NARCOLEPSY AND AUTOIMMUNITY IN MICE

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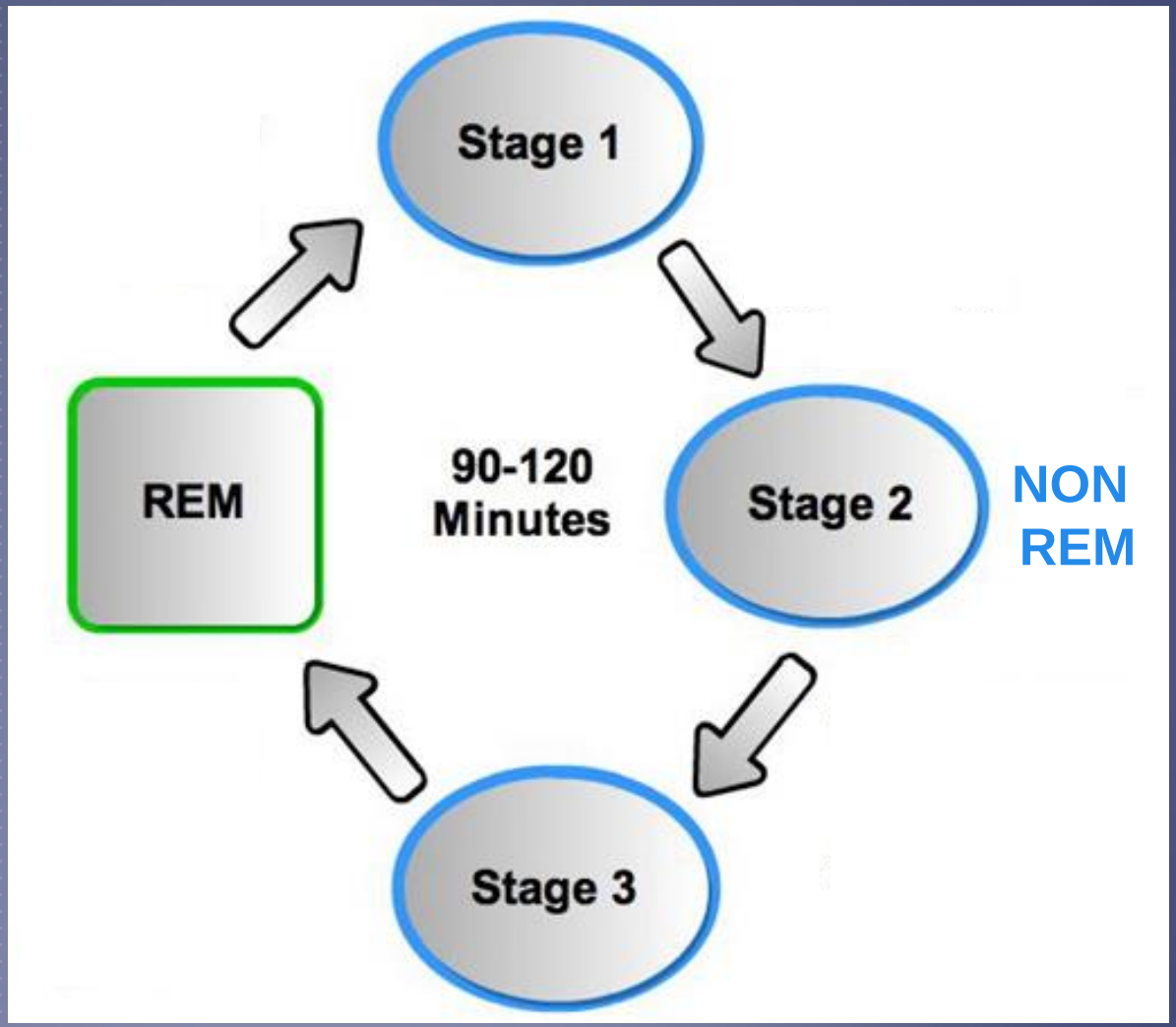
NARCOLEPSY

- Sleep/Wake Dysregulation
- Daytime Sleepiness
- Sudden onset cataplexy (loss of muscle tone)
  - 50% of patients
- Attacks are often triggered by emotion or exertion
- Affects 25-50 people per 100,000
- A difficult condition to live with

