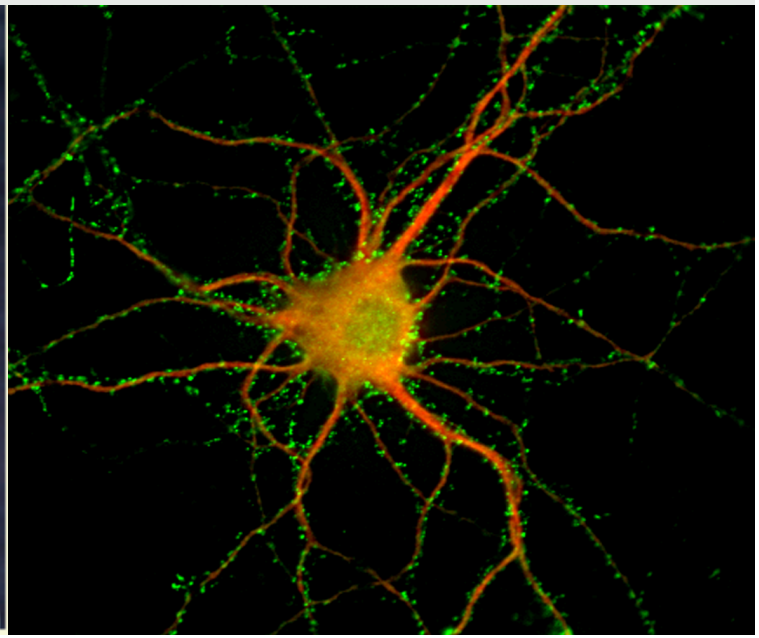
Understanding Research and Findings Associated with Learning and Memory

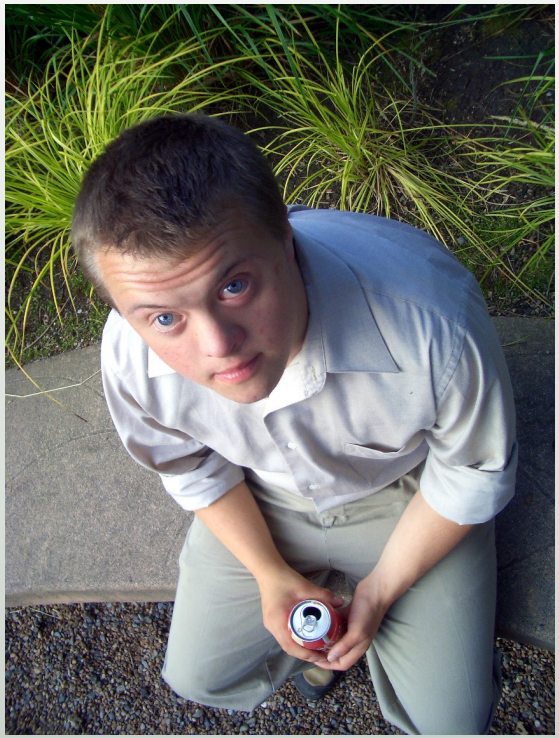
Craig Heller
Stanford University
Down Syndrome Connection of the Bay Area

May 31, 2017



From research findings to approved drug

translational research and clinical development for treatment of individuals with Down syndrome

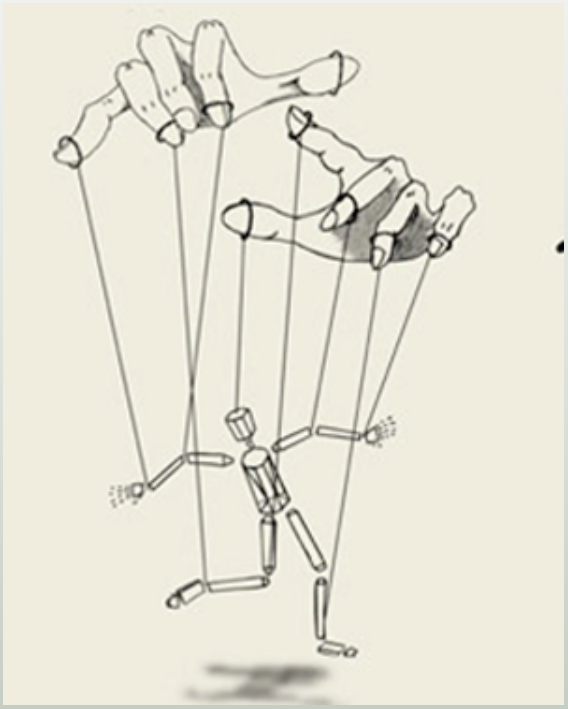


Brains not functioning optimally can be improved

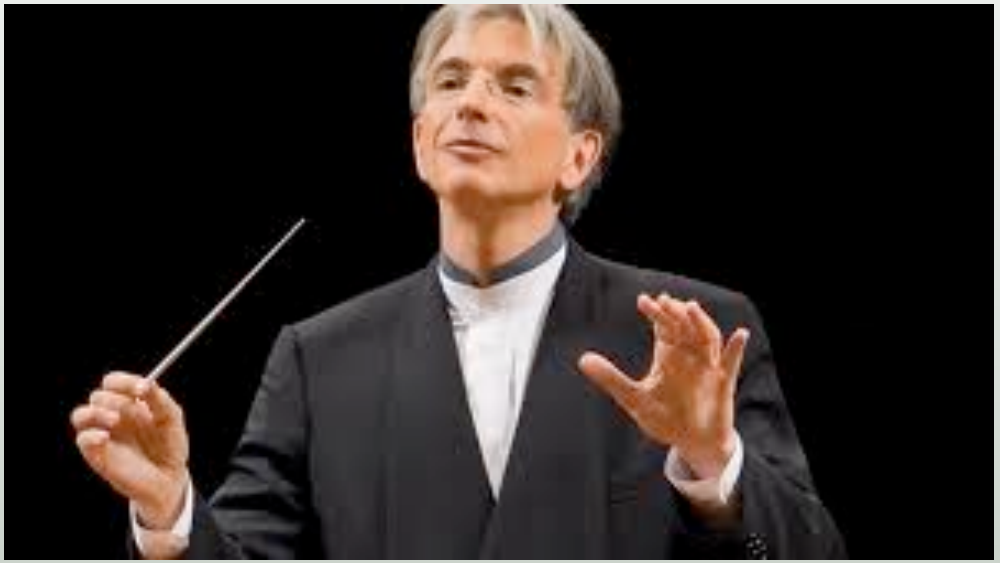
How do our brains work?

A different concept of brain function than is commonly held even by neurobiologists.

The brain is not like a puppet master just pulling the strings.

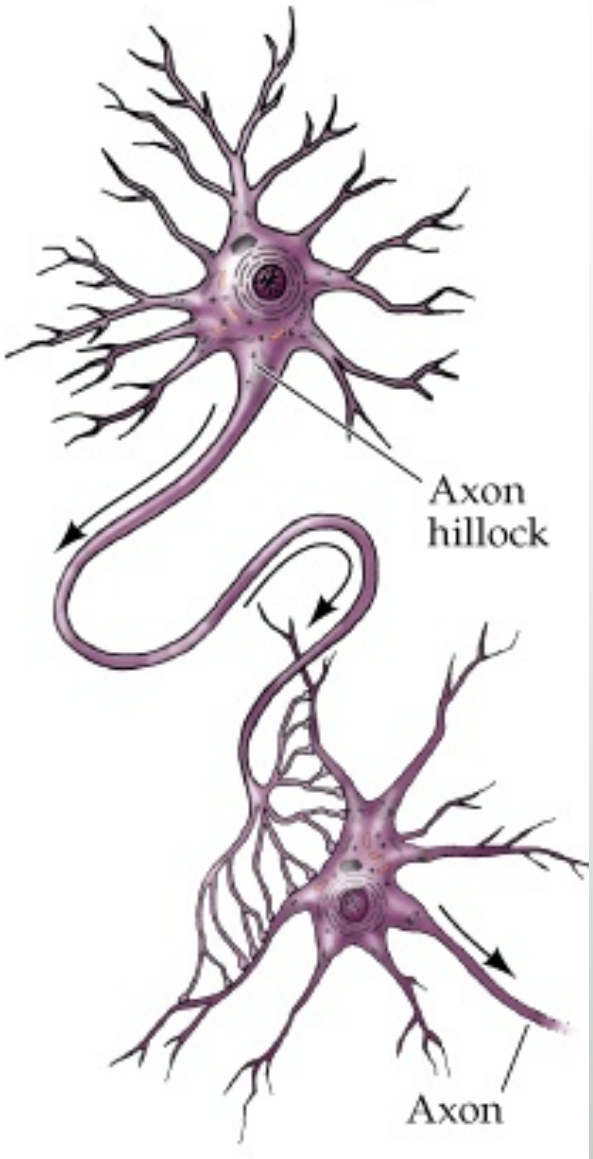


The brain is more like a symphony conductor, speeding up, slowing down, making some sections louder and others softer.



Let's review some basics.

Short course on Neurobiology.



Brains are made up of billions and billions of nerve cells or neurons.

They send information to each other by electrical signals -- nerve impulses -- that travel along extensions of these cells.

At the end of these processes there are connections between the cells called synapses. But, there are gaps between the presynaptic and postsynaptic cells that the electrical signals cannot cross.

The information is carried across the gaps by chemicals called neurotransmitters released by the pre-synaptic cell and received by the post-synaptic cell.

These chemicals are neurotransmitters and they can either excite or inhibit their target cells.

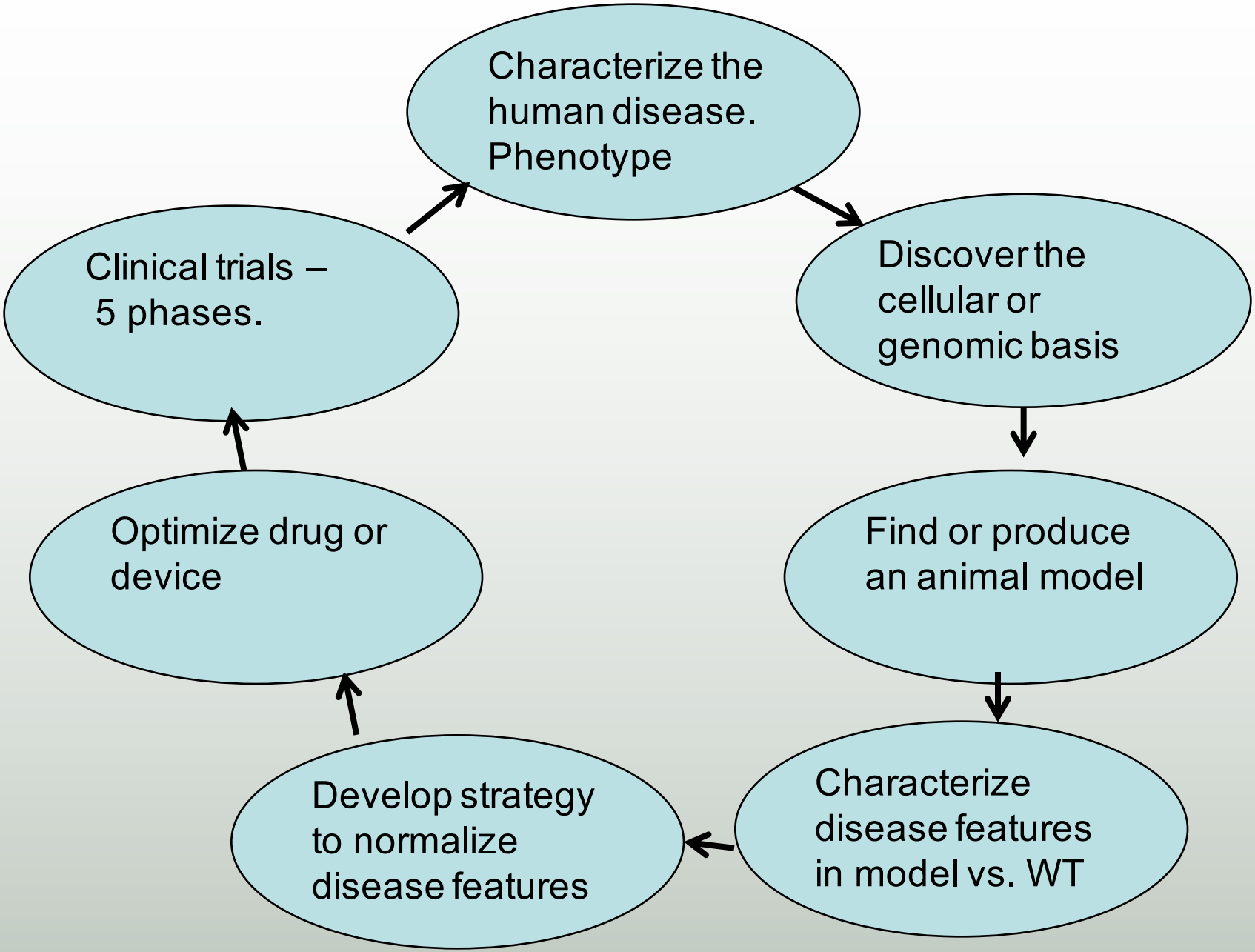
What is different in the electrical/chemical symphony in the brain of a person with Down syndrome?

