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16.45 - 17.05: Sleep and metabolism: Potential for anti-obesity drug developments

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Integration of Multiple Systems in the Hypothalamus

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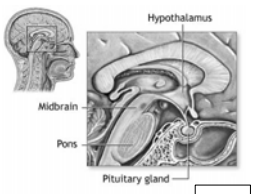
- Sleep
- Circadian Rhythms
- Feeding - Appetite
- Metabolism
- Sexual Behaviour - Puberty
- Reproduction
- Stress
- Inflammation
- Growth
- Lactation



Integration of Multiple Systems in the Hypothalamus

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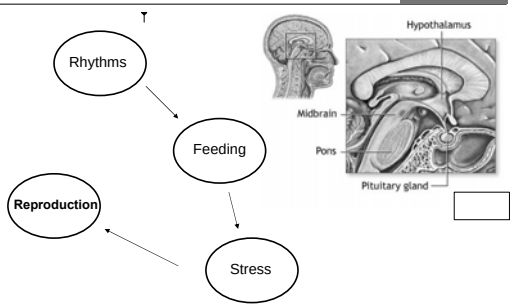

Integration of Multiple Systems in the Hypothalamus

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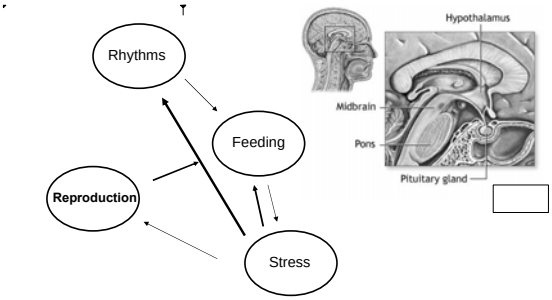

Pharmacological Treatments and the Hypothalamus: Target of Action outside.

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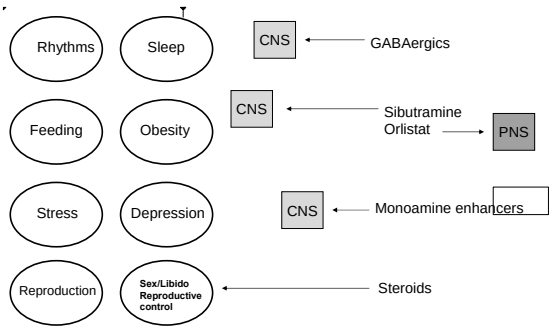

Strategies for Finding new Treatments

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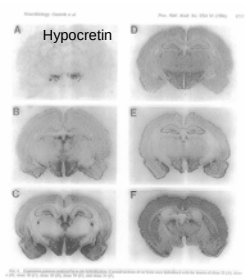

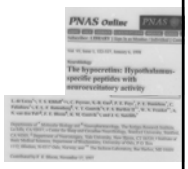

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How hypocretin got its name

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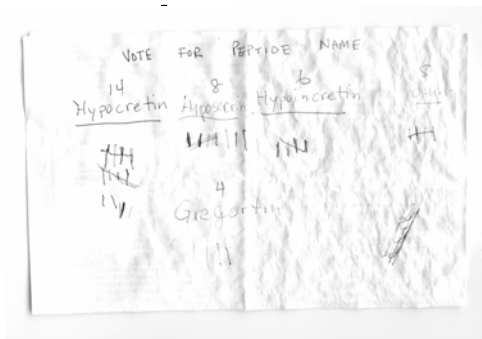

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Target Identification: Neuronatin

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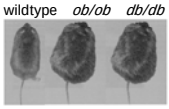
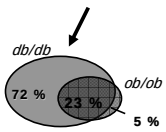
wildtype *ob/ob* *db/db*



Genotype	Glucosekonc. [mM]
C57BL/6J (n=4)	3.1± 1
C57BL/6J:Ob (n=4)	10± 1
C57BL/6J:Db (n=4)	22± 10

Expression analysis



Found genes

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Bioinformatic analysis/prediction of the found gene product: Neuronatin

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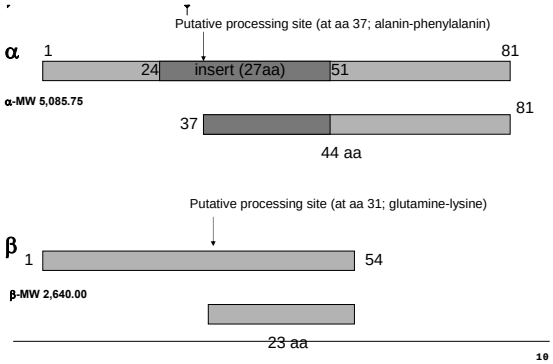

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Neuronatin Inhibits Food Intake in the Rat

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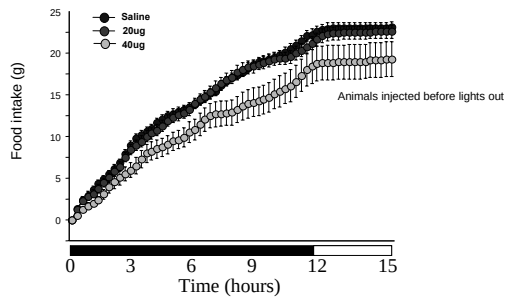

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Target Discovery in Animals

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Pro

Detectable
Measurable
Phenotype using transgene animals

Con

Involvement in Pathophysiology

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Narcolepsy-cataplexy – An Autoimmune disease?

