Epilepsy in the Developing Brain

Focus on Neuropathology Research
Past, Present and Future.

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The Past → Present

Neuropathology (cellular based / tissue studies) have played a vital part in understanding interactions of seizures and the developing brain.
The Doublecortin Story

Subcortical Laminar heterotopia

Pathology described ~1936

First neuroimaging reports ~1981

Livingstone and Aicardi
A Novel CNS Gene Required for Neuronal Migration and Involved in X-Linked Subcortical Laminar Heterotopia and Lissencephaly Syndrome

Vincent des Portes,¹ Jean Marc Pinard,² Pierre Billuart,¹ Marie Claude Vinet,¹ Annette Koulakoff,³ Alain Carrié,¹ Antoinette Gelot,⁴ Elisabeth Dupuis,⁵ Jacques Motte,⁶ Yoheved Berwald-Netter,³ Martin Catala,⁷ Axel Kahn,¹ Cherif Beldjord,¹ and Jamel Chelly¹,⁸

Doublecortin gene identified - 1998
Doublecortin (DCX) and normal brain development

**Fetal**

- DCX Human 15 days gestation
- http://www.hudsen.org/

**Birth**

- Day 2, layer II cortex

**Adult**

- Age 2, Epilepsy
- Age 42, Epilepsy

Srikandarajah, 2009
Epileptogenesis mechanisms, 2007

Doublecortin – experimental replacement, 2008

Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder

Jean-Bernard Manenti, Yu Wang, YoonJeung Chang, Murugan Paramasivam & Joseph J LoTurco

Bataglia, 2007
‘An immature brain is not a small adult brain’

Granule cell dispersion in Hippocampal sclerosis / TLE

Martinian, 2012
GABAergic excitation after febrile seizures induces ectopic granule cells and adult epilepsy

Ryuta Koyama1, Kentaro Tao1,2, Takuya Sasaki1,2, Junya Ichikawa1, Daisuke Miyamoto1, Rieko Muramatsu1, Norio Matsuki1 & Yuji Ikegaya1

Epilepsy-Induced Motility of Differentiated Neurons

Xuejun Chai1,2, Gert Münzner2,3, Shanting Zhao1, Stefanie Timnes2, Janina Kowalski2, Ute Häussler2, Christina Young1, Carola A. Haas3,4 and Michael Frotscher1,5
Natural history of refractory mTLE/hippocampal sclerosis

Febrile seizures → Granule Cell Dispersion → Epilepsy and Hippocampal sclerosis → MRI → Surgery
Febrile seizures → Granule Cell Dispersion → Epilepsy and Hippocampal sclerosis → MRI → Surgery

Detection on MRI

Reversal with targeted treatments with Reelin / anti-GABA agents

Future: Natural History refractory mTLE/HS?
mTOR Pathway Activation in Tubers and Focal Cortical Dysplasia

mTOR Cascade Activation Distinguishes Tubers from Focal Cortical Dysplasia

Marianna Baybis, MS,1 Jia Yu, MD,1 Allana Lee, BA, Jeff A. Golden, MD,2 Howard Weiner, MD,3 Guy McKhann II, MD,4 Eleonora Aronica, MD,5 and Peter B. Crino, MD, PhD6

Detection of Human Papillomavirus in Human Focal Cortical Dysplasia Type IIb

Julie Chen, BA,1 Victoria Tsai, MS,1 Whitney E. Parker, BA,1 Eleonora Aronica, MD, PhD,2,3 Marianna Baybis, MS,1 and Peter B. Crino, MD, PhD1,4

panHPV

mTOR Pathway

Cell growth and metabolism

ATP

LKB1

AMPK

REDD1

p306

PKB

p308

p473

FOXO

Rheb

GTP

GDF

GEF

Rheb

GTP

TSC1

TSC2

mTOR

QBL

raptor

PRAS40

4EBP-1

eIF4E

S6K

eIF4E2K

PDCD4

Cell growth and metabolism

TRENDS in Molecular Medicine
Epilepsy neuropathology studies in the developing brain have highlighted

1. Reciprocal influences between seizures and continuing brain development
2. Pro-epileptogenetic processes
3. New biomarkers and novel treatment pathways
Time course of brain development

