Basic Mechanisms Underlying Seizures and Epilepsy

American Epilepsy Society
Definitions

- **Seizure**: the clinical manifestation of an abnormal and excessive synchronization of a population of cortical neurons.

- **Epilepsy**: a tendency toward recurrent seizures unprovoked by any systemic or acute neurologic insults.

- **Epileptogenesis**: sequence of events that converts a normal neuronal network into a hyperexcitable network.

*Revised per Ruteckie 7/06*
Types of Seizures

- **Partial**: Seizures arising from a focal area of the brain.
  - Simple partial: focal seizures without loss of consciousness
  - Complex partial: focal seizures with loss of consciousness

- **Generalized**: Seizures which involve the entire brain.
  - Types of generalized seizures: absence seizures, myoclonic seizures, clonic seizures, tonic seizures, tonic-clonic seizures, atonic seizures.
Types of Epilepsies

- **Idiopathic:** Idiopathic epilepsy arises from an unknown cause thought to have a strong genetic basis.

- **Symptomatic:** Epilepsy is called symptomatic when it arises from a known cause, which may include:
  - Head injury
  - Brain tumor
  - Stroke
  - Etc.
In Vivo Models of Epilepsy

- Numerous genetic models of epilepsies.
- Acquired focal models (lesions, injury)
- Chemoconvulsant models of seizures (kainic acid, PTZ, picrotoxin)
- Chemoconvulsant models of status epilepticus (kainic acid, pilocarpine)
- Electrical stimulation (kindling)
- Models of epileptogenesis (kainic acid, pilocarpine)
In Vitro Models of Epileptiform Activity

- Resected tissue from patients with epilepsy
- Cell culture models
- Acute slice models (4-AP, low Mg2+, high KCl)
- Organotypic slices
Temporal Lobe Epilepsy

- Temporal lobe epilepsy (TLE) refers to an epilepsy syndrome with seizure arising from the temporal lobe with a high degree of involvement of the hippocampus.

- TLE is the most common form of epilepsy.

- A great deal of basic epilepsy research has focused on temporal lobe epilepsy. Therefore, studies of hippocampal pathology and pathophysiology during epileptogenesis will be a focus of this tutorial.
Basic Mechanisms Underlying Seizures and Epilepsy

- Feedback and feed-forward inhibition, illustrated via cartoon and schematic of simplified hippocampal circuit

Epilepsy—Basic Neurophysiology

- Causes of Hyperexcitability:
  - excitatory post synaptic potentials (EPSPs)
  - inhibitory post synaptic potentials (IPSPs)
  - changes in voltage gated ion channels
  - alteration of local ion concentrations
Epilepsy—Basic Neurophysiology

- Major Neurotransmitters in the brain:
  - Glutamate
  - GABA
  - Acetylcholine
  - Dopamine
  - Serotonin
  - Histamine
  - Other modulators: neuropeptides, hormones
Epilepsy—Glutamate

- The brain’s major excitatory neurotransmitter

- Two groups of glutamate receptors
  - Ionotropic—fast synaptic transmission
    - Three subtypes – AMPA, kainate, NMDA
    - Glutamate-gated cation channels
  - Metabotropic—slow synaptic transmission
    - G-protein coupled, regulation of second messengers (cAMP and phospholipase C)
    - Modulation of synaptic activity

- Modulation of glutamate receptors
  - Glycine, polyamine sites, Zinc, redox site
Epilepsy—Glutamate

Diagram of the various glutamate receptor subtypes and locations

From Takumi et al, 1998
Epilepsy—GABA

- Major inhibitory neurotransmitter in the CNS
- Two types of receptors
  - $\text{GABA}_A$—post-synaptic, specific recognition sites, linked to $\text{Cl}^-$ channel
  - $\text{GABA}_B$—presynaptic autoreceptors that reduce transmitter release by decreasing calcium influx, postsynaptic coupled to G-proteins to increase $\text{K}^+$ current
Epilepsy—GABA

Diagram of the GABA_A receptor

From Olsen and Sapp, 1995

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Cellular Mechanisms of Seizure Generation

- **Excitation (too much)**
  - Ionic—inward Na\(^+\), Ca\(^{++}\) currents
  - Neurotransmitter—glutamate, aspartate

- **Inhibition (too little)**
  - Ionic—inward Cl\(^-\), outward K\(^+\) currents
  - Neurotransmitter—GABA
Neuronal (Intrinsic) Factors Modifying Neuronal Excitability

