Characterizing the Melatonergic System After Brain Injury

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Acknowledgements

Dissertation Committee
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Funding Sources

[Logos of funding sources]
Impact of Brain Injury

Every 18.5 seconds in the USA (Scudellari, 2010)
Many Faces of TBI

Image Credit: Ohio Department of Aging
Image Credit: Technologist
Image Credit: Ellie Downie
Image Credit: Ellie Downie
Image Credit: The Expert Institute
Image Credit: Active.com
Image Credit: Healthcare Online
Melatonin

Molecule

Receptors

Original Artwork: M. Farmer & N. Osier
Controlled Cortical Impact vs. Sham
Pilot 1: MEL Therapy

![Graph showing Ipsilateral Hemispheric Volume Normalized to Sham]
Pilot 2: MT1 ↓ in Mice

% Change in Densometric Units

Post-operative Day 13

Sham (n = 3)
CC I (n = 3)
p = 0.04
Design & Sample

- Pre-clinical
- Sprague-Dawley
- N=25
- Homogeneous

• 2x2 Design
  • Randomized, blinded

<table>
<thead>
<tr>
<th>Injury Exposure</th>
<th>Point (Post-Surgery)</th>
<th>Sacrifice Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>N=6</td>
<td>6 hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 hr</td>
</tr>
<tr>
<td>CCl (2.8 mm)</td>
<td>N=6</td>
<td>6 hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 hr</td>
</tr>
</tbody>
</table>
Methods
# Results: Raw Data

## 6 hr Ipsilateral Hippocampus

<table>
<thead>
<tr>
<th>Exposure Group</th>
<th>MT1</th>
<th>MT2</th>
<th>Beta Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S = Sham</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I = Injury</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**MW, kDa**

- 198
- 98
- 62
- 49
- 38
- 28
- 17
- 14
- 6
- 3

*CCI 2.8mm*
Results: Summary
Conclusions

• Endogenous MEL receptors altered after TBI
• May correlate with TBI outcomes
  • Follow up KO mouse- no effect on outcomes chosen

Future Directions
• Do lower receptors affect response to melatonin Tx?
• Biomarker utility of melatonin?
• Role of genetic polymorphisms?
• Effect on sleep outcomes?
Brain injury results in lower levels of melatonin receptors subtypes MT1 and MT2.

Osler ND¹, Pham L², Pugh BJ³, Puccio A⁴, Ren D⁵, Conley YP⁶, Alexander S⁷, Dixon CE⁸.

Abstract

BACKGROUND: Traumatic brain injury (TBI) is a devastating and costly acquired condition that affects individuals of all ages, races, and geographies via a number of mechanisms. The effects of TBI on melatonin receptors remains unknown.

PURPOSE: The purpose of this study is to explore whether endogenous changes in two melatonin receptor subtypes (MT1 and MT2) occur after experimental TBI.

SAMPLE: A total of 25 adult male Sprague Dawley rats were used with 6 or 7 rats per group.

METHODS: Rats were randomly assigned to receive either TBI modeled using controlled cortical impact or sham surgery and to be sacrificed at either 6- or 24- hours post-operatively. Brains were harvested, dissected, and flash frozen until whole cell lysates were prepared, and the supernatant fluid aliquoted and used for western blotting. Primary antibodies were used to probe for melatonin receptors (MT1 and MT2), and beta actin, used for a loading control. ImageJ and Image Lab software were used to quantify the data which was analyzed using t-tests to compare means.

RESULTS: Melatonin receptor levels were reduced in a brain region- and time point- dependent manner. Both MT1 and MT2 were reduced in the frontal cortex at 24hours and in the hippocampus at both 6hours and 24hours.

DISCUSSION: MT1 and MT2 are less abundant after injury, which may alter response to MEL therapy. Studies characterizing MT1 and MT2 after TBI are needed, including exploration of the time course and regional patterns, replication in diverse samples, and use of additional variables, especially sleep-related outcomes.

CONCLUSION: TBI in rats resulted in lower levels of MT1 and MT2; replication of these findings is necessary as is evaluation of the consequences of lower receptor levels.
Thank You! Questions?!
Repeated Measures ANOVA:
Main Effect for Injury (p=0.002)
Main Effect for Time (p<0.0005)
No Interaction Effect (p>0.05)
Probe Trial

Sham vs. CCI

Sham Mean = 36.6 seconds

CCI Mean = 28.5 seconds

Sham/CCI vs. Chance

Sham (n = 9) vs. CCI (n = 12)

all p = 0.08

Mean Percent Time in Target Quadrant

Sham Mean = 36.6 seconds vs. CCI Mean= 28.5 seconds
Beam Balance Task

Group Mean (seconds)

- **Sham (n = 10)**
- **CCl (n = 10)**

Day 1 $p = 0.002$

- **Pre**
- **No Variability**
- **Post-operative Day 1**

- **Sham (n = 10)**
- **CCl (n = 10)**

Day 1 $p = 0.002$
Novel Object Recognition

Discrimination Ratio
Post-operative Day 19

Discrimination Index

Novel Object Recognition Group Mean
p = 0.049

Sham (n = 7)
CC I (n = 7)

p = 0.049
MEL & Circadian Rhythm

A. Pineal gland
   - Inhibition
   - Stimulation

B. Melatonin (pg/ml)
   - Time of Day

MT1 → Gi → AC → cAMP → PKA → DNA → Circadian Rhythm
MT2 → Gq → PLC → DAG → PKC → Protein kinase cascades → Circadian Rhythm

Clock genes (immediate early genes)

Phase Advance/Phase Delay
Sleep in TBI

- TBI survivors experience sleep issues (Vermaelen et al. 2015)

- Sx common after TBI of all severities (Duclos et al. 2014)

- Sleep issues exist; hard to assess (Foreman et al. 2015)

- Common problems
  - Insomnia / Hypersomnia
  - Trouble maintaining sleep
  - Sleep disordered breathing
  - Nightmares (esp. w/ PTSD)

Hou et al. 2013 doi: 10.1371/journal.pone.0076087
Other Peer-Reviewed Publications as a Pre-Doc