How can we improve the performance of that orchestra in the head?

What I will tell you

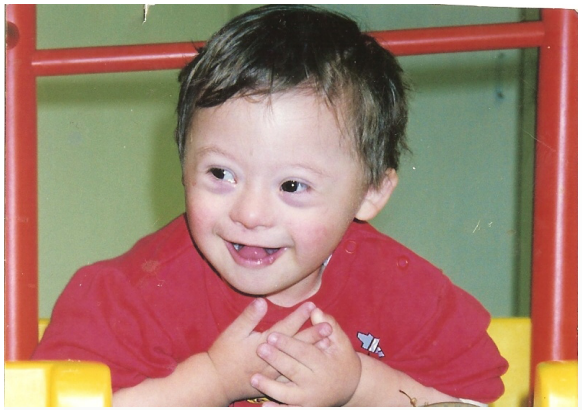
1. Over-inhibition in the brain can be the cause of learning disability.
2. Over activity of the neurotransmitter GABA can be responsible.
3. GABA is important in controlling sleep and daily rhythms.
4. We approach the problem through animal models:
   A. A mouse model of DS
   B. A hamster model of learning disability
5. Modifying sleep and rhythms can restore learning.
6. Promising clinical trials are underway.
7. But, the path to an approved therapy is long and difficult.

The Translational Cycle



Clinical Studies

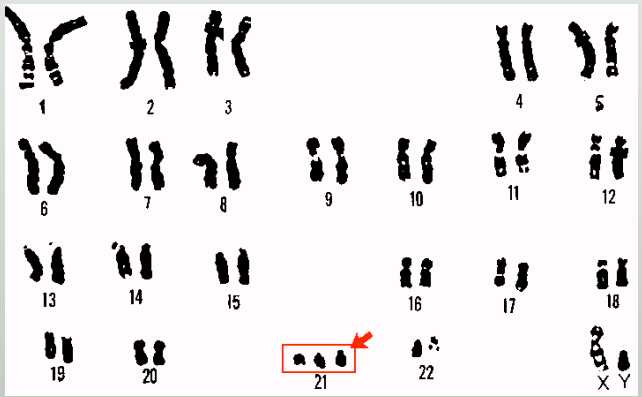
- Phase 0 – First in humans: pharmacodynamics and pharmacokinetics
- Phase 1 – Safety, Small group (20-80), dosage, side effects
- Phase 2 – Safety and Efficacy, larger group (100-300), comparison with a placebo
- Phase 3 – Efficacy, large group (1000-3000), comparison with best alternative
- Phase 4 – Continuing safety studies during marketing.



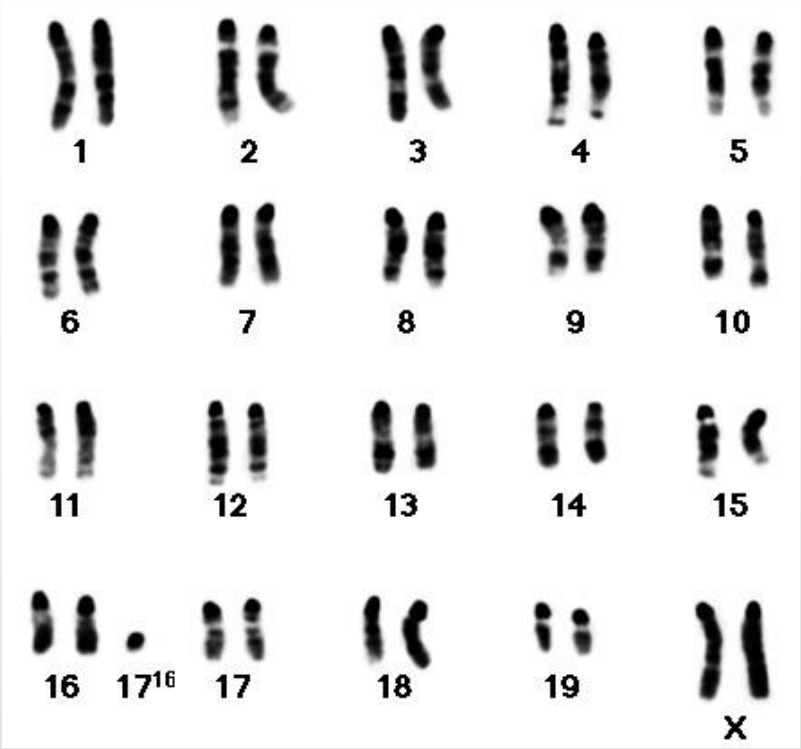
Reducing the cognitive disability associated with Down syndrome

DS is a very common cause of intellectual disability:
350,000 in US 500,000 Europe.

- Incidence > 1/700 live births
- Trisomy 21 (nondisjunction) three copies of chromosome 21. About 250 genes
- More common in mothers >35 y/o
- Down syndrome (DS) is a complex, clinically heterogeneous disorder
  - Deficits in speech, language, declarative short-term and long-term memory
  - Progressive cognitive decline
  - Early onset AD



Meet the Ts65Dn Mouse: Our Workhorse, our Hero.



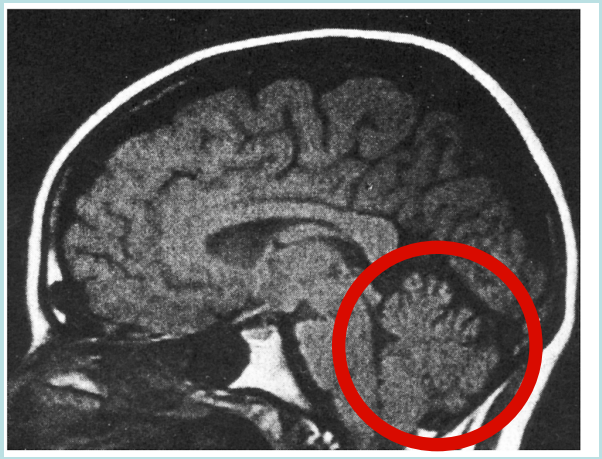
Karyotype analysis

(visual display of the chromosomes grouped by their size, number and shape)

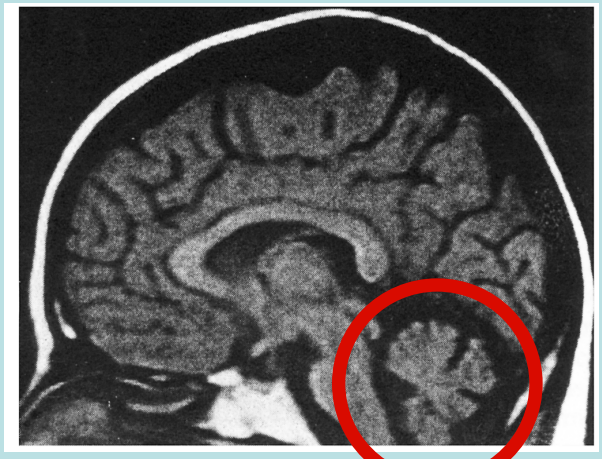
TS Mouse **WT or 2N Mouse**

Anatomy

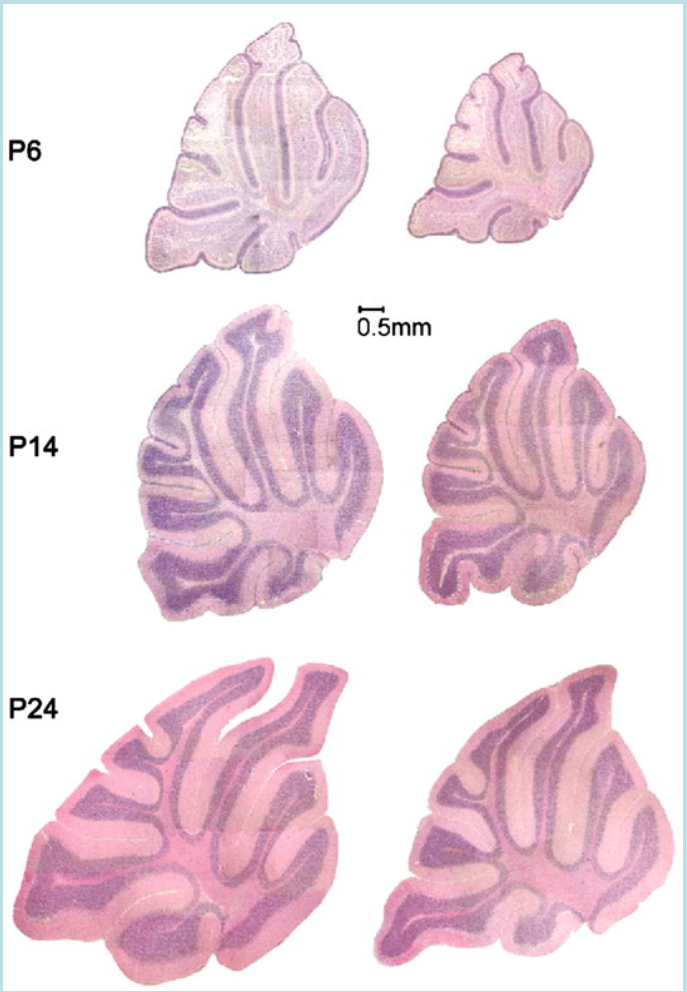
2N



DS



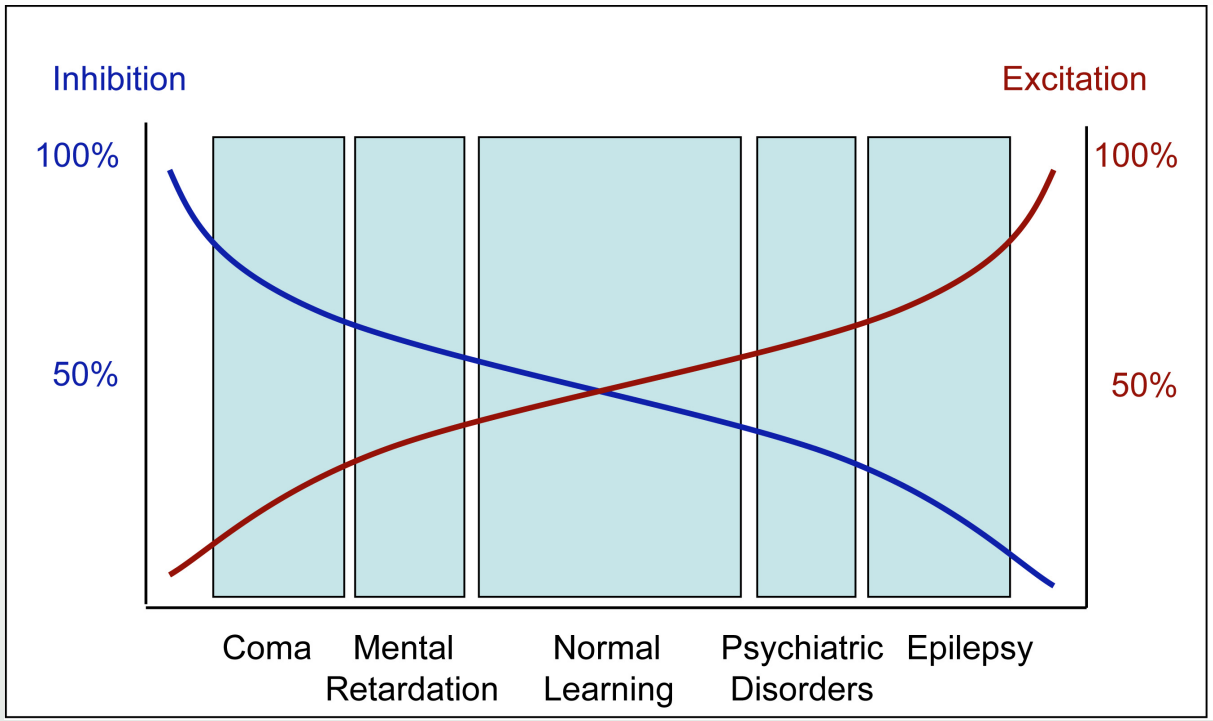
2N Ts65Dn

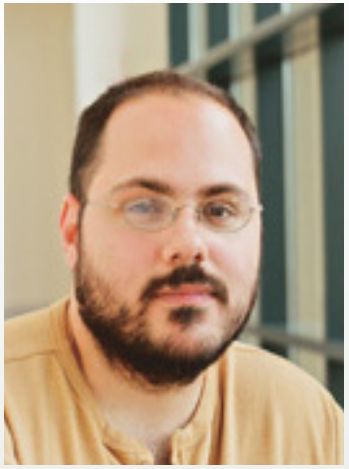


Kesslak et al. *Neurology* 1994; Roper et al. *PNAS* 2006

Hypothesis:

Learning disability is due to over-inhibition in the CNS





Fabian Fernandez

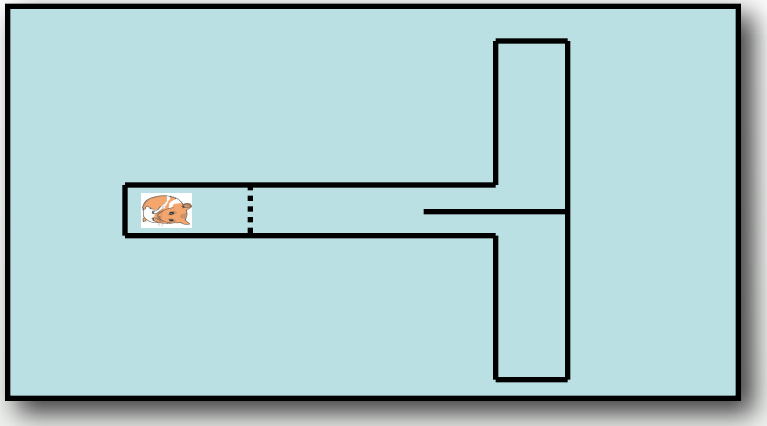
Over-inhibition impairs the transfer of Short Term Memory to Long Term

Major inhibitory system in brain is GABA… (very much involved in Sleep and Circadian Rhythms).

