### Background

#### Traumatic Brain Injury (TBI)
- Traumatic brain injury (TBI) facts:
  - Significant global health problem
  - Emergency room visits:
  - Care: expensive, intensive care, rehabilitation, and/or outpatient
  - High personal and economic cost
- Some of the science for TBI
  - Extensive research into pathophysiology of 1’ and 2’ injury
  - TBI symptom profile is well-characterized
  - Despite extensive care, morbidity remains high
- Treatment:
  - Thousands of successful pre-clinical trials
  - Several thousand clinical trials
  - Many standardized models available
- Experimental TBI models
  - Critical tool for understanding TBI and testing novel therapies
  - No new FDA-approved therapies
  - Despite extensive care, morbidity remains high
- TBI symptom profile is well-characterized
- Personal & economic cost
  - Everyone is at risk (all ages; sexes; etc.)
  - Significant global health problem

#### Melatonin (MEL)
- Enzyme substrate produced in the brain and elsewhere
- MEL levels are deranged after TBI
  - Salivary MEL production higher in controls (Schloemer, 2010)
  - CSF MEL, up to day 2, is minimally detectable on day 8
  - For all days but 0, 1, & 4, MEL higher after TBI vs. control (Solman, 2008)
  - Blood MEL lower after TBI than established ranges (Papageorgopoulos, 2006)
- May be involved in response to TBI but insufficient for full neuroprotection
  - Exact mechanism is unknown
  - Anti-inflammatory effects extensively studied
- Has not led to clinical trials
  - Anti-inflammatory effects well-characterized
  - G-protein receptor dependent mechanism poorly understood
- MEL-specific receptors
  - MT1 and MT2 found in the brain
  - Role in TBI remains unknown
  - Gap to target: Characterize MT1 and MT2 after experimental TBI

#### Data Quantification and Analysis
- Use of the least sentient animal appropriate for the study
- Animal Welfare Act
  - Protocol 1307238 and Protocol 14012346
  - Approved by the University of Pittsburgh Animal Care & Use Committee
- Also conducted in accordance with state/national laws & ethical standards
- Confirmed gel ran without error
  - Gel incubated in GelCode Blue for 1 hr to stain for protein
- Examination of gel after transfer
  - Invitrogen Bolt (Generation 1) device used w/ PVDF membrane (7 min)
- Semi-dry transfer
  - Gel running conditions: 165 Volts for approximately 30 minutes
  - Biorad blotting system used with 15 well Criterion 4-12% Bis-Tris Gel
  - Samples centrifuged, supernatant fluid collected & aliquoted for analysis

### Methods, Results, and Discussion

#### Treatment of Animals
- Approved by the University of Pittsburgh Animal Care & Use Committee
- Protocol 1307238 and Protocol 14012346
- Also conducted in accordance with state/national laws & ethical standards
- Animal Welfare Act
- Guide for the Care & Use of Laboratory Animals
- Use of the least sentient animal appropriate for the study

#### Wet Laboratory Methods
- Tissue processing
  - Whole cell lysates prepared using lysis buffer & a sonicator
  - Samples centrifuged, supernatant fluid collected & dispersed for analysis
- BCA assay
  - 2 μL of protein per well (diluted 5-fold with 8 μL of deionized water)
  - Compared to 8 protein standards to determine μg protein per μL sample
- Western blot
  - Blotting system used with 15 well Criterion 4-12% Bis-Tris Gel
  - Gel running conditions: 165 Volts for approximately 30 minutes
- Semi-dry transfer
  - Invitrogen Bolt (Generation 1) device used w/ PVDF membranes (7 min)
- Examination of gel after transfer
  - Gel visualized in GelCode Blue for 1 hr to stain for protein
- Confirmation gel ran without error
- Membrane treatment
  - Washed in TBS-T, blocked in 5% milk, incubated in 1% antibody overnight
  - MT1 (Abcam ab144013)
  - MT2 (Abcam ab215346)
  - Cet (Cell Signal Technology 1ME3)- marker of cell death
  - Anti-Sigma (Sigma Aldrich a016)
  - Washed in TBS-T, incubated in 1% milk with 2% antibody for 1 hr
  - Stepped with Restore Striping Buffer and washed between 1st antibodies

#### Summary of Main Findings
- MT1 and MT2 downregulated 24 hr after TBI
- CCI (n = 3)
  - p = 0.04
- MT1 downregulated 24 hr after TBI
  - In rats
  - Cortical tissue (p= 0.011)
  - Hippocampal tissue (p= 0.003)
  - MT2 downregulated 24 hr after TBI
  - In rats
  - Cortical tissue (p= 0.010)
  - Hippocampal tissue (p= 0.003)
- Not tested in mice
- Cytosch C showed apoptotic changes
- However, methodological limitations
- Compiles interpretation
- Still, model is well-validated

### Discussion

#### Results
- MT1 and MT2 downregulated after TBI
- Supports use of control for gel loading
  - β-actin
  - MT1 downregulated 24 hr after TBI
  - In rats
  - Cortical tissue (p= 0.011)
  - MT2 downregulated 24 hr after TBI
  - In rats
  - Cortical tissue (p= 0.003)
- MT1 and MT2 found in the brain
  - Role in TBI remains unknown
  - Gap to target: Characterize MT1 and MT2 after experimental TBI

#### Conclusions and Future Directions

### Implications
- Although preliminary, a role of MT1 and MT2 in TBI is apparent
- Endogenous receptors are downregulated
- Effects depend on brain region and time point after TBI
- Effect on receptor correlates with apoptosis but not functional outcomes

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### Conclusions
- Additional research
  - Preclinical: Female animals? Other ages? MEL therapy? Effect in transgenics?
  - Sex: Male?
  - Animals and treatment
  - Receptor downregulation? Effect of polymorphisms? Clinical trials of MEL
  - Clinical translation (if the evidence warrants it)

### Future Directions
- Additional research
  - Preclinical: Female animals? Other ages? MEL therapy? Effect in transgenics?
  - Sex: Male?
  - Animals and treatment
  - Receptor downregulation? Effect of polymorphisms? Clinical trials of MEL
  - Clinical translation (if the evidence warrants it)
- Personalized and precise medicine:
  - Identify patients most likely to benefit from melatonin therapy
  - Tailor dose based on genotypes

### Purpose
- To gain proficiency in basic and bench science methods, via completing a dissertation study characterizing the melatonergic system after experimental TBI, modeled in mice and rats.