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Excessive daytime sleepiness "EDS" and sleep attacks

Cataplexy
(muscle atonia triggered by positive/negative emotions, especially laughter, jokes and anger)

Sleep paralysis
(atonia at transition between sleep/awakening)

Hypnagogic hallucinations
(visuel, auditive, tactile episodes at transition between sleep/awakening)

Stine Knudsen[1], Jens D. Mikkelsen[2], Poul Jennum[1]
[1]Danish Center for Sleep Medicine, Glostrup Hospital, and [2]Neurosearch A/S, Denmark

Onset of Symptoms in Narcolepsy

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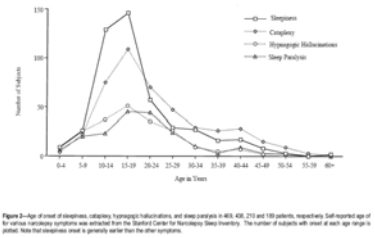

Figure 2—Age of onset of sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis in 409, 408, 210 and 169 patients, respectively. Self-reported age of for various narcolepsy symptoms was extracted from the Stanford Center for Narcolepsy Sleep Inventory. The number of subjects with onset at each age range is plotted. Note that sleepiness onset is generally earlier than the other symptoms.

Sleep. 2002 Feb 1;25(1):27-35.

Strong Genetic Factor in Narcolepsy

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- **5-7% of patients have relatives with narcolepsy, (1. degree relatives: 20-40% increased risk of narcolepsy)**
- **90% with cataplexy og 56% without cataplexy have at specific HLA-type (DQB1*0602), indicating a strong autoimmune component.**

Sleep 1994;17(suppl 8):54-59 Lancet 1989;2:1376-79 Am J Hum Genet 2001;68:686-99

Specific Death(?) of Hypocretin neurons

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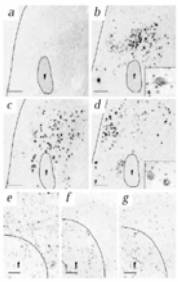
- 85-100% reduction in hypocretin neurons in the hypothalamus
- Ca. 90% of narcolepsy-cataplexy have low levels of (<110 pg/ml) hypocretin-1 in the cerebrospinal fluid



Nature Medicine 6, 991 - 997 (2000)

The Hypothesis

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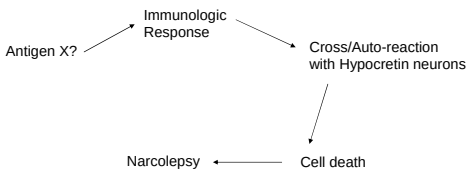

Treatment Opportunities

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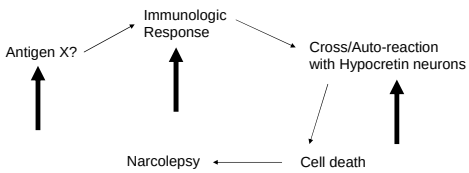

The Danish Study – 9 patients for IgG tests

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HLA DQB1*0602-positive/negative and CSF-hypocretin

4. Danish laboratory data	narcolepsy-cataplexy	narcolepsy without cataplexy
HLA-type		
HLA-DQB1*0602-positive	100% (25/25)	35.71 % (5/14)
CSF-hypocretin		
mean value (pg/ml)	40.34±98.67	379.47±155.61 *(p=<0.0001)*
<110 pg/ml	96.43% (27/28)	14.29% (2/14)
100-200 pg/ml	0% (0/28)	0% (0/14)
> 200 pg/ml	3.57% (1/28)	85.71 % (12/14)

Narcolepsy: Autoantibodies against hypocretin neurons in serum/CSF?

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Methods:

Serum (diluted 1:20 or 1:100) and CSF from 9 of our HLA-DQB1*0602-positive HCRT-deficient Narcolepsy-cataplexy patients and 9 age and gender matched controls.

Patient data: age 14-63 years, disease duration 3-41 years,
MSLT: sleep latency < 8 min and ≥2 SOREMPS
CSF-hypocretin-1: <110 pg/ml

Samples were each applied onto 6 coronal sections of paraformaldehyde-fixed adult male rat hypothalamus.

Immunostaining was evaluated blind to the nature of the sample. Double-staining with anti-HCRT-IgG was done if positive immunostaining was found.

NeuroReport. 18(1):77-79, (2007)

Serum from a DQB1-0602-positive patient with narcolepsy-cataplexy binds to rat hypocretin neurons

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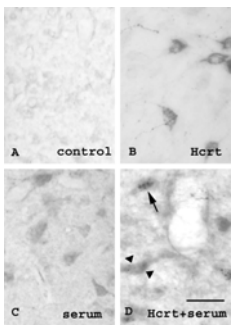

NeuroReport. 18(1):77-79, (2007)

Results:

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Immunostaining of HCRT-containing neurons was found in serum of one (of 9) narcolepsy patient. - Not in the CSF?!

Same result confirmed in an additional sample from the same patient taken 1 year and 3 months later.

NeuroReport. 18(1):77-79, (2007)

Discussion and Perspectives

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- Not the right cellular target (neurons/axons outside hypothalamus)?
- Antibody levels are too low for detection?
- Antibodies are mainly present at disease onset?
- It is not humoral (IgG) autoimmunity (but cellular)?
- No Cross-reactivity between rat and human antigens
- Fixation has destroyed the antigen

Thanks

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Danish Center for Sleep Medicine, Glostrup Hospital,

Rheoscience A/S