Transcriptomics Signature of Type 1 Narcolepsy (T1N)

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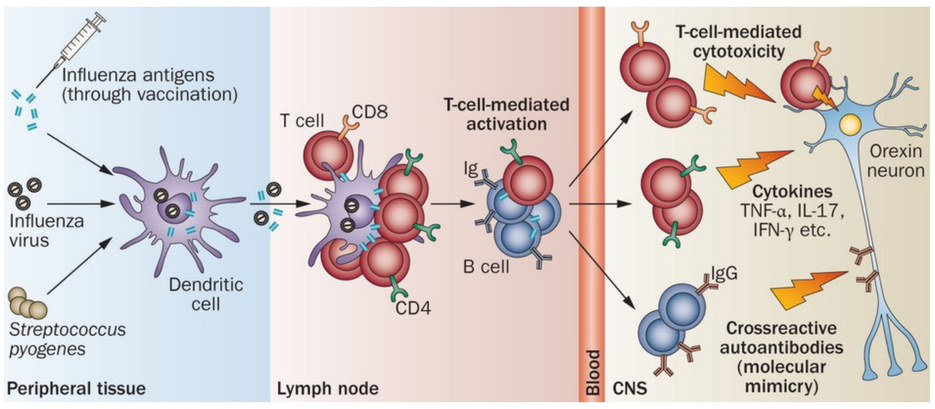
• Introduction

Narcolepsy (type 1) is a rare and severe sleep disorder characterized with the exclusive and extensive destruction of orexin-producing hypothalamic neurons. The patients exhibit cataplexy (episodes of partial or complete paralysis of the voluntary muscles), sleep paralysis, hypnagogic hallucinations, as well as excessive daytime sleepiness and fragmented nocturnal sleep. Orexin-A and -B are small peptide neurotransmitters produced only by a cluster of neurons in the lateral hypothalamus which stimulate target neurons that promote wakefulness, regulate appetite and conserve energy. The mechanisms involved in the selective destruction of orexin neurons are not yet elucidated but its association with certain environmental triggers (vaccine against flu virus - Pandemrix), genetic susceptibility (strong association with the human leukocyte antigen – HLA), T-cell receptor (TCR) and other immune loci (such as CTSH, P2RY11, ZNF365, IFNAR1, TNFSF4) implicate an immunopathological process.

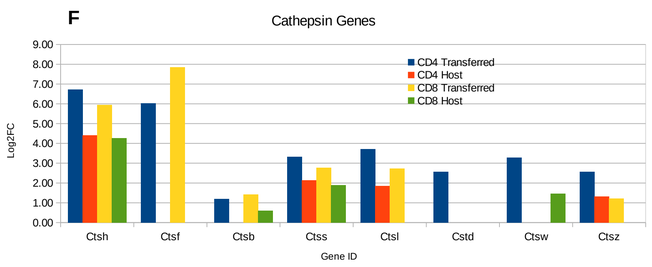
• Hypothesis

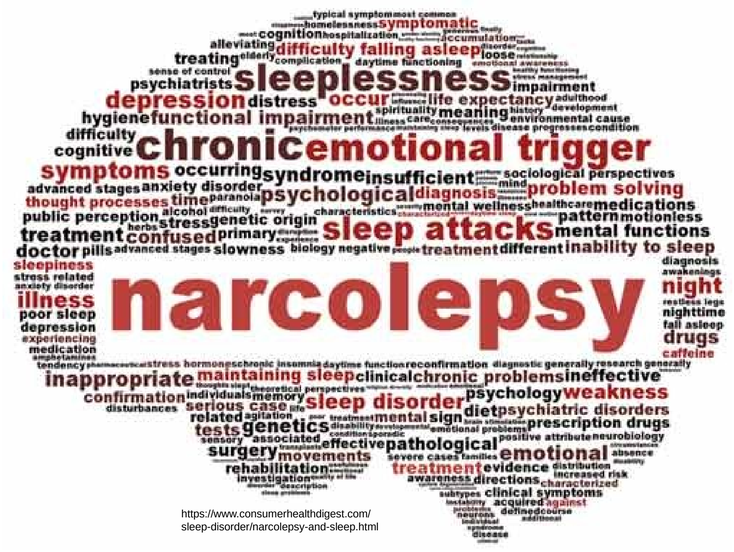
Our hypothesis is that microbial antigens (from an infection or vaccination), which share similarities with self antigens from orexin neurons, are taken up by dendritic cells in peripheral tissues. These dendritic cells then mature and migrate to local lymph nodes where they present antigen-specific epitopes to T cells that express CD4 or CD8. Activated T cells migrate across the blood brain barrier (BBB) into the central nervous system (CNS). In the brain, an (auto)immune response mediated by these activated T cells contributes to the selective destruction of orexin-producing neurons in the hypothalamus, whereas nearby non-orexin neurons are left largely unharmed.

Figure 1
Graphical summary of hypothetical model leading to an (auto)immune response resulting in the destruction of orexin-producing neurons in the hypothalamus.



Source: Partinen et al., Narcolepsy as an autoimmune disease: the Role of H1N1 infection and Vaccination; Lancet Neurol., 2014 13(6):600-13





• Objective

To investigate whether an (auto)immune process could lead to narcolepsy development and to decipher the mechanisms involved in the selective loss of orexin neurons, we have developed a mouse model of immune-mediated narcolepsy (Orex-HA) to determine the molecular profiles of the hypothalamus infiltrating T-cells (Figure 2). The Orex-HA mice are genetically engineered to express hemagglutin (HA) of the H1N1 influenza virus as a self-antigen selectively on orexin neurons.