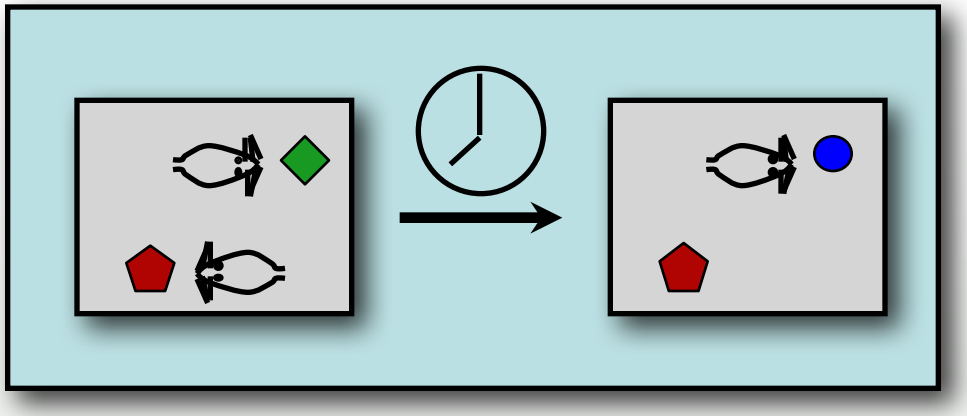
Can GABA antagonists restore learning and memory in TS mice?
If so, is the action through modulation of sleep and/or circadian systems?

Behavioral tests

Working memory



Long-term memory



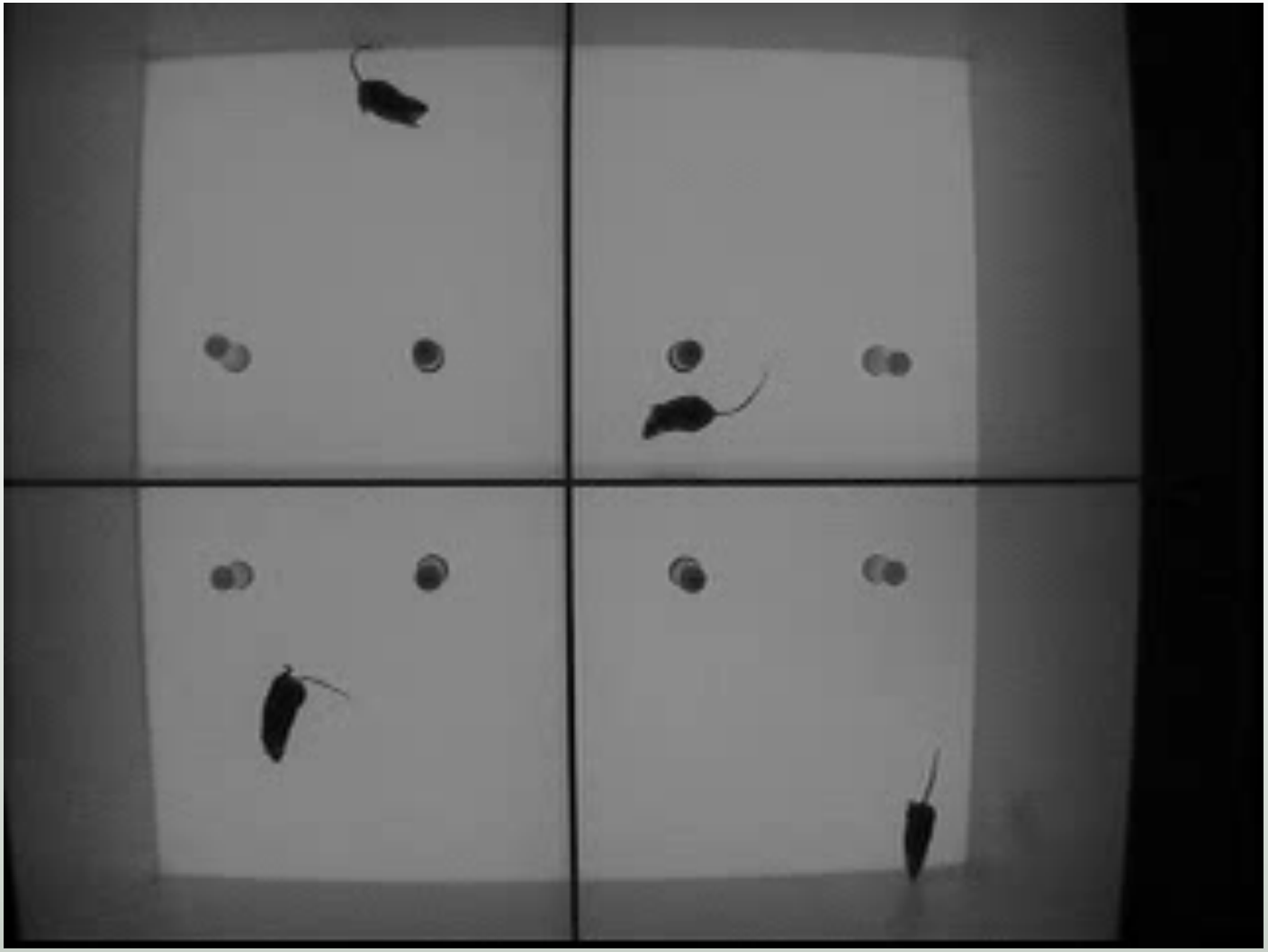
Motor learning

Social behaviors

As in humans, Ts mice exhibit learning and memory impairments

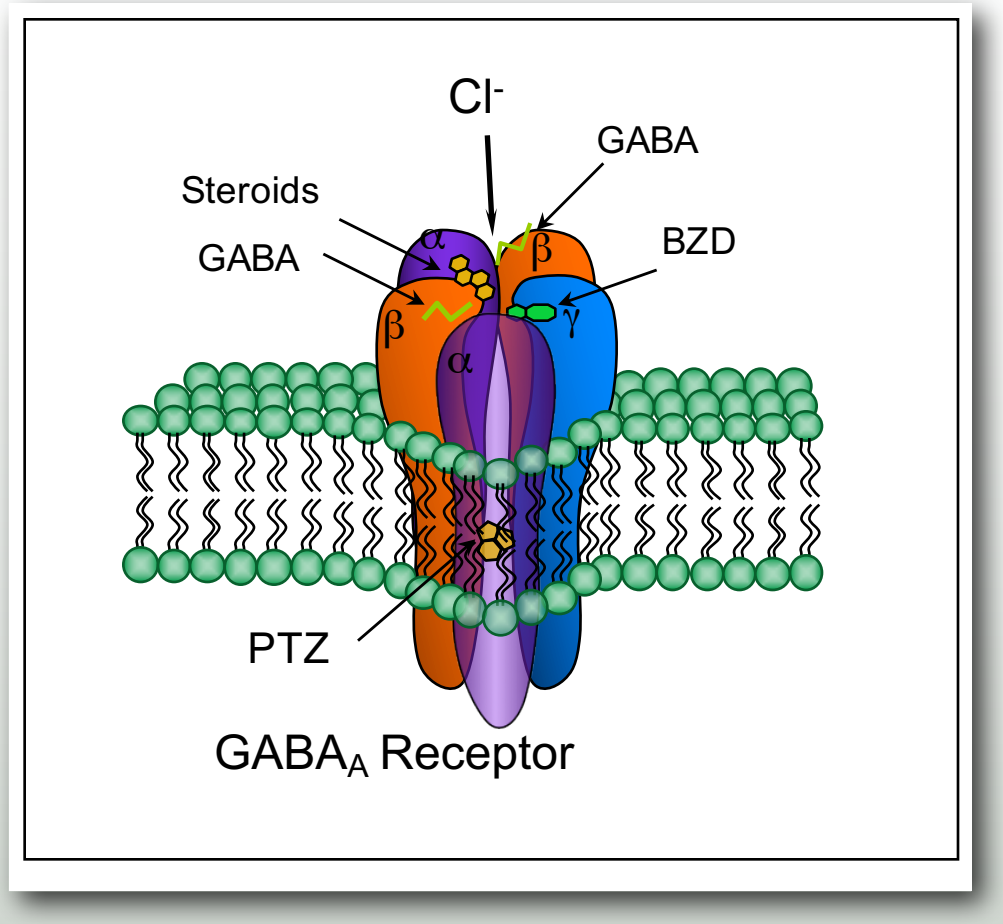
How do you assess the ability of mice to learn and form long-term memories?

The Novel Object Recognition (NOR) Test Provides Excellent Biometric for Cognition



Other tests: open field, T-maze, hole board, fear conditioning, Barnes maze.

The $GABA_A$ receptor mediates inhibition and has a rich pharmacology

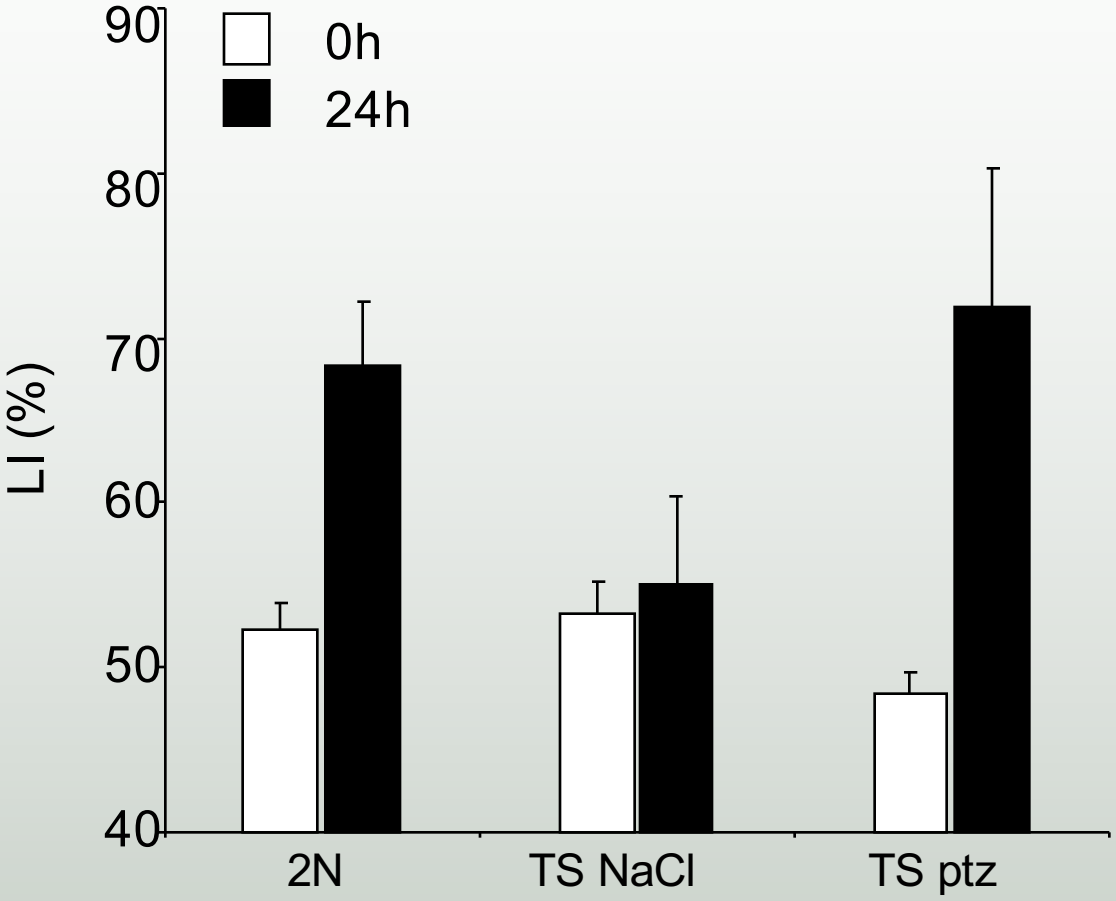


$GABA_A$ Receptor Antagonists Tested and Shown to be Efficacious

- Picrotoxin:
  - Pros: Potent compound (IC50 1uM), excellent bioavailability
  - Cons: narrow therapeutic window
- Bilobalide:
  - Pros: Potent compound (IC50 2uM), excellent bioavailability, good therapeutic window
  - Cons: currently available in plant extract only (Gingko Biloba), difficult synthesis.
- Pentylenetetrazole:
  - Pros: Excellent pharmacokinetic values, oral delivery, excellent bioavailability, good therapeutic window, long history in humans
  - Cons: Currently not approved by FDA
- Alpha5 inverse agonist:
  - Pros: Excellent pharmacokinetic values, oral delivery, excellent bioavailability, good therapeutic window. Specific for a subset of hippocampal $GABA_A$ receptors.
  - Cons: currently not approved by FDA
- Flumazenil:
  - Pros: Excellent pharmacokinetic values, good therapeutic window, approved by FDA for the treatment of benzodiazepine overdose
  - Cons: poor oral bioavailability, acute IV administration

