Astrocyte Plasticity Revealed by Adaptations to Severe Proteotoxic Stress

Rehana Leak, Ph.D.
Plasticity

- Short duration stress
- Low dose stress
- Long duration stressors of very low dose
- Quantified by response to a second hit
- Plasticity in neurodegenerative disease models?
Vehicle pretreatment

Cortex

Striatum

Accumbens
6-OHDA toxicity is blunted by preconditioning

* $p \leq 0.05$ vs Vehicle
What happens if the stress is severe?

**Dual-hit hypotheses**
- Severe stress potentiates response to second hit

**Alternative dual-hit hypothesis**
- Severe stress may leave behind resistant cells
- These survivors may be harder to kill
- A new type of plasticity
The 26S proteasome

- Proteasome activity is inhibited in PD in nigra
  - Aggregated synuclein clogs proteasome
- Astrocytes contain aggregated synuclein
  - Both glia and neurons undergo protein-misfolding stress in PD

2 Hit Model in Primary Astrocytes

Plate Astrocytes

0 h

1st MG132 hit (pretreatment)

24 h

2nd MG132 hit (post-treatment)

48 h

Assay

72 h
Severely stressed glia resist 2\textsuperscript{nd} hit

\[ p \leq 0.05 \text{ vs } 0 \mu M \text{ post-treatment}; + p \leq 0.05 \text{ vs } 0 \mu M \text{ pretreatment} \]
Severe stress reduces ATP loss in glia

* $p \leq 0.05$ vs 0 µM post-treatment
Very severe stress blocks ATP loss in glia

* $p \leq 0.05$ vs 0 µM post-treatment; + $p \leq 0.05$ vs 0 µM pretreatment
Alternative Interpretations

• Remaining cells are simply refractory to MG132
  – Would not respond to 2\textsuperscript{nd} hit either

• Cells are still responsive to 2\textsuperscript{nd} hit, but do not die
  – 1\textsuperscript{st} hit elicits adaptations (supported by ATP data)
2nd hit still has an impact on stressed cells

Ubiquitin-conjugated proteins / β-actin

MG132 post-treatment (μM)

MG132 pretreatment (μM)

Ubiquitin-conjugated proteins

β-actin

* $p \leq 0.05$, *** $p \leq 0.001$ vs 0 μM post-treatment; ++ $p \leq 0.01$ vs 0 μM pretreatment
2nd hit still has an impact on stressed cells

** p ≤ 0.05, *** p ≤ 0.001 vs 0 μM post-treatment; + p ≤ 0.05 vs 0 μM pretreatment
Impact of 2\textsuperscript{nd} hit on Hsp70 is blunted

\[ \text{MG132 (\(\mu\text{M}\))} \]

\begin{tabular}{c|c|c|c|c}
Pre: & 0 & 0 & 0.4 & 0.4 \\
Post: & 0 & 80 & 0 & 80 \\
\end{tabular}

\[ \text{Hsp70} \]

\[ \beta-\text{actin} \]

*** \(p \leq 0.001\) vs 0 \(\mu\text{M}\) post-treatment; + \(p \leq 0.05\) vs 0 \(\mu\text{M}\) pretreatment
1st hit prevents loss of glutathione

• Does BSO elicit vulnerability to 2nd hit?

** $p \leq 0.01$ vs 0 μM post-treatment; ++ $p \leq 0.05$ vs 0 μM pretreatment; ^^^ $p \leq 0.001$ vs no BSO
Glutathione loss makes stressed cells vulnerable to 2nd hit

** $p \leq 0.01$, *** $p \leq 0.001$ vs 0 $\mu$M post-treatment; +++ $p \leq 0.001$ vs 0 $\mu$M pretreatment
Can neurons also adapt to severe proteotoxicity?

Can neurons adapt to severe oxidative toxicity?

Next questions
Severely stressed primary neurons resist 2\textsuperscript{nd} hit
Severe stress reduces ATP loss in primary neurons.

The bar chart shows the percentage of ATP remaining after H$_2$O$_2$ treatment at different concentrations. Arrows indicate significant reductions in ATP loss compared to controls.
Astrocytic responses to severe stress
Conclusions

• Astrocytes become progressively harder to kill
• Adaptation is glutathione dependent
• Adaptation may be fueled by a rise in ATP
• Adaptation is not dependent on autophagy

• Can stressed astrocytes retain their neurosupportive roles in disease states?
Leak Laboratory

Amanda Titler
Jessica Posimo
Hailey Choi
Yiran Jiang
Ajay Unnithan
Sree Pulugulla
Jenn Rumble
MG132 post-treatment (µM)

% DRAQ5 + Sapphire

MG132 pretreatment (µM)