- White matter volume
- Gray matter volume

Weeks post-conception: 0, 8, 16, 24, 32

Birth

Years of age: 1, 2, 5, 10, 15, 20

- Neurogenesis
- Immune System
- Gliogenesis
- Synaptogenesis
- Myelination
- Synaptic pruning

Semple 2013
Developmental genes
Migration
Cell specification
Regional expression

Seizures

'EPILEPTOGENESIS'
Neurotransmitters
Cell signalling
Astroglia
Microglia
BBB
Structural changes

Gene mutation
Epigenetic factors

Neuronal proliferation
Neuronal migration
Myelination
Synaptogenesis
Apoptosis
Neurochemical maturation
Cortical cytoarchitecture

4 8 12 16 20 24 28 32 36 Birth 4 months Adolescence Adulthood
Dysmyelination in Focal Cortical Dysplasia (FCDIIIB)

Myelin basic protein

Dysplasia

Normal

Shepherd 2013

% Reduction of labelling in ROI 1 compared to ROI 3

Duration (years)
Possible mechanisms of epileptogenesis acting in focal epilepsy:

- **Neuronal loss** ? essential for epileptogenesis
- **Neuronal hypertrophy & altered morphology** (in tumours and dysplasias)
- **Alteration of extracellular matrix**
- **Axonal sprouting**
- **Interneuronal alterations**
  - Loss
  - Sprouting
  - Dormancy
  - Altered networks
- **Neurogenesis**
  - Integration of new neurones into circuits
  - Abnormal physiology
- **Blood brain barrier alteration**
  - Angiogenesis
- **Gliosis**
  - Physical interruption of axon repair
- **Channelopathy**
  - Acquired changes to ligand-gated and receptor-gated ion channels
  - Emergence of ‘Pacemaker cells’
- **Astrocyte dysfunction**
  - Glutamate metabolism and transport
  - Alteration of K+ homeostasis
  - Inflammatory mediators
  - Synaptic transmission
- **Epidephlogistic factors**
  - Neuronal degeneration
  - Axonal sprouting
  - Dendritic and synaptic plasticity
  - Inflammatory cells
    - Interleukins, cytokine synthesis, complement
- **Background genes**
- **Developmentally regulated genes**
- **Epigenetic factors**

**Functional outcome**

- Epilepsy
- Memory impairment
- Drug resistance
- Developmental delay

**Epileptogenesis mechanisms!**
Current and future tissue technologies

- Proteomics
- Fresh
  - Cell culture / slice culture / electrophysiology
  - Functional properties
  - Immunohistochemistry, Confocal ISH, FISH

- Fixed
  - Laser cell microdissection
  - Lineage Differentiation Distribution
  - Morphology
  - Visualising synapses

- Tissue 9T MRI

- Cell gene expression

Yuki Goda
Value of human tissue research

• Exploration of complexity
  – Compared to animal models of epilepsy

• Localisation
  – Cell subtype, cortical layer, region, networks

• Greater resolution
  – Compared to neuroimaging

• Effects of local environment
  – ECM, glia, inflammatory cells, BBB

• Pathology diagnosis and classification
  – Benchmark or ‘gold standard’
‘Fine tuning’ neuropathology diagnostic criteria.
ILAE neuropathology task force 2010-13

FCD and HS - reclassification

Virtual microscopy

Teaching / training our workforce

http://community.ilae-epilepsy.org/diagnosticmethods/MEMBERS/NeuropathologyTaskForce/
Value of post-mortem brain tissue in epilepsy research

- Can compare epileptogenic and normal regions in focal epilepsies
- Enables the study of ‘non-surgical’ epilepsies
- Investigations into SUDEP
- Study of secondary or long term effects of seizures and co-morbidities
Epilepsy Brain/Tissue Banks

Problems
Few dedicated epilepsy brain banks
Decline in autopsy rate
Public perception of organ retention
Collection of atypical cases

Advantages
Enables collection/sharing of rare pathologies
Specific brain regions sampled, collected relevant to condition
Relevant clinical data collected
<table>
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<th>Epilepsy Syndromes in Neonatal Period – Total Post Mortem studies last 15 years</th>
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Clinical syndrome
Prevention of epilepsy
Acknowledgments

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Members of the Neuropathology Task Force of ILAE