A HUNDRED YEARS OF NARCOLEPSY RESEARCH

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The emergence of a clinical syndrome:

The first convincing descriptions of narcolepsy-cataplexy were reported in Germany by Westphal (1877) (100) and Fisher (1878) (25). The unique association of episodes of muscle weakness triggered by excitement and sleepiness were described in these two reports. In both cases, hereditary factors were noted, with the mother of Westphal’s patient and one sister of Fisher’s patient presenting narcolepsy symptoms. The leading hypothesis to explain narcolepsy at the time was to refer to the well publicized case of von Zastrow. Von Zastrow was a recently arrested pathological rapist widely believed to experience pathological sleepiness as a result of repressed homosexuality and excessive masturbation.

Gélineau (1880) (28, 29) is widely recognized for giving narcolepsy its name and for recognizing the disorder as a specific clinical entity. Its description of a wine cask maker with narcolepsy in la Gazette des hôpitaux de Paris was classical but Gélineau did not strictly differentiate muscle weakness episodes and sleep attacks triggered by emotions. Rather, Gélineau suggested a common physiology for these two symptoms. Loewenfeld (1902), was the first to give a name to muscle weakness episodes triggered by emotions or “cataplexy” (53).

The 1917-1927 epidemic of encephalitis lethargica led to a renewed interest in narcolepsy and sleep research but also added much confusion to the nosological definition of narcolepsy. Encephalitis lethargica often presented initially with somnolence and the term “narcolepsy” was often used to describe any form of daytime sleepiness. The association of somnolence and oculomotor paralysis also led to the pioneer work of Von Economo (1930) and the recognition of the posterior hypothalamus as a critical region for the promotion of wakefulness (98). In fact, Von Economo should be credited as being one of the first investigators to correctly propose that a region in the posterior hypothalamus was lesioned in human narcolepsy. In 1930, he wrote: “it is very probable, though not proved, that the narcolepsy of Gelineau, Westphal and Redlich has its primary cause in an yet unknown disease of that region” (98). As narcolepsy with cataplexy was also observed in some cases of encephalitis lethargica, clinicians long debated the existence of idiopathic narcolepsy and the importance of cataplexy for defining the clinical entity. Large case series of narcolepsy-cataplexy were reported by Addie (1926) (1), Wilson (1927) (101) and Daniels (1934) (17). The review by Daniels is considered by many as one of the most insightful clinical reviews published to date. Further work at the Mayo Clinic, by Yoss and Daly (103) and in Prague by Bedrich Roth (91), then led to the classic description of the narcolepsy tetrad.

Various methods were initially proposed in the treatment of narcolepsy, including intrathecal injection of air, cerebrospinal fluid removal and X-ray irradiation of the hypothalamic region. Ephedrine treatment was generally used as the only marginally effective treatment for daytime sleepiness until Prinzmetal and Bloomberg introduced amphetamines in 1935 (87). Yoss and Daly introduced methylphenidate in the 1960s (104). Shortly after the discovery of tricyclic antidepressants in 1957, Akimoto, Honda and Takahashi used imipramine in the treatment of human cataplexy (2), establishing the dual stimulant/antidepressant pharmacological treatment most commonly used in narcoleptic patients to this date.

Narcolepsy and its relationship with REM sleep:

The early descriptions of REM sleep and its associated atonia led multiple investigators to study sleep onset in
narcolepsy. Vogel (1960) was the first to report REM sleep at sleep onset in a narcoleptic patient (99), an observation extended by Rechtschaffen and Dement a few years later (88). Together with Hishikawa who studied the EEG of narcoleptic subjects during sleep paralysis and hypnagogic hallucinations (34), these authors articulated the now classical hypothesis of dissociated REM sleep to explain some of the symptoms of narcolepsy (88). This discovery also led to the establishment of the multiple sleep latency test as a standard diagnostic test for narcolepsy (15, 90).

In spite of this progress, the early postwar period was fraught with psychoanalytical explanations for narcolepsy-cataplexy. Together with Kleitman, who believed narcolepsy was an organic disorder involving abnormal carotid stimulation reflexes (46), Daniels, was among the few to believe in the organic nature of narcolepsy. In a letter to Kleitman dated July 21, 1948, Daniels wrote: "I was particularly interested in your views regarding the significance of narcolepsy, because I came to the same conclusion early in the course of my studies and have been decidedly annoyed by recent attempts of the psychosomaticists to revive the old idea that narcolepsy is simply a form of escape".

