT1. Different underlying mechanisms are responsible for the development of the disease, which is probably a mix of genetic and environmental factors [5].

Animal models are the basis for developing and testing new treatments, aiming to develop a cure for the disease. As mentioned, findings on neuronal circuits in narcolepsy derived from current mouse models do not explain why cataplexy in humans often develops later than other sleep-wake disturbances. The current models merely focus on Hcrt deficiency, but the involvement of other cell groups might help to find more clarity about the disease course. New models using optogenetic techniques or the MCH-CreOX-KO model are examples of promising investigations of multiple cell group interactions. Additionally, as the aetiology of narcolepsy is revealed in more detail, such as the apparent disappearance of paraventricular CRH neurons [95], other immune-mediated models should be developed to provide further insights into preventing, treating, or arresting the onset of narcolepsy.

Thus far, Hcrt has been regarded as the sole candidate antigen to be targeted by the auto-immune response leading to NT1. However, information on the expression of genes in human Hcrt-producing neurons, or even in the hypothalamus, is lacking. Whether other proteins are unique to these neurons is largely unknown. With the emergence of *in silico* data analysis methods, it becomes possible to re-evaluate existing open-access histological and genetic data on the human hypothalamus. Single-nucleus RNA sequencing and spatial transcriptomics methods that are quickly evolving make unlocking the genetic and protein fingerprint of Hcrt-producing neurons an attainable goal. Proteins or peptides (relatively) unique to Hcrt-producing neurons could then serve as promising alternative candidate antigens targeted by the immune system.

The multiple-hit model [5] is an exciting hypothesis to target with future studies. New studies systematically investigating the infection history of people with recent-onset NT1 with immunological laboratory confirmation could provide critical new insights into all environmental triggers that potentially lead to NT1. The search for genetic predisposition in combination with multiple environmental triggers (e.g. infections) might yield important information for the pathophysiology of NT1 and explain individual differences between patients.

Recently, an intriguing hypothesis was introduced, which states that in NT1, Hcrt-producing neurons are not selectively destroyed by an auto-immune process but epigenetically silenced by hypermethylation [177]. This means that methyl groups bind to the promotor region of the *Hcrt* gene preventing gene transcription. This leads to the absence of Hcrt-production, while the Hcrt-producing neurons are still present [177]. The prepublication shows that Hcrt-producing neurons in the LH not only co-express dynorphin and NARP, but also express pyroglutamylated RFamide peptide (QRFP). In rodent knock-out models, QRFP expression is unaltered, like the expression of dynorphin and NARP. Destruction of Hcrt-producing neurons, however, does lead to a reduction in QRFP expression (and similarly dynorphin/NARP). In post-mortem human hypothalamus sections, there is an expected loss of Hcrt, dynorphin and NARP staining, but surprisingly QRFP staining remained similar. In these sections, the *Hcrt* promotor region was hypermethylated at two specific CpG-sites (regions in the DNA where a guanine nucleotide follows a cytosine nucleotide and DNA-methylation can take place), inhibiting expression of prepro-Hcrt. This was not the case in blood cells from NT1 subjects. This may point to epigenetic silencing of Hcrt neurons, which hypothetically might even be reversible. However, many pieces of evidence are still missing to prove this hypothesis. For example, QRFP is produced by many hypothalamic neurons, not only by Hcrt producing neurons. The total amount of hypothalamic QRFP expression is thus the result of the activity of many types of neurons. The methylation data obtained in the human post-mortem NT1 sections represents data from numerous neurons in the LH. This is thus not representative of only the possibly silenced/-surviving Hcrt-producing neurons. Future studies should use more detailed techniques, such as single-nucleus RNA sequencing and spatial transcriptomics, to hopefully identify the genetic fingerprint of Hcrt neurons and assess if they are genuinely lost or still present in a silent state.

Whether Hcrt-1 deficiency is part of a larger secondary cascade of pathophysiological mechanisms or whether the widespread brain differences reported could be explained by disease chronicity remains up for investigation. Future neuroimaging studies in NT1 should clarify inconsistencies in reported results through harmonized analysis strategies in larger homogeneous patient cohorts. Direct comparisons with central hypersomnolence diagnoses without Hcrt-1 deficiency should also be performed.

