Center for Neuroscience and Regeneration Research
A Collaboration of the Paralyzed Veterans of America with Yale University

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*A Collaboration of the Paralyzed Veterans of America with Yale University
Harnessing the molecular revolution to preserve and restore function after injury to the brain and spinal cord
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Major Research Programs

Molecular Repair of the Injured Spinal Cord and Brain

Cell Transplantation / Adult Stem Cell Technology

Neuroprotection

Neuropathic Pain and Spasticity
Targeting the Memory Trace:
A New Perspective in Spasticity following SCI
Are mechanisms involved in memory also involved in spasticity?
Central Premise:
Learning & memory in the spinal cord underlies normal motor/muscle control.

Abnormal learning/memory mechanisms in the spinal cord may explain the persistent, hard-to-treat nature of spasticity?
Spasticity
Unmet Medical Need

• Quality-of-life issue, disruptive
• Common in patients with SCI and MS
• Uncontrollable “jerking” movement and abnormal motor control whereby muscles continually contract
• Standard treatment can be “hit-or-miss”
  – I.e., baclofen, botox injections, etc.
  – Narrow therapeutic window

(Skold et al., 1999; Bennett et al., 2004; Eaton et al. 2003).
What is Spasticity?

- Clinically defined:
  - Velocity-dependent increase in tonic stretch reflexes with exaggerated tendon jerks, resulting from hyperexcitability of the spinal stretch reflex (e.g., H-reflex)

**H-reflex**
- Very simple, very fast
- One synapse, two neurons
- All complex movements use this circuit
- Experimentally accessible
Mechanisms of Spasticity?

- Loss of local and descending inhibition/regulation

- Increased excitability of motor neuron properties
  - Down regulation of the KCC2 co-transporter
  - Sodium channel misexpression

- **Neuroplasticity in the spinal cord**
  - Structural reorganization, fiber and synaptic plasticity

(Thompson et al., 1998; Li et al., 2004; Nielsen et al., 2007; Halter JA et al., 1995; Bennett et al., 2004; Boulenguez et al., 2010; Tan et al., 2012)
Neuroplasticity in SCI

- After SCI, the body tries to repair neuronal structure
  - Reactive neuroplasticity
  - Organic

- Neuron structure contributes to function
  - Structure = Function
Learning (early synaptic-basis)

- Lasts for minutes to hours
- Increased number of post-synaptic receptors
Long-Term Memory

Formation and elaboration of **dendritic spines**

- AMPA receptor
- NMDA receptor

Stabilize & strengthen synaptic connections
Dendritic Spines in Memory

before

after

Postsynaptic

Presynaptic

After Learning
“Activity”-Dependent Spine Remodeling

1. Spine density changes...

2. Spine shape changes...

- **Thin** (unstable, immature)
- **Mushroom** (stable, mature)
Hypothesis:
Dendritic spine dysgenesis contributes to spasticity after SCI
Structure-Function in Memory and Spasticity

**Rationale:**
If abnormal dendritic spine profiles represent new or stronger synaptic connections associated with “spasticity-memory”, then disrupting dendritic spine remodeling will reduce SCI-induced spasticity.
Study Design

- All Subjects
  - Sham (no injury)
    - Sham
  - SCI
    - SCI (above injury)
    - SCI (below injury)

Dendritic spine structure analysis
Reflex Testing
SCI Model

Cervical

SCI

T12

Lumbar
Abnormal Dendritic Spines on α-Motor Neurons after SCI

Dendritic spine remodeling
Digital Reconstruction

Spinal motor neuron
(leg muscle control)
Dendritic Spine Density

Increased dendritic spine density after SCI **below the injury, not above injury**
Sholl’s Analysis
Dendritic spines are distributed closer to the soma after SCI. Clustering is more pronounced in SCI below the injury.
Test for Spasticity:
H-Reflex Responsiveness

M-wave – occurs earlier because it results from stimulation of the motor neuron that directly produces a muscle response (signals travel a shorter distance)

H-reflex wave – occurs later because it results from the signal that travels through the spinal cord, across a synapse, and back into the muscle (signals travel through the longer circuit loop)