**Canine narcolepsy and neurochemical models in narcolepsy:**

Dr. William Dement pursued his interest in narcolepsy research after moving from Chicago to Stanford in 1963. In 1964, after running a small advertisement in the San Francisco Chronicle, a large number of narcoleptic patients was identified, suggesting that the clinical entity was more frequent than anticipated. A narcolepsy clinic was initially opened at Stanford in 1964-1965 and more permanently in 1970, when Christian Guillenault first visited Stanford. The first careful epidemiological studies were performed by Roth (91) and Dement (20, 21), indicating a prevalence of 0.02-0.05% for narcolepsy-cataplexy. Canine narcolepsy was described in 1973 by Knecht (47) and Miller (72). In the following few years, Drs. Dement and Miller visited veterinarians in more than 50 cities and spoke at many US colleges of veterinary medicine. A small number of animals of various breeds with narcolepsy were identified and a small canine colony was established. Poodles and Beagles were bred (backcrosses included) but genetic transmission was not established in these breeds (9, 26). In 1975, two affected littermate Dobermans and one unrelated Doberman with narcolepsy were donated to the colony, (9, 26). The breeding of these animals led to the first successful genetic transmission of narcolepsy, with a litter of affected animals born at Stanford on July 29, 1976. Multiple cases of Labradors with narcolepsy were subsequently reported and the trait was found to be transmitted as a single autosomal recessive gene (9, 26). Canine narcolepsy was further characterized at the clinical level (48, 55, 73, 80) but many investigators refused to believe these animals had narcolepsy.

The study of monoaminergic and cholinergic transmission in the regulation of sleep was emerging as a leading research avenue (43). Thanks to Jouvet’s pioneer lesion experiments (41), the pons was initially considered as a logical first candidate region for a narcolepsy abnormality. The levels of acetylcholine, various monoamines, metabolites and receptors were measured in the cerebrospinal fluid and various brain region of narcoleptic animals and humans (3, 4, 5, 12, 13, 24, 44, 44, 59, 71). This led to the general hypothesis of a pontine monoaminergic-cholinergic imbalance in narcolepsy (8). In this model, narcolepsy was the result of cholinergic hyperactivity and monoaminergic hypoactivity in the pons, a concept paralleling the Hobson and McCarley model for REM sleep regulation (35, 43). A primary role of the amygdala was also proposed based on the observation of consistent dopaminergic abnormalities in this brain region (59, 71). The involvement of this structure was also attractive conceptually as it potentially explained why cataplexy was triggered by emotions. A systematic pharmacological dissection of the mode of action of currently prescribed narcolepsy treatments was also initiated (80). This led us to demonstrate that presynaptic activation of adrenergic mediated the anticataleptic effects of antidepressants (67, 78). We also
established that wake promoting effects of amphetamine stimulants and modafinil was mediated by a presynaptic activation of dopaminergic transmission (66, 79). Other studies emphasized the importance of cholinergic hypersensitivity in the basal forebrain area (82), mesolimbic dopaminergic hypoactivity (89) and neuronal degeneration in the amygdala and the forebrain (94) as important pathophysiological abnormalities in canine narcolepsy.

**HLA, narcolepsy and autoimmunity:**

In 1981, together with Drs. Asaka and Juji, Dr. Honda initiated a research study on narcolepsy as part of a larger program on various intractable diseases. A significant increase in HLA-B35 frequency was observed in narcoleptic patients and was followed two years later by the discovery that all narcoleptic patients typed at the HLA-DR level were HLA-DR2 positive, when compared to 30% in controls (37, 38, 42). This discovery was quickly confirmed by British (49), German (75), French (11) and Canadian (86) investigators.

As most HLA associated disorder are autoimmune in nature, this discovery led to the hypothesis that human narcolepsy too was an autoimmune disorder (14, 68, 83). This possibility was addressed by Matsuki (58), Frederikson (27) and Rubin (92) but all results were negative, leading Matsuki to conclude “Narcolepsy is not an autoimmune disease” (58). The possibility that HLA-DR2 was only a linkage marker for another, non-immune related, narcolepsy gene located within the HLA complex was suggested (“the sleep gene hypothesis”) (58). The existence of non-HLA-DR2 positive patients with narcolepsy was hotly debated, with Dr. Honda stressing the importance of carefully defining cataplexy to diagnose narcolepsy (30, 57). The finding that African-American narcoleptic patients were frequently DR2 negative added to the confusion (77).