SLEEP CYCLE

Awake State

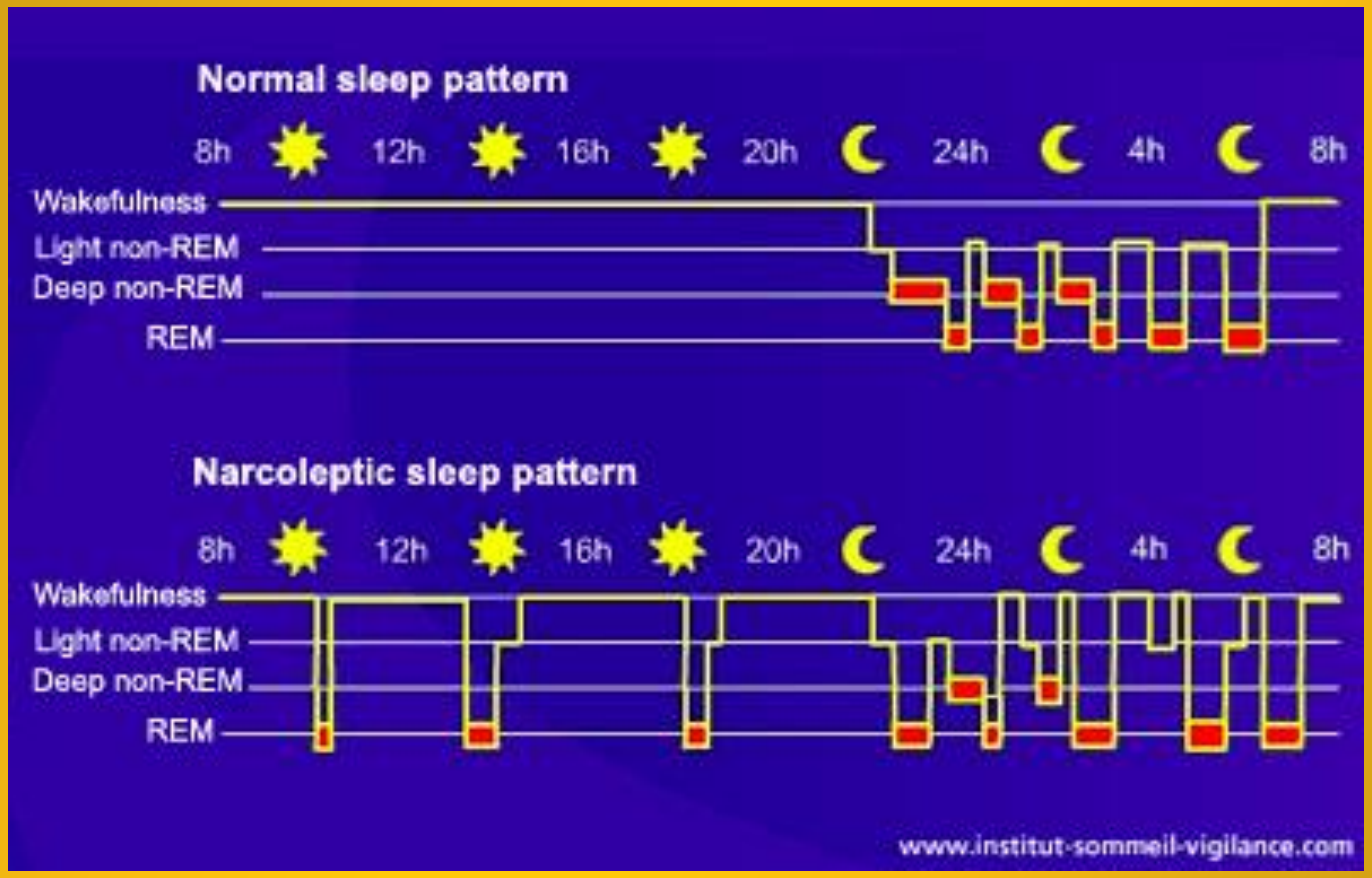


	Brain Activity	Skeletal Muscles
NON REM	↓	↑
REM	↑	↓

Rapid Eye movement

Huang W, Ramsey KM, Marcheva B, Bass J (2011) Circadian rhythms, sleep, and metabolism. J Clin Invest 121: 2133-2141

SLEEP CYCLE


Normal sleep pattern
8h
12h
16h
20h
24h
4h
8h
Wakefulness
Light non-REM
Deep non-REM
REM
Narcoleptic sleep pattern
8h
12h
16h
20h
24h
4h
8h
Wakefulness
Light non-REM
Deep non-REM
REM
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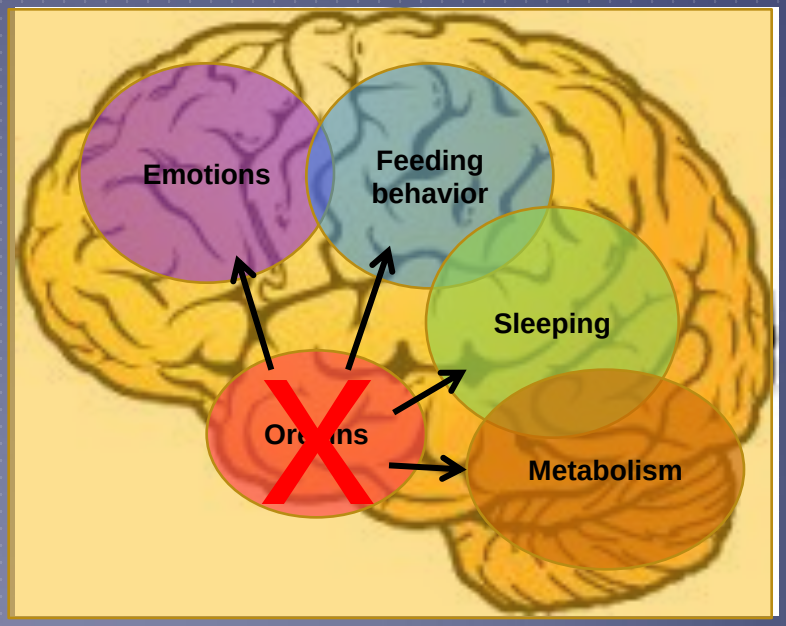
ETIOLOGY