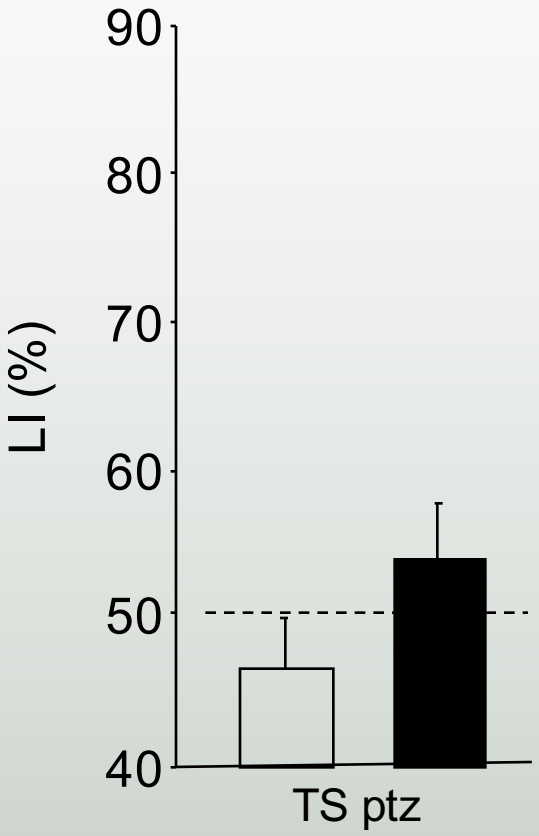
Single doses of GABA antagonist, Pentylenetetrazole (PTZ) improves learning in TS mice, but effects do not last.

ptz 3mg/kg ip 10 min prior training



N=7 4 to 6mo old

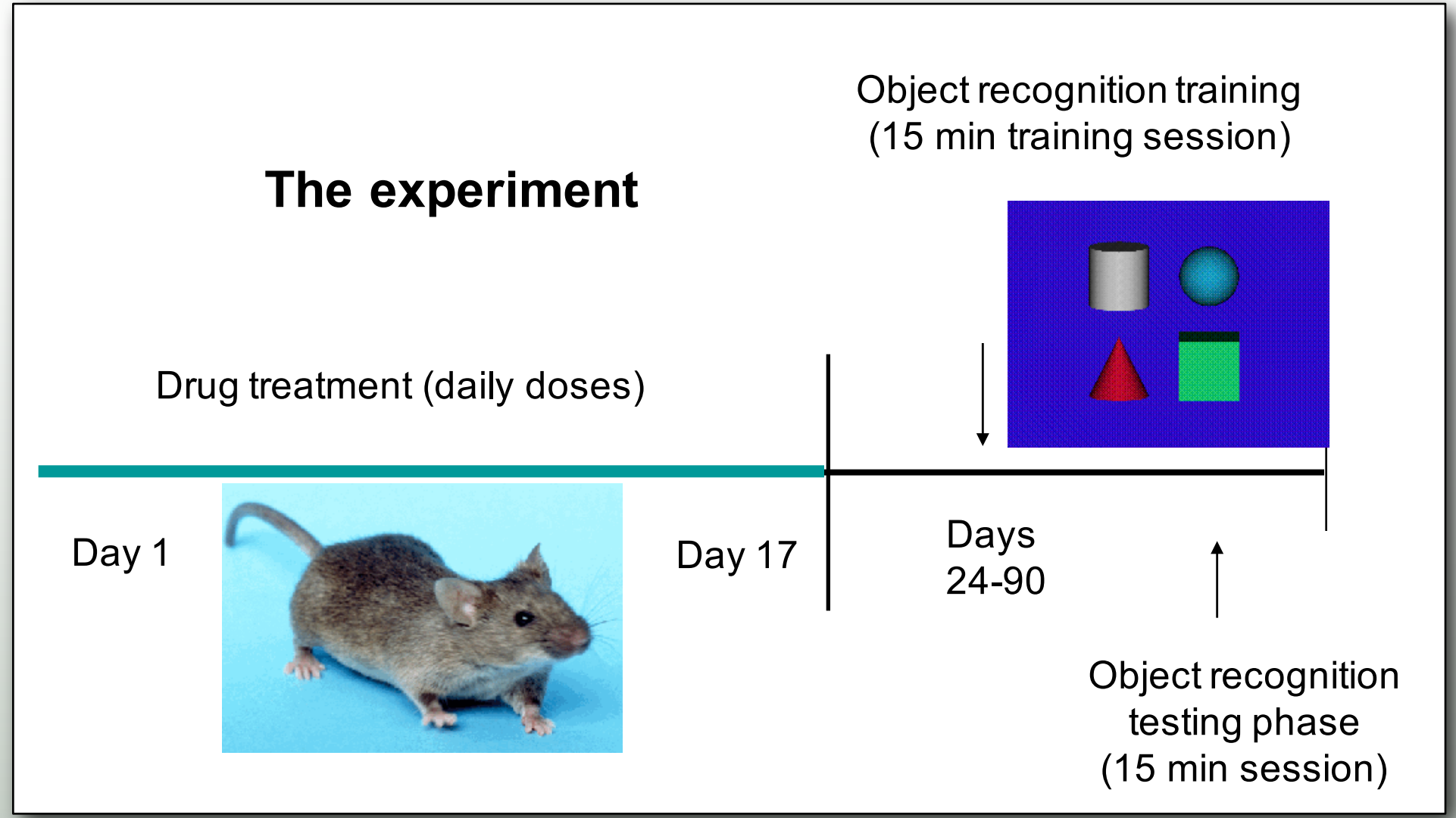
Training/Testing 1 week later



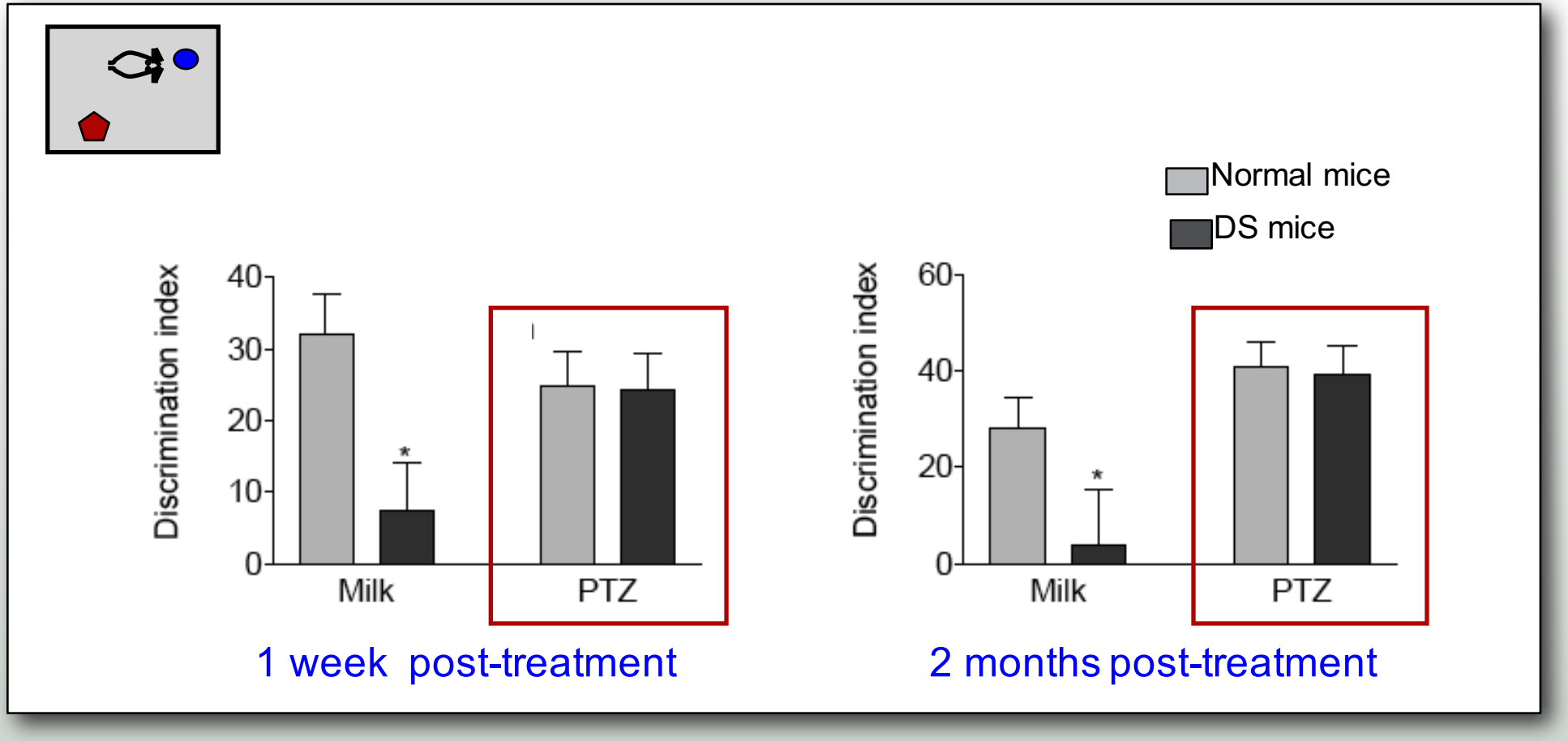
N=10 4 to 6mo old

Colas, Chuluun et al 2013.

It gets better! Short-term chronic treatment with PTZ at low doses induces a very long lasting improvement in learning and memory.



Memory improvement is long-lasting after daily pentylenetetrazole (PTZ) dose



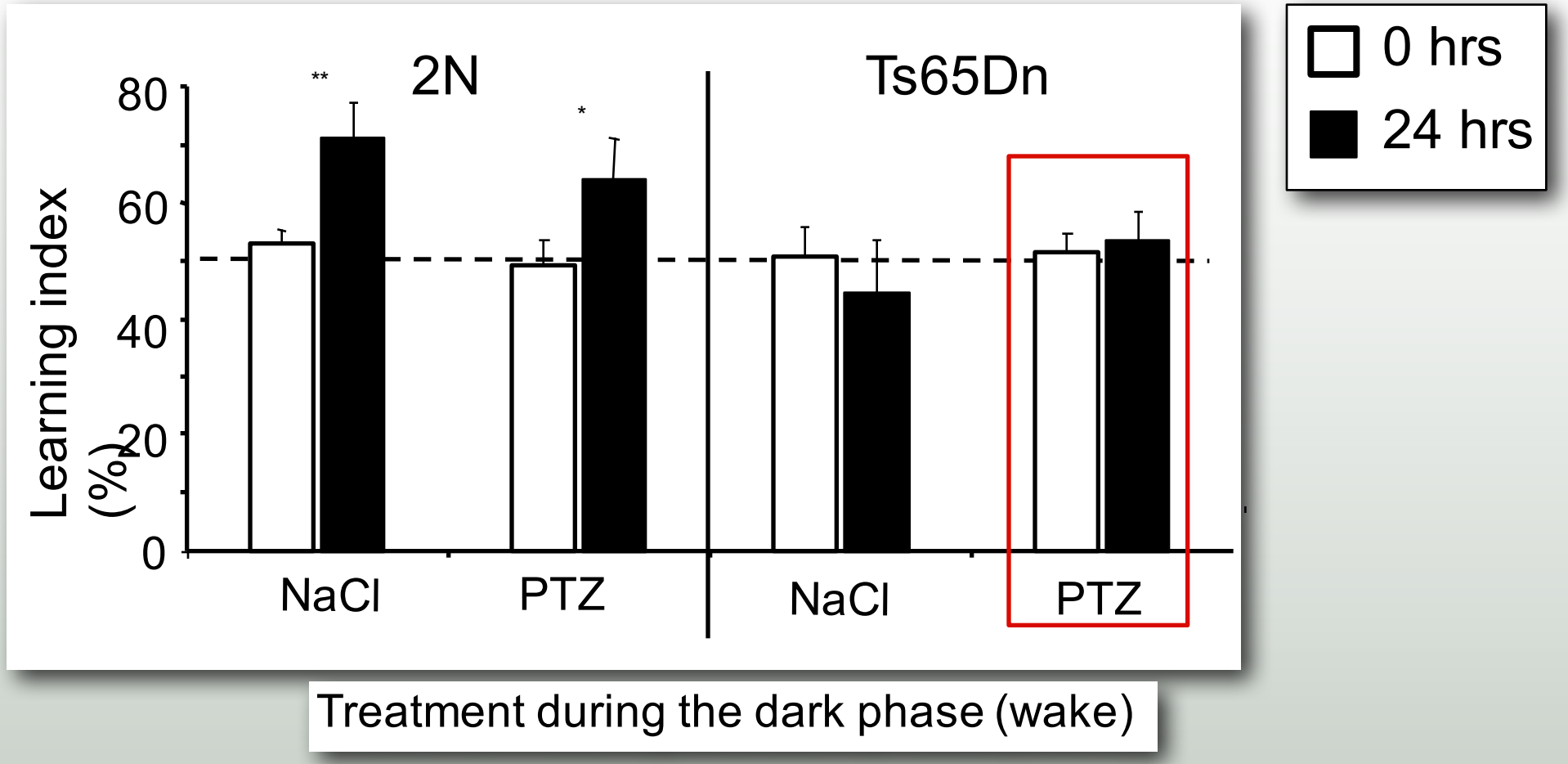
Fernandez & Garner, 2007

PTZ rescues learning also in 5, 8, 12, and 18 months old TS mice, Hence, independent of developmental or neurodegenerative processes.

n=10 TS mice, 12-13 months old, were tested for long term (24h) NOR before and after a 2 weeks daily regimen of PTZ (0.3mg/kg i.p. light period). Wt mice did not respond to (0.3mg/kg).