Further studies in African-Americans showed that the HLA association was tighter with another HLA gene allele, HLA-DQB1*0602 (56, 61, 62, 63). HLA-DQB1 is another polymorphic HLA class II gene located very close to HLA-DRB1 on human chromosome 6 (6p21). This observation explained the lower association reported with HLA-DR2 in African-American patients by Neely et al. (77) but did not explain the rare reports of HLA-DR2 and DQB1*0602 negative cataplectic patients by other clinicians (6, 30, 31, 62, 64, 95).

Investigators continued to search for potential narcolepsy genes in the Major Histocompatibility complex (MHC) region. Genomic sequencing studies in the HLA-DQ region indicated that no other expressed genes were present in the narcolepsy susceptibility region (23). Complex HLA-DQ genetic effects, with HLA-DQB1*0602 homozygotes (84) and DQB1*0602/DQB1*0301 (65) carrying the highest relative risks for narcolepsy susceptibility were reported. These data, paralleling those observed in other HLA associated diseases, suggested that HLA-DQ itself rather than an unknown gene in the MHC region was the susceptibility gene. Even using the improved DQB1*0602 genetic marker, however, a few cataplectic patients without the marker were reported (6, 30, 31, 62, 64, 95) suggesting possible heterogeneity in the etiology of narcolepsy. The high frequency of HLA-DQB1*0602 in the general population (12-38%, depending on the ethnic group) (61), also indicated that factors other than HLA-DQ had to be involved to produce narcolepsy in most cases.

**Positional cloning studies in narcolepsy:**

In the early part of the century, human narcolepsy was frequently believed to be a familial disorder. More recent studies have shown that human narcolepsy is not a simple genetic disorder. Monozygotic twins are most frequently discordant for narcolepsy, indicating environmental factors in narcolepsy susceptibility (70). In fact, familial clustering of narcolepsy-cataplexy is the exception rather than the rule. Only 1-2% of first degree relatives of patients with narcolepsy have narcolepsy-cataplexy (10, 31, 36, 70). This indicates a 20-40 fold increased relative risk when compared to the general population (70).

The complex picture in human narcolepsy led us to focus our genetic work in canines. In contrast to the human situation, narcolepsy is a simple autosomal recessive disorder in Dobermans and Labradors (26) thus making positional cloning theoretically possible in this species.
Backcrosses were carried out and a genetic linkage study initiated in 1989. Our first focus was to exclude potential candidate genes. Canine narcolepsy was shown not to be associated (19, 74) or tightly linked with Dog Leukocyte Antigen (DLA) polymorphisms (69). This result suggested the canine narcolepsy gene was not an MHC gene. Other candidate genes and minisatellite probes were used in a second stage. Using a candidate gene approach, a band crossreacting with the human immunoglobulin μ-switch segment on a southern blot was shown to cosegregate with canine narcolepsy in 1991 (69). This result initially suggested an immunoglobulin/immune involvement in canine narcolepsy. Further studies however demonstrated that this linkage marker was only a crossreacting sequence of no functional significance (60).

Considering the relatively small number of animals tested, the actual narcolepsy gene was likely to be located at a large genetic distance from our initial μ-switch like marker. Chromosome walking in the vicinity of the identified marker was difficult using available phage and cosmid genomic libraries. These libraries have small genomic inserts and chromosome walking is very slow, if not impossible. In 1997, a large insert Bacterial Artificial Library (BAC) genomic library was built in collaboration with Dr. De Jong (50). Fluorescence In Situ Hybridization (FISH) was also established in our laboratory and the canine narcolepsy marker was found to be located on dog chromosome 12 (51). We also found that the μ-switch like marker was on the same chromosome as the Dog Leukocyte Antigen (DLA) but that both loci were at a large genomic distance from each other (51).