- Not completely understood
  - Low levels of Orexin in the CSF
- Orexins are produced in hypothalamus
  - Orexins have stimulatory effect over the mono-aminergic neurons
  - Control release of neurotransmissors associated with alert states.
- Loss of orexin (hypocretin) neurons



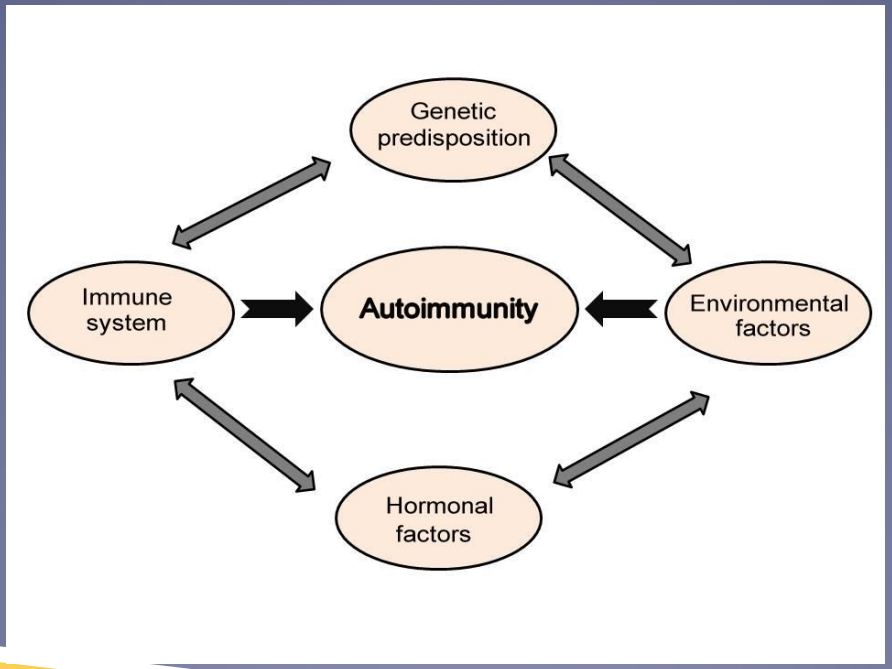
The main issue in narcolepsy is the loss of orexin neurons

Autoimmunity?

Ohno K, Sakurai T (2008) Orexin neuronal circuitry: role in the regulation of sleep and wakefulness. Frontiers in neuroendocrinology 29: 70-87

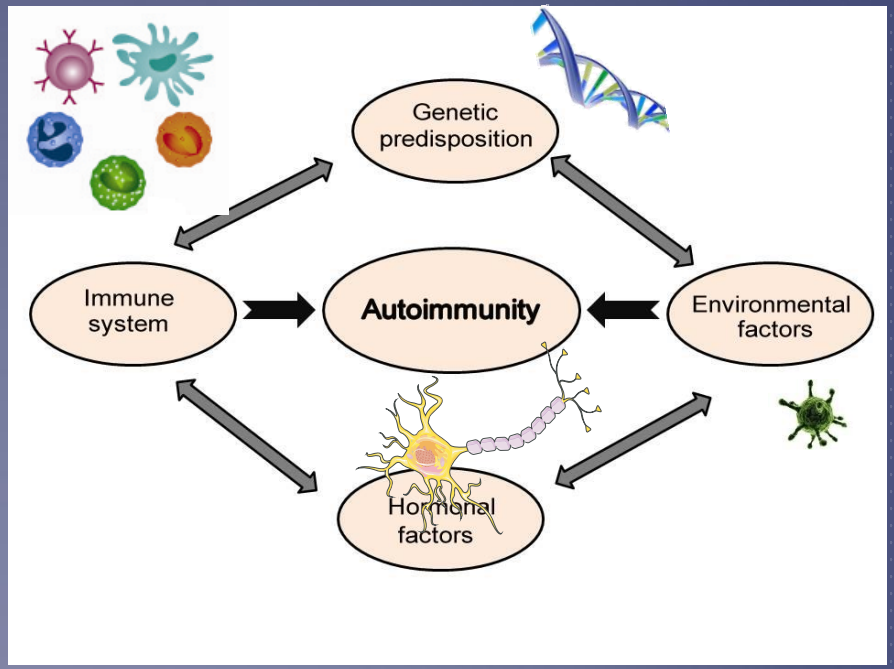
AUTOIMMUNE DISEASES

- Disease where the immune system attacks ones body.
- Develops in predisposed individuals.