Colas et. al. 2013

$GABA_A$ receptor antagonist effects are circadian phase dependent

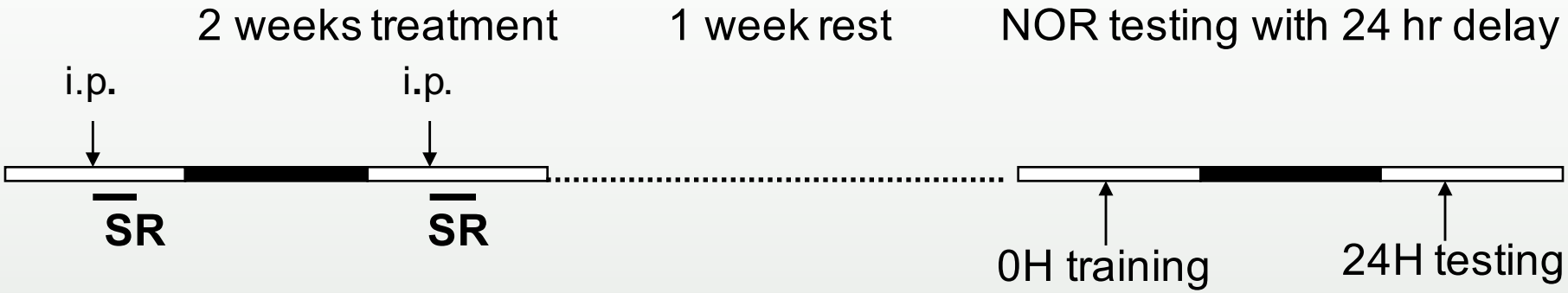


They are most affective during sleep

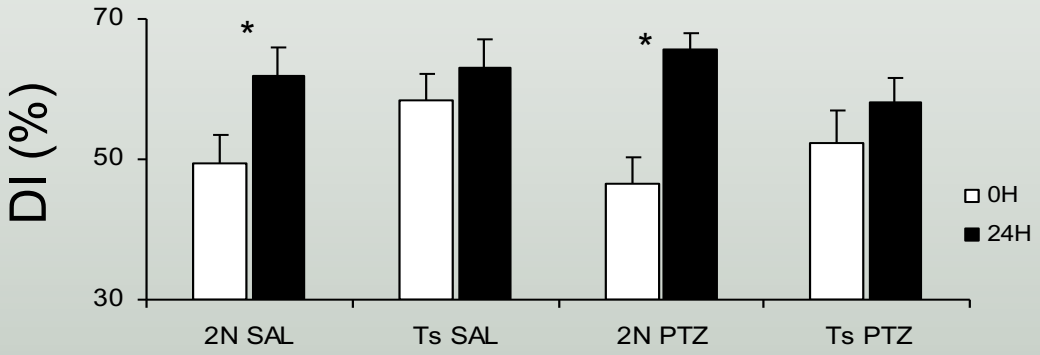
Colas & Chuluun

Sleep during the circadian sleep phase is necessary for the pro-cognitive effect of PTZ

Ts65Dn mice treated during the light phase with daily doses of PTZ (0.3 mg/kg) for two weeks, but each treatment followed by 150 min. of sleep restriction.



Sleep restriction induced by providing new nesting material and toys.



Treatment/Genotype

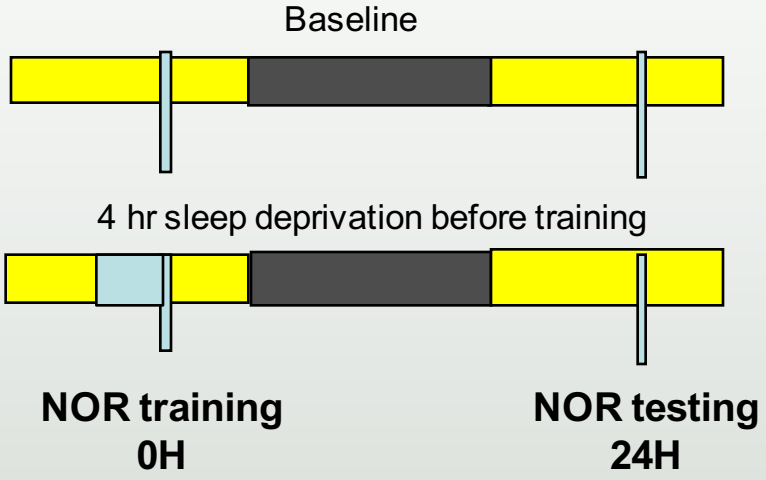
n=8/9

Results: TS mice showed no improvement on the NOR test unlike results when there was no SR following treatment.

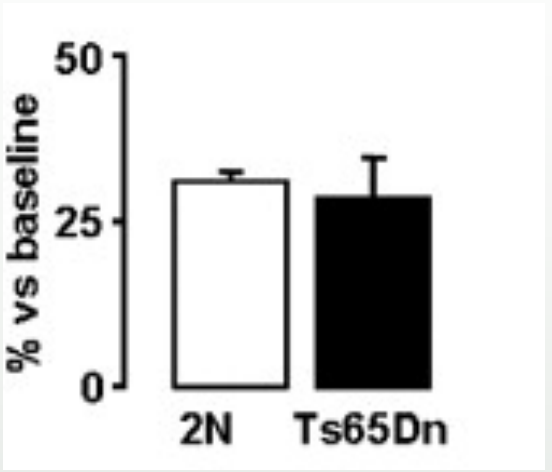
Colas, Chuluun et al. unpublished

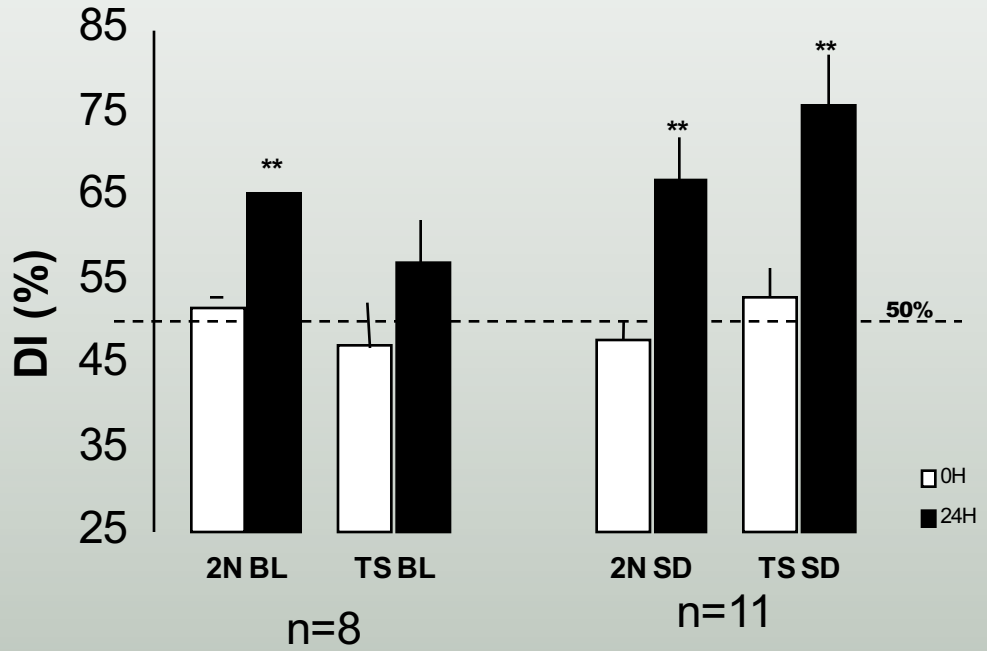
The Converse: **Enhancing SWA in Ts65Dn mice during the circadian sleep phase improves their learning and memory without GABA antagonists.**

Sleep deprivation for 4 hrs prior to training.



Normalization of delta power following SD



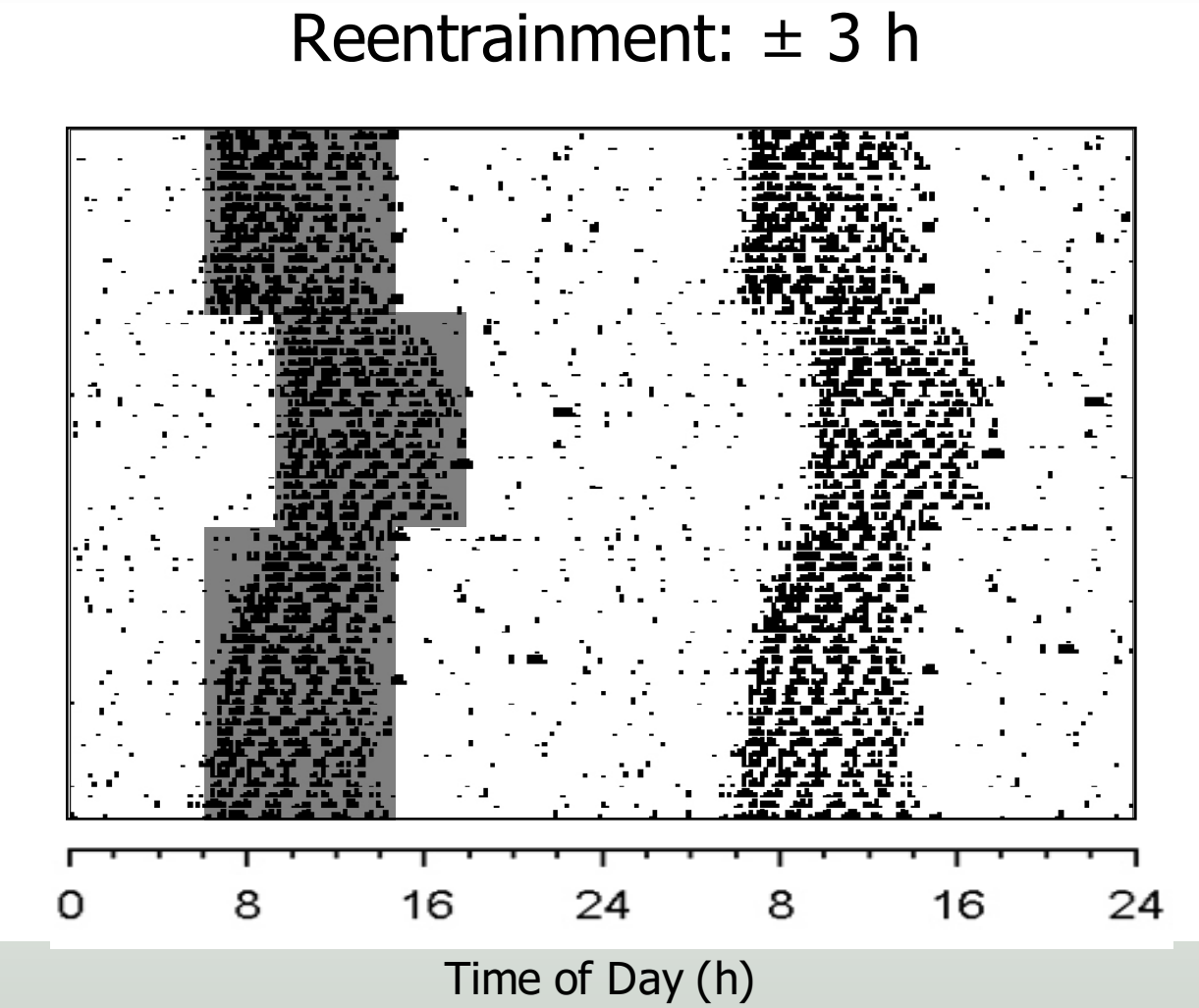


Colas et al. unpublished

The Siberian Hamster Reveals the Importance of CR's in Learning and Memory

A model system for research on circadian rhythms and photoperiodicity.