Chromosome walking was initiated using the newly available BAC library. In this process, high density grided library filters are hybridized with Polymerase Chain Reaction (PCR) products derived from end BAC sequences. The new clones are then isolated, PCR tested, their ends sequenced and the filters rehybridized to extend the contig. Minilibraries are prepared and screened with microsatellite repeats to develop polymorphic markers. These markers are then tested in canine backcrosses to confirm genetic linkage and map possible recombinant animals (51). BAC end sequences are also analyzed using the BLAST algorithm to identify putative known genes. In 1998, one of the BAC end sequences was shown to contain Myosin VI (MYO6), a gene known to map on the long arm of human chromosome 6 (6q13). The finding that both DLA and MYO6 were on the same dog and human chromosomes led us to suspect a large region of conserved synteny between dog chromosome 12 and human chromosome 6. This result was a turning point as it gave us direct access to the human and mouse maps in the region. Human Expressed Sequenced Tags (ESTs) known to map between MHC and MYO6 on the human map were then used as probes to screen the canine BAC library. The resulting canine BAC clones were then hybridized on canine metaphase spreads to verify localization onto dog chromosome 12 (51). Together with chromosome walking and microsatellite marker development and genetic testing in backcrosses, the process was refined until the canine narcolepsy gene was flanked in a small genetic segment known to contain only two potential genes. These two genes were tested for potential abnormalities and an abnormal hybridization pattern observed with one of the two ESTs, the hypocretin receptor 2 gene (Hcrtr2) (51). Further analysis then demonstrated that in both Labradors and Dobermans with autosomal recessive narcolepsy, the hypocretin receptor transcripts were disrupted by distinct exon splicing mutations (51). This report was the first to implicate hypocretins in the cause of canine narcolepsy.

**Hypocretins, orexins and their receptors:**

Hypocretins/orexins were identified almost simultaneously by DeLecea (18) and Sakurai (93) in 1998. DeLecea et al. identified the preprohypocretin transcript using a directional tag PCR subtraction technique (18). Their aim was to identify novel hypothalamic specific transcripts. The identified hypocretin clone was shown to be only expressed in the lateral hypothalamus and to encode a precursor molecule for two related peptides having a possible homology with secretin (this weak homology is disputed by others). Based on the preferential expression of the gene in the lateral hypothalamus and their homology...
with the gut hormone secretin, the peptides were called hypocretin-1 and 2 by DeLecea (18), who also demonstrated neuroexcitatory properties for hypocretin-2 and suggested a possible role in feeding regulation based on the neuroanatomical localization in the lateral hypothalamus. The existence of hypocretins was independently confirmed by Sakurai a few weeks later (93). These authors also identified and mapped two receptors for these peptides (Hcrt1 and Hcrt2). In this elegant work, a series of orphan G-Protein coupled receptors (e.g., receptor genes having no identified endogenous ligand identified) were expressed in cell lines (the “orphange”) and the resulting cell lines used to screen High Pressure Liquid Chromatography (HPLC) purified tissue fractions for biological activity (93). Cell lines containing the orphan receptor HFGAN72 (later shown to be the hypocretin receptor-1) were found to strongly react with purified brain fractions. These fractions were shown to evoke a calcium transient, suggesting activation of the G-Protein coupled receptor by an endogenous ligand. The resulting activity was purified and shown to be a 33 amino acid peptide that Sakurai et al. called orexin-A (93). Another weaker activity was also isolated and shown to be a 28 aminoacid sharing 13/28 aminoacid identity with orexin-A; this second peptide was called orexin-B (92). Both peptides were then shown to be processed from the same precursor, a transcript identical to DeLecea’s previously reported preprohypocretin mRNA molecule (18). Hypocretin-1 and 2 and Orexin-A and B are thus identical with the caveat that DeLecea reported a 6 amino acid longer sequence for hypocretin-1 versus orexin-A. The latter author mentioned that the N-terminal of the hypocretin-1 peptide could not yet be established at the time (18). Sakurai et al. (93), also noted that hypocretin-2/orexin-B had a lower affinity for the hypocretin receptor-1 and found that another unknown EST had high nucleotide homology with HFGAN2. This receptor was expressed in CHO cell lines and was shown to bind and mobilize calcium in the presence of hypocretin-1 and 2. This second receptor was called the Orexin receptor 2 (officially called the hypocretin receptor-2).