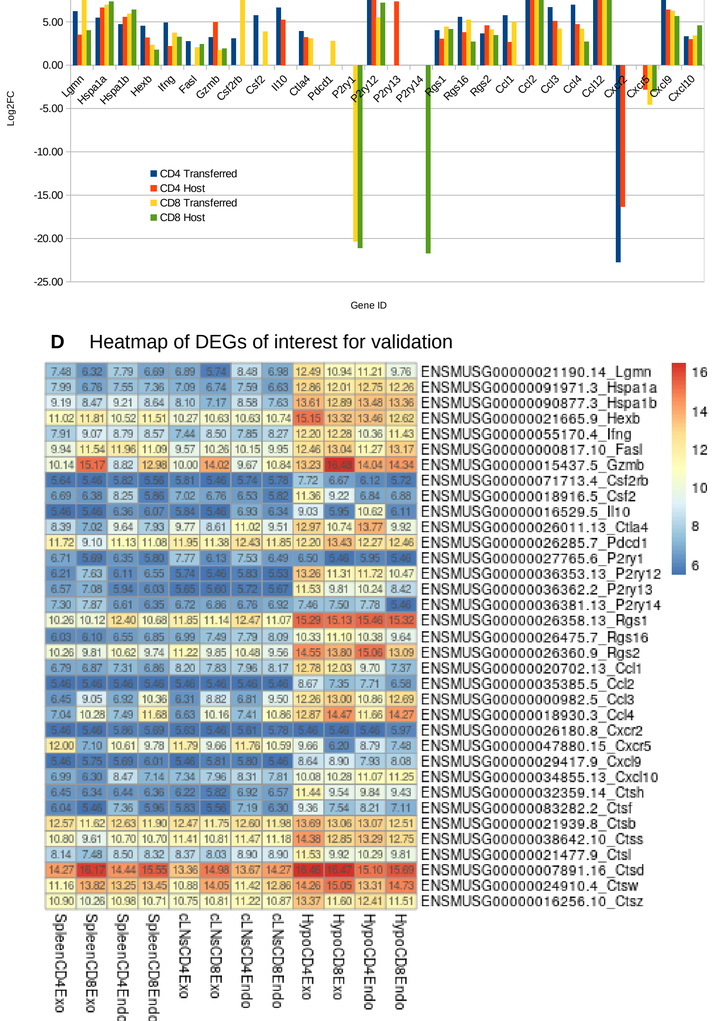


Figure 3: Results
a. Principle Component Analysis (PCA) showing a clear separation between samples from the hypothalamus and periphery (spleen and lymph nodes) tissues (along PC1) as well as a separation between CD4 and CD8 T cell populations (PC2).
b. Global view of the differentially expressed genes along each chromosome for the CD8 transferred population, comparing Brain vs Periphery
c. Global view of the differentially expressed genes along each chromosome for the CD4 transferred population, comparing Brain vs Periphery
d. Heatmap of the genes of interest for validation; numbers represent the mean of the variance stabilized counts across four experiments; sample names are represented by the columns and gene names are represented by the rows
e. Bar graph displaying the log2fold change of each gene of interest for the four different T cell populations, calculated by comparing Brain vs Periphery tissues.
f. Bar graph displaying the lod2fold change of the cathepsin genes for the four different T cell populations, calculated by comparing Brain vs Periphery tissues.

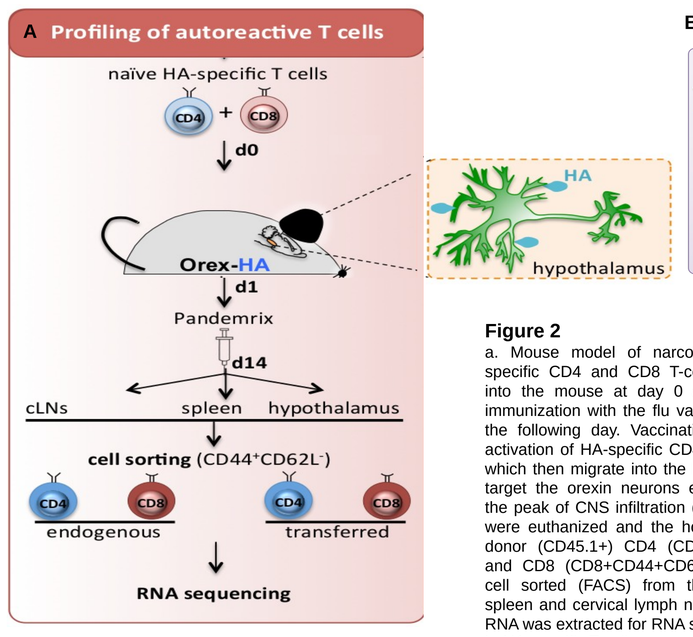


Figure 2
a. Mouse model of narcolepsy: naive HA-specific CD4 and CD8 T-cells were injected into the mouse at day 0 and activated via immunization with the flu vaccine (Pandemrix) the following day. Vaccination results in the activation of HA-specific CD4 and CD8 T-cells which then migrate into the hypothalamus and target the orexin neurons expressing HA. At the peak of CNS infiltration (day 14), the mice were euthanized and the host (CD45.1-) and donor (CD45.1+) CD4 (CD4+CD44+CD62L-) and CD8 (CD8+CD44+CD62L-) T-cells were cell sorted (FACS) from the hypothalamus, spleen and cervical lymph nodes (cLNs). Total RNA was extracted for RNA sequencing.
b. HA-specific T cells

• Results