A mouse model of extended hippocampal kindling: features of spontaneous recurrent seizures

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Kindling model

Modelling temporal lobe epilepsy
Focal seizures and secondary generation

Classic kindling
Evoked stage-5 seizures after 30-40 stimuli

Extended kindling
Spontaneous recurrent seizures after >100 stimuli
No gross brain injury but subtle hilar cell loss (vs. post status models)
Reliable and reproducible

Laborious
Spontaneous recurrent seizures previously observed in extended kindling models

Rats
- Milgram et al., 1995, Brain Res 670(1):112-120.

Cats

Monkeys
Objectives

- To establish a mouse model of extended hippocampal kindling with spontaneous recurrent seizures

- To characterize EEG and pharmacological features of these seizures

- To explore effects of potential antiepileptic treatments (drugs, brain stimulation or others) and ictal genic mechanisms
Experimental protocol

- C57BL, 8 or 11-12 months-olds;
- Electrodes: bilateral CA3; unilateral CA3-perietal, CA3-piriform or CA3-entorhinal
- 24-h EEG and video, for up to 11 consecutive days
- Hippo. stim. twice daily, at 60 hz for 2 s, 125% of after-discharge threshold
Spontaneous seizures: Concurrent EEG ictal discharges and motor convulsions

![EEG and motor convulsions](image)

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Spontaneous recurrent seizures detected after 80-120 stimulations in most of mice kindled (n=45).
Durations and intervals of EEG ictal discharges

A

hippocampal EEG ictal discharges

B

hippocampal EEG ictal discharge
(n=244 events from 8 mice)

count

inter-ictal intervals (hour)

0 5 10 15 20 25

C

ictal discharge durations (sec)

0 20 40 60 80 100 120 140

count

clustering
Regional EEG ictal discharges

Discharges from one region – no; separation of discharges from motor seizures - no
## Effects of antiepileptic and other drugs on spontaneous recurrent seizures

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Doses and administrations</th>
<th>Effects on seizures</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>20mg/kg, single ip.</td>
<td>abolished</td>
<td>7/7</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>100 mg/kg, 3 ip, every 4 hours</td>
<td>incidences decreased</td>
<td>2/5</td>
</tr>
<tr>
<td></td>
<td>400mg/kg, 3 ip, every 4 hours</td>
<td>abolished</td>
<td>7/7</td>
</tr>
<tr>
<td>Valproate</td>
<td>300mg/kg, 3 ip, every 4 hours</td>
<td>abolished</td>
<td>6/6</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>100mg/kg, 3 ip, every 4 hours</td>
<td>abolished</td>
<td>5/5</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>1.5mg/kg, single ip</td>
<td>motor seizure suppressed</td>
<td>7/7</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10mg/kg, 3 ip. every 4 hours</td>
<td>no suppression</td>
<td>4</td>
</tr>
<tr>
<td>NBQX</td>
<td>40mg/kg, 3 ip, every 4 hours</td>
<td>no suppression</td>
<td>4</td>
</tr>
<tr>
<td>Perampanel</td>
<td>10mg/kg</td>
<td>solubility problem</td>
<td>3</td>
</tr>
</tbody>
</table>
An example of phenytoin’s effects

Phenytoin 25mg/kg, single ip
General features of spontaneous recurrent seizures in mice following extended hippocampal kindling

Generalized seizures: EEG ictal discharges involving multiple structures and motor convulsions of stages 3-5

Relatively stable in seizure incidence (3-10/day) and severity

Responded to AEDs (but in an all-or-none manner)

In all seizure events detected (>45 mice, >15-120 seizures/mouse), there were no isolated regional discharges (i.e., hipp. or piriform only) or EEG discharges without motor convulsion.

Focal onset with secondary generation?
Performance of extended kindled and handling control mice in water maze task

Improved performance after AED treatment?
An example of hippocampal stimulations on spontaneous recurrent seizures

**A**

- **no stimulation**
- **contralateral hippoc. stim.**
  - 1 Hz
  - 5 Hz
  - 100 Hz

**B**

- **no stimulation**
- **1 Hz stim.**
- **5 Hz stim.**
- **100 Hz stim.**
No gross brain injury but hippocampal hilar cell loss in extended kindled mice
Paired pulse inhibition of the dentate gyrus was diminished in slices of extended kindled mice.

No epileptiform activity was observed from the dentate gyrus area of kindled mice under baseline conditions.
Similar evoked IPSCs in CA3 pyramidal neurons of control and extended kindled mice

No epileptiform activity was observed from the CA3 area of kindled mice under baseline conditions
Ictal-like events induced by alkaline artificial cerebrospinal fluid in brain slices of extended kindled mice

Huberfield et al, Nature Neurosci. 2011, (pH 7.8)
Propensity of ictal-like events induced in brain slices of control and extended kindled mice

**controls**
- hipp. CA3: 28 slices/7 min
- pirif. cort.: 15 slices/6 mice
- entor. cort.: 11 slices/6 mice

**extended kindled**
- ictal: 26 slices/6 mice
- 16 slices/5 mice

- durations of ictal-like discharges (sec)
  - hippocampal CA3: * *
  - entorhinal cortex
  - piriform cortex

- intervals of ictal-like discharges (sec)
Future experiments

- To examine deeper subcortical inputs in genesis of spontaneous recurrent seizures

- To examine local circuitry and cellular activities related ictal genesis

- To assess behavioral performance in water maze, fear conditioning, forced swimming and sucrose preference tasks

- To further test the effects of brain electrical stimulation or potential antiepileptic drugs on spontaneous recurrent seizures
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