Perspective of Basic Science: Is There Life Outside the Hippocampus?

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American Epilepsy Society | 2013 Annual Meeting
Learning Objectives

- The neocortex is not the hippocampus

- *Reverse Translational Research* and *Systems Biology* to understand and develop treatments for human neocortical epilepsy
The neocortex is not the hippocampus
The neocortex is not the hippocampus
The neocortex is not the hippocampus
Number of Articles in PubMed:

“Epilepsy + Hippocampus” → 12,464 hits

“Epilepsy + Neocortex” → 1,620 hits
The neocortex is not the hippocampus

- Many Lesions Associated with Neocortical Epilepsy
- Epileptogenic Zones are most often normal.
Interictal spiking is far more frequent than seizures.
Interictal spiking is far more frequent than seizures.


Systems Biology - “The ability to obtain, integrate and analyze complex data from multiple experimental sources using interdisciplinary tools.”

Epilepsy is a disease of recurrent seizures that affects up to 1% of the world’s population. Yet it remains one of the least understood human disorders in the most complicated of human organs, the brain. As a means to understand and develop improved diagnostic and treatment strategies, we have formed an interdisciplinary collaborative project that uses the power of systems and computational biology to understand human epilepsy through its electrical, anatomical, and molecular features.

This work has lead to the discovery of a ‘final common pathway’ of genes that are consistently induced at human epileptic foci and now serve as invaluable molecular markers and drug discovery targets. We have recently been funded by the President of Wayne State University to build a user-friendly database of human epilepsy that links electrical, anatomical, and molecular features of human epilepsy that brings the latest advances in bioinformatics workflow. All of this work truly reflects the human condition as it is made possible through our far-sighted patients that have allowed us to study portions of their brains removed for the treatment of medically refractory epilepsy.
Systems Biology of Epilepsy Project (SBEP)

Clinical Research
- Epilepsy Surgery Program
  - Clinical Database
  - Quantitative Electrophysiology
  - Imaging
  - 3-Dimensional Mapping

Human Tissue Molecular Research
- Genomics
- Proteomics
- Metabolomics
- Tissue Histology
- In Situ Hybridization

Animal Research

Diagnostics

Biomarkers

Therapeutics
What is different about neocortical regions that produce seizures?

**Internal Control:** Epileptic vs. “Less-Epileptic”


Genomics:
Genome-wide transcriptome analysis

**Microarray:** Each spot measures mRNA levels of a single gene from a sample of tissue.

Covers 43,000 genes across the entire Human Genome!
Area of Overlap Suggests Common Pathophysiology
Seizure Onset  vs.  Interictal Spiking

Seizure Onset vs. Interictal Spiking

Validation:

1. Where in the human genome?

2. What Pathways are involved?

3. Where in the neocortex?
   - Layers
   - Cells

4. Are they responsible for seizures?
HOTSPOTS of gene transcription
Validation:

1. Where in the human genome?

2. What Pathways are involved?

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   - Layers
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Seizure Onset vs. Interictal Spiking

# Common Pathway Activation in Human Seizure Onset Neocortex

## Ontology

<table>
<thead>
<tr>
<th>Impact Factor</th>
<th>Pathway</th>
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</thead>
<tbody>
<tr>
<td>90</td>
<td>MAPK signaling</td>
</tr>
<tr>
<td>80</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>70</td>
<td>Focal adhesion</td>
</tr>
<tr>
<td>60</td>
<td>Regulation of actin cytoskeleton</td>
</tr>
<tr>
<td>50</td>
<td>Cytokine receptor interaction</td>
</tr>
<tr>
<td>40</td>
<td>Toll-like receptor signaling</td>
</tr>
<tr>
<td>30</td>
<td>Complement and coagulation</td>
</tr>
<tr>
<td>20</td>
<td>Neuroactive ligand-receptor interaction</td>
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<tr>
<td>10</td>
<td>TGF-beta signaling</td>
</tr>
<tr>
<td>0</td>
<td>Jak-STAT signaling</td>
</tr>
<tr>
<td></td>
<td>Circadian rhythm</td>
</tr>
<tr>
<td></td>
<td>Notch signaling</td>
</tr>
<tr>
<td></td>
<td>Wnt signaling</td>
</tr>
</tbody>
</table>