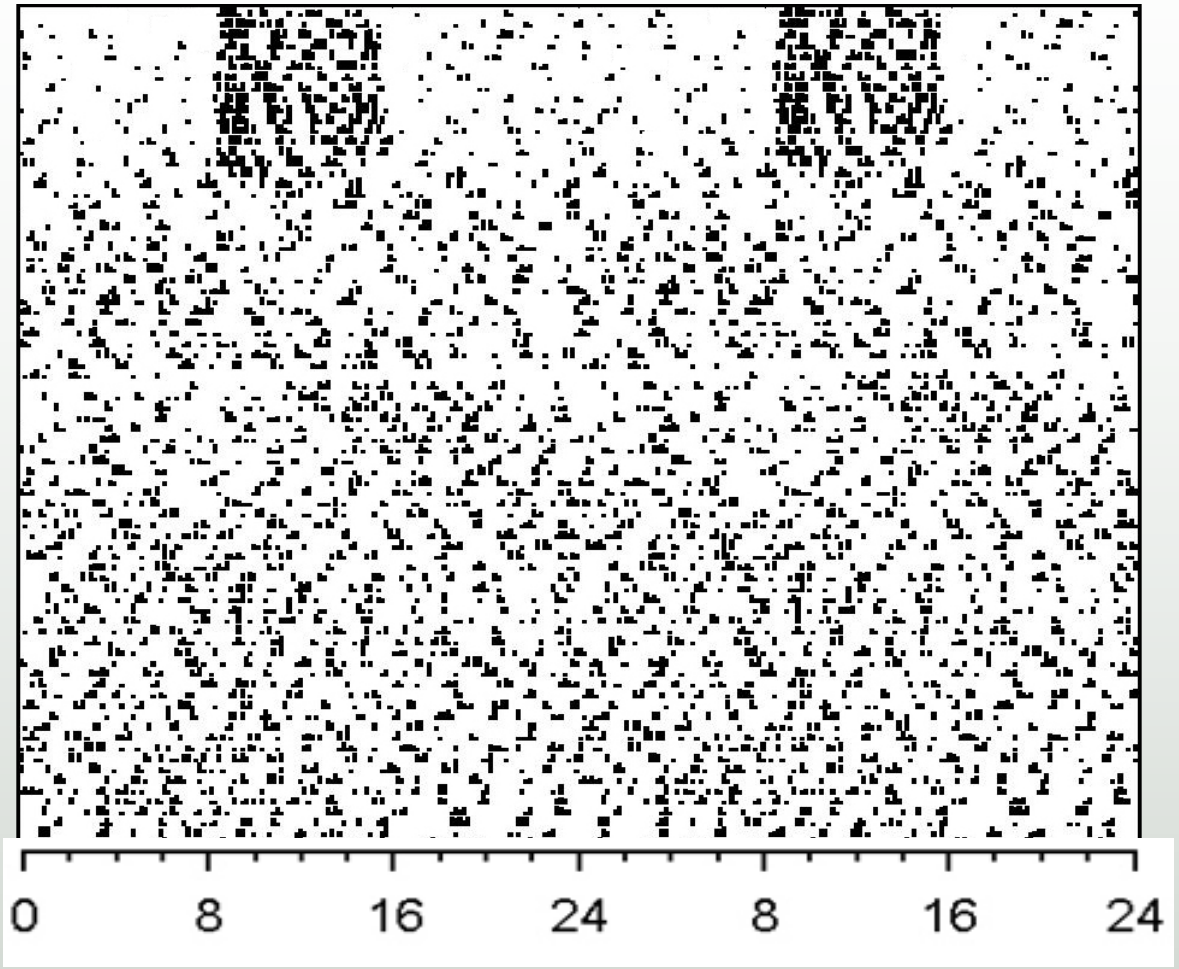
Normal reentrainment to short shifts in photocycle



Ruby et al.

But, if we give them a 5 or 6 hr phase shift,

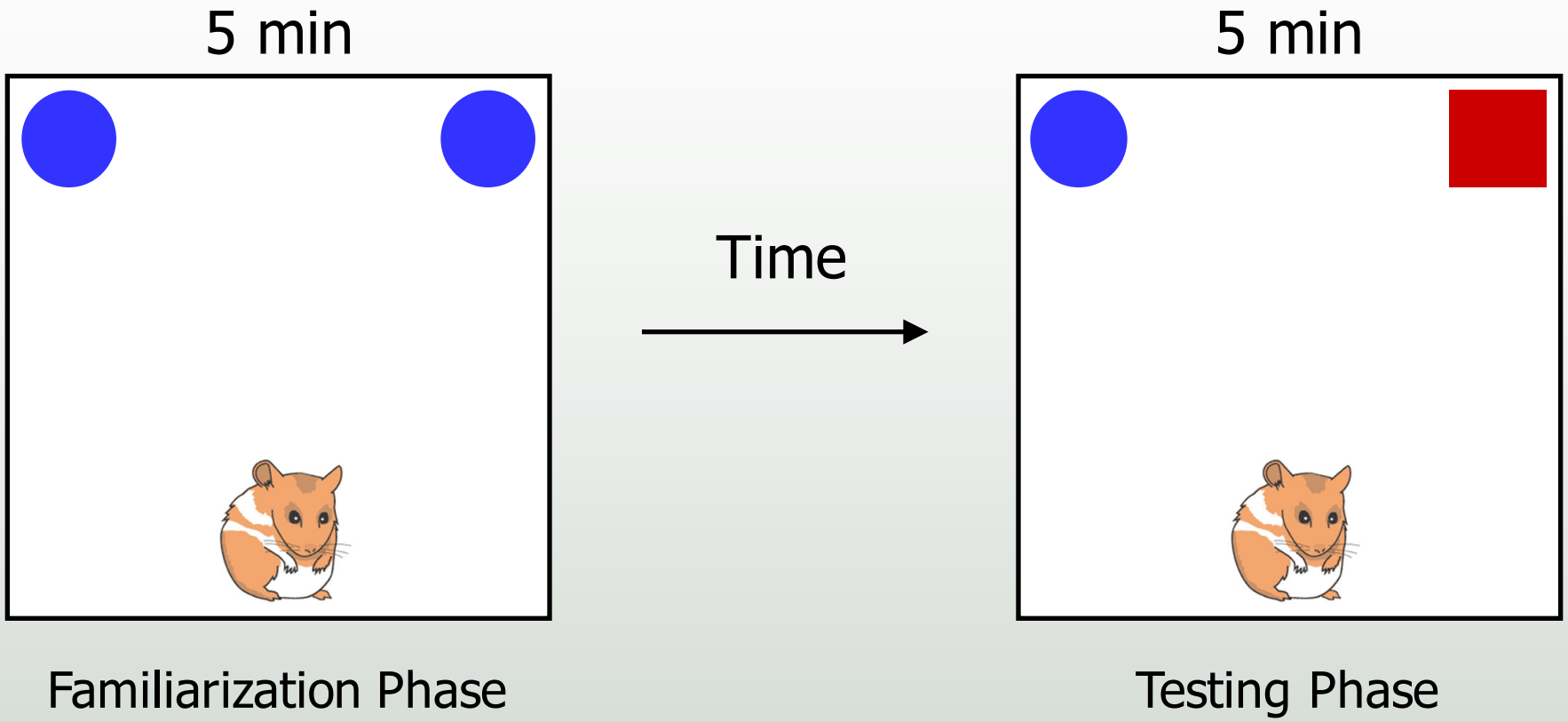
Circadian Rhythms are eliminated….for life.



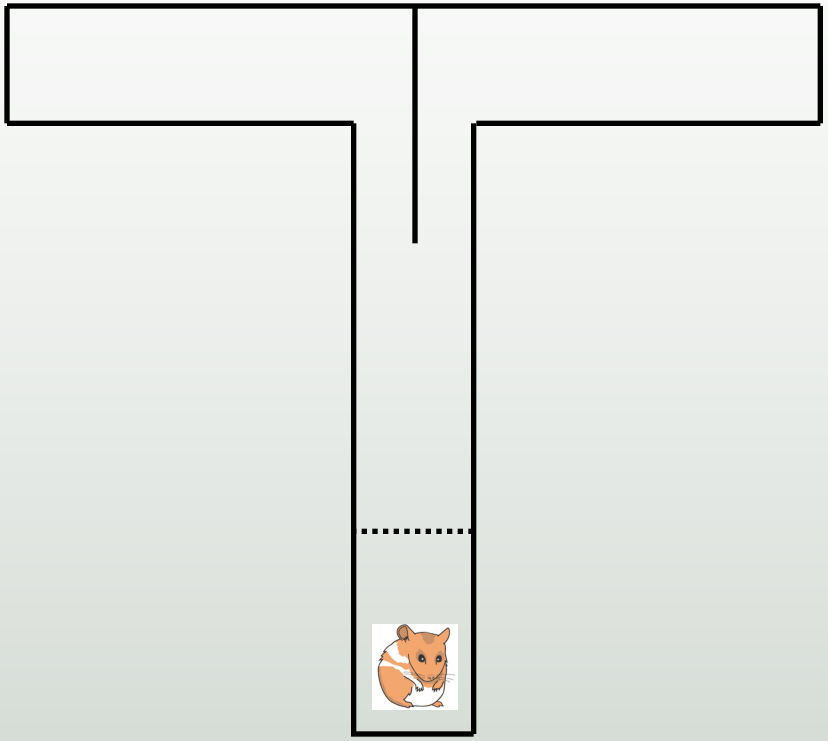
A small percentage of animals re-entrain.

Ruby et al. 1996

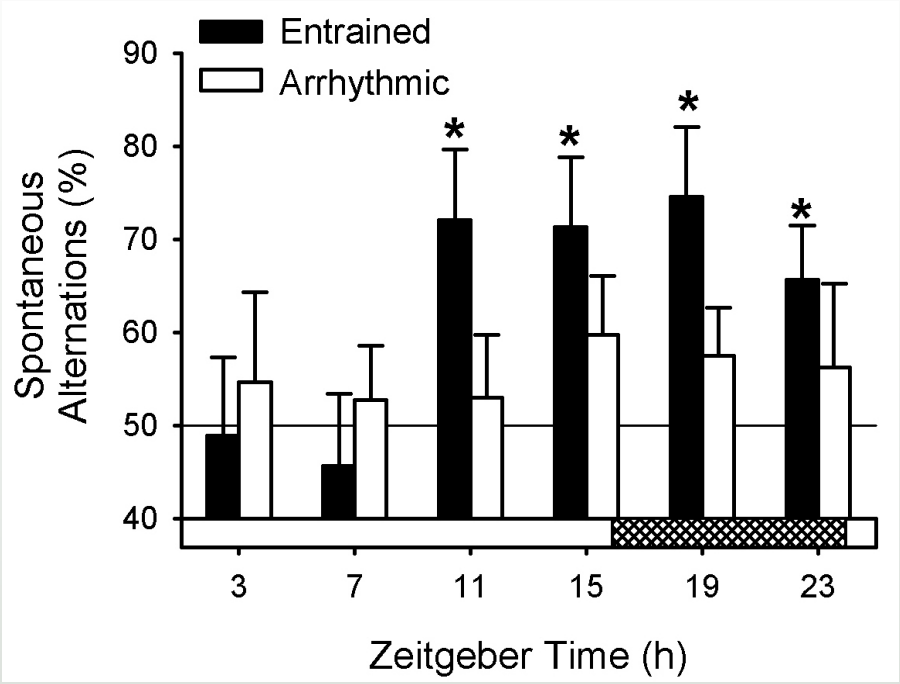
Arrhythmia has huge effects on long-term Learning and Memory as assessed by the novel object recognition test (NOR)

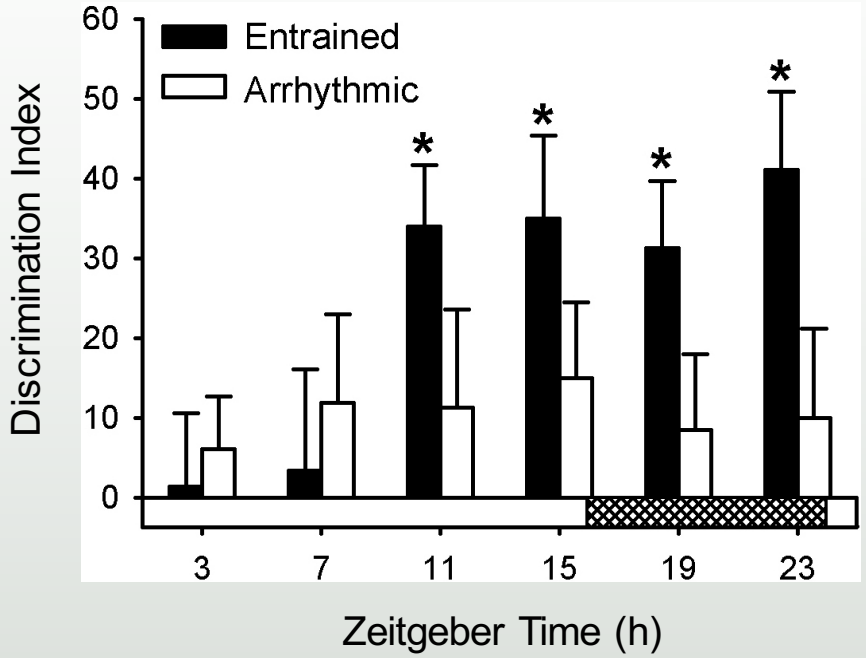


And, short-term working memory as assessed by spontaneous alternation in a T-maze