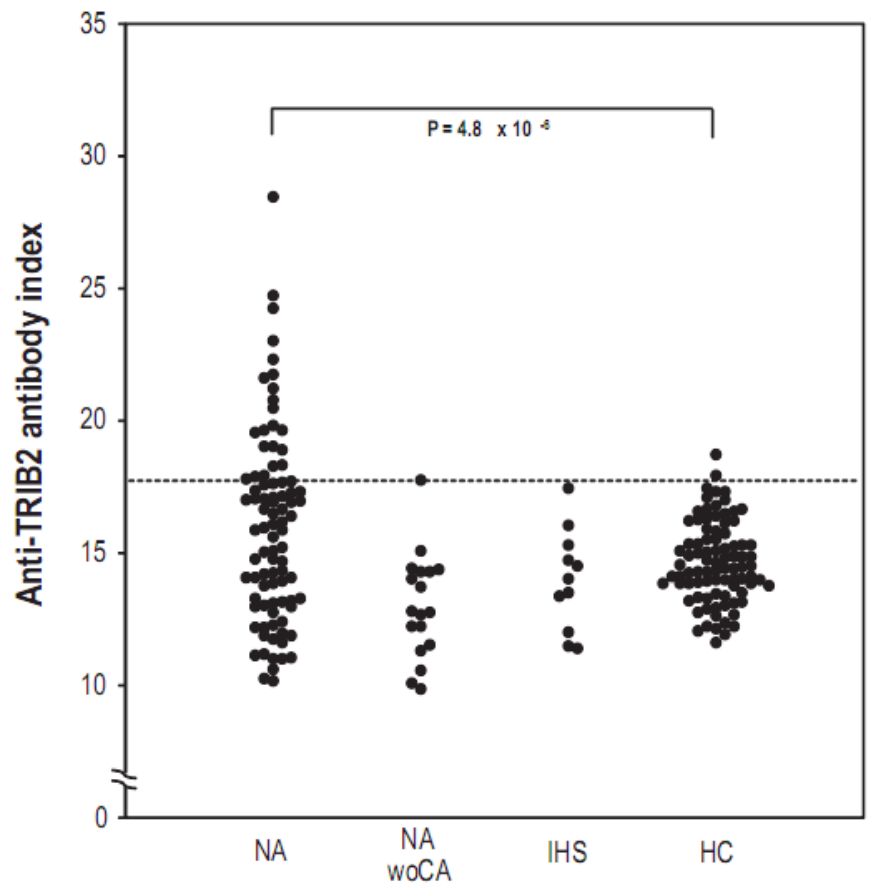
WHY NARCOLEPSY?

- Autoimmunity Hypothesis
  - Several clues lead researchers to believe that the mechanism behind narcolepsy is autoimmunity
- Genetics
  - HLA-DQB1*0602 allele is present in 82-99% of narcoleptic patients
- Environmental
  - Evidence of cases of Narcolepsy with Cataplexy after vaccination against H1N1.
  - Streptococcus infections (antibodies against Strep, found in patients with recent onset narcolepsy)



Arango MT, Kivity S, Shoenfeld Y. Is narcolepsy a classical autoimmune disease? Pharmacol Res 2015 Feb;92:6-12.
Kornum BR, Faraco J, Mignot E (2011) Narcolepsy wit hypocretin/orexin deficiency, infections and autoimmunity of the brain. Current Opinion in Neurobiology

ANTIBODY FOUND?



- Autoantibody anti Tribbles homologue 2 (Trib2) has recently been found in the serum of afflicted individuals
- 26.1% of Japanese patients suffering from narcolepsy had higher levels of anti -Trib2 compared to 2.3% of healthy controls

Kawashima M, Lin L, Tanaka S, Jennum P, Knudsen S, et (2010) An9-Tribbles homolog 2 (TRIB2) autoantibodies in narcolepsy are associated with recent onset of cataplexy. Sleep 33: 869.

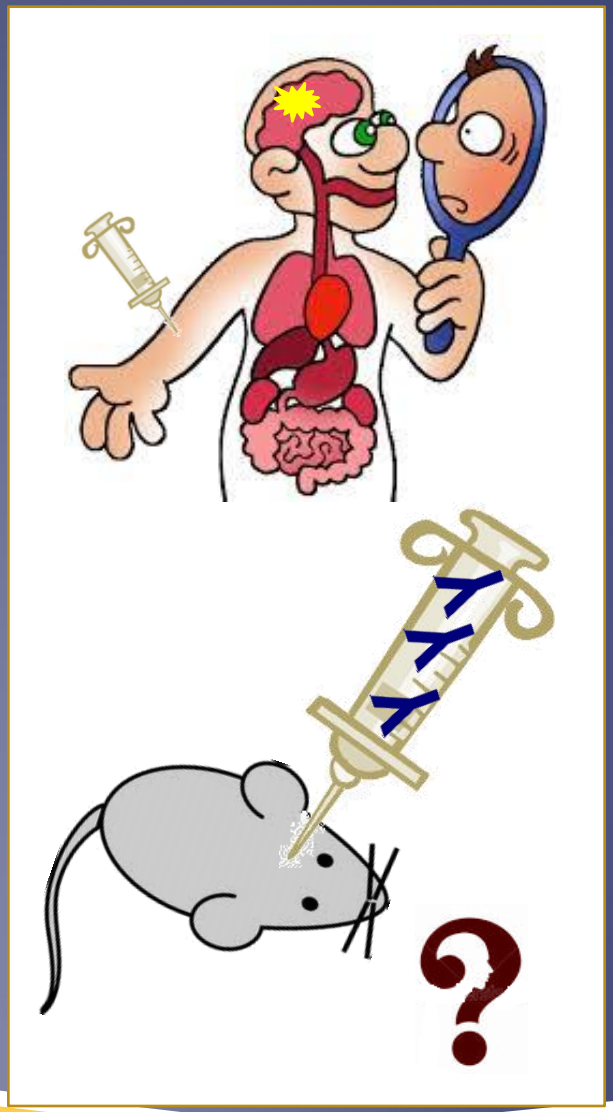
Witebsky's Postulates

- Criteria to establish a causal link between an autoantibody and a disease:
  1. **Identification of the autoantigen**
  2. **Reproduction of the disease**
  3. **Active immunization with the autoantigen**
  4. **Passive transfer of T cells or autoantibodies**

Lim ASP, Scammell TE (2010) The trouble with Tribbles: do antibodies against TRIB2 cause narcolepsy? Sleep 33: 857.