- Ion channel type, number, and distribution
- Post-translational modification of channels (phosphorylation, etc).
- Activation of second-messenger systems that affect channel function (e.g. G proteins)
- Modulation of gene expression of ion channels
Epilepsy and Channelopathies

- Inherited
  - Voltage-gated ion channel mutations
  - Ligand-gated ion channel (neurotransmitter receptor) mutations
  - Different mutations in the same gene can result in radically different types of seizures and epilepsy

- Acquired
  - Auto-immune (anti-potassium channel antibodies)
  - Changes in channel expression after seizures
Ion Channel & Neurotransmitter Receptors Mutated in Epilepsy - I

Voltage-gated Sodium Channel Gene Mutations

- *SCN1A*
  - Generalized Epilepsy & Febrile Seizures Plus (GEFS+) type 2
  - Severe Myoclonic Epilepsy of Infancy (SMEI)

- *SCN1B*
  - GEFS+ type 1

- *SCN2A1*
  - GEFS+
  - Benign Familial Neonatal-Infantile Seizures (BFNIS)
Ion Channel & Neurotransmitter Receptors Mutated in Epilepsy - II

Voltage-gated Chloride Channel Gene Mutations

- *CLCN2A*
  - Juvenile Absence Epilepsy (JAE)
  - Juvenile Myoclonic Epilepsy (JME)
  - Epilepsy with Grand Mal upon Awakening (EGMA)
Voltage-gated Potassium Channel Mutations

- *KCNQ2, KCNQ3*
  - Benign Familial Neonatal Convulsions (BFNC)

- *KCND2*
  - Temporal Lobe Epilepsy (TLE)

- *KCNMA1*
  - Generalized Epilepsy with Paroxysmal Dyskinesia (GEPD)
Neurotransmitter Receptor Mutations

- *GABRG2* (GABA-receptor gamma-2 subunit)
  - GEFS+ type 3
- *GABRA1* (GABA-receptor alpha-1 subunit)
  - JME
- *CHRNA4* (nicotinic acetylcholine receptor alpha-4 subunit)
  - Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE) type 1
- *CHRNBJ2* (nicotinic acetylcholine receptor beta-2 subunit)
  - ADNFLE type 3
Synaptic Factors Modifying Neuronal Excitability

- Alterations in expression of transmitter gated ionotropic channels
- Post-translational changes in neurotransmitter channels
- Remodeling of synapse location or configuration (deafferentation, sprouting)
- Changes in gap-junction synaptic function
Non-synaptic (Extrinsic) Factors Modifying Neuronal Excitability

♦ Changes in extracellular ion concentration

♦ Changes in extracellular space

♦ Modulation of transmitter metabolism or uptake by glial cells
Mechanisms of Generating Hyperexcitable Networks

- Excitatory axonal “sprouting”
- Loss of inhibitory neurons
- Loss of excitatory neurons “driving” inhibitory neurons
- Change in neuronal firing properties (channelopathies)
Electroencephalogram (EEG)

- Graphical depiction of cortical electrical activity, usually recorded from the scalp.
- Advantage of high temporal resolution but poor spatial resolution of cortical disorders.
- EEG is the most important neurophysiological study for the diagnosis, prognosis, and treatment of epilepsy.
10/20 System of EEG Electrode Placement
Physiological Basis of the EEG

- Extracellular dipole generated by excitatory post-synaptic potential at apical dendrite of pyramidal cell
Physiological Basis of the EEG (cont.)

- Electrical field generated by similarly oriented pyramidal cells in cortex (layer 5) and detected by scalp electrode
Electroencephalogram (EEG)

- Clinical applications
  - Seizures/epilepsy
  - Sleep
  - Altered consciousness
  - Focal and diffuse disturbances in cerebral functioning
EEG Frequencies

- Gamma: 30-60 Hz
- Beta: 13-30 Hz
- Alpha: 8 to ≤ 13 Hz
- Theta: 4 to under 8 Hz
- Delta: < 4 Hz
EEG Frequencies

Awake - eyes open - beta - 18-25 cycles per second (cps)

Relaxed - eyes closed - alpha - 8-13 cps

Stage 1 - theta - 4-8 cps

Stage 2 - 12-14 cps - sleep spindles and K complexes

Radtke, in Ebersole and Pedley, 2003
EEG Frequencies

Stages 3 and 4 - delta (or slow wave sleep) waves 1-4 cps > 75uV

REM sleep - 3-7 cps (like theta or stage 1)

