Evaluation of Postural Steadiness before and after Propofol Sedation

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Fitness for Ambulation

- Important criterion for discharge
- Impaired by sedation/anesthesia
- Recovery rates different
- No objective test
Postural Steadiness

- Complex interaction of multiple systems
- Complexity gives rise to chaotic behavior
- Chaos can be measured by nonlinear tools such as entropy
Previous Work

- AP sway acquired from Nintendo Wii® via Bluetooth
- Measurement of chaos via Fuzzy Sample Entropy (FSE)
- Can distinguish pre- and post- sedation states with Midazolam, Sleep Deprivation

Hypothesis

FSE of postural sway can detect return towards baseline state during recovery from procedural sedation with propofol.
Methods

• IRB approval, informed consent
• 131 patients undergoing colonoscopy/EGD
• Assessed at 3 times:
  • Prior to procedure (PRE)
  • When first able to stand (POST1)
  • Appx. fifteen minutes later (POST2)
• Propofol administration obtained from EMR
• Propofol effect site estimates via Cortinez model
• Postural sway measured by FSE, compared by paired T test

## Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.3 ± 14.5</td>
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<tr>
<td>Height (cm)</td>
<td>170.39 ± 11.44</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.94 ± 23.27</td>
</tr>
<tr>
<td>Time to POST (min)</td>
<td>33.9 ± 12.1</td>
</tr>
<tr>
<td>Time to POST2 (min)</td>
<td>53.1 ± 14.2</td>
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Propofol Effect Site Estimates (ug/mL)

Peak: 2.98
POST: 0.29
POST2: 0.16
Fuzzy Sample Entropy (Individual)

*$P < 0.05$ vs PRE
†$ P < 0.05$ vs POST2
Fuzzy Sample Entropy (Change)

Change in FSE

- PRE-POST
- POST-POST2
- PRE-POST2

*P < 0.05, Paired T, N=93
Results

- FSE was decreased from PRE at POST
- FSE was increased from POST to POST2
- FSE at POST2 is still below PRE
- NO Correlation between
  - $\Delta$ FSE and Age, Height, Weight
  - $\Delta$ FSE and Propofol peak, POST, POST2
  - Time to POST and Age, Height, Weight
Discussion

• Propofol is associated with a marked decrement in FSE of postural sway.
• This effect is still measurable at time of discharge.
• Implications for risk of falls unknown.
• There is significant variation between patients not attributable demographic differences.
• No subgroups stood out as being remarkably fast or slow at regaining stability.
Conclusions

- Fuzzy Sample Entropy shows promise for tracking recovery of postural steadiness.
- Recovery may not be as simple as measuring the propofol concentration.
- The technology permits inexpensive and safe collection of large amounts of data.
Thank You

- FAER
- Department of Anesthesiology & Critical Care, Perelman School of Medicine at the University of Pennsylvania
- Jeff E Mandel MD MS
Macrophages and Long Noncoding RNA
Objectives

- Investigate long noncoding RNA that are differently expressed in classically activated macrophages (M1), and myelin-laden macrophages.
- More specifically examine the expression of long noncoding RNA, TUG1, which is known to have a repressive effect on classical macrophage activation.
Macrophage

- Elimination of dust, allergens and microorganisms
- Clearance of pathogens and toxins
- Bone resorption and osteoclast
- Bone marrow macrophage
- Intraocular macrophage
- Alveolar macrophage
- Kupffer cell
- Liver
- Lymph node
- Antigen capture and presentation to B cells
- Subcapsular sinusoidal macrophages and medullary macrophages
- Clearance of senescent red blood cells by red pulp macrophages
- Clearance of blood-borne particulate antigens by marginal zone macrophages
- Recognition and removal of enteric pathogens
- Tolerance to food antigens and microbiota
Macrophage Activation

Monocyte

IFN-γ, LPS
Classic activation

NO, IL-1, TNF-α
M1
Killer
cytotoxic and inflammation function
Neural destructive

IL-4, IL-13, IL-10
Alternative activation

M2
Healer
Tissue repair, immune suppression and tumor progression
Neural protective

Therapeutic agent
inhibit
promote
Scanning Electron Micrograph of a Macrophage Infected with *Francisella tularensis*  

Checroun *et al.* PNAS 2006 103 (39) 14578
Dead men may tell no tales, but dead cells certainly do, the macrophage having the last word. ------Sir John Savill

TLR Activation Promotes Pro-inflammatory Gene Activation
Response to Toll Like Receptor Stimulation