AIM of Study at The Zabludowicz Center for autoimmune diseases

- The aim of this study is to further confirm the theory that narcolepsy is an autoimmune disorder.
- Induction of narcolepsy in mice through passive transfer of total-IgG (including Trib2 autoantibodies) purified from patients with narcolepsy.



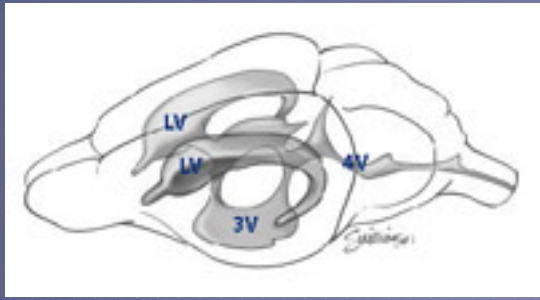
METHODS

- Evaluate the effect of injecting total-IgG from narcoleptic patients to mice brain.

- Mice evaluated for:
  - Sleep behavior
  - Neurocognitive behavior
  - Brain histology

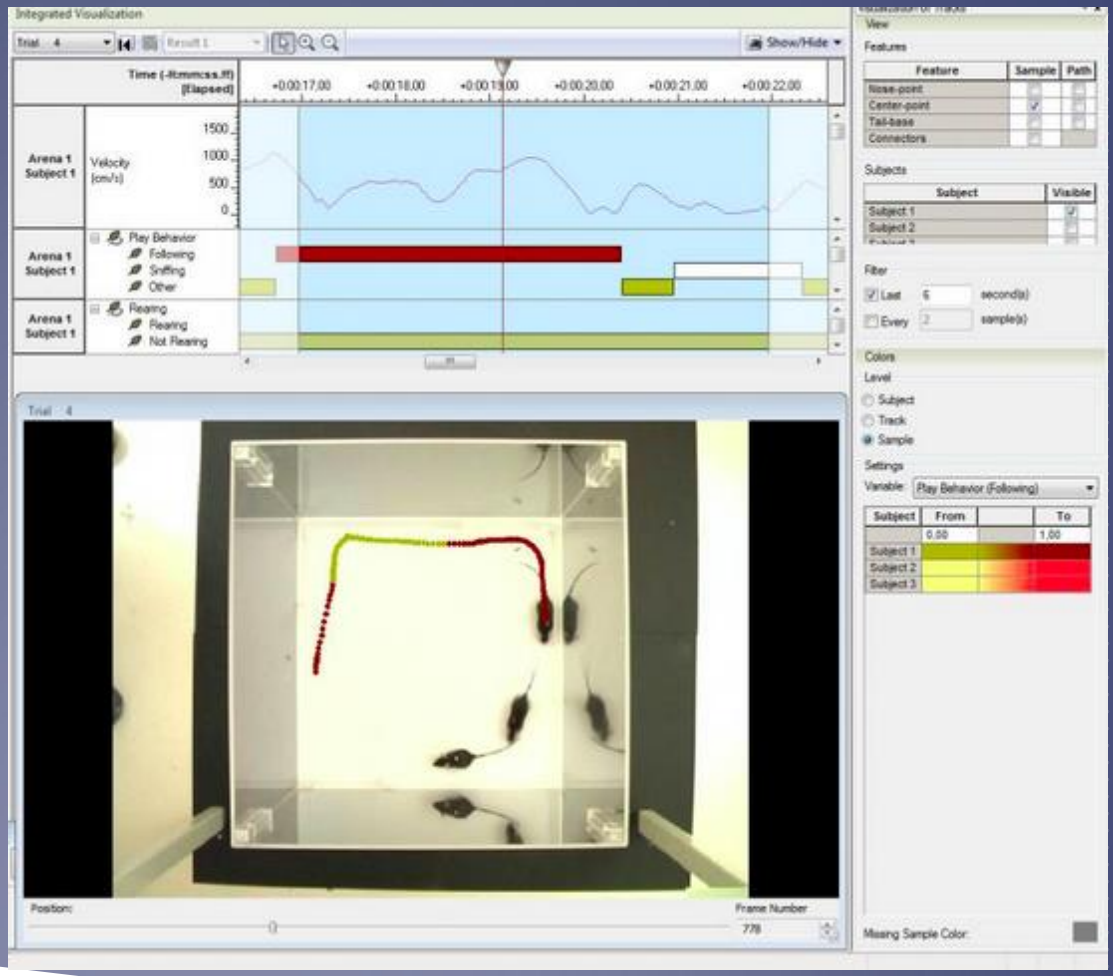
INJECTION OF MICE

1. Mice are anesthetized
2. Skull exposed
3. Injection given in lateral vetricle by entering 2mm anterior and lateral to lambda suture



SLEEP PATTERN

- The sleep behavior is analyzed by looking for freezing events by using EthoVision software®.
- Freezing events defined as an abrupt transition from an obvious motor activity, with the resumption of obvious purposeful motor activity.