Memory deficits in SA and NOR in arrythmic hamsters

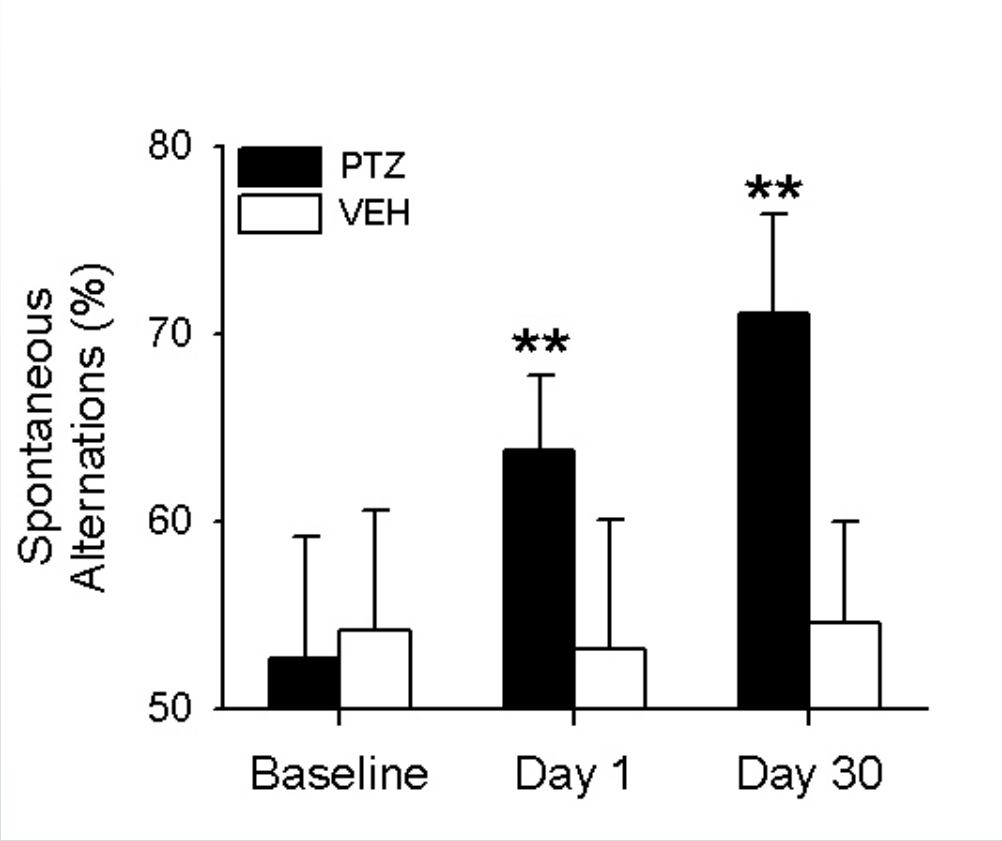


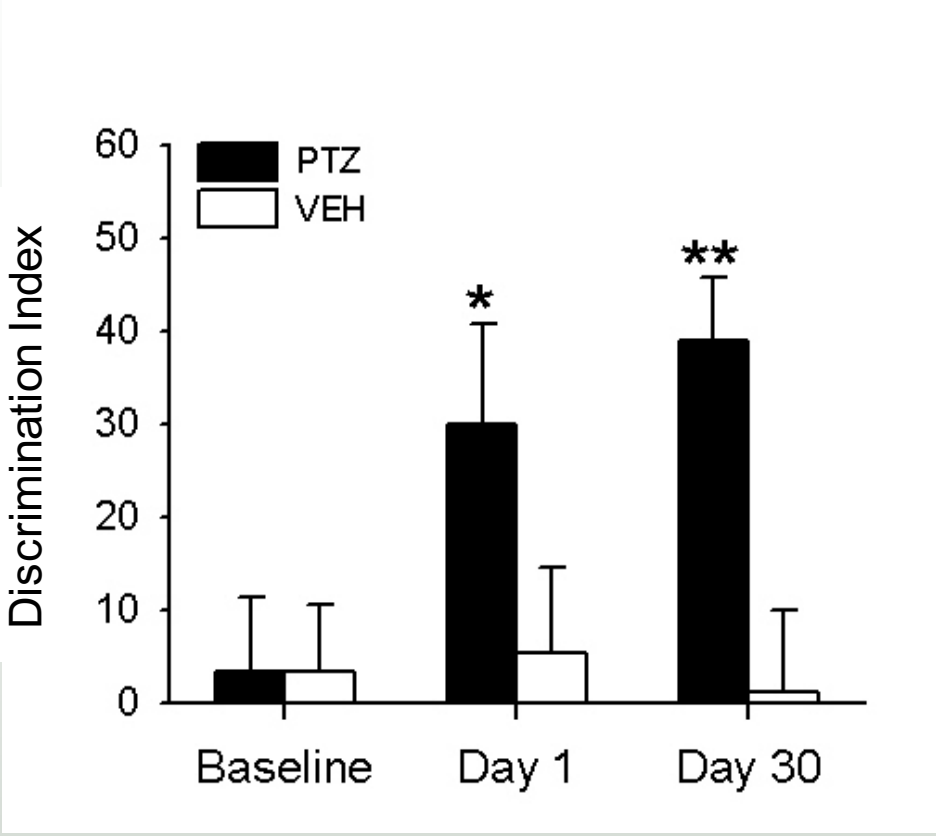


Entrained animals show a circadian rhythm of memory, but arrhythmic animals do not.

Ruby et al. 2008

Memory is rescued by the chronic treatment protocol with the $GABA_A$ receptor antagonist PTZ, but animals remain arrhythmic



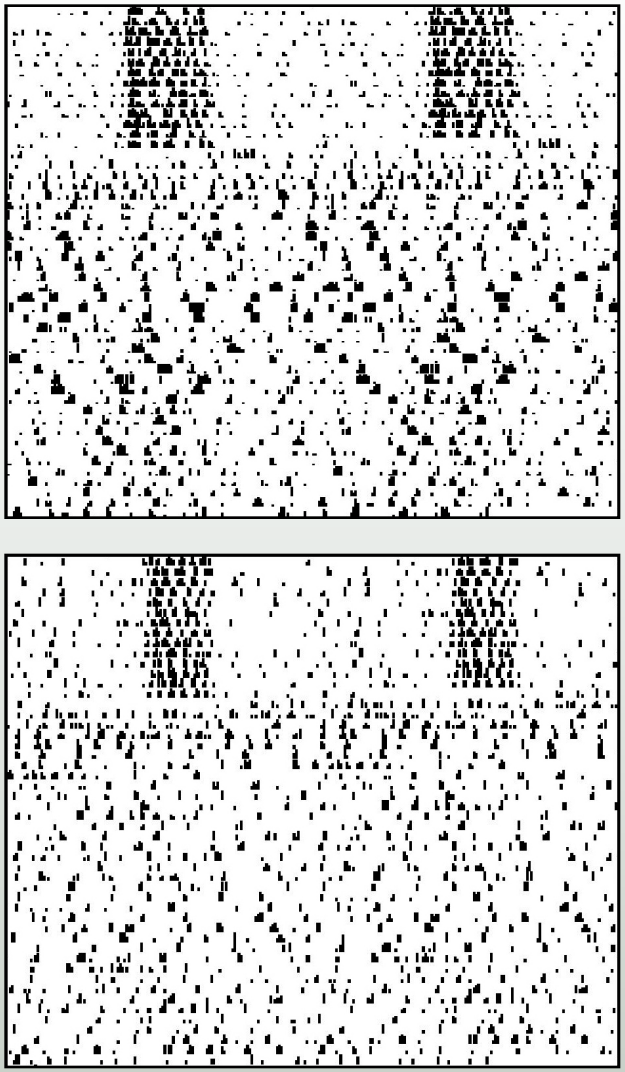


The SCN is a GABAergic nucleus, could constant SCN activity inhibit learning and memory?

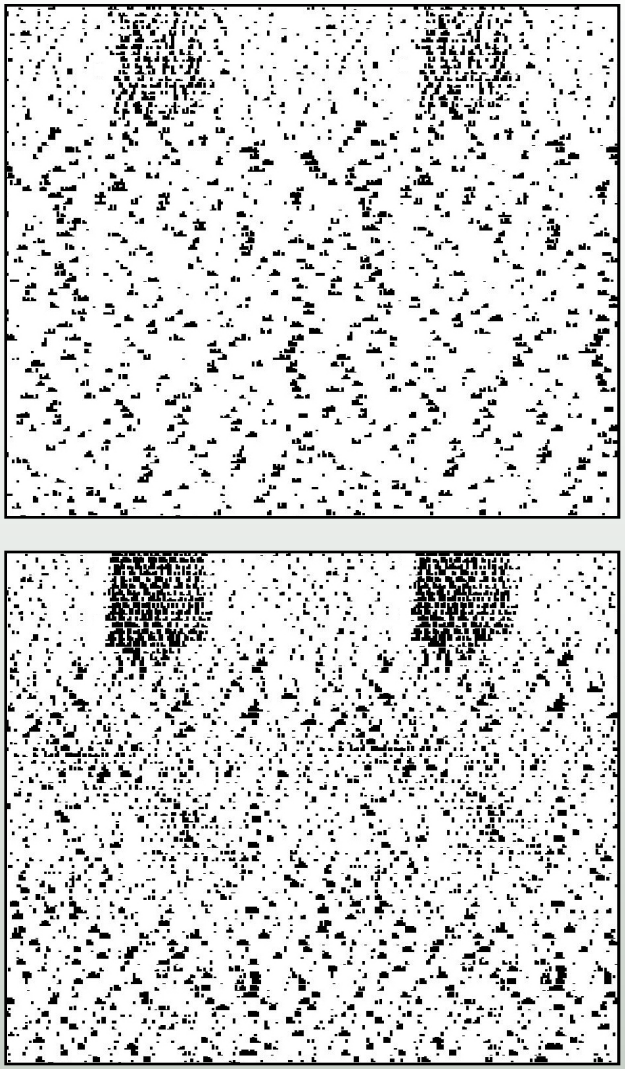
Ruby et al. 2013

Arrhythmia can also be induced by SCN ablation

SCN Lesion (SCNx)

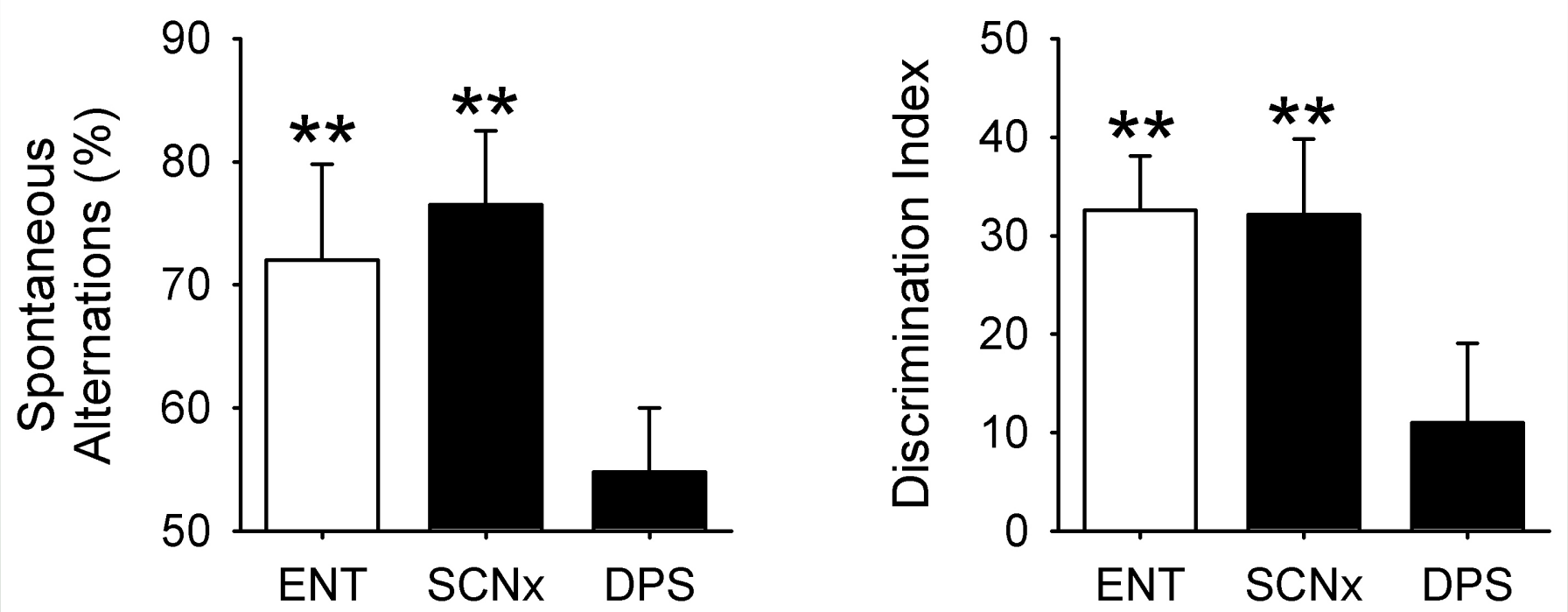


Disruptive Phase Shift (DPS)