The discrete localization of these peptides in the lateral hypothalamus suggested a role for hypocretins in feeding behavior. In their initial publication, Sakurai (93) reported a stimulation of feeding after central administration of hypocretins/orexins and an increased preprohypocretin mRNA expression after fasting. The authors speculated that a main physiological function for these molecules could thus be the regulation of energy homeostasis (93). Further work indicated more minor and variable effects on feeding (22, 33, 40, 54, 96, 102). Neuroanatomical work indicating widespread projections for hypocretin neurons also suggested more complex physiological functions (76, 85). Of note, dense projections to monoaminergic cell groups such as the locus coeruleus suggested a possible involvement in sleep regulation (39). In 1999, a few weeks after canine narcolepsy was shown to be due to hypocretin receptor mutations, a knockout mouse for the preprohypocretin gene was generated and shown to have sleep abnormalities reminiscent of narcolepsy (16), thus independently suggesting a role for hypocretin in the sleep disorder.

Hypocretins in human narcolepsy

The potential role of hypocretins in the human disorder is still under investigation. Hypocretin-1 levels were measured in the cerebrospinal fluid of 9 narcoleptic subjects and 8 controls by Nishino et al. (81). Seven narcoleptic subjects were found to have undetectable hypocretin-1 levels. Two narcoleptic patient had normal and very elevated levels of hypocretin-1 respectively. Hypocretin-1 levels were detectable in all controls. This result suggests that human narcolepsy is caused by a deficiency in hypocretin production (81). A simple explanation might be that hypocretin producing cells are destroyed by an autoimmune process in HLA-associated narcolepsy. Only a few thousand cells in the hypothalamus produce hypocretins and a discrete lesion in this area might not have been detected in previous neuropathological studies.

Perspectives:
The observation that human narcolepsy is associated with low/absent hypocretin levels opens novel therapeutic
and diagnostic perspectives. Measuring hypocretin levels in the CSF might become a standard diagnostic procedure. Hypocretin levels are undetectable in plasma using current technology but the existence of hypocretin and receptors in the gut (45) and on the adrenal medulla (52) suggests that very low levels might be circulating in the periphery. These could one day be measured. Novel therapies are also likely to be developed. Hypocretin-1 has recently been shown to promote wakefulness in vivo (32). Hypocretin receptors are probably functional in most cases of human narcolepsy. It might thus be possible to administer analogues or receptor agonists to supplement a deficient hypocretin neurotransmission. Hypocretin antagonists might also have desirable hypnotic properties.

The finding that hypocretins are centrally involved in narcolepsy also opens new research avenues in basic sleep research. The excitatory neurotransmitter system is uniquely positioned at the neuroanatomical level to modulate monoaminergic and cholinergic transmission. Some of these projections are likely to be more important than others and a challenge of the next decade will be to establish functional relevance. The respective role of Hcrtr1 and Hcrtr2 (97) and the relationship with reported neuronal degeneration in the basal forebrain and the amygdala of narcoleptic dogs (94) also needs further clarification. Narcolepsy being primarily characterized by disrupted sleep state organization, hypocretin cells might be critical coordinators of the sleep cycle via their monoaminergic projections.

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Table 1: A few milestones in narcolepsy research and therapy

<table>
<thead>
<tr>
<th>Year</th>
<th>Milestone</th>
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<tbody>
<tr>
<td>1877</td>
<td>First description in the medical literature (100)</td>
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<tr>
<td>1880</td>
<td>Gelineau called the disorder “narcolepsy” (28)</td>
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<tr>
<td>1902</td>
<td>Loewenfeld coined the term “cataplexy” (53)</td>
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<tr>
<td>1935</td>
<td>First use of amphetamines in the treatment of narcolepsy (87)</td>
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<tr>
<td>1960</td>
<td>Description of Sleep Onset REM periods in a narcoleptic subject (99)</td>
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<tr>
<td>1970</td>
<td>Description of the Multiple Latency Test (15, 90)</td>
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<tr>
<td>1973</td>
<td>First report of a narcoleptic dog (47, 72)</td>
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<td>1983</td>
<td>Association of narcolepsy with HLA-DR2 (37)</td>
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<tr>
<td>1985</td>
<td>Monoaminergic and cholinergic imbalance in narcolepsy (7, 80)</td>
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<tr>
<td>1992</td>
<td>Association of narcolepsy with HLA-DQB1*0602 (56, 63)</td>
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<tr>
<td>1998</td>
<td>Identification of hypocretins/orexins and their receptors (18, 93)</td>
</tr>
<tr>
<td>1999</td>
<td>Hypocretin mutations cause narcolepsy in mice and dogs (16, 51)</td>
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<tr>
<td>2000</td>
<td>Human narcolepsy is also associated with an hypocretin deficiency (81)</td>
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References


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