Paired-pulse test
• Rate-dependent changes in M- and H-response amplitudes
• 10 – 2000ms interpulse stimulation intervals
H-Reflex Response

Sham
(no injury)

SCI
(above injury)

SCI
(below injury)

~20-30 traces averaged for each trial
• **Normal** – As stimulation activity increases, H-reflex amplitude decreases
• **Spasticity** (increased excitability of spinal reflex arc) – As stimulation increases, H-reflex amplitude decreases at a lesser rate or remains stable
Abnormal dendritic spine profiles below the level of SCI accompany spasticity

– Conversely, the lack of such spine profiles above the level of injury correspond with the lack of spinal reflex hyperexcitability
Reduce Spasticity?
Target dendritic spine remodeling

If abnormal dendritic spine profiles underlie a “spasticity-memory” mechanism, then disrupting dendritic spine remodeling will reduce SCI-induced spasticity.
What is Rac1?

1. **Highly-studied**
   - Belongs in a family of Rho GTPases, i.e., Rac, cdc42, Rho

2. **Molecular “switch”**
   - Exists in a dynamic equilibrium between active and inactive state

3. **Regulates dendritic spine structure**

1. **Disease relevant**
   - Activated after injury/stress

(Dubriel et al. 2003; Tzima et al. 2002; Wong et al. 2000)
NSC23766 treatment (100mM) decreases the number of dendritic spines on cultured dorsal horn neurons.
Does disrupting dendritic spine remodeling will reduce SCI-induced spasticity?
Anti-Rac1 treatment disrupts SCI-induced dendritic spine dysgenesis below the injury.
Disruption Rac1-regulated dendritic spines restores close-to-normal H-reflex activity

- Treatment reduces electrophysiological evidence of spasticity

~20-30 traces averaged for each trial
• Structure-function link between dendritic spines and motor control
• Dendritic spines contribute to a “spasticity-memory” mechanism
• Molecules involved in classical learning and memory may be considered promising targets for addressing chronic complications after SCI
• Rac1 signal pathway include other downstream molecules that may have therapeutic utility in modifying motor reflex hyperexcitability
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THANK YOU
No changes in cell body and dendritic branch morphology across groups

<table>
<thead>
<tr>
<th></th>
<th>Maximum cell diameter (µm)</th>
<th>Number of primary dendrites</th>
<th>Total dendrite length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>54.6 ± 19.4</td>
<td>8.16 ± 3.69</td>
<td>1073.0 ± 671.4</td>
</tr>
<tr>
<td>SCI (above injury)</td>
<td>46.7 ± 11.7</td>
<td>5.73 ± 1.95</td>
<td>815.10 ± 451.9</td>
</tr>
<tr>
<td>SCI (below injury)</td>
<td>51.4 ± 17.4</td>
<td>4.80 ± 1.23</td>
<td>739.30 ± 280.6</td>
</tr>
<tr>
<td>SCI + anti-Rac</td>
<td>46.1 ± 8.7</td>
<td>4.38 ± 1.15</td>
<td>1085.8 ± 307.9</td>
</tr>
</tbody>
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Experimental effects of SCI and treatment occurred for dendritic spines only
Future Steps/Perspective

• **Off target effects of pharmacological inhibition of Rac1?**
  
  – Rac1 inhibition does not significantly affect inflammation within the injured spinal cord (Tan et al., 2011)
  
  – Rac1 inhibition (at analgesic dose) fails to affect dendritic spine structure on primary motor neurons (Tan et al., 2013)

• **Rac1 effects on normal, uninjured animals?**
  
  – Subtle change in dendritic spine structure, and unaffected nociception (Tan et al., 2012)

• **Time-course study of dendritic spine remodeling?**
  
  – Abnormal dendritic spines appear only in the presence of significant neuropathic pain, not temporally synced with progressive diseases, i.e., diabetes (Tan et al., 2012)

• **Clinical translational of targeting dendritic spine remodeling?**
  
  – Molecules involved in classical learning and memory are now promising targets for novel therapeutics for chronic pain
  
  – Rac1 signaling pathway includes a stream of promising target molecules for developing novel therapies for neuropathic pain