Radtke, in Ebersole and Pedley, 2003
EEG Abnormalities

- **Background activity abnormalities**
  - Slowing not consistent with behavioral state
  - May be focal, lateralized, or generalized
  - Significant asymmetry

- **Transient abnormalities / Discharges**
  - Spikes
  - Sharp waves
  - Spike and slow wave complexes
  - May be focal, lateralized, or generalized
Focal seizure generation

- Seizure initiation
  - burst of action potentials, i.e. paroxysmal depolarizing shift
  - hypersynchronization of neighboring cells
- Propagation
  - activation of nearby neurons
  - loss of surrounding inhibition
Sharp Waves

An example of a left temporal lobe sharp wave (arrow)
The “Interictal Spike and Paroxysmal Depolarization Shift”

Intracellular and extracellular events of the paroxysmal depolarizing shift underlying the interictal epileptiform spike detected by surface EEG

Ayala et al., 1973
Generalized Spike Wave Discharge
EEG: Absence Seizure
Circuitry Underlying Generalized Epilepsies

McCormick and Contreras, 2001
Causes of Acquired Epilepsy

- Severe head injury
- Cerebral hemorrhage
- Brain tumor
- CNS infection
- Early life febrile seizures
Development of acquired epilepsy

Acute → Latent → Chronic

Insult to CNS → Epileptogenesis → Epilepsy
Development of acquired epilepsy

- Acute
- Latent
- Chronic

Insult to CNS → Epileptogenesis
- Altered ion channels
- Altered connectivity, neurotrophins, etc.

Seizure → Epilepsy
Possible Mechanism of Delayed Epileptogenesis

- Kindling model: repeated subconvulsive stimuli resulting in electrical afterdischarges
  - Eventually lead to stimulation-induced clinical seizures
  - In some cases, lead to spontaneous seizures (epilepsy)
  - Applicability to human epilepsy uncertain
The hippocampus: a model-systems approach to understanding epilepsy

- The following slides will focus on the pathophysiology and circuitry changes in the hippocampus during epileptogenesis.
- These processes may have direct relevance to temporal lobe epilepsy, but also may provide insight into the development of other types of epilepsy.
- The hippocampus does present a special case, however, because of the high rate of lifelong neurogenesis identified in this region.
Epileptogenesis

- Epileptic Focus
- Cortical Seizures
  - Early propagation
  - Late

- Epileptogenic Zone

- Hippocampus
  - Seizures
    - Enhanced Susceptibility To Further Seizures
    - Neuronal Injury
    - Increased Excitability
    - Rewiring of Neuronal Networks

Cavazos and Cross, 2006
Hippocampal Anatomy

From Chang and Lowenstein, 2003
Newborn granule cells in rodent epilepsy models contribute to three distinct anatomical alterations in the epileptic brain

1. Mossy fiber sprouting
2. Ectopic granule cell migration
3. Hilar basal dendrite sprouting
Mossy fiber sprouting is observed animal models and human temporal lobe epilepsy

In control rats, Timm staining of mossy fibers is restricted to the hilus and the CA3 stratum lucidum. (B) A profound mossy fiber sprouting response in the kainate-induced status epilepticus group of rats, with mossy fibers traversing the dispersed granule cell layer into the inner molecular layer. There is some sprouting of the mossy fibers into the stratum oriens of the CA3 subfield. Brain Research 1066 (1-2)129-146.

Photomicrographs illustrating the differences in neo-Timm's staining in non-sclerotic (non-HS) and sclerotic (HS) human hippocampi. (B) Normal distribution of human mossy fibers limited to hilus and CA3. In the HS specimen. (D) Aberrant mossy fiber sprouting in the FD inner molecular layer (IML) of an HS specimen. FD, fascia dentata; h, hilus; hf, hippocampal fissure, SUB, subiculum complex; alv, alveus. Scale bar: 500 μm. Ying et al. Brain Research 798; (1-2) 239-246.
Hippocampal Circuit Changes With Hippocampal Sclerosis

Chang and Lowenstein, 2003
Neuronal “birthdating” with BrdU reveals increased generation of new neurons in the hippocampal dentate gyrus during epileptogenesis.