## Model

- **Epileptic Activity**
- **MAPK**
Common Pathway Activation in Human Seizure Onset Neocortex

Pathway Analysis

Model

Epileptic Activity

MAPK

CREB activation

Immediate Early Genes

Synapse strengthening and plasticity
Validation:

1. Where in the human genome?

2. What Pathways are involved?

3. Where in the neocortex?
   - Layers
   - Cells

4. Are they responsible for seizures?
Why Layer 2/3 Neurons?

Hypothesis: Layer 2/3 neurons have cortical connections that lead to hypersynchrony
Validation:

1. Where in the human genome?

2. What Pathways are involved?

3. Where in the neocortex?
   • Layers
   • Cells

4. Are they responsible for seizures?
The neocortex is not the hippocampus

- Many Lesions Associated with Neocortical Epilepsy
- Epileptogenic Zones are most often normal.
Seizure Onset  vs. Interictal Spiking

- **Seizure Onset**
  - 5 Patients
  - Seizure Onset Genes (137)

- **High Spiking**
  - 15 Patients
  - High Spiking Genes (1700)

- **Control**
  - Low Spiking
  - High Spiking
Gene correlation profiles

Cell A

Cell B

Profile of gene 2

Profile of gene 1
IDENTIFY CELL TYPES USING PUBMED

Cell Types

- leukocyte
- oligodendrocyte
- astrocyte
- schwann
- endothelial
- ependymal
- radial
- satellite
- neuron
- macrophage
- platelet

Gene Set

- CD55
- ATP2A3
- ADAMTS9
- ACTG2
- COL14A1
- COL3A1
- CPA3
- LUM
- OR51E2
- RBPMS
- SVIL
- THBD
- VCAM1

Score

0.00 - 0.10
0.10 - 0.20
0.20 - 0.30
0.30 - 0.40
0.40 - 0.50
0.50 - 0.60
0.60 - 0.70
0.70 - 0.80
0.80 - 0.90
0.90 - 1.00
Cluster Analysis of Gene Expression Patterns Predicts 11 Cellular Changes in High Spiking Human Neocortex
Dendrogram of how cell types change relative to each other.
Dendrogram of how cell types change as a function of spiking.
Downregulated cells are represented on the negative vertical axis.

Dendrogram of how cell types change relative to each other.
BLOOD VESSELS

LOW SPIKING

HIGH SPIKING
DENDROGRAM OF HOW CELL TYPES CHANGE AS A FUNCTION OF SPIKING
Cluster of 280 genes downregulated

The layer IV gene *RorB* is part of this cluster

The NeuN gene is part of this cluster

Predicts a decrease in layer IV NeuN neurons in high spiking brain
INVERSE RELATIONSHIP BETWEEN NEURON (1) AND MICROGLIA (1)

Correlation of fold change profiles = -0.86
Microlesions
‘Microlesions’

NeuN Neurons

H&E LFB Histology
Microlesions
Neocortical Epilepsy (reverse translation)

Activation of MAPK-CREB in superficial layers linked to ongoing interictal spiking

Highly associated with ‘microlesions’ in deeper layers

Is MAPK-CREB required for spiking? (forward translation)
Validation:

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Tetanus Toxin Somatosensory Cortex Interictal Spiking Model

Setup

Record
Focal interictal spiking

Chronic and progressive
CREB is phosphorylated on spiking side in layers 2/3.
Identification of drug candidates to test in this model:

pERK1/2  SL327
MAPK Inhibitor Blocks the Development of Epileptic Spiking (Interictal)

MAP Kinase inhibition blocks the development of interictal spiking

“Morning after pill”
Conclusions

• The hippocampus is not the neocortex

• *Reverse Translation*: Systems biology of well-characterized human cortex generates new hypotheses

• *Chromosomal Hotspots* of epileptic gene transcription

• Biomarkers of epileptic spiking localized to Layer 2/3

• A new gene clustering method predicts novel ‘Microlesions’ in deeper layers of epileptic spiking

• Animal models (forward translation) turn biomarkers into new *Drug Targets*
Wayne State Collaborators

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