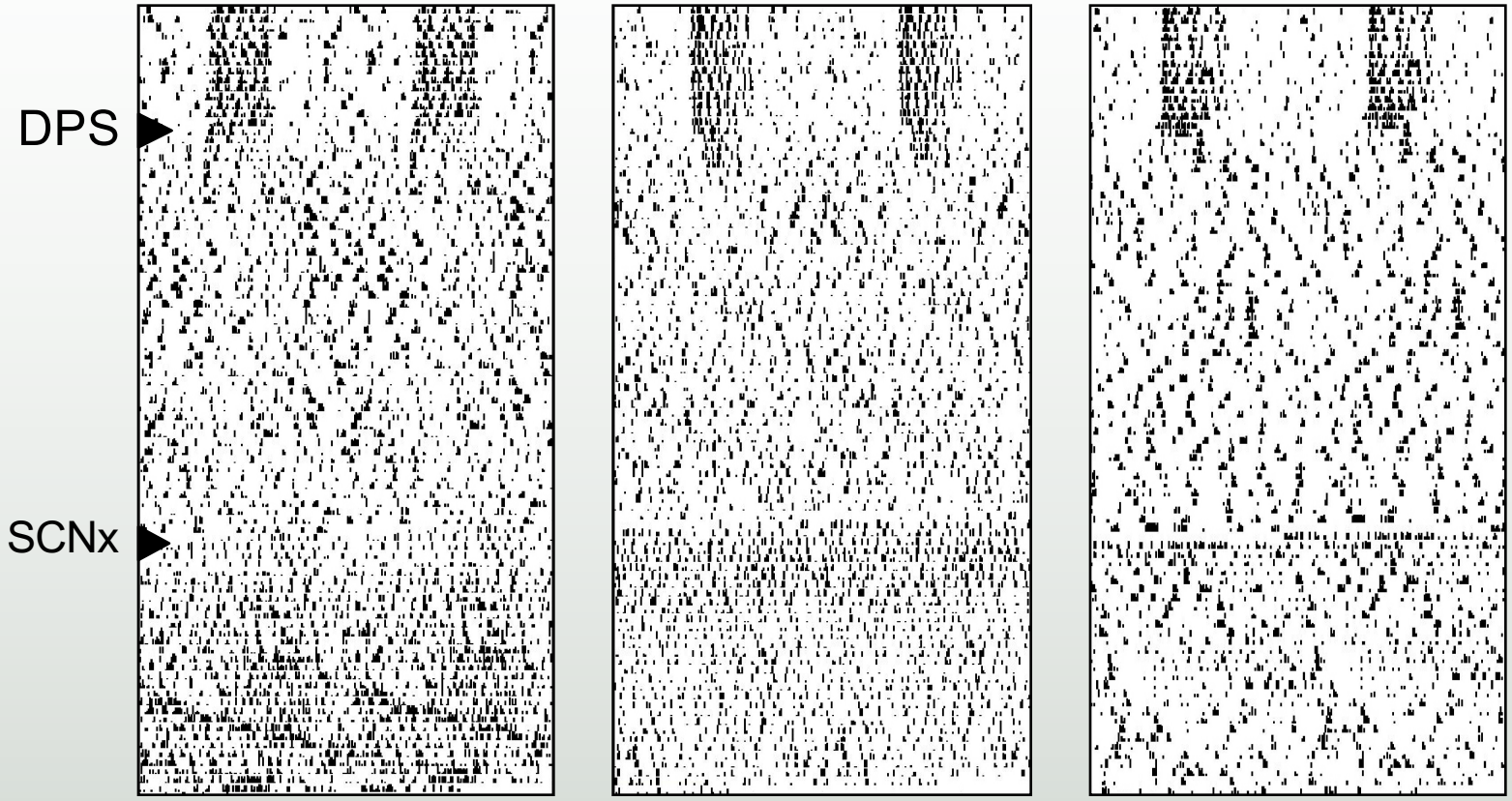
Ruby et al 2014

SCN ablation has no effect on SA or NOR



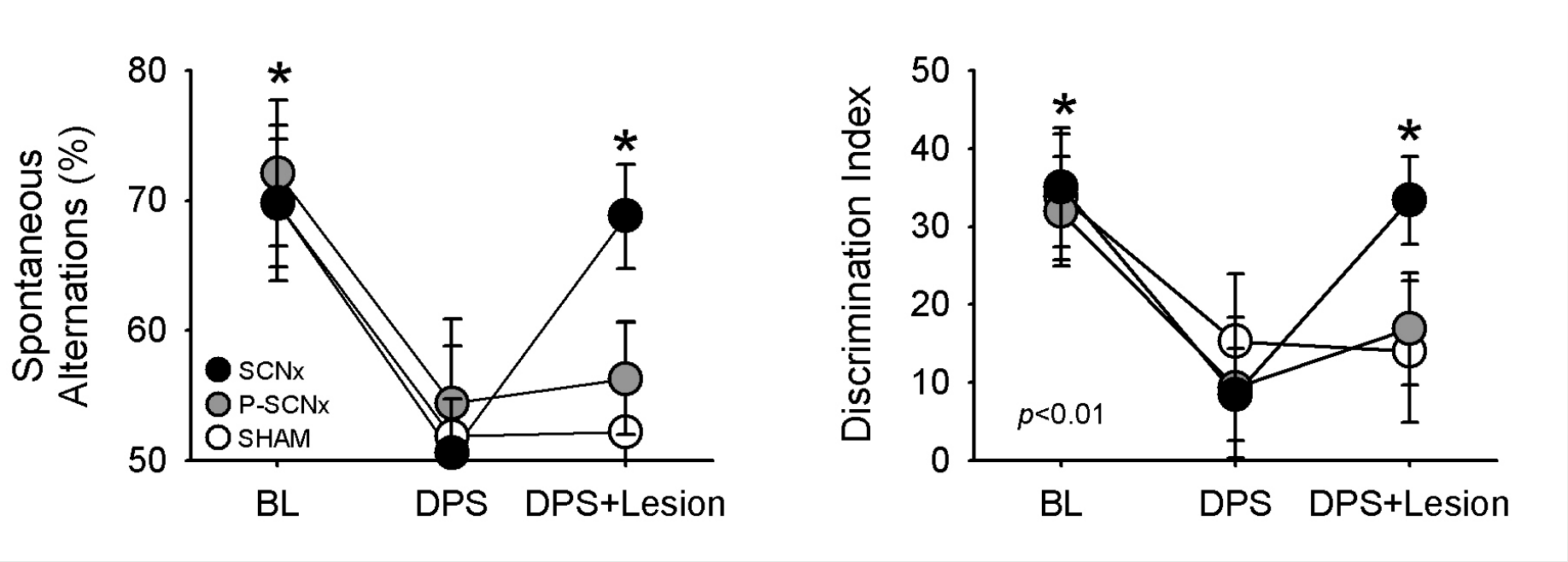
Ruby et al. unpublished

So, Can SCN ablation rescue memory in DPS hamsters?



Ruby et al. unpublished

Yes, SCN ablation rescues memory in arrhythmic hamsters



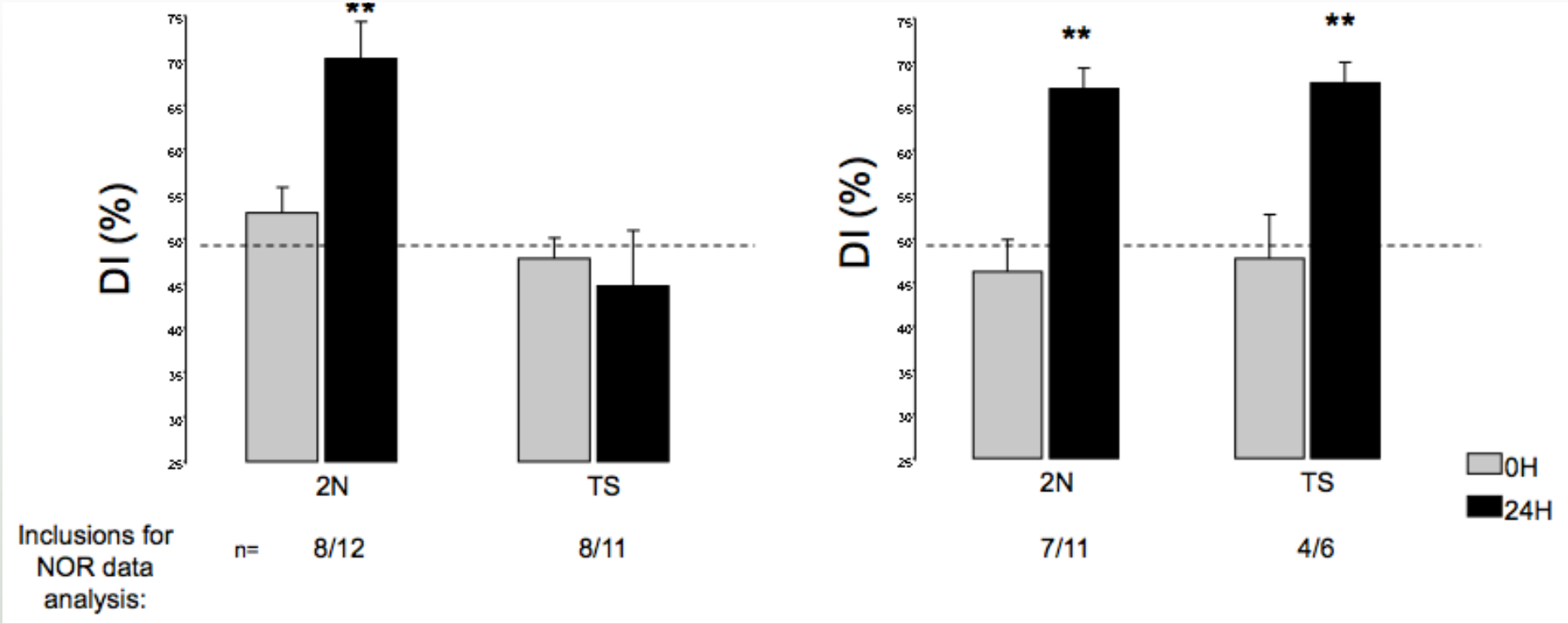
Evidence that the SCN actively suppresses neuroplasticity at a particular circadian phase.

Fernandez, Ruby et al. unpublished

And also in Down Syndrome model mice…

Before SCN lesion

After SCN lesion



Heller, Colas et al. unpublished

Why does the SCN limit neuroplasticity?

A Bold Hypothesis

When short term memory is being transferred to long term memory during sleep, the circadian system suppresses neuroplasticity to protect the fidelity of the memory transcripts.

Compose Study
(**Co**gnition and **M**emory in **P**eople with D**o**wn **S**yndrom**e**)

- Phase II placebo controlled study to evaluate safety and tolerability of BTD-001 in adolescents and young adults with Down Syndrome (13-35 yrs)
- Study designed to assess improvements in cognitive processes (memory, reaction time and language), daily activities and behavior.
- Study included 99 subjects at 4 sites in Australia.
- For more info see Http://compose21.com
- PI: Dr Robert Davis, Monash University
- Sponsored By Balance Therapeutics Inc
- Study completed, results will be available soon

Key points to take home:

- The development of approved drug therapies is slow and expensive. The Translational Cycle defines the steps in this process.
- Research in animal models improves our understanding of brain function and permits principled design of therapies.
- Significant progress is being made for drug therapies for neurodevelopmental disorders such as DS.
- We believe investment in basic research is the best value for advancing the Translational Cycle.

Thanks to LuMind and Matthew Foundations and to NIMH and individual donors for support, and to you for listening!

Current Treatment Strategies for Cognitive Impairment in DS

Drug	Description	Trial	Outcome	Adverse
Vitamin supplement	Antioxidants, folinic acid, vitamins A, C, E… and more	Numerous, including placebo-controlled	No significant benefit	
Vasopressin	Peptide hormone	One trial: short, placebo-controlled	No significant benefit	N/A
Piracetam	Nootropic, GABA derivative. Site of action unknown.	One trial: placebo-controlled	No significant benefit	Various, common
Donepezil	Acetylcholinesterase inhibitor	Various. DS + AD, adults, children. Large trial ongoing	Mixed. No clear significant benefit for non-AD.	Various, common
Rivastigmine	Cholinesterase inhibitor.	2 trials: DS + AD placebo-controlled; adolescents open label	No benefit DS + AD, small improvement adolescents	7/11 in adolescents
Memantine	NMDA-R inhibitor	1 placebo-controlled Trial in DS	No significant benefit	Various

None have been shown to be effective