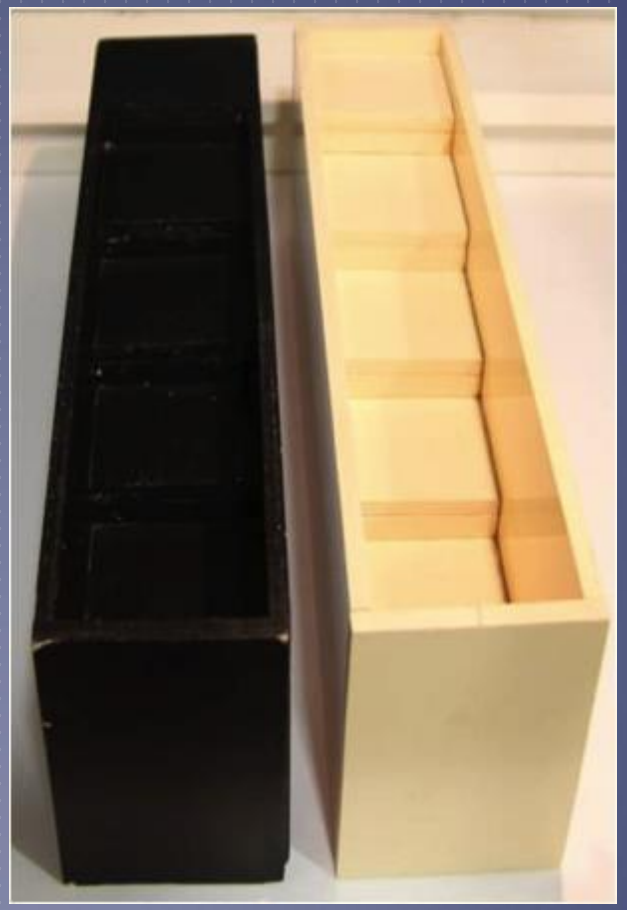


SLEEP PATTERN

- Sleep pattern changes have been observed in some mice
- Patterns are still being analyzed

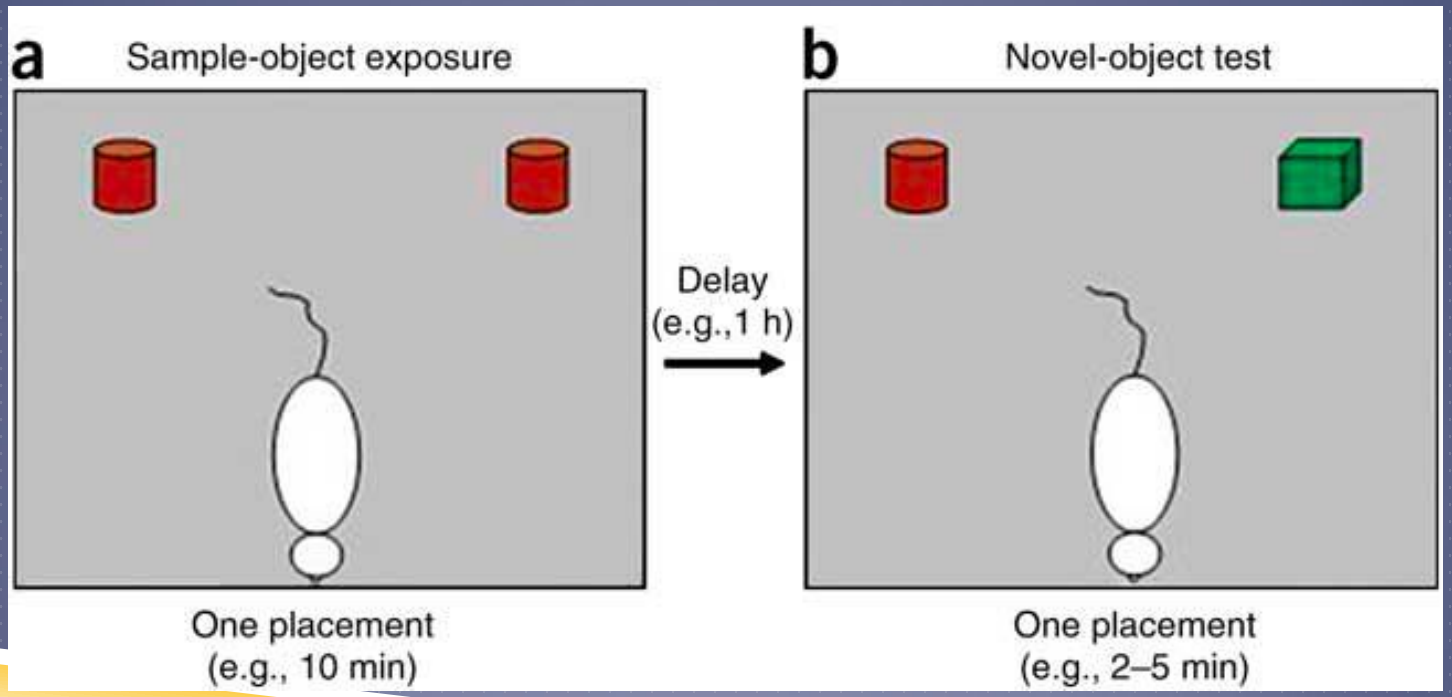
NEUROCOGNITIVE TESTS

- Staircase Test
- Assesses the level of anxiety (rears) and exploratory activity (stairs) of each mouse
- Total number of rears and stairs climbed are counted