5.2. Novel hypocretin agonists

Currently, used treatment options are only symptomatic. Since discovering that Hcrt deficiency causes narcolepsy symptoms, research has focused on the unmet need for compounds directly targeting the Hcrt system. Hcrt peptides are too large to cross the blood-brain-barrier, which makes it an unsuitable candidate for activating the Hcrt system in people with NT1. However, targeting Hcrt receptors with agonists are of interest. HcrtR2 is the most important receptor type for alleviating the sleep-related symptoms of NT1 (i.e. EDS, cataplexy, hypnogogic hallucinations, and disturbed nocturnal sleep). Other typical NT1 phenomena – like obesity, depression, and anxiety – probably rely more on HcrtR1, or a combination of both receptor types [7]. A few small molecule HcrtR2-specific agonists have shown promising results in pre-clinical and clinical trials [178]. Danavorexton (TAK-925) promotes wakefulness during the active phase and suppresses weight gain in narcoleptic mice [179]. Phase I studies in people with NT1 shows a decrease in EDS and cataplexy after administration of danavorexton [179] (ClinicalTrials.gov identifiers: NCT03332784 and NCT03748979). Because danavorexton enhances wakefulness in healthy men, the compound may also benefit hypersomnolence disorders without Hcrt deficiency [180]. Danavorexton is administered intravenously (IV) but another HcrtR2-specific agonist (TAK-861) can be administered orally and is currently under investigation in a phase II study (NCT05687903). The effect of a third HcrtR2-specific agonist (TAK-994), has been investigated in multiple phase I and II studies (NCT04551079, NCT03933488, NCT04096560, NCT04445129, NCT04667338, NCT04820842) but the ongoing trials have been prematurely terminated in October 2022 due to safety concerns. Despite people with NT1 showed improvements regarding their sleepiness and cataplexy symptoms, the use of this HcrtR2-specific agonist was also associated with drug-induced liver injury [181]. A fourth HcrtR2-specific agonist (YNT-185) currently under investigation ameliorates cataplexy and promotes wakefulness in various narcoleptic mouse models. Also, no increase in the cataplexy-like episodes have been observed after multiple injections of the compound [182]. A dual HcrtR1 and HcrtR2 agonist (RTOXA-43 or compound 40) investigated in mice shows reduced sleep fragmentation and longer wake time [183].

The Hcrt agonists are currently investigated in pre-clinical and clinical studies. In the upcoming year, results of some phase II studies are expected. The development of various types of Hcrt agonists to replace the shortage of Hcrt peptides in narcolepsy yields promising results for future drug developments in the treatment of NT1.

6. Conclusions

Hcrt deficiency causes NT1, but its exact aetiology remains unclear. As NT1 is strongly associated with the HLA-DQB1*06:02 and several other haplotypes and an increased NT1 incidence was related to the 2009 H1N1 pandemic, a possible auto-immune cause of the disease has been extensively investigated. The search for autoantibodies and autoreactive T-cells has not yielded conclusive evidence for the auto-immune hypothesis. Recently, other mechanisms like shortage of paraventricular CRH cells and potential epigenetic silencing are considered to impact NT1 possibly. Diagnosis and suitable treatment of NT1 in the early stages of the disease is essential, especially in children to prevent impaired cognitive, emotional and social development. Early treatment is also vital in light of possible auto-immune mechanisms as the cause of Hcrt deficiency. The development of HcrtR2-specific or dual HcrtR1 and HcrtR2 agonists shows promising results in pre-clinical and clinical trials. These agonists can potentially become the first drugs that directly target the Hcrt system and replace the shortage of Hcrt in NT1.

([184–195])

Research Agenda.

1. Structured international collaboration focused on identifying the influence of environmental factors could help the field forward.
2. The search for (remnants of) an autoimmune response should focus on the hypothalamus itself or as close to it as possible.
3. Hcrt receptor agonists show promising results in preclinical and clinical studies and should be further pursued. These compounds would be the first drugs to treat narcolepsy type 1 symptoms that directly target the hypocretin system.

Practice Points.

1. Narcolepsy type 1 is a chronic sleep-wake disorder caused by the loss of the neuropeptide hypocretin.
2. The strong association with HLA-DQB1*06:02, the increased incidence of narcolepsy type 1 after the H1N1 pandemic, and the identification of hypocretin-specific T-cells strongly suggest an autoimmune response leading to narcolepsy type 1, but conclusive evidence for the autoimmune hypothesis of narcolepsy type 1 is lacking.
3. According to the multiple hit model, the combination of a genetic predisposition and different environmental factors might better explain the pathophysiology of narcolepsy type 1 and differences between individuals with narcolepsy type 1.
4. Early recognition and treatment of narcolepsy type 1, especially in children, is needed to prevent impaired cognitive, emotional and psychosocial development of people with narcolepsy type 1.

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