Control

Post-Status Epilepticus

Parent et al. J. Neurosci 1999
New neurons generated during epileptogenesis integrate abnormally

Hilar ectopic granule cells in a rat model of temporal lobe epilepsy. (A) Nissl-stained section showing the hippocampus in the transverse axis. The boxed area is shown in part B. (B) A section is stained with an antibody to Prox1, a specific marker of GC nuclei in the dentate gyrus. In the saline-treated control, Prox1-labeled cells in the subgranular zone (SGZ) and hilus (HIL) are rare. In the pilocarpine-treated epileptic rat, there are numerous hilar and SGZ cells (arrows). MOL, molecular layer; GCL, granule cell layer; HIL, hilus. Calibration = 250 lm (A) and 150 lm (B). Epilepsia, 53(Suppl. 1):98–108, 2012.
New neurons generated during epileptogenesis integrate abnormally

Danzer, The Neuroscientist., 2008
Ectopic granule cells are also seen in human temporal lobe epilepsy and is associated with granule cell layer dispersion.

**Ectopic granule cells**
Ectopic granule cells in the hilus of the dentate gyrus in epilepsy related to hippocampal sclerosis in humans. **A:** Ectopic granule cells (arrows) in the hilus of the dentate gyrus labeled with NeuN. **B:** Individual ectopic granule cells (arrows) in the hilus revealed with calbindin-staining.

**Granule cell layer dispersion**
C: In epilepsy associated with hippocampal sclerosis, a large part of the granule cell layer appears as a bi-layer. Boxed area in C is shown at higher magnification in D. **D:** Granule cells forming two relatively compact cell layers with a gap between them.

Abraham et al. Brain Research 1399; 66-78.
Ectopic cells contribute to mossy fiber sprouting

Ectopic cells have abnormal physiology

New neurons generated during epileptogenesis integrate abnormally
BrdU Birthdating of newborn cells with basal dendrites

5-week-old DGCs -- born 3 weeks after SE

Walter et al., J. Neuroscience, 2007
Basal dendrites receive excitatory MF input

Summary of synapses with granule cell basal dendrites from epileptic rats. Proximal is up, distal is down. Synapses are indicated by markers. Most synapses are with GABA-negative spines. Thind et al., The Journal of Comparative Neurology; 509:190–202 (2008)

Schematic diagram of granule cells in the dentate gyrus of the hippocampal formation
A shows a normal granule cell with its dendrites in the molecular layer (ML), cell body in the granule cell layer (GL) and axon terminals (filled circles) in the hilus (H). B is a granule cell from an epileptic rat showing mossy fiber sprouting (3 axon terminals in the ML). C shows a granule cell with a hilar basal dendrite (HBD) in the hilus (arrow) that is postsynaptic to mossy fiber collaterals (7 axon terminals) and sprouted mossy fibers in the ML (3 axon terminals). Reprinted from Shapiro and Ribak, Brain Res Reviews. 2005;48:43–56.
Juvenile, immature and newborn granule cells underlie aberrant neuronal integration in rodent TLE models. Mature cells are comparatively normal.

Abnormally interconnected granule cells impair the dentate gate.
Formation of recurrent excitatory connections in the epileptic dentate gyrus is hypothesized to impair the dentate gate.

Figure 3–57. Summary of the serial and parallel pathways through the hippocampal formation. Although the intrinsic hippocampal circuitry is largely unidirectional, it contains both serial and parallel projections. See text for details. The Hippocampus Book, Oxford U. Press
Summary of abnormal integration of newborn granule cells and their putative role in seizures

1. Newborn granule cells sprout mossy fibers axons that result in aberrant integration into Hippocampal circuitry.

2. Newborn granule cells sprout hilar basal dendrites that receive mossy fiber synapses in the hilus, further altering the hippocampal circuitry.

3. Newborn granule cells migrate into the hilus, resulting in abnormal granule cell layer morphology.

These 3 anatomical changes are hypothesized to alter hippocampal physiology, resulting in a pro-epileptogenic circuitry. These functional changes are shown in the next two slides.
Summary

- Seizures appear to represent an attractor state for the brain wherein slight disruptions in normal brain circuitry or physiology arising from many different situations can result in a final common pathway.

- Known causes of epilepsy are genetic and acquired and can range from slight modifications of single channels to reorganization of whole brain regions.

- A combination of molecular, anatomical, physiological, and behavioral tools are used by investigators to study the epileptogenic process, seizure triggers, and seizure disruption.

- Animals ranging from zebrafish to primates are used to model seizures and epilepsy.

- Currently, the study of epileptogenesis in the hippocampus of rodents (rats and mice) are the most common.

- Hippocampal-derived epileptogenesis presents a special case because of the lifelong neurogenesis in this region.