Regulation of ncRNAs in THIOs

Dr. Joshua Stender UCSD
Known Role of Non Coding RNA

- Cis-Acting non coding RNA (ncRNA)-
  - local silencer
- Trans-long non coding RNA (lncRNA)-
  - Transcriptional regulator
- ncRNA as Histone Modifier Scaffolds
- Enhancer related RNAs
Myelin

- Composed of **lipids** and **proteins** (myelin basic protein, MBP; proteolipid protein, PLP; myelin-associated glycoprotein, MAG; myelin-oligodendrocyte glycoprotein, MOG)

- Myelin debris is an inhibitory signal for regeneration

- No direct evidence that myelin-debris can stimulate inflammation
1. Extract hematopoietic stem cells from mice bone marrow.
2. Culture the hematopoietic stem cells in a medium that promotes macrophage differentiation.
3. Culture a sufficient number of macrophages for multiple trials of experiment.
4. Culture macrophages with IFN-gamma, LPS and myelin debris. The myelin debris is to represent myelin after traumatic spinal cord injury.
5. Isolate RNA from the cytoplasm of each group at 3, 6, and 12 hours.
6. Analyze the RNA by use of quantitative real time PCR.
TUG1 Expression

![Bar graph showing TUG1 expression levels at different time points and conditions.](image-url)
TUG1 expression has anti-inflammatory effect
TUG1 silencing increases the expression of several pro-inflammatory proteins
In LPS exposed and myelin laden macrophages, TUG1 expression is repressed. This suggests that myelin-laden macrophages have pro-inflammatory characteristics.
This also suggests that TUG1 is an important mediator for the pro-inflammatory state of macrophages at sites of spinal cord injury
Future Directions

- The next step in the project would be to observe the phenotypical changes involved with TUG₁ silencing and overexpression.
Acknowledgements

- FSU College of Medicine and the Division of Research, Graduate and Undergraduate Programs
- Dr. Ren and the Ren Lab
Differential Incorporation Rates of the S-Phase Markers Bromodeoxyuridine and Ethynyldeoxyuridine

Grand Rounds 2014
Princess Urbina
Eric Laywell, Ph.D.
Thymidine

BrdU

EdU
BrdU and EdU delay tumor progression in rodent models of glioma

BrdU

Control
Pre-treated

syngeneic rat glioma

EdU

50mg/kg
25mg/kg QD
25mg/kg QOD
10mg/kg
Control

human GBM in mouse xenograft
• BrdU has a long history of use as an s-phase marker in cell birthdating studies and proliferation assays.

• EdU is beginning to replace BrdU as the preferred s-phase marker, since it is faster and easier to detect.

• Both BrdU and EdU have shown potential as tumor inhibitors.

• Therefore, studies of their uptake kinetics will provide needed information that may influence their use as both experimental s-phase markers, and as possible adjunctive cancer therapeutics.
BrdU/sytox green
EdU contains an alkyne which reacts with an azide (Alexa fluor 488), forming a very stable covalent bond.
Goal

Compare the rates of BrdU and EdU incorporation in cells in vitro and in two areas of persistent neurogenesis in vivo.
1) Treat: 10uM **BrdU** or **EdU**

2) Fix

3) Stain

**BrdU** Immunostaining or **EdU** Click-It Kit

4) Quantify
EdU incorporates more slowly than BrdU in vitro

ANOVA
*p<0.001
**Flow Cytometry**

% of Total Cells Positive for BrdU or EdU

- **BrdU**
- **EdU**

1 min, 10 min, 30 min, 120 min, 360 min

1-Cells AND DAPI+
2-Cells AND DAPI+
3-Cells AND DAPI+
4-Cells AND DAPI+
5-Cells AND DAPI+

BrdU

EdU
1) Treat: BrdU or EdU

2) Sacrifice, collect, section

3) Stain

4) Quantify

In Vivo

Adult Male C57BL/6 Mice

s.c. inj. (50mg/kg)

BrdU Immunostaining

EdU Commercial Labeling Kit
Mouse Dentate Gyrus

Mouse SEZ
Mouse
Dentate
Gyrus

Mouse SEZ
EdU incorporation lags behind BrdU in vivo

Dentate Gyrus

Subependymal Zone

Duration of “Chase” Interval
(survival time after injection)
Conclusions

• EdU incorporation consistently and substantially lags behind that of BrdU in SaoS cells in vitro and in newly-generated cells in vivo.

• Failure to appreciate these differential uptake kinetics when designing cell birthdating and proliferation index experiments may result in a drastic underestimation of DNA synthetic events.

• Conversely, from a chemotherapeutic approach, one risks overestimating EdU uptake.
Future Directions

- Manuscript in progress
- Exact mechanism requires further investigation
  - Entry of analogs into cell?
  - Phosphorylation states?
  - Polymerase efficiency?
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Thank you!

Questions?