Excessive inhibitory tone also suppresses learning and memory function in Down Syndrome.
Central hypothesis

Excessive inhibition of critical circuits contributes to reduced cognitive function in Down syndrome.

This imbalance blocks learning and may be fixed with drugs.
Down Syndrome

Excitation / inhibition ratio

Neural circuit performance

2N
Down syndrome

Excitation / inhibition ratio

Neural circuit performance

2N

Down syndrome
Down syndrome

Excitation / inhibition ratio

Neural circuit performance

2N

GABA_A antagonist

Down syndrome
Down syndrome

Excitation / inhibition ratio

GABA\textsubscript{A} antagonist

Neural circuit performance

2N

GABA\textsubscript{A} antagonist

Down syndrome
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
Object recognition in children with Down syndrome
Novel Object Recognition Game

• **Mice are curious:** mice explore new environments and remember what they learn.

• **Learning and memory games for mice:** Games allow us to measure what a mouse learns and how long it remembers. Do therapies improve performance? Short term? Long term?

• **Game rules:**
  – Place mouse in arena with 2 or 3 objects (toys).
  – Mouse explores the objects.
  – At a later time, mouse is placed back in the arena, but one object has been changed. Can it identify the novel object?

Two Different Familiar Objects  One Familiar Object + One Novel Object
Playing the Game
Single doses of GABA antagonist, Pentylenetetrazole improves learning in TS mice, but effects do not last.
It gets better! Short-term chronic treatment with PTZ at low doses induces a very long lasting improvement in learning and memory.

The experiment

Drug treatment (daily doses)

Day 1

Day 17

Days 24-90

Object recognition training (15 min training session)

Object recognition testing phase (15 min session)
Memory improvement is long-lasting after daily pentylenetetrazole (PTZ) dose

1 week post-treatment

2 months post-treatment

Fernandez & Garner, 2007
Goals of recent studies

• Preclinical development of PTZ
  – Dose, safety, age, pharmacokinetics

• Investigation of mechanism of drug therapy
  – Dosing strategy
  – Developing New Biometrics
  – Understand Mechanism

• Initiate Clinical Trials
  – FDA approval to move forward
  – Design clinical trial
Is PTZ effective in young (2m) and older mice?

13 months old Ts65Dn mice

24h NOR

Discrimination index (%)

before PTZ

after 2 weeks PTZ

Colas & Chuluun
A 100 fold lower dose of PTZ (0.03mg/kg once-a-day for two weeks) normalizes Long-term Memory in 3mo old Ts65DN mice

Conclusion: drug therapy is very safe.

Colas & Chuluun
GABA\textsubscript{A} receptor antagonist effects are circadian phase dependent

They are most affective during sleep

Colas & Chuluun
Learning and memory processes occur during sleep. Is sleep different in DS mice, and can it be improved?

We record the EEG and EMB of Ts mice.

And, we find differences in comparison to wild-type mice that are partially remediated by PTZ treatment.

But, what do these differences mean?
One current goal is to understand how the brain processes of memory consolidation during sleep are altered in DS, and then to fix them.

During wake, information is acquired through our senses and is placed in short term storage.

During sleep, some of the information in short term storage is transferred to long term storage.

These events are organized by specific brain electrical oscillations that we can measure.

**Which ones are abnormal in DS, and how can we fix them?**
Summary of PTZ characteristics

Pharmacokinetics of PTZ are consistent with expectation of short half-life (~1-2 hours).

Is effective at extremely low doses 0.03-3mg/kg

Long history of safe use in humans
  Chronic: 6-8 mg/kg orally, three-four times daily for weeks to 10 years. No epileptogenesis reported

Effective in young and aged animals

Time-of-day dosing influences effectiveness of therapy

GABA drugs act to normalize sleep architecture
Conclusions

Over-inhibition contributes to memory deficits in Ts65Dn mice

GABA_A antagonism with PTZ is a promising therapy

Other drugs could be effective or complementary
- Other GABA_A drugs (e.g. flumazenil or alpha5IA’s)
- L-DOPS or risperidone for ADHD-like behaviors
- Fluoxetine & NAC for hippocampal neurogenesis defects
- Rapamycin for over-activation of mTOR pathways
The Translational Cycle

1. Human phenotype
   - characterization of disease
   - cognitive strengths and weaknesses

2. Human genotype
   - gene(s), mutations
   - inheritance, XCI
   - second-hit mutations

3. Animal genotype
   - transgenic mice and flies
   - inducible or tissue-specific
   - knockin of human gene(s)

4. Animal phenotype
   - behavioral tests of cognitive, motor, and social function
   - anatomy, neurophysiology

5. Therapeutic strategy
   - improve efficacy and safety in animal models
   - test various compounds

6. Drug development
   - modify lead compound for specificity, bioavailability, PK
   - dosing strategy

7. Clinical trials
   - safety, dosing, open-label and placebo-controlled trials
   - NDD-appropriate endpoints
# Current Treatment Strategies for Cognitive Impairment in DS

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

None have been shown to be effective
Findings in animal models suggest therapies to address dysfunction.

1. Overinhibition addressed with GABA$_A$ antagonist

Fernandez & Garner 2007

![Diagram showing neural circuit function vs. excitation/inhibition ratio with Down Syndrome

Pentylenetetrazole, Bilobalide, Balance Therapeutics

Alpha5 inverse agonist, Roche, Merck
Compose Study
(Cognition and Memory in People with Down Syndrome)

- 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 will also assess improvements in cognitive processes (memory, reaction time and language), daily activities and behavior.
- Study is currently enrolling at 4 sites in Australia.
- For more info see Http://compose21.com
- PI: Dr Robert Davis, Monash University
- Sponsored By Balance Therapeutics Inc
Findings in animal models suggest therapies to address dysfunction

2. Reduced neurogenesis, increased cell death and memory impairment can be addressed with SSRIs or drugs that reduce ROS

Fluoxetine (prozac)

N-AcetylL-Cysteine (NAC)
Findings in animal models suggest therapies to address dysfunction

3. Reduced neuromodulatory function addressed with various drugs, e.g. ADHD medications
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.
Learn more about your child’s sleep!

We are looking for families interested in taking part in a Stanford University study investigating sleep in children.

Department of Psychiatry and Behavioral Sciences
Stanford University School of Medicine
Study Coordinator: Sean Berquist
Phone: 650-723-2795
DS Clinical Research at Stanford

Stanford Vision and Neurodevelopment Lab
Vision in Down Syndrome

Vision in typically developing infants and children

Using vision to understand general properties of brain development in special populations

Professor Tony Norcia, Director
650-736-2793  Svndl.stanford.edu
DS Clinical Research at Stanford

STANFORD UNIVERSITY

Children Needed for Intellectual/Developmental Disorder Research Study

*Does your child have an intellectual disorder or developmental delay and a significant language delay?*

Researchers at Stanford University are currently recruiting children to participate in a research study examining the effectiveness of Pivotal Response Treatment (PRT) in targeting language skills in children with intellectual disorders or developmental delay.

Professor Antonio Hardan  
650 736-1235

Lucile Packard Children’s Hospital
### Acknowledgements

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<tr>
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Thanks!