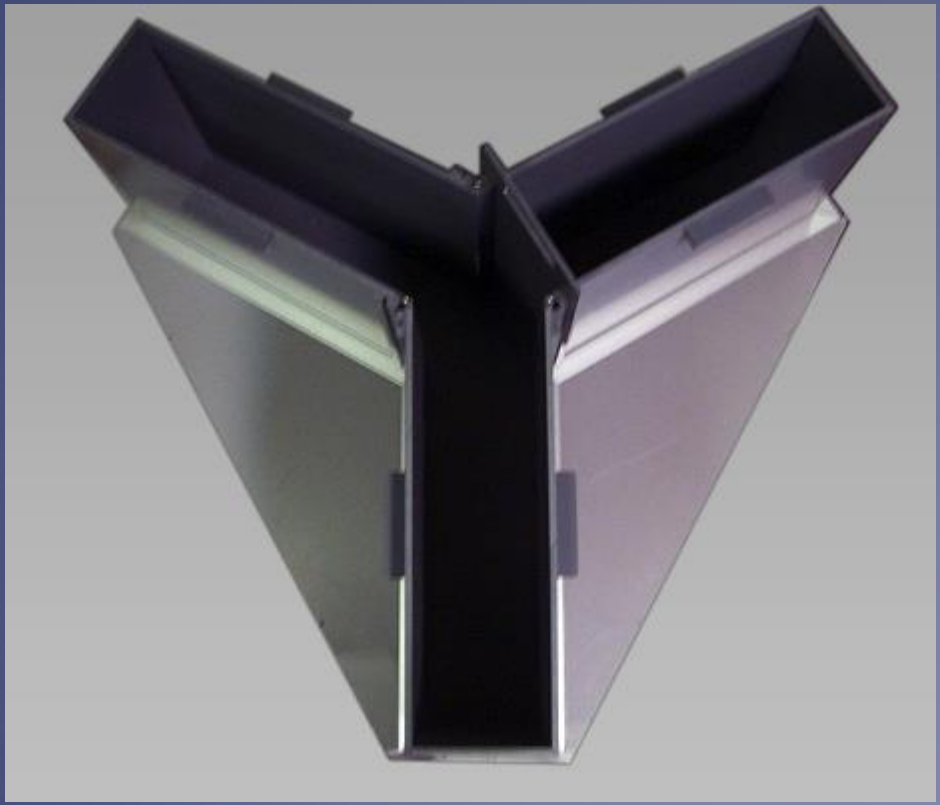


NEUROCOGNITIVE TESTS

- Novel Object Recognition (NOR)
  - Evaluates any long-term memory deficits

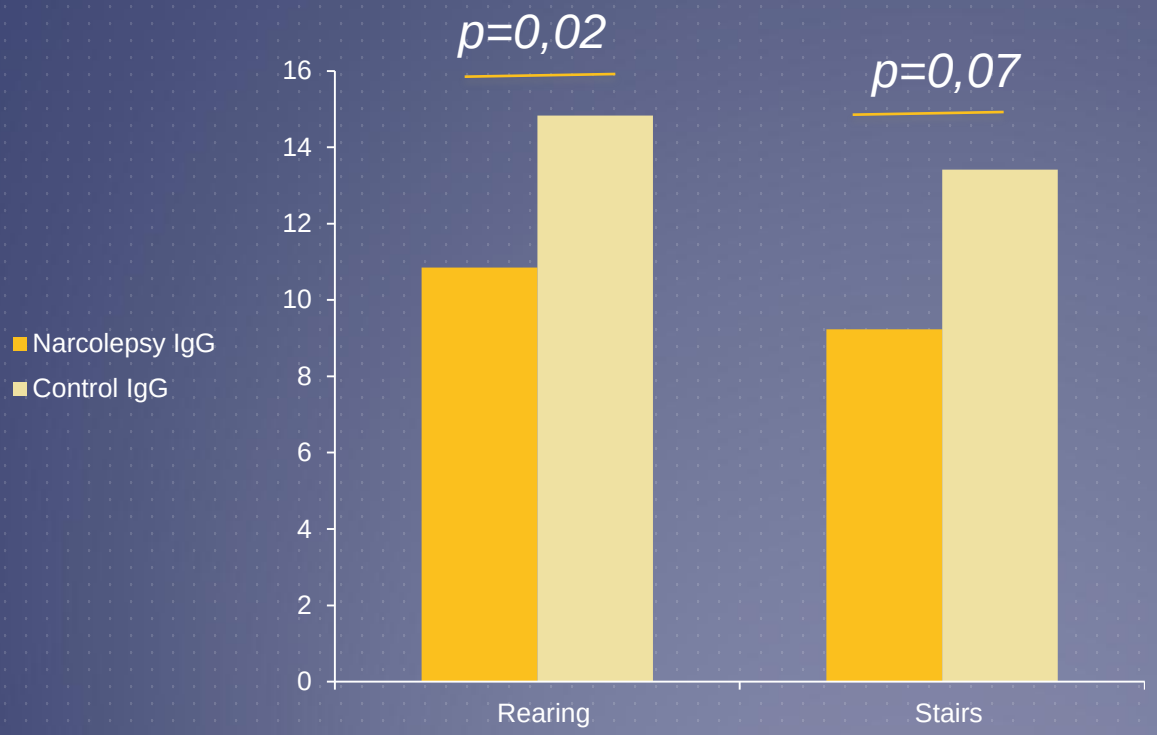


NEUROCOGNITIVE TESTS



- Y-maze
  - Evaluates spatial short-term memory

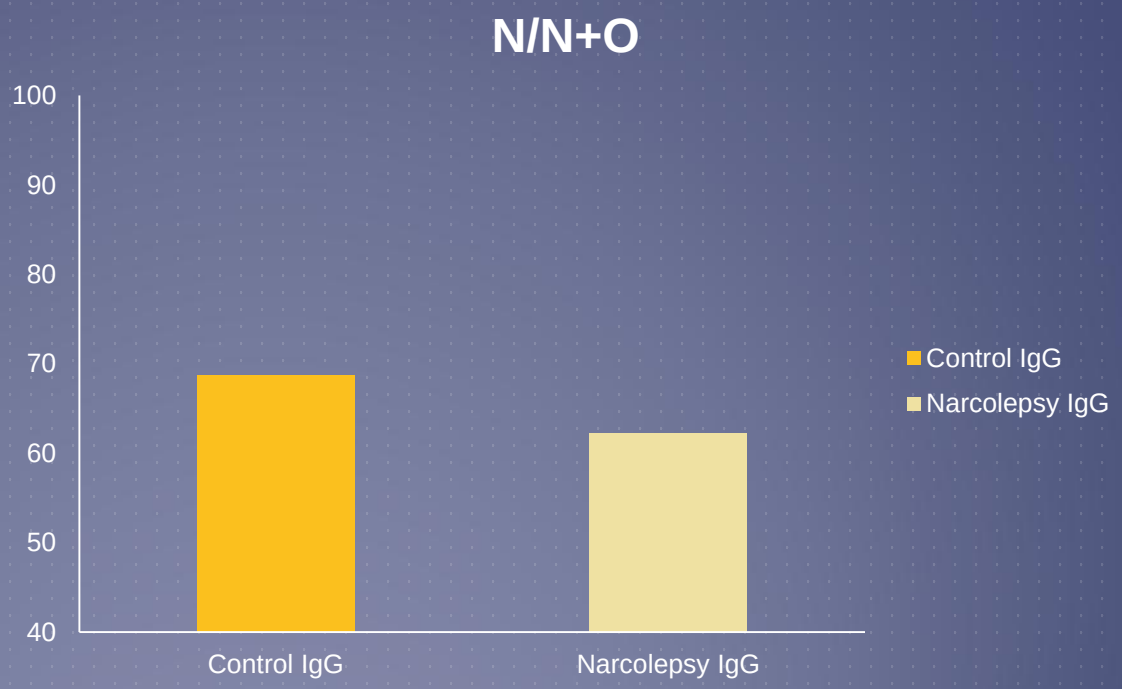
RESULTS



- Staircase Test
  - Narcoleptic IgG injected mice were slightly less active than normal IgG injected mice.
  - Narcoleptic mice less axnious

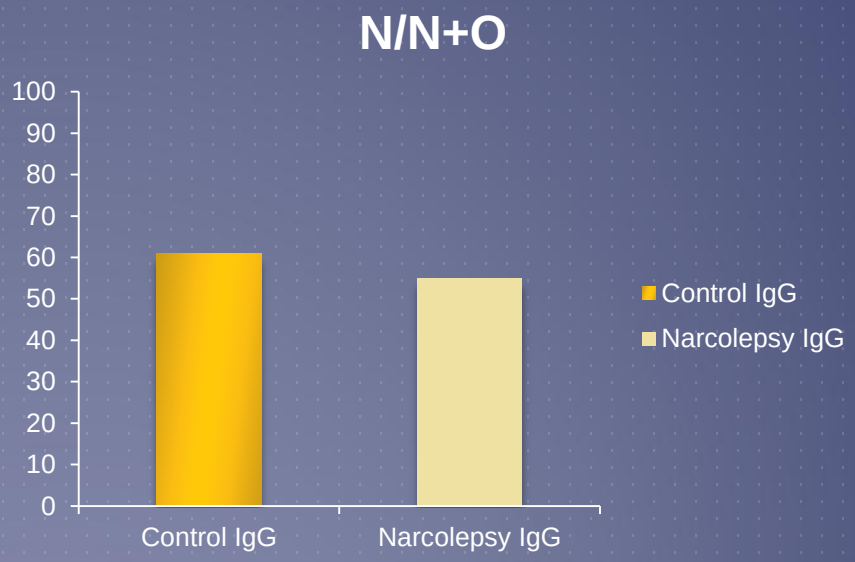
RESULTS

- NOR
  - No difference between Narcolepsy IgG injected mice and Control IgG injected mice.
  - Normal time spent with novel objects is >50%. *(p=0,3*)



RESULTS

- Y-maze
  - No difference between Narcolepsy IgG injected mice and Control IgG injected mice.
  - Normal time spent with new arm is >50%. *(p=0,07)*



CONCLUSION

- Autoimmunity may be involved in the pathophysiology of Narcolepsy.
- Research is being done to prove this so that treatment can be better understood and developed in the future.